

**Using the DGT to investigate the soil chemistry of P and Cd in sandy loam soil after the application of phosphorus fertilizers**

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Using the DGT to investigate the soil chemistry of P and  
Cd in a sandy loam soil after the application of  
phosphorus fertilizers

by

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B.Sc (M.Sc)

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## Abstract

A recently developed DGT with a mixed binding layer of Chelex and Metsorb ( $\text{TiO}_2$ ) was successfully deployed in fresh and seawater to simultaneously measure available cations and anions. But, to date, no studies have used similar DGTs in soils. This study used the Metsorb Gel in two types of DGTs the Metsorb Chelex Mixed Binding layer (MBL-DGT) and the Metsorb Chelex Double Binding layer (DBL-DGT) or sandwich DGT to determine the concentration of available P and Cd ( $C_{\text{DGT}}$ ) in soil.

Three experiments were conducted to assess the accuracy, sensitivity, and capacity of the new DGTs to measure the availability of P and Cd ( $C_{\text{DGT}}$ ) in a sandy loam soil. The study also investigated the relationship between the  $C_{\text{DGT}}$  of P and Cd in the soil and concentrations of available P and Cd in plants and leachate water. Three scenarios were prepared for the assessment of the DGTs. First, the MBL-DGT was deployed in a large-scale flume filled with sandy loam soil, which was fertilized with Single Super Phosphate (SSP) of three different doses (0.01, 0.1, and 0.5 mg P L<sup>-1</sup>). Second, a biological experiment was conducted in a greenhouse using the tomato plant (*Lycopersicon esculentus*) as an indicator to assess the reliability of the  $C_{\text{DGT}}$  of the available P and Cd measured in soil solution and that absorbed by the plants. This experiment used the same type of sandy loam soil, with three types of P fertilizers: SSP, Egyptian Reactive Phosphate (ER) and phosphorus soft rock from Nutri-tech Solution (NTS) applied at four dosages (0, 100, 200 and 250 kg P ha<sup>-1</sup>). Third, three consecutive doses of the three fertilizers were added to the sandy loam soil to investigate the capacity of the MBL-DGT. The repeated fertilizer application simulated the repeating application of P fertilizer in the field cycle.

The mobility of available P and Cd to the leachate water was monitored by deploying the DGTs at different depths in the soil. This technique was used to evaluate the sensitivity of the DGTs when deployed in the soil for 4, 7 and 14 days. In addition, the new DGT measurements were compared with conventional measurements of P and Cd, using the Olsen and  $\text{NH}_4\text{Cl}$  extraction techniques, respectively. Results of all three experiments confirmed that the MBL-DGT and the DBL-DGT were versatile measures of P and Cd availability and mobility in soil. The Metsorb mixed binding layer uptake of

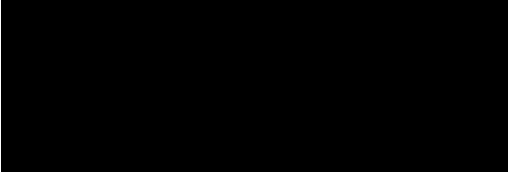
P and Cd rose with increasing P fertilizer dose. The new DGTs were sensitive to fertilizer type due to the differences in the solubility properties of the three fertilizers.

The new DGTs showed that the excessive use of P fertilizers in the sandy loam soil increased the mobility of available P and Cd to the leachate water.  $P_{DGT}$  and  $Cd_{DGT}$  had stronger correlations with P and Cd content in the plants than the conventional P and Cd extraction methods. These findings indicated that the DBL-DGT mimicked the process of plant P uptake. The MBL-DGT was also able to measure the most plant accessible form of Cd from the soil solution. MBL-DGT measurements of P and Cd were also better correlated with total P and Cd than those of conventional extraction methods.

The capacity of the MBL-DGT for Cd uptake did not exceed the effective capacity of the MBL-DGT previously recorded by Panther et al. (2010). On the other hand, the MBL-DGT capacity for available P uptake was 42,000 ng per disc which was slightly higher than that recorded by Panther et al. (2014). Quantitative assessment of available P in the soil which has recently received high doses of P fertilizer will require MBL-DGTs with higher capacity. Further research is needed on different soil types and different crops to obtain a more comprehensive understanding of when the MBL-DGT can be practically applied in the field.

## Statement of Originality

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.



Rawaa Abdul Jabbar

October 31, 2018

Date

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## List of Abbreviations

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ANZECC	Australian- New Zealand Environment of Conservation
ARMCANZ	Agriculture and Resource Management Council of Australia and New Zealand
Cd	Cadmium
Cd <sub>DGT</sub>	Cadmium measured by DGT
Cd <sub>EX</sub>	Cadmium extracted from the soil by chemical extractant (NH <sub>4</sub> Cl)
C <sub>DGT</sub>	The concentration of analyte measured by DGT
C <sub>EX</sub>	The concentration of analyte measured by chemical extraction
DBL-DGT	Double Binding Layer DGT
DGT	Diffusive Gradient in Thin Films
DTPA	Diethylenetriaminepentaacetic acid
EDTA	Ethylenediaminetetraacetic acid
ER	Egyptian phosphate rock
HPR	Hamrawin phosphate rock
MBL-DGT	Metsorb Chelex Mixed Binding layer -DGT
M <sub>EFC</sub>	Metal, Effective Capacity
Na <sub>2</sub> EDTA	Ethylenediaminetetraacetic acid disodium salt dihydrate
NaOAc	Sodium Acetate
NCPR	North Carolina phosphate rock
NTS	Nutri-Tech Solution
P	Phosphate
PAPR	Partially acidulated phosphate rock
P <sub>DGT</sub>	Phosphate measured by DGT
P <sub>EX</sub>	Phosphate measured by Olsen method
SSP	Single Super Phosphate
TEA	Triethanolamine
WHC	Water holding capacity in soil

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# Chapter 1-. Literature review

## 1.1 Introduction

Phosphorus (P) is a macronutrient required in large amounts by crops, second only to nitrogen (N). The concentration of available P in soils can limit agricultural production. Therefore, P fertilizers are extensively used to compensate for P deficiency to achieve optimal crop production. However, P oversupply may lead to the environmental pollution of the associated watersheds. It is well recognized that continuous application of P, more than crop requirements, leads to a build-up of soil P levels (Campos et al., 2018). Accumulation of available P in agricultural soil may enhance P loss by leaching into groundwater and surface water which is a major concern for water quality (Divya and Belagali, 2012). Consequently, the risk of eutrophication of waterways will be increased (Boitt et al., 2018). Currently, phosphate rock reserves could be depleted by 2033 (Cordell and White, 2011) and will be exhausted by around 2100 (Reijnders, 2014; Roberts and Johnston, 2015; Pantano et al., 2016; Luo et al., 2017). In addition, many commercial P fertilizers contain different levels of non-nutritive metals, such as Cd, arising from its sources including parent material (Egle et al., 2016; Weissengruber et al., 2018). The input of Cd in agricultural soils through the application of phosphate fertilizers has been well documented in both Australia and New Zealand (de Vries and McLaughlin, 2013; Six and Smolders, 2014). Therefore, soil testing prior and post-fertilization is important for identification of accurate P and Cd levels in soils to establish a comprehensive plan for economic and environmental P management systems.

Bioavailable P, which is readily available for plant use, is only a small portion of the total soil P concentration (Lemos et al., 2009). Several measurement techniques have been used to determine the concentration of bioavailable forms of P, Cd, and other heavy metals in soils. Lemos et al. (2009) recorded that changing the extraction technique for the same soil samples led to different results. For example, Lemos et al. (2009) compared the Mehlich 1 method and the ionic exchange resin method to extract P from the same soil. The results showed that Mehlich 1 had a higher extraction capacity of  $17 \pm 0.4 \text{ mg P} - \text{PO}_4^{3-} \text{ dm}^{-3}$  because of its acidic characteristics which caused overestimations of the concentration of available P. The amount of extracted P determined using ionic exchange resin for the same soil was lower,  $8.5 \pm 0.7 \text{ mg P} - \text{PO}_4^{3-} \text{ dm}^{-3}$ , because the method depends on the resin exchange capacity and ionic concentration of the soil solution

(Lemos et al., 2009). Valladares et al. (2001) observed that the P concentration determined by Mehlich 3 (HCl + NH<sub>4</sub>F) complex, underestimated the available P content compared to other extraction methods such as Mehlich 1 (double-acid solution), 0.12 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> and 0.05 mol L<sup>-1</sup> HCl. Mehlich 3 extract removes the P complexed to aluminum from the soil, which does not reflect the accurate bioavailable portion of P to plants (Lemos et al., 2009). Therefore, standardization and regional calibration of each individual method are necessary (Lemos et al., 2009).

Chemical extraction involves the separation of the solution and solid phases causing a potential distribution between the two phases (Zhang and Davison, 1995; Zhang and Davison, 2000; Hooda, 2003; Hooda and Zhang, 2008; Dai et al., 2017). These types of procedures provide limited information about bioavailable P and Cd in soil (Hooda and Zhang, 2008). Hence, development of soil sampling and extracting P and Cd from the soil is an essential part of determining their bioavailability. The total concentration of metals in soil is a poor indicator of their availability to plants (Marr et al., 1999; McLaughlin et al., 2000a). Conventional procedures involve soil sampling, drying, grinding and treating with chemical solutions crucially changing the soil field condition. These changes modify the chemical structure of the soil but, the kinetics of metal resupply from the solid phase to the solution is not considered (Zhang and Davison, 1995; Hooda, 2003). Therefore, chemical extraction methods are incapable of accurately evaluating bioavailable forms of metals due to technical issues (Piper, 1943; Zhang and Davison, 2000; Zhang et al., 2001c; Peters et al., 2003).

Measurement of labile metal species, which reflect the bioavailable portion of metals, is of vital importance (McGrath and Lane, 1989; McGrath et al., 1999). The best way to achieve accurate P and heavy metal assessment is by using *in situ* procedures which do not involve the separation of solution from solid phase (McGrath et al., 1999; Dai et al., 2017). In the last two decades, a new technique, Diffusive Gradients in Thin Films (DGT), has been developed by Davison and Zhang (1994) for *in situ* samplings of heavy metals and P (Almås et al., 2017; Duboc et al., 2017). This technique provides an accurate measurement of the labile fraction of P and heavy metals in water (Piper, 1943; Zhang, 1995; Zhang, 2000; Sangi, 2002; Hooda, 2003; Peters, 2003), sediments (Zhang and Davison, 1995), measuring the flux of metals from solid phase to soil solution and in soils (Scally et al., 2003; Nowack et al., 2004; Nolan et al., 2005; Mason et al., 2008;

Duquène et al., 2010; Roulier et al., 2010), which shows significant correlations between DGT measurements and plant uptake (Hooda et al. 1999; Zhang et al. 2001).

A Mixed Binding Layer DGT (MBL-DGT) was used in this study to simultaneously measure P and Cd in the soil for the first time. This technique has previously been successfully used in water (Panther et al., 2014). Further research into the use of MBL-DGT in heterogeneous soils in field or semi-field conditions was needed. In this study, use of the MBL-DGT to understand the fate of available P and Cd after the application of P fertilizers to sandy loam soil was carried out by inserting the DGTs directly into the soil at different depths to track the mobility of available P and Cd through the soil depth down to the leachate water. Tomato plants were used to examine the accuracy of the Metsorb Binding Layer to detect the available P and Cd that was taken up by the tomato plant roots. Finally, comprehensive studies of the mobility of available P and Cd through the soil body were carried out to monitor the contamination of soil and leachate water. Accumulation of available P and available Cd concentrations in soil and leachate water is expected due to the excessive use of P fertilizers for crop production. The developed procedure to measure available P and available Cd concentrations in the soil using one DGT device will reduce effort and cost. Using the MBL-DGT before fertilization will provide an indication of the accurate amount of P fertilizer needed by plants thereby enhancing fertilizer use efficiency and allowing monitoring of soil contamination with available Cd. Commonly used methods (i) determine the bioavailability and mobility of P and Cd in soils and (ii) investigate important soil, plant, and, water interactions, which will be reviewed in the next sections of this chapter.

## **1.2 Phosphorus in Australian soils**

In Australia, there are large areas of low fertile soil, with P deficiency being a particular problem (Perverill et al., 2003). Perverill et al. (2003) stated that P deficiency has been diagnosed in soil, but was especially prevalent in sandy soil due to the coarse-textured parent materials. Sandy soils occupy around 18 million hectares of Australia, excluding desert regions (Mann and Ritchie, 1995). Majority of Australian soils are P deficient due to the geological history of the land (Peverill et al., 2003). Additionally, farming practices have enhanced the chemical weathering processes that contribute to the depletion of various nutrients in Australian soils, thus expanding the problem of infertile

soils (Williams and Raupach, 1983; Stritsis et al., 2014). Therefore, the application of P fertilizers is essential for profitable production (Peverill et al., 2003). The application of P fertilizers to correct the nutrient deficiency, however, creates serious environmental problems such as environmental pollution (Loneragan, 1997; Luo et al., 2017; Bai et al., 2013).

The amount of P absorbed by plants and permanently removed from the soil is small compared to the amount of P fertilizers added to the soil (Loneragan, 1997). This observation is attributed to the P fixation by most soils which creates the need for the extra application of P fertilizer to meet plant requirements for optimum economical production (Loneragan, 1997; Johnston et al., 2014). Phosphorus fixation occurs through a variety of chemical reactions usually involving cations such as  $Al^{+3}$  and  $Fe^{+3}$  in acidic soil and  $Ca^{+2}$  in alkaline soils (Iqbal, 2009). These cations act as “sinks” for P. The reactions of P fixation depend on several soil factors including the content of hydrous oxides, Fe and Al hydroxides, soil pH, drainage conditions of the soil, amount and type of clay minerals and the degree of soil weathering (Ch'ng et al., 2016). Extensive and continuous use of P fertilizers leads to P accumulation in the soil which may be removed by soil erosion or leaching into groundwater. Increasing P concentrations in agricultural land are potentially causing an increase in P concentration in surface waters (Williams and Raupach, 1983; Weatherley et al., 2011).

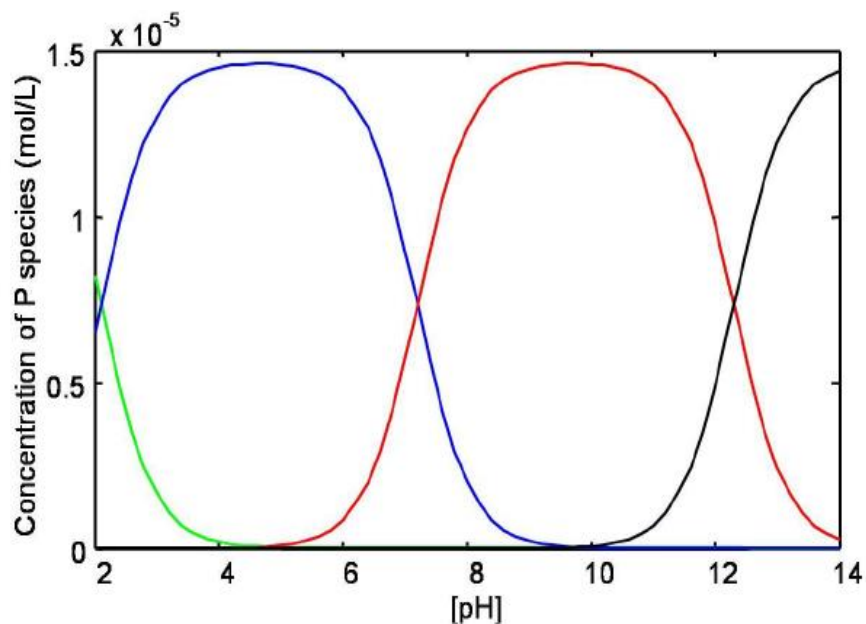
Furthermore, as P fertilizers contain impurities of different non-nutritive trace elements, such as Cd, the long-term use of P fertilizers can lead to the accumulation of Cd in soil. Cadmium can be absorbed by plants and cause health problems to humans and animals consuming the plants (Bingham et al., 1976; Anderson and Hahlin, 1981; Chaney and Hornick, 1987; Bramley, 1990; Hao et al., 2008). The impact of Cd and soil processes are described in detail in section 1.7.

### **1.3 Sources and forms of phosphorus in soil**

Sources of P in soils can be anthropogenic and/or pedogenic including the use of inorganic P fertilizers, decomposition of organic materials and weathering of soil parent materials (Scherer and Sharma, 2002; Verma et al., 2005; Vrana et al., 2005). Phosphorus in soils is found in two forms, mineral or inorganic P ( $P_i$ ), largely orthophosphate ions, and organic P ( $P_o$ ) such as inositol phosphate, nucleic acids and phospholipids (Degryse

et al., 2003). These forms can come from either natural or anthropogenic sources such as fertilizers and pesticides.

There are 200 different phosphate-bearing minerals in the Earth's crust (Tate, 1985), which tend to be found in higher concentrations within sedimentary rocks. Native  $P_i$  in the soil is derived during the soil forming processes from primary P bearing minerals such as strengite ( $FePO_4 \cdot 2H_2O$ ), variscite ( $AlPO_4 \cdot 2H_2O$ ) and the apatite group  $M_{10}(XO_4)_6Y_2$  where M can be Ca, Sr, Ba, or Pb, X can be P, V, or Si and Y can be OH, F, or Cl (Stevenson and Cole, 1999; Brady and Weil, 2000; Luo et al., 2010). Apatite exists as part of igneous, metamorphic and sedimentary rocks. It can also be found as biotical and biological forms (Anderson et al., 1985). Dissolution of apatite by organic acids is higher than its dissolution by inorganic acids (Feng et al., 2011; Calvaruso et al., 2013). Plants, bacteria and ectomycorrhizal fungi are able to accelerate the release of P, Ca and trace elements from apatite by excreting inorganic and organic acids (Smits et al., 2012). Chemical and physical weathering processes on P minerals and other more stable geological materials release soluble forms of anion P which are controlled by soil pH (Figure 1-1).

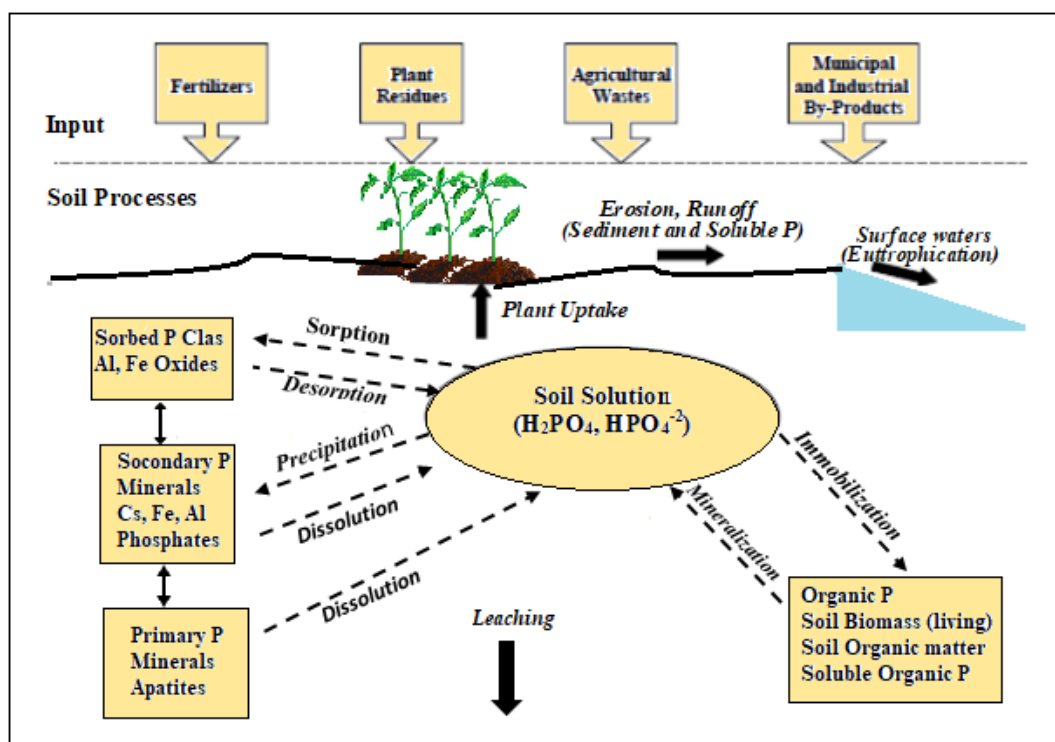


**Figure 1-1 Distribution of orthophosphate ions (expressed as mole fraction of total P) in solution as a function of pH. adopted from (Hinsinger, 2001). Different colours represent different orthophosphate forms.**



For example, when the pH ranges from 2.2-7.2  $\text{H}_2\text{PO}_4^-$  is the dominant orthophosphate form whereas  $\text{HPO}_4^{2-}$  is the dominant orthophosphate form in the pH range of 7.2-12.3 (Figure 1-1). Therefore, phosphate forms diagram can be an elucidation for pH dependence of P removal take advantage of adsorption reactions.

Regardless of the source, once P has been converted to orthophosphate forms in the soil, several key processes can occur as part of the P biogeochemical cycle in soils. These processes are summarized in Figure 1-2 including the various sources discussed above.



**Figure 1-2 A conceptual model for an enhanced phosphorus cycle in soil highlighting the physical, chemical and microbiological processes affecting the availability of P to plants and P transport in runoff or leachate in soils that have a low buffering index (PBI). Modified from Pierzynski (2000) and Gachon (1969)**

The concentrations of orthophosphates are comparatively low in soil solution because  $\text{P}_i$  is removed by either strongly binding (adsorbing) to soil minerals (Hinsinger, 2001), precipitating as slightly soluble salts or is leached from the soil by gravitational water. Orthophosphate P is also taken up by plants as (labile) P.  $\text{H}_2\text{PO}_4^{-1}$  is available in acidic soils and  $\text{HPO}_4^{-2}$  in alkaline soils (Brady, 2000; Hinsinger, 2001) as shown in Figure 1-1. Important physicochemical reactions controlling P concentration in soil solution include adsorption, desorption, precipitation, and dissolution of labile P (Sanyal

and Datta, 1991; Matar et al., 1992; Hinsinger, 2001). Biological reactions such as mineralization and immobilization also affect P concentration in soil solution (Figure 1-2).

When P is solubilized in soil by chemical and physical weathering processes, it is accumulated by plants and animals, returns to stable form in the soil from plant residues and animal waste or is eroded from soils and deposited as sediment in freshwater or oceans. The forms and solubility of inorganic P are strongly affected by other soil factors such as the concentration of calcium (Ca), aluminum (Al), and iron (Fe), as well as soil texture, organic matter, clay content and mineralogy (Pierzynski et al., 2005).

The labile P pool is a function of the anion exchange capacity of the soil. The quantity of labile P is limited by the solubility product and dissolution kinetics of P secondary minerals. Olsen and Khasawneh (1980) found that the equilibrium between labile P and soluble P is rapidly established. Therefore, when soluble P is removed by leaching, plant uptake, or conversion to secondary P minerals, the labile P pool is depleted to replenish P in the soil solution (Smeck, 1985).

Organic P in soil solution can be taken up by the plant root after its mineralization (He et al., 2018), immobilized by the soil microorganisms, adsorbed to soil mineral surfaces or precipitated to form secondary P (Hinsinger, 2001). Precipitation and adsorption are ongoing reactions for P retention (Figure 1-2). The essential stages of the P cycle are described below.

### **1.3.1 Adsorption and desorption of soil phosphorus**

Adsorption is a chemical reaction in the soil that tends to remove P ions ( $\text{H}_2\text{PO}_4^{-1}$  and  $\text{HPO}_4^{-2}$ ) from the soil solution. The adsorbed P sits on the solid phase of soil particles thereby decreasing P availability for plant uptake (Yan et al., 2006). Major P adsorbents contain hydroxyl groups (Fe and Al oxides), carboxyl groups (organic matter) or silanol (clay) groups (Hinsinger, 2001). In acidic soil, P is adsorbed to Al and Fe hydroxides and in alkaline soil, to calcite (Smith and Jackson, 1998; Turner and Haygrat, 2000). Adsorbed P is slowly available and gradually replenishes the soil solution in response to plant uptake. The sorption and desorption reactions are one of the soil processes maintaining equilibrium between the solid P phases and solution P and buffering any

changes in the soil solution (Moody et al., 2013). This equilibrium is termed P buffering capacity and is measured as the quantity of P that is adsorbed per unit change in solution P concentration. The number of sorption sites on the soil particles and the sorption energy are the main factors affecting the amount of P available to the plant (Borling et al., 2004; Singh et al., 2006). Soil with a high natural P sorption capacity may have reduced the available P, requiring more fertilizer inputs to maintain profitable crop production.

P adsorption and desorption in the soil are commonly studied by using isotherm experiments. It is typically conducted through batch experiments where soils are equilibrated with a series of P standards with varying concentrations (e.g., 0– 50 mg P L<sup>-1</sup>) (Dou et al., 2009). The amount of P sorbed or desorbed can then be calculated as the difference between the concentration of P standards and equilibrium P concentration in soil solution, from which the P adsorption and desorption characteristics can be modeled (Dou et al., 2009). Several models have been proposed to evaluate the dynamics of P adsorption and desorption in soil. Langmuir and Freundlich are the two main models used to fit back sorption data and describe P adsorption to soils (Graetz and Nair, 2000; Villapando and Graetz, 2001; Barrow, 2008). The Langmuir model can estimate the soil maximum capacity to sorb P ( $S_{max}$ ) and the P bonding energy constant ( $k$ ), therefore, it is most commonly used in environmental-related P adsorption studies (Graetz and Nair, 2000; Villapando and Graetz, 2001; Barrow, 2008). The Langmuir model can be presented as:

$$S = S_{max} \times k \times C / 1 + (k \times C)$$

where  $S$  (mg kg<sup>-1</sup>) is the total amount of P sorbed in soils per unit mass of soil,  $S_{max}$  (mg kg<sup>-1</sup>) is the maximum soil capacity,  $k$  (L mg<sup>-1</sup>) is the bonding energy constant, and  $C$  (mg L<sup>-1</sup>) is the equilibrium P concentration in solution (Zhang et al., 2009). The fitting of  $S_{max}$  and  $k$  allows estimation of soil buffering capacity and the potential for soil P loss (Zhang et al., 2009). In comparison, the Freundlich model can be presented as:

$$\log S = \log K_f + \frac{1}{n} \times \log C$$

where  $S$  (mg kg<sup>-1</sup>) is the total amount of P sorbed in soils per unit mass of soil,  $K_f$  (L kg<sup>-1</sup>) is the rate of P sorption,  $n$  is the slope parameter, and  $C$  (mg L<sup>-1</sup>) is the equilibrium

P (Toor and Sims, 2015). The  $K_f$  parameter is mostly used to provide an estimation of the rate of soil P sorption.

Soils differ widely in their adsorption capacity (Van Ranst and Gent, 1999; Pierzynski et al., 2000). Generally, highly weathered soils adsorb more P because they contain more clay and Al and Fe oxide. Sandy soil has much lower adsorption capacity due to the low amounts of P-retentive materials. Therefore, sandy soils are the most susceptible to leaching of P due to the application of organic or chemical P fertilizers (Moharami and Jalali, 2014). However, the relatively low adsorption ability of coarse-textured particles can be increased by Fe and Al coatings on those particles (Cohen, 1992). The low retention of P in sandy soils leads to increased algal blooms, eutrophication and water quality deterioration (Pierzynski et al., 2000). In general, soils that have low P concentration, are acidic, and high in clay or Fe and Al oxide (particularly non-crystalline oxides), have the greatest P adsorption capacity (Cox, 2000; Mason, 2010; Shapiro, 2013). In calcareous soils,  $\text{CaCO}_3$  and Fe oxides have the greatest P adsorption capacities.

Desorption is the reverse reaction to adsorption. It refers to the release of P from the soil solid phase to the soil solution. The desorption reaction occurs in soil when the P concentration is depleted to a very low level in the soil solution by plant uptake, runoff or leaching. P desorption kinetics is first estimated using anion exchange resin and / or anionic extractant to investigate the availability of soil P to plant roots (Sharpley et al., 1981). The amount of P desorption is highly affected by the time allowed for desorption and the water (solution)/ soil ratio during desorption (Barrow, 1979). It was observed that the P desorption reactions occur very fast and then decrease when approaching equilibrium (Vadas and Sims et al., 2002). Only a small fraction of adsorbed P in most soils is readily absorbable (Pierzynski et al., 2000). Increasing the concentration of competing ions for organic ligands (e.g. oxalate and citrate) contributes to a significant amount of desorbed P (Hinsinger, 2001).

### **1.3.2 Precipitation and dissolution of phosphorus**

Precipitation is the reverse process of mineral dissolution in soil. Insoluble compounds of P in soil solution lead to the precipitation of P in soil. The most common forms of precipitated P include the products of reactions between soluble, ionic P and Ca,

Al and Fe. In acidic soil, soluble Al and Fe dominate soluble cations and are the major P precipitates. The presence of amorphous or partially crystalline Fe and Al oxides in acidic soil retains P in crystal structures such as Fe-P and Al-P (Pierzynski et al., 2000). In neutral and calcareous soils,  $\text{Ca}^{+2}$  is the dominant soluble cation. The addition of soluble P results in the formation of dicalcium phosphate ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) which converts over time to octacalcium phosphate ( $\text{Ca}_8\text{H}(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ ) and over the long term to apatite ( $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$ ) (Samadi and Gilkes, 1998; Bertrand et al., 2003). Additionally, precipitation can occur due to the addition of chemical fertilizers or manure. Soluble P is rapidly and irreversibly “fixed” and is difficult to desorb. This process is referred to as “adsorption hysteresis”. Adsorption hysteresis reflects the transformation of soluble P into very unavailable forms by processes such as precipitation (Cohen, 1992; Pierzynski, 2000; McCmaller, 2015). The dissolution of precipitates in acidic and calcareous soils is highly dependent on soil pH. The solubility and availability of P for plant uptake, but also loss by runoff and leaching, is generally greatest under slightly acidic soil conditions (pH 6.0 to 6.5) (Hinsinger, 2001; Bera et al., 2006).

### **1.3.3 Mineralization and immobilization of soil phosphorus**

Phosphorus mineralization and immobilization are simultaneous reactions in the soil system. Mineralization is the conversion from organic P forms to inorganic soluble P forms. Immobilization is the conversion of the inorganic form of P, potentially plant-available P into organic P (unavailable P). Both conversions require microbial organism activity. The rate of organic P mineralization was measured at between 1.4 and 2.5 mg P  $\text{kg}^{-1}$  per day in arable soils (Frossard et al., 2011).

A number of soil factors determine whether P is mineralized or immobilized such as soil OM, soil temperature, soil moisture, soil texture, and soil pH. These factors affect the soil microorganism activity to decompose organic matter and facilitate P release. However, the C:P ratio of the material being decomposed by soil microorganisms is the most important determinant affecting soil P availability and P pools (Dalal and Hallsworth, 1976) as the C:P ratio of the organic matter influences whether there will be initial net mineralization or initial immobilization of soil P. It was proposed that the C:P ratio of organic matter can be a good indication of P availability (Gagnon and Simard, 1999). Alamgir et al. (2012) reported that a C: P ratio of 200 is typically used as an indication of the threshold for mineralization and

immobilization: a C: P ratio > 200 will induce P immobilization and depletion of soil P pools, whereas a C: P ratio < 200 will result in mineralization and increase P availability and P pools (Alamgir et al. 2012).

Organic P in soils and P added to soils as organic wastes or crop residues represent an important source of P for plant growth. Organic P is also a potential source of soluble P that can be lost in runoff or leachate. Mineralization refers to the decomposition of organic matter by soil microorganisms to release inorganic forms of P into soil solution (Pierzynski et al., 2000; Hinsinger, 2001). Immobilization refers to the conversion of mineral P by soil microorganisms into biochemical compounds essential for microbial metabolism. The amount of P in organic residues is another important factor for regulating the quantity of soluble P in the soil (Griffin et al., 2003; Azeez and Van Averbek, 2010).

There is a critical P content of 0.2-0.3% above which there is no net immobilization from organic matter (Laoski and Lamb, 2003; Gichangi et al., 2009). The release of orthophosphate from organic or inorganic P anions (Figure 1-2) is affected by mineralization-immobilization, sorption-desorption processes, and soil pH. The fluctuation of soil moisture (drying and rewetting process) can affect microbial activity and biomass which in turn affects P mineralization (Erinle et al., 2018).

#### **1.4 Procedures to measure bioavailable phosphorus in the soil**

Phosphorus has a dynamic behavior in soil. Scientists have confirmed that moving soil samples from farms and processing them in laboratory conditions can result in inaccurate measurements of different forms of P. It has been suggested that it is preferable to do available P measurement *in situ* (Menon et al., 1989; Menon et al., 1991; Zhang et al., 1998b). The assessment of P concentration in soil has been commonly made by *ex-situ* methods, such as the Olsen-P method (Olsen, 1954), the Colwell method (Colwell, 1963), the Bray phosphate method (Bray and Kurtz, 1945) and the lactate-extraction phosphate method which is the most common method used in Australia

**Table 1-1 Examples of common soil P extraction methods and their conditions. Adopted from Moody and Bolland (2003).**

<b>Method</b>	<b>Extractant</b>	<b>Soil/extractant ratio</b>	<b>Extraction period</b>	<b>Reference</b>
Ammonium lactic acetic acid	0.1 Ammonium lactate+ 0.4M acetic	1:2	30 min	Egner et al. (1960)
Bray1	0.03 M NH <sub>4</sub> F in 0.025 M HCl	1:7	60 s	Bray and Kurtz (1945)
Bray 2	0.03 M NH <sub>4</sub> F in 0.1 M HCl	1:7	40 s	Egner et al. (1960)
BSES	0.005 M H <sub>2</sub> SO <sub>4</sub>	1:20	16 h	Keer and Von Stieglitz (1938)
Calcium acetate lactate	0.1 M calcium lactate + 0.01 M calcium + 0.3 M acetic acid	1:20	2 h	Schuller (1969)
Calcium chloride	0.005 M	1:5	18 h	Moody et al. (1983)
Colwell	0.5 M NaHCO <sub>3</sub> . pH 8.5	1:100	16 h	Colwell (1963)
Equilibrium P concentration	0.01 M CaCl <sub>2</sub>	1:10	20 min	Moody (1963)
Lactate	0.02 M calcium lactate m 0.01 M HCl	1:50	90min	Holford et al. (1985)
Flurite	0.5 NH <sub>4</sub> F	1:50	90 min	Holford et al. (1985)
Mehlich 1	0.05M H <sub>2</sub> SO <sub>4</sub>	1:5 m	5 min	Mehlich (1953)
Olsen	0.5M (NaHCO <sub>3</sub> ) pH 8.5	1:20	30	Olsen et al. (1954)
P1	Iron Oxide impregnated in 0.01 M CaCl <sub>2</sub>	1:40	5 min	Menon et al. (1989)
Troug	0.001 M H <sub>2</sub> SO <sub>4</sub> + 0.3% (NH <sub>4</sub> ) SO <sub>4</sub>	1:200	30 min	Troug (1930)

(Holford et al., 1985). Solutions used in these extraction methods contain a competing ion that substitutes exchangeable orthophosphate. The reagents used in these various tests in the soil are listed in Table 1-1

Fixen and Gorven (1990) reported that the extractants listed in Table 1-1 can also reflect soil P quantity or intensity to identify soil P buffering capacity. The quantity of available P is a dynamic fraction that can only be approximately estimated by any extractant or chemical measurement.

It has been noted that these competing ions, in some conditions, overestimate the concentration of available P. Extraction solutions used in *ex situ* methods also solubilize more inert forms of P (non-labile P or stable forms of P) or the soluble portion of total P from the soil (Young et al., 1996). In some conditions, the extraction solutions fail to extract the plant available P because they may not be the most suitable solutions for some soil properties and soil types (Tessier and Turner, 1995) as in the Bray (Bray and Kurtz, 1945) and Mehlich methods (Mehlich, 1978). Significant variation in soil types and pH provides challenges for each soil test. Clay soils can behave differently to sandy soils in response to soil: solution dilution ratios in different soil extraction procedures (Mason et al., 2013). P extracted by acid reagents in extraction methods such as Bray-1 (Bray and Kurtz, 1945) and Mehlich-3 (Mehlich, 1984) tend to extract large amounts of P from calcareous soil (Six et al., 2012). The reagents using bicarbonate ions such as Olsen-P (Olsen et al., 1954) and Colwell-P (Colwell, 1963) may release P from Al-P and Fe-P complexes which are stable and unavailable forms for plant uptake (Six et al., 2012). The bicarbonate extraction methods of Olsen (Olsen, 1954) and Colwell (Colwell, 1963), were initially developed for calcareous soils, though are being used for both acid and alkaline soils (Tessier and Turner, 1995; Menzies et al., 2005). However, it has been reported that the Olsen method was less effective than the acidic extractants in predicting available P in acidic soils (Chen and Ma, 1998). The hydrolysis of organic P to orthophosphate is increased by long exposure to heat and colour development which occurs in the Cowell-P test (Murphy and Riley, 1962).

Soil P buffering capacity is another parameter used to estimate the available P in the soil. After available P, buffering capacity (PBC) is potentially the most useful soil P measurement that influences the amount of P fertilizer available for plant adsorption (Burkitt et al., 2002). PBC can be defined as the resistance of the concentration of P solution to change (Holford, 1997). Therefore, it can be described as buffering P solution concentration against increasing solution P after fertilization or buffering the



concentration of solution P against decreasing its concentration after plant uptake (Holford, 1997). PBC is important in characterizing fertilizer effectiveness and characterizing P desorption and the diffusion of labile P (Holford, 1997). Generally, PBC is determined from the slope of sorption curve by adding a range of known P concentrations to the soil. The amount of sorbed P is measured after a period of equilibrium. Then, the sorbed P data are fitted to Freundlich and Langmuir equations to present the P sorption curve. In order to accurately fit the sorption curve, between 5 to 10 different additions of known P concentrations are often used. This method was criticized because it is time-consuming, labour-intensive and not commercially viable in Australian soil testing laboratories (Burkitt et al., 2002).

Another parameter, the P buffering index (PBI), has been proposed to provide broader information to improve the interpretation of Colwell P results (Moody, 2007). The PBI measurement gives an indication of soil tendency to fix P (Mason et al., 2010). It is a quicker measurement than producing the P sorption curve, which requires a range of P application rates to measure the soil PBC. The PBI was found to be highly correlated with the soil PBC measurement (Burkitt et al., 2002). The PBI can be measured from the difference between the amount of P added to the soil and the amount of P remaining in the solution, which gives the amount of P sorbed. Moody (2007) combined PBI with Colwell P to describe the forms of P pools in soil as the following: Pool (1) is the solution P (P-Intensity) which is the most available P form for plant uptake, Pool (2) is the P-Quantity, which represents the form of P that will become available when the intensity pool decreases, and finally Pool (3) the fixed P or unavailable P which is unavailable for plant uptake. In soils having low PBI (< 15) the proportion of P between pools favors the solution P (Pool 1) relative to the soil that has high PBI (>840) where the supply from Pool 2 to Pool 1 is smaller. Therefore, the higher PBI soil will need larger Pool 2 P (the quantity to provide the same amount of available P in Pool 1). Although the combination of the critical concentration of Colwell P and the PBI to adjusted Colwell P measurement, it appeared that the DGT technique better predicts the plant response to the application of P fertilizers, regardless of soil type (Mason et al., 2010). The PBI measurements depend on the Colwell method using the bicarbonate extractant, which extracts the adsorb-desorb P form and the dissolved Ca-P compounds by reducing  $\text{Ca}^{2+}$  ion activity. This reaction can displace the stable forms of P associated with Ca in alkaline soils and Fe and Al in

acidic soils through a similar process. Therefore, P measured by this extractant may provide overestimated results (Bertrand et al., 2003).

As a result, the resin absorption method has been used as an alternative for extracting labile P from soil (Fixen and Grove, 1990; Abrams and Jarrell, 1992; Cooperband and Logan, 1994). Soils are shaken with anion exchange resins strips in distilled water for 16 h. P ions are then extracted from the soil on to the resin (McLaughlin et al., 1993). Then, the resin is separated from resin strips by rinsing with distilled water. P ions within the resin are then eluted with an acid or salt solution. The phosphate retained on the anion exchangeable strip is measured by shaking the strip directly with colorimetric phosphate reagent. The amount of phosphate accumulated by the resin is positively correlated with P uptake by plants (Saggar et al., 1990; Abrams and Jarrell, 1992).

The conventional soil extraction methods (acid, bicarbonate, resin extraction, and  $\text{CaCl}_2$  extraction) provide an indication of available phosphate to plants. However, these methods also include the soluble portion of total P in soil (Mason and McNeill, 2005b). For example Mehlich-1 (Moya et al., 1993) and Bray-1 (Bray and Kurtz, 1945) both use acid extractants to acidify soil samples, which can probably cause the release of the stable carbonate form of P.

It was reported that P extracted by the Colwell method will estimate the concentration of available P in soil (Moody and Bolland, 2003). But, Bertrand et al. (2003) suggested that extraction reagents of Colwell method may dissolve the fixed P in the solid-phase in calcareous soils. In tropical acidic soils, the conventional extraction methods of P from soil including oxalate, Olsen, Colwell, Bary-1 and Mehlich-3 methods which are also measured significant amounts of non-labile P. Other criticisms of the conventional P soil tests are that the ratio of solution to soil is very small and that the reagents used, such as bicarbonate solutions, can solubilize a stable form of P which is not available for plant uptake. Additionally, most soil analyses are conducted by taking surface soil samples from a depth of 0-15 cm. However, plant roots usually penetrate beyond this depth and based solely on soil surface samples may underestimate available P and other nutrients available for plant use. Therefore, subsoil contributions in supplying nutrients to labile pools should not be ignored (Jungk, 1991). Comprehensive soil tests involving various soil depths would provide better results (McLaughlin et al., 2000a).

The development of better soil test methods for plant available P will maximize fertilizer efficiency and reduce the negative impacts of over-fertilizing. Therefore, there is a critical need for a rapid, accurate and simple method to measure the bioavailable P concentration in soils. This can be used to predict better fertilizer regimes which may reduce economic costs as well as soil and water pollution (Colwell, 1963; Holford, 1985; Moody, 1990; Bertrand, 2003; McBeath, 2005; Mason, 2010).

## **1.5 Phosphorus percolation and connection with surface waters**

Phosphorus deficiency in soil is usually compensated for by adding organic or inorganic P fertilizers (Withers and Lord, 2002). When P input to the soil exceeds the needs of the crop, most of the excess P will be accumulated in the soil (Hansen et al., 2002). Some of this accumulated P will be transformed into inactive or less active forms over time though will be susceptible to transport processes (Feng et al., 2011). Studying the P balance in the soil gives an indication of P losses from the soil to surface water or - groundwater (Feng et al., 2011). The link between P balance in the soil, P losses and P received by water is a complicated process due to the strong tendency of soil to bind P (Hansen et al., 2002). Most research efforts have focused on the surface run-off of P from agricultural lands into surface water resources (Heathwaite et al., 2005; Withers and Haygarth, 2007). It is generally considered that the mobility of P through the soil is limited due to the adsorption of P onto metal oxyhydroxides which retain the majority of P in the soil solution and limit its supply to groundwater (Kilroy and Coxon, 2005; Heathwaite et al., 2006). In particular, P is unable to leach through the profile of clay soils because P will be adsorbed to iron and aluminum hydroxides and oxides (Rajan et al., 1974; Bolt, 1976). However, recent research has proved that leaching could increase P mobility from soil to the leachate water then to the surface water (Holman et al., 2008). This kind of P transfer could take place in special conditions such as sandy soils or soil with high organic matter or soil saturated with P from long term P fertilizer application.

Holman et al. (2008) presented the first international assessment of P concentrations in groundwater by using data from Ireland, Northern Ireland, Scotland, England and Wales. Their results suggest that groundwater P concentration might be a more important contributor to surface water than was previously assumed. However, the

main sources of P contamination in groundwater systems are in agricultural lands and water purification/ distribution systems.

Agricultural land is considered a substantial contributor to groundwater pollution by plant nutrients. This is particularly the case with agricultural practices which include large inputs of P fertilizers combined with irrigation on coarse soil and shallow groundwater (Jalali and Kolahchi, 2008). As a result of the lack of information about accurate P requirements for crops, P is usually added in excess quantities (Sims et al., 2000). It is well documented that the excessive application of P fertilizers has led to P accumulation in many soils and subsequent increased risk of leaching loss (Heckrath et al., 1995; Sharpley, 1995). Losses of P are related to the concentration of P in the soil which is strongly influenced by P additions from fertilizers and manure (McDowell et al., 2001). Researchers over the last three to four decades have pointed out that organic P is mobile in the soil profile and a significant proportion of organic P can exist in the leachate water from grasslands (Hannapel et al., 1964; Frossard et al., 1989).

The ability of groundwater to deliver P to the surface water was shown by Foy (2005) especially in temperate regions during maximum biological demand (House, 2003). Holman et al. (2008) confirmed that in some cases groundwater P has the potential to increase the concentration of available P in the surface water and cause deterioration of ecosystem quality. Recent studies on bio-available P concentrations in leachate water suggest that this transfer is more active than previously assumed given that the amount of P transferred to leachate water is normally ignored (Kitagishi and Obata, 1981; Wood et al., 2005).

## **1.6 Sources of phosphorus fertilizers**

The global green revolution hugely increased global demand for higher crop production. As P is the limiting nutrient for plant growth and production, P fertilizers are substances that can be applied to the soil to increase crop yields and quality (Pierzynski et al., 2000). The main source of P in nature is the weathering of P minerals in rocks and organic soil P (biological accumulation by plant and soil microbial biomass). Phosphate-rich parent materials are commonly classified into the following three apatite groups (Borlaug and Dowswell, (1994):

- Fluorapatite,  $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$ , found mainly in soils derived from igneous and metamorphic environments.
- Hydroxyapatite,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , found in biogenic layers like bone deposits in addition to igneous and metamorphic environments.
- Carbonate - hydroxyapatite,  $\text{Ca}_{10}(\text{PO}_4\text{CO}_3)_6(\text{OH})_2$ , found mainly in caves and on islands as part of bird and bat excrements and guano.
- Carbonate Hydroxyl fluorapatites  $\text{Ca}_{10}(\text{PO}_4, \text{CO}_3)_6\text{F}_{2-3}$  (Peltzer et al., 2010).

Eighty per cent of the world's phosphate rock production is derived from deposits of sedimentary marine origin, with some 17% derived from igneous rocks and their weathered derivatives. The remainder comes from sedimentary and guano type deposits. More than 40% of these deposits are used as raw material for manufacturing P fertilizers (Zapata and Roy, 2004). The procedure of manufacturing superphosphate fertilizers [triple superphosphate (TSP) and the single super phosphate (SSP)] uses wet acidulation of P rocks. This process enriches the P fertilizer and increases its solubility to become more available and accessible to plant roots (Kuo et al., 2007). However, the acidulation process also enriches Cd and enhances Cd availability to plants in the P fertilizer product (Huang et al., 2004). The chemical composition of Cd in phosphate fertilizers has not been clearly identified but is assumed to be reacted with Ca in both the phosphate and sulfate components of the fertilizers. Phyto-availability of Cd in triple superphosphate (TSP) and diammonium phosphate (DAP) was compared with a known Cd compound. The comparison study revealed that Cd in phosphoric fertilizers has a similar phyto-availability to  $\text{Cd}(\text{H}_2\text{PO}_4)_2$  or  $\text{CdHPO}_4$  or a mixture of these two salts (Mortvedt and Osborn, 1982).

