

**Diffusive Gradients in Thin Films (DGT) as a technique  
to predict nutrient availability to plants**

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## Abstract

The soils of Australia have extensive macro and micronutrient disorders varying greatly in their capacities to provide the chemical nutrients essential for plant growth. Assessment of nutrient availability in soils is important in order to maximise fertilizer efficiency and crop yields and to minimise environmental pollution associated with over fertilisation. Nutrient availability has proven difficult to assess due to the complexity of trace element soil chemistry and plant uptake mechanisms. The relatively new method, Diffusive Gradients in Thin Films (DGT), provides the potential to become an alternative soil test that could accurately predict nutrient availability.

To date, DGT technology has only been designed for separate assessment of anionic and cationic species in waters or soils typically at concentrations characteristic of highly contaminated systems. In this study a new mixed binding gel (MBL) was developed capable of simultaneous assessment of cations and anions in a single assay at concentrations more representative of uncontaminated agricultural soils, sediments and waters. The MBL has the potential to eliminate measurement errors associated with very fine spatial scale changes in element concentrations in these environments.

The MBL consisted of ferrihydrite and Chelex-100 cation exchange resin combined together in a binding gel. Results from the MBL were comparable to experiments performed using individual Chelex gels and ferrihydrite gels that have been shown to work successfully for DGT methodology. To facilitate combined analysis of P and cations by ICP-MS, HCl (1 M) was used for gel elution to minimise interferences from  $^{14}\text{N}^{16}\text{OH}$  or  $^{15}\text{N}^{16}\text{O}$  on  $^{31}\text{P}$ . All elements tested (Cd, Cu, Mn, Mo, P and Zn) were bound successfully to the MBL. DGT measurements obtained using the MBL on agricultural soils correlated well ( $r^2 = 0.95$ ) with measurements obtained using pure Chelex and ferrihydrite binding layers. This suggests that the MBL could be used for simultaneous measurement of cationic and anionic element availability in soils.

Performance of the Diffusive Gradient in Thin Films (DGT) technique was compared with three other common testing methods (Colwell, Olsen, Resin) for available soil P in terms of the ability of each to predict wheat, canola, lupin and barley responsiveness to applied P on 21 Australian agricultural soils. DGT accurately predicted plant responsiveness in > 90 % of the soils used. In contrast the other soil testing methods failed to correctly predict plant response to P on numerous occasions. These observations reveal that the DGT technique with the newly developed MBL can predict plant available P on these soils with greater accuracy than other traditional soil P testing methods and could become a useful tool for predicting P fertilizer requirements.

The DGT method using the MBL was also used to test Zn deficiency thresholds for canola and wheat in a manufactured soil (acid washed sand). DGT successfully determined the threshold for Zn deficiency in this soil, overcoming detection limit issues usually accompanying such low levels of Zn. This method also provides that potential to assess other micronutrients (Mn, Cu) and with further modification potentially assess K.

Before DGT can become established as an alternative soil testing method, validation of the performance is required under field conditions. This study has shown that it out performs current common soil testing methods in glasshouse conditions but questions still remain if this will be reflected out in the field.

## Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person except where due reference has been made in the text.

I give consent for this thesis, when deposited in the University Library, to be available for loan and photocopying.

**Sean David Mason**

**Date**

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## Abbreviations

A	Area (cm <sup>2</sup> )
AAS	Atomic Adsorption Spectrometer
AEM	Anionic Exchange Membrane
Ag	Silver
Al	Aluminium
As	Arsenic
ASV	Anodic Stripping Voltammetry
Ba	Barium
Ca	Calcium
CaCl <sub>2</sub>	Calcium Chloride
CaCO <sub>3</sub>	Calcium Carbonate
Cd	Cadmium
C <sub>DGT</sub>	Concentration DGT
C <sub>diff</sub>	DGT interfacial concentration (diffusion only case)
C <sub>E</sub>	Effective concentration (DGT)
C <sub>e</sub>	Concentration of the eluted gel (DGT)
C <sub>i</sub>	DGT interfacial concentration of labile analyte species
C <sub>ls</sub>	Liabile element pool on the solid phase
Cl	Chloride
cm	Centimetre
Co	Cobalt
CoSO <sub>4</sub> .7H <sub>2</sub> O	Cobalt Sulfate
CO <sub>2</sub>	Carbon Dioxide
Cr	Chromium
CSIRO	Commonwealth Scientific Industrial and Research Organisation
C <sub>soln</sub>	Soil solution concentration (diffusion-only case)
Cu	Copper
CuSO <sub>4</sub> .5H <sub>2</sub> O	Copper Sulfate
D	Diffusion gradient of element through the diffusive layer
DGT	Diffusive Gradients in Thin Films
DI	Deionised
DIFS	DGT Induced Fluxes in Sediments and Soils
D <sub>o</sub>	Element diffusion coefficient in water
DOC	Dissolved Organic Carbon
dp	Density of soil particles (2.65 g cm <sup>-3</sup> )
D <sub>s</sub>	Diffusion gradient of element through soil
DTPA	Diethylenetriamenepentaacetate acid
EC	Electrical Conductivity
ETDA	Ethylenedinitrilotetraacetic acid
F	Fluoride
F	Flux of analyte through diffusive and filter layers (DGT)
FA	Fluvic Acid
Fe	Iron
F <sub>e</sub>	Elution factor (DGT)
FeSO <sub>4</sub> .7H <sub>2</sub> O	Iron Sulfate
g	Gram
GFAAS	Graphite Furnace Atomic Adsorption Spectrometer
h	Hour

H	Hydrogen
ha	Hectare
H <sub>2</sub> SO <sub>4</sub>	Sulfuric acid
H <sub>3</sub> BO <sub>3</sub>	Boric acid
H <sub>3</sub> PO <sub>4</sub>	Phosphoric acid
HCL	Hydrochloric acid
HCO <sub>3</sub> <sup>-</sup>	Carbonate
Hg	Mercury
HNO <sub>3</sub>	Nitrate
<i>I</i>	Intensity (soil solution concentration)
ICPMS	Inductively Coupled Plasma Mass Spectrometry
ICPOES	Inductively Coupled Plasma Optical Emission Spectrometry
IS	Ionic Strength
IUPAC	International Union of Pure and Applied Chemistry
K	Potassium
K <sub>2</sub> SO <sub>4</sub>	Potassium Sulfate
K <sub>2</sub> PO <sub>4</sub>	Potassium Phosphate
kg	Kilogram
l	Litre
M	Molar
m	Metre
M	Measured accumulated mass (DGT)
MBL	Mixed Binding Layer (DGT)
MDL	Method Detection Limit
Mg	Magnesium
MgSO <sub>4</sub> .7H <sub>2</sub> O	Magnesium Sulfate
mg	Milli-gram
MgCl <sub>2</sub>	Magnesium Chloride
ml	Milli-litre
mM	Milli-molar
mm	Milli-metre
Mn	Manganese
MnO <sub>2</sub>	Manganese Oxide
MnSO <sub>4</sub> .7H <sub>2</sub> O	Manganese Sulfate
Mo	Molybdenum
N	Nitrogen
Na	Sodium
NaCl	Sodium Chloride
NaHCO <sub>3</sub>	Sodium Bicarbonate
NaNO <sub>3</sub>	Sodium Nitrate
NaOH	Sodium Hydroxide
Na <sub>2</sub> SO <sub>4</sub>	Sodium Sulfate
ng	Nanno-gram
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Ammonium Sulfate
NH <sub>4</sub>	Ammonium
NH <sub>4</sub> F	Ammonium Fluoride
NH <sub>4</sub> NO <sub>3</sub>	Ammonium Nitrate
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> .4H <sub>2</sub> O	Ammonium Molybdate
Ni	Nickel
NO <sub>3</sub>	Nitrate
NO <sub>3</sub> N	Nitrate-Nitrogen

O <sub>2</sub>	Oxygen
P	Phosphorus
Pb	Lead
P <sub>c</sub>	Particle concentration
Q	Quantity
QLD	Queensland
r	Correlation coefficient
r <sup>2</sup>	Coefficient of determination for a fitted regression curve
R <sub>diff</sub>	Ratio between C <sub>DGT</sub> and C <sub>E</sub> (DGT), calculated using 2D DIFS
S	Sulfur
S.A.	South Australia
SO <sub>4</sub>	Sulfate
Sr	Strontium
t	Time (secs) DGT
TDS	Total Dissolved Solids
TEMED	Tetramethylethylenediamine
μg	Micro-gram
μm	Micro-metre
μmol	Micro-molar
μS	Micro-Siemens
V <sub>e</sub>	Volume of the acid added to elute the gel (DGT)
V <sub>g</sub>	Volume of the binding layer (DGT)
VIC	Victoria
W	Watt
W.A.	Western Australia
WHAM	Windermere Humic Aqueous Model
WHC	Water Holding Capacity
Zn	Zinc
ZnCl <sub>2</sub>	Zinc Chloride
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	Zinc Sulfate
Φ	Porosity
Δg	Diffusive layer thickness

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## 1. Introduction/ Literature review

### 1.1. Nutrient deficiency issues in Australian soils

Nutrient deficiency is becoming an increasing problem in Australia due to agricultural practices. The soils of Australia and New Zealand have extensive micronutrient disorders (Tiller 1963) and they vary greatly in their capacities to provide the chemical nutrients essential for plant growth (Williams et al. 1983). Borlaug and Dowsell (1994) emphasized the importance of plant nutrition in the future – “We believe without doubt that the single most important factor limiting crop yields in developing nations worldwide - and especially among resource poor farmers – is soil fertility”. Limited areas of Australia have highly fertile soils (e.g. black earth soils in eastern Australia) and many areas have moderate fertility; however, a high proportion of Australian soils are nutrient deficient. Areas of nutrient deficiency vary with soil type and with locations around Australia. Reported nutrient deficiencies in Australian soils are summarized in Table 1.1.

**Table 1.1** Soil types and locations in Australia experiencing nutrient deficiency (Peeverill et al., 2003).

**NOTE:**

This table is included on page 1 of the print copy of the thesis held in the University of Adelaide Library.

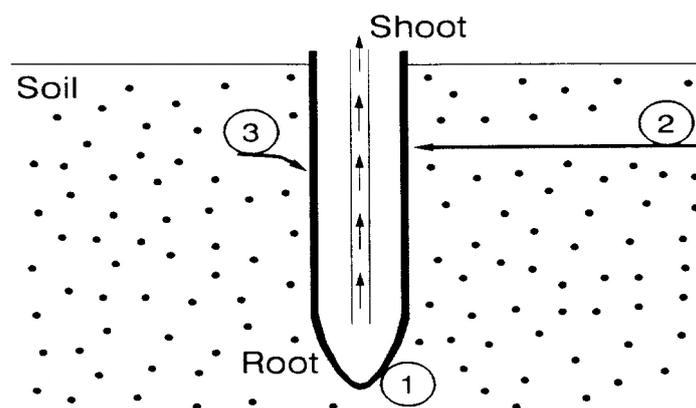
Chemical weathering processes enhanced by farming practices have led to the depletion of several nutrient elements in many regions of the continent, resulting in large

areas of infertile soils (Williams et al. 1983). An estimated average P value in Australian soils of 300 mg/kg indicates generally lower P content when compared to soils in other developed countries (Wild 1958). Available P levels in Australian soils are also comparably low with average grain levels of 0.26% (Williams et al. 1983) compared to values ranging from 0.36 to 0.42% for American and English grain (Woodroffe et al. 1953). Acute deficiencies of N and P are common, and frequently there are associated deficiencies of other nutrients, especially S and K and the trace elements Mo, Cu, Zn, and Mn (Williams et al. 1983). Areas suitable for agriculture in Australia constitute less than 20% of the continent and are confined mainly to the southern coastal areas where rainfall is sufficient enough to support growth of crops (Loneragan 1997). The introduction of fertilizers to correct for nutrient deficiencies can cause problems for the environment. Overuse of fertilizers to compensate for low fertility, has led to serious problems of nutrient pollution (Loneragan 1997). Phosphorus is a major example, as the amount of P taken up by the plant and permanently removed from the soil is small compared to the amount of P applied. This is due to a high P fixation by most soils that require the addition of a large excess of P fertilizer to meet crop requirements for good production (Loneragan 1997). Teakle et al. (1943) reported that the average recovery of P from wheat in Western Australia was less than one third of that added. Other studies have shown recoveries of approximately 20% of P in South Australia (Woodroffe et al. 1953) and less than 5% from pastures grazed by sheep in New South Wales (Donald 1954). Residual P accumulated in the soil may be removed by erosion, thus accumulating in waterways where adverse affects can arise (Williams et al. 1983). A balance between fertilizer input and crop demand is essential to minimize possible environment contamination. Williams et al. (1983) concluded that increased productivity must be attained by more efficient use of fertilizers, by more precise recognition of nutrient deficiencies, and by more accurate estimation of the amounts of fertilizer nutrients

required for optimum crop production. Supporting Williams's conclusions, Loneragan (1997) stated that plant nutritional research could raise productivity by diagnosis of nutrient deficiencies and toxicities of crops on previously unfertilized soils and their correction with minimal fertilizer and remediation costs.

## 1.2. Macro-and micronutrient availability in soils.

Nutrient availability to plants is determined by factors that affect the ability of the soil to supply nutrients and factors that affect the ability of plants to utilise the nutrients supplied (Corey et al. 1973). Three processes which provide nutrients to the root surface have been identified, 1) root interception, 2) mass flow and 3) diffusion (Figure 1.1).



**Figure 1.1** Schematic presentation of the movement of mineral elements to the root surface of soil grown plants. 1) Root interception: soil volume displaced by root volume. 2) Mass flow: transport of bulk soil solution along water potential gradient (driven by transpiration). 3) Diffusion: nutrient transport along the concentration gradient.

• = available nutrients. (Marschner, 1995)

### 1.2.1 Root interception

Continued growth of roots through the soil causes an increase in soil density in the immediate vicinity as the root replaces space previously occupied by the soil (Barber et al. 1963). Root surfaces may thus intercept nutrients during this displacement process. Enhanced concentration gradients, caused by an increase in nutrient concentrations due to

the increase in bulk density of the soil, promote diffusion of ions to the roots (Marschner 1995). As a result of this diffusion component, root interception is included as one of the diffusion processes relevant to solute movement in the soil-root system (Barber 1984, Jungk 1991). In contrast, as conditions vary quite considerably from the rhizosphere (see Section 1.3.2) to the bulk soil, these conditions are insufficiently described by a mechanistic model treating roots primarily as a sink for mineral nutrients supplied by mass flow and diffusion processes (Marschner 1995). The quantity of nutrients supplied by root interception is taken as the quantity present in the volume of soil equal to the root volume (Barber 1984). More realistically the maximum amount of nutrient intercepted is that estimated to be “available” in the soil volume occupied by the roots (Corey et al. 1973). Estimations of the contribution of root interception also incorporate the proportion of the total soil volume occupied by pores that is dependent on the soil bulk density. Root volume of annual crops in the topsoil is on average 1% of the soil’s volume, therefore only about 1% of the available soil nutrients could be supplied by root interception (Barber 1984). Corey et al. (1973) suggested that about 50% of the total soil volume is composed of pores and as roots occupy 1% of the total soil volume, roots therefore occupy about 2% of the total pore space.

### *1.2.2 Mass flow*

Mass flow involves the movement of nutrients dissolved in soil solution to the root surface by convective transport from the bulk soil. Transpiration from the plants shoots drives the transport; therefore the contribution of mass flow to nutrient supply is dependent on the amount of water transpired and the concentration of nutrients in the soil solution (Marschner 1995). The percentage of the nutrient requirement which can be satisfied by mass flow depends on: a) the plant nutrient requirements; b) concentration of the nutrient in soil solution; c) amount of water transpired per unit weight of tissue, and;

d) the effective volume of water which comes in contact with the root surface as it moves in response to potential gradients (Corey et al. 1973). The significance of mass flow can be assessed by comparing the rate of supply with the rate of influx. Alternatively, mass flow can be calculated by multiplying water use per plant by the concentration in the soil solution (Barber 1984). Differences with water use attributed to crop varieties, climate, and moisture conditions cause variations in the significance of mass flow. Lorenz et al. (1994) calculated mass flow ( $M$ ) supply of elements in rhizosphere soils in the following way:

$$Mn = (Tn + Pn)Sn + Mn^{-1} \quad (1)$$

where  $Tn$  is the water transpired between two sampling dates,  $n^{-1}$  and  $n$ ,  $Pn$  is the volume of water absorbed during the same sampling time, calculated as the difference between fresh and dry plant matter after sampling period and  $Sn$  is the solution concentration of an element at date  $n$ .  $Mn^{-1}$  represents potential uptake via mass flow at the previous sampling date  $n^{-1}$ . Ratios of mass flow to actual uptake into leaves calculated by Lorenz et al. (1994) revealed a higher uptake rate of K, Cd and Zn than could be supplied by mass flow, while mass flow of Ca and Mg was in excess to plant requirements.

### 1.2.3 Diffusion

When a particular nutrient is not supplied in sufficient quantities to the root by mass flow and root interception, the concentration of nutrients in the soil at the root surface is decreased due to continued uptake. A concentration gradient perpendicular to the root surface is formed and nutrients subsequently diffuse along the gradient towards the root surface (Barber 1984). The distance for diffusive-nutrient movement through the soil to the root is usually in the range of 0.1 to 15mm (Marschner 1995). Diffusion is the main mechanism for the movement of at least P and K to the root surface (Marschner 1995). The balance between nutrient uptake by roots, replenishment from the soil, and

mobility of ions by diffusion influences the shape of depletion profiles as they develop with time. Nutrient transport to the root can also exceed the requirement of the plant leading to an accumulation of a nutrient at the root surface (Lorenz et al. 1994, Brewster et al. 1970). Common elements that can accumulate at the root surface are Ca, Mg, and Na. This accumulation can lead to a concentration gradient away from the root and consequent diffusion back into the bulk soil.

### 1.3. Factors affecting nutrient availability

#### *1.3.1 Soil factors*

The rate of nutrient uptake by a plant depends not only upon the demand imposed by its roots, but also on the mobility/availability of the nutrient in the soil (Nye 1979). Nutrient availability is a function of the dissolved nutrient species in the soil solution and the ability of the soil to buffer nutrient concentrations in the soil solution (McLaughlin et al. 2000b). Tiller et al. (1972) described these two components as Intensity (*I*) relating to the concentration in the soil solution and Quantity (*Q*) the amount in the solid phase capable of readily entering solution. Major influences changing soil conditions have on nutrient availability have been studied by numerous authors and are summarised in Table 1.2 for P and cationic micronutrients (e.g. Cu, Mn, Zn).

**Table 1.2** Influence of soil properties on nutrient availability in the rhizosphere (adapted from Marschner 1993 and McLaughlin et al. 2003).

Soil factors	Nutrient movement	
	Phosphorus	Cations
Increasing pH	Decrease > 7	Decrease
Decreasing pH	Decrease < 6	Increase
Increasing clay content	<i>na</i>	Decrease
Increasing Phosphorus	<i>na</i>	Decrease
Low soil temperature	Decrease	Decrease
High bicarbonate	Decrease	Decrease
High organic matter (chelators)	Increase	Increase
High microbial activity	Increase	Increase

*na* = not applicable

#### 1.3.1.1 Soil pH

Of the soil factors, pH has the most marked influence on nutrient availability. For example, in the pH range 5.5-7.0, the equilibrium concentration of pore water Zn may decrease 30 to 45 times for each unit increase in soil pH (Barber 1994). Therefore diffusion coefficients of Zn in calcareous soils may be up to 50 times lower than in acidic soils (Barber 1994). Liming of acidic soils would therefore act to decrease the available cationic micronutrients but also increase dissolved organic carbon (DOC), which has a positive effect on available nutrients. In comparison P availability decreases with decreasing soil pH due the increased availability of Fe and Al that form complexes/precipitates with P.

#### 1.3.1.2 Buffering capacity

As dissolved nutrients are in equilibrium with those of the solid phase, any change in the concentration of a nutrient in the soil solution will result in release/absorption of that nutrient from the solid phase. This process of buffering tends to stabilize the concentration of nutrients in the soil solution (Jungk 1991). The buffering capacity of the

soil determines the rate of replenishment of nutrients from the solid phase. Effective diffusion coefficients of elements tend to decrease with increasing soil-buffering capacity (Jungk 1991). The buffering capacity of each nutrient is dependent on different soil properties. For example the buffering capacity of phosphate depends on the quantity and the properties of ferric iron at the surface of soil colloids, while the mineralogical nature of the clay and its percentage in the soil determines the buffer capacity of potassium (Jungk 1991). Cation exchange reactions on negatively charged surfaces and specific cation/anion adsorption reactions with electron donor/acceptor sites also influence nutrient availability (Corey 1990). These adsorption reactions are dependent on pH and the relative bonding strengths under varying pH situations are summarized in Table 1.3.

**Table 1.3** Relative strength of bonding of cations normally found in soils to cation exchange sites under varying pH and redox status. Cations normally present in significant amounts are underlined (Corey 1990).

<p>NOTE: This table is included on page 8 of the print copy of the thesis held in the University of Adelaide Library.</p>
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#### *1.3.1.3 Chelates*

Numerous reactions including dissolution, precipitation, desorption, adsorption, or exchange can affect nutrient availability to roots and a slight change in soil properties can influence these reactions. Plant nutrients, in particular trace metals, can form complexes with organic ligands (Corey 1990). When the metal ion acts as an acceptor of two or more electron pairs donated by a single organic ligand molecule, the latter are

called chelates. Organic matter can increase available nutrients by desorption reactions that form soluble complexes, increasing solution concentrations of some nutrients and transport to plant roots. It has been shown that the presence of chelating ligands results in a greater transport rate to the root than would be found with the same free-ion concentration but no chelate (Corey 1990). The ultimate effect of chelates on nutrient availability in soils will depend on whether the increased rate of transport to the root offsets the decrease in free-metal-ion activity in the bulk solution (Corey 1990). The increase in Zn concentration in the soil solution that occurs with increased soil temperature is suggested to be an expression of enhanced mobilization of Zn from insoluble forms by biologically produced chelators (Linehan et al. 1989).

#### *1.3.1.4 Other soil factors*

##### *Soil water-holding capacity*

Also contributing to nutrient availability is the soils water holding capacity, as it is an important factor affecting the diffusion of elements. The pore size distribution of a soil, which is a function of soil texture, determines the water-holding capacity of that soil (Jungk 1991). As diffusion takes place in the water-filled pores, diffusion coefficients increase with increasing soil water-holding capacities. Compared with sandy soils, fine textured soils with high clay content have higher water-holding capacities. Jungk (1991) used this data to explain, at least in part, high natural fertility of loamy soils compared to sandy soils and the increase in nutrient supply to plants under high soil moisture conditions. However in flooded soils elevated bicarbonate levels can inhibit plant growth and activity and therefore reduce ion uptake. Low Zn contents in rice plants grown on soils high in pH and organic matter are a result of these increased bicarbonate concentrations and the formation of precipitates with S (e.g. ZnS, see Table 1.4) (Marschner 1993).

### *Soil bulk density*

Soil compaction can affect the availability of most nutrients. Increasing the bulk density decreases the larger sized pore space and the mechanical resistance is increased. The increase in mechanical resistance may indirectly lead to an increase in mobility of some nutrients through chemical reduction to more soluble forms (Marschner 1995). It is assumed that the mobility of nutrients is impaired by the increase in bulk density as a result of reduced number of larger pores and changes in water-holding capacities. Although a study by Hoffmann et al. (1995) revealed the overall diffusion coefficient of P increased with soil compaction mainly due to enhanced P concentrations per volume soil. Enhanced P uptake efficiencies were reported from the roots and substituted for the smaller root/shoot ratio observed due to increased bulk densities (Hoffmann et al. 1995).

### *Precipitation*

Under certain soil conditions, precipitates can form and hence associated nutrients become unavailable for plant uptake (Corey 1990). Common precipitates found in soil systems are summarised in Table 1.4. The underlined elements form the common precipitates and the others are usually found as co-precipitated minor constituents within the major precipitates (Corey 1981).

**Table 1.4** Common precipitates in soils (underlined) and other elements that can form co-precipitates with common precipitates (Corey 1990).

NOTE:

This table is included on page 11 of the print copy of the thesis held in the University of Adelaide Library.

*Nutrient competition*

Nutrient deficiency symptoms can be easily addressed if they are a direct result of a nutrient deficiency. However, symptoms are often the result of interactions with other nutrients that limit the availability of the required nutrient (Berry 2002). Mineral nutrients that are chemically similar or have similar uptake mechanisms can compete with each other for plant uptake. For example, transition metals such as Cu, Zn, Cr and Ni compete with Fe and each other for plant uptake (Berry 2002). Of the macronutrients, excessive amounts of available Mg can compete with K for plant uptake and can also restrict Ca uptake (Berry 2002).

Precipitated Fe and Mn plaques on plant roots can restrict the root uptake of P in aquatic plants where the formation of Fe and Mn plaques is favorable. Iron and Mn plaques are formed in reduced organic sediments that have high concentrations of Fe and Mn. These oxidized compounds have a high capacity for P binding therefore restricting

the plant P access and uptake (Christensen and Sand-Jensen 1998). However, Christensen and Wigand (1998) also stated that the mycorrhizal association of the plant might be important in the solubilization of the metal bound P in the plaques. The Fe and Mn plaques also have the ability to act as Cu (Ye et al. 2001) and Zn (Zhang et al. 1996) beneficial reservoirs through the absorption of these heavy metals. Zhang et al. (1996) revealed through absorbing and immobilizing Zn, these plaques could also act as barriers to Zn uptake and toxicity by rice depending on the amount of Fe plaque.

### *1.3.2 Plant factors*

The rhizosphere is a term used to describe the soil-root interface of a plant, an area of high importance when considering metal and nutrient uptake by plants. Rhizosphere soil solution properties and composition have been found to differ considerably when compared to the bulk soil (Hamon et al. 1995). Although chemical properties in the bulk soil are very important for root growth and nutrient availability, conditions in the rhizosphere and the extent to which roots can modify these conditions play a very decisive role in mineral and micronutrient uptake (Marschner et al. 1986b, Marschner 1991a). While plant roots act as sinks for mineral nutrients transported to the root surface by mass flow and diffusion (Marschner 1995), depletion or accumulation of ions can occur in the rhizosphere with preferential uptake of either ions or water.

#### *1.3.2.1 Rhizosphere pH*

The pH of the rhizosphere soil may differ from that in the bulk soil by more than two units (Marschner et al. 1987, Marschner et al. 1996). Both soil and plant factors contribute to the degree of pH change and the extension of pH change from the rhizosphere to the bulk soil, while only plant factors influence the direction (increase or decrease) of pH changes (Marschner et al. 1996). The important soil factors include buffering capacity and initial bulk soil pH. Buffering capacity is at its lowest around pH

5, therefore root induced changes in the rhizosphere are maximal at bulk soil pH between 5 and 6 and decrease in magnitude outside these values (Nye 1986, Schaller 1987). Plant factors include excretion or reabsorption of  $H^+$  or  $HCO_3^-$  due to an imbalance between cation and anion uptake, evolution of  $CO_2$  by root respiration and release of low molecular weight root exudates (Marschner 1996). Contributions to pH changes in the rhizosphere from microbial production of organic acids and  $CO_2$  from root organic carbon release are also common. In aerated soils the influence of  $CO_2$  is minimal as it diffuses through air-filled pores (Nye 1986). Also affecting rhizosphere pH is the form of nitrogen supply. Marschner and Romheld (1996) presented a representative example of the role of nitrogen supply to rhizosphere pH for soybean. Rhizosphere pH was much lower compared to the bulk soil with ammonium supply ( $NH_4^+$ ). Conversely the pH of the rhizosphere can be considerably higher, especially in basal roots with nitrate supply ( $NO_3^-$ ), when compared to the bulk soil. A change in pH at the rhizosphere strongly affects solubility, concentration in the soil solution, ionic form, mobility and thus availability of micronutrients for uptake by plants. As a general rule the availability of Zn, Mn, and Fe increases with decreasing pH, whereas Mo availability decreases (Marschner et al. 1996). No clear relationship for Cu has been established due to it being predominantly bound to organic matter in both solution and solid phase (Sanders 1982).

Rhizosphere acidification can arise from deficiencies in a particular element. Phosphorus deficiency induced acidification mobilizes sparingly soluble P in the rhizosphere of calcareous soils and improves P nutrition (Hoffland et al. 1992). White lupins enhance P uptake by forming clusters of roots that release citrate. This strongly acidifies the surrounding soil mobilizing sparingly soluble phosphates (Marschner et al. 1986b).

### *1.3.2.2 Plant genotype*

Plant genotype can also affect the degree of pH changes in the rhizosphere (Marschner et al. 1986b, Romheld 1986). In different legume species supplied with  $\text{NO}_3^-$  as the only source of nitrogen, the degree of change in rhizosphere pH depends strongly on the geographical origin of the species (Romheld 1986). Romheld (1986) revealed that no rhizosphere acidification occurred with species originating from acid soils of the humid tropics, while moderate acidification of the rhizosphere occurred with species from temperate climates and strong acidification in species from semiarid climates. Individual plants can also have different influences on nutrient availability and varying success in utilizing available nutrients. The roots of many plant species behave differently from others in terms of effective rooting depth and ability to modify soil properties (McLaughlin et al. 2003). All soil chemical tests for agricultural purposes are therefore empirical approximations of plant root action, and are often element, plant, soil, or region specific (Elliot et al. 1997).

### *1.3.2.3 Redox potential and root exudates*

The rhizosphere can also influence the redox potential of the soil by its consumption or release of  $\text{O}_2$ . Reliable data on redox potentials in the rhizosphere are lacking due to the difficulty of making precise measurements of bulk soil redox potentials in well-aerated soils (Marschner and Romheld 1996). There is evidence that plants adapted to water logging (e.g., lowland rice) maintain high redox potentials in the rhizosphere, which are needed to immobilize excessive levels of soluble Mn and Fe in the rhizosphere (Marschner et al. 1987). In well-aerated soils an increase in the reducing capacity of roots can often be observed in response to iron deficiency (Marschner and Romheld 1996). The Fe deficiency-induced root responses can lead to increase Mn uptake by enhancement of Mn mobilization in the rhizosphere (Marschner et al. 1986).

Roots release appreciable amounts of organic carbon into the rhizosphere. The amount of root exudates released is dependant upon numerous factors including plant age, mechanical impedance of the substrate, plant species, and nutritional status of plants (Marschner et al. 1987). Effects of root exudates are mainly due to low-molecular-weight organic compounds, a result of either “leakage” or decomposing cells and tissues. Increased uptake of mineral nutrients can occur from this organic carbon, directly (e.g. by chelation) or indirectly (by stimulation of microbial activity) (Marschner et al. 1987). Other types of root exudates can also improve plant nutrient acquisition. Graham et al. (1981) showed that enhancement of sugar and amino acid exudation with P deficiency is correlated with enhanced root infections of mycorrhizae. As a consequence utilization of sparingly soluble phosphates would be enhanced. In white lupin, P deficiency induces an increase in citric and malic acid exudation and corresponding acidification of the rhizosphere (Marschner et al. 1996, Marschner et al. 1987).

#### *1.3.2.4 Rhizosphere microorganisms*

Due to the higher organic carbon levels supplied by roots, populations of bacteria are increased in the rhizosphere by a factor of 5-50 (Marschner et al. 1996). Non-infecting rhizosphere microorganisms may affect mineral nutrition of plants through their influence on (1) the growth and morphology of roots, (2) the physiology and development of plants, (3) the availability of nutrients, and (4) the nutrient uptake process (Bowen et al. 1991). The end products of microbial activity (e.g., organic acids, phenolics, siderphores) are speculated to have effects on micronutrient mobilization similar to low molecular weight root exudates (Marschner et al. 1996). An example is the root induced increases in P availability in the rhizosphere in relation to high phosphatase activity in the rhizosphere. The phosphatase is presumed to be of microbial or cell wall origin (Marschner et al. 1987).

The role of mycorrhizae in nutrient supply is clearer. Arbuscular mycorrhizal (AM) fungi form mutually beneficial (mutualistic) symbioses with the roots of around 80 % of vascular plants, often increasing P uptake and plant growth (Smith et al. 2003). By supplying the AM fungi with sugars and other derivatives of the photosynthetic processes, plants improve their ability to scavenge for scarce and immobile nutrients, particularly P, through the fungi (Smith et al. 2003). Apart from P, AM fungi can also increase micronutrient availability and it has been shown that in mycorrhizal plants, the uptake of Cu and Zn is usually markedly higher compared to nonmycorrhizal plants (Lambert et al. 1991). The role of mycorrhizae is particularly evident in delivering Zn to plants. Root colonization by mycorrhizae increases Zn availability resulting in mycorrhizal plants usually having higher Zn contents (Marschner 1993). Mycorrhizal plants are also less sensitive to Zn deficiency compared to non-mycorrhizal plants (Marschner 1993).

#### 1.4. Predicting nutrient availability

The interpretation of soil analyses has been seen as an essential component in the development of sustainable agriculture in many countries (McLaughlin et al. 2003). Most soil analyses are performed on samples taken from surface soil, typically 0-15 cm. However plant roots have been shown to access nutrients below these depths; therefore the role of the sub-soil in supplying nutrient to the labile pools is often ignored (Jungk 1991). The function of a nutrient soil test is to estimate the available pools of nutrient elements to plants (McLaughlin et al. 2003) therefore analyses performed solely on surface soils may underestimate nutrient availability. A comprehensive test involving soil at depth would provide the most relevant results.

Element bioavailability in soils has proven difficult to assess (Blair et al. 1993, Rayment 1993, Jones 1998, Conyers 2003, McLaughlin et al. 2003, Nolan et al. 2003b)

due to the complexity of trace element soil chemistry and plant uptake mechanisms. While trace element availability has commonly been linked to total concentrations in soil solution, this measurement does not give a true indication of the fraction of element available to soil-exposed organisms. In the soil system there is a pseudo-equilibrium between the solid and solution phases as a result of a number of processes operating as sources and sinks for trace elements. Consequently, methods that involve separation of solution and solid phases can disrupt this distribution (Hooda et al. 1999). Ideally the best method of measuring metal and nutrient bioavailability to plants would be a technique that does not involve disturbance of the pseudo-equilibrium.

It is widely recognized that the total metal content and total nutrient content of soil embraces large fractions that are unavailable to plants, microorganisms, or soil fauna (Rayment 1993, Marschner 1995, McLaughlin et al. 2000a, Zhang et al. 2001, McLaughlin et al. 2003, Nolan et al. 2003b). For example soil tests based on determination of the most available metal pools, while not perfect, appear to better indicate toxicity of metals in soil than total metal concentrations or metals removed by strong extractants (McLaughlin et al. 2000a). This suggests that the nutrient availability should also be assessed with methods based on the most available nutrient pools. Figure 1.2 summarizes the pathways and reactions of elements involved in the availability of nutrients in soil. It is clear a direct measure of nutrient concentration is inadequate to predict nutrient availability as it depends on both components Q and I and also the rate of resupply from the soil solid phase (see Section 1.3.1). Current methods of soil analysis measure variable proportions of Q and/or I, with the following section representing a summary of common techniques used for measuring nutrient availability.

NOTE:  
This figure is included on page 18  
of the print copy of the thesis held in  
the University of Adelaide Library.

**Figure 1.2** Conceptual diagram of major nutrient pools and pathways in soil (McLaughlin et al., 2003).

#### *1.4.1 Extraction of soil solution*

Soil solution, or soil interstitial pore water, is defined as “the aqueous liquid phase of the soil and its solutes” (<http://www.soils.org/sssagloss/index.html>). This term is recognised as the critical pool through which pass virtually all of the elements taken up from the soil by plants (Marschner 1995). Procedures for determining total nutrient concentrations in soil solution are relatively simple, however nutrient concentrations in the soil solution are insufficient to sustain plant growth and replenishment is required from the solid phase. Soil solution concentrations can underestimate nutrient availability

in the longer term and also overestimate nutrient availability if the solution is poorly buffered (Nolan et al. 2003a).

The simplest procedures to obtain soil solution include centrifugation (Thibault and Sheppard 1992), by Rhizon soil solution samplers (Tiensing et al. 2001, Menzies and Guppy 2000). These methods have the advantage of being low cost, easy to use and reliable with the samplers having an added advantage of being non-destructive. Centrifugation tends to extract higher concentrations of most solutes in soil solution when compared to other methods particularly rhizon samplers (Tiensing et al. 2001, Lorenz et al. 1994). Rhizon samplers are considered to better represent the solution extracted by plants, as a lower, constant matrix potential is applied; solution is extracted preferentially from the larger pores replicating plant mechanisms (Tiensing et al. 2001, Menzies and Guppy 2000). The sample also remains intact so the physical structure of the soil is not disturbed. Comparisons of concentrations of metals in the soil solution with uptake in plants have shown that in some instances soil solution correlates well with uptake when considering one soil (Hamon et al. 1995). Other studies have also revealed poor correlations using different soils (McLaughlin et al. 1997) and a discrepancy with correlations between elements (Lorenz et al. 1997).

#### *1.4.2 Extraction methods*

Due to their simplicity, soil extractions are still the most common method used for predicting element availability in soils, although they are not without their problems. Ideally, a chemical extraction procedure should remove both the active, available form of the element of interest on a short-term basis, and a constant fraction of the reserve form that replenishes the former over time (Levesque et al. 1988). Many attempts have been made to find an extractant that can remove the labile fraction of elements from soil and to relate this fraction to plant availability. However, to date no one has succeeded in finding

an extractant applicable to all soils, under all soil conditions. Many extractants have been used and these can be summarised in three main groups 1) weak replacement of ion salts ( $\text{MgCl}_2$ ,  $\text{CaCl}_2$ ,  $\text{NH}_4\text{NO}_3$ ), 2) dilute solutions of weak or strong acids and 3) chelating agents (DTPA, EDTA). Weak salts are able to release into solution the element associated with exchange sites on the soil solid-phase and hence are often considered to release the bioavailable element of the soil (McLaughlin et al. 2000b). Using neutral salts has the advantage of minimizing changes in pH and they match more closely the ionic strength of the soil, simulating conditions during plant uptake (McLaughlin et al. 2000b). Weak and strong acids, while extracting labile pools, change the soil conditions in relation to the conditions experienced during plant uptake unless they reflect rhizosphere acidification. Chelating agents form complexes with free metal ions in solution and in response metal ions desorb from soil surfaces or from labile solid phases (McLaughlin et al. 2000b). The amount of complexed metals that accumulate in solution during the extraction is a function of both the activity of the metals in the soil and the ability of the soil to replenish those metals (Lindsey et al. 1978). Chelating agents can also extract the non-plant available pools of trace metals, overestimating their plant availability. Both sequential and single extraction methods have been used to quantify the concentration of elements available for plant uptake (Maiz et al. 2000, and reviews in Rayment 1993). The effectiveness of soil extractions with 0.01 M  $\text{CaCl}_2$  in predicting the bioavailability of Cd, Zn, Cu and Pb has been compared to other common techniques (Nolan et al. 2003c, see Section 1.8). There was a strong relationship comparing plant uptake with the extraction method for Cd ( $r^2 = 0.89$ ) although the relationship decreased for Zn ( $r^2 = 0.55$ ) and Pb ( $r^2 = 0.77$ ) in comparison with other soil testing methods. Relationships with Cu uptake were poor for all soil testing methods used.

#### *1.4.3 Free ion activities and analytical speciation techniques*

Aquatic toxicological studies have suggested that the main chemical species controlling the availability and toxicity of metals to organisms is the free metal ion (Campbell 1995, Allen and Hansen 1996). These studies showed that the fraction of dissolved metal in solution bound to dissolved organic matter was not readily bioavailable. There have been several studies on the relationship between free metal ion activity in soil or nutrient solutions and plant availability (e.g. Bell et al. 1991, Smolders and McLaughlin 1996, Parker et al. 1997, Cheng et al. 2001, Zhang et al. 2001, Nolan et al. 2005). The complex nature of soils compared to water has a major influence on free ion activity and its relationship to plant bioavailability. The soil solid-phase component buffers metal solubility, making the reactions of free metal ions more complex. Soil solution chemistry is controlled by adsorption-desorption reactions on the surface of the solid phase, exchange reactions with the solid organic matter component, and complexation with dissolved organic and inorganic ligands (Sauve et al. 1998). The amount of metal actually bioaccumulated by soil organisms has been shown to be considerably larger than the free metal pool (Sauve et al. 1996), therefore if free metal activity relates to availability then replenishment of the free metal pool has a considerable influence on bioavailability.

Free ion activity measurements are performed on important cationic nutrients. Plant uptake of many metals has been shown to have a marked dependence on the aqueous speciation of the metal, and these responses often correlate best with the activity of the free metal ion (Parker et al. 1997). It has also been shown that free metal activity improves the prediction for toxic effects on crops, soil organisms, or soil microbial processes (Sauve et al. 1998). The free metal ion is believed to be in rapid equilibrium with cell surface-binding sites and metal species that are not able to react with cell

surface-binding sites, including colloidal metals and those that complexed strongly with organic ligands, are considered biologically inactive (Morel 1983).

Numerous analytical techniques have been used to measure metal speciation but all suffer from mainly chemical interferences, disturbance of solution equilibria, or poor limits of detection (Nolan et al. 2003a). Measurements of trace metal speciation in soil solution should be performed as soon as possible after extraction due to changes in the stability of the sample, for example degassing of CO<sub>2</sub> leads to a higher pH value in the soil solution (Suarez 1986). Potentiometric techniques utilizing ion-selective electrodes, voltammetric techniques such as anodic stripping voltammetry, ion-exchange resins, chromatographic methods, competitive chelation, filtration and ultrafiltration and dialysis have all been used with varying success (Nolan et al. 2003a). Development of a dialysis technique known as the Donnan equilibrium method by Cox et al. (1984) has minimized these errors as it is free from interferences from neutral anionic species and minimizes equilibrium perturbations. It has also been applied to soils successfully (Checkai et al. 1987, Fitch and Helmke 1989, Salam and Helmke 1998, Nolan et al. 2003b)

#### *1.4.4 Soil testing for Phosphorus*

Phosphorus is the most expensive major nutrient used in broadacre cropping in Australia (McBeath et al. 2005) and is also very important elsewhere in the World (Raij et al. 2002). Therefore there is a major requirement to develop a soil test capable of accurately predicting the P status of a soil, which in turn will maximise fertilizer efficiency and reduce the risks of environmental pollution due to over fertilising.

The major purpose of soil P analysis is to determine the quantity of supplemental P required to prevent economic loss of crop value experienced with P deficiency (Fixen and Grove 1990). Assessment of P availability is not a straightforward procedure as available P varies with plant root characteristics and environmental conditions that affect

soil and plant properties (Fixen et al. 1990). Soil P can exist in numerous chemical forms which can contribute a greater or less extent to the plant available fraction.

Phosphorus concentrations in soils have been commonly assessed by extraction methods. The most common methods include the Colwell-P extraction (Colwell 1963) and the Olsen-P method (Olsen et al. 1954). Others that have been in routine use in Australian laboratories are the lactate-extractable phosphate (Holford et al. 1985) and the Bray 1 phosphate method (Bray et al. 1945). Extraction solutions typically contain a competing ion that displaces the exchangeable forms of P from the soil. Common chemicals used as extractants in soil testing laboratories both present and past are listed in Table 1.5. It has been reported that these competing ions in some cases can displace non-labile and relatively stable forms of P from the soil consequently over predicting availability (Menon et al. 1990). These solutions may also fail to extract plant available P if they are used on soil types for which they are inappropriate (Menon et al. 1990). For example acid extractants (Bray 1 (Bray and Kurtz 1945), Mehlich 1 (Mehlich 1984)) acidify the system, which can potentially cause relatively stable carbonate forms of P to become available. Menon et al. (1990) along with Menzies et al. (2005) have suggested that the bicarbonate extraction methods (Olsen (Olsen et al. 1954), Colwell (Colwell, 1963)) originally developed for calcareous soils are suitable for use on both acid and alkaline soils. However, Shuman et al. (1988) reported that the Olsen method is less efficient than the acidic extractants at predicting P response on acidic soils.

The analytical measurement for orthophosphate-P has also been reported to affect the apparent result. For example, evidence presented by Murphy et al. (1962) suggested that hydrolysis of organic P to orthophosphate is increased by long periods of heat and colour development which is incorporated in the Cowell-P test. The extractants listed in Table 1.5 are reported to better reflect a soils P quantity and buffering capacity compared to the soils P intensity (Fixen et al. 1990). Fixen et al. (1990) stated that the quantity of

plant available P in a soil is a dynamic entity that can only be crudely estimated by any soil extractant.

The resin method is an alternative method for extracting labile P from soils (Abrams et al. 1992, Cooperband et al. 1994). Anion exchange resins are shaken with soil in distilled water and the ions are extracted from the soil onto the resin (McLaughlin et al. 1993). The ion on the resin is then eluted with an acid/salt solution. Saggart et al. (1990) used a slightly alternative method using strips of resin. The phosphate retained on the anion exchange strip was determined by shaking the strip directly with phosphate reagent. Phosphate accumulated by the resin has been found to correlate well with P uptake by plants (Abrams et al. 1992, Saggart et al. 1998) and plant yields (Menzies et al. 2005). Alternative plant available P assessment methods such as resin desorption while effective, have not proven uncomplicated enough in practice, theory, or interpretation to make serious inroads into common soil test usage (Fixen and Grove 1990).

