DEVELOPMENT OF A RAPID SCREENING TECHNIQUE FOR CONTAMINANTS IN ENVIRONMENTAL MONITORING AND REGULATION

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Declaration

I hereby declare that except where specific reference is made to the work of others, the contents of this dissertation are original and have not been submitted in whole or in part for consideration for any other degree or qualification in this, or any other university. This dissertation is my own work and contains nothing which is the outcome of work done in collaboration with others except as specified in the text and Acknowledgements.

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Abstract

Rapid screening technique is important and efficient for routine monitoring of chemical pollutants, risk assessment and decision making in dealing with contaminants in waters and soils. The focus of this thesis is on developing simple and rapid screening methods based on the diffusive gradients in thin films (DGT) technique to assess the concentration of phosphorus and metals qualitatively and quantitatively. Firstly, a rapid detection technique for phosphorus based on Metsorb DGT devices and a colour imaging method using the conventional molybdenum blue were developed and fully tested under different conditions. The fully quantitative interpretation of the P concentration can be assessed in the linear range of 0.1 to 1.02 μg cm^{-2} device that corresponds to the concentration range of 9 to 98 μg L^{-1} if the deployment time is 24 hours and the water temperature is 20^{o} C. Secondly, digital colorimetric analysis using a flat-bed scanner was utilised to quantify the Cu, Ni, and Co in water following the DGT uptake of metals by Chelex resin gel without involving further reactive reagents. The fully quantitative interpretation of the Cu, Ni, and Co concentration can be assessed in the linear range of 1.5 to 165 μg cm^{-2}, 2.7 to 153 μg cm^{-2}, and 1.6 to 159.2 μg cm^{-2}, respectively, which correspond to the concentration range of 0.05 to 5 mg L^{-1} for all three metals if the deployment time is 24 hours and the water temperature is 20^{o} C. Thirdly, a rapid screening technique for Cr(VI) using DGT and a high-resolution CID

base on the surface colouration of the N-Methyl-D-glucamine (NMDG) binding gel has been developed. The relationship between the accumulation of Cr(VI) in NMDG gels and the corresponding change in grayscale intensity was well fitted using a quintic polynomial. The fully quantitative interpretation of the Cr(VI) concentration can be assessed in the linear range of 0.31 to 2.47 μg cm^{-2} which correspond to the concentration range of 12.5 to 150 μg L^{-1} if the deployment time is 24 hours and the water temperature is 20^{o} C.

This study has formulated a DGT deployment guide list to determine whether the concentration of metals has exceeded the maximum contaminant level allowed based on the regulation standards in different countries and regions. The use of both a simple visual inspection and a scanner for DGT devices at different deployment times and different temperatures will be considered for this list. Moreover, the rapid screening technique has been evaluated in water and soil from five regions in China.

Furthermore, a novel approach with biological material incorporated in the DGT (Bio-DGT) was developed to measure the concentrations and toxicity of metals at the same time in water and soil. The new method immobilised a whole-cell toxicity bioreporter, ADPWH_recA, into a thin layer of agarose gel to replace the polyacrylamide gel that is commonly used in DGT. The test results indicated that the concentrations of metals measured by Bio-DGT and the cell free DGT have no significant differences during a 7-day deployment in synthetic water. A positive metal exposure relationship was shown between Bio-DGT accumulation and biological

response. Bio-DGT showed a stable response to heavy metals under a wide range of pH and ionic strength. The bioluminescent signal of Bio-DGT was maintained at a high level during up to 30 days of storage. The deployment of Bio-DGT devices in field soils collected from China allowed the measurement of both the available concentration and the toxicity of metals. It indicated that the new Bio-DGT can assess the bioavailability and toxicity of metals at the same time.

The newly developed rapid screening technique for P and metals were applied in waters and soils in situ in 5 different regions of China. It showed the concentrations of P in most of the monitored waters in Beijing were low and the quality of the waters has reached the Chinese water quality standards for surface water. The concentration of DGT-measured P in the two main rivers run through Tianjing were higher than the national water standard in China. The concentrations of Cu in monitored aquatic systems of all field areas have also reached the Chinese water quality standards for surface water.

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List of Nomenclature

δ	The thickness of diffusive boundary layer
Δg	Length of diffusive layer
A	Sampling area of DGT devices
D	Diffusion coefficient of relevant analyte in diffusive layer
M	Accumulated mass in a binding gel
C_{DGT}	DGT calculated concentration
C_{sol}	Concentration of analyte in solution
J	The flux of analyte through a diffusive layer
f_e	Elution efficiency of analyte from a binding gel
t	DGT deployment time
V_e	Volume of the eluent used to release the analyte from a binding gel
V_g	Volume of gel in the binding phase

List of Abbreviations

AAS	Atomic absorption spectroscopy
AMD	Acid mine drainage
As	Arsenic
ATP	Adenosine triphosphate
Bio-DGT	Biological diffusive gradients in thin films
Ca	Calcium
Cd	Cadmium
CID	Computer Imaging Densitometry
CN	China
Co	Cobalt
Cr	Chromium
Cu	Copper
DBL	Diffusive boundary layer
DET	Diffusive equilibration in thin films
DGT	Diffusive gradients in thin films
DNA	Deoxyribonucleic acid
DPC	Diphenylcarbazide
DTPA	Diethylenetriaminepentaacetic acid

EDTA	Ethylenediaminetetraacetic acid
EPA	Environmental Protection Agency
EU	European Union
Fe	Iron
HCl	Hydrochloric acid.
$HClO_4$	Perchloric acid
HNO_3	Nitric acid
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma atomic emission spectroscopy
LB	Lysogeny broth
MCLG	Maximum contaminant level goal
MDL	Method detection limit
MEP	Ministry of Environmental Protection of the People's Republic of China
Mg	Magnesium
Mn	Manganese
MQ	Milli-Q
$NaHCO_3$	Sodium hydrogen carbonate
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide
Ni	Nickel

NMDG	N-Methyl-D-glucamine
OM	Organic matter
P	Phosphorus
Pb	Lead
PTFE	Polytetrafluoroethylene
RLU	Relative Light Units
RNA	Ribonucleic acid
RSD	Relative standard deviation
SRP	Soluble reactive phosphorus
TEMED	N,N,N′,N′-Tetramethylethylenediamine
TWA	Time-weighted average
UK	United Kingdom
US	United States
WHO	World Health Organization
XRF	X-ray fluorescence
Zn	Zinc
ZrO	Zirconium oxide

List of Units

m	milli 1 x 10^{-3}
µ	micro 1 x 10^{-6}
n	nano 1 x 10^{-9}
ppm	parts per million
ppb	parts per billion
w/v	weight/volume
v/v	volume/volume
g	gram
kg	kilogram
ml	Millilitre
L	litre
mm	millimetre
cm	centimetre
m	metre
h	hour
s	second
min	minute
d	day

M	mole
˚C	degree Celsius

Chapter 1 Introduction

1.1 Rational of the Study

For everything we take from the earth, there is always a by-product or consequence. Environmental pollution is perhaps a prefiguration of the imbalance of nature. It occurs when the natural environment is incapable of destroying an element without creating hazard and damage to itself. Some profit from the Earth whereas many others, not only humans but also wildlife, suffer from disease, destitution, and harm due to the resulting pollution. The problem of the environment must be taken seriously as it poses a threat to the essential natural elements such as water, soil, and air, which all living creatures rely on for existence on the planet. In today's world, about 40% of deaths are caused by environmental pollution (Lang, 2007). It has been suggested as the cause of the majority of human cancers (Tomatis, 1990). Soil and water pollution were also taken as encitonmetal carcinogens (Kasprzak et al., 2003). The carcinogenic chemicals even exist in the daily food intake such as the residuum of pollutants used in processed food products or pesticides from crops and vegetables. Aside from humans, environmental pollution has an enormous impact on plants and animals too. For example, nitrogen and phosphates in water could cause toxic algae blooms, which reduce the growth of plants in littoral zones, while also decreasing the success of predators that need light to pursue and catch prey by reducing the light penetration (Chislock et al., 2013). The heavy metals in polluted soil could replace the essential nutrients at cation exchange sites of

plants (Chibuike and Obiora, 2014). The hazard of soil pollution could destroy the microorganisms, which are the crucial layers of ecosystems and cause more negative effects on the upper layers (Ramakrishnan et al., 2011). Contaminants exist in the modern environment, many from anthropogenic and sustained release (Rhind, 2009). Industrial activities, dumping solid waste, combustion of fossil fuels, agricultural waste, and transportation are the major sources of anthropogenic chemical pollutants in the environment (Hoffmann, 1993).

With the constant appearance of the negative issues in the environment, such as climate change, people have realised the seriousness of pollution. The 1972 Stockholm Declaration proclaimed that 'the protection and improvement of the human environment is a major issue which affects the well-being of peoples and economic development throughout the world; it is the urgent desire of the people of the whole world and the duty of all Governments' (UNEP, 1972). In 1992, the largest ever first Earth Summit, the United Nations Conference on Environment and Development (UNCED, 1992), also known as the Rio de Janeiro Earth Summit, was convened to identify ways to halt the disruption of irreplaceable natural resources and root pollution of the planet (UNCED, 1992).

With the advance of science and technology, a variety of methods and techniques were developed to solve the problem of environmental pollution. Preventing potentially harmful substances from reaching the harmful level of pollution is an efficient way to prevent pollution entirely, which requires the monitoring of areas at risk. However, once

prevention has proven unsuccessful, the remediation must be performed in the polluted area, accompanied by further monitoring to verify recovery. Therefore, monitoring is a key element in the protection of the environment, which is essential in preventing and remediating the pollution. A critical part of the monitoring process is collecting the representative samples of the environment to ensure the accuracy of the monitoring and to quantify the contamination (Strobl and Robillard, 2008). Generally, pollutants can be measured in flux or concentration, with *in situ*, on-site, automatic, and grab sampling. The choice of sampling methods are usually based on budget constraints, the availability of people, and the goals of the assessment programme (Erickson et al., 2013).

Grab sampling is when the samples are manually collected in the field and transported back to a laboratory for analysis; it is the most common sampling technique in water and soil monitoring. This method is the cheapest and the easiest to operate. However, the main drawback of this approach is that only a snap shot of the concentration of pollutants is provided during a small amount of time, and due to the concentration of analytes in the environment varying over time it can produce an inaccurate measurement. Some unpredictable variations may also occur in samples during transportation and storage. Due to the issues associated with grab sampling, *in situ* sampling as a more accurate and quicker alternative to the traditional approach. In *in situ* measurement, sensors or probes are placed in the environment and accumulate pollutants continuously. *In situ* measuring devices provide time-series data that reproduce the natural process at the maximum extent.

The *in situ* passive sampling technique of diffusive gradients in thin-films (DGT) has been used in various fields of research, including water quality monitoring (Schintu et al., 2010b, Sherwood et al., 2009), chemical speciation in solution (Balistrieri and Blank, 2008, Pesavento et al., 2009), dynamic processes in waters and soils (Town et al., 2009, Warnken et al., 2007, Dahlqvist et al., 2007, Oporto et al., 2009, Ernstberger et al., 2005), and bioavailability in waters and soils (Ferreira et al., 2008, Bradac et al., 2009, Cattani et al., 2009, Pérez and Anderson, 2009). Compared to the commonly used active sampling methods, the DGT technique has specific advantages, including its convenient deployment for obtaining time-integrated concentrations of analytes and its low effective detection limits for trace species (Panther et al., 2008, Guan et al., 2015). Although DGT has been successfully developed in many fields, most of the methods still need the complicated and expensive instrumental analysis in a laboratory, especially for metals. Therefore development of DGT combined with other techniques such as the rapid screening technique, rather than the conventional analysis method, is important for the further use of DGT in more visible, efficient, and cost-effective *in situ* monitoring.

1.2 Research Aim

The aim of this study was to develop a rapid screening technique combining the DGT device with colour development and colour intensity measurements for phosphorus and metals contaminants, with a view to them being used routinely in monitoring and risk

assessment of waters and soils. It is necessary to test out various binding phases and colour reagents with DGT devices that may perform differently in P and metals measurements. Once a suitable binding phase has been found, DGT is able to assess P and metals quantitatively by colour response with or without colour reagents. Instead of analysis by ICP-MS or other complicated instruments in the laboratory, the concentration of P and metals were determined by a flat-bed scanner directly.

In order to implement a rapid and cost-effective monitoring of P and metals in water, this technique was used to determine if the concentration of P and metals reach the safe concentration level of regulations and standards set by different countries. As well as using a scanner, visual inspection was also considered in order to achieve easier and quicker monitoring.

In addition to developing DGT combined with the colorimetric method, this study also investigated the feasibility of using DGT to measure in situ labile metal concentration and toxicity simultaneously. A novel Bio-DGT has been developed by immobilising bioreporters in the diffusive gel layer of the DGT devices, various conditions of bioreporter immobilisation and viability, and environmental factors need to be tested in order to ensure the stable performance of Bio-DGT.

The newly developed rapid screen technique was applied in waters and soils for the chemical monitoring by combining DGT and colorimetry, *in situ* in different environmental conditions of waters and soils. As the research developed, it became clear that the rapid screening devices based on the DGT technique for assessing P and

metal concentrations qualitatively and quantitatively is a reliable tool in environmental monitoring.

1.3 Structure of the Thesis

The thesis has seven chapters.

Chapter 1 is an introduction to the thesis. It sets out the rationale of the study and the research aim. The serious problem of environmental pollution is introduced briefly. The harmful effects of environmental pollution have received more attention in the last few decades, and actions and responses have been undertaken around the world. The introductory chapter describes the importance of using suitable monitoring methods in order to prevent environmental pollution.

Chapter 2 presents the background literature, including a brief review of the environmental role of trace metals and P, their toxicity in high concentration, existing *in situ* monitoring techniques and rapid screening techniques in environmental monitoring, DGT theory and application, development in colorimetric DGT, and methodological needs for risk assessment.

In Chapter 3, a rapid screening and detection technique for phosphorus based on well-tested Metsorb DGT devices and a colour imaging method using the conventional molybdenum blue has been developed and fully tested under different conditions. Precise interpretation and quantification of the phosphorus concentrations are carried out in this chapter. Comparison between the pervious DGT method, using the similar

scanning technique and this rapid detection technique, has been undertaken. It demonstrated how this technique was simplified and modified. Finally, the developed approach was applied to the *in situ* monitoring of P in natural water.

Chapter 4 demonstrates a rapid screening device based on the DGT technique for assessing metal concentration qualitatively and quantitatively. The potential of using Chelex DGT and high resolution Computer Imaging Densitometry (CID) to make a rapid estimation of metal concentrations in waters was investigated. Since a distinctive colour will appear on the binding gel when the amount of metals (copper, nickel and cobalt) accumulate at a certain level, no further colour reagent was involved in this study. The performance of the technique was tested in different conditions. A DGT deployment guide list was formulated to determine if the concentration of metals has exceeded the Maximum Contaminant Level, based on the regulation standards in different countries and regions. Additionally, a rapid screening technique for Cr (VI) using DGT and a high resolution CID base on the surface colouration of the N-Methyl-D-glucamine (NMDG) binding gel reacting with the diphenylcarbazide in an acidic solution was developed.

In Chapter 5, a novel approach with biological material incorporated in the DGT (Bio-DGT) was developed to measure *in situ* labile metal concentrations and toxicity simultaneously. Whole-cell toxicity bioreporter ADPWH_recA was immobilised in the diffusive gel. The performance of the technique under different environmental conditions was fully tested in laboratory solutions and in soil samples collected from

China.

Chapter 6 focuses on the application of the rapid screening technique in field sites. Different types of DGT were applied in five regions of China. The field applicability of the DGT with colorimetry approach for measuring P and metals was investigated. The efficiency and accuracy of DGT devices in distinguishing different degrees and types of contamination in various water systems was also evaluated.

The work is concluded in the final chapter (chapter 7) by summarising major findings from each chapter. The limitations of the study are discussed, and the potential solutions are recommended along with the avenues for further research.

Chapter 2. Literature Review

2.1 Heavy Metals in Water and Soil

Heavy metals exist in waters and soils naturally. However, they can be toxic or poisonous at high concentrations. The main threats heavy metals pose to human health are associated with exposure to lead, cadmium, mercury, and arsenic(Jarup, 2003). Some metals may be beneficial to living organisms in trace amounts, since they are used to stabilize protein structures, facilitate electron transfer reactions and catalyze enzymatic reactions. For example, copper, zinc, and iron are all essential constituents of the catalytic sites of several enzymes (Ash and Stone, 2003). Other metals, however, such as lead, mercury, and cadmium may displace or take the place of an essential trace metal, and interfere with the proper functioning of enzymes and associated cofactors. Heavy metals are bioaccumulative, which makes them hazardous to biotic systems. Heavy metals are widely used in electronics, machines and other artefacts of everyday life. Other high-tech applications also rely on their various chemical properties to function. As a result, they are able to enter into aquatic systems and food chains from numerous anthropogenic sources as well as from the natural geochemical weathering of soil and rocks.

Metals are usually present at low or very low concentrations in the oceans, however, in coastal waters, metals can occur at much higher concentrations, probably due to inputs from river systems (Torres et al., 2008). Mining wastes, landfill leaches, civil

wastewater, urban runoff and industrial wastewaters are the main sources of contamination in water (Gautam et al., 2015). With an increasing demand for new and better technologies and increasing industrial development, the metal pollution as a result of waste disposal is becoming more and more serious. Many aquatic environments are confronted with metal concentrations that exceed the water quality standards formulated to protect the environment, animals and human beings. For example, New Caledonia is among the five major nickel producers in the world and extended portions of its fringing reefs are impacted by extensive nickel mining activities (Wantiez, 2008), which contribute primarily to metal discharges (Fichez et al., 2005, Hédouin et al., 2006, Metian et al., 2008). Among these metals, cobalt is associated with nickel in the laterites of the mining sites and the most recent mines in New Caledonia, and throughout the world, launch the mining of cobalt, as a by-product of the treatment of nickel.

The heavy metal pollution of aquatic environments has attracted much attention due to its environmental toxicity, abundance and persistence (Islam et al., 2015), Generally, heavily polluted aquatic sites are impoverished or completed denuded in flora and fauna, thus the pollution adversely affects aquatic biodiversity(Kelly et al., 2012). Some heavy metals were used as a pesticide such as copper sulphate which can kill bacteria, algae, molluscs, and fungi that demonstrated it is highly toxic to plants and aquatic organisms (Rojik et al., 1983, Domogalla, 1956). The toxicity of copper sulphate relies on the copper content. In fact, copper is one of the most toxic metals to aquatic organisms and ecosystems (Solomon, 2009)

Bioaccumulation of heavy metals in tissues of animals has received considerable attention because of the lethal and sublethal effects of such accumulation(Burger et al., 1994). Birds have treated as early warnings for a Varity of environmental contaminants such as DDT, pesticides and heavy metals. Their feathers are ideal for assessment of heavy metals because they accumulate certain heavy metals in proportion to blood levels at the time of feather formation (Burger, 1993). Except harmful effect on fauna, the heavy metals in polluted soil could replace the essential nutrients at cation exchange sites of plants (Chibuike and Obiora, 2014). The hazard of heavy metals could destruct the microorganisms, which was the crucial layers of ecosystem and causing more negative effect on the upper layers (Ramakrishnan et al., 2011, Giller et al., 1998).

In addition, heavy metals and metalloids may accumulate in soils through emissions from the rapidly expanding industrial areas; mine tailings; disposal of high metal wastes; use of leaded gasoline and paints; application of fertilisers and animal manures; sewage sludge; pesticides; wastewater irrigation; coal combustion residues; spillage of petrochemicals; and atmospheric deposition(Chen et al., 2005, Zhang et al., 2010). For example, Democratic Republic of Congo (DRC) produces about half of the world's cobalt and is Africa's largest copper producer. Water in DRC is unfit for human consumption and agriculture because soil and water in the immediate vicinity of the mines are polluted by discharges of wastewater. Precious research has shown that people living close to DRC's mines had 43 times the level of cobalt in their urine than is considered normal (Fleur et al., 2016).

Soils may become contaminated by the accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition (Khan et al., 2008, Zhang et al., 2010) .

In recent years, heavy metal contamination in China's urban and agricultural soils is rapidly getting worse with the development of industrial activities. According to Bulletin on National Survey of Soil Contamination, jointly issued by the Ministry of Environmental Protection and the Ministry of Land Resources nearly 4 million of hectares of arable lands have been contaminated moderately or severely, which accounts for about 2.9% of China’s arable lands. 16.1% of soils were exceeds environmental standard. Most of soil contamination is inorganic (82.8%), seconded by organic, and the third is complex contamination. The main contaminants in arable land soil are Cd, Ni, Cu, As, Hg, Pb, DDT and PAHs. (Su et al., 2014b).

When the concentration of metals is higher than the required range, even essential trace elements might have a harmful effect on the human body. For example, high-level exposure to copper dust can cause nose, eyes and mouth irritation and may cause nausea and diarrhoea. Continuous exposure to such conditions may result in kidney damage and even death. Copper is also toxic to various aquatic living beings, even at very low concentrations. Another essential element, zinc, can cause nausea and vomiting in

children when they are exposed to it in large amounts. In higher concentrations, zinc may cause anaemia and cholesterol problems in human beings (Gautam et al., 2015).

There are many other toxic effects caused by heavy metals. The diseases named Itai-itai and Minamata, which are found in Japan, were caused by relatively low concentrations of Cadmium and Mercury. Chromium, commonly used in leather and tanning industries as well as paper and rubber manufacturing applications, is also toxic and exposure to it adversely affects the central nervous system and may result in liver and kidney damage or skin ulceration (Gautam et al., 2015). Extensive use of chromium compounds in industrial applications has created a dramatic increase in the amount of wastewater discharged into aquatic systems, which contains toxic chromium species.

2.2 Phosphorus in Water and Soil

Phosphorus (P) is a key element in biological reactions. Consequently, changes in phosphorus availability could have great influences on the function and structure of an ecosystem(Tiessen, 2001). Phosphorus moves in a cycle through rocks, water, soil, sediments and organisms. Unlike many other biogeochemical cycles, the atmosphere does not play a significant role in the movements of phosphorus because phosphorus and phosphorus-based compounds are usually solid within the typical temperature and pressure ranges found on Earth. Most naturally occurring phosphorus takes the form of phosphate (PO_4). Phosphates are a component of DNA, RNA, ATP and the phospholipids, which makes phosphorus an essential element for life. A reduced

concentration of phosphate in the blood serum is a disorder known as hypophosphatemia. Phosphorus deficiency may cause bone diseases such as rickets in children and osteomalacia in adults. An improper balance of phosphorus and calcium may cause osteoporosis (Darshana, 2010).

Phosphorus is also an essential element in modern agriculture on account of its importance to plant growth and seed production (USDO, 2011). In Agriculture, the most common use of P is as a fertiliser for crops. The appropriate use of phosphorus leads to higher grain production, improved crop quality, greater stalk strength, increased root growth and earlier crop maturity (Douglas and Philip). In the last five years over 40 million tonnes of phosphorus fertiliser has been used yearly to support crop production systems all over the world (FAO, 2015). As a result, Phosphorus cannot be substituted by any other element in these biological functions. It has also been used extensively in fertilisers to meet food production requirements, which continue to increase due to the tremendous growth of the global population. Early sources of P used in agricultural production were organic materials. In the past, bones and guano (seabird droppings) were the most important sources of fertiliser within the commercial industry (Jacob, 1964). Since then, the use of phosphorous in soils for crop production has become increasingly widespread. Now, phosphate rock is the main raw material used in the production of practically all phosphate fertilisers in modern agriculture (Sims and Sharpley, 2005).

It takes about one tonne of phosphate to produce 130 tonnes of grain, although this

figure is highly variable depending on soil conditions, farm history, crop type, and fertilising efficiency (Vaccari, 2009). This figure, coupled with the importance of phosphorus as an agricultural resource, highlights the immense pressure the world's mineral reserves are placed under. About two-thirds of the world's phosphate rock is mined by only three countries: China, the United States and Morocco. China imposed a 135% export tariff.in 2008, and the reserves of the United States are expected to deplete within the next 25 years (Vaccari, 2009). Both countries are two of the largest consumers of phosphorus. The largest reserves of phosphorus are controlled by Morocco, a country that, contrary to the United Nations resolutions, annexed the Western Sahara in the 1970s to gain control of these reserves. Since the global production rate of phosphate rock is expected to increase incrementally from 223 million tonnes in 2015 to 255 million tonnes in 2019 (Stephen, 2016) and the U.S. Geological Survey (USGS) estimates global phosphate reserves at 71,000Mt (Jasinski, 2012), it is estimated that current production levels will exhaust these reserves in less than 80 years.

In natural environments, phosphorus is mainly present in its particulate form as a mineral with low solubility. Many aquatic ecosystems are controlled by a restricted availability of phosphorus, which represents one important factor for high biodiversity. In upland waters, where there is limited P input from diffuse or point discharge, the concentration of soluble reactive P (SRP) can be less than 10 $\mu g\ L^{-1}$ which restricts biological productivity (Mainstone and Parr, 2002). However, the anthropogenic

discharge of phosphorus into fresh water bodies (lakes, rivers, and reservoirs) and coastal waters increases the level of nutrients in these waters, which has caused undesirable changes in their ecology and the balance of species (plants, fish, etc.). For example, P concentration can dramatically increase the productivity of phytoplanktonic algae. The decaying algae uses up vast quantities of dissolved oxygen, which affects fish populations and can produce low-oxygen dead zones (Mark, 2009). This phenomenon results in a dense growth of algae, which is known as eutrophication. The main anthropogenic sources of phosphorus within the aquatic environment are municipal and industrial waste waters, drainage from agricultural land, excreta from livestock, and diffuse urban drainage (Helmut Kroiss, 2011). Most particularly, human excreta, phosphorus containing household detergents, and some industrial and trade effluents constitute the main sources of phosphorus contamination in wastewater. Run-off P from over-fertilised and manure-rich farmland is the main source of P contamination in water bodies (Chen et al., 2014, Sharpley et al., 2003). It is also important to note that excessive application of P-fertiliser is a key contributor to the major phosphorus pollution of agriculture soil. As well as undermining farmers' ability to produce sustainable food and energy from their fields, the use of fertiliser contributes towards air pollution, soil acidification and degradation, water eutrophication, and crop yield reduction (Carpenter, 2008). Since the early 1980s, pH has declined from 0.2 to 0.8 across China, mostly due to the overuse of fertiliser (Guo et al., 2010).

Thus, although some of these metals are essential for life, high concentrations of

heavy metals and phosphates in water or soil can cause serious environmental problems if their levels exceed the admissible range. Consequently, heavy metal and phosphate concentrations need to be monitored for assessment and remediation purposes.

2.3 Importance of Environmental Monitoring

Water pollution is one of the major environmental problems in today's world. It poses a threat to human welfare and obstructs the sustainable development of both society and economy. According to the United Nations World Water Assessment Programme, every day, 2 million tonnes of sewage, industrial and, agricultural waste are discharged into the world's water (UN, 2010). In addition, unsafe or inadequate water, sanitation, and hygiene cause approximately 3.1% of all deaths and 3.7% of DALYs (disability adjusted life years) worldwide (WHO, 2002). In 2012, over 800,000 deaths worldwide were caused by contaminated drinking water, inadequate hand-washing facilities, and inappropriate sanitation services. In the seas and oceans, there is a growing number of de-oxygenated dead zones caused by the discharge of untreated wastewater. They affect an estimated 245,000 km^2 of marine ecosystems, impact fisheries, peoples' livelihoods, and various food chains (UN, 2017).

Water pollution is not the only threat to the environment. Soil, food, and water contamination is caused by the toxic chemicals used in and created by agriculture, as well as ever increasing amounts of domestic and industrial solid waste. According to the EU, the most frequent contaminants of Europe are heavy metals and mineral oil,

and it is estimated that approximately 3 million sites have been affected by activities that can pollute the soil (Science Communication Unit, 2013). Of these sites, approximately 250,000 are in need of urgent remediation. More seriously, arable land is turning to desert and becoming non-arable at ever-increasing rates. This is due, in part, to global warming and the use of agricultural fertilisers and pesticides. In 1990s, the United Nations Food and Agricultural Organisation states that 75 billion tonnes of fertile soil, the equivalent of nearly 10 million hectares of arable land, is lost to erosion, waterlogging and salination every year. Another 20 million hectares is abandoned because its soil quality has been degraded (Pimentel and Burgess, 2013). A two-year study further reports that if the current rate of soil loss in China continues over the next 50 years, food production will decrease by 40% (Jie, 2010) . However, the chemical pollution of soil is an insidious risk because it is harder to observe than many other soil degradation processes, such as those caused by mining and industrial activity or by sewer and waste mismanagement. The hazards posed by the chemical pollution of soil depend on how soil's properties affect the behaviour of the chemicals and the speed at which they enter ecosystems.

The existence of heavy metals, chemical toxins, and organic or inorganic pollutants in water and soil needs to be monitored constantly to protect the population's supply of clean drinking water and control the impact of these pollutants on the environment and the ecosystem (Wang, 2013). Here, 'monitoring' is defined as a routine assessment of environmental quality, which involves using a sound experimental design to measure

causes (pollutants) and effects (ecosystem impact) over a number of years (Karydis, 2013).

Since a number of international conventions, treaties and laws were passed to protect and govern regional seas and coastal areas, monitoring has become a powerful and decisive tool in environmental policy (DiMento, 2012). Laws and regulations such as the Urban Waste Water Treatment Directive (C.E.C., 1991) and the Water Framework Directive (C.E.C, 2002) have proved successful in protecting the aquatic environment largely as a result of good monitoring systems. There is a wide variety of methods that can be used to monitor water quality and identify a range of different water pollutants in the aquatic environment. Accurate, intensive and long-term data is key to assessing the circumstances of the world's water resources and creating a fully functioning and successful preservation or renovation program.

Pollutants found in soil are generally more difficult to measure than those found in water because of the way that the contaminants interact with soil particles (Aelion, 2004). The best techniques, generally speaking, are those that are inexpensive and relatively easy to carry out using field-sampling instruments. However, the accuracy and reliability of the measurements by using those instruments may far less than using more difficult, time consuming and expensive techniques. Conventional methods, such as the extraction methods used in laboratories, often involve time-consuming sample preparations, which are no longer attractive for monitoring of contaminated soils in situ. Some fast and novel methods have been investigated, such as the infrared reflectance

spectroscopy, and X-ray fluorescence, which was used to estimate heavy metal pollution in soils (dos Anjos et al., 2000, Shi et al., 2014).

2.4 Importance of *in situ* Monitoring

Over years, a variety of monitoring methods and techniques have been developed to meet the problem of heavy metals and P pollutions assessment. Traditionally, water-sampling techniques are mostly based on taking water samples away from their environment and into a laboratory for analysis. Since this technique can only measure the concentration of contaminants in the sampled water at the time of analysis, use of this approach is not suitable for monitoring purpose owing to temporal and spatial restrictions. For example, the levels of heavy metal or phosphorus found in water bodies will fluctuate temporally depending on natural occurrences, in response to human activity, or natural changes within the population of aquatic plants and animals (Goldman, 1983). Increasing sampling frequency is not always feasible because of its high cost and impracticality when accessing remote sites. Furthermore, the quality of samples may be compromised by inaccurate manual operation caused by holding the samples for too long before analysis or avoidable wastage in transportation. These operational mistakes may result in changes to chemical speciation. If only total concentrations are required, speciation changes are not a big issue. However, further information on chemical speciation is required, as it is known to have an impact on mobility, bioavailability and related eco-toxicity of elements (Yuan et al., 2011).

To overcome the shortcomings mentioned above, there has been a recent increase in the number of monitoring programs developed for in situ measurement. Many new in situ hydrological technologies have been developed that can be interfaced with cost-effective, real-time monitoring networks. These techniques use a range of deployment times and allow for constant monitoring so that changes and trends of metals and nutrients can be detected rapidly (Divis et al., 2005, Glasgow et al., 2004). Compared with the traditional monitoring techniques, this approach has several advantages. Streamlining the data collection process conceivably reduces human errors and working time, decreasing the total cost of data collection, and increasing the quantity and quality of temporal and spatial scales. Another major advantage of in situ measurement is its ability to measure any slight fluctuations caused by the dynamic processes, which contribute and sustain the concentrations of heavy metals and phosphorus. Various in situ sensors have been conspicuous in real-time monitoring owing to their splendid properties and rapid development in last decades. For example, Electrochemical-based instruments are among the most widely used devices for in situ chemical analysis and include conductometric, potentiometric and Amperometric/Voltammetric electrode systems (Denuault, 2009). The Voltammetric *In-situ* Profiling System (VIP System) was developed for continuous, real-time monitoring of trace elements in fresh and seawater at up to 500 meters depth. The VIP System allows one to perform direct *in-situ* measurement of the mobile fractions of Cu^{II}, Pb^{II}, Cd^{II} and Zn^{II} as well as Mn^{II} and Fe^{II} using either Square Wave Anodic Stripping Voltammetry (SWASV) or Square Wave

Cathodic Sweep Voltammetry (SWCSV) (Howell et al., 2003, Tercier-Waeber and Taillefert, 2008). In addition, a high resolution in situ UV spectrophotometer was used to measure nitrate, bisulphite, and bromine in the sea (since these species have distinctive UV absorption spectra) and can be deployed for over three months at the depths of 2km without any degradation of performance due to biofouling or instrumental drift (Johnson and Coletti, 2002, Johnson et al., 2006). Moreover, there are plenty of electrochemical sensing techniques that have been investigated to determine phosphate in aqueous solutions as well (Villalba et al., 2009, Warwick et al., 2014). Additionally, a Molecularly Imprinted Polymer (MIP) based receptor, which can selectively bind phosphate, has been developed (Warwick et al., 2014) to overcome the poor selectivity of most sensors when measuring the range of P concentrations in wastewater (0.1-15mg L^{-1}) (Modi et al., 2011, Tafesse and Enemchukwu, 2011, Kumar et al., 2010).

Other than chemical monitoring, biomonitoring has become a beneficial and widely used technique that is based on the sensitivity of organisms to subtle currents or chronic exposure to heavy metals. The deleterious effects of altered biochemical and/or physiological states of organisms are reflected faster and at lower levels. These techniques employ biomonitoring or bioindicators with attractive advantages compared to traditional methods (Zhou et al., 2008, Sures, 2004, Nachev et al., 2010). Aquatic insects and other benthic invertebrates are the most widely used organisms in freshwater biomonitoring of human impact. For instance, talitrus saltators and barnacles are used

as biomonitors to detect trace metals (Cd, Hg, Cr, Cu, Fe, Mn and Zn) in coastal waters (Fialkowski et al., 2009, Kuklina et al., 2013). Fish, crayfish, and mussels are also been used as bioindicators to monitor water quality (Brumbaugh et al., 2005, Kuklina et al., 2013). There is reported use of plants for monitoring trace metals and inorganic substances as well (Whitton and Kelly, 1995, Demars and Thiebaut, 2008). There are a number of factors can influence the results of these kinds of tests, including the metabolism, depuration rates, excretion, stress, viability, and condition of the test organism. Biomonitoring is limited by confounding factors that are not related to pollution and these should be carefully considered when interpreting biomarker data (van der Oost et al., 2003). Furthermore, the extraction of analytes from the tissue of animals prior to instrumental analysis is complex (Vrana et al., 2005).

In the future, in situ methods are expected to be taken for environmental monitoring and thus improve scientific understanding of ecosystems and protect the environment.

2.5 Passive Sampling

A critical part of the monitoring process is collecting the representative samples of the environment to ensure the accuracy of the monitoring and to quantify the contaminations (Strobl and Robillard, 2008). Heretofore, many alternatives have been sought to overcome some of the difficulties of on-site monitoring. Of these, passive sampling methods have shown much promise as tools for measuring aqueous concentrations of a wide range of priority pollutants.

2.5.1 The Principle of Passive Sampler

Passive sampling is based on the unassisted molecular diffusion of sample matrix (analytes) through a diffusive surface to a receiving phase (Greenwood et al., 2007). After sampling, the adsorbed analytes are desorbed from the adsorbent by a solvent or through thermal desorption. Put simply, a passive sampling device is placed in the environment and accumulates analytes based on the interaction of the bulk solution with the collection medium of the device. Unlike active sampling, passive samplers require no electricity, have no moving parts and are simple to use.

