Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Department of Soil and Environment

Prediction of plant available copper, zinc and phosphorus in arable soils

– Comparison of diffusive gradients in thin film (DGT) technique with soil extraction methods

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Abstract

Nutrient deficiencies in crop production are today a worldwide problem. To maximize fertilizer efficiency and crop yields it is important to be able to assess the accurate amount of available nutrients in the soil (Mason et al., 2005). There are different methods to assess plant available nutrients in the soil and asses the risk of nutrient deficiency. Soil extraction analyses like Aqua regia (HNO_3 + HCl), HNO_3, EDTA, DTPA, $CaCl_2$ and P-AL are often used in purpose to assess available nutrients (Tandy et al., 2011). Copper, zinc and phosphorus are all essential nutrients which the plant requires. Diffusive gradients in thin films (DGT) is a fairly new method, a gel technique which accumulates metals and phosphates in soil (Zhang, 2003). This study was carried out in the middle to Southern part of Sweden, fourteen different soils from agricultural fields were chosen with cultivated wheat (*Triticum aestivum*). All the laboratory work was done at Swedish university of agriculture sciences (SLU) in Uppsala. The objective with this study was to investigate and compare the DGT technique with three conventional extraction methods: HNO_3, $CaCl_2$ and P-AL. The concentration of copper (Cu), phosphorus (P) and zinc (Zn) was measured by all methods and was then compared with the plant Cu, P and Zn concentration, to see which method that correlated best with the plant uptake. All methods predicted Cu concentration significantly but DGT technique was the most accurate method (R^2=0.64). Extracted Zn and P were not significantly correlated to the Zn or P concentration in the plant, or of DGT or any other extraction method. Copper and phosphorus concentration measured by the DGT technique showed significant correlation between the extracted Cu and P by P-AL, HNO_3 and $CaCl_2$. Zinc measured by DGT did neither prove significant correlations to the Zn plant concentration or to extracted Zn concentrations by HNO_3 and $CaCl_2$.

It was concluded that DGT was found to be the most accurate method for predicting plant available Cu but not for P or Zn. Further research has to be done before DGT can become one of the conventional trustworthy methods.

Keywords: Diffusive gradients in thin film technique (DGT), Cu, Zn, P, HNO_3, $CaCl_2$, P-AL, nutrient availability.

Sammanfattning
Näringsbrist i lantbruksgrödor är idag ett problem över hela världen. För att undvika näringsbrist och maximera gödslingseffekten och skördepotentialen är det därför viktigt att kunna bedöma de växttillgängliga näringsämnena i marken (Mason et al., 2005). Det finns idag en rad olika metoder att använda sig utav för att bedöma mängden växttillgängliga näringsämnen i marken och därmed bedöma risken för näringsbrist. Några utav de vanligaste extraktionsmetoderna för att extrahera växtnäringsämnen ifrån jorden är till exempel Aqua regia (HNO_3 + HCl), HNO_3, EDTA, DTPA, $CaCl_2$ och PAL (Tandy et al., 2011). Koppar (Cu), fosfor (P) och zink (Zn) är alla tre essentiella näringsämnen för lantbruksgrödor och det är därför intressant att bedöma dess växttillgänglighet i jorden. En ny metod som är uppbyggd av en så kallad gelteknik är *diffusive gradients in thin films* (DGT). DGT är en teknik som ackumulerar metaller och fosfater i jorden via diffusion (Zhang, 2003). Den här studien var utförd ifrån södra Skåne till mellersta Uppsala i Sverige. Fjorton olika fält där höstvete odlades (*Triticum aestivum*) valdes ut. På varje fält togs fyra stycken jordprover samt växtprover. Alla laborationer utfördes på Sveriges Lantbruksuniversitet (SLU) i Uppsala. Syftet med studien var att undersöka och jämföra geltekniken DGT med tre konventionella extraktionsmetoder (HNO_3, $CaCl_2$ och P-AL) för att se vilken metod som bäst korrelerade med upptaget i grödan. Av de olika metoderna visade DGT mest signifikant korrelation mellan upptag av gröda och tillgänglig Cu i jord (R^2=0.64). Plantans upptag av P och Zn korrelerade inte signifikant med någon utav extraktionsmetoderna. Slutsatsen i denna studie var att DGT-metoden visade sig vara den mest exakta metoden för att bedöma växttillgänglig koppar men inte för fosfor (P) eller zink (Zn). Ytterligare studier med DGT-metoden behöver göras innan DGT-metoden kan räknas som en stabil och konventionell metod.

Populärvetenskaplig sammanfattning

Näringsbrist i grödor som vete, havre och korn är idag ett problem i hela världen som orsakar minskad skörd. Några essentiella näringsämnen för grödan är tillexempel kväve, kalium, fosfor, koppar och zink. Utan dessa näringsämnen kan inte grödan tillväxa. För att veta hur mycket näringsämnen grödorna tar upp från jorden, finns det olika extraktionsmetoder att använda, till exempel HNO_3, EDTA, DTPA, $CaCl_2$ och P-AL. Alla metoder består av att en extraktionslösning som skakas tillsammans med sönderdelad jord. Med hjälp av dess metoder kan mängden växttillgängliga näringsämnen sedan bedömas. En ytterligare metod är den så kallade *Diffusive gradi-ents in thin films* (DGT) som är en ganska ny metod. DGT är så kallad gel-teknik som ackumulerar metaller och fosfater från jorden som sedan rör sig via en diffusionsgradient igenom en gel på en DGT-platta som är omgiven av plast. DGT är alltså ingen extraktionsmetod.

Denna studie utfördes på Sveriges lantbruksuniversitet, Uppsala. Datain-samlingen skedde från södra delen i Sverige upp till Uppsalatrakten och studien undersökte hur mycket lättillgänglig fosfor, koppar och zink som grödan höstvete kan ta upp från jorden. Syftet med studien var att ta reda på om den nya gelmetoden DGT gav ett exaktare näringsupptag av grödan jämfört med extraktionsmetoderna: HNO_3, $CaCl_2$ and P-AL. Koncentration-en av fosfor, koppar och zink i grödan jämfördes med koncentrationen av näringsämnena i respektive jordprov. På detta sätt kunde det jämföras vilken metod som korrelerade bäst med hur mycket fosfor, koppar och zink grödan faktiskt hade tagit upp ute i fält. Därefter jämfördes alla extraktionsme-toderna med den nyare metoden DGT för att se om DGT extraherade lik-nande mängd näringsämnen eller skiljde sig åt jämfört med de traditionella extraktionsmetoderna. Det visade sig att resultatet skiljde sig både emellan de olika näringsämnena samt metoderna. De proven som var gjorda för att se hur mycket koppar som fanns i jorden och som grödan tagit upp var alla signifikanta, dock så var DGT-metoden den mest exakta metoden (R^2=0.64), för att visa växttillgänglig koppar. DGT verkar alltså vara den bästa metoden för att förutsäga hur mycket lättillgängligt koppar som grödan tar upp ifrån Jorden. Tyvärr visade varken proven med fosfor och zink signifikanta resultat, varken för HNO_3, $CaCl_2$, P-AL eller DGT-metoden. Utav de tre valda näringsämnena verkar zink vara det ämne som är svårast att förutse växttillgänglig mängd av, det gav inga signifikanta korrelationer för någon metod. En utav felkällorna i mätningarna är att det är svårt att jämföra så olika metoder som P-AL och $CaCl_2$, då de är olika starka och ger lätt olika utslag. Slutsatsen I denna studie är att DGT-metoden var den bästa metod för att förutsäga växttillgängligt koppar men inte fosfor och zink. Ytterligare studier krävs innan DGT-metoden kommer att bli en utav de konventionella metoderna som P-AL och HNO_3.

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Abbreviations

Aqua Regia – A mix of HCl and HNO_3, a strong extraction method

$CaCl_2$- Calcium chloride

C_E - The effective concentration,

C_{DGT} – Time-averaged concentration in solution at the surface of

the DGT device

Cu- Copper

DGT - Diffusive Gradients in Thin films

EDTA – Ethylenediaminetetraacetic acid

DTPA - Diethylene Triamine Pentacetic acid

ICP-MS - Inductively Coupled Plasma Mass Spectrometer

ICP-OES - Inductively Coupled Plasma Optical Emission Spec-

trometer

MBL - Mixed Binding Layer

P-AL

P- Phosphorus

Zn- Zinc

WHC - Water Holding Capacity

Table of contents

1 Introduction

Nutrient deficiencies in crop production is today a worldwide problem (Tandy et al., 2011). To maximize fertilizer efficiency and crop yields it is important to be able to assess the accurate amount of available nutrients in the soil (Mason et al., 2005). There are different methods to assess plant available nutrients in the soil and asses the risk of nutrient deficiency. Soil extraction analyses like Aqua regia (HNO_3 + HCl), HNO_3, EDTA, DTPA, $CaCl_2$ and P-AL are often used in purpose to assess available nutrients (Tandy et al., 2011). However, these methods often show both available and not direct available nutrients for the crop, which is a limitation in predicting nutrient deficiency (Six et al., 2013, Schifman et al., 2012). Phosphorus is a macronutrient where the conventional extraction methods often fail to give an accurate assessment of its availability to crops (Mason et al., 2005). There is therefore a need for developing new methods that better shows the accu-rate amount of available nutrients to crops. One new method is diffusive gradient in thin films (DGT), which is a diffusive method that accumulates dissolved met-als, sulphides and phosphates (Zhang, 2003). According to Tandy et al. (2011), DGT can in a better way show plant available nutrients compared to the standard extraction methods, which show both plant available and not direct plant available nutrients. The objective with this study was to investigate and compare the new DGT technique with three conventional extraction methods: HNO_3, $CaCl_2$ and PAL. The concentration of Copper (Cu), phosphorus (P) and zinc (Zn) was meas-ured in fourteen different soils in Sweden with different methods for soil analysis and correlated to the concentration of Cu, P and Zn in wheat plants.