**Table 1.5** Examples of common soil P extractions and conditions (Moody 2003)

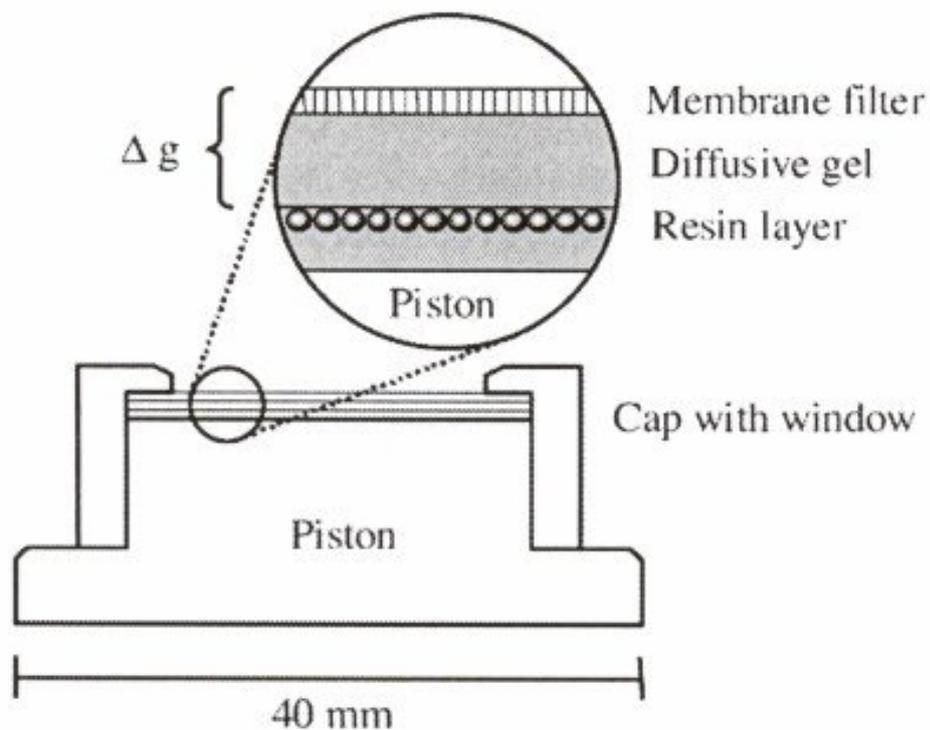
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the University of Adelaide Library.

## 1.5. Principles of Diffusive Gradients in Thin Films

### *1.5.1 The DGT device*

Diffusive Gradients in Thin Films (DGT) has been developed to assess element availability of some elements in waters (Zhang and Davison 1995, Zhang and Davison 2000, Sangi et al. 2002, Dunn et al. 2003, Gimpel et al. 2003, Peters et al. 2003), sediments (Zhang et al. 1995a) and more recently in soils (Zhang et al. 2001, Degryse et

al. 2003, Nowack et al. 2004, Nolan et al. 2005) but to date it has only been tested in contaminated scenarios. The technique has been recently adapted to assess P concentrations in waters (Zhang et al. 1999). DGT is a simple, robust analytical tool comprising of a plastic backing plate and a plastic front plate with an exposure window that acts as the surface sampling area. Two layers of polyacrylamide gel are placed between the two plates along with a membrane filter. The first layer of gel is impregnated with an ion-exchange resin (the resin layer) and is placed on the backing plate along with the diffusion gel layer (see Figure 1.3).

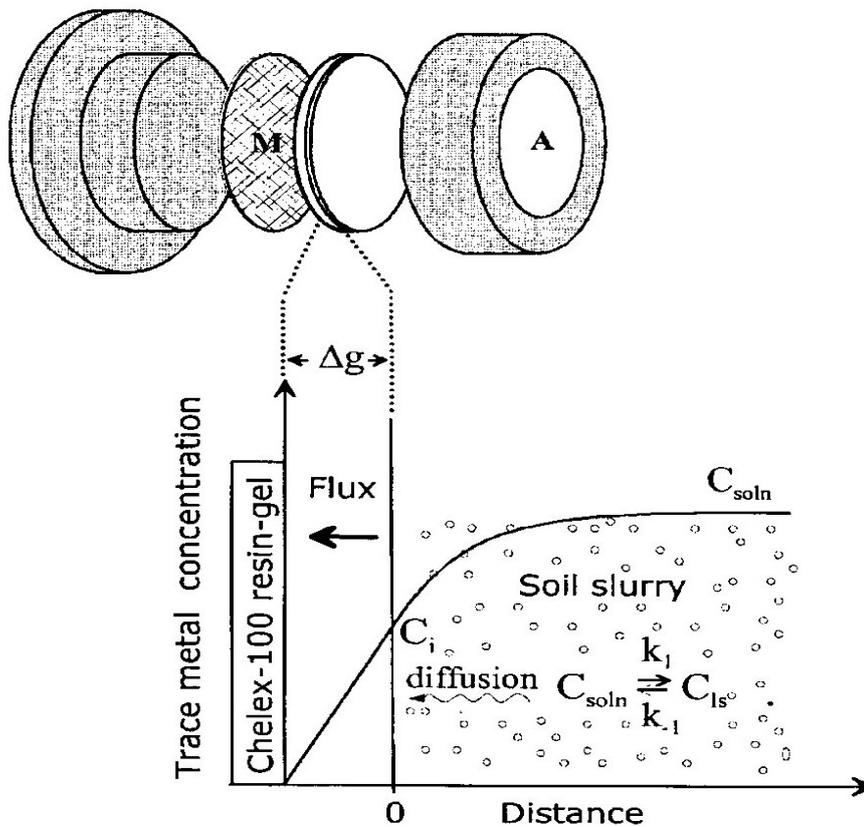


**Figure 1.3** Schematic representation of a cross section through the DGT device and hydrogel layers (Dahlqvist et al. 2002).

### 1.5.2 Mechanics of DGT

During deployment of the DGT device in either waters or soils, the dissolved analytes in the water or porewater diffuses through the filter and the diffusive gel layer. On contacting the binding layer the analyte is removed from the solution (Harper et al.

1998). The rate of accumulation of analyte on the resin is determined by the concentration gradient established in the diffusive gel (Figure 1.4). The gradient depends on the thickness of the diffusive layer ( $\Delta g$ ) and the interfacial concentration of labile analyte species ( $C_i$ ) (Ernstberger et al. 2002). After the required deployment time, the binding layer is eluted and the mass of analyte accumulated on the binding layer is measured (Zhang et al. 1995). A time-averaged flux of element accumulation on the binding layer from the system can be calculated (Zhang et al. 2001). In a soil system, the DGT is considered to mimic plant behavior by locally lowering element concentrations in the soil solution (Zhang et al. 2001). It responds to the re-supply of element concentrations from labile sources in the solution ( $C_{\text{soln}}$ ) and also the labile element pool on the solid phase ( $C_{\text{ls}}$ ). The measured element flux from the soils is quantitatively related to the new term, effective concentration or  $C_E$  (Zhang et al. 2001).



**Figure 1.4** Processes induced by deployment of a DGT probe in soil slurry. The mass (M) of metal is accumulated by diffusion across the diffusion layer of exposed interfacial area (A) (Ernstberger et al. 2002). Further description is given in the text.

### 1.5.3 Calculation of $C_E$ from DGT measurements

Calculations of  $C_E$  integrate both free ion activity in solution (I) as well as the fraction associated with the resupply of elements from the solid phase. A major advantage of having the diffusive layer in the DGT device is that it simplifies calculations and allows interpretation of the measured mass without resorting to numerical simulations (Zhang et al. 2001). As DGT locally lowers the element concentration, the element will be depleted near the surface of the DGT device from its initial concentration, i.e. from  $C_{\text{soln}}$  down to  $C_{\text{diff}}$  if the analyte is solely supplied from the soil solution (diffusion-only case). A pseudo steady-state is established after a few minutes, as the diffusion coefficient in the gel and the filter paper is the same (Zhang et al, 1995). Using Fick's first law in the form,

$$J = -D \frac{\delta C}{\delta x} \quad (2)$$

the flux goes in the opposite direction to the concentration gradient, thus the flux through the diffusive and filter layers is (eq 3).

$$F = DC_{\text{diff}}/\Delta g \quad (3)$$

Where  $D$  ( $\text{cm}^2\text{s}^{-1}$ ) is the diffusion coefficient in the diffusive layer of thickness  $\Delta g$  (cm), and  $C_{\text{diff}}$  ( $\text{moles cm}^{-3}$ ) is the instantaneous concentration at the surface of the device (Zhang et al. 2001). The mean flux,  $F$ , can also be calculated using the time of deployment ( $t$ ), the measured accumulated mass of element on the resin,  $M$  (moles), and the exposed area of the gel,  $A$  ( $\text{cm}^2$ ) (eq 4).

$$F = M/(At) \quad (4)$$

Combining equations 3 and 4, the mean interfacial concentration can be calculated in the diffusion-only case (eq 5).

$$C_{\text{diff}} = M\Delta g/(DA t) \quad (5)$$

However, since the measured accumulated mass of element on the binding layer in a soil system incorporates the re-supply of element from the solid phase as well as the

diffusional supply, these contributing factors would cause a larger measured mass compared to a solely diffusional supply. Therefore  $C_{DGT}$  is the mean interfacial concentration used to reflect the supply from both solution and solid phases (eq 6).

$$C_{DGT} = M\Delta g/(DA t) \quad (6)$$

This equation is used for the measurement of trace elements in aqueous solutions. Measured accumulated mass ( $M$ ) is calculated using the mass of diffused ions in the binding layer (eq 7),

$$M = C_e (V_g + V_e)/f_e \quad (7)$$

where  $C_e$  is the concentration of the eluted gel,  $V_g$  is the volume of the gel in the resin layer,  $V_e$  is the volume of the acid added to elute the gel and  $f_e$  is the elution factor, as only a fraction of the bound element is eluted (Zhang et al. 1995). The terms  $\Delta g$ ,  $A$ , and  $t$  from equation 5 are measured and  $D$  is the value of the molecular diffusion coefficient in water at a given temperature.

The ratio of the mean interfacial concentration due to resupply by diffusion only ( $C_{diff}$ ) to the initial or bulk concentration,  $C_{soln}$ , can be expressed as  $R_{diff}$  (Zhang et al. 2001) (eq 8).

$$R_{diff} = C_{diff}/C_{soln} \quad (8)$$

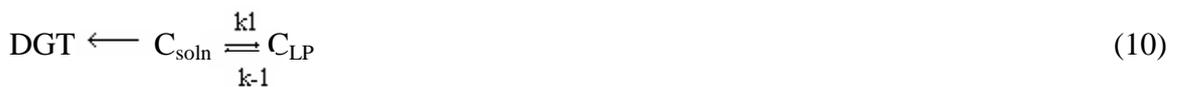
$R_{diff}$  is determined by the geometry of the device, deployment time, and soil tortuosity (see section 1.5.4). Providing that the condition of an infinite sink is maintained,  $C_{soln}$  does not represent the effective concentration that a sink like the DGT device or a plant root experiences due to solid phase and solution re-supply. A new concept of effective concentration  $C_E$ , has been recently proposed that incorporates the soil solution concentration and its re-supply from the solid phase. The effective concentration can be obtained by simply dividing  $C_{DGT}$  by  $R_{diff}$  (eq 9), as performed to convert  $C_{diff}$  to  $C_{soln}$  (eq 8).

$$C_E = C_{DGT}/R_{diff} \quad (9)$$

The effective concentration can be directly related to the uptake in plants as it is based on the knowledge of rhizosphere diffusional processes. Measurement of  $C_E$  from the DGT is largely determined by the soil properties but is totally independent of the surface area of the device (Zhang et al. 2001). However,  $C_E$  increases with deployment time and decreases with increasing diffusive layer thickness  $\Delta g$ , and therefore these are stated at all times. This is because the solely diffusional supply decreases with time and a greater concentration is required as the diffusional supply becomes less effective. Effective concentrations ( $C_E$ ) decrease with increasing thickness of the diffusive layer due to the lower demand of solution resupply placed on the soil.

#### *1.5.4 Calculation of $R_{diff}$ using DGT Induced Fluxes in Soils and Sediments (DIFS)*

DIFS is a software tool used for investigating and interpreting trace element measurements made by DGT. This numerical model describes in one and two dimensions the time-dependant interaction of a DGT device of variable geometry with soils and sediments that have various properties (Harper et al. 1998, Zhang et al. 2001). It simultaneously considers, at any location and point of time, diffusion of elements in the soil solution in response to the induced concentration gradients that occur with DGT deployment (Zhang et al. 2001). The exchange of elements between solid phase and solution is assumed to be governed by first-order kinetics (eq 10) (Zhang et al. 2001).



$C_{\text{soln}}$  is the concentration of elements in soil solution and  $C_{\text{LP}}$  is the element concentration associated with the labile solid phase. The rate of resupply depends on both the transfer rate constant and the concentration of the labile element in the solid-phase reservoir (Zhang et al. 2001).

The conversion of  $C_{DGT}$  to  $C_E$  requires the calculation of  $R_{diff}$  (eq 8), which expresses the extent of concentration depletion at the interface of the device and the soil for the diffusion-only case and is calculated using DIFS (see Section 1.5.3). This parameter,  $R_{diff}$ , can be obtained by providing the input parameters of particle concentration,  $P_c$ , soil porosity,  $\Phi$ , and diffusion coefficient in soil,  $D_s$ , (eqs 11, 12, 13) into the DIFS model (Nolan et al. 2005).

$$P_c = m / V \quad (11)$$

$$\Phi = d_p / (P_c + d_p) \quad (12)$$

$$D_s = D_o / (1 - \ln \Phi^2) \quad (13)$$

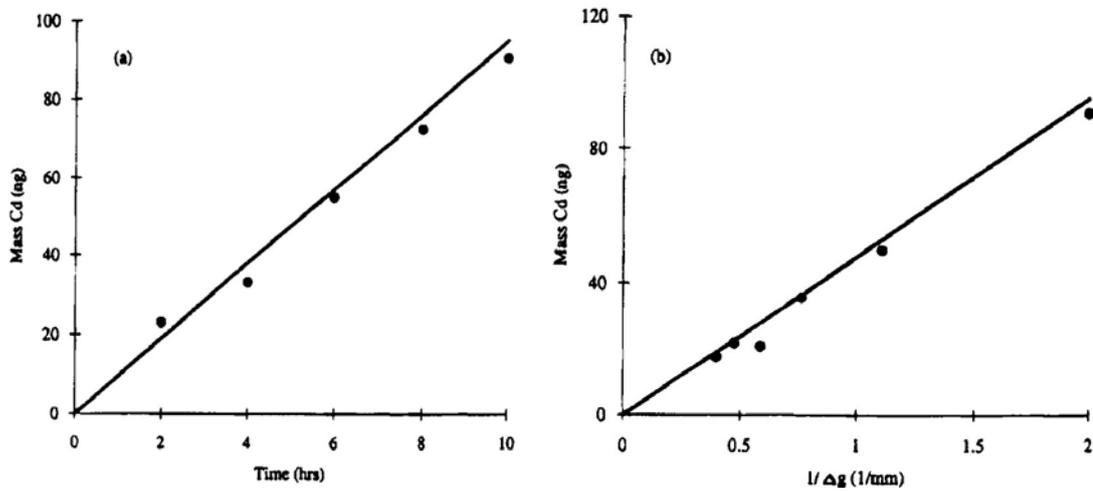
where  $m$  is the total mass of soil particles,  $V$  is the pore water volume in a given volume of soil,  $d_p$  is the density of the soil particles, which is commonly assumed to be  $2.65 \text{ g cm}^{-3}$  (Biielders et al. 1990). The term  $D_o$  is the diffusion coefficient in water.

Previously DIFS was a one-dimensional model and a correction factor was required to obtain a value of  $R_{diff}$  for two-dimensional diffusion (Harper et al. 1998). Recently a new version of DIFS has been developed that incorporates a 2D soil/ water system as opposed to the 1D system of the original program and therefore no correction factor is required (Sochaczewski et al. 2007).

### 1.5.5 Theory verification

Experiments designed to test the theoretical eqs 2-5 describing DGT processes were performed by Zhang et al. (1995) with the results confirming the principles behind DGT. For example the measured mass of Cd increased linearly with increasing solution concentration and the measured mass that accumulated on the binding layer was inversely proportional to the thickness of the diffusive layer. Studies that have investigated other elements have all reported similar results. Gimpel et al. (2001) also performed a time series experiment with Cu and Cd (Figure 1.5). A linear increase in measured mass on the

resin was obtained with time and is consistent with theoretical prediction from eq 5, indicating gel stability.

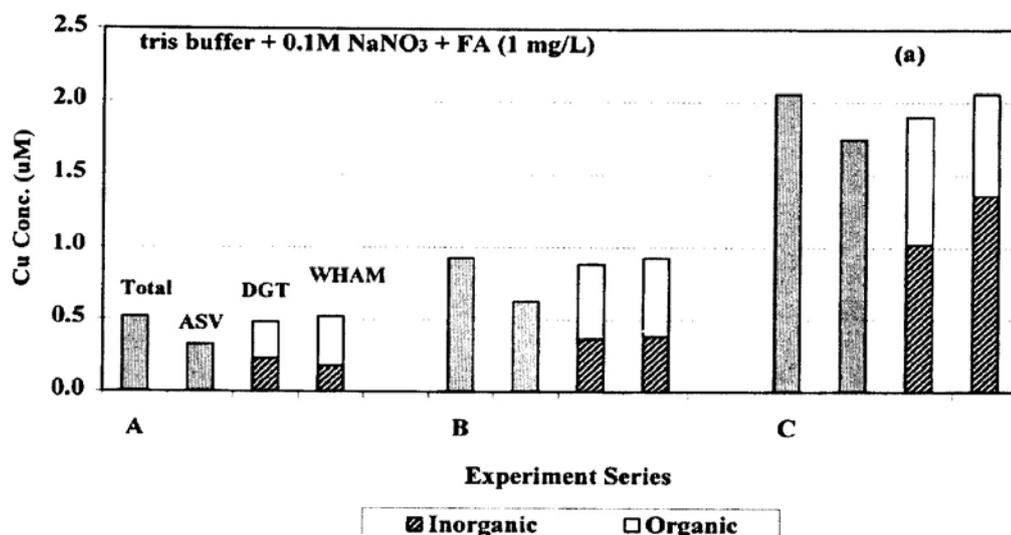


**Figure 1.5** (a) Examples of the linear relationship of increasing measured mass on the resin with time, and (b) relationship of measured mass on the resin with diffusion gel thickness (Gimpel et al., 2001).

#### 1.6. Predicting trace element bioavailability using DGT

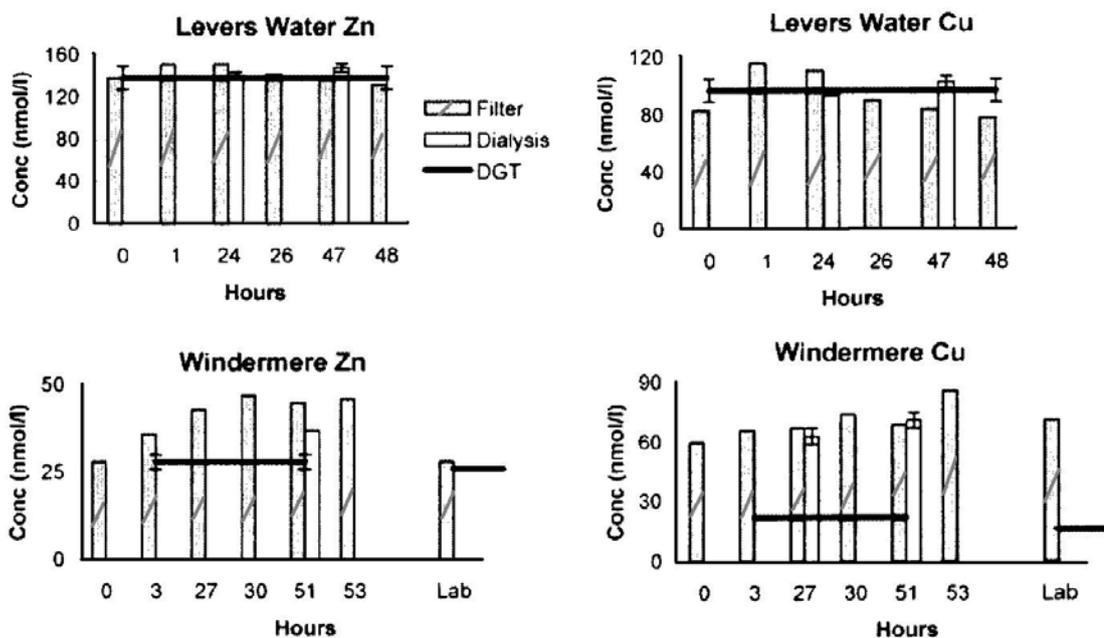
Previous analytical techniques used to predict bioavailability of trace elements have shown some correlations with metal uptake in plants (e.g. Lindsay 1978, Levesque 1988, Jones 1998, Delgado et al. 2001, Nolan et al. 2003a, Nolan et al. 2005). It seems beneficial that when predicting trace element bioavailability, a technique is used that best simulates the environment of a soil grown plant. Previously, most techniques that have been used have not focused on these aspects as they analyse bulk soil concentrations or fractions of the intensity and quantity factors relating to nutrient availability. The prediction of metal bioavailability in soils using DGT appears to accurately reflect metal uptake by plants (Zhang et al. 2001, Nolan et al. 2003c). DGT has many advantages over conventional techniques; it can be placed on top of the soil with minimal disturbance of the soil, *in situ* measurements minimize changes in metal speciation with time, and it can provide an infinite sink and promote resupply of ions from labile species in the solid

phase thereby mimicking plant behavior (Zhang et al. 2001). The performance of DGT has been tested on a wide variety of solutions ranging from model solutions to direct *in situ* analysis of lakes, river systems and sediments. Numerous elements have been investigated with successful results. Trace metals including Al, Cd, Co, Cu, Fe, Ni, Pb and Zn have been assessed in model solution work and real soil samples and sludges (Davison et al. 1994a, Zhang et al. 1995a, Zhang et al. 1995b, Harper et al. 1998, Denney et al. 1999, Hooda et al. 1999, Zhang et al. 2000, Gimpel et al. 2001, Ernstberger et al. 2002, Jansen et al. 2002, Peters et al. 2002, Sangi et al. 2002) and recently it was extended to measure Ca and Mg (Dahlqvist et al. 2002). These studies revealed that the performance of DGT was both reliable and reproducible. DGT accurately predicted solution concentrations of various elements (Gimpel et al. 2001). DGT has also been used to assess speciation of trace metals in natural waters by controlling the pore size of the diffusive layer (Zhang and Davison 2000). Small inorganic species diffuse freely through all gels but organic complexes associated with fulvic and humic acids diffuse less freely (Zhang and Davison 2000). Reported results from Zhang and Davison (2000) are shown in Figure 1.6 (fulvic acid only) and indicate correlations between WHAM calculations and measured values from DGT. The measured labile inorganic species can be used as an input parameter in speciation programs along with the major ions and cations, allowing the prediction of the concentrations of the species present.



**Figure 1.6** Comparison of different measurements of Cu in three solutions, A, B, and C, in the presence of fulvic acid (FA). “Total” was the analytical measurement on the solution made by AAS. “ASV” was measured by anodic stripping voltammetry. Inorganic and organic labile species were measured separately using DGT devices with different gels and were also calculated using WHAM speciation code using “Total” concentration as an input parameter (Zhang and Davison, 2000).

Using multiple gels of differing pore size also allowed for determination of labile metal associated with large molecules. Denney et al. (1999) compared measurements made by DGT to dissolved metal concentrations to determine the degree of complexation of the metals with organic matter in a river system. Gimpel et al. (2003) predicted speciation of lake waters by comparing in situ measurements by dialysis and DGT to on-site filtered samples. From the observed differences in these measurements it was inferred that a degree of complexation by organic matter or colloids was present in the lake waters. Complexation with humic and fulvic substances caused substantial differences to measurements made by dialysis and DGT. The importance of an *in situ* measurement of trace metals has been highlighted (Gimpel et al. 2003). They observed slightly lower DGT measurements of metals, especially Cu, from filtered samples taken back to the lab, compared to *in situ* measurements made by DGT (Figure 1.7).

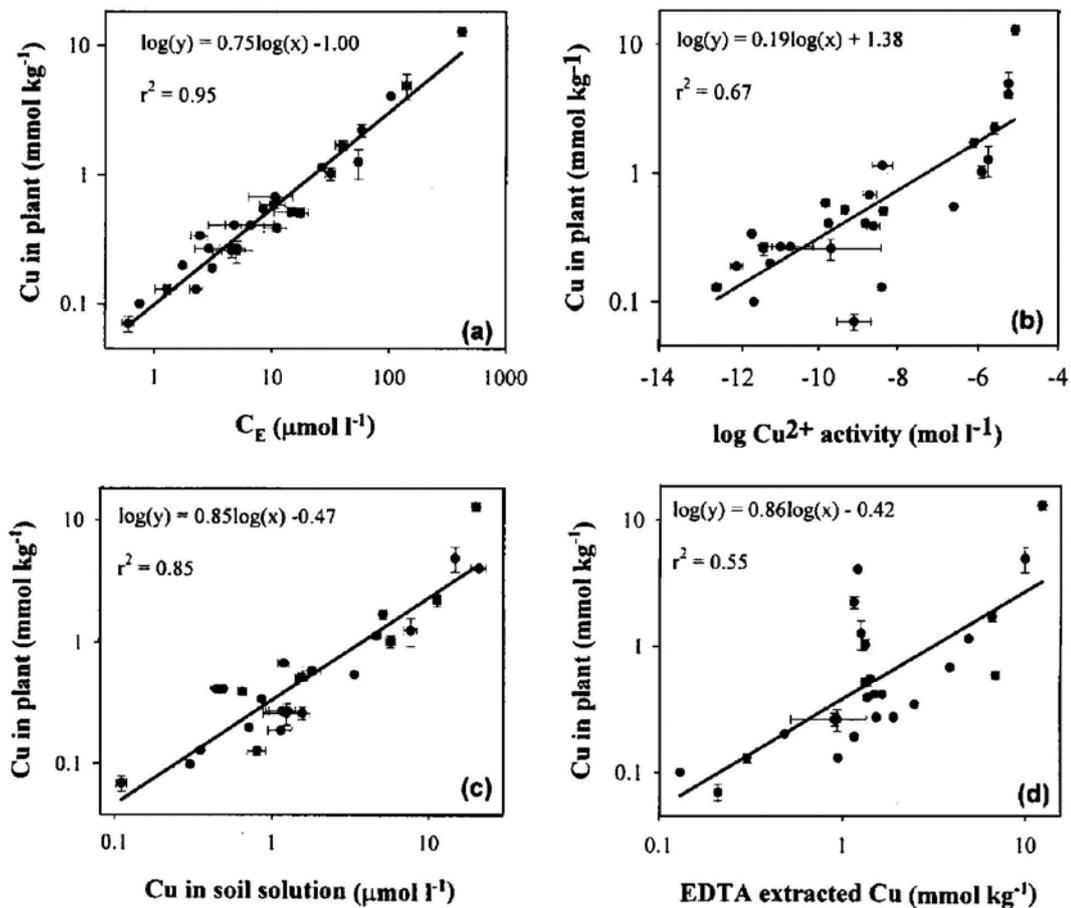


**Figure 1.7** Concentrations of Cu and Zn in two lakes measured by DGT, dialysis, and filtration. Additional DGT and filtration measurement were made on a sample taken back to the lab (Gimpel et al., 2003).

### 1.6.1 Effective concentration correlation with plant uptake

Evidence of the performance of DGT as a technique for predicting metal bioavailability in soils to plants was reported in a recent paper by Zhang et al. (2001). Comparison of effective concentration  $C_E$ , with chemical extraction techniques and free metal measurements in relation to uptake in plants showed that  $C_E$  measurements using the DGT device provided a more accurate prediction of metal bioavailability (Nolan et al. 2005, Zhang et al. 2001). Copper was investigated closely by Zhang et al. (2001) in a comparison of different measures of Cu and its uptake in the plant *Lepidium heterophyllum*. Analysis of the soil included measurements of  $C_E$  (DGT), free  $Cu^{2+}$  activity, soil solution concentration and also an EDTA extraction. Results showed that the plant concentrations were linearly related and highly correlated with  $C_E$  compared to the free activity, soil solution concentrations and extractions, which were more non-linear and scattered. A log-log plot of  $C_E$  ( $\mu\text{mol L}^{-1}$ ) vs Cu in the plant ( $\text{mmol kg}^{-1}$ ) revealed an

$r^2$  value of 0.95. This was compared to the log transformed data for  $\text{Cu}^{2+}$  activity; Cu in soil solution and EDTA extracted Cu, which produced  $r^2$  values of 0.67, 0.85 and 0.55 respectively (Figure 1.8). In this study deployments of the DGT device were conducted after harvesting of the plant. For nutrient assessment, it is perhaps better to deploy the device before any plant growth and possibly on a sub-sample to measure all the available nutrients before the plant has utilized them. Results of  $C_E$  before harvest and  $C_E$  after harvest from Nolan et al. (2005) did show that measurements after harvest were a little lower compare to measurements from deployment before harvest.



**Figure 1.8** Dependence of log concentrations of Cu in above ground plant tissue on the log of (a) effective concentration,  $C_E$ , (b) free  $\text{Cu}^{2+}$  activity, (c) soil solution concentration, and (d) EDTA extracted Cu concentration (Zhang et al. 2001).

Recently a comprehensive study by Nolan et al. (2005) supported the improved prediction of plant metal availability using DGT. Nolan et al. (2005) assessed the correlation of DGT  $C_E$ , chemical speciation and  $\text{CaCl}_2$  extraction techniques with plant metal uptake with three other metals, Zn, Cd, and Pb. Uptake in the plant was assessed using wheat (*Triticum aestivum*). Overall the best predictive measure for three of the four metals was  $C_E$  measured by DGT. Coefficient of determinations averaged over a range of 12 soils, for log-log plots of plant metal concentrations vs  $C_E$  for Zn, Cd, and Pb were calculated to be 0.87, 0.87, and 0.92 respectively (Table 1.6). A low  $r^2$  value of 0.47 for Cu was proposed to be possibly due to the phytotoxic effects of high available Zn concentrations in some soils affecting Cu uptake in the low concentration range (Nolan et al., 2005).

In summary, the studies of Zhang et al. (2001) and Nolan et al. (2005) showed that overall the best predictor of metal availability in soil to the respective plants was  $C_E$  measured from the DGT device. It is possibly that in some of the soils used in these studies, the supply of metals to the plant was not diffusion limited due to their high metal concentrations. These results indicate that DGT may also provide an accurate and reproducible method of assessing metal bioavailability in non-diffusion limited soils, where the DGT device is then simply providing a reliable measure of soil solution concentrations.

**Table 1.6** Coefficient of determinations of the regression fit between plant material concentration and soil measurement concentrations (determined for 12 soils) (Nolan et al. 2005).

NOTE:  
This table is included on page 38  
of the print copy of the thesis held in  
the University of Adelaide Library.

Based on the theory DGT should be a better predictive measure of nutrient availability under conditions where the diffusion of nutrients to a sink like a plant is limited as it quantifies the resupply factor off the soil solid phase. Therefore the performance of DGT with respect to plant uptake/ response needs to be assessed on nutrient responsive soils where they are diffusion limitations.

## 1.7. Limitations of DGT (Chelex Resin)

### *1.7.1 Recovery efficiencies*

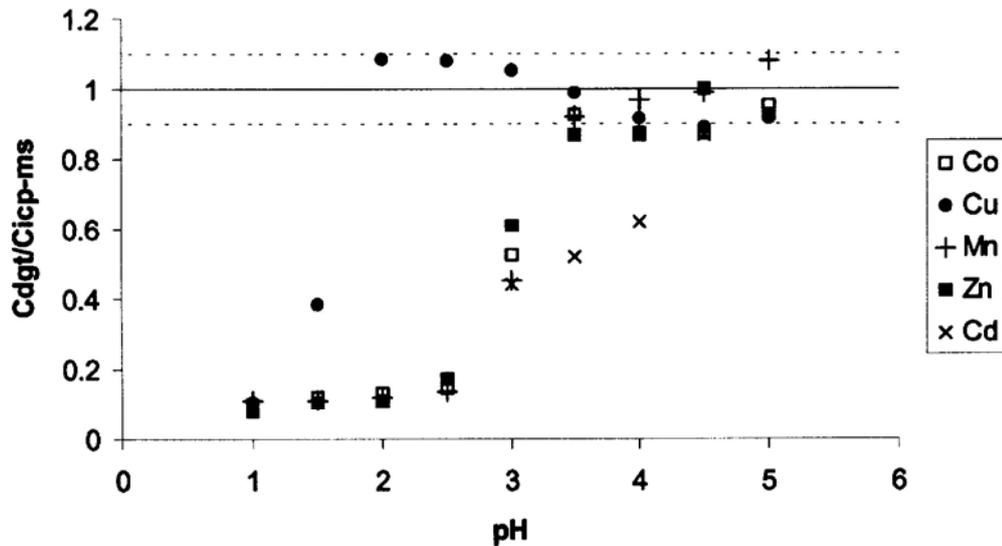
Recovery efficiency tests on the elution of metals have revealed that indeed the elution of metals from the chelex gel is not 100% effective. Results showed that recoveries were as follows: Zn,  $80.3 \pm 5.5\%$ ; Cd,  $83.9 \pm 2.7\%$ ; Cu,  $79.3 \pm 6.4\%$ ; Ni,  $81.6 \pm 6.9\%$ ; Mn,  $81.4 \pm 2.2\%$ ; and Fe  $69.7\% \pm 5.0\%$  (Zhang et al. 1995). Within the standard deviation boundaries all elements had elution efficiencies of 80% with the exception of Fe, which may be explained by its partial oxidation to  $\text{Fe}^{3+}$ . An elution factor,  $f_e$ , of 0.8 is then used in equation 4 to account for the metal still bound to the gel after elution.

### 1.7.2 Effect of temperature, pH, and ionic strength

The rate of mass transport through the diffusive gel depends not only on the concentration gradient but also on the temperature, through the variation of the diffusion coefficient,  $D$ , with temperature (Zhang et al. 1995). The gel structure may vary in both its structure and dimensions with temperature, thereby affecting diffusional processes. DGT performance was tested at a series of temperatures from 5-35°C and correlation with theoretical values proved that DGT measurements made in aqueous environments within this temperature range could be theoretically interpreted using molecular diffusion coefficients measured in water (Zhang et al. 1995).

The pH dependence of the DGT technique was analyzed by Zhang et al. (1995) but was performed only for Cd and they found that DGT measurements were effective in the pH range 4.5 to 10. Gimpel et al. (2001) revealed that certain elements bind better to the chelex gel under changing pH conditions. The study compared measurements of  $C_{DGT}$  with the trace metals Co, Cu, Mn, Zn, and Cd. The performance of the Chelex gel was scrutinized by comparing  $C_{DGT}$  with the analyzed concentration of the model solutions. Theoretically the ratio of  $C_{DGT}$  to  $C_{soln}$  should equal 1 if the DGT is performing to its potential i.e. no pH dependence. DGT was exposed to a series of solutions in the pH range of 2-12. These results showed there are some major limitations of some elements summarized in Figure 1.9. DGT measurements ( $C_{DGT}$ ) for Cu were in good agreement with  $C_{soln}$  under pH conditions between 2-10, but ratios of  $C_{DGT}/C_{SOLN}$  dropped to 0.4 below pH 2. Cadmium ratios dropped considerably below pH 4.5 but were consistently close to 1 up to pH 10. A sharp drop in performance of DGT was seen at pH below 3.5 for Mn, Zn and Co. Above pH 10, it is believed that the metal species are in a colloidal or relatively inert form or that they simply absorb onto the gel holder and fail to reach the resin. In practice, DGT performance would therefore appear to be robust for most soils as typically soil pH ranges from 4.5-8.5. There is clearly a problem in the analysis of metal

availability in very low pH soils, however, this is unlikely to be a major constraint for use of DGT for agronomic purposes as very acidic soils ( $\text{pH} < 5$ ) are not optimal for plant growth due primarily to Al/Mn toxicity, and therefore are either not used for growing crops, or are limed before use.



**Figure 1.9** Effect of pH on the measurement of Cd, Co, Cu, Mn and Zn by DGT, determined by the ratio of metal concentrations measured by DGT ( $C_{\text{DGT}}$ ) to the concentrations obtained by direct ICP-MS measurement in solution ( $C_{\text{ICP-MS}}$ ) (Gimpel et al. 2001).

The DGT technique has also been tested against a wide range of ionic strengths by adjusting solutions with  $\text{CaCl}_2$  ( $1\mu\text{M}$  to  $1\text{mM}$ ) or  $\text{NaNO}_3$  ( $0.1\text{mM}$  to  $0.8\text{M}$ ) (Peters et al. 2003). Ratios of Cd and Cu concentrations measured by DGT with concentrations measured directly from solution were in good agreement for solutions with  $(\text{Ca}) > 0.1\text{mM}$ . The lower limit of ionic strength where reliable DGT results are obtained was  $100\mu\text{mol}^{-1}$ ; below this limit results were erratic and thought to be caused by interactions of the trace metals with the diffusive gel when there were insufficient excess cations present (Peters et al. 2003). Implications are that DGT assessment of soils with ionic strengths below this value could prove inaccurate. Sangi et al. (2002) used a bisacrylamide cross-

linked polyacrylamide diffusion gel replacing the agarose cross-linked gel usually used. Results revealed that diffusing trace metal cations were weakly and reversibly bound to the diffusion gel layer and measurements were not independent of variations in ionic strength. As ionic strength increased, competition between cations binding to the diffusion gel sites was responsible for a decrease in the amount of metal absorbed by the resin gel layer. Therefore agarose cross-linked gel is recommended for diffusion gels. Recently Warnken et al. (2005) have established that the reduced performance of DGT below ionic strengths of 0.1 mM (Peters et al. 2003) were due to high pH values in the diffusive gel associated with residual TEMED (N,N,N',N'-Tetramethylethylenediamine) still present as a result of inadequate hydrating procedures. The performance of DGT has been subsequently verified at very low ionic strengths of 0.01 mM with improved hydrating procedures (Warnken et al. 2005).

### *1.7.3 Capacity of DGT*

The DGT technique has the potential to be used for long-term deployment to obtain an integrated record of trace metal concentrations (Zhang et al. 1995a). However, the capacity of the binding layer determines the maximum length of period of deployment before unreliable results are obtained. The capacity of DGT has been tested by deploying the devices in solutions containing high concentrations for short periods of time (Zhang et al. 1995). The capacity of the Chelex binding layer was reported by Zhang et al. (1995) to be 0.63mg of Cd and considering the concentrations of metals in seawater, predicted the maximum deployment time possible to be for 2.5 years and 3 months for coastal seawater. Gimpel et al. (2001) also investigated the capacity of the chelex gel by deploying DGT devices for periods of up to 33 days and exposed in solutions containing Cd. The performance over that time remained consistent and the mass of accumulated Cd increased linearly with time. The capacity of DGT devices deployed in natural waters and

soils may be reduced as all trace metals present will contribute and there is the possibility of problems associated with biofouling (Gimpel et al. 2001).

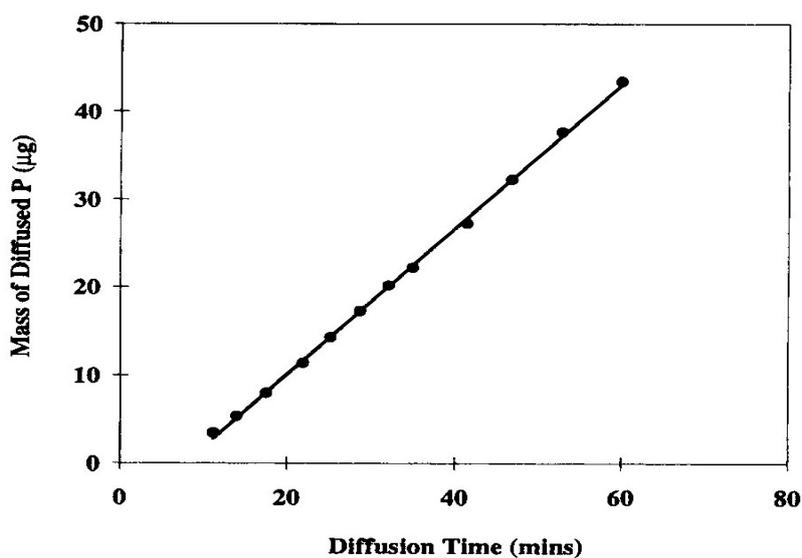
#### *1.7.4 Competition*

The effect of calcium competition on the Chelex resin has proven to have little influence on the binding of Cd and Cu at typical concentrations of calcium in seawater and hardwater (0.01M) (Zhang et al. 1995, Gimpel et al. 2001). It was revealed that Cd and Cu had a higher affinity for the resin and therefore should be able to displace Ca on the resin. Experiments to investigate the effect of calcium on metal uptake at a concentration obtainable in an agricultural soil have not yet been performed.

#### 1.8. Potential of DGT for assessment of nutrient availability

DGT has been utilised for analysis of trace metals and P in water, sediments and soil systems (Davison et al. 1994a, Davison et al. 1994b, Zhang et al. 1995, Zhang et al. 1995a, Denney et al. 1999, Hooda et al. 1999, Zhang et al. 2000, Gimpel et al. 2001, Zhang et al. 2001, Li et al. 2002, Dahllqvist et al. 2002, Sangi et al. 2002, Twiss et al. 2002, Dedryse et al. 2003, Gimpel et al. 2003, Peters et al. 2003, Nolan et al. 2005). While there has been some manipulation of the dimensions of the device and the size/thickness of the diffusive layer (Zhang et al. 1995, Sangi et al. 2002), limited testing has been performed on the binding resin. Chelex embedded resin has been popular and little manipulation is probably needed for trace metals due to its successful performance. Recently a study by Li et al. (2002) used a cellulose phosphate ion exchange membrane as a binding phase for trace metals, which produced equally reliable results. Benefits include easier preparation, reusability and a preferential binding of transition metals rather than matrix ions that are competitive species in natural waters (Li et al. 2002). Another important measurement in natural waters is P. Resin gels and anion-exchange

membranes have been used to measure labile P to provide an indication of nutrient bioavailability (Abrams et al. 1992, Cooperband et al. 1994). Zhang et al. (1998) reported their studies using ferrihydrite embedded in the gel as the binding agent. Verification for the use of the ferrihydrite gel to accurately measure P in solution is shown in Figure 1.10. Zhang et al. (1998) demonstrated that DGT could be used to measure P species *in situ* in natural waters. *In situ* measurement of P is important due to its dynamic interactions in natural systems, hence the concentrations of individual species may change when samples are stored (Zhang et al. 1998). Importantly, this work showed the potential application of DGT in the measurement of bioavailable P and other nutrients in soils and the possibility of relating  $C_E$  to plant uptake for nutrients. Recently Menzies et al. (2005) compared the performance of DGT with plant response (Tomato) to heavily applied P. The DGT method proved to be more successful in comparison with extraction methods but there was no significant improvements compared to the resin method. It is unclear in this study if DIFS was used to convert DGT measurements to an effective concentration ( $C_E$ ).



**Figure 1.10** Measured mass of P on ferrihydrite gel as a function of deployment time (Zhang et al., 1998).

### 1.9. Demands for an improved soil test for soil nutrient availability

Typical soil testing methods provide a good indication of the nutrient status of the soil only if it is either acutely deficient or abundantly supplied with nutrients. Particularly in the upper part of the response curve relating nutrient supply to plant growth, soil chemical analysis alone is unsatisfactory for predicting the effects of fertilizer application (Marschner 1995).

Past and present methods of soil analysis measure variable proportions of Quantity and/or Intensity and therefore give an indication of the capacity of the soil to supply nutrients to a plant in some cases. Studies performed to correlate soil measurements with plant uptake have had varying degrees of success (see Section 1.4). Due to their simplicity, extraction methods are still commonly used as soil tests for assessment of available P. Many attempts have been made to find an extractant that can remove the labile fraction of trace elements and relating this fraction to plant availability; However, to date no one has succeeded in finding an extractant applicable to all soils, under all soil conditions. Extraction solutions typically contain a competing ion that displaces the exchangeable forms of P from the soil and problems can arise from the choice of competing ion (see Section 1.4.4).

Many of the remaining soil methods are performed on the intensity proportion of nutrient availability (i.e. analysis on soil solution) and fail to incorporate the resupply of nutrients from labile phases associated with the solid phase. DGT has been shown to be a good predictor of metal bioavailability to plants (see Section 1.6.1) and therefore provides the potential to predict nutrient availability, as  $C_E$  (measured by DGT) incorporates the soil solution concentration and its re-supply from the solid phase. Other advantages of DGT are the relative ease of sampling and the benefits of multi-element testing in a single assay. Importantly DGT has the potential to become an alternative

method to extraction techniques, providing accurate reliable results on a wide range of soils with varying properties.

#### 1.10. Conclusions and Project Aims

The new measurement of effective concentration  $C_E$ , measured by the technique diffusive gradients in thin films (DGT), has been shown to provide an effective prediction of trace metal availability in highly contaminated soil systems. There is a need for an accurate measurement of element bioavailability in soil due to the inability to find a soil test that accurately predicts available concentrations of elements. Often, techniques either overestimate or underestimate available forms of elements in a soil system. DGT is believed to best mimic a plant system by providing an infinite sink for labile ions. While DGT has successfully measured trace metals in soils under contaminated scenarios and related these measurements to plant uptake, another important requirement for soil assessment is nutrient availability. Potentially, DGT can measure P availability and relationships between  $C_E$  and plant responsiveness to P need to be investigated.