The overall composition of phosphate rock mostly depends on its type and origin. Sedimentary rocks contain high concentrations of heavy elements, such as the rare earth elements uranium (U), thorium (Th) and rhenium (Re) (Hurst, 1987). In addition, these rocks may contain elements such as cadmium (Cd), arsenic (As), antimony (Sb), vanadium (V), zinc (Zn), copper (Cu) and nickel (Ni), many of which are micronutrients at low concentrations but are all toxic at higher concentrations. The amounts of these hazardous elements vary widely not only among various phosphate rock sources but also within the same deposits (Stoica et al., 1997). Currently, there are no commercial means of completely removing these pollutants during the P fertilizer manufacturing process;

the only controlled way is to use pure phosphate rock to make fertilizer (Stoica et al., 1997). There is a worldwide concern that persistent fertilizer uses causes soil and environmental pollution. Currently, the main phosphate rocks for the manufacturing of phosphoric fertilizers in Australia are from oceanic sedimentary guano-based sources that have Cd concentration ranging from 42-100 mg Cd kg<sup>-1</sup> P (McLaughlin et al., 1997b). Generally, these sources of phosphate rock have higher Cd concentrations than rocks found in other countries (McLaughlin et al., 1997b), as indicated in Table 1-2.

Approximately 100 million tonnes of phosphate rock have been imported to Australia over the last 100 years to supply approximately 15 million tonnes of fertilizer (McLaughlin et al., 1992). Based on this amount, it could be estimated that more than 6000 tonnes of Cd have been added to Australian soils through the use of phosphoric fertilizer in the last 90 years (McLaughlin et al., 1992).

**Table 1-2 Cadmium and phosphorus contents of phosphate rocks from different countries (McLaughlin et al., 1996)**

Phosphate rock	Cd µg kg <sup>-1</sup>	P%	Cd mg kg P <sup>-1</sup>	Reference
Australia (Christmas Island)	43	15.3	275	David et al. (1978)
Australia (Nauru)	100	15.6	641	Syers et al. (1986)
Egypt (Hamrawein)	9	12.7	6.7	McLaughlin et al. (1996)
Syria	5	13.1	38	McLaughlin et al. (1996)
Australia (Duchess)	7	13.9	50	Williams (1974)
Peru	11	13.1	84	Syers et al. (1986)
Western USA	60-340	NR	NR	Auer (1977)

At present, the phosphorus resources industry is facing several problems, such as shortages of phosphate resources, the expansion of phosphorus fertilizer production capacities and serious environmental pollution problems resulting from industrial development (Al Rawashdeh and Maxwell, 2011).

## 1.7 Cadmium in soils and its accumulation through phosphorus fertilizer application

Cadmium (Cd) is a non-essential element potentially accumulated in plant tissues (Lambert et al., 2007). The main sources of Cd in nature include natural weathering of pedagogical rocks, anthropogenic activities such as mining industries, sewage sludge application, and the use of phosphate fertilizers (Devkota and Schmidt, 2000). Table 1-3 presents the concentration of Cd in some P fertilizers widely used in Australia. Although P fertilizers improve plant growth and yield quality, P fertilizers are a major source of trace element enrichment in soils especially Cd (Mann et al., 2002; McLaughlin et al., 2011; Jiao et al., 2012) (Table 1-3).

**Table 1-3 Concentration of Cd and P in commonly used P fertilizers (McLaughlin et al., 1997b)**

Fertilizer	Cd (mg kg <sup>-1</sup> )	P%	Cd:P (mg Cd kg <sup>-1</sup> P)
SSP	15.1	10	151
NCPR	40.3	12.9	312
PAPR	32	10.8	296
HPR	5.3	12.5	42

Note: SSP: Single superphosphate, NCPR: North Carolina phosphate rock, PAPR: partially acidulated phosphate rock and HPR: Hamrawin phosphate rock

Inorganic phosphorus fertilizers usually have between 0-150 mg kg<sup>-1</sup> Cd because Cd is a common impurity in phosphoric fertilizers (Wild, 2001; Mapanda et al., 2005; Pe'rez and Anderson, 2009; Tijani, 2009). It has been reported that the Cd content in the original phosphate rocks is not changed by the P fertilizer manufacturing processes. Therefore, Cd content in the original rock transfers to the final fertilizer product in the same proportion (Williams and David, 1977; David et al., 1978). The main rock P sources are oceanic sedimentary, guano-based deposits, containing between 42-99 mg Cd kg<sup>-1</sup> (McLaughlin, 1991). Generally, these island sources of phosphate rock have higher Cd concentrations than rocks found in other countries (McLaughlin, 1991). There is limited data available about the Cd status in virgin (unfertilized) soil in Australia (Table 1-4).

**Table 1-4 Comparison of cadmium concentration (mg kg<sup>-1</sup>) in unfertilized and fertilized Australian soils (Mclaughlin et al., 1996)**

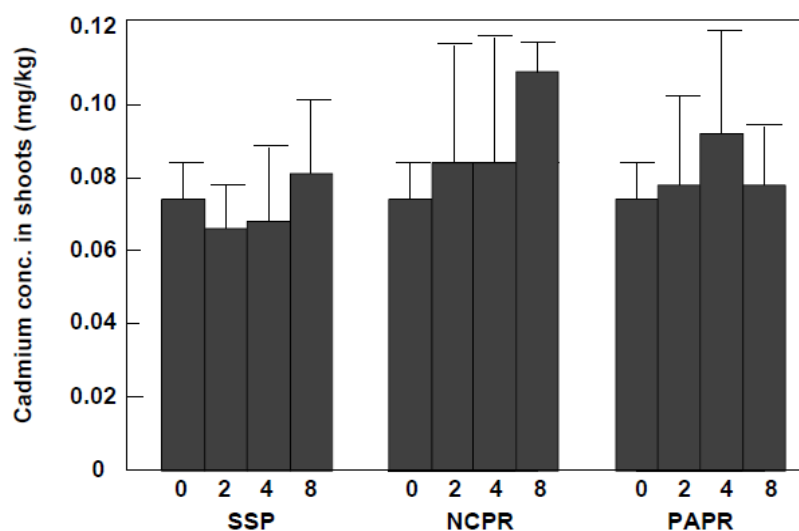
Reference	Cd (mg kg <sup>-1</sup> )	Cd range (mg kg <sup>-1</sup> )	Extraction method	Notes
<b>Unfertilized soil</b>				
Williams and David (1976)	0.05	0.02-0.13	1.0 N HCl, 18 h	NSW paired site study
Hanson (1988)	0.97	0.06 -2.02	0.05 M EDTA, pH 6, 4 h	VIC soil, 0- 2.5 mm
	0.51	0.02 – 7.02		VIC soil, 0 -100 mm
Merry and Tiller (1991)	0.11	0.01 -0.29	0.1 m EDTA, pH 6, 168 h	SA soils, 0 -50 mm
	0.06	0.01 – 0.20		Sa soils, 50 -100 mm
Olszowny et al. (1993)	< 0.50	<0.50	HNO <sub>3</sub> /H <sub>2</sub> O <sub>2</sub> / HCl	Soil in Old National Park, 0-150 mm
<b>Fertilized soils</b>				
Hilliard et al. (1988)	0.056	0.2 – 13.9	0.05 M EDTA, pH 6, 4 h	0 – 100 mm
Hanson (1988)	0.82	0.2 – 13.9	0.05 M EDTA, pH 6, 4 h	0 – 100 mm
Merry and Tiller (1991)	0.18	0.01 – 0.73	0.1 EDTA, pH 6.0, 168 h	0 – 50 mm
	0.09	0.01 – 0.49		50 – 100 mm



Table 1-4 presents a comparison of the Cd concentrations in the soil before and after P fertilization in seven sites (Williams and David, 1976).

Phosphorus fertilizers have elevated soil Cd concentrations to levels of concern, due to its accumulation behavior in soil (McLaughlin et al., 1996; McLaughlin et al., 2000) and the long persistence period of Cd in soils (José L.R. Gallego et al., 2002). The fertilizer type and dose also affect the concentration of Cd in soil and plants. Hamon et al. (1998) investigated the changes in total and available concentration of Cd in soil that had received Cd from the application of SSP fertilizer at different doses since 1948. The results showed that the concentration of Cd was highly correlated with the application dose of SSP.

McLaughlin et al. (1997b) carried out a study to investigate the relationship between P fertilizer types and the concentration of Cd in clover herbage cultivated on Australian soils. Three types of P fertilizers were used: SSP, North Carolina phosphate rocks (NCPR) and PAPR (an experimental batch manufactured by 50% acidulation of NCPR with sulfuric acid) on five different sites, together with an unfertilized site as a control. The results demonstrated that (i) there were notable differences in Cd concentrations in the soils and clover shoots of the fertilized sites compared with the control and (ii) an obvious difference in Cd concentration in the clover tissues between different P fertilizer types and dose (Figure 1.3).



**Figure 1-3** The effect of phosphate fertilizer type (SSP, NCPR, and PAPR) and dose on Cd concentration in clover herbage, adopted from McLaughlin et al. (1997b).

Cadmium persists in soils for long periods and has the capacity to be transferred into food chains (Judith et al., 2008; Malizia et al., 2012). Cadmium transfer from soil to plant tissues or the edible parts of the plants depends on soil properties, plant species and cultivation management. Soil properties, such as soil pH, organic matter, percentage of other nutrients, clay content, and type of clay mineral, Fe and Mn oxide, all affect the amount and dose of Cd taken up by the plant (Holm et al., 2003; Chen et al., 2007). Plant factors affecting Cd uptake are plant species and plant age while soil management factors include soil preparation, liming, and crop rotation (Mench, 1998). Crops grown on metal-contaminated soils accumulate metals in quantities that may cause clinical problems for both animals and humans once consumed (Tiller et al., 1988). The physiological response of each plant controls the degree of metal uptake and its bioaccumulation. Roberts et al. (1994) and Lee et al. (1996) noted that 20% of the kidneys of grazing animals tested in New Zealand were found to contain a Cd concentration of 1 mg kg<sup>-1</sup> fresh weight. The maximum permitted the concentration of Cd set by ANZECC (1995) in soil quality was 3 mg kg<sup>-1</sup> (ARMCANZ, 1995). Also, it was advised that kidneys from older animals should be excluded from shipments for human consumption because Cd accumulation in the kidneys increases with an animal's age (Morcombe et al., 1994). Once Cd is in the food chain, human ingestion of foods containing small concentrations of Cd is unavoidable (Kabata-Pendias and Mukherjee, 2007; Chaney and Donald, 2012; Chen et al., 2014).

Soil is not only where Cd accumulates; it is also the possible transport pathway of Cd to groundwater (Chen and Ma, 1998; Chen et al., 2014). The low mobility of Cd in the soil had led to the assumption that Cd was unlikely to contaminate groundwater (Loganathan and Hedley, 1997). This assumption was confirmed by Hamon et al. (1998) that there was no offsite movement of Cd, either through surface or lateral flow or via leaching water in clay loam soil. However, Bramley (1990) and Loganathan et al. (2003) found that most Cd applied to undisturbed soils remain in the topsoil near the active plant roots. Taylor (1997) found that over 80% of the Cd added to the soil via phosphate fertilizers may stay in the topsoil. Tijani (2009) reported that there is a high possibility of Cd and heavy metals transfer to shallow groundwater through infiltration and leaching from the topsoil. Mann and Ritchie (1995) reported that there is a great risk from the leaching of Cd to groundwater, especially in sandy soils. These contradictory results

indicate that the measurement methods used to evaluate the concentration of available Cd in soil and water were not applicable to all soil types and conditions.

### **1.8 Measuring trace metals concentrations and bio-availability in soil**

It has been reported that the measurement of total concentrations of trace elements does not give a reliable prediction of their bioavailable pool due to the complexity of their soil chemistry and plant uptake mechanisms (Sauvé et al., 1998; McGrath et al., 1999; Zhang et al., 2001a). In soil systems, there is pseudo-equilibrium between the soil solid phase and soil solution phase as a result of processes operating as sources and sinks for the elements. Consequently, methods that involved the separation of the solution and solid phases can disrupt this distribution of nutrient species (Hooda, 2003). The best method of measuring metal or nutrient bioavailability to plants would be a technique that does not involve any disturbance of field soil conditions (McLaughlin et al., 2000a).

### **1.9 Measurement of cadmium in soil**

Precisely measuring the labile Cd in soil solution is the first priority for health risk assessments and crop production quality (Tiller et al., 1988). Many methods have been proposed for Cd assessment in the soil; most of these are *ex-situ* methods. In the past, research work was hindered by the inability of analytical instrumentation to precisely determine labile Cd (available Cd) in soil (McLaughlin et al., 2000b). Usually, the concentration of labile Cd in the soil is low, thus some of the early instruments were unable to detect the low concentration of labile Cd or the changes in its concentration (McLaughlin et al., 2000b). For this reason, measurement of labile Cd in soil was built on the principle of extracting labile Cd using strong extractant solutions. Table 1-5 shows some of these extractants which have different reactions with Cd.

**Table 1-5 Extractants used to extract the available form of Cd in the soil (Wang et al., 2016; Wang et al., 2017)**

<b>Extractant</b>	<b>Strength (M)</b>	<b>Solution: soil ratio</b>	<b>pH</b>	<b>Shaking time (h)</b>	<b>References</b>
<b>Complexant- based extractions</b>					
DTPA +CaCl <sub>2</sub> + TEA	0.1	10:20	7.3	2	Lindsay and Norvell,(1978)
NH <sub>4</sub> OAc	1	5:1	4.8	1	De Vries et al., ((2005)
HOAc	0.11	5:1		16	Quevauviller (1998)
EDTA	0.05	10:1	7.0	2	Feng et al. (2005)
NaOAc	0.05	5:1		2	Kaplan et al. (2009)
Na <sub>2</sub> EDTA	0.1	2.5:1	7.3	168	Clayton and Tiller (1979)
<b>Inorganic salt- based extractions</b>					
CaCl <sub>2</sub>	0.01	40: 1		3	Qasim et al. (2015)
Ca(NO <sub>3</sub> ) <sub>2</sub>	0.1	5:1		2	Mench et al.,(1994)
NH <sub>4</sub> NO <sub>3</sub>	1	10:1	-	16	Smolders and Jelle (2013)
MgCl <sub>2</sub>	1	8:1		2	Krishnamurti et al. (1995)
NH <sub>4</sub> Cl	1	6:1		16	Krishnamurti et al. (1995)
<b>Inorganic acid – based extractions</b>					
HNO <sub>3</sub>	0.5	5:1		0.5	Van Ranst and Gent (1999)
HCl	0.1	25:1		2.5	Fizman et al. (1984)
<b>Distilled water soil solution</b>					
Distilled water		1:10		16	Milićević et al. (2017)
Distilled water		1:10		2	Pueyo et al. (2004)

Initially, strong chelating agents including ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepenta acetate (DTPA) (De Vries et al., 2005) or strong acids such as HNO<sub>3</sub> and HCl (Van Ranst and Gent, 1999) were used. The use of weaker extractants was subsequently proposed for measuring Cd in soil. Some of these extractants are listed in Table 1-5 such as 0.01 M CaCl<sub>2</sub> (Van Ranst and Gent, 1999) or NH<sub>4</sub>NO<sub>3</sub> (Udawatta et al., 2006). Solution concentration methods (Luo et al., 2010) using 1 M NH<sub>4</sub>NO<sub>3</sub>, the free ion activity model, single or sequential extraction methods (Menzies et al., 2005; Menzies et al., 2007; Feng et al., 2011) and the isotope dilution exchange method are now widely applied to investigate Cd bioavailability in soil solution.

The isotopic technique estimates the chemically reactive soil metal pool. The isotopic dilution procedure was used to determine the isotopically exchangeable (labile) pool. In this technique, the radioisotope of Cd is mixed thoroughly with the soil. Plants taking up metal solely from the labile pool will have some isotopic ratio as labile Cd reservoir in the soil. This quantity is called the L-value of the soil which is the biological estimate of labile Cd (Hamon et al., 1997). The E-value is the chemical estimate of the radiolabile Cd pool. It was found that the E-value is greater than the L-value by a factor of 1.05 and 1.4, suggesting that rhizosphere conditions caused solubilization of the non-labile soil metal fraction. However, Hutchinson et al. (2000) found that the Cd E-value had no relation with the concentration of Cd in the plant tissue. This suggests that in the soils, plants did not mobilize non-labile soil Cd by producing root exudates or altering rhizosphere pH. Nolan et al, (2005) concluded that the DGT technique was the best in describing Cd adsorption in the wheat plant when CaCl<sub>2</sub> extraction and isotopic dilution techniques were used as well. These approaches have simple operation procedures and are less time-consuming.

The *ex-situ* methods are not of great significance when evaluating Cd bioavailability in a static manner (Hooda et al., 1999b; Black et al., 2011). These equilibrium-based approaches do not consider the depletion of analytes at the root-soil interface and depletion-induced resupply from the solid phase (Feng et al., 2005; Menzies et al., 2007). The concentration of Cd in soils is expected to increase due to the excessive use of P fertilizers (McLaughlin et al., 2011; Senila et al., 2012). Therefore, the assessment of Cd content in soil and the estimation of its transfer dose to vegetation are of great interest (Malizia et al., 2012).

Most of the quality guidelines in the National Environment Protection Measure (NEPM) are based on total Cd in soils (McLaughlin et al., 2000a), which means the

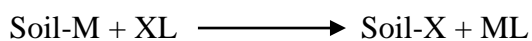
content of both bioavailable and non-bioavailable fractions (McLaughlin et al., 2000a; Zhang et al., 2001b; Ahumada et al., 2011). In general, conventional procedures for determining total Cd concentration are based on single and sequential extractions rather than continuous measurement (Kaplan et al., 2011; Senila et al., 2011). In addition, the pre-treatment of these procedures affects the physio-chemical equilibriums of Cd in soil samples (Zhang et al., 1998a). All these constraints lead to concerns about the quality of the measurements of labile Cd in soil. The conventional procedures used to determine total Cd concentration are described below.

### **1.9.1 Dissolution**

Dissolution processes are defined as metals being released to the soil solution from the soil solid phase. In this technique, hot concentrated acids are used and mineral and organic particles are solubilized or oxidized to release metals for analysis (McLaughlin et al., 2000). This method may over-estimate the metal concentration by releasing the structural form of the metal (Helmke et al., 1997) or it may form a mixed mineral phase in the soil, co-precipitated with Fe or Al oxides thus solubility may not represent true mineral behavior (McLaughlin et al., 2000b).

### **1.9.2 Complexation**

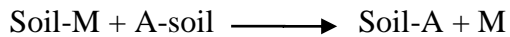
The mechanism of complexation is when the metal (M) is paired with a ligand supplied by a metal-ligand salt (XL). The effective charge on the metal will be changed, allowing metal desorption. This helps to retain the metal ion in complex form for subsequent analysis:



The effectiveness of this reaction is dependent on the reactive association constant between metal and complexing ion for the soil sorption surface. The most widely used examples are DTPA (diethylenetriamine pentaacetate) and EDTA (Table 1-5). Clayton (1979) mentioned that the saturation of the chelate could happen in some highly polluted soils and suggested that the concentration of the chelate be doubled.

### 1.9.3 Desorption/ion exchange

The basis of this procedure is that the metal (M) is removed from the soil solid phase by swapping it with a desorbing cation (A). This reaction then removes the metal from the soil surface and brings it into a solution for analysis as in the following equation:



Examples of these extractants are neutral salt solutions such as 1.0 M  $\text{NH}_4\text{NO}_3$  (Symeonides and McRae, 1977), 0.1 M  $\text{NaNO}_3$  (Gupta and Aten, 1993; Hani, 1996), 0.01/0.1 M  $\text{CaCl}_2$  or  $\text{Ca}(\text{NO}_3)_2$  (Fujii et al., 1983; McLaughlin et al., 1997a) and  $\text{MgCl}_2$  (Shuman, 1979; Krishnamurtia et al., 1995). This method usually extracts very low analyte concentrations due to the strong bonding of the metal to soil mineral and organic surfaces (McLaughlin et al., 2000). The Cl salt extracts a relatively high concentration of metal, due to the complexation of free metal ions by Cl salts compared with  $\text{NO}_3$  salts. Therefore, this procedure is more suitable to measure free metal activities in solution (Fujii et al., 1983) rather than metal bioavailability (McLaughlin et al., 2000b).

### 1.9.4 Oxidation/reduction

In this procedure, several extractants are used to extract metals bound to organic matter or metal sulfides by oxidation using peroxide, hypochlorite or hot concentrated acid solutions ( $\text{HNO}_3$ ,  $\text{HCl}$ ,  $\text{HClO}_4$  or  $\text{HF}$ ) (Shuman, 1979) (Table 1-5). These kinds of solutions are used for metals which are strongly bound to oxides and hydroxides of Al, Fe, and Mn.

Reducing agents are used to solubilize these minerals and release the associated heavy metals (Bernhard et al., 1986). Some of these extractants are acidified hydroxylamine ( $\text{NH}_2\text{OH}\cdot\text{HCl}$ ), acidified oxalate and dithionite/citrate solutions which often form part of the sequential fractionation scheme for heavy metals (McLaughlin et al., 2000b). The oxidation/reduction procedure extracts high concentrations of heavy metals from soils because it extracts the total metal concentration. The use of  $\text{HF}$  or caustic fusion tends to dissolve silicate and release metals trapped in these minerals (Pickering, 1986). McLaughlin et al. (2000b) concluded that the risk assessment of heavy metals based on the total concentration of the metal is not accurate.

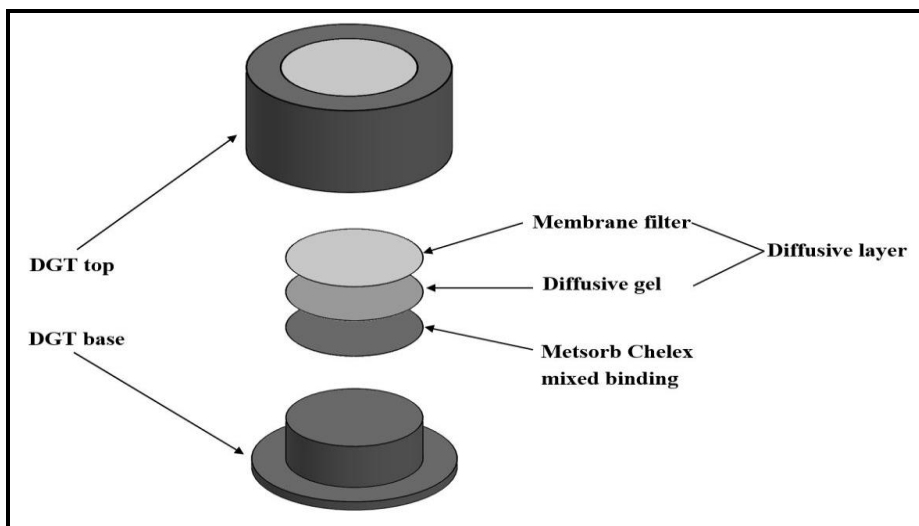
## **1.10 Diffusive Gradients in Thin Films (DGT)**

DGT is a diffusive passive sampling device developed for *in situ* quantitative determination of labile metals in the environment (Davison and Zhang, 1994; Buffle and Horvai, 2000; Zhang and Davison, 2000b). The DGT was initially developed in 1994 by Davison and Zhang (1994) and was used to measure different kinds of elements in water, soil and sediments (Zhang et al., 1998a and b; Bennett 2011). It has been successfully used for measuring trace element fluxes from the solid phase of soil and sediment to solution (Zhang, 2004), for measuring metal speciation (Unsworth et al., 2005), as a kinetic tool (Scally et al., 2003), for nutrient bioavailability measurement (Royset et al., 2005) and for routine environmental monitoring (Dunn et al., 2003). The DGT has been used successfully for P measurements in sediments and recently in soils under laboratory conditions, and homogenized soils (Zhang and Davison, 1995; Zhang et al., 1998a; Dahlgqvist et al., 2002; Zhang et al., 2004; Royset et al., 2005). The DGT device is deployed in an environmental system such as soil, for a period of time ranging from days to months. Labile species are accumulated *in situ* after passing through a hydrogel (diffusive layer) of known thickness and being immobilized by an adsorbent layer. The adsorbed species are taken to the laboratory for analysis using suitable analytical techniques.

### **1.10.1 The DGT components**

The effective parts of the DGT device are the diffusive layer and the binding layer. A binding agent, usually a resin selective for the solute species being measured, is embedded in a layer of the hydrogel, which is called the binding layer. The binding layer is separated from the solution by the diffusive gel and both are placed in a small simple plastic DGT unit (see Figure 1-4).





**Figure 1-4** The components of the DGT unit from top to bottom are: the DGT cup, the DGT membrane filter, the diffusive gel, the Metsorb Chelex mixed binding layer, and the DGT base.

The unit consists of a plastic piston and a 2 cm diameter cap with a window. This window exposes the DGT to the solution and controls the diffusion area (Zhang and Davison, 1995). Once the diffusive gel and resin gel are assembled in the DGT unit, the unit is deployed in the media being monitored. The amount of the analyte taken up by the DGT binding layer often represents the concentration of the available analyte in the bulk solution (Davison and Zhang, 1994).

### 1.10.2 The diffusive layer and filter membrane

The diffusive layer is an acrylamide-based hydrogel, cross-linked with a patented agarose-derived crosslinker (DGT Research Ltd. UK). The diffusive gel allows analyte species to pass from the solution to the binding layer, and to generate a concentration gradient. The pore size of the diffusive gel used in the DGT permits free ions and inorganic and small organic metal complexes to diffuse through to the binding layer (Zhang and Davison, 1995; Zhang and Davison, 1999). To measure the metal complexes, the complexes must dissociate during the transport process through the diffusive layer. Therefore, the DGT measures dissolved labile ion species of molecular size smaller than the hydrogel pore size (Zhang and Davison, 1995; Gimpel et al., 2001). The diffusive layer was covered by a 100  $\mu\text{m}$  thick, 0.45  $\mu\text{m}$  pore size, cellulose nitrate membrane filter in order to prevent the particles in the pore water from adhering to the diffusive layer surface and to control the transport of the analytes to the DGT device.

### 1.10.3 The binding layer

The binding layer usually comprises an adsorbent material cast within a polyacrylamide hydrogel, which accumulates analyte species that diffuse into the DGT device. The binding agent is responsible for the binding of the target ions. The binding agent used is selective to the species being measured. Chelex-100 is the first binding layer used for the DGT and it is the most common binding agent used to measure metal species in a wide range of natural waters and soil (Zhang and Davison, 1995; Zhang et al., 1998a; Dahlgvist et al., 2002; Zhang et al., 2004; Royset et al., 2005). Chelex resin takes up trace metals because it contains paired iminodiacetate ions which act as chelating groups in binding polyvalent metals ions (Zhang et al., 2014). Zhang et al. (1998) then used ferrihydrite as a binding layer. Ferrihydrite (FeOOH) is iron hydroxyl-oxide and is a very reactive agent due to its OH group. The OH groups can bind both cation and anions. The same chemical reaction is applied as Metsorb (TiO<sub>2</sub>) with orthophosphate as illustrated in Figure 1-5.

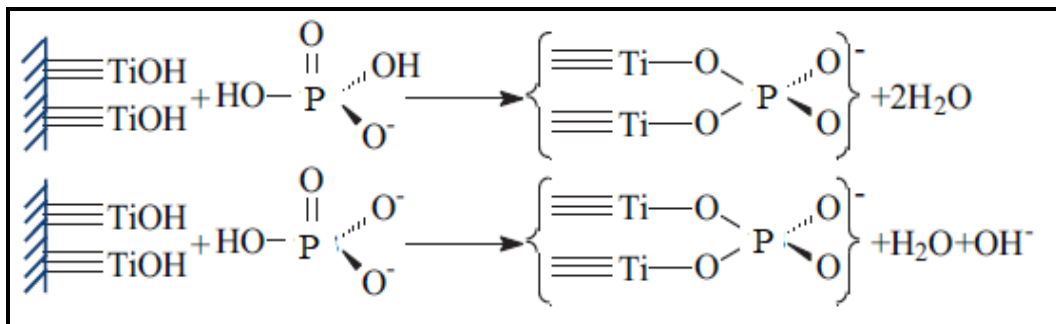
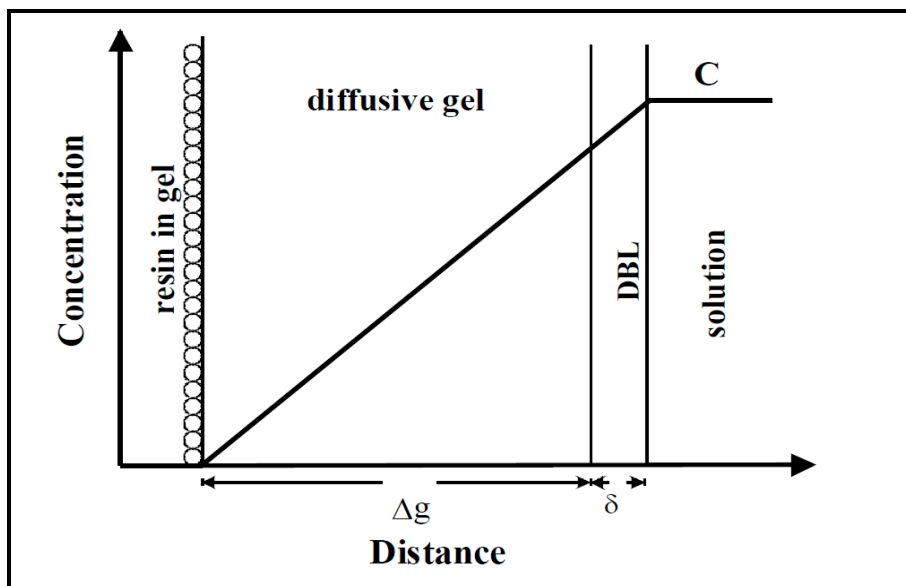


Figure 1-5 Schematic illustration of the reaction of orthophosphate with Metsorb (TiO<sub>2</sub>) adopted from (Guan et al., 2012; Chen et al., 2015).

### 1.10.4 DGT theory and calculation of analyte concentration

The binding layer adsorbs analyte species from the environment. The adsorbent binding layer is separated from the bulk solution by a permeable gel (see Figure 1-6). A diffusive boundary layer (DBL) of thickness  $\delta$  will be formed between the bulk solution and the diffusive layer membrane.



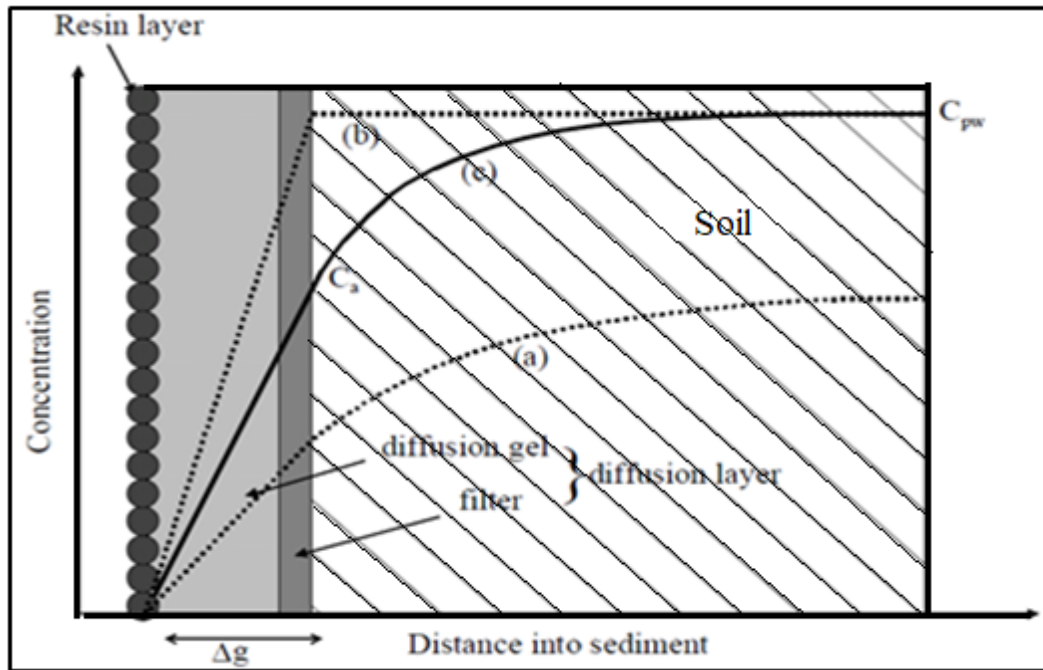
**Figure 1-6 Conceptual views showing a cross-section through a DGT device in contact with the solution, showing the study-state concentration gradient. The diffusive layer is shown as a single layer of gel, but it can include a gel layer and filter. Concentration (C), diffusive layer thickness ( $\Delta g$ ), including 0.45  $\mu\text{m}$  pore size cellulose nitrate membrane thickness, diffusion boundary layer ( $\delta$ ), analyte accumulated (M), diffusion coefficient (D), and cross-section area (A). Adopted from (Zhang, 2003)**

The analyte species will be transported from the bulk solution to the binding layer by molecular diffusion through the diffusive gel and the DBL. The rate of analyte mass transport to the binding layer is generally controlled by the diffusive layer regardless of the hydrodynamics of the bulk solution.

The principle of the DGT technique is based on Fick's First Law of Diffusion. Equation 1-1 shows the flux, F, of the analyte species to the binding layer, where D is the diffusion coefficient of analyte through the diffusive gel,  $C_b$  is the concentration of the analyte species in the bulk solution,  $C'$  is the concentration of the species at the interface between the binding gel and diffusive gel, and  $\Delta g$  and  $\delta$  are the thicknesses of diffusive gel and DBL, respectively. A conceptual illustration of this is shown in Figure 1-7.

$$F = D (C_b - C') / \Delta g + \delta \quad \text{Equation 1-1}$$

If the analyte species rapidly equilibrates with the binding layer and the interaction between them is strong enough,  $C'$  will effectively become zero, provided that the adsorbent layer is not approaching saturation. Assuming that  $\delta$  is negligibly small compared to  $\Delta g$  (as in well-mixed solution) then equation 1-1 will be simplified to equation 1-2:



**Figure 1-7** Schematic cross-section through the DGT device and adjacent solute from the solid phase or soil solution (Davison et al., 2007). Pseudo-steady state concentration gradients are illustrated for three cases: (a) unsustained, (b) sustained and (c) the concentration between the soil and DGT device. Adopted from Zhang (2003)

$$F = D C_b / \Delta g \quad \text{Equation 1-2}$$

According to the diffusion flux, the mass (M) diffused through the area (A) after a period of time (t) represents the flux, therefore (M /At) can substitute for F.

$$F = M / At \quad \text{Equation 1-3}$$

Combining and rearranging equations 1-2 and 1-3 will form equation 1-4:

$$C_b = M \Delta g / DAt \quad \text{Equation 1-4}$$

Equation 1-4 is well known as the DGT equation. After completion of the DGT deployment, the binding gel is removed from the DGT device. The accumulated analyte is then eluted from the binding layer. Elution of P and Cd usually can be achieved by using 1-2 mol L<sup>-1</sup> NaOH and HNO<sub>3</sub>, respectively (Davison and Zhang, 1994; Zhang and Davison, 1995; Warnken et al., 2006). The concentration of the analyte in the eluent is then measured by an appropriate analytical technique. The accumulated mass of analyte (M) on the binding layer can be calculated by using equation 1-5:

$$M = C_e (V_e + V_g) / E_f \quad \text{Equation 1-5}$$

Where  $V_e$  and  $V_g$  are the volume of the eluent and binding, respectively, and  $E_f$  is the elution efficiency.  $C_e$  is the concentration of analyte determined in the eluent sample (Zhang and Davison, 1995).

#### 1.10.5 DGT mechanism in soil

The theory and measurement principles of using DGT in water are also applicable to soil and sediments (Davison et al., 2000; Warnken et al., 2006). However, there are two significant differences between using DGT in water and in soil. In soil, a DGT device acts as a sink that induces a flux of analytes from the soil to the binding layer of the DGT. The sampled interstitial water is not well-mixed and therefore interpretation requires more care. The analyte flux into the deployed DGT depends on the concentration of the labile species in soil solution  $C_a$  (Figure 1-7) and how desorption or dissolution responds to depletion of the  $C_a$ . The reduction of the analyte concentration in soil solution can induce the release of more analyte from the solid-phase (Davison et al., 2007). To interpret the DGT measurements in the soil it is important to keep in mind that there are two significant differences from the use of the DGT in water:

Firstly, the lack of mixing, in general, leads to pore water concentration adjacent to the DGT becoming depleted as in cases (a) and (c) in (Figure 1-7). If the supply of the solute is by diffusion, the area of depletion adjacent to the DGT device increases with time. The flux of solute to the DGT device, which is driven by concentration gradient through the binding layer, therefore, gradually decreases with time.

Secondly, if it is assumed that  $C_a$  remains relatively constant during the deployment due to a constant resupply from the soil solid phase, the theory developed for solution can be adapted to deployments in soil, using the interfacial pore water concentration between the DGT device and soil,  $C_a$ , rather than the bulk solute concentration (Davison et al., 2007). The resupply process will not be part of the current study however, it is still necessary to understand the range of responses observed.

The DGT concentration, ( $C_{DGT}$ ) will be less than or equal to the concentration of labile analytes in the pore water ( $C$ ). Where the independent measurements can be interpreted in terms of the ratio  $R$ :

$$R = C_{DGT} / C, 0 < R < 1$$

Equation 1-6

R can be obtained experimentally and used to characterize the deployment as one of the three cases descriptive below. The three cases are illustrated in Figure (1-7), which shows a cross-section through a DGT device during deployment. The three cases are:

- a) Unsustained case ( $R = R_{diff}$ ). There is no resupply of analyte to the pore water. That means, the DGT device is supplied only by the diffusion of analytes through the pore water, which becomes gradually depleted. The exact value of the  $R_{diff}$  depends on the analyte diffusion coefficient in soil solution, the design of the DGT device, and the deployment time. The estimated value for R for a typical sampler design in high porosity soil is  $R_{diff} = 0.1$  (i.e.  $C_{DGT} = 0.1C$ ) for 24 hour deployment (Harper et al., 1998).
- b) Sustained Case ( $R > 0.95$ ). The pore water concentration adjacent to the DGT device is sustained at the bulk pore water concentration throughout the deployment. This condition can occur if (i) analytes mixing rates in the soil are fast for example, from tidal pumping, bio-irrigation, or (ii) the rate of resupply from solid phase is fast compared to the rate of removal to DGT device, and that the capacity of the solid phase to resupply the pore water is large. The theoretical upper limit,  $R = 1$ , is never fully achieved as it would require instantaneous resupply from an infinite capacity reservoir adjacent to the DGT device
- c) Partially sustained case ( $R_{diff} < R < 1$ ). Significant resupply of the analytes from the soil solid phase occurs, but it is insufficient to fully sustain pore water concentrations. The precise value of R is a quantitative measure of the depletion induced by the DGT sink.

#### 1.10.6 Varieties of DGT binding layers

Different types of resin binding layers have been used to increase the DGT selectivity of element species. Chelex-100 was the first binding material used for the DGT, it contains paired iminodiacetate ions which act as chelating groups polyvalent metal ions (Davison and Zhang, 1994; Zhang and Davison, 1995). The DGT device with this kind of binding layer showed significant agreement with theory and its performance did not deteriorate, even after four consecutive uses. Then, the ferrihydrite gel which has a strong affinity for P was used to measure labile P (Zhang et al., 1998a; Ding et al., 2011). The Chelex Binding Layer was used with the different types of binding layers as described below.

A Polyacrylamide–polyacrylic acid copolymer hydrogel is used for selective binding of  $\text{Cu}^{2+}$  (Li et al., 2002a). This kind of resin shows a strong affinity for binding  $\text{Cu}^{2+}$  under competitive binding conditions. Other types of the resin include ion-specific resins using copolymer hydrogels composed of polyacrylamide–polyacrylic acid which selectively binds  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  over alkali and alkaline earth metals, within the hydrogel structure (Li et al., 2002c).

A diffusive layer was proposed for DGT devices containing a liquid binding phase, poly 4-styrene sulphonate (PSS) aqueous solution and a cellulose dialysis membrane (CDM), with the intention of excluding copper-organic matter complexes from the device (Li et al., 2002b; Li et al., 2003b).

Li et al. (2005) investigated the possibility of using DGT devices with different binding phases. The performance of different DGTs in accumulating labile fractions of Cd and Cu in laboratory solutions and in natural waters was determined. Several binding phases were studied, including conventional Chelex-100 resin imbedded polyacrylamide hydrogel (Chelex) and several recently developed binding phases such as poly acrylamide-co-acrylic acid (PAM-PAA) gel, Whatman P81 cellulose phosphate ion-exchange membrane (P81), and poly 4-styrene sulfonate (PSS) aqueous solution. Results showed that the DGT labile metal concentrations measured by different binding phases can be significantly different. It was concluded that the DGT labile metal fractions were dependent on the binding strength of the binding phase. By designing binding phases that can compete with different natural water complex ligands to varying extents, it is possible to use these different DGT devices to measure metal speciation in natural waters.

Phosphorus has been measured by DGT in freshwater using a binding layer of ferrihydrite embedded in the gel (Zhang et al., 1998b). This type of binding layer has also been used for arsenic (As) measurement (Panther et al., 2012; Dai et al., 2018)

In addition, a mixed binding layer (MBL) of both Chelex-100 and ferrihydrite was developed by Mason et al. (2005) to measure cations, such as Mn, Cu, Zn and Cd, and anions, such as molybdate and phosphate, in a single device.

A new DGT adsorbent (Metsorb) has been developed for the measurement of As, Se and dissolved reactive phosphate (DRP) in fresh and marine water. The new DGT employs a titanium dioxide-based adsorbent as a binding layer (Bennett et al., 2010; Panther et al., 2010). Metsorb has also been used to measure inorganic uranium (U) species in fresh and marine water (Hutchins et al., 2012; Turner et al., 2012). Recently,

mixed binding layer (MBL) consisting of Chelex-100 and Metsorb titanium dioxide has been used as the DGT adsorbent for simultaneous measurement of trace metals (Mn, Co, Ni, Cu, Cd and Pb) and oxyanions (V, As, Mo, Sb, and P) (Panther et al., 2014). The results showed that all measurements of trace metals and oxyanions by the MBL-DGT were independent of pH (5.03-8.05) and ionic strength ( $I = 0.001-0.7 \text{ mol L}^{-1}$ ). The new MBL-DGT measurements were accurate in freshwater. In addition, the Chelex-Metsorb DGT proved that it has significant advantages over the previous Chelex-ferrihydrite DGT described by (Mason et al., 2005). Some of these advantages are the commercial availability of both Metsorb and Chelex-100, the high accuracy of Metsorb for measuring some oxyanions in freshwater and seawater, and the possibility of measuring Fe, which would be impossible with the Chelex-ferrihydrite layer (Panther et al., 2011).

A DGT based on hydrous zirconium oxide (Zr-oxide) has been developed by Sun et al. (2013) for the measurement of dissolved reactive phosphate (DRP). It was found that the presence of anions such as  $\text{Cl}^{-1}$ ,  $\text{SO}_4^{2-}$ ,  $\text{AsO}_4^{3-}$  and  $\text{AsO}_3^{3-}$  in solution had a negligible effect on DRP measurements. The DRP measurement by Zr-oxide decreased with the increase of solution pH. It was reported that the lowest capacity of Zr-oxide ( $95 \mu\text{g P cm}^{-2}$  at pH 9.2) was greater than the other DGT techniques usually used for measurement of DRP ( $2-12 \mu\text{g P cm}^{-2}$ ). Also, it was found that the Zr-oxide binding gel could be stored for up to two years without any aging effect. In contrast, the storage age for other DGT gels, like ferrihydrite, is only 6 months.

#### **1.10.7 The Metsorb DGT**

A DGT technique developed by Bennett et al. (2010) uses a titanium dioxide based adsorbent. A commercially available titanium dioxide-based adsorbent (Metsorb, <http://www.gravertech.com>) is a highly effective, low-cost adsorbent for reduction of As III and Vanadium (V) and a wide variety of heavy metals. Therefore, it was identified as a potential binding agent for the DGT technique. Metsorb is an agglomerated nanocrystalline (anatase) titanium dioxide powder of  $50 \mu\text{m}$  particle size which is used primarily as an adsorbent for both small and large-scale water treatments.

A comparison study was carried out between ferrihydrite and Metsorb DGT by Panther et al. (2011). The Metsorb had significant advantages over the ferrihydrite adsorbent used by many researchers (Zarcinas and Nable, 1992; Zhang and Davison, 1995; Zhang et al., 2001b; Zhang, 2004; Mason et al., 2005a). Ferrihydrite requires



careful laboratory synthesis under highly controlled conditions otherwise formation of a less reactive mineral phase and slower adsorption of analytes could be produced (Zhang et al., 1998a). In another comparison study where Metsorb and ferrihydrite DGTs were deployed in a simple solution, the results showed that both DGTs produced linear trends between the mass of analyte accumulation and deployment time. Also, both DGTs demonstrated predictable uptake across the range of pH values (4.0-8.3) and ionic strengths (0.001-1 mol L<sup>-1</sup> NaNO<sub>3</sub>) investigated (Panther et al., 2010). It was found that the total capacity of the Metsorb binding layer (~ 40000 ng P) was 2.5-5 times higher than the recorded total capacity of a ferrihydrite binding layer (Panther et al., 2010).

The new Metsorb DGT was evaluated for the determination of dissolved inorganic As and Se (Bennett et al. 2010). The uptake efficiencies of the new Metsorb DGT were 96.5-100% and eluted efficiencies were 81.2%, 75.2% and 88.7% for As III, As V, and Selenium VI, respectively. Laboratory experiments showed a linear increase in mass uptake over time (Bennett et al., 2010). It was concluded that the new Metsorb was a reliable and robust adsorbent for measurement of inorganic As and selective measurement of Se VI with high levels of accuracy. Titanium dioxide has a high affinity for the inorganic species of As and the reduced Se oxyanion (Bang et al., 2005; Pena et al., 2005).

Recently, Panther et al. (2014) used a new binding layer technique with a mixed binding layer (Chelex-100 and the titanium dioxide-based adsorbent Metsorb) (MBL-DGT) to measure labile concentrations of a large number of analytes including P and Cd in seawater and freshwater. The new DGT was capable of Chelex-DGT and Metsorb-DGT techniques individually and exceeded the performance of Chelex-DGT. The passive DGT sampler is an *in-situ* technique that selectively accumulates labile analyte species in stable form. The DGT method usually decreases the chemical interference between labile nutrient forms and chemicals and simulates plant nutrient uptake from the soil. The newly developed Metsorb was tested as a DGT binding layer and thoroughly evaluated for the determination of soluble inorganic P, As and Se. It was investigated over a range of pH and ionic strengths that could be encountered in natural waters. The new MBL-DGT was successfully used for the measurement of dissolved reactive phosphorus (DRP) and large varieties of trace metals in freshwater and seawater (Panther et al., 2014). Given the success of the MBL-DGT in water sampling, this study will investigate the performance of the Metsorb binding layer as a mixed binding layer and a double binding layer with Chelex for the first time in soil.

## **1.11 Summary**

Intensive agricultural practices have led to increased addition of P fertilizers to soils with the associated environmental problems of increased runoff and leaching of P into surface and groundwaters. In addition, many P fertilizers used in Australia and elsewhere also contain high concentrations of the heavy metal Cd, which has caused concern regarding Cd accumulation in soils, plants, water and in the food chain. Since fertilizer applications are frequently based on soil P test results, an improved prediction of plant-available P in soils may help to minimize the excessive application of P fertilizer, thus reducing pollution and improving fertilizer economics. Current test methods to measure plant-available P have many limitations. They rely on time-consuming sample collection and measurement methods which often under- or over-estimate the soluble and labile forms of inorganic P required by plants due to limitations of the extraction solution or technique. The new Diffusive Gradient in Thin-films (DGT) technology offers an improved technique for measuring labile ions such as P and Cd in soils. DGTs are passive sampling devices developed for the quantitative determination of labile metals and oxyanions in the environment and they can be inserted into water or soil. They comprise of a filter, a diffusive layer, and an absorbent or binding layer. Various single and mixed diffusive and binding gel materials have been evaluated for measuring different elements in soil and water, with differing levels of success. However, recently, a Metsorb Chelex Mixed Binding Layer (MBL-DGT), which contains a mixed binding layer of Chelex and a Metsorb layer to bind cations anions spontaneously, has been successfully used to in the water. To date, it has not been investigated for use in soils.