Despite its relatively long history (over 20 years), passive sampling is still developing and in the last few years, remarkable progress has been made in passive device design, calibration methods, and quality commitment. Publications on passive sampling have grown substantially since the 1990s, with over 200 journal publications a year as of 2008 (Zabiegala et al., 2010). There are two general sampling regimes that determine analyte uptake in passive samplers, and they are the equilibrium-base and kinetic-base samplers. The sampling process is similar for both types of samplers (Figure 2.1) (Mayer et al., 2003, Vrana et al., 2005).

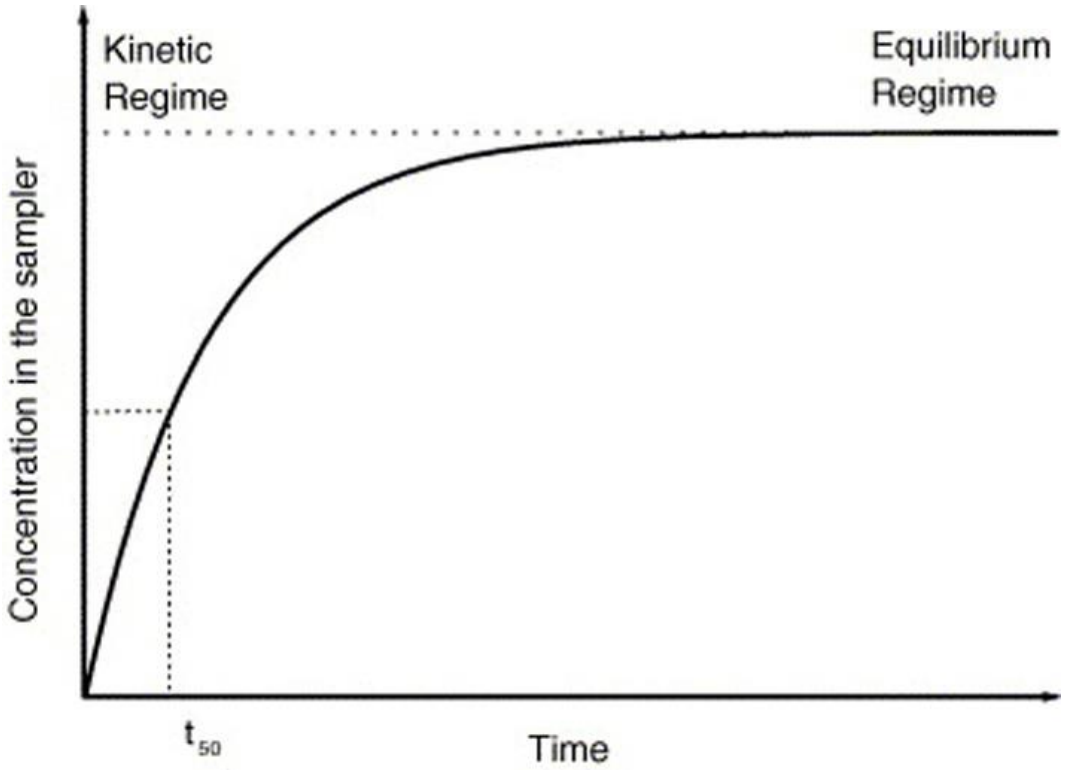


Figure 2.1 Concentration of passive samplers in two sampling regimes.

Once they are exposed to the medium being examined, they collect the analyte molecules that reach the collecting medium by diffusion through a static layer of the examined medium, which is contained in the well-defined openings of the sampler, or by penetrating a nonporous membrane. In both cases, the driving force for the transport is the difference in chemical potential of the analyte on both sides of the barrier. Ultimately equilibrium is obtained between the collecting medium and the bulk solution, and then the collecting medium is removed and analysed. Equilibrium passive sampling does not provide quantitative information on the concentrations of the pollutants in the environment, but it indicates the level of contamination in the monitored compartment of the environment. The first passive sampling methods were created for aquatic systems in 1974 and used to monitor the concentrations of

dissolved trace metals in natural water by measuring equilibrium concentrations in the water enclosed by the dialysis membranes (Beneš and Steinnes, 1974). Since this experiment, Semipermeable Membrane Devices (SPMD) have become the most frequently used equilibrium passive sampler in water quality control. In 1990 it was reported that SPMD were able to indicate the bioavailability of organic pollutants (Huckins et al., 1990). It also has been applied in soils to determine the relationship between the partial pressure and mobility for monoaromatic and polyaromatic pollutions (Hayes and Soni, 2006). A screening methodology based on SPMD to determine the bioavailable petroleum hydrocarbons (BPHs) has many benefits, including a reduced reliance on the use of live test organisms and reduced cost of estimating the bioavailability of non-polar organic contaminants in soils (Lanno et al., 2000)

On the other hand, kinetic-based samplers do not reach equilibrium; instead, they assume that the sampling rate maintains constant throughout the period of sampling and the relationship between the concentration of target analytes in the sample matrix and the amount of analytes extracted is linear. Under the kinetic regime, a passive sampler provides the Time-weighted Average concentration (TWA) of the analyte in the sampled environment over a known period of time (Zabiegala et al., 2010). A crucial advantage of kinetic based passive sampling is the use of an *in situ* pre-concentration of the analyte, which improves the detection limit of the method used for analysis. In addition, kinetic-based samplers can response to pollution from

occasional events normally not detected with spot sampling. This characteristic of the method is a valid improvement to the accuracy of monitoring areas where the concentration of contaminants in water are variable (Vrana et al., 2005). When a receiving phase, such as a chelating resin, which has a high affinity for the species measurement, was added to equilibrium dialysis, the diffusion rate is theoretically directly proportional to the concentration of metals in the water being sampled (Beneš, 1980). If the receiving phase is selected properly, the bioavailable metal species can be separated. In these circumstances, diffusion through the dialysis membrane may imitate metal transport processes through biological barriers. Chelex 100, which is typically used as the chelating resin, showed an efficient, measurable uptake of soluble heavy metal at very low contamination concentrations. Besides, Supported Liquid Membrane devices (SLM), chemcatchers and Gradient in Thin-film (DGT) devices are the kinetic-based passive samplers that are most frequently used in water monitoring for inorganic compounds.

2.5.2 Frequently-used Passive Samplers for Inorganic Pollutants

Liquid Membranes (LMs) is a sample pre-treatment technique based on Liquid-Liquid Extraction (LLE) and Solid-Phase Extraction (SPE) techniques. LMs borrow from these two techniques for their abilities to perform analyte enrichment and sample-matrix separation compared to the two former techniques, LMs have multiple advantages such as lower sample volumes, decreased cost and time of analysis, higher

analyte-enrichment factors and selectivity (Jonsson and Mathiasson, 2001, Keith et al., 2007). LMs are generally classified into three basic types: bulk LMs (BLMs); emulsion LMs (ELMs); and supported LMs (SLMs) (Lopez-Lopez et al., 2010). The SLM process was a promising technology since it possesses many attractive features. It has high selectivity and combines extraction and stripping into one single stage. The SLM is a non-dispersive type of LM, whose membrane phase is immobilized in the pores of a porous polymer. The polymeric support, which usually consists of microporous hydrophobic polymers, does not play an active role in the separation, but provides a structural support for the membrane phase (organic extractants), which is the active component in the separation (Parhi, 2013). It is a promising separation and pre-concentration technique that is well suited for trace metal speciation in natural water (Ndungu et al., 2005). SLM devices have been used to measure metals (Zn, Ni, Co, Cd, Mn, Cu and Pb) in natural waters. Effects of turbulence, pH and concentration variations on the performance of SLM devices have been reported (Parthasarathy et al., 1999, Slaveykova, 2004, Parthasarathy et al., 2004). The Permeation Liquid Membrane technique (PLM), which is based on the carrier-mediated transport of metals across a hydrophobic membrane and developed to further improve SLMs, is used to determine trace metal speciation and concentrations (Slaveykova, 2004). However, its stability is one of the biggest limitation of SLM. It also suffer from the liquid phase evaporation (Dżygiel and Wieczorek, 2010).

Another passive sampler, Chemcatcher, followed the principle and design of the DGT

(diffusive gradient in thin-films) and developed in the early 2000. It comprises a polypropylene body with a receiving phase (47 mm diameter disk), a cellulose acetate diffusion limiting membrane, and a protective, open mesh. A chelating resin or bound chromatographic stationary phase (e.g., C_8 or C_{18}) is used to monitor metal species in aquatic environments (Persson et al., 2001). The working principle of the chemcatcher is based on Fick's first law of diffusion: the sorbent is supposed to be sufficiently high to maintain a zero concentration at the surface of the receiving phase.

$$\mathrm{m} = -D\frac{dC}{dx} \tag{2.1}$$

Where m is the mass flux (g $cm^{-2}s^{-1}$), D is the diffusion coefficient (cm^2s^{-1}), C is the concentration of the compound (g cm^{-3}), x is the distance in the axial direction (cm), and dC/dx is the concentration gradient across the diffusion path x (cm).

It follows that mass transfer across an area can be described by equation 2.2.

$$\dot{M} \equiv \frac{dM}{\mathrm{dt}} = -\mathrm{D}\frac{\mathrm{dC}}{dx}A \tag{2.2}$$

Where $\dot{M}$ is the mass flow (g s^{-1}), A is the cross-sectional area (cm^2) of diffusion, and M is the mass (g). The chemcatcher has been used to monitor the time-averaged concentrations of trace metals (Pb, Cd, Cu, Hg etc.) and inorganic compounds like nitrate (NO_3^-) and phosphate ($HPO4^{2-}$) in a wide range of aquatic environments, such as storm water and wastewater (Aguilar-Martinez et al., 2008, Sanchez-Bayo et al., 2013). However, this technique is relatively sensitive to variation in water turbulence which may cause inaccuracy in water monitoring. The physical conformation (cavity) and relatively thin diffusion membrane of the latter suggest that changes in the thickness

of the diffusive boundary layer ensuing from variations in turbulence may result in significant changes in uptake rates (Allan et al., 2008, Allan et al., 2007).

Of these techniques, DGTs are one of the most flexible and accurate for aqueous metal sampling (Sigg et al., 2006).

2.6 DGT Application

2.6.1 Principles of DGT

Sampling for organic compounds in aquatic environments gained more attention in the late 1990s when there was an increasing number of publications on the subject. The application of passive samplers to inorganic species has been slower, but the development of Diffusive Gradients in Thin-film (DGT) samplers in 1994 accelerated scientific interest in the passive sampling of inorganic chemicals, and since then there has been widespread interest in such monitoring techniques. The DGT technique provides in situ quantitative speciation measurements in waters, soils, and sediments.

Typically, the DGT device comprises a filter membrane, a diffusive gel, and a binding phase, as shown in Figure 2.1. During a given time, ions are continuously accumulated in the DGT and a steady-state linear concentration gradient is established between the solution and the binding phase. This drives solutes to move from the bulk solution to an effective zero concentration at the interface between the diffusive gel and the binding phase. These three layers are then assembled into a plastic, water-sealed device comprised of a cap and a base, which has a 3.14 cm^2 or 2.54 cm^2 window that exposes

the filter membrane to the solution or soil. After diffusing through the filter membrane and the diffusive gel, the solutes irreversibly taken by the binding phase. The diffusion of large molecules is partly impeded by the hydrogel in DGT, but simple metal ions can diffuse almost freely with the molecular diffusion coefficient from solution.

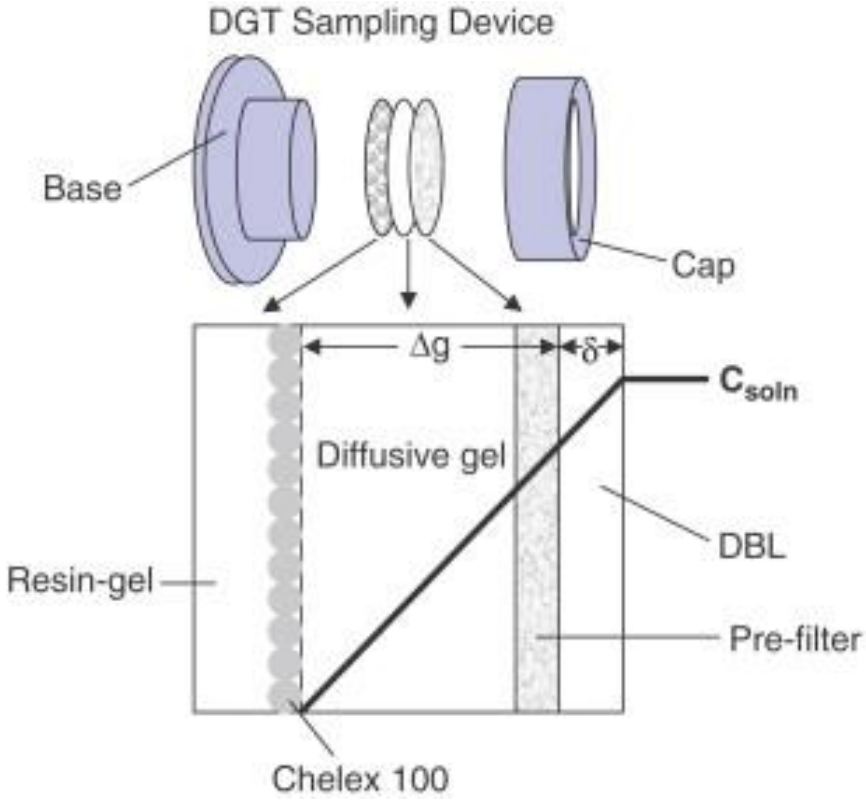


Figure 2.2 The DGT device consists of a base and cap, which contains the pre-filter, diffusive gel and binding phase. The Diffusive Boundary Layer (DBL) extends out from the device's face into the bulk water where the concentration is C_{sol}.

The DGT technique is based on Fick's first law of diffusion. A binding agent such as a resin, selective to the target ions in the solution, is immobilised in a thin layer of hydrogel (binding-gel). It is separated from the bulk solution by an ion-permeable gel membrane of thickness Δg as shown in Figure 2.2. Between the gel layer and the bulk solution, there is a Diffusive Boundary Layer (DBL), of thickness δ, where the transport of ions is solely by molecular diffusion. If δ is negligibly small compared to Δg, the

flux, J, of metal ions diffusing through the gel layer to the resin can be expressed by equation 2.3.

$$J = D\frac{C - C'}{\Delta g} \tag{2.3}$$

Where D is the diffusion coefficient in the gel, C the free concentration of a metal ion in bulk solution, and C' is the free concentration of the metal ion in the binding phase. If the free metal ions are in rapid equilibrium with the resin, with a large binding constant, C' is effectively zero providing the binding phase is not saturated. The DBL is a water film between the bulk solution and the DGT's surface, where ion transfer is solely by diffusion. The thickness of the DBL depends on the fluid velocity and the shape and dimensions of the sampler. For accurate interpretations its thickness needs to be considered, the Δg is effectively increased when there is an extended diffusion layer.

Direct measurement of the total mass accumulated in the binding phases is possible with techniques capable of analysing solids, such as laser ablation inductively coupled plasma mass spectroscopy (LA-ICP-MS), or X-ray fluorescence (XRF). Alternatively, metal ions in the resin layer can be eluted using a known volume of acid or base (V_e). The concentration of ions in the eluent, C_e, can then be measured by ICP-MS or UV-spectrophotometry. In practice, only a fraction of the bound metal is eluted. The ratio of the eluted to bound metal is known as the elution factor, f_e. Taking the elution factor into account, the accumulated mass, M, of ions in the binding phase can be calculated from equation 2.4 where V_g is the volume of gel in the binding phase.

$$M = \frac{C_e\ (V_e + V_g)}{f_e} \tag{2.4}$$

M can be used to calculate the flux through the known sampling area, *A*.

$$J = \frac{M}{At} \tag{2.5}$$

By equating equations 2.3 and 2.5 and rearranging the solute concentration in the bulk solution can be obtained by equation 2.6:

$$C_{DGT} = \frac{M\Delta g}{DAt} \tag{2.6}$$

As the known value of the sampling area, *A*, the diffusive layer thickness, Δg, and the relationship between *M* and bulk water concentration, *C*, is only controlled by the deployment time, *t*, and the diffusion coefficient, *D*, of solute in the diffusive layer.

2.6.2 Application of the DGT Technique in Environmental Monitoring

There is a need to develop new monitoring tools for the evaluation of chemicals in water that are able to comply with the requirements of the Water Framework Directive (WFD). In this sense, DGTs provide an alternative that overcomes the shortcomings of traditional water sampling. DGTs have been used for the evaluation of trace metals in rivers (Divis et al., 2007, Dragun et al., 2008),lakes (Gimpel et al., 2003), estuaries(Dunn et al., 2003, Wallner-Kersanach et al., 2009, Wu et al., 2011) and coastal waters(Schintu et al., 2008, Slaveykova et al., 2009, Schintu et al., 2010a). After a series of studies, it is recognized that trace metal bioavailability and toxicity depends on their speciation (Alfaro-De la Torre et al., 2000, Meyer, 2002, Peijnenburg and Jager, 2003). Therefore, the time-integrated concentration measured using DGTs refers to the labile metal fraction, which includes free ions, inorganic complexes, and labile organic

complexes (Zhang and Davison, 2000, Twiss and Moffett, 2002).The labile metal fraction is considered a better proxy for determining potential biological adverse effects than the total dissolved metal concentrations measured using spot sampling. The speciation of metals with the DGT device relies on two effects: the relative difference in diffusion coefficients, and the relative difference in affinity of the binding agent and the species to be characterised. It is possible to differentiate between inorganic labile species and organic labile species by systematically using a variety of different diffusion gels with different pore sizes. This results in a size-discriminating uptake, which is similar to voltammetry. However, the diffusion coefficients of the model species have to be determined individually so that accurate measurements of the concentration of labile species can be taken (Zhang and Davison, 2000). Over the past few years, DGTs are increasingly being used for the determination of metal speciation in a wide variety of media, such as natural waters and wastewater. According to available reports, DGTs were applied to a variety of labile metal fractions currently monitored in waterworks, such as Al， Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn (Meylan et al., 2004, Gaabass et al., 2009, Liu et al., 2013). Moreover, it has been reported that there is potential for DGTs to predict metal accumulation in organisms and their possible toxicity (Tusseau-Vuillemin et al., 2004, Royset et al., 2005). In addition, DGTs helped select an adequate sampling strategy for the monitoring of transitional water bodies (Montero et al., 2012).

DGT provide an indirect way to measure the maximum available concentration of pollutants in soil and water, and consequently an estimation of potential uptake by plants

when exposed to soil solution (Zhang et al., 1998b, Lombi et al., 2002, Zhang et al., 2001, Degryse et al., 2009). Comparing the DGT assessed fraction (DGT estimated concentration, C_E) with soil solution (soluble concentration, C_s) has been related to the resupply rate, R_{diff}, which increases as the soil is able to resupply elements to the soil solution upon depletion (Williams et al., 2011). It is a stimulating parameter in agricultural soils where plants are continuously exploiting available nutrient resources from the soil (Menezes-Blackburn et al., 2016). Besides, compared to other techniques, such as soil extraction, the DGT technique was one of the most widely applicable methods for the assessment of heavy metal bioavailability in various soils affected by different sources metal pollution (Soriano-Disla et al., 2010).

DGTs have also been used to assess phosphorus in water and soils widely. The first binding agent for measuring phosphorus with a DGT device was the ferrihydrite gel, which had a strong affinity for phosphorus and was used to measure labile phosphorus (Zhang et al., 1998a). Following this, a titanium dioxide gel-assembled DGT was used to measure phosphorus, arsenic and other metals simultaneously (Panther et al., 2010, Bennett et al., 2010, Panther et al., 2013). A Zr oxide gel was developed to measure phosphorus and inorganic arsenic with high capacities (Ding et al., 2010, Sun et al., 2014). Since the development of a binding gel for P analyses, DGTs have been used to assess P levels in soils in numerous studies, and this technique has successfully demonstrated the effect that P fertilisers have on the yield of crops like wheat, tomato, and maize (McBeath et al., 2005, Mason et al., 2010, Menzies et al., 2005). Recently,

the DGT technique used an iron oxide based binding gel to successfully determined both DRP and low molecular weight organic P species such as adenosine monophosphate (AMP) (Mohr et al., 2015).

2.7 Colourimetric Application

In situ quantification, i.e. measurement of a response in the binding phase, would provide an opportunity to further simplify the passive sampling technique. For example, the incorporation of colourimetric reagents into the passive sampler, to permit direct colourimetric quantification of the adsorbed analyte, would enable in-field quantification of the passive samplers. In the late 1970s, Yoshimura et al. have proposed "ion-exchanger absorptiometry" as a way of increasing the sensitivity of colourimetric analyses, with the sample species being adsorbed onto a resin, and absorption spectrophotometry measurements made directly on the resin-bound complexes (Yoshimura et al., 1978). Colourimetric spot tests and semi-quantitative tests have long been used(Feigl and Anger, 1972), but unfortunately, many of these commercial test strips are effective at mg L^{-1} concentrations, whereas environment guidelines require measurements at μg L^{-1} concentrations (Takahashi et al., 2006). As a commercial example, the Merckoquant® Test measures a selection of common inorganic ions through the use of 'dip sticks', in which colour intensity is dependent on analyte concentration. The results can be obtained to mg L^{-1} levels when it is combined with a hand-held reflectance spectrophotometer (Wetselaar et al., 1998).

Combining this type of technique with the pre-concentration abilities of the DGT technique has the potential to improve detection limits significantly. It can also provide an accurate in situ analysis of time-weighted average metal concentrations, without further time and financial expenses of taking analyses in the laboratory. Numerous colour-responding metal dyes have been found and discussed (Cheng et al., 1982). DGT samplers enable in situ colourimetric quantification of the metal ions accumulated in the DGT binding phase by using resin-bound dyes that provide a metal-specific response. Cu(II) has been successfully determined by Methylthymol blue (MTB) and the immobilisation of diphenylcarbazide(DPC), both of which are widely used for the detection of many metal ions (McGifford et al., 2010, Feng et al., 2011). Furthermore, it has been reported that a combined DGT-colourtimetric DET technique has been used to investigate the distributions of inorganic As and Fe(II) (Bennett et al., 2012).

Recently, a colourimetric sensor, which can eliminate the need for analytical instruments, has attracting a lot of attention (Wallace, 2009, Moores and Goettmann, 2006). The development of colourimetric metal ion sensors is commonly based on manipulating Au NPs, which possess intrinsically strong surface plasmon resonance (SPR) absorptions, with extremely high extinction coefficients (10^8–10^{10} M^{-1} cm^{-1}) in the visible wavelength range and different sensing elements such as DNA, enzymes, proteins (Wang and Ma, 2009). Since these colourmetric sensors require several steps of preparation before they can be used, the technique is complex and relatively expensive to undertake. Modification-free Au NP-based colourimetric sensors have

been developed to simplify the detection process (Nath and Chilkoti, 2004, Lee et al., 2008). For example, a label-free alkanethiol/Au NPs-based sensor was developed for selective colourimetric detection of Hg, Ag, and Pb in water and soil by recording their UV-vis absorption spectra using a μ-Quant microplate reader (Hung et al., 2010). Moreover, a β-galactosidase(B-GAL)-based colourimetric paper sensor for rapid, selective, and sensitive detection of heavy metals has been highly discussed (Hossain and Brennan, 2011). This sensor is a solid-phase bioactive lab-on-paper sensor that was inkjet printed with sol–gel entrapped reagents to allow colourimetric visualisation of the enzymatic activity of B-GAL. The colour intensity of the sensing areas was monitored either by the naked eye, by obtaining a digital camera or by using a flat-bed scanner, with ImageJ software used to analyse the Jpeg images in the latter two cases.

2.8 XRF and Computer Imaging Densitometry Analysis as Rapid Scanning Techniques

Traditionally, the levels of trace elements in soils and waters have been measured using Atomic Absorption Spectrometry (AAS), or Inductively Coupled Plasma Mass Spectrometry (ICP-MS), or Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) after acid digestion of the samples (Han et al., 2006, Butcher, 2010). These methods are very sensitive and relatively accurate, nevertheless, hazardous water was produced, and there were significant time and high running costs, particularly when large numbers of samples were tested.

The X-ray Fluorescence spectrometer (XRF), which is considered to be a rapid screening tool for locating hot spots of uncontaminated field soils and sediment, has attracted much more attention (McComb et al., 2014). Though XRF analysers are not as sensitive in detecting most and trace elements, such as ICP-OES or ICP-MS, they can provide non-destructive, faster, cleaner and more affordable results for a wide range of elements, which require limited soil sample preparation (Marina and Lopez, 2001). As a result, the Total Reflection X-ray Fluorescence (TXRF) has become a versatile tool for a fast and simple screening of heavy metals and trace elements in contaminated water, soil, and sediment samples (Stosnach, 2005). Good correlations between the certified and measured values were achieved for Mg, S, Cl, K, Ca, V, Cr, Mn, Co, Ni, Cu, As, Se, Br, Rb, Sr, Ba, Pb in water. In addition, the XRF technique was used to determine the average concentrations of toxic metals in the region, and the associations between different toxic elements and their spatial distribution (Gowd et al., 2010). It has also been reported that this technique is a rapid, safe and accurate procedure for the simultaneous, non-consumptive analysis of Si and phosphorus in as little as 0.1 g dried and ground plant material using a portable X-ray Fluorescence Spectrometer (P-XRF) (Reidinger et al., 2012). Apart from the advantages of XRF mentioned above, the P-XRF was able to analyse smaller amounts of plant material, as well as being very compact and easy to store. The P-XRF was a particularly valuable instrument for many laboratories because of its ability to accurately analyse elemental levels in soil as well as plants, both *in situ* and *in vitro*. However, XRF analyses cannot distinguish ions of

the same element in different valence states. In practice, most commercially available instruments are very limited in their ability to precisely and accurately measure the abundances of elements with Z<11 in most natural earth materials (Wirth, 2017).

In addition to the laboratory instruments, a conventional flat-bed scanner was used in a rapid scanning technique to determine the concentration of analyte related to the colour intensity, such as the B-GAL-based colourimetric paper sensor mentioned above. The computer-imaging system used to analyse the colour 'density' was termed 'Computer Imaging Densitometry' (Teasdale et al., 1999). 'Density', in this case, refers to the amount of 'colour' in a system, for example in a greyscale range, white is considered as no density and black was maximum density. The inverse of this concept is luminosity, the quantifying value for CID work, where a white sample provides maximum luminosity. A desktop scanner was first used to quantify the colour in the National Institute of Health (NIH) Image by using the luminosity value obtained when the image was examined by the computer-imaging system. Following this, Shishkin and co-worker took solid-phase colourimetric analysis a step further by investigating the possibility of using a desktop scanner and digital processing software to quantify coloured substances adsorbed on polyurethane foam (Shishkin et al., 2004). In the past few decades, the conventional flat-bed scanner was combined with readily available image manipulation software to provide a simple, flexible, and effective way to analyse colour intensity (Adu et al., 2014, Göröcs and Ozcan, 2014). In a similar manner, measuring dissolved sulphide concentrations quickly has been achieved using a grey-

scale measurement of the AgS formed by an AgI impregnated DGT sampler (Teasdale et al., 1999, Naylor et al., 2004). This has recently been extended to measuring sulphide and Fe (II) using colourimetric techniques, with the colour being quantified using a conventional flat-bed scanner and image processing software (Jezequel et al., 2007). The DGT technique also successfully determined the accumulated mass of labile phosphorus in sediments and soils by measuring the greyscale intensity on the gel surface using CID (Ding et al., 2013).

2.9 Summary

The importance of *in situ* monitoring and passive sampling was demonstrated in this review. A variety of methods and techniques have been discussed for monitoring heavy metals and phosphorus in waters and soils. The developemnt of a novel rapid screening technique based on DGT and colorimetry has been the ultimate goal of this work. The most appropriate method for achieving this goal would be considered and tested with the wide variety of DGT methods and screening techniques. The methods evaluated and the results of the development are discussed in the following chapters.

Chapter 3. Rapid in situ Detection of Available Phosphorus in Waters and Soils by Combining DGT and Colourimetry

3.1 Introduction

3.1.1 Phosphorus Contamination in Waters and Soils

The use of chemical phosphorus fertiliser has had a dual effect. On the one hand, as a critical component of the "green revolution", the dramatic increase in production and use of phosphorus fertiliser has contributed considerably towards increasing agricultural productivity and reducing hunger worldwide (Tilman, 1998). One of the side effects of using a chemical phosphorus fertiliser is phosphoric acid, a chemical compound which acidifies the soil. When Chinese farmers acidify their soil by applying fertiliser they impede crop growth by restricting themselves to growing acid tolerant species and varieties, thus reducing profitable market opportunities. Since the early 1980s, pH has declined from 0.2 to 0.8 across China, mostly due to the overuse of fertiliser (Guo et al., 2010). Beginning in the 1970s, ever-increasing amounts of fertiliser were used across China to achieve the Chinese farmers' dream of bigger harvests. However, this increased use of fertilizer has led to rising in water pollution (Hvistendahl, 2010). Phosphorus is rarely found in high concentrations in freshwaters as it is actively taken up by plants. However, the transfer of P from agricultural systems to surface water exacerbates the potential for eutrophication (Cooper et al., 2002).

In the Midwestern United States, over-fertilisation was the norm from the 1970s until the mid-1990s. During that period, tonnes of excess nitrogen and phosphorus entered the Mississippi River Basin and drained into the Gulf of Mexico, where the large influx of nutrients has triggered huge algal blooms. The decaying algae use up vast quantities of dissolved oxygen, producing a seasonal low-oxygen dead zone in the Gulf that reaches a size bigger than the state of Connecticut in some years (Mark, 2009). In China, livestock seems to be the largest contributor to run-off pollution and is responsible for 56% of phosphorus discharges. This excess of phosphorus has caused eutrophication in many of China's lakes, coastal waters and rivers (Qiu, 2010a, Qiu, 2010b). The International Lake Environment Committee (ILEC), in cooperation with the United Nations Environment Programme (UNEP), undertook a project entitled "Survey of the State of the World Lakes". In it, almost all 217 lakes show increasing eutrophication, including Lake Dianchi and Lake Taihu in China. These lakes suffer from the most extreme eutrophication; almost all native water plants and many fish species have been killed. Even large lakes like Lake Victoria in Africa, or Lake Baikal, the largest freshwater body in the world, show signs of eutrophication (UNEP, 1994). Increased algae production in rivers and lakes has had a huge ecological impact, reducing the diversity of flora and fauna, reducing oxygen in water and cause death of fishes during the summer time. Also, the growth of more toxic microbial species can be hazardous and pose a considerable threat to livestock and human health (Sharpley et al., 2003).

Since the balance of environmental quality and crop yield is teetering on a point,

precision agriculture has become a hot field of research in international agriculture science (Chandrajith et al., 2010, LJ and TL, 2005). Precision agriculture combines existing agricultural production measures with new, specialised technology, such as Global Positioning System (GPS), Geographic Information System (GIS), miniaturised computer components, automatic control, in-field and remote sensing, mobile computing, advanced information processing, and telecommunications (Jess, 2004, Gibbons, 2000). Precision agriculture aims to optimise field-level management with regards to crop science, environmental protection and economics, for example, fertilisation decision, irrigation water control, crop planting density (Zhang et al., 2002).

3.1.2 Monitoring Phosphorus in Waters and Soils

Therefore, monitoring phosphorus is important as an early warning system which highlights signs of water eutrophication and plays an irreplaceable role in precision agriculture (Hakanson et al., 2007, Sims et al., 1998). In addition, given the accumulation of P in soils and its corresponding environmental threat, the development of effective soil monitoring has become necessary (Lemercier, 2008).

The soil tests used to measure P levels vary according to land uses, cropping regimes, soil types (Wheeler et al., 2004, Skinner and Todd, 1998, Cahoon and Ensign, 2004). Olsen, Colwell, Bray P1, Mehlich I and Mehlich III are the most commonly used soil P test methods (Wolf and Baker, 1985, Quirine and Pete, 2010, Sarker et al., 2014, Moody, 2007). The advantages and disadvantages of these extraction procedures and their

effects on the resolution of ^{31}P NMR spectra, which enables the quantitative identification of various P species in environmental samples (Cade-Menun and Liu, 2014, Turner et al., 2003, Vestergren et al., 2012) have been discussed extensively in previous studies (Cade-Menun and Preston, 1996, Turner et al., 2005, Cade-Menun and Liu, 2014). Nowadays, one of the most common extraction protocols involves shaking the soil with 0.25 mol L^{-1} NaOH and 0.05 mol L^{-1} Na_2EDTA for 16h, followed by lyophilisation. However, as with any alkaline treatment, this protocol risks sample alteration. The alteration include the distortion of the original distribution of P compounds in the samples because of selective extraction of certain P species, compound-specific vulnerability to alkaline hydrolyses (especially digester), possible mobilisation of unavailable orthophosphate from soil P minerals (*e.g.*, Ca-, Fe-, and Al-phosphates) and P complexed at surfaces which would result in an overestimation of the soil orthophosphate pool as a result of strongly alkaline conditions (Kruse et al., 2015). It has also been identified that measuring the total concentration of phosphorus in soils does not provide sufficient information to assess its bioavailability. Established soil testing methods used to assess the availability of P, such as Colwell and Olsen, have difficulties reliably predicting plant P requirements within a range of soil types (Holford et al., 1985, McBeath et al., 2005, Mason et al., 2010). Owing to the current failure of existing soil test methods to predict plant responsiveness to applied P, it is necessary to find a more accurate test for plant-available P (Tandy et al., 2011).

One of the newly developed technique is Diffusive Gradients in Thin-film (DGT).

DGT has been developed to assess bioavailable trace elements, P in waters and soils (Zhang et al., 1998b, Zhang et al., 2001, Menzies et al., 2005, Zhang et al., 2014). The first binding agent for phosphorus DGT was the ferrihydrite gel which had a strong affinity for phosphorus and was used to measure labile phosphorus (Zhang et al., 1998a). Following this, a titanium dioxide gel-assembled DGT has been used to measure phosphorus, arsenic and metals simultaneously (Panther et al., 2010, Bennett et al., 2010, Panther et al., 2013), and Zr-oxide as binding gel was developed to measure phosphorus and inorganic arsenic with higher capacities (Ding et al., 2010, Sun et al., 2014). Since the development of the DGT technique for P, it has been used to assess P in soils in a wide range of studies. The technique has successfully demonstrated its effectiveness in predicting use of P fertilisers on obtaining the optimum yields of crops like wheat, tomato and maize (McBeath et al., 2005, Mason et al., 2010, Menzies et al., 2005).

For phosphorus in water, monitoring commonly involves two basic steps, collecting water samples, and analysing them in the field or in laboratories. This seems to be a simple methodology, however, many studies have shown that the accuracy of the calculated P load is highly dependent on the choice of sampling protocols, analytical protocols, and sampling frequency (Sims and Sharpley, 2005). Furthermore, the commonly used analytical method is based on the molybdate phosphate complex formation whose ultraviolet-visible absorbance corresponds to the phosphate concentrations rather than on a true reflection of the P lability (Reynolds and Davies, 2001). The fact that nonpoint P sources contribute significantly to P levels in some

aqueous systems makes measuring and regulating P even more difficult and challenging (Carpenter et al., 1998). Most aquatic monitoring programmes depend on collecting discrete spot samples of water at a given time. With the frequent garb sampling, the representative flows and the fluctuant concentrations of phosphorus may be captured. However, grab sampling has limitations. For example, in water systems that are prone to flash flooding, the lack of instantaneous water sampling means that the polluting effects of flooding cannot be measured easily and are often missed (Cassidy and Jordan, 2011, Vrana et al., 2005). In addition, it is difficult to measure naturally occurring phosphate concentrations which may be very low (W. et al., 2000). There are methods for measuring phosphorus on-site and frequently such as Flow injection-capillary electrophoresis (FI-CE) systems and the autonomous microfluidic sensor, which were developed to improve sampling protocols, but these methods are still limited in the following areas: automation, continuity and detection (Kuban et al., 2004, Andrew et al., 1994, Cleary et al.). In the past few decades, alternatives have been sought to overcome these difficulties. The diffusive gradients in thin films (DGT) has been successfully developed for determining kinetically labile dissolved phosphorus in aquatic systems including both freshwaters and marine waters (Pichette et al., 2009, Li et al., 2014, Huo et al., 2014, Panther et al., 2010)

However, these DGT methods require an elution of phosphate from the binding gels by placing the gel in acid or base overnight before measuring the pre-concentrated amount of phosphate in DGT devices. This step and the subsequent analysis make the

process more complex and delay data acquisition. The advantages of DGT, such as accumulation and fixation through binding on the resin gels, and the colour development for P analysis can have the potential to eliminate the slow processes mentioned above. Some research have been successfully conducted and reported in combining DGT methods with a scanning technique to directly measure the analytes in the binding gels, such as DGT with silver iodide for sulphides (Teasdale et al., 1999) and DGT with Methylthymol blue absorbed Dowex 1×8 for Cu (McGifford et al., 2010). Another method of measuring P by computer imaging densitometry based on Zr-oxide DGT was also developed recently (Ding et al., 2013). However, the process of making Zr-oxide gel was complicated with poor reproducibility and the sample pre-treatment for colour development required 5 days, unrealistically long time for using in monitoring.