The aims of the following work are to focus on accurately predicting P availability in a wide range of soils using DGT. Phosphorus is an important nutrient worldwide (Raij et al. 2002) and is the most expensive major nutrient used in broadacre cropping in Australia (McBeath et al. 2005). However, DGT provides the potential for multi-element testing that include other important micronutrients (Cu, Mn, Mo and Zn). Therefore the aim is to develop a binding layer capable of simultaneously measuring anions and cations in a single assay. Validation of the new binding layer for use with DGT technology is required and the constraints of the binding layer will be assessed against variable conditions obtainable from agricultural soils. The effectiveness of the DGT method in predicting plant response to P will be determined along with other established soil testing methods. Prediction of plant response to P using DGT will be also

assessed with various crop species (wheat, barley, canola and lupin). The capabilities of DGT in predicting Zn and Mn availability using a single sensor will also be explored. Successful outcomes of these experiments could see its application to agricultural soils throughout Australia and increase the efficiency of fertilizer use on agricultural areas.

## 2. Performance of a mixed binding layer (MBL) for measuring anions and cations in a single assay using the diffusive gradients in thin films (DGT) technique

### 2.1. Introduction

To date, DGT technology has only been designed for separate assessment of anionic and cationic species in waters or soils, typically at concentrations characteristic of highly contaminated systems. Development of a single sensor for anions and cations offers a significant benefit, particularly for soil, which is highly heterogenous at very fine spatial scales, in terms of limiting measurement comparability problems that arise from the need to use two different assays to measure these two categories of analyte. The ferrihydrite contained in the binding layer used for assessment of P in water systems (Zhang et al. 1999) has been reported to bind divalent cations through an ion exchange process in which the absorbed cations replace bound protons (Violante et al. 2003, Cornell and Schwertmann 1996). Therefore, the ferrihydrite binding layer alone might be a suitable sink for cations using DGT technology. The aim of this work was to investigate the capabilities of the ferrihydrite binding layer to simultaneously assess cations and anions in a single assay in soils and waters. If unsuccessful the development of a new binding layer that incorporates both ferrihydrite (P assessment) and Chelex-100 (Cation assessment) may fulfil our aim. The binding system will be developed to measure elements at concentrations more representative of uncontaminated agricultural soils. This work will focus on measurement of Cu, Mn, Mo, P and Zn, as they are important plant nutrients that can be assessed by DGT. Cadmium was also included, in view of its importance to food quality (McLaughlin et al. 2000).

## 2.2. Materials and methods

### 2.2.1 Gel preparation and DGT devices

The gel solution used for making diffusive gels as well as for the tested binding layers was composed of 15 vol. % acrylamide (Boehringer) and 0.3 vol. % agarose derived cross linker obtained from DGT Research Ltd ([www.dgtresearch.com](http://www.dgtresearch.com)). Both the diffusive and binding gels were cast according to published procedures (Zhang et al. 1995). Plastic DGT devices designed for soil deployment comprised a backing plate and a front plate with an exposure window ( $A = 2.52 \text{ cm}^2$ ). Diffusive gel thicknesses of either 0.4 mm or 0.8 mm were used depending on the objective of the experiment. A 0.13 mm thick cellulose nitrate filter ( $0.45 \mu\text{m}$ ) was placed on top of the diffusive gel for protection. The filter has been found to behave as an extension of the diffusive layer (Zhang et al. 1995).

The MBL consisted of a combination of the binding agents ferrihydrite and cation exchange resin Chelex-100 (Na form, 200 - 400 wet mesh) incorporated in 10 ml of gel solution. The maximum combined amount of binding agents that could be incorporated in 10 ml of gel solution was approximately 4.5 g. Incorporation of larger amounts of binding agents was found to hinder casting procedures. The binding gel thickness was increased from 0.4 mm as reported in previous studies (Zhang et al. 1995) to 0.6 mm to allow a greater quantity of binding agents to be incorporated in the MBL and also to improve its strength.

### 2.2.2 MBL Elution and analysis

For all experiments DGT assemblies once removed from the experimental treatments, were rinsed with ultra pure water (Milli-Q) and prised open. The binding layers were then removed and placed in 1 ml of 1 M HCl for at least 24 hours. All other experimental solutions were acidified to pH 1-2 with HCl prior to analysis. DGT eluants

and solution aliquots were analysed by inductively coupled plasma-mass spectrometry (ICP-MS). Previous DGT method development studies have deployed devices in solutions containing nitrate salts of metals and eluted the binding layer in 1M HNO<sub>3</sub>. However in order to allow analysis of <sup>31</sup>P by ICP-MS it was necessary to eliminate sources of <sup>14</sup>N<sup>16</sup>O<sup>1</sup>H or <sup>15</sup>N<sup>16</sup>O from the experimental protocols as these molecular ions can significantly interfere with <sup>31</sup>P (Wilbur and McCurdy 2001). In the current study only chloride metal salts were used in the standard solutions and the eluting acid was 1 M HCl. Chloride was found to have minimal effect on the analysis of the other elements by ICP-MS. Kinetic energy differentiation was applied to further minimise potential isobaric interferences from <sup>14</sup>N<sup>16</sup>O<sup>1</sup>H or <sup>15</sup>N<sup>16</sup>O. This was achieved by setting up a bias potential between the collision cell (−3 V) and the quadrupole detector (+1 V). To assist in resolving <sup>31</sup>P from the <sup>14</sup>N<sup>16</sup>O<sup>1</sup>H and <sup>15</sup>N<sup>16</sup>O interferences instrument resolution was set at 0.4 at 10 % peak height. Background noise levels were further minimised by use of the plasma at 1600 W.

### *2.2.3 MBL Detection limits and sample handling*

Detection limits were determined by collating blank analyses from each of the individual experiments using the MBL reported in this study (total = 12). Blank analyses were assessed as follows: DGT devices (3 replicates per experiment) containing a MBL and 0.8 mm diffusive gel were assembled and placed in plastic bags. After 24 hours, DGT assemblies were dismantled and the MBL eluted as a normal sample (see Section 2.2.2). To minimise contamination in all samples, DGT devices, centrifuge tubes and sample tubes were acid washed (10 % HCl) followed by numerous rinses with ultra pure H<sub>2</sub>O (Milli-Q, 18.2 MΩ cm). All sample handling and drying was carried out in a laminar flow class-100 clean bench. Standard solutions were prepared from AnalaR grade

salts and AnalaR grade HCl was used for acid baths and other prepared acid solutions (1 M, 2 %).

#### *2.2.4 Diffusion coefficient measurement*

The diffusion coefficients necessary for calculation of  $C_{DGT}$  (equation 1) were taken from published results available from DGT Research Ltd ([www.dgtresearch.com](http://www.dgtresearch.com)) for all elements except Mo. Although Mo has been measured by DGT in solution (Garmo et al. 2003) the diffusion coefficient has not been determined independently. The diffusion coefficient for this element was therefore determined in a preliminary experiment using the procedure of Chang and co-workers (Chang et al. 1998).

#### *2.2.5 Characterisation of DGT performance*

##### *2.2.5.1 Uptake and elution efficiencies*

Simple mass balance experiments were performed to test whether the binding of elements to the MBL was affected by the quantity and properties of the two binding agents incorporated in the gel. Gels with three different ratios of ferrihydrite to Chelex (3 g:1 g, 2 g:2 g, 1 g:3 g) were used. The MBL disks were exposed to known amounts of elements by immersing them in 10 ml solutions containing Cd, Cu, Mn, Zn, Mo and P at concentrations of either 50 or 100  $\mu\text{g l}^{-1}$  with continuous end over end mixing for 24 hours. Element concentrations in the solutions were analysed at the start and end of each experiment to determine the uptake efficiency. The masses of elements that could be eluted from the MBL disks were determined by eluting with 1 M HCl and measuring the concentration in the eluant by ICP-MS.

### 2.2.5.2 Kinetics of binding

Mass balance experiments were conducted to evaluate the kinetics of element binding onto the MBL. Either MBL or pure Chelex binding layer disks (4 replicates) were placed in separate vials containing 50 mL of multi-element solutions ( $50 \mu\text{g l}^{-1}$  Cu, Mo, Mn, P, Zn). Element concentrations remaining in the solutions were measured at the start of deployment and at the following time intervals 3, 6, 10, 20, 40 min, 1, 2, 5 and 24 hours.

### 2.2.5.3 Binding layer verification

When DGT devices are deployed in well-stirred solutions of large enough volume for the analyte not to be significantly depleted by the device during the time of deployment,  $C_{\text{DGT}}$  will equate to the original analyte concentration in solution providing: 1) solution parameters do not cause any precipitation of analytes to occur, 2) the binding layer is functioning as an infinite sink for the analyte, and 3) that dissociation of any analyte complexes do not provide a rate-limiting step to diffusion across the diffusive layer or binding to the binding layer. Measurement of  $C_{\text{DGT}}$  in simple solutions can therefore be used to assess the functionality of different types of binding layers (Zhang et al. 1995, Zhang et al. 1998). When DGT devices are deployed in soils  $C_{\text{DGT}}$  provides an integrated measure of analyte contributions from both diffusional supply through the solution and from re-supply of the element from the solid phase (Zhang et al. 2001).

In order to firstly test the functionality of the pure ferrihydrite binding layer DGT assemblies (3 replicates) were immersed in a 2 L multi-element solution at pH 6.5 containing Cd, Cu, Mo ( $50 \mu\text{g l}^{-1}$ ), Mn, Zn ( $100 \mu\text{g l}^{-1}$ ) K ( $10 \text{mg l}^{-1}$ ) and P ( $400 \mu\text{g l}^{-1}$ ) for 24 hours while stirring vigorously. DGT performance was also assessed in two additional solutions containing the same element concentrations but with the pH adjusted to 4.5 and 7.5 using  $\text{HNO}_3$  or  $\text{NaOH}$ . The same experiment was used to test the MBL but

solutions contained Cu, Mo, Mn, P and Zn (each at  $50 \mu\text{g l}^{-1}$ ) at pH 6 only and DGT assemblies were immersed for 6 hours. This solution was prepared in 0.01 M NaCl to eliminate variability reported in solutions of very low ionic strength (Gimpel et al. 2001) due to the low element concentrations. The values of  $C_{\text{DGT}}$  obtained by the ferrihydrite binding layer and the MBL were calculated from the concentrations in the eluant using equation 1. Analyte concentrations in solution were measured at the beginning and end of the deployment.

Gel elution and analysis for the MBL was performed as explained in Section 2.2.2. The ferrihydrite gels were removed and eluted in 1 ml of 1 M  $\text{HNO}_3$  for at least 24 hours. Eluted solutions were analysed by inductively coupled plasma-mass spectrometry (ICP-MS) for cation concentrations excluding K and by inductively coupled plasma-optical emission spectrometry (ICP-OES) for K and P.

#### *2.2.5.4 Effect of pH and ionic strength*

To investigate the effect of pH on DGT response, DGT assemblies were immersed in multi- element solutions containing Cu, Mo, Mn, P and Zn (each at  $50 \mu\text{g l}^{-1}$ ), which were prepared to cover a pH range from 2 to 9. The pH of the solutions was adjusted using dilute (1 %) HCl or NaOH and solutions were made up to 0.01 M NaCl. Three DGT devices containing the MBL were deployed for 6 hours in such solutions.

To test DGT performance at low ionic strengths (0.001 M and 0.01 M), DGT assemblies (3 replicates) were exposed for 24 hours to multi element solutions ( $50 \mu\text{g l}^{-1}$  Cu, Mo, Mn, P and Zn) with appropriate additions of NaCl to give  $I = 0.001$  and 0.01 M. The pH of these solutions were 6.7.

#### *2.2.5.5 Capacity*

To determine the capacity of the MBL to adsorb anions and cations, DGT assemblies were immersed in well-stirred solutions containing P and Mn at various

concentrations (0.2 to 400 mg l<sup>-1</sup> Mn and 50 µg l<sup>-1</sup> to 2 mg l<sup>-1</sup> P). The diffusive gel thickness was 0.8 mm and devices were deployed for 24 hours.

Manganese was chosen as an indicator for cation capacity as it has the lowest selectivity for the Chelex with our elements of interest. To test the capacity of the MBL for Zn and Cu compared with Mn, 3 separate solutions of Zn, Cu and Mn (each at 100 mg l<sup>-1</sup>) were prepared. Three DGT assemblies with diffusive gel thickness of 0.8 mm were deployed in each solution for 24 hours to ensure capacity had been reached for each element.

#### *2.2.5.6 Ageing effect*

The shelf life of the MBL was investigated by deploying DGT assemblies in well-stirred multi-element solutions containing Cu, Mo, Mn, P and Zn (each at 50 µg l<sup>-1</sup>) for 24 h using 0.8 mm diffusive gel thickness. The experiment was repeated at selected times (10, 23, 50, 60, 86 and 146 days) following production of the MBL.

#### *2.2.5.7 DGT recoveries with diffusive gel thickness*

DGT measurements using two different gel thicknesses (0.4 mm and 0.8 mm) were investigated by deploying DGT devices with a MBL in well-stirred multi-element solutions containing Cu, Mn, P and Zn (each at 50 µg l<sup>-1</sup>). Four assemblies were deployed per solution, two containing 0.8 mm diffusive gels and two containing 0.4 mm diffusive gels for 6 hours.

#### *2.2.5.8 DGT deployment in soils*

To assess the ability of the MBL to measure cationic and anionic species in soil, DGT assemblies were deployed on 8 agricultural soils sampled from Western and South Australia. Results were compared to results obtained from DGT assemblies containing either standard Chelex or standard ferrihydrite binding layers deployed in the same soils.

Selected soils were spiked with Cu, Mn, Zn and Mo or P (concentrations of elements are shown in Table 2.1) in an attempt to achieve a reasonable spread of nutrient concentrations. The soils were left to incubate at 50 % water holding capacity (WHC) for 2 weeks and then the moisture content was increased to 80 % WHC the day prior to DGT deployment. Two DGT devices for each binding layer were deployed on the soil for 24 hours and a diffusive gel thickness of 0.8 mm was used.

**Table 2.1** The pH and selected total nutrient concentrations of soils used for DGT deployment prior to amendment with additional nutrients as indicated.

<b>Sample ID</b>	<b>pH (H<sub>2</sub>O)</b>	<b>Cu mg kg<sup>-1</sup></b>	<b>Mn mg kg<sup>-1</sup></b>	<b>Mo mg kg<sup>-1</sup></b>	<b>Zn mg kg<sup>-1</sup></b>	<b>P mg kg<sup>-1</sup></b>
Bal*	5.8	2.6	32.7	0.8	3.9	69.4
Yal*	6.2	3.1	39.8	0.6	4.0	92.9
West York*	5.4	1.7	40.5	0.5	4.4	42.1
Northam	4.3	6.3	60.3	0.6	7.3	76.5
Northam*	4.3	6.3	60.3	0.6	7.3	76.5
Wongan <sup>#</sup>	5.2	0.7	24.9	0.4	18.2	27.0
Cunderdin <sup>#</sup>	6.1	6.2	148.8	0.5	12.5	76.3
Salmon Gums	8.3	6.7	67.4	0.2	18.6	97.2

\* 20 mg kg<sup>-1</sup> Cu, Mn, Zn and 5 mg kg<sup>-1</sup> Mo added

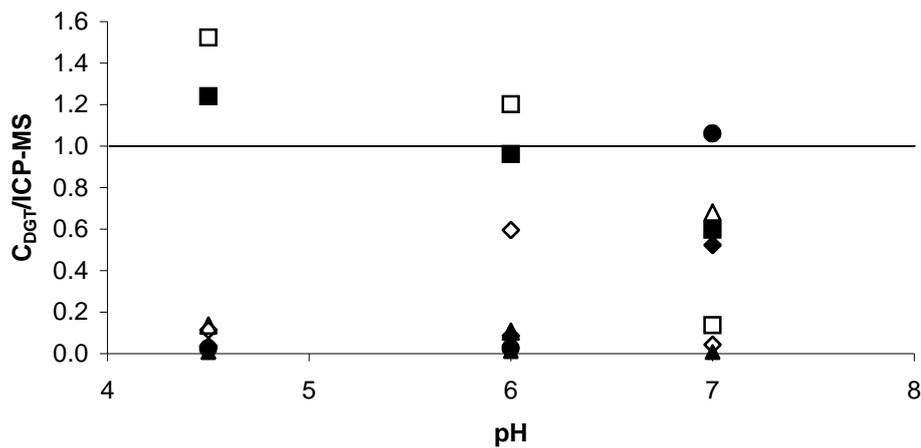
<sup>#</sup>20 mg kg<sup>-1</sup> P added

## 2.3. Results and discussion

### 2.3.1 Solution measurements using DGT with ferrihydrite binding layer

Solution concentrations measured by DGT using the pure ferrihydrite binding layer ( $C_{DGT}$ ) were quite variable when compared to actual solution measurements for P and Mo at all solution pHs (Figure 2.1). This variability could be due to a significant residual charge in the diffusive gel that is caused by inadequate washing procedures (see Section 2.3.7, Warnken et al. 2005). On consultation with colleagues at Lancaster University the washing procedure was not adequate and was consequently changed by increasing the volume of water used in the washing step. It appears that the ferrihydrite binding layer is acting as an infinite sink for P and Mo at pH 6 in accordance with results

produced by Zhang et al. (1999). However, DGT recoveries for P and Mo dropped considerably at pH 7 and the mechanism for this observation remains unclear. Solution concentrations measured by DGT are considerably lower than actual solution concentrations for all cations with the exception of Mn at pH 7. This suggests that although the pure ferrihydrite binding layer is able to bind cations to some extent it is not sufficient enough to act as an infinite sink. Sorption capacity of the ferrihydrite for the cations appears to increase with pH. From this variability and along with the poor agreement with solution concentrations it can be concluded that the ferrihydrite binding layer is not adequate for simultaneous anion and cation assessment.



**Figure 2.1** Measurement of multi-element solutions containing Cd ( $\Delta$ ), Mn ( $\bullet$ ), Zn ( $\blacklozenge$ ), Mo ( $\square$ ), Cu ( $\diamond$ ), K ( $\blacktriangle$ ) and P ( $\blacksquare$ ) with the ferrihydrite binding layer. Concentrations assessed by DGT ( $C_{DGT}$ ) are compared to the direct measurement of concentrations in the solution by ICP-MS.

The following experiments details the method development of a Mixed Binding Layer (MBL) that allows simultaneous assessment of both anions and cations. Potassium sorption onto the ferrihydrite binding layer was minimal possibly due to K being a monovalent cation. It has also been reported (Garmo et al. 2003) that the Chelex-100 used as the binding agent for cations is unable to bind K in quantifiable amounts.

Therefore at this current stage assessment of available K in soils using DGT appears unachievable. There is some scope that DGT could be used simply as a resin technique for assessment of K in order to get some information from a measurement. It appears necessary for the quantifiable measurement of K that a new binding layer needs to be developed that incorporates an agent that can bind K in sufficient amounts to act as an infinite sink. This type of work was outside time restraints for this project.

### 2.3.2 DGT (MBL) blanks and detection limits

Blank values, expressed as ng per binding disc are given in Table 2.2. Blank concentrations of P in the MBL were higher than concentrations of other elements (apart from Zn), suggesting minor P contamination in the production of ferrihydrite and/or mass interferences associated with the ICP-MS analysis. Phosphorus contamination has recently been significantly reduced with improved contamination controls and instrument settings. Contamination with Zn has been observed by other workers (Gimpel et al. 2001) and is difficult to eliminate completely.

**Table 2.2** Calculated blanks and method detection limits (MDL) for selected elements using the mixed binding layer (MBL) (refer to text for the method used to calculate the MDL).

<b>Element</b>	<b>MBL Blank (ng/device)</b>	<b>MBL MDL (ng/device)</b>	<b>MBL MDL (<math>\mu\text{g l}^{-1}</math> solution)*</b>
P	28.1 $\pm$ 7.0	49.2	4.76
Mn	3.2 $\pm$ 1.6	8.0	0.77
Cu	2.3 $\pm$ 0.8	4.6	0.45
Mo	1.5 $\pm$ 0.8	4.0	0.38
Zn	15.4 $\pm$ 4.3	28.3	2.74
Cd	0.05 $\pm$ 0.1	0.2	0.02

\*Required solution concentration to obtain MBL MDL for a 24 hour deployment using a 0.8 mm diffusive gel.

The minimum detection limits (MDL) for the MBL are also given in Table 2.2, calculated in accordance with the IUPAC definition as the mean value of the blank plus three times its standard deviation (Thompson et al. 1987). To assist comparison with anticipated experimental data for soils, the MDL values have also been converted to the solution concentration ( $\mu\text{g l}^{-1}$ ) which would be indistinguishable from the detection limit for a 24 hour deployment using a 0.8 mm diffusive gel (Table 2.2). It can be seen that the levels of contamination observed for P and Zn are equivalent to soil pore water concentrations of  $< 5$  and  $3 \mu\text{g l}^{-1}$  respectively. As mentioned above, blank concentrations for P have been decreased in more recent experiments resulting in soil pore water equivalent concentrations of  $< 1 \mu\text{g l}^{-1}$ . Detection limits for the other elements were all well below  $1 \mu\text{g l}^{-1}$  (Table 2.2). Examples of soil solution concentrations for Cd, Cu, Mn, P and Zn that might be expected in agricultural soils are shown for comparison in Table 2.3, which consists of unpublished data from the CSIRO archives collected from 35 different agricultural soils across Australia. These data suggest that the detection limits achievable with MBL DGT are unlikely to pose a constraint to use of this technique for assessment of nutrient availability in a wide spectrum of agricultural soils. It should also be noted that much of the variability in the blank values were caused by fluctuations in low-level contamination between each experiment. Substantially lower MDL values were obtained within a given experiment.

**Table 2.3** Soil solution concentrations for selected elements from a wide range of agricultural soils throughout Australia.

<b>Soil Areas</b>	<b>State</b>	<b>Cd µg l<sup>-1</sup></b>	<b>Cu µg l<sup>-1</sup></b>	<b>Mn µg l<sup>-1</sup></b>	<b>P µg l<sup>-1</sup></b>	<b>Zn µg l<sup>-1</sup></b>
Berrigan	NSW	1.0	nd	1760	3120	93
Emerald Black	Qld	nd	nd	nd	331	63
Emerald Brown	Qld	nd	nd	nd	490	77
Ingham - Hamleigh	Qld	0.2	nd	1290	250	57
Innisfail - Innisfail	Qld	0.1	nd	1220	409	85
Innisfail - Liverpool	Qld	nd	nd	424	163	38
Innisfail - Pin Gin	Qld	0.1	nd	21	152	31
Innisfail - Toobana	Qld	0.2	nd	1840	228	56
Kingaroy Coolabunia	Qld	0.1	8	229	247	45
Kingaroy Goodger	Qld	nd	12	243	268	47
Tully - Banyan	Qld	0.4	nd	858	62	58
Boloto Trial	SA	1.0	47	121	2510	61
Booloroo	SA	0.1	nd	64	1510	50
Bordertown	SA	1.4	nd	636	478	65
Bute	SA	0.2	nd	nd	1950	66
Cooke Plains	SA	0.8	nd	243	2460	53
Freeling	SA	4.5	nd	15300	1370	85
Inman Valley	SA	1.2	nd	5720	1800	112
Kapinnie	SA	0.8	nd	1150	1300	54
Kapunda	SA	0.6	51	1340	1080	42
Kelly Trial	SA	2.3	nd	63	266	50
Lower SE	SA	1.7	17	416	2110	295
Martindale	SA	0.8	60	6010	2390	83
Minnipa	SA	nd	nd	nd	380	34
Mintaro	SA	1.1	nd	6910	1390	89
Mt Shank	SA	1.2	14	688	567	84
Mundulla	SA	5.5	nd	3240	33300	442
Nangari	SA	0.1	nd	25	534	47
Pinnaroo	SA	0.1	nd	nd	542	44
Siekmann Trial	SA	1.0	25	84	20	45
Tarlee	SA	4.4	9	9850	962	82
Tepko	SA	0.6	10	103	1940	49
Clarke Hill	Vic	0.7	nd	7680	489	43
Kooweerup	Vic	2.8	nd	1630	353	114
Thorpedale	Vic	0.9	nd	1960	518	70
	<b>Mean</b>	1.2	21	2370	1880	80
	<b>Minimum</b>	0.1	8	21	20	31
	<b>Maximum</b>	4.5	60	15300	33300	442

nd\* = Below GF-AAS detection limit (Cd) or ICP-OES detection limit (Cu, Mn, Zn)

*CSIRO Land and Water, PMB 2, Glen Osmond, SA 5064, Australia*

### *2.3.3 Diffusion of Mo in diffusive gel*

The measured value of the diffusion coefficient for Mo (converted to 25 °C) obtained using the standard diffusive gel was  $6.48 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ , which is 66 % of the published value in water at 25 °C ( $9.91 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ ) (Li and Gregory 1974). This result is consistent with that found for other anionic species, for example the diffusion coefficient of P through the diffusive gel has been reported as 71 % of the value in water (Zhang et al. 1998).

### *2.3.4 Uptake and elution efficiencies*

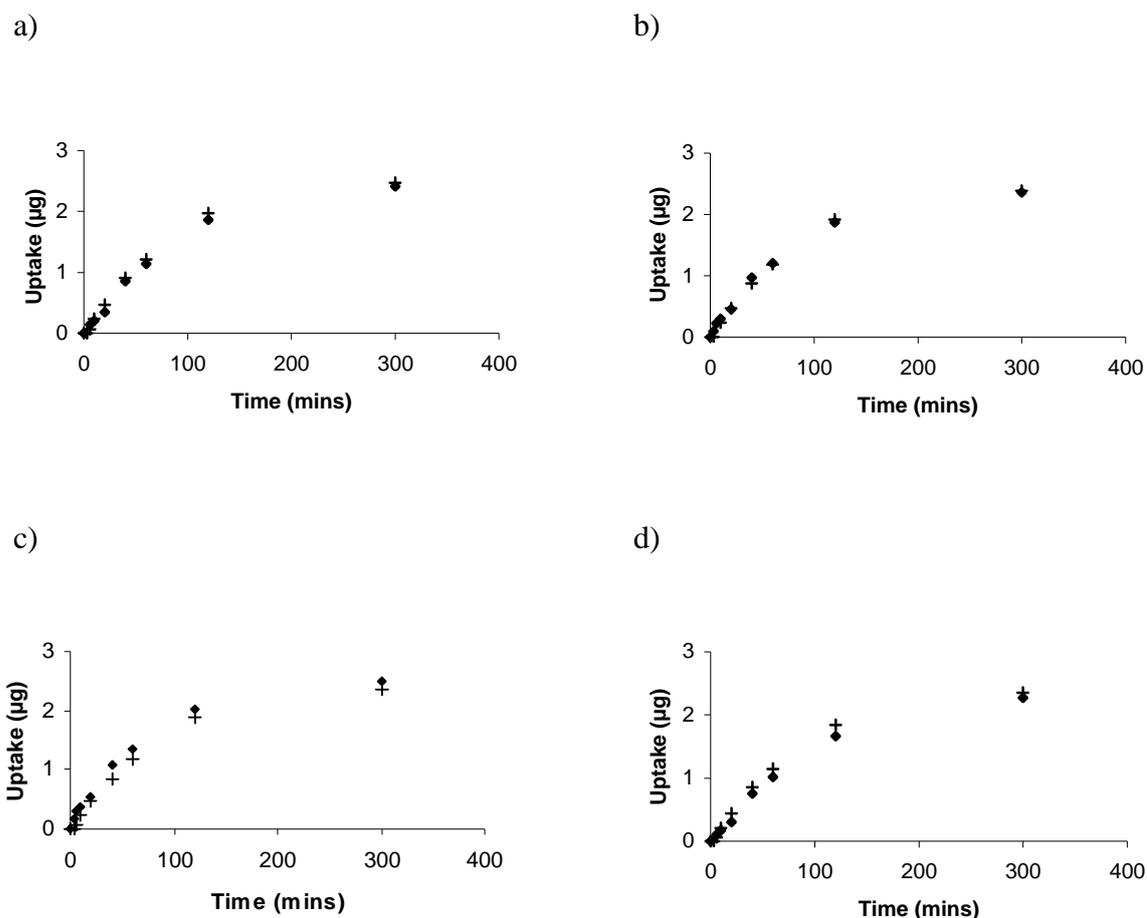
Uptake efficiencies of P were found to be dependent on the amount of ferrihydrite incorporated in the MBL. The MBL (0.4 mm thick), which contained 3 g of ferrihydrite and 1 g of Chelex per 10 ml gel solution, had the greatest uptake efficiency (> 98 %) for the anions tested. Uptake efficiencies dropped slightly with decreased amounts of ferrihydrite. Uptake efficiencies for selected cations (Cd, Cu, Mn and Zn) were unaffected by the amount of Chelex used in the gel as they were consistently > 98 % for the three different types of MBL. We increased the total amounts of binding agents incorporated as part of the binding layer to maximise the capacity of the MBL. To preserve the physical strength of the gel, this necessitated increasing the total gel thickness to 0.6 mm. With 3 g ferrihydrite and 1.5 g Chelex per 10 ml gel solution, uptake efficiencies for all elements (Cd, Cu, Mn, Mo, P and Zn) were > 98 %. This MBL was used for all subsequent experiments.

Elution efficiencies were: P  $91.9 \pm 4.6 \%$ ; Mn  $94.2 \pm 2 \%$ ; Cu  $92.3 \pm 3.4 \%$ ; Zn  $92.2 \pm 3.3 \%$ ; Mo  $79.3 \pm 5.4 \%$  and Cd  $92.1 \pm 3 \%$ . All elements investigated except Mo had a recovery of 92 % within the error limits, irrespective of the amount of element bound. This is higher than the established value of 80 % (corresponding to an elution factor of 0.8) using 1 M HNO<sub>3</sub> and a binding layer composed of entirely Chelex (Zhang

et al. 1995). Using 1 M HNO<sub>3</sub> for elution from the MBL also gave values close to 0.8, suggesting that elution is more efficient with HCl. An elution factor of 0.92 (0.79 for Mo) was adopted for the calculation of element concentrations in bulk solutions.

### *2.3.5 Kinetics of binding*

The rate of binding of the cation to the MBL was similar to the rate for the Chelex binding layer (Figure 2.2). Uptake of elements increased linearly for both types of binding layer for the first hour of exposure. Beyond the first hour, uptake by the binding layers decreased as the solution approached total depletion. If there were a kinetic limitation to binding to the MBL, i.e. if the MBL did not act as an infinite sink, then the rate of uptake by the MBL should be significantly decreased in comparison to the Chelex binding layer. Since this was not observed, it is concluded that there was no kinetic limitation.



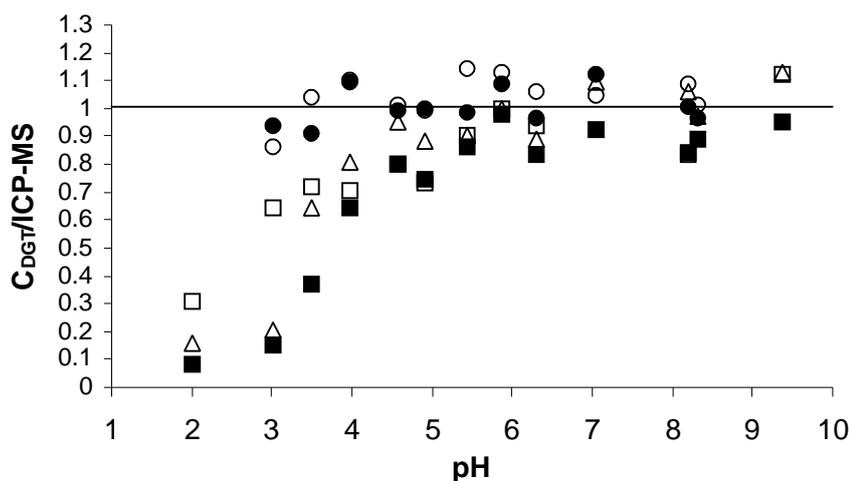
**Figure 2.2** Comparison of cation uptake with Chelex binding layers (+) and MBLs (◆) placed in multi element solutions ( $50 \mu\text{g l}^{-1}$ ) for 5 hours, a) Mn, b) Cu, c) Zn and d) Cd.

### 2.3.6 Solution measurements using DGT with MBL

Solution concentrations for both P and cations predicted by DGT using MBL (i.e.  $C_{\text{DGT}}$ ) were in good agreement with the concentrations directly measured in the solution. Mean values of  $C_{\text{DGT}}/\text{ICP-MS}$  for P, Mn, Cu and Zn were  $1.02 (\pm 0.06)$ ,  $0.95 (\pm 0.04)$ ,  $0.91 (\pm 0.04)$  and  $0.93 (\pm 0.04)$  respectively. This demonstrates that the new MBL can allow simultaneous, quantitative measurement of selected anions (P and Mo) and cations (Cd, Cu, Mn and Zn) by DGT. There is also potential for the measurement of other analytes using the MBL that have previously shown to be successful using the chelex binding layer (see section 1.6).

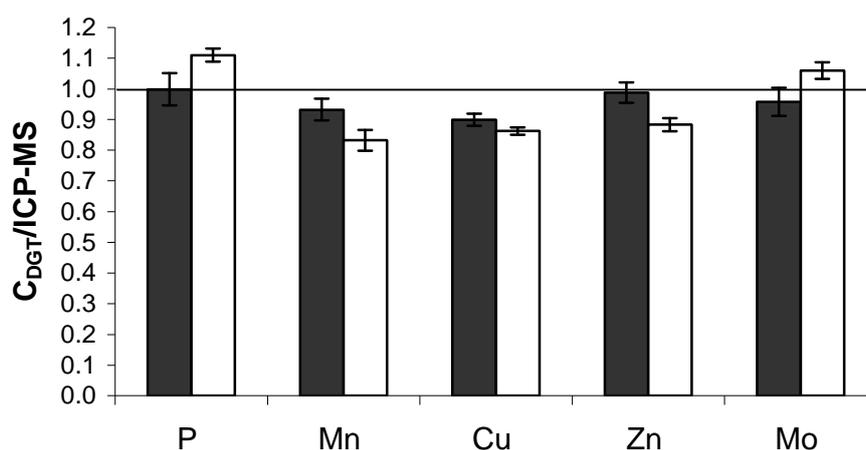
### 2.3.7 Effect of pH and ionic strength

Element concentrations determined by DGT agreed well with bulk solution measurements between pH 5 and 9 (Figure 2.3). Below pH 5 the DGT measurement of cations declined, but DGT measurements of anions were independent of pH. Slightly lower recoveries (90 %) for the cations at pH 5 are possibly due to  $\text{Fe}^{3+}$  from the iron oxide competing with these cations for binding sites on the Chelex. Supporting this observation are the poor DGT recoveries of Cu at lower pH. Gimpel et al. (2001) previously found that pH had no effect on DGT measurements for Cu down to pH 2 when Chelex alone was used as the binding layer. Caution should therefore be exercised in using DGT with the MBL for measuring cations in natural waters and soils when the pH is below 5. However, this is unlikely to be a major constraint for use of DGT for agronomic purposes as very acidic soils ( $\text{pH} < 5$ ) are not optimal for plant growth due primarily to aluminium/manganese toxicity, and therefore are either not used for growing crops, or are limed before use. Furthermore, while P deficiencies in acidic soils may be common, deficiencies of cationic trace elements are unlikely due to greater solubility of cations under acidic conditions.



**Figure 2.3** Effect of pH on the measurement of multi-element solutions containing P (●), Mn (■), Cu (□), Zn (Δ), and Mo (○) ( $50 \mu\text{g l}^{-1}$ ) with the MBL. Concentrations assessed by DGT ( $C_{\text{DGT}}$ ) are compared to the direct measurement of concentrations in the solution by ICP-MS.

At ionic strengths of 0.001 M and 0.01 M, DGT measurements agreed well with bulk solution concentrations (Figure 2.4), which concurs with previous work (Peters et al. 2003). Several studies have reported inaccurate measurements of elements by DGT at ionic strengths less than 0.001 M (Peters et al. 2003, Alfaro-Del la Torre et al. 2000, Sangi et al. 2002), which have been attributed to modification of the diffusion of ions through the gel layer. However recent work has shown that there may be significant residual charge in the diffusive gel if it is not washed properly. The charge may affect diffusion of ions in very low ionic strength solutions by introducing a Donnan potential at the surface of the gel (Yezek et al. 2004, Warnken et al. 2005). Warnken et al. (2005) demonstrated that measurements with good precision and accuracy could be made by DGT in low ionic strength solutions ( $I < 0.001$  M) when gels were washed adequately. In soil pore waters, ionic strength is typically reported to be  $> 0.002$  M (Gilman 1981, Dolling and Ritchie 1985), hence the reported ionic strength effects are unlikely to be an issue for assessment of elements in soils.



**Figure 2.4** Effect of ionic strength (0.001 M (■), 0.01 M (□)) on the ratio of DGT measurements ( $C_{DGT}$ ) using the MBL with direct measurement of solution concentrations (ICP-MS). Error bars represented  $\pm 1$  standard error.

### 2.3.8 Capacity

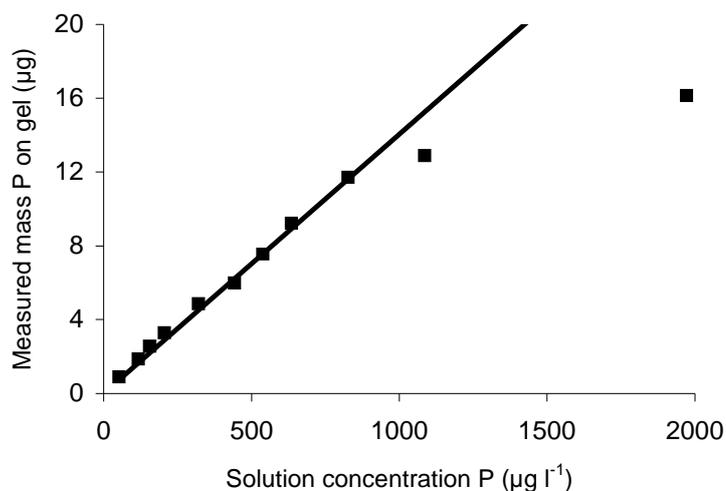
#### 2.3.8.1 *P* (anion) capacity

The MBL gave a linear and theoretically predictable response with concentration up to  $850 \mu\text{g l}^{-1}$  P (Figure 2.5a). The capacity of the MBL is effectively limited to about  $12 \mu\text{g}$  of P. Pure ferrihydrite gels that incorporated the same amount of ferrihydrite per 10 ml gel solution were also deployed. Deviation from linearity occurred at a similar concentration (data not shown) suggesting that the added Chelex does not interfere with the capacity of ferrihydrite to sorb P.

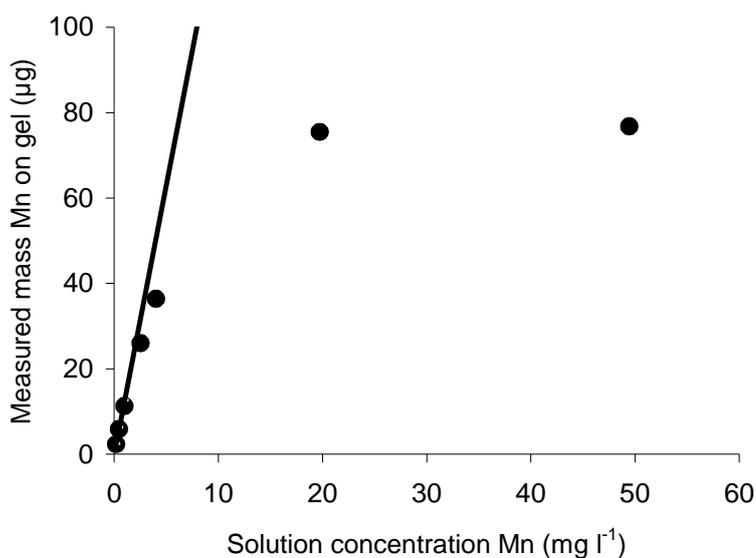
#### 2.3.8.2 *Mn* (cation) capacity

A linear and theoretically predictable response with solution concentration was obtained up to  $3 \mu\text{g ml}^{-1}$  of Mn using the MBL. The capacity of the MBL is effectively  $75 \mu\text{g}$  of Mn (Figure 2.5b). The capacity of pure Chelex binding layers was also considerably lower, at  $80 \mu\text{g}$  measured mass of Mn (data not shown) compared to the literature value of  $0.56 \text{ mg}$  for Cd (Zhang et al. 1995). This suggests that the capacities of the Chelex gels are element dependent. As the selectivity of Chelex for Mn is particularly low, and considerably lower than that for Cd which has the next lowest selectivity of the analytes investigated in this study, the capacity of the MBL can be expected to be higher than Mn for other metals. During this experiment there were no precautions taken in preventing  $\text{Mn}^{2+}$  oxidising to  $\text{MnO}_{2(s)}$ . Any oxidation that did take place has not affected capacity measurements, as these appear stable at  $75 \mu\text{g}$  Mn when the Mn solution concentration was increased to  $50 \mu\text{g ml}^{-1}$ .

a)



b)

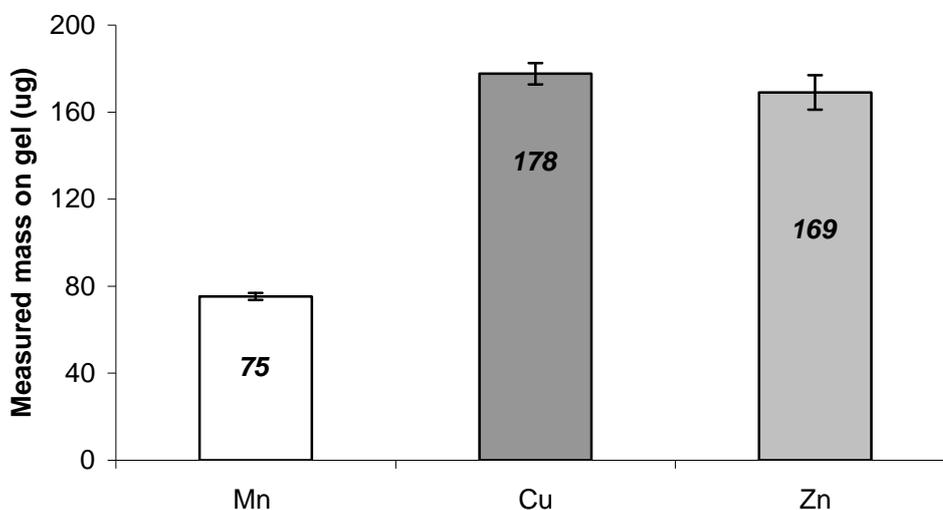


**Figure 2.5** a) Measured mass of P on the MBL with increasing solution concentration. b) Measured mass of Mn on the MBL with increasing solution concentration. Solid lines represent the theoretical mass that should be obtained using eqn 7.

### 2.3.8.3 MBL cation capacity comparison

Measured amounts of metal obtained from the MBL were 75 µg of Mn, 178 µg of Cu and 169 µg of Zn (Figure 2.6). The reproducibility of Mn capacity on the MBL and the higher amounts obtained for Zn and Cu suggests that cation capacity on the Chelex is

element dependent and corresponds with individual metal selectivity for the Chelex, although amounts obtained for Cu and Zn were not significantly different ( $p < 0.05$ ).

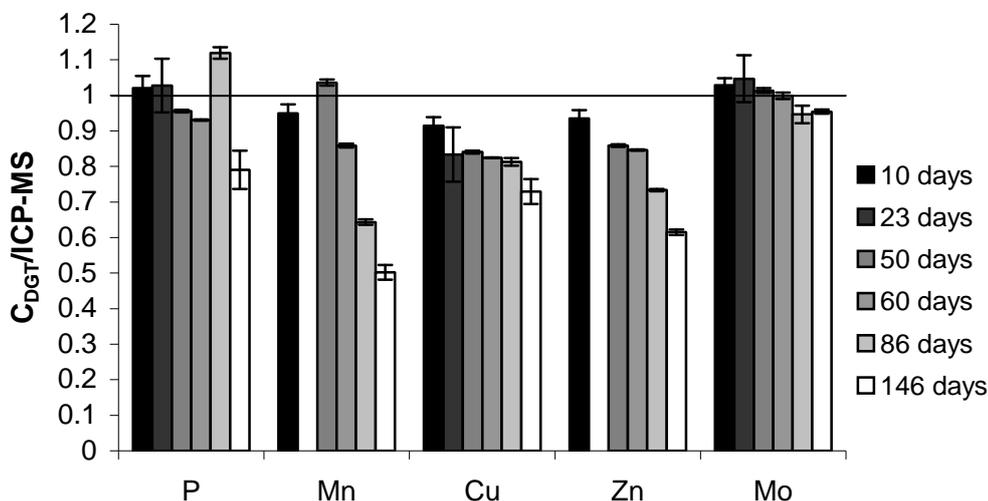


**Figure 2.6** Measured mass of Mn, Cu and Zn on the MBL at capacity. Error bars represent one standard error of the mean (3 reps).

### 2.3.9 Ageing effect

There appeared to be a trend of decreasing Mo and P with age of the MBL (Figure 2.7). However, this trend did not translate to a significant difference in the results for Mo ( $p < 0.05$ ) over the whole time course of the experiment, and a significantly lower concentration of P was found only at the 146 day sampling point. In contrast, concentrations of cations measured by DGT using MBLs that had been stored for 2 months were only 85 % of concentrations measured directly in solution (Figure 2.7). Results for Mn and Zn at 23 days were removed due to a large standard deviation between replicates, which contributed to unexpectedly low DGT recoveries. The decrease in performance in cation assessment could indicate that with time, Fe dissociates in the gel and binds to the Chelex, leading to competition for binding sites with the other elements. These results suggest that the MBL should be used within two weeks of production to obtain a DGT response within 10 % of solution concentrations for all

elements tested. The performance of each batch of the MBL (beyond 2 weeks) should be checked to provide a calibration rather than relying solely on the theoretical DGT equation.