1.1 Hypothesis

i) DGT shows a more accurate concentration of plant available Cu and Zn in the soil than HNO_3.

ii) DGT shows a more accurate concentration of plant available P in the soil than P-AL.

iii) The amount of accurate concentration of plant available Cu, P and Zn will decrease in following order: DGT, $CaCl_2$ and HNO3/P-AL.

iv) Both P-AL and HNO_3 extract larger amounts of nutrients compared to DGT technique and $CaCl_2$.

2 Background

2.1 Plant nutrients

Crops demand both macro- and micronutrients for good growth. Potassium (K) nitrogen (N), and phosphorus (P) are considered to be the most yield limiting essential macronutrients (may be needed in large quantities). Essential micronutrients are boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni) and zinc (Zn) (needed in smaller quantities). From the crops point of view none of the nutrients are secondary, deficiency of one single nutrient can mean a large reduction in yield (Hamnér et al., 2012). It is important to know the limiting factors to succeed with an optimized crop production. One limiting nutrient cannot be replaced by a not-limiting nutrient. This relationship is known as "law of the minimum" and states that the growth is controlled by the most limiting nutrient. Liebig's law illustrates the minimum paradox well (Figure 1: (Mengel, 1987). High concentrations of N contributes to high yields, but e.g. P, K, Mg, S, Zn and Cu are at least as important to create fertile soil and good conditions to grow in. This study was restricted to only investigate Cu, P and Zn. These elements were chosen due to their high dependency of release from the solid phase to become plant available and due to their movement via diffusion in the soil. These two properties are necessary when measuring element with DGT. Phosphorus is a nutrient which often is analyzed to assess the need of fertilization. But today there is no method suited for all kind of soils and there is an interest to find a good method to analyze P. There is also an interest to find a good method to analyze Cu and Zn to assess the risk of deficiency (Tandy et al., 2011).

2.2 Copper

Copper is an essential micronutrient and has a role in photosynthesis, respiration and protection against oxidative stress (Marschner, 2012). Copper is mostly found in the soil in divalent forms, Cu^{2+}, which are stable complexes with low solubility. But also found in monovalent forms, $CuOH^+$ and $CuCl^+$ (Eriksson et al., 2011). The copper concentration is in general low in the soil solution, 98% of the Cu in the soil solution is bound to organic matter (Kirkby, 1987). Copper complexes binds hard to clay particles, Fe-oxides and organic matter (Eriksson et al., 2011). Due to the hard binding of Cu is Cu very immobile. Compared to other cations like Zn^{2+} more strongly bound to the organic matter which often regulates how mobile and plant available Cu is. Copper concentration in the soil solution de-creases with an increased pH (Kirkby, 1987). Copper deficiency take place in peat soils that bind Cu^{2+} hard and therefore is poor in plant available Cu, sandy or silty soils which are low in Cu in general. Cereal crops like wheat and oat are more sensitive to Cu deficiency than e.g. leguminous (Marschner, 2012). The threshold for critical nutrient deficiency of Cu is approximately 1.3 mg/kg (RobinsonJ.B., 2008). The crops takes up Cu in small amount, and can conquer with other nutri-ents. Typical symptoms of Cu deficiency are distortion of young leaves and necro-sis/chlorosis starting at the apical meristem and continue down the leaf margins (Marschner, 2012). During Cu deficiency the leaves often rolls together, twisting and drying out. During severe Cu deficiency the ax becomes deformed and the flowers sterile (Yara, 2015). The risk for serious diseases increases during severe Zn deficiencies (Marschner, 2012).

Figure 1. Symptoms of Cu deficiency in winter wheat (Yara, 2015).

2.3 Phosphorus

Phosphorus is a non-renewable source from rock phosphate and an essential mac-ronutrient which is one of the components in the DNA, RNA, ATP molecules and in cell membrane phospholipids (Marschner, 2012). Phosphorus can be found in both organic and inorganic forms in the soil. Organic P is located in humus and plant residues, while inorganic P is released from rocks by weathering and after adsorption on soil particles (Eriksson et al., 2011). The most common forms of P in the soil solution are $H_2PO_4^-$ and HPO_4^{2-} (inorganic forms) (Marschner, 2012). Phosphorus ions are strongly pH dependent and are often bound hard to the inner sphere complex and has the highest solubility around pH 6. After nitrogen, phosphorus is the second most frequently limiting macronutrients for plant growth. Water content, pH, humus content and clay content are factors which affect reactions with P. So many factors involved complicates the prediction of plant available P (Schachtman et al., 1998). In arable soils with cereal production and the easily accessible P (P-AL) is below 4,0 mg/100g there is often a need for P fertilization (Eriksson et al., 2011). In heavy clays with very low pH are the risks for P deficiencies high and requires larger amount of P fertilization (Marschner, 2012). Different crops are different sensitive to phosphorus deficiency where both maize and wheat are relatively sensitive to P deficiency. Phosphorus deficiency is common in clayey soils with very high or very low pH. Deficiency symptoms of P are leaves or stem turning into purple colors and stunted plants with weak root devel-opment. Since P is a mobile nutrient the older leaves are affected first and P is then rearranged and stored in the younger leaves. It is important that the plant has access to P in its developing state so the plant can develop a big root system (Yara, 2015).

Figure 2. Symptoms of phosphorus deficiency in winter wheat (Yara, 2015).

2.4 Zinc

Zinc is an essential micronutrient and is required for plant growth, present in several enzymes, involved in carbohydrate- and protein metabolism as well as in hormones (Eriksson et al., 2011). Zinc is found in the soil solution as divalent cations Zn^{2+} and in complexes as $ZnOH^+$, $ZnCl^+$, and $ZnNO3^+$. Zinc is adsorbed to clay and humus particles, in living organisms or in organic material (Fogelfors, 2001). The average total concentration of Zn in Swedish arable soils amount to 10-300 mg/kg but only 1-4% of the total amount of Zn is usually plant available

(Eriksson et al., 2011). Zinc deficiency is common in calcareous soils and highly weathered soils with high pH, due to the adsorption to $CaCO_3$ in calcareous soils. In zinc deficient plants, the rate of protein synthesis is strongly reduced (Marschner, 2012). A symptom of Zn deficiency can be a mature leaf that exhibits irregular light brown lesions, bordered by a dark brown margin. Chlorosis on older leaves can also indicate Zn deficiency (Yara, 2015). During Zn deficiency, shoot growth is usually more inhibited than root growth (Marschner, 2012). Zinc defi-ciency is not that common in Sweden. There is however a higher risk of Zn defi-ciency at farms that only practice crop production due to lack of manure rich of Zn (which can be supplied from fodder) (Eriksson et al., 2011).

Figure 3. Symptoms of Zn deficiency in winter wheat (Yara, 2015).

2.5 Uptake of nutrients

It is of great interest for the farmer to optimize crop production, get the maximal yield and thereby obtain the largest possible profit. Through e.g. precision fertili-zation, application in good weather conditions and right timing, the nutrient defi-ciencies will decrease and the production will increase. Severe nutrient deficien-cies at an early growth stage of the plant will lead to serious reduction in biomass production (Bussink and Temminghoff, 2004). To maximize fertilizer efficiency and crop yields it is important to be able to accurately assess the amount of availa-ble nutrients in the soil (Mason et al., 2005). To avoid nutrient deficiencies it is important to investigate how much nutrients the soil contains and how much the crops takes up. The total amount of nutrients may be large in the soil, but this does not necessarily results in high levels of plant available nutrients (Degryse et al., 2009, Schifman et al., 2012). Different ions can vary in how hard they are bound to the soil particles, through adsorption or surfaces complexes. Ions that are bound to the surfaces of particles are strongly bound and is therefore not as plant-available as the ions next to the particle surfaces. Uptake of ions by the roots leads to a shift of the equilibrium (Eriksson et al., 2011). There are different mecha-nisms for the crops to take up nutrients; mass flow, diffusion and a more active uptake by the secretion of root exudates. Mass flow is driven by transpiration which means the content of dissolved nutrients in the soil solution will be transported from the soil solution in the plant via the roots. Nitrogen e.g. is transported by mass-flow and therefore dependent on the rate of water flow and on the average nutrient concentration. The amount of nutrients may vary due to the supply of nutrients and actual root uptake (Kirkby, 1987). Diffusion is the process where ions move along a concentration gradient, from a higher to a lower concentration to even out the concentration differences. The plant takes up nutrients through diffusion when the concentration is lower around the root. Ions mobility is defined as diffusion coefficient and differs between nutrients (Syers et al., 2008). When the roots are taking up the ions it results in a decreased concentration (Mengel, 1987). The size of the root volume is crucial for how much nutrients the plant can take up, the bigger volume, the larger uptake of nutrients is possible (Syers et al., 2008). Micronutrients like Cu and Zn and macronutrient P is to a high degree driven by diffusion (Mengel, 1987). The diffusion coefficient of $H_2PO_4^-$ and for most metal ions are 5.42E^{-6} cm^2/sec (0.53m^2/min) (Zhang, 2003).