3.1.3 Aim of This Work

In this study, a rapid detection technique for phosphorus based on well tested DGT devices and a colour imaging method using the conventional molybdenum blue has been developed and fully tested under different conditions. Precise interpretation and quantification of the phosphorus concentrations were carried out. This paper has also demonstrated how this technique was simplified and modified. The developed approach was applied to the in situ monitoring of P in waters and the measurement of labile P in soils.

3.2 Materials and Methods

3.2.1 Reagents, Materials and Solution

Milli-Q water was used to prepare all solutions. Potassium dihydrogen phosphate was used to prepare the P stock solution (1000 mg L^{-1}) for carrying out all the testing experiments. Metsorb gels were prepared in Lancaster University laboratory. Metsorb is an agglomerated nanocrystalline titanium dioxide based adsorbent was identified by Bennett and co-workers as an alternative binding phase for the DGT measurement of inorganic arsenic and selenium in water (Bennett et al., 2010). Plastic containers were used for experimental work and for the preparation and storage of solutions. All DGT components (including materials used to prepare DGT gels) were acid-cleaned in 10% (v/v) HNO_3 (AR grade, VWR) and rinsed thoroughly with deionized water prior to use. All chemicals used to prepare solutions were AR grade or higher.

The mixed reagent used to determine P was the molybdenum blue method based on Murphy and Riley (Murphy and Riley, 1962). The proportion of the mixed reagent was slightly adjusted to increase the sensitivity of coloration. It was prepared by using 24g ammonium molybdate tetrahydrate and 0.14 g potassium antimonyl tartrate each in 100ml MQ water. Aliquot of 99.2 mL concentrated sulfuric acid (18M) was slowly mixed well with the above solution. After cooling to room temperature, the mixed solution was diluted to 1000 ml using deionized water. Prior to colorimetric analysis, 1.76 g ascorbic acid was added to 100ml deionized water. It must be prepare freshly and used within 2h. For 10mL final mixed reagent for P determination, 3mL ascorbic acid

and 7ml mixed stock solution are needed. This final reagent contained 0.017 M MoO_4^{2-} and had a pH value of 0.5±0.02.

3.2.2 Preparation of the DGT Assemblies

The Metsorb binding gels were prepared according to (Bennett et al., 2010). The gel sheet was cut into discs with a diameter of 2.5 cm. The piston-type DGT holder with a 2-cm diameter exposure window and space for 0.04 cm diffusive gel was supplied from DGT Research Limited (Lancaster, UK). The diffusive gel was omitted in this work to enhance the sensitivity of the technique. A 0.04 cm thickness PTFE spacer which was acid-cleaned in 10% (v/v) HNO_3 (AR grade, VWR) was used to fill the gap and to make sure the window edge was sealed. In the DGT assembly, the binding gel was placed in the middle of two 0.14-mm hydrophilic polyethersulfone filter membranes (0.45 μm pore size, Acrodisc). The PTFE spacer was placed underneath binding gel and the filter membranes to make sure the binding gel was tightly held in the DGT device. The membrane filters were cleaned three times using MQ Water before use. Zr-oxide binding gels were tested for comparison. The details of the two preparation procedures for preparation of Zr-oxide gel has been described previously (Ding et al., 2010). ZrO DGT was assembled with the same procedure as for Metsorb DGT. DGT samplers were assembled in a Class 100 laminar flow cabinet within a Class 1000 clean room.

3.2.3 General Procedures

3.2.3.1 The Uptake of P by DGT

The DGT devices were deployed into 2L well-stirred solutions containing 0.01 mol L^{-1} $NaNO_3$ and with different concentration of P (20 μg L^{-1}, 200μg L^{-1}, 2000μg L^{-1}) for different length of time (up to 24h), depending on the purposes of each respective experiment. This process was carried out in triplicate for each binding gel at each P concentration.

3.2.3.2 Colour Development

The deployed DGT devices were rinsed with deionized water before being placed in a 100 mL straight Polystyrene sample tube containing 20 mL of mixed reagent for direct colour development without disassemble the device as pervious study (Ding et al., 2013). Different reaction time (10 min, 20 min and 30 min) were test at room temperature (20-22 ^{o}C)

3.2.3.3 CID Analysis

After retrieval from the mixed reagent, the binding gel discs and the downside filter membrane were peeled from the DGT. The Metsorb gel discs were rinsed using cool deionized water and immersed in cool water for 5 min to stop further colour development. The water adhering to the surface of the gels was removed gently using hydrophilic polyethersulfone filter paper. Both Metsorb and ZrO gel discs were then

scanned using a flat-bed scanner (HP G3110) at a resolution of 300 dpi with the side of the gel surfaces where titanium dioxide and Zr-oxide had settled. The grayscale intensity of the scanned images corresponding to the open window of the solution DGT unit was finally analysed with Image J 1.48.

3.2.4 Calculation

The concentrations of P were calculated using the DGT equation 2.6. Specifically, the mass, M, in this study could be calculated from the calibration curve (Figure 3.1) using the directly measured grayscale intensity on the gel surface and also can be calculated by equation 2.4 with a known eluted volume of 1M NaOH (V_e).

3.2.5 Validation of Metsorb DGT and Colouration Technique

3.2.5.1 Calibrating the P Measurement.

Calibrating the P measurement was performed by establishing the relationship between the grayscale intensities of the Metsorb gel and its accumulated masses of P after the DGT deployments. It was tested by deploying eight sets of six replicate DGT devices in 2L of well-stirred solutions containing 0.01mol L^{-1} $NaNO_3$ with different concentrations of P (20 μg L^{-1}, 200μg L^{-1}, 2000μg L^{-1}). The pH of the solution was adjusted and maintained at pH 6.5±0.5, and the solution samples were taken after the removal of each set of the DGT devices. They were removed after 6 h, 12 h and 24 h deployments, then rinsed with deionized water. Three Metsorb gels were eluted in 1.5

ml of 1mol L^{-1} NaOH for 24 h with an elution efficiency of 92±5% (Panther et al., 2010). The other three DGT units were immersed in 20 ml of the mixed reagent at room temperature (20-22 ^{o}C) for 20 minutes. After a brief rinse with MQ water, the binding gel discs were retrieved from the devices and placed on a flat scanner for the grayscale intensities measurement using CID, as described earlier. The relationship between the masses of P accumulated in the gels and their corresponding grayscale intensities was fitted using an exponential equation for the whole range and a linear equation for the lower mass part.

3.2.5.2 DGT Detection Limits

DGT detection limits were calculated as three times the standard deviation of the DGT blanks (n=12). DGT blanks were prepared in the same protocol as the deployed DGT devices expect for deployment. Blank analyses were assessed as follow: 12 DGT devices were placed in a deployment tank with 8 L of 0.01 M $NaNO_3$ solution for 24h. 6 Metsorb binding gels were eluted in 1M NaOH for mass measurement and the grayscale intensities on the surface of the other 6 gels were analysed following the same colouration and CID method of deployed DGT samplers.

3.2.5.3 Reaction Time of Colour Development

The optimal time of colour development was tested by deploying five sets of triplicates DGT devices in 2L of well-stirred solutions containing 0.01mol L^{-1} $NaNO_3$

with different concentrations of P (20 μg L^{-1}, 200μg L^{-1}). The mass of accumulated P in DGT is in a range of 0.16 to 3.2 μg after 6 h, 12 h and 24 h deployments. Each of the Metsorb disc was placed in the 100 mL sample tubes containing 20 mL of mixed reagent. Different reaction time (10 min, 20 min and 30 min) were tested at the room temperature (20-22 ^{o}C).

3.2.5.4 Effect of pH on P Measurements

The effect of solution pH on the accumulation of P was tested by deploying quadruplicate DGT devices in solutions of different pH. Devices were deployed for 24 h in 2L of continuously stirred 0.01 M $NaNO_3$ containing 20 μg L^{-1} of P. The measurements were carried out at pH 4.0, 6.0 and 8.0 and the effect of the pH was assessed. Diluted HNO_3 or $NaHCO_3$ was used to adjust solutions to the desired pH.

3.2.5.5 Potential Interference from Oxyanion Metals

To investigate the possible interference of oxyanion metals on the measurements of P, As was chosen as a testing element. Interference that could affect the colouration of P uptake by Metsorb DGT, the performance of the DGT devices, in terms of uptake and colour development, was tested in 200 μg L^{-1}P solutions (0.01 mol L^{-1} $NaNO_3$) containing As in a range of 40-1000 μg L^{-1} at pH 6±0.5 and at room temperature(20 ^{o}C) for 6 h.

3.2.5.6 Comparison of the Performance of the ZrO and Metsorb Binding Agents in Colouration.

The Zr-oxide DGT devices were prepared by Ding and co-workers (Ding et al., 2013). Zr-oxide DGT was immersed in 2L well-stirred solutions containing 0.01mol L^{-1} $NaNO_3$ and a certain concentration of P (20 μg L^{-1}, 200μg L^{-1}, 2000μg L^{-1}) at pH 6.0±0.5. Pistons were removed after 6 h, 12 h and 24 h deployments and the binding gel disc was placed in the 100 ml vessel (with the side where Zr-oxide settled facing up) containing the 20mL of the mixed reagent for colouration. The vessel was kept at room temperature (20-22 ^{o}C) for 45 min.

3.2.6 Field Application.

DGT samplers were deployed in situ at three field sites in Tianjin, China. The first field site included five freshwater streams. The second site included three fish and shrimp farms. The third site was a reservoir for collecting wastewater from a hog farm. Three DGT were deployed at each place for approximately five hours to measure the concentration of P in the waters. The deployment time was calculated based on the variant of Equation 3.1. Where the mass, M, set as 0.64 μg which is the minimum amount of P on gel surface can be observed distinctly and homogeneously. The predefined concentration of P is 0.2 mg L^{-1} which is the standard level of P of Chinese surface water. With a known exposure area, A, the thickness of the diffusive layer, Δg, and the diffusion coefficient in the gel, D, the deployment time can easy to obtain. After

development, the Metsorb DGT devices were rinsed with deionized water and stored in clean plastic bags at 4°C in a fridge until analysis. The sample treatment and analysis are the same as described above for laboratory testing DGT devices. The mass of P accumulated on each device was obtained using the calibration provided and the concentration of P in water was calculated using equation 2.6. Water samples were collect at the beginning and end of DGT deployments. The turbid water samples were pre-filtered through a 0.45μm membrane filters prior to acidification with Sulphuric Acid (pH<2). Samplers were then kept at 4°C until analysis.

3.3 Results and Discussion

The DGT technique of phosphorus colorimetric analysis based on gel surface colouration using the conventional molybdenum blue method has been reported using Zr-oxide binding gel (Ding et al., 2013). Although this technique offers advantages, since colouration equipment is common in most laboratories and the preparation of colouration is simple and easily learned, it still has some drawbacks. First, the process of making Zr-oxide gel is complicated. It's difficult to manufacture using common equipment found in chemical laboratories. Secondly, the pre-treatment of Zr-oxide gel disc is demanding. A 5-day of heating gel disc before CID analysis and a repaid technique is inconformity. In this study, a similar but more practical and simpler combination of DGT with CID is developed by using a Metsorb binding gel in water.

3.3.1 Calibration

The calibration standard in mass of P and the corresponding colour on each binding gel and grayscale intensity measurements are presented in Figure 3.1. It shows that there is a logarithmic increase of grayscale intensity with the accumulating mass of P in the binding gel. The grayscale intensity increased rapidly first and then slower down and reach a plateau. At the accumulated mass < 3.23 μg P, the calibration demonstrates a linear relationship between grayscale intensity and the accumulated mass of P in the Metsorb binding gel. The calibration ranges of grayscale intensity increases from 28 at the background level to 186 at the saturation level. There is no further obvious increase in grayscale intensity with increasing P loading above a mass of 35 μg. The capacity of the Metsorb binding phase for DGT, calculated according to Panther and co-workers (Panther et al., 2011), was ~37μg P per device (3.14 cm^2), which is about 12 μg cm^{-2}.

The relationship between the accumulation of P in Metsorb binding gels and the corresponding changes in greyscale intensity was fitted using an exponential equation, as shown in Figure 3.1. The Relative Standard Deviation (RSD) of grayscale intensities were in the range of 1% to 11%. The colouration was uniformly distributed on the gels with a low RSD, mostly within 4%. However, for the fully quantitative interpretation of P concentration in water using this approach, the linear calibration is necessary. The linear range of 0.3 μg to 3.2 μg correspond to the concentration range of 9 to 98 μg L^{-1} if the deployment time is 24 hours and the water temperature is 20^{o}C. This is for the DGT devices without diffusive gel, which means the diffusion layer thickness is

0.014cm (the filter membrane thickness only). If longer deployment time is needed, for example one week, the standard DGT devices with 0.08cm diffusive gel could be used. The concentration range can be detected fully quantitatively is 9 to 94μg L^{-1}. This range can be extended to 62 to 661μg L^{-1}using shorter deployment time of 24 hours with the standard DGT devices. In summary, the linear range for fully quantitative measurement of P is sufficient for environmental monitoring of water with low P of a few ppb level to waters with high P of several hundreds of ppb P.

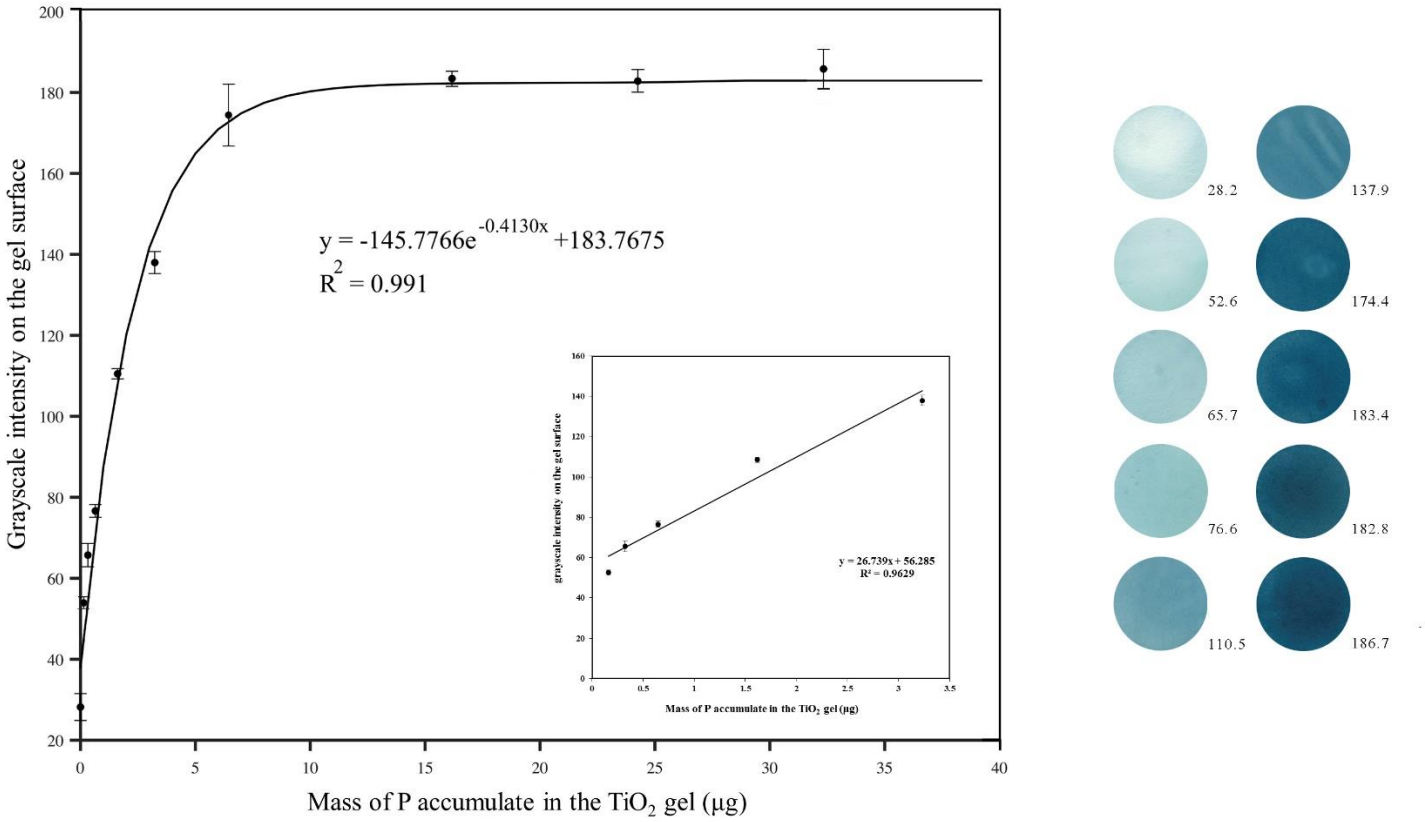


Figure 3.1 The grayscale intensity on the gel surface as a function of the mass of P accumulated by Metsorb gel (left). Mass of P accumulated by DGT in a range of 0.3~3.2μg, a liner equation is presented in the thumbnail (R^2=0.96). Examples of scanned images of the coloured P-loaded gels (right). The numbers on the right corner of each gel image show their grayscale intensity values. The uncertainties associated with each datum point is the standard deviation of the mean from triplicate DGT samples.

3.3.2 Detection limits and Precision

The DGT measured blank for P was 0.75±0.11μg L^{-1}. The blank of grayscale intensity on the gel surface was 28.2±2.7. The method detection limit (MDL) of the DGT technique was calculated as three times the standard deviation of the blank value was 0.44 μg L^{-1}. This detection limits are specific to DGT combined with the molybdenum-blue detection method used in this study. The method precision for data obtained by DGT-measured mass was 9 % and grayscale intensity on gel surface was 13%.

3.3.3 Reaction Time of Colour Development

The grayscale intensity on gel surface increased with the exposure time up to 20 min and followed by a constant state. A liner range of 0.3 μg to 3.2 μg was inspected in reaction time of colour development (Figure 3.2A). Since the grayscale intensity on the high P-loaded gel surface was reached the near maximum of coloration (186) at 20 min (Figure 3.2B), the optional time for colour development was set as 20 min.

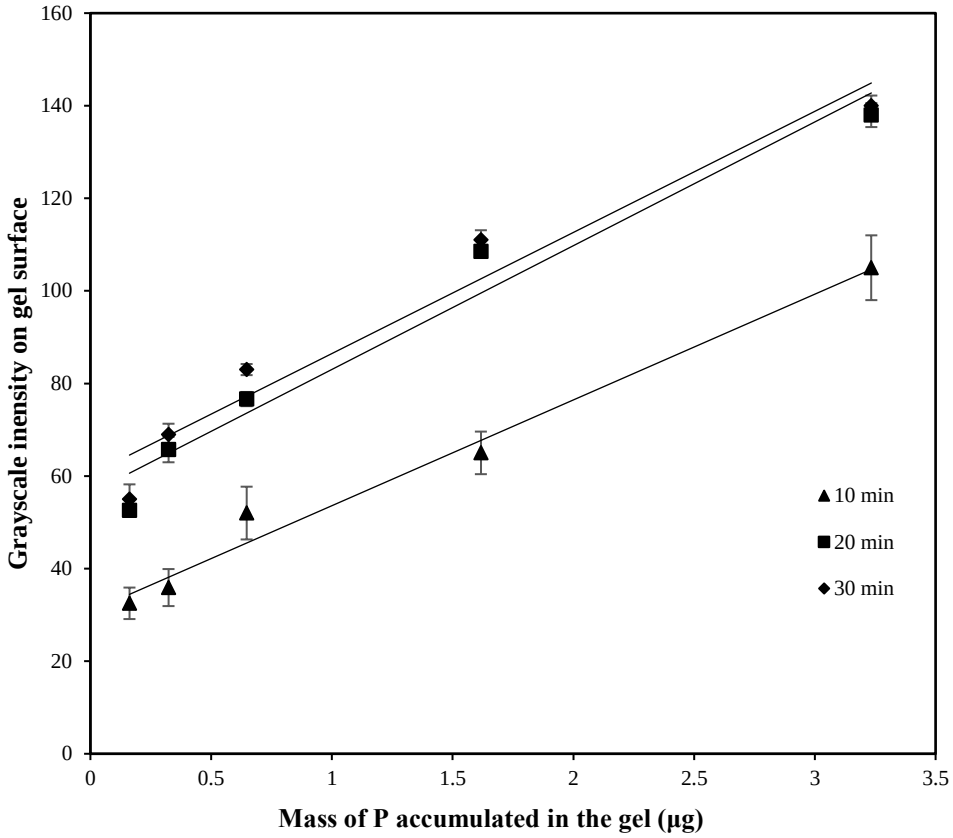


Figure 3.2A Optimization of the reaction time of colour development for coloration based on the changes of grayscale intensity on the surface of the Metsorb gels (0.3 µg to 3.2 µg). The uncertainties associated with each datum point is the standard deviation of the mean from triplicate DGT samples.

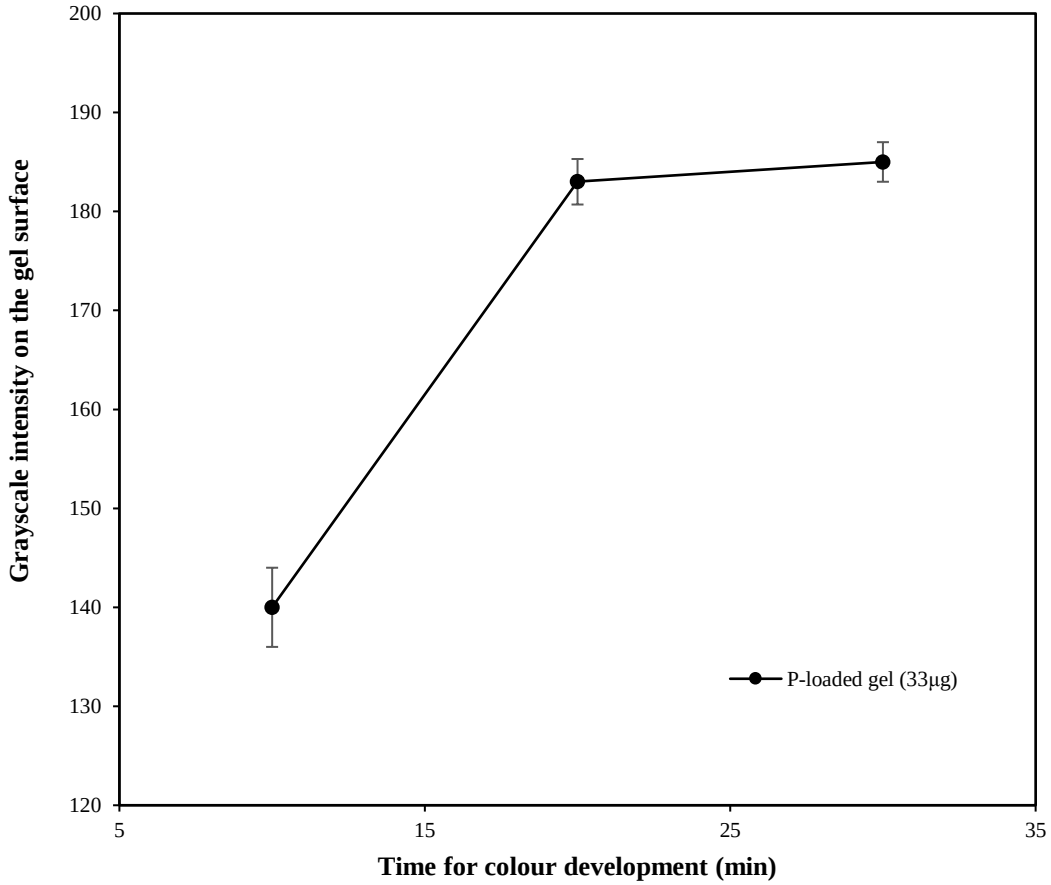


Figure 3.2B Optimization of the reaction time of colour development for coloration based on the grayscale intensity on the surface of the Metsorb gels (33µg). The uncertainties associated with each datum point is the standard deviation of the mean from triplicate DGT samples.

3.3.4 Effect of pH Colouration and Measurements

The effect of pH on the colour development and on the P measurements have been investigated in solutions with different pH. The results were presented in Figure 3.4, showing the greyscale intensity of P-loaded binding gel at different pH values. No significant change has been observed within the pH range of 4-8, indicating the measurements and colour development are not affected by the pH of the water body.

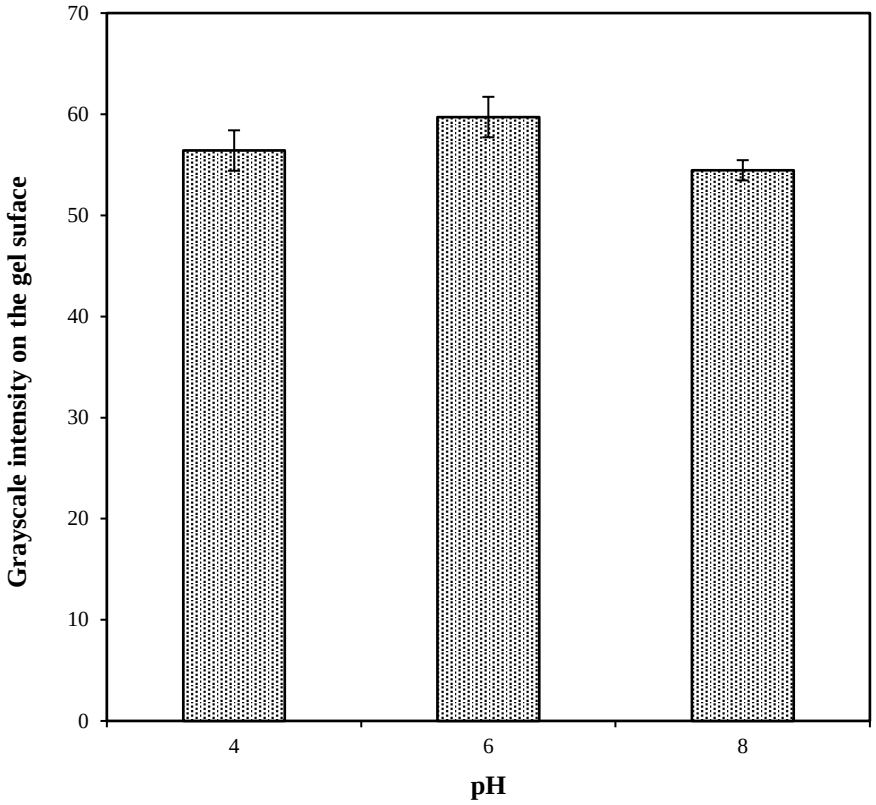


Figure 3.3 The grayscale intensity on the gel surface after it was deployed for 24 h in 2L of continuously stirred 0.01 M NaNO3 containing 20 μg L^{-1} of P at pH 4.0, 6.0 and 8.0 and kept at room temperature (20 ^{o}C).

3.3.5 Potential Interference of Oxyanion Metals

Arsenic and phosphorus are both in group VA of the periodic table and they have the similar chemical properties. Arsenate was found to form blue complexes with ammonium molybdate under similar conditions as phosphate. An over estimation of the concentration would be obtained for the determination of phosphate until arsenate was removed before forming the phosphomolybdenum complex (Pett, 1933, Harvey, 1948).

The results of greyscale intensity measured on the DGT binding gels loaded with P in the presence of different concentrations of As are presented in Figure 3.3. No

significant various were observed, suggesting As does not interfere with the colour development and the P measurements at high concentration of 1000 μg L^{-1} level. However, the China's standard determining method of P suggested that if the concentration of As > 2mg L^{-1} in solution, the interference need to be eliminated by $Na_2S_2O_3$ (MEP, 1990).

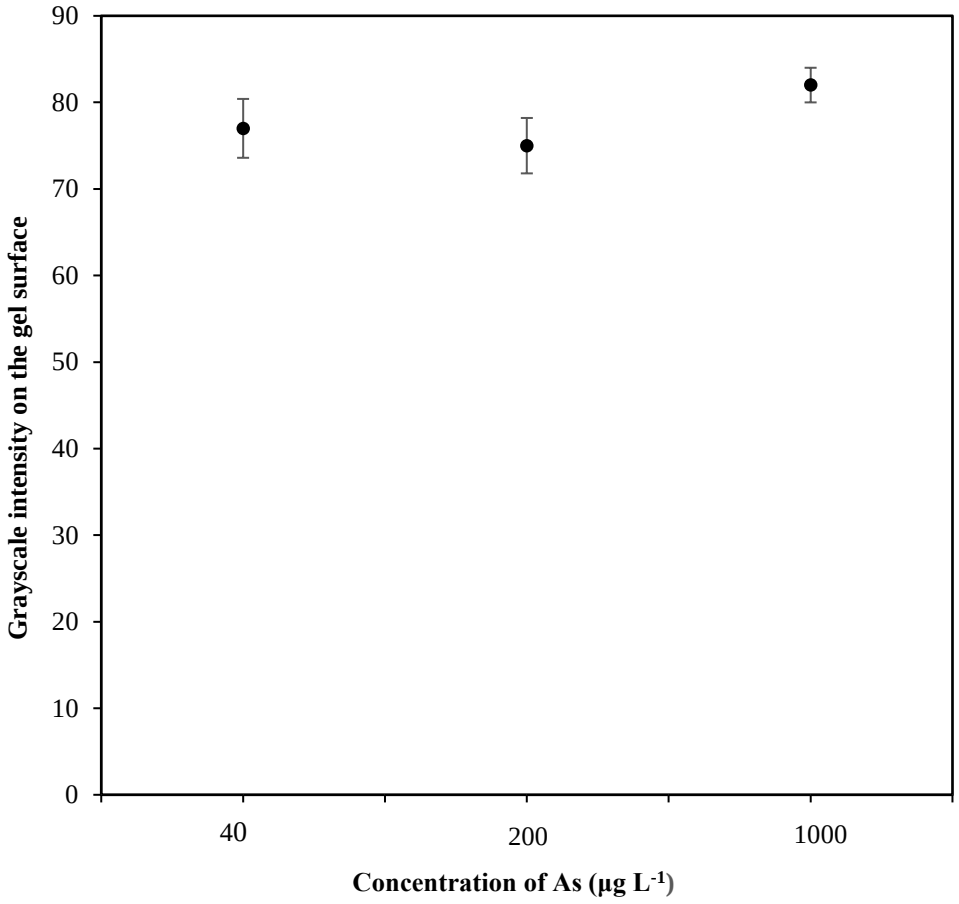


Figure 3.4 The grayscale intensity on the gel surface after it was deployed for 6h in 200 μg L^{-1} P solutions (0.01 mol L^{-1} $NaNO_3$) containing As in a range of 40-1000 μg L^{-1} at pH 6 and kept at room temperature(20°C).

3.3.6 Comparison of Zr-oxide DGT and Metsorb DGT

ZrO DGT was the first DGT technique for high-resolution imaging of labile

phosphorus based on the surface coloration of the binding gel. However, it only applied in sediment and soil. It is interesting to compare the performance of ZrO DGT and Metsorb DGT in water. All pre-treatment which required in pervious ZrO DGT experiments (Ding et al., 2013) was been omiited. Firstly, the grayscale intensity on the blank ZrO gel was reported to 65 (Ding et al., 2013) which is more than twice of the Metsorb gel in this study. Compared the performances between Metsorb DGT and ZrO DGT in the linear range of calibration, the gradient of colour was significant different (Figure 3.5). The grayscale intensity of ZrO gel and Metsorb gel were from 75 to 110 and 55 to 137 respectively. If the colour on the gel surface cannot be distinguished obviously, the potential error will increase (Appendix1). The result indicated that the Metsorb DGT has more reliability and operability of P measurement in natural water. With a higher capacity of P, ZrO DGT was reported to assess the measurement in sediment and soil combined the screening technique with a long time pre-treatment. However, it is difficult to ensure the accuracy of measurement when using a non-linear calibration. In addition, the 5 days heating treatment prior to coloration, makes the ZrO DGT method opposite to the rapid screening technique.

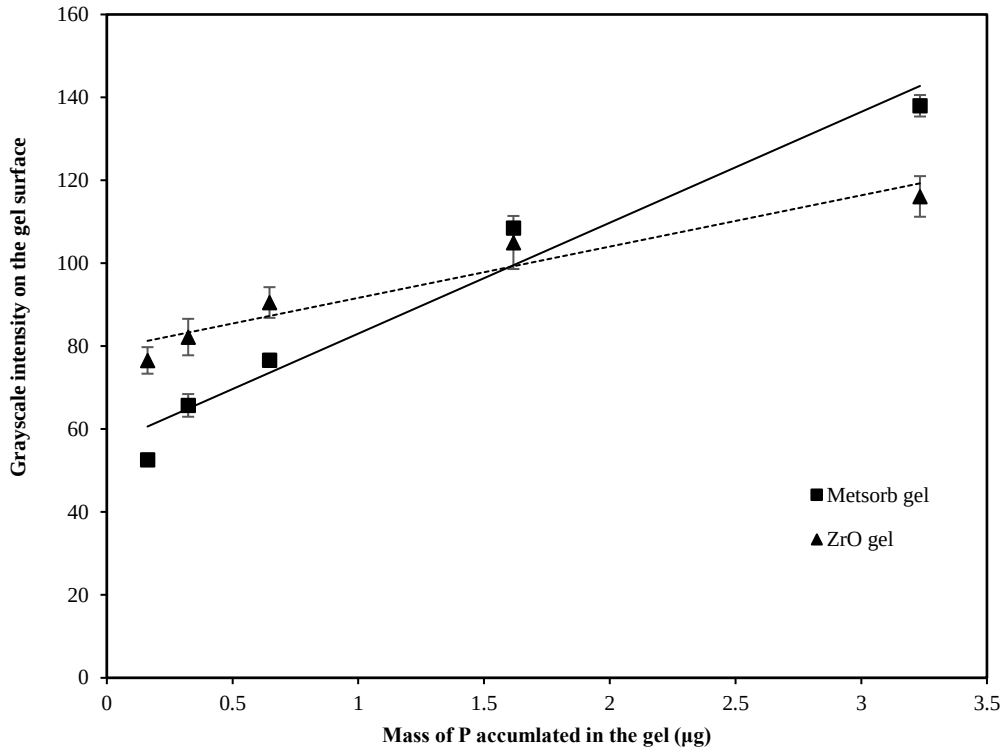


Figure 3.5 The grayscale intensity on the gel surface as a linear function of the mass of P accumulated by Metsorb and ZrO gel. The scope of the linear trend line is 27 and 12 of Metsorb gel and ZrO gel respectively. The uncertainties associated with each datum point is the standard deviation of the mean from triplicate DGT samples.

3.3.7 Field Application

DGT samplers were deployed at three sites: freshwater streams, fish farms and a reservoir in Tianjin, China. The standard level of Chinese surface water of P is 0.2 mg L^{-1} (SEPA and AQSIQ, 2002). As the diffusive gel layer was omitted for enhancing the sensitivity, the diffusive boundary layer thickness, δ, cannot be assumed as negligibly small and it has to be considered in calculation of the C_{DGT}. The measured concentration, *C*, is, therefore, given by

$$C_{DGT}=\frac{M(\Delta g+\delta)}{DAt} \quad (3.1)$$

A thickness of 0.39mm was suggested in previous work for typical flow 0.02 m s^{-1} in

rivers and streams, and the effective resin gel area was used as 3.80 cm^2 in this case (Warnken et al., 2006, Zhang et al., 1998a). In this work, the same parameters were adopted.