## **1.12 Knowledge gap**

This study is the first to examine the use of the two types of binding layers in DGTs, the Metsorb, and Chelex, in soil. Two different scenarios will be used: (i) Metsorb Chelex Mixed Binding Layer DGT (MBL-DGT) which comprises both Metsorb and Chelex mixed in one single binding layer and (ii) the Metsorb Chelex Double Binding Layer (DBL-DGT) which contains two binding layers in one device (Metsorb and Chelex). Both of those binding layer types have not yet been evaluated for measurement of labile anions or cations in soils. Compared to water, soils have many additional chemical and physical complexities that affect the quantities of labile elements. These may include ion exchange, fixation, and dissolution with soil particles, plant uptake, major changes in

salinity and pH, and large fluctuations in air/water contents. Therefore, specific knowledge about the performance of these Metsorb-Chelex DGTs in soils is required.

Additionally, the use of *in situ* DGTs deployed for long periods of time (more than 24 hours) in soils has not been evaluated. *In situ* sampling devices are exposed to variations in water and air content due to wetting, drainage, plant uptake, and leaching as well as variations in the above-mentioned soil chemical parameters. These changes in soil properties may also be depth-dependent. It is not certain how these DGT devices will perform with these changes and if their performance remains consistent with time.

This research, therefore, examined the performance of the two Metsorb and Chelex DGTs deployed *in situ* in soils. Given the previously discussed concerns with P fertilizers, this work focused on the measurement of labile P and Cd. The MBL-DGT *in situ* performance was examined in large soil volumes in a flume installed outdoors to mimic field conditions. The DBL-DGT was deployed in large pots with tomato plants in greenhouse experiments. The first greenhouse experiment, tomato plant cultivation, investigated the capability of the DBL-DGT to predict the bioavailability of P and Cd to plants. The second greenhouse experiment was conducted without plants in the pots but using successive applications of three types of P fertilizer to mimic the rotatory P fertilizer applications in agricultural practices. This allowed the investigation of available P and Cd accumulation in soil and leachate water. DGTs were deployed at three depths 15 cm and 45 cm in the flume experiment and 15 cm 45 cm, and 60 cm in the greenhouse experiment to evaluate DGT performance at different depths of the soil profile. Different deployment times of 4, 7, and 14 days were used in the flume experiment. The most suitable time was used for the next two pot experiments. Comparisons of  $P_{DGT}$  and  $Cd_{DGT}$  results with those determined by traditional techniques in the laboratory and with a mass balances approach was used to evaluate the performance of the DGTs.

### **1.13 Research questions**

The general questions are as follows:

- 1- Does the MBL-DGT effectively and accurately predict available P and Cd in sandy loam soil as it does when used in water?
- 2- How do the MBL-DGT measurements of available P and Cd compare with traditional chemical extraction methods of measuring P and Cd?

- 3- Can the Metsorb layer of the MBL-DGT be used as a plant surrogate to determine the bioavailable P and Cd concentrations, the form most accessible to plants?
- 4- Does the MBL-DGT have sufficient capacity if deployed in soil with prolonged use of P fertilizers?

### **1.14 Research objectives**

- 1- Use the MBL-DGT to investigate the influence of P fertilizer runoff in water containing different concentrations of soluble SSP fertilizer on sandy loam soil. A flume experiment will be set up to investigate the changes in the concentration of available P and Cd through the soil profile by deploying the DGTs in the flume at two different depths.
- 2- Investigate the influence of short-term P fertilization on the availability and mobility of P and Cd in sandy loam soil. Pot experiments will be carried out in greenhouse where DBL-DGTs are deployed in sandy loam soil in which tomato plants are grown. Three types of P fertilizers will be added at different doses.
- 3- Determine the capacity of the MBL-DGT to adsorb available P in long-term fertilization and understand the effect of this process on the availability Cd in sandy loam soil. This will be done using a pot experiment.
- 4- Evaluate the accuracy of the new MBL-DGT by comparing MBL-DGT measurements from the three previous experiments to the Olsen and  $\text{NH}_4\text{Cl}$  methods for measuring available P and Cd in the soil.

### **1.15 Thesis outline**

Chapter 1 provides a general introduction to the study. It also reviews the relevant literature relating to phosphorus and cadmium in soil, the different methods used to assess P and Cd in soil and the use of Diffusive Gradient in Thin Film (DGT) techniques to determine concentrations of P, Cd, and other metals in soil. By identifying knowledge gaps, the chapter also presents the research questions and objectives and outlines the thesis structure.

Chapter 2 outlines the general materials and methods used throughout the study. These include DGT preparation and deployment, analyses and calculations of P and Cd concentration. More specific materials and methods are provided in each data chapter.

Chapter 3 is the first data chapter and presents the study on the application of the Metsorb MBL-DGT to measure the concentration and behavior of P and Cd in leachate water. The MBL-DGTs were deployed in outdoor flumes to simulate field conditions.

Chapter 4 is the second data chapter evaluating the relationship between plant absorption of available P and available Cd in the soil and the concentration of available P and Cd accumulated on the DBL-DGT. This was done using tomato plants under greenhouse conditions.

Chapter 5 is the third data chapter which studies the changes in labile P and Cd concentrations after multiple applications of P fertilizer using the Metsorb MBL-DGT.

Chapter 6 provides a general overview of the study conclusions, discusses the limitations of the research, and highlights the implications of the results for P and Cd sampling in soil, particularly in relation to soil contamination due to excessive fertilizer application in agricultural areas. It also discusses potential future areas of research to build on the results of this study.

## **Chapter 2-. Materials and methods**

### **2.1 The general methodology for experiments**

Three experiments were carried out at Griffith University Nathan Campus where the glasshouse and the flume facilities are located. Most of the laboratory work was performed at the Gold Coast Campus. Handling procedures applicable to phosphorus (P) and cadmium (Cd) were employed at all times.

Deionized water (Milli-Q Element 18.2 m  $\Omega$ . cm) was used for the preparation of all standards and solutions and for the washing of all materials. Powder-free latex gloves, disposable caps, and shoes were worn at all times. Phosphorus measurements were performed with spectrophotometrically a Shimadzu UV-1800 utilizing the standard molybdenum-blue method. Phosphorus standard solutions of 0.01-2000  $\mu\text{g P L}^{-1}$  were prepared from 10 mg  $\text{P L}^{-1}$  which was originally prepared from 1000 mg  $\text{L}^{-1}$  high purity Certified Merck phosphate test kit (EPA 365.2, APHA 4500-PE). Cadmium analyses were performed using an Agilent ICP-MS 7500a with scandium, yttrium, and indium as internal standards. All containers and DGT devices used in this study were washed and stored in 10%  $\text{HNO}_3$  acid (AR grade, Merck) and then rinsed several times in deionized water prior to use.

### **2.2 Preparation of the DGT**

All preparations of DGT probes, gel, binding layers and disassembly of DGT probes were conducted in an ISO class 5 laminar flow cupboard within a class 6 cleanroom.

The diffusive gel layers were prepared as per the procedures described by Zhang and Davison (1995). A gel stock solution was made by mixing 18.75 mL of 40% acrylamide solution (Bio-Rad, electrophoresis purity reagent), 7.5 mL DGT gel cross-linker (DGT Research Ltd, UK) and 23.75 mL of Mill-Q water in a clean plastic bottle. This solution was stored in the refrigerator  $< 4^\circ\text{C}$  until used.

Diffusive gels were prepared by adding 10 mL of gel stock solution to a 50 mL plastic centrifuge tube containing a magnetic stirrer, followed by 70  $\mu\text{L}$  of freshly made

10% ammonium persulfate solution (AR Grade, Aldrich) and 50  $\mu\text{L}$  of N,N,N',N'-tetramethylethylenediamine (TEMED; Merck) catalyst. The solution was mixed well and immediately pipetted into a gel mold consisting of two glass plates, separated by an inert plastic spacer of 0.05 cm thickness and clipped firmly together to prepare diffusive gel of 0.08 cm thickness when hydrated. Before use, the glass plates and the plastic spacers were all acid-washed and rinsed thoroughly by deionized water and left to dry in the fume hood in the cleanroom. The gel was then allowed to polymerize at 44°C for approximately 60 min. Upon removal, the plates were separated and the gel hydrated in deionized (Milli-Q) water for 24 h with at least three to four water changes to diffuse any contaminants and remaining reagents out of the gel. The diffusive gel had a thickness of 0.08 cm and was stored in 0.01 M  $\text{NaNO}_3$  at room temperature until required for use.

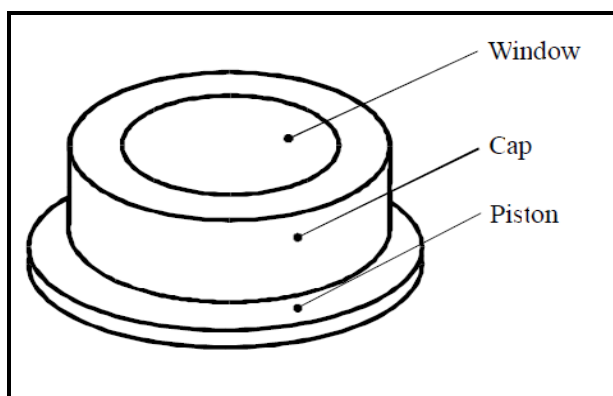
The Metsorb binding layer gel was prepared as described by Bennett et al. (2010). Briefly, 1 g of  $\text{TiO}_2$  was added to 10 mL of gel stock solution. The solution was sonicated for approximately 5 min to ensure the  $\text{TiO}_2$  powder was completely mixed with the gel solution. This was followed by the addition of 60  $\mu\text{L}$  of 10% ammonium persulfate solution (Aldrich, AR Grade) and 20  $\mu\text{L}$  of TEMED (Bio-Rad). The solution was mixed well using a magnetic stirrer bar and immediately pipetted into a gel mold consisting of two slightly offset acid-washed glass plates separated by a 0.025 cm thickness plastic spacer to prepare 0.04 cm thickness when hydrated. The gel was put in the oven to set at 44°C for approximately 60 min to allow setting of the binding agent on one side of the gel. The gel was stored in Milli-Q water in the refrigerator at  $< 4^\circ\text{C}$ .

The Chelex binding layer was used to adsorb Cd. To prepare the Chelex binding layer, 2 g of ion exchange resin Chelex-100 (Na form, 100-200 wet Mesh) was mixed in 10 mL of gel solution. Then, 60 mL of ammonium persulfate initiator (10%) solution and 20 mL of TEMED catalyst were added to the Chelex solution to prolong the setting process. The solution was mixed with a magnetic stirrer. It was then immediately cast between two glass plates separated by plastic spacers of 0.025 cm thickness to prepare 0.04 cm thickness when hydrated. It was allowed to set in the oven at 45°C for 45 min (Zhang and Davison, 1995).

The mixed binding layer MBL-DGT is a new technique developed by Panther et al. (2014). It was prepared by mixing 2 g of dry Chelex-100 (Bio-Rad, 100-200 dry mesh) and 1 g of Metsorb ( $\text{TiO}_2$  dry powder) obtained from Graver Technologies (<http://www.gravertch.com>) (Bennett et al., 2010) with 10 mL of gel solution. The mixture

was sonicated for 5 min to ensure the Chelex and TiO<sub>2</sub> powder was properly mixed with the gel solution. Then 60 mL of 10% ammonium persulfate solution and 20 mL TEMED were added. The solution was mixed well using a magnetic stirrer and immediately pipetted into the gel mold. The gel mold consisted of two glass plates separated by plastic spacers of 0.025 cm thickness to prepare the Metsorb Chelex Mixed binding layer of 0.04 cm. The gel was put in an oven horizontally at 45°C for 60 min to allow setting of the binding agent on one side of the gel. After removal from the oven, the plates were separated and the gel hydrated in deionized water for 24 h with at least three water changes to allow any remaining reagents to diffuse out of the gel. The hydrated binding layers were stored in deionized water at < 4°C until used.

The double binding layer DGT (termed sandwich DGT) device consisted of two adsorbent layers (Figure 2.1), a titanium dioxide layer (Metsorb layer) to adsorb P and a Chelex layer to adsorb Cd. The procedures for preparation of the polyacrylamide diffusive gels, the Chelex adsorbent layers and the Metsorb binding layer are the same as described previously by Zhang and Davison (1995) and Bennett et al. (2010).



**Figure 2-1 DGT components - the piston, the cap, and the window**

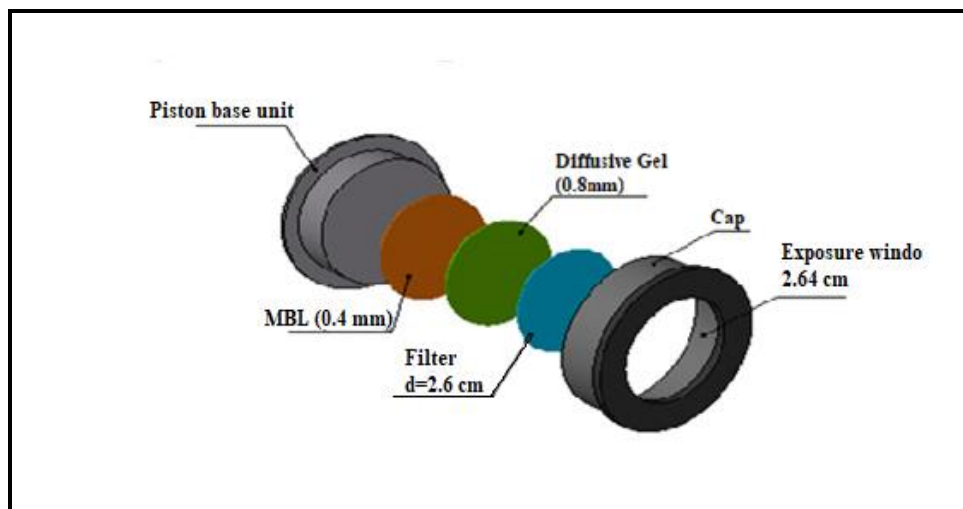
### **2.3 The DGT assembly**

Once prepared, the diffusive and binding gels were used to assemble the DGT devices for each experiment. The DGT devices were purchased from DGT Research Ltd (Lancaster, UK). All DGT devices were soaked in 10% HNO<sub>3</sub> acid solution for 24 h and rinsed in deionized water. The DGT devices were assembled in class 100 laminar flow cabinet in a class 1000 cleanroom.

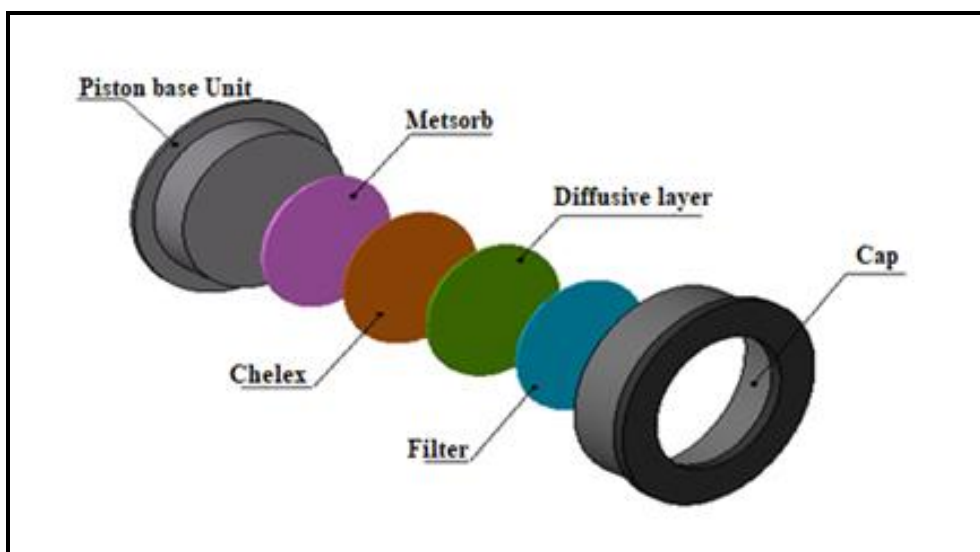
The MBL-DGT was assembled as follows: the binding gel (the Metsorb Chelex mixed binding layer) was laid onto the piston first, followed by a diffusive gel and a



membrane filter of 0.45  $\mu\text{m}$  cellulose nitrate as seen in Figure 2-2. The sandwich DGT was assembled as shown in Figure 2.3.



**Figure 2-2 MBL-DGT assemblies with the MBL laid on the piston, followed by the diffusive layer and the filter membrane and finally the cap.**



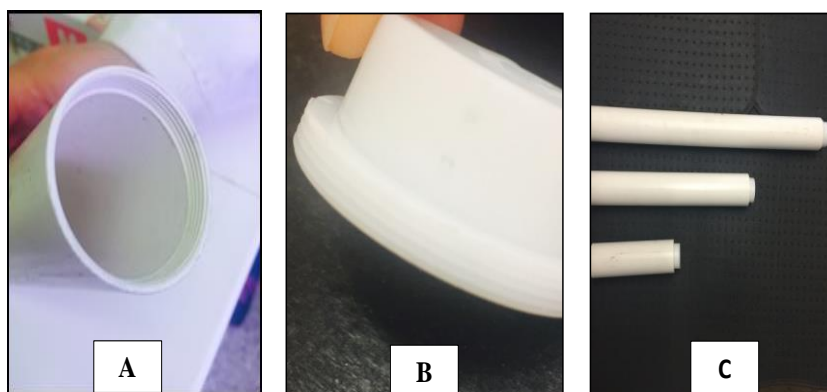
**Figure 2-3 The DBL-DGT components of the Sandwich DGT: from the left - the DGT base piston, the  $\text{TiO}_2$  binding layer (Metsorb), the Chelex binding layer, the diffusive gel, the filter, and the cover cap.**

The Metsorb layer was first placed on top of the cylinder of the piston, and then covered with the Chelex layer, the diffusive gel layer and finally the filter membrane. The front cap was then pressed down tightly until a good seal was formed in the DGT. Both types of prepared DGTs were stored in a plastic zip bag covered with a little Milli-Q water to protect the DGT membranes from drying. They were kept under cold conditions until required.

A filter membrane of 0.45  $\mu\text{m}$  cellulose nitrate was used to protect the diffusive gel. The membrane filters were soaked in deionized water for 24 h then rinsed and stored in deionized water at room temperature. The combined thicknesses of 0.09 cm for the filter and the diffusion gel were used for DGT calculations.

## 2.4 DGT deployment

PVC tubes of three different lengths (20 cm, 50 cm, and 65 cm) were prepared. They were 5 cm longer than the planned depth in all experiments to prevent water or soil from getting in behind the DGTs. The PVC tubes were threaded on one side (Figure 2-4A). The base edges of the DGT pistons were threaded as well (Figure 2-4B). The PVC tubes were soaked in 10% 1 M  $\text{HNO}_3$  before being attached to the DGT probes. The DGTs were then screwed onto the PVC tubes as shown in Figure 2.4C.



**Figure 2-4 DGT deployment. (A) PVC tubes threaded at one end, (B) Threaded base edge of a DGT, (C) DGT secured to PVC tube.**

A hole was opened in the soil using a spare PVC tube to insert the DGT smoothly into the soil. DGTs were attached to the PVC tubes then inserted into the holes leaving 5 cm of the tube above the soil surface to prevent the soil collapsing into the hole or into the tube. The depth of the DGTs in the soil and the deployment time were set according to each experiment as described in the Methods section in each of the chapters.

## **2.5 Elution and analysis of the Metsorb-Chelex Mixed Binding Layer (MBL-DGT)**

After removing DGTs from the soil, all DGT devices were first rinsed thoroughly with deionized water to remove any soil particles on the probe before being disassembled in the clean room. In both types of DGTs, the diffusive gel layer and the filter membrane were discarded. The Metsorb-Chelex mixed binding layers were eluted in 1 mL 1 M NaOH for at least 24 h for P recovery followed by elution in 1 mL 1 M HNO<sub>3</sub> for 24 h for Cd recovery. The eluent solutions were diluted 10-fold before the chemical analyses.

In regard to the sandwich DGT (Double binding layer DGT), the two binding layers were eluted separately. The Metsorb gel layer was immediately soaked in 5 mL of Milli-Q water overnight to remove any extra Ca<sup>2+</sup> or Mg<sup>2+</sup> on the gel. Then it was eluted in 1 mL 1 M NaOH for 24 h. The Chelex layers were eluted in 1 mL 1 M HNO<sub>3</sub> for 24 h. The eluent solutions were diluted 10-fold before P and Cd chemical analyses. All eluent solutions were labeled and stored at < 4°C until analysis.

## **2.6 Phosphorus analyses**

The original available P in the soil was extracted from the soil by collecting soil samples before fertilization. Soil samples were taken after DGTs were removed from the soil. The samples were taken from 30 cm of soil depth using a small auger hole. Soil samples were dried and gently ground with a wooden mortar and pestle to pass through a 2 mm sieve. Available P was then extracted by 0.5 M NaHCO<sub>3</sub> solution (pH 8.5) at a 1:20 soil: solution ratio. The suspension was then shaken for 30 min (Olsen, 1954). The samples were centrifuged at 2500 RPM for 5 min. The supernatant was filtered by syringe through 0.45 µm nitrocellulose. Available P was determined spectrophotometrically using the molybdenum-blue method with a Merck phosphate test kit (EPA 365.2, APHA 4500-PE) as described above. Phosphorus standard solutions of 0.01-2000 µg P L<sup>-1</sup> were prepared from 10 mg P L<sup>-1</sup> which was originally prepared from 1000 mg L<sup>-1</sup> high purity Certified Merck phosphate test kit (EPA 365.2, APHA 4500-PE). Samples and standard solutions were measured by spectrophotometer (Shimadzu UV-160A). The standard solutions were run in the spectrometer every 20-30 eluent samples.

Total P measurements were performed by grinding soil samples and sieving through a < 0.2 mm sieve followed by extraction of total P from the soil using perchloric acid. The digestion was performed on a 50-place block digester with an AIM 500 controller attached to control the temperature. Total P concentration was then measured spectrophotometrically as described above. Phosphorus concentration in the leachate water samples was determined after water samples were digested by potassium persulphate as described by Hosomi and Sudo (1986). Phosphorus concentration was determined spectrophotometrically as described above.

Eluent samples of Metsorb and the Metsorb Mixed Binding layer were tested for available P concentration based on the standard molybdenum–blue colour method using a Merck phosphate test kit and analyzed by spectrophotometer (Shimadzu UV-1800).

For quality assurance purposes, freshwater certified reference material, (Queensland health scientific Services) were used. Typical recovery for freshwater was 107 % (Table 2-1).

**Table 2-1 Recovery of labile P from freshwater certified reference materials (CRM). n=6, ± 1 standard error.**

CRM <sup>a</sup>	Certified DRP Concentration ( $\mu\text{g L}^{-1}$ ) <sup>b</sup>	Measured DRP Concentration ( $\mu\text{g L}^{-1}$ ) <sup>b</sup>	Recovery
ENCT- Round 13- bottle 3 <sup>c</sup>	84 ±7	90 ±5 (n =6)	107%

a: CRMs were obtained from Queensland health Scientific Services. b: Uncertainties are ±1 standard error.

## 2.7 Cadmium analyses

Available Cd in the soil was extracted using a 1:6 ratio of soils: 1 M NH<sub>4</sub>Cl extractant (Krishnamurti et al., 1995, Krishnamurti et al., 1999). The Cd extract was diluted 10-fold by adding 2% (v/v) HNO<sub>3</sub> matrix. Quality control standards at 10  $\mu\text{g L}^{-1}$  were analyzed every 20-30 samples. An internal standard solution then measured by inductively coupled plasma mass spectrophotometry (ICP-MS, Agilent 7500A) using scandium, yttrium and Indium as internal standards to account for instrument drift.

Total Cd was also measured from the digested soil solution as explained in section 2.6. Total Cd was then measured using the ICP-MS procedure described above. The

concentration of Cd in the leachate water was determined by taking 2 mL of the digested water with internal standard and 2% HNO<sub>3</sub> and analyzing using ICP-MS.

## 2.8 Calculation of the concentration of P and Cd accumulated by DGT (C<sub>DGT</sub>)

The DGT technique measures the concentration (C<sub>DGT</sub>) ng mL<sup>-1</sup> of analytes within an external solution by accumulating analytes within the binding layer after diffusional transport across a layer of known thickness. This process is described by Fick's First Law of Diffusion from which the DGT equation is derived (Zhang and Davison, 1995):

$$C_{DGT} = M \Delta g / D A T \quad \text{Equation 2-1}$$

D is referring to the diffusion coefficient in the diffusive layer (cm<sup>-2</sup> s<sup>-1</sup>). D = 6.38×10<sup>-6</sup> and 6.43×10<sup>-6</sup> for P and Cd at 27°C, respectively, where the diffusion coefficients of P and Cd were determined in water under range of temperatures (Zhang, 2003). Δg is the diffusive layer thickness A is the exposed area of the gel (cm<sup>2</sup>), M is the accumulated mass (ng). The accumulated mass of the diffused ions (P and/or Cd) in the binding layer is calculated as follows:

$$M = C_e (V_g + V_e) / f_e \quad \text{Equation 2-2}$$

C<sub>e</sub> is the concentration of the eluted gel. V<sub>e</sub> is the volume of the acid solution or NaOH added to the elute of the gel, V<sub>g</sub> is the volume of the gel in the resin layer, f<sub>e</sub> is the elution factor. Elution factors of 0.98 and 0.93 were used for P and Cd, respectively (Panther et al., 2012; Panther et al., 2014).

## 2.9 Blank gel DGTs and method detection limits

The C<sub>DGT</sub> of P and Cd detection limits were determined by analyzing the blank DGTs in each of the individual experiments (see Table 2-1). Six blank DGTs were used in each experiment. The blank DGTs were assembled at the same time as the soil DGTs using the same procedure. The blank DGTs were placed in clean zipped plastic bags and were kept at < 4°C. After 24 h the blank DGTs were dismantled and the binding layers eluted exactly as described in section 2.5. The blank eluent was analyzed for P and Cd similar to procedures for normal samples (see section 2.6). Data collected from the blank analyses were calculated according to equation 2.2. The standard deviation of the six

blanks was multiplied by 3 and applied in equation 2.1 to find  $C_{DGT}$  of the six blanks. The  $C_{DGT}$  of the blanks was subtracted from the  $C_{DGT}$  of the normal sample. All experiments had very low detection limits.

**Table 2-2 Concentrations of P and Cd detection limit in blank DGT gels n = 6, ± 1 standard error.**

Analytes	MBL-DGT (ng/ml)	Metsorb (DGT) (ng/ml)	DBL-DGT (ng/ml)	Chelex DGT (ng/ml)
P	0.05 ± 0.01	0.021 ± 0.012	0.05 ± 0.02	
Cd	0.04 ± 0.1		0.05 ± 0.02	0.05 ± 0.01

## 2.10 Soil Properties

A single soil type was used in each of the experiments within this study. The soil was a podsol soil with sandy loam texture collected from the A-horizon (0-30 cm) of Toohey Forest (27° 35' S, 135° 05' E) in Queensland, Australia. The soil was passed through a 1 cm sieve to remove large pieces of gravel, roots and other unwanted substances. Once placed inside the flume, the soil was lightly compacted to a density of 1.4 g cm<sup>-3</sup>. Two kg of the processed soil was preserved and used for soil testing.

Soil chemical properties were determined: exchangeable cations Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, and K<sup>+</sup> were extracted using 1 M ammonium acetate adjusted at pH 7 (Rayment and Higginson, 1992) and concentrations measured by flame atomic absorption (GBC AVANTA sigma with a GBC SDS- 270 samplers). Cation exchange capacity was calculated from the sum of these four cations in cmol kg<sup>-1</sup>. The exchangeable sodium percentage (ESP) was calculated (Soil Survey Staff, 1996) as:

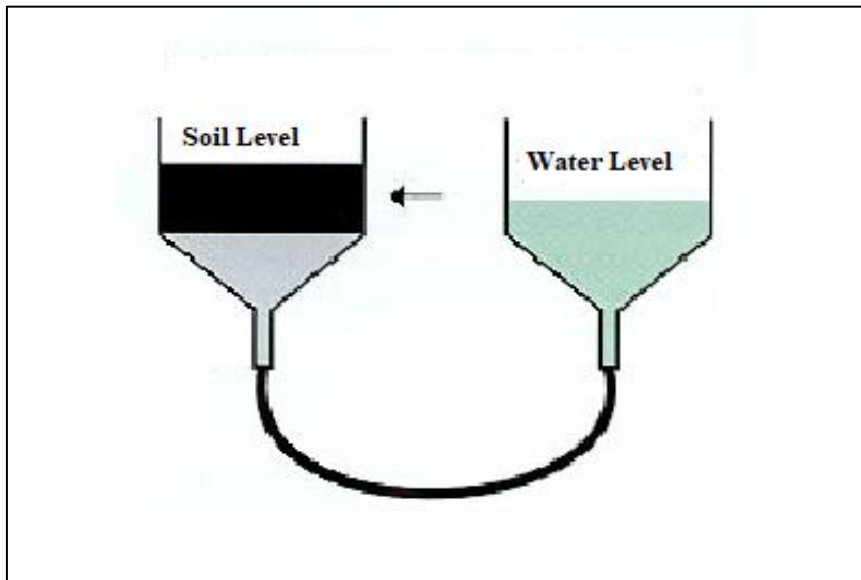
$$ESP = [(Na)/(Ca + Mg + K + Na)] \times 100 \quad \text{Equation 2-3}$$

Soil EC and soil pH of 1:5 soil: water suspensions were measured as instructed by Rayment and Higginson (1992). The % organic carbon was determined by the adapted Walkley Black method (Rayment and Higginson, 1992).

Physical properties of the soil were determined at the beginning of the experiments. Soil particle size analysis (sand, silt, and clay) was conducted according to the US Salinity Lab (1954). The water holding capacity (WHC) of the soil and the amount of water required to achieve 80% of WHC was determined according to AGVISE Laboratories (1998) using the 0 Bar method. The apparatus used for the 0 Bar water

holding capacity method is shown in Figure 2-5. In this method, a soil sample is saturated with water from an adjacent container, with the water level being kept in the middle of the soil (Figure 2-5). Once the equilibrium in this system is reached, the soil sample is weighed. The water holding capacity is calculated based on the weight of the water held in the sample vs. the dry weight of the sample

**Figure 2-5 The apparatus for ( 0 Bar ) method used for measuring the soil water holding capacity as described by AGVISE laboratories for soil testing and laboratory services.**



## 2.11 Statistical analyses

All data for the three experiments were statistically analyzed using SPSS version 23. All soil DGTs were designed as randomly blocked design and the leachate water data as repeated analyses design. Multiple regression was used to investigate the significant difference between variables. The correlations between the DGT measurements and chemical extraction measurements and the correlations between the extraction methods and P and Cd concentration in the plants were carried out using Pearson correlations.

## **Chapter 3-. Application of Metsorb Mixed Binding Layer DGT in flume experiments to investigate the changes in available P and Cd in sandy loam soil after the application of SSP fertilizer**

### **3.1 Introduction**

Sandy soils occupy around 18 million hectares in Australia, excluding the desert regions, with 52% of this land used for crops (Mann and Ritchie, 1995). Phosphorus (P) is commonly deficient in such soils, especially in western Australian (Ozanne and Shaw, 1967) and in similar agricultural land worldwide (McLaughlin et al., 2011). High application doses of P fertilizers are therefore required to maintain soil fertility and high crop production (Ozanne and Shaw, 1967). Such agricultural practices often result in stormwater runoff of orthophosphate and undissolved fertilizer (McDowell and Sharpley, 2002), and leaching of orthophosphate into groundwaters (Sinaj et al., 2002; Sharpley et al., 2008). These processes contribute to eutrophication of receiving or recharge zone surface waters (Kilroy and Coxon, 2005; Jalali and Kolahchi, 2008; Divya and Belagali, 2012).

Phosphorus fertilizers frequently contain heavy metals (McLaughlin et al., 2000b), such as cadmium (Cd) which can accumulate within crops (Li et al., 2003a; Lombi et al., 2003; Menzies et al., 2005). Cadmium is highly toxic and can produce adverse effects even at low concentrations (Jensen and Brorasmussen, 1992). Ingestion of Cd in contaminated food can cause both chronic and acute health effects such as bone fractures, kidney dysfunction, hypertension and cancer (Nordberg et al., 2002; Kursad et al., 2003). The link between P fertilizers, the accumulation of Cd in soils/plants and the associated health risks have been well established (Rothbaum et al. 1986; McLaughlin et al., 1997b; de Meeûs et al., 2002; McGrath and Tunney, 2010; Jiao et al., 2012).

These findings indicate a clear need to monitor soils for elements like P and Cd resulting from long term fertilizer use. There are several *ex-situ* extractions used to mobilize P (Abrams and Jarrell, 1992) and heavy metals (Manouchehri et al., 2011; Krishnamurtia et al., 1995) in soils to predict concentrations available to plants or for environmental assessment.

Over recent decades, the *in situ* diffusive gradients in a thin film (DGT) technique (Davison and Zhang, 1994; Zhang et al., 1998a; Zhang et al., 1998b) has successfully been



applied to measure and investigate biogeochemical processes involving heavy metals and P in soil systems (Mason et al., 2005a; Mason and McNeill, 2005; Mason et al., 2008; Senila et al., 2012) and to predict bioaccumulation in plants (Tandy et al., 2011; Six et al., 2014). DGT has been demonstrated to obtain equivalent or superior results to measurements made by extractions over a wide range of conditions (Mason et al., 2005a; Mason, 2007; Six et al., 2012; Six et al., 2013) and will, therefore, be the focus of this study. Most DGT deployments have been done in small containers (Mason et al., 2008; Mason et al., 2010; Mason et al., 2013) or occasionally in lysimeters (Nowack et al., 2004). Another experimental approach used to investigate soil-water processes is a flume (Chaplot and Le Bissonnais, 2003), which allows investigation of vertical processes. To date, flumes have not been used with DGT techniques. DGT probes are deployed within the sample matrix for a period of time, during which analytes diffuse through a layer of a known thickness (a polyacrylamide hydrogel and membrane cover) and accumulate on a binding layer, which is usually a commercial resin incorporated within a polyacrylamide hydrogel. These conditions are defined by Fick's First Law of Diffusion, from which the DGT equation has been derived (Zhang et al., 1998b) (see methods section 2.8 for more detail). The binding layer material normally used with DGT to measure heavy metal ions is Chelex-100 resin (Davison and Zhang, 1994). Several binding layers have been used with DGT to measure P, including ferrihydrite (Zhang, 1998; Mason, 2007; Mason, et al., 2013; Shapiro, 2013), Metsorb resin (Panther et al., 2008; Bennett et al., 2010; Panther et al., 2010; Bennett et al., 2011; Panther et al., 2012) which contains titanium dioxide, and zirconium dioxide (Jensen and Brorasmussen, 1992; Jamieson et al., 2003). Mixed binding layer DGT techniques have been used to combine measurements of metals and P, including Chelex and ferrihydrite (Mason et al., 2005a; Huynh et al., 2012) and Chelex and Metsorb (Panther et al., 2013). The Chelex-Metsorb mixed binding layer has undergone thorough characterization in water (Panther et al., 2014) but has not been applied to soils.

The aims of this study were to (i) study the ability of the MBL-DGT to measure available P and Cd in the soil after different doses of SSP fertilizer runoff, (ii) compare MBL-DGT measurements against the chemical extraction procedures for measuring available P and Cd in soil and (iii) use a mass balance approach to validate the MBL-DGT measurements.

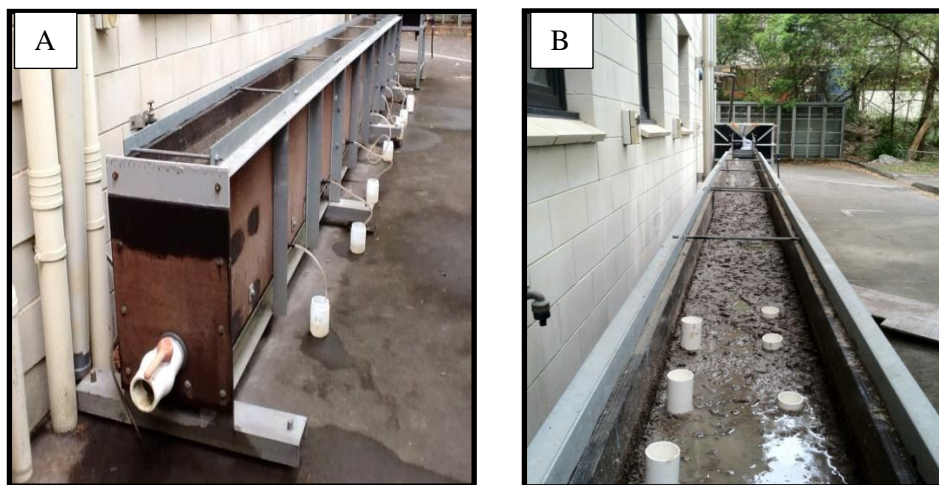
The flume experiment simulates the effect of surface run-off of fertilizer solutes interacting with and percolating through soil. DGT devices were systematically deployed within the soil at different locations along the flume, depths and times. Relationships between various fertilizer solute concentrations, representing different fertilizer application doses, and the accumulation of P and Cd in the soil were investigated. Phosphorus and Cd concentrations

were also measured in the water draining from the flume to simulate leaching of contaminants into groundwater. A mass balance was determined for P and Cd for each experiment. This study will improve understanding of soil-water processes and demonstrate the use of the DGT technique for field investigations of the effect of surface run-off within soils adjacent to agricultural areas.

## 3.2 Material and Methods

### 3.2.1 Flume preparation

The flume used in this study was 8 m long, 0.3 m wide and 0.8 m deep and set at a slope of 1% for all experiments (Chaplot and Le Bissonnais, 2003) (Figure 3-1). Drainage tubes were installed 45 cm below the soil surface at nine positions along the length of the flume. The main drainage hole was located at the exit end of the flume with a mechanism to control water drainage. The exit end was left off at all times during the experiment and the leachate water was collected from the front drain holes as shown in Figure 3-1A.



**Figure 3-1 (A) Flume set up showing the main drainage hole at the end, (B) Systematic DGT deployment in the flume**

A water reservoir of 1 m<sup>3</sup> at the upstream end of the flume delivered water containing various concentrations of dissolved Single Super Phosphate (SSP) to the flume. Further details are provided in section 3.2.3.

### 3.2.2 Soil preparations

The soil was a podzol soil, sandy loam collected from the A-horizon (0-30 cm) of Toohey forest in the Nathan suburb of Brisbane (27° 35' S, 135° 05' E), Queensland, Australia (Asadi et al., 2007). The soil was passed through a 1 cm sieve to remove large pieces of gravel, roots and other unwanted substances. Then, 1848 kg of 1.4 gm cm<sup>-3</sup> of soil was loaded in the flume. Soil volume was 1320 cm<sup>3</sup> and soil porosity (0.4%) was determined as described by Sarkar (2005). The volume of pore water ( $V_p$ ) cm<sup>3</sup> was calculated as in equation 3-1.

$$V_p = \Phi \times \text{soil volume} \times \% \text{ of soil moisture} \quad \text{Equation 3-1}$$

Where  $\Phi$  is soil porosity and % soil moisture which was 80%.

Before fertilization, soil samples were collected from the flume soil at 30 cm soil depth using a small auger hole to measure the initial P and Cd in the soil. Soil samples were also collected at the same time of DGT devices were removed from the soil. The samples were dried and gently ground with a woodmen pestle to pass through a 2 mm sieve. Available P was then extracted by 0.5 M NaHCO<sub>3</sub> solution (pH 8.5) at a 1:20 soil:solution ratio. The suspension was shaken for 30 min (Olsen, 1954) using an end to end shaker. Samples were then centrifuged at 2500 g for 5 min. The supernatant was filtered by syringe through 0.45  $\mu$ m nitrocellulose. Available P was determined spectrophotometrically using the molybdenum-blue method (Hylander et al., 1996) using a Merck phosphate test kit (EPA 365.2, APHA 4500-PE). Phosphorus standard solutions of 0.01-2000  $\mu$ g P L<sup>-1</sup> were prepared from 10 mg P L<sup>-1</sup> which was originally prepared from 1000 mg L<sup>-1</sup> high purity Certified Merck phosphate test kit (EPA 365.2, APHA 4500-PE). The samples and standard solutions were then measured by spectrophotometer (Shimadzu UV-160A).

Available Cd in the soil was extracted by 1:6 soil: NH<sub>4</sub>Cl extractant (Kitagishi and Obata, 1981; Krishnamurtia et al., 1995). The Cd was measured by ICP-MS (Agilent 7500A) with all solutions prepared in a 2% (V/V) HNO<sub>3</sub> matrix using a 1 mg L<sup>-1</sup> internal standard (final concentration 10 $\mu$ g L<sup>-1</sup>). Independently certified QC standards and matrix matched QC standards at 10  $\mu$ g L<sup>-1</sup> were analyzed every 20-30 samples.

Total P and Cd concentrations in the soil was extracted by soil digestion using a Perchloric, Nitric and Sulfuric Acid digest as described by Melaku et al. (2005). The digest was performed on a 50 place block digester with an AIM 500 controller attached to control the temperature. Total P concentration was measured spectrophotometrically and Cd measured by ICP-MS as described above.

### **3.2.3 Flume preparation and experimental design**

Single superphosphate (SSP) fertilizer was used for this study with P and Cd concentrations of 88,000 mg kg<sup>-1</sup> (8.8%) and 25 mg kg<sup>-1</sup>, respectively. Three sub-experiments were conducted within the flume experiment by applying a different dose of SSP fertilizer to the flume soil in each experiment. SSP fertilizer was dissolved in tap water and applied sequentially to the soil of the flume: 0.01 mg P L<sup>-1</sup>, 0.1 mg P L<sup>-1</sup>, and 0.5 mg P L<sup>-1</sup> each concentration was added to the flume soil in one experiment lasted for 14 days. These concentrations were selected based on the findings of Holtan et al. (1988), Zheng et al. (2004) and Mankin et al. (2007) concerning the P concentration in agricultural runoff. It was reported that the average P concentration in surface runoff water from agricultural land was 0.3 mg L<sup>-1</sup> (Zheng et al., 2004). The tap water had a negligible concentration of both P (<0.001 mg L<sup>-1</sup>) and Cd concentration (below the ICP-MS detection limit value of 0.024 µg L<sup>-1</sup>).

Prior to the first experiment, the soil was brought up to 80% of field capacity moisture content with tap water. For each experiment, 180 L of the prepared water and fertilizer as described above was applied at a steady dose from the reservoir; it took about 45 min for the 180 L of water to enter the flume. No overflow or drainage from the flume was permitted initially and the flume was left for a 24 h period to allow the water to percolate into the soil. After 24 h the DGTs were inserted and the leachate taps were opened to allow water to drain out of the flume. The three experiments were conducted over 10 weeks (including 10 days between each experiment for drying the soil, mixing the soil and refilling the flume. The soil was mixed after each experiment to simulate the tillage process in the agricultural field.

### **3.2.4 Preparation of Metsorb-Chelex Mixed binding layer DGTs (MBL-DGT)**

The MBL-DGTs were prepared as described in section 2.2 in Chapter 2.

### **3.2.5 MBL-DGT deployment**

For each experiment, 18 DGTs were deployed systematically at 75 cm distances between each DGT along the flume as seen in Figure 3-1B. Nine DGTs were deployed at 15 cm depth and nine at 45 cm. Deployment of the DGTs in soil was done as described in section 2-4 and Figure 2-4 in Chapter 2.

### 3.2.6 Elution and analysis of DGTs

At each sampling time, a total of six MBL-DGTs were randomly removed from the flume by taking three DGTs from the 15 cm depth and three from the 45 cm depth. DGTs were collected after 4, 7 and 14 days making a total of 18 DGTs after every run. The DGT disassembly and elution were performed as described in section 2.5 in Chapter 2.

Concentrations of  $P_{DGT}$  and  $Cd_{DGT}$  were calculated as described in section 2.8 in Chapter 2. The concentration of blank gel DGTs was measured as described in section 2-10 and are presented in Table 2-1 in Chapter 2. The deployment time of the DGTs during each experiment were 4, 7, and 14 days. In Figure 3-2 and Figure 3-3, the intervals between each experiment had been taken into account in plotting these graphs. accordingly, the first P dose of  $0.01 \text{ mg kg}^{-1}$  deployment time was 4,7, and 14. the second dose of  $0.1 \text{ mg kg}^{-1}$  the deployment time was 28, 31 and 38 days by adding 10 days to the last deployment time (14 days). The third dose of  $0.5 \text{ mg kg}^{-1}$  was 52, 55, and 62 days by adding 20 days to the 28 days of the second dose. These calculations were done to show the accumulation of the labile P and Cd on the MBL-DGT clearer after each dose of P fertilizer.

### 3.2.7 Mass balance calculation of P and Cd in the flume

Mass balance calculations for the P and Cd in the flume were calculated on the basis of the following equations (Withers and Lord, 2002):

$$\text{Input of analytes} = \text{Output of analytes} \quad \text{Equation 3-2}$$

This can be rewritten as follows:

$$M_{In} + M_{Fr} = M_L \quad \text{Equation 3-3}$$

Where  $M_{In}$  is the initial mass of P or Cd in the flume soil before each run,  $M_{Fr}$  is the amount of P or Cd added to the soil from the SSP fertilizer dissolved in 180 L of tap water.  $M_L$  is the mass of P or Cd in the leachate water calculated as:

$$M_L = C_{P \text{ or } Cd} \times \text{leachate volume} \quad \text{Equation 3-4}$$

The concentration of P and Cd were determined every 24 h from the collected leachate water. Therefore, the mass was calculated daily from equation 3-4 depending on the volume of leachate water collected. The sum of the  $M_L$  on day 14 of each experiment was considered the mass of P and Cd lost in the leachate water.

The final mass of P and Cd left in the soil after each run was then calculated as in equation 3-5 and considered the total concentration of P and Cd for the next experiment:

$$M_T = (M_{In} + M_{Fr}) - M_L \quad \text{Equation 3-5}$$

Where  $M_T$  represents the mass of total P or Cd left in the soil.

As the MBL-DGT measures labile P and Cd in soil pore water, the  $M_{DGT}$  was calculated from equation 3-6:

$$M_{DGT} = V_p \times C_{DGT} \quad \text{Equation 3-6}$$

$V_p$  is the volume of pore water  $\text{cm}^3 = \Phi \times \text{soil volume} \times \% \text{ of soil moisture}$ .  $\Phi$  is % of soil porosity. % soil moisture which was 80% in this study.

The various parameters in equation 3-5 were calculated from the measured concentrations of the available and total P or Cd before the first flume run. Then, the available fraction of P or Cd was determined by the extraction methods (Olsen procedure for P and  $\text{NH}_4\text{Cl}$  for available Cd) after 14 days of each flume run (Table 3-1). The flume soil was assumed to be at equilibrium at 14 days as there was no further loss of P or Cd in the leachate waters (total leachate water volume collected was 176 L).  $C_{In}$  concentrations at the start of the second and third runs were assumed to be equal to the concentration left in the soil at the end of the first and second runs, respectively, as the soil was not replaced after each run.

### 3.2.8 Leachate water analyses

From the 10 drain holes located at the lower base of the flume (Figure 3-1A), leachate water was collected every 24 h and mixed in a big bucket. The total volume of the leachate water was measured to calculate P or Cd mass (equation 3-3). Three triplicates of leachate water samples were taken from the big water bucket. Total water volume was measured every 24 h for 14 days after each experiment. Water samples were digested and analyzed for available P and Cd on the same day of collection as described in section 2-6 and measured as described in section 2-7 in Chapter 2.