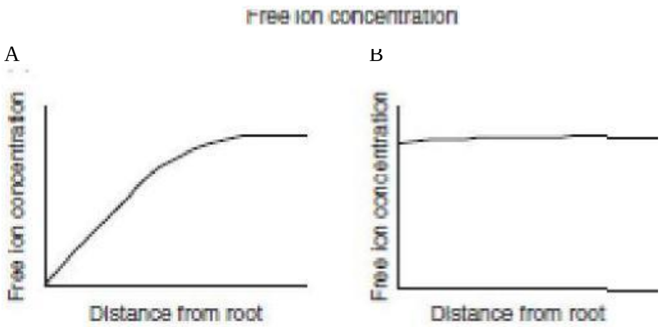

Figure 4. A)Low concentration and diffusion limitations, B)high concentration without any diffusion limitation (Degryse et al., 2009).

At low concentrations of ions in the soil solution, the uptake of nutrients is limited by diffusion. Under diffusion limitations, an increase in the diffusion flux will lead to increased plant uptake. This phenomenon is illustrated by the equation Michae-lis-Menten. K_M (Michaelis constanst) represent the free ion concentration, Fmax is the maximal rate of the flux and α is the root absorbing power (equation 5).

P_{max}

Equation 5. $a = K\ M$

The Michaelis-Menten equation shows how the reaction rate depends on the substrate concentration and measures the uptake at different free ions concentrations. Concentration in the soil solution and its buffering capacity plays a major role in the plant uptake (Degryse et al., 2009).

2.6 Extraction methods for soil analyses

Today there are plenty of different methods to extract nutrients from soils. Some methods are better suited to extract certain nutrients than others and the performances can also differ between different crops (Tandy et al., 2011). All the meth-ods requires shaking mixed soil with an extractant (solution) (Wünscher et al., 2013). Amount of extracted nutrients decreases in following order with the different extraction methods: Aqua regia (HNO3 +HCl) > HNO_3 >EDTA > NH_4NO_3 > $CaCl_2$ > DGT (Hamels et al., 2014). As mentioned earlier it is an uncertainty when the total amounts of nutrients are measured since not only the plant available nu-trients are estimated. Therefore it is of great interest to develop and improve meth-odologies for predicting plant-available nutrients, especially that can be used under field conditions in field trails (Tandy et al., 2011, Wünscher et al., 2013). It is in-teresting to compare strong acids extractions with weak acids extractions, to see how they differ from each other and which one that best can predict plant uptake.

2.7 Strong extraction methods

Some commonly used strong acid extraction methods are: Aqua regia (HNO_3 + HCl), HCl, HNO_3, P-Olsen, mehlich, P-AL, EDTA and DTPA. P-Olsen (Na-HCO3) and Mehlich 3 (0.2 M acetic acid (CH3COOH), 0.25 M ammonium nitrate (NH4NO3), 0.015 M ammonium fluoride (NH4F), 0.013 M HNO3 and 0.001 M ethylenediaminetetraacetic acid (abbreviated as EDTA) ((HOOCCH2)2 NCH2CH2N (CH2COOH)2) are a commonly used extraction methods to predict P concentration in Denmark and Europe. In Sweden is P-AL(acetate lactate) the most common method (Wünscher et al., 2013). According to Mason et al. (2005), Mehlich-3 only demonstrated poor to moderate correlations between extracted Cu and Zn and plant uptake. In this study P-AL was used as the strong acid extraction method to estimate the amount of P in the soil. Due to its common usage in practical farming it is easy to relate and compare the results with farmers P-AL values, and recent studies has shown significant correlations (Tandy et al., 2011).

EDTA and DTPA are grouped as moderately strong extraction methods. According to Hamels et al. (2014) EDTA was the most robust method for Cu-contaminated soils(Hamels et al., 2014). The study of Koster et al. (2005) also showed that Cu correlated best with EDTA. In other studies, poor or no correlations between extracted Cu by EDTA or DTPA and plant shoot uptake of Cu been seen (Tandy et al., 2011). There are no clear trend of which extraction methods that shows the best correlations.

HCl, HNO_3 and Agua regia are classified as the strongest acid extraction methods which extract a large amount of nutrients, which is better suited to measure metal ions than P since it builds complexes with e.g. Cu^{2+}. Previous studies have shown a strong correlation between plants uptake of Cu and HCl extracted Cu. Strong extraction methods will extract a lot more nutrients (both available and not plant available) than the weaker once. It works therefore very well as a reference to a weaker extraction method (Koster et al., 2005). HNO_3 is also one of the most common used extraction methods in Sweden to extract Cu and Zn. In this study HNO_3 was used as the strong acid extraction method to estimate the amount of Cu and Zn in the soil.

2.7.1 Weak extraction methods

Some common weak extraction methods are: H_2O, $CaCl_2$ and extracted soil solutions. Wünscher's et al. (2013) showed that H2O and CaCl2 extractable P correlated best with the nutrient concentration in plants compared to other extraction methods. $CaCl_2$ seems to be a promising extraction method, due to its ability to predict the actual available amount of Zn in different soils (Sauerbeck and Styperek, 1985: Hamels, 2014). $CaCl_2$ is a multi-nutrient extraction method which are able to extract both macro- and micronutrients. Soil to liquid ratio differs a lot between different studies. Sauerbeck and Styperek (1985) used a ratio of 4:10 while Hamels (2014) used the ratio 2:10. Ratio 1:10 has shown good results, have been used in many studies and seems to be a successful soil to liquid ratio and was therefore chosen in this study (Koster et al., 2005, Six et al., 2013, De Groot et al., 1998).

2.8 Diffusive gradients in thin films (DGT)

Diffusive Gradients in Thin films (DGT) is a fairly new method for estimation of plant available nutrients in soils. It is a gel technique which measures ions in soil, water or sediment, but in this study the focus was on ions in soils (Zhang, 2003). The DGT technique have are also suitable to measure toxicity of elements like Cd and Zn and bioavailability in sediments (Zhang et al., 1995). The technique consists of a so called DGT device which contains of a membrane filter, a diffusive gel and a resin gel, see figure 6 (Tian et al., 2008). When the DGT device is ar-ranged into the soil surface the diffusive layer simulates the interference of a root and measures the diffusive supply of elements through acting like an infinite sink. Diffusive gradients in thin films technique mimics the main mechanism of plant uptake by lowering the concentration locally and inducing diffusive supply and release from the solid phase. This is a dynamic process that depends on both the diffusion rate and resupply from the solid phase (Tandy et al., 2011). If the pore water concentrations are controlled by adsorption/desorption processes will it suit better to DGT measurement compared to solubility coprecipitation processes (Zhang and Davidson, 1995). The ions in the soil first diffuses through the mem-brane filter and diffusive gel to get to the resin gel, where the ions precipitates (Tian et al., 2008, Degryse et al., 2009). Ions from the soil solution become avail-able by desorption from the solid phase and will move towards the DGT device (Koster et al., 2005). A constant concentration gradient establishes in the diffusive layer which forms the source for measuring the concentration of ions in the soil solution. The diffusions layer is an important factor to measure the correct flux of metals or concentration of nutrients in the soil. The diffusion coefficient (cm^2/s) is dependent on temperature and differ between different elements (Zhang and Da-vidson, 1995). Diffusive gradients in thin films are possible to be deployed in situ in natural soil conditions. At extreme high and low pH will the accumulating ca-pacity decrease of the resin gel, but it depends on which measured element (Gimpel et al., 2001).

Elements which are possible to measure are: aluminum (Al), arsenic (As), cadmi-um (Cd), cesium (Cs), cobalt (Co), chromium (Cr), copper (Cu), ion (Fe), manga-nese (Mn), nickel (Ni), lead (Pb), phosphorus (P), sulfur (S) and zinc (Zn). Phos-phorus is driven by diffusion and is therefore suitable to be measured with the DGT device. Nitrogen which is moving by mass flow will not create concentration gradient and is therefore not suitable to be measured with the DGT technique (Hamnér, 2015). There are different gels that suits different elements differently well. A chelex (chelating material with ability to bind metal ions) gel is used for the metals and a Fe-oxide gel is used to bind P (Zhang, 2003).

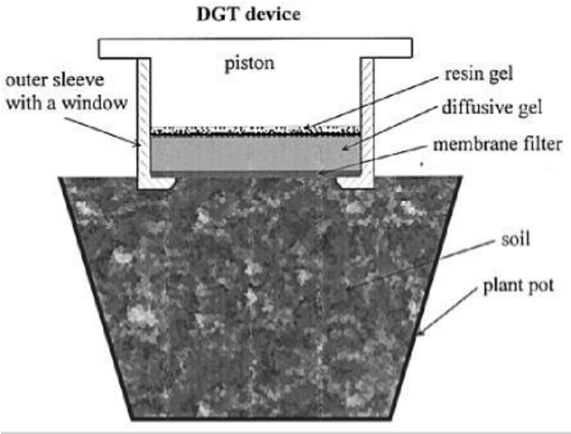

Figure 5. Cross section through a DGT device with the resin gel, diffusive gel and membrane filter closest to the soil. The figure is not proportionally correct, the DGT device is much larger than the pot (Zhang et al., 2001).