Since conventional monitoring for a water system on a large scale is time consuming, costly, non-representative and non-reflective, the DGT samples were deployed for 4 to 5 h for a fast in-situ pre-measurement before further quantitative analysis. The grayscale intensity, mass and the concentration of P measured by DGT was demonstrated in Table 3.1. The example images of P-loaded gels were given in Figure 3.6. The grayscale intensity of sample 2 was 184, exceeding the maximum intensity of 183 that can be meaningfully measured. Therefore, the concentration of the P cannot be accurately estimated from the grayscale intensity. Meanwhile, the grayscale intensity of sample 1 and sample 3 were beyond the linear range of calibration (intensity of 137 and mass up to 3.3μg) and the concentrations obtained may not be accurate.

Table 3.1 The grayscale intensity, mass[a] and C_{DGT} of samples, the C_{sol} in water system analysis by Continuous flow analysis(CFA) and Ammonium molybdate spectrophotometry

Sample[b]	Grayscale intensity	Mass (μg)	C_{DGT} (mg L^{-1})	C_{sol} (mg L^{-1})
1	141.81	3.20	0.37	1.12
2	183.95	/	/	2.24
3	151.65	3.57	0.41	0.97
4	129.02	2.72	0.31	0.51
5	66.88	0.40	0.05	0.24
6	104.91	1.82	0.21	0.48
7	93.43	1.39	0.16	0.36
8	89.84	1.26	0.14	0.28
9	86.42	1.13	0.13	0.37

[a] Mass of P accumulated on the gel surface was calculated using the linear calibration ;
[b] Sample 1,6,7 are fish and shrimp farms; sample 3,4,5,8,9 are freshwater streams in Tianjin City and Sample 2 is the wastewater reservoir.

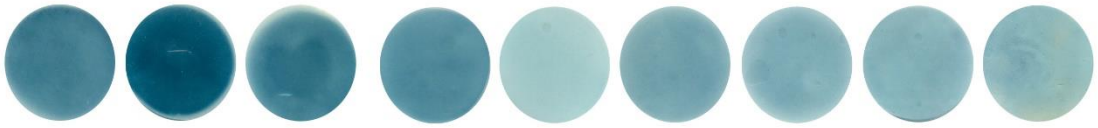


Figure 3.6 Examples images of the coloured P-loaded gels. Three DGT were deployed at each point as triplicate The Relative Standard Deviation (RSD) of the grayscale intensities were in the range of 2% to 9%.

Mass of P accumulated on the gel surface enable to obtain from the linear calibration directly when the grayscale intensity on the gel surface within the range. As the grayscale intensity measured in samples 4 to 9 are all in the linear range, the mass of P in these samples can be accurately calculated. With a known mass, M, the concentration of DGT-measured P can be obtained from the equation 3.1. Compared to the concentration of P in water samples, the concentration of DGT-measured P were

generally lower. It's mainly because of the P species in the water system. Metsorb DGT could only measure the dissolved reactive phosphorus (DRP) (also called soluble or filterable reactive phosphorus) in the natural water. The Relative Standard Deviation (RSD) of the grayscale intensities were in the range of 2% to 9%. Therefore, the RSDs of the concentration of DGT-measured P was within 9%.

3.4 Conclusion

This chapter has demonstrated the feasibility of combining the Metsorb DGT method with colour development and computer imaging densitometry (CID) as a rapid screening technique to assess the phosphorus levels in natural water. The calibration standard in mass of P and the corresponding colour on each binding gel and grayscale intensity measurements which fitting an exponential equation was presented. The calibration ranges of grayscale intensity increases from 28 at the background level to 186 at the saturation level. There is no further obvious increase in grayscale intensity with increasing P loading above a mass of 16 μg. The colouration was uniformly distributed on the gels with a low RSD, mostly within 4%. The fully quantitative interpretation of P concentration can be assessed in the linear range of 0.3 to 3.2μg per device. The effect of pH and interference of oxyanion metals (As) colour development and the DGT measurements are insignificant.

The comparison of the performance of the ZrO and Metsorb binding agents in colouration was made since the former is also being used in DGT technique combined with CID of P measurement. As a long-term treatment prior to colouration was required

and less sensitivity was performed in P measurements, ZrO DGT was not suitable for rapid screening technique in natural water.

The field evaluation of the suitability of this technique for monitoring P in natural waters was carried in three waterbodies in Tianjin, China. Provided the mass of P accumulated on the gel were within the linear range of the colour calibration, the concentration of P can be easily and accurately obtained by Metsorb DGT. The RSDs of the concentrations of DGT-measured P were between within 2% to 9%.

This newly developed approach provides several advantages over the traditional DGT technique. Firstly, in contrast with the use of UV-spectrophotometer or ICP-MS in previous studies, this method uses a scanner, equipment commonly found in most laboratories. Secondly, a more efficient analysing process of P measurement has been provided as the elution step was eliminated. The future work may put interest on enhance the capacity of Metsorb binding phase, for example, pressing the resin into an adhesive paper disc directly as new approach of binding layer preparation. To further enhance the efficiency of the technique, smart phone may be used for colour intensity recording and data processing and transferring.

Chapter 4. Rapid Screening Technique for Heavy Metal Pollution and Risk Assessment in Water and Soils

4.1 Introduction

4.1.1 Water Standards and Regulations

Water is essential to sustain life, and an adequate, safe and accessible supply must be available to all. Improving access to safe drinking water can lead to definite health benefits. Thus, every effort should be made to achieve an access to clean water. With this purpose in mind, over the past fifty years, the World Health Organization (WHO, 2011) has built on water quality guidelines which are widely accepted by nation across the world (WHO, 2011). The nature and form of drinking water standards may vary among countries and regions. Approaches that may work in one country or region will not necessarily work in other countries or regions. Therefore, it is essential that each country reviews its needs and capacities when developing a regulatory framework.

In addition, WHO formulates the standards and regulations for other classes of water as well. For example, standard quality of surface water, ground water, irrigation water, integrated wastewater discharge, etc. are strictly established around the world.

4.1.2 Monitoring of Cu(II), Ni(II), Co(II) and Cr (VI) in Waters and Soils

In order to prevent or reduce the health risks or hazards that heavy metal pollution

poses to humans and other living creatures in the ecosystem (the review of metal contaminations and toxicity see Chapter 2), it is important to explore the various techniques that could efficiently determine the occurrence and the concentrations of heavy metals in the environment. A number of approaches developed by several regulatory agencies and research laboratories are applied routinely to monitor and assess the quality of water, soil and sediments.

Generally heavy metals are analysed by discrete sampling of waters either manually or by automatic samplers. However, discrete samples only provide information regarding the sampled source at the certain time of sampling which is not suitable for monitoring and evaluating the full extent of the problem owing to temporal and spatial limitation. Voltammetric sensors are promising candidates for real-time, simultaneous measurements, as they can acquire continuous information with minimal or limited sample pre-treatment (Tercier-Waeber and Taillefert, 2008). However voltammetric sensors suffer from some drawbacks, such as lack of specificity and reproducibility, formation of intermetallic compounds in measurements, and the doubts of sensibility and stability (March et al., 2015). A variety of extraction methods, including simple and sequential have been proposed for measuring the forms of heavy metals in soil (Garcia et al., 1996, Abollino et al., 2002, Feng et al., 2005). However those extraction methods have limitations such as inaccuracy of quantitative sampling, redistribution of metals during fractionation, requirements of complemented analytical technique, and may not be suitable for phytoavailable metal contents evaluation investigations (Rao et al., 2008,

Zimmerman and Weindorf, 2010).

4.1.3 Application of DGT for Cu(II), Ni(II), Co(II) and Cr (VI) Measurement

As mentioned in Chapter 2, DGT is a robust *in situ* monitoring device that can be easily applied in remote areas, provide time-integrated concentration and speciation information.

The DGT was first used to measure metals (Cd, Zn, Cu, Ni, Mn, Fe) in an aqueous solution using Chelex-100 as binding layer (Zhang and Davison, 1995). Then, the first fully quantitative in situ measurements were taken of the potential resupply flux of metals (Cd, Zn, Cu, Ni)) from soil to solution, provided by Chelex-100 DGT (Zhang et al., 1998b). The fluxes of trace elements were first measured by DGT in a sludge-treated soil to evaluate the effect of soil moisture on DGT performance in soils (Hooda et al., 1999).

The expanding demand for DGT as an in situ environment monitoring technique has resulted in the development of numerous binding phases for the measurement of different metals and their speciation. The Whatman P81 cellulose phosphate ion exchange membrane has been successfully used as a binding phase for DGT applications to determine Cu and Cd. This overcomes many of the problems that arose from using hydrogel based binding phases (Li et al., 2002). Suspended particulate reagent-iminodiacetate (SPR-IDA) was chosen as an alternative binding agent due to its smaller bead size to achieve a more homogeneous distribution than the Chelex-100 in resin gel layer (Warnken et al., 2004).

Using polyvinyl alcohol as a liquid binding phase with the liquid-DGT devices provided a selective measurement of Cu (Fan et al., 2009). Another liquid-type DGT which can strongly coordinate to Ni with sodium poly (aspartic acid) was designed (Chen et al., 2013). Additionally, direct colourimetric detection of Cu(II) was achieved using the DGT technique by employing methylthymol blue as a chelating and chromogenic agent (McGifford et al., 2010). Although various binding material have been developed to make measurements of cations, DGT with Chelex-100 resin as binding layer remains the most conventional and authoritative method for measuring divalent metals.

However, as a strong binding phase for metals, Chelex-100 has difficulty to absorb Cr(VI). The first selective measurement of Cr(VI) in water was developed using polyquaternary ammonium salt (PQAS) as a binding agent combined with ICP-MS analysis. Subsequently a high capacitive N-Methyl-D-glucamine (NMDG) functional resin was incorporated into the DGT binding phase for selective measurement of Cr(VI). It successfully provided an accurate measurement of time-averaged Cr(VI) in both uncontaminated natural and contaminated waters (Pan et al., 2015).

4.1.4 The Aim and the Objectives

The aim of this work was to develop rapid screening devices based on DGT technique to assess metal concentrations qualitatively and quantitatively. Copper, nickel and cobalt, which are highly soluble in water, can exist in waters and soils at high concentrations. They accumulate on the binding gel of DGT device with deployment

time. When the amount of a metal on the binding gel reaches certain level, a distinctive colour will appear on the gel. The intensity of the colour will be directly proportional to the amount of metal accumulated. The intensity can be quantified using a computer-imaging densitometry (CID). Base on the calibration, a relationship between the amount of metal on the gel and the colour intensity, the mass accumulated by DGT can be fully quantified and the concentration of metal measured by DGT can be calculated using the DGT equation. The objectives of this work were:

1) Investigate the potential of using Chelex-100 type DGT and high resolution CID measurement for rapid estimation of metal concentrations in waters.
2) Test the performance of the technique under different conditions.
3) Base on the regulation standards in different countries and regions, formulate a DGT deployment guide list to determine if the concentration of metals has exceeded Maximum Contaminant Level. Using both a simple visual inspection and a scanner for DGT devices at different deployment times and different temperatures will be considered for this list.
4) Develop a rapid screening technique for Cr (VI) using DGT and high resolution CID base on the surface colouration of the N-Methyl-D-glucamine (NMDG) binding gel reacting with the diphenylcarbazide in an acidic solution.

4.2 Materials and Methods

4.2.1 General Chemicals

All experimental and reagent solutions were prepared using Milli-Q water (18MΩ). The metal salts used were all of analytical grade and were (with water of hydration omitted): $CuSO_4$， $Ni(NO_3)_2$, $Co(NO_3)_2$, Na_2CrO_4 (VWR, UK). Chelex-100 resin was obtained from Bio-Rad, US. NMDG was purchased from Sinopharm Chemical Reagent Co. Ltd., China. All containers, glass plates and sample tubes were acid-washed using a 10% v/v HNO_3 bath and rinsed thoroughly with Milli-Q water prior to use.

4.2.2 Preparation of Gels and Assembling DGT Devices

Diffusive gel was prepared by mixing 3.75ml of acrylamide solution (40% w/w) (BDH, Electran) and 4.75 mL of MQ water with 1.5 mL of DGT cross-linker (0.3%, measured by weighing 1.5 g). This 10 mL of gel solution was well mixed and then 70μL of freshly made ammonium persulfate solution (10% w/v) (BDH Electran) and 25 μL of *N,N,N'N'- tetramethylenediamine* (TEMED) (99%) (BDH Electran) were added. After well mixed using a pipette, it was immediately cast between two glass plates which were separated by a 0.5 or 0.25 mm plastic spacer (for a hydrated gel of 0.78 mm or 0.4mm thickness) and held together with plastic clips. The assembly was immediately placed in oven at 45 ºC for at least 1 hour. Once the gel was completely set, it was removed from the glass plates and placed into a deionized water (two gel sheets per litre of deionized water, which was changed repeatedly until all the excess

polymerization products were removed, for example the pH of the washed solution is equal to that of the deionized water stored in. Finally, the gel was stored in 0.03 mol L^{-1} $NaNO_3$ solution until its use (Warnken et al., 2005).

The Chelex 100 resin-gel used in this work (for Cu, Co, Ni) was prepared by mixing 4g Chelex 100 resin (200-400 mesh) (wet weight) available from Bio-Rad (UK) into 10 mL of gel solution consisting of 3.75ml of acrylamide solution (40% w/w) (BDH Electran) and 4.75 mL of MilliQ (MQ) water with 1.5 mL of DGT cross-linker. Sixty microliter of ammonium persulfate solution (10% w/v) and 15 μL of TEMED (99%) were then added to the mixture. After mixing them well, the resin-gel solution was immediately cast between two glass plates, which separated by a 0.25mm plastic spacer (for a hydrated gel of 0.04mm). Gel setting, hydration and storage procedures then followed those for the diffusive gel. However, the Chelex gels were only washed once before storage.

The NMDG binding gel used in this work (for Cr) was prepared by bis(acrylamide)–cross-linked polyacrylamide instead of the conventional agarose cross-linked polyacrylamide to achieve a uniform distribution of the NMDG resin. Dry weight of 2.5 g of NMDG resin was added to 10 mL gel solution consisting of 28.5% acrylamide solution (w/v) (BDH Electran) and 1.5% *N,N-methylene* bis(acylamide). After mixing them well, 250 μL of freshly made ammonium persulfate solution (10% w/v) (BDH Electran) and 10 μL of TEMED (99%) (BDH Electran) were added. Mixing them properly again, the mixture was immediately cast between two glass plates separated

by a 0.4 mm plastic spacer. The glass plate assembly was placed horizontally in a freezer at 4 ºC for 30 min to allow the NMDG resin to settle by gravity to one side of the gel, and then transferred to an oven at 50 ºC for 1h. Hydration procedures then followed those for the diffusive gel and storage the NMDG resin gel in a fridge at 4 ºC (Pan et al., 2015). The DGT assembling procedure has been described in Chapter 3.2.2.

4.2.3 Metal Uptake by DGT

The DGT devices were deployed in 2 L well stirred solution, containing 0.01 mol L^{-1} $NaNO_3$ and different concentrations of Cu, Ni, Co (up to 5 mg each metal ions L^{-1}) separately for different deployment times (up to 24 h), depending on the purpose of the respective experiments. Three replicates were deployed in all experiments.

The uptake of Cr(VI) was evaluated by developing the NMDG-DGT in 8 L of solution containing 50 μg L^{-1} of Cr(VI) in 0.01mol L^{-1} $NaNO_3$ for 6 h to 144 h. Six replicates were deployed in Cr(VI) measurements.

4.2.4 Colour Development for Cr(VI)

For the determination of hexavalent chromium, spectrophotometric method with diphenylcarbazide (DPC) was adopted. Cr(VI) reacts with DPC to form reddish violet complex and the reaction is selective and very sensitive for chromium.

It was prepared by dissolving 250 mg 1,5-diphenylcarbazide in 100 mL acetone(analytical reagent grade). This stock solution was stored in a brown bottle and

discarded when it was discoloured. Prior to colorimetric analysis, 100 mL of 2.5 mol L^{-1} H_2SO_4 solution was slowly added to 100 mL of stock solution of DPC, after which the solution was diluted to 400 mL with deionized water. The pH of the solution was about 2.

The DGT devices were rinsed with deionized water after deployment. For colour development, carefully peeled off the filter membrane and reassembled the DGT device. Placed the reassembled DGT sampler upside down in a 100 mL plastic pot containing 20 mL of mixed reagent for 10-15 min at room temperature until full colour development.

4.2.5 CID Analysis

After DGT deployment in solutions, the Chelex gel discs and the downside filter membranes were retrieved from the DGT device. They were rinsed using cool deionized water stored in fridge and the water adhering to the surface of the gels was removed gently using poly filter paper. After transferred into a clean plastic bag, the side of the gel surface where the Chelex beads had settled was then scanned using a flat-bed scanner (HP G3110) at a resolution of 300 dpi. The grayscale of the scanned images corresponding to the exposure of the DGT devices was analysed with ImageJ 1.48(download from https://imagej.nih.gov/ij/).

Similar to the procedure of Chelex-DGT, the NMDG gel disc and the down side filter membrane were retrieved form the DGT device after colour development in the

mixed reagent solution. The NMDG gel discs were rinsed using cool deionized water and then immersed in cool water for 5 min to stop further colour development. The water adhering to the surface was removed using poly filter paper. The side of the gel surface where NMDG resin had settled was then scanned using the same flat-bed scanner (HP G3110) at a resolution of 300 dpi. The grayscale intensity of the scanned images corresponding to the open window of the solution DGT device was analysed with ImageJ 1.48.

4.2.6 Calibrations for Quantification

4.2.6.1 Calibrations of Cu, Co, Ni

The calibration curves of Cu, Co and Ni in standard solution were obtained by establishing the relationship between the grayscales intensity of Chelex gel and its accumulated massed of metal ions after the DGT deployments. It was tested by deploying seven sets of six replicate DGT devices in a 2 L of well-stirred solution containing 0.01 mol L^{-1} $NaNO_3$ and different concentration of Cu, Co, Ni (500μg L^{-1}, 2mg L^{-1}, 5mg L^{-1} respectively). The pH of the solution was adjusted and maintained at 6.0±0.5, and solution samples were taken at the beginning and end of the deployment. The deployment time varied from 2.4h to 24h. After rinsed with deionized water, the gel discs were retrieval from the DGT devices. Three Chelex gel discs were eluted in 1ml of 1 mol L^{-1} HNO_3 for 24h followed by ICP-MS analysis (Zhang and Davison, 1995). The grayscale of the gel surfaces of other three Chelex discs were measured

using CID, as described earlier. The relationship between the metal mass of accumulated in the DGT and their corresponding grayscale amount was fitted using a linear equation.

4.2.6.2 Calibration of Cr (VI)

The calibration of Cr(VI) in standard solutions was obtained by establishing the relationship between the grayscale intensities of the NMDG resin gel and its accumulated massed of Cr(VI) after the DGT deployments. It was carried out by deploying eight sets of six replicate DGT in two of 8 L well-stirred 0.01mol L^{-1} $NaNO_3$ solution spiked with 50μg L^{-1} Cr(VI). Each set of six DGT replicates were removed at deployment time of 6, 12, 24, 48, 72, 96, 120 and 144 hours. To confirm the stability of Cr species, samples of solution were taken after the removal of each set of devices and analysed for total Cr using ICP-MS and for Cr(VI) using the DPC method. Chromium accumulated in the three binding gels was eluted using 1 mL of 1 mol L^{-1} HNO_3. The other three DGT units were immersed in 100 ml plastic pot containing 20 ml of the mixed reagent after removed the filter membrane as described in the section for colour development. The DGT units were kept in the mixed reagent at room temperature (20 ^{o}C) for 15 minutes. After a brief rinse with MQ water, the gel discs were peeled off and scanned. The relationship between the masses of Cr accumulated in the gels and their corresponding grayscale intensities was fitted using both a linear equation and an exponential equation.

4.2.7 Calculation of DGT

The concentrations of metals were calculated using the DGT equation 2.6. The corresponding accumulated mass can also be calculated by equation 2.4 as described in section 2.5.1.

4.3 Results and Discussion

4.3.1 Calibration of Cu

The calibration standard in mass per unit area (μg cm^{-2}) for Cu and the corresponding colour on chelex-100 binding and grayscale intensity measurements are presented in Figure 4.1. It shows that there is a linear increase of grayscale intensity with the accumulating mass of Cu in the binding gel. The calibration ranges of grayscale intensity increase from 28.7 to 117.1. The linear range of 1.5μg cm^{-2} to 165μg cm^{-2} correspond to the concentration range of 0.05 to 5 mg L^{-1} if the deployment time is 24 hours and the water temperature is 20^{o}C. This is for the DGT devices without diffusive gel, which means the diffusion layer thickness is 0.014cm (the filter membrane thickness only). If longer deployment time is needed, for example one week, the standard DGT devices with 0.08cm diffusive gel could be used (same as Ni and Co).

The DGT measured blank for Cu was 0.44 ng cm^{-2}. The blank of grayscale intensity on the gel surface was 13. The method detection limit (MDL) of the DGT technique and CID analysis were calculated as three times the standard deviation of the blank value. The MDL of DGT was 0.13μg L^{-1} when DGT devices are deployed for 24

hours and 0.018 μg L^{-1} if the deployment time is 7 days. The MDL of CID analysis was calculated as three times the standard deviation of the blank value was 1.9 in grayscale intensity. The method precision for data obtained by DGT-measured mass was 5 % and grayscale intensity on gel surface was 11%.

In this work, the colour of Cu, Ni and Co on the gel surface was detected by both scanner and visual inspection. The detection limit by visual inspection depends on person and may also affected by different light. The colour of Cu could be detected by visual inspection when the mass accumulated reach around 16 μg cm^{-2} as a reference.

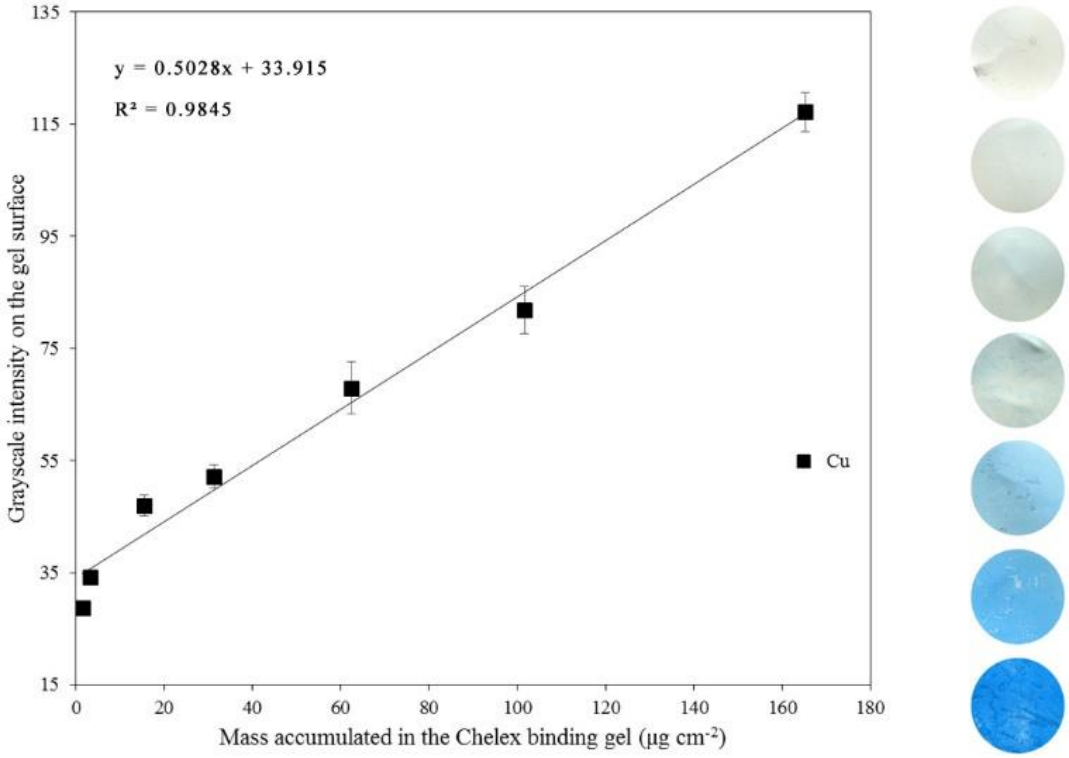


Figure 4.1 The DGT colour calibration curve for Cu, in standard solution scanning by the flat-bed scanner in 300dpi (left). Examples of scanned images of the coloured metal ions-loaded gels (right). The uncertainties associated with each datum point is the standard deviation of the mean from triplicate DGT samples.

4.3.2 Calibration of Ni

The calibration standard in μg cm^{-2} mass of Ni and the corresponding colour on chelex-100 binding and grayscale intensity measurements are presented in Figure 4.2. It demonstrated that there is a linear increase of grayscale intensity with the accumulating mass of Ni in the binding gel. The calibration range of grayscale intensity increase from 16.9 to 33.3. The linear range of 2.7 μg cm^{-2} to 153 μg cm^{-2} correspond to the concentration range of 0.05 to 5 mg L^{-1} if the deployment time is 24 hours and the water temperature is 20^{o}C.

The DGT measured blank for Ni was 0.31μg cm^{-2}. The grayscale intensity on the blank gel surface was 13. The MDL of the DGT technique was 0.10 μg L^{-1} for 24 hours deployment and 0.014 μg L^{-1} for 7 days deployment at 20^{o}C. The MDL of the CID analysis was 1.9 in grayscale intensity. The method precision for data obtained by DGT-measured mass was 5 % and grayscale intensity on gel surface was 7 %.

The colour of Ni could be detected by visual inspection when the mass accumulated reach around 32 μg cm^{-2} as a reference.

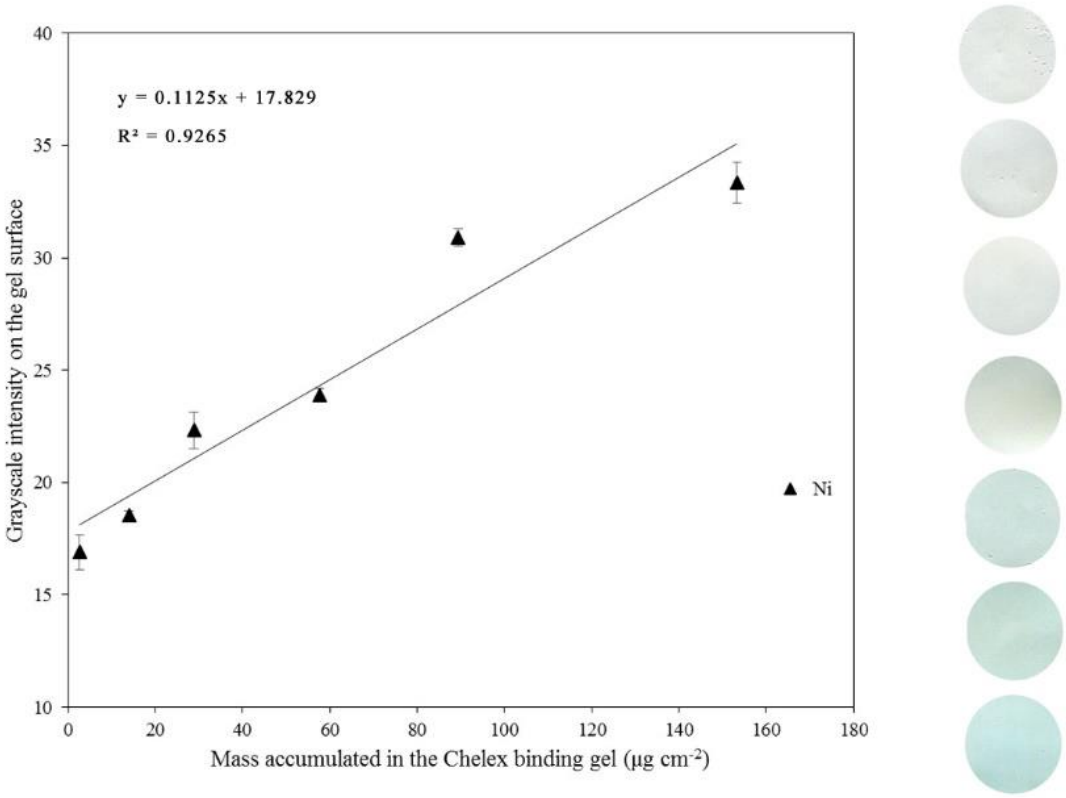


Figure 4.2 The DGT colour calibration curve for Ni, in standard solution scanning by the flat-bed scanner in 300dpi (left). Examples of scanned images of the coloured metal ions-loaded gels (right). The uncertainties associated with each datum point is the standard deviation of the mean from triplicate DGT samples.

4.3.3 Calibration of Co

The calibration standard in µg cm^{-2} mass of Co and the corresponding colour on chelex-100 binding and grayscale intensity measurements are presented in Figure 4.3. It indicated that there is a linear increase of grayscale intensity with the accumulating mass of Co in the binding gel. The calibration ranges of grayscale intensity increase from 23 to 44. The linear range of 1.6 µg cm^{-2} to 159.2 µg cm^{-2} corresponds to the concentration range of 0.05 to 5 mg L^{-1} if the deployment time is 24 hours and the water temperature is 20 ºC.

The DGT measured blank for Co was 0.019µg cm^{-2}. The blank of grayscale

intensity on the gel surface was 13. The MDL of the DGT measurement of Co was 0.006 μg L^{-1} for 24 hours deployment and 0.001 μg L^{-1} for 7 days deployment at 20 ºC. The MDL of the CID analysis was 1.9 in grayscale intensity. The method precision for data obtained by DGT-measured mass was 4 % and grayscale intensity on gel surface was 14 %.

The colour of Co could be detected by visual inspection when the mass accumulated reach around 32 μg cm^{-2} as a reference.

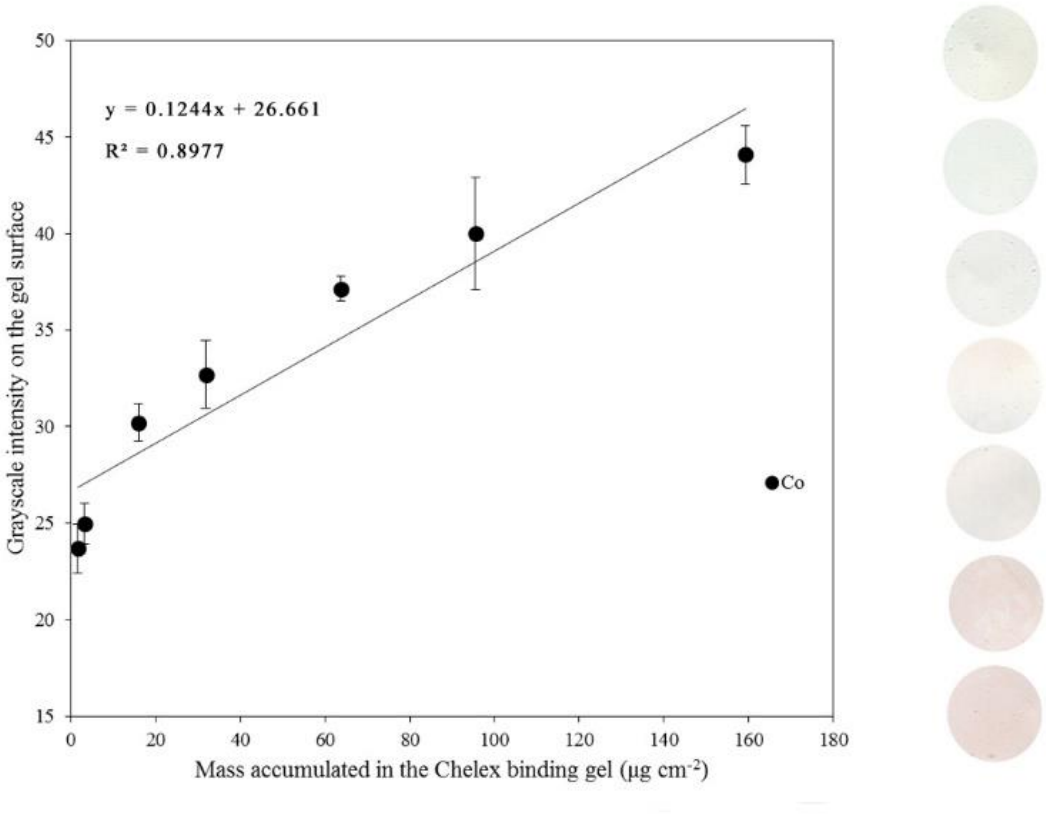


Figure 4.3 The DGT colour calibration curve for Ni, in standard solution scanning by the flat-bed scanner in 300dpi (left). Examples of scanned images of the coloured metal ions-loaded gels (right). The uncertainties associated with each datum point is the standard deviation of the mean from triplicate DGT samples.

4.3.4 Calibration of Cr (VI)

The relationship between the accumulation of Cr(VI) in NMDG gels and the corresponding change in greyscale intensity on the gel surface was fitted using a quintic polynomial in the whole range and fitted using a linear equation when the accumulated mass of Cr(VI) was below 2.47μg cm^{-2} as shown in Figure 4.4. The calibration ranges of grayscale intensity increases from 15 at the background level to 96 when the mass of Cr (VI) is 13.7 μg cm^{-2} on the gel disc. The calibration range of grayscale intensity for the linear increase from 15 to 63. Due to the DPC method is being very sensitive, the performance of coloration on the gel surface is unstable in higher mass accumulation.

The accumulated mass of Cr(VI) in the blank gel was 0.59 ng cm^{-2} after 72h. The MDL for NMDG-DGT was calculated by three times standard deviations of 12 blank measurements was0.16 μg L^{-1} of 24 hours deployment and 0.002 μg L^{-1} for 7 days deployment at 20 ºC. The detection limit of DPC method for Cr (VI) measurement was approximately 5 μg L^{-1} (Babel and Kurniawan, 2004). Three NMDG gel disc was been analysis by CID after colouration at each point. The RSD of grayscale intensities were in the range of 1% to 10%. The colouration was uniformly distributed on the gels with a low RSD, mostly within 5%.

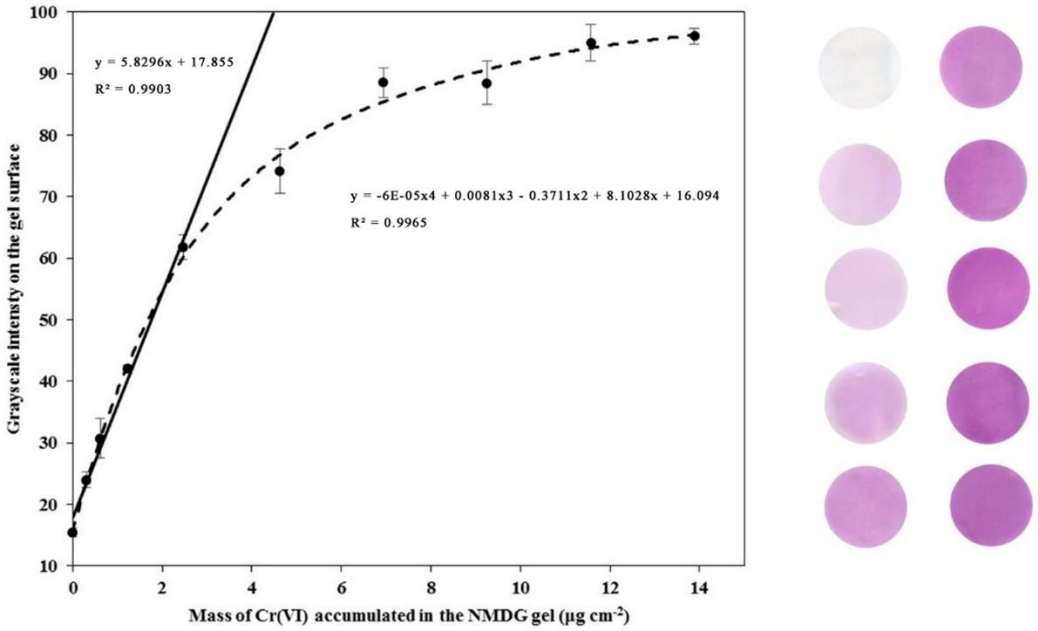


Figure 4.4 The grayscale intensity on the gel surface as a function of the mass of Cr(VI) accumulated by NMDG gel (left). Mass of Cr(VI) accumulated by DGT in a range of 0.31-13.9 μg cm^{-2}. Examples of scanned images of the coloured Cr(VI)-loaded gels (right). The uncertainties associated with each datum point is the standard deviation of the mean from triplicate DGT samples.