### 3.3 Results and discussion

Soil particle size analysis indicated that the soil texture was sandy loam soil. It was composed of 9.7% clay, 7.3% silt and 83% sand (Table 3-1).

**Table 3-1 Chemical and physical properties of the soil**

Soil properties	Unit	Value
<b>Chemical Properties</b>		
pH		5.5
Electrical conductivity (EC)	$\mu\text{S cm}^{-1}$	148
Organic carbon (OC)	% Carbon (by weight)	1.32
Ca <sup>2+</sup>	cmol kg <sup>-1</sup>	1.68
Mg <sup>2+</sup>	cmol kg <sup>-1</sup>	0.49
Na <sup>+</sup>	cmol kg <sup>-1</sup>	0.2
K <sup>+</sup>	cmol kg <sup>-1</sup>	1.3
Cation exchange capacity	cmol kg <sup>-1</sup>	3.67
Exchangeable sodium percentage ESP	%	5.4
Available Phosphorus Olsen P	$\mu\text{g kg}^{-1}$ (dry wt.)	1.13
Total P	$\mu\text{g kg}^{-1}$ (dry wt.)	39.1
Available Cadmium (Cd) (1 M NH <sub>4</sub> Cl)	$\mu\text{g kg}^{-1}$ (dry wt.)	BDL* = 0.02
Total Cd	$\mu\text{g kg}^{-1}$ (dry wt.)	0.46
<b>Physical properties</b>		
Sand	% by wt.	83
Silt	%	7.3
Clay	%	9.7
WHC	% (mm water per 100 mm soil)	12.1
Hydraulic conductivity (K <sub>sat</sub> )	(m. Sec <sup>-2</sup> )	7.45x10 <sup>-2</sup>
Soil porosity ( $\Phi$ )	%	0.4

\*BDL: Below Detection of limit of available Cd of 0.04  $\mu\text{g kg}^{-1}$

The soil is acidic with a pH of 5.5 (Queensland DPI) and is non-saline as the electrical conductivity is 1.48  $\mu\text{S cm}^{-1}$ . Water holding capacity is low at 12.7% due to the coarse soil texture. Organic carbon is also low at 1.3% C. Cation exchange capacity is low at 3.67 cmol kg<sup>-1</sup> due to the sandy nature of the soil and the soil is non-sodic as the exchangeable sodium percentage (ESP) is less than 6% according to the definition of sodic soil by the Department of

Primary Industries and Regional Development (Overheu et al., 2019). The low cation exchangeable capacity and organic carbon indicate a possible low potential to adsorb or chelate cations whilst the low pH may indicate a tendency to fix P (Cox et al., 2000). The initial concentration of total P and Cd extracted by digestion of soil samples were very low at 39.1  $\mu\text{g kg}^{-1}$  and 0.46  $\mu\text{g kg}^{-1}$ , respectively. Available P and Cd chemically extracted from the soil were also very low at 1.13  $\mu\text{g kg}^{-1}$  and 0.02  $\mu\text{g kg}^{-1}$  (BDL of 0.04  $\mu\text{g L}^{-1}$ ), respectively. This soil was used because it was available in large amounts in the local area and the soil adsorption characteristics will not interfere the MBL-DGT adsorption capacity.

### 3.3.1 Flume experiment results for P

Total P was initially 39.0  $\mu\text{g kg}^{-1}$  and did not increase greatly with the addition of 10  $\mu\text{g L}^{-1}$  or 100  $\mu\text{g L}^{-1}$  P (39.5 and 43.8  $\mu\text{g P kg}^{-1}$ , respectively) but did increase considerably after the addition of 500  $\mu\text{g L}^{-1}$  P (53.1  $\mu\text{g kg}^{-1}$ ) (Table 3-2). The amount of available P extracted by  $\text{NaHCO}_3$  increased to 80% of the total P which is 135% of the initial total P and about 97% of total P from experiment 2 (the addition of 100 mg P  $\text{L}^{-1}$ ).

Available P changed considerably, with P becoming more extractable. The chemically extractable P measured initially (0.04  $\mu\text{g kg}^{-1}$ ) was 2.9% of the total P (39.1  $\mu\text{g kg}^{-1}$ ) and changed considerably in all three experiments by 43%, 65%, and 80%, respectively, after 14 days.

**Table 3-2 Concentration and mass of total P and available P in soil and the leachate water after 14 days of SSP fertilizer dissolved in water and added in three consecutive applications of 0.01, 0.1 and 0.5 mg P  $\text{L}^{-1}$ . For each concentration, n = 6  $\times$  3 replicates  $\pm$  1 standard error.**

P Dose ( $\mu\text{g PL}^{-1}$ )	Added P $\mu\text{g}$ per Flume $\times 10^3$	P concentration			Mass of P ( $\mu\text{g}$ )		
		Total soil P ( $\mu\text{g kg}^{-1}$ )	Extracted available soil P ( $\mu\text{g kg}^{-1}$ )	Leachate water P ( $\mu\text{g L}^{-1}$ )	Total soil P $\times 10^3$	Extracted Soil P $\times 10^3$	Leachate water P $\times 10^3$
Initial	0.0	39.1	1.13 $\pm$ 0.01	BDL	72.3	2.09	BDL
10	1.8	39.5	16.8 $\pm$ 0.21	40 $\pm$ 2	73.0	31.0	0.90
100	18	43.8	28.3 $\pm$ 1.5	189 $\pm$ 3	81.0	52.3	10.1
500	90	53.1	42.5 $\pm$ 2.0	1,840 $\pm$ 152	98.1	78.5	72.9

In the first experiment, 1800  $\mu\text{g}$  of P was added to the flume with about half (49.8%) lost in the leachate (900  $\mu\text{g}$ ). Most interestingly, the large increase was in the available P after



the first flume experiment, which increased from 2.09  $\mu\text{g}$  to 31.0 mg, while the total P was virtually unchanged. This suggests that the physical conditions of the first experiment induced a substantial change in the lability of P. The flume was covered to maintain soil moisture at 80% of the soil water holding capacity prior to the experiment and to protect the soil from rain and air contamination. The addition of a large volume of water that was not permitted to drain from the flume for 24 hours seemed to 'activate' over 40% of the soil P over the 14 days of this experiment. The total P was therefore in a form that was readily converted to exchangeable P. This result indicates that the major effect that even brief floods may have on sandy loam soil, which is consistent with previous studies (Cerdan et al., 2002; Jamieson et al. 2003).

The second experiment added 18 mg of P to the soil with more than half (56%) lost to percolation through the soil. After this experiment 64% of the total P in the soil was extractable available P. In the third experiment, 90 mg of P was added to the flume, with about 81% loss in leachate water. Application of the dissolved SSP fertilizer suggested various reactions can occur in the soil. The concentration of P added to the soil by P fertilizers involves a variety of reactions. Four P pools can be generated: the soil solution P, soil surface P, mineral compound P and micro-organism P. Each pool has sub-reactions which could be slow or fast cycling. Individually, each pool could be described by its capacity (size), affinity (strength of binding P) and flux (rate of movement to and from the pool) (Ritchie and Weaver, 1993). Soil content of iron and aluminum oxides, organic matter and clay minerals affect the properties of each individual pool in respect to the capacity, affinity, and flux. Phosphorus adsorption of the sandy loam soil was severely limited because the constituents that contribute to increasing the capacity and the affinity were also limited. Therefore, increasing soluble P from the SSP fertilizer will occupy the majority of adsorption sites and the cycling between pools reaches equilibrium. Consecutive input of P in the sandy loam soil favors an equilibrium shift towards the liquid phase by the loss of P to the leachate water. This also indicates the amount of P that will get into leachate water may be connected to surface water.

Adding P to the flume soil every 14 days lead to saturation of the adsorption sites of this soil type which has low clay and low organic matter. Alternatively, it could lead to the precipitation of phosphate minerals, such as di-calcium phosphates dehydrate (DCPD), which is sparingly soluble depending on  $\text{Ca}^{2+}$  activity in the soil and P fertilizer (Nash and Murdoch, 1997). Generally, it was affirmed that  $\text{PO}_4^{2-}$  in the soil can be either adsorbed by calcite or Fe (hydro)oxides, clay minerals, and precipitation on to calcium phosphate minerals. At low concentration  $\text{PO}_4^{2-}$  can be adsorbed by calcite or precipitates at high concentration. The

increased P loading leads to saturation increases the formation of the DCPD (Perassi and Borgnino, 2014; Li et al., 2017) and the results of the extracted P by Olsen method are evidence of the formation of the DCPD. Hansen et al. (2002) found that DCPD precipitates about 20-34% of total P. Consequently, when SSP fertilizer was added to the soil, P arbitrarily divided into two pools varying in their ability to release P into soil solution: slowly released P (SRP) and rapidly released P (RRP). Phosphorus in these two pools constantly changes in relation to each other based on fertilizer input, soil moisture and P output by leaching or plant uptake (Weaver and Wong, 2011).

Phosphorus behavior also varies under aerobic and anaerobic soil conditions due to the changes brought about by ferric oxyhydroxide. After the first experiment ( $10 \mu\text{g P L}^{-1}$ ), low concentrations of soluble P added to the flume soil led to the adsorption of half of the added P to soil particles under soil reduction conditions. After the second and third experiments ( $100 \mu\text{g P L}^{-1}$  and  $500 \mu\text{g P L}^{-1}$ ) the soil pore water was close to P saturation due to continuous moist conditions (80% WHC) which created anaerobic conditions that can increase P sorption when high soluble P levels are in soil solution (Jamieson et al., 2003). However, in this sandy loam soil, all the adsorbed P was recovered in equilibrium solution under reduced conditions and the high mobility of P to the leachate water suggested this soil has limited capacity to adsorb P. Concentration of P in the leachate water was measured every 24 h (Table 3-3). The loss of P was greatest in the first 24 h after opening the drainage holes because of the large volume of leachate water draining out (Table 3-3).

After the first 24 h, P concentration in the leachate water decreased gradually and stabilized after 8 days except in the third experiment where P concentration stabilized after 12 days. The phosphorus loss was governed mainly by the solubility of the recently applied SSP fertilizer and the volume of water leached and collected every 24 h. The available P concentration in the leachate water was due to the SSP fertilizer dose and time of collecting the leachate water sample (ANOVA:  $F_{2,6} = 153$ ,  $P < 0.0001$ ) (Appendix 2B).

**Table 3-3 Mean P mass in the leachate water after the application of the SSP fertilizer dissolved in water. Three different P concentrations, 0.01 mg P L<sup>-1</sup>, 0.1 mg P L<sup>-1</sup> and 0.5 mg P L<sup>-1</sup>, were added to the flume soil. n = 42 × 3 replicates ± 1 standard error.**

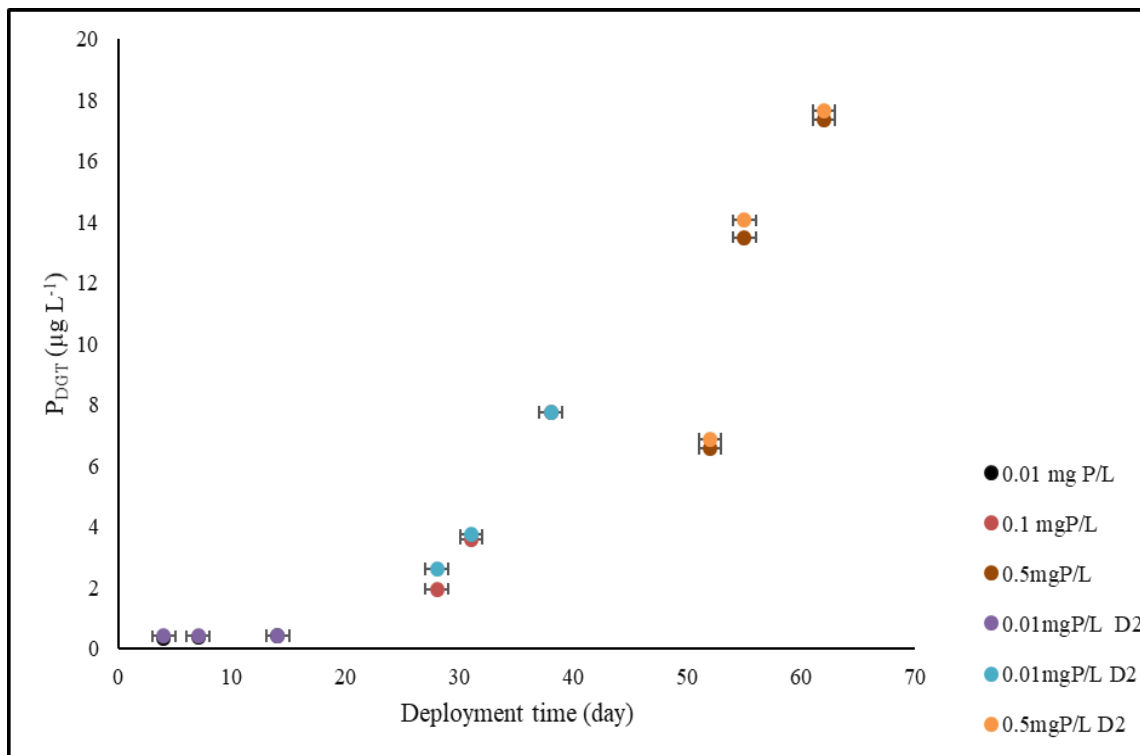
Time (h)	Water volume (L)	0.01 (mg P L <sup>-1</sup> )		0.1 (mg P L <sup>-1</sup> )		0.5 (mg P L <sup>-1</sup> )	
		P (µg L <sup>-1</sup> )	P Mass (µg)	P (µg L <sup>-1</sup> )	P Mass (µg)	P (µg L <sup>-1</sup> )	P Mass ×10 <sup>3</sup> (µg)
24	68.00	8.00 ± 0.10	517.0	117 ± 7.45	7,945	651 ± 5.21	44.24
48	39.00	4.00 ± 0.12	172.0	45.0 ± 3.21	1,739	460 ± 0.61	17.96
72	22.00	4.00 ± 0.01	88.00	11.0 ± 2.15	251.0	290 ± 0.84	6.373
96	18.00	3.00 0.01	57.00	6.00 ± 0.11	102.0	146 ± 2.10	2.624
120	10.00	3.00 ± 0.02	25.00	3.00 ± 0.01	25.00	100 ± 2.45	997.0
144	6.000	2.00 ± 0.05	14.00	2.00 ± 0.01	10.00	69.0 ± 3.38	415.0
168	3.000	1.00 ± 0.02	4.000	1.00 ± 0.00	3.000	47.0 ± 0.68	142.0
192	3.000	2.00 ± 0.01	5.000	1.00 ± 0.00	3.000	36.0 ± 0.56	108.0
216	2.000	1.00 ± 0.02	3.000	1.00 ± 0.00	1.000	13.0 ± 0.84	27.00
240	1.000	2.00 ± 0.03	2.000	1.00 ± 0.01	1.000	10.0 ± 0.22	10.00
264	1.000	2.00 ± 0.01	2.000	1.00 ± 0.00	1.000	9.00 ± 0.09	9.000
288	1.000	1.00 ± 0.00	1.000	1.00 ± 0.01	1.000	5.00 ± 0.05	5.00
312	1.000	3.00 ± 0.01	3.000	1.00 ± 0.01	1.000	4.00 ± 0.21	4.000
336	1.000	3.00 ± 0.02	3.000	1.00 ± 0.00	1.000	3.00 ± 0.11	3.000
Total	Total		Total		Total		Total
2520	176.0		896.0		10.08		72.91

### 3.3.2 DGT measurements of P in soil

The concentration of P<sub>DGT</sub> increased with each SSP dose and deployment time (Table 3-4). The results also show that P<sub>DGT</sub> at 45 cm was slightly higher than at 15 cm (Table 3-4 and Figure 3-2). It proves that deeper layers in the flume had more anaerobic conditions and therefore supports Fe<sup>3+</sup> reduction as a key mobilization mechanism. The concentration of P<sub>DGT</sub> significantly varied with the soluble fertilizer dose added to the soil (ANOVA: F<sub>2,12</sub> = 382, P < 0.001). P<sub>DGT</sub> was also significantly affected by the deployment time (ANOVA: F<sub>2,12</sub> = 114.9, P < 0.001) and depth (ANOVA: F<sub>1,12</sub> = 0.113, P < 0.001) (Appendix 2A).

**Table 3-4 Mean  $P_{DGT}$  concentration after using SSP fertilizer of three different concentrations,  $0.01 \text{ mg P L}^{-1}$ ,  $0.1 \text{ mg P L}^{-1}$  and  $0.5 \text{ mg P L}^{-1}$ . Each fertilizer application,  $n = 6 \times 3$  replicates  $\pm 1$  standard error.**

Time (day)	Depth (cm)	P ( $\mu\text{g L}^{-1}$ )		
		Fertilizer concentration ( $\text{mg L}^{-1}$ )		
		0.01	0.1	0.5
4	15	$0.361 \pm 0.01$	$1.96 \pm 0.60$	$6.58 \pm 0.60$
	45	$0.420 \pm 0.40$	$2.62 \pm 0.20$	$6.89 \pm 1.20$
7	15	$0.402 \pm 0.05$	$3.61 \pm 0.30$	$13.5 \pm 0.40$
	45	$0.423 \pm 0.10$	$3.75 \pm 0.20$	$14.1 \pm 0.41$
14	15	$0.423 \pm 0.07$	$7.75 \pm 0.20$	$17.3 \pm 0.30$
	45	$0.440 \pm 0.02$	$7.76 \pm 0.01$	$17.7 \pm 0.10$



**Figure 3-2  $P_{DGT}$  concentration measured by MBL-DGT after the application of  $0.01 \text{ mg P L}^{-1}$ ,  $0.1 \text{ mg P L}^{-1}$  and  $0.5 \text{ mg P L}^{-1}$  dissolved SSP fertilizer at different deployment time (4, 7 and 14 days). The interval times between each P fertilizer application were included for plotting the graph. When  $0.1 \mu\text{g L}^{-1}$  P fertilizer was added, deployment times were 28, 31, and 31 days including interval time of 10 days after the 14 days. For the third concentration of  $0.5 \mu\text{g L}^{-1}$ , deployment times were 52, 55, and 62 days including the interval time of 20 days at two depths 15 cm and 45 cm (D2).  $n = 18 \times 3$  replicates  $\pm 1$  standard error.**

The adsorption of labile P onto the MBL-DGT increased sharply with increasing fertilizer dose and deployment time (Figure 3-2). The increasing concentration of labile P over the MBL-DGT deployment time is an unusual result. The DGT system introduces a sink for solutes in the soil. This creates a local concentration gradient due to the depletion of the solutes by DGT adsorption (Zhang et al., 1998b; Zhang et al., 2001c; Zhang and He, 2009).

Concentrations of solutes in soil solution adjacent to DGT devices therefore normally decrease with deployment time. In this experiment, the application of three increasing consecutive doses of soluble SSP fertilizer to the flume soil over six weeks provided more available P to the soil solution.

No depletion may be noticed Figure (3-2) due to the large size of the soil sample used in the flume experiment and the long deployment time compared with previous studies where small soil samples were used, such as 10-30 g of soil (Six et al., 2013). In small samples, the solid phase reservoir solutes are rapidly depleted due to the small size and mass of the soil sample. When the solid phase reservoir size and mass are large, the depletion of solute in the solid phase will take longer (Zhang and He, 2009). In addition, maintaining the water content of the flume soil at 80% of water holding capacity increased DGT uptake. Increasing soil water content caused decrease tortuosity of the diffusion pathway, and an increase in the effective area of contact with the MBL-DGT surface as the area of air-filled pores contacting the DGT surface was reduced (Hooda, et al., 1999).

MBL-DGT responded to the increase in available P over time in a similar way to the chemically extracted P (Olsen) values, although the actual measurements were very different. The Olsen P concentration measurements were much higher than  $P_{DGT}$  concentrations (Table 3-2 and Table 3-4) but, a high correlation was found between the two methods (more information about the Olsen and DGT method is in section 3-3-5 and Table 3-11). These findings corresponded with other studies (Zhang and Tillman, 2007; Ahmad et al., 2008; Mason et al., 2010) that found positive correlations between Olsen P and  $P_{DGT}$  despite the high concentration of the Olsen P measurements. Olson P extraction using sodium bicarbonate in pH 8 is a strong extractant which solubilizes P fractions strongly adsorbed on to soil particles. This fraction of P, called fixed P is normally considered unavailable P therefore, Olsen P overestimated the available P in the soil (Six et al., 2012; Mason et al., 2013).

### 3.3.3 Cd concentration in the soil

The concentration of Cd in the soil before application of P fertilizer was  $0.025 \mu\text{g L}^{-1}$  which was below the detection limit of the ICP-MC ( $0.042 \mu\text{g L}^{-1}$ ) and was 0.05% of the total Cd ( $0.460 \mu\text{g kg}^{-1}$ ). The percentage of the total Cd present in the soil as available Cd changed over the three experiments to 26%, 34% and 35% ( $0.12$ ,  $0.159$ , and  $0.162 \mu\text{g kg}^{-1}$ ) after the application of  $10 \mu\text{g L}^{-1}$ ,  $100 \mu\text{g L}^{-1}$  and  $500 \mu\text{g L}^{-1}$  P fertilizer, respectively (Table 3-5).

Total Cd was initially  $0.460 \mu\text{g kg}^{-1}$  and did not increase much with the addition of  $10 \mu\text{g P L}^{-1}$ ,  $100 \mu\text{g P L}^{-1}$  or  $500 \mu\text{g P L}^{-1}$  ( $0.460$ ,  $0.461$  and  $0.462 \mu\text{g Cd kg}^{-1}$ , respectively). Most of the added Cd moved to the leachate water. Concentrations of Cd in the leachate water were 65%, 69% and 88% of the added Cd ( $0.5 \mu\text{g L}^{-1}$ ,  $5.1 \mu\text{g L}^{-1}$  and  $26 \mu\text{g L}^{-1}$ ), after the application of  $10 \mu\text{g P L}^{-1}$ ,  $100 \mu\text{g P L}^{-1}$  and  $500 \mu\text{g P L}^{-1}$  P fertilizer, respectively (Table 3-5).

**Table 3-5 Concentration and content of total Cd and available Cd in soil and in the leachate water after the addition of dissolved SSP at  $0.01 \text{ mg P L}^{-1}$ ,  $0.1 \text{ mg P L}^{-1}$  and  $0.5 \text{ mg P L}^{-1}$ , for each concentration,  $n = 18 \times 3$  replicates  $\pm 1$  standard error.**

P dose $\mu\text{g kg}^{-1}$	Cd added $\mu\text{g}$	Cd Concentration ( $\mu\text{g kg}^{-1}$ )			Mass of Cd ( $\mu\text{g}$ )		
		Total soil Cd	Extracted soil Cd	Leachate water Cd ( $\mu\text{g L}^{-1}$ )	Total soil Cd	Extracted soil Cd	Leachate water Cd
	0	$0.46 \pm 0.11$	BDL	BDL	850	46.2	BDL
10	0.500	$0.46 \pm 0.01$	BDL	BDL	851	222	0.33
100	5.100	$0.46 \pm 0.02$	$0.16 \pm 0.01$	BDL	852	293	3.50
500	26.00	$0.46 \pm 0.03$	$0.16 \pm 0.01$	$0.60 \pm 0.01$	854	299	22.9

Increasing the concentration of Cd in the leachate water indicated that the sandy loam soil in these experiments had no tendency to absorb Cd. The concentration of Cd in the leachate water was significantly increased with increasing fertilizer dose (ANOVA:  $F_{2,6} = 912$ ,  $p < 0.0001$ ) (Appendix 2B). The Cd concentrations in the leachate water were highest after 24 h of fertilizer application then decreased rapidly over the next three days in the highest P fertilizer dose of  $0.5 \text{ mg L}^{-1}$  (Table 3-6).

These results are consistent with Laboski and Lamb (2004) and Sidle and Kardos (1977). In a different type of soils, such as loamy sand soils, Cd movement is different. Liu et al. (2015) found low mobility of Cd in soils while Mahdavi et al. (2013) found that Cd accumulated near soil surfaces. Lee et al. (1996) also observed that Cd remains at or near the soil surface and did not move deeper. Loganathan (1995) concluded that Cd concentration decreases with soil depth due to the higher pH of soil surfaces and higher adsorption due to the higher organic matter content of the topsoil.

**Table 3-6 Mean Cd mass in the leachate water after the application of the SSP fertilizer dissolved in water. Three different SSP concentrations, 0.01 mg P L<sup>-1</sup>, 0.1 mg P L<sup>-1</sup> and 0.5 mg P L<sup>-1</sup>, were added to the flume soil. n = 42 × 3 replicates ±1 standard error.**

Time (h)	Water volume (L)	0.01 mg P L <sup>-1</sup>		0.1 mg P L <sup>-1</sup>		0.5 mg P L <sup>-1</sup>	
		Cd (µg L <sup>-1</sup> )	Cd Mass (µg)	Cd (µg L <sup>-1</sup> )	Cd Mass (µg)	Cd (µg L <sup>-1</sup> )	Cd Mass (µg)
24	68.0	BDL	0.209	BDL	1.836	0.234 ± 0.010	15.23
48	39.00	BDL	0.025	BDL	0.975	0.142 ± 0.011	4.680
72	22.00	BDL	0.017	BDL	0.373	0.063 ± 0.013	1.385
96	18.00	BDL	0.015	BDL	0.276	0.057 ± 0.011	1.024
120	10.00	BDL	0.012	BDL	0.122	0.048 ± 0.012	0.480
144	6.000	BDL	0.004	BDL	BDL	0.011 ± 0.00	0.066
168	3.000	BDL	0.001	BDL	BDL	0.017 ± 0.011	0.052
192	3.000	BDL	0.001	1.000	BDL	BDL	0.026
216	2.000	BDL	0.001	BDL	BDL	BDL	0.017
240	1.000	BDL	0.001	BDL	BDL	BDL	0.005
264	1.000	BDL	0.001	BDL	BDL	BDL	0.002
288	1.000	BDL	0.003	BDL	BDL	BDL	0.002
312	1.000	BDL	0.002	BDL	1.000	BDL	0.002
336	1.000	BDL	0.001	BDL	0.000	BDL	0.001
Total	Total		Total		Total		Total
2520	176.0		0.330		10.08		23.00

### 3.3.4 Cd measurement by the MBL-DGT

The Cd concentrations were measured using the MBL-DGT and calculated using the DGT equation (Zhang and Davison, 1995). Cd<sub>DGT</sub> concentrations are presented in Table 3-7.

Labile Cd concentration significantly increased with increasing P fertilizer dose (ANOVA:  $F_{2,12} = 38.42$ ,  $P < 0.0001$ ) and MBL-DGT deployment time (ANOVA:  $F_{2,12} = 13.25$ ,  $P < 0.005$ ) (Figure 3-3) (Appendix 2A).

**Table 3-7 Mean  $Cd_{DGT}$  concentration after using SSP fertilizer at three doses, 0.01 mg P L<sup>-1</sup>, 0.1 mg P L<sup>-1</sup> and 0.5 mg P L<sup>-1</sup>. n = 18 × 3 replicates ± 1 standard error.**

Time (day)	Depth	Cd ( $\mu\text{g L}^{-1}$ )		
		Fertilizer dose (mg L <sup>-1</sup> )		
		0.01	0.1	0.5
4	15	BDL	BDL	0.049 ± 0.00
	45	BDL	BDL	0.086 ± 0.00
7	15	BDL	0.052 ± 0.00	0.089 ± 0.02
	45	BDL	0.064 ± 0.00	0.111 ± 0.00
14	15	BDL	0.074 ± 0.01	0.128 ± 0.02
	45	BDL	0.070 ± 0.01	0.145 ± 0.01

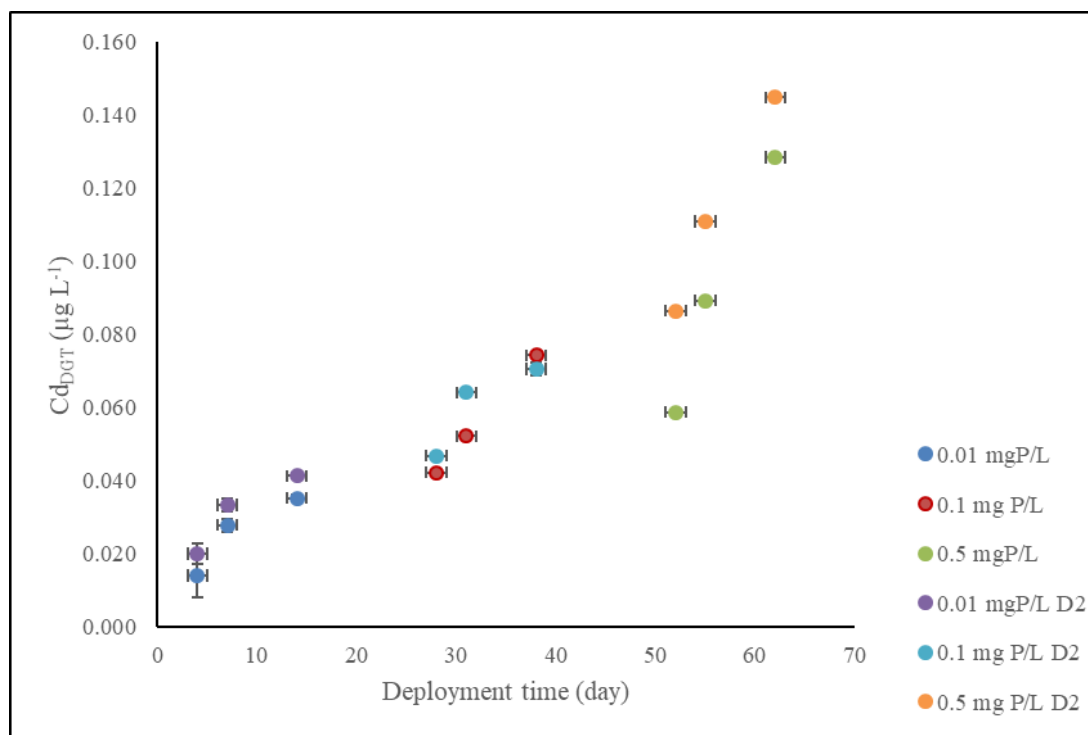
The successive application of increasing P fertilizer doses over six weeks led to increased  $Cd_{DGT}$  concentration in the flume soil (Figure 3-3). Similar to  $P_{DGT}$ ,  $Cd_{DGT}$  at 45 cm was higher than the  $Cd_{DGT}$  concentration at 15 cm. There was a significant change in  $Cd_{DGT}$  with deployment depth of the MBL-DGT (ANOVA:  $F_{2,12} = 0.922$ ,  $P < 0.05$ ) Appendix 2A indicates that Cd was moving down to the leachate water.

$Cd_{DGT}$  adsorption onto the MBL-DGT increased with deployment time in all treatments and at both depths (Figure 3-3). The highest increase was in the third experiment (0.5 mg P L<sup>-1</sup>). Although labile Cd was extremely low, the MBL-DGT had a higher selectivity for detecting labile Cd. The concentration of Cd chemically extracted by  $\text{NH}_4\text{Cl}$  and the  $Cd_{DGT}$  using the MBL-DGT demonstrated large differences between the two procedures (see Table 3-5 and Table 3-7).

The labile Cd concentration did not deplete around the DGT (Zhang and Davison, 1995) due to soil conditions in the three experiments. Maintaining water saturation of the soil (80% WHC) over the experimental period provided more readily available Cd (Zhang et al., 2001a), provided a shorter diffusion pathway to the MBL-DGT surface and increased the effective area of contact with the DGT surface because the air-filled pores around the DGT surface are



reduced (Hooda et al., 1999). Dissolution of the SSP fertilizer allowed the Cd embedded within the crystal lattice of the fertilizer to be available in the pore water.



**Figure 3-3**  $Cd_{DGT}$  concentration measured by MBL-DGT after the application of 0.01 mg P L<sup>-1</sup>, 0.1 mg P L<sup>-1</sup> and 0.5 mg P L<sup>-1</sup> dissolved SSP fertilizer at different deployment time (4, 7 and 14 days). The intervals time between each P fertilizer application was included for plotting the graph. when 0.1  $\mu\text{g L}^{-1}$  P fertilizer was added, deployment times were 28, 31, 31 days including intervals time of 10 days after the 14 days. For the third concentration of 0.5  $\mu\text{g L}^{-1}$  deployment times were 52, 55, and 62 days including the intervals time of 20 days at two depths 15 cm and 45 cm (D2). each depth,  $n = 18 \times 3$  replicates  $\pm 1$  standard error.

### 3.3.5 Comparison between chemical extraction and MBL-DGT results

The concentration and mass of available P and Cd extracted chemically ( $C_{EX}$ ) and ( $M_{EX}$ ), respectively, and the concentration and mass of available P and Cd extracted *in situ* by the MBL-DGT ( $C_{DGT}$ ) and ( $M_{DGT}$ ), respectively, are presented in Table 3-8.

The concentration of the available P and Cd increased with increasing fertilizer dose. Although both procedures targeted the same fractions of P and Cd in the soil (labile P and Cd), they produced very different results. Available P and Cd determined by the chemical extraction methods were higher than the available P and Cd determined by the MBL-DGT method.

**Table 3-8 Concentration and mass of P and Cd measured by two methods: MBL-DGT and chemical extraction.  $C_{EX}$  is the P or Cd concentration from chemical extraction,  $EX_{mass}$  is the extracted P or Cd mass,  $C_{DGT}$  is the P or Cd concentration from DGT,  $DGT_{mass}$  is the P or Cd mass accumulated on the DGT.  $n= 18 \times 3$  replicates and  $\pm 1$  standard error.**

Analytes	P fertilizer added ( $\mu\text{g L}^{-1}$ )	$C_{EX}$ ( $\mu\text{g L}^{-1}$ )	$EX_{mass}$ ( $\mu\text{g}$ )	$C_{DGT}$ ( $\mu\text{g L}^{-1}$ )	$DGT_{Mass}$ ( $\mu\text{g}$ )
P	10	$16.80 \pm 4.021$	31.05	$0.390 \pm 0.020$	177.1
	100	$28.32 \pm 3.120$	52.34	$0.440 \pm 0.010$	2,016
	500	$42.47 \pm 5.346$	78,49	$12.47 \pm 2.581$	5,662
Cd	10	$0.080 \pm 0.000$	147.0	$0.023 \pm 0.000$	10.44
	100	$0.120 \pm 0.000$	214.0	$0.060 \pm 0.000$	27.24
	500	$0.140 \pm 0.000$	264.0	$0.110 \pm 0.000$	49.94

A multiple linear regression (MLR) model (Microsoft Excel 10) was undertaken for the three P measurement methods ( $P_{Total}$ ,  $P_{DGT}$ , and  $P_{EX}$ ) as dependent variables with fertilizer dose entered as an independent variable to investigate the effect of increasing P fertilizer dose on labile P extracted from the soil by using  $\text{NaHCO}_3$  (Olsen method), labile P measured by MDL-DGT methods and total P in the soil (Table 3-9 and Table 3-10).

**Table 3-9 Summary output of multiple regression model statistical results for the fertilizer dose effect on P measurements ( $P_{Total}$ ,  $P_{EX}$ , and  $P_{DGT}$ ).  $n= 18 \times 3$  replicates and  $\pm 1$  standard error.**

Variables	$R^2$	Standard Error	$df$	Significant F- value
X= Fertilizer dose Y= $P_{Total}$ , $P_{EX}$ and $P_{DGT}$	0.98	0.03	53	> 0.0001

**Table 3-10 Multiple Linear Regression statistics of P fertilizer dose as the independent variable effect on  $P_{Total}$ ,  $P_{DGT}$  and  $P_{EX}$  measurements as dependent variables in the soil.  $n= 18 \times 3$  replicates and  $\pm 1$  standard error.**

Variables	Coefficients	SE	P-value
$P_{DGT}$	0.003	0.001	> 0.0001
$P_{EX}$	0.009	0.002	> 0.0001

The results show a highly significant difference in P concentrations measured by the three methods with increasing P fertilizer dose ( $R^2 = 0.98$ ,  $P < 0.001$ ) for all variables (Table 3-10, and Table 3-11). The results were consistent with the correlation between fertilizer dose and both of the  $P_{Total}$  and  $P_{DGT}$  concentrations ( $r = 0.92$ ,  $P < 0.05$  and  $r = 0.96$ ,  $P < 0.05$ ) respectively (Table 3-11). In contrast, a moderate correlation was found between the fertilizer dose and the concentration of labile P measured by Olsen method ( $r = 0.68$ ,  $P < 0.05$ ). Table 3-11 shows a very high correlation between  $P_{Total}$  and  $P_{DGT}$  ( $r = 0.92$ ,  $P < 0.05$ ), whereas the correlation between  $P_{Total}$  and  $P_{EX}$  was weak ( $r = 0.51$ ,  $P < 0.05$ ).

**Table 3-11 The correlation between P measurements methods (Olsen, DGT and total P) in the soil. n= 18 × 3 replicates and ± 1 standard error.**

	$P_{Total}$ μg /kg	$P_{EX}$ μg/L	$P_{DGT}$ μ g/L	Fertilizer mg/kg
Total (mg / kg)	1.00			
$P_{EX}$ μg/L	0.51*	1.00		
$P_{DGT}$ μg/L	0.92**	0.68*	1.00	
Fertilizer mg/ kg	0.96**	0.68*	0.96**	1.00

\*Correlation is significant at 0.01 level, \*\*Correlation is significant at the 0.05 level

Based on these results a multiple regression model was undertaken with  $P_{Total}$  as an independent variable and the two labile P measurements ( $P_{DGT}$  and  $P_{EX}$ ) as dependent variables (Table 3-12 and Table 3-13). The statistical results in Table 3-12 are consistent with the source of labile P from total P in the soil system ( $R^2 = 0.86$ ,  $P < 0.0001$ ).  $P_{DGT}$  is significantly affected by the concentration of total P ( $P < 0.0001$ ) but  $P_{EX}$  did not show the same response ( $P > 0.0001$ ) (Table 3-13). The results also showed a moderate correlation between  $P_{EX}$  and  $P_{DGT}$  which means that both methods are targeting the same P form.

**Table 3-12 The multiple regression model statistical results for  $P_{Total}$  and labile P measured by Olsen method and DGT method. (n= 18 × 3 replicates and ± 1 standard error).**

Variables	$R^2$	Standard Error	df	Significant F-value
X= $P_{Total}$	0.86	2.27	53	> 0.0001
Y= $P_{EX}$ and $P_{DGT}$				

**Table 3-13 Multi Linear Regression statistics of P<sub>Total</sub> as the independent variable with P<sub>DGT</sub> and P<sub>EX</sub> measurements as dependent variables in the soil. (n= 18 × 3 replicates and ± 1 standard error).**

Dependent variables	Coefficients	SE	P value
P <sub>DGT</sub>	1.053	15.36	> 0.0001
P <sub>EX</sub>	-0.123	-3.168	0.003

To confirm these statistical results a Pearson's Correlations was calculated among the three P measurements (P<sub>Total</sub>, P<sub>EX</sub> and P<sub>DGT</sub>) and fertilizer dose (Table 3-14). A significant correlation was found between P<sub>DGT</sub> with fertilizer dose and total P in the soil ( $r = 0.95$ ,  $P < 0.01$  and  $r = 0.90$ ,  $p < 0.01$ ) respectively. By comparison, P<sub>EX</sub> had a moderate correlation with fertilizer dose and a significant correlation with total P in the soil ( $r = 0.79$ ,  $P < 0.01$  and  $r = 0.88$ ,  $P < 0.01$ ) (Table 3-14). Also, Table 3-14 shows a strong correlation between P<sub>DGT</sub> and P<sub>EX</sub> ( $r = 0.85$ ,  $P < 0.01$ ), which corresponded with Burkitt et al., (2016) who found a strong correlation between log P<sub>DGT</sub> method and Olsen method.

**Table 3-14 Pearson 's R correlations between P<sub>DGT</sub>, P<sub>Olsen</sub>, total P and fertilizer dose. n= 18 × 3 replicates and ± 1 standard error.**

Correlations	Dose	P <sub>DGT</sub>	P <sub>EX</sub>	Total P
Dose	1.00	0.95**	0.79*	0.95**
P <sub>DGT</sub>	0.95**	1.00	0.85*	0.90**
P <sub>EX</sub>	0.79*	0.85*	1.00	0.84*
Total P	0.95**	0.90**	0.88*	1.00

\*\* . Correlation is significant at the 0.01 level (2-tailed); \* . Correlation is significant at the 0.05 level (2-tailed)

After the application of SSP fertilizer at 10, 100 and 500  $\mu\text{g L}^{-1}$  doses, available P values measured by the chemical extraction method, constituted 40%, 90% and 80% of the total P in the soil respectively. Phosphorus extracted by the Olsen method using  $\text{NaHCO}_3$  extractant was high compared to the available P measured by the MBL-DGT which was only 0.2% of the total P concentration. It is well known that in soil systems available P rarely makes up more than

20% of total P concentration at any particular point in time (Wild, 1958; Moody and Bolland, 2003). Using NaHCO<sub>3</sub> extraction increased the solubility of non-labile P forms in the soil and produced an overestimate measurement of labile P.

Similarly, Cd<sub>DGT</sub> and Cd<sub>EX</sub> were assessed to validate the MBL-DGT method. A linear regression was undertaken to investigate the effect of P fertilizer dose on the concentration of available Cd measured by extracted method (C<sub>EX</sub>), available Cd measured by DGT method (C<sub>DGT</sub>) and the concentration of total Cd in the soil. Increasing fertilizer dose had a significant effect on elevating the concentration of available Cd and total Cd in the soil ( $R^2 = 0.89$ ,  $P < 0.001$ ) (Table 3-15).

**Table 3-15 Summary output of multiple regression model statistical results for the fertilizer dose effect on Cd measurements (Cd<sub>Total</sub>, Cd<sub>EX</sub>, and Cd<sub>DGT</sub>). n= 18 × 3 replicates and ± 1 standard error.**

Variables	R <sup>2</sup>	Standard Error	df	Significant F- value
X= Fertilizer dose	0.88	0.07	53	> 0.0001
Y= Cd <sub>Total</sub> , Cd <sub>EX</sub> and Cd <sub>DGT</sub>				

**Table 3-16 Multiple Linear Regression statistics of P fertilizer dose effect on Cd<sub>Total</sub>, Cd<sub>DGT</sub> and Cd<sub>EX</sub> measurements in the soil. n= 18 × 3 replicates and ± 1 standard error.**

Variables	Coefficients	Standard Error	P value
Total P	225.05	21.012	> 0.0001
P <sub>DGT</sub>	0.5472	0.463	> 0.0001
P <sub>EX</sub>	0.1175	0.3001	> 0.0001

A significant correlation was found between fertilizer dose and C<sub>DGT</sub> of Cd ( $R^2 = 0.82$ ,  $P < 0.05$ ), whereas a medium correlation was recorded between the fertilizer dose and C<sub>EX</sub> of Cd ( $R^2 = 0.69$ ,  $P < 0.05$ ) (Table 3-17). C<sub>DGT</sub> of Cd showed a significant correlation with total Cd in the soil ( $R^2 = 0.84$ ,  $P < 0.05$ ) but, Cd<sub>EX</sub> had only a low correlation with total Cd in the soil ( $R^2 = 0.59$ ,  $P = 0.05$ ) (Table 3-17).

**Table 3-17 The correlation between Cd measurement method (CEX, CDGT and total Cd in the soil) and P fertilizer dose. (n = 54 ×3replicates ).**

	Cd <sub>Total</sub> (µg/kg)	Cd <sub>EX</sub> (µg/L)	Cd <sub>DGT</sub> (µ g/L)	Fertilizer dose (mg/kg)
Cd <sub>Total</sub> µg/L	1.00			
Cd <sub>EX</sub> µg/L	0.59*	1.00		
Cd <sub>DGT</sub> µg/L	0.81**	0.58*	1.00	
Fertilizer mg / kg	0.93**	0.69*	0.89**	1.00

\*Correlation is significant at 0.01 level, \*\*Correlation is significant at the 0.05 level

Available Cd measured by MBL-DGT (Cd<sub>DGT</sub>) has a stronger correlation with total Cd than with available Cd extracted by NH<sub>4</sub>Cl (Cd<sub>EX</sub>) (Table 3-10). A moderate correlation was found between Cd<sub>EX</sub> and Cd<sub>DGT</sub> (Table 3-18).

**Table 3-18 Pearson's R correlations between Cd<sub>DGT</sub>, Cd<sub>EX</sub>, total Cd and fertilizer dose (n= 18 × 3 replicates and ± 1 standard error).**

Factors	Dose	Cd <sub>DGT</sub>	Cd <sub>EX</sub>	Total Cd
Dose	1.00	0.96**	0.66*	0.90**
Cd <sub>DGT</sub>	0.65*	1.00	0.60*	0.93**
Cd <sub>EX</sub>	0.66*	0.60*	1.00	0.45*
Total Cd	0.90**	0.93**	0.45*	1.00

\*\* Correlation is significant at the 0.01 level (2-tailed); \*. Correlation is significant at the 0.05 level (2-tailed)

Likewise, available Cd extracted by NH<sub>4</sub>Cl constituted 40-50% of total Cd in the soil after the application of SSP fertilizer. Cd<sub>DGT</sub> constituted only 0.06, 0.1 and 0.2% of total Cd after the application of SSP fertilizer at 10 µg L<sup>-1</sup>, 100 µg L<sup>-1</sup> and 500 µg L<sup>-1</sup>, respectively. The high ratios of extracted Cd do not reflect the real concentration of the available Cd because the concentration of available Cd in soil solution is only a very small fraction of total Cd in the soil (Auer, 1977; Menon et al., 1991). These findings were consistent with the MBL-DGT with Metsorb mixed binding layer being superior to the Olsen and NH<sub>4</sub>Cl extraction methods in determining how available P and Cd concentration change in soil after the addition of SSP fertilizer of different concentrations.

The Metsorb Mixed Binding Layer is capable of describing changes in labile Cd in soil despite the low soil concentrations of Cd. Although only a small increase in labile Cd occurred

during the experiment, the accumulation of Cd in the flume soil was well predicted by the MBL-DGT technique.

### 3.4 Conclusions

Phosphorus fertilizers are the most common supplements added to soil to improve agricultural production. However, two important environmental problems arise from the successive application of P fertilizers. The first problem is eutrophication of surface water caused by the accumulation of labile P due to P runoff from agricultural lands. The second issue is the accumulation of labile Cd in soil and water due to impurities in P fertilizers. Therefore, accurate determination of labile P and Cd is needed to monitor changes in the concentration of P and Cd throughout the soil profile and in leachate water.

The Metsorb mixed binding layer DGT (MBL-DGT) is the most accurate *in situ* method to detect P and Cd in synthetic freshwater and seawater (Panther et al., 2014) and natural seawater (Shiva, et al., 2016). This study showed that the MBL-DGT was able to simultaneously determine P and Cd concentrations in the soil and was more accurate in expressing the relationship between labile and total concentrations of P and Cd than traditional chemical extraction methods.

The results also showed that both labile P and Cd were prone to leaching when different concentrations of SSP fertilizer were added to sandy loam soil. Accumulation of available P and Cd in soil and leachate water is expected due to the repetitive use of P fertilizers. As such, the MBL-DGT technique is an accurate, cost-effective and multifunctional *in situ* sampling device suitable for research and monitoring of vulnerable soils and ecosystems leading to more sustainable and healthier agricultural processes.

## **Chapter 4-. Evaluation of Metsorb-Chelex Double Binding Layer DGT (DBL-DGT) for estimating the labile concentration of phosphorus and cadmium following the addition of P fertilizers**

### **4.1 Introduction**

Excessive application of P fertilizer will increase available P content in soils above plant requirements. The surplus P will be transferred with water runoff or water leaching from the soil body to waterbodies. Increasing P concentration in water will cause algal growth in waterbodies (eutrophication) leading to the deterioration of water quality for use by other organisms (Carpenter, 2008; King et al., 2015).