Copper, P and Zn are strongly dependent on kinetics of release from the solid phase, therefore DGT is expected to show more accurate plant uptake than the conventional extraction methods (Tandy et al., 2011). When measuring nutrients with DGT technique the concentration (C_e) of nutrients in the solution (1M HNO3 or 1M HCl) is given in the unit µg/l. To see the average concentration of measured metals and phosphates over time (C_{DGT}) further calculations has to be cone (chap-ter 4.2.1). C_{DGT} is normally expressed in µg/l (Zhang et al., 2001). C_{DGT} reflects supply from both solid phase and solution and is maintained approximately con-stant, providing the kinetically labile source close to the device is not really de-pleted (Zhang, 2003). Diffusive gradients in thin films technique integrates factors like intensity, buffering capacity and quantity into the parameter C_E (Tandy et al., 2011). How much nutrients that accumulates on the resin gel depends on the con-centration of the element in the soil pore water and on the rate of the resupply (Mason et al., 2005). Several surveys have been done to predict Cu and Zn by DGT but slightly fewer studies has been made to predict P by the DGT technique. Menzies el al. (2005) and Mason et al. (2010) where both studies were made to predict growth and yield response of P, showed a good correlation between con-centration of P in the soil and in plant. There is a need for further studies to predict plant concentrations of nutrients in arable soil by DGT technique (Tandy et al., 2011).

3 Materials and methods

3.1 Study site and sample preparation

This study was carried out in Uppsala in Sweden. All the laboratory work was done at Swedish university of agriculture sciences (SLU) in Uppsala. Fourteen different soils from agricultural field trials were chosen from the middle to south-ern part of Sweden, where the field work was done. The fields were cultivated with wheat (*Triticum aestivum*). Each site were fertilized with a normal dose of nitrogen (40kg+ 120kg). The fields in Kårby (22kg P/ha) and Mellerud (16.5kg P/ha) were further fertilized with P. At each field four replicates of soil samples were collected. Only the topsoil (0-20cm) was collected, the soil samples were taken with an auger. From each field also four replicates of plant samples were collected. Both the soil- and plant samples were randomly collected at each field. The soil samples was a heterogenic material which differs from trial pots in a greenhouse with homogeny soil material. With a heterogenic material is the uncer-tainty much larger than in a homogeny material.

The plant samples were gathered near the edge of each experimental plot, (approx-imately 30cm x 12cm) and the wheat was cut off two centimeters above ground. The wheat was sampled at stage 37 according to the DC scale. After collecting all the 56 soil samples (14soils x 4 replicates) and plant samples they were all dried for approximately 48 hours at 40 ºC. Then about 450 gram soil were sieved to <2mm and stored in cans in room temperature. The plant samples were then grind-ed into small pieces in a grinder (Retsch GM200) using a titanium blade to avoid contamination and about 45 gram wheat was stored in a warm cabinet and then prepared for further analyses in the lab (see section 3.3).

3.2 Soil analyses

The methods DGT, $CaCl_2$, P-AL and HNO_3 were used to extract copper, phosphorus and zinc from the soils (table 1). The different extraction methods are not equally strong. It's therefore discussable that more equal strong methods should have been used. P-AL is a much weaker extractions method compared with HNO_3, which might lead to an uncertain result.

Table 1. The used extraction methods and measured nutrients.

Element	Weak extraction method	Strong extraction method	Diffusive method
P	$CaCl_2$	P-AL	DGT
Cu	$CaCl_2$	HNO_3	DGT
Zn	$CaCl_2$	HNO_3	DGT

3.2.1 DGT

The DGT measurement was performed according to "the practical guide for using DGT in soils" provided by DGT research Ltd., Lancaster, HK, where the DGT devices also were ordered from. A mixed binding layer (MBL) was used instead of using two different resin gels (one gel that was needed for P and another for the metals Cu and Zn). The DGT samples were run in two rounds because of uncertainties whether the method would work or not. The first batch was done with two replicates from each soil and eluted in HCl. The second batch was done with the two remaining replicates from each soil and eluted in HNO_3. Two DGT blanks were done per batch without contact with the soil, the DGT device was kept in the plastic bag just before adding the resin gel to the elution solution.

Approximately 50.0 g of dry soil was weighted and placed in 100 ml containers and then Milli-Q water was carefully added until the soil reached the saturation point, approximately 68.0ml (+ - 6.5ml) per container. The containers were left calibrate for 24 h in room temperature (21.2 ^{C}C). In DGT batch 1 were the lids slightly ajar (so the process would not be anaerobic) and in DGT batch 2 were the lids closed because the soils got too dry. The DGT devices were stored in the fridge but were left to acclimatize to room temperature a few hours before de-ployment into the soils. The exposure window of the DGT was smeared with moist soil just before it was pushed to the soil in the containers just to ensure good contact with the soil (figure 6A). The exposure window was smeared with a knife and cleaned between every sample. The DGT devices were left in the soil for about 24 h.

Then the devices were removed from the soil, any soil that remained were rinsed with Milli-Q water and dried easily with tissues. Thereafter the cap of the DGT devices were open by a sharp knife and removed, see figure 6B. Also here were the knife cleaned with ethanol between every sample, it is very important to work in a clean environment so the DGT devices not get contaminated. The binding gel was then removed by a tweezer and eluted in 1 ml 1M HCl (DGT batch 1) and in 1M HNO_3 (DGT batch 2: figure 6C). The gels were removed from the elution solution after 24 h and were sent to ALS lab in Luleå for analyses by ICP-MS. Because of too high values of the first blanks were new blanks prepared with a plastic tweezer (instead of both metals and plastic) and HCl as elution solution and sent to ALS lab in Luleå for analyses by ICP-MS.

Figure 6. A) DGT device deployed into the soil sample, B) DGT device with only the resin gel left (removed plastic cap, front window plate and membrane filter, C) The resin gel was removed from the DGT device into the elution solution. Photos: Andersson, M. (2015).

Further calculations were done according to the practical guide for using DGT in soils (Zhang, 2003), to get the mean concentration of *C*DGT.

Equation 1. $M= \frac{Ce(VHCl/VHNO3+Vgel)}{fe}$

Where M is the mass of accumulated ions in the resin gel layer, C_e is the concentration of metals in the 1M HCl/ 1M HNO_3 elution solution in µg/l, V_{gel} is the volume of the resin gel (0.15ml), $V_{HNO3/VHCl}$ is the volume of HCl added to the resin gel, fe is the elution factor for each metal (1=P and 0.8=Cu and Zn).

Equation 2. F= _____ iti(tA)

F is the flux measured by DGT, t (s) is deployment time in the soil and A is the exposure area of the DGT window ($A=3.14cm^2$).

Δ

Equation 3. C_{DGT}= D

Δg is the thickness of the diffusive gel (0.8mm) plus the thickness of the filter membrane (0.14mm) and D is the diffusion coefficient of the metals or phos-phates, see table 1, page 26 in (Zhang, 2003). The diffusion coefficient is tempera-ture dependent and in this study was the average temperature 21°C in the laborato-ry, according to Zhang, (2003) was the diffusion coefficient for Cu 5.58E^{-6} cm^2/sec, Zn5.44E^{-6} cm^2/sec and P 5.42E^{-6} cm^2/sec. In this way was the concentra-tion of the amount measured metals by the DGT calculated. The concentration of metals that had accumulated on the resin gel after a certain time C_{DGT} had the unit µg/l. After the calculations were the data compared to other studies, to see if similar results had been achieved.

3.2.2 $CaCl_2$

In this method was a solid-to-liquid ratio by 1:10 used, 10 gram dry soil was weighed and placed in tubes. Then 100ml of 0.01M $CaCl_2$ added (8.82 g $CaCl_2$ was dissolved in 6 liters of Milli-Q water) into the tubes and did shake in a shaker for two hours. Thereafter centrifuged with 2800 rpm about seven minutes, hence each sample was filtered through Munktell V 00A filter into plastic bottles. All the samples were done at the same time plus two $CaCl_2$ blanks. The first blanks showed too high values of Cu and Zn. Six new blanks were done; two blanks with a new $CaCl_2$ solution (from 2015), two with the old $CaCl_2$ solution (from 1993) with shaking and two blanks with the old $CaCl_2$ solution (from 1993) but without shaking.

3.2.3 P-AL

Three gram of soil was weighted and placed in tubes with a lid, then 60 ml ammo-nium lactate solution was added. The solution was shaken for 90 minutes and the filtered through Munktell V 00A filter in plastic bottles, and analyzed with an ICP OES (Optima 7300DV).

3.2.4 HNO_3

Two gram soil was weighed in tubes hence added 10 ml 65% HNO_3 (make sure that the entire sample is washed down) and left to stand overnight, 24 hour. There-after the samples were briefly shaken and boiled in three steps: 60 degrees in two h, 100 degrees in one hour and 130 degrees in two hours. The samples were cooled down and diluted with 50 ml Milli-Q water and then the samples was filtered through Munktell V 00A filter into plastic bottles. As reference samples wheat flour and rice and flour were used. Afterwards the samples were analyzed with an ICP-OES spectrometer.

3.3 Plant analysis

One gram of grinded wheat was weighed in tubes and then 10 ml 65% HNO_3 was added and left to stand overnight, 24 hour. Thereafter the samples were briefly shaken and boiled in three steps: 60 degrees in two h, 100 degrees in one hour and 130 degrees in two hours. The samples were cooled down then added with further five ml 65% HNO_3. The samples were thereafter boiled in two hours at 130 degrees, cooled down and diluted with 50 ml Milli-Q water. The samples were then filtered through Munktell V 00A filter into plastic bottles. As reference samples wheat flour and rice flour were used. Afterwards the samples were analyzed with an ICP-OES spectrometer.

3.4 Calculations and statistics

The collected data was first written and calculated in Excel, then transferred to Minitab (Minitab 17 Statistical software). In Minitab was an analysis of regression and analysis of variance (ANOVA) done with a significance level at 5% (p=0.05). The analytics uncertainty is quite big, because of the low numbers of samples and of analyses.