According to Pan and co-worker (Pan et al., 2015) the good performance over a wide pH range of 3-10 indicates that both $HCrO_4^-$ and CrO_4^{2-} can be effectively measured by NMDG-DGT. DGT measurements were within 10% of predicted values when the supporting electrolyte of $NaNO_3$ was in the range of 0.1–50 mmol L^{-1}.

The Cr reaction with DPC is usually free from interferences (EPA). However, certain substances may effect if the concentration of Cr is relatively low. Mo(VI) and Hg also react to form colour with the reagent, but the red-violet intensities produced are much lower than those for Cr at the specified pH. The optimized pH is 2 ± 0.4. If the pH is too low, the reaction will be unstable. Conversely, the colour development won't complete if the pH is higher.

4.3.5 Using DGT as a Diagnosis Tool for Contamination Level

With increasing urban populations and aging infrastructure, poor water quality can have a large impact on public health. Modern water management requires more reliable and quicker characterization of contaminants, to allow for a more timely response. DGT combined with CID analysis can serve as a rapid screening technique and can estimate metal concentrations in waters. It can provide a quick and visual measurement of metals without complicated and expensive analysis in laboratory. The elution step of conventional DGT measurement has also been eliminated which has dramatically decreases the response time of monitoring. If the colour on the gel surface was detected by visual inspection, this technique can achieve the goal of *in situ* and on site assessment of heavy metals (Cu, Ni, Co) pollution in water systems.

In this study, the new DGT approach was used to estimate whether the concentration of Cu, Ni, Co in the water meet the acceptable level of different standards and regulations in UK/EU, US and China for waters. The deployment time of DGT at different temperature was calculated using equation 2.6. C_{DGT}, is set as the standard concentration of metals in different water standard and regulation. With the different temperature in water, different diffusion coefficient, D, was used in calculating (see Appendix 2). At the end of the deployment, any colour observed either by naked eyes (visually) or by a scanner will indicate the concentration of trace metals in the water body has exceeded the Maximum Contaminant Level (MCL) based on each regulation or standard (see Appendix 3). Different temperature were discussed in two modalities,

visual and scanning (Table 4.1-4.4). As shown in Table 4.1, the lower the standard concentration of metals is the longer deployment time of DGT device require. In order to meet the goal of rapid screening technique, the deployment time should be rational (few hours to 1 day). Therefore, this technique may not be suitable for determining the concentration of Cu in drinking water in UK. The UK MCL of Cu in drinking water is 20 times lower than of China. The Cu concentration in UK's drinking water may be detected by using a scanner because of the low detection limit.

In table 4.2, the DGT deployment time needed for determining Ni and Co in drinking and surface waters were too long due to the strict standard level of Ni and Co in both UK and China. DGT can assess the concentration of Ni and Co in the effluent in both UK and China by deploying the devices for 24 hours. If there is any colour observed by naked eyes on the gel surface, the concentration of Ni and Co was exceeded the MCL of regulation standards of these two countries.

Compared to results in Table 4.1 and 4.2, when using a scanner to detect colour change on surface, the deployment time of DGT devices were much shorter than using visual inspection (Table 4.3 and Table 4.4). Due to the initial establishment of the steady state in the DGT diffusive gel, the minimum deployment time is one hour (W Davison, 1998). Therefore, a diffusive layer should be added into the DGT device to extend the deployment time when determining Cu concentration in drinking water of US and China, and the Co concentration in China's effluent. The deployment time could be recalculated by equation 2.6.

The results in table 4.1-4.4 indicate that DGT can be used for rapid scanning of Cu in all cases considered, except for UK/EU drinking water when the colour of the samples was inspected visually. For Co and Ni, this method only adapted to detecting the contamination in Chinese effluents in which the Maximum Contaminant Level is higher compared to other water standards. When the scanner is used for colour detection, DGT could be used for monitoring Co in UK and China drinking water.

Table 4.1 DGT deployment time (hours) needed for determining Cu concentration exceeding regulation standards using visual inspection of colour change

	UK /EU standard [a]	US [b]	CN [c]	
Temp(℃)	Drinking water	MCLG	Drinking water	Effluent
6	8.9	14.8	21.5	35.5
7	8.6	14.2	20.7	34.3
8	8.3	13.7	20.0	33.2
9	8.0	13.2	19.3	32.1
10	7.8	12.8	18.6	31.0
11	7.5	12.4	18.0	30.0
12	7.3	11.9	17.4	29.1
13	7.0	11.5	16.8	28.2
14	6.8	11.2	16.3	27.3
15	6.6	10.8	15.8	26.5
16	6.4	10.5	15.3	25.7
17	6.2	10.1	14.8	24.9
18	6.1	9.8	14.3	24.2
19	5.9	9.5	13.9	23.5
20	5.7	9.2	13.5	22.8
21	5.5	9.0	13.1	22.2
22	5.4	8.7	12.7	21.6
23	5.2	8.5	12.3	21.0
24	5.1	8.2	12.0	20.4
25	5.0	8.0	11.6	19.9
26	4.8	7.8	11.3	19.4
27	4.7	7.6	11.0	18.8
28	4.6	7.3	10.7	18.4
29	4.5	7.2	10.4	17.9
30	4.4	7.0	10.1	17.4

[a] UK standard: Drinking water: Cu: 2.0mg/L. Water Supply (Water Quality) Regulations 2000 EU standard: Human Consumption: Cu: 2.0mg/L Council Directive on the quality of water intended for human consumption (Drinking Water Directive)

[b] US standard: National Primary Drinking Water Regulations MCLG: Maximum Contaminant Level Goal Cu: 1.3mg/L.

[c] CN standard: Drinking Water: Cu: 1.0mg/L. Drinking water quality standards GB5749－2006

Effluent: Cu: 0.5mg/L Emission standard of pollutants for copper, nickel, cobalt industry GB 25467-2010

Table 4.2 DGT deployment time (hours) needed for determining Ni and Co concentrations exceeding regulation standards using visual inspection of colour change

	Ni			Co	
	UK/EU [a]		CN [b]	UK [a]	CN [b]
Temp(˚C)	Drinking water	Effluent	Surface Water	Drinking water	Effluent
6	1918.5	38.4	372.7	745.4	37.3
7	1853.2	37.1	360.0	720.1	36.0
8	1791.1	35.8	348.0	695.9	34.8
9	1731.9	34.6	336.5	672.9	33.6
10	1675.5	33.5	325.5	651.0	32.6
11	1621.7	32.4	315.1	630.1	31.5
12	1570.3	31.4	305.1	610.2	30.5
13	1521.3	30.4	295.6	591.1	29.6
14	1474.4	29.5	286.5	572.9	28.6
15	1429.6	28.6	277.7	555.5	27.8
16	1386.8	27.7	269.4	538.8	26.9
17	1345.8	26.9	261.5	522.9	26.1
18	1306.5	26.1	253.8	507.6	25.4
19	1268.8	25.4	246.5	493.0	24.7
20	1232.7	24.7	239.5	479.0	23.9
21	1198.1	24.0	232.8	465.5	23.3
22	1164.9	23.3	226.3	452.6	22.6
23	1133.0	22.7	220.1	440.2	22.0
24	1102.4	22.0	214.2	428.3	21.4
25	1072.9	21.5	208.4	416.9	20.8
26	1044.6	20.9	202.9	405.9	20.3
27	1017.4	20.3	197.7	395.3	19.8
28	991.2	19.8	192.6	385.1	19.3
29	966.0	19.3	187.7	375.3	18.8
30	941.7	18.8	183.0	365.9	18.3

[a] UK standard: drinking water: Ni: 0.02mg/L. Water Supply (Water Quality) Regulations 2000 EU standard: drinking water: Ni: 0.02mg/L, Co: 0.1mg/L Council Directive on the quality of water intended for human consumption (Drinking Water Directive)

[b] CN standard: Drinking Water: Ni: 0.02 mg/L. Drinking water quality standards GB5749－2006 Co: 0.05mg/L Quality standard for ground water GB/T 14848-9

Effluent: Ni: 1mg/L, Co: 1mg/L Emission standard of pollutants for copper, nickel, cobalt industry GB 25467-2010 Surface water: Co: 0.1mg/L Council Directive on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community (Dangerous Substances Directive) - List II substance

Table 4.3 DGT deployment time (hours) needed for determining Cu concentration exceeding regulation standards using scanner for detecting colour change

	UK /EU standard	US	CN	
Temp(˚C)	Drinking Water	MCLG	Drinking water	Effluent
6	0.9	1.5	2.2	3.6
7	0.9	1.4	2.1	3.4
8	0.8	1.4	2.0	3.3
9	0.8	1.3	1.9	3.2
10	0.8	1.3	1.9	3.1
11	0.8	1.2	1.8	3.0
12	0.7	1.2	1.7	2.9
13	0.7	1.2	1.7	2.8
14	0.7	1.1	1.6	2.7
15	0.7	1.1	1.6	2.6
16	0.6	1.0	1.5	2.6
17	0.6	1.0	1.5	2.5
18	0.6	1.0	1.4	2.4
19	0.6	1.0	1.4	2.4
20	0.6	0.9	1.3	2.3
21	0.6	0.9	1.3	2.2
22	0.5	0.9	1.3	2.2
23	0.5	0.8	1.2	2.1
24	0.5	0.8	1.2	2.0
25	0.5	0.8	1.2	2.0
26	0.5	0.8	1.1	1.9
27	0.5	0.8	1.1	1.9
28	0.5	0.7	1.1	1.8
29	0.4	0.7	1.0	1.8
30	0.4	0.7	1.0	1.7

Table 4.4 DGT deployment time (hours) needed for determining Ni and Co concentrations exceeding regulation standards using scanner for detecting colour change

	Ni			Co		
	UK	CN		UK	CN	
Temp(℃)	Drinking Water	Drinking water	effluent	Drinking Water	Drinking water	effluent
6	191.8	191.8	3.8	18.6	37.3	1.9
7	185.3	185.3	3.7	18.0	36.0	1.8
8	179.1	179.1	3.6	17.4	34.8	1.7
9	173.2	173.2	3.5	16.8	33.6	1.7
10	167.5	167.5	3.4	16.3	32.6	1.6
11	162.2	162.2	3.2	15.8	31.5	1.6
12	157.0	157.0	3.1	15.3	30.5	1.5
13	152.1	152.1	3.0	14.8	29.6	1.5
14	147.4	147.4	2.9	14.3	28.6	1.4
15	143.0	143.0	2.9	13.9	27.8	1.4
16	138.7	138.7	2.8	13.5	26.9	1.3
17	134.6	134.6	2.7	13.1	26.1	1.3
18	130.6	130.6	2.6	12.7	25.4	1.3
19	126.9	126.9	2.5	12.3	24.7	1.2
20	123.3	123.3	2.5	12.0	23.9	1.2
21	119.8	119.8	2.4	11.6	23.3	1.2
22	116.5	116.5	2.3	11.3	22.6	1.1
23	113.3	113.3	2.3	11.0	22.0	1.1
24	110.2	110.2	2.2	10.7	21.4	1.1
25	107.3	107.3	2.1	10.4	20.8	1.0
26	104.5	104.5	2.1	10.1	20.3	1.0
27	101.7	101.7	2.0	9.9	19.8	1.0
28	99.1	99.1	2.0	9.6	19.3	1.0
29	96.6	96.6	1.9	9.4	18.8	0.9
30	94.2	94.2	1.9	9.1	18.3	0.9

4.4 Conclusions and Implication

One of the significant advantage of DGT is it passively accumulates labile species from solution while deployed *in situ* and therefore contamination problems associated with conventional water sample collection and filtration procedures are eliminated. DGT can be used as a rapid screening technique. Compare to the existing DGT method for metals, this study provided a more practicable and time saving approach for environmental monitoring and risk assessment.

The calibration curves of copper, cobalt and nickel demonstrated the linear increases in the grayscale intensities with the accumulation metal mass. The calibration ranges of grayscale intensity of Cu increase from 28.7 to 117.1. The calibration ranges of grayscale intensity of Ni increase from 16.9 to 33.3. The calibration ranges of grayscale intensity of Co increase from 23 to 44. The linear range of mass accumulation of Cu , Ni and Co were 1.5μg cm^{-2} to 165μg cm^{-2}, 2.7 μg cm^{-2} to 153 μg cm^{-2}, 1.6 μg cm^{-2} to 159.2 μg cm^{-2} correspond to the concentration range of 0.05 to 5 mg L^{-1} if the deployment time is 24 hours and the water temperature is 20 ^{o}C respectively. The method precision for data obtained by DGT-measured mass was 5% and grayscale intensity on the gel surface is in a range of 7% to 14%. Subsequently, a guide list for using DGT to determine if the concentration of metals has exceeded Maximum Contaminant Level based on regulation standards set by different countries and regions. Because of the intense coloration on the gel surface, both using simple visual inspection and using a scanner for DGT devices at different deployment time and different

temperature have been considered in the list. DGT applying to the guide list presented a simple and fast *in situ* pre-measurement before further complicated and costly quantitative analysis in drinking water and effluent monitoring. In this guide list, DGT can be used for rapid scanning of Cu in all cases considered. For Co and Ni, this method only adapted to determining the contamination in Chinese effluents in which the Maximum Contaminant Level is higher compared to other water standards. When a scanner is used for colour detection, DGT could be used for monitoring Co in UK and China drinking water. Due to the Chelex binding phase was able to accumulate various trace metal cations, this method may not adapt to complex polluted environment. It was more suitable for deploying in the single targeted and extremely contaminated area such as copper mining area (see Chapter 6). The following work may focus deploying selective binding phase for Cu, Ni and Co, in order to improve the performance of this rapid screening technique in complex environment.

For Cr(VI), the relationship between the accumulation of Cr(VI) in NMDG gels and the corresponding change in grayscale intensity was perfectly fitted using a quintic polynomial in whole range and fitted using a linear equation when the mass of Cr(VI) up to 2.47μg cm^{-2} on the gel surface. With its good selectivity for Cr(VI) and strong reddish colour appeared on the white opaque gel, NMDG-DGT combined with colorimetric method will be very useful in monitoring of Cr(VI) in aquatic systems. Further work can be carried out to improve the stability of the coloration in higher Cr(VI) concentration.

Chapter 5. Determination of Heavy Metal Toxicity by Biological Diffusive Gradient in Thin Films (Bio-DGT) Using *Acinetobacter* Whole-cell Bioreporters

5.1 Introduction

5.1.1 Heavy Metal Contamination and Toxicity

Due to the rapid industrial development and urbanization to cope with large world population, heavy metal pollution has become an increasingly significant environmental issue, posing a threat to human health and ecosystem because of their toxicity, accumulation in the food chain and persistence in nature (Jarup, 2003b). The main sources of anthropogenic heavy metals in natural environments are industrial wastes, mining activities, sewage irrigation, atmospheric deposition, fertilizers and pesticides (DeVolder et al., 2003, Jones and Jarvis, 1981, Khan et al., 2008a, Kumar Sharma et al., 2007, Zhang et al., 2010b, Wuana and Okieimen, 2011, Zhang et al., 2011, Satarug et al., 2003). Heavy metal contamination is widely distributed and has pervaded globally, especially in developing countries such as China and India. In China, the average concentrations of Cu, Zn, Cd and As in agricultural soils are 10, 2.5, 13 and 24 times than the standard soil quality (Yang and Sun, 2009, Wang et al., 2011).

The enzymatic activities and metabolic activities of microbes can reflect the quality of soils sensitively, which is suggested that low concentrations of heavy metals can stimulate the microbial biomass, while excess amounts can lead to dramatic decrease of

microbial biomass (Fliepbach et al., 1994, Lee et al., 1996). The toxicity of soil heavy metals in plants varies with metal concentrations, metal species and plant species (Nagajyoti et al., 2010). Heavy metals in low concentration, no matter the heavy metal is important or unimportant for plant growths, possess little effects on plants (Su et al., 2014). Some heavy metals including arsenic, mercury and cadmium can destroy the metal-sensitive enzymes, which results in the growth inhibition or even death of plants. Heavy metal contamination is generally considered to be carcinogenic, mutagenic and teratogenic to mammals (Jarup, 2003a). Hazardous impacts of heavy metals on human can be enlarged by ecological accumulation through food chains. Inorganic arsenic can lead to cardiovascular disturbances, central nervous systems disruption and gastrointestinal synptoms (Jarup, 2003b, Bissen and Frimmel, 2003). Higher than 0.01 ppm time-weight average (TWA) concentration of cadmium can result in renal tubular damage and blood pressure increase. TWA concentration Cadmium at 2-3 mg per kg in creatinine leads to kidney damage (Buchet et al., 1990).

The existence of heavy metal varies under different environmental conditions. Particularly in soil, the availability and toxicity of metals are significantly dependent on soil physical and chemical characteristics, like pH, redox conditions, organic matters, carbonate and clay contents (Zhang and Zhang, 2007, Satarug et al., 2003, Kabata-Pendias, 1992). Chemical processes, such as adsorption-desorption, precipitation-dissolution, mineralization-immobilization determine the behaviour of metals in soils (Buekers, 2007, Levy et al., 1992, Shiowatana et al., 2001). This makes it difficult to

evaluate the real formation and impacts of heavy metal contamination in soils. The occurrence and fate of metal contaminants need to be monitored. The appropriate concentrations which reflect bioavailability need to be measured for controlling pollution and protecting the environment.

5.1.2 Chemical Sensors and Biosensors in Environmental Monitoring

Some chemical sensors based on electrochemistry (Hulanicki et al., 1991) and Chemiluminescence (CL) have been developed in recent years order to provide better tools for environmental monitoring. In addition, they have not been used for *in situ* measurement in soils.

The use of biosensor in environmental pollution monitoring has been a growing interest in the last decade, as these devices using whole-cell bioreporter to evaluate bioavailability and toxicity of contaminants via living microorganisms (Rodriguez-Mozaz et al., 2006, Farre et al., 2010, Lagarde and Jaffrezic-Renault, 2011). With genetically engineered bacteria, yeast, fungi, or animal cells, the biological signals of whole-cell bioreporter are initiated by phenotypic colour (lacZ), fluorescent (gfp/yfp) or bioluminescent (luc/lux) genes (Sanseverino et al., 2005, Van Dyk et al., 2001, Dunlap, 2014). It offers highly sensitive, rapidly analytic, easy operation and cost-effective feasibility for in situ pollutants assessment (D'Souza, 2001). Some whole-cell bioreporters specifically sense the heavy metal molecules (Rasmussen et al., 2000) or their cytotoxicity/genotoxicity (Rodriguez-Mozaz et al., 2006) However, most of the

bioreporters suffered from the low limit of detection and far away from application in the field situations. Meanwhile, whole-cell bioreporter can only assess the synergetic effects of all the pollutants, not able to distinguish the toxicity of heavy metals from the others. It is important to broaden the feasibility of whole-cell bioreporters and achieve selective assessment of heavy metal toxicity.

Since the assessment of the metal toxicity requires consideration of their speciation and bioavailability, the most advanced in situ speciation technique, DGT has the potential of combining with biosensor to form a unique new bio-chemical sensor. The DGT technique can quantitatively measure labile species in situ in waters, soils and sediments with high selectivity, high precision and low detection limits (W. et al., 2000, Alcock et al., 2003, Zhang et al., 1998b). Pervious study have shown that bacteria can be effectively incorporated into modified DGTs and the impact on the measured accumulated metal was generally small (Baker et al., 2015).

5.1.3 Aim of This Work

The aim of this study is to develop a novel biological diffusive gradient in thin films (Bio-DGT) by immobilizing whole-cell toxicity bioreporter ADPWH_recA in the diffusive gel to measure in situ labile metal concentrations and toxicity of metals simultaneously. The performance of the technique under different environmental conditions will be fully tested in laboratory solutions and in soil samples.

5.2 Materials and Methods

5.2.1 Bioreporter Strain and Cultivation

The whole-cell bioreporter ADPWH_recA was constructed with the *luxCDABE* gene fused on the chromosome of *Acinetobacter baylyi* ADP1 host (Song et al., 2009). It was utilised as an effective biological device to assess the cytoxicity and genotoxicity of specific chemicals or environmental samples (Zhang et al., 2013). After cultivated in Luria-Bertani (LB) medium supplemented with 10 μg mL^{-1} kanamycin at 30°C overnight, the 1.0 mL bioreporter strain was harvested by 10 minutes centrifugation at 3000 rpm. The bacterial pellet was resuspended in 10.0 mL fresh LB medium for direct toxicity measurement or in 1.0 mL sterile deionized water for Bio-DGT immobilization.

5.2.2 Preparation of Gels and Bio-DGT

All chemicals were analytical grade reagents from Sigma-Aldrich without specific statement. All glass plates, spacers and DGT devices had been soaked in 10% HNO3 acid for several hours and then thoroughly rinsed in ultrapure water (MQ at 18.2 MΩ cm) until pH 6.

An agarose suspension (1.2%, w/v) was prepared by adding 1.2 g agarose in 100 mL deionized water and autoclave at 121°C for 15 min. For the diffusive gel discs of Bio-DGT, after cooled to 50°C, the 0.9 mL agarose gel solution was mixed with 0.1 mL ADPWH_recA whole-cell bioreporter suspension which was washed and resuspended in deionized water. The mixture was stirred for 1 min and then injected between two

pre-heated glass plates separated by a 0.5 mm PTFE spacer. After set at the room temperature, the discs (2.2 cm in diameter and 0.5 mm thickness) were cut from the gel sheet. They were gently eased off the glass plate into MQ water. For the diffusive gel discs of control DGT, they were prepared as previously described except the immobilization of whole-cell bioreporters. Preparing procedure of Chelex gels and DGT assembly have been described in section 4.2.2.

5.2.3 Bio-DGT Deployment and Measurements

Both control DGT devices and bio-DGT devices were deployed in triplicates in either 2 L or 8 L stirred solutions of different pH, ionic strengths and for different times. They were carefully rinsed with MQ water after retrieval. The devices were disassembled and the Chelex-100 binding gels were removed and eluted in 1ml of 1M HNO_3 overnight before carrying out analysis by ICP- MS (Thermo Scientific). The eluents were diluted at least 10 times to a final volume of 1ml to a final concentration in the range of ICP-MS calibration.

For the biological response, the agarose diffusive gel discs from Bio-DGT and control DGT were placed into the 12 well cluster flat bottom culture plates containing 2ml LB medium in each well. The bioluminescent signals of each well were measured by a FLUOstar Omega microplate reader (BMG Labtech, UK). Throughout the measurement process, the bioreporters were incubated at 30°C with continuous shaking at 100 rpm and the relative bioluminescence unit (RLU) was measured every 10 min.

The bioluminescent induction was obtained by averaging the RLU from 250 minutes to 300 minutes, which was the best responsive time for ADPWH_recA bioreporter. The bioluminescent response ratio was evaluated to estimate the cytoxicity and genotoxicity of targeting samples by the ratio of the bioluminescent induction of samples to that of control treatment (non-induced samples).

For direct measurement of heavy metal toxicity, 180 μL of ADPWH_recA bioreporter suspension (washed and resuspended in LB medium) was transferred into the well of black clear-bottom 96-well microplate (Corning Costa, USA). After addition of 20 μL of mitomycin C (standard genotoxin) or heavy metal solution, the plate was cultivated at 30°C for 8 hrs with continuous shaking at 100 rpm. Both RLU and OD_{600} were measured every 10 minutes. The normalized RLU was calculated by dividing induced RLU by OD_{600}. Three biological replicates were undertaken for each sample.

5.2.4 Bio-DGT Performance and Validation

Without specific statement, all the performance test and validation experiments were carried out in 0.01M NaNO3 solution containing 10 μg L^{-1} Cd.

5.2.4.1 Whole-cell Bioreporter Immobilization and Viability

To evaluate bioreporter viability and achieve the optimal immobilization temperature, when casting the diffusive gel, the agarose suspension was cooled to 100°C, 80°C, 60°C, 50°C and 40°C respectively before mixing with the bioreporter suspension. The

obtained agarose diffusive gel discs were then placed into the 12-well flat bottom cell culture plates containing 2 mL of LB medium and exposed to 1 μM mitomycin C. The following bioluminescent signal measurement followed the same procedure as described above.

5.2.4.2 Bioluminescent Distribution of Bio-DGT

To test the uniformity of bioluminescent signals of Bio-DGT, the agarose diffusive gel discs were placed into the 6-well flat bottom cell culture plates containing 5 mL of LB medium. After adding ultrapure water (negative control) or mitomycin C to final concentration of 1 μM, the plate was cultivated at 30°C for 8 hrs without shaking. The bioluminescent signals were obtained every 10 min by the FLUOstar Omega microplate reader via the scanning mode with 625 measurement points per disc (25×25). Control DGT gel discs were used as the blank control. Triplicated biological were undertaken for each treatment.

5.2.4.3 Effect of Deployment Time

The effect of bioreporter on the general performance of DGT and the effect of deployment time on bioreporter were investigated by deploying triplicate bio-DGT together with control DGT devices in 8 litres of Cd solution. The pH of the solution was maintained at 6.0 ± 0.5 using 1M NaOH and 1M HNO_3. Solution samples were taken at the beginning, the middle and the end of the experiments. After 24, 48, 72, 96, 120, 144, 168 hours of deployment, Bio-DGT and control DGT devices were removed and

rinsed with MQ water.

5.2.4.4 Effect of pH and Ionic Strengthen

The effect of solution pH on the uptake of metal by bio-DGT and on the stability of bioreporter were tested by deploying DGT devices in the Cd solution with different pH of 4, 6 and 8. The effects of ionic strength were investigated by deploying DGT devices in Cd solution with different ionic strength of 0.001, 0.01, 0.1 and 0.5M using NaNO3. Four devices were used for each tests and the deployment time was 48 hours.

5.2.4.5 Effect of Storage Time

The viability and sensitivity of the whole-cell bioreporter ADPWH_recA in a long storage time were evaluated by placing the agarose discs with live cells in 0.01M $NaNO_3$ and stored at <4 ^{o}C for at least 60 days. The bioluminescent response of the bioreporter were measured initially and then at day 5, 10, 20, 30, 40 and 60.

5.2.4.6 Detection Limits and Precision

The detection limits of DGT were first calculated as three times the standard deviation of the DGT blanks, expressed in ng/disc of resin gel. It is then calculated using DGT equation, for certain temperature and deployment, to obtain the detection limits in concentration (ng L^{-1} or μg L^{-1}). The blank DGT devices were assembled at the same time using the same batch of gels as DGT devices for the experiments. They were kept in plastic bags and the resin gels were retrieved and eluted at the same time following

the same procedure as for devices being deployed in solutions. The precision of Bio-DGT was determined by measuring triplicate devices in each experiment and presented as the relative standard deviation (RSD).

5.2.5 Application in Soils

Four soil samples were collected from Guangzhou, China. Samples were air dried and passed through a 2mm mesh sieve for the DGT measurements.

The DGT deployments were carried out using a standard procedure (Ernstberger et al., 2002) on soil slurries and the soil moisture was maintained at about 80% of water holding capacity (WHC) during the experiment. The soil slurries were prepared two days before DGT deployment to allow equilibrium between the soil solution and solid phase. The slurries were divided into three portions and transferred into 60mm petri dishes. One DGT device was placed on the surface of the each soil slurry carefully, making sure that good contact between soil and DGT device. After 8 hours of deployment, DGT devices were retrieved from the soil slurries and rinsed with MQ water to remove soil particles. After disassembling the devices, Chelex gel discs were eluted in 1ml of 1M HNO3 solution and subsequently measured by ICP-MS. The bioluminescent response in diffusive gel discs (with cell) were measured by microplate reader (V_{LB} = 3mL).

The soils were of the same geological origin but differ in terms of soil properties. Soil properties, including pH, texture, cation exchange capacity (CEC), organic matter (OM),

total concentration of Cd and other metals were measured using standard soil analysis methods recommended by Chinese MEP (MEP, 1995) (Table 5.1).

Table 5.1 Properties of soil samples and DGT measured concentration of metals.

Soil samples	1	2	3	4
Texture	Sandy Clay	Sandy Clay	Silt Loam	Silt Loam
pH	5.73	4.86	6.59	5.77
CEC(cmol kg^{-1})	3.72	4.63	3.42	3.43
OM(g kg^{-1})	50.72	66.01	10.48	26.37
Cd_{tot}(mg kg^{-1})	4.58	3.01	1.24	1.09
Pb_{tot}(mg kg^{-1})	240.89	39.78	59.58	30.27
Mn_{tot}(mg kg^{-1})	41.17	27.42	281.07	193.61
Fe_{tot}(g kg^{-1})	7.29	6.94	13.06	13.67
Cd_{DGT} (μg L^{-1})	1.79	17	0.44	8.58
Pb_{DGT} (μg L^{-1})	4.06	62.18	0.64	8.20
Mn_{DGT} (μg L^{-1})	30.08	56.44	94.68	87.02
Fe_{DGT} (μg L^{-1})	153.45	177.77	1.41	31.85

Besides, to eliminate the potential contribution of Mn to the toxicity of Cd, a simple test was carried out using the diffusive agarose gels (with cell) in solution of Mn, Cd and the mixture of the two. The agarose gel was placed in 20 ml of i) 100 μg L^{-1} Cd

solution, ii) 100 mg $L^{-}1$ Mn solution and iii) the mixture of Cd/Mn solution with 100 μg L^{-1} Cd and 100 mg L^{-1} Mn, respectively. After eight hours immersion, the bioluminescent response on the gels was measured by microplate reader.

5.2.6 Data Analysis

The DGT measured concentration of metals was calculated using the standard DGT equation 2.6. The 0.05 cm agarose gel used as a diffusive gel. Previous studies have shown that there is an assumption that cells have negligible effect on the diffusion coefficient, *D*, within the agarose gel (Westrin, 1990). The mass of metal accumulated on the resin gel of a DGT device was calculated from the ICP-MS measurement by equation 2.4 (details see Chapter 2).

The bioluminescent response was predicted by the previously developed gene regulation model (Al-Anizi et al., 2014, Zhang et al., 2012) with some modifications to simulate the heavy metal accumulation and flux in the film with bioreporter immobilization. SOS response represents the global response of bacterial cells to carcinogens and the subsequent process of DNA repair (Sancar, 1996), including the three key steps as mutagenesis by heavy metals, single stranded DNA (ssDNA) stimulation and DNA repair activation (Foster, 2007, Krishna et al., 2007). The formation of methylated or alkylated (K_{metal}) double stranded DNA (dsDNA) consequently resulted in the synthesis of ssDNA (k_{metal}) and the cleavage of LexA-like SOS repressor (*LSR*, $cell^{-1}$). In the transcriptional cross-regulation model (Zhang et al., 2012), the DNA damage and SOS box promotion is expressed in Equation 5.1 and

5.2, where k_{ssDNA} represents the cleavage reaction constant of LSR dimer by RecA protein, and the equilibrium of LSR dimer (K_{dLSR}) and monomer (K_{sLSR}) determines SOS expression rate (k_{dSLR} and k_{sLSR}, respectively). The SOS response level is expressed in Equation 5.3.

$$[dLSR] + [SOS - box] \overset{K_{dLSR}}{\Longleftrightarrow} [dSLR - SOS] \overset{k_{dSLR}}{\longrightarrow} SOS\ response\ repression \tag{5.1}$$

$$[sLSR] + [SOS - box] \overset{K_{sLSR}}{\Longleftrightarrow} [SOS] \overset{k_{sLSR}}{\longrightarrow} SOS\ response\ expression \tag{5.2}$$

$$SOS_s = \left(\frac{k_{ssDNA} \cdot k_{dSLR}}{1+k_{ssDNA}} \cdot [LSR]_{total}\right) \cdot \frac{[dLSR]}{K_{dLSR}^{-1}+[dLSR]} + \left(\frac{k_{dSLR}}{2\cdot(1+k_{ssDNA})} \cdot [LSR]_{total}\right) \cdot \frac{[sLSR]}{K_{sLSR}^{-1}+[sLSR]} \tag{5.3}$$

$$SOS_{r,s} = 1 + \left(\frac{k_{dSLR}}{2\cdot(1+k_{ssDNA})} \cdot [LSR]_{total}\right) \cdot \frac{\int_{t=0}^{n}[Metal]dt}{(K_{sLSR}\cdot K_{metal}\cdot k_{ssDNA}\cdot k_{metal})^{-1}+\int_{t=0}^{n}[Metal]dt} \tag{5.4}$$

Here, $dLSR$ (cell^{-1}) and $sLSR$ (cell^{-1}) refer to LSR dimer and monomer respectively, showing unique repression and activation on the SOS box. $[LSR]_{total}$ (cell^{-1}) represents the total amount of SOS protein in terms of monomer. SOS_s represents the intensity of SOS response (cell^{-1}). Compared with the bioluminescent baseline absence of genotoxins, the relative SOS response ratio, $SOS_{r,s}$, is expressed in Equation 5.4. The SOS response coefficient is defined as $K_{sLSR} \cdot K_{MO} \cdot k_{ssDNA} \cdot k_{MO}$, referring to the combined efficiency of genotoxin DNA damage, ssDNA recognition and SOS box promotion. $K_{Genotoxicity}$ is the SOS responsive intensity of specific carcinogens and refers to the coefficient of genotoxicity impacts, representing

$\frac{k_{dSLR}}{2\cdot(1+k_{ssDNA})} \cdot [LSR]_{total}$.

The cytoxicity is caused by direct inhibition effects of cytotoxic compounds, consequently resulting in the suppression of cell activities. Various types of cytotoxic effect can be identified, such as membrane integrity loss as the result of cell lysis and protein activity inhibition. Particularly, with the dynamic cytotoxic coefficient ($k_{cytoxicity}$), the protein inhibition can be expressed in the following Equation 5.5.

$$[Metal] + [protein] \overset{k_{cytoxicity}}{\Longleftrightarrow} [inhibited\ protein] \tag{5.5}$$

$$K_{cytotoxic\ ity} = \exp(-k_{cytoxicity} \int_{t=0}^{n} [Metal]dt) \tag{5.6}$$

Considering the equilibrium reaction state, the cytotoxic inhibition ratio is therefore associated with the exposed cytotoxic chemicals, as expressed in Equation 5.6. Consequently, the cytoxicity also affects cell metabolism, causing damage to enzymes and cell activities, or even apoptosis (Joiner et al., 1999). The remaining cell activities, as defined in Equation 5.7, are characterised by the constant expressed *luxCDABE* gene on the chromosome of *Acinetobacter baylyi* ADP1, which is linked to the function of cellular proteins.

$$SOS_{r,s} \cdot K_{cytotoxic\ ity} = [1 + \left(\frac{k_{dSLR}}{2\cdot(1+k_{ssDNA})} \cdot [LSR]_{total}\right) \cdot \frac{\int_{t=0}^{n}[Metal]dt}{(K_{sLSR}\cdot K_{metal}\cdot k_{ssDNA}\cdot k_{metal})^{-1}+\int_{t=0}^{n}[Metal]dt}] \cdot \exp(-k_{cytoxicity} \int_{t=0}^{n} [Metal]dt) \tag{5.7}$$

5.3 Results and Discussions

5.3.1 Whole-cell Bioreporter Immobilization and Viability

The viability of whole-cell bioreporter ADPWH_recA remained satisfactory when immobilized in agarose thin film at different temperatures (Figure 5.1A). At 100ºC, all the ADPWH_recA bioreporter cells were dead without producing any bioluminescent signal or response. When the immobilization temperature was 60 ºC or less, the bioreporter viability ranged from 60% to 85%. Meanwhile, the bioluminescent response of ADPWH_recA to 1 μM mitomycin C was positively correlated with the immobilization temperature (Figure 5.1B), attributing to the heat-shock inducible SOS activation (Benndorf et al., 1999). Above 60ºC, the bioreporter strains were seriously damaged and its SOS response was inhibited. As a soil bacterium, *Acinetobacter baylyi* is extremely robust to environmental variation, including temperature.