Cadmium (Cd) accumulates in soil due to the application of P fertilizers and other soil amendments in agricultural soils (Loganathan et al., 1995; Mingxia et al., 2010). Many contaminants are associated with P fertilizers, such as selenium (Se), cobalt (Co), molybdenum (Mo) and lead (Pb), however, Cd is singular of major concern in regards to contamination of the soil or plants (Mclaughlin et al., 1996; Chaney and Donald, 2012). The high concentration of Cd in Australian superphosphate fertilizer was first identified by Walkley (1940). This high Cd concentration in P fertilizer resulted from the use of high Cd phosphate rock for fertilizer manufacture (Tiller et al., 1997). The environmental consequences of these high Cd levels for soil and food quality were first highlighted by Williams and David (1973).

It is important to assess the movement of the labile forms of P and Cd in the soil as they are the most accessible form of the two solutes in soil to the plant tissues through plant absorption processes (Mann and Ritchie, 1995; Hooda et al., 1999; Pierzynski et al., 2000; Menzies et al., 2005). Many attempts have been made to precisely measure the concentration of bioavailable P and Cd in soil using conventional chemical extractant methods. To date, Colwell (1963) and Olsen et al. (1954) methods have been used for available P while methods including soil solution, HOAc, EDTA, CaCl<sub>2</sub>, NH<sub>4</sub>Cl, and NaOAc, have been used for available Cd. The available or labile form of P and Cd is a dynamic species in soil, therefore, *ex-situ* measurements are generally unreliable, partly because the chemical solution may change the species during sampling and extraction (Hooda et al., 1999a; Menzies et al., 2005; Mason et al., 2013; Six et al., 2014). The accuracy of the measurement of these methods depends on the chemical and physical properties of the soil such as temperature, soil pH, redox conditions, organic matter, and clay type. Moreover, the combination of sorption and



desorption processes, resulting in pseudo-equilibrium between solid and solution phases is sensitive to predominant conditions. *Ex situ* procedures involving separation of the solution and solid phases can disrupt this distribution (Hooda et al., 1999; Menzies et al., 2005).

Traditional methods of evaluating bioavailability of P and Cd using different soil extractions methods do not simulate plant uptake by these chemicals (Murdock et al., 2001; Mann et al., 2002; Mason et al., 2005b; Divis et al., 2007; Mansour and Gad, 2010; Mason et al., 2010), therefore an accurate and integrated method is required. A new *in situ* DGT technique capable of quantitative measurement has been developed to measure labile metal species in soil (Zhang et al., 1998a; Zarrouk et al., 2014). This technique has successfully been used to measure the available P and Cd in soil. This technique could eliminate the analytical error resulting from the re-sorption and re-dissolution common with traditional methods (Wang et al., 2016). The DGT is a passive sampler making it possible to determine *in situ* weighted average concentrations. In this technique, the analyte from pore water diffuses through the diffusion layer to the binding phase (Davison and Zhang, 1994; Zhang and Davison, 1995). The DGT acts as a plant surrogate, lowering the analyte concentration in the pore water, while simultaneously enhancing desorption of the analyte from the soil solid phase to the pore water (Wang et al., 2016). Research has shown that DGTs can measure the analyte's labile form throughout the whole soil system (Zhang et al., 2001b).

The DGT developed by Bennett (2010) used a Metsorb binding layer instead of the ferrihydrite binding layer which was first developed by Zhang et al. (1998). Both types of binding layers have been used successfully in water and soil. The Metsorb Chelex mixed binding layer DGT was developed to measure anions and cations (Panther et al., 2014). To date, the Metsorb DGT and the Metsorb Chelex mixed binding layer have not been used together in soil. Therefore, the experiment in this chapter will use the double binding layer of Metsorb and Chelex in the DGT device as a sandwich Metsorb DGT.

The overall aim of this study was to investigate the potential impact of three different P fertilizers on the concentration and mobility of P and Cd in soil. The main goal of this study was to evaluate the capacity of a new Metsorb and Chelex double layer DGT (DBL-DGT) to investigate P and Cd accumulation in tomato tissues. This experiment was conducted in pots in a greenhouse, providing bulk soil conditions, as previous experimental work using DGTs in soils has been carried out under laboratory conditions using only small samples (~10-50 g) of soils. In addition, Metsorb DGTs have never been used in studies of the performance of DGTs at soil depths greater than 5 cm. To investigate the mobility of P and Cd in the soil, DBL-DGTs

were deployed at three soil depths. DGTs were deployed for short periods of time in soil (~24-48 hours) in previous studies which may be insufficient time for longer-term adsorption and desorption reactions to occur. Therefore, this study left DGTs in the soil for up to 7 days. Concentrations of P and Cd measured by the new DBL-DGT in the soil were investigated with plant uptake and compared with P and Cd concentrations measured using the Olsen method as a conventional soil extraction test.

## **4.2 Materials and methods**

### **4.2.1 Soil type and preparation**

A sandy loam soil was used in this study; details of the soil collection location, classification and characterization are described previously in Chapter 2 (Section 2.10 and Table 2.2). Total and available P in the soil were  $39.0 \mu\text{g P kg}^{-1}$  and  $1.13 \mu\text{g kg}^{-1}$ , respectively. Total and available Cd in the soil were  $0.46 \mu\text{g kg}^{-1}$  and  $0.02 \mu\text{g kg}^{-1}$ , respectively.

The soil was sieved through a 10 mm diameter mesh to exclude gravel, large roots, and other unwanted substances. Three replicates were prepared for each treatment: in total 36 large pots (22 cm x 22 cm x 75 cm) were filled with soil and contained a 75 cm tube for collecting leachate water. To prevent blockages, the bottom bases of the pots were lined with three successive layers of a fine screen net; a filtering fabric; and a 1 cm layer of fine gravel. Approximately 47 kg of soil was then packed into each pot. Soil depth was 70 cm and the soil density was approximately  $1.4 \text{ g cm}^{-3}$ . The experiment commenced on 27 April 2011. Each pot received 5.6 L of distilled water to achieve soil moisture of 80% of water holding capacity (WHC) as described in section 2-10. No leaching was permitted at this stage via lifting up the ends of the drainage tubes. The soil in the pots was then covered by medium gravel to prevent evaporation and left for 48 h to allow the uniform distribution of the water throughout the soil profile.

### **4.2.2 Fertilization**

Three types of fertilizers were used in this pot experiment: Single Super Phosphate (SSP), Egyptian Reactive Phosphate (ER) (Rock Stock) and phosphorus soft rock from Nutri-tech Solution (NTS). These three P fertilizers have different P and Cd concentrations as shown in Table 4-1. NTS fertilizer was used in this experiment to confirm that this type of fertilizer has no Cd. Four doses of each P fertilizer (0, 100, 200, and  $250 \text{ kg P ha}^{-1}$ ) were applied in the second week of the experiment. The P fertilizer doses were chosen within and above the

standard fertilization dose for agricultural production of tomatoes in the field as recommended by the DPI tomato kit of 70- 100 kg P ha<sup>-1</sup> in soils that have low P (> 0.5 mg Kg<sup>-1</sup> P concentration (Fullelove et al., 1998).

**Table 4-1**Types of P fertilizers and application doses used in the glasshouse experiment. Initial available P in the soil before fertilization was 0.134 µg L<sup>-1</sup> while initial available Cd was below the detection limit of the instrument. Initial total P in the soil was 39.9 µg P kg<sup>-1</sup> and initial total Cd was 0.46 µg Cd kg<sup>-1</sup>.

Fertilizer type	% P in the fertilizer	Cd (mg kg <sup>-1</sup> )	Fertilizer dose (kg P ha <sup>-1</sup> )	Fertilizer added (kg ha <sup>-1</sup> )	Fertilizer (g pot <sup>-1</sup> )	P (g pot <sup>-1</sup> )	Cd (mg pot <sup>-1</sup> )
SSP	13	25	0.0	0.0	0.0	0.0	0.00
			100	1.14	13.4	1.18	0.33
			200	2.27	26.7	2.35	0.67
			250	2.84	33.4	2.94	0.83
NTS	9.08.8	0.0	0.00	0.00	0.00	0.00	0.00
			100	1.11	13.1		0.00
			200	2.22	26.1	2.35	0.00
			250	2.78	32.6	2.94	0.00
ER	10	8.8	0.00	0.00	0.00	0.00	0.00
			100	769	9.0	1.18	0.09
			200	1.54	18.1	2.35	0.18
			250	1.92	22.6	2.94	0.23

The fertilizers were mixed into the soil surface to a depth of 5 cm. All treatments received the same basic amount of nitrogen (N) and potassium (K) and other nutrients (Fullelove et al., 1998). Nitrogen was added as urea to each pot (6 g N pot<sup>-1</sup>) by dividing into three portions and applying directly to the soil surface three times over the growth period of the tomatoes (planting, flowering and at the beginning of fruiting). Potassium was added as K<sub>2</sub>SO<sub>4</sub> (9 g per pot split into three portions) directly to the soil with the N fertilizer at planting, flowering, and fruiting. In addition, Ca (60 µg g<sup>-1</sup>), Mg (50 µg g<sup>-1</sup>), S (40 µg g<sup>-1</sup>), Zn (4 µg g<sup>-1</sup>), Cu (4 µg g<sup>-1</sup>), Mn (1.25 µg g<sup>-1</sup>), B (0.2 µg g<sup>-1</sup>), and Mo (0.3 µg g<sup>-1</sup>) were applied at the start of the pot trial. Three tomato plants (*Lycopersicon esculentus*) were grown in each pot using two-week-old seedlings and thinned to one plant per pot two weeks later. The tomato plant was chosen for this experiment because it is a high consumer of soil nutrients and solutes so it is a good indicator of Cd accumulation in plant tissues. It is also a common plant in the human diet. The

pots were irrigated with 500 mL of deionized water every second day during the first three weeks of plant growth and increased to 1000 mL every day thereafter, due to increases in plant water consumption. This amount of irrigation water was necessary to keep soil moisture at the required 80% of WHC during the seven days of the DGT deployment. After seven days of deployment, the DGTs were removed from the soil. The greenhouse has, on average, ~14 hours sunlight per day and a temperature range of 22-29°C. The tomato fruits were harvested 120 days after planting the tomato seedlings.

### **4.2.3 Soil analyses**

The available concentrations of P or Cd in the soil were measured after harvesting the plants, using conventional procedures of Olsen extraction (Olsen, 1954) for available P and  $\text{NH}_4\text{Cl}$  extraction (Krishnamurtia et al., 1995) for the available Cd as described in sections 2.6 and 2.7 in Chapter 2. The total concentration of P and Cd were measured by microwave soil digestion by adding 3 mL of 65%  $\text{HNO}_3$  and 9 mL of 37%  $\text{HCl}$  to 5 g of soil (Smith et al., 2001) then measured as described in section 2.7.

### **4.2.4 DGT assembly and deployment**

All DGTs were prepared as described in sections 2.2 and 2.3. The DGT devices were provided by DGT Research Ltd. and assembled as described by Zhang et al. (1998). The double binding layer DGT was assembled as shown in Figure 2.3. The DBL-DGT deployment was conducted as described in section 2.4 using the PVC tubes (Figure 2.4) to deploy the DGTs at the targeted depths.

Elution of the binding layers of the DBL-DGT was carried out as presented in section 2.5. Each binding layer of the double binding layer was eluted separately. The concentrations of P and Cd were determined as described in sections 2.6 and 2.7, respectively, and  $C_{\text{DGT}}$  was calculated as explained in section 2.8 using Equation 2.1 and 2.2.

### **4.2.5 Leachate water sampling and analysis**

The leachate water from the drainage tubes (section 4.5.1) was initially collected from each pot in 1000 mL polyethylene containers every second day. When the plants grew larger, water was collected continually over 2-3 days. Water samples were collected until the

completion of the experiment. Each leachate sample collected was analyzed on the day of collection. Immediately, after collecting leachate water, samples were filtered by a syringe filter and were digested by the persulphate method (Hosomi and Sudo, 1986). P and Cd were measured as previously described in sections 2.6 and 2.7, respectively. The remainder of each sample was preserved in the freezer without filtering.

#### 4.2.6 Plant analysis

The tomato plant leaves, stems, and fruits were harvested, weighed and washed thoroughly with Milli-Q water. A total of 72 plant samples of each plant part were collected, representing three replicates for each treatment group of 3×4 (three types of fertilizers × four doses of each fertilizer). Each replicate consists of two composite samples: one for tomato fruit and the second for the vegetative parts of the plant (stem and leaves). The samples were cut into pieces and freeze-dried at -12°C for one week utilizing a VirT Freeze Drier, model 2K. Subsequently, the dry weight was determined and the samples were finely ground and sub-sampled producing one composite sample for each pot. The samples were acid digested as described by Martinie and Schilt (1976). The P and Cd concentrations in the plant sample were determined as described in sections 2.6 and 2.7.

#### 4.2.7 Calculation of P and Cd mass in soil, plant and leachate water

The mass of each analyte form was calculated to compare the two extraction methods. The mass of analyte in the soil was calculated from multiplying the analyte concentration by the soil mass. The mass of analytes from the DGT ensured that there was an approximate balance between the mass input and mass output of P and Cd in the soil as described by (de Vries and McLaughlin, 2013). The P and Cd absorbed by the DGTs were compared with the concentrations of P and Cd remaining in the soil after subtracting the concentration of P and Cd in the tomato plants plus the concentration of P in the leachate water (see equation 4.1).

$$M_{In} + M_{Fr} = M_L + M_P + M_T + M_{CDGT} \quad \text{Equation 4.1}$$

Where  $M_{In}$  is the initial mass of P or Cd in the soil,  $M_{Fr}$  is the amount of P or Cd added to the soil in each pot from the SSP, NTS, and ER fertilizer.  $M_L$  is the mass of P or Cd in the leachate water.  $M_P$  is the mass of P or Cd in the plant.  $M_T$  can be calculated as all other parameters are known.

#### 4.2.8 Statistical analyses

SPSS Version 23 was used for statistical analyses. ANOVAs were used to compare the methods, with P and Cd concentration the dependent variables and fertilizer type and doses the independent variables. Spearman's Rho coefficient was used to find the correlation between P experiment factors such as fertilizer type, fertilizer dose, and soil depth with each of the P or Cd measurement methods used.

### 4.3 Results and discussion

#### 4.3.1 Phosphorus status assessment by chemical extraction

Measurements of the extracted available P using the Olsen method and total P after the application of the different P fertilizers doses are presented in Table 4-2. The concentration of available P initially in the soil was  $0.001 \text{ mg kg}^{-1}$  or 2.9% of total P ( $0.039 \text{ mg kg}^{-1}$ ). P fertilizer was added to the soil at three doses 100, 200 and  $250 \text{ kg ha}^{-1}$ , which are equivalent to 25, 50 and  $63 \text{ mg kg}^{-1}$ , respectively (Table 4-2). Accordingly, the masses of P added to the soil were 1175 mg, 2350 mg and 2938 mg of P per pot (47 kg mass of soil in each pot).

Extracted P, which represents the concentration of available P, increased with fertilizer dose regardless of the fertilizer type (Table 4-2). However, pots that were treated with SSP fertilizer showed the highest increase in concentration and mass of total and available P (Table 4-2). SSP fertilizer application increased the extracted available P from  $0.001 \text{ mg kg}^{-1}$  to  $16.7 \pm 1.28 \text{ mg kg}^{-1}$  and total extracted P from  $0.039 \pm 0.00 \text{ mg kg}^{-1}$  to  $41.9 \pm 0.770 \text{ mg kg}^{-1}$  when added at  $250 \text{ kg ha}^{-1}$ . The results indicated that P fertilizer type affected the concentration and mass of available P released to the soil solution. More soluble P was released to the soil solution from SSP fertilizer because it has the highest solubility of the three fertilizers. The ER fertilizer resulted in higher available P concentration than NTS application (Table 4.2). The addition of P fertilizers also increased the concentration and mass of P moving to the leachate water. SSP fertilizer increased P concentration in the leachate water from  $0.017 \pm 0.00 \text{ mg L}^{-1}$  to  $32.0 \pm 3.24 \text{ mg L}^{-1}$ . Similar trends were noticed for the ER and NTS fertilizers, however, were at a lower level.

**Table 4-2 Concentration and amount of total P, labile P and P in the leachate water after the application of three types of fertilizers added in four doses (0, 100, 200 and 250 kg ha<sup>-1</sup>). n = 120 × 3 replicates ± 1 standard error.**

Fertilizer type	P dose kg ha <sup>-1</sup>	P added mg kg <sup>-1</sup>	P concentration				P mass (mg per pot)		
			Total P (mg Kg <sup>-1</sup> )	Extracted P (mg Kg <sup>-1</sup> )	P in the leachate water (mg L <sup>-1</sup> )	Mass of P added	Total P mass (mg)	Extracted P mass (mg)	P mass in the leachate water (mg)
	0	0.00	0.039 ± 0.0	0.001 ± 0.00	0.02 ± 0.00	0.000	1.83 ± 0.00	0.047 ± 0.0	0.27 ± 0.0
ER	100	25	18.2 ± 2.15	5.50 ± 0.38	8.56 ± 2.11	1,175	844 ± 101	256 ± 62.2	300 ± 91.3
	200	50	34.5 ± 0.83	10.4 ± 0.97	20.1 ± 3.25	2,350	1,62 ± 38.9	488 ± 45.7	698 ± 103
	250	63	39.6 ± 1.81	15.4 ± 0.61	29.8 ± 3.52	2,938	1,86 ± 60.4	724 ± 28.7	1,033 ± 245
SSP	100	25	20.1 ± 1.04	5.87 ± 0.72	8.64 ± 1.02	1,175	946 ± 48.8	276 ± 101	302 ± 1.06
	200	50	34.2 ± 0.65	11.3 ± 1.25	23.7 ± 3.22	2,35	1,607 ± 30.8	529 ± 58.9	821 ± 135
	250	63	41.9 ± 0.77	16.7 ± 1.28	32.0 ± 3.24	2,938	1,862 ± 60.4	786 ± 60.4	1,109 ± 89
NTS	100	25	20.4 ± 1.76	4.91 ± 1.13	5.70 ± 0.25	1,175	961 ± 82.9	231 ± 53.2	201 ± 39
	200	50	34.8 ± 1.37	10.2 ± 0.76	14.9 ± 10.6	2,350	1,635 ± 64.7	480 ± 35.9	518 ± 34.3
	250	63	38.9 ± 1.02	13.6 ± 3.31	22.3 ± 22.4	2,938	1,827 ± 48.0	638 ± 156	875 ± 57.7

### 4.3.2 The response of tomato dry matter yield to available P added from P fertilizers.

The tomato dry matter yield (DMY) and P accumulation in the plant were influenced by the application of the three P fertilizers (Table 4-3). The DMY increased dramatically with P fertilizer dose (ANOVA:  $F_{3,24} = 493.5$ ,  $P < 0.0001$ ) (see Appendix 3-C for more details). However, the zero-fertilizer treatment produce a tomato dry matter higher than the NTS of 100 kg ha<sup>-1</sup> application. This could be attributed to the application of other nutrients to all pots which show a production even in unfertilized pots.

**Table 4-3 P content in plants after the application of P fertilizers. Three types of P fertilizers (SSP, NTS, and ER) were applied in four doses (0, 100, 200, and 250 kg ha<sup>-1</sup>). Dry matter weight in (g) was reported for each treatment. n = 12 × 3 replicates ± 1 standard error for the vegetative (stem, leaves) and tomato fruit samples.**

Fertilizer Type	Dose kg ha <sup>-1</sup>	Dry matter (g)		P Mass (µg)		Total P in the plant (µg)
		Vegetative n=36	Fruit n=36	Vegetative	Fruit	
SSP	0	14.9 ± 1.25	7.67 ± 1.35	0.72 ± 0.05	0.19 ± 0.05	0.91
	100	21.0 ± 3.16	16.0 ± 1.11	13.5 ± 0.62	11.6 ± 1.41	25.1
	200	23.0 ± 6.32	17.7 ± 0.85	36.8 ± 4.16	29.5 ± 2.44	66.3
	250	27.0 ± 4.70	18.0 ± 0.52	44.3 ± 3.36	37.2 ± 5.10	81.5
NTS	100	14.7 ± 0.56	14.3 ± 0.42	7.07 ± 0.52	8.72 ± 1.06	15.8
	200	19.7 ± 2.24	12.7 ± 0.55	14.4 ± 2.33	12.9 ± 0.53	27.9
	250	21.7 ± 4.22	14.0 ± 1.02	22.8 ± 1.23	17.3 ± 1.02	40.2
ER	100	19.7 ± 2.10	12.0 ± 1.21	12.6 ± 0.43	3.83 ± 0.11	16.45
	200	21.7 ± 1.00	13.0 ± 1.0	17.9 ± 1.11	15.7 ± 2.10	33.7
	250	24.7 ± 1.31	16.7 ± 0.82	18.5 ± 2.33	23.7 ± 2.11	42.4

In addition, the interaction effect of fertilizer type and dose showed highly significant differences (ANOVA;  $F_{6, 24} = 16.31$ ,  $P < 0.0001$ ) (Appendix 3-C). The DMY of the ER fertilizer treatments increased from 40% to 53% and 80% at 100, 200 and 250 kg ha<sup>-1</sup>, respectively, compared to the control treatment (no P fertilizer). Similarly, NTS fertilizer resulted in an increase of 41%, 43% and 58% DMY at the three doses, respectively. However, plants treated with the SSP fertilizer exhibited 63%, 80% and 99% DMY increases with respect to the control treatment (Table 4-3). The DMY of tomatoes was significantly higher in pots that had received SSP fertilizer, especially at the highest dose (Table 4-3). These results indicate that SSP fertilizer provided appreciable levels of available P which produced



significant differences in DMY compared to ER and NTS fertilizers. Moreover, the highest response of tomato plant in pots receiving SSP fertilizer could be attributed to the fact that SSP fertilizer has the highest percentage of Ca and S (34.3% and 11%, respectively). In general, the tomato plant growth and production were influenced by the nutrients which were added evenly to all of the experiment pots (36 pots).

P content in the plant also significantly increased with increasing fertilizer dose (Table 4-3). Fertilizer type and dose considerably affected P mass in the plant (ANOVA, fertilizer type:  $F_{2,24} = 24.12$ ,  $P < 0.0001$ , fertilizer dose:  $F_{3,24} = 548.2$ ,  $P < 0.0001$ ) (Appendix 3-D). The mass of P in plants treated with SSP was the highest among the three treatments which indicated that the SSP fertilizer provided a greater concentration of available P to the soil. In addition, increasing plant production indicates that more available P was provided to the plant. SSP fertilizer released the highest amount of available P to the soil which increased in the dry matter to 81.5  $\mu\text{g}$  when SSP was added to the soil at a dose of 250 kg P ha<sup>-1</sup> (Table 4-3). Increasing plant uptake of available P was expected due to the high solubility of SSP fertilizer and P deficiency in the soil. ER and NTS fertilizers had lower solubility than SSP therefore, the rate of available P released to the soil was slower and lower plant production occurred.

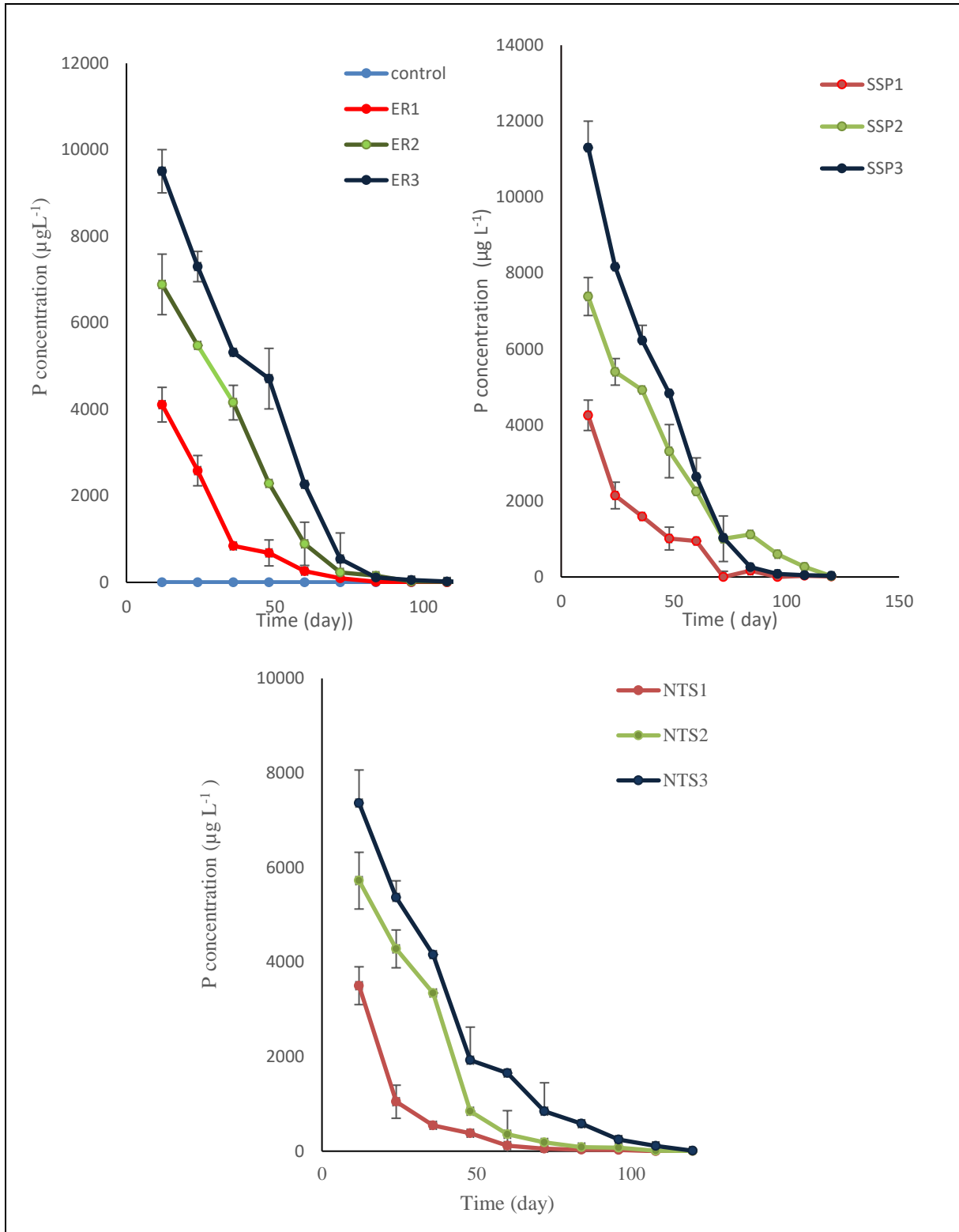
#### **4.3.3 Labile phosphorus (P) in the leachate water**

Leachate water samples were collected every 24 h to measure P and Cd concentration immediately on the day of collection for the entire 120 days of tomato growth. Measurements of P were conducted as described in section 2.7. Time had a significant effect on the concentration of available P in the leachate water (ANOVA:  $F_{9,216} = 975$ ,  $P < 0.0001$ ) (Appendix 3B). In addition, it was noticed that the interaction effect of fertilizer type, fertilizer dose and the time had a highly significant effect on the changes occurred in available P concentration in the leachate water (ANOVA:  $F_{54,216} = 7.27$ ,  $P < 0.0001$ ) (Appendix 3B). The aim was to investigate the performance of the DBL-DGT in detecting the movement of available P through the depth of the soil. Figure 4-1 shows the trends in P concentration of each fertilizer type and dose over 120 days. Twenty-four hours after the application of the P fertilizers, concentrations of available P increased in the leachate water. After 24 h of SSP fertilizer application, the highest concentration of available P was 15.30 mg L<sup>-1</sup> at the highest dose (250 kg ha<sup>-1</sup>) (Appendix 4). Concentrations then declined over the next 72 h and continued to decline gradually until the end of the experiment (120 days). At the end of the

120 days, the highest concentration of available P in the leachate water was  $0.03 \text{ mg P L}^{-1}$  recorded from the application of SSP fertilizer at a dose of  $250 \text{ kg ha}^{-1}$  (Appendix 4). There was a significant difference among the three fertilizer types and highly significant effect of P fertilizer dose on the concentration of available P in the leachate water (ANOVA, fertilizer type:  $F_{2, 24} = 991$ ,  $P < 0.0001$ , fertilizer dose:  $F_{3, 24} = 137$ ,  $P < 0.0001$ ) (Appendix 3B).

P concentration in the leachate water was in the following order  $\text{SSP} > \text{ER} > \text{NTS}$  (Appendix 4). The total accumulative mass of P in the leachate water after the application of the four different doses of the three P fertilizers is presented in Appendix 4. The  $100 \text{ kg P ha}^{-1}$  dose resulted in an accumulated mass of  $300 \text{ mg P}$  and  $302 \text{ mg P}$  for ER and SSP fertilizer, respectively. The  $200$  and  $250 \text{ kg P ha}^{-1}$  doses produced the highest accumulative mass of available P in the leachate water of  $820 \text{ mg P}$  and  $1111 \text{ mg P}$  for ER and SSP fertilizer, respectively (Appendix 4) and Figure 4-1.

The interaction between fertilizer type and dose had a significant effect on the concentration of available P in the leachate water (ANOVA:  $F_{6,24} = 237$ ,  $P < 0.0001$ ) (Appendix 3B). Increasing available P in the leachate water is apparently caused by the solubility rate and the dose of the P fertilizer. SSP fertilizer has the highest solubility of the three fertilizers followed by ER then NTS. It is presumed that the addition of P fertilizers to the soil produces two P forms: the majority was soluble P in soil solution and some exchangeable P was weakly retained by the soil particles. The soil property is coarse texture and the low hydraulic conductivity enhanced the downward movement of the water. Therefore, most of the soluble P rapidly moved to the leachate water in the first 24 hours. Then, the exchangeable P was released gradually to the soil solution and then to the leachate water depending on the strength of its bond to the soil adsorption site. SSP fertilizer released the highest concentration and mass of P to the soil resulting in the greatest movement of available P to the leachate water. The behaviour of SSP fertilizer in the soil was consistent with Sinclair et al. (1998) who studied the effectiveness of SSP fertilizer compared with six other types of P fertilizers and found that SSP was the most effective regarding fertilizer solubility and available P released. In addition, the soil texture and low adsorption capacity reduced the retention of available P in the soil and contributed to declining P concentrations over the duration of the experiment.



**Figure 4-1 Concentration of labile P (mg L<sup>-1</sup>) in the leachate water over the 120 days of the application of ER, SSP and NTS fertilizers at four doses (control = 0, ER1, SSP1 and NTS1 = 100 kg ha<sup>-1</sup>, ER2, SSP2 and NTS2 = 200 kg ha<sup>-1</sup> and ER3, SSP3 and NTS3 = 250 kg ha<sup>-1</sup>). n = 120 × 3 replicates, ±1 standard error.**

#### 4.3.4 The concentration of P using the DBL-DGT

The results of available P measurements using the DBL-DGT ( $P_{DGT}$ ) are presented in Table 4-4. Significant differences were observed between  $P_{DGT}$  concentrations in the control treatment (no P fertilizer added) and all other treatments of the fertilizer types and doses.

**Table 4-4**  $P_{DGT}$  concentration in relation to three types of phosphorus (SSP, NTS, and ER) applied in four doses (0, 100, 200, 250 kg ha<sup>-1</sup>) at three deployment depths (15, 45 and 60 cm). n = 12 × 3 replicates 3, ± 1 standard error.

Depth	Fertilizer dose (kg ha <sup>-1</sup> )			
	0	100	200	250
<b><math>P_{DGT}</math> in the soil from SSP fertilizer (<math>\mu\text{g L}^{-1}</math>)</b>				
15	1.03 ± 0.02	56.8 ± 1.50	80.5 ± 1.62	92.4 ± 3.03
45	1.04 ± 0.00	80.2 ± 19.6	118 ± 4.29	125 ± 17.5
60	1.03 ± 0.03	90.2 ± 3.00	125 ± 0.53	144.9 ± 1.52
<b><math>P_{DGT}</math> in the soil from NTS fertilizer (<math>\mu\text{g L}^{-1}</math>)</b>				
15	1.03 ± 0.05	21.5 ± 2.00	52.9 ± 0.30	75.3 ± 0.89
45	1.03 ± 0.03	26.1 ± 1.50	58.6 ± 1.89	83.9 ± 4.72
60	1.01 ± 0.07	31.4 ± 0.20	73.7 ± 0.30	108 ± 1.52
<b><math>P_{DGT}</math> in the soil from ER fertilizer (<math>\mu\text{g L}^{-1}</math>)</b>				
15	1.02 ± 0.04	37.8 ± 1.60	63.9 ± 0.96	84.4 ± 1.38
45	1.04 ± 0.10	54.3 ± 1.50	79.6 ± 7.84	96.4 ± 1.09
60	1.03 ± 1.20	59.5 ± 11.0	101 ± 3.44	127 ± 1.02

Fertilizer type and dose have a highly significant effect on  $P_{DGT}$  concentration (ANOVA, type:  $F_{2,72} = 170$ ,  $P < 0.0001$ , dose:  $F_{3,72} = 1.03$ ,  $P < 0.0001$ ) (Appendix 3A). The concentration of available P recovered from the Metsorb layer of the DBL-DGT at 15 cm depth was  $92.40 \pm 3.03 \mu\text{g P mL}^{-1}$  in the SSP fertilizer treatment. Available P increased 100-fold in this treatment compared to the control treatment where the available P was  $1.03 \text{ ng P mL}^{-1}$  (Table 4-4). NTS and ER fertilizers had similar effects however, concentrations of available P in these two treatments were lower than the SSP treatment of the same dose. The  $P_{DGT}$  ranged from  $1.03 \pm 0.02$  (control, 15 cm) to  $144.96 \pm 1.52 \text{ ng P mL}^{-1}$  in the 250 kg ha<sup>-1</sup> P SSP treatment at 60 cm depth (Table 4-4).

The variation in  $P_{DGT}$  concentrations among the three fertilizer treatments is due to the difference between the solubilities of the three fertilizers. The properties of each fertilizer (Appendix 1) show that the proportion of critical insoluble P is 0.2% in SSP, 7% in ER while the NTS fertilizer is slowly solubilized in soil water (according to manufacturer's specifications). That means the SSP fertilizer has the highest solubility among the three fertilizers used in this experiment. These results were confirmed by the positive significant correlation was found between fertilizer dose and the concentration of available P measured by the DBL-DGT (Spearman's correlation:  $r = 0.93$ ,  $P < 0.01$ , Table 4-5). The correlation between  $P_{DGT}$  and SSP fertilizer was the highest among the three fertilizers (Spearman's correlation:  $r = 0.59$ ,  $P < 0.01$ , Table 4-5).

**Table 4-5 Spearman's Rho coefficients (r) for correlations between available P extracted from soil by MDB-DGT from the soil with different P fertilizers (ER, SSP, and NTS) treatments applied at different doses. n = 12 × 3 replicates ± 1 standard error.**

Fertilizer type	ER	SSP	NTS	Dose (kg ha <sup>-1</sup> )	$P_{DGT}$ (µg L <sup>-1</sup> )
ER	1.00	ns	ns	ns	0.45*
SSP	ns	1.00	ns	ns	0.59**
NTS	ns	ns	1.00	ns	0.25*
Dose	ns	ns	ns	1.00	0.93**
$P_{DGT}$ (µg L <sup>-1</sup> )	0.15*	0.235*	0.250**	0.93**	1.00

\* Correlation is significant at the 0.05 level (2-tailed).

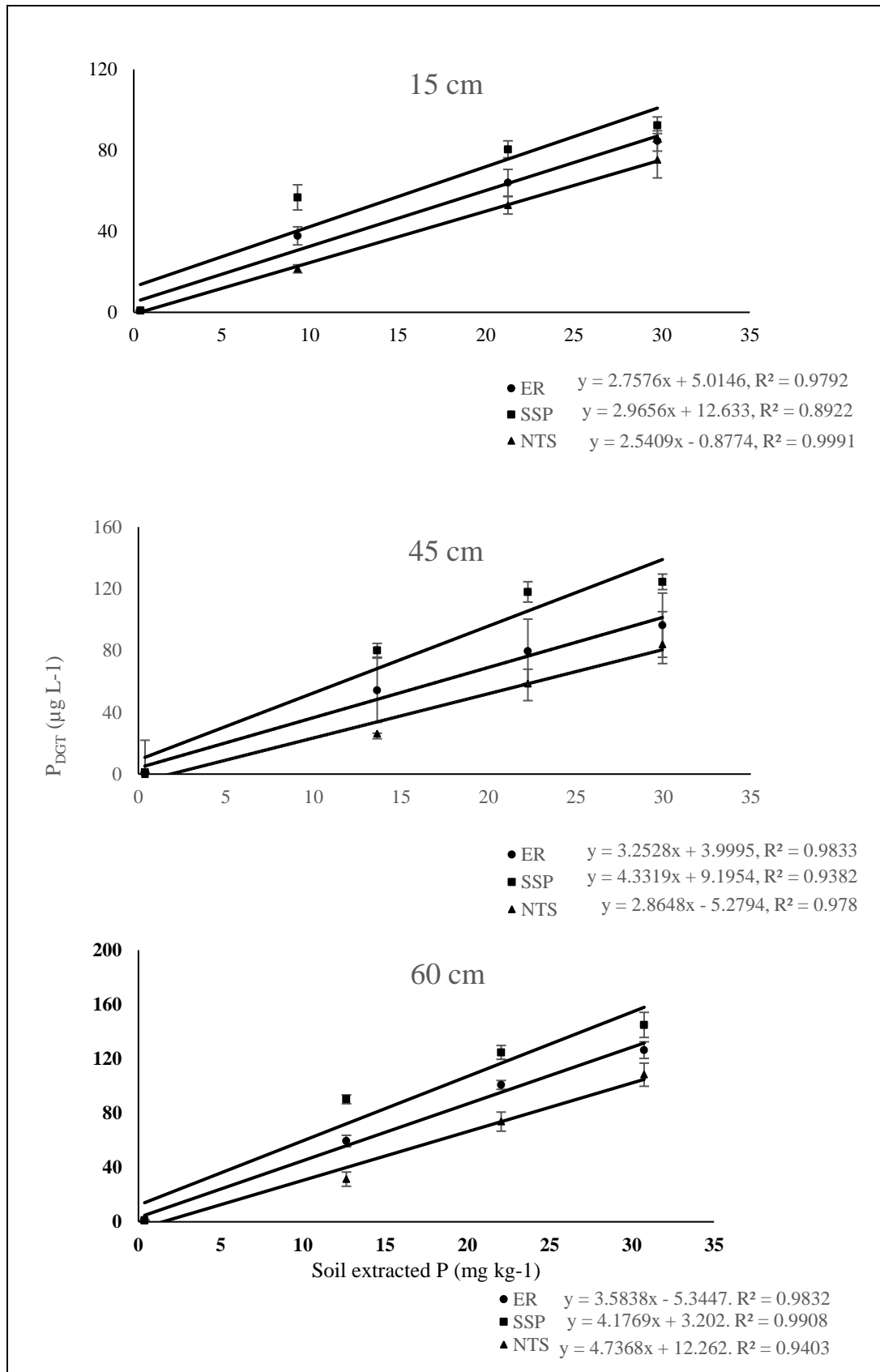
\*\* Correlation is significant at the 0.01 level (2-tailed).

The SSP caused the highest increase in  $P_{DGT}$  concentration relative to the control treatment with a 56 to 141-fold increase at 15 cm and 60 cm when the SSP fertilizer dose increased from 100 to 250 kg P ha<sup>-1</sup>. Uptake of available P by the DBL-DGT deployed at different depths detected the mobility of the available P through the soil depth (Table 4-4) as seen by the significant variance between treatments of  $P_{DGT}$  at different depths (ANOVA:  $F_{2, 72} = 102$ ,  $P < 0.0001$ ) (Appendix 3-A). Therefore, these results were integrated with the leachate water results. SSP fertilizer produced the greatest amount of available P which then easily moved from the soil to the leachate water.

The penetration dose of available P passing through the front layers of the DGT was influenced by the solubility of each P fertilizer. A fertilizer with greater solubility will increase

the P uptake of the Metsorb layer by providing further P to pass through the diffusive and Chelex layers (Arvajeh et al., 2013) and on to the Metsorb binding layer. The relationship between  $P_{DGT}$  and fertilizer dose indicates that the Metsorb binding layer of the DBL-DGT is sensitive to differences in available P concentration released from different fertilizers at different doses.

The concentration of available P measured by DBL-DGT ( $P_{DGT}$ ) was plotted against concentrations of available P measured by the Olsen extraction method (Olsen, 1954) to investigate the validity of the DBL-DGT method compared with the Olsen method in measuring the available P in the soil. Spearman's Rho coefficients were also conducted to investigate the relationship between the two methods (Figure 4-2 and Table 4-6). In both methods, concentrations of available P increased with increasing fertilizer dose (Figure 4-2). However, the concentration of available P extracted by the Olsen method was greater than the available P determined by DBL-DGT. The Olsen method utilizes sodium bicarbonate at pH 8.5 in the extraction solution followed by 30 min shaking. This method induces the release of the inert P in the soil (Mason and McNeill, 2005; Six et al., 2012; Mason et al., 2013). The DBL-DGT employs the Metsorb binding layer which is highly specific for available P absorption (Panther et al., 2010). The diffusion layer of the DGT limits the flux of P and indicates the equilibrium between the solid and solution phase, which is determined by the buffering properties of soil. In comparison with the Olsen bicarbonate extraction method, the DGT technique does not change the soil pH or dilute the soil with the extracting solution.



**Figure 4-2**  $P_{DGT}$  concentration at three deployment depths of the DBL-DGTs and soil sampling (15, 45 and 60 cm) as affected by P fertilizer type and dose. All three P fertilizers were added at doses of 0, 100, 200 and 250 kg P ha<sup>-1</sup>. For each depth, n = 12 × 3 replicates ± 1 standard error).

Linear relationships between  $P_{DGT}$  and the  $P_{EX}$  of soil extracted by the Olsen method for the same fertilizer types, doses and soil depths are presented in Figure 4-2. A positive correlation was found between  $P_{DGT}$  and  $P_{EX}$  (Table 4-6).

**Table 4-6 Spearman's Rho coefficients (r) for correlations between available P extracted by MDB-DGT and available P extracted by the Olsen method from the soil with different P fertilizer (ER, SSP, and NTS) treatments applied at different doses. n = 12 × 3 replicates ± 1 standard error.**

Fertilizer type	ER	SSP	NTS	Dose	$P_{DGT}$	$P_{EX}$	$P_{Plant}$
ER	1.00	ns	ns	ns	0.46*	0.37	0.76
SSP	ns	1.00	ns	ns	0.59**	0.21	0.81**
NTS	ns	ns	1.00	ns	0.25*	-0.17	0.73
Dose	ns	ns	ns	1.00	0.93**	0.88**	0.89**
$P_{DGT}$ ( $\mu\text{g L}^{-1}$ )	0.46*	0.59*	0.25*	0.93**	1.00	0.83**	0.95**
$P_{EX}$ ( $\mu\text{g kg}^{-1}$ )	0.37*	0.21	-0.17	0.88**	0.83**	1.00	0.62*
Plant	76.3*	80.5**	72.6	89.58**	95.3**	62.1*	1.00

\* Correlation is significant at the 0.05 level (2-tailed).

\*\* Correlation is significant at the 0.01 level (2-tailed).

The concentration of available P extracted by DBL-DGT was significantly and positively correlated with fertilizer type (ER:  $r = 0.46$ ,  $P < 0.05$ ; SSP:  $r = 0.59$ ,  $P > 0.01$ ; NTS:  $r = 0.25$ ,  $P < 0.05$ ) (Table 4-6). The Olsen extraction method had lower correlation with fertilizer type than the DBL-DGT method (ER:  $r = 0.37$ ,  $P < 0.05$ ; SSP:  $r = 0.21$ ,  $P > 0.01$ ; NTS:  $r = -0.17$ ,  $P < 0.05$ ). Positive correlations were also found between fertilizer dose and both methods of available P extraction from the soil (DBL-DGT ( $P_{DGT}$ ):  $r = 0.93$ ,  $P < 0.01$ ; Olsen ( $P_{EX}$ ):  $r = 0.88$ ,  $P < 0.01$ ) (Table 4-6). In both procedures, concentrations of available P increased with increasing fertilizer dose (Figure 4-2).

The correlation between the P concentration measured by the two methods with the concentration of P in the plant was also investigated. The concentration of available P extracted by both methods showed a positive correlation with the concentration of P in the plants ( $P_{Plant}$ ). However, concentration of  $P_{DGT}$  was highly correlated with  $P_{Plant}$  ( $r = 0.95$ ,  $P < 0.01$ ) whereas, a moderate correlation was found between  $P_{EX}$  and  $P_{Plant}$  ( $r = 0.62$ ,  $P < 0.01$ ) (Table 4-6). This finding has proven that the DBL-DGT was mimicking the plant mechanism



of taking up available P from soil. This means that the DBL-DGT measured the identical P form that is required by the plant. The  $P_{EX}$  and  $P_{DGT}$  methods were significantly correlated ( $r = 0.83$ ,  $P < 0.01$ ) (Table 4-6). Correlations between MBL-DGT and soil independent factors (depth, fertilizer dose and fertilizer type) were stronger ( $r = 0.92$ ,  $P < 0.0001$ ) than those between the Olsen method and the same soil independent factors ( $r = 0.62$ ,  $P < 0.0001$ ) (Table 4-7). The results confirmed that increasing P addition to the soil using different types of P fertilizers clearly shows the variation between the two methods. These findings corresponded with Burkitt et al., (2016) who found a strong correlation between log  $P_{DGT}$  method and Olsen method.

**Table 4-7 Spearman’s correlations between independent variables (depth, fertilizer dose, and fertilizer type) and available P extracted by DGT or by the Olsen method.  $n = 12 \times 3$  replicates  $\pm 1$  standard error.**

P extraction method	Independent variables	r	Probability
MCB-DGT	Depth	0.91	$P < 0.0001$
	Dose of P fertilizer ( $\text{kg ha}^{-1}$ )		
	And Fertilizer Type		
Olsen	Depth	0.62	$P < 0.0001$
	Dose of P fertilizer ( $\text{kg ha}^{-1}$ ) and		
	Fertilizer Type		

The concentration of available P extracted by DBL-DGT was lower than P determined by the Olsen method for all three fertilizers as the chemical method of P extraction induces the release of inert P in the soil. Increasing the fertilizer dose caused significant increases in the available P concentration in soil solution and therefore, P flux in the soil solution increased with increasing fertilizer dose. Comparing  $P_{DGT}$  with  $P_{EX}$  explains the available P condition in the soil: if  $P_{DGT} = P_{EX}$  then the concentration of available P in the soil solution is fully sustained. This means that P absorbed by the DGT and removed from the soil solution is rapidly resupplied from the soil solid phase and P concentration in the soil solution is effectively buffered. If  $P_{DGT} < P_{EX}$ , the soil solution is partially sustained or unbuffered and when  $P_{DGT} > P_{EX}$ , P concentration in the soil solution is high (Zhang et al., 1998b). According to these conditions, the concentration of available P in our soil is partially sustained and unbuffered. The results of the  $P_{DGT}$  can be interpreted as the P fertilizers elevated the concentration of available P concentration in the soil (Table 4-4). However, the soil has a weak tendency to fix P on soil particles due to its low clay percentage, low organic matter,

and coarse texture. When available P was lost from the soil due to plant uptake, leaching and/or consumed by soil microbes then available P will decline. These conditions apply to the soil used in this experiment, therefore, the DBL-DGT provides information not only on the concentration of available P but explains soil conditions for buffering available P when exhausted.