3.5 Literature study

A literature study was done with focus on plant nutrition and to understand the different extraction methods and the DGT technique. Because DGT is a rather new method were not so many articles found.

4 Results

The majority of the soils contained moderate amount of clay (25-40%) with moderate organic matter. Strömsholm was the only heavy clay (40-60%).The pH values did not differ so much between the soils (6.1-7.3). There was a broad variation between how high concentrations of Cu, Zn and P were extracted between different methods and the locations contained (Table 2, 3 and 4). There was a wide variation between the different soils (a mean value was calculated of the four replicates from each field), see table 3. All the values of the concentrations were distributed with a normal distribution. Some soils showed a broad variation of nutrients content both within each site and between the fields. The grown wheat species were: Julius, Mariboss and Ellvis.

4.1 Copper

The amount of extracted Cu differed both between locations and the different methods of analysis. The highest concentration of plant Cu was obtained at Strömsholm (5.5 mg/kg ts) closely followed by Glyttinge, Kårby and Grillby with values from 5.2-5.5 mg/kg ts. DGT extracted the maximum amount of Cu at the soil in Strömsholm. $CaCl_2$ and HNO_3 both showed the highest extracted concentra-tion of Cu at site Grillby 0.0075mg/kg ($CaCl_2$) and 32.78 mg/kg (HNO_3). (The raw data can be found in Appendix I). None of the plants displayed symptoms of Cu deficiency and the concentration were all above the approximate deficiency threshold of 1.3 mg/kg. The probability values for the different methods correlated to the plant concentration of Cu, P and Zn are shown in table 4.

4.1.1 Correlation between plant uptake and soil extractable Cu

HNO_3, $CaCl_2$ and DGT measurements all had a significant correlation with plant Cu uptake ($p < 0.05$). The R^2-value differed however between the methods where. HNO_3 (R^2=0.395) and $CaCl_2$ (R^2=0.368) showed a poorer correlation than DGT (R^2=0.639,figure 7). The green lines represent standard deviation in figure 7 and 8.

Table 2. Locations were the soil and wheat samples were collected (OM= organic matter).

Location	Soil type	Species	pH
Grillby/Mällby (Uppland)	Moderate OM content, clay (25-40%)	Julius	6.4
Nybble/Vintrosa (Närke)	Moderate OM, clay (25-40%)	Ellvis	6.6
Sörby/Skultuna (Västmanland)	Little OM, silty clay (15-25%)	Julius	6.7
Strömsvik/Strömsholm (Västmanland)	Moderate OM, Heavy clay (40-60%)	Ellvis	6.0
Kårby (Östergötland)	Moderate OM: Silty clay (15-25%)	Mariboss	6.8
Glyttinge (Östergötland)	Little OM, Silty clay (15-25%)	Mariboss	6.8
Karlsfält/Mellerud (Dalsland)	Rich in OM, clay (25-40%)	Julius	6.1
Skofteby/Lidköping (Västergötland)	Low in OM, clayey silty soil	Mariboss	6.3
Forshall/Grästorp (Västergötland)	Low in OM, clay (25-40%)	Julius	6.5
Torebo/Falkenberg (Halland)	Low in OM, clayey fine sand	Ellvis	7.3
Tjustorp/Hammenhög (Skåne)	Low in OM, sandy clay (15-25%)	Mariboss	6.3
Höjagården/Ängeholm (Skåne)	Low in OM, clay (25-40%)	Mariboss	7.1
Klagstorp (Skåne)	Low in OM, sandy clay (15-25%)	Mariboss	7.5
Trä/Teckomatorp (Skåne)	Moderate OM, sandy clay (15-25%)	Ellvis	7.6

Table 3. Locations with the highest and lowest extracted concentration of copper.

	Maximum of copper	**Minimum of copper**
Plant Cu (mg/kg)	Strömsholm (5.47)	Grästorp (2.27)
$CaCl_2$ extracted Cu (mg/kg)	Grillby (0.075)	Mellerud (0.013)
HNO_3 extracted Cu (mg/kg)	Grillby (32.78)	Mellerud (6.81)
DGT extracted Cu (µg/l)	Strömsholm(0.026)	Grästorp (0.0038)

4.1.2 Correlations between measured Cu by DGT vs. $CaCl_2$ and HNO_3

Cu measured by DGT technique showed a strong and significant correlation with both $CaCl_2$ extractable Cu (Figure 8; $p<0.001$; $R^2=0.754$) and HNO_3 extractable Cu (Figure 8; $p<0.001$; $R^2=0.737$). The probability values of the correlations between DGT measurements of Cu, P and Zn versus $CaCl_2$, HNO_3 and P-AL are shown in table 5.

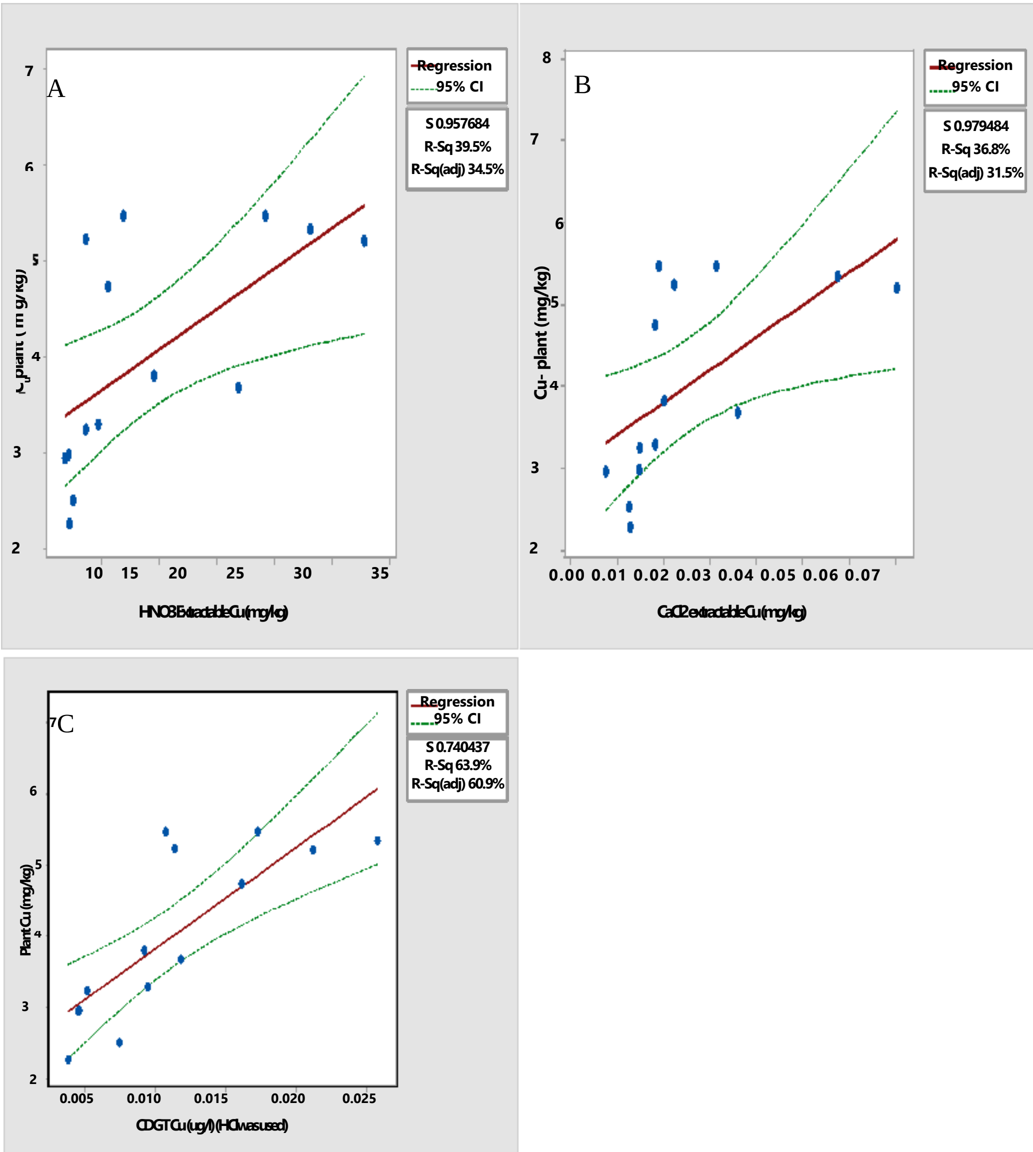

Figure 7. Cu plant concentration versus A) extracted Cu by HNO_3, B) extracted Cu by $CaCl_2$, C) soil Cu measured by DGT with HCl as solution (DGT batch 1)