**Figure 2.7** Performance of MBL with time after initial production. DGT devices (3) deployed in solutions containing P, Mn, Cu, Zn, and Mo ( $50 \mu\text{g l}^{-1}$ ) for 24 hours using 0.8 mm diffusive gels. Solution pH ranged from 6.6 to 6.7.

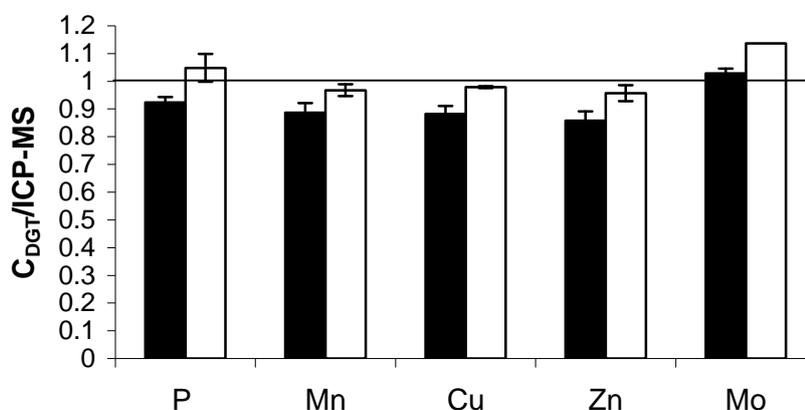
Throughout experiments with the MBL, cation recoveries were consistently lower ( $\sim 6\%$  for Zn, 9-10% for Cu and Mn) compared to recoveries for both P and Mo (Table 2.4). Chelex beads will be spaced further apart in the MBL than in the pure Chelex gels, due to the lower concentration of Chelex used. Furthermore, when ferrihydrite is added to the Chelex as a random distribution of particles, some particles of ferrihydrite will inevitably lie between the Chelex beads at the gel surface. On approaching the MBL a cation may have to travel further and bypass a ferrihydrite particle before binding to a Chelex particle. The extra diffusion pathway may result in an underestimation of the diffusion layer thickness ( $\Delta g$ ) and consequently result in an underestimate of  $C_{\text{DGT}}$ .

**Table 2.4** Mean calculated  $C_{DGT}/ICP-MS$  ratios for elements P, Mn, Cu, Zn and Mo using the mixed binding layer (MBL) for all experiments.

$C_{DGT}/ICP-MS$	P	Mn	Cu	Zn	Mo
Mean	1.00	0.90	0.91	0.94	1.05
Standard dev.	0.08	0.06	0.07	0.09	0.06
% RSD	7.55	6.46	8.08	9.41	6.11

### 2.3.10 DGT recoveries with diffusive gel thickness

There was no significant difference in DGT measurements of P, Mo, Mn, Cu or Zn with use of either a 0.4 mm or 0.8 mm diffusive gel ( $p < 0.05$ ) (Figure 2.8). Thinner diffusive gels could therefore be an option for deployments on nutrient deficient soils, improving detection limits by increasing the masses of elements accumulated by DGT for a given deployment time.

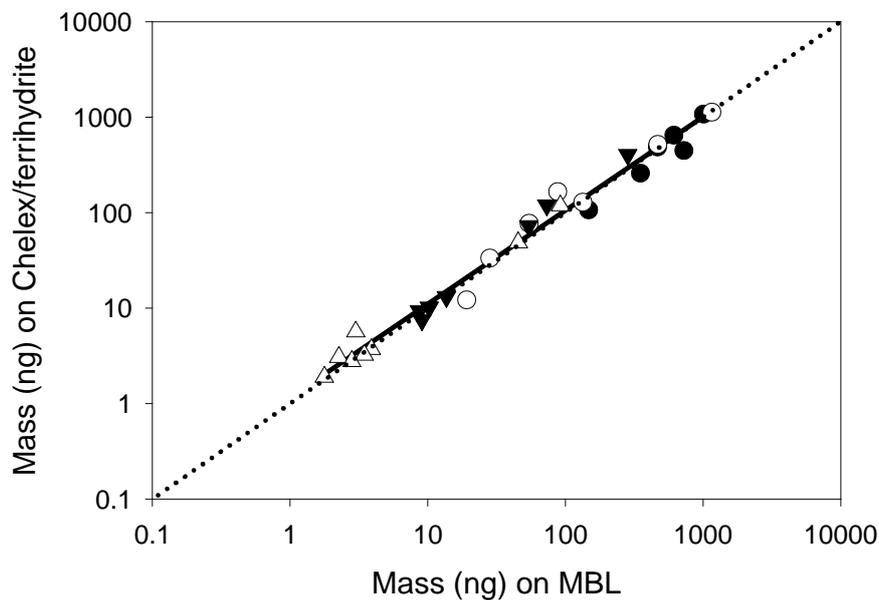


**Figure 2.8** Measurements made by DGT devices (4) containing MBL with either 0.4 mm (■) or 0.8 mm (□) diffusive gels immersed in solutions containing P, Mn, Cu, Zn ( $50 \mu\text{g l}^{-1}$ ). Solution pH was 6.6.

### 2.3.11 DGT deployment in soils

Measurements made using DGT assemblies containing the MBL on agricultural soils from Western and South Australia were comparable to measurements made with

devices containing either pure ferrihydrite or pure Chelex binding gels (Figure 2.9). Combining the whole dataset for P, Cu, Mn and Mo measurements resulted in a slope of 0.97 with a correlation coefficient ( $r^2$ ) of 0.95 (Figure 2.9). Reliable Zn results were not obtained due to contamination that caused values to fall below the analytical detection limit for this experiment. It appears that the addition of ferrihydrite and Chelex together in a binding gel does not hinder its ability to act as an infinite sink for both cation and anion measurement in soils.



**Figure 2.9** Measured mass (ng) obtained with the MBL compared with measured mass (ng) obtained with the ferrihydrite binding layer for P (●) and Mo (Δ) and the Chelex binding layer for Cu (▼) and Mn (○). Dotted line represents the 1 to 1 line and the solid line represents the regression fit for the data. Soil pH ranged from 4.3 to 8.3 (see table 2.1), two DGT devices for each type of binding layer were deployed.

#### 2.4. Overall analysis

This study has evaluated the performance of DGT devices containing a new binding layer. The mixture of ferrihydrite and Chelex in the binding gel (MBL) allows simultaneous measurement of both anions and cations in a single assay.

The performance of DGT containing the MBL has been tested over a range of solution and deployment conditions. At pH values < 5, cations were not measured quantitatively, possibly due to Fe<sup>3+</sup> within the gel dissociating and competing with the other cations for binding to Chelex. The MBL DGT was able to quantitatively measure both P and Mo between pH 3-8 and all cations between pH 5-9. Caution will be necessary when measuring cations on soils and waters with pH values below 5. Measurements of cations with the MBL were found to be independent of ionic strength above 0.001 M.

The capacity of the MBL gel for quantitative measurement of P is effectively limited to 12 µg. For Mn the capacity is effectively limited to 75 µg. Capacities of the MBL for other metals with a greater affinity for the Chelex resin should be substantially higher as obtained for Cu and Zn in section 2.3.8.3.

Use of the MBL enables the analysis of all elements together using ICP-MS. Analysis of P was made possible by eliminating nitrates from DGT preparation and processing steps which minimised potential N interferences with P. With 1 M HCl as the eluting agent, the elution factor was 0.92 for all elements except Mo, which had an elution factor of 0.79.

The MBL performed effectively when deployed on soils. Good correlations between the MBL and either pure Chelex or ferrihydrite gels were obtained for P, Cu, Mn and Mo. With use of the MBL, DGT could become a very important tool for defining nutrient deficient soils and, therefore, fertilizer requirements.

### 3. Investigating chemical constraints to the performance of DGT and Resin methods in the assessment of soil phosphorus.

#### 3.1. Introduction

The increasingly popular method which uses anion exchange membranes (AEM), the “resin P” test, has been shown in some studies to be an improvement over extraction methods as a soil test for predicting plant yield (van Raij et al. 1986, Kumar et al. 1992, Saggar et al. 1992, Saggar et al. 1999, Bissani et al. 2002, McBeath et al. 2005, Menzies et al. 2005). The Diffusive Gradients in Thin Films (DGT) method was also found to be an improvement over the extraction methods for assessing plant available P in a recent study (Menzies et al. 2005). These methods are likely to be improvements on the extraction methods as they potentially mimic plant P uptake by creating a well-defined sink for P. These tests lower the concentration of P in the solution phase, promoting resupply of the soil solid phase. For the resin P method, an AEM is placed in a soil solution while the DGT method, which uses a ferrihydrite binding gel as the sink, is placed *in situ* directly on the soil.

Numerous authors have studied both the resin P method and a similar method that, instead of an AEM, uses an iron oxide impregnated paper strip as the sink (Menon et al. 1989, Menon et al. 1990, Myers et al. 1995, Guo et al. 1996). Some limitations to the resin method have been evaluated during the method development phase of the soil test. In one of the earliest studies using resin beads in nylon bags, Sibbesen (1978) found that the amount of P extracted was dependent on the anionic counterion associated with the resin. In a calcareous soil he found that resin beads in the  $\text{HCO}_3^-$  form extracted more  $\text{HPO}_4^{2-}$  than resin beads in the  $\text{Cl}^-$  form. Another study by Sibbesen and Rubaek (1994) also reported similar observations using a sandy loam with a pH of 4.6. Sibbesen (1978) therefore recommended that resins should be used in bicarbonate form. However, when

Sibbesen (1978) compared resin P values with the uptake of P by ryegrass there was little difference between the relationship when the resin was in  $\text{HCO}_3^-$  form ( $r^2 = 0.9$ ) versus the  $\text{Cl}^-$  form ( $r^2 = 0.88$ ). In contrast, Bache and Ireland (1980) and Sherrod et al. (2003) found little difference between using  $\text{HCO}_3^-$  or  $\text{Cl}^-$  as the counter ions on the resin to assess P in a calcareous soil. One proposed advantage of using  $\text{HCO}_3^-$  form is that the  $\text{HCO}_3^-$  displaced by the nutrient ions may mimic  $\text{HCO}_3^-$  produced by the rhizosphere due to the excretion of  $\text{CO}_2$  by plant roots and micro-organisms (Qian and Schoenau 2002). Numerous authors have used the bicarbonate form as it also maintains low Ca concentrations in solution and has been reported to enhance P extraction from the soil (van Raij et al. 1986, Saggar et al. 1990, Qian et al. 1992). Arguments for using the  $\text{Cl}^-$  form are that  $\text{HCO}_3^-$  can increase the supernatant pH, it also complicates the acid desorption step through  $\text{CO}_2$  evolution and the membranes must be converted to  $\text{HCO}_3^-$  each time for re-use (McLaughlin et al. 1993). However based on the observations of Sibbesen (1978), Sherrod et al. (2003) stated that the best resin counter ion for P testing is a function of inherent soil properties.

In recent studies, the use of an AEM has been preferred over the use of resin beads (Saggar et al. 1990, Schoenau and Huang 1991, Saggar et al. 1992, Saggar et al. 1999) due to the procedural disadvantages of using either resin beads mixed with soil (Amer et al. 1955) or resin beads in nylon bags (Sibbesen 1978, Binkley 1984, Gibson 1986). The AEM is not specific for binding P (Van Der See et al. 1987) and experiments have been performed using the AEM to measure N and S concentrations in soils (Qian et al 1992, Collins and Allinson 1999). The capacity of the AEM will therefore be an important property ensuring it is acting as an infinite sink for the quantitative extraction of available P. Sherrod et al. (2003) stated that despite the use of resins for more than 40 years, resin use among researchers is not uniform and interpretive differences between methods are poorly understood. Reported capacities of the AEM for P are lacking,

possibly due to these numerous variations with the resin method. Discussion and studies on the potential competitive effects of common environmental anions on the assessment of P by resins has also been limited. In one of the few studies looking at the effect of potential competitive anions on the measurement of P by resins, Cooperband and Logan (1994) reported  $\text{NO}_3\text{-N}$  concentrations of 50 and 100  $\text{mg l}^{-1}$  reduced AEM P sorption by 50 and 75 % respectively. They also reported that  $\text{SO}_4\text{-S}$  concentrations of 500 and 1000  $\text{mg l}^{-1}$  reduced AEM P sorption by 98 %. Turrion et al. (1999) assessed the influence of  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  on the extraction of P using AEM in  $\text{HCO}_3^-$  form. They found that as both  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  concentrations increased, the AEM P extraction decreased to approximately 60 % at 50  $\text{mg N/L}$  and at 30  $\text{mg S/L}$ . Also when the P concentration in the solution increased, the extraction of P decreased in the presence of these anions, possibly indicating that capacity had been reached. In both these studies the concentrations of anions used have not been put into an agricultural context and the possible effect of  $\text{Cl}^-$  concentrations in solution was not examined. Uusitalo and Ekholm (2003) reported decreases in P recovery by 15 – 20 % when anion ( $\text{Cl}^-$ ,  $\text{NO}_3^-$ , or  $\text{SO}_4^{2-}$ ) equivalent concentrations exceeded about 4  $\text{mmol L}^{-1}$  using resin beads in nylon bags. The concentrations tested were based on those obtained from selected water systems, hence also not necessarily relevant to a soil fertility context. The effect of possible competition by  $\text{HCO}_3^-$  in solution on resin P measurements has also not been investigated.

Competitive limitations to anion uptake by the DGT method have not yet been investigated in any study. The ferrihydrite used as the binding agent for P in DGT technology has a high affinity for P, and phosphates are rapidly adsorbed onto these surfaces (Menon et al. 1990). Similar to the ferrihydrite binding layer is the iron oxide-impregnated paper used as the P sink in the  $\text{P}_i$  method. The P measured by the  $\text{P}_i$  method has been shown to correlate well with maize yield and P concentrations in plant herbage

(Menon et al. 1990), but has been shown to overestimate P when compared to the AEM method (Bissani et al. 2002), possibly due to problems with contamination by adhering soil material. DGT consists of a selective binding layer and also contains a diffusive gel and filter paper that eliminates any source of particulate contamination.

The aims of this study were to investigate the potential effects of the anions ( $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ , and  $\text{HCO}_3^-$ ) on the sorption of P to the AEM (resin method) and mixed binding layer (DGT method), to compare resin P and DGT measurements obtained on agricultural soils, and to assess the performance of the resin test using an AEM with two different counter ions ( $\text{HCO}_3^-$  and  $\text{Cl}^-$ ).

## 3.2. Materials and methods

### 3.2.1 *Diffusive Gradients in Thin Films*

Detailed information regarding the preparation and initial testing of the MBL is given in Chapter 2. The MBL was the binding layer used in this current work. DGT assemblies contained a 0.8 mm diffusive gel plus a protective filter membrane and were deployed for 24 h in all experiments. Elution and analysis followed the procedure described in Section 2.2.2.

### 3.2.2 *Resin P*

Anion exchange membranes were obtained from BDH and cut into strips (62.6 mm x 25 mm). The AEM strips were pre-treated as described by Saggari et al. (1990), but substituting  $\text{Cl}^-$  instead of  $\text{HCO}_3^-$  by immersing them in a solution of 0.1 M HCl and 0.1 M NaCl (Hamon and McLaughlin 2002). To determine resin P, one strip was immersed in experimental solutions and the solutions shaken for 17 h (Saggari et al. 1990). The AEM strips were removed and rinsed with DI water and the P was extracted from the strip by placing it in a solution of 0.1 M NaCl/HCl for at least 2 h. Ammonium molybdate reactive P concentrations were determined employing a modified version of

the Murphy and Riley method (Murphy and Riley 1962) using a segmented flow analyser. Prior to each analysis the colour reagent containing 2.4 g l<sup>-1</sup> ammonium molybdate, 4 g l<sup>-1</sup> ascorbic acid, 0.2 g l<sup>-1</sup> potassium antimony tartrate and 1 M H<sub>2</sub>SO<sub>4</sub> was prepared. The colour reagent and sample were allowed to react for 2 m and 15 s before passing through a spectrometer where P absorbance was measured at 880 nm. Calibration standards were prepared using the same matrix as the resin P eluant solution.

### *3.2.3 P Capacity on the resin strip*

To determine the capacity of the resin strips for P, the strips were placed in 30 ml solutions covering a range of P concentrations up to 105 mg l<sup>-1</sup>.

The capacity of DGT using the MBL has been previously assessed (Mason et al. 2005).

### *3.2.4 Effect of pH*

The influence of pH on DGT measurement of P using the MBL was investigated previously (Mason et al. 2005).

For direct comparison between the two methods, the effect of pH on P binding to the resin strips was investigated by placing resin strips in solutions with pH ranging from 3 – 9. Two concentrations (0.2 and 2 mg l<sup>-1</sup>) of P were used at each pH to represent concentrations relevant to a P responsive soil (low available P) and a non-responsive soil (high available P) with the addition of P fertiliser (McBeath et al. 2005).

### *3.2.5 Competition experiments*

#### *3.2.5.1 DGT*

To investigate the competitive effect of other anions binding onto the MBL, three DGT assemblies were deployed in 3 L (500 ml for HCO<sub>3</sub><sup>-</sup>) solutions composed of a range of concentrations of the competing anions of interest. Phosphorus concentrations in the

solutions were kept constant at  $0.2 \text{ mg l}^{-1}$ . Concentrations of the other anions in solution were as follows:  $\text{SO}_4^{2-}$  (as  $\text{Na}_2\text{SO}_4$ ) ranged from 0 to  $600 \text{ mg l}^{-1}$ ,  $\text{NO}_3^-$  (as  $\text{NaNO}_3$ ) from 0 to  $1200 \text{ mg l}^{-1}$ ,  $\text{Cl}^-$  (as  $\text{NaCl}$ ) from 0 to  $15000 \text{ mg l}^{-1}$  and  $\text{HCO}_3^-$  (as  $\text{NaHCO}_3$ ) from 0 to  $93000 \text{ mg l}^{-1}$ . Due to the large amounts of added  $\text{NaHCO}_3$  required, solution volumes for DGT deployment were reduced to 500 ml. For standard DGT deployment the soil moisture content is typically increased to 80-100% of the soil water holding capacity (WHC), and the WHC of soils is generally in the range of  $150 - 500 \text{ ml kg}^{-1}$ , hence soil to water ratios for DGT deployment are very roughly 3:1. These concentrations of anions were selected to cover the range of concentrations likely to be found in 3: 1 (soil: water) extracts of agricultural soils (see Results and Discussion, below).

#### 3.2.5.2 Resin P

For a direct comparison with the performance of DGT, resin strips were deployed in 50 ml solutions containing increasing concentrations of  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{Cl}^-$  and  $\text{HCO}_3^-$ . For each concentration of  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{Cl}^-$  and  $\text{HCO}_3^-$  two concentrations of P were used, one at  $0.2 \text{ mg l}^{-1}$  typical of a P responsive soil and the other at  $2 \text{ mg l}^{-1}$  reflective of a P non-responsive soil (McBeath et al. 2005). Anion concentrations in the solutions ranged from 0 to  $12 \text{ mg l}^{-1}$   $\text{SO}_4^{2-}$ , 0 to  $40 \text{ mg l}^{-1}$   $\text{NO}_3^-$ , 0 to  $700 \text{ mg l}^{-1}$   $\text{Cl}^-$  and 0 to  $22000 \text{ mg l}^{-1}$   $\text{HCO}_3^-$ . These concentrations are reflective of the possible range of concentrations found in solution in 1:30 (soil: water) extracts of agricultural soils (see Results and Discussion, below) which is a common soil to water ratio used in the determination of resin P (Saggar et al. 1992, McBeath et al. 2005).

#### 3.2.6 DGT and resin performance on soil

Results obtained by the DGT and resin methods were compared for 8 Australian agricultural soils. Selected soil chemical properties are given in Table 3.1. Soil preparation for DGT deployment consisted of incubating air-dried, sieved ( $< 2 \text{ mm}$ ) soil

at 50 % water holding capacity (WHC) for 2 days and then the moisture content was increased to 80 % WHC the day prior to DGT deployment. DGT devices were deployed on the soil for 24 hours and a diffusive gel thickness of 0.8 mm was used. Measurements were converted to  $C_E$  (effective concentration) using 2D DIFS (Sochaczewski et al. in press). The effective concentration ( $C_E$ ) measurement incorporates both the diffusional supply of P from soil solution and the resupply from the soil solid phase. Detailed explanation of the theory of  $C_E$  has been published previously (Zhang et al. 2001, refer to Chapter 1) and will not be discussed here.

For the resin method 1 g of air-dried, sieved soil was used per 30 mls of DI water. The procedure for the measurement of P was the same as described above. Resin P measurements were also performed in these soils at different soil (g) to water (ml) ratios (0.1: 30, 0.3: 30, and 0.6: 30). One AEM was placed in each solution and samples were run in duplicate for each soil.

**Table 3.1** Selected soil properties of the 8 Australian soils used in this study.

<i>Site</i>	<i>Location</i>	<i>Soil Classification</i>	<i>pH</i> ( $H_2O$ )	<i>EC<sub>1:5</sub></i> $\mu S/cm$	<i>P (total)</i> $mg\ kg^{-1}$	<i>Cl<sup>-</sup></i> $mg\ kg^{-1}$	<i>IC (1:5)</i> $mg\ kg^{-1}$	<i>DOC (1:5)</i> $mg\ kg^{-1}$	<i>TOC</i> %
<b>Mt Damper</b>	<b>S.A.</b>	<b>Grey Calcareous</b>	8.0	203	93	4	8	20	0.6
<b>Birchip</b>	<b>VIC</b>	<b>Calcarosol</b>	8.8	204	242	32	16	26	1.3
<b>Horsham</b>	<b>VIC</b>	<b>Vertosol</b>	8.3	279	185	10	16	19	1.2
<b>Kalkee</b>	<b>VIC</b>	<b>Vertosol</b>	8.5	265	196	27	23	23	1.3
<b>Lower Norton</b>	<b>VIC</b>	<b>Red-Brown Earth</b>	7.9	210	181	14	11	65	1.5
<b>Mildura</b>	<b>VIC</b>	<b>Calcarosol</b>	8.0	129	121	12	7	44	0.7
<b>Gillingarra</b>	<b>W.A.</b>	<b>Podosol</b>	5.5	108	270	11	0.4	40	2.5
<b>Meckering</b>	<b>W.A.</b>	<b>Chromosol</b>	5.9	122	160	22	0.8	47	1.1
		<i>Minimum</i>	<b>5.5</b>	<b>108</b>	<b>93</b>	<b>4</b>	<b>0.4</b>	<b>19</b>	<b>0.6</b>
		<i>Maximum</i>	<b>8.8</b>	<b>279</b>	<b>270</b>	<b>32</b>	<b>23</b>	<b>65</b>	<b>2.5</b>

### 3.2.7 AEM counter ion

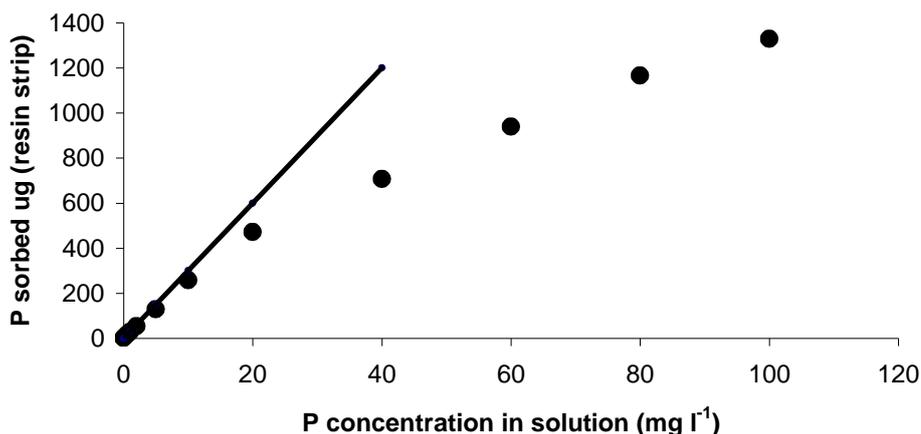
The Performance of the AEM prepared as either the  $Cl^-$  or  $HCO_3^-$  form (Saggar et al. 1992) was investigated using 6 different Australian agricultural soils (see Table 3.1)

using 1 g of soil per 30 ml of DI water. The effect of changing the soil to solution ratio on P sorption with the AEM in both counter ion forms was also assessed on the 6 Australian agricultural soils. Two different soil weights (0.3 g or 0.1 g) were used per 30 ml of DI water.

### 3.3. Results and discussion

#### 3.3.1 Capacity of resin strips

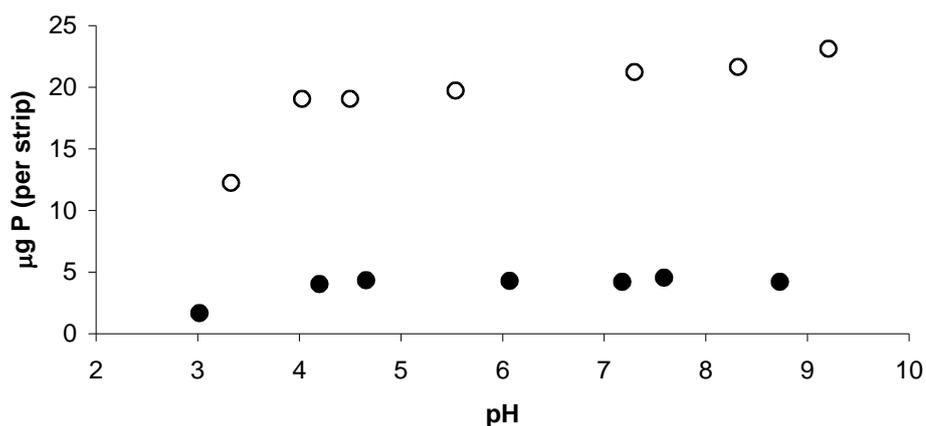
The accumulated P on the resin strip increased linearly with the concentration of P in solution up to a concentration of  $4.8 \text{ mg l}^{-1}$ , which corresponds to a resin P value of  $126 \text{ }\mu\text{g}$  per strip (with specific dimension of  $62.6 \text{ mm} \times 25 \text{ mm}$ ) (Figure 3.1). Beyond this P concentration resin P values deviated from linearity indicating that resin capacity had been reached. The solution concentration at which capacity is reached using the standard 1 g of soil in 30 ml of DI water would therefore be  $4.8 \text{ mg l}^{-1}$ . This capacity value thus corresponds to a resin P value of  $126 \text{ mg/kg}$  (1: 30 soil to water ratio). Previous studies using even heavily P fertilised soils have observed the majority of resin P measurements to be well below this value (Saggar et al. 1999, Menzies et al. 2005). Hence the capacity of the AEM for P would be unlikely to be a limitation for measuring P in P responsive and non-responsive soils. The capacity of the DGT device using a MBL was found to be considerably lower at  $12 \text{ }\mu\text{g}$  P per device (Mason et al. 2005).



**Figure 3.1** Measured mass of P on the resin strip with increasing solution concentration. Solid line represents the linear relationship from the first 4 solution concentrations.

### 3.3.2 Effect of pH

Phosphorus deficiencies are common in acidic soils due to the very high sorption capacity of Al and Fe hydrous oxides for P at low pH (Haynes and Mokolobate 2001). This highlights the importance of a soil test that functions well under acidic conditions. DGT performance for measuring P has shown to be unaffected by pH within the range 3 – 9 (Mason et al. 2005). The binding of P onto the AEM strip was also found to be unaffected significantly by pH within the range of 4 – 9 (Figure 3.2). Below pH 4 the resin P concentrations decreased significantly. However, this is unlikely to be a limitation to the use of the resin method for determining P for agronomic purposes as very acidic soils (pH < 5) are not optimal for plant growth due primarily to aluminium/manganese toxicity, and therefore are either not used for growing crops, or are limed to increase pH before use (Peverill et al. 2003, Mason et al. 2005).



**Figure 3.2** Effect of pH on the recovery of resin P values. Open circles represent a typical available P concentration of a P non-responsive soil and solid circles represent a typical available P concentration of a P responsive soil.

### 3.3.3 Selection of anion concentrations for competition experiments

Only a few studies have assessed the potential for competitive effects of some anions on resin P measurement (see Introduction) and the results have not been placed in a soil P testing context. No studies have yet been performed to assess possible competitive effects on DGT P, mainly due to the early development stage of this method. Possible anions and their concentrations that the DGT method may encounter are discussed below.

Potential sources of chloride deposition in soils include rainwater, fertilizer applications (KCl), irrigation waters, sea spray, dust, and air pollution (White and Broadley 2001). The concentration of NaCl in irrigation water can be as high as 1600 mg l<sup>-1</sup> (Weggler et al. 2004) and when rich irrigation waters were applied to soils, Cl<sup>-</sup> soil solution concentrations at field capacity reached 160 mM (5670 mg l<sup>-1</sup>) (Weggler et al. 2004). Rainwater Cl<sup>-</sup> concentrations have been reported to be as high as 3000 mg l<sup>-1</sup> (Xu et al. 2004) and are largely dependent on proximity to salt water (Weggler et al. 2004). Even irrigation with water of low salinity can easily deposit 1000 kg Cl<sup>-</sup> ha<sup>-1</sup> year<sup>-1</sup> (Xu et al. 2004). A study on acid sulfate soils reported Cl<sup>-</sup> concentrations up to 10289 mg kg<sup>-1</sup>

(McElnea et al. 2002) although these soils are very hostile environments and not conducive to agricultural production. A review from Eynard and Wiebe (2005) reported that salt-affected soils where plant growth is limited by an excess of salts consist of saline soils in which electrical conductivity is  $> 4 \text{ dSm}^{-1}$  which equates to  $> 2200 \text{ mg kg}^{-1}$  (Hunt and Gilkes 1992). Bower and Wilcox (1965) reported that the yields of many crops are restricted at Total Dissolved Salt (TDS) concentrations of  $250 - 500 \text{ mg l}^{-1}$  ( $1250 - 2500 \text{ mg kg}^{-1}$ ) in a 1 to 5, soil to water extract. They also reported that only a few salt tolerant plants yield satisfactorily at TDS concentrations of  $500 - 1000 \text{ mg l}^{-1}$  ( $2500 - 5000 \text{ mg kg}^{-1}$ ) again in a 1 to 5, soil to water extract. It appears there is potential for severely decreased crop yields at Cl concentrations anywhere from 1250 to 5000  $\text{mg kg}^{-1}$ . Therefore to cover the most extreme case, the DGT method was tested at chloride solution concentrations up to  $15000 \text{ mg l}^{-1}$  (i.e.  $5000 \text{ mg Cl kg}^{-1}$  at 3:1 soil: water ratio).

Nitrogen fertilizers are applied each year at rates dependant on the type of soil and crop grown at each locality and range from less than 20 to  $> 100 \text{ kg N ha}^{-1}$  ( $100 \text{ kg N ha}^{-1}$  is approximately  $80 \text{ mg N kg}^{-1}$  or  $350 \text{ mg NO}_3 \text{ kg}^{-1}$ ) (e.g. Rasiyah and Armour 2001). However, there is typically minimal nitrate absorption by soils and significant nitrate losses through volatilisation, leaching and crop uptake, are also prevalent in agricultural soils (Black and Waring 1979, Anderson et al. 1998, Raun and Johnson 1999). In a study by Anderson et al. (1998) nitrogen concentrations from deep sands in Western Australia at the start of the growing season were up to  $106 \text{ kg N ha}^{-1}$  and were dependent on the previous crop variety grown. The leaching of excess nitrate fertilizer can lead to nitrate accumulation in the subsoil as, for example, was evident in the Johnstone River catchment in Queensland (Donn and Menzies 2005). However, typically a soil test is performed on the top 10 cm of the soil profile and it appears unlikely that significant nitrate accumulation would occur at the surface due to its propensity for leaching and other losses. Analysis of the soluble  $\text{NO}_3\text{-N}$  (1: 30, soil: water) concentration in 21

Australian agricultural soils found it to range from  $< 2$  to  $68.4 \text{ mg kg}^{-1}$  (Table 3.2), which equates to a maximum soluble  $\text{NO}_3$  concentration of approximately  $300 \text{ mg kg}^{-1}$ .

The previous studies that found that nitrate affected the performance of the resin P method focused on nitrate concentrations up to  $100 \text{ mg N l}^{-1}$  in 40 ml solutions (Cooperband and Logan 1994). This is equivalent to up to  $17700 \text{ mg NO}_3 \text{ kg}^{-1}$  soil in a 1:40 soil: water resin test, which according to the above discussion is unlikely to be present in agricultural soils; we therefore assumed a 'worst case' scenario of  $400 \text{ mg NO}_3 \text{ kg}^{-1}$  soil. This corresponds to a concentration of  $1200 \text{ mg l}^{-1} \text{ NO}_3$  for testing the performance of DGT assuming a 3 to 1, soil to water ratio, and approximately  $15 \text{ mg l}^{-1} \text{ NO}_3$  for testing the performance of the resin in a 1:30, soil:water system.

Literature on range of concentrations of  $\text{SO}_4^{2-}$  likely to be found in agricultural soils is limited. Analysis of the soluble S (1: 5, soil: water) concentration in 21 Australian agricultural soils found it to range from 1.7 to  $62.9 \text{ mg kg}^{-1}$  (Table 3.2). Assuming that the entire soluble S was in the form of  $\text{SO}_4^{2-}$ , this equates to a maximum  $\text{SO}_4^{2-}$  concentration of approximately  $180 \text{ mg kg}^{-1}$ . These concentrations are significantly lower than those found in acid sulfate soils (McElnea et al. 2002), but such soils are unlikely to be agriculturally productive. We therefore assumed a maximum  $\text{SO}_4^{2-}$  concentration of  $200 \text{ mg kg}^{-1}$ , equivalent to solution concentrations of  $600 \text{ mg l}^{-1} \text{ SO}_4^{2-}$  for DGT testing and approx  $7 \text{ mg l}^{-1} \text{ SO}_4^{2-}$  for the resin method.

The concentration of carbonate in soils used for agricultural purposes is variable, but can be very high. For example soils from Southern Australia used for grain growing are renowned for having very high concentrations of calcium carbonate, with percentages ranging up to 90 % (McBeath et al. 2005). Studies have been performed on the chemical characteristics of P from these soils but the actual amount of soluble  $\text{HCO}_3^-$  in solution in these highly calcareous soils has not been measured. We used concentrations up to 1 g of  $\text{HCO}_3^-$ , as soluble  $\text{NaHCO}_3$ , per 30 ml of DI water to test the resin method. This assumes

a soil composed of 100% carbonate with the majority of the carbonate free in solution, which is obviously a significant overestimate in comparison to soils where carbonate solubility is generally controlled by sparingly soluble  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  carbonate precipitation. Corresponding  $\text{HCO}_3^-$  concentrations used for DGT testing were up to  $93 \text{ g l}^{-1}$ , though at the highest rates, the solubility product was exceeded.

**Table 3.2** Soluble  $\text{NO}_3\text{-N}$  (1: 30, soil: water) and S (1: 5, soil: water) concentrations from 21 Australian agricultural soils. Detection limits were  $0.02 \text{ mg l}^{-1}$   $\text{NO}_3\text{-N}$  in solution ( $0.6 \text{ mg kg}^{-1}$  in a 1:30 dilution) and  $0.054 \text{ mg l}^{-1}$  S ( $0.27 \text{ mg kg}^{-1}$  in a 1:5 dilution).

Soil Site	State	$\text{NO}_3\text{-N}$ (1:30) $\text{mg kg}^{-1}$	S (1:5) $\text{mg kg}^{-1}$
Mt.Damper	S.A	1.6	<i>nd</i>
Pt. Kenny	S.A	14.8	24
Waramboo	S.A	2.9	11.7
Birchip	VIC	8.8	7
Birchip B	VIC	22.6	62.9
Horsham	VIC	68.4	7.2
Kalkee	VIC	23.9	8.8
Lower Norton	VIC	3.1	14.7
Mildura	VIC	5.5	5.6
Rupanyup	VIC	18.3	44.7
Balkuling	W.A	<i>nd</i>	6
Cunderdin	W.A	1.6	1.7
Gillingarra	W.A	4.8	11.6
Kojonup	W.A	0.9	6.1
Lancelin	W.A	1.2	3.4
Meckering	W.A	4.1	8.7
Waranine	W.A	2	7
West York	W.A	<i>nd</i>	3.8
Wodjil	W.A	<i>nd</i>	21.3
Wongan	W.A	2.4	4.7
Yalenbee	W.A	1.7	8.5
Minimum		0.9	1.7
Maximum		68.4	62.9

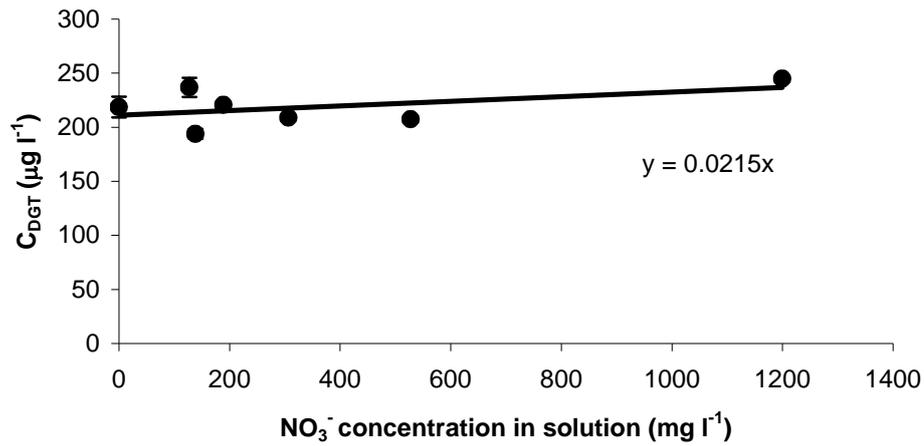
*nd\** = Below detection limit

### 3.3.4 Effect of anion competition on DGT P

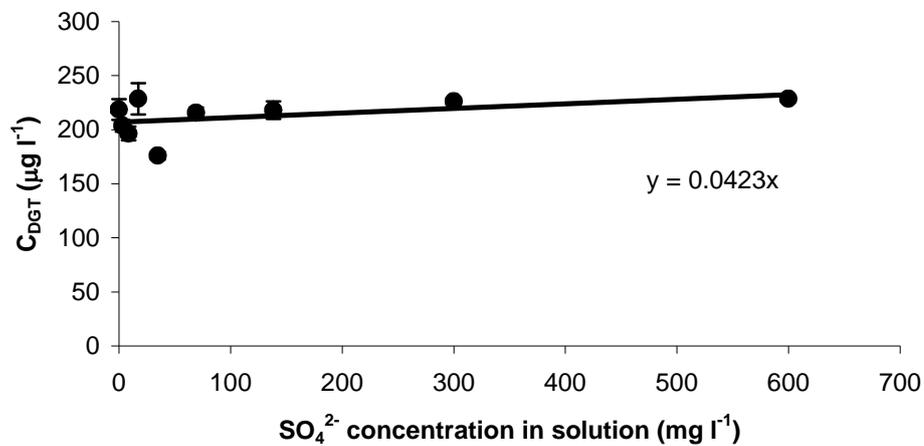
The concentration of P in solution as assessed by DGT ( $C_{\text{DGT}}$ ) agreed well with the concentration of P that was actually in solution (200 – 220  $\mu\text{g l}^{-1}$ ). Furthermore, there was no effect of increasing concentrations of  $\text{Cl}^-$  (Figure 3.3a),  $\text{NO}_3^-$  (Figure 3.3b) or  $\text{SO}_4^{2-}$  (Figure 3.3c) on the concentration of P as assessed by DGT. Increasing bicarbonate concentrations in solution had a marked effect on DGT P recovery (Figure 3.3d). The concentration of P measured by DGT ( $C_{\text{DGT}}$ ) decreased to 51  $\mu\text{g l}^{-1}$  at  $\text{HCO}_3^-$  concentration of 93  $\text{g l}^{-1}$ . However, this was in a system containing only Na as the cation and as discussed above, the solubility of carbonate is much lower in calcareous soils where  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  control carbonate solubility. Soluble carbonate concentrations obtainable in a closed system at 4 different pH levels (charge balanced using  $\text{Cl}^-$ ), where solubility is controlled by  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$ , were modelled using GEOCHEM (Parker et al. 1995). The model gave maximum soluble carbonate (as  $\text{CO}_3^{2-}$ ,  $\text{HCO}_3^-$  and  $\text{CaCO}_3$  species) concentrations in solution of 1800, 350, 100, and 20  $\text{mg l}^{-1}$  at pH 6, 7, 8, and 9 respectively using calcium as the associated cation (Figure 3.4). These conditions were used to investigate the ‘worst-case’ scenario for agricultural soils, although it is highly unlikely that high concentrations of carbonate would be associated with a soil of pH 6. At these concentrations, minimal reduction in P absorption using DGT occurs. The largest concentration of  $\text{CO}_3^{2-}$  species (pH 6) relates to only a 7 % decrease in DGT P sorption (Table 3.3a, Figure 3.3d). Using  $\text{Mg}^{2+}$  as the associated cation increased the maximum possible concentrations of soluble  $\text{CO}_3^{2+}$  species in comparison to  $\text{Ca}^{2+}$ , however, even in this extreme example there would only be a decrease in P sorption by DGT of 18 % (Table 3.3b, Figure 3.3d). Tyler and Olsen (2001) obtained  $\text{HCO}_3^-$  concentrations of approximately 1 mM (63  $\text{mg l}^{-1}$ ) in soil solution after a soil had been applied with 15  $\text{g kg}^{-1}$  of  $\text{CaCO}_3$ . At these values it appears there is little effect of  $\text{HCO}_3^-$  on DGT P measurements.

As the range of concentrations chosen for these elements is based on the possible obtainable concentrations from agricultural soils we can therefore assume that minimal (up to ~10 % in the presence of  $\text{HCO}_3^-$ ) competitive or interfering effects of anions on measurements of P using DGT are likely in the majority of agricultural soils.

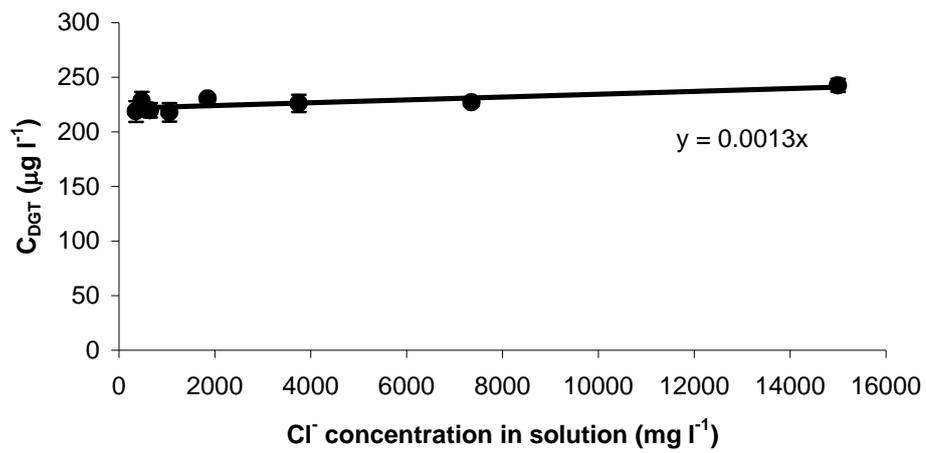
a)



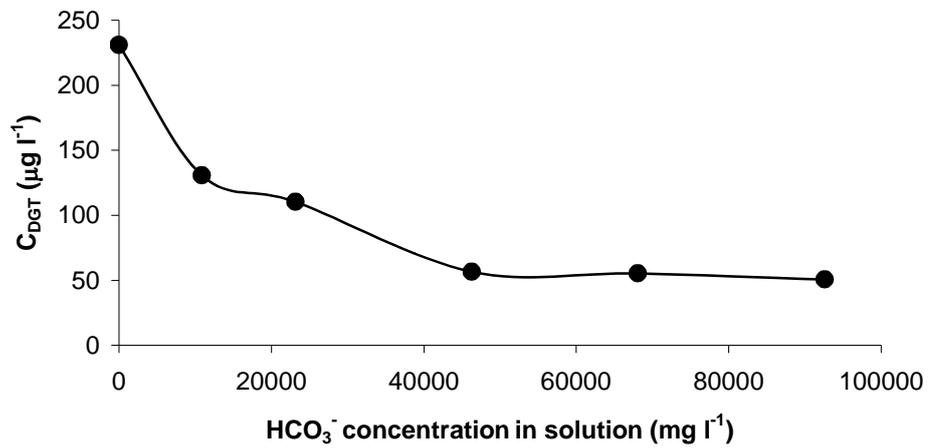
b)



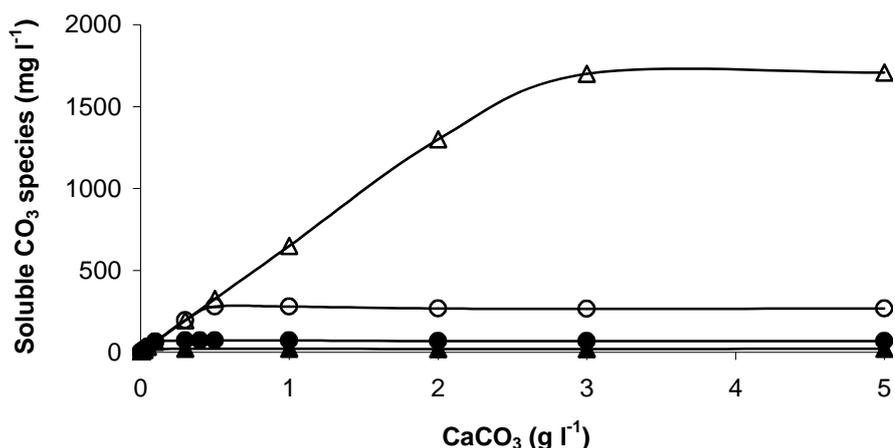
c)



d)



**Figure 3.3** DGT measurements (converted to  $C_{DGT}$ ) with increasing anion solution concentrations, a) Nitrate, b) Chloride, c) Sulfate and d) Bicarbonate.