#### 4.3.5 Labile cadmium in the soil measured by NH<sub>4</sub>Cl

Several extractant salts have been trialed for measuring the concentration of bioavailable Cd in soil. Using 1 M NH<sub>4</sub>Cl neutral salt to extract bioavailable Cd from the soil is the most appropriate method to measure phytoavailable Cd. Krishnamurtia et al. (1995) demonstrated that soil Cd complexed with the chloride ions (Cl<sup>-</sup>) of NH<sub>4</sub>Cl ( $\log K_{CdCl^+} = 1.96$ ) at an ionic strength of 1.0 in 1 M NH<sub>4</sub>Cl solution and brought into soil solution was closely related to Cd absorbed by plants grown on natural soil and accumulated in plant tissues.

Concentrations of available Cd extracted by NH<sub>4</sub>Cl (Cd<sub>EX</sub>), Cd in soil solution (Cd<sub>Sol</sub>) and total Cd (Cd<sub>T</sub>) are presented in Table 4.8. The concentration of Cd<sub>Sol</sub> is considerably lower than Cd<sub>EX</sub> and total Cd. All Cd species in the soil increased with fertilizer dose and varied with fertilizer type. The concentration of Cd in the leachate water also increased with fertilizer dose. The percentage of Cd lost ranged from 34-51% when ER fertilizer dose increased from 100-250 kg P ha<sup>-1</sup>. When SSP fertilizer dose increased from 100 kg P ha<sup>-1</sup> to 250 kg P ha<sup>-1</sup>, Cd loss ranged from 43-49%. The concentration of Cd species of C<sub>sol</sub> and C<sub>EX</sub> in the NTS treatments were below the detection limit of the ICP-MS of 0.04µg L<sup>-1</sup>. Therefore, no comparisons were made for NTS treatments (Table 4-8).

The addition of P fertilizers to the soil changed the Cd species in the soil, for example, total Cd in the soil increased from 0.46 µg kg<sup>-1</sup> to 5.42 µg kg<sup>-1</sup> in ER fertilizer treatments and from 0.46 µg kg<sup>-1</sup> to 9.16 µg kg<sup>-1</sup> in SSP fertilizer treatments. The initial concentration of available Cd in soil was below the detection limit (BDL). After application of P fertilizers, available Cd extracted by NH<sub>4</sub>CL increased to 40% of total Cd when ER and SSP fertilizers were added to the soil at 250 kg ha<sup>-1</sup>. The percentage of Cd in soil solution (C<sub>Sol</sub>) increased from BDL in the control to 24% in the highest dose of SSP fertilizer treatment. It was previously reported that extraction methods for metals from soil overestimate the concentration of available Cd in soil solution. Degryse et al. (2003) concluded that the concentration of available Cd extracted by neutral and buffered salts is larger than the

**Table 4-8 Cd concentration and mass extracted by NH<sub>4</sub>Cl in the soil and leachate water. BDL = below detection limit of 0.04 µg L<sup>-1</sup>. n = 72 × 3 replicates, ± 1 standard error.**

Fertilizer Type	Cd added µg kg <sup>-1</sup>	Cd concentration				Cd Mass (µg)		
		C <sub>Sol</sub> (µg kg <sup>-1</sup> )	Total Cd (µg kg <sup>-1</sup> )	Cd <sub>Ex</sub> (µg kg <sup>-1</sup> )	Cd in leachate water (µg L <sup>-1</sup> )	Total Cd	labile Cd	Cd in leachate
ER0	0.47	BDL	0.46 ± 0.1	BDL (0.014)	BDL	22.00	BDL	BDL
ER <sub>1</sub>	90.0	0.32 ± 0.01	1.56 ± 0.0	0.80 ± 0.01	0.910	73.50	5.640	31.39
ER <sub>2</sub>	180	0.54 ± 0.03	3.15 ± 0.1	1.04 ± 0.02	2.600	100.9	48.88	89.74
ER <sub>3</sub>	225	0.64 ± 0.01	5.42 ± 0.1	1.56 ± 0.04	3.380	113.9	73.32	116.6
SSP <sub>1</sub>	333	0.63 ± 0.04	4.31 ± 0.1	0.87 ± 0.02	4.080	202.0	14.10	144.2
SSP <sub>2</sub>	668	1.32 ± 0.05	7.96 ± 0.1	1.02 ± 0.04	8.650	374.0	48.13	299.0
SSP <sub>3</sub>	834	2.11 ± 0.02	9.16 ± 0.2	3.65 ± 0.02	11.93	430.0	171.5	411.8

concentration of Cd in the pore water. These types of extractants extracted 11 times the amount of Cd than available Cd found in soil solution. Neutral salts, such as  $\text{NH}_4\text{Cl}$ , extract Cd species including soluble, exchangeable Cd and Cd associated with calcium carbonate and other minerals (Degryse et al., 2003). Increasing total Cd after the application of P fertilizers indicated that sorption of the added Cd was accrued despite low clay and organic matter. Smolders and Jelle (2013) inferred that soil texture has weak effects on Cd sorption by showing higher sorption on sandy loam soil than loamy sand soil. Soil organic matter, oxyhydroxides of Al, Fe and Mn and clay minerals are the three main Cd adsorbents in soil.  $\text{Cd}^{+2}$  binds to surface oxygen atoms of humic substances or to surface hydroxyl groups on oxyhydroxides (Smolders and Jelle, 2013)

#### 4.3.6 Cadmium concentration in the tomato plant samples

Cadmium content in plant samples of the vegetative part of the plant (combined stems, leaves and tomato fruit) varied significantly with fertilizer type of ER and SSP fertilizer (ANOVA:  $F_{2,24} = 21.54$ ,  $P < 0.0001$ ) (Appendix 3 -C) and fertilizer dose (ANOVA:  $F_{3,24}$ ,  $p = 5.65$ ) (Appendix 3C).

**Table 4-9 Cadmium (Cd) concentration and mass in the vegetative parts of the plant (leaves and stem) and the tomato fruit after the application of three fertilizers (SSP, NTS, and ER) at four doses (0, 100, 200 and 250)  $\text{kg ha}^{-1}$ . For each part,  $n = 12 \times 3$  replicates  $\pm 1$  standard error.**

Fer. Type	Dose ( $\text{kg ha}^{-1}$ )	Dry matter yield (g)		Cd concentration ( $\mu\text{g g}^{-1}$ )		Cd mass in plant ( $\mu\text{g}$ )	
		Vegetative n=36	Fruit n=36	Vegetative	Fruit	Vegetative	Fruit
SSP	0	15.0 $\pm$ 0.21	7.67 $\pm$ 0.21	BDL	BDL -	BDL	BDL
	100	21.0 $\pm$ 0.11	16.0 $\pm$ 0.31	0.18 $\pm$ 0.11	0.12 $\pm$ 0.11	3.78 $\pm$ 0.11	1.92 $\pm$ 0.12
	200	25.0 $\pm$ 0.32	17.5 $\pm$ 0.21	0.23 $\pm$ 0.21	0.15 $\pm$ 0.20	4.83 $\pm$ 0.21	2.63 $\pm$ 0.02
	250	27.0 $\pm$ 0.11	18.0 $\pm$ 0.20	0.25 $\pm$ 0.30	0.25 $\pm$ 0.10	6.75 $\pm$ 0.11	4.50 $\pm$ 0.11
NTS	0	11.7 $\pm$ 0.31	10.7 $\pm$ 0.23	BDL	BDL	BDL	BDL
	100	14.6 $\pm$ 0.12	14.2 $\pm$ 0.22	BDL	BDL	BDL	BDL
	200	19.7 $\pm$ 0.22	12.5 $\pm$ 0.35	BDL	BDL	BDL	BDL
	250	21.5 $\pm$ 0.51	14.0 $\pm$ 0.21	BDL	BDL	BDL	BDL
ER	0	15.0 $\pm$ 0.30	6.67 $\pm$ 0.31	BDL	BDL	BDL	BDL
	100	19.6 $\pm$ 0.22	8.68 $\pm$ 0.12	0.15 $\pm$ 0.10	0.12 $\pm$ 0.30	2.94 $\pm$ 0.11	1.04 $\pm$ 0.10
	200	21.6 $\pm$ 0.21	13.0 $\pm$ 0.2	0.15 $\pm$ 0.1	0.14 $\pm$ 0.1	3.24 $\pm$ 0.2	1.82 $\pm$ 0.2
	250	24.6 $\pm$ 0.21	16.6 $\pm$ 0.1	0.22 $\pm$ 0.1	0.15 $\pm$ 0.1	5.41 $\pm$ 0.2	2.49 $\pm$ 0.1

Table 4-9 shows the concentration and mass of P and Cd in the tomato plants' dry matter (vegetative and fruit). The SSP fertilizer has the greatest effect on Cd content in tomato plant samples as it has the highest Cd content of 25 mg Cd kg<sup>-1</sup>; ER fertilizer has 10 mg Cd kg<sup>-1</sup>, while NTS has no measurable Cd.

The mass of Cd in the tomato plants was calculated by multiplying the vegetative or fruit dry matter weight by the concentration of Cd in each plant sample. When SSP fertilizer was added at 250 kg ha<sup>-1</sup>, the Cd content in the plant increased from BDL to 6.75 ± 0.1 µg and 4.50 ± 0.1 µg in vegetative and fruit, respectively. When ER fertilizer was added at 250 kg ha<sup>-1</sup>, Cd content increased from BDL to 5.41 ± 0.02 µg and 2.49 ± 0.1 µg in vegetative and fruit, respectively. Cd content in the vegetative parts of the plant was higher than in the fruit which is consistent with Meers et al. (2007). Labile Cd is the most accessible form of Cd to the root, with labile Cd uptake by the plant controlled by the diffusive transport of free Cd<sup>+2</sup> ions from the roots (Degryse and Smolders, 2012). Therefore, targeting labile Cd in measurements will provide important information for Cd risk assessment in the soil.

There was a strong positive correlation between available Cd in the soil and fertilizer dose for both SSP (r = 0.98, P < 0.01) and ER (r = 0.87, P < 0.01) fertilizers (Table 4-10).

**Table 4-10. Spearman's Rho coefficients (r) for correlations between available Cd extracted from soil by DBL-DGT and extracted by the NH<sub>4</sub>Cl method with different P fertilizers (ER, SSP, and NTS) applied at different doses. n = 12 × 3, replicates ± 1 standard error.**

Fertilizer type	ER	SSP	Dose (kg ha <sup>-1</sup> )	Cd <sub>NH<sub>4</sub>Cl</sub> (µg kg <sup>-1</sup> )	Cd <sub>Plant</sub> (µg kg <sup>-1</sup> )
ER	1.00	ns	0.87**	0.47	0.76**
SSP	ns	1.00	0.98**	0.51	0.92**
Dose (kg ha <sup>-1</sup> )	0.87**	0.92**	1.00	0.66	0.85**
Cd <sub>NH<sub>4</sub>Cl</sub> (µg kg <sup>-1</sup> )	0.47	0.51	0.66*	1.00	0.71*
Cd <sub>Plant</sub> (µg kg <sup>-1</sup> )	0.76*	0.92**	0.85**	0.71*	1.00

\* significant at P < 0.05

\*\* significant at P < 0.01; ns: not significant.

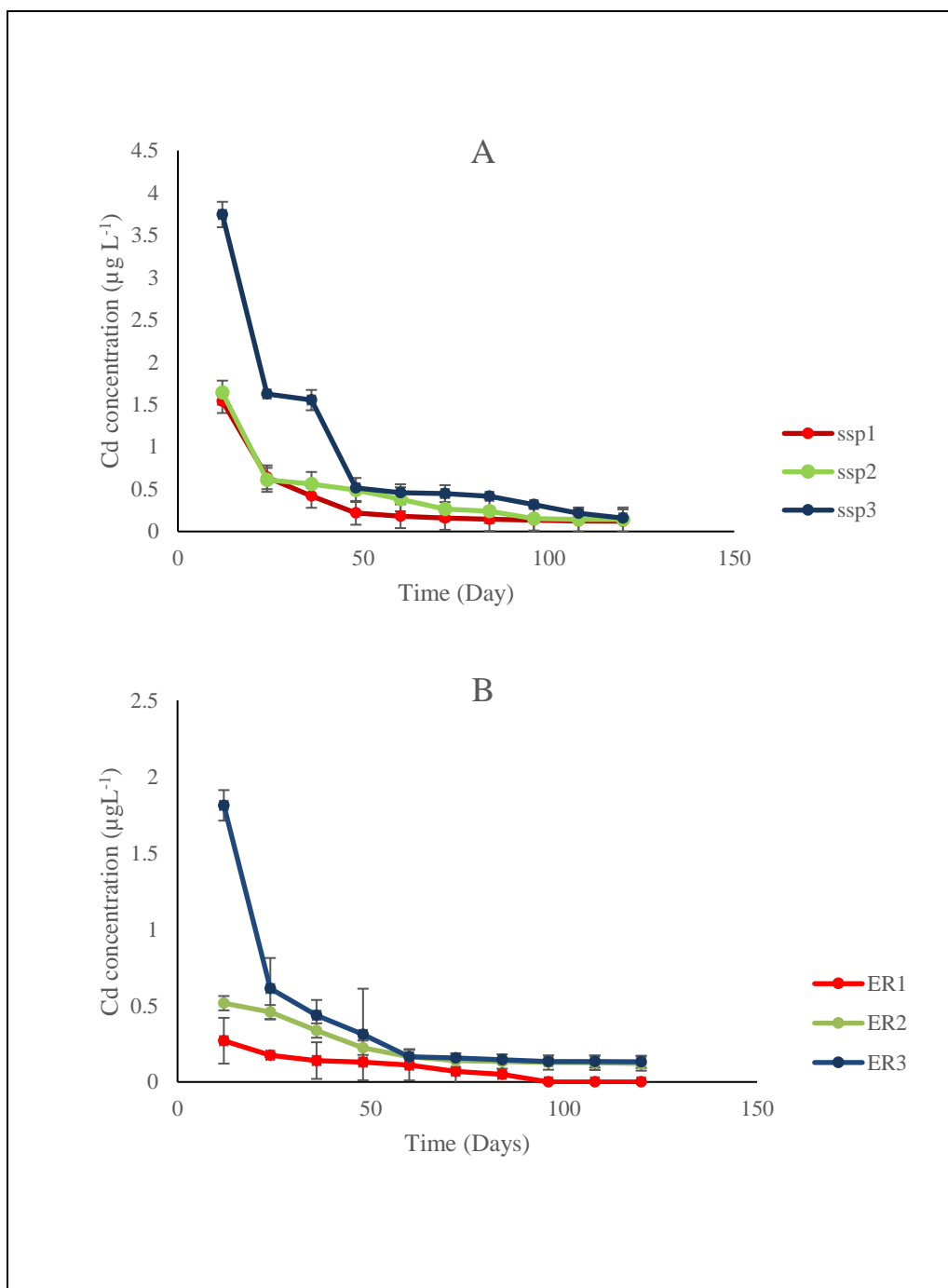
There were strong correlations between the concentration of available Cd in the tomato plants and fertilizer dose for both SSP fertilizer (r = 0.92, P < 0.01) and ER fertilizer (r = 0.76, P < 0.05). Although the concentration of Cd in the plants was below the maximum limit (ML) of Cd (0.1 mg kg<sup>-1</sup>) according to Australian food regulations (FSANZ, 2005), in the long term it is likely to increase with high fertilizer application doses. Increasing concentrations of labile

Cd in the soil are associated with greater risk of increasing Cd concentration in tomato fruits, especially from the SSP fertilizer.

#### **4.3.7 Cadmium movement to the leachate water**

Leachate water samples for measuring labile Cd were collected as described in section 4.2.5 and labile Cd concentrations were measured immediately after collecting the sample. Figure 4-3 shows increase Cd concentration in the leachate water over time. The concentration of labile Cd in the leachate water significantly increased with increasing fertilizer dose (ANOVA:  $F_{3, 24} = 7.582$ ,  $P < 0.0001$ ) and highly significant affected by the P fertilizer type (SSP and ER) (ANOVA:  $F_{2, 24} = 23.82$ ,  $P < 0.0001$ ) (Appendix 3-B).

Cd mass added to the soil varied according to P fertilizer type and dose due to the different Cd contents of each fertilizer: ER has  $10 \text{ mg Cd kg}^{-1}$ ; SSP has  $25 \text{ mg Cd kg}^{-1}$ . After 20 days, the concentrations of labile Cd in all treatments became very low then steadily decreased until the end of the plant growth period of 120 days (Figure 4-3). When ER fertilizer was added at doses of 100, 200 and  $250 \text{ kg P ha}^{-1}$ , the amount of Cd applied to the soil was  $90.00 \text{ } \mu\text{g}$ ,  $180.0 \text{ } \mu\text{g}$ , and  $225.0 \text{ } \mu\text{g}$ , respectively. The amount of Cd applied to the soil was  $333.0 \text{ } \mu\text{g}$ ,  $667.0 \text{ } \mu\text{g}$  and  $834.0 \text{ } \mu\text{g}$  from the application of the same doses of SSP fertilizer. SSP fertilizer addition resulted in labile Cd content in the leachate water increasing to  $3.7 \text{ } \mu\text{g}$  which was calculated from Cd concentration  $\times$  volume of water (L) collected on the day of sampling. All doses of SSP fertilizer produced higher labile Cd content in the leachate water than the equivalent doses of ER fertilizer. The accumulative mass of available Cd in the leachate water from the highest dose of SSP fertilizer over the growth cycle of the tomato plants was  $412.0 \text{ } \mu\text{g Cd}$ . The accumulative mass from the same ER fertilizer dose was  $117.0 \text{ } \mu\text{g Cd}$  over the course of 120 days (Figure 4-3).



**Figure 4-3 Cd concentrations ( $\mu\text{g L}^{-1}$ ) of available Cd in the accumulative leachate water collected every 24 h for 120 days following the application of SSP (A) and (B) ER fertilizers added at four doses (0, 100, 200, and 250  $\text{kg P ha}^{-1}$ ).  $n = 72 \times 3$  replicates,  $\pm 1$  standard error.**

The solubility of P fertilizer (Appendix 1) is the key factor affecting the increase of labile Cd in the leachate water. SSP fertilizer has the highest solubility among the three fertilizers, followed by ER (Appendix 1). The behaviour of SSP fertilizer in the soil corresponded with Sinclair et al. (1998) who found that SSP fertilizer was the most effective P fertilizer regarding increasing Cd availability in the soil in comparison with other six types of

P fertilizer. In addition, soil properties and plant root biological activity had a direct influence on soil pH and P fertilizer solubility. The experimental conditions possibly enhanced Cd mobility to the leachate water. This includes the high soil moisture at 80% of WHC during the DGT deployment, low soil pH, high soil hydraulic conductivity, low organic matter and low cation exchange capacity of the soil.

In fact, mobility of Cd is influenced by the quantity of Cd added to the soil from chemical fertilizers; the local environment conditions including soil type, soil properties, land use, and climate; and the interaction between soil and the added Cd. Leaching of Cd, therefore, could be possible in some areas of Australia, for instance, horticultural areas, where high quantities of P fertilizers are used. Moreover, Cd leaching may be possible in soils with a light texture, such as those found in sandy coastal areas, which have a low retention capacity for Cd. In addition, sandy soils are often heavily fertilized as they are suitable for vegetable production such as carrots, onions, and potatoes (Mclaughlin et al., 1996). Another hypothesis reported by Mann and Ritchie (1995) is that most of the Cd added to sandy soils from P fertilizers could stay as soluble and exchangeable forms which enables Cd to move to the leachate water or to be taken up by plants.

The combined DBL-DGT results for the Cd concentration at different soil depths and concentration of Cd in the leachate water confirm the hypothesis that Cd added to the soil from P fertilizers increases Cd mobility to the leachate water (Mann et al., 2002).

#### **4.3.8 Cadmium (Cd) concentration using the DBL-DGT**

Concentrations of  $Cd_{DGT}$  are shown in Table 4-11. NTS fertilizer treatments were BDL of  $0.04 \mu\text{g L}^{-1}$  and below the deduction line of the ICP-MS instrument of  $0.024 \mu\text{g L}^{-1}$  (section 2-2 to 2-7). Therefore,  $Cd_{DGT}$  measurements for the NTS treatments were excluded from the comparisons. The concentration of  $Cd_{DGT}$  increased from BDL in the control treatment to  $0.30 \pm 0.00 \mu\text{g L}^{-1}$  and  $1.18 \pm 0.02 \mu\text{g L}^{-1}$  when ER and SSP fertilizers, respectively, were added at  $250 \text{ kg ha}^{-1}$ .



**Table 4-11 Cd<sub>DGT</sub> concentrations in relation to three types of phosphorus fertilizers (SSP and ER) applied in four doses (0, 100, 200 and 250 kg P ha<sup>-1</sup>) at three deployment depths (15, 45 and 60 cm). For each depth, n = 12 × 3 replicates ± 1 standard error. NTS measurements were excluded.**

Depth (cm)	Fertilizer dose (kg ha <sup>-1</sup> )			
	0	100	200	250
<b>Cd<sub>DGT</sub> in the soil from SSP fertilizer (µg L<sup>-1</sup>)</b>				
15	BDL	0.49 ± 0.00	0.75 ± 0.00	1.03 ± 0.11
45	BDL	0.49 ± 0.01	0.75 ± 0.00	1.16 ± 0.01
60	BDL	0.49 ± 0.01	0.96 ± 0.03	1.18 ± 0.02
<b>Cd<sub>DGT</sub> in the soil from ER fertilizer (µg L<sup>-1</sup>)</b>				
15	BDL	0.11 ± 0.01	0.25 ± 0.02	0.28 ± 0.04
45	BDL	0.15 ± 0.00	0.25 ± 0.00	0.30 ± 0.01
60	BDL	0.15 ± 0.00	0.26 ± 0.00	0.30 ± 0.00

The Cd<sub>DGT</sub> concentration in soil varied significantly depending on fertilizer type and dose (ANOVA, type:  $F_{2,72} = 818.2$ ,  $P < 0.0001$ , dose:  $F_{3,72} = 1.61$ ,  $P < 0.0001$ ) (Appendix 3-A). Spearman's Rho correlations demonstrated significant correlations between available Cd<sub>DGT</sub> concentration in soil and fertilizer dose for SSP ( $r = 0.98$ ,  $P < 0.001$ ) and ER ( $r = 0.87$ ,  $P < 0.001$ ) (Table 4-12).

**Table 4-12 Spearman's Rho coefficients (r) for correlations between available Cd extracted from soil by the DBL-DGT method with different P fertilizer (ER, SSP) treatments applied at different doses. Each depth, n = 12 × 3 replicates ± 1 standard error. NTS measurements were excluded.**

Fertilizer type	ER	SSP	Dose (kg ha <sup>-1</sup> )	Cd <sub>DGT</sub> (µg L <sup>-1</sup> )
ER	1.00	ns	0.87**	0.85**
SSP	ns	1.00	0.98**	0.99**
Dose (kg ha <sup>-1</sup> )	0.87**	0.98**	1.00	0.93**
Cd <sub>DGT</sub> (µg L <sup>-1</sup> )	0.85**	0.93	0.93**	1.00

\* significant at  $P < 0.05$

\*\* significant at  $P < 0.01$ ; ns: not significant.

Both extraction methods, DBL-DGT and NH<sub>4</sub>Cl, had positive correlations with soil factors (DBL-DGT:  $r = 0.95$ ,  $P < 0.0001$  and NH<sub>4</sub>Cl:  $r = 0.64$ ,  $P < 0.0001$ ) (Table 4-13).

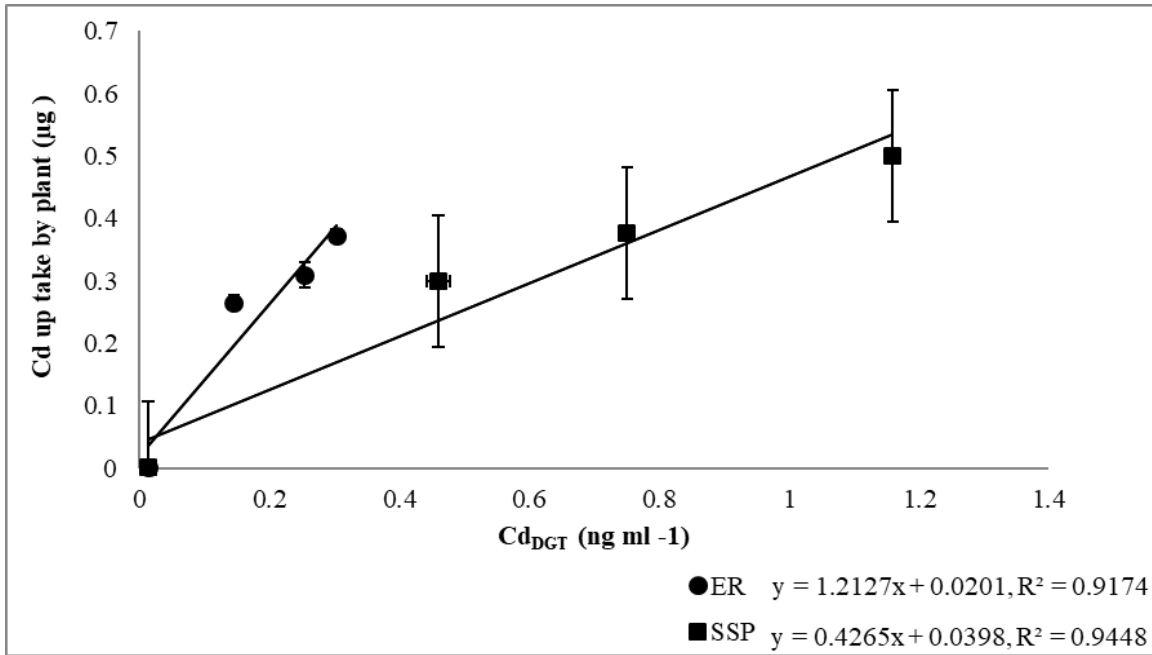
**Table 4-13 Influence of soil factors (depth, fertilizer dose, and fertilizer type) on available P extracted by DBL-DGT or NH<sub>4</sub>Cl methods. Each depth,  $n = 12 \times 3$  replicates  $\pm$  1 standard.**

<b>Cd extraction method</b>	<b>Independent variables</b>	<b>r</b>	<b>Probability</b>
MCB-DGT	Depth	0.95	$P < 0.0001$
	Dose of P fertilizer (kg ha <sup>-1</sup> )		
	Fertilizer Type		
NH <sub>4</sub> Cl	Depth	0.64	$P < 0.0001$
	Dose of P fertilizer (kg ha <sup>-1</sup> )		
	Fertilizer Type		

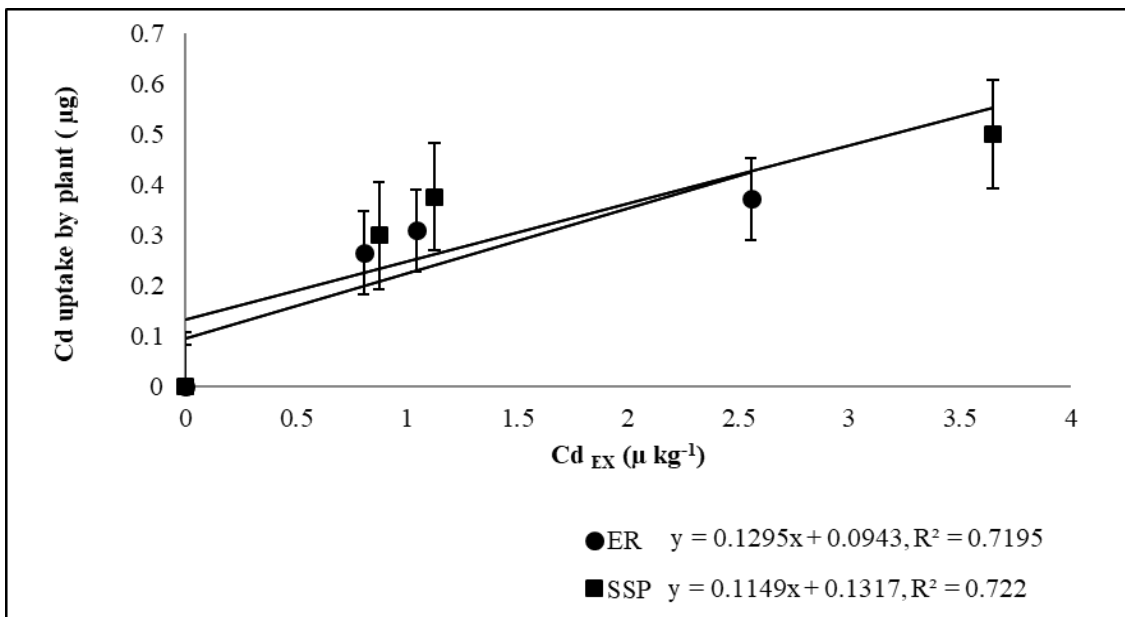
Results indicate that DBL-DGT was sensitive to the changes in available Cd concentration in soil pore water after the application of P fertilizers. These results confirm that DBL-DGT is a better method for measuring Cd in the soil after the application of different types of P fertilizers and doses than the NH<sub>4</sub>Cl method. There were significant differences between Cd<sub>DGT</sub> concentrations at the three deployment depths of the DBL-DGT (ANOVA:  $F_{2,72} = 14.9$ ,  $P < 0.0001$ ) (Appendix 3A). This finding demonstrated the ability of the DBL-DGT to detect the mobility of Cd to a depth of 60 cm and highlights a new concern about Cd transference to the leachate water. The DBL-DGT method is able to track the movement of Cd into deeper layers of soils and to leachate water which corresponded to the hypotheses of Mann et al. (2002). Although Cd<sub>DGT</sub> concentration was very low in the soil, the available Cd in the soil will increase with fertilizer dose, especially in SSP treatments, which may result in the accumulation of available Cd in the soil over time and Cd becoming a major soil contaminant.

#### **4.3.9 Correlation between DBL-DGT measurements and Cd in tomato**

The relationships between Cd concentration in tomato plants (vegetative and fruit) and available Cd concentration in the soil measured by DBL-DGT and NH<sub>4</sub>Cl are shown in Figure 4-4 and Figure 4-5. Both methods (DBL-DGT and NH<sub>4</sub>Cl) were positively related to the concentration of Cd in the plant but varied in their ability to determine the available Cd in the soil (DBL-DGT for SSP:  $R^2 = 0.94$  and ER:  $R^2 = 0.92$ ; NH<sub>4</sub>Cl for SSP:  $R^2 = 0.72$  and ER:  $R^2 =$



**Figure 4-4 Relationships between measured Cd<sub>DGT</sub> concentration in the soil and Cd in tomato samples. n=12 × 3 replicates ± 1 standard error.**



**Figure 4-5 Relationships between Cd concentration extracted from the soil by NH<sub>4</sub>Cl and Cd concentration in tomato samples. n = 12 × 3 replicates ± 1 standard.**

0.71). To confirm these results Spearman's Rho coefficients ( $r$ ) for the correlations between Cd<sub>DGT</sub> or Cd<sub>EX</sub> and Cd in tomato plants were calculated. There was a strong correlation between Cd<sub>DGT</sub> and Cd concentration in plants ( $r = 0.92$ ,  $P < 0.01$ ) and moderate correlation between Cd<sub>EX</sub> and concentration of Cd in plants ( $r = 0.71$ ,  $P < 0.01$ ) (Table 4-15). These results

indicate that the DBL-DGT used in this study is acting similar to the plant root by removing labile Cd from the soil. The flux of Cd from the soil to the plant roots is similar to the flux of Cd from the soil to the diffusion layers of the DBL-DGT. To confirm this hypothesis Spearman Rho correlations were calculated for Cd<sub>DGT</sub> and Cd<sub>EX</sub> with fertilizer dose, fertilizer types and Cd concentration in the plant in all treatments (Table 4-14).

**Table 4-14 Spearman’s Rho coefficients (r) for correlations between P concentration in plants (P<sub>plant</sub>) and available Cd extracted from soil by DBL-DGT and NH<sub>4</sub>Cl methods for different P fertilizer treatments (ER, SSP, and NTS) applied at different doses. n = 12 × 3 replicates ± 1 standard error. NTS measurements were excluded.**

Fertilizer type	ER (µg kg <sup>-1</sup> )	SSP (µg kg <sup>-1</sup> )	Dose (kg ha <sup>-1</sup> )	Cd <sub>DGT</sub> (µg L <sup>-1</sup> )	Cd <sub>EX</sub> (µg kg <sup>-1</sup> )	P <sub>Plant</sub> (µg kg <sup>-1</sup> )
ER	1.00	ns	0.87	0.85**	0.61	0.76**
SSP	ns	1.00	0.98	0.99**	0.64	0.82**
Dose	0.87**	0.98	1.00	0.93**	0.66*	0.85**
Cd <sub>DGT</sub> (µg L <sup>-1</sup> )	0.85**	0.99**	0.93**	1.00	0.53*	0.94**
Cd <sub>EX</sub> (µg kg <sup>-1</sup> )	0.64	0.61**	0.66*	0.53*	1.00	0.71*
Cd <sub>Plant</sub> (µg kg <sup>-1</sup> )	0.76	0.92**	0.85**	0.94**	0.71*	1.00

\* significant at P < 0.05

\*\* significant at P < 0.01

Extraction methods using salts, such as NH<sub>4</sub>Cl, do not usually consider the effects of the soil characteristics because these methods do not include kinetics of Cd uptake by plants, and thus, only reflect the equilibrium state of soil metal (Dai et al., 2017). The extraction method is sensitive to soil pH and organic matter content (Oborn et al., 1995; Li et al., 2009). Therefore, soil properties should be considered when using *ex-situ* chemical extraction methods in the evaluation of Cd bioavailability. The DBL-DGT technique includes contributions from the liquid and solid phases of soil as well as the exchange dynamics between the two phases (Luo et al., 2010; Wang et al., 2017). The exchange dynamic from the solid to the liquid phase is an important factor influencing Cd uptake thus, the Cd measured by the DBL-DGT reflects these processes.

The correlation between the concentration of Cd<sub>EX</sub> and the concentration of Cd<sub>DGT</sub> was positive but low (r = 0.53, P < 0.05) (Table 4-14). The Cd concentration in the soil reduced close to the surface of the DGT device, creating a gradient in the soil solution and diffusive

gel. This process produces a flux from the solid to the liquid phase, which induces natural depletion. These processes are identical to metal uptake mechanisms by roots in the soil (Zarrouk et al., 2014). Previous ferrihydrite DGT studies concluded that this type of *in situ* method is better than the conventional extraction method for predicting Cd absorbed by plants (Pe'rez and Anderson, 2009; Williams et al., 2012; Six et al., 2013; Zarrouk et al., 2014; Sun et al., 2014; Wang et al., 2017).

The Metsorb double binding layer has not been previously used in soil to assess Cd bioavailability. The  $\text{NH}_4\text{Cl}$  method of available Cd extraction from soil has not been used to compare the performance of DBL-DGT with chemical extraction in greenhouse experiments. Thus, the DBL-DGT technique has the potential to evaluate metal bioavailability in soil under greenhouse conditions. Although greenhouse experiments have some limitations, it is easier to control the environmental conditions, such as temperature and humidity, but several studies have reported that the results of greenhouse experiments can be verified by field tests (Ding et al., 2015). The results of this study proved that DBL-DGT better described the available Cd in the soil for tomato plant absorption than the  $\text{NH}_4\text{Cl}$  method. The better performance of the DBL-DGT method involves the effects of soil properties and exchange dynamics between the solid and liquid phases and the absence of disturbance to the soil sample. DBL-DGT is a promising device for assessing Cd bioavailability in soil, however, more research is needed on the suitability of the DBL-DGT in different soil types and with different crops in field conditions.

#### 4.4 Conclusion

A new DGT technique, Metsorb Chelex Double Binding Layer (DBL-DGT), was used to determine available P and Cd concentrations for the first time in soil. The results showed that the concentration of  $\text{P}_{\text{DGT}}$  and  $\text{Cd}_{\text{DGT}}$  in the soil increased with increasing fertilizer dose but varied between fertilizer type. The results also showed that  $\text{P}_{\text{DGT}}$  and  $\text{Cd}_{\text{DGT}}$  increased with soil depth indicating the movement of labile P and Cd by leachate water. The concentration of P and Cd in the tomato plants correlated well with the concentration of  $\text{P}_{\text{DGT}}$  and  $\text{Cd}_{\text{DGT}}$  in all treatments.

The comparison between DBL-DGT and the chemical extraction method of measuring labile P and Cd in the soil demonstrated that the DBL-DGT measurements of both analytes were better correlated with their actual concentrations in plants than those measured by chemical extraction.

Accumulation of P and Cd occurred in the treated pots which had received the highest dose of P fertilizer, particularly for the SSP fertilizer. Application of high quantities of P fertilizers can result in loss of nutrients (P) from the soil before absorption by plants and the contamination of soil and water by non-nutrients (Cd). The DBL-DGT provides suitable information about the movement, accumulation, and accessibility of P and Cd added to sandy loam soil from different types of P fertilizers. More work is needed using different soil types and crops in field conditions.

## **Chapter 5-. Studying changes in labile P and Cd concentration due to the consecutive application of P fertilizers using the Metsorb Mixed Binding layer DGT**

### **5.1 Introduction**

This chapter examines the impacts of the ongoing application of phosphorus fertilizers to sandy loam soil on increasing availability of Cd in soil and movement of available Cd to the leachate water. In the experiment, three consecutive treatments of three types of P fertilizer were applied to the soil to investigate the impact of the successive application of P fertilizer on the accumulation available P and Cd in the soil using the MBL-DGT. Despite the fact that available P will be increased due to the consecutive application of P fertilizers, the capacity of the MBL-DGT to adsorb P was also investigated. As the emphasis was on the accumulation of labile Cd concentrations in the soil and leachate water, no plant was involved in this experiment. Concentrations of labile Cd in the soil were monitored using Metsorb–Chelex mixed binding layer DGTs (MBL-DGT) and compared with the chemical extraction methods (Olsen et al., 1954; Krishnamurtia et al., 1995) used to measure the available P and available Cd. Sequential leachate water analyses were used to investigate the impact of continuous application of P fertilizers on the mobility of available P and Cd to the leachate water.

#### **5.1.1 Impact of P fertilizers on water quality**

There are several risks associated with excessive and continuous application of phosphorus (P) fertilizers in horticultural and pastoral production (Zhao et al., 2009). Most critical is the degradation of water resource quality through leaching and runoff. Many studies stressing that the deterioration of water quality is principally due to the application of chemical fertilizers to soils (Sharpley and Pekolainen, 1997; Hansen et al., 2002; Foy, 2005; Holman et al., 2008; Jalali and Kolahchi, 2008; Sharpley et al., 2008). The magnitude of such degradation is difficult to assess because agricultural pollution is a non-point source in nature.

Loss of P from agricultural lands through runoff is the most common mechanism of P arriving into water bodies followed by leachate water (Divya and Belagali, 2012). Although P loss by leaching is often considered minor compared with surface runoff (Gartley and Sims,

1994; Fortune et al., 2005), there are some soil conditions which make P loss by leaching more significant. These conditions include high soil P concentration associated with drainage ditches, high water table and sandy soil texture (Gartley and Sims, 1994; Fortune et al., 2005). For example, P mobility to leachate water in sandy soils can be 10-20 cm deeper than P mobility in clay soils (Ohalloran, 1993). Previous chapters of this thesis demonstrated that increasing concentration of available and soluble P in soil solution increases the potential for P transfer from soil to the leachate water. Chapter 4 also showed that an imbalance between P input and output over time may lead to excessive P accumulation in soil and increase P mobility to ground and surface water.

The main risk associated with the transfer of available P to leachate water or runoff then into natural water bodies is the creation of eutrophication (Carpenter, 2008; King et al., 2015). Soils treated with high levels of P fertilizer have the potential to release bioavailable P to surface waters which promotes algal growth (Pote et al., 1996). P losses through leaching depend mainly on the saturation levels of P in the soil, the extent of phosphate adsorbing mineral surfaces, the species of P in solution and the method of transport through the soil profile. The mobility of P through the soil profile can happen by preferential flow through cracks and macropores or via artificial drainage channels (Simard et al., 2000).

The second concern emerging from the downward movement of P fertilizers to the leachate water is the accumulation of cadmium (Cd) in soil and leachate water. Divya and Belagali (2012) concluded that the application of P fertilizers can have major impacts on the presence of Cd in water bodies. Cd associated with P fertilizers leaches into ground and surface waters causing global environmental concern for public health. Cd is a nonessential element for plants, animals and humans and its presence in the soil and water can have severe impacts on the ecosystem, human and animal health (Kachenko and Singh, 2006).

Cd is at the top of the “Big Three” toxic heavy metals category (cadmium, lead, and mercury) because of its acute toxicity to humans and animals. Consuming Cd-contaminated food and drinking water can cause stomach irritation, vomiting, and diarrhea in humans (Ochiai, 1987; Zhang et al., 2012). The oral lethal dose of Cd for humans is a few milligrams per liter (Lussier et al., 1985; Li et al., 2014). It has been found that 15-30 mg of Cd from acidic food can result in acute gastroenteritis (Rehman et al., 2017). Human uptake of Cd is mainly through food with Cd-accumulating in the kidneys and liver. Also, it has been stated that Osteomalacia (softening of the bone) originates from acute Cd poisoning and elderly women who have had many children can be affected more than others (Li et al., 2014). In addition to



Cd impacts on human and animal health, Cd may also have deleterious effects on plant growth through phytotoxicities (McLaughlin et al., 2000a; Kachenko and Singh, 2006).

The movement of Cd has been studied in pasture soil to predict the impacts of long term application of P fertilizers (McGrath and Lane, 1989), and to examine Cd distribution in pasture soil after the application of P fertilizers (Loganathan et al., 1995). Some studies have examined Cd movement to leachate water after long term P fertilizer application (Loganathan and Hedley, 1997) where it was reported that downward movement of Cd was unlikely to pollute groundwater due to the slow movement of Cd in soil (Loganathan and Hedley, 1997). However, Divya and Belagali (2012) studied Cd movement in a yellow podzolic soil under clover plants in Australia and demonstrated downward Cd movement for more than 100 mm.

Research evaluating Cd concentrations in Australian rivers showed that high Cd concentrations existed in a number of rivers, including King River, South Esk River and Derwent River Estuary in Tasmania; and Captain Flat and Molonolo River in New South Wales (Bloom and Ayling, 1977; Stark, 1998) due to the leaching process and surface runoff of chemical fertilizers from agricultural practices. These contradictory results may, in part, be due to the method of measuring available P and Cd in soil and water.

Implementing accurate P measurements to control P fertilizer application in the field is recommended to minimize eutrophication (Sharpley and Pekolainen, 1997). Available P and Cd at different soil depths is critical when assessing P and Cd mobility while measuring the concentration of available P in the leachate water. These investigations are also important for protecting natural water bodies from P and Cd pollution (Zhanga et al., 2013). A universal, simple and *in situ* method is therefore needed to simultaneously investigate P and Cd concentrations and mobility in these circumstances.

This chapter examines the impacts of the ongoing application of P fertilizers to sandy loam soil on (i) increasing availability of Cd in soil, (ii) movement of available Cd to leachate water, and (iii) the limit of the MBL-DGT capacity for adsorbing P or Cd from the soil.

## **5.2 Material and methods**

### **5.2.1 Soil Preparation**

A sandy loam soil was used for this experiment (see description of soil characteristics in Chapter 2 and Table 3.1). The treatments were applied in the same order and to the same pots

in the glasshouse (see section 4.2.1 for details). The last measured levels of available  $P_{DGT}$  and  $Cd_{DGT}$  in the pots (see Table 4-4 and 4-11) were considered the initial available P and Cd levels for this experiment. The soil in each pot was emptied onto a separate clean plastic mat and later replaced in the same pot. The soil on the mat was left to air dry in the glasshouse, cleaned of tomato plant remains and roots, mixed thoroughly and then replaced in the same pot. A composite soil sample was taken from each pot to measure the initial available and total concentrations of P and Cd using chemical extractants 0.5 M  $NaHCO_3$  and 1 M  $NH_4Cl$  as described in sections 2.6 and 2.7, respectively, before commencing this experiment.

The bottom of each pot was lined by three successive layers of a fine screen net, a filtering fabric, and a 1 cm layer of fine gravel to avoid blockage of the drainage holes. Each pot was filled with 47 kg of soil packed to a depth of 70 cm. To achieve uniform soil density of  $1.4 \text{ g cm}^{-3}$ , the soil was put into the pots in several layers and gently hammered with a wooden hammer (square base with the same dimensions as the inner cross-section of the pots). These preparations for each pot were repeated after each P fertilizer application in the second and the third cycle to simulate the plowing process in the field after the harvesting of each crop in the agricultural cycle. Soil moisture was brought to 80% of soil water holding capacity (WHC) by adding distilled water to each pot before DGT deployment. When the soil achieved 80% WHC, the weight of each pot was measured using a digital scale and recorded on the pot. Pot weight was maintained during the 7-day MBL-DGT deployment time by adding distilled water to the pots when required to maintain soil moisture in each pot at 80% WHC. No leaching was permitted for 48 h to allow soil equilibrium after application of P fertilizers. Soil samples were taken from the pots to chemically extract available P using the Olson method (as described in Olsen et al., 1954) and available Cd by using 1 M  $NH_4Cl$  (as described in Krishnamurtia et al., 1995) after the DGTs were removed. This allowed comparisons of the measurement of available P and Cd by the two methods (DGT and chemical extraction).

### **5.2.2 Fertilizer application**

Three consecutive cycles of fertilization were conducted in this experiment. In each cycle, three types of phosphorus fertilizers were added to the soil in the pots: Single superphosphate (SSP), Nutri-Tech Solution (NTS) and Egyptian phosphate rock (ER). The fertilizer properties are described in Appendix 1. The fertilizers were applied to the soil at the same doses used for tomato plant cultivation in Chapter 4 (0, 100, 200, and  $250 \text{ kg P ha}^{-1}$ ) (see Table 4-2). The fertilizer type and dose treatments were randomly distributed in 36 pots in a

randomized block design greenhouse experiment. After applying each fertilizer by spreading it on the soil, the fertilizer was covered with a very thin layer of the soil. The pots were left for 48 h with no drainage to allow soil and fertilizer equilibrium, after which time, the MBL-DGTs were deployed and drainage holes opened. The MBL-DGT deployment period in the pots was seven days. These processes were repeated three times. Each fertilizer application time represented one cycle of fertilizing the soil. Seventeen days were spent on unloading the pots, drying the soil and refilling the pots. The aim of this process was to simulate the tillage process in the field after each cycle. The total interval between each cycle of fertilizer application was 45 days. Three consecutive applications of the same dose were applied to each pot to simulate three agricultural cycles of crops. The schedules of P fertilizer applications and DGT deployment are presented in Table 5.1.

**Table 5-1 Schedule of P fertilizer application during four weeks of each cycle, MBL-DGT deployment in the soil, and MBL-DGT removal from the soil.**

<b>Fertilizer cycle</b>	<b>Date of fertilizer application</b>	<b>Date of DGT deployment</b>	<b>Date of DGT removal from the soil</b>	<b>End of cycle</b>
First	17- 4-2012	19-4-2012	26-4-2012	15-5-2012
Second	1-6-2012	3-6-2012	10-6-2012	29-6-2012
Third	16-7-2012	18-7-2012	25-7-2012	13-8-2012

### **5.2.3 Soil chemical extraction**

Total and available P and Cd concentrations were extracted and measured twice in this experiment. Firstly, before commencing the experiment, composite samples were taken from each pot (36 pots) to measure the status of P and Cd in the soil of each pot after the tomato experiment. Secondly, soil samples were collected from each pot with a small auger at a depth of 30 cm after removal of the MBL-DGTs in this experiment. Samples were oven-dried at 50 °C then sieved through a 2 mm sieve for available P and Cd measurement. For total P and Cd measurements, soil samples were ground and sieved through a < 0.2 mm sieve and prepared for extraction as described in section 2.6. Available P was extracted by 0.5 M NaHCO<sub>3</sub> as described by Olsen et al. (1954). Available Cd was extracted by 1 M NH<sub>4</sub>Cl as described by Krishnamurtia et al. (1995).