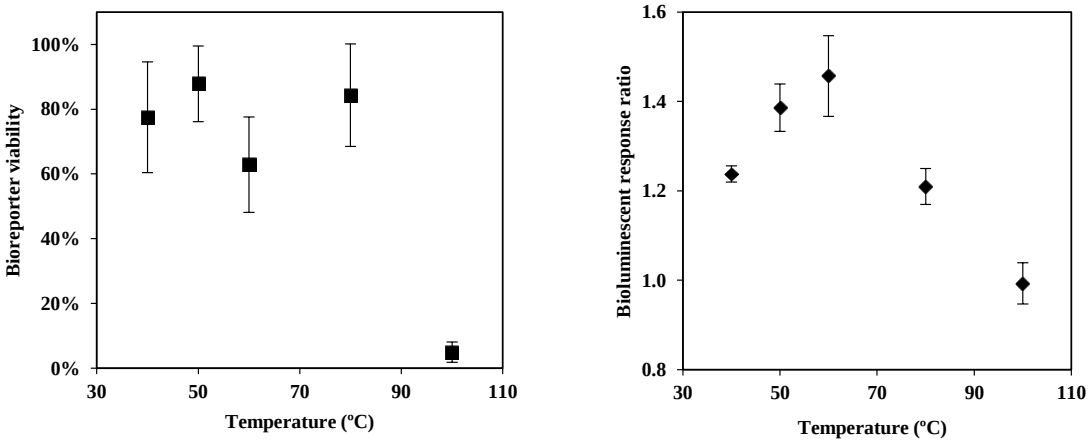


Figure 5.1 (A) Bioreporter viability test for Bio-DGT immobilization at different temperatures. (B) Bioreporter response to mitomycin C (1 μM) after Bio-DGT immobilization at different temperatures.

Post exposure to 1 μM mitomycin C, Bio-DGT showed high uniformity in bioluminescence throughout the induction time (Figure 5.2). No significant bioluminescent signals were detectable for negative control (DGT discs), and they followed the normal distribution between 0 to 300 RLU which was the same as the background. In blank treatment (no mitomycin C exposure), a weak bioluminescent signal (5,000-10,000 RLU) was detected and it gradually increased with time. After exposure to 1μM mitomycin C, Bio-DGT showed the strong bioluminescence, averaging 13,535±1,620 RLU, with the normal distribution pattern across the film. The responsive ratio 2.3 compared to the blank.

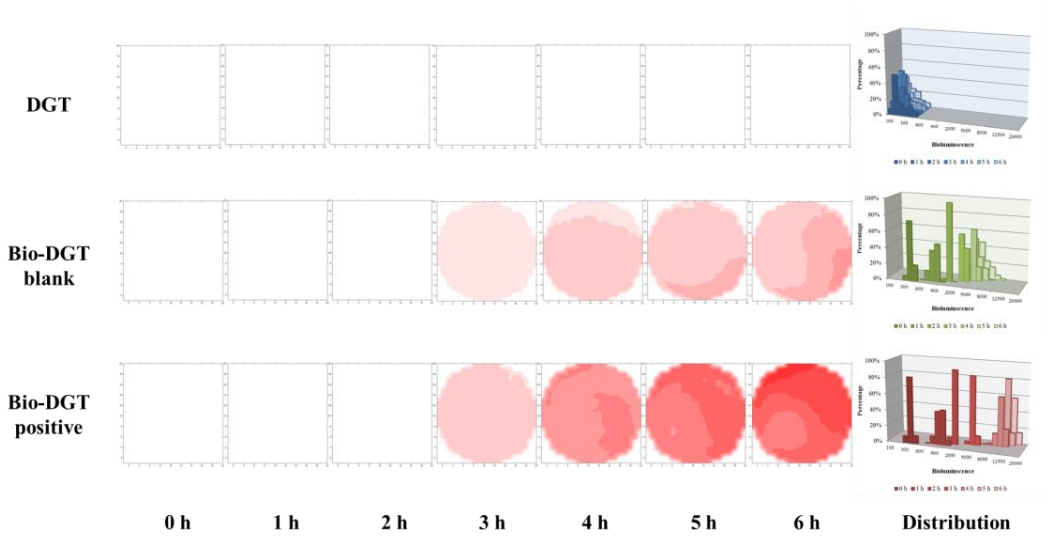


Figure 5.2 Bioluminescent distribution of control DGT, blank Bio-DGT and Bio-DGT postexposure to 1 μM mitomycin C

5.3.2. Deployment Time and Mass Accumulation

The mass of Cd accumulated on the resin gels of Bio-DGT and control DGT devices were measured and compared for different deployment times up to 7 days. The mass increased linearly with deployment time and there was no significant difference

between Bio-DGT and control DGT, as demonstrated in Figure 5.3. The results obtained by both DGT devices are very close to theoretical line predicted by DGT equation. Though slight deviations are observed for longer deployment times, they are all less than 10%. These indicate that the live cells in the diffusive gel layer did not affect the performance of the Bio-DGT devices. The Cd concentration obtained by DGT (C_{DGT}) calculated using DGT equation were 4.93 μg L^{-1}, very close to the Cd concentration (C_{soln}) in solution (5.35 μg L^{-1}). The concentration of Cd that was measured by Bio-DGT containing cells grown in LB was slightly higher, 5.12 μg L^{-1}, compared with the Cd concentration measured by control DGT devices. The ratios of C_{DGT} and C_{soln} were 0.92 and 0.95, within the acceptable range that indicating good performance of the Bio-DGT devices.

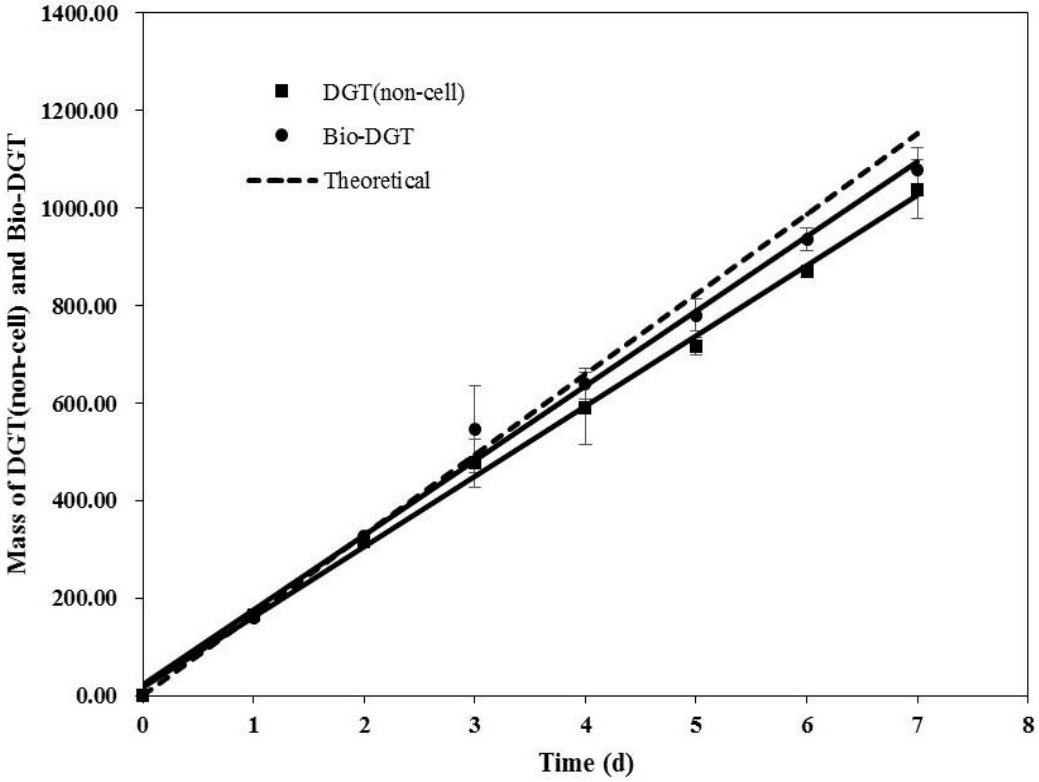


Figure 5.3 Mass of Cd accumulated by control DGT and Bio-DGT devices as a function of time in solution. Both DGT devices were immersed in a well-stirred solution of 10 μg L^{-1} Cd(II) (I= 0.01mol L^{-1} $NaNO_3$, pH=6.0, 25±0.5℃).

5.3.3 Response Dynamics and Performance of Bio-DGT

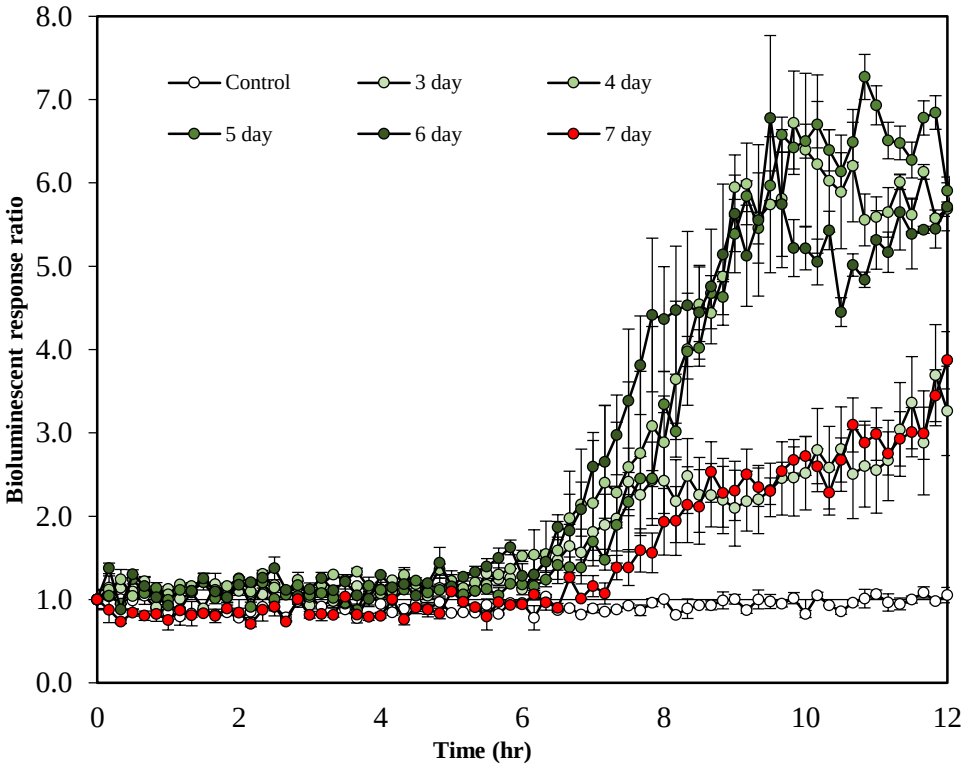
The real-time bioluminescent curve of Bio-DGT was illustrated in Figure 5.4A. There was low biological signal within 6 hours detection. The immobilized bioreporter cells needed a certain time to adapt the cultivation medium and activate their gene expression. In this period, the recA gene was not activated and the bioluminescent signal represented the viability of whole-cell bioreporter cells. It was therefore used to evaluate the cytoxicity of heavy metals on bioreporter cells. In the present study, low cytoxicity of Cd was observed in Bio-DGT. There was no significant cytoxicity identified for all

the treatments, except for the 7-day deployment treatment in which a slight activity reduction (13%) of the Bio-DGT device was found. For DGT without bioreporter immobilization, no significant bioluminescent signal variation is observed in the treatments of different deployment time.

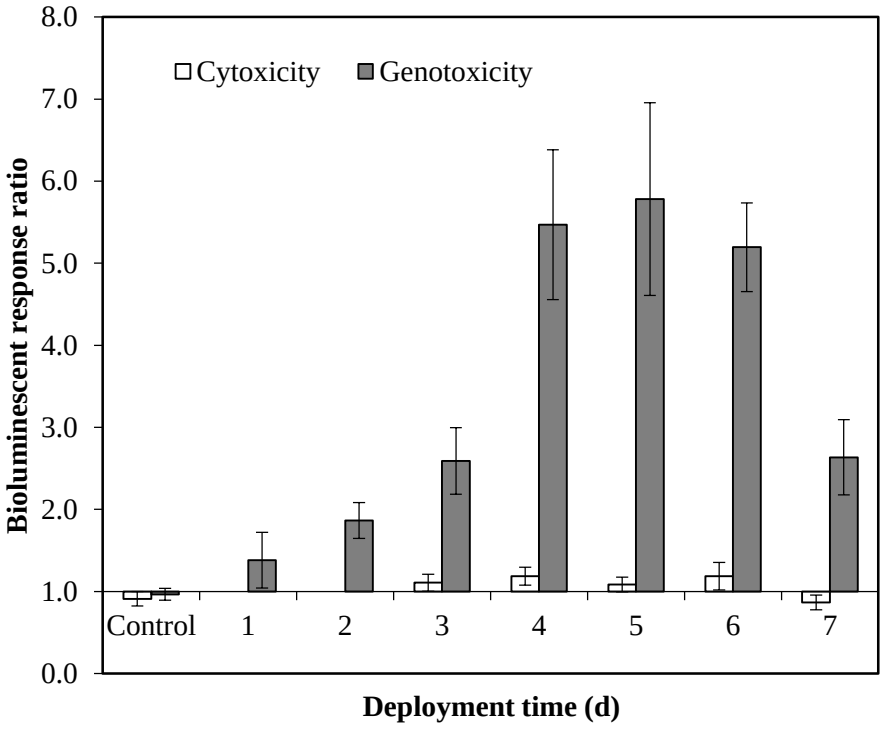
The response of bioreporter ADPWH_recA followed the toxicity gene expression model, which was the combination of cytoxicity and genotoxicity (Figure 5.4B). The significantly increasing bioluminescence from 6 to 10 hours indicated the expression of *recA* gene, triggered by the heavy metal genotoxicity and DNA damage. Figure 5.4B showed significant genotoxicity effects with different deployment time. Genotoxicity is positively related to the deployment time from 0 to 5 days, showing the accumulated Cd causing more severe DNA damage and genotoxicity. After 6-day deployment, the biological response becomes saturated and decreased, hinting the over-exposure causes less activity and death of bioreporter cells, fitting with the cytoxicity assessment. Without bioreporter immobilization, DGT has minimal bioluminescent signal (< 25,000 RLU) for all the treatments. The bioluminescence of Bio-DGT ranges from 3,700 RLU to 9,900 RLU at 0 h, increasing to over 300,000 RLU after 10 h induction. The significant bioluminescence inhibition in the first 6 hours attributed to the cytoxicity effects of heavy metals on bacterial activities, and the positive response from 8 h to 10 h was caused by the heavy metal genotoxicity and the activation of SOS response and *recA* gene expression. After 10 hours detection, the bioluminescent response ratio maintained stable, indicating the late exponential phase and saturated expression of *recA*

gene.

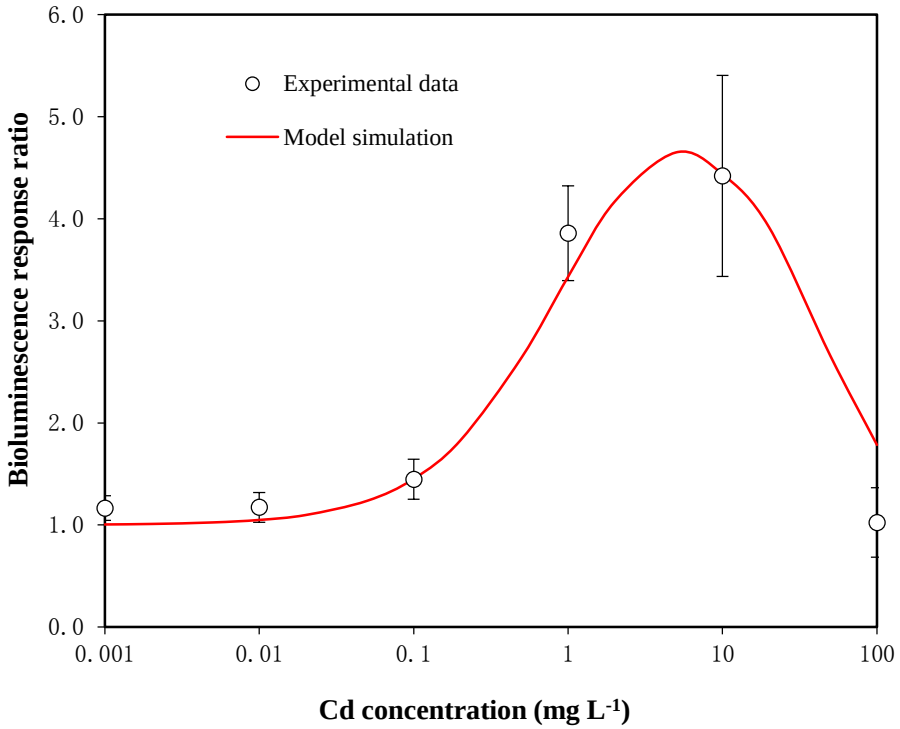
The increasing bioluminescent response of Bio-DGT with deployment time was attributed to the integrals of heavy metal flux. The activation of SOS-related *recA* gene was regulated by the ssDNA caused by DNA damage, and it reflected the impacts of heavy metal exposure and could be quantified by the total flux of heavy metal. In Bio-DGT treatment, the increasing bioluminescent signals was used to calculate the integral heavy metal flux at different deployment time. The good fitting curve (Figure 5.4C) suggested the positive heavy metal exposure relationship between Bio-DGT accumulation and biological response.



(A)



(B)



(C)

Figure 5.4 Bioluminescence dynamics (A) and bioluminescent response ratio (B) of Bio-DGT under different deployment treatments (0 to 7 days). (C) Dose-effect correlation between bioluminescent response ratio and heavy metal (Cd) concentrations.

5.3.4 Effects of pH and Ionic Strength

The wide variety of environmental variables in real samples might affect the performance of bioreporter. The feasibility needs to be validated for environmental sample detection. The robustness of Bio-DGT has been tested under different pH and ionic strength conditions.

The effect of pH on bioreporter response was examined in the pH range of 4 to 8, most relevant to natural waters and soils (Fig. 5.5). The difference of bioluminescent signals of pH 4.0 to 8.0 were within 7%, similar to the previous research on the pH influence on *A. baylyi* ADP1 and ADPWH_recA that *Acinetobacter* based bioreporter could tolerate large pH variation (Jia et al., 2016, Li et al., 2009). The ratios of Cd concentration measured by Bio-DGT to the concentration measured by ICP-MS were illustrated in Figure 5.5A for each tested pH. All three results were in the accepted range of 0.9 to 1.1 confirming the accuracy of Bio-DGT measurements under natural environmental pH ranges for waters and soils. The bioluminescent response of Bio-DGT did not change significantly under different pH, attributing to the robustness of the *Acinetobacter* strain.

The effect of ionic strength was investigated by deploying Bio-DGT devices in solutions containing 1mM, 10mM, 0.1M and 0.5M $NaNO_3$. High ionic strength significantly inhibited the bioreporter viability and reduced the response sensitivity, as illustrated in Figure 5.6. The bioreporters are difficult to survive in high salinity environment may due to plasmolysis appeared in the cells. The Cd concentrations

measured by DGT and directly by ICP-MS in deployment solutions were in good agreement for all ionic strengths, similar results as previously published work (Figure 5.6) (Gimpel et al., 2001).

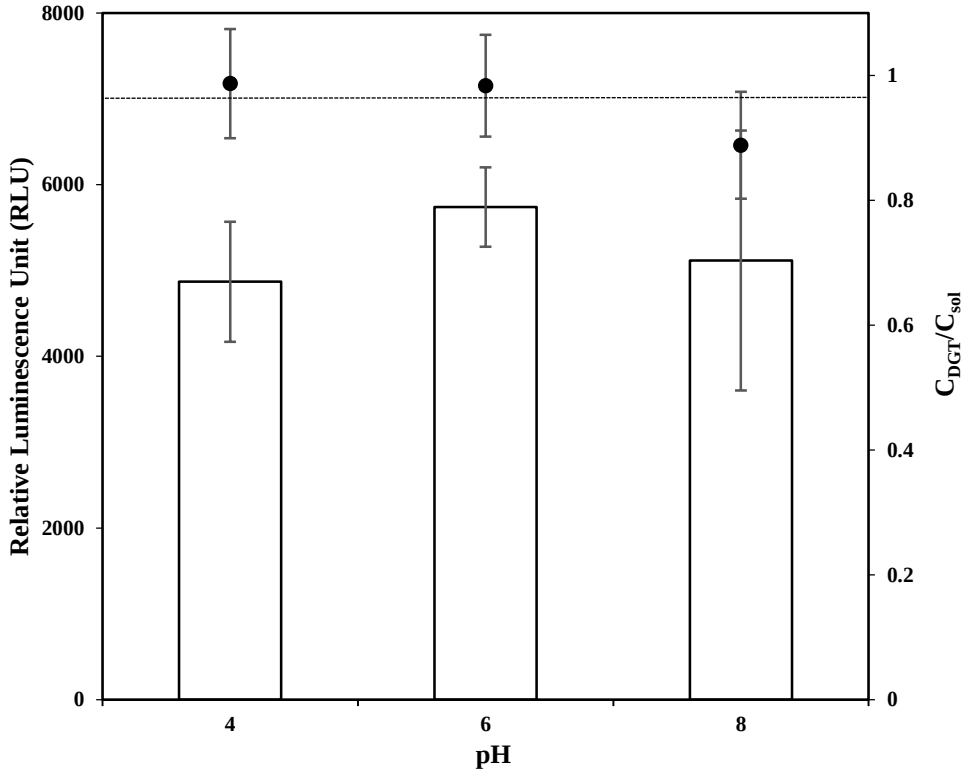


Figure 5.5 Effect of pH on Bio-DGT performance. Deployment solution containing 10μg L^{-1} of Cd(II); I=0.01mol L^{-1} $NaNO_3$ at various pH levels, and pH=6.0 at various ionic strengths; t=2 d; T=25±0.5℃.

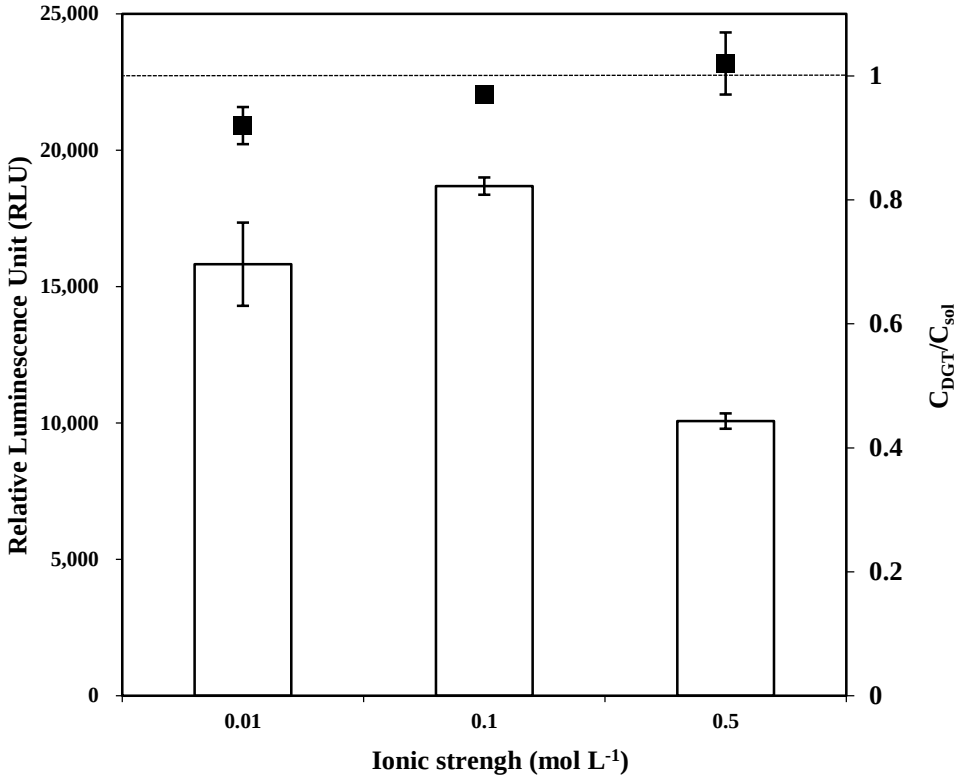


Figure 5.6 Effect of ionic strength on Bio-DGT performance. Deployment solution containing 10μg L^{-1} Cd(II); I=0.01mol L^{-1} $NaNO_3$ at various pH levels, and pH=6.0 at various ionic strengths; t=2 d; T=25±0.5℃.

5.3.5 Effect of Storage Time on Performance

As DGT devices have been used for environmental monitoring of heavy metals in aquatic systems, the newly developed Bio-DGT has the great potential to improve the efficiency of our monitoring practice. Therefore, it is important to investigate the performance of the device after storage and determining the shelf life base on the metal toxicity assessment, which is the determine factor. The whole-cell bioreporter ADPWH_recA is reported to remain viability and sensitivity after 30 days storage. The bioluminescent signal of Bio-DGT device maintained at high level up to 30 days storage

(Figure 5.7) before significant reduction at 40 days. The test was carried out for 60 days. Slight reduction of bioluminescent signal with time was observed after 30 days. The results indicate the robustness and reasonable long shelf life of the Bio-DGT, making it viable for applications in environmental monitoring.

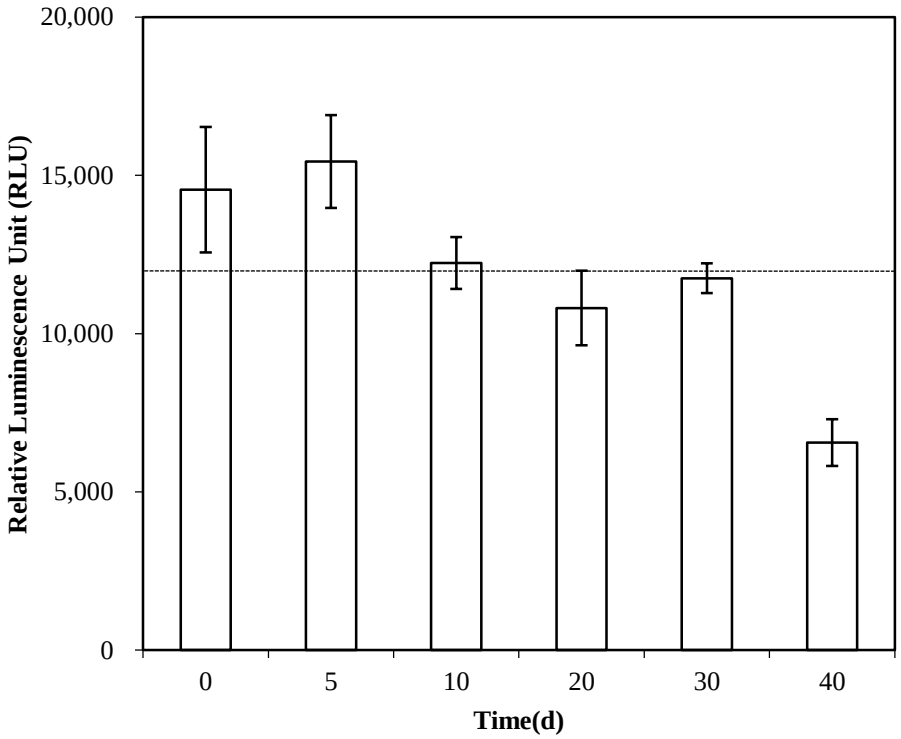


Figure 5.7 The bioluminescent signal and viability of Bio-DGT devices in response to storage time for up to 40 days at 4°C. They were measured in 0.01 M $NaNO_3$.

5.3.6 Detection Limits and Precisions

As DGT is an accumulation technique, the method detection limit (MDL) varies with deployment time. The absolute method detection limit (MDL) of the technique for Cd based on three times the standard deviation of the blank DGT resin gel value is 0.16ng/disc and 0.19ng/disc for control DGT and Bio-DGT respectively. If the DGT

devices are deployed for 1 day at 20 °C, the MDLs for control DGT and Bio-DGT would be 0.15 and 0.20 μg L^{-1}, respectively. For 7 days deployment at 20℃, the MDLs would be 0.02 and 0.03μg/L, respectively. The method precision for data obtained by Bio-DGTs was between 1% to 10% in all performance test experiments and 6% to 17% in soil applications presented in section 5.3.8.

5.3.7 Bio-DGT Application in Soils

The results of bioluminescent response in the diffusive gels of the Bio-DGT devices and the DGT measured Cd concentrations in soil samples are presented in Figure 5.8. The bioluminescent response ratio was calculated by dividing the RLU of Bio-DGT which deployed in soil samples by the blank Bio-DGT. A positive correlation between the bioluminescent response ratio and the concentration of Cd measured by Bio-DGT can be observed in Figure 5.8. The soil with greater available Cd concentration has higher response ratio indicating more toxic situation with possible DNA damage to the cells in Bio-DGT.

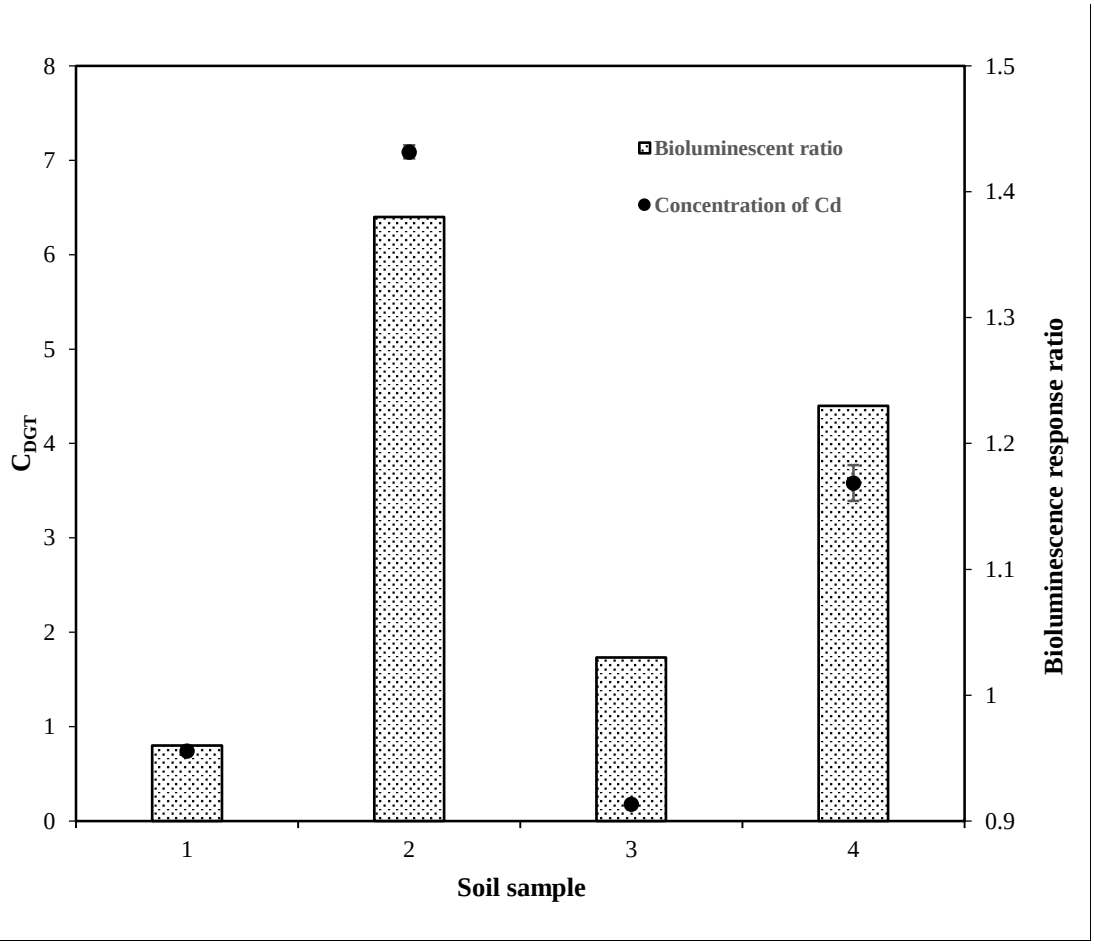


Figure 5.8 The bioluminescent response in the diffusive gels of the Bio-DGT devices and the DGT measured Cd concentrations in soil samples.

In this group of soils, the concentrations of Mn are significantly higher and dominating as these soils are laterite, with high Fe, Al and Mn content, which collected in Southwest of China (Gidigasu, 2012). To investigate the effect of high Mn concentration on the response of Cd toxicity, a simple experiment of exposing the cell loaded agarose gels to the mixed solution was carried out. The bioluminescent response of bioreporter on gels being exposed to three different solutions showed no significant difference even though the concentration of Mn was 1000 times higher than that of Cd. There was lack of literature on Mn affects the bioreporter and no direct evidence shows Mn will damage RecA gene.

5.4 Conclusions

This study was to develop a new DGT technique, Bio-DGT that can measure in situ labile metal concentrations and toxicity of metals simultaneously. As a robust and adaptable bacteria, ADPWH_recA was successfully grow in agarose gel as the diffusive layer during the deployment. Bio-DGT showed high uniformity in bioluminescence throughout the induction time and the optimal immobilization temperature was set as 50 ºC. It also demonstrated that the metal ions associated with ADPWH_recA have negligible effect on the measurements of metals by DGT. The novel Bio-DGT has achieved a reliable and stable measurement of metals in a wide range of pH and ionic strength except the high salinity.

The application in soils, showed the Bio-DGT can provide a simple and effective way to measure toxicity and concentration of heavy metals at same time in the same location. A positive correlation was obtained between the bioluminescent response ratio and the concentration of Cd measured by Bio-DGT in soils with a wide range of properties. Although there are more tests need to be done on combining microorganisms and DGT, this work has demonstrated a significant step forward from the previous study (Baker et al., 2015). It successfully build a bridge between chemical monitoring and biological monitoring. Future studies could be focused on developing more selective and sensitive biosensor to improve the effectiveness of Bio-DGT. Bio-DGT could be used widely for continuous monitoring of heavy metals in both contamination level and toxicity effect to biota in the environment.

Chapter 6 Field Applications of DGT as Rapid Screening Technology for Metals and P

6.1 Introduction

6.1.1 Field Evaluation of DGT

Passive sampling, as an alternative to overcome some of difficulties of traditional sampling, has been widely used for monitoring a variety of pollutants (Seethapathy et al., 2008). Passive samplers avoid many of the problems mentioned above, since they collect the target analytes *in situ* and without affecting the bulk solution. Diffusive gradients in thin films (DGT) is a *in situ* passive sampling technique, which has been successfully applied in a variety of environmental monitoring projects on hazardous radionuclides, trace metals, nutrients, organic contaminants and pharmaceuticals (Mengistu et al., 2012). DGT samplers have also been deployed to assess levels of aluminium pollution in the fish populations of Norway and to determine the source of elevated the metal successfully. DGT measured Al made a better prediction of the fish gill uptake and the aluminium-induced physiological stress responses of fishes than laboratory-determined labile monomeric aluminium (Royset et al., 2005). Monitoring the changes in heavy metal concentrations using DGT measurements also demonstrates how various cycles and events within estuaries are related. DGT measurements were taken at twelve sites in Broadwater, south-east Queensland, Australia for 6 hours over a 4 week period (Dunn et al., 2007). By choosing the probes deployment period

carefully, changes in heavy metal concentration due to estuarine events (storm water run-off, recreational boating season) and cycles (tidal currents and flushing) were observed using the DGT technique. In addition, DGT samplers have been used to evaluate the concentration of metals in the water of thirteen estuaries in the south-eastern Bay of Biscay, France (Montero et al., 2012). The results demonstrate that DGTs can measure labile metal concentrations in a reproducible way, providing a representative average labile metal concentration in highly fluctuating systems, such as estuaries. This reaffirms the potential of using DGT for the chemical evaluation of transitional water bodies within the Water Framework Directive (WFD). Moreover, DGT has been confirmed as reliable tool to evaluate metal fractionation in wastewater which successfully distinguished between labile and inert forms of metals in wastewater (Buzier et al., 2006). The DGT technique was also effective at taking *in situ* measurements of reactive phosphorus in freshwater aquaculture. However, the high concentration of phosphorus and suspended matter in aquaculture freshwaters can lead to biofilm build up on the surface of the filter membrane of DGT samplers. As a result, DGT must only be deployed for a maximum of four days to ensure the technique yields a good response (Pichette et al., 2009). Besides, in other scenarios, the DGT technique excels. For example, when determining the bioavailability of heavy metals in wheat fields where the soils are affected by different sources of metal pollution, the DGT technique is by far the most widely applicable method used to assess heavy metal bioavailability in soils. It has successfully predicted the in root concentration of Cu, Ni,

Pb and Zn, and it was able to distinguish between low and high Cr concentration (Soriano-Disla et al., 2010).