**Figure 3.4** GEOCHEM calculations of concentrations of soluble CO<sub>3</sub><sup>2-</sup> species with increasing CaCO<sub>3</sub> concentrations at four different pH levels 6 (Δ), 7 (○), 8 (▲) and 9 (●). The model was charge balanced with addition of Cl<sup>-</sup> and run as a closed system to reflect the ‘worst-case’ scenario.

**Table 3.3** Concentration of total soluble CO<sub>3</sub><sup>2-</sup> species calculated by GEOCHEM for a closed system at different pH values with charge balanced using Cl<sup>-</sup> for a) Ca and b) Mg as the associated cation with the corresponding decrease in P sorption that would be anticipated (refer to Figures 3.3d and 3.5d) for both the DGT and resin methods.

a)

Ca		% reduction in P sorption	
pH	CO <sub>3</sub> (mg l <sup>-1</sup> )	DGT	resin
6	1707	7	61
7	266	0.9	11
8	68	0.4	2.6
9	21	0	0.8

b)

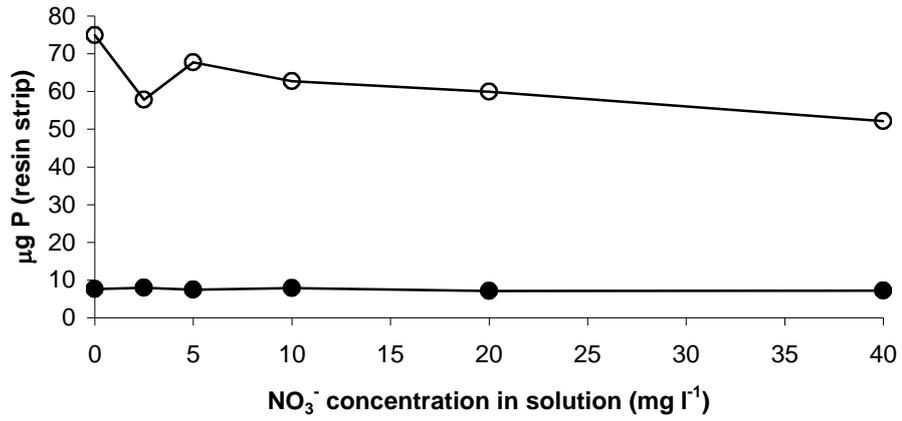
Mg		% reduction in P sorption	
pH	CO <sub>3</sub> (mg l <sup>-1</sup> )	DGT	resin
6	3901	18	88
7	1505	6.5	58
8	356	1.3	15
9	105	0.4	4.1

### 3.3.5 Effect of anion competition on resin P

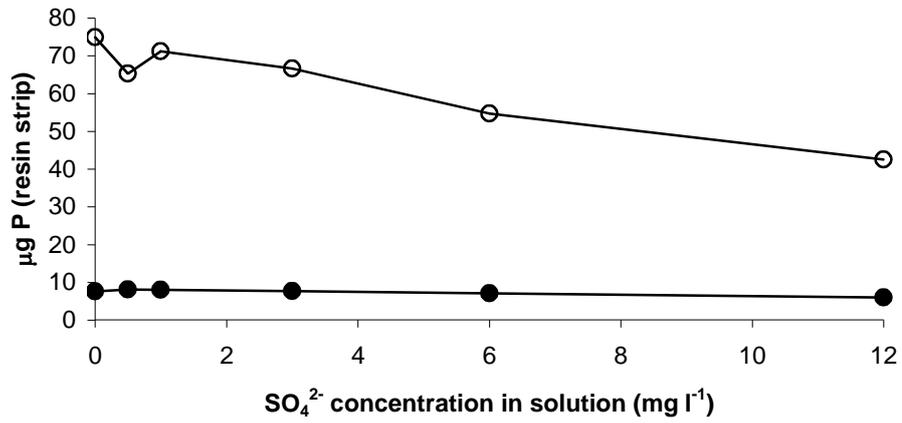
The same anion concentrations were used to assess the performance of the resin method for measuring P as for DGT but adjusted for the 1: 30 soil to water ratio typically used (i.e. diluted 30 x).

Phosphorus sorption onto the AEM was slightly affected by increasing  $\text{NO}_3^-$  concentrations up to  $40 \text{ mg l}^{-1}$  (Figure 3.5a). At the highest  $\text{NO}_3^-$  concentration P sorption was reduced by 24 % at  $2 \text{ mg l}^{-1}$  P and by 6 % at  $0.2 \text{ mg l}^{-1}$  P. Increasing the sulfate concentrations decreased the P sorption onto the AEM by 47 % at  $12 \text{ mg l}^{-1} \text{ SO}_4^{2-}$  for a P concentration of  $2 \text{ mg l}^{-1}$  (Figure 3.5b). This value decreased to 27 % for the corresponding P concentration at  $0.2 \text{ mg l}^{-1}$ . Increasing the  $\text{Cl}^-$  and  $\text{HCO}_3^-$  concentration in solution had a large impact on P sorption due to the higher concentrations of  $\text{Cl}^-$  and  $\text{HCO}_3^-$  used compared to  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$  (Figure 3.5c and d). Phosphorus sorption was reduced by 100 % at a concentration of  $300 \text{ mg l}^{-1} \text{ Cl}^-$  at a P concentration of  $2 \text{ mg l}^{-1}$ . At a P concentration of  $0.2 \text{ mg l}^{-1}$  P sorption was reduced by 85 % and 92 % at  $\text{Cl}^-$  concentrations of  $300 \text{ mg l}^{-1}$  and  $700 \text{ mg l}^{-1}$  respectively. At the  $\text{Cl}^-$  concentration of  $40 \text{ mg l}^{-1}$  which corresponds to a concentration of  $1200 \text{ mg kg}^{-1}$  (30 x dilution) in a soil, where no visible reduction in yield is observed (Bower and Wilcox 1965), P sorption was reduced by 62 % at a P concentration of  $2 \text{ mg l}^{-1}$  and by 27 % at a P concentration of  $0.2 \text{ mg l}^{-1}$ . The possible concentrations of soluble carbonate species in solution as modelled using GEOCHEM reveals that there could be a potential problem of carbonate interference decreasing the performance of the resin P method (Table 3.3, Figure 3.5d), however, the competitive effect is not as significant as for the other anions. A cumulative effect of various anions in solution, on the measurement of P using the resin method also cannot be ruled out.

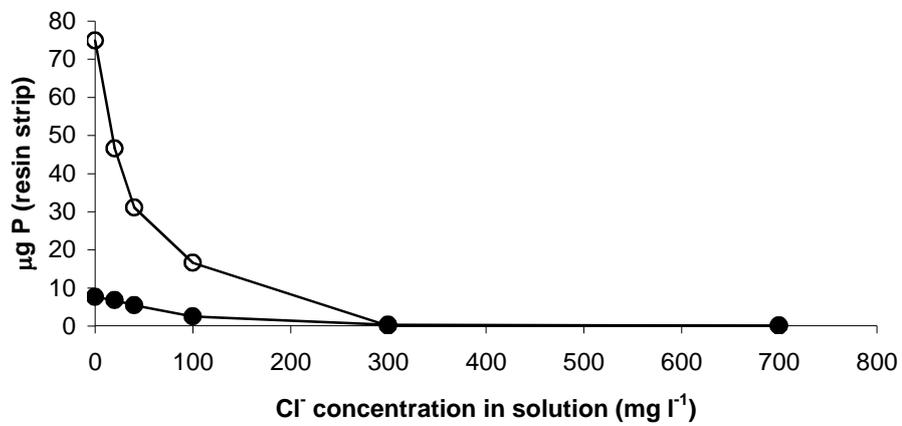
a)



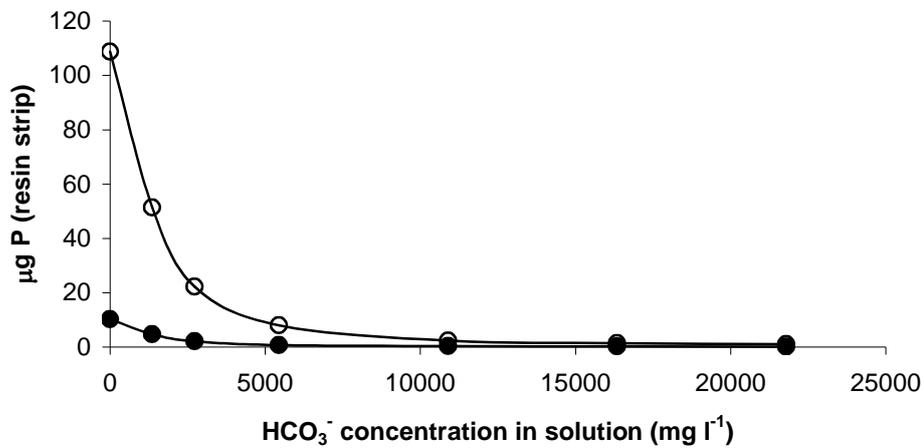
b)



c)



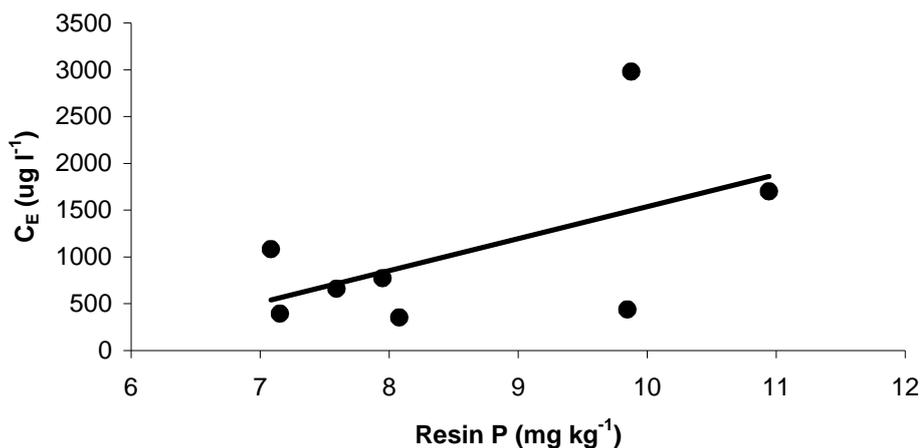
d)



**Figure 3.5** Resin P values with increasing anion solution concentrations, a) Nitrate, b) Sulfate, c) Chloride and d) Bicarbonate. Open circles represent P concentration typical of a P non-responsive soil and solid circles are representative of a P responsive soil concentration.

### 3.3.6 DGT and resin performance on soil

Comparison of DGT and resin P measurements on 8 agricultural soils revealed some major discrepancies between the two methods (Figure 3.6). The poor regression ( $r^2 = 0.31$ ) suggests the pool of P measured by DGT is different from the pool measured by the resin method. The determination of the effective concentration ( $C_E$ ) from DGT measurements on these soils is outlined in table 3.4. These parameters are further explained in section 1.5.3 and 1.5.4.



**Figure 3.6** Relationship between DGT measurements ( $C_E$ ) and resin P measurements (1:30 soil to water ratio) on 8 Australian agricultural soils.

**Table 3.4** Calculation of effective concentration ( $C_E$ ) from DGT measurements by calculating  $R_{diff}$  using 2D DIFS. Input parameters for  $R_{diff}$  calculation include the density of the soil particles ( $\rho_p$ ), soil porosity ( $\Phi$ ), diffusion coefficient in soil ( $D_s$ ), and particle concentration ( $P_c$ ). Refer to section 1.5.4 for detailed calculations of these parameters.

Soil Site	$\rho_p$	$\Phi$	$D_s$	$P_c$	$R_{diff}$	$C_{DGT}$ ( $\mu\text{g l}^{-1}$ )	$C_E$ ( $\mu\text{g l}^{-1}$ )
Gillingarra	2.65	0.39	1.84E-06	4.08	0.0337	51	786
Meckering	2.65	0.32	1.62E-06	5.56	0.0262	166	2741
Horsham	2.65	0.50	2.2E-06	2.67	0.0466	24	325
Kalkee	2.65	0.47	2.1E-06	2.99	0.0430	28	395
Lower Norton	2.65	0.47	2.09E-06	3.03	0.0429	32	455
Birchip	2.65	0.38	1.79E-06	4.35	0.0324	109	1698
Mildura	2.65	0.31	1.58E-06	5.88	0.0251	39	645
Mt Damper	2.65	0.32	1.62E-06	5.56	0.0262	63	1042

As discussed above, increasing anion concentrations in solution can have a marked impact on P sorption onto the AEM due to competition. Therefore, the possible competitive impact of anions in the soils on the resin method was explored by increasing the soil: water ratio to ‘dilute’ out any interfering anion. Resin P measurements did indeed increase significantly with decreasing soil to water ratios (Table 3.4). For each of the 8 soils the increase in resin P values were not uniform. As discussed previously (see Introduction) some studies have shown that resin P measurements decrease when the soil to water ratio increases (Guo et al. 1996, Sibbesen, 1978). In contrast McLaughlin et al. (1994) found that increasing the amount of soil from 1 g to 10 g using 100 ml of water

had no effect on resin P results using resin beads in nylon bags. However that study also increased the amount of resin in accordance with the increase in soil, which may have overcome any interference or competitive effect.

**Table 3.5** Resin P values ( $\text{mg kg}^{-1}$ ) obtained for solutions containing varying amounts of the 8 soils per 30 ml of DI water.

Resin P ( $\text{mg kg}^{-1}$ ) Soil	Amount soil (g) per 30 ml H <sub>2</sub> O			
	0.1	0.3	0.6	1
Gillingarra	17.7	10.7	8.7	7.9
Meckering	19.8	12.5	11.2	9.9
Horsham	13.1	9.8	8.9	8.1
Birchip	24.5	18.0	13.7	10.9
Kalkee	12.4	11.2	8.0	7.2
Lower Norton	15.0	10.0	10.2	9.8
Mildura	13.6	9.4	8.0	7.6
Mt. Damper	16.2	11.3	9.3	7.1

While the increase in resin P measurements with decreasing soil: water ratios could indicate that there is an interference or competitive effect on P sorption onto the AEM, analysis of the  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  and inorganic carbon (IC) concentrations in these soils revealed that this is possibly not the direct cause; with  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  and IC concentrations generally lower than  $1.1 \text{ mg kg}^{-1}$  and  $2.1 \text{ mg kg}^{-1}$  and  $3.8 \text{ mg kg}^{-1}$  respectively in a 30 x dilution (converted from Tables 3.2.1 and 3.3.1). However, the possible competitive effect of DOC on P sorption by the resin has not been investigated, and this could also be a contributing factor.

Alternatively, the increase in resin P measurements with increasing soil: water ratio could simply reflect P resupply from the soil solid phase that occurs at higher dilutions as the soil buffers the solution P concentrations. Schoenau and Huang (1991) similarly observed an increase in water-extractable P with widening soil to water ratios. They suggested that when the soil to water ratio is high only the most soluble Ca-P precipitates dissolve and enter the solution. At lower soil to water ratios further

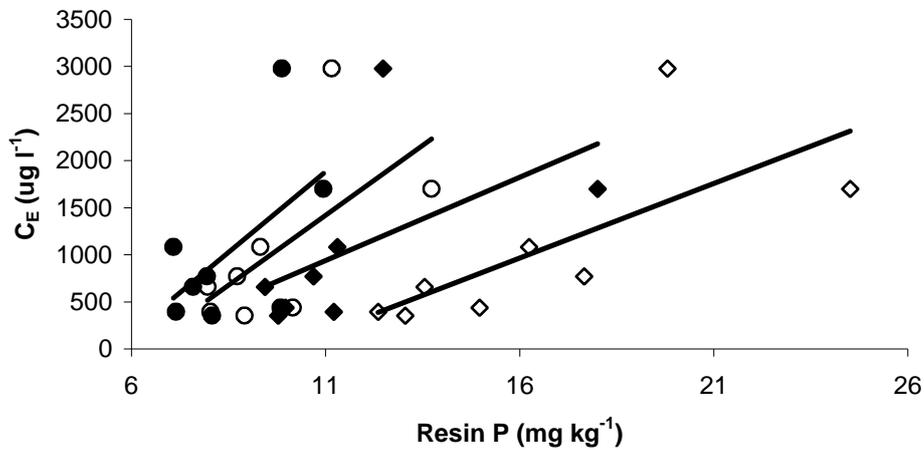
dissolution occurs as the most soluble forms dissolve completely and less soluble forms begin to enter the solution (Schoenau and Huang 1991). Measurements by DGT devices placed in soil solutions containing the soil: water ratios used for the resin P experiment also increased with decreasing amounts of soil used per 30 ml of DI water (Table 3.5). This supports the theory of solution replenishment as the cause for increased P at lower soil to water ratios as the DGT method was shown to be free from competitive interference (Figure 3.3) by anions most likely to be in solution. The dependence of the resin P method on the soil to water ratio is likely to be dependent on the P buffering capacity of the individual soil. Therefore, there is considerable doubt over which soil to water ratio will most accurately reflect plant available P. In contrast, DGT devices are placed on the soil *in situ* at water contents, which more closely reflect the environment of the rhizosphere.

**Table 3.6** DGT measurements ( $\mu\text{g kg}^{-1}$ ) when placed in solutions containing varying amounts of the 8 soils per 30 ml of DI water.

DGT ( $\mu\text{g kg}^{-1}$ ) Soil	Amount soil (g) per 30 mls H <sub>2</sub> O			
	0.1	0.3	0.6	1
Gillingarra	5.4	5.8	4.3	3.9
Meckering	6.2	5.0	5.7	5.4
Horsham	6.7	4.5	3.2	2.2
Birchip	14.7	10.9	7.4	4.8
Kalkee	5.2	3.7	2.3	1.8
Lower Norton	6.2	5.8	4.3	3.7
Mildura	10.7	4.6	4.0	3.2
Mt. Damper	4.5	3.5	3.1	2.9

There was no improvement in the correlation between DGT and resin P measurements in mixtures with different resin P soil to water ratios (Figure 3.7). Regression coefficients ( $r^2$ ) using 0.6, 0.3 and 0.1 g soil per 30 mls of DI water were 0.41, 0.30 and 0.52 respectively. Results have shown that the resin method is a more accurate measurement of plant available P compared to extraction methods (Saggar et al.

1999, McBeath et al. 2005, Menzies et al. 2005). DGT has been shown to predict both plant P (Menzies et al. 2005) and plant metal availability with great accuracy (Zhang et al. 2001). However the discrepancies between the two methods in terms of assessing soil P found in the current study suggests that they are assessing different pools of P and therefore a comparison of DGT and resin P measurements with plant responsiveness is required in order to be able to assess which of the two methods is the more reliable test for plant available soil P.

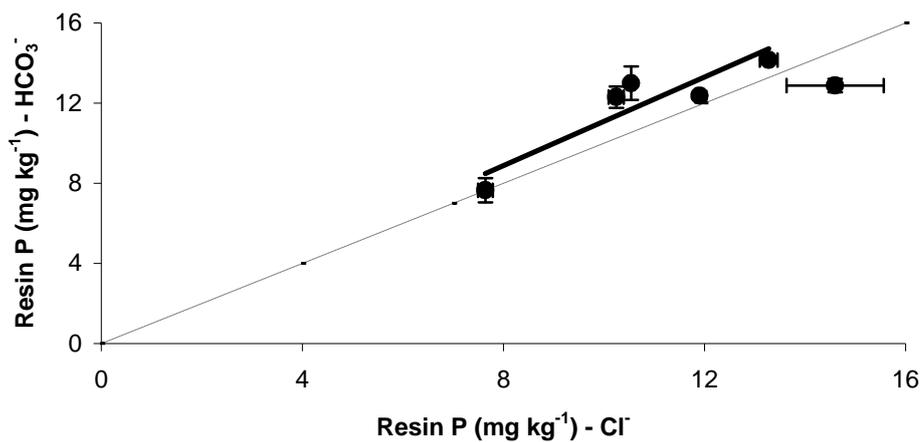


**Figure 3.7** Relationships between DGT ( $C_E$ ) and resin P measurements with different soil to water ratios. 1 g in 30 ml (●), 0.6 g in 30 ml (○), 0.3g in 30 ml (◆), 0.1g in 30 ml (◇). Note that the 1 g in 30 ml data is taken from Figure 3.6.

### 3.3.7 AEM counter ion

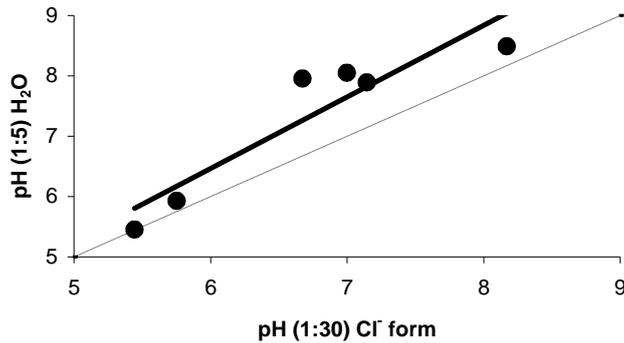
Previous studies have suggested that the resin counter ion can affect the amounts of P sorbed onto the resin. Since Sibbesen (1978) most studies have used the resin/AEM in bicarbonate form. We investigated the effect of two counter ions on the AEM for sorption of P from 6 agricultural soils with varying pH (5.4 – 8.5). Previously work has shown 1) significantly increased sorption of P to resin in nylon bags when  $\text{HCO}_3^-$  rather than  $\text{Cl}^-$  is used as a counter ion (Sibbesen, 1978, Sibbesen et al. 1994) and 2) more consistent pH values obtained for the  $\text{HCO}_3^-$  form with changing soil to water ratios,

attributed to the buffering capability of  $\text{HCO}_3^-$  (Sibbesen 1978). However, in the current study the overall recovery of P from the AEM in  $\text{HCO}_3^-$  form was not significantly different to the AEM in  $\text{Cl}^-$  form (Figure 3.8). When the resin P values using each counter ion were compared a slope of 1.11 and a regression coefficient of 0.83 (excluding outlier with large standard error), revealed that there was no bias with P sorption using either counter ion form of AEM. The slopes of plots of pH obtained from the 1: 30 soil: water solutions after AEM removal against the original pH values of the soils were 1.19 ( $r^2 = 0.86$ ) ( $\text{Cl}^-$  form) and 1.88 ( $r^2 = 0.69$ ) ( $\text{HCO}_3^-$  form) (Figure 3.9a and b). The AEM in  $\text{Cl}^-$  form has less of an influence on the solution pH compared to the  $\text{HCO}_3^-$  form.

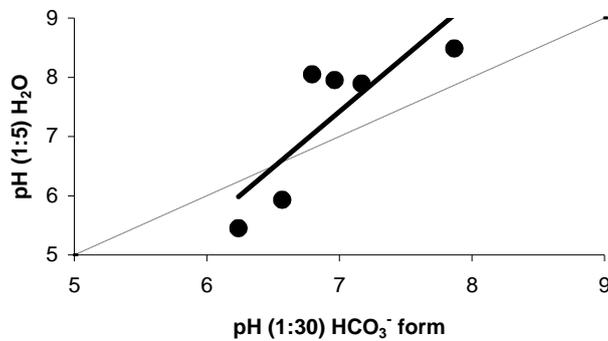


**Figure 3.8** Regression between resin P values obtained using AEM in  $\text{Cl}^-$  form and those obtained with AEM in  $\text{HCO}_3^-$  form for the 6 soils. Errors bars represent the standard error from 2 replicates. Dotted line represents the 1 to 1 line and the solid line represents the regression fit for the data.

a)



b)



**Figure 3.9** Comparison of pH values obtained with the 1: 30 soil to water solutions after removal of a) AEM in Cl<sup>-</sup> form and b) AEM in HCO<sub>3</sub><sup>-</sup> form, with pH values obtained at the typical 1: 5 soil to water ratio. Dotted lines represent the 1 to 1 line and the solid lines represent the regression fit for the data.

#### 3.4. Overall analysis

Comparison of the DGT and Resin P methods for measuring P in solutions of different compositions identified potential problems with use of the resin method using an AEM. DGT performance has previously been shown to be independent of pH (3-9) and was unaffected by anions (SO<sub>4</sub><sup>2-</sup>, NO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup>) present in solution at concentrations relevant to agricultural soils. Bicarbonate did affect DGT performance, but only at extremely high concentrations of soluble carbonate. Such high concentrations are unrealistic in soils due to precipitation with Ca or Mg. Resin P performed well at all pH values down to pH 4, below which, measured values were appreciably lower. Solutions

containing increasing  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$  concentrations had a limited effect on the P sorption at the highest concentrations relevant to agricultural soils. Increasing  $\text{Cl}^-$  concentrations however had a marked effect on sorption of P onto the AEM. Increasing  $\text{HCO}_3^-$  concentrations also reduced P sorption onto the resin strip significantly at concentrations that could exist in agricultural soils. Resin P measurements increased when the soil: water ratio was increased. The increases in resin P values were not uniform for the 8 agricultural soils used in this experiment, which indicates that the increase is due to the inherent soil properties. DGT measurements also increased with increasing soil: water ratios, suggesting the increase is in response to the replenishment of P from solid phases to solution. There were major discrepancies observed between DGT and resin measurements using 8 agricultural soils, which suggest that they are measuring different pools of available P. Further studies will need to be performed comparing DGT P and resin P against plant responsiveness to assess whether either of these two methods accurately reflect plant available P.

## 4. Critical soil thresholds of phosphorus deficiency for wheat: assessment using diffusive gradients in thin films

### 4.1. Introduction

Phosphorus is the most expensive major nutrient used in broadacre cropping in Australia (Mcbeath et al. 2005) and is also very important elsewhere in the World (Raij et al. 2002). Therefore, there is a major requirement to develop a soil test capable of accurately predicting the P status of a soil, which in turn will maximise fertilizer efficiency and reduce the risks of environmental pollution due to over fertilising. The quantities of P exported from agricultural land are in small proportion to that applied in fertilizers and manures (1-5 %) (Nash and Murdoch 1997), however, these quantities have significant impact on receiving waters (Webster et al. 2001). Nutrients including P that enter water systems stimulate the growth of plants including undesirable algae. This stimulation of plant growth commonly referred to as eutrophication limits the potential uses of the affected water (Nash and Halliwell 1999) and is a worldwide problem (Cooke et al. 1993). Algal blooms in Australia have resulted in human illness and stock deaths (Department of Natural Resources and Environment 1996).

Failure of established methods to predict plant P requirements has generated interest in new test procedures. Substantial variations in soil types and soil pH provide challenges for any soil P test. Diffusive Gradients in Thin Films (DGT) is a new technique for measuring P that has been applied successfully to aquatic systems (Zhang et al. 1998). It has recently been used for predicting tomato response to applications of P (Menzies et al. 2005). As for the resin method, the DGT method attempts to mimic the physico-chemical uptake of solutes by plant roots by providing a sink for the free ion through a ferrihydrite binding gel. The main differences between the DGT and the resin technique is the placement of a diffusive gel in front of the binding layer for the DGT

method and the soil to solution ratios used for each method. Possible advantages the diffusive layer may offer the DGT technique are: a) limitation of the maximum flux of P onto the binding layer, b) facilitation of precise flux calculations, and c) prevention of contamination of the binding layer with particulate material (Zhang et al. 2001). The DGT device is placed directly on to the soil and so does not require separation of soil and solution. The amount of P accumulated onto the binding layer depends on the concentration of P in the soil pore water as well as the rate at which P is supplied from the soil solid phase into the pore water, i.e. the rate of re-supply. The resin method involves placing a resin strip into a soil solution and as shown in Chapter 3 the resin P measurement is dependent on the ratio of soil to water employed.

The aims of this work were to compare ‘available P’ as estimated by four soil-testing techniques with actual available P as assessed by plant response. The four soil-testing techniques assessed were DGT, resin P, and two bicarbonate extraction methods (Olsen (Olsen et al. 1954), Colwell (Colwell 1963)). The resin P method is increasing in popularity and is used widely in Brazil (Chapter 3). It is important to test if the chemical constraints to the resin P method outlined in Chapter 3 has any influence on the methods capability to predict plant available P. Since Colwell (1963) developed the Colwell P extraction procedure it has been widely used in Australia and the Olsen P (Olsen 1954) extraction method has been very popular in North America since its development.

## 4.2. Material and methods

### 4.2.1 Soils

Soil samples (0-10 cm) were collected from 21 sites in Australia. Locations were representative of land used for grain production. In total 10 soils represent the southwest region of Western Australia with the majority (7) obtained within the wheat belt. In South Australia, 3 soils were collected from the Eyre Peninsula and the remaining 8 soils were

obtained in the Wimmera (5), Central Mallee (2), and Northern Mallee (1) regions of Victoria. There were a wide variety of soil types, ranging from Vertosols in Victoria to Tenosols from W.A. (Table 4.1).

#### *4.2.3 Soil preparation*

Soils were dried at 40°C for 1 week and then sieved (< 2 mm) prior to any soil testing or nutrient application.

Total element concentrations in the soils were determined by aqua regia digest with analysis performed using inductively coupled plasma optical emission spectroscopy (ICP-OES) (Zarcinas et al. 1996). Soil pH and EC was measured in samples containing 1: 5, soil: water (Rayment et al. 1992). Approximate water holding capacities were determined using an established procedure (Jenkinson et al. 1976).

The chemical characteristics of the soils used in this experiment are summarized in Table 4.1. Soil pH ranged from 5.5 to 8.8 and EC values from 81 to 993  $\mu\text{S}/\text{cm}$ .

The pot experiment was designed to determine the response of Wheat (*Triticum aestivum* L., cv frame) to an application of P. Basal nutrients were applied as solution to samples of each soil (dry weight), comprising 57  $\text{mg kg}^{-1}$  Nitrogen ( $\text{NH}_4\text{NO}_3$ ), 45  $\text{mg kg}^{-1}$  Potassium ( $\text{K}_2\text{SO}_4$ ), 5.7  $\text{mg kg}^{-1}$  of Zinc ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) resulting in an application of 21  $\text{mg kg}^{-1}$  Sulfur ( $\text{K}_2\text{SO}_4$ ,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ). These concentrations equated to the equivalent rate of 50 kg/ha N, 40 kg/ha K, and 5 kg/ha Zn. Phosphorus (30  $\text{mg kg}^{-1}$ ) was applied to two of the bulk soil samples (400 g) as phosphoric acid ( $\text{H}_3\text{PO}_4$ ). The moisture content of all the soils was then increased to 50 %, WHC; the samples were thoroughly mixed and then left to incubate for 2 days. Soil bulk samples were then split into two replicates, resulting in eight 200 g soil samples for each soil, four replicates without P addition and four with P addition. Sub-samples of soil (10 g) were taken for the other soil tests (see below) and DGT devices were deployed on the remaining soil.

**Table 4.1** Location and chemical characteristics of the soils collected for the purpose of this study.

<i>Site</i>	<i>State</i>	<i>Soil Type</i>	<i>pH(H<sub>2</sub>O)</i>	<i>EC<sub>1:5</sub></i> <i>µs/cm</i>	<i>P (total)</i> <i>(mg kg<sup>-1</sup>)</i>
Mt Damper	S.A.	Grey Calcareous	7.9	203	49
Pt. Kenny	S.A.	Grey Calcareous	9.1	323	374
Waramboo	S.A.	Grey Calcareous	9.0	202	295
Birchip	VIC	Calcarosol	6.9	993	173
Birchip Barbers	VIC	Calcarosol	8.8	204	242
Horsham	VIC	Vertosol	8.3	279	185
Kalkee	VIC	Vertosol	8.5	265	196
Lower Norton	VIC	red brown earth	7.9	210	181
Mildura	VIC	Calcarosol	8.0	129	121
Rupanyup	VIC	Vertosol	8.2	404	156
WRS	VIC	Vertosol	8.7	261	227
Balkuling	W.A.	Kurasol	5.6	129	69
Cunderdin	W.A.	Ferrasol	6.6	81	76
Gillingarra	W.A.	Podosol	5.5	108	270
Kojonup	W.A.	Tenosol	6.0	93	72
Lancelin	W.A.	Tenosol	6.0	86	13
Meckering	W.A.	Chromosol	5.9	122	160
Waranine	W.A.	Chromosol	7.5	111	183
West York	W.A.	Kurasol	7.4	238	42
Wongan	W.A.	Kandosol	5.9	87	27
Yalabee	W.A.	Tenosol	6.1	91	93
Wodjil	W.A.	Kandosol	4.9	138	73
Northam	W.A.	Chromosol	5.4	82	77
		<i>Minimum</i>	<b>5.5</b>	<b>81</b>	<b>13</b>
		<i>Maximum</i>	<b>9.1</b>	<b>993</b>	<b>374</b>

#### 4.2.4 Soil testing methods

For all soil tests except DGT, 10 g equivalent sub-samples were taken from each pot of soil and dried in an oven (40°C, 72 h) before analysis. This was performed to enable replication of all soil tests.

#### 4.2.5 Resin method

Resin-exchangeable P was determined by shaking soil solutions (1: 30, soil: water) for 17 h (Saggar et al. 1990) with an Anion Exchange Membrane (AEM) obtained from BDH and cut into strips (62.6 mm x 25 mm). The resin strips were pre-treated as described by Saggar et al. (1990) in Cl<sup>-</sup> form by immersing the AEM strips in a solution of 0.1 M HCl and 0.1 M NaCl (Hamon et al. 2002). The resin strip was removed and

rinsed with DI water and the P extracted from the strip by placing it in a solution of 0.1 M NaCl/HCl for at least 2 h. Phosphorus concentrations were determined colorimetrically using an auto analyzer and the same procedure reported in Chapter 3.

#### *4.2.5.1 Resin measurements on wet and dry soil*

Resin measurements were repeated on moist soil samples (50 % WHC) for 18 of the 21 soils used. Dried soils were weighed (1 g) and increased 50 % WHC and left to incubate for 4 days. Before AEM deployment 30 ml of DI water was added as performed on the dried soils.

#### *4.2.6 Bicarbonate extractions (Colwell P, Olsen P)*

Both Colwell P (Colwell 1963, Rayment et al. 1992) and Olsen P (Olsen et al. 1954) use 0.5 M NaHCO<sub>3</sub> (pH 8.5) to extract the soil. The main differences between the two methods are the extraction period and the soil to extractant ratios. The extraction time for the Olsen method is 30 min compared to 16 h for the Colwell method while soil to extractant ratios for the Colwell method are 1 to 100 compared to 1 to 20 for Olsen. For both methods, supernatant aliquots were filtered through 0.45 µm filter papers. Solutions were neutralized with 1 M H<sub>2</sub>SO<sub>4</sub> and P concentrations were determined colourimetrically using a spectrometer (Olsen P) and an auto analyzer (Colwell P).

#### *4.2.7 Diffusive Gradients in Thin Films (DGT)*

##### *4.2.7.1 Gel preparation and DGT devices*

A binding layer containing both ferrihydrite and Chelex-100 (Mason et al. 2005) was used for the DGT test. Properties of this mixed binding layer (MBL) have been discussed previously in detail (Chapter 2, Mason et al. 2005).

Both the diffusive and binding gels were cast according to published procedures as described in Chapter 2. Plastic DGT devices designed for soil deployment comprised a backing plate and a front plate with an exposure window ( $A = 2.52 \text{ cm}^2$ ). Diffusive gels with a thickness of 0.8 mm were incorporated on top of the MBL. A 0.13 mm thick cellulose nitrate filter ( $0.45 \mu\text{m}$ ) was placed on top of the diffusive gel for protection. The filter has been found to behave as an extension of the diffusive layer (Zhang et al. 1995).

Two days after the addition of nutrients to each pot of soil, the moisture content of the soils were increased to 80 % WHC the day prior to DGT deployment. During the morning of the next day DGT assemblies were deployed on each 200 g soil replicate (4 controls, 4 with P addition). In total 8 DGT assemblies were deployed on each soil. After 24 hours the DGT assemblies were removed and rinsed with ultra pure  $\text{H}_2\text{O}$  (Milli – Q,  $18.2 \text{ M}\Omega \text{ cm}$ ) to remove any adhering soil particles. DGT assemblies were prised open and gel elution and analysis followed previously published procedures (Mason et al. 2005).

#### 4.2.7.2 Calculation of Effective Concentration, $C_E$

The conversion of concentrations resulting from the eluant solutions to a DGT measured concentration ( $C_{\text{DGT}}$ ) has been discussed previously (Zhang et al. 1995). The conversion of  $C_{\text{DGT}}$  to  $C_E$  was performed by calculating  $R_{\text{diff}}$  (Zhang et al. 2001) using a 2D numerical model of the DGT-soil system called 2D-DIFS (DGT Induced Fluxes in Sediments and Soils) (Sochaczewski et al. 2007). The theory behind the  $C_E$  calculation has been previously discussed (Chapter 1, Zhang et al. 2001). Input parameters for the calculation of  $R_{\text{diff}}$  used previously (Chapter 1, Nolan et al. 2005) were applied to this work and are detailed in table 4.2.

**Table 4.2** Calculation of effective concentration ( $C_E$ ) from DGT measurements by calculating  $R_{diff}$  using 2D DIFS. Input parameters for  $R_{diff}$  calculation include the density of the soil particles ( $dp$ ), soil porosity ( $\Phi$ ), diffusion coefficient in soil ( $Ds$ ), and particle concentration ( $Pc$ ). Refer to section 1.5.4 for detailed calculations of these parameters.

<i>Site</i>	<i>State</i>	<i>dp</i>	$\Phi$	<i>Ds</i>	<i>Pc</i>	$C_{DGT}$ ( $\mu\text{g l}^{-1}$ )	$R_{diff}$	$C_E$ ( $\mu\text{g l}^{-1}$ )
<b>Mt Damper</b>	<b>S.A.</b>	2.65	0.33	1.63E-06	5.43	64	0.0591	1081
<b>Waramboo</b>	<b>S.A.</b>	2.65	0.45	2.03E-06	3.24	14	0.0665	205
<b>Pt. Kenny</b>	<b>S.A.</b>	2.65	0.37	1.78E-06	4.72	18	0.0618	286
<b>Birchip</b>	<b>VIC</b>	2.65	0.52	2.28E-06	2.49	23	0.0722	317
<b>Birchip Barbers</b>	<b>VIC</b>	2.65	0.38	1.79E-06	4.35	109	0.0643	1698
<b>Horsham</b>	<b>VIC</b>	2.65	0.50	2.21E-06	2.64	25	0.0714	351
<b>Kalkee</b>	<b>VIC</b>	2.65	0.49	2.17E-06	2.76	27	0.0701	392
<b>Lower Norton</b>	<b>VIC</b>	2.65	0.48	2.13E-06	2.89	30	0.0693	438
<b>Mildura</b>	<b>VIC</b>	2.65	0.33	1.62E-06	5.51	40	0.0591	671
<b>Rupanyip</b>	<b>VIC</b>	2.65	0.53	2.32E-06	2.36	34	0.0722	476
<b>WRS</b>	<b>VIC</b>	2.65	0.48	2.14E-06	2.86	90	0.0714	1266
<b>Balkuling</b>	<b>W.A.</b>	2.65	0.38	1.81E-06	4.23	25	0.0640	390
<b>Cunderdin</b>	<b>W.A.</b>	2.65	0.33	1.63E-06	5.48	2.7	0.0601	46
<b>Gillingarra</b>	<b>W.A.</b>	2.65	0.41	1.89E-06	3.86	50	0.0645	771
<b>Kojonup</b>	<b>W.A.</b>	2.65	0.35	1.71E-06	4.90	5.2	0.0618	84
<b>Lancelin</b>	<b>W.A.</b>	2.65	0.33	1.64E-06	5.44	1.2	0.0602	20
<b>Meckering</b>	<b>W.A.</b>	2.65	0.34	1.68E-06	5.10	179	0.0601	2977
<b>Waranine</b>	<b>W.A.</b>	2.65	0.42	1.93E-06	3.64	9.41	0.0654	144
<b>West York</b>	<b>W.A.</b>	2.65	0.41	1.9E-06	3.82	1.1	0.0657	17
<b>Wongan</b>	<b>W.A.</b>	2.65	0.31	1.58E-06	5.88	3.7	0.0581	65
<b>Yalenbee</b>	<b>W.A.</b>	2.65	0.42	1.92E-06	3.68	3.1	0.0662	46

#### 4.2.8 Pot experiments.

After DGT deployment five pre-germinated wheat seedlings (*Triticum aestivum* L., cv frame) were sown at an approximate depth of 1 cm in the 4 replicates without and 4 replicates with P added. Plants were thinned to three at the 2-leaf growth stage, which occurred 1 week after sowing. At this stage 20 g of alkathene granules were applied to the surface of the soil to minimize evaporation. Pots were watered with ultra pure H<sub>2</sub>O (milli – Q, 18.2 MΩ cm) and kept at 50 - 60 % WHC throughout the experiment. The experiment was performed in a controlled environment growth room at 20 °C with 12 h day and 12 h night. The pots were placed in a completely randomised arrangement that was frequently changed throughout the course of the experiment.

Four weeks after germination the above ground plant material was harvested and rinsed with ultra pure H<sub>2</sub>O (milli – Q, 18.2 MΩ cm). Plant samples were bulked and dried in an oven at 60 °C for 72 h by which time a consistent dry weight was obtained. Plant dry weight in each replicate pot was recorded.

#### *4.2.9 Statistical comparison*

Microsoft Excel was used to perform statistical analysis between dry weight means (4 replicates) using a simple T test (assuming unequal variances) to determine whether an increase in dry matter with P application was significantly different ( $p > 0.05$ ) to the control. Any increase in dry matter was expressed as a % increase in yield over the control.

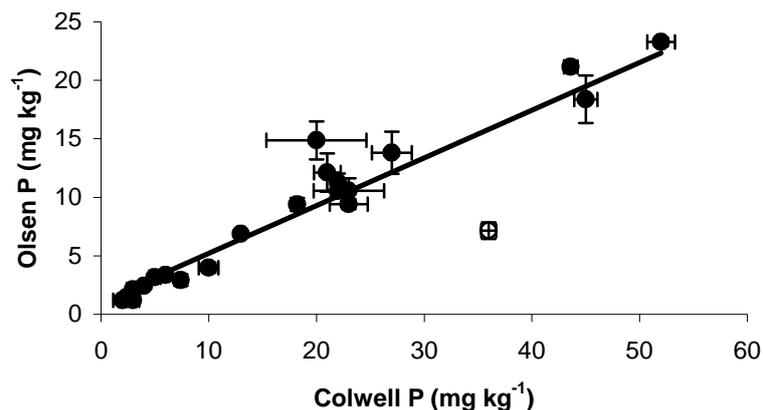
### 4.3. Results and discussion

#### *4.3.1 Comparison of soil testing methods*

In the past it has been common practice to compare the performance a new soil test against another more established method to determine if it is a suitable soil testing method (e.g. Mehlich 1984, Saggar et al. 1990, McLaughlin et al. 1994). This is a potentially dangerous process as there are too many presumptions of the original soil test used for the comparison. Firstly, it is assumed that the original test is providing a correct measurement of element (P) availability and, secondly, there is no comparison with either plant uptake or response, which is the ultimate indication of element availability in a soil. Therefore, questions arise if two soil-testing methods are poorly correlated. Which soil test is accurately predicting plant availability? Do you eliminate the new soil testing method, as the other soil test is an established method? Below is the comparison of the 4 soil tests used in this experiment based purely on their measurements without any correlation with plant uptake or response as an example

#### 4.3.1.1 Colwell vs Olsen

The Colwell P method is a modified version of the bicarbonate extraction first described by Olsen and co-workers used to improve reproducibility (Colwell 1963). The improvement of reproducibility with the Colwell method compared to the method of Olsen was suggested to be possibly due to decreased interference of organic matter coloring in the extraction solution, due to the wide dilution and more reproducible equilibrium conditions associated with the longer shaking period (Colwell 1963). Comparison of the two methods on these soils produced a strong correlation ( $r = 0.97$  ( $r^2 = 95$ )) (Figure 4.1). There is one outlier associated with a calcareous soil from S.A. (Pt. Kenny), removing this outlier further improves the correlation. The effect of the longer extracting time results in a larger fraction of P extracted as indicated by the slope ( $y = 0.41$ ) but essentially both extractions are in fact measuring a similar form of available P. There is no evidence from these results that reproducibility is improved using the Colwell P method (Figure 4.1).

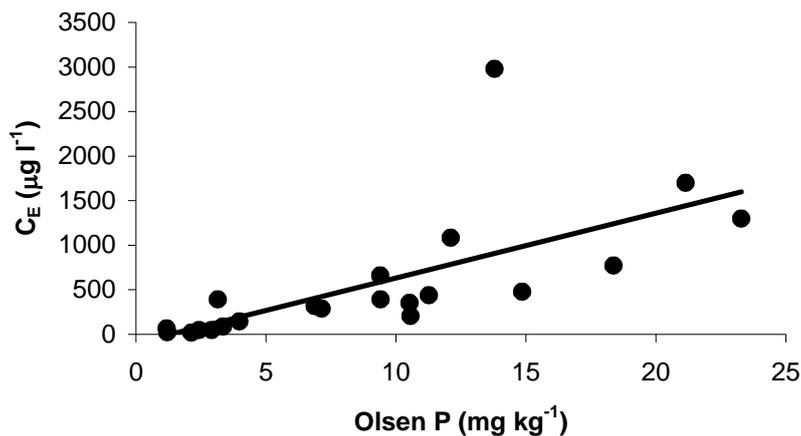


**Figure 4.1** Relationship between Colwell P and Olsen P measurements. Open circle represents the outlier mentioned in Section 4.3.1.1.