#### **5.2.4 Leachate water sampling**

Leachate water was collected every day during the 7-day of DGT deployment and for the remainder of each cycle. The water samples were digested as described in section 2-6. P and Cd concentration were determined in the digested water samples as described in sections 2.6 and 2.7, respectively.

#### **5.2.5 Mixed Binding Layer DGT (MBL-DGT) assembly and deployment**

The MBL-DGTs were assembled as described in section 2-3 and Figure 2-2 and then deployed in the soil for seven days at three depths of 15 cm, 45 cm, and 60 cm as described in section 2.4. Available P and Cd concentrations were measured in the eluents as described in sections 2-6 and 2.7, respectively.

In this experiment, the Metsorb Chelex Mixed Binding Layer (MBL-DGT) was used as there was no difference observed between the Metsorb Chelex Double Binding Layers (DBL-DGT) and the MBL-DGT in terms of adsorption capacity of P and Cd (see Chapter 4). However, the MBL-DGT was more easily prepared.

### **5.3 Results and discussion**

#### **5.3.1 Changes in available and total P concentrations in the soil after repeated application of P fertilizer**

In this experiment, three consecutive applications (cycles) for three types of P fertilizer, each applied at four doses (0, 100, 200 and 250 kg P ha<sup>-1</sup>) to the soil. Before commencing this experiment, the pots received different P treatments from the previous tomato planting experiment (Chapter 4), therefore, the starting point of available P concentration varied between pots (Table 5-2). Pots that received the SSP fertilizer treatment in the tomato experiment (Chapter 4) had the highest concentrations of extracted available P (2.125 mg kg<sup>-1</sup>) when 250 kg ha<sup>-1</sup> of SSP fertilizer had been added to the soil in (Chapter 4) (Table 5-2). Total P concentration in the soil ranged between 10.45-31.85 mg kg<sup>-1</sup> in the ER and SSP fertilizer treatments. Total P concentration substantially increased in comparison to the initial total concentration of the soil before the commencement of any experiment 0.039 mg kg<sup>-1</sup> (see Table 3-1).

**Table 5-2 Concentration of available P extracted using Olsen methods before commencing the experiment. n = 12 × 3 replicates ± 1 standard error.**

Dose (kg ha <sup>-1</sup> )	SSP (mg kg <sup>-1</sup> )		NTS (mg kg <sup>-1</sup> )		ER (mg kg <sup>-1</sup> )	
	Available	Total	Available	Total	Available	Total
0	BDL	0.02 ± 0.0	BDL	0.02 ± 0.00	BDL	0.02 ± 0.00
100	1.99 ± 0.00	16.8 ± 0.10	1.34 ± 0.00	10.8 ± 0.11	1.68 ± 0.01	10.5 ± 0.20
200	2.09 ± 0.10	30.8 ± 4.20	1.46 ± 0.00	29.8 ± 4.21	1.87 ± 0.10	30.2 ± 0.41
250	2.13 ± 0.10	31.9 ± 3.40	1.45 ± 0.20	31.9 ± 3.40	1.98 ± 0.01	31.5 ± 0.30

These results indicate that some adsorption occurred in the soil after the application of P fertilizers. The soil properties in Table 3-1 are not compatible with the adsorption factors which increase soil adsorption affinity to P such as clay content, organic matter, calcium carbonate or other soil colloids. Therefore, it is presumed that the presence of undissolved fertilizer particles remained in the soil from the previous experiment and this has resulted in the increase in total P concentration.

The control treatment which did not receive P fertilizer resulted in a slightly lower concentration of total P than the original total P concentration in the soil (Table 3-1). Available P was below the detection limit (Table 5-2) as in the original soil. Total P decreased due to the soil moisture conditions, leaching and plant absorption in the control treatment.

The P<sub>DGT</sub> concentration in the soil from the tomato experiment was considered the initial P<sub>DGT</sub> concentration of this experiment in each treatment (Table 5-3). The difference between the results of the chemical extraction method and the MBL-DGT method for measuring available P suggests that the chemical extraction method may have extracted non-available forms of P or undissolved fertilizer particles thus giving erroneous results as some of the reported available P cannot be accessed by plant root.

**Table 5-3 Concentration of the initial available P measured by the MBL-DGT at three different depths (15, 45 and 60 cm) after the application of three types of fertilizer (SSP, ER and NTS) at four different doses (0, 100, 200 and 250 kg P ha<sup>-1</sup>) from the previous tomato experiment. For each depth, n = 12 × 3 replicates ± 1 standard error.**

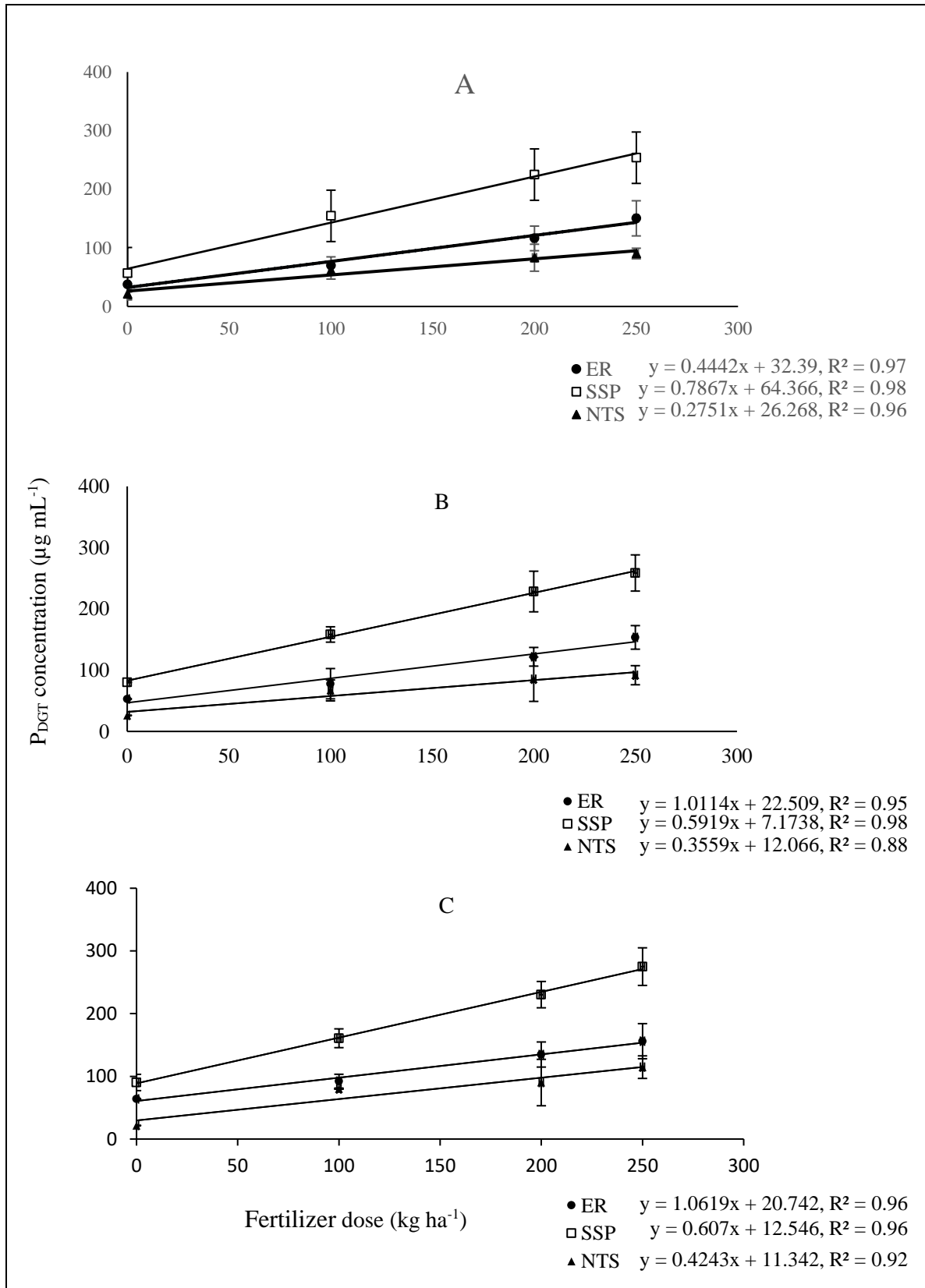
Soil depth (cm)	Fertilizer dose (kg ha <sup>-1</sup> )			
	0	100	200	250
<b>Available P from SSP Fertilizer (µg L<sup>-1</sup>)</b>				
15	1.01 ± 0.00	52.4 ± 1.40	77.2 ± 4.10	88.5 ± 5.10
45	1.04 ± 0.00	77.1 ± 8.40	113 ± 2.30	118 ± 7.20
60	1.04 ± 0.00	88.4 ± 4.20	119 ± 0.31	138 ± 11.20
<b>Available P from NTS Fertilizer (µg L<sup>-1</sup>)</b>				
15	1.01 ± 0.00	18.3 ± 1.10	47.1 ± 0.71	69.2 ± 7.80
45	1.04 ± 0.00	21.2 ± 5.50	53.5 ± 2.91	75.7 ± 3.90
60	1.04 ± 0.00	28.4 ± 2.10	68.5 ± 1.80	97.1 ± 1.20
<b>Available P from ER Fertilizer (µg L<sup>-1</sup>)</b>				
15	1.01 ± 0.0	33.95 ± 7.1	58.16 ± 5.1	76.1 ± 4.20
45	1.04 ± 0.0	47.94 ± 3.2	64.58 ± 3.3	82.8 ± 0.10
60	1.04 ± 0.0	51.48 ± 8.2	92.75 ± 2.4	116 ± 3.00

The correlation between the concentration of absorbed P in tomato plants and the P<sub>DGT</sub> concentration (Chapter 4) proved that the DGTs were measuring available P the most accessible P form to the plant root. In the first cycle, there was a notable increase in P<sub>DGT</sub> concentration caused by P fertilizer addition to the pots in all treatments (Appendix 6-A). The mean concentration of P<sub>DGT</sub> at the three soil depths increased 108%, 113% and 56% after the application of 100 kg P ha<sup>-1</sup> of SSP, ER and NTS fertilizer, respectively. The highest P<sub>DGT</sub> value (274.97 ± 2.7 µg L<sup>-1</sup>) was recorded for the highest dose of SSP fertilizer (Appendix 1). The second cycle of P fertilizer application contributed additional available P adsorbed by the MBL-DGT with the highest P<sub>DGT</sub> (280.8 ± 0.8 µg L<sup>-1</sup>) measured in pots which received the highest dose of SSP fertilizer (Appendix 6-B). During the third cycle of P fertilizer application, greater labile P was adsorbed onto the mixed binding layer (Appendix 6-C). The results confirmed that the SSP fertilizer produced the highest available P adsorbed by the MBL-DGT

which concurs with the previous results of the flume and the tomato experiments (Chapters 3 and 4).

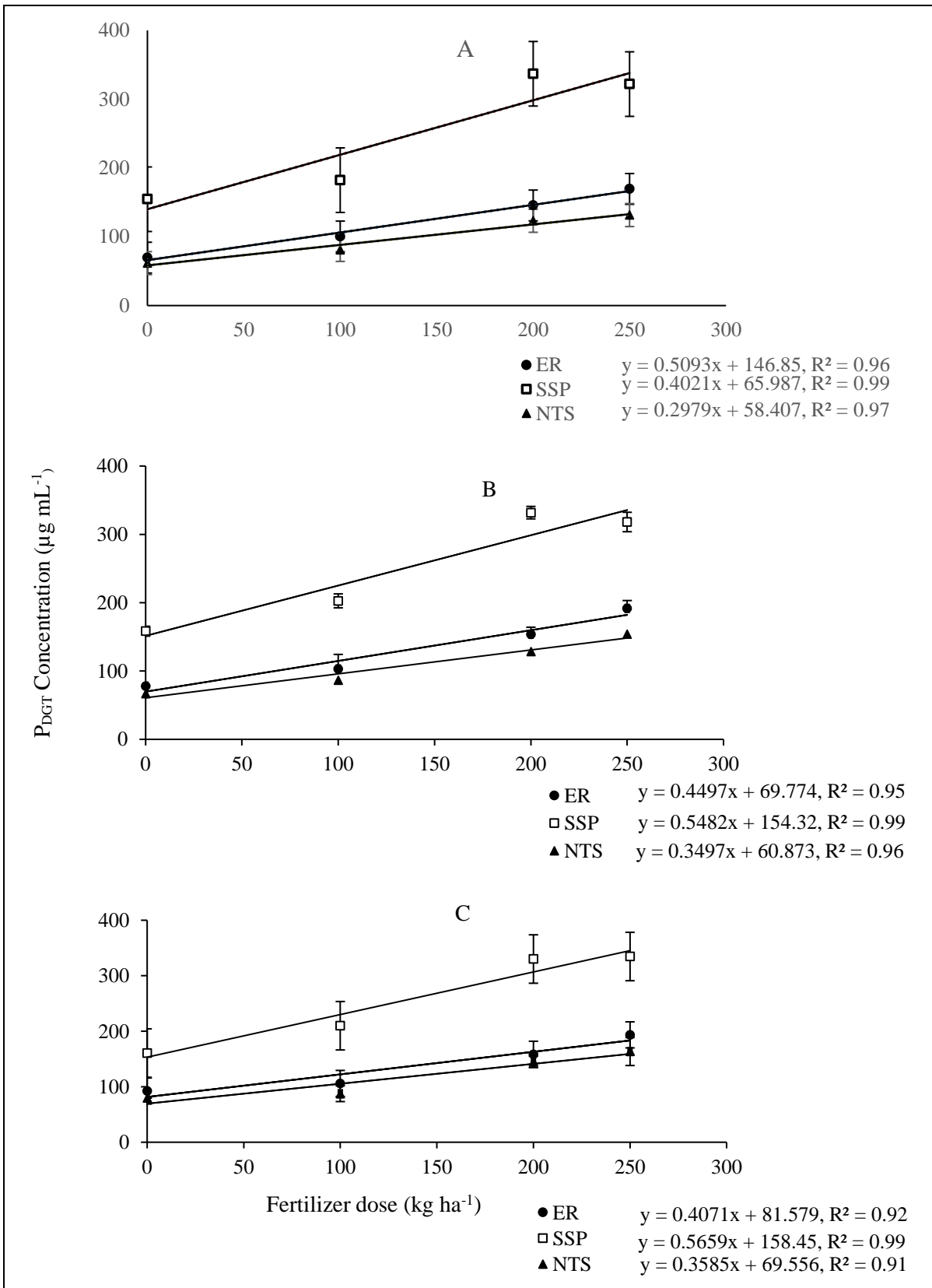
In the first cycle, the adsorption of labile P onto the MBL layer increased linearly with increasing fertilizer dose for all three fertilizer types in the order SSP > ER > NTS (Figure 5-1). In the second and third cycles, application of P fertilizers caused further increases in the concentration of the labile P uptake by the MBL however, the increase of labile P was not as strong as in the first cycle (Figure 5-2 and Figure 5-3). In the third cycle, the  $P_{DGT}$  trends had lower gradients than the first two cycles and were no longer linear, especially at the highest dose of P fertilizer (Figure 5-3). This suggests that P uptake by the MBL plateaued after P saturation of the binding sites on the MBL of the DGT.

The SSP fertilizer has the highest water solubility amongst the P fertilizer types used in this experiment. The mean concentration of  $P_{DGT}$  of the three deployment depths increased 117% in the first cycle due to the application of 250 kg P ha<sup>-1</sup> of SSP fertilizer, however, only 19% and 5.8% in the second and the third cycle, respectively, when applying the same dose of the same fertilizer. The application of the highest dose of the ER fertilizer resulted in an increase in the mean concentration of  $P_{DGT}$  at the three depths of 15%, 86% and 106% in the first, second and third cycles. The NTS fertilizer had the lowest solubility in water, with the mean  $P_{DGT}$  concentration increasing linearly from 10%, 51% to 72% after the first, second and third cycles.

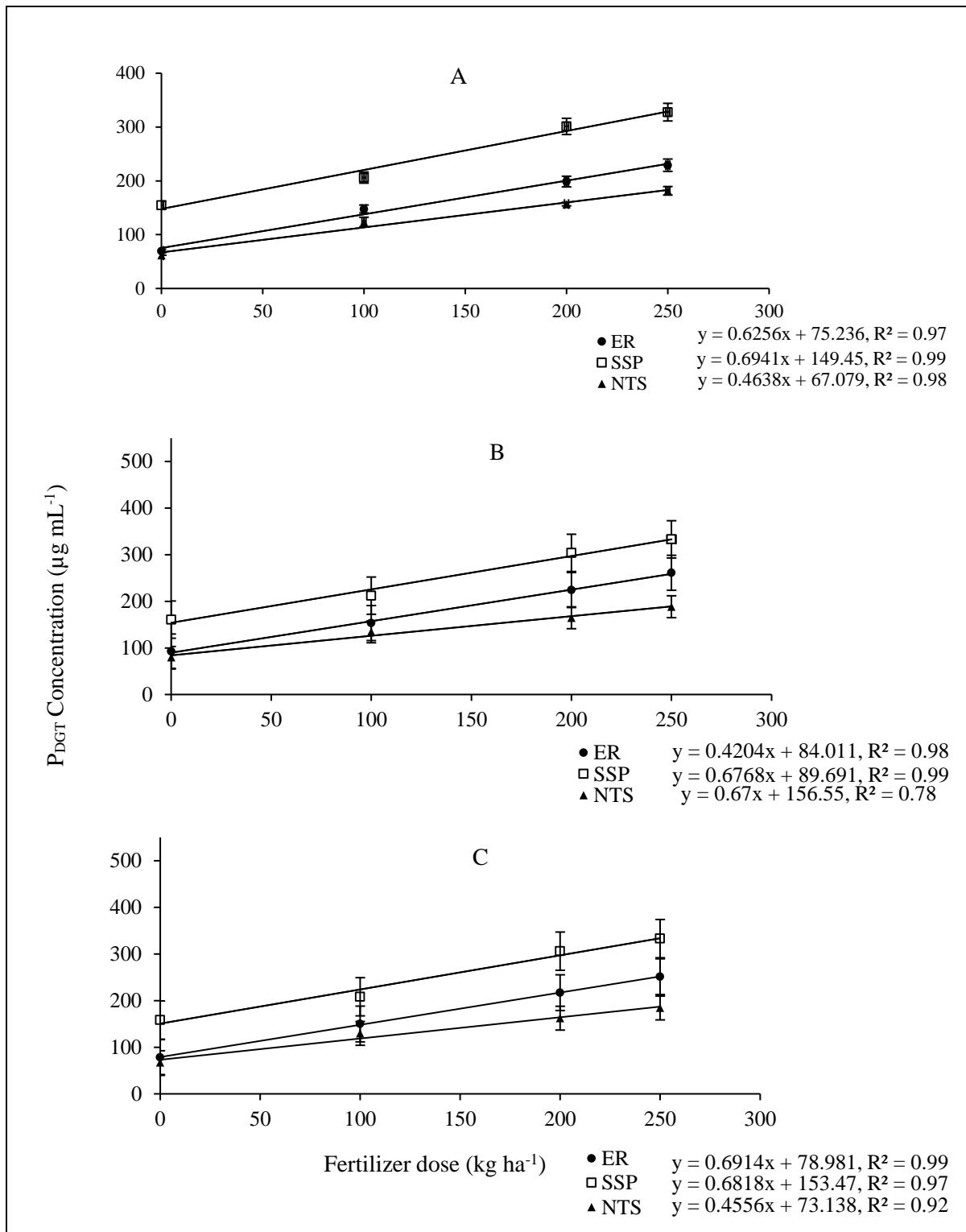


**Figure 5-1** The relationship between  $P_{DGT}$  concentration and fertilizer dose in the first cycle of applying three types of fertilizer (SSP, NTS, and ER) at the rate of 0, 100, 200, and 250 kg ha<sup>-1</sup> at three different depths (A = 15 cm, B = 45 cm and C = 60 cm). The concentration of each treatment at the end of each cycle was considered the initial concentration of the next cycle. For each depth  $n=12 \times 3$ , replicates  $\pm 1$  standard error.





**Figure 5-2 Relationship between  $P_{DGT}$  concentration and fertilizer dose in the second cycle of applying three types of fertilizers (SSP, NTS, and ER) at rate of 0, 100, 200, and 250  $kg\ ha^{-1}$  at three different depths (A = 15 cm, B = 45 cm and C = 60 cm. For each depth,  $n=12 \times 3$ , replicates  $\pm 1$  standard error.**



**Figure 5-3 Relationship between  $P_{DGT}$  concentration and fertilizer dose in the third cycle of applying three types of fertilizers (SSP, NTS, and ER) at rate of 0, 100, 200, and 250 kg ha<sup>-1</sup> at three different depths (A = 15 cm, B = 45 cm and C = 60 cm). For each depth, n=12× 3, replicates ± 1 standard error.**

The gradient of the increasing trend then decreased in the SSP fertilizer treatment from 64.36 to 3.47 in P uptake by the MBL varied among P fertilizer treatments due to the variable solubility product of each fertilizer. The solubility of the ER and NTS fertilizers are low therefore, available P was released gradually to replenish available P lost by leaching and DGT uptake to maintain the level of available P in the soil. Furthermore, MBL-DGT uptake of labile P depends on the adsorption capacity of the Metsorb Chelex mixed binding layer. When MBL-DGTs were deployed in the soil after the application of the first cycle, the concentration of labile P in the eluent increased linearly (Figure 5-1) and more increase was noticed after the second cycles (Figure 5-2). the gradient of an increasing trend then decreased in SSP fertilizer treatment from 64.36 to 3.47 in Figure 5-3 C. However, this did not occur in the ER and NTS treatments (Figure 5-3 A and B) for the reason that both fertilizers release available P gradually into the soil. The available P uptake by MBL in the ER and NTS treatments depended on the solubility product of P fertilizer in water and the released dose of available P from each individual P fertilizer to the soil solution.

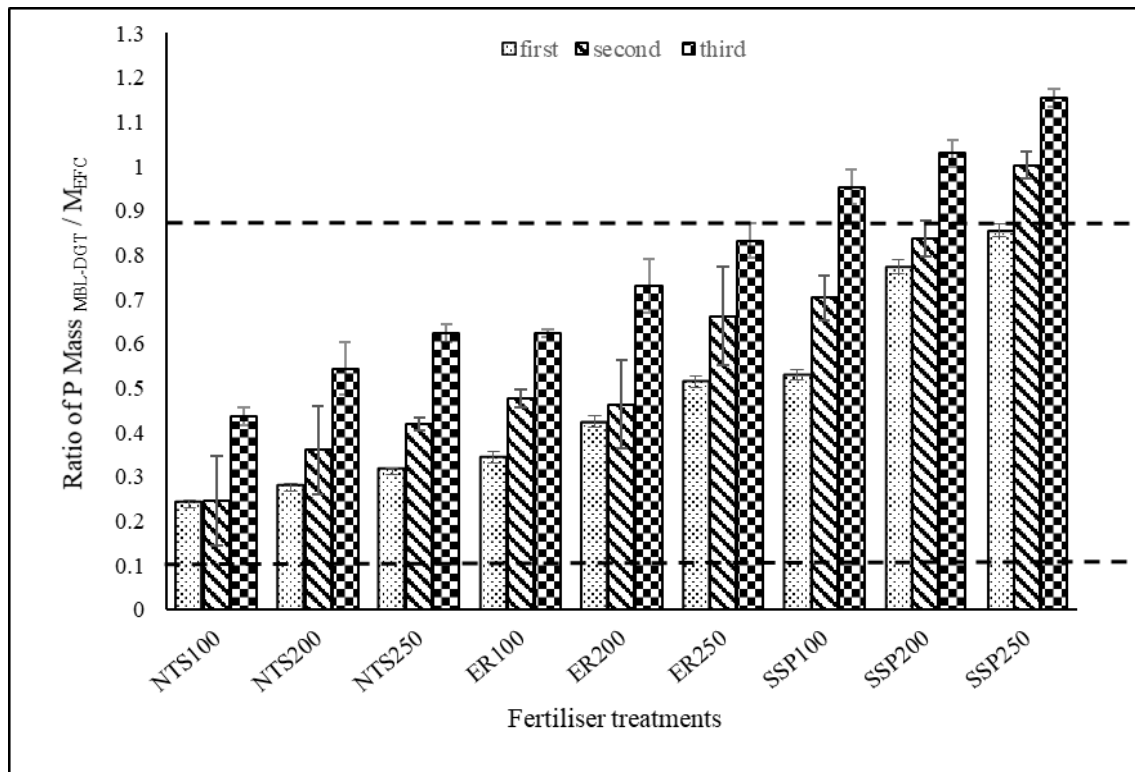
### **5.3.1 Mass of P accumulated on the mixed binding layer (MBL-DGT)**

The MBL-DGTs were collected after seven days of deployment in each fertilization cycle. The mass of P accumulated on the MBL-DGTs increased correspondingly with increasing P fertilizer dose (Appendix 6A). The highest P uptake by the MBL-DGT was recorded in the DGTs deployed in pots treated with SSP fertilizer (see Appendix 6-A). However, the increasing trend of available P mass in the high dose treatment of SSP plateaued after 42,525 ng P per disk (Appendix 6-D). The highest dose of SSP fertilizer increased MBL-DGT uptake to 54% in the first cycle then decreased to 16.5% and 4.9% in the second and third cycles, respectively. The highest dose of ER fertilizer caused an increase of 23%, 25% and 27% in the first, second and third cycles, respectively. The same doses of NTS fertilizer caused an increase in available P mass of 10%, 34% and 42% in the first, second and third cycles, respectively. The NTS fertilizer has the lowest solubility in water hence, resupply of available P to the soil was the slowest among the P fertilizers after the reduction of available P due to the leaching process and MBL adsorption. Increasing linear relationships were found between P mass accumulated on the adsorbent sites of the MBL and fertilizer dose over the seven days of MBL-DGT deployment. The mass of P was 32,945 ng P per DGT disc after the first cycle of SSP fertilizer. The mass of available P then increased to 39,459 and 42,552 ng P per disc in the second and third cycle s, respectively.

The DGT technique has the ability to be deployed for periods of more than one week to collect an integrated record of analytes concentration (Zhang et al., 1998b). To accurately measure available P (or any analyte in soil solution) using the DGT technique, the capacity of the adsorbent layer must not be exceeded during the deployment time. The effective capacity of the DGT reflects the mass of analyte accumulating on adsorbent linearly as a function of time (Panther et al., 2010). The effective capacity is normally lower than the total capacity (Zhang et al., 1998a; Mason et al., 2005b; Panther et al., 2010). Although the effective capacity of the MBL was not determined in this experiment, Panther et al. (2010) reported that the effective capacity of the Metsorb binding layer for available P was approximately 40,000 ng and there was no statistical difference between the P concentration measured by Metsorb binding layer DGT or MBL-DGT (Panther et al., 2014).

When soil solution contains available P below the range of the MBL maximum capacity, the eluted mass of P increased linearly up to 40,000 ng P per disc. This condition was applicable to the ER and NTS fertilizers during the deployment time (see Appendix 6-D). Deviation of the adsorption trend from linearity indicates that the effective capacity of the binding gel was exceeded. SSP fertilizer has high solubility, therefore, high doses released greater available P to the soil solution and the amount of available P in the binding gel continued to increase correspondingly with the increasing concentration of available P in soil solution. However, in the third cycle, the trendline of the SSP fertilizer (Appendix 6-C, 6-D) had a lower gradient and was no longer linear as observed in Appendix 6A-D. The results indicated a marginally greater mass of P on the MBL of 42,552 ng P per disc than the corresponding results of the Metsorb layer reported by Panther et al. (2010) from the application of high doses of SSP fertilizer to the soil after the third cycle.

To assess the validation of the current MBL-DGT capacity for P uptake in the soil, the ratio of the current results of P mass on the MBL-DGT ( $M_{\text{MBL-DGT}}$ ) in soil and the maximum effective capacity of the Metsorb reported by Panther et al. (2010) ( $M_{\text{EFC}}$ ) were calculated as ( $M_{\text{MBL-DGT}}/M_{\text{EFC}}$ ) (Figure 5-4).



**Figure 5-4 Ratio of P  $M_{\text{MBL-DGT}}$  / P  $M_{\text{EFC}}$  as a function of P fertilizer dose. All P mass ratios above 0.9 are semi-quantitative, between 0.1 to 0.9 are quantitative, and less than 0.5 are not quantitative.). For each depth,  $n = 12 \times 3$ , replicates  $\pm 1$  standard error.**

The performance of the MBL-DGT was evaluated relative to the effective capacity (see dotted lines in Figure 5-4). If the P mass ratio was above 0.9, the MBL-DGT measurements are semi-quantitative. If it was between 0.1 and 0.9, then available P measurements are quantitative. If it was less than 0.1, the P measurements are non-quantitative (Panther et al., 2011; Panther et al., 2014).

The measurements of the MBL-DGT for the third cycle of SSP application at 250 kg ha<sup>-1</sup>, the third cycle of SSP at 200 kg ha<sup>-1</sup> and the third cycle of SSP at 100 kg ha<sup>-1</sup> were semi-quantitative because the ratios were above the effective capacity (Figure 5-4). All other measurements of the first and second cycle of SSP fertilizer and all P fertilizer treatments (ER and NTS) in the three cycles were quantitative as the ratios were between 0.2 and 0.9. (Figure 5-4). The capacity of the MBL-DGT depended primarily on the concentration of labile P added to the soil, however, was similarly affected by soil conditions and P mobility in the soil.

This experiment was conducted in an open dynamic soil system which allows P leaching and other soil physio-chemical reactions and biological activity to result in P loss from soil. The low mass ratio of P is attributed to the lack of P retention in the soil and high hydraulic

conductivity due to the soil sandy loam texture. Furthermore, the loss of P to the leachate water increased by maintaining soil moisture condition (80% WHC) and competing ions in the soil such as  $\text{HCO}_3^{-1}$ .

The binding mechanism of P in the MBL-DGT is dependent on exposure to the soil solution, with P concentration below or above the concentration corresponding to the reported effective capacity of the Metsorb binding gel. Below the effective capacity of available P, mass transport is diffusion-based, induced by the available P concentration gradient, where the Metsorb particles in the MBL act as an infinite sink over the exposure period. Consequently, Fick's First Law of Diffusion can be applied to calculate the mass flow into the binding gel during the application time and hence the concentration at the interface of the DGT device and solution ( $C_{\text{DGT}}$ ). Above the critical capacity, the concentration gradient occurring between the binding layer and soil solution results in an equilibrium between soil solution and the binding layer of the device. The Metsorb particles provide adsorption sites on the MBL and increase the P uptake by the MBL. However, these sites could be less accessible and therefore do not function as infinite sinks to maintain the surface concentration. ER fertilizer released available P gradually to the soil it has slower solubility in water than SSP fertilizer. Therefore, P uptake by the Metsorb binding layer increased steadily after each cycle (Figure 5-3).

When SSP fertilizer was exposed to water, the MBL attracted the most available species of labile P in the soil solution. Adding the same dose in the second and third cycle resulted in more available P in the soil, however, the MBL uptake did not increase at the same rate as in the first cycle. The SSP gradient decreased in the second and third cycle (Figure 5-3 C). The highest P mass accumulated on the Metsorb particles of the MBL was slightly higher than 40,000 ng P per disc reported by Panther et al. (2010). ER fertilizer and NTS fertilizer gradients of P mass increased linearly as both are less soluble in water than SSP fertilizer (Figure 5-3 A, B, and C)

These results indicated that the MBL-DGT used in this experiment had limited capacity to adsorb available P on the Metsorb Mixed Binding Layer. An agricultural soil that used to having high P fertilizer input, may require a DGT with different specifications, such as larger diameter or larger DGT size, thicker Metsorb mixed binding layer, increase the amount of Metsorb and longer deployment times to increase the capacity of the MBL-DGT.

### 5.3.2 Phosphorus loss in the leachate water

Consecutive addition of P fertilizers to the soil for three cycles lead to increased P lost to the leachate water (Table 5-4). The total mass of P lost during the three cycles was 52%, 66% and 67% when SSP was applied at 100, 200 and 250 kg ha<sup>-1</sup>, respectively. ER fertilizer lost P to the leachate water of 31%, 36% and 36% at doses of 100, 200 and 250 kg ha<sup>-1</sup>, respectively (Table 5-4). NTS fertilizer resulted in the lowest loss of available P mass to the leachate water (Table 5-4). These results support the results of available P accumulated in the leachate water from Chapters 3 and 4. The main factor affecting P lost to the leachate water is soil texture and the excessive use of P fertilizers over three consecutive applications. Moreover, fertilizer solubility had an effect as the highest loss of available P to the leachate water occurred in SSP fertilizer treatments which have the highest P content and highest solubility in water.

The MCB-DGT accurately predicted the outcome of available P added to the soil from each fertilizer. Increasing P<sub>DGT</sub> concentration with depth indicated that available P in the soil solution moved to the leachate water (Appendix 6-A, B and C). The sensitivity of the MBL also demonstrated the differences between fertilizer type (solubility) which increased available P. The new MCB-DGT technique is easy to use in the field prior to planting crops and it could be suitable for any soil type to accurately investigate the nutrient status of the soil. Using this method will be efficient and economical for growers, as well as to reserve P resources. In addition, the accuracy of MBL-DGT will protect the soil and water environment from P and heavy metal contamination by controlling and limiting excessive P fertilizer use.

**Table 5-4 Mass of P loss in the leachate water after the application of the same dose of P fertilizer in three consecutive cycles using three types of fertilizer of SSP, NTS, and ER at four application doses of 0, 100, 200 and 250 kg ha<sup>-1</sup>. For each fertilizer type, n = 90 × 3 replicates ± 1 standard error.**

Fertilizer Type	The total mass of P added in three cycle s (µg)	Mass of P in the leachate water (µg)			Total P lost (µg)	P lost%
		First cycle	Second cycle	Third cycle		
0	BDL	BDL	BDL	BDL	BDL	BDL
SSP1	3525 ± 325.0	353.6 ± 75.01	606.0 ± 55.00	876.5 ± 16.00	1836 ± 163.0	52
SSP2	7050 ± 245.0	974.7 ± 25.00	1705 ± 124.0	1987 ± 214.0	4667 ± 249.0	66
SSP3	8813 ± 300.0	1545 ± 31.00	1947 ± 252.0	2425 ± 163.0	5918 ± 214.0	67
NTS1	3525 ± 264.0	275.1 ± 95.00	396.3 ± 365.0	323.5 ± 141.0	994 ± 175.0	28
NTS2	7050 ± 752.0	525.9 ± 135.0	712.7 ± 425.0	970 ± 216.0	2209 ± 351.0	31
NTS3	8813 ± 224.0	887.1 ± 226.0	1107 ± 200.0	1205 ± 166.0	3199 ± 415.0	36
ER1	3525 ± 384.0	353.2 ± 18.00	413.0 ± 11.01	447.0 ± 16.00	1214 ± 133.0	34
ER2	7050 ± 652.0	743.9 ± 62.00	999.0 ± 213.0	1289 ± 59.00	3032 ± 146.0	43
ER3	8813 ± 452.0	994.7 ± 33.50	1474 ± 168.0	1631 ± 66.00	4100 ± 115.0	46



### 5.3.3 Changes in Cd species concentration after three cycles of P fertilizer application

The consecutive application of P fertilizers, in particular ER and SSP fertilizers, altered all Cd species over the duration of the experiment. Table 5-5 shows the concentration of each species of Cd (soluble, available and total) after each cycle of P fertilizer application. The NTS fertilizer was excluded from the comparison as all Cd species concentrations of NTS were below the detection limit of the ICP-MS instrument ( $0.04 \mu\text{g L}^{-1}$ ).

**Table 5-5 Concentration of Cd species extracted from soil samples after each cycle. Soluble Cd was extracted by water, available Cd extracted by  $\text{NH}_4\text{Cl}$  and total Cd by acid extraction. Fertilizer doses were ER1 and SSP1 =  $100 \text{ kg ha}^{-1}$ , ER2 and SSP2 =  $200 \text{ kg ha}^{-1}$ , ER3 and SSP3 =  $250 \text{ kg ha}^{-1}$ . The control treatment is zero fertilization. For each cycle.  $n = 12 \times 3$  replicates  $\pm 1$  standard error.**

Application cycle	Fertilizer Type	Added Cd ( $\mu\text{g kg}^{-1}$ )	Soluble Cd ( $\mu\text{g L}^{-1}$ )	Total Cd ( $\mu\text{g L}^{-1}$ )	Available Cd ( $\mu\text{g L}^{-1}$ )
First cycle	Control	0.47	BDL	$0.46 \pm 0.01$	BDL
	ER1	90.0	$0.33 \pm 0.02$	$1.56 \pm 0.14$	$0.80 \pm 0.21$
	ER2	180	$0.56 \pm 0.1$	$3.16 \pm 0.11$	$1.10 \pm 0.11$
	ER3	225	$0.65 \pm 0.11$	$5.46 \pm 0.21$	$1.62 \pm 0.13$
	SSP1	333	$0.63 \pm 0.01$	$3.36 \pm 0.24$	$0.88 \pm 0.12$
	SSP2	668	$1.02 \pm 0.01$	$3.99 \pm 0.02$	$1.13 \pm 0.14$
	SSP3	834	$3.13 \pm 0.03$	$9.18 \pm 0.11$	$3.67 \pm 0.20$
Second cycle	ER0	0.47	BDL	$0.30 \pm 0.13$	BDL
	ER1	90.0	$0.54 \pm 0.01$	$1.60 \pm 0.10$	$0.85 \pm 0.16$
	ER2	180	$0.59 \pm 0.10$	$3.20 \pm 0.15$	$1.13 \pm 0.14$
	ER3	225	$0.86 \pm 0.03$	$3.51 \pm 0.21$	$1.16 \pm 0.13$
	SSP1	333	$1.67 \pm 0.21$	$3.65 \pm 0.14$	$1.91 \pm 0.12$
	SSP2	668	$2.41 \pm 0.23$	$4.02 \pm 0.15$	$3.17 \pm 0.22$
	SSP3	834	$4.12 \pm 0.24$	$4.19 \pm 1.03$	$5.72 \pm 0.15$
Third Cycle	ER0	0.47	BDL	$0.22 \pm 0.10$	BDL
	ER1	90.0	$0.83 \pm 0.13$	$1.65 \pm 0.21$	$0.91 \pm 0.14$
	ER2	180	$1.04 \pm 0.21$	$3.22 \pm 0.23$	$1.34 \pm 0.13$
	ER3	225	$1.65 \pm 0.13$	$5.54 \pm 0.31$	$1.71 \pm 0.21$
	SSP1	333	$4.48 \pm 0.22$	$9.45 \pm 0.11$	$4.93 \pm 0.11$
	SSP2	668	$7.59 \pm 0.16$	$11.0 \pm 0.21$	$6.19 \pm 0.22$
	SSP3	834	$9.61 \pm 0.22$	$14.2 \pm 0.14$	$8.71 \pm 0.21$

Total Cd increased from  $0.46 \pm 0.01 \mu\text{g L}^{-1}$  to  $14.2 \pm 0.14 \mu\text{g L}^{-1}$ , soluble Cd increased from BDL to  $9.61 \pm 0.22 \mu\text{g L}^{-1}$  and available Cd increased from BDL to  $8.71 \pm 0.21 \mu\text{g L}^{-1}$ . Available Cd increased 100-fold after the application of the third fertilization cycle particularly

after the 250 kg ha<sup>-1</sup> dose of SSP fertilizer. The initial concentration of Cd species concentration varied among pots due to the variation in the P fertilizer doses and the concentration of Cd in each fertilizer composition. In addition, the concentrations of Cd in the soils after harvesting the tomato plants (Chapter 4) varied among the fertilizer treatments both in fertilizer type and dose. Table 5-6 displays the initial concentration of available Cd measured by MBL-DGT (Cd<sub>DGT</sub>) in the soil at the end of the greenhouse experiment (Chapter 4).

**Table 5-6 Mean initial concentration of Cd<sub>DGT</sub> in the soil over the 7-day deployment before the first cycle of fertilization. For each depth, n = 12 ×3 replicates ± 1 standard error. NTS samples were excluded.**

Soil depth (cm)	Fertilizer dose (kg ha <sup>-1</sup> )			
	0	100	200	250
<b>Available Cd from SSP Fertilizer (µg L<sup>-1</sup>)</b>				
15	BDL	0.490 ± 0.100	0.752 ± 0.001	1.033 ± 0.100
45	BDL	0.491 ± 0.100	0.753 ± 0.001	1.161 ± 0.000
60	BDL	0.421 ± 0.000	0.965 ± 0.000	1.082 ± 0.000
<b>Available Cd from ER Fertilizer (µg L<sup>-1</sup>)</b>				
15	BDL	0.112 ± 0.000	0.25 ± 0.00	0.281 ± 0.000
45	BDL	0.150 ± 0.000	0.25 ± 0.00	0.301 ± 0.000
60	BDL	0.153 ± 0.000	0.26 ± 0.00	0.331 ± 0.000

The mean concentration of Cd<sub>DGT</sub> increased by 21%, 86% and 106% after ER fertilizer was added to the soil in the first, second and third cycle, respectively. The same doses of SSP fertilizer caused an increase in the mean concentration of Cd<sub>DGT</sub> of 14%, 58% and 131% after the first, second and the third cycle, respectively. The second and third cycle of fertilization resulted in greater increases in the Cd<sub>DGT</sub> concentration in the soil from the SSP fertilizer (Table 5-7 and Table 5-8)

The Metsorb mixed binding layer exhibited high sensitivity to the source of labile Cd added to the soil with respect to fertilizer type and dose. It clearly demonstrates that SSP fertilizer significantly increased Cd<sub>DGT</sub> in the soil solution when compared with Cd<sub>DGT</sub> in soils treated with ER fertilizer at the same dose and in the same cycle of fertilization. For example, SSP increased from an initial Cd concentration of 1.033 ± 0.100 µg L<sup>-1</sup> (Table 5-6) to 4.661 ± 0.082 µg L<sup>-1</sup> at the end of the third cycle of fertilization (Table 5-9).

**Table 5-7 Concentration of Cd<sub>DGT</sub> after the application of SSP and ER fertilizer in the first cycle of fertilization. P fertilizer doses are 0, 100, 200 and 250 kg ha<sup>-1</sup>. For each depth, n = 12 ×3 replicates ± 1 standard error. NTS measurements were excluded.**

Soil depth (cm)	Fertilizer dose (kg ha <sup>-1</sup> )			
	0	100	200	250
<b>Available Cd from SSP Fertilizer (µg L<sup>-1</sup>)</b>				
15	BDL	0.592 ± 0.000	0.892 ± 0.000	1.232 ± 0.100
45	BDL	0.594 ± 0.000	0.901 ± 0.000	1.235 ± 0.000
60	BDL	0.641 ± 0.000	1.094 ± 0.000	1.282 ± 0.000
<b>Available Cd from ER Fertilizer (µg L<sup>-1</sup>)</b>				
15	BDL	0.152 ± 0.000	0.212 ± 0.000	0.312 ± 0.000
45	BDL	0.183 ± 0.001	0.283 ± 0.000	0.373 ± 0.000
60	BDL	0.184 ± 0.001	0.314 ± 0.000	0.374 ± 0.000

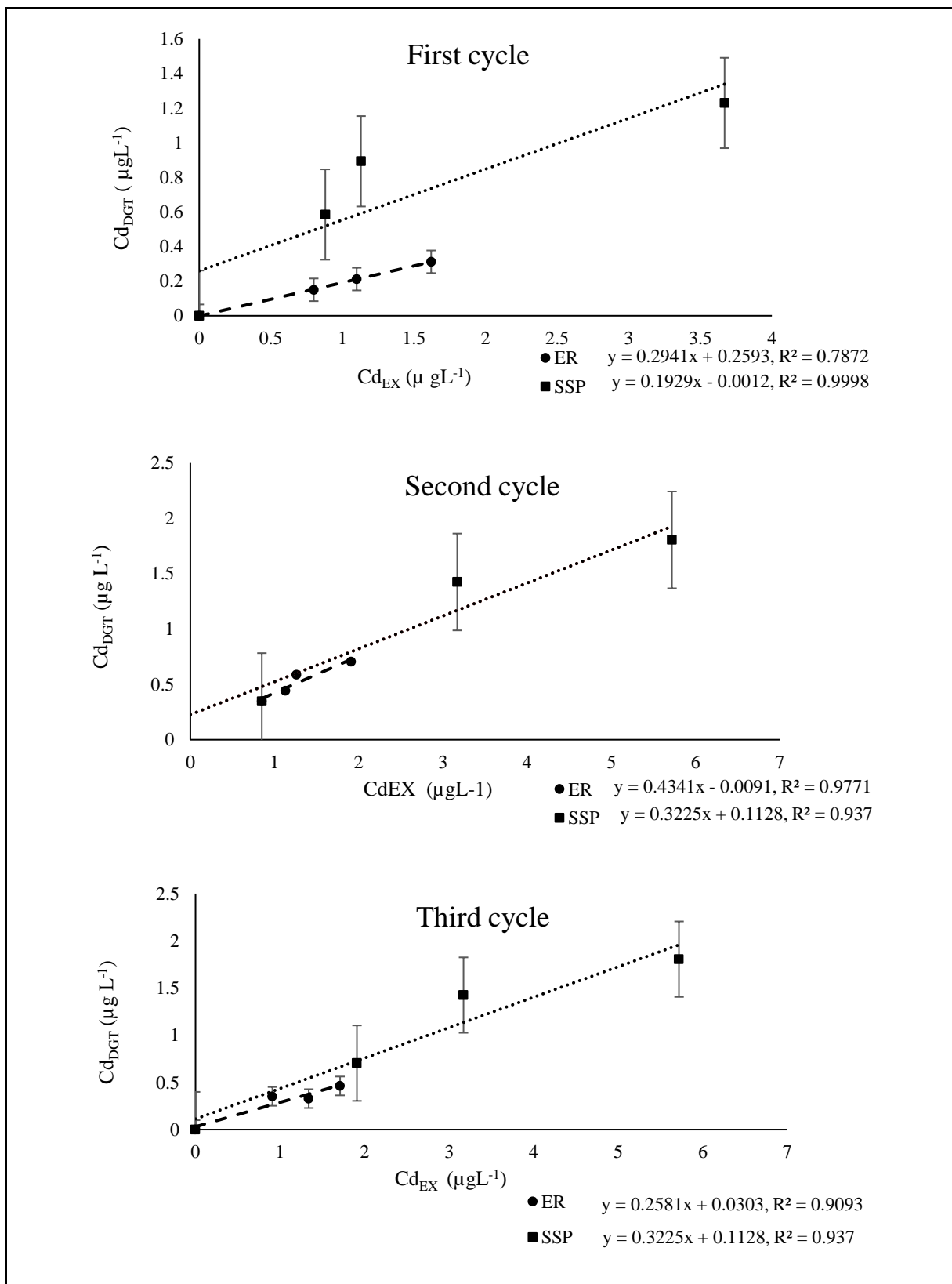
**Table 5-8 Concentration of Cd<sub>DGT</sub> after the application of SSP and ER fertilizer in the second cycle of fertilization. P fertilizer doses are 0, 100, 200 and 250 kg ha<sup>-1</sup>. For each depth, n = 12 ×3 replicates ± 1 standard error. NTS measurements were excluded.**

Soil depth (cm)	Fertilizer dose (kg ha <sup>-1</sup> )			
	0	100	200	250
<b>Available Cd from SSP Fertilizer (µg L<sup>-1</sup>)</b>				
15	BDL	0.702 ± 0.040	1.433 ± 0.040	1.813 ± 0.020
45	BDL	0.813 ± 0.020	1.385 ± 0.020	1.954 ± 0.060
60	BDL	0.833 ± 0.000	1.474 ± 0.030	2.143 ± 0.130
<b>Available Cd from ER Fertilizer (µg L<sup>-1</sup>)</b>				
15	BDL	0.355 ± 0.010	0.444 ± 0.010	0.592 ± 0.130
45	BDL	0.342 ± 0.010	0.502 ± 0.000	0.713 ± 0.040
60	BDL	0.355 ± 0.010	0.523 ± 0.000	0.764 ± 0.030

**Table 5-9 Concentration of Cd<sub>DGT</sub> after the application of SSP and ER fertilizer in the third cycle of fertilization. P fertilizer doses are 0, 100, 200 and 250 kg ha<sup>-1</sup>. For each depth, n = 12 ×3 replicates ± 1 standard error. NTS measurements were excluded.**

Soil depth (cm)	Fertilizer dose (kg ha <sup>-1</sup> )			
	0	100	200	250
<b>Available Cd from SSP Fertilizer (µg L<sup>-1</sup>)</b>				
15	BDL	1.577 ± 0.0	2.493 ± 0.0	4.447 ± 0.093
45	BDL	1.666 ± 0.1	2.543 ± 0.0	4.501 ± 0.104
60	BDL	1.781 ± 0.1	3.194 ± 0.0	4.661 ± 0.082
<b>Available Cd from ER Fertilizer (µg L<sup>-1</sup>)</b>				
15	BDL	0.469 ± 0.0	0.819 ± 0.0	1.072 ± 0.109
45	BDL	0.494 ± 0.0	0.888 ± 0.2	1.382 ± 0.180
60	BDL	0.495 ± 0.0	0.907 ± 0.0	1.555 ± 0.010

The relationship between the concentration of Cd<sub>DGT</sub> and the concentration of Cd extracted by the NH<sub>4</sub>Cl method (Cd<sub>EX</sub>) is presented in Figure 5-7. Both methods of Cd measurement established that labile Cd in the soil increased linearly with fertilizer dose and continue for three cycles at the same dose ( $R^2 > 0.90$ ). SSP fertilizer had a greater effect on available Cd concentration in soil due to the higher percentage of Cd in SSP fertilizer 25% (W/W) of SSP fertilizer (Figure 5-7).