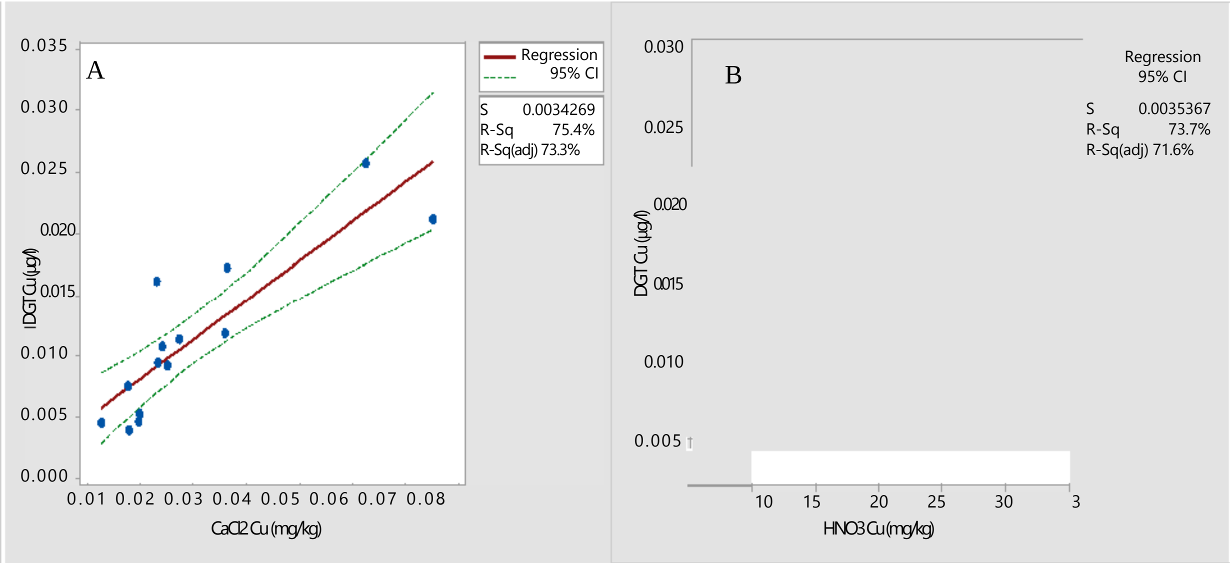

Figure 8. Relationship between extractable Cu by DGT (µg/l) versus extractable Cu by A) $CaCl_2$ and B) HNO_3 (mg/kg).

Table 4. Probability values of the correlations between Cu, P and Zn plant uptake and extracted Cu P and Zn by HNO_3, P-AL, $CaCl_2$ and DGT($p < 0.05$ is significant).

Plant uptake	**HNO_3/P-AL**	**$CaCl_2$**	**DGT (HCl)**
Cu	0.016	0.018	0.001
Zn	0.569	0.01	0.39
P	0.58	0.422	0.503

Table 5. Probability values of the correlations between DGT measurements of Cu, P and Zn versus extracted Cu, P and Zn by $CaCl_2$, HNO_3 and P-AL ($p < 0.05$ is significant).

	HNO_3/P-AL	**$CaCl_2$**
DGT Cu	<0.001	<0.001
DGT Zn	0.647	0.878
DGT P	*0.016*	*<0.001*

4.2 Zinc

The amount of extracted Zn differed both between locations and the different methods of analyses. The highest plant Zn concentration was obtained at Strömsholm (24.21 mg/kg ts) closely followed by Skofteby (24.05 mg/kg ts).

The blanks that were done in DGT batch 2 showed much lower values (almost zeroed) compared to the blanks made in DGT batch 1. Diffusive gradients in thin films technique extracted the maximum amount of Zn (0.57µg/l) in Kårby and the least Zn (0.13µg/l) in Grästorp. Whereas $CaCl_2$ extracted the most Zn (0.16 mg/kg) in Strömsholm and the least (0.01 mg/kg) in Teckomatorp. The highest concentration of Zn extracted by HNO_3 was in Skultuna (103.67 mg/kg) closely followed by Strömsholm (102.85 mg/kg) and the least amount of extracted Zn in Mellerud (28.88 mg/kg). The lowest plant Zn concentration was obtained in Grästorp (10.00mg/kg ts) closely followed by Nybble (11.68 mg/kg) and Teckomatorp (11.30 mg/kg; table 7). (The raw data can be found in Appendix I). None of the plants displayed symptoms of Zn deficiency but Grästorp, Vintrosa and Teckomatorp all had lower concentration of Zn than the approximate deficien-cy threshold of <14 mg/kg.

Table 6. *The locations with the highest and lowest extracted concentration of zinc.*

	Maximum of zinc	**Minimum of zinc**
Plant Zn (mg/kg)	Strömsholm (24.21)	Grästorp (10.00)
$CaCl_2$ extracted Zn (mg/kg)	Strömsholm (0.16)	Teckomatorp (0.01)
HNO_3 extracted Zn (mg/kg)	Skultuna (103.67)	Mellerud (28.88)
DGT extracted Zn (µg/l)	Kårby (0.57)	Grästorp (0.13)

4.2.1 Correlations between plant uptake and extractable Zn

There were no significant correlation between DGT and HNO_3 measurements with plant Zn uptake (Figure 9; $p>0.05$). $CaCl_2$ on the other hand showed a significant correlation with Zn plant uptake ($p<0.01$; $R^2=0.436$; figure 9). None of the blanks in DGT batch 1 were deducted due to too high value so these results are very un-certain. Hopefully will the results in DGT batch 2 show a positive significant cor-relation between Zn plant uptake and measured Zn concentrations by DGT (still waiting for the last result).

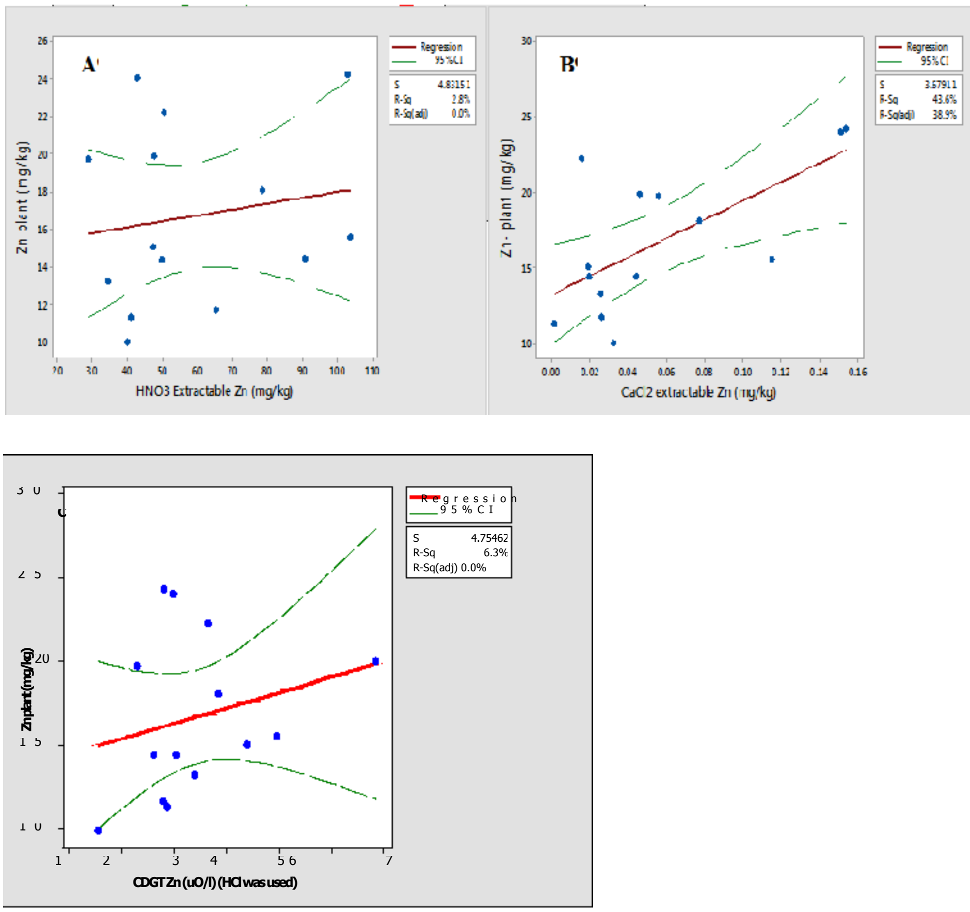

Figure 9. Zn plant concentration versus A) extracted Zn by HNO_3, B) extracted Zn by $CaCl_2$, C) soil Zn measured by DGT with HCl as solution (DGT batch 1)

4.2.2 Correlations between measured Zn by DGT vs. $CaCl_2$ and HNO_3 Diffusive gradients in thin films technique extractable Zn did not show a signifi-cant correlation with $CaCl_2$ or HNO_3 extracted Zn ($p>0.05$; figure 10).

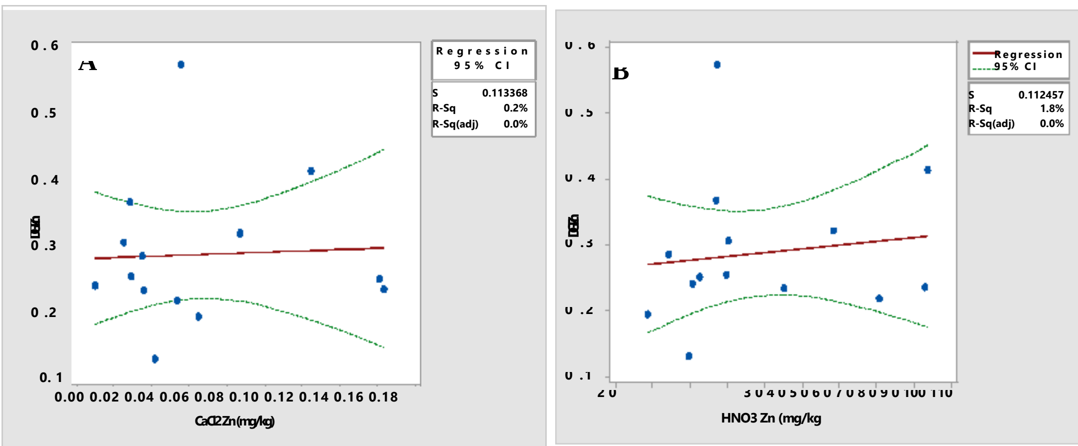

Figure 10. Relationship between extractable Zn by DGT (μg/l) versus extractable Zn by A) $CaCl_2$ and B) HNO_3 (mg/kg).