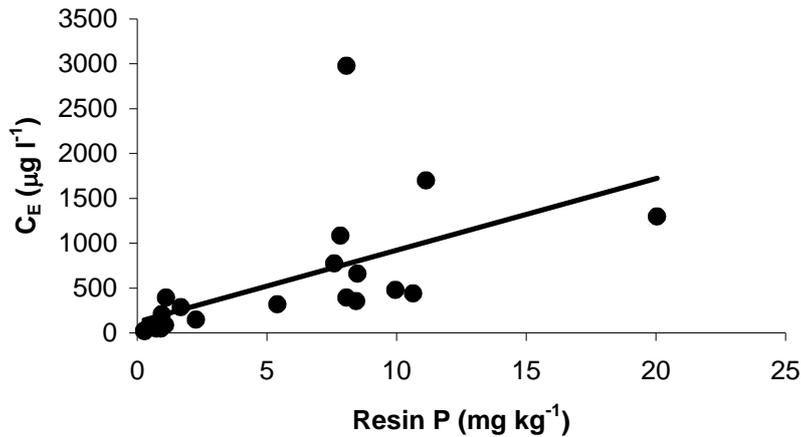
#### 4.3.1.2 DGT with other soil testing methods

Comparison of DGT with Resin P and both extraction measurements on these soils resulted in only poor to moderate correlations. The DGT method had the highest correlation with Olsen P resulting in a correlation coefficient ( $r$ ) of 0.81 ( $r^2 = 0.67$ ) compared to resin P 0.77 ( $r^2 = 0.60$ ) and Colwell P 0.77 ( $r^2 = 0.60$ ) (Figure 4.2). The correlations between the soil tests were significant apart from the correlation between Colwell and DGT ( $p < 0.05$ ). The decreased correlation between DGT and resin P measurements compared to DGT and Olsen was an unexpected result, as the resin P method has more similarities to the DGT method compared with the extraction methods. This observation is also in contrast to published work from Menzies et al. (2005), who reported a higher regression fit ( $r^2 = 0.87$ ) between DGT and resin P. This result was obtained with no mention of DIFS and therefore DGT values were not reported as  $C_E$  or  $C_{DGT}$ .

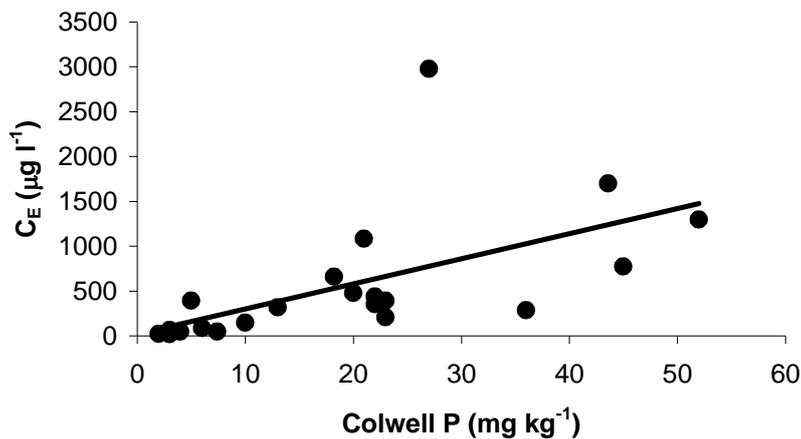
a)



b)



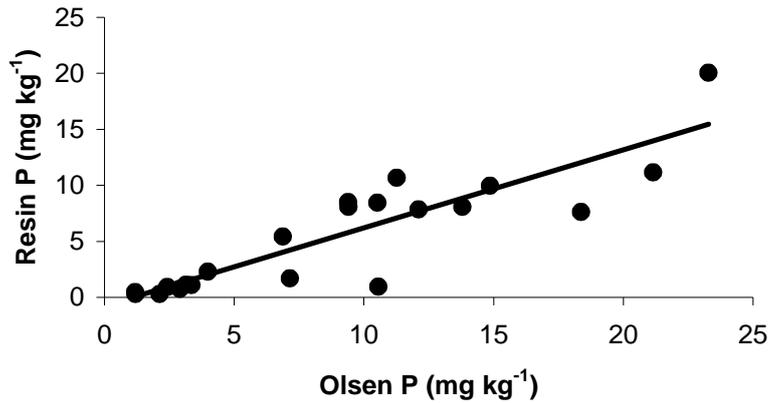
c)



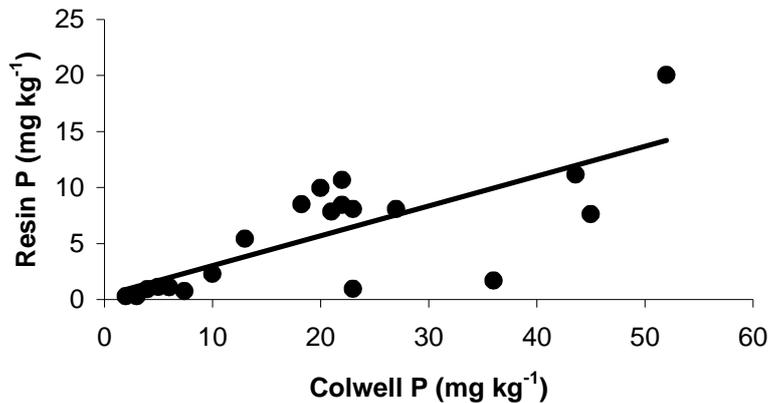
**Figure 4.2** Relationships of C<sub>E</sub> values (measured by DGT) with a) resin P, b) Olsen P, and c) Colwell P. Open circles represent the outlier mentioned in Section 4.3.1.2.

Moderately good correlations were obtained from comparisons of resin P measurements with both the extraction methods, with correlation coefficients ( $r$ ) of 0.95 ( $r^2 = 0.90$ ) (Olsen P) and 0.90 ( $r^2 = 0.81$ ) (Colwell P) (Figure 4.3). In each comparison the correlation was significant ( $p < 0.05$ ). Overall, lower correlations were observed between DGT and any other method. Therefore on the basis of comparing soil tests would you eliminate the DGT method as a potential indicator of plant available P in a soil due to the lower correlations obtained with other established soil tests?

a)



b)



**Figure 4.3** Relationship between resin P values and a) Olsen P and b) Colwell P.

#### 4.3.2 Dry matter yield of wheat

Of the 21 soils used in this experiment, 12 soils had no statistically significant increase ( $p < 0.05$ ) in the production of wheat dry matter with applied P while 9 soils were responsive to applied P (Table 4.2). Dry matter was expressed as increase in yield, using eqn 13.

$$\% \text{ Increase in yield} = (Y_{(\text{applied P})} - Y_{(\text{control})}) / Y_{(\text{control})} \times 100 \quad (13)$$

Where  $Y_{(\text{applied P})}$  is the yield (g) of wheat obtained in soil to which P was added and  $Y_{(\text{control})}$  is the yield (g) of wheat obtained in soil to which no additional P was added.

Using a small number of replicates (4) the T test at 95 % confidence interval was very sensitive to any variability with yields. Although this is potentially a disadvantage, there is a positive side, as good replication and precise data was a requirement for the determination of responsiveness.

**Table 4.3** Yield data (g/pot) of wheat grown in 21 agricultural soils (control + P application).

<i>Site</i>	<i>State</i>	<i>Yield*</i> (g/pot)	<i>% Increase</i> <i>in yield from control</i>	<i>P responsive</i> ( <i>p</i> < 0.05)
<b>Mt Damper</b>	S.A	0.36		
<b>Mt Damper +P</b>		0.40	10	No
<b>Pt. Kenny</b>	S.A	0.23		
<b>Pt. Kenny +P</b>		0.36	55	Yes
<b>Waramboo</b>	S.A	0.17		
<b>Waramboo +P</b>		0.24	42	Yes
<b>Birchip</b>	VIC	0.26		
<b>Birchip +P</b>		0.40	51	Yes
<b>Birchip B</b>	VIC	0.61		
<b>Birchip B +P</b>		0.69	14	No
<b>Horsham</b>	VIC	0.49		
<b>Horsham +P</b>		0.72	47	Yes
<b>Kalkee</b>	VIC	0.48		
<b>Kalkee +P</b>		0.60	25	Yes
<b>Lower Norton</b>	VIC	0.53		
<b>Lower Norton +P</b>		0.63	19	No
<b>Mildura</b>	VIC	0.31		
<b>Mildura +P</b>		0.34	10	No
<b>Rupanyip</b>	VIC	0.58		
<b>Rupanyip +P</b>		0.48	-17	No
<b>WRS</b>	VIC	0.37		
<b>WRS +P</b>		0.39	4	No
<b>Balkuling</b>	W.A	0.27		
<b>Balkuling +P</b>		0.31	11	No
<b>Cunderdin</b>	W.A	0.21		
<b>Cunderdin +P</b>		0.27	28	Yes
<b>Gillingarra</b>	W.A	0.33		
<b>Gillingarra +P</b>		0.37	13	No
<b>Kojonup</b>	W.A	0.23		
<b>Kojonup +P</b>		0.30	29	Yes
<b>Lancelin</b>	W.A	0.17		
<b>Lancelin +P</b>		0.35	107	Yes
<b>Meckering</b>	W.A	0.36		
<b>Meckering +P</b>		0.38	6	No
<b>Waranine</b>	W.A	0.20		
<b>Waranine +P</b>		0.24	23	No
<b>West York</b>	W.A	0.24		
<b>West York +P</b>		0.30	25	No
<b>Wongan</b>	W.A	0.22		
<b>Wongan +P</b>		0.36	65	Yes
<b>Yalenbee</b>	W.A	0.24		
<b>Yalenbee +P</b>		0.24	2	No

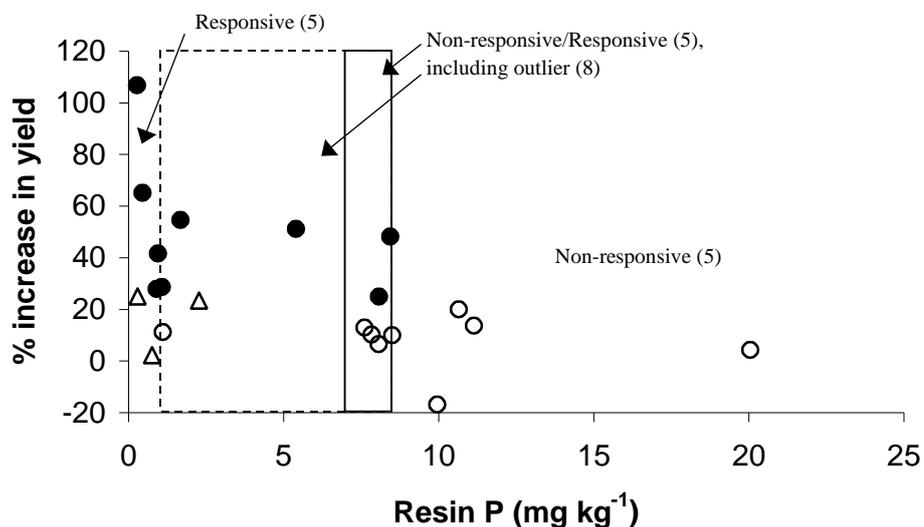
*\*mean of dry matter yield (3 plants) obtained from 4 replicate pots of soil*

### 4.3.3 Soil testing methods with wheat responsiveness

#### 4.3.3.1 Resin P

The resin P method appears to have failed to definitively separate P responsive and non-responsive soils (Figure 4.4). For resin P values in the range 1.1 and 8.5 mg kg<sup>-1</sup>

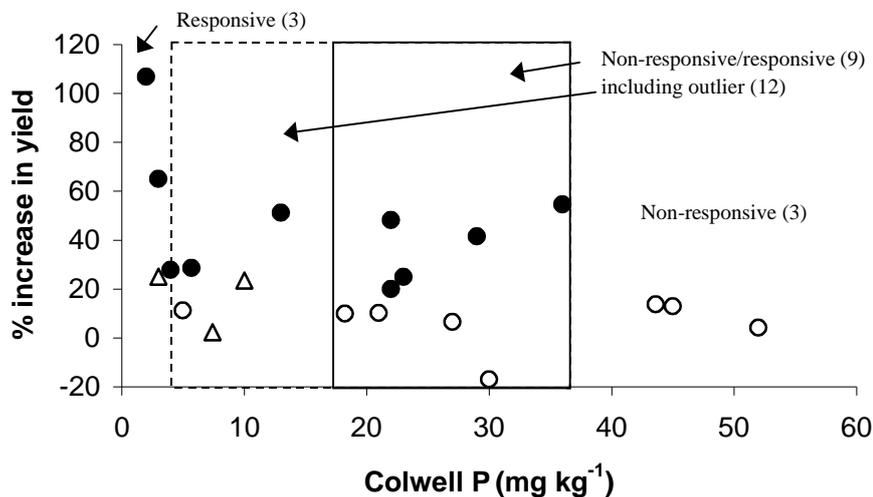
soils could be either responsive or non-responsive. Supporting this observation, McBeath et al. (2005) found that increases in yields with applied P ranged from < 10 % up to approximately 100 % between resin P values of approximately 7 and 14 mg kg<sup>-1</sup>. Three soils indicated by triangles on figure 4.4 have resin P values < 3 mg kg<sup>-1</sup> and were statistically non responsive to applied P. These three soils are not included in this assessment, as they have fixed most of the P applied in this experiment (see discussion in Section 4.3.3.3). Overall resin P correctly predicted 10 out of 18 soils (56%) considered in this assessment. The 8 soils that were deemed to be incorrectly predicted are indicated within the boxes on figure 4.4. This indicates that within this resin P region the soils were either responsive or non-responsive. There was a marked outlier, Balkuling: a non-responsive soil with a low resin P value of 1.1. Excluding Balkuling the resin method correctly predicted the responsiveness of 13 out of the 18 soils (72 %). The soils incorrectly predicted are highlighted within the solid box in figure 4.4.



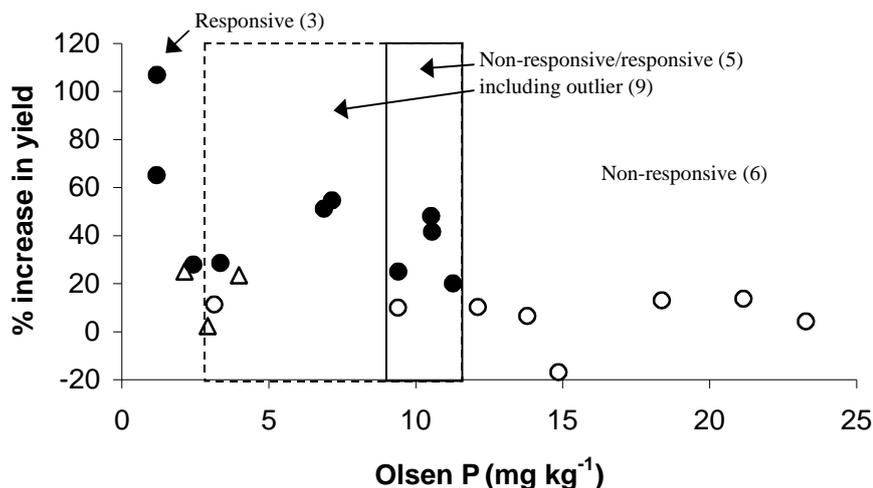
**Figure 4.4** Percentage increase of dry matter response to applied P over the control as a function of resin P measurements. (o) non-responsive soils (●) responsive soils (Δ) outliers discussed in Section 4.3.3.3. Dashed box includes the region of non-responsive and responsive soil overlap with respect to resin P values. Solid box excludes outlier mentioned in the text.

#### 4.3.3.2 Extraction techniques

Both bicarbonate extraction techniques failed to discriminate between responsive and non-responsive soils between 18 - 36 mg kg<sup>-1</sup> for Colwell and between 9.4 – 11.3 mg kg<sup>-1</sup> for Olsen, excluding the Balkuling soil at 5 mg kg<sup>-1</sup> (Colwell) and 3.2 mg kg<sup>-1</sup> (Olsen). Colwell P overall correctly predicted 9 out of the 18 soils (50 %) (soils that fall outside of the box which indicates area of responsive/ non-responsive overlap) or 6 out of the 18 soils (33 %) by including Balkuling (Figure 4.5). Olsen P improved the prediction correctly predicting 13 out of 18 soils (72 %) or 9 out of 18 soils (50%) by including Balkuling (Figure 4.6). Inconsistent results are common for these methods, which have often shown to correlate poorly with plant yield (Saggar et al. 1999, McBeath et al. 2005, Menzies et al. 2005). Those studies found that resin P values correlate better with % increase in yield, counter to the findings of this study where the resin P method was only as effective as extraction methods for predicting P responsive soils.



**Figure 4.5** Percentage increase of dry matter response to applied P over the control as a function of Colwell P. (o) non-responsive soils (•) responsive soils (Δ) outliers discussed in Section 4.3.3.3. Dashed box includes the region of non-responsive and responsive soil overlap with respect to Colwell P values. Solid box excludes outlier mentioned in the text.



**Figure 4.6** Percentage increase of dry matter response to applied P over the control as a function of Olsen P. (o) non-responsive soils (●) responsive soils (Δ) outliers discussed in Section 4.3.3.3. Dashed box includes the region of non-responsive and responsive soil overlap with respect to Olsen P values. Solid box excludes outlier mentioned in the text.

#### 4.3.3.3 DGT

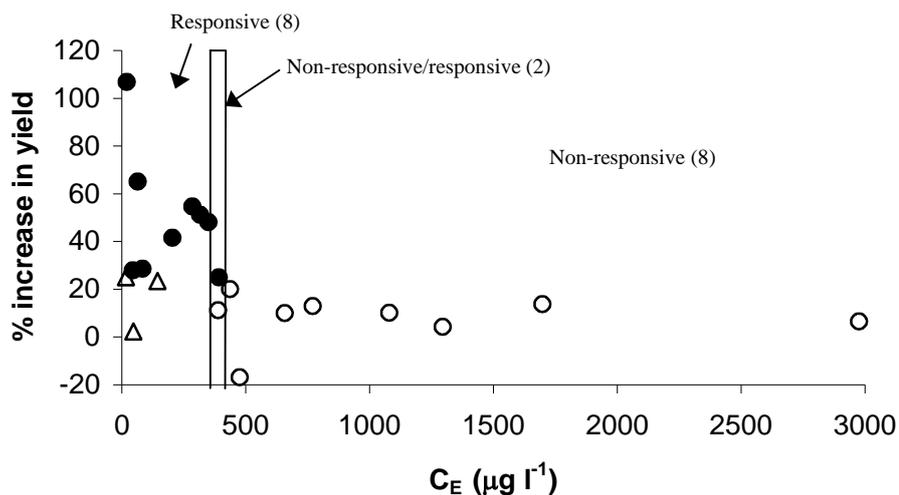
In contrast to the other techniques DGT successfully distinguished the non-responsive soils from the responsive soils (Figure 4.7). The results indicate that the threshold of P deficiency corresponds to a  $C_E$  value of approximately  $390 \text{ ug l}^{-1}$  (value at which responsive and non-responsive soils are separated) (Figure 4.7). Soils with lower  $C_E$  values generally were responsive, although there are a few exceptions. Three soils (Yalenbee, Waranine and West York) that were statistically non-responsive to applied P all had low soil test values for the untreated soil, suggesting they should be P responsive soils. However, the  $C_E$  values obtained from DGT deployment on these soils at the rate of P applied in this experiment revealed that there was a negligible increase in ‘available’ P (Table 4.3). It appears these soils have a high P fixing capacity and therefore the applied P ( $30 \text{ mg kg}^{-1}$ ) was insufficient to result in any plant response. These soils with initially low ‘available P’ also had very small plant yields, which contributed to a large variation between replicates and even yield increases of  $> 20 \%$  were statistically non-significant

(Table 4.2). Further experiments have shown that when a higher rate of P was applied on two of these soils (the third soil was not available for further experiments) there was a significant response in dry matter (Chapter 5). After excluding these three non-responsive soils, DGT correctly predicted the wheat response for 16 out of the 18 (89 %) soils used in this study. The two overlapping soils (1 responsive and 1 non-responsive) were both labeled as incorrectly predicted by DGT as it is unsure which soil DGT has overestimated or underestimated.

Recent work that suggested that DGT did not significantly improve predictions of plant yield in comparison to resin P was performed on highly fertilized soils (Menzies et al. 2005). In this study a one off application rate of P was used more typical of field conditions ( $30 \text{ mg kg}^{-1}$ ), with the highest resin P value obtained from the control soil being only  $20 \text{ mg kg}^{-1}$ . This corresponds to a very small section of values obtained by Menzies et al (2005) where resin P values ranged up to  $300 \text{ } \mu\text{g g}^{-1}$  with the majority of samples  $> 20 \text{ } \mu\text{g g}^{-1}$  due to higher applications of P. A similar study performed by Saggart et al. (1999) using the resin method as an indicator of yield increases, also obtained a wide range of resin P values with the majority between 20 and  $80 \text{ } \mu\text{g g}^{-1}$ . The soils in this study correspond to only a very small section of their correlation with yield and more sensitive to errors in tests for distinguishing non-responsive soils and responsive soils.

**Table 4.4**  $C_E$  values (measured by DGT) of the control and with applied P for the 3 highly P fixing soils.

Soil	$C_E$ (control) $\mu\text{g l}^{-1}$	$C_E$ (applied P) $\mu\text{g l}^{-1}$
Waranine	144	294
West York	17	63
Yalenbee	46	125



**Figure 4.7** Percentage increase of dry matter response to applied P over the control as a function of C<sub>E</sub> (measured by DGT). (o) non-responsive soils (●) responsive soils (△) outliers discussed in Section 4.3.3.3. Solid box includes the region of non-responsive and responsive soil overlap with respect to C<sub>E</sub> (DGT) values.

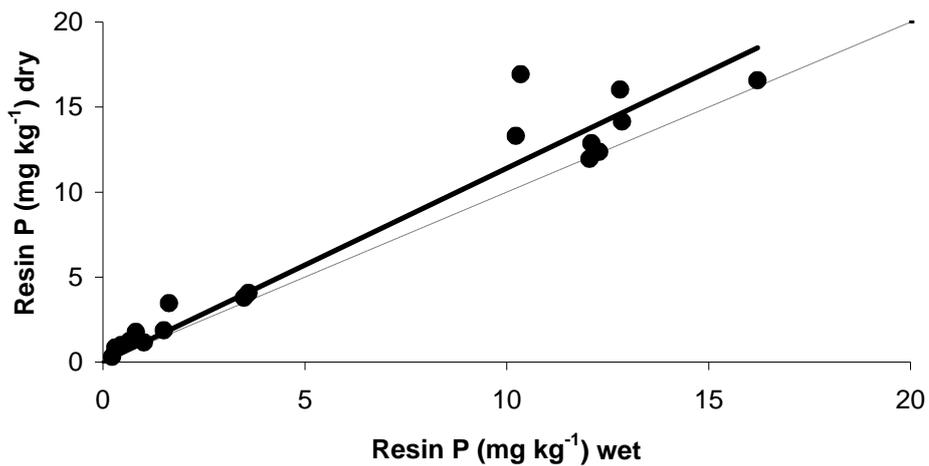
#### 4.3.4 Resin measurements on wet and dry soils

Previous studies have shown that a dried soil can have different chemical properties compared to the corresponding moist sample (Turner and Haygarth 2003, Sparling et al. 1985, Olsen and Court 1982, Barlett and James 1980, Turner 2005, Chepkwony et al. 2001, Haynes and Swift 1985). Soil samples taken for resin P measurements for comparison with wheat response to P were dried prior to analysis. Therefore, the poor performance of the resin method in predicting plant response to P could be due to the soil samples changing chemically from the moist state in the pot to the dried soil used for resin P measurements. To determine if drying the soil had an effect on available P measured by the resin P method, resin P measurements were performed on 18 of the 21 agricultural soils used in this experiment (due to soil availability) with two different soil pretreatments, 1) oven dried soil (40°C) and 2) soil incubated at 50 % WHC. Resin P measurements were generally higher from the oven dried soil with increases ranging from 0 to 171 % (Table 4.4). Comparison of the resin P measurements obtained from the dried soil compared to the wet soil revealed an excellent correlation (r

= 0.99,  $r^2 = 0.98$ ) and a slope of 1.09. This suggests that the proportionally large increases from the oven-dried soil at low P concentrations did not significantly affect this relationship (Figure 4.8). In a similar experiment by Olsen and Court (1982) increases in resin P measurements on dried soil samples were normally less than 20 % with a maximum increase of 37 %. The difference in the Olsen and Court (1982) study was that the dried samples were subjected to 11 wetting and drying cycles before analysis. Turner and Haygarth (2003) reported increases in bicarbonate extractable inorganic P from dried soil samples between 11 and 165 % compared to moist samples. In accordance with the results from Table 4.4 they also reported that the greatest increases were associated with soils with low P concentrations. Chemical changes to different sources of P have been documented to cause the increase in resin P or extractable P in dried and rewetted soil samples. Sparling et al. (1985) reported that increases in extractable inorganic P between 0 and 186 % from air-dried samples compared to the corresponding moist samples were derived from killed microbial cells. Chepkwony et al. (2001) suggested that increases in bicarbonate extractable P, Bray -1, and resin P measurements in soil samples that had been dried and rewetted compared to moist soil samples was due the stimulation of soil organic P mineralization. In highly weathered, P deficient soils, organic P often accounts for 50 %, or more, of their total P content (Chepkwony et al. 2001). Therefore increases in available P measurements due to organic P mineralization from dried soil samples would be associated with P deficient soils due to their overall greater composition of organic P.

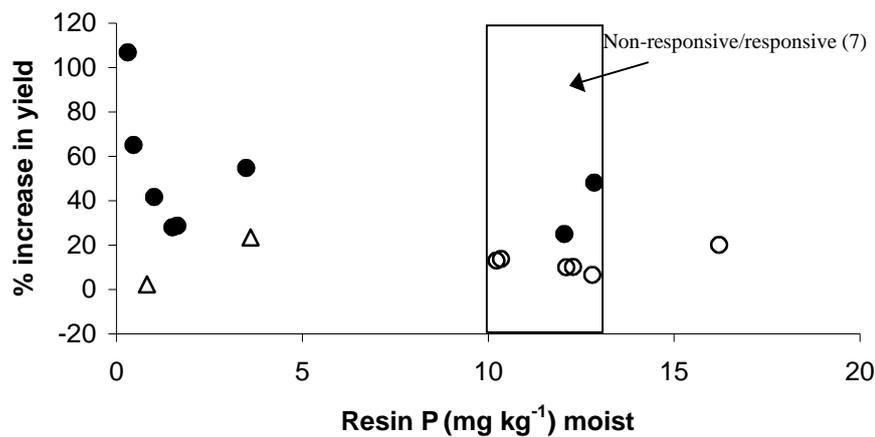
**Table 4.5** Resin P measurements on 18 agricultural soils that were pretreated prior to resin P analysis by either incubating at 50 % WHC for 4 days (wet) or oven-dried (dry) (40°C).

Site	Wet mg kg <sup>-1</sup>	Dry mg kg <sup>-1</sup>	% Increase
Birchip	10.3	16.9	63
Cunderin	1.5	1.9	22
Gillingarra	10.2	13.3	30
Horsham	12.9	14.1	10
Kalkee	12.1	12.0	0
Kojonup	1.6	3.5	110
Lancelin	0.3	0.8	171
Lower Norton	16.2	16.6	2
Meckering	12.8	16.0	25
Mildura	12.1	12.9	6
Mt Damper	12.3	12.4	1
Northam	0.7	1.2	85
Pt Kenny	3.5	3.8	8
Waramboo	1.0	1.1	12
Waranine	3.6	4.1	12
Wodjil	0.2	0.3	25
Wogan	0.5	1.0	115
Yalenbee	0.8	1.8	115
Mean	5.9	7.0	



**Figure 4.8** Relationship between resin P measurements performed on dry and moist soil samples. Dotted line represents the 1 to 1 line and the solid line represents the regression fit for the data.

Substituting the resin P measurements from the oven-dried samples for those obtained from the corresponding incubated moist soils did not improve the resins predictive capability of wheat response to applied P (Figure 4.9). It appears with these soils that the effect of oven drying the soil is insignificant due to the major increases in resin P measurements being associated with highly P deficient soils.



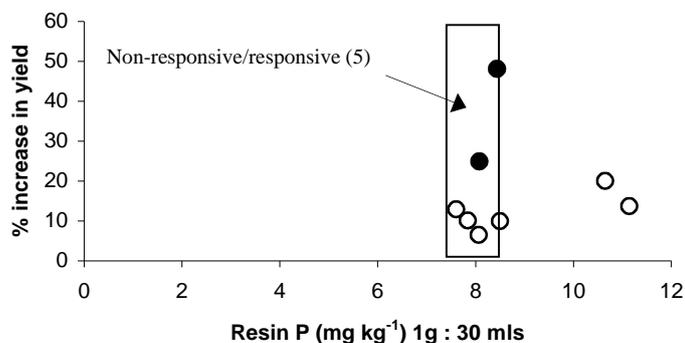
**Figure 4.9** Relationship between resin P values obtained from incubated moist soil samples with wheat yield response to P. Solid box includes the region of non-responsive and responsive soil overlap with respect to resin P values.

#### 4.3.5 Improving resin P prediction of plant response

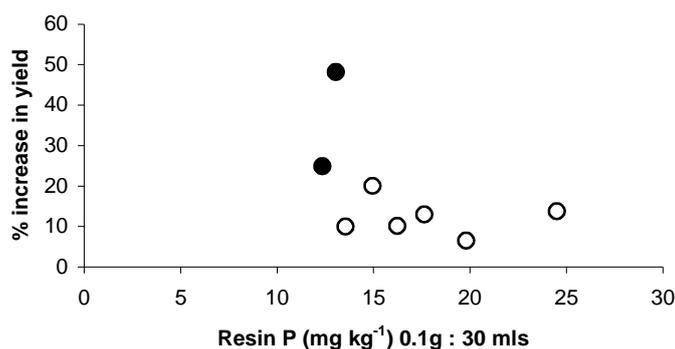
Experiments performed in Chapter 3 revealed that the resin P measurements increase significantly by decreasing the amount of soil used in a set amount of DI water (30 ml). It appears that the increase in resin P measurements is associated with the increased resupply of the soil solid phase in response to the greater dilutions. By substituting the resin P measurements obtained from the greatest dilution (Chapter 3) for the resin P values used in this study, there is a slight improvement in the prediction of plant response to P for the selected soils (Figure 4.10). It appears that the increase in P resupply of the soil solid phase created by diluting the soil solutions and measured by

resin P, in this case, is a better reflection of plant available P than with larger soil: water ratios.

a)



b)



**Figure 4.10** Percentage increase of dry matter response to applied P over the control as a function of resin P a) normal 1: 30 soil: water ratio and b) 0.1: 30 soil: water ratio. (o) non-responsive soils (●) responsive soils. Solid box includes the region of non-responsive and responsive soil overlap with respect to resin P values.

#### 4.4. Overall analysis

The results obtained in this experiment suggest that the DGT method offers a potential improvement on the other soil tests for predicting P deficiency in a soil. There are several possible reasons why DGT should be a better indicator of ‘available’ P. Both the resin and DGT methods attempt to mimic the physico-chemical uptake of solutes by

the plant (Mason et al. 2006). However, soil properties that probably influence the P status of the soil are included in the calculation of  $C_E$ . Input parameters, including soil porosity and particle concentration, can influence the  $R_{diff}$  value and the consequent  $C_E$  value appreciably. The resin method is performed by placing an AEM in a soil solution (1: 30 soil: water) and therefore does not incorporate these parameters. DGT by incorporating a diffusive gel places a limit on the P flux to the binding gel. It appears the ability of DGT to quantify the rate of resupply from the soil solid phase is very important. The resin P method is also subject to possible interferences/competition with other anions (Chapter 3). Comparison of DGT and resin P results revealed there to be major discrepancies in the measurement of 'available' P in some soils. It is becoming generally accepted that extraction methods are inaccurate for determining plant available P over a wide range of soils (Saggar et al. 1990, McBeath et al. 2005, Menzies et al. 2005). The extraction methods in some soils appear to extract sources of P that are unavailable to plants. This was evident for the two calcareous soils (notably Colwell) when compared to wheat response to applied P (Figure 4.5).

The DGT method potentially offers an accurate assessment of the P status of agricultural soils, improving the prediction of fertilizer use and efficiency. This study has been performed on 21 soils with a wide range of soil properties and locations. However, overall validity of the DGT method needs to be established by performing further comparisons using a larger set of different soils to confirm these potentially exciting results. Furthermore comparison of the performance of DGT needs to be assessed with plant yields obtained under field conditions (Chapter 8) before the full benefits of the DGT can be determined.

## 5. Predicting soil P fertilizer requirements using DGT and resin methods

### 5.1. Introduction

The importance of accurately predicting plant P requirements in any given soil cannot be underestimated. Efficient use of P fertilizers is essential for reducing unnecessary expense and environmental problems associated with over fertilisation. Authors have assessed the ability of a range of soil testing methods to predict plant responsiveness to applied P (e.g. Saggar et al. 1992, Saggar et al. 1999, McBeath et al. 2005, Menzies et al. 2005) and all have had varying results. A general conclusion from these studies has been that the extraction methods of Colwell, Olsen, and Bray are inappropriate for predicting plant P requirements. One major problem associated with extraction methods has been that the reagents used extract different proportions of the different forms of P from soils (Menon et al. 1989, Menon et al. 1990). For example in Western Australia, Colwell P calibration curves relating to plant yield differ substantially across different soil types, plant species, and soils that have been treated with different types of fertilizer (Bolland et al. 1989). Kumar et al. (1992) suggested that this was due to different forms of fertilizers leaving different types and amounts of P residues in the soil. Ideally a soil test should not be dependent on soil type used but simply measure the forms of P that are available to the plant.

The resin method has been shown in some studies to be an improved soil test compared to extraction methods for predicting plant available P (Menzies et al. 2005, Saggar et al. 1992, Saggar et al. 1999). However, other studies have shown the inability of the resin method to predict plant response with different fertilizer types (McBeath et al. 2005) and plant yield with applied P (Kumar et al. 1992).

In an ideal situation a soil test should indicate to growers if 1) they need to apply P fertilizer and 2) how much P fertilizer do they need to supply in order to produce

maximum yields and maximise fertilizer efficiency. The DGT method has been shown to be more accurate at predicting plant P response compared to other soil testing methods when one rate of P was applied (Chapter 4). Therefore DGT can provide an accurate indication of whether a grower needs to apply P or not to obtain a yield response. However, a one off application rate of 30 mg kg<sup>-1</sup> P used in the experiment described in Chapter 4 will in many cases not result in enough available P in soil to produce the maximum yield obtainable, especially in highly P deficient soils. It is therefore difficult to provide accurate information to growers on how much P fertilizer they need to apply in order to achieve maximum yields from a one off P application rate. In this chapter the aim is to determine the capabilities of both DGT and resin in predicting P requirements in order to obtain the maximum yield possible on several P responsive soils with different P buffering capabilities. This was achieved by applying several rates of P as opposed to a one off application rate in order to produce yield response curves with P application rates and comparing these to measurements made by the two soil tests.

## 5.2. Materials and methods

### 5.2.1 Soils

Eight soils were chosen from the previous experiment (Chapter 5) that had varying degrees of response to applied P (30 mg kg<sup>-1</sup>). These soils all have different propensities for attenuation of available P as shown by DGT measurements on the soil with applied P (Table 5.1). For soil preparation see Section 4.2.3.

**Table 5.1** Percentage increase in DGT measurements ( $C_E$ ) on soils with the addition of P ( $30 \text{ mg kg}^{-1}$ ) compared to the control soil and the resulting increase in yield (Chapter 4).

Soil	Classification	Location	% increase in $C_E$ with P application	% increase in yield
Pt. Kenny	Grey Calcareous	S.A.	326	55
Waramboo	Grey Calcareous	S.A.	112	42
Birchip	Calcarosol	VIC	145	37
Cunderdin	Ferrasol	W.A.	1474	28
Kojonup	Tenasol	W.A.	204	29
Lancelin	Tenasol	W.A.	11240	107
Waranine	Chromosol	W.A.	104	23
Yalenbee	Tenasol	W.A.	172	2

### 5.2.2 Pot experiments

The pot experiment was designed to determine the response of Wheat (*Triticum aestivum* L., cv frame) to a series of P application rates. Basal nutrients were applied as solution to each 200 g sample of dried soil, comprising of  $100 \text{ mg kg}^{-1}$  nitrogen ( $\text{NH}_4\text{NO}_3$ ),  $75 \text{ mg kg}^{-1}$  potassium ( $\text{K}_2\text{SO}_4$ ),  $10 \text{ mg kg}^{-1}$  of zinc ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) which resulted in an application of  $36 \text{ mg kg}^{-1}$  sulfur ( $\text{K}_2\text{SO}_4$ ,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ). Phosphorus was applied as phosphoric acid ( $\text{H}_3\text{PO}_4$ ) at 10 different rates, (0 (control), 5, 10, 20, 30, 50, 70, 100, 200,  $300 \text{ mg kg}^{-1}$ ), to each 200 g sample for the 8 soils. Application of P in a liquid form meant that DGT and resin methods could measure the increase in P with increasing application rates. The soil moisture was increased to 50 % WHC, the samples were thoroughly mixed and then left to incubate for 2 days. Prior to DGT deployment, sub-samples (10 g) were taken for resin P analysis and DGT devices were deployed on the remaining soil in the pots (see below).

After DGT deployment five pre-germinated wheat seedlings (*Triticum aestivum* L., cv frame) were sown at an approximate depth of 1 cm. Plants were thinned to three at the 2-leaf growth stage, which occurred 1 week after sowing. At this stage 20 g of alkathene granules were applied to the surface of pots to minimize evaporation. Pots were watered with ultra pure  $\text{H}_2\text{O}$  (milli – Q,  $18.2 \text{ M}\Omega \text{ cm}$ ) and kept at 50 - 60 % WHC

throughout the experiment. The experiment was performed in a controlled environment growth room at 20 °C with 12 h day and 12 h night. The pots were placed in a completely randomised arrangement, which was frequently changed throughout the course of the experiment.

Four weeks after germination the above ground plant material was harvested and rinsed with ultra pure H<sub>2</sub>O (milli – Q, 18.2 MΩ cm). Plant samples were dried in an oven at 60 °C for 72 h by which time a consistent dry weight was obtained.

For each soil the percent increase in yield caused by the rate of P application was calculated as (see Chapter 4):

$$\% \text{ Increase in yield} = (Y_{(\text{applied P})} - Y_{(\text{control})}) / Y_{(\text{control})} \times 100 \quad (14)$$

where  $Y_{(\text{applied P})}$  is the dry matter yield from the corresponding P application rate and  $Y_{(\text{control})}$  is the yield from the control pot without any applied P. This increase in yield was converted to % relative yield by assigning the yield from the highest P application rate a value of 100 %.

The relationship between the relative yield (%) and soil P test value for all soils could be fitted using a Mitscherlich-type equation:

$$y = a + b(1 - e^{-cx}) \quad (15)$$

where  $y$  is the % increase in yield with applied P,  $x$  is the soil test value,  $(a + b)$  is the maximum yield and  $c$  is the curvature coefficient (Saggar et al. 1999).

### 5.2.3 Soil testing methods

#### 5.2.3.1 DGT

Soil P status was measured by using mixed binding layers (MBL) in DGT devices with the same procedure explained in Chapter 4 (see Section 4.2.7). Input parameters for the calculation of  $R_{\text{diff}}$  are detailed in table 5.2.

**Table 5.2** Calculation of  $R_{diff}$  values using 2D DIFS. Input parameters for  $R_{diff}$  calculation include the density of the soil particles ( $dp$ ), soil porosity ( $\Phi$ ), diffusion coefficient in soil ( $Ds$ ), and particle concentration ( $Pc$ ). Refer to section 1.5.4 for detailed calculations of these parameters.

<i>Site</i>	<i>State</i>	<i>dp</i>	$\Phi$	<i>Ds</i>	<i>Pc</i>	$R_{diff}$
<b>Pt Kenny</b>	<b>S.A.</b>	2.65	0.44	1.99E-06	3.41	0.0675
<b>Waramboo</b>	<b>S.A.</b>	2.65	0.46	2.06E-06	3.14	0.0690
<b>Birchip</b>	<b>VIC</b>	2.65	0.49	2.18E-06	2.72	0.0715
<b>Cunderdin</b>	<b>W.A.</b>	2.65	0.33	1.63E-06	5.48	0.0602
<b>Kojonup</b>	<b>W.A.</b>	2.65	0.41	1.91E-06	3.74	0.0640
<b>Lancelin</b>	<b>W.A.</b>	2.65	0.41	1.91E-06	3.74	0.0640
<b>Waranine</b>	<b>W.A.</b>	2.65	0.44	2.00E-06	3.34	0.0679
<b>Yalenbee</b>	<b>W.A.</b>	2.65	0.44	2.00E-06	3.37	0.0677

After the soils had incubated with added nutrients for 2 days, the moisture content of the soils was increased to 80 % WHC the day prior to DGT deployment. During the morning of the next day, DGT assemblies were deployed on each 200 g soil sample. In total 2 DGT assemblies were deployed at each P rate for each soil. After 24 hours the DGT assemblies were removed and rinsed with ultra pure H<sub>2</sub>O (milli – Q, 18.2 MΩ cm) to remove any adhering soil particles. DGT assemblies were prised open and gel elution and analysis followed previously published procedures (Mason et al. 2005).

### 5.2.3.2 Resin method

Sub-samples (10 g) were taken from each 200 g pot of soil and oven dried at 40°C for 72 h before resin P analysis. Resin-exchangeable P was determined by shaking soil solutions (1: 30, soil: water) for 17 h (Saggar et al. 1990) with an Anion Exchange Membrane (AEM) in Cl<sup>-</sup> form (same dimensions reported in Chapter 4). The resin strip was removed and rinsed with DI water and the P extracted from the strip by placing it in a solution of 0.1 M NaCl/HCl for at least 2 h. Phosphorus concentrations were determined colourimetrically using an auto analyzer and the same procedure reported in Chapter 3.

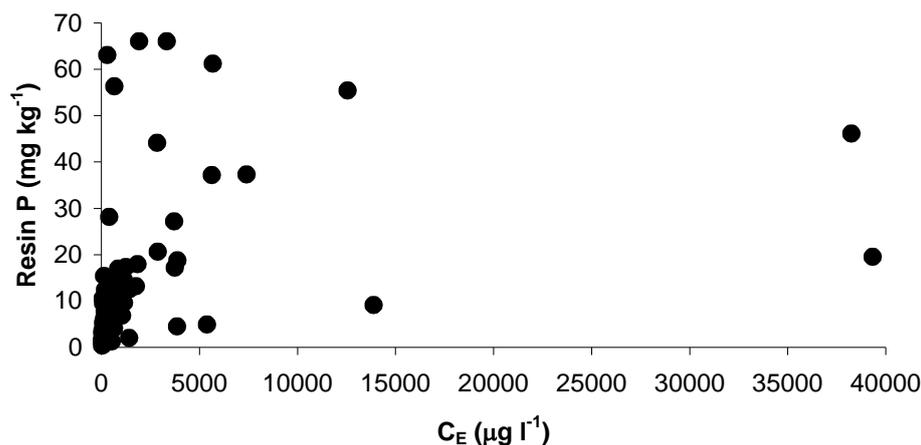
#### *5.2.4 Resin measurements on wet/dry soil samples*

Large increases in resin P measurements were obtained in P deficient soils on dried soil samples compared to moist soil samples (see Chapter 4). In the experiment described in Chapter 4 only the resin measurements made on the control soils were used for comparison with the response of wheat to applied P. For this experiment resin P measurements will be used on soil samples that have had freshly applied P and compared to wheat response. To determine if drying the soil with freshly applied P had any effect on resin P measurements, three P deficient soils were used from the P rate experiment, Lancelin, Kojonup, and Waranine. These soils were chosen to cover the range of % increase in  $C_E$  values (measured by DGT) with applied P obtained in the experiment described in Chapter 4 (see Table 5.1). Samples (50 g) were spiked with P at two different rates (30 and 70 mg kg<sup>-1</sup>) plus the control soil, the samples were thoroughly mixed and then left to incubate for 3 days at 50 % WHC. After the incubation period, sub samples (10 g) were dried at 40 °C for 24 h. Resin P measurements were performed on both dried and moist samples simultaneously using the method described in Section 5.2.3.2, and each rate was replicated.

### 5.3. Results and discussion

#### *5.3.1 DGT and resin P measurements*

Significant differences were observed between DGT and resin P measurements on some soils. There was no significant relationship between measurements from the two soil testing methods (Figure 5.1). Differences between the two methods were also observed in Chapter 3 and 4, which confirms that the available P measured by DGT might not be necessarily the same pool of available P measured by the resin method. Problems associated with the resin method have been highlighted in Chapter 3 and these could have contributed to the discrepancies observed here.