**Figure 5-7 Relationship between  $Cd_{DGT}$  and  $Cd_{EX}$  in the soil after the application of P fertilizers SSP and ER of (0,00,200, and 250)kg a<sup>-1</sup> doses for three cycles. The concentration of  $Cd_{DGT}$  and  $Cd_{EX}$  in the (A) first cycle, (B) second cycle, and (C) third cycle of fertilizer application due to the accumulation of labile Cd on the MBL-DGT after the application of SSP and ER fertilizer. For each fertilizr n = 12 × 3 replicates ± 1 standard error. NTS measurements were excluded).**

Spearman's Rho coefficient confirmed that there were highly significant correlations between Cd<sub>DGT</sub> with fertilizer type, total Cd, soluble Cd, leachate water and repeating fertilization cycle. Both fertilizers had high correlation with Cd<sub>DGT</sub> however, SSP fertilizer had higher correlation with Cd<sub>DGT</sub> ( $r = 0.95$ ,  $P < 0.01$ ) than did ER fertilizer ( $r = 0.85$ ,  $P < 0.01$ ) (Table 5-10).

**Table 5-10 Spearman's Rho coefficients (r) for correlations between available Cd extracted from the soil by MBL-DGT and NH<sub>4</sub>Cl for different P fertilizer treatments (ER and SSP). <sup>1</sup>For each Cd species, n = 12 × 3 replicates ± 1 standard error in each cycle. (NTS samples were excluded).**

Fertilizer type	Cd <sub>DGT</sub>	Cd <sub>EX</sub>	ER	SSP	Soluble Cd	Total Cd	Leachate water	Number of cycle s
ER	0.85**	0.46	1.00	ns	0.51*	0.37	0.78*	0.86**
SSP	0.95**	0.42	ns	1.00	0.45	0.35	0.88**	0.87**
Soluble Cd	0.89**	0.60	0.51	0.45	1.00	0.25	0.73**	0.79*
Cd <sub>DGT</sub>	1.00	0.72*	0.85**	0.95**	0.89**	0.24	0.87**	0.80**
Cd <sub>EX</sub>	0.72**	1.00	0.46	0.42	0.60*	0.45	0.60*	0.80**
Total Cd	0.24	0.45	0.37	0.35	0.25	1.00	0.21	0.79*
Leachate water	0.87**	0.60*	0.78*	0.88**	0.73*	0.21	1.00	0.85**
Number of cycles	0.80**	0.80**	0.86**	0.87**	0.79*	0.79*	0.85**	1.00

\*\* . Correlation is significant at the 0.01 level (2-tailed).

\* . Correlation is significant at the 0.05 level (2-tailed).

A positive correlation was found between Cd<sub>DGT</sub> and Cd<sub>EX</sub> ( $r = 0.72$ ,  $P < 0.01$ ) due to the continuous application of the SSP and ER fertilizers which resulted in the release of available Cd from these fertilizers (Table 5-10). Total Cd had stronger positive correlation with Cd<sub>DGT</sub> ( $r = 0.74$ ,  $P > 0.05$ ) than with Cd<sub>EX</sub> ( $r = 0.25$ ,  $P < 0.05$ ). Moreover, soluble Cd had stronger correlation with Cd<sub>DGT</sub> ( $r = 0.80$ ,  $P < 0.05$ ) than with Cd<sub>EX</sub> ( $r = 0.60$ ,  $P < 0.05$ ). The results indicated the MBL-DGT better explained the changes in available Cd concentration in the soil after the application of P fertilizers than the chemical extraction method.

The MBL-DGT established a local concentration gradient at the soil and DGT interface, which facilitated the natural depletion of Cd in soil solution. This process is identical to the process of metal depletion in soil by plant roots uptake as described in the greenhouse experiment and correspond with Zarrouk et al. (2014). Therefore, the kinetically labile metal in soil solid phase plays an important role in plant uptake and is included in the MBL-DGT measurement. The chemical extraction method does not fully reflect the soil solid phase resupply processes and the re-adsorption of metals during the extraction process (Dai et al., 2017).

To determine the ability of the MBL-DGT to explain the capacity of the soil to resupply soil solution with available Cd, a comparison was made between soluble Cd (Table 5-5) and  $Cd_{DGT}$  extracted by MBL-DGT (Zhang et al., 1998). If the concentration of soluble Cd = concentration of  $Cd_{DGT}$ , then the concentration of Cd in soil solution is fully sustained by the soil. This means that the available Cd removed from the soil solution by the MBL-DGT device was rapidly resupplied from the soil solid phase or the concentration of available Cd in soil solution is effectively buffered to a constant value. If the concentration of  $Cd_{DGT} <$  the concentration of soluble Cd, the soil solution is partially sustained or unsustainable. That is, there is no resupply from the soil to the solution. Most of the Cd supply to the MBL-DGT device is solely by diffusion from the soil solution, which is progressively depleted over time. If  $Cd_{DGT}$  is less than 10% of soluble Cd in soil solution the flux can primarily be accounted for by simple diffusion. That means the soil solution is unsustainable and there is effectively no resupply to soil solution in this case (Zhong et al., 1995),

The concentrations of  $Cd_{DGT}$  in the third cycle in all treatments were less than the soluble Cd (Table 5-5, Table 5-9) indicating that available Cd resupply from the soil after MBL-DGT uptake did not occur. This result accurately reflects the soil properties and Cd status in the used soil. The  $Cd_{EX}$  concentrations in Table 5-5 were greater than or similar to soluble Cd which indicated that available Cd in soil solution is sustained or partially sustained by the soil solid phase which does not correspond with the soil chemical characteristics. In addition, the control and NTS treatments demonstrated that no resupply occurred during the experiment time, which proves that available Cd in the soil was unsustainable. The chemical extraction method does not fully reflect the soil solid phase resupply processes to soil solution and the re-adsorption of metal during the extraction process. Therefore, MBL-DGT is a more suitable and accurate method. The MBL-DGT includes contributions from the liquid and solid phases of soil as well as exchange dynamics between soil solid phase and soil solution (Dai et al., 2017).

The dynamic exchange of Cd from the soil solid phase to the liquid phase is an important factor that influences Cd uptake, and Cd concentrations measured by the MBL-DGT reflect these processes. The MBL-DGT is a promising technique for assessing Cd bioavailability because soil properties do not need to be further considered when using this method.

### 5.3.4 Cd in the leachate water

The results indicate that Cd moved from the soil to the leachate water after each application of P fertilizer (Table 5-11).

**Table 5-11 Mass of P loss in the leachate water after the application of the same dose of P fertilizer in three consecutive cycles. The fertilizers (SSP and ER) were applied at four doses (0, 100, 200 and 250 kg ha<sup>-1</sup>). For each cycle, n = 12 ×3 replicates ± 1 standard error. NTS samples were excluded.**

Fertilizer Type	Total Cd add in three cycles (µg)	Mass of Cd in the leachate water (µg)			Total Cd lost(µg)	% of Cd lost
		First cycle	Second cycle	Third cycle		
Control	BDL	BDL	BDL	BDL	BDL	BDL
SSP1	1001	131.8 ± 18.20	203.7 ± 2020	214.5 ± 0.321	549.9	55
SSP2	2003	254.7 ± 20.11	363.7 ± 10	525.7 ± 21.11	1144	57
SSP3	2503	380.21 ± 12.21	401.8 ± 10	684.3 ± 13.23	1466	60
ER1	271.2	21.18 ± 14.12	23.89 ± 06	77.37 ± 6.120	122.4	45
ER2	542.3	43.30 ± 10.24	85.38 ± 12	155.1 ± 10.123	283.7	52
ER3	677.9	62.30 ± 7.214	127.1 ± 10	206.4 ± 10.001	395.8	59

Total Cd added to the soil from the SSP and ER fertilizers during the three cycles was varied because SSP has 25% Cd and ER fertilizer has 10% Cd. The highest dose of SSP fertilizer caused 60% of the added Cd to move to the leachate water. Similarly, the highest concentration of ER fertilizer resulted in 59% of the added Cd to be removed to the leachate water. The high solubility of SSP fertilizer caused more Cd to be added to the soil solution. The physical properties of the soil, such as the coarse texture, high permeability, and high hydraulic conductivity, present some reasons for the Cd movement. In addition, chemical properties of the soil such as low CEC due to the low clay and organic matter content were

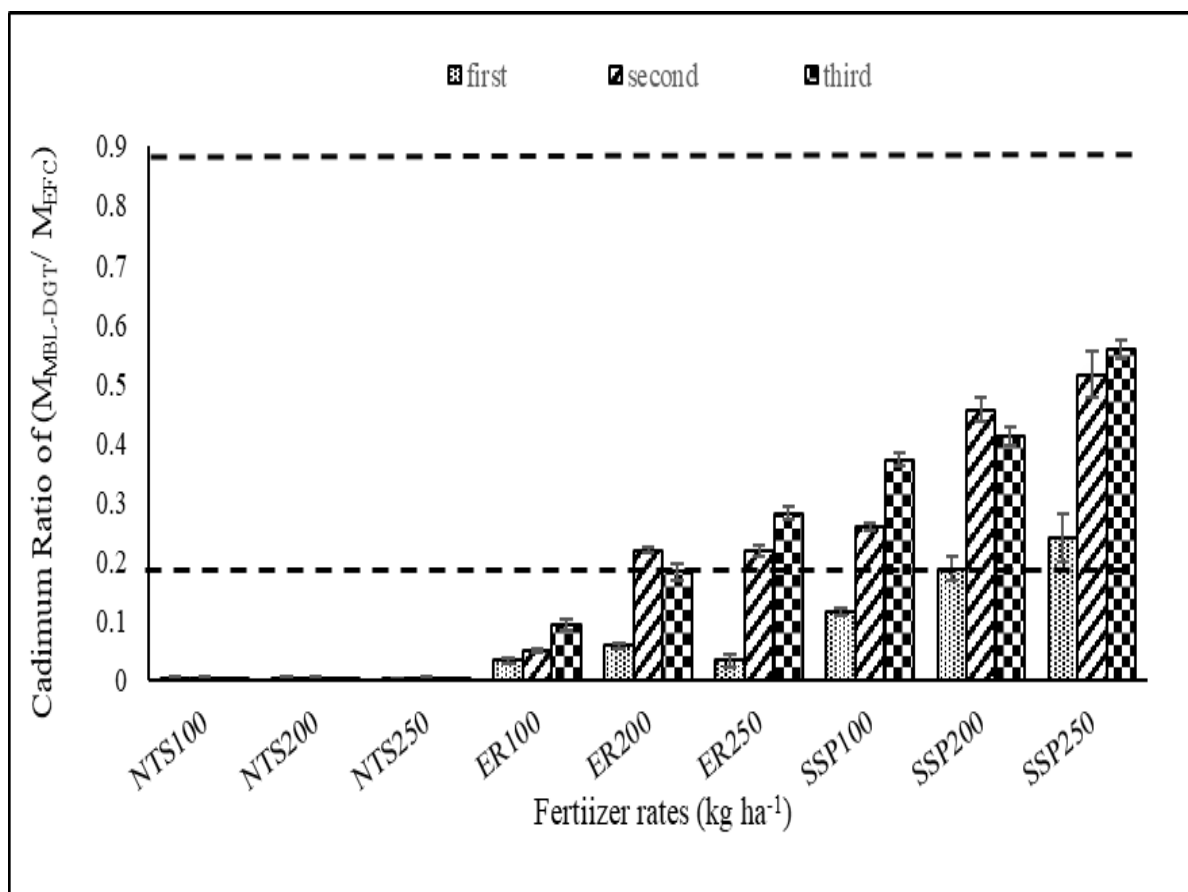


responsible for low retention of Cd in the soil. These results provide a cautionary note about the risk of Cd pollution from continuous and excessive use of P fertilizer, particularly in coarse-textured soils. The accumulation of Cd in the leachate water may lead to the deterioration of the quality of ground and surface waters as well as the soil itself. Furthermore, field studies are necessary regarding the use of the MBL-DGT in leachate water to monitor and measure Cd concentration and mass to protect aquatic environments.

### **5.3.5 Validation of $Cd_{DGT}$ measurements using the MBL-DGT in soil**

The principles of the DGT technique were applied to the new MBL-DGT. Similar to other DGTs, the MBL-DGT technique can be used for long term deployment of more than four days (Panther et al., 2014). Accumulation of analyte mass increases linearly according to the DGT equation  $C = M \Delta g / DtA$  (as described in Equation 2-1) until the accumulated mass exceeds the effective capacity of the absorbent (Zhang and Davison, 1995; Zhang et al., 1998). The effective capacity of the MBL-DGT was not studied in this experiment, however, Panther et al. (2014) reported that the effective capacity of the MBL-DGT for Cd was approximately 900 ng per disc in water. Figure 5-8 shows the ratio of available Cd mass accumulated on the MBL-DGT ( $M_{MBL-DGT}$ ) for different fertilizer treatments to the maximum effective capacity ( $M_{EFC}$ ) of the MBL-DGT identified by Panther et al. (2014).

The performance of the MBL-DGT was evaluated relative to the maximum effective capacity. If the ratio of available Cd mass accumulated on the mixed binding layer was above 0.9 the measurement was semi-quantitative, if the ratio between 0.1- 0.9 the measurement is quantitative but if the ratio was less than 0.1, the measurement then is non- quantitative. So, if the ratio was 10% -80%, less than the effective capacity, then available Cd measurements are quantitative and if the ratio was more than 90% of the effective capacity then the  $Cd_{DGT}$  measurements are semi-quantitative. (Figure 5.8).



**Figure 5-8** Ratio of available Cd mass accumulated on the MBL-DGT to the effective capacity of 900 ng when three fertilizers (SSP, NTS, and ER) are applied to the soil at 0, 100, 200 and 250 kg ha<sup>-1</sup> after the first, second and third cycles of P application. For each depth n = 12 × 3, replicates ± 1 standard error.

Results indicated that Cd mass uptake by the MBL-DGT increased with increasing fertilizer dose. Figure 5-8 shows that all NTS treatments were non-quantitative, but, the other treatments were quantitative as the ratios were between 0.1-0.6 and did not achieve a ratio of 0.9 because Cd is a trace element in the soil. These findings explain that MBL-DGT was capable of measuring available Cd in soil accumulated from the application of P fertilizers. Most of the Cd added to the sandy loam soil from the P fertilizers became readily available for plant uptake. This finding indicated that the MBL-DGT could be suitable for measuring Cd risk assessment in the soil. Further investigations need to be conducted on different soil types and different agricultural practices.

## 5.4 Conclusion

The aims of this experiment were to investigate (i) the accumulation of available Cd in soil and leachate water and (ii) the capacity limit of the MBL-DGT to absorb available P in sandy loam soil receiving continuous high doses of P fertilizer applications.

$Cd_{DGT}$  was relatively influenced by fertilizer type, fertilizer dose and regular application of P fertilizers. Repeated application of P fertilizer with high Cd content will increase the concentration of available Cd in the soil and the risk of moving available Cd to the leachate water. The results indicated that the MBL-DGT was able to measure the labile concentration of Cd in the soil and determine the changes of labile Cd due to the fertilizer dose, type, and consecutive application of P fertilizer.

The results also indicated that significant increases in available P concentration occurred after each cycle of fertilization. Available P uptake by MBL-DGT varied among fertilizer treatments due to the variable solubility products of individual fertilizers. The solubility of the ER and NTS fertilizers are low therefore, available P is released slowly from the fertilizer to replenish available P lost by leaching and MBL-DGT uptake to maintain the level of available P in the soil. The MBL-DGT uptake of available P depends on the adsorption capacity of the binding layer. To accurately measure available P, or any analyte, in soil solution using the MBL-DGT, the capacity of the adsorbent must not be exceeded during the deployment time. Application of repetitive high doses of SSP fertilizer to the soil exceeded the available P adsorption by the MBL-DGT over the effective capacity recorded by Panther et al. (2010) of 40,000 ng. Therefore, the treatment of SSP fertilizer at the 100, 200 and 250 kg ha<sup>-1</sup> dose after the third cycle was considered semi-quantitative. All other treatments of P fertilizers and all the repeated fertilization cycle s were between 0.2 -0.9 of the effective capacity and the MBL-DGT measurements were quantitative. Hence, these results indicated that MBL-DGT with the characteristics identified and used in this experiment had limited capacity for available P adsorption on the MBL layer. Higher P fertilizer inputs to agricultural soils will require different DGT diameters and MBL thicknesses along with longer deployment times. Available P in the leachate water increased with increasing fertilizer dose due to the coarse soil texture and the excessive use of P fertilizers over three consecutive applications.

A comparison between the MBL-DGT and the chemical extraction methods for available P and Cd was conducted. The results showed that the MBL-DGT measurements were highly correlated with P and Cd species concentrations in the soil. Unlike the chemical extraction

methods for measuring available P and Cd, which are mainly based on equilibrium concentrations related to the molarity of the extraction reagent, the MBL-DGT technique established a local concentration gradient at the soil and the DGT interface, which facilitated the natural depletion of available P and Cd in soil solution. This process is identical to the metal uptake by plant roots from soil (Zarrouk et al., 2014a).

## Chapter 6-. Conclusions and recommendations

### 6.1 A general summary of the results

This research project was the first to evaluate the Mixed Binding Layer DGT (MBL-DGT) and the Metsorb Chelex Double Binding layer (DBL-DGT) techniques for measuring important nutrients and contaminants in soil. The research included a series of experiments used to compare the accuracy of both DGT techniques against the traditional chemical extraction methods (Olsen method and  $\text{NH}_4\text{Cl}$ ) for measuring the availability and mobility of P and Cd in soil. Furthermore, a comparison between the measurement of the two methods (the DGT techniques and traditional methods) was used to assess plant uptake of both P and Cd in three different experiments:

#### 6.1.1 The flume experiments

The main aims of this experiment were to (i) use the MBL-DGT to simultaneously measure available P and Cd using one device in sandy loam soil treated with different doses of SSP fertilizer and (ii) determine the ability of the MBL-DGT to track the mobility of available P and Cd through the soil depth during deployment times longer than 4 days as reported by (Panther et al., 2014).

In summary, the main results are:

- $C_{\text{DGT}}$  for both available P and Cd was slightly higher at 45 cm depth than at 15 cm confirming that both available P and Cd moved down through the soil. Roughly 88% of the added Cd and 64% of added P were lost through the soil to the leachate water.
- The  $C_{\text{EX}}$  for both P and Cd was higher than  $C_{\text{DGT}}$ . However, the correlations between fertilizer dose and  $C_{\text{DGT}}$  were stronger than those with  $C_{\text{EX}}$ . Therefore, the MBL-DGT measurement was more closely related to the availability of P and Cd in soil than traditional extraction methods.
- MBL-DGT uptake increased over the 14 days of deployment.

In previous work the Metsorb Chelex Mixed Binding Layer DGT was applied successfully in synthetic freshwater, seawater (Panther et al., 2014) and natural water (Shiva et al., 2016), however, the performance of the MBL-DGT had never been investigated in soil.

The flume experiment results show that the MBL-DGT is applicable for simultaneous prediction of available P and Cd in soil. Measurements of available P and Cd compared well with results obtained previously using Chelex or Metsorb individually (Panther et al., 2010; Price et al., 2013; Panther et al., 2014; Shiva et al., 2016). However, there were some differences between MBL performance in soil compared to natural water, artificial freshwater and seawater, such as the ability of the MBL to act effectively in soil over 7-14 days (compared to 4 days in water) (Panther et al., 2014). In addition, the results of the flume experiment show that concentrations of available P and Cd did not deplete over the 14 days of deployment indicating that the adsorption capacity of the MBL did not reach the saturation under the flume experiment conditions.

### **6.1.2 The greenhouse experiments**

The major aim of this experiment was to investigate the validity of the Metsorb Chelex Double Binding Layer DGT (DBL-DGT) for measuring available P and Cd in soil and the relationship with the concentration of P and Cd in tomato vegetative parts. The Metsorb Chelex Double Binding Layer DGT was used for the first time in the soil in a greenhouse experiment.

The findings in relation to P are as follows:

- Extracted P using the Olsen method increased with increasing fertilizer dose. The highest concentration of available P was found in all SSP fertilizer treatments. A positive correlation was found between fertilizer dose and type with increasing available P concentration in the leachate water.
- The dry matter yield (DMY) increased dramatically with P fertilizer dose and varied between fertilizer types. The highest DMY was found in the highest SSP fertilizer treatments.
- The Metsorb binding layer of the DBL-DGT was capable of measuring the concentration of available P in the soil. The DBL-DGT for P measurements was evaluated relative to the performance of the Metsorb Binding Layer-DGT (MBL-DGT) as described by Bennett et al. (2010) and Panther et al. (2010).
- The concentration of  $P_{DGT}$  significantly increased with increasing P fertilizer dose, especially in SSP fertilizer treatments. The sensitivity of the Metsorb layer of the DBL-DGT was proven by the increasing  $P_{DGT}$  concentration with increasing fertilizer dose and deployment depth consistent with the Metsorb DGT action in water (Panther et al., 2010; Price et al., 2013).

- Significant correlations were found between  $P_{DGT}$  and soil depth. Consequently, more than 50% of the added P was lost in the leachate water.
- Linear and positive relationships were found between  $P_{DGT}$  and  $P_{EX}$  for each fertilizer type, dose, and depth.
- The available P concentration measured by the two methods showed a positive correlation with the concentration of P in plants ( $P_{Plant}$ ) but was more strongly correlated to  $P_{DGT}$ . This suggests that the DBL-DGT measured the identical P form required by the tomato plant. The correlation of soil independent factors (depth, fertilizer dose, and fertilizer type) was stronger with MBL-DGT measurements than the Olsen method.

Available Cd was below the detection limit in the original soil and total Cd was very low. The concentration of Cd species increased after the application of P fertilizers with the exception of pots which were treated with NTS fertilizer. Therefore, NTS treatments were excluded from the comparisons. The results of Cd showed the following:

- Increased doses of ER and SSP fertilizers caused significant increases in the concentration of  $Cd_{EX}$  in the soil and in leachate water.
- The DBL-DGT was able to detect the concentration of available Cd in the soil and describe the changes that occurred in the soil after the application of SSP and ER fertilizers.
- A significant correlation was found between  $Cd_{DGT}$  and SSP fertilizer dose. This finding confirmed that the Chelex Binding Layer of DBL-DGT was sensitive to the changes in available Cd concentration in soil pore water after the application of P fertilizers. The results were compatible with the Chelex-DGT results when it was deployed in seawater and freshwater (Price et al., 2013; Panther et al., 2014).
- $Cd_{DGT}$  significantly increased with soil depth therefore, the DBL-DGT method is able to track the movement of available Cd deep into soil and leachate water.
- There was a strong correlation between  $Cd_{DGT}$  and concentration of Cd in tomato plants. In contrast,  $Cd_{EX}$  concentration was poorly correlated with Cd in tomato plants. Therefore, the DBL-DGT acted like the plant root by removing Cd from the soil.
- The DBL-DGT technique includes contributions from the liquid and solid phases of soil as well as the exchange dynamics between the two phases which is an important factor influencing Cd uptake by plants.

### 6.1.3 The successive and continuous application of P fertilizer

The main aim of this experiment was to investigate the capacity of MBL-DGT for adsorbing available P and Cd in heavily fertilized sandy loam soil after consecutive application of P fertilizers over three cycles. It also aimed to investigate the accumulation of available P and Cd in the soil and leachate water. The results showed that:

- The successive application of SSP and ER fertilizers for three consecutive cycles increased the concentration of available Cd in the soil and leachate water. Therefore, the risk of transportation of available Cd and available P to the leachate water is highly expected especially with the highest dose of SSP fertilizer.
- Concentrations of available P dramatically increased by repeating the same doses for three cycles. SSP fertilizer caused the highest increase of available P among the treatments.
- The Metsorb Chelex mixed binding layer has the highest P accumulated mass of 42,000 ng P per disc in 7 days of deployment. This was slightly higher than the effective capacity of the Metsorb binding layer of 37,000-40,000 ng P per disc recorded by Panther et al. (2010, 2011). Also, it was much higher than the adsorption capacity of the mixed binding layer of 2000 ng P per disc recorded by Panther et al. (2014). But, non-quantitative uptake was noticed in the third cycle of SSP fertilizer application indicating that the effective capacity of the binding gel was exceeded. Therefore, in order to apply the MBL-DGT in heavily fertilized soil, a new modification must be considered, such as increasing the amount of Metsorb in the binding layer and increasing the diameter of the DGT.
- Most of the  $P_{DGT}$  measurements of ER and NTS treatment were quantitative. The successive application of SSP and ER fertilizers led to increased  $P_{DGT}$  in the soil. There was also a radical increase in the concentration of available Cd in leachate water.

## 6.2 Limitations of the study

In heavily or newly fertilized soils the capacity of the current MBL-DGT will be a limiting factor for accurate P measurements. As it was only used on sandy loamy soil, different results may be obtained when used in different soils or under different field conditions.



### 6.3 The implications of the results

The results of this study have several implications for research and field applications. These have been discussed in the relevant chapters and are summarized below:

- MBL-DGTs enable *in situ* simultaneous measurements of oxyanions and cations in soil.
- MBL-DGTs can be used to monitor the accumulation of P and heavy metals in the soil after each fertilizer application.
- The MBL-DGT method is more accurate and less complex than chemical extraction methods.
- MBL-DGTs are suitable for use in soil and for risk assessments of Cd in soil.
- The accurate MBL-DGT measurements of the level of available P in the soil will reduce the excessive use of P fertilizer and reduce P source rock depletion.

### 6.4 Recommendations for future work

Based on the results of this study several recommendations for future work can be made:

- Further studies should compare the performance of MBL-DGT and the DBL-DGT with the Metsorb-DGT and Chelex-DGT individually in the soil to confirm the validation of the MBL-DGT of Metsorb and Chelex in soil.
- The diameter and thickness of the MBL layer should be increased to maximize the capacity for available P uptake in heavily fertilized soils.
- Further studies should test the suitability and effectiveness of the MBL-DGT in different soil types such as clay, loamy silt, or loamy sand soils as well as in leachate water.
- Further research should attempt to improve the MBL-DGT to enable simultaneous extraction of multiple analytes from soil, e.g. nitrogen (N) or potassium (K) as a macronutrient and zinc (Zn), manganese (Mn) or lead (Pb) as a trace element associated with fertilizers, to reduce time, effort, monitor contamination, and expense.

In addition, this study has demonstrated that the use of low soluble P fertilizer, such as NTS, is better for healthier agricultural soil because it reduces the loss of available P in the leachate water, provides an adequate amount of available P for plants, and has no Cd added.

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## Appendix 1 – Properties of P fertilizers used in the experiments

<b>Fertilizer properties</b>	<b>SSP</b>	<b>ER</b>	<b>NTS</b>
Total P %	13	10	8.8
P water soluble %	8.08	< 1.0	Not soluble
P critical insoluble %	0.28	7	Not soluble
S%	11	1	0.45
Carbon %	-	2.8	0.16
Ca %	19.0	34.3	20
Mg %	-	0.1	0.66%
Cl %	-		0.026
Cd mg Kg <sup>-1</sup>	25	8.8	0

## Appendix 2 – Additional information for Chapter 3

### A: Available phosphorus and Cd measured by the MBL-DGT in flume soil

The examination of the concentration of phosphate in flume soil involved three doses of fertilizer (fertilizer 0.01, 0.1 and 0.5 mg P L<sup>-1</sup>), three replicates two depths (depth 15 and 45 cm) and three deployment times of 4 days, 7 days and 14 days. The three deployments time were entered into ANOVA (SPSS) as a repeated measurement dependent variable (DV), and level of fertilizer and depths were entered as between-groups independent variables (IVs). n= 18 × 3 replicates.

Source	df	P		Cd	
		F	Sig.	F	Sig.
<b>Fertilizer dose</b>	2	382.2	0.001	38.42	0.000
<b>Depth</b>	1	0.113	0.007	0.922	0.036
<b>Fertilizer * Depth</b>	2	0.936	0.042	0.064	0.028
<b>Deployment time (days)</b>	2	114.9	0.001	13.25	0.003
<b>Deployment time * Fertilizer</b>	4	32.03	0.000	0.670	0.032
<b>Deployment time * Depth</b>	2	3.796	0.027	0.013	0.005
<b>Occasion * Fertilizer * Depth</b>	4	1.333	0.030	0.142	0.001
<b>Error</b>	12	-	-		

### B: Available phosphate and available Cd concentration in leachate water

The 14 test occasions were entered into ANOVA (SPSS) as a repeated measurement dependent variable (DV), and the level of fertilizer was entered as a between-groups independent variable (IV). n = 42 × 3 replicates.

Source	df	P		Cd	
		F	Sig.	F	Sig.
<b>Fertilizer dose</b>	2	153	0.0001	912	0.0001
<b>Time of sampling</b>	13	199	0.0001	980	0.0001
<b>Occasions * Fertilizer</b>	26	128	0.0001	650	0.0001
<b>Error</b>	6				
<b>Total</b>					



### Appendix 3 – Additional information for Chapter 4

**A: Between-group effects for fertilizer, dose, depth, and their two-way and three-way interaction on  $P_{DGT}$  and  $Cd_{DGT}$  concentrations in the soil. Each depth ( $n = 12 \times 3$  replicates) for P and Cd measurements.**

Source	df	Available P		Available Cd	
		F	Sig.	F	Sig.
Fertilizer	2	170	0.0001	8.18	0.0001
Dose	3	1.03	0.0001	1.61	0.000
Depth	2	102	0.0001	14.9	0.0001
Fertilizer * Dose	6	21.7	0.0001	1.27	0.0001
Fertilizer * Depth	4	6.26	0.0001	9.01	0.0001
Dose * Depth	6	14.9	0.0001	11.7	0.0001
Fertilizer * Dose * depth	12	0.970	0.487	12.2	0.0001
Error	72				
Total	107				

**B: Repeated measures ANOVA (SPSS) for P and Cd in the leachate water as a dependent variable (DV), with the type of fertilizer and dosage of fertilizer entered as between-groups independent variables (IVs). n = 120 × 3 replicates For P and Cd measurements.**

Source	df	Available P		Available Cd	
		F	Sig.	F	Sig.
<b>Fertilizer type</b>	2	991	0.0001	23.82	0.0001
<b>Dose</b>	3	137	0.0001	7.582	0.0001
<b>Fertilizer* Dose</b>	6	237.	0.0001	4.132	0.0001
<b>Error</b>	24				
Time and fertilizer dose as repeated measurements					
<b>Time</b>	9	975.	0.0001	616	0.0001
<b>Time * Fertilizer</b>	18	12.9.	0.0001	332	0.0001
<b>Time × dose</b>	27	176		113	0.0001
<b>Time ×fertilise× dose</b>	54	7.27		70.2	0.0001
<b>Error</b>	216				

**C: ANOVA (SPSS) concentration of P and Cd in the plant after the application of P fertilizers. The effect of P fertilizer type and fertilizer dose on tomato dry matter yield (DMY). n = 12 × 3 replicates.**

Source	df	P in plant		Cd in plant	
		F	Sig.	F	Sig.
<b>Fertilizer type</b>	2	136.1	0.0001	21.54	0.0001
<b>Dosage</b>	3	493.5	0.0001	5.653	0.0001
<b>Fertilizer type * dosage</b>	6	16.31	0.0001	8.241	0.0001
<b>Total dry mater</b>	35	12.54	0.0001	7.452	0.0001
<b>Error</b>	24				

**D: ANOVA (SPSS) Mass of P and Cd in the plant after the application of P fertilizer. The effect of P fertilizer type and dose on tomato dry matter yield (DMY). n = 12 × 3 replicates.**

Source	df	P in plant		Cd in plant	
		F	Sig.	F	Sig.
Fertilizer type	2	24.12	0.0001	20.17	0.0001
Dosage	3	548.2	0.0001	3.245	0.0001
Fertilizer type * dosage	6	32.51	0.0001	3.354	0.0001
Error	24				
Total Dry mater	35	21.54	0.0001	5.67	0.0001

## Appendix 4 – Concentration of available P in leachate water collected every 24 h

Each set of collections represents 12 days. The total of collection time was 120 days after the application of P fertilizers (ER, NTS, and SSP) at four doses (0, 100, 200 and 250 Kg P ha<sup>-1</sup>). The results of available P are the mean of three triplicates. n = 90 × 3 replicates. ± 1 standard error= 0.01-0.03

No. of collection set	Water volume (L)	P in leachate water (mg L <sup>-1</sup> )											
		ER <sub>0</sub>	ER <sub>1</sub>	ER <sub>2</sub>	ER <sub>3</sub>	NTS <sub>0</sub>	NTS <sub>1</sub>	NTS <sub>2</sub>	NTS <sub>3</sub>	SSP <sub>0</sub>	SSP <sub>1</sub>	SSP <sub>2</sub>	SSP <sub>3</sub>
1	36	0.04	4.109	8.889	11.51	0.005	3.50	5.72	8.36	0.004	5.86	10.38	15.30
2	34	0.02	2.583	6.479	8.302	0.003	1.91	4.28	6.37	0.002	3.55	7.40	10.17
3	34	BDL	0.843	4.156	6.986	BDL	0.55	3.35	5.16	0.00	0.99	2.72	8.22
4	34	BDL	0.681	2.288	4.710	BDL	0.38	2.84	4.92	0.00	0.68	3.31	4.84
5	34	BDL	0.260	0.891	1.267	BDL	0.12	1.27	1.65	0.00	0.35	3.25	0.64
6	34	BDL	0.093	0.231	0.542	BDL	0.05	0.88	0.85	0.00	0.11	1.01	0.43
7	34	BDL	0.015	0.154	0.111	BDL	0.03	0.18	0.58	0.00	0.06	1.12	0.26
8	34	BDL	0.008	0.021	0.053	BDL	0.02	0.08	0.25	0.00	0.00	0.01	0.08
9	34	BDL	0.001	0.014	0.021	BDL	0.00	0.01	0.11	0.00	0.03	0.26	0.04
10	34	BDL	0.001	0.000	0.012	BDL	0.00	0.00	0.01	0.00	0.01	0.02	0.03
Total	342	0.06	8.59	23.12	33.51	BDL	6.57	18.62	28.26	0.007	11.64	29.50	40.01
Total mass (mg)		2.12	300	698	1033	0.27	BDL	518	772	0.24	302	820	1111

## Appendix 5 – Concentration of available Cd in leachate water collected every 24 h

Each set of collections represents 12 days. The total collection time was 120 days after the application of P fertilizers (ER, NTS, and SSP) at four doses (0, 100, 200 and 250 kg P ha<sup>-1</sup>). The results of available Cd are the mean of three triplicates. n = 90 × 3 replicates. ± 1 standard error= 0.01-0.02.

Order of collection	Water volume (L)	Cd in the leachate water (µg L <sup>-1</sup> )											
		ER0	ER1	ER2	ER3	NTS0	NTS1	NTS2	NTS3	SSP0	SSP1	SS2	SSP3
1	36	BDL	0.26	0.67	0.86	BDL	BDL	BDL	BDL	0.021	1.2	2.8	3.1
2	34	BDL	0.14	0.62	0.74	BDL	BDL	BDL	BDL	0.015	1.11	2.4	2.5
3	34	BDL	0.121	0.41	0.52	BDL	BDL	BDL	BDL	0.002	1.01	1.4	1.2
4	34	BDL	0.11	0.21	0.35	BDL	BDL	BDL	BDL	0.001	0.32	0.7	1.3
5	34	BDL	0.1	0.19	0.2	BDL	BDL	BDL	BDL	0.001	0.2	0.5	1
6	34	BDL	0.06	0.17	0.18	BDL	BDL	BDL	BDL	0.001	0.12	0.3	0.9
7	34	BDL	0.05	0.12	0.16	BDL	BDL	BDL	BDL		0.1	0.2	0.8
8	34	BDL	0.05	0.08	0.14	BDL	BDL	BDL	BDL	0	0.01	0.2	0.620
9	34	BDL	0.009	0.07	0.12	BDL	BDL	BDL	BDL	0	0.008	0.1	0.41
10	34	BDL	0.008	0.06	0.11	BDL	BDL	BDL	BDL	0	0.005	0.05	0.1
Total		BDL	0.908	2.6	3.38	BDL	BDL	BDL	BDL	0.042	4.083	8.65	11.93
Total Mass (µg)	342	BDL	31	90	117	BDL	BDL	BDL	BDL	1.6	141	299	412

## Appendix 6 – Additional information for Chapter 5

**A: Mean  $P_{DGT}$  concentration after the first cycle (application) of three different P fertilizers (SSP, NTS, and ER) to the soil at four different doses (0, 100, 200 and 250 kg ha<sup>-1</sup>) in three depths (15, 45 and 60 cm. Each depth n= 12 × 3, ± 1 standard error.**

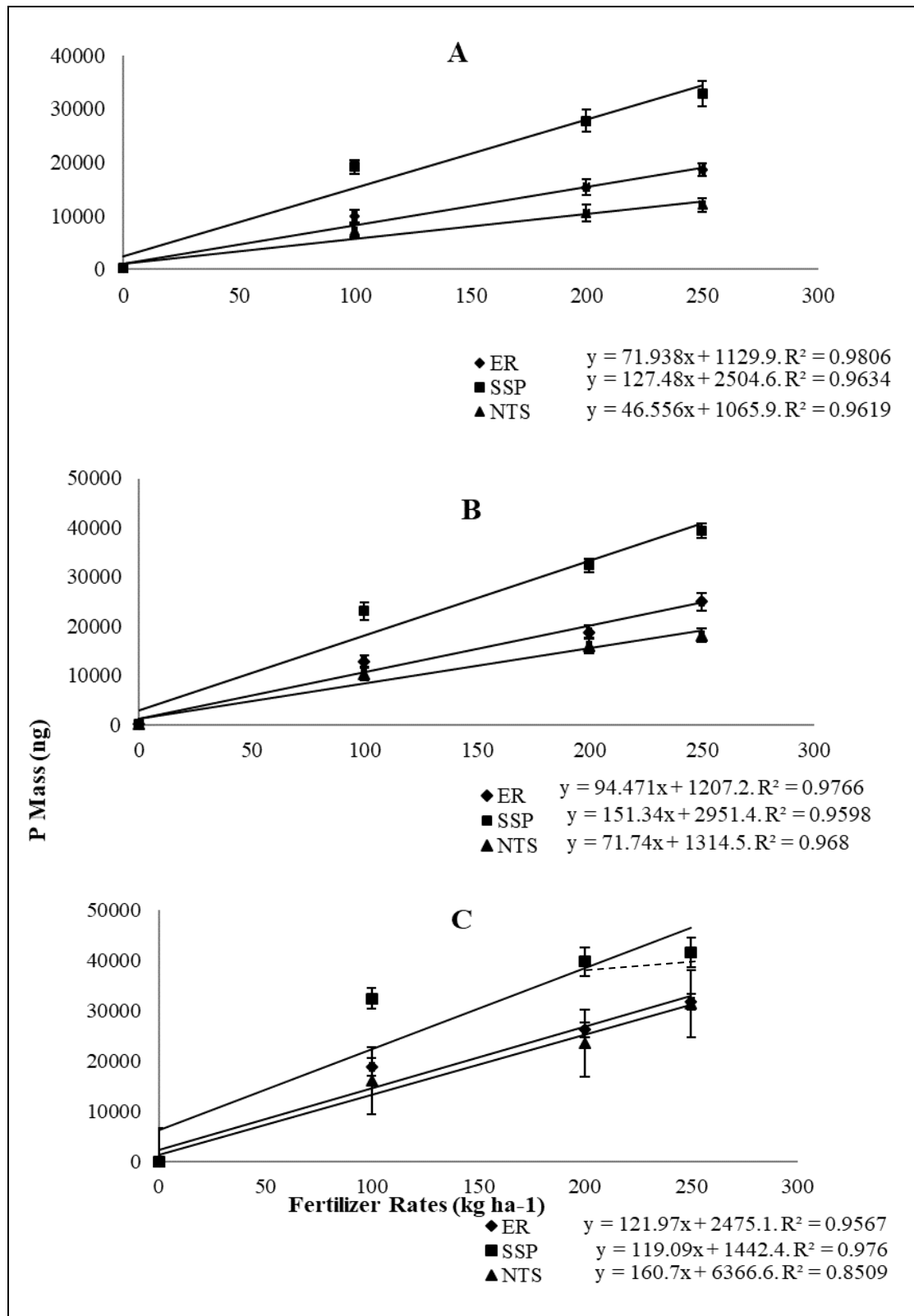
Soil depth cm	Fertilizer dose (kg ha <sup>-1</sup> )			
	0	100	200	250
<b>Available P from SSP Fertilizer (µg L<sup>-1</sup>)</b>				
15	0.840 ± 0.1	154.6 ± 0.9	225.0 ± 0.1	253.8 ± 5.5
45	0.900 ± 0.0	158.3 ± 1.2	228.4 ± 0.5	258.6 ± 0.7
60	0.860 ± 0.0	160.7 ± 0.4	230.3 ± 2.2	274.9 ± 2.7
<b>Available P from NTS Fertilizer (µg L<sup>-1</sup>)</b>				
15	0.990 ± 0.1	55.26 ± 2.1	83.17 ± 0.9	90.37 ± 0.7
45	0.900 ± 0.0	56.91 ± 1.7	85.22 ± 0.4	91.78 ± 0.6
60	0.950 ± 0.1	62.46 ± 0.2	90.07 ± 0.4	114.6 ± 1.9
<b>Available P from ER Fertilizer (µg L<sup>-1</sup>)</b>				
15	0.850 ± 0.0	69.66 ± 0.8	116.2 ± 0.9	150.3 ± 1.4
45	0.880 ± 0.1	76.03 ± 2.5	121.9 ± 4.0	153.5 ± 0.6
60	0.960 ± 0.0	92.28 ± 0.4	134.8 ± 11	156.0 ± 1.8

**B: Mean  $P_{DGT}$  concentration after the second cycle (application) of three different P fertilizers (SSP, NTS and ER) to soil at four different doses (0, 100, 200 and 250 kg ha<sup>-1</sup>) in three depths (15, 45 and 60 cm). Each depth n= 12 × 3, ± 1 standard error.**

Soil depth cm	Fertilizer dose (kg ha <sup>-1</sup> )			
	0	100	200	250
<b>Available P from SSP Fertilizer (µg L<sup>-1</sup>)</b>				
<b>15</b>	0.341 ± 0.3	182.3 ± 0.6	256.5 ± 1.4	272.8 ± 1.4
<b>45</b>	0.260 ± 0.01	192.8 ± 12	263.7 ± 5.9	297.9 ± 1.0
<b>60</b>	0.300 ± 0.0	195.8 ± 9.9	275.6 ± 3.7	280.9 ± 0.8
<b>Available P from NTS Fertilizer (µg L<sup>-1</sup>)</b>				
<b>15</b>	0.315 ± 0.0	80.95 ± 4.2	123.3 ± 0.3	131.6 ± 0.6
<b>45</b>	0.274 ± 0.0	86.57 ± 1.5	128.4 ± 0.7	153.9 ± 9.1
<b>60</b>	0.306 ± 0.0	87.50 ± 4.1	144.0 ± 0.4	163.4 ± 0.7
<b>Available P from ER Fertilizer (µg L<sup>-1</sup>)</b>				
<b>15</b>	0.351 ± 0.0	100.4 ± 5.5	145.3 ± 7.9	201.2 ± 4.5
<b>45</b>	0.235 ± 0.0	103.0 ± 1.4	153.8 ± 2.4	203.9 ± 7.9
<b>60</b>	0.302 ± 0.0	105.9 ± 1.3	158.5 ± 1.1	212.0 ± 2.9

**C: Mean  $P_{DGT}$  concentration ( $\pm 1$  SE,  $n = 3$ ) after the third cycle (application) of three different P fertilizers (SSP, NTS and ER) to soil at four different doses (0, 100, 200 and 250 kg ha<sup>-1</sup>) in three depths (15, 45 and 60 cm). Each depth  $n = 12 \times 3 \pm 1$  standard error.**

Soil depth cm	Fertilizer dose (kg ha <sup>-1</sup> )			
	0	100	200	250
<b>Available P from SSP Fertilizer (<math>\mu\text{g L}^{-1}</math>)</b>				
15	0.060 $\pm$ 0.0	268 $\pm$ 0.82	323 $\pm$ 5.81	328 $\pm$ 4.31
45	0.072 $\pm$ 0.0	263 $\pm$ 0.51	334 $\pm$ 111	330 $\pm$ 2.92
60	0.072 $\pm$ 0.0	270 $\pm$ 2.50	320 $\pm$ 8.32	334 $\pm$ 0.41
<b>Available P from NTS Fertilizer (<math>\mu\text{g L}^{-1}</math>)</b>				
15	0.061 $\pm$ 0.0	127 $\pm$ 1.41	180 $\pm$ 7.91	225 $\pm$ 1.23
45	0.070 $\pm$ 0.0	130 $\pm$ 1.91	199 $\pm$ 0.22	266 $\pm$ 0.11
60	0.065 $\pm$ 0.0	141 $\pm$ 142	204 $\pm$ 0.21	286 $\pm$ 18.2
<b>Available P from ER Fertilizer (<math>\mu\text{g L}^{-1}</math>)</b>				
15	0.062 $\pm$ 0.0	147 $\pm$ 0.71	198 $\pm$ 0.61	248 $\pm$ 0.52
45	0.072 $\pm$ 0.0	152 $\pm$ 2.11	217 $\pm$ 3.21	263 $\pm$ 11.2
60	0.074 $\pm$ 0.0	153 $\pm$ 1.21	224 $\pm$ 1.10	274.8 $\pm$ 2.21



**D:** Mass of available P accumulated on the MBL-DGT after (A) the first cycle, (B) second cycle and (C) the third cycle of fertilization of three different P fertilizer (SSP, NTS and ER) to soil at four different doses (0, 100, 200 and 250 kg ha<sup>-1</sup>). Each depth n= 12 × 3, ± 1 standard error for each cycle.