4.3 Phosphorus

The amount of extracted P differed both between locations and the different methods. The highest plant P concentration was obtained at Lidköping (3983.30 mg/kg ts) and the lowest concentration of P at Grästorp (2376.91 mg/kg ts). $CaCl_2$, P-AL and DGT measurements of P all had highest concentration of P in Skultuna ($CaCl_2$=2.79 mg/kg, P-AL=164.9 mg/kg and DGT= 1.67 µg/l). Both $CaCl_2$ (0.38 mg/kg) and DGT (0.21 µg/l) showed the lowest concentration of P in Ängelholm, while lowest P concentration with P-AL was obtained in Kårby. (The raw data can be found in Appendix I). None of the plants displayed symptoms of P deficiency but Grästorp had lower concentration of P than the approximate deficiency threshold of <2400mg/kg.

Table 7. *The locations with the highest and lowest extracted phosphorus (P) concentrations.*

	Maximum of P	**Minimum of P**
Plant P (mg/kg)	Lidköping (3983.30)	Grästorp (2376.91)
$CaCl_2$ extracted P (mg/kg)	Skultuna(2.79)	Ängelholm (0.38)
P-AL extracted P (mg/kg)	Skultuna (164.9)	Kårby (37.9)
DGT extracted P (µg/l)	Skultuna (1.67)	Ängelholm (0.21)

4.3.1 Correlation between plant uptake and extractable Zn

There were no significant relationships between any of the methods for soil analysis (HNO_3, $CaCl_2$ and DGT) and plant uptake of P, (p>0.05). The R^2 value were low for all of the methods (R^2=0.026-0.051), see figure 11.

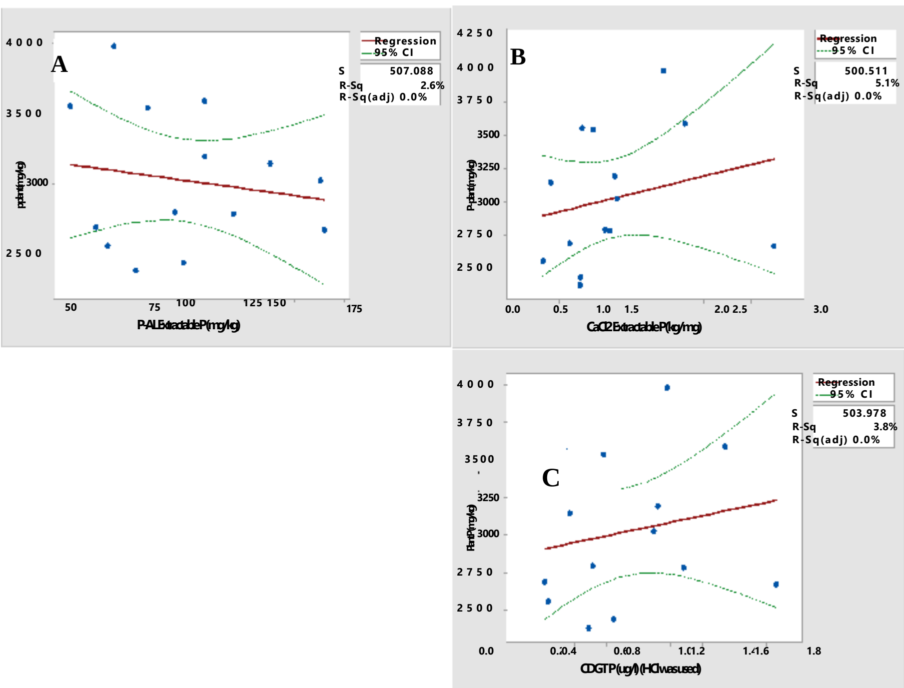

Figure 11. Phosphorus plant concentration versus A) extracted P by P-AL, B)extracted P by $CaCl_2$ and C) P concentration in the soil measured by DGT.

4.3.2 Correlations between measured P by DGT vs. $CaCl_2$ and P-AL.

Diffusive gradients in thin film technique extractable P showed a strong and significant relationship with $CaCl_2$ extracted P ($p<0.001$) and with P-AL extracted P ($p<0.05$, figure 12).

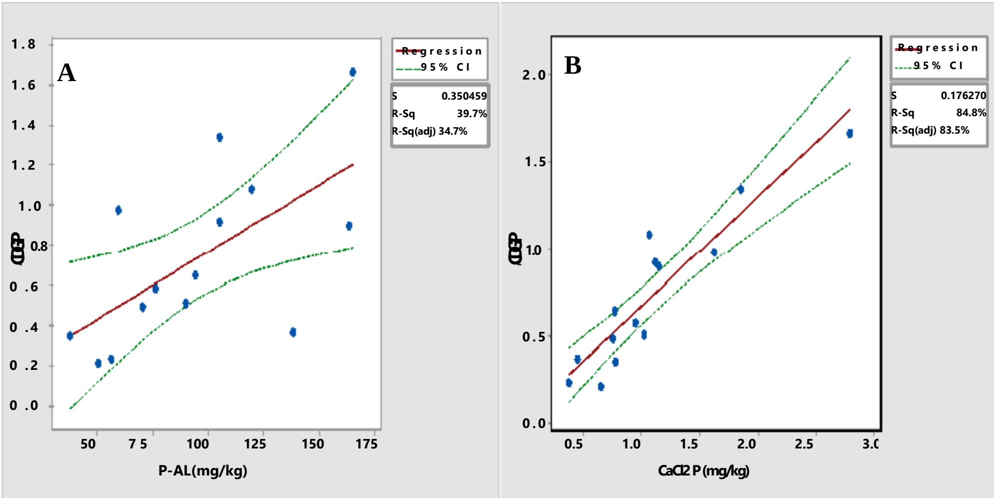

Figure 12. Correlation between extractable P by DGT (µg/l) vs extractable P by A) P-AL (mg/kg) and B) $CaCl_2$ (mg/kg).

5 Discussion

5.1 Comparison of copper, phosphorus and zinc

5.1.1 Copper

Diffusive gradients in thin films measured Cu correlated well to the plant Cu uptake (R^2=0.64; $p<0.01$), the highest concentration of Cu was in Strömsholm and the lowest concentration of Cu in Grästorp (table 4). The soil in Strömsholm is a heavy clay with moderate organic matter content and pH 6 while the soil in Grästorp had both lower percentage of humus and clay content and pH6.5. Copper is strongly correlated to clay content which may explain the high Cu content in Strömsholm.

The extracted concentration Cu by DGT, HNO_3 and $CaCl_2$ was significantly correlated to Cu plant uptake. The DGT measurement had the strongest significant correlation (R^2 =0.64; figure 7). Copper appeared to be an element which was easy to assess the plant-available concentration of, irrespective of the method used. However DGT showed the highest correlation of the used methods which was consistent with previous studies made by Tandy et al. (2011) and Zhang et al. (2001). They concluded significant correlations between plant Cu uptake and measured Cu concentration by DGT (R^2=0.9 and R^2=0.95). Mason et al. (2005) also stated that the DGT method predicted Cu with significantly larger accuracy compared to the conventional extraction methods.

A few soil samples showed very high plant Cu concentrations but low extractable amount of Cu in the soil and therefore deviated from the significant correlation for all methods (figure 7). When the two samples from Glyttinge and Kårby were removed, the R^2 value increased radically (from 0.64 to 0.86). A R^2 value of 0.86 indicates a very strong correlation and would be a promising result for DGT. A reason why these two soils showed low concentration of measured Cu might have been some error in the collecting phase or that they simply do not correlate strongly for some unknown reason. Mason et al. (2010) removed a few samples in his study and the R^2 value increased as well. Diffusive gradients in thin films technique showed a significant correlation with HNO_3 (R^2=0.754) and $CaCl_2$ (R^2=0.737) extractable Cu. It is interesting to see that the methods correlated well, which proved DGT as a good method. On the other hand, if DGT and HNO_3 correlate well it would not be necessary to run DGT as they would than show more or less the same values as the extraction methods. The aim with DGT was to find a method that assessed plant available nutrients better than the conventional methods, so it is discussable what a too good correlation means.

5.1.2 Phosphorus

No correlation was found between P-AL, $CaCl_2$ or DGT extracted P and plant P concentration in the plant ($p>0.05$). Neither in Six et al. (2013) showed a good correlation between P plant uptake and extractable P. Skultuna contained highest concentration extractable P by DGT, P-AL and $CaCl_2$ and the lowest concentration of P varied a lot between the different sites (table 6). The soil in Skultuna was low in organic matter, low in clay content (15-25%) with a pH of 6.7. Mason et al. (2010) concluded in his Australian study that DGT appeared to be a robust technique that better measured the accurate plant available P irrespective of soil type. It was also stated that DGT had a great potential to improve prediction of fertilizer requirements and efficiency in all types of agriculture. The fact that he succeeded might have to do with different climate, microclimate and different soil types. Phosphorus concentration measured by DGT was also well correlated to plant available P in Six et al. (2013) study. But was not confirmed in this study in Uppsala, SLU.