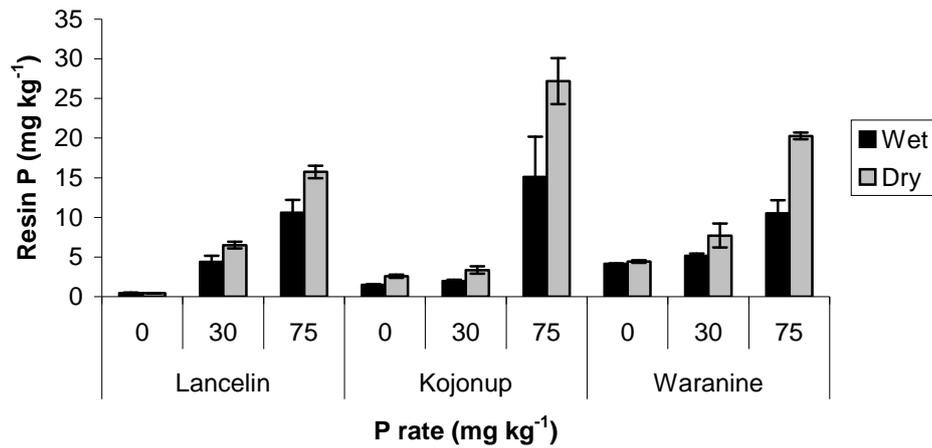
**Figure 5.1** Relationship between DGT ( $C_E$ ) and resin P measurements.

### 5.3.2 Resin measurement on wet/dry samples

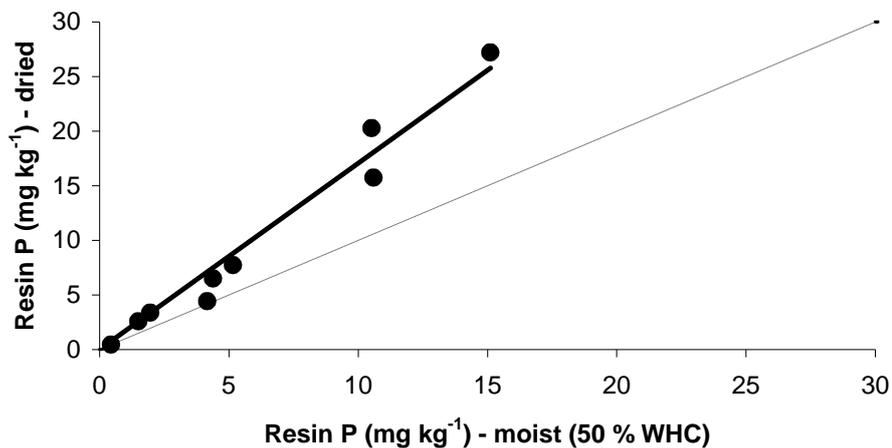
Another contributing factor that could have affected the resin P values obtained in this experiment was the drying of soil samples that took place before resin P analysis. In Chapter 4 it was highlighted that measurements made on moist soil samples were lower than the corresponding dried samples but primarily only on the highly P deficient soils (< 3.5 mg kg<sup>-1</sup> resin P). The difference in this experiment is that resin measurements made on soils that have had P recently applied are used and compared to plant response. Therefore, it is unclear what affect drying the soil would have on the resin P measurement.

Comparison of results for resin P on dried versus moist soil samples showed higher resin P measurements for the dried soil samples compared to the moist samples (Figure 5.2). However, comparing resin P measurements made from the dried and moist soil revealed an excellent correlation between the two measurements ( $r = 0.99$ ) and a slope of 1.7 (Figure 5.3). The increase in resin P measurements appears uniform over the three different soils and for the two different P application rates. These results are not in accordance with results obtained in Chapter 4 that showed the increases in resin P from

dried soil samples were not uniform for all 18 soils used but these measurements were obtained from the control soils only. It appears that drying the soil before resin P analysis will not affect the comparison of resin P measurements from soils that have had recent applications of P with wheat response.



**Figure 5.2** Resin P measurements obtained on pre-wet and dried soil samples with applied P.



**Figure 5.3** Comparison of the resin P values obtained on the moist and dried soil samples. Dotted lines represent the 1 to 1 line and the solid lines represent the correlation fit for the data.

### 5.3.2 Plant response

Significant increases in yield to applied P were experienced for all the soils used (Table 5.3.1). Large rates of P were applied in this experiment in order to obtain the maximum yield of wheat from each soil. For three soils (Waranine, Kojonup, and Yalenbee) with the highest P available attenuation capabilities (shown by the lowest % increases in DGT measurements from applied P, Table 5.1), maximum plant yields were potentially not reached as increases in yield still occurred at the highest P application rate (300 mg kg<sup>-1</sup>) (i.e. the yield plateau had not been reached).

**Table 5.3** Increases in yield (%) from the control at the corresponding P application rate for the 8 soils used.

P rate mg kg <sup>-1</sup>	% increase in yield from control							
	Waranine	Lancelin	Kojonup	Cunderin	Yalenbee	Pt Kenny	Waramboo	Birchip
0	0	0	0	0	0	0	0	0
5	21	36	-4	27	10	21	8	-13
10	26	4	36	25	6	22	2	7
20	23	63	42	49	13	70	-5	25
30	34	136	26	99	23	56	6	40
50	18	147	79	124	67	70	21	45
70	77	127	50	149	69	105	23	70
100	72	138	68	110	65	95	23	62
200	157	141	152	136	184	149	136	74
300	191	163	194	135	235	139	120	74

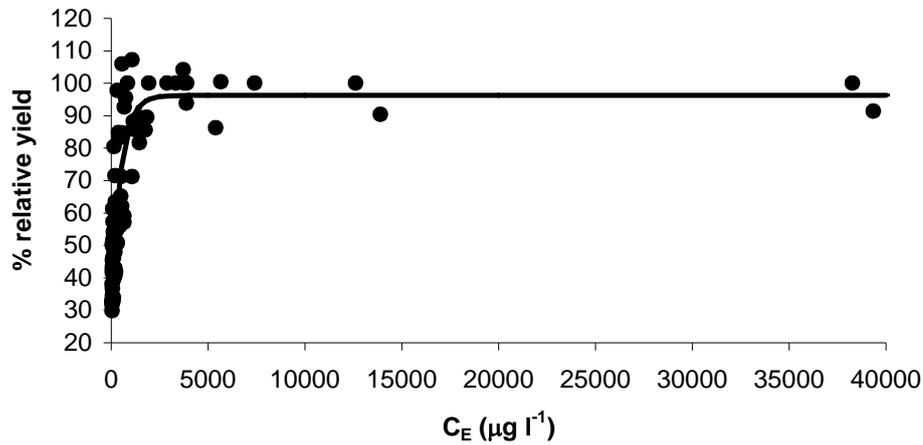
### 5.3.3 Soil testing methods with plant response

#### 5.3.3.1 DGT

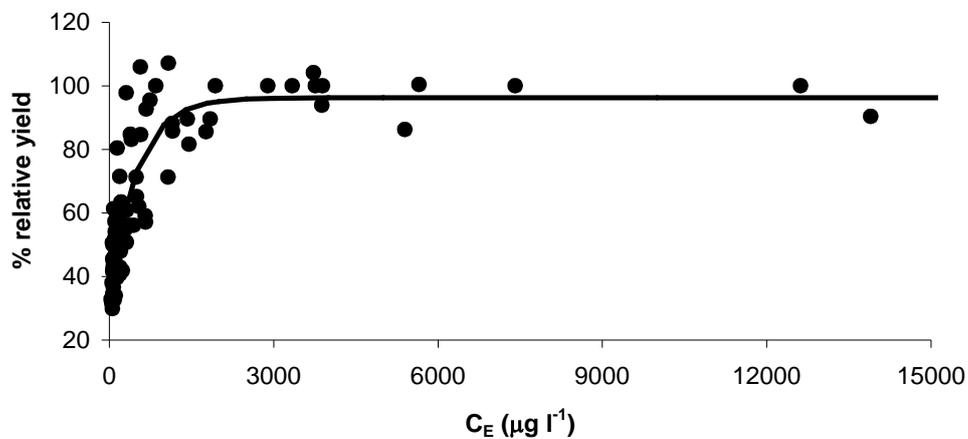
Comparison of DGT measurements and plant response to applied P (expressed as % increase in yield) resulted in a coefficient of determination ( $r^2$ ) for the fitted regression curve of 0.78 using a Mitscherlich type equation (eq. 15) confirming DGT accuracy for predicting P availability (Figure 5.4a). The good coefficient of determination could be heavily dependent on two large  $C_E$  values at approximately 40000 ug l<sup>-1</sup>. Removing these two large DGT values still resulted in a relationship of  $r^2 = 0.78$  confirming the coefficient of determination for the fitted regression curve is true (Figure 5.4b). Using the

relationship parameters from the Mitscherlich equation revealed that the critical thresholds of wheat deficiency for a yield of  $\leq 80\%$  of maximum yield is  $< 677 \mu\text{g l}^{-1} C_E$  and for a yield  $\leq 90\%$  of maximum yield is  $< 1154 \mu\text{g l}^{-1} C_E$ .

a)



b)



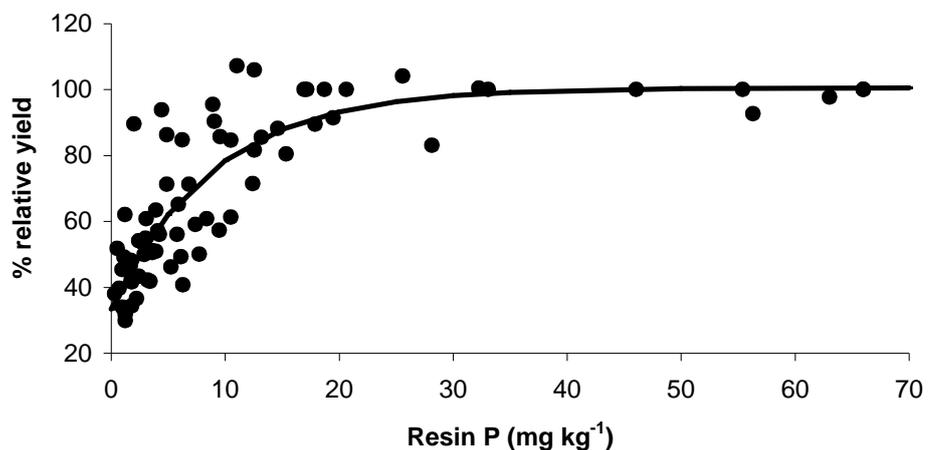
**Figure 5.4** Relationship between relative dry-matter yield of wheat and effective concentration ( $C_E$ ) measured by DGT, a) all data, b) two largest  $C_E$  measurements removed.

### 5.3.3.2 Resin P

The relationship between resin P measurements and wheat P response (% relative yield) resulted in a slightly lower coefficient of determination ( $r^2 = 0.74$ ) for the fitted

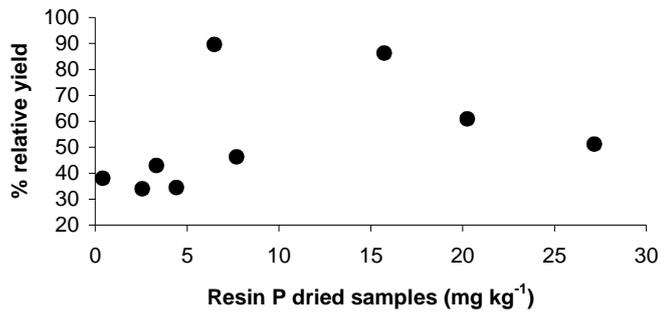
regression curve using a Mitscherlich type equation (eq. 15) (Figure 5.5). This indicates more variability with the resin P method for predicting plant response on different soil types compared to the DGT method. Critical P thresholds using the resin method using the relationship parameters are  $< 10.6 \text{ mg kg}^{-1}$  for a yield  $\leq 80 \%$  of the maximum yield and  $< 16.6 \text{ mg kg}^{-1}$  for a yield  $\leq 90 \%$  of the maximum yield. There visually appears to be more uncertainty with these thresholds for the resin method compared to DGT.

In Section 5.3.2 it was shown that resin P values obtained from the dried soil samples were higher than the corresponding moist soil sample. Substituting the moist resin values for the dried values did not improve the relationship between resin P values and the % increase in yield for the 3 soils tested (Figure 5.6). The increase in resin values using the dried soil samples is consistent between the 3 soils with each rate of P (Figure 5.3) and would therefore not improve the relationship with plant response. Substituting the resin values for  $C_E$  values (measured by DGT) for this data set alone resulted in a coefficient of determination ( $r^2$ ) for the fitted regression curve of 0.95 (Figure 5.6c).

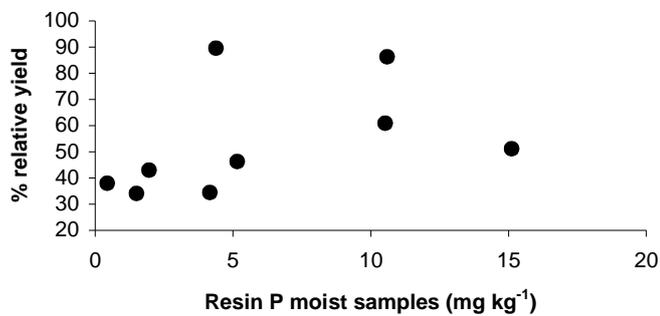


**Figure 5.5** Relationship between relative dry-matter yield of wheat and resin P measurements ( $\text{mg kg}^{-1}$ ).

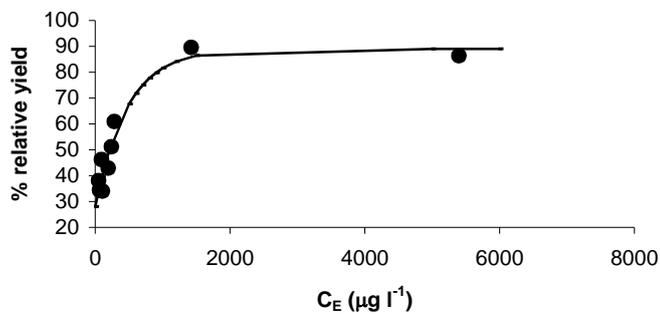
a)



b)



c)



**Figure 5.6** Relationship between relative yield (%) with P application for 3 soils (Waranine, Lancelin, Kojonup) and resin P measurements (mg kg<sup>-1</sup>) made on, a) dry soil samples, b) moist (50 % WHC) soil samples and c) the corresponding effective concentration (C<sub>E</sub>) measured by DGT.

#### 5.4. Overall analysis

In the only known study comparing the performance of the DGT method and the resin method with plant response (Tomato) to applied P, Menzies et al. 2005 showed

DGT was a slightly improved soil test for predicting plant response. Comparison between the soil tests with relative yield (%) resulted in a correlation coefficient of 0.93 for DGT and 0.83 for the resin method (Menzies et al. 2005). The DGT method provided good discrimination between soils around the critical soil test value (Menzies et al. 2005). This experiment yielded a similar result with DGT out performing the resin method on the basis of the relationship with relative plant yield (%). The main difference with the study by Menzies et al. (2005) was the large resin P values they obtained by assessing plant response on heavily fertilised soils. Resin extractable P values ( $\mu\text{g g}^{-1}$ ) ranged from 0 up to approximately 220 with one sample yielding a resin P value of approximately 320  $\mu\text{g g}^{-1}$ . In this experiment the highest resin P value obtained was 66  $\text{mg kg}^{-1}$ . It is likely that the improved relationships between soil tests and relative yield (%) obtained by Menzies et al. (2005) was due to the larger spread of soil test values. The soils used in this experiment were all P deficient and therefore generally had lower soil test values.

In a study by Saggar et al., (1999) it was revealed that the resin method was an improved technique compared to extraction methods for predicting permanent pasture response to P. A coefficient of determination of 0.81 was obtained for the fitted regression curve comparing the resin method with % increase in yield, compared to 0.65 for the Olsen P method and 0.49 for the Colwell P method. The majority of resin values obtained from Saggar et al (1999) were above 20  $\mu\text{g g}^{-1}$  which appears to be above the critical P threshold for wheat using results from this experiment. It also appears to be above the threshold for the permanent pasture used by Saggar et al. (1999) as beyond 20  $\mu\text{g g}^{-1}$  resin P the % increase in yield was generally below 20.

In this experiment the resin and DGT method has been tested on soils that contain highly deficient P soils and are therefore more focussed around the critical P threshold value for wheat. The DGT method provides a more accurate assessment of plant available P compared to the resin method at soil test values more relevant to the P critical threshold

of wheat. Therefore, it appears the DGT method will be able to predict fertilizer requirements from any given soil more accurately than the resin method.

The critical P threshold obtained in this experiment ( $677 \mu\text{g l}^{-1} \text{C}_E$ ) is considerably higher of that obtained in Chapter 4 ( $390 \mu\text{g l}^{-1} \text{C}_E$ ). This indicates that the application rate of  $30 \text{ mg kg}^{-1} \text{P}$  used in Chapter 4 was insufficient at obtaining the full yield potential for the soils used. Extending P application rates to provide excessive plant P requirements in the soil has revealed a more accurate indication of critical P threshold for wheat. It was reported from Chapter 4 that 3 soils showed non-responsive characteristics to P although they all had relatively low soil P as indicated by all soil tests. From DGT measurements it was concluded that these three soils had a high capacity to fix any P applied to the soil. Two of those soils were used in this experiment (Waranine and Yalenbee) and both showed a high response to applied P at the higher application rates (see Table 5.3). This confirms previous observations that these soils should be responsive to P and highlights potential problems of performing growth response experiments to P if using low P application rates. After P addition, the soils were both thoroughly mixed in both these experiments. This could also contribute to the fixation of P by exposing P to more fixation sites on the soil solid phase as apposed to supplying P as a granual or as a liquid in a concentrated region.

## 6. Phosphorus deficiency thresholds determined by DGT for three crop species.

### 6.1. Introduction

Barley is a major food and animal feed crop. In 2005, barley ranked fourth in quantity produced and in area of cultivation of cereal crops in the world behind maize, wheat and rice (<http://faostat.fao.org>). Canola and lupin are also important cash crops for the agricultural industry and used in rotation with cereals, they can increase soil nitrogen levels, soil fertility and improve disease breaks for the other two crops (Brennan and Bolland 2001). Different crop species vary in their response to P deficiency with some more successful than others. Brennan and Bolland (2001) revealed by comparing the response of canola and wheat to P that canola is more efficient at accessing P in the soil. This is due to canola possessing fine roots and abundant root hairs that increase in length and density under P deficient conditions (Foehse and Jungk 1983). Canola roots also acidify the rhizosphere just behind the root tip dissolving insoluble forms of P and increasing P uptake from the soil (Moorby et al. 1988, Hoffland et al. 1989 and Hoffland et al. 1989b).

Different lupin species behave differently under P deficient scenarios. Comparing fertilizer P requirements for lupin (*L. angustifolius*) with canola and wheat, Brennan and Bolland (2001) showed that this Lupin variety required 60 % more P to reach maximum yield than canola and 10 % more than wheat. Their explanation was that lupin has a thicker, less extensive but deeper roots compared to wheat. In contrast, Bolland et al. (1999) revealed lower grain yield responses to P for a different lupin species (*Lupinus albus*) compared to wheat and canola. In response to P deficiency, *L. albus* develops proteoid roots and these roots secrete citrate and malic acid along with hydrogen ions,

which dissolve insoluble sources of P, enhancing P uptake (Marschner et al. 1987, Uhde-Stone et al. 2003).

Comparison of the response of barley to P with other crop species has not yet been investigated possibly due to the similarities between the root systems of barley and wheat. It is quite possible that these two cereals have similar P deficiency thresholds. However, root hair variations have been observed between cereal cultivars in response to P deficiency and these root hairs can play a significant role in P acquisition (Gahoonia et al. 1997).

Previous experiments (Chapters 4 and 5) have revealed that the DGT method is a more accurate and reliable assessment tool for predicting wheat available P in comparison to three other routinely used soil tests for P. It is therefore a requirement for the progression of DGT technology to test its capabilities in assessing available P with other plant species that potentially have different critical P thresholds due to differences in the way they combat P deficiency. This experiment replicates the experiment performed in Chapter 4 but canola, barley and lupin (*L. albus*) have been substituted for wheat. *L. albus* was chosen due to its increase use and adaptation to fine textured neutral to alkaline soils in which narrow leaf lupin (*Lupinus angustifolius*) grow poorly on (Bolland et al. 1999). The DGT method was compared to the resin method only, since the inability of the extraction methods to measure plant available P has already been shown (see Chapter 4).

## 6.2. Materials and methods

### 6.2.1 Soils

Ten soils from the wheat experiment (Chapter 4) were chosen on the basis of providing an even spread of  $C_E$  values and the availability of the soils (see Table 6.1). All soils were collected and prepared as described in Section 4.2.3.

### 6.2.2 Resin method

The resin method was performed on oven dried 10 g sub samples in accordance with the outlined procedure in Section 4.2.5.

### 6.2.3 Diffusive Gradients in Thin Films

The method of DGT preparation, deployment and calculations for these experiments is described in Section 4.2.7. Input parameters for the calculation of  $R_{diff}$  are detailed in table 6.1.

**Table 6.1** Calculation of effective concentration ( $C_E$ ) from DGT measurements by calculating  $R_{diff}$  using 2D DIFS for a) canola, b) barley and, c) lupin. Input parameters for  $R_{diff}$  calculation include the density of the soil particles ( $dp$ ), soil porosity ( $\Phi$ ), diffusion coefficient in soil ( $Ds$ ), and particle concentration ( $Pc$ ). Refer to section 1.5.4 for detailed calculations of these parameters.

<i>Canola</i>						$C_{DGT}$		$C_E$
<i>Site</i>	<i>State</i>	<i>dp</i>	$\Phi$	<i>Ds</i>	<i>Pc</i>	$\mu\text{g l}^{-1}$	<i>R diff</i>	$\mu\text{g l}^{-1}$
Mt. Damper	S.A.	2.65	0.274	1.47E-06	7.02	53	0.0596	643
Pt. Kenny	S.A.	2.65	0.437	1.98E-06	3.42	28	0.0709	396
Waramboo	S.A.	2.65	0.437	1.98E-06	3.42	5.8	0.0709	82
Birchip	VIC	2.65	0.493	2.18E-06	2.72	27	0.0751	366
Kalkee	VIC	2.65	0.493	2.18E-06	2.72	23	0.0751	303
Lower Norton	VIC	2.65	0.490	2.17E-06	2.76	23	0.0749	310
Mildura	VIC	2.65	0.281	1.49E-06	6.78	31	0.0601	523
Gillingarra	W.A.	2.65	0.415	1.91E-06	3.74	52	0.0695	753
Lancelin	W.A.	2.65	0.281	1.49E-06	6.78	1.1	0.0601	19
Meckering	W.A.	2.65	0.307	1.57E-06	5.97	134	0.0619	2170
Wongan	W.A.	2.65	0.281	1.49E-06	6.78	2.5	0.0601	42

<i>Barley</i>						$C_{DGT}$		$C_E$
<i>Site</i>	<i>State</i>	<i>dp</i>	$\Phi$	<i>Ds</i>	<i>Pc</i>	$\mu\text{g l}^{-1}$	<i>R diff</i>	$\mu\text{g l}^{-1}$
Mt. Damper	S.A.	2.65	0.237	1.36E-06	8.51	42	0.0525	791
Pt. Kenny	S.A.	2.65	0.391	1.83E-06	4.12	28	0.0625	444
Waramboo	S.A.	2.65	0.445	2.01E-06	3.31	14	0.0661	217
Birchip	VIC	2.65	0.540	2.36E-06	2.26	14	0.0727	188
Kalkee	VIC	2.65	0.540	2.36E-06	2.26	28	0.0727	383
Walpeup	VIC	2.65	0.391	1.83E-06	4.12	230	0.0625	3682
Cunderin	W.A.	2.65	0.338	1.66E-06	5.19	1.2	0.0591	21
Gillingarra	W.A.	2.65	0.437	1.98E-06	3.42	42	0.0655	649
Kojonup	W.A.	2.65	0.391	1.83E-06	4.12	4.8	0.0625	76
Meckering	W.A.	2.65	0.338	1.66E-06	5.19	156	0.0512	3046

<i>Lupin</i>						$C_{DGT}$	$C_E$	
<i>Site</i>	<i>State</i>	<i>dp</i>	$\Phi$	<i>Ds</i>	<i>Pc</i>	$\mu\text{g l}^{-1}$	<i>R diff</i>	$\mu\text{g l}^{-1}$
<b>Mt. Damper</b>	<b>S.A.</b>	2.65	0.320	1.61E-06	5.63	39	0.0571	686
<b>Mt. Shank</b>	<b>S.A.</b>	2.65	0.537	2.35E-06	2.29	57	0.0715	798
<b>Pt. Kenny</b>	<b>S.A.</b>	2.65	0.453	2.04E-06	3.20	47	0.0657	722
<b>Waramboo</b>	<b>S.A.</b>	2.65	0.445	2.01E-06	3.31	13	0.0651	193
<b>Birchip</b>	<b>VIC</b>	2.65	0.537	2.35E-06	2.29	22	0.0715	304
<b>Kalkee</b>	<b>VIC</b>	2.65	0.522	2.29E-06	2.42	30	0.0726	414
<b>Walpeup</b>	<b>VIC</b>	2.65	0.338	1.66E-06	5.19	278	0.0582	4784
<b>Gillingarra</b>	<b>W.A.</b>	2.65	0.415	1.91E-06	3.74	50	0.0651	771
<b>Meckering</b>	<b>W.A.</b>	2.65	0.301	1.55E-06	6.15	125	0.0557	2247
<b>Wongan</b>	<b>W.A.</b>	2.65	0.274	1.47E-06	7.02	2.0	0.0539	36

#### 6.2.4 Pot experiments and glasshouse trials

The pot experiments were designed to determine the response of canola (*Brassica napus*, *ATR-Beacon*), barley (*Hordeum vulgare*, *Schooner*) and lupin (*Lupinus albus*) to an application of P as in Chapter 4. Basal nutrients were applied as per Section 4.2.3. The P application rate in these experiments was increased to 100 mg kg<sup>-1</sup> to remove any uncertainty of non-responsive soils due to their high P fixing capacity. During the course of the lupin experiment it became apparent that the lupin variety used had a high seed P reserve or was highly efficient at accessing soil P. At the 28-day growth stage there was no visual response to applied P for all soils that included highly P deficient soils from Chapter 4. It was decided to extend the growth period until 56 days after seeding to attempt to obtain a response. After the 28-day growth period an additional application of basal nutrients at the same concentrations was applied to the lupin plants. All other protocols were performed in accordance with the experiment using wheat (see Section 4.2.3, 4.2.8).

## 6.3. Results and discussion

### 6.3.1 Soil chemical characteristics

Identity and chemical characteristics of the soils used in these experiments are summarized in table 6.1 (from Table 4.1). Due to availability not all soils had the response to P assessed for all three crop species.

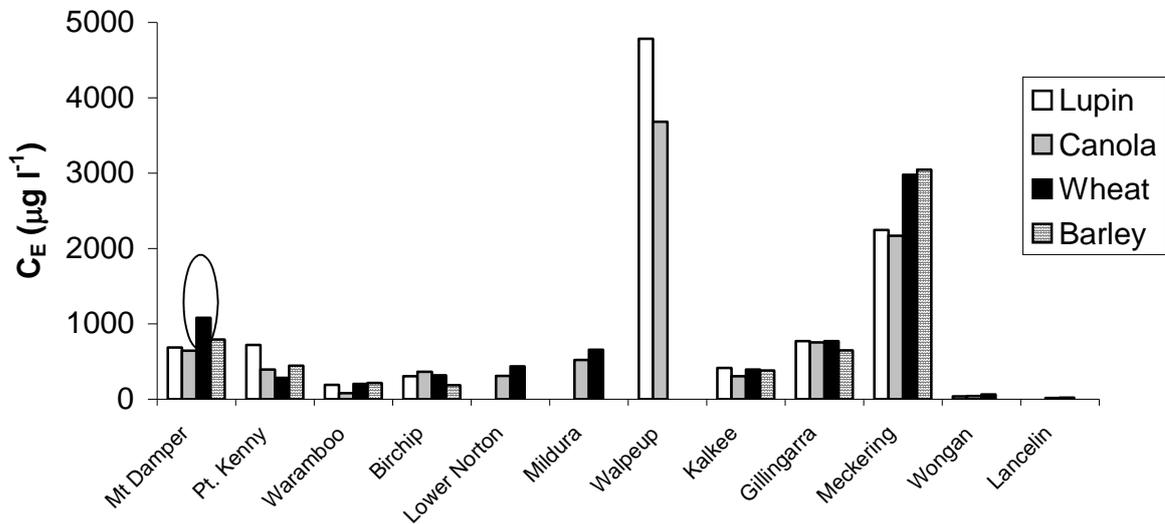
**Table 6.2** Location and chemical characteristics of the soils used for the purpose of this Chapter. Asterisks highlight which soils were used for each crop species.

<i>Site</i>	<i>State</i>	<i>Soil Type</i>	<i>Canola</i>	<i>Lupin</i>	<i>Barley</i>	<i>pH (H<sub>2</sub>O)</i>	<i>EC<sub>1:5</sub> us/cm</i>	<i>P (total) (mg kg<sup>-1</sup>)</i>
<b>Mt Damper</b>	<b>S.A.</b>	<b>Grey Calcareous</b>	*	*	*	7.9	203	49
<b>Pt. Kenny</b>	<b>S.A.</b>	<b>Grey Calcareous</b>	*	*	*	9.1	323	374
<b>Waramboo</b>	<b>S.A.</b>	<b>Grey Calcareous</b>	*	*	*	9.0	202	295
<b>Birchip</b>	<b>VIC</b>	<b>Calcarosol</b>	*	*	*	6.9	993	173
<b>Kalkee</b>	<b>VIC</b>	<b>Vertosol</b>	*	*	*	8.5	265	196
<b>Lower Norton</b>	<b>VIC</b>	<b>red brown earth</b>	*			7.9	210	181
<b>Mildura</b>	<b>VIC</b>	<b>Northern Mallee</b>	*			8.0	129	121
<b>Walpeup</b>	<b>VIC</b>	<b>Northern Mallee</b>		*	*			
<b>Cunderdin</b>	<b>W.A.</b>	<b>Ferrasol</b>			*	6.6	81	76
<b>Gillingarra</b>	<b>W.A.</b>	<b>Podosol</b>	*	*	*	5.5	108	270
<b>Kojonup</b>	<b>W.A.</b>	<b>Tenosol</b>			*	6.0	93	72
<b>Lancelin</b>	<b>W.A.</b>	<b>Tenosol</b>	*			6.0	86	13
<b>Meckering</b>	<b>W.A.</b>	<b>Chromosol</b>	*	*	*	5.9	122	160
<b>Wongan</b>	<b>W.A.</b>	<b>Kandosol</b>	*	*		5.9	87	27
		<i>Minimum</i>				<b>5.5</b>	<b>81</b>	<b>13</b>
		<i>Maximum</i>				<b>9.1</b>	<b>993</b>	<b>374</b>

### 6.3.2 DGT replication

Comparison of DGT measurements ( $C_E$ ) through each experiment reveals that all components of the DGT method including deployment conditions, MBL production, elution/analysis and measurement calculations (DIFS modelling) are reproducible and provided excellent replication between experiments (Figure 6.1). The circled outlier is likely to be a result of the soil being resampled from the field after the wheat experiment (Chapter 4). The freshly collected soil used to assess the response of canola, lupin and

barley to P appears to have a lower  $C_E$  value as measured by DGT indicating lower available P compared to the first batch of soil that was sampled in the field (and used for the wheat experiment).



**Figure 6.1** Mean DGT measurements ( $C_E$ ) for various soils used in each plant P responsive experiment for lupin, canola, wheat and barley.

#### 6.3.4 Dry matter yield of canola, lupin and barley

Of the 11 soils used to assess canola response to P, 2 soils had no statistically significant increase ( $p < 0.05$ ) in dry matter of canola with applied P and 9 soils were responsive to applied P. Dry matter was expressed as increase in yield, using eqn 13.

In contrast, of the 10 soils used to assess lupin response to P none of the soils had significant response to P application. This may indicate the efficiency of the root system to access originally insoluble P within the soil by secreting citrate and malic acid. Bolland et al. (1999) also attributed the minimal yield responses of lupin to applied P (*Lupinus albus*) to their larger seeds and therefore greater P contents. This was especially noticeable with the nil-P treatments as lupin yields were 2 to 7 times greater compared to the smaller seeded species of canola and wheat (Bolland et al. 1999). Previous studies have revealed that increasing the P concentration in the lupin seed can increase grain

yields on deficient soils (Bolland et al. 1989). It is uncertain in this experiment the contribution of P originally in the seed compared to the ability of lupin to access soil P.

There was a statistically significant response ( $p < 0.05$ ) in dry matter of barley to the addition of P for 8 of the 10 soils used for this experiment while the remaining 2 soils were non-responsive.

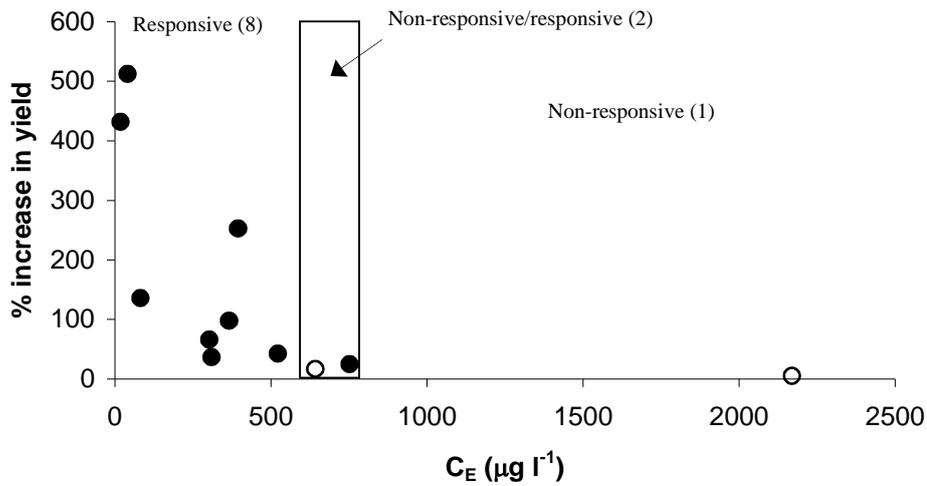
### *6.3.5 DGT and resin methods with plant responsiveness*

#### *6.3.5.1 Canola*

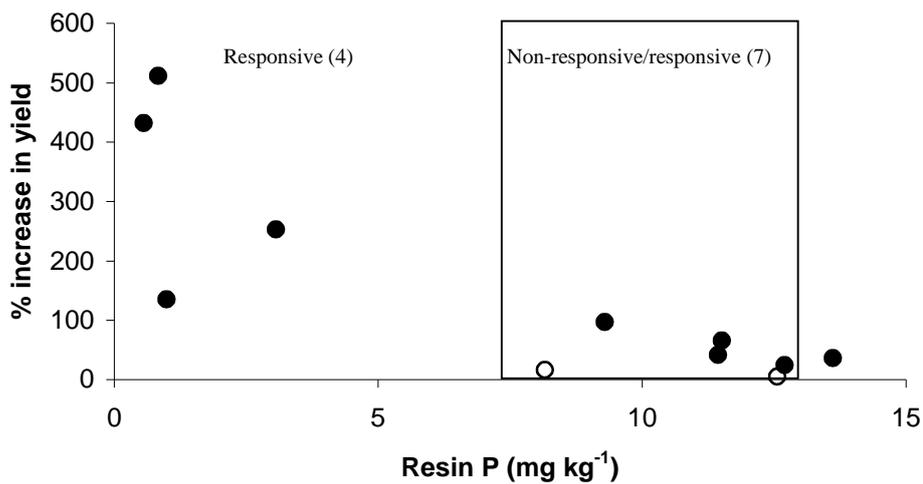
DGT accurately predicted dry matter response of canola to added P for 9 out of the 11 soils (82 %). The 2 soils that were incorrectly predicted are indicated within the box on figure 6.2a. This indicates that within this resin P region the soils were either responsive or non-responsive. The threshold of canola P deficiency appears to be in the region of 640 and 755  $\mu\text{g l}^{-1} \text{C}_E$  (Figure 6.2a). This threshold is lower than the corresponding deficiency threshold for wheat at 1154  $\mu\text{g l}^{-1} \text{C}_E$  for yields  $< 90\%$  of the maximum yield (Chapter 5). It has been shown previously through its root system (see Section 6.1); canola is more efficient at accessing P in a given soil (Brennan and Bolland, 2001) and therefore the decreased deficiency threshold for canola compared to wheat obtained in this experiment further support the observations of Brennan and Bolland (2001).

The resin P method correctly predicted only 4 out of the 11 soils (36 %) (Figure 6.2b) used in this experiment highlighting again the improved performance of DGT in predicting P availability on these selected soils. The 7 soils that were incorrectly predicted are indicated within the box on figure 6.2b. This indicates that within this resin P region the soils were either responsive or non-responsive. The critical threshold of canola determined by using the resin measurements obtained from this experiment is anywhere in the range of 8 - 14  $\text{mg kg}^{-1}$ .

a)



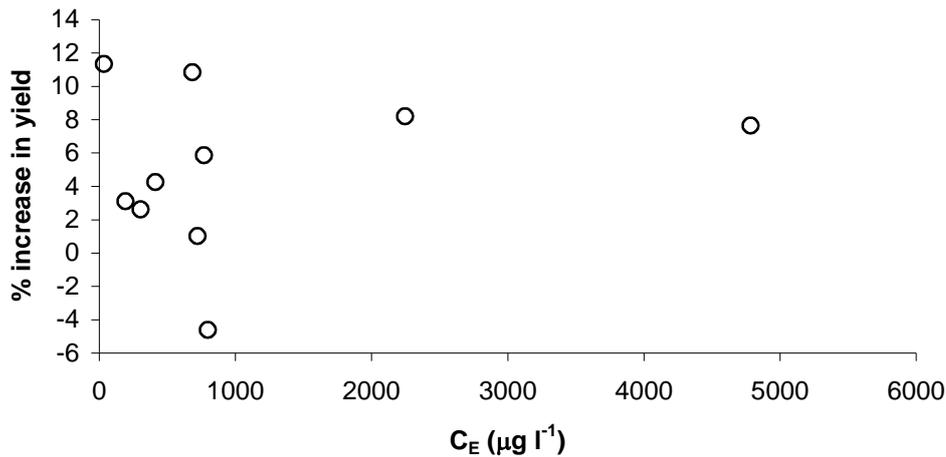
b)



**Figure 6.2** Percentage increase of dry matter response of canola to applied P over the control as a function of a)  $C_E$  (measured by DGT) and b) Resin P. (o) non-responsive soils (●) responsive soils

### 6.3.5.2 Lupin

Due to no significant increases in dry matter of lupin to applied P for the 10 soils used (Figure 6.3), comparative discussion of soil testing methods and % increases in yield is not applicable.



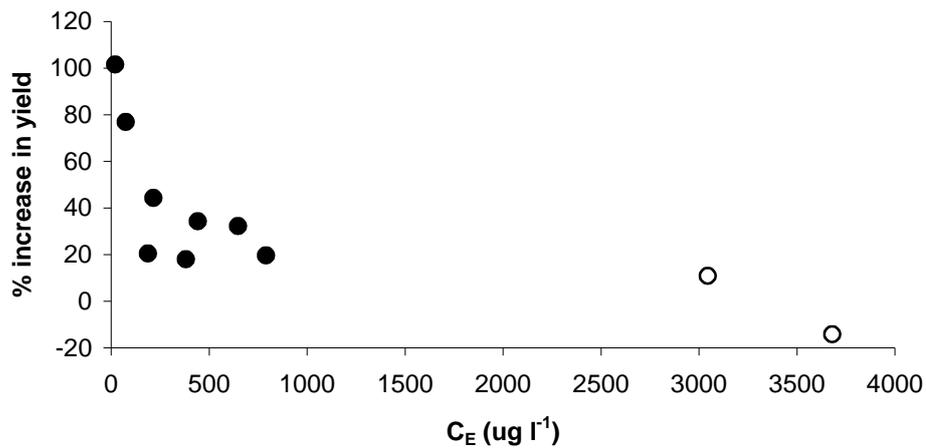
**Figure 6.3** Percentage increase of dry matter response of lupin to applied P over the control as a function of  $C_E$  (measured by DGT). (o) non-responsive soils (•) responsive soils

#### 6.3.5.3 Barley

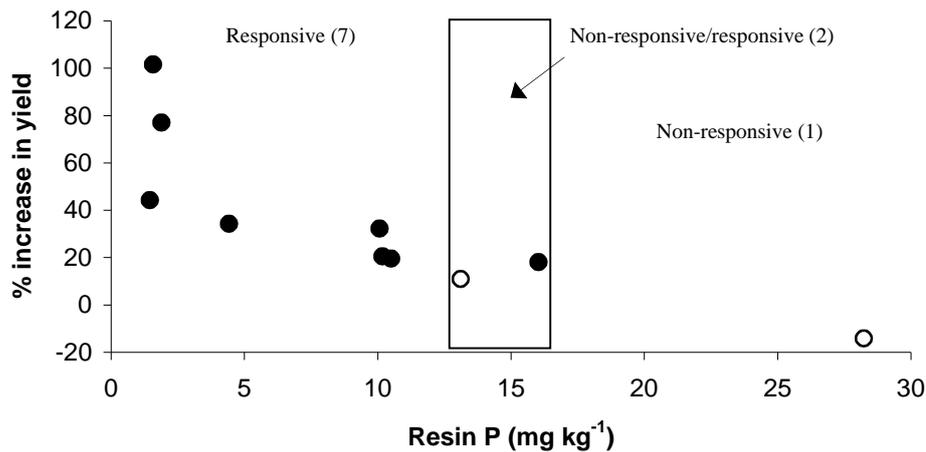
DGT accurately predicted all 10 soils on the basis of responsiveness and non-responsiveness of barley to applied P (Figure 6.4a). Unfortunately the separation  $C_E$  values between the responsive and non-responsive soils is considerable large and therefore an accurate deficiency threshold value is hard to interpret. It can be suggested that this deficiency threshold is beyond  $790 \mu\text{g l}^{-1} C_E$  which is slightly larger than that of canola but possibly comparable to wheat. The root system of both barley and wheat are very similar (see Section 6.1) and this is the first known study that directly compares the response of barley and wheat to P, possibly due to their similarities. Therefore critical thresholds of barley and wheat would be expected to be similar on the basis that they will have similar root mechanisms in P deficient situations. Canola is more effective at accessing P in a given soil compared to wheat and therefore should also be more effective than barley. This observation is supported by the larger critical threshold obtained for barley compared to canola in this experiment.

The resin P method correctly predicted 8 out of the 10 soils (80 %) used in this experiment (Figure 6.4b), which is an improvement from previous experiments. Using these measurements from the resin method, the critical threshold for barley is anywhere between 13 and 16 mg kg<sup>-1</sup>.

a)



b)



**Figure 6.4** Percentage increase of dry matter response of barley to applied P over the control as a function of a)  $C_E$  (measured by DGT) and b) Resin P. (o) non-responsive soils (●) responsive soils

#### 6.4. Overall analysis

Comparative experiments between two soil testing methods, DGT and resin P, with plant response to applied P using three different crop species has supported results observed in Chapter 4 and 5 that indicate that DGT is a more accurate soil assessment method for measuring available P. The DGT method can accurately predict P deficiency thresholds for different plant species that have various methods in combating P deficiency.

The thresholds of P deficiency predicted using DGT for each crop type were in the order of wheat < = > barley > canola > lupin. Accurate determination of the critical threshold of barley using DGT was not possible due to a large separation between responsive and non-responsive soils. To accurately obtain the critical threshold for barley this experiment would need to be repeated using soils with DGT P measurements between 800 and 3000  $\mu\text{g l}^{-1} \text{C}_E$ . Due to the uncertainty of the critical threshold for barley direct comparison with the critical threshold determine for wheat was not possible but is likely to be very similar based on the root systems of the two crop species. Higher critical thresholds obtained for wheat compared to canola is in accordance with previous published results (Brennan and Bolland 2001). These observations are a result of canola processing a more effective root system for accessing P sources from a given soil (Foehse and Jungk 1983, Moorby et al. 1988, Hoffland et al. 1989 and Hoffland et al. 1989a). Critical thresholds of P deficiency predicted using resin P for each crop type followed a similar trend to thresholds predicted by DGT. However, the thresholds predicted by the resin technique are more difficult to determine due to reduced accuracy. The critical P thresholds were in the order of wheat (16.6  $\text{mg kg}^{-1}$ ) > barley (13-16) > canola (8-14) > lupin.

From this experiment it is clear that the lupin species used (*Lupinus albus*) is highly effective either in accessing forms of P through its root system that are not

available to wheat, barley or canola, and/or provides a large initial contribution of P from its seed.

## 7. Predicting plant micronutrient (Zn, Mn) deficiency thresholds using DGT

### 7.1. Introduction

Zinc and Mn are two of the eight essential trace elements required for normal healthy growth and reproduction of crop plants. Referred to as micronutrients these elements are only required in relatively small concentrations in the plant tissues (5 – 100 mg kg<sup>-1</sup>) (Alloway 2004).

Zinc deficiency is a serious problem worldwide, threatening world food production. Field trial results indicate that Zn deficiency can occur on most major soil types (Takkar et al. 1993). Alloway (2004) reported soils that have a high risk of causing Zn deficiency in crops include sandy soils, calcareous soils, saline soils, soils with neutral or alkaline pH, waterlogged soils and soils with inherently low nutrient status that have received heavy applications of P fertilisers.