6.1.2 Aim of Work

In this chapter, Metsorb DGT, ZrO DGT and Chelex DGT have been applied in five regions of China, including upstream of a water reservoir, in fish and shrimp farms, in agricultural soil, and in mining areas. The concentration of P was measured by Metsorb DGT and ZrO DGT followed by the conventional molybdenum blue method as described in Chapter 3. The concentration of metals was measured by Chelex DGT as described in Chapter 4. The aim of the study was to apply the newly developed rapid screen technique, for the chemical monitoring by combining DGT and colorimetry, in situ in different environmental conditions. The specific objectives of this work were (i) to investigate the field applicability of the new DGT approach with colorimetry for measuring metals and P, (ii) to assess how efficient DGT devices are at distinguishing different concentrations and types of contamination in various aquatic systems.

6.2 Field Sites

To test the applicability of the DGT devices, five different field sites in different parts of China were chosen.

6.2.1 Rivers in Suburb of Beijing

The River Chao is located in Miyun County, which is eighty kilometres northeast of

downtown Beijing. The Miyun Reservoir divides the river into two parts. The upstream of River Chao stretches 24 kilometres and the drainage area, which is located in a hilly area, is 234.5 kilometres square. The eight sites were distributed across two different villages, two bridges and a hydrometric station. The Miyun Reservoir is one of Beijing's main sources of potable water. It has a catchment area of 15,788 square kilometres and a maximum storage capacity of 4.375 billion cubic metres.

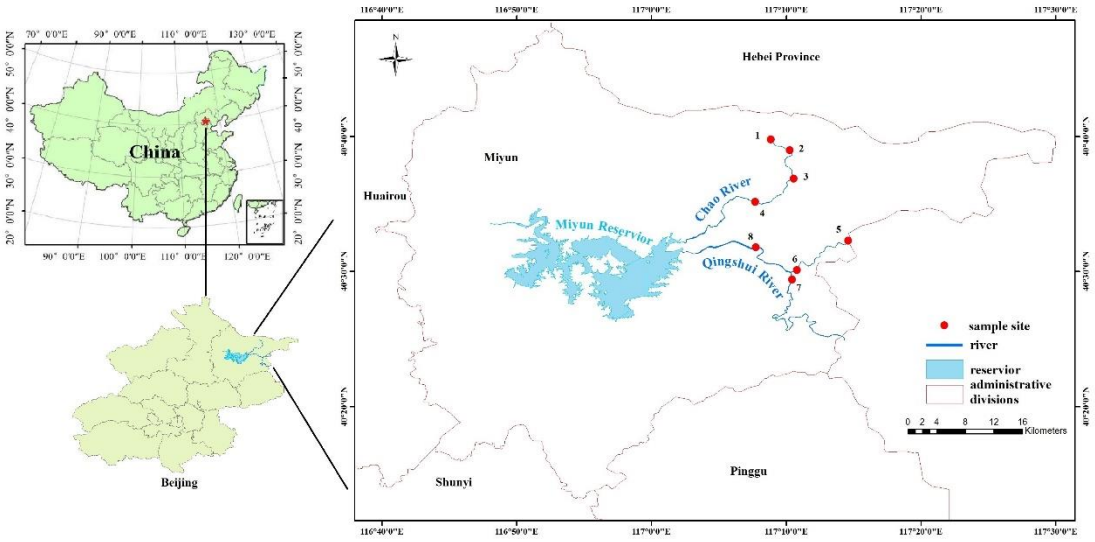


Figure 6.1 Location of field sites where phosphorus was monitored by ZrO-DGT in Beijing, China. Sites 1-4 are located upstream in the River Chao and sites 5-8 on the River Qingshui. River Qingshui is a tributary of the River Chao. The latter goes to the Miyun Reservoir.

6.2.2 Rivers near Tianjing

The Yongding, Chaobai and Jiyun rivers are tributaries of the Hai River, which flows into the Bohai Sea. The Yongding River is 650 kilometres in length and drains an area of 47,016 kilometres square. It emerges from Shanxi province and flows northeast into Inner Mongolia, then heads southeast into Hebei Province. Well known as the largest river to flow through Beijing, the water quality of the Yongding River has

received a lot of attention. Many researchers reported P pollution in the Yonding River being very severe (Xiaowen, 2010, Tao). The Chaobai River originates in Beijing at the confluence of its two main tributaries, the Chao and Bai River, about 3 kilometres south of the town of Miyun and 16 kilometres south of the Miyun Reservoir, Beijing. Sites 4-6 are located on the new Chaobai River, which is the southeast branch of the Chaobai River, which meets the Yongding River at Tianjin before it empties into the Bohai Sea. The Chaobai River has a length of 275 kilometres and the New Chaobai River has a length of 180 kilometres.

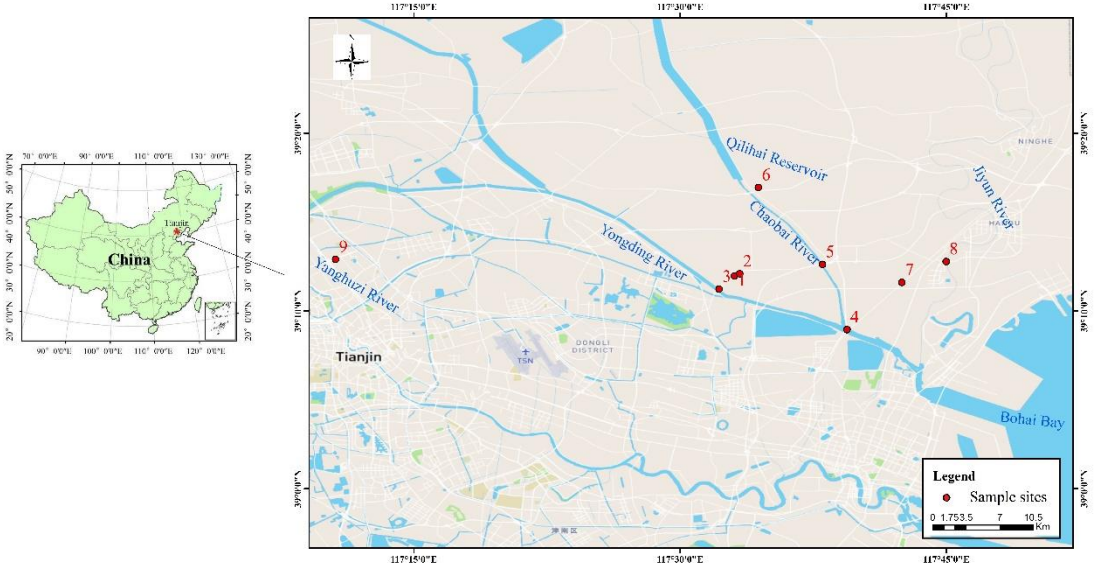


Figure 6.2 Location of field sites for monitoring of phosphorus by Metsorb DGT in Tianjing, China. Site 1 and 7 are located on fish and shrimp farms. Site 2 is located on a wastewater reservoir. Site 3 is located on the Yongding River. Site 4 is situated on the confluence of the Yongding and Chaobai River. Site 5 is located on the Chaobai River. Site 6 is located on the Qilihai Reservoir, which is filled by the Chaobai River. Site 8 is located on the Jiyun River. Site 9 is located on the Yanghuzi River.

6.2.3 Lakes and Fsh-farms near Yueyang

Dongting Lake is a large, shallow lake located in the northeast of Hunan province, China (28° 30'-30° 20' North, 110° 40' - 113° 10' East). It is a flood basin of the Yangtze River. Hence, the size of lake depends on the season. The surface usually is 2,820 kilometres square and may increase to 20,000 kilometres square during flood season (July to September).

Due to the rapid development of industries in the surrounding areas, 100 million tonnes of wastewater from over 200 paper manufacturers is dumped into the lake each year (2011a). Before 2003, the water from this lake mostly belonged in class III of the water quality identification index, but after 2003, the water quality dropped into class IV (LI Zhongwu, 2013).

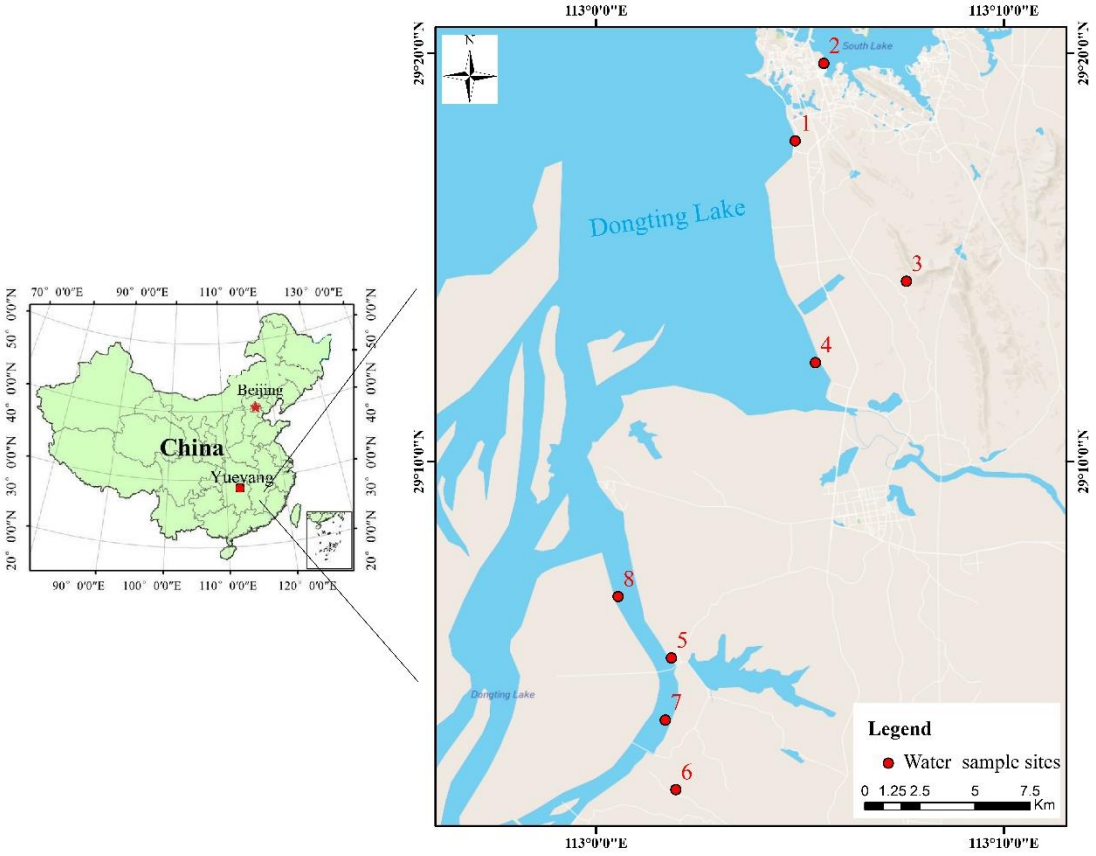


Figure 6.3 Location of field sites for monitoring Copper in Yueyang, Hunnai, China. Site 1 is located on the Dongting Lake. Site 2 is located on the Nan Lake. Sites 3-9 are located in the fish farms of different villages in Yueyang.

6.2.4 Mining Sites in Yunnan

Dongchuan copper ore field is the third largest copper mining area in China. It extends over an area of about 660 kilometres square around Dongchuan city, about 120 kilometres north of Kunming (26° 10' 48" N, 103° 3' 35" E). It includes the Tangdan, Luoxue, Yinmin, Xintang, Lanniping-Baixila, Lanniping-Suoyipo and Shijiangjun copper mines, of which the Tangdan and Luoxue mines are the largest. It is estimated that the Tangdan mine alone reaches almost 5 million tonnes of copper ore deposit.

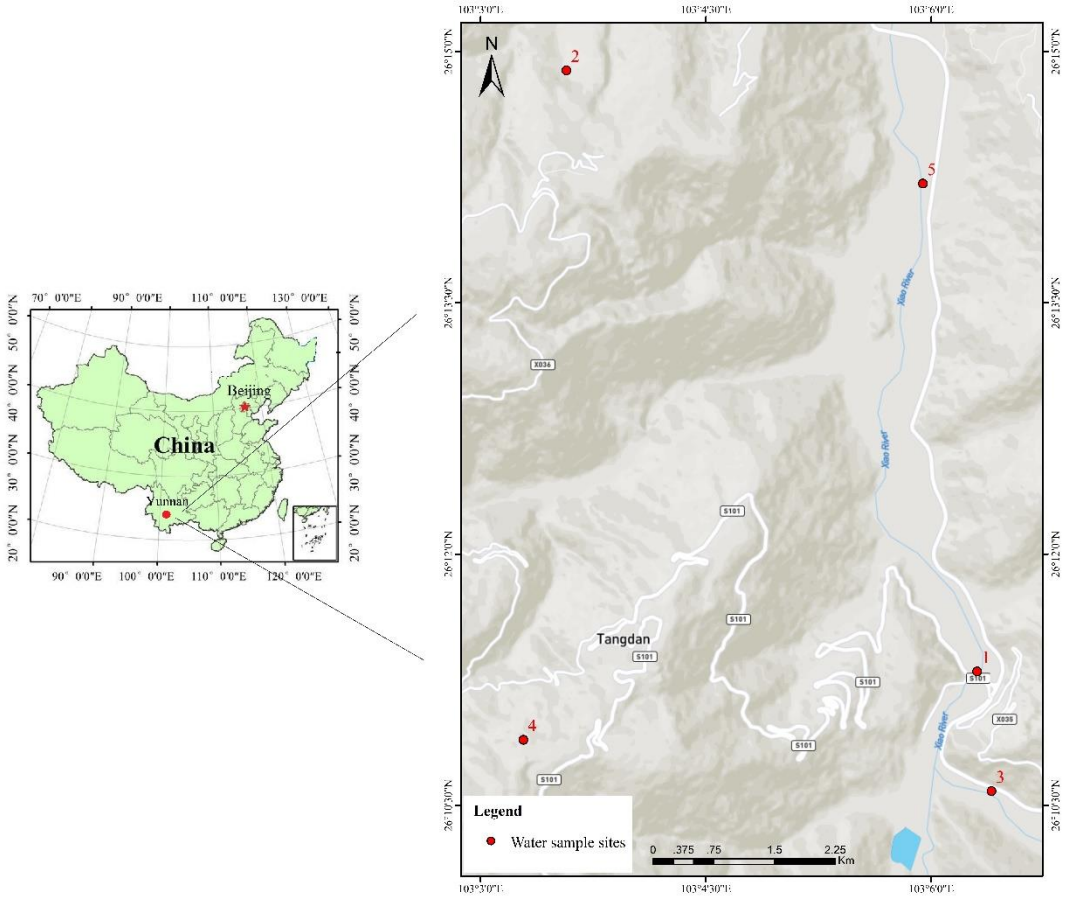


Figure 6.4 Location of field sites for monitoring Cu in water in Tangdan Copper ore field, Dongchuan, Yunan, China. Sites 1 and 4 are located on tailing ponds. Sites 2 and 3 are located on streams which run through the Copper ore field. Site 5 is located on the freshwater stream in Tangdan Town.

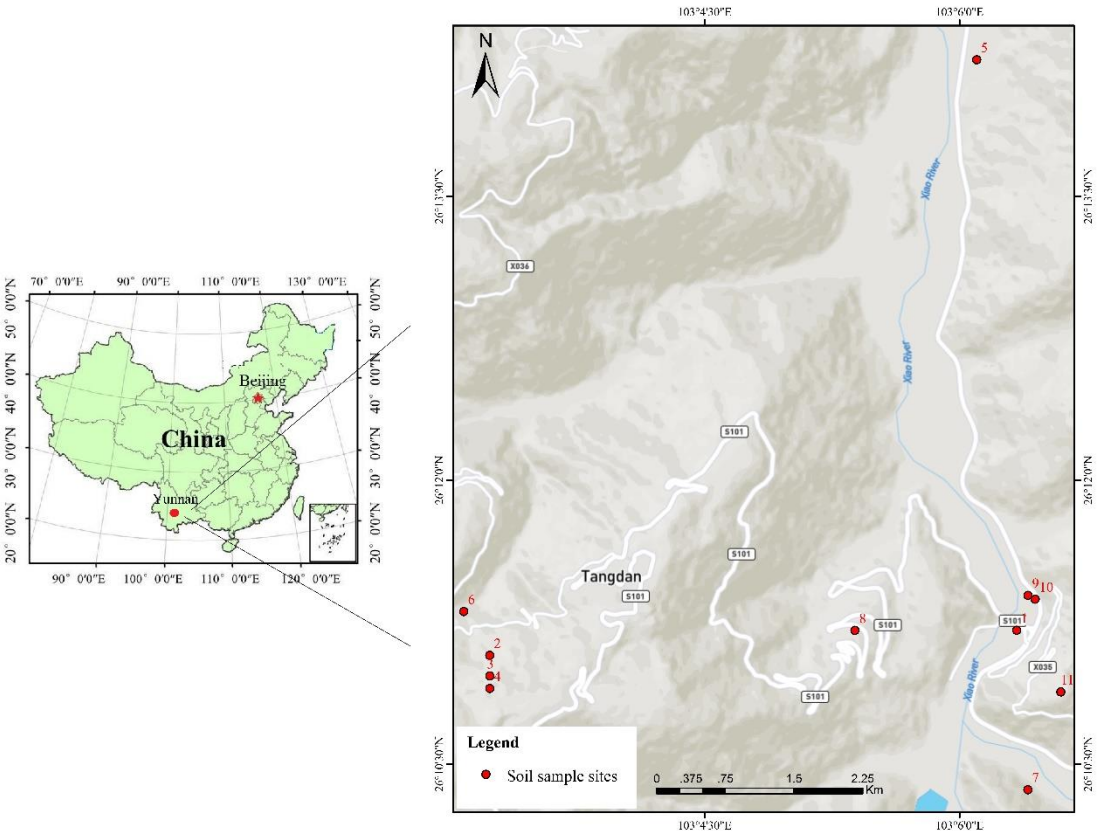


Figure 6.5 Location of field sites for monitoring Cu in Soil in Dongchuan Copper ore field, Yunan, China. Sample 1 was collected at tailings of Tangdan copper mines. Samples 2-4 were collected at Tangdan in the opencast mining area. Sample 5 was collected in a hole of the ore field. Samples 6-11 were collected from the agricultural soil near the ore field. Sample 11 was collected in the green area near the Tangdan concentrator plant.

6.2.5 Agriculture Field Sites.

Eight soils were collected from Zhangliang experimental station, Tianshui municipal agricultural science institute, Tianshui, Gansu province (34°05′ N， 104°05′ E). In this area the highest temperature reaches 35 °C and the lowest temperature -19 °C. The average annual temperature of this sample site is 11 °C. The texture of the soil was silty clay and the pH of the soil was around 8. Eight soils were collected from different split-plot experimental areas. Four soils had 0 tonnes km^{-2} (no fertilizer added), 7.5 tonnes km^{-2}, 15 tonnes km^{-2}, 22.5 tonnes km^{-2} P fertilizer applied. Another four soils had the same gradients of P fertilization but with a 750 tonnes km^{-2} chicken manure added.

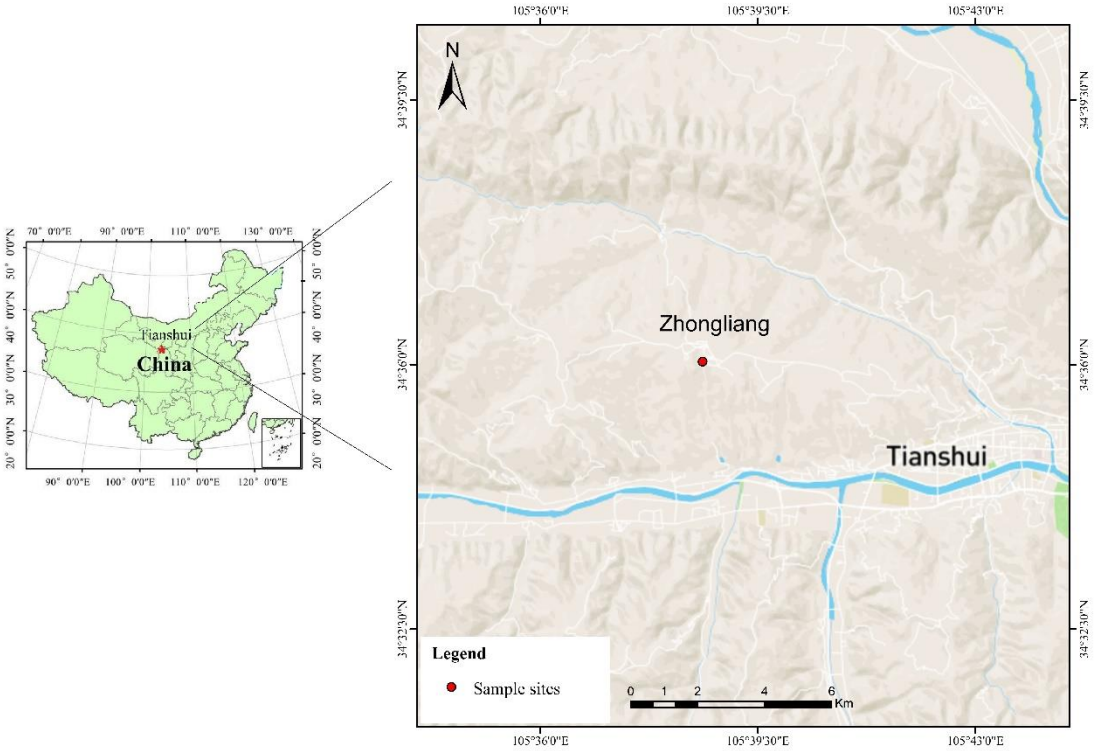


Figure 6.6 Location of field sites for monitoring P in Soil in Tianshui, Gansu, China.

6.3 The Design for Field Deployment

The DGT devices were deployed in several fish and shrimp farms in Tianjing and Yueyang. To prevent fish from interfering with the measurements, the DGT devices were deployed between two plastic baskets (the DGT devices were fixed on one basket and another basket covered it to form a sphere) (Figure 6.7), attached to a rope and float, then weighted to the river bed. These DGT stations were deployed 2-3 metres from the river bed.

Figure 6.7 (Left) Photograph of DGT units held in place by a plastic basket. Prior to deployment, another plastic basket was used to cover the devices, thus forming a spherical shape. The basket held up to six devices at each sampling site. (Middle) DGT deployment in fish farm in Tianjing. (Right) DGT deployment in the fish farm in Yueyang.

6.4 Materials and Methods

6.4.1 Materials and Preparation of DGT

All experimental and reagent solutions were prepared using MQ water (18MΩ). Chemicals were of analytical grade or higher. Sampling bottles and containers were acid-washed using a 10% v/v HNO_3 bath and rinsed thoroughly with MQ water prior to use.

The production of diffusive gel and Chelex-100 resin gel was described in Chapter 4.2.2. The Metsorb binding gel was prepared as described in Chapter 3.

The ZrO binding gel was prepared according to published procedure (Ding et al., 2010). Briefly, 2 g of the half-dried Zr-oxide was added to 4 ml of the gel solution composed of 28.5% acrylamide (w/v) and 1.5% N, Ń-methylene bisacrylamide (w/v). This mixture was then dispersed in an ultrasonic disruptor after it was thoroughly ground in an agate mortar. After removing the settled particles, 3.0 μL TEMED catalyst and 75 μL freshly prepared ammonium persulfate initiator (10%, w/v) were added to the mixture. The solution was cast between glass plates, which were separated by 0.4-mm plastic spacers. The glass plate assembly was placed in an incubator at 10 ± 1 °C for 30 minutes to allow the Zr-oxide to settle to one side of the gel. It was then transferred to an oven at 45 ± 1 °C to polymerize for 30 minutes. The gel sheet removed from the glass plates was soaked in MQ water for at least 24 h (the water was replaced 2−3 times) and stored in MQ water prior to use.

The devices were assembled using the method detailed in Chapter 4 and stored at 4 °C in zip lock plastic bags, containing 0.5 mL of 0.01 M $NaNO_3$ solution (ionic strength matched to freshwater deployment site) to ensure the diffusion properties of the gels were not altered, and to prevent the gels from drying out.

6.4.2 DGT Deployment in Waters

Different types of DGT devices were deployed to determine P and metals in waters in China (Table 6.1)

Table 6.1 Information of DGT deployment in water

Site location	Water type	DGT type	Average Temperature (°C)	Deployment time
Beijing	Rivers	ZrO-DGT	23	2 d
Tianjing	Rivers and fish-farms	Metsorb-DGT	25	5 h
Yueyang	Lakes and fish-farms	Chelex-DGT	17	8 h
Dongchuan	mining site	Chelex-DGT	16	18 h

ZrO DGT devices were deployed in streams for two days in September 2015 in River Chao and River Qingshui in Beijing, China. The DGT devices were deployed at around 0.5 m depth. The water temperatures were 21 to 25 °C. Metsorb DGT devices were deployed in Yongding, Chanbai, Jiyun and Yanghuzi River, and fish and shrimp farms for approximately 5 hours in May 2017 in Tianjing, China. The water temperatures were 20 to 29 °C. The deployment times for P were calculated based on the Chinese Environmental Quality Standards for P for surface water. Chelex-DGT devices were deployed in lakes and fish farms at 1 m of depth and collected after approximately 10 h of deployment in September 2016 in Yueyang, China. The water temperatures were 16 to 20 °C. Further Chelex-DGT devices were deployed in streams that flowed through copper ore fields for approximately 18 h in May 2017 in Dongchuan, China. The water

temperatures were 16 to 17 °C. Three DGT devices were deployed at each sampling site. At each deployment and retrieval of the DGT devices, water samples were collected into 1L PE bottles and their temperatures were recorded. When retrieved, the DGT exposition window was cleaned with MQ water to remove any particles adhered to the surface, and each device was stored in an individual zip lock plastic bag. The DGT devices were transported to the laboratory and stored at 4 °C until further sample treatment and analysis.

6.4.3 DGT Deployment in Soils

Surface soil samples were collected from the surface of the sites. They were air dried and then passed through a 2mm mesh sieve for the DGT measurements. All DGT deployments were carried out using a standard procedure was described in Chapter 5.2.5.

6.4.4 Colour Development on Binding gels

The mixed reagent used to determine P was the molybdenum blue method based on Murphy and Riley's research(Murphy and Riley, 1962), which is described in Chapter 3. The Metsorb DGT was rinsed with MQ water before being placed in a 100 mL container with 20 mL of mixed reagent. It was then tested for colouration directly without disassembling the device. They were kept at room temperature (20-22 °C) for 20 min.

For the ZrO DGT devices, the binding gel disc was removed from the DGT sampler

and rinsed with MQ water before being placed in a 100 mL container with 20 mL of mixed reagent for colour development.

6.4.5 CID Analysis

The steps of CID analysis using flat-bed scanner and Image J 1.48 were described in Chapter 3 and Chapter 4.

6.4.6 Gel Elution

The steps of gel Elution and elution efficiency was described in Chapter 3 and Chapter 4.

6.4.7 Water Sampling during DGT Deployments

Water sampling was carried out by collecting 1 L of water at a depth of 0.5m from the surface as the same depth of DGT deployment for all sites at the beginning and end of DGT deployments. The PE sample bottles were filled with 0.1% HCl to reduce the risk of metals binding to the container walls during transportation into the field. Prior to sampling, the 0.1% HCl was discarded and the bottles were rinsed thoroughly with sample water to ensure that all acid was removed. At each site during DGT deployment and retrieval, the waters' temperatures were measured. The turbid water samples were pre-filtered through a 0.45μm membrane filters prior to acidification with Sulphuric Acid (pH<2). Samplers were then kept at 4°C until analysis.

Based on the Chinese water environment quality standards, the total level of phosphorus in the water was determined by the ammonium molybdate spectrophotometric method. The total level of copper in the water was determined by ICP-MS.

6.4.8 Soil Analysis

Soil samples for chemical analyses were dried at 35 °C in an air drying cabinet. They were then ground in a roller mill until it could pass through a 2-mm sieve. Where a test uses a small sample weight, a more homogeneous subsample was prepared by further grinding using a ring and puck mill. When measuring P in the soil, the soil was ground until it could pass through a 0.25-mm sieve. When measuring Cu in the soil, the soil was ground until it could pass through a 0.18-mm sieve.

The total level of copper in the soil was determined by flame atomic absorption spectrometry after HCl- HNO_3-$HClO_4$ digestion (MEP, 1995). The total level of P in the soil was determined by the alkali fusion-Mo-Sb Anti spectrophotometric method and the total level of available P was determined by Mo-Sb anti spectrophotometric method (molybdenum blue method) after the Sodium hydrogen carbonate solution extraction (Protection, 2011, Protection, 2014).

6.5 Results and Discussions

6.5.1 Assessing the Concentration of P in Rivers using ZrO DGT Samplers

ZrO-DGT samplers were deployed for two days at eight sites in two rivers that run through four different villages before flowing into a reservoir. After deployment, their grayscale intensities were obtained according to the procedure described earlier. The results of grayscale intensity, mass and the concentration of P obtained by DGT and by direct water sample analysis are presented in Table 6.2. The scanned images of the coloured P-loaded gels of each sample are shown in Figure 6.8.

As the diffusive gel layer was omitted for enhancing the sensitivity, the diffusive boundary layer thickness, δ, cannot be assumed as negligibly small. The measured concentration, *C*, is, therefore, calculated by equation 3.1.

A Diffusive Boundary Layer (DBL) thickness of 0.39mm was indicated in previous work for a typical flow of river and stream of 0.02 m sec^{-1}, and the effective resin gel area was used as 3.80 cm^2 in this case (Warnken et al., 2006, Zhang et al., 1998a). As the water flow of the two rivers were similar to the typical flow, DBL of 0.39 mm and an effective surface area of 3.80 cm^2 were used for calculations in this work. It is worth noting that site 5 was lost because the river was closed on the day of retrieval to clean up of the upstream river bed. The grayscale intensity of sample 1 and sample 7 were out of the linear range of calibration (up to 116) and the results may not be accurate.

Table 6.2 The grayscale intensity, mass[a] and C_{DGT} of samples, the C_{sol} in water system analysis by Continuous flow analysis(CFA) and Ammonium molybdate spectrophotometry

Site[b]	Grayscale intensity	Mass (μg)	C_{DGT} (mg L^{-1})	C_{sol} (mg L^{-1})
1	168.14	7.18	0.078	0.201
2	104.97	2.08	0.022	0.042
3	122.88	3.52	0.038	0.042
4	112.69	2.70	0.029	0.046
6	106.95	2.24	0.024	0.043
7	209.08	10.49	0.113	0.43
8	117.07	3.05	0.033	0.046

[a] Mass of P accumulated on the gel surface was calculated using the linear calibration ;
[b] Sites 1-4 are located upstream in the River Chao and sites 6-8 on the River Qingshui. River Qingshui is a tributary of the River Chao. The latter goes to the Miyun Reservoir.

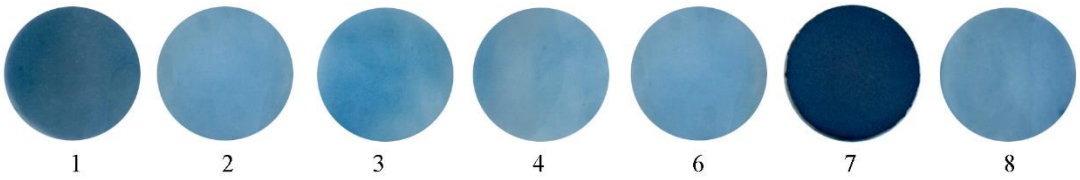


Figure 6.8 The scanned images of the coloured P-loaded gels of each sample after colouration treatment. Three DGT devices were deployed at each site. The Relative Standard Deviation (RSD) of the grayscale intensities ranged between 4% and 11%.

Compared to the concentration of P in water samples, the concentration of DGT-measured P were generally lower. There are two possible explanations for this: i) DGT was assessing the measurement of bioavailable P rather than total P, ii) the larger size particles may exist in a different aquatic system and they were not able to diffuse into the DGT because the much smaller pore size of the gel that coated the surface of the resin.

In these two rivers, the concentration of P was mostly up to national standard except

for site 7. Site 7 was located in the vicinity of a village so it is possible that the concentration of P in the stream was higher due to runoff from agricultural land with excessive P fertilisation and treated or untreated sewage. Additionally, the rivers where the samples were taken run through the Hebei province and up to Beijing, where there are numerous big farms and industries. The colour on the gel surfaces deployed in site 1 and site 7, which were located upstream near Hebei province, were significantly darker than other sites. This was mainly caused by contamination from the Hebei province. However, the amount of P found in other sites were below the regulation limits set by Chinese authority, which is 0.2 mg L^{-1}. The concentration of P in the rivers have been effectively reduced by an ecological purification system in Beijing (Figure 6.9).

Figure 6.9 Photo of eco-purification of Chao River in Beijing, China. Bulrush and reeds extract phosphorus from the river. Weirs retain the water in a sequence of basins while the ecosystem cleans the water of impurities. Water is aerated upon exit to increase the amount of oxygen dissolved in the water.

6.5.2 Assessing the Concentration of P in Waters and Soils Using Metsorb DGT Samplers

Metsorb DGT devices were used to measure P in rivers in Tianjing for a duration of between 4-5 h. The exact length of time the DGT deployment varied according to the different water temperatures. The time of monitoring was determined according to the Maximum Contaminant Level of P based on the standard which was explained in chapter 3.4.7. The results of grayscale intensity, mass and the concentration of P measured by DGT and by direct water sample analysis are presented in Table 6.3. The example images of P-loaded binding gel was given in Figure 6.10. The grayscale

intensity of sample 2 exceeded the maximum intensity of colour development where the intensity was no long respond to the P mass. Therefore, the mass accumulated on the surface of gel cannot be obtained from the grayscale intensity. The grayscale intensity of sample 1 and sample 3 were outside of the linear range of the calibration (up to 3.3μg) and the results obtained may not be accurate. The calculation of mass of P in sample 4 to 9 is eligible as they are all within the linear range of the calibration. With a known mass, M, the concentration of DGT-measured P can obtain from the equation 2.6. The scanned images of the coloured P-loaded gels have obvious differences from each sites.

Table 6.3 The grayscale intensity, mass[a] and C_{DGT} of samples, the C_{sol} in water system analysis by Continuous flow analysis(CFA) and Ammonium molybdate spectrophotometry

Sample[b]	Grayscale intensity	Mass (μg)	C_{DGT} (mg L^{-1})	C_{sol} (mg L^{-1})
1	141.81	3.20	0.37	1.12
2	182.95	/	/	2.24
3	151.65	3.57	0.41	0.97
4	129.02	2.72	0.31	0.51
5	66.88	0.40	0.05	0.24
6	104.91	1.82	0.21	0.48
7	93.43	1.39	0.16	0.36
8	89.84	1.26	0.14	0.28
9	86.42	1.13	0.13	0.37

[a] Mass of P accumulated on the gel surface was calculated using the linear calibration ;
[b] Sample 1,6,7 are fish and shrimp farms; sample 3,4,5,8,9 are freshwater streams in Tianjin City and Sample 2 is the wastewater reservoir.

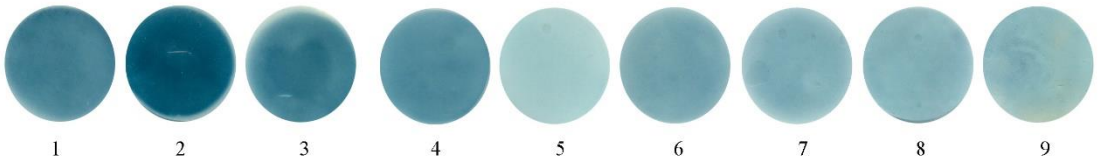


Figure 6.10 The scanned images of the coloured P-loaded gels from each sample. Three DGT units were deployed at each site as triplicate. The Relative Standard Deviation (RSD) of the grayscale intensities were in the range of 2% to 9%.

Sites 1-3 were 1.5 to 2 times higher than the national water standard in China. According to DGT-measured result, P concentration of other sites is slightly higher or just about the standard limit. As reported in the Telegraph, the harmful effects of nutrient pollution from fish farms is as serious as sewage (Clover, 2000). Phosphorus (P) is the main end-product of fish loading, and it can affect not only the rearing water, but also the whole environment (Lazzari and Baldisserotto, 2008). The concentration of P in the two main rivers which run through Tianjing (Yongding and Chaobai River) were higher than other streams. The untreated household sewage from the dense residential areas by riverside may become the main resource of the P contamination in the two rivers.