Extracted P by DGT showed a strong significant correlation with P extracted from P-AL and $CaCl_2$. There were thus a stronger correlation between DGT and $CaCl_2$ (R^2= 0.848) than DGT and P-AL (R^2= 0.397) which was expected due to the fact that the weaker extraction methods extracted less nutrients than the stronger ones. Worse correlation between extracted phosphorus by DGT and P-AL shown in previous studies was also confirmed in this these studies (Mason et al., 2005: Tandy et al., 2011). A reason for poor correlations might be because there are so many different forms of P in the soil and the plant-available form has not yet been correlated with some method (Mason et al., 2005). It is however interesting that the relationship between DGT and $CaCl_2$ correlated better than DGT and P plant uptake. This indicated that the methods follow each other but not necessarily predict an accurate value of phosphorus.

5.1.3 Zinc

$CaCl_2$ extracted Zn correlated significantly to the Zn plant uptake but did not show a strong correlation (R^2=0.44: p<0.005). DGT measured Zn and HNO_3 extracted Zn did not correlate significantly with the plant uptake of Zn (p>0.05). Other studies have shown that DGT measurement of Zn were strongly correlated to plant Zn (R^2= 0.87) (Mason et al., 2005, Tandy et al., 2011). Koster et al. (2005) indi-cated that DGT-extractable Zn correlated well to extracted Zn plant uptake which hence not was confirmed in this study. Measured Zn concentration by DGT showed an interesting correlation (R^2= -0.455), with a negative regression line. The results in this study may be caused by errors that occurred in the lab (section 5.2). Zn seems to be an unpredictable element and therefore difficult to assess the accurate plant available concentrations. The major problem seems to be that Zn easily become contaminated from various sources which disturbed the results, so the poor results may not necessarily be due to the used methods.

5.2 Sources of error

5.2.1 DGT

During the performance of DGT there were plenty of things that could have gone wrong and that could have contributed to sources of error. It was difficult to reach the maximum water holding capacity (WHC) without letting the soil samples get too wet or dry. If the soil samples got too dry would there be air through the diffusive passage instead of nutrients, which would lead to lower concentration of nutrients. In DGT batch 1 the lid was ajar that made them dry out a little. One ml Milli-Q water was added to wet the soils to the WHC before the DGT devices was placed into the soil. When the DGT batch 2 was prepared the lid was left closed and they did therefore not dry out. It was therefore good to do two batches of DGT samples when the procedure could be repeated and the mistakes could be avoided. It was a learning process through the whole lab session.

The blanks of Zn in DGT batch 1 was higher (0.35 μg/l) than the lowest Zn values (0.13 μg/l) and was not deducted to avoid negative values. The Zn blank showed higher values in more than half of the samples and for that reason was the Zn blanks not deducted. This might be one of the reasons why none of the Zn values was significantly correlated to the plant concentration of Zn and not correlated with the other methods. This indicated some kind of Zn contamination during the process of the blanks. It is confusing because the blanks had not contact with the soil, but were left in plastic bags right before the resin gel were removed to the elution solution (HCl). Many factors could have contributed to too high blank values of Zn. The tweezer broke down during batch 1, so there was a possibility that the metal part of the tweezer were in contact with the soil sample and contributed with Zn and affected the result. Another factor that could have affected the results of the DGT measurement was the fact that mixed binding layer (MBL) was used instead of separate binding layers. Mason et al., (2005) used MBL in his study, for a more efficient result instead of using several DGT devices with different resin gels. To save time and be able to measure both metals and phosphate at the same time MBL was also used in this study. The DGT batch 1 was HCl used to elute the resin gel like in Mason et al. (2005) study, which confirmed good results. The poor outcome of the measured Zn in this study was therefore unexpected. New blanks eluted with HNO_3 resulted in much lower Zn concentrations, they decreased from 49.4µg/l to 4.3µg/l (C_e values not C_{DGT}), which indicated that the HCl solution that first was used not was clean enough. This shows that at DGT is a sensitive method and small errors can have major impact on the results. If the same study would be carried out again, it would recommend to use HNO_3 as elution and to use a plastic tweezer in an extreme clean environment to avoid contamination. The time when the DGT devices were attached into the soil was also an important factor to be taken into account. According to Six et al. (2012) the best deployment time of the DGT devices differed from 30 minutes up to 48h, to be sure that enough of nutrients accumulated and at the same time avoid saturation. In this study was 24 h used as deployment time. The standard thickness of the diffusive gel was used as recommended (0.8mm) by Zhang et al. (2001), but there were also thicker gels that could have been done. The thicker diffusive gel, the longer time is required for accumulation of the nutrients. It required high precision and accuracy when DGT was performed, the environment had to be extremely clean to not contaminate the samples. The fact that not so many studies have been made on DGT from field trials contributed to further uncertainties for the method (Koster et al., 2005).

In both Ängelholm and Klagstorp the resin gel were broken when moving the gel into the elution solution, which may have affected the result in lower concentrations of nutrients. However it rather indicated that the soil itself contained low concentrations of P as $CaCl_2$ also showed low values of P.

5.2.2 $CaCl_2$, HNO3 and P-AL

The $CaCl_2$ method correlated well to both Cu and Zn plant uptake and seemed to work better as extraction method thanHNO_3. It was a simple method that was easy to use. One possible source of error was the old $CaCl_2$ solution (from 1993) that was used to the first solutions. When the $CaCl_2$ solution from 2015 was used in-stead of the old one from 1993, the blank concentrations decreased from 8.35µg/l to <2 µg/l for Zn, 3.7 µg/l to <1 µg/l for Zn but the blank values for P remained the same <10 µg/l. There could also have been something wrong with the shaking process or contaminated bottles which also may contribute to higher concentrations of nutrients. The most likely source of error was however the old $CaCl_2$ solution that probably had been contaminated. It was however confusing that the old solution not showed increased values in the second batch with blanks. This might be the reason why the $CaCl_2$ did not correlate so well with the plant uptake of nutrients. Another reason why the correlation not was so good could be due to many other factors. Factors such as leaching of nutrients, or the plants had different root growth that can affect the uptake. In a recent study, the weaker extraction method $CaCl_2$ correlated better to the Cu and Zn plant uptake compared to stronger methods like HNO_3 (Menzies et al., 2005) In this study it was only confirmed that plant Zn uptake correlated better to the $CaCl_2$ extracted Zn than to HNO_3 extracted Zn, thus Cu showed equally good correlations to both $CaCl_2$ and HNO_3. It is not optimal to use differently strong extraction methods, the more similar they are the better result it will show.

The conventional extraction methods required shaking of the soil samples, which is a process that disturbs the natural ratios. The shaking may contribute to more extracted nutrients and lead to an incorrect result regarding plant available nutrients. Diffusive gradients in thin films technique does not contain any shaking moments which means there are no distraction of the soil. This factor has to be taken into consideration when comparing the different methods (Koster et al., 2005). Diffusive gradients in thin films technique was more costly, required more laboratory experience and generally took longer time than the conventional extraction methods which is a drawback.

All the soil samples were dried and grinded into small fractions, which means that the natural soil conditions changes and homogenizes the soil. This factor may have affected the result. All factor that disturbs the soil conditions affects the result. To best measure the plant available nutrients should be to do the analysis out in the field. It is difficult to see the connection between plant uptake and available nutrients in a heterogenic soil material. It could be nutrients in spots so if the soil samples were taken in a spot with lack of phosphorus the result will be subsequently.

5.3 The future of DGT

Mason et al. (2010) concluded that DGT appeared to be a robust technique that better measured the plant available P irrespective of soil type. The authors also stated that DGT has a great potential to improve prediction of fertilizer requirements and efficiency. Tandy et al. (2011) showed that agricultural soils (not pot trails) correlated better to the Cu and Zn plant concentrations than the convention-al extraction methods. In Sweden the diffusive gradients in thin films technique is not a established method like in Australia and further research has to be done be-fore the technique could be used in practical agriculture. It would also be interest-ing to evaluate the potential to assess the plant availably amount of other nutrients with the DGT technique. To achieve good results in plant prediction of nutrient it is important to increase the knowledge about the DGT technique (Koster et al., 2005). Many other studies have measured Cd which have shown significant rela-tionships with plant uptake. There are thus potential of DGT to predict concentra-tions of other nutrients than Cu, Zn and P and e.g. avoid toxicity in the soil (Degryse et al., 2009, Mason et al., 2010)). If the same study would be carried out again, it would recommend to use HON3 as elution solution, plastic tweezer in an extreme clean environment.

In the future it would be interesting if the DGT technique could be used directly out in the fields and assess the amount of plant-available nutrients direct. I think DGT has a great potential to become one of the standard methods and there is certainly the potential to develop the method further.

6 Conclusion

The objective with this study was to predict and compare plant available Cu, Zn and P concentrations on 14 different soils in Sweden by comparing the DGT technique with HNO_3, $CaCl_2$ and P-AL. It was confirmed that DGT showed more accurate concentration of plant available Cu than HNO_3 but not of plant available Zn and P. Copper plant concentration showed significant correlation to extracted Cu by all the methods (DGT, HNO_3, $CaCl_2$ and P-AL) but DGT had the strongest correlation. It was also confirmed that P-AL and HNO_3 extracted larger amounts of nutrients compared to DGT technique and $CaCl_2$. Diffusive gradients in thin films technique correlated well to $CaCl_2$, HNO_3 and P-AL.

As previous studies have shown significant correlations between plant Cu, P and Zn uptake and extracted nutrients by DGT has DGT a potential to become one of the used conventional methods both in Sweden and in other countries. Further studies of the DGT technique is however recommended.

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6.2 **Oral references**

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