In Australia extensive areas of Zn deficiency have been reported. Soil groups that have been associated with Zn deficiency are calcareous sands from Victoria; Black Earths, Solodic soils and grey, brown, and red clays from Queensland; Rendzinas, Terra Rossa and Red Brown Earths from both Queensland and South Australia; Solonized Brown soils from South Australia; Grey-Brown and Brown podsollic soil from Western Australia; and Lateritic podsollic soils from both Western Australia and Queensland (Tiller 1983).

Manganese is an essential plant nutrient that is involved in important growth processes (Husted et al. 2005). Manganese deficiency may occur consistently in certain crops grown on particular soil types over broad geographical regions and may also occur locally in small patches in otherwise healthy crops (Reuter et al. 1988). Main soil

properties commonly associated with low available Mn are high organic matter and carbonate content, high pH, low bulk density and low content of readily reducible Mn in the soil (Reuter et al. 1988). In Australia Mn deficiency has been associated with a large range of soil types including fen soils, sodic soils, podzols and podzolic soils and soils associated with carbonates (Donald et al. 1975).

Certain crop types are more susceptible to micronutrient deficiency than others due to their different root morphology and variations in other factors including the reductive capacity of the roots, production of root exudates and rhizosphere chemistry (Reuter et al. 1988). Oats and wheat species have been reported to be among the most susceptible crop plants to micronutrient deficiencies compared to maize and pasture grasses (Lucas et al. 1972, Graham et al. 1993, Alloway 2004).

Testing for micronutrient concentrations in soils have been mainly restricted to the use of extractions to remove the 'available fraction' from a given soil (see Chapter 1). These soil tests have required the incorporation of measurements of other soil properties (e.g. pH, clay, organic carbon) for improved performance (Brennan et al. 1993). The most common extraction methods used for the simultaneous assessment of the micronutrient status of soils have been, EDTA (Trierweiler et al., 1969) and DTPA (Lindsay et al., 1978) (Brennan et al. 1993). The extraction method of DTPA has been shown to correlate well with plant response to applied Zn from selected Australian soils (Brennan and Gartrell 1990, Brennan et al. 2005). Assessment of Mn availability has been less successful with the use of numerous types of extraction methods. In a review by Reisenauer (1988) only poor to moderate correlations have been obtained between plant Mn uptake and soil Mn extracted from several greenhouse studies.

The DGT method has been shown to be an improved method in comparison to selected extraction techniques (EDTA,  $\text{CaCl}_2$ ) for accurately measuring plant available

metal under contaminated scenarios (Zhang et al. 2001, Nolan et al. 2005) (see Chapter 1). In this chapter, the aim is test the DGT methods capabilities to predict Zn and Mn availability in deficient scenarios. Numerous benefits would be obtained by developing a method that simultaneously reflects both micronutrient (Zn, Mn) and macronutrient (P) plant availability in deficient soils. These benefits include a quicker, more accurate and cost effective analysis by using a single sensor (DGT).

## 7.2. Material and methods

### 7.2.1 *Substitute soil*

In order to accurately obtain the Zn deficiency threshold for wheat and canola, Zn concentrations in the soil needed to be as low as possible. Preliminary trials with field soils failed to find one which was Zn responsive. Therefore a substitute soil was used consisting of an acid washed sand obtained from Mt. Compass in South Australia. The sand was initially washed with acid (1 % HNO<sub>3</sub>/ HCl) and then rinsed repeatedly with DI water until a neutral pH was obtained. Before any addition of nutrients, CaCO<sub>3</sub> (1 %) was added to the sand with thorough mixing

### 7.2.2 *Diffusive Gradients in Thin Films*

The method of DGT preparation, analysis and calculations for these experiments is described in Section 4.2.7. The deployment method used in these experiments was adjusted slightly and is explained for each separate experiment below.

### 7.2.3 *Decreasing DGT detection limits*

In order to assess micronutrient deficiency thresholds in soils the DGT detection limit reported in Chapter 2 would most likely need to be improved. Therefore in addition to improved decontamination methods an additional DGT preparation step was introduced. It is possible in the preparation of gels and assembling the DGT devices that

slight contamination may occur. Therefore the day before deployment, assembled DGT devices were placed in a dilute acid solution (0.1 % HCl) and left overnight to remove any contamination that occurred during the preparation. The following day DGT devices were removed from the acidic solution and rinsed several times with ultra pure water (milli-Q) before deployment.

The DGT devices that had been subjected to an extra acid wash were tested along with normally prepared DGT devices to determine if the extra acid washing step had interfered with the MBL ability to bind either cations or anions. Three acid washed DGT devices and two normal DGT devices were immersed in multi-element solutions containing P ( $50 \mu\text{g l}^{-1}$ ) Cu, Mo, Mn and Zn (each at  $25 \mu\text{g l}^{-1}$ ) for 24 hours. Elution and analysis was performed in accordance with the methods outlined in Chapter 2.

#### *7.2.4 Predicting Zn deficiency thresholds using DGT*

Pot experiments were designed to determine the response of canola (*Brassica napus var. oleifera* – ATR beacon) and wheat (*Triticum turgidum*) to increasing applications of Zn. Canola was selected as it has a very small seed and therefore low initial Zn resources in the seed compared to larger seed plant varieties. Durum wheat was chosen as it has previously been reported to be more sensitive to Zn deficiency than other plant and wheat varieties (Alloway 2004). Basal nutrients were applied to each pot comprising  $350 \text{ mg kg}^{-1}$  nitrogen ( $\text{NH}_4\text{NO}_3$ ),  $70 \text{ mg kg}^{-1}$  potassium ( $\text{K}_2\text{SO}_4$ ,  $\text{K}_2\text{PO}_4$ ),  $33 \text{ mg kg}^{-1}$  phosphorus ( $\text{K}_2\text{PO}_4$ ),  $9 \text{ mg kg}^{-1}$  magnesium ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ),  $1.13 \text{ mg kg}^{-1}$  manganese ( $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ ),  $0.5 \text{ mg kg}^{-1}$  of copper ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ),  $0.7 \text{ mg kg}^{-1}$  cobalt ( $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ ),  $0.7 \text{ mg kg}^{-1}$  of iron ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ),  $0.57 \text{ mg kg}^{-1}$  boron ( $\text{H}_3\text{BO}_3$ ),  $0.27 \text{ mg kg}^{-1}$  of molybdenum ( $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ ) resulting in an application of  $35 \text{ mg kg}^{-1}$  sulphur. Finally Zn was added to each pot at 10 different rates (0, 0.01, 0.02, 0.05, 0.1,

0.5, 1, 5, 10, 20 mg kg<sup>-1</sup>) as ZnCl<sub>2</sub>. Each Zn rate was replicated and the sand was left to incubate for 1 week at 50 % WHC.

After DGT deployment 10 canola seedlings were planted at the approximate depth of 1 cm. Plant seedlings were thinned to three after 1 week when 40 g of alkathene granules were applied to minimize evaporation. Sand moisture was kept at 50 % WHC with additions of ultra pure water H<sub>2</sub>O (milli – Q, 18.2 MΩ cm). The experiment was performed in an Environmental Simulator at 20 °C with 12 h day and 12 h night. All pots were set up in a completely randomised arrangement which was frequently changed throughout the course of the experiment. After 4 weeks, above ground plant material was removed, rinsed and dried as reported in previous chapters.

To assess wheat response to applied Zn the same pots were used as for the canola experiment. Canola roots and alkathene granules were removed leaving 250 g of acid washed sand in each pot. Basal nutrients were applied as stated above and the soil was left to incubate for 1 week. After DGT deployment, 10 wheat seedlings were planted at approximately 1 cm depth and the course of the experiment was run as described for canola above.

In order to overcome any detection limit issues for Zn, DGT devices were deployed for extended deployment times to accumulate more Zn on the MBL. In addition to the customary 1-day deployment, the DGT devices were also deployed for 2 and 5 days in each pot for both experiments assessing canola and wheat response to applied Zn. Pre-acid washed DGT devices (see Section 7.2.3) were used for the 2-day deployment time before wheat was sown to assess if the extra acid washing step of the DGT influenced measurements on the sand and improved detection limits.

DGT detection limits were determined by collating blank analyses from each experiment. Two DGT blanks were used for each deployment time (1, 2 and 5 days) for

assessing canola response to applied Zn. Blank numbers were extended to 4 for each deployment time for the wheat response experiment to reduce detection limits by improving the standard deviation of the mean.

#### *7.2.5 Predicting Mn deficiency thresholds using DGT*

The same experimental set up was used to determine wheat response (*Triticum turgidum*) to applications of Mn as used to determine canola response to Zn applications (see above). Pots containing 300 g of sand + CaCO<sub>3</sub> had basal nutrients and Mn (as MnCl<sub>4</sub>·4H<sub>2</sub>O) applied in the exact same concentrations as mentioned above.

DGT assemblies were deployed for 1 day only as the detection limit for Mn using DGT (see Chapter 2) should prove low enough not to have to extend deployment times.

#### *7.2.6 Predicting Zn deficiency in soils*

To assess the capabilities of DGT to determine wheat response to Zn applications in true soils, 6 soils from around Australia were used, 5 of which had characteristics mentioned in Section 7.1 that could lead to Zn deficiency. Basal nutrients were applied to 8 pots (200 g dry weight) of each soil in the same concentrations mentioned above. Zinc (as ZnCl<sub>2</sub>) was applied at 10 mg kg<sup>-1</sup> to 4 of the 8 pots for replication and left to incubate for 1 week at 50 % WHC.

Soil moisture was increased to 80 % WHC the day prior to DGT deployment. One pre acid washed DGT device was deployed for 2 days on each pot (4 reps control, 4 reps added Zn) along with one untreated DGT device that was deployed for 5 days on each pot for each of the 8 soils. DGT detection limits were determined by collating the analysis of 4 blanks for each deployment time. Retrieval, elution and analysis of the DGT devices were performed in accordance with methods outlined in Chapter 2.

Ten wheat seedlings (*Triticum turgidum*) were sown after DGT deployment at approximately 1 cm depth and were thinned to three after the 1-week stage. All other plant growth conditions and harvesting were performed in accordance to methods outlined in this chapter.

### 7.3. Results and discussion

#### 7.3.1 Improving DGT detection limits

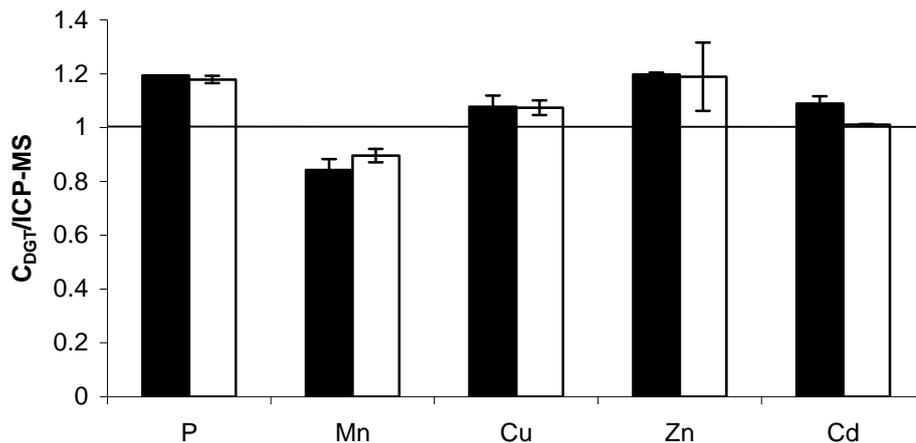
In order to accurately predict micronutrient deficiency, DGT detection limits must be as low as possible. Therefore an additional acid washing step was included in the DGT preparation process.

Blank values decreased significantly for the acid washed DGT devices compared to the DGT devices prepared as usual for Mn, Cu and Zn (Table 7.1). The extra acid washing step did not compromise the performance of the MBL to act as an infinite sink as the acid washed devices, along with non treated devices, accurately predicted concentrations of analytes in solution (Figure 71).

**Table 7.1** Calculated blanks for selected elements for DGT devices with additional acid wash and normally prepared DGT devices.

	<b>P</b> (ng/device)	<b>Mn</b> (ng/device)	<b>Cu</b> (ng/device)	<b>Zn</b> (ng/device)	<b>Mo</b> (ng/device)	<b>Cd</b> (ng/device)
<b>DGT (acid washed)</b>	<i>b.d.</i>	1.4 ± 0.2	1.5 ± 0.2	22 ± 4.7	0.7 ± 0.1	0 ± 0
<b>DGT device</b>	<i>b.d.</i>	3.2 ± 0.8	2.4 ± 0.6	57 ± 29.5	0.8 ± 0.1	0.02 ± 0

*b.d.* = below ICP-MS detection limit for P ( $1 \mu\text{g l}^{-1} = 9 \text{ ng/device}$ )



**Figure 7.1** Ratio of DGT measurements ( $C_{DGT}$ ) with direct measurement of solution concentrations (ICP-MS) for acid washed DGT devices (■) and normally prepared DGT devices (□).

### 7.3.2 Predicting Zn deficiency using DGT

#### 7.3.2.1 Canola

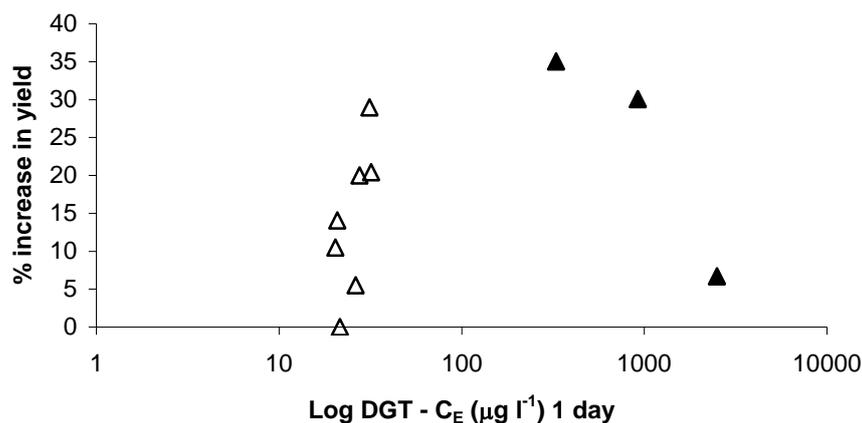
Dry matter yields of canola in substitute soil generally increased with increasing Zn application to reach a maximum yield increase of 35 % at an application rate of 5 mg kg<sup>-1</sup> (Table 7.2). Yields obtained at the highest Zn application rate were significantly ( $p < 0.5$ ) decreased probably indicating Zn toxicity.

**Table 7.2** mean % increase in yield (canola) with standard deviation (2 replicates) at each Zn application rate.

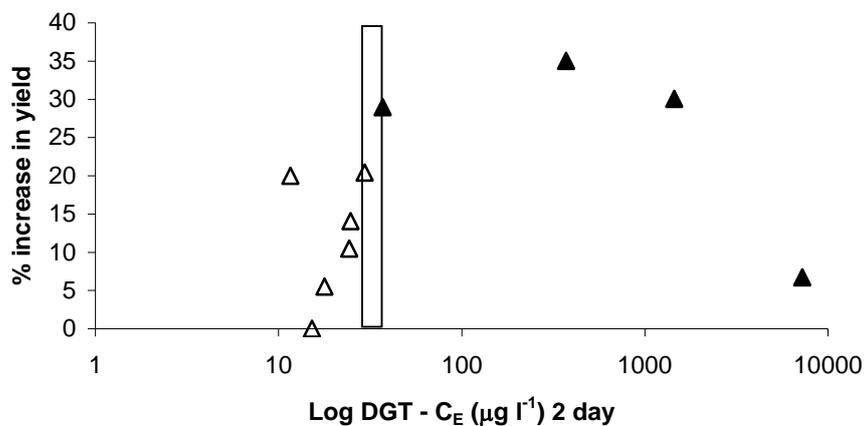
Zn rate mg kg <sup>-1</sup>	% increase in yield
0	6 ± 3.4
0.01	0
0.02	10 ± 11.6
0.05	14 ± 4.4
0.1	20 ± 0.6
0.5	20 ± 4.3
1	28 ± 1.7
5	35 ± 6.9
10	30 ± 11.8
20	7 ± 24.6

DGT detection limits, calculated in accordance with the IUPAC definition as the mean value of the blank plus three times its standard deviation, improved with the extended deployment times. The ability of DGT to determine the Zn deficiency threshold of canola also improved with extended DGT deployment times. Comparing DGT measurements (1 day) with canola response resulted in values below DGT detection limits (represented by open triangles) overlapping the maximum yield response (deficiency threshold) (Figure 7.2a) that corresponds to a Zn application rate of  $0.1 \text{ mg kg}^{-1}$  (Table 7.2). The deficiency threshold was successfully determined by extending DGT deployment times to 2 days (Figure 7.2b). The 5-day deployment time provided further confidence that the deficiency threshold exceeded DGT detection limit (Figure 7.2c). Approximate deficiency thresholds predicted by DGT assuming maximum canola response was reached at the Zn application rate of  $1 \text{ mg kg}^{-1}$  are  $37 \text{ } \mu\text{g l}^{-1} \text{ C}_E$  (2 day deployment) and  $54 \text{ } \mu\text{g l}^{-1}$  (5 day deployment).

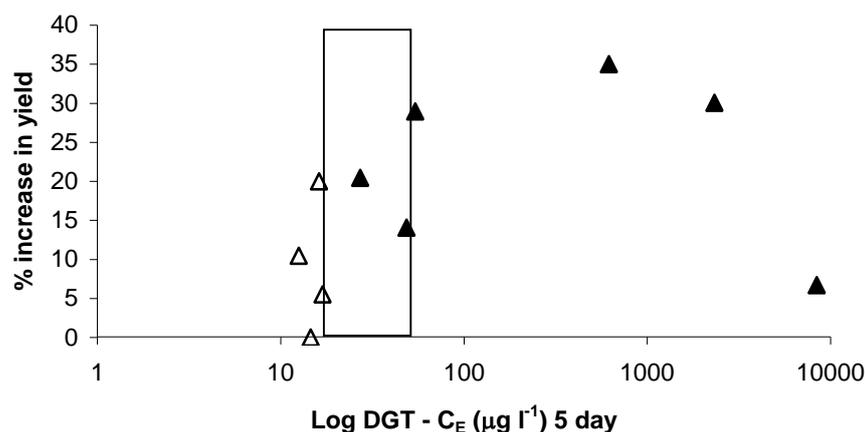
a)



b)



c)



**Figure 7.2** Comparison of  $C_E$  values (measured by DGT) with increase in yield (%) of canola to Zn application for different DGT deployment times, a) 1 day, b) 2 day and c) 5 day. (▲)  $C_E$  values above DGT detection limit, (Δ)  $C_E$  values below DGT detection limit. The area inside the rectangle illustrates the difference in  $C_E$  values from the detection limit to the deficiency threshold.

### 7.3.1.2 Wheat

Dry matter yields of wheat sharply increased between a Zn application rate of 0.5 mg kg<sup>-1</sup> and 1 mg kg<sup>-1</sup> (Table 7.3). Yields were quite variable at the higher Zn application rates.

**Table 7.3** mean % increase in yield (wheat) with standard deviation (2 replicates) at each Zn application rate.

Zn rate mg kg <sup>-1</sup>	% increase in yield
0	0
0.01	2 ± 3.8
0.02	3 ± 6.4
0.05	6 ± 5
0.1	2 ± 4.1
0.5	7 ± 3.2
1	39 ± 2.3
5	20 ± 7
10	33 ± 27.7
20	15 ± 16.9

Detection limits of the DGT method were lower than the corresponding canola experiment. There was no significant improvement to DGT detection limits using the acid washed devices (2-day deployment) compared to the originally prepared DGT devices (1 and 5-day deployment) (data not shown). This observation was not in accordance with results shown in section 7.3.1. The MDL for this set of DGT blanks (without acid washing) used in this experiment was considerably lower than that shown in section 7.3.1 and therefore further acid washing could not improve the MDL.

DGT measurements (1 day) were unable to separate the maximum yield value from lower yields (< 10 %) and therefore an approximate deficiency threshold could not be established (Figure 7.3a). Approximate Zn deficiency thresholds of < 25 ug l<sup>-1</sup> C<sub>E</sub> (2 day deployment) (Figure 7.3b) and < 17 ug l<sup>-1</sup> C<sub>E</sub> (5 day deployment) (Figure 7.3c) for wheat are lower than corresponding canola deficiency thresholds indicating that canola in this case is more susceptible to Zn deficiency. Currently this data is quite limited as there is only one point that experiences an increased yield without also experiencing a mild Zn toxicity effect. Accurate determination of the critical threshold for wheat would require additional rates of Zn to provide confidence at which rate the maximum yield would be

obtained. Variability in yields obtained at the higher Zn application rates also adds uncertainty to the determination of maximum yield.

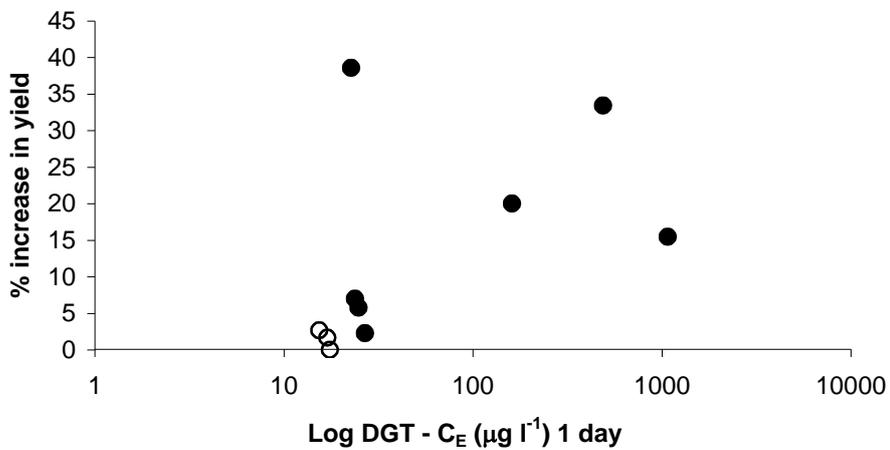
These factors could have contributed to the lower critical threshold for wheat than canola, a result that is in contrast to a study by Brennan and Bolland (2002) that showed canola used indigenous, current and incubated Zn more effectively than durum wheat. Brennan and Bolland (2002) harvested after 40 days growth as apposed to the 28 day growth period used in this study. Plant yields grown for shorter periods would likely to be more variable and Zn deficiency symptoms would be less severe. Zinc seed reserves could also be a contributing factor to the determination of the critical threshold as seeds with larger Zn contents produce bigger roots and shoots during early growth before seed reserves are exhausted (Rengal 2001). The Zn seed reserves of wheat used in this experiment could have been larger than the comparative study of Brennan and Bolland (2002).

Canola acidifies the rhizosphere just behind the root tip (see section 6.1) dissolving insoluble forms of P and potentially Zn. The root exudates from canola may not necessarily enhance metal (Zn) mobilisation in soil or in this experiment a substitute soil. Zhao et al. (2001) reported a significant increase in Zn mobilization from Zn loaded resins by root exudates from hydroponically grown wheat (*Triticum aestivum*) but in comparison there was no significant increase in Zn mobilization by root exudates from canola (*Brassica napus*). The increased mobilisation of Zn by root exudates from wheat compared to root exudates from canola was also observed in a contaminated soil (Zhao et al. 2005). Therefore, in this experiment a lower Zn deficiency threshold for wheat could be attributed to the increased mobilization of Zn in the substitute soil by root exudates from wheat to that of canola. Alloway (2004) suggested that variations in reaction to Zn deficiency of plant species might in fact be due to the differences in the plant species

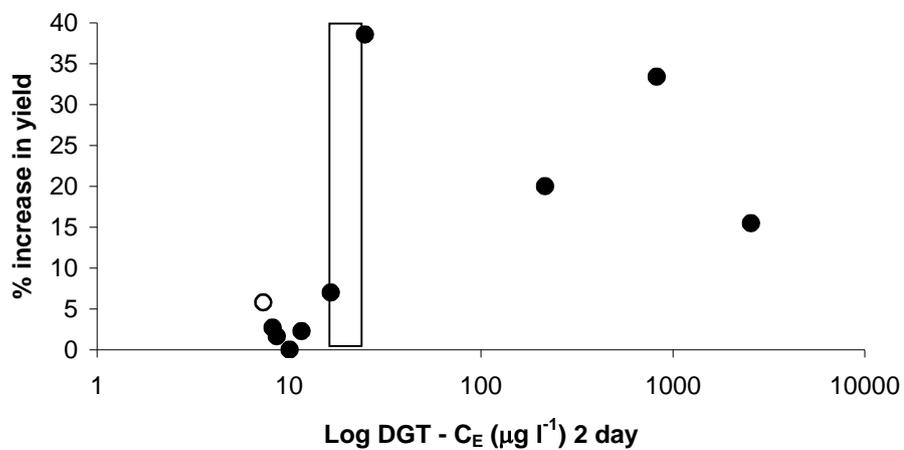
capacity to release Zn mobilising phytosiderophores. Phytosiderophores released by roots enhance Zn solubility and mobility by chelation of sparingly soluble Zn found in calcareous soils (Cakmak et al. 1994).

In this experiment a substitute soil (sand + 1 % CaCO<sub>3</sub> + nutrients) was used and therefore the mechanism of canola and wheat in accessing Zn may be different to that in a true soil.

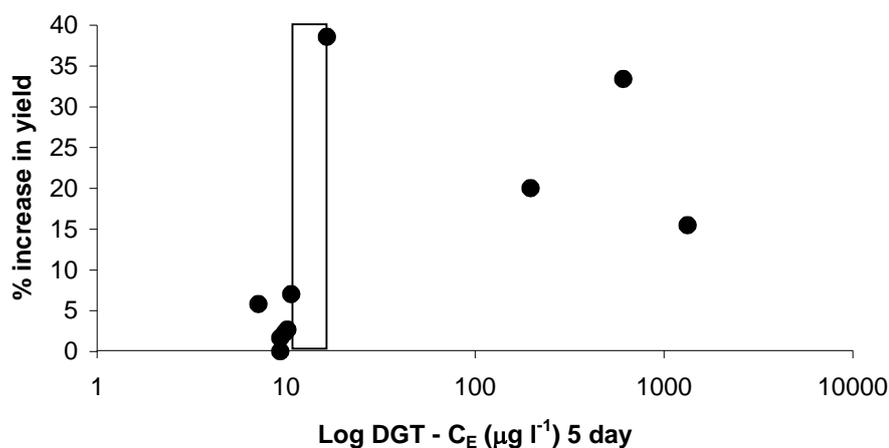
a)



b)



c)



**Figure 7.3** Comparison of  $C_E$  values (measured by DGT) with increase in yield (%) of wheat to Zn application for different DGT deployment times, a) 1 day, b) 2 day and c) 5 day. (●)  $C_E$  values above DGT detection limit, (○)  $C_E$  values below DGT detection limit. The area inside the rectangle illustrates the region of the deficiency threshold in regard to  $C_E$  values.

### 7.3.3 Predicting Mn deficiency using DGT

Dry matter yields of wheat increased corresponding to increasing rate of Mn application to reach a maximum yield increase of 59 % at an application rate of 1 mg kg<sup>-1</sup> Mn (Table 7.4).

**Table 7.4** mean % increase in yield (wheat) with standard deviation (2 replicates) at each Mn application rate.

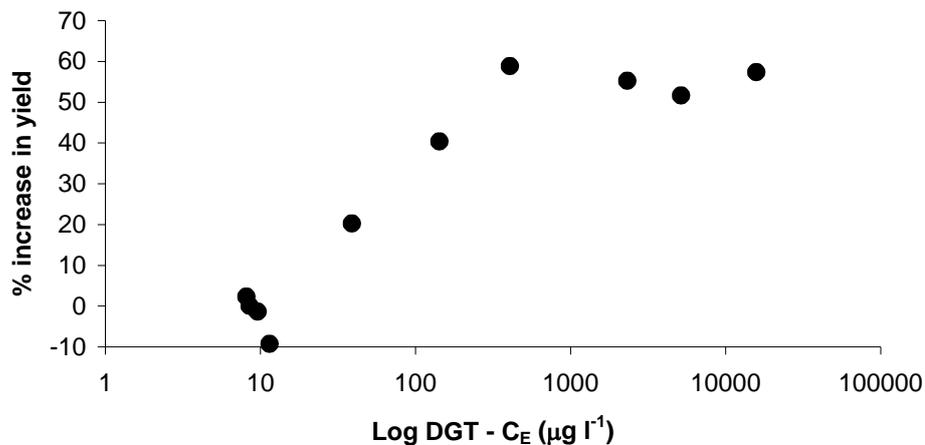
Mn rate mg kg <sup>-1</sup>	% increase in yield
0	0
0.01	(-1) ± 2.3
0.02	2 ± 8.2
0.05	(-9) ± 0
0.1	20 ± 4.3
0.5	40 ± 17.3
1	59 ± 1.3
5	55 ± 2.1
10	52 ± 11.8
20	57 ± 17.9

Comparison of DGT measurements and plant response to applied Mn (expressed as % increase in yield) revealed an excellent relationship ( $r^2 = 0.97$ ) using a Mitcherslich type equation (see Section 5.2.2, eqn. 14) (Figure 7.4a). Converting yields to relative yield (%) provided an accurate assessment of the Mn deficiency threshold of durum wheat. The deficiency threshold that corresponds to 80 % of the relative yield is  $161 \mu\text{g l}^{-1} C_E$  (Figure 7.4b). All DGT measurements were substantially over the detection limit for Mn calculated using the IUPAC definition (see Section 7.3.2.1) (Table 7.5).

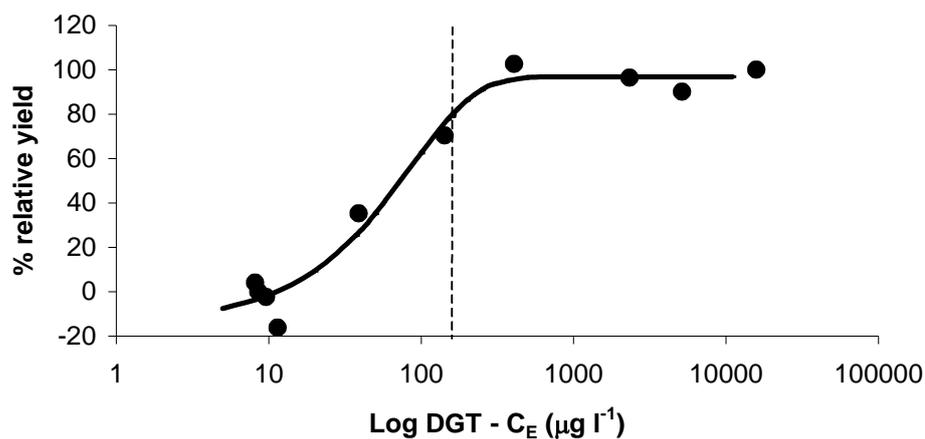
**Table 7.5** Method detection limit (MDL) and sample measurements of DGT for Mn. MDL = mean  $C_{DGT}$  (Blank) + 3\*Stdev(3 Blanks).

<b>Rate</b> <b>mg kg<sup>-1</sup></b>	<b>C<sub>DGT</sub></b> <b>μg l<sup>-1</sup></b>	<b>Mean</b>	<b>Stdev</b>	<b>MDL</b>
Blank 1	0.06			
Blank 2	0.05			
Blank 3	0.05	0.05	0.007	<b>0.07</b>
0	0.32			
0	0.33	0.32		
0.01	0.36			
0.01	0.37	0.36		
0.02	0.32			
0.02	0.29	0.31		
0.05	0.49			
0.05	0.37	0.43		
0.1	1.5			
0.1	1.5	1.5		
0.5	5.8			
0.5	5.0	5.4		
1	17			
1	14	15		
5	87			
5	89	88		
10	213			
10	176	195		
20	473			
20	723	598		

a)



b)



**Figure 7.4** Comparison of DGT measurements ( $C_E$ ) with a) % increase in yield in response to increasing Mn applications, b) % relative yield, dashed line indicates deficiency threshold corresponding to 80 % of the relative yield on the fitted Mitscherlich curve.

Manganese measurements made by DGT ( $C_E$ ) on 21 agricultural soils that were used to determine the response of wheat to applied P (Chapter 4) reveal that the majority of soils are not Mn deficient assessed by using the deficiency threshold determined above (Table 7.6). Only two calcareous soils from S.A. had values lower than  $160 \mu\text{g l}^{-1}$  and therefore a minimal yield increase to applied Mn would be expected on these soils.

**Table 7.6** Manganese measurements obtained by DGT ( $C_E$ ) on 21 Australian agricultural soils.

<b>Site</b>	<b>Location</b>	<b><math>C_E</math> <math>\mu\text{g l}^{-1}</math></b>
<b>Balkuling</b>	<b>W.A</b>	1080
<b>Cunderdin</b>	<b>W.A</b>	509
<b>Gillingarra</b>	<b>W.A</b>	2492
<b>Kojonup</b>	<b>W.A</b>	1113
<b>Lancelin</b>	<b>W.A</b>	271
<b>Meckering</b>	<b>W.A</b>	1687
<b>Waranine</b>	<b>W.A</b>	7828
<b>West York</b>	<b>W.A</b>	270
<b>Wongan</b>	<b>W.A</b>	3525
<b>Yalenbee</b>	<b>W.A</b>	336
<b>Birchip</b>	<b>VIC</b>	792
<b>Birchip B</b>	<b>VIC</b>	1709
<b>Horsham</b>	<b>VIC</b>	288
<b>Kalkee</b>	<b>VIC</b>	592
<b>Lower Norton</b>	<b>VIC</b>	231
<b>Mildura</b>	<b>VIC</b>	514
<b>Rupanyip</b>	<b>VIC</b>	561
<b>WRS</b>	<b>VIC</b>	1107
<b>Mt Damper</b>	<b>S.A</b>	519
<b>Pt. Kenny</b>	<b>S.A</b>	135
<b>Waramboo</b>	<b>S.A</b>	101

#### 7.3.4 Predicting Zn deficiency in agricultural soils

Comparison of DGT measurements with increases in yields to Zn applications on a substitute soil, indicated that the critical Zn threshold for durum wheat was between 17 – 25  $\mu\text{g l}^{-1} C_E$  (Section 7.3.2.2). From this estimation, DGT measurements obtained on 6 agricultural soils from Australia indicated that 5 of the 6 soils are potentially Zn deficient (Table 7.7). Comparison of DGT measurements from the 2 different deployment times (2 and 5 day) were generally in agreement apart from two exceptions. Considerably lower DGT measurements were obtained for the 5 day deployment on the Mt. Damper soil for both the control soil and the Zn applied soil. Lower DGT values obtained on the control soil for the extended deployment time could be attributed to the exhaustion of available Zn in solution and off the soil solid phase with time. The mechanism of lower DGT

values with deployment time on the Zn applied soil could be attributed to the fixation of Zn with time (see below).

Zinc deficient soils as indicated by DGT measurements did not relate to a response of durum wheat to applied Zn in 3 of the 5 soils (Table 7.7). DGT correctly predicted significant yield responses for Streaky Bay and Mt. Damper (5 day deployment). It is unclear as to why there were no significant yield responses from potentially Zn deficient soils (Waramboo, Pt. Kenny, Birchip).

One possible explanation is that Zn applied in liquid form and homogenised through the soil could with time become unavailable due to the properties of these soils. In Alkaline soils Zn can be absorbed onto clays and organic matter through chemisorption and complexation by organic ligands (Alloway 2004). Zinc can be absorbed onto calcium carbonates by a chemisorption mechanism and this reaction due to some Zn being irreversibly fixed to the soil (Kiekens 1995). Three soils used in this experiment (Mt. Damper, Pt. Kenny and Waramboo) were sampled from Eyre Peninsula in South Australia where soils contain large amounts of  $\text{CaCO}_3$  (McBeath et al. 2005).

DGT measurements were performed before seeding, 1 week after Zn + nutrient application and therefore by the time the plant had emerged and used up all Zn resources from the seed and soil, the applied Zn had become unavailable. This hypothesis could have been confirmed had DGT devices been deployed on the soils after plant harvest and this is certainly something that would be recommended for future experiments. Future experiments may also need to involve looking at applying Zn as a foliar spray so the plant can access the Zn before it is tied up in the soil.

**Table 7.7** DGT measurements ( $C_E$ ) for two deployment times (2 and 5 day) on 6 Australian agricultural soils (control + Zn applied ( $10 \text{ mg kg}^{-1}$ )) and the corresponding % increase in yield.

<b>Site</b>	<b>Zn applied <math>\text{mg kg}^{-1}</math></b>	<b><math>C_E</math> (2 day) <math>\mu\text{g l}^{-1}</math></b>	<b><math>C_E</math> (5 day) <math>\mu\text{g l}^{-1}</math></b>	<b>% increase in yield</b>
Streaky Bay	0	7.1	3.5	
Streaky Bay	10	<b>34</b>	<b>35</b>	18.5
Waramboo	0	<b>13</b>	<b>7.1</b>	
Waramboo	10	<b>43</b>	<b>47</b>	0.4
Pt Kenny	0	4.3	3.6	
Pt Kenny	10	<b>44</b>	<b>48</b>	-4.0
Birchip	0	6.2	3.8	
Birchip	10	<b>21</b>	<b>23</b>	-1.3
Mt Damper	0	<b>24</b>	7.6	
Mt Damper	10	<b>507</b>	<b>251</b>	21.7
Mt Shank	0	<b>26</b>	<b>22</b>	
Mt Shank	10	<b>270</b>	<b>222</b>	-10.1

Deficiency threshold between 17-25  $\mu\text{g l}^{-1} C_E$   
*Below DGT detection limit*  
**Above DGT detection limit**

#### 7.4. Overall analysis

DGT has shown to be capable of predicting micronutrient (Mn, Zn) deficiencies in a manufactured soil. Detection limits were improved by extending deployment times beyond 1 day for Zn. There were no DGT detection limit issues for measuring Mn and DGT measurements were highly correlated with % increase in yield.

The failurer of DGT to predict Zn deficiencies in agricultural soils may be due to the Zn application method which may not provide a significant increase in available Zn in the soil by the time the plant has utilised all other Zn resources and, therefore, there is no significant response. Applying the Zn as a solution directly on the soil and then homogenising the soil would allow greater exposure of the soil particles to the Zn and therefore greater fixation potential as opposed to applying the Zn in a concentrated area (e.g. granular form). Foliar applications might also be necessary to allow the plant access to sufficient Zn and overcome any Zn deficiencies.

The ability of DGT to predict Mn deficiencies on agricultural soils needs to be assessed and might be subjected to similar difficulties as for Zn in applying Mn to soils that remains available to a plant.

## 8. Conclusions and recommendations

The DGT method has been modified with the development of a new Mixed Binding Layer (MBL). The MBL allows simultaneous assessment of particular cations and anions in a single assay. Development of the MBL provides slightly quicker and cheaper analysis of analytes (if requiring analysis of cations and anions) and eliminates potential errors of using two different sensors in a heterogeneous environment like a soil. Use of the MBL has been validated under a wide range of solution parameters and has met the requirements for use on nutrient deficient soils.

DGT technology has been extensively tested for predicting plant response to applied P for various crop types. Consistently DGT has provided an accurate assessment of plant available P and has outperformed other standard soil testing procedures. The improved performance of DGT can be attributed to a number of factors. DGT provides an in situ assessment of nutrient availability in a soil without disturbing the dynamic pseudo equilibrium between solution and solid phases. Placement of the diffusive layer in front of the binding layer allows precise calculation of fluxes and places a limit on the maximum flux. The use of ferrihydrite as a sink for P is a selective binding agent for P and has been shown in this study to be mostly free from other anionic interferences under most soil conditions.

The resin method as with the DGT method provides a sink for P that has been related to plant uptake mechanisms. Therefore it was a surprise that resin P measurements were poorly related to DGT measurements and failed to predict plant response to P on several different soils. The resin method is dependent on the soil to solution ratio employed in the method and it remains unclear what ratio best reflects plant available P. The other major drawback is the Anion Exchange Membrane (AEM) used in the resin

method. The AEM is not a specific sink for P and also binds other anions that can affect the net absorption of P through competition for binding sites.

DGT methodology provides the potential to assess micronutrient deficiencies in agricultural soils. The challenge with micronutrient deficiency is that deficiency signs occur at very low concentrations and therefore a sensitive soil testing method is required to assess these disorders. Using a MBL, the DGT method has shown to be capable of determining Mn and Zn deficiency in manufactured soil through very low detection limits. DGT technology also allows for flexibility in extended deployment times to assess micronutrient deficient soils if detection limits are an issue. To prove the ability of DGT to assess micronutrient deficiency, further work is required with agricultural soils. The MBL provides potential to assess other micronutrient deficiencies that include Cu and Mo.

This work has established that the relatively new soil test (DGT) is more reliable than other traditional soil testing methods in predicting plant response to P in controlled conditions (glasshouse). There are numerous questions that still need to be examined before DGT can become an established soil test. Ideally a soil test needs to reflect nutrient availability in any given soil under field conditions. Correlation with crop and grain yields obtained in the field is vitally important for any soil test, as the grain yield is the product that actually contributes to the economic value of the crop and to the grower. There are many contributing conditions in the field that can alter nutrient availability compared to a controlled environment like a glasshouse. Therefore plant responses obtained in a glasshouse over 4 weeks may not actually reflect an increase in grain production at harvest if conditions in the field become unfavourable for crop growth (see below). McDonald (2006) stated that crop growth is dynamic and reflects the interactions between the growing crop, soil properties, rainfall and temperature during the growing

season. Some examples of factors that may affect plant yields in the field are described below.

Typical soil tests are measured on the top section of the soil usually < 15 cm. Plant roots have been shown to reach below these depths and therefore nutrient availability could be overestimated or underestimated if only assessed in the top section of the soil profile (Jungk 1991). This raises the question of where to actually test in the soil profile. Early growth of a crop is dependent on the properties of the topsoil but as the season progresses the crop becomes more dependant on the moisture content of the subsoil to sustain growth (McDonald 2006). The properties of the subsoil will therefore assume greater importance to grain yield and the effect these soil properties will have on growth and yield is influence by the rainfall pattern during the growing season (McDonald 2006). As an example, McDonald (2006) revealed that addition of P promoted early dry matter production of wheat and barley in two successive growing seasons on Eyre Peninsula, S.A. but in both years this was not translated into increased growth at anthesis or maturity. McDonald (2006) suggested that the early season benefits could have diminished through the growing season as plant growth was limited by other soil properties e.g. high pH or high EC. Borges and Mallarino (2000) also found there was no relationship between early growth responses of soybean to P and grain yield from long term field trials in Iowa. They suggested that the environmental growing factors later in the season might have precluded any influence of increased early growth on plant yield. Rengasamy et al. (2003) discussed numerous subsoil chemical and physical constraints in Australian dry land soils that could affect any early season growth benefits by reducing overall crop yields. These include hardsetting, crusting, compaction, salinity, sodicity, acidity, alkalinity, nutrient deficiencies, and toxicities due to high concentrations of boron, carbonates and aluminium (Rengasamy et al. 2003).

Another aspect of a crop cycle is how long after fertiliser application should you test the soil and at what stage of the growing season would testing best reflect plant nutrient availability. As discussed in Section 4.1 laboratory studies have revealed that P availability is also controlled by microbial activity within the soil as a response to varying temperatures and moisture content. This raises the question of the possibility of correlating a controlled laboratory soil test (e.g. DGT) with variable soil moisture conditions that occur in the field.

In preparing soils for glasshouse trials typically soils are either oven or air dried and sieved < 2 mm which can affect the overall nutrient status of the soil. Death of microbes as a result of soil drying can release nutrients not normally available to plants (Sparling et al. 1985) and the contribution of the larger soil particles to the overall nutrient status of the soil would be unclear due to their removal. Another major difference from glasshouse experiments performed in this study compared to field conditions is that the P added was homogenised throughout the soil for DGT assessment. This could increase the fixation of P to soil particles due to greater exposure of P to absorption sites on the soil compared to the field where P is typically added in granular form and in a band. In regards to fertiliser recommendations will the amount ( $\text{mg kg}^{-1}$ ) of nutrient applied and homogenised in a pot required for maximum yield reflect the amount ( $\text{mg kg}^{-1}$ ) required in the field taking into consideration that in the field P is typically applied in a band.

DGT has the potential to accurately measure nutrient availability in agricultural soils maximising fertiliser efficiency and reduce potential environmental pollution problems associated with over fertilisation. However, in order for DGT to provide this service results obtained from the glasshouse need to be validated under field conditions.

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## Appendix A

Chapter 2. Performance of a mixed binding layer (MBL) for measuring anions and cations in a single assay using the diffusive gradients in thin films (DGT) technique

### Statement of Contributions

Chapter 2 has been published (Mason et al., 2005):

Mason S. D., Hamon R. E., Nolan A. L., Zhang H. and Davison W. (2005) Performance of a mixed binding layer for measuring anions and cations in a single assay using the diffusive gradients in thin films technique. *Anal. Chem.* **77**, 6339-6346.

and is attached as appendix A

The role of R. E. Hamon, A. L. Nolan, H. Zhang and W. Davison in this publication was the revision of drafts of the manuscript prior to publication and intellectual input into experimental design. As primary author I was responsible for the overall experimental design and set up, experimental procedures, data collection, collation and analysis and the primary preparation of the manuscript.

This manuscript is presented in Chapter 2 with only alterations to formatting to suit that of the thesis plus an additional experiment (section 2.3.8.3) that took place after the paper had been accepted but was deemed appropriate for the subject of the chapter. The abstract and part of the introduction has been removed for consistency between chapters.

Mason, S., Hamon, R., Nolan, A., Zhang, H. and Davison, W. (2005) Performance of a Mixed Binding Layer for Measuring Anions and Cations in a Single Assay Using the Diffusive Gradients in Thin Films Technique.  
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