Compared to the concentration of P in water samples, the concentration of DGT-measured P were generally lower. It's mainly because of the P species in the water system. Metsorb DGT could only measure the dissolved reactive phosphorus (DRP) (also called soluble reactive phosphorus) in the natural water. Therefore, when the C_{DGT} is close to the standard level, it is necessary to using other analysis method to ensure the total P concentration.

For soil application, Metsorb DGT devices were deployed for 24 h in eight soils collected from Tianshui, Gansu. The soils correspond to a wide range of P statuses,

which is reflected in the parameters shown in Table 6.4. Olsen P ranged from 21.7 to 115.7 mg kg^{-1}. CK is original soil without fertilization. P5, P10, P15 were with the P fertilizer applied at the amount of 7.5 tonnes km^{-2}, 15 tonnes km^{-2}, 22.5 tonnes km^{-2} P fertilizer applied respectively. M, MP5, MP10, MP15 had the same gradients of P fertilization but with a750 tonnes km^{-2} chicken manure addition.

After obtaining the grayscale intensity of each gel surface by scanning it, the mass of P accumulated in the gel was calculated using the equation of calibration mentioned in Chapter 3. The concentration of DGT measured P was calculated using equation 2.6.

Table 6.4 Phosphorus Concentrations in P_{tot}, P_{Olsen}, P_{DGT}

Soil	CK	P5	P10	P15	M	MP5	MP10	MP15
C_{POlsen}(mg kg^{-1})[a]	21.7	39.7	56.8	68.8	61.5	67.4	115.7	103.6
C_{Ptot} (mg kg^{-1})[b]	867.1	929.0	1053.6	1066.9	987.4	1031.2	1168.1	1182.5
C_{PDGT}(mg L^{-1})[c]	0.09±0.01	0.14±0.02	0.24±0.1	0.31±0.07	0.17±0.01	0.22±0.06	0.35±0.07	0.37±0.1

[a] C_{POlsen} is measured by the sodium hydrogen carbonate solution-Mo-Sb anti spectrophotometric method.
[b] C_{Ptot} is measured by the alkali fusion-Mo-Sb anti spectrophotometric method
[c] C_{PDGT} is calculated from the grayscale intensity of the gel surface. Three DGT units were deployed at each site as a triplicate.

The difference between P_{DGT} and P_{Olsen} is not surprising. The DGT measured P includes P from the solution and resupply from the solid phase. However, P extraction methods just consider P to maintain an equilibrium with a constant solid to solution ratio (Menezes-Blackburn et al., 2016).

There is a significant relationship between the phosphorus concentration in P_{DGT} and P_{Olsen} (Figure 6.11) as they share a similar trend. The amount of available phosphorus is very low compared with the total amount of phosphorus in the soil. However, the bioavailable P measured by DGT and Olsen P extraction displayed a similar tendency towards the gradients of fertilization.

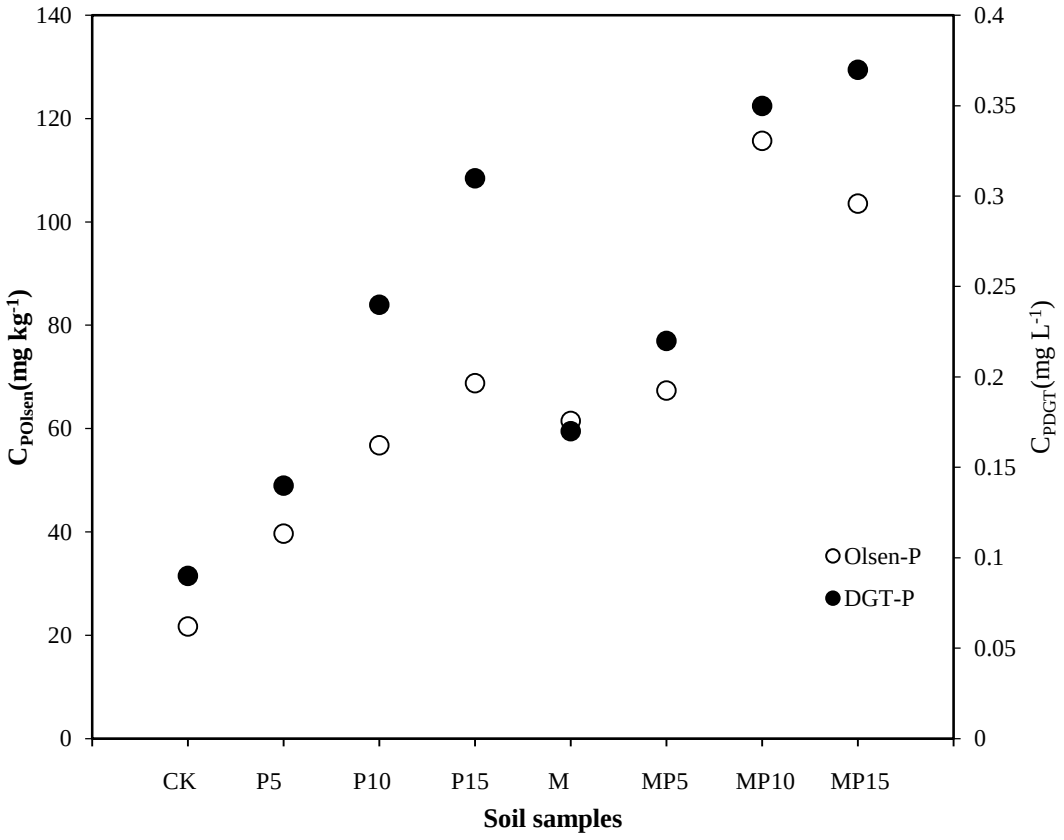


Figure 6.11 The concentration of Olsen-P and DGT-measured P in different soil samples.

Compared to the deployment of DGT units in freshwater, the grayscale intensity on the gel surface varied slightly where there were high concentrations of P in the soil. The Relative Standard Deviation (RSD) of the grayscale intensities ranged between 4% and 17%, which was considerably higher than measurements taken in the freshwater stream.

The RSD of concentration in P_{DGT} ranged between 9% and 23%.

6.5.3 Assessing the Concentration of Cu in Waters and Soils Using Chelex-DGT Samplers

The Chelex-DGT devices were deployed in various aquatic system including fish farms, lakes and freshwater streams for a certain amount of time, which varied according to the temperature of the water. The deployment time was determined by using the guild standard list (Table 4.1). Two things were needed to assess whether or not the concentration of Cu in the water exceeded standard regulations: the DGT deployment time (hours) and the visual inspection of colour change, formulated and described in Chapter 4.

After recommended deployment time, there was no visible blue colour or other colour observed on all gel discs. This means all the concentrations of Cu were below the standard regulation level. To identify the exact concentrations of Cu measured by DGT, the gel discs were eluted by 1M HNO_3 and determined by ICP-MS. Water samples taken at the time of deployment were analyzed for total dissolved Cu. Results show considerable variability in both Cu concentration and speciation (Figure 6.12, Figure 6.13).

Triplicate Chelex-DGT devices were deployed for approximately 8 h at an average temperature of 17 °C in Yueyang, Hunan, China. All DGT-labile Cu accounted for over 75% of the total dissolved concentrations and dominated the dissolved fraction. The

RSD of the concentration of DGT-liable Cu ranged between 0.9% and 12%, with an average of 6%. The concentrations of copper reached the Environmental Quality Standards for Surface Water of China (<10 μg L^{-1} for fish farming), which was consistent with the result obtained from the rapid screening technique.

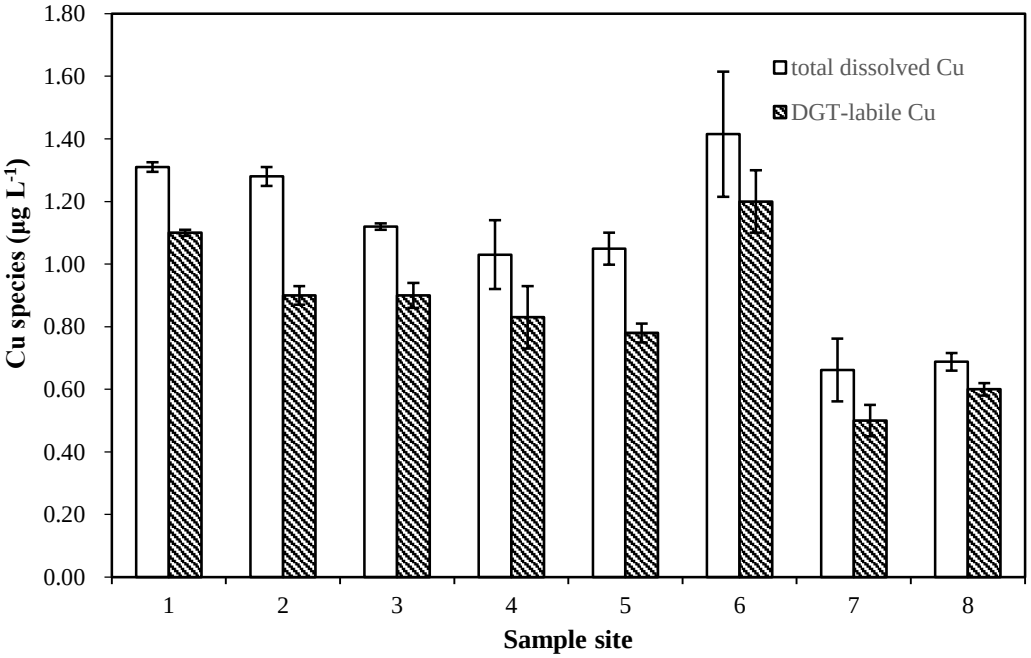


Figure 6.12 Total dissolved and DGT-labile Cu in lakes, ponds, and fish farms in Yueyang, Hunan in September 2015. Sites 1 and 2 are located on a lake; sites 3-8 are located on fish farms.

Triplicate Chelex-DGT devices were deployed for 17 to 18 h at an average temperature of 16 °C in Dongchuan, China and the results are presented in Figure 6.13. In the tailing ponds and the streams that flow through the copper ore field, DGT-labile Cu accounted for more than 70% of the total concentrations and dominated the dissolved fraction. The DGT-labile Cu in the freshwater stream that flows through the town only accounts for 7% of the total dissolved concentrations. DGT measures labile metals species that can pass through the pores of the diffusive layer. Both small

inorganic metal species and complex metal substances can pass through the gel, but large colloidal species cannot.

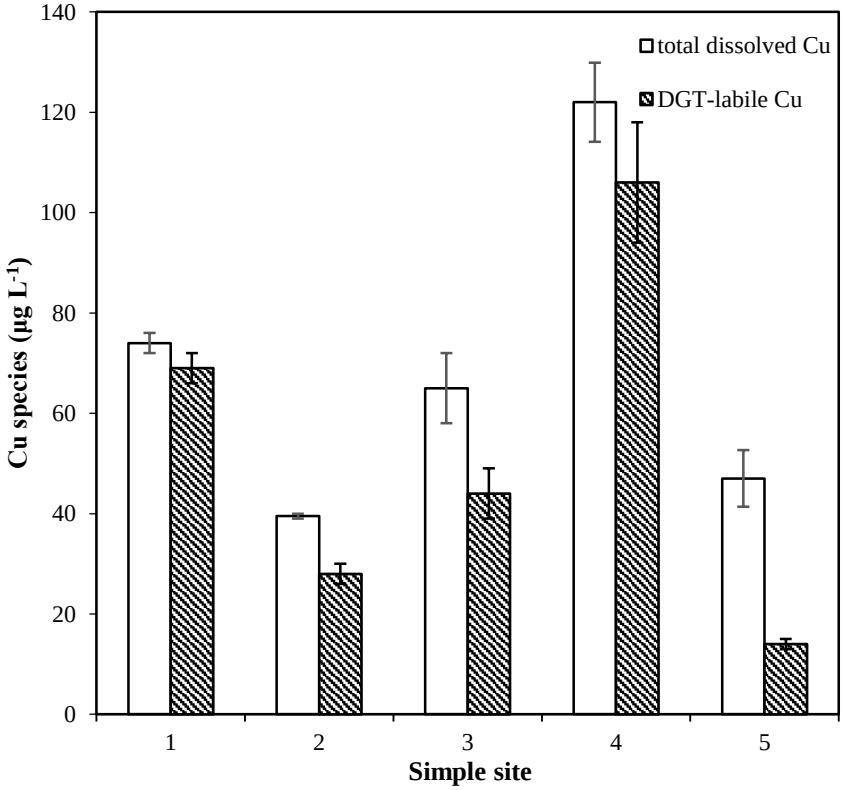


Figure 6.13 Total dissolved and DGT-labile Cu in different bodies of water in the Tangdan copper ore field in May 2017. Sites 1 and 4 are located on the tailing ponds. Sites 2 and 3 are located on streams that flow through the copper ore field. Site 5 is located on the freshwater steam in Tangdan Town, Dongchuan, China.

A precision of 10% or better has been claimed for the DGT method(Zhang and Davison, 1995). The biggest uncertainty is usually associated with the handling of samples (sample treatment, elution, dilution and analysis), but there are also uncertainties in the diffusion coefficient (*D*), the proportion of metal extracted from the resin (*fe*) and the gel thickness (*Δg*). The RSD of the concentration of DGT-liable Cu was within 11%, with an average of 8%.

The concentrations of copper were within the Environmental Quality Standards for

Surface Water of China (<1 mg L^{-1}), which is consistent with the result obtained from the rapid screening technique.

Soils The soils collected for this study correspond to a wide range of Cu statuses, which is reflected in the parameters displayed in Table 6.4. Available Cu measured by DETA extraction ranges from 8 to 1309 mg kg^{-1}. In this study half of the soils contained very high available Cu (>200mg kg^{-1}). All of the soils had intense colour of dark brown except for site 1 (tailings). After a 24 h deployment, the blue colour was only observed on the surface of the gel in sample 1. The concentration of DGT-measured Cu is 1.87 mg L^{-1} (29 μM). Since generally the Cu concertation beyond 10.5 μM was toxic to plant growth (Cook et al., 1998), soils in site 1 should not be exposed outside mining site without treatment. For other samples, only the brown colour were observed on the surface of the gel which transformed from the soils with dark brown colour, possibly due to high OM content (Figure 6.14).

Table 6.5 Copper concentration in Cu-$_{tot}$ and Cu-$_{EDTA}$

Sample	Texture	OM (g kg^{-1})	pH	$C_{Cu\text{-}tot}$ (mg kg^{-1})	$C_{Cu\text{-}EDTA}$(mg kg^{-1})*
1	Sand	6.8	8.66	1803	310
2	Sandy Clay	12.5	7.8	6509.2	665
3	Sandy Clay	14.8	8.08	7226.8	858
4	Loam	9.8	7.42	1318.4	60
5	Clay Loam	13.6	7.35	4531	1309
6	Loam	27.0	7.18	394.3	31
7	Loam	47.6	7.2	380.1	112
8	Clay Loam	32.2	7.53	1147	214
9	Clay Loam	8.4	7.92	151.6	19
10	Silt Loam	15.7	7.42	106.6	8
11	Silty Clay	51.1	7.81	918.8	158

* Soil test for plant-available Cu is extracted with EDTA solution.

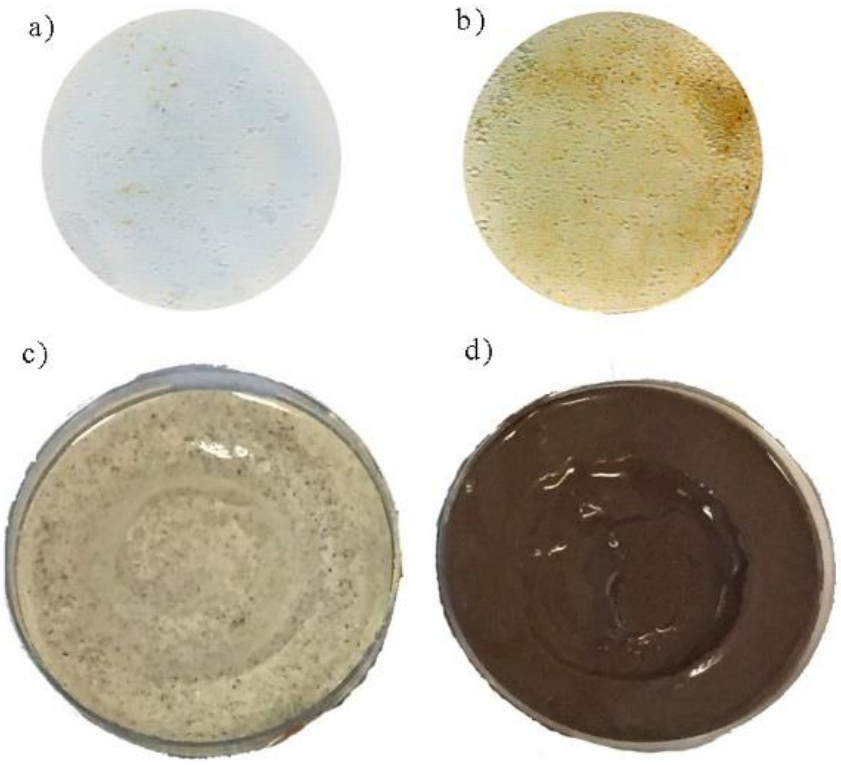


Figure 6.14 a) The scanned image of the coloured Cu-loaded gels from sample 1. b) A sample of scanned images of gel deployed in other soils (site 5). c) Photo of soil slurry found in site 1. d) Photo of soil slurry found in site 5.

A possible explanation of the result may be attributed to the dissimilarity of soil texture. The influence of soil texture on metal solubility in soils is best expressed in terms of the division of soils into clay, silt and sand fractions. In previous studies, the extractability of Cu by ammonium acetated was always lower in loamy soils than in sandy soils (Scokart et al., 1983). Besides, in the study of soil texture in relation to the extractable (0.1 M HCl and DTPA) concentration of Cu, which was generally enriched in the clay fraction. By comparison, relatively large amounts of available Cu was recorded in the fine sand fraction (Qian et al., 1996). Moreover, a high degree of extractability was also observed in the sand fractions of the soil. This was attributed to the low binding strength of these fractions (Rieuwerts et al., 1998). In addition, the

bioavailability of metals from soil decreases with increasing pH (Morel, 1997). Therefore, the bioavailability of copper may be suppressed due to the high pH in these soil samples.

6.6 Conclusions

It is important to develop new monitoring tools for water and soil chemical evaluation that are able to comply with the demands of the environmental quality standards or regulations of different countries. DGT provides an alternative that overcomes the deficiencies of traditional water sampling.

All DGT devices were successfully deployed in situ in waters and soils in field sites selected in China.

The deployment of ZrO DGT devices in suburb of Beijing showed the P concentration in upstream near Hebei province were exceed the water standard in China due to the contamination from Hebei province. After an ecological purification system in Beijing, the P concentration in rivers was significantly decreased and reach the water standard.

The deployment of Metsorb DGT devices in Tianjing demonstrated the concentration of P in fish farms and rivers near area with dense population were much higher. Therefore, fish farms and sanitary wastewater may be the main source of P contamination in rivers which run through Tianjing.

Compared to the concentration of P in water samples, the concentration of DGT-

measured P were generally lower due to speciation. The larger size particles may exist in water samples and they were not able to diffuse into the DGT because the much smaller pore size of the gel that coated the surface of the resin. The RSD of grayscale intensities in ranged from 2% to 11%.

Metsorb DGT devices were deployed in soils from Tianshui, Gansu. Though no significant relationship between the phosphorus concentration in P_{DGT} and P_{Olsen} was demonstrated, the bioavailable P measured by DGT and Olsen P extraction displayed the similar tendencies for the gradients of fertilization. The RSD of the grayscale intensities and DGT measured concentrations were significantly higher than those of deployment in waters. .

Chelex DGT devices were deployed in Yueyang and Dongchuan to assess rapidly the contamination level of Cu in extremely polluted waters and effluents using colour directly from metal ion itself due to accumulation of ions at high concentrations. No colour was observed on the surface of the gel after deployment in the water, indicating that the quality of the monitored water reached the acceptable water standards. DGT-labile Cu accounted for more than 70% of the total dissolved concentrations. The results produced an average precision for 7% of Cu measured in different rivers, lakes and ponds.

Deployment of Chelex DGT devices on soils showed the possibility of using the colour directly from the Cu ions to assess the concentration in sandy soils. Soils with dark colour may affect the direct colour reading and may not be suitable for using the

rapid screening method developed in this work. One possible solution is adding diffusive layer to DGT device since there is no diffusive layer (only filters) used in this work. The diffusive layer can resist some organic matters such as humic acid which contributed to the dark brown colour in soil. The overall results from the field measurements showed the concentrations of P in most of the monitored waters in Beijing were low and the quality of the waters has reached the Chinese water quality standards for surface water. The concentrations of Cu in monitored aquatic systems of all field areas have also reached the Chinese water quality standards for surface water.

Chapter 7. Conclusions and Future Work

With the booming use of diffusive gradient in thin films (DGT) in recent years, a variety of novel techniques based on DGT have been developed to enhance the diversity and feasibility of DGT application. A number of new types of resin phase (Bennett et al., 2010, Baker et al., 2015, Guan et al., 2015, Pan et al., 2015) and analysis methods (McGifford et al., 2010, Ding et al., 2013, Kruse et al., 2015) of phosphorus and metals have been developed successfully.

In this study, two novel DGT techniques were developed. Firstly, a rapid screening technique based on DGT devices and colour imaging method for assessing phosphorus and metal concentrations qualitatively and quantitatively has been developed. Secondly, a novel technique with biological material incorporated in the DGT (Bio-DGT) has been developed by immobilizing whole-cell toxicity bioreporter ADPWH_recA in the diffusive gel to simultaneously measure *in situ* labile metal concentrations and toxicity of metals.

7.1 Rapid *in situ* Detection of Available Phosphorus and Metals in Waters and Soils by Combining DGT and CID.

This newly developed approach provides several advantages over the traditional DGT technique. Firstly, in contrast with the use of UV-spectrophotometer or ICP-MS in previous studies, this method uses a scanner, equipment commonly found in most

laboratories. Secondly, a more efficient analysing process of P and metals measurement has been provided as the elution step was eliminated.

7.1.1 Phosphorus

The feasibility of combining the Metsorb-DGT method with colour development and computer imaging densitometry (CID) as a rapid screening technique to assess the phosphorus levels in natural water has been demonstrated in this study. The calibration standard in mass of P and the corresponding colour on each binding gel and grayscale intensity measurements were fitting with an exponential equation. The calibration ranges of grayscale intensity increases from 28 at the background level to 186 at the saturation level. There is no further obvious increase in grayscale intensity with increasing P loading above a mass of 16 μg. The colouration was uniformly distributed on the gels with a low RSD, mostly within 4%. The fully quantitative interpretation of P concentration can be assessed in the linear range of 0.3 to 3.2 μg per device. The effect of pH and interference of oxyanion metals (As) colour development and the DGT measurements are insignificant.

The comparison of the performance of the ZrO and Metsorb binding agents in colouration was made since the former is also being used in DGT technique combined with CID of P measurement. ZrO-DGT was seems not suitable for rapid screening technique in natural water due to its 5-day pre-treatment for colour developed and less sensitive P calibration.

The field evaluation of the suitability of this technique for monitoring P in natural waters was carried in three waterbodies in Tianjin, China. Provided the mass of P accumulated on the gel were within the linear range of the colour calibration, the concentration of P can be easily and accurately obtained by Metsorb-DGT. The RSDs of the concentrations of DGT-measured P were 2% to 9%.

7.1.2 Copper, Nickel and Cobalt

A Chelex-100 type DGT and high resolution CID measurement for rapid detection of metal concentrations in waters has been investigated. Since the amount of copper, nickel and cobalt on the binding gel reaches certain level, a distinctive colour will appear on the gel, no colour reagent was involved in this technique.

The calibration curves of copper, cobalt and nickel demonstrated the linear increases of the grayscale intensities with the accumulation mass of each metal ion. The calibration ranges of grayscale intensity of Cu increase from 28.7 to 117.1. The calibration ranges of grayscale intensity of Ni increase from 16.9 to 33.3. The calibration ranges of grayscale intensity of Co increase from 23 to 44. The linear range of mass accumulation of Cu, Ni and Co were 1.5μg cm^{-2} to 165μg cm^{-2}, 2.7 μg cm^{-2} to 153 μg cm^{-2}, 1.6 μg cm^{-2} to 159.2 μg cm^{-2}, respectively, correspond to the concentration range of 0.05 to 5 mg L^{-1} for all three metals if the deployment time is 24 hours and the water temperature is 20^{o}C respectively. The method precision for data obtained by DGT-measured mass was 5% and grayscale intensity on the gel surface is in a range of 7% to

14%.

7.1.3 Cr(VI)

A rapid screening technique for Cr (VI) using DGT and high resolution CID base on the surface colouration of the N-Methyl-D-glucamine (NMDG) binding gel reacting with the diphenylcarbazide in an acidic solution has been developed.

The relationship between the accumulation of Cr(VI) in NMDG gels and the corresponding change in grayscale intensity was perfectly fitted using a quintic polynomial in whole range and fitted using a linear equation when the mass of Cr(VI) up to 2.47μg cm^{-2} on the gel surface. With its good selectivity for Cr(VI) and strong reddish colour appeared on the white opaque gel, NMDG-DGT combined with colorimetric method will be very useful in monitoring of Cr(VI) in aquatic systems.

7.1.4 Use the Simple Screening Technique in Monitoring and in Risk Assessment in Waters and Soils

A guide list for using DGT at different deployment times has been produced to determine if the concentration of metals has exceeded Maximum Contaminant Level based on regulation standards set by different countries and regions. Because of the intense coloration on the gel surface, both using simple visual inspection and using a scanner for DGT devices at different deployment time and different temperature have been considered in the list. DGT applying to the guide list presented a simple and fast *in situ* pre-measurement before further complicated and costly quantitative analysis in

drinking water and effluent monitoring. In this guide list, DGT can be used for rapid scanning of Cu in all cases considered, except for UK and EU drinking water when detecting by visual inspection. For Co and Ni, this method is adapted to determining the contamination in Chinese effluents in which the Maximum Contaminant Level is higher compared to other water standards. When a scanner is used for colour detection, DGT could be used for monitoring Co in UK and China drinking water.

This newly developed rapid screen technique also has been applied for *in situ* monitoring in different waters and soils in China. The overall results from the field measurements showed the concentrations of P in most of the monitored waters in Beijing were low and the quality of the waters has reached the Chinese water quality standards for surface water. The concentrations of Cu in monitored aquatic systems of all field areas have also reached the Chinese water quality standards for surface water.

The deployment of ZrO-DGT devices in suburb of Beijing showed the P concentration in upstream near Hebei province were exceed the water standard in China due to the contamination from Hebei province. After an ecological purification system in Beijing, the P concentration in rivers was significantly decreased and reach the water standard.

Metsorb-DGT devices were deployed in soils from Tianshui, Gansu. Though no significant relationship between the phosphorus concentration in P_{DGT} and P_{Olsen} was demonstrated, the bioavailable P measured by DGT and Olsen P extraction displayed the similar tendencies for the gradients of fertilization. The RSD of the grayscale

intensities and DGT measured concentrations were significantly higher than those of deployment in waters.

Chelex-DGT devices were deployed in copper ore area to assess rapidly the contamination level of Cu in extremely polluted waters and effluents using colour directly from metal ion itself due to accumulation of ions at high concentrations. No colour was observed on the surface of the gel after deployment in the water, indicating that the quality of the monitored water reached the acceptable water standards. DGT-labile Cu accounted for more than 70% of the total dissolved concentrations. The results produced an average precision for 7% of Cu measured in different rivers, lakes and reservoirs.

Deployment of Chelex-DGT devices on soils showed the possibility of using the colour directly from the Cu ions to assess the concentration in sandy soils. Soils with dark brown colour may affect the direct colour reading and may not be suitable for using the rapid screening method developed in this work. One possible solution is adding diffusive layer to DGT device since there is no diffusive layer (only filters) used in this work. The diffusive layer can resist some organic matters such as humic acid which contributed to the dark brown colour in soil.

7.2 Bio-DGT, a Bridge Between Chemical Monitoring and Biological Monitoring.

This study has developed a new DGT technique, Bio-DGT that can measure in situ

labile metal concentrations and toxicity of metals simultaneously. As a robust and adaptable bacteria, ADPWH_recA was successfully grow in agarose gel as the diffusive layer during the deployment. Bio-DGT showed high uniformity in bioluminescence throughout the induction time and the optimal immobilization temperature was set as 50 ºC. It also demonstrated that the metal ions associated with ADPWH_recA have negligible effect on the measurements of metals by DGT. The novel Bio-DGT has achieved a reliable and stable measurement of metals in a wide range of pH and ionic strength except the high salinity.

The application in soils, showed the Bio-DGT can provide a simple and effective way to measure toxicity and concentration of heavy metals at same time in the same location. A positive correlation was obtained between the bioluminescent response ratio and the concentration of Cd measured by Bio-DGT in soils with a wide range of properties. Although there are more tests need to be done on combining microorganisms and DGT, this work has demonstrated a significant step forward from the previous study.

7.3 Future Work

This study has provided strong evidence that the new DGT technique can be used in a wide range of condition encountered in natural environments for determined metals and phosphate. However, there are still some works to improve. For phosphorus, it may put interest on enhance the capacity of Metsorb binding phase, for example, pressing the resin into an adhesive paper disc directly as new approach of binding layer

preparation. For Chelex-DGT measured metals, it may focus deploying colours with chemical reagents for Cu, Ni and Co, in order to improve the sensitivity and performance of this rapid screening technique in complex environment. For Cr(VI)，the stability of the coloration in higher Cr(VI) concentration needs to be improved.

In routine monitoring, where precision is not the first priority, the colour developed on binding gels could be scanned by smartphone and analysed by mobile applications. Rapid cost-efficient monitoring system as seen in Figure 7.1.

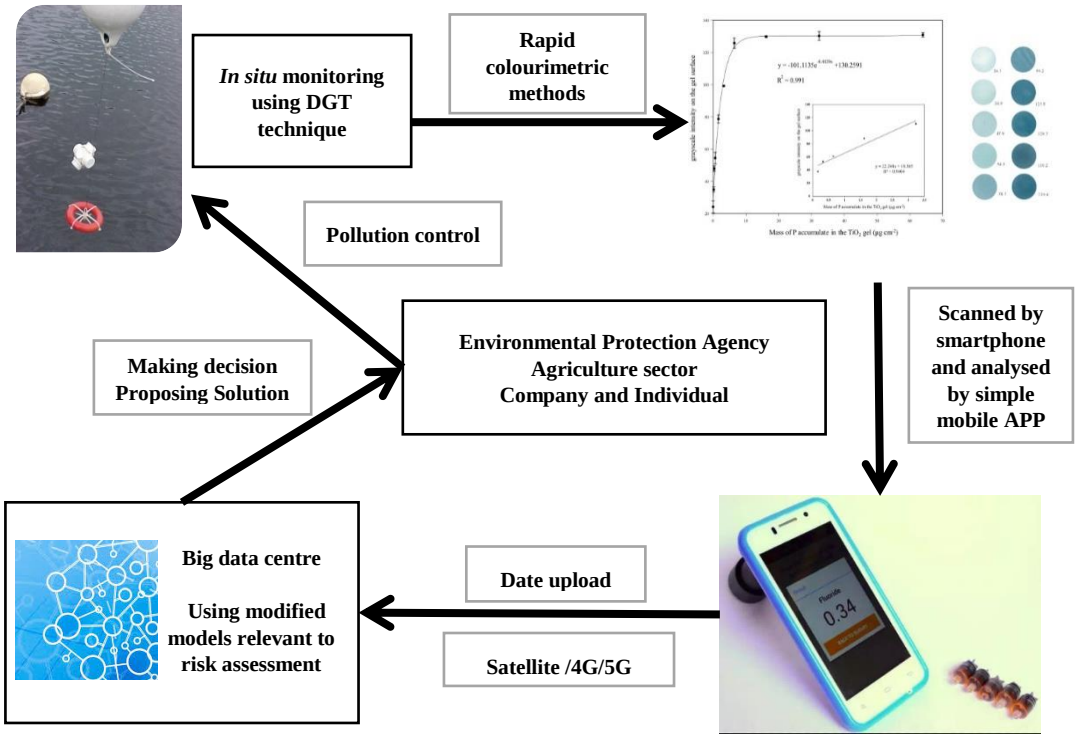


Figure 7.1 A rapid cost-efficient monitoring system combined with rapid scanning technique and mobile data networks.

In addition, the more selective and sensitive biosensor to improve the feasibility of Bio-DGT need be investigated. Also the study of performance of Bio-DGT samplers in complex environment samples need to be done. Positively, Bio-DGT could be used widely for monitoring of heavy metals in both quantitative and toxicity measurements.

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Appendix

Appendix 1 Examples images of the coloured P-loaded Metsorb gel discs and ZrO gel discs.

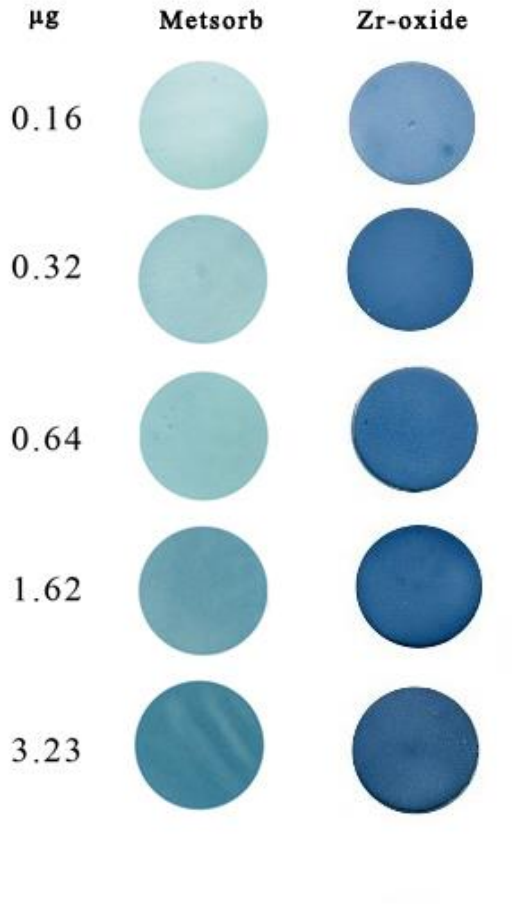

Figure A1 Examples images of the coloured P-loaded Metsorb gel discs and ZrO gel discs.

The gradients of colour development have a huge difference between the gel surfaces of Metsorb and ZrO discs. The colour changed on Metsorb gel discs was visible to naked eyes. The difference of colour on ZrO gel was difficult to distinguish, to the contrary.

Appendix 2 The Diffusion coefficients of Cu, Ni, Co in DGT gel (open pore) at different temperatures from 6 to 30 ºC

	D (E-6 cm^2/sec)		
T (ºC)	**Cu**	**Ni**	**Co**
6	3.48	3.23	3.32
7	3.61	3.34	3.44
8	3.73	3.46	3.56
9	3.86	3.58	3.68
10	3.99	3.70	3.80
11	4.12	3.82	3.93
12	4.26	3.94	4.06
13	4.39	4.07	4.19
14	4.53	4.20	4.32
15	4.68	4.33	4.46
16	4.82	4.47	4.60
17	4.97	4.60	4.74
18	5.12	4.74	4.88
19	5.27	4.88	5.02
20	5.42	5.02	5.17
21	5.58	5.17	5.32
22	5.74	5.32	5.47
23	5.90	5.47	5.63
24	6.06	5.62	5.78
25	6.23	5.77	5.94
26	6.40	5.93	6.10
27	6.57	6.09	6.27
28	6.74	6.25	6.43
29	6.92	6.41	6.60
30	7.10	6.58	6.77

Appendix 3 The water regulation standards in different countries and regions

Countries	Type of Water	Cu (mg L^{-1})	Ni (mg L^{-1})	Co (mg L^{-1})
UK	Drinking water	0.05		
	Human Consumption	2.0	0.02	
EU	Human Consumption	2.0	0.02	0.1
US	Drinking water	1.3		
CN	Drinking water	1.0	0.02	0.05
	Effluent (Surface water)	0.5	1.0	1.0 (0.1)