

**FATE AND TRANSPORT OF GLYPHOSATE AND
DEGRADATION BYPRODUCTS: IMPLICATIONS FOR
REMEDICATION AT CKD_u ENDEMIC AREAS IN SRI
LANKA**

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(158008R)

Degree of Master of Engineering

Department of Civil Engineering

University of Moratuwa
Sri Lanka

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Thesis submitted in partial fulfillment of the requirements for the
degree Master of Engineering in Civil Engineering

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DECLARATION

“I declare that this is my own work and this thesis does not incorporate without acknowledgement any material previously submitted for a Degree or Diploma in any other University or institute of higher learning and to the best of my knowledge and belief it does not contain any material previously published or written by another person except where the acknowledgement is made in the text.

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Name of the co-supervisor: Prof. J. M. A. Manatunge

Signature of the supervisor:

Date:

Abstract

Glyphosate, which is commercially available as Roundup®, was the widely used herbicide in Sri Lanka until 2015 and is suspected to be one of the causal factors for Chronic Kidney Disease of unknown etiology (CKDu). This research, therefore, focuses on investigation of glyphosate and Aminomethylphosphonic acid (AMPA), the major degradation product of glyphosate, immobilization in top soil and subsequent mobilization to water and their effective and efficient removal. A field study and a series of mesocosm studies were performed to investigate the immobilization of glyphosate in the top soil and the mobilization of glyphosate and AMPA to water. Further, a long term batch experimental study was carried out to study the degradation of glyphosate in water in the absence and presence of hardness, for 240 days. Finally, the applicability of Ozonation process for glyphosate removal from water was studied using batch experiments. Glyphosate and AMPA were analyzed using LC/MS and GC/MS. It was evident that glyphosate persistence in the environment was high, especially due to the sorption of glyphosate to soil. Mobilization of glyphosate to water was minimal and it was catalyzed by the event of first precipitation after the application of glyphosate and application of Triple Super Phosphate (TSP) to the soil. Furthermore, glyphosate degradation was hindered and its persistence was increased due to the presence of hardness in water and surfactants in commercial grade glyphosate. Ozonation process rapidly degraded the glyphosate present in water both in the absence and presence of hardness to levels less than 700 µg/L which is the USEPA Maximum Contaminant Level for drinking water. In conclusion, this study provides the insight that the dominant mechanism of glyphosate in the environment is adsorption of glyphosate to the topsoil and mobilization to water is minimal. Further, Ozonation is an effective and efficient method to remove glyphosate in water in CKDu prevalent areas despite the presence of hardness and surfactants.

Key words: Adsorption, AMPA, Desorption, Hardness, Ozonation.

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LIST OF ABBREVIATIONS

Abbreviation	Description
AMPA	Aminomethylphosphonic acid
CKDu	Chronic Kidney Disease of unknown etiology
EPSP	5_enolpyruvylshikimate_3_phosphate synthase
EPSPS	Enolpyruvyl shikimate-3-phosphate synthase
GC/MS	Gas Chromatography/Mass Spectrometry
GFR	Glomerular filtration rate
LC/MS	Liquid chromatography/Mass Spectrometer
PEP	Phosphoenolpyruvate
POEA	Polyethoxylated tallowamine
S3P	5-hydroxyl of shikimate 3-phosphate
TFAA	Trifluoro-acetic anhydride
TFE	Trifluoroethanol
TSP	Triple Super Phosphate
UCSC	Unified Soil Classification System
USEPA	United States Environmental Protection Agency

1. INTRODUCTION

1.1. Introduction

Chronic Kidney Disease of Unknown Etiology (CKDu) is one of the most critical health issues prevailing in Sri Lanka and found to be widely spreading in the North Central Province. To date, the extent of the CKDu prevalent area is estimated to be approximately 17,000 km² (Noble, et al. , 2014) with the affected population over 400, 000 and approximately 20, 000 deaths have been recorded (Jayasumana, et al., 2014). The majority of the CKDu affected group is males above 40 years, whose occupation is farming (Wanigasuriya, 2012). Previous studies have proposed that CKDu is a multi-factorial disease. Despite many studies carried out on the potential causal factors of CKDu over the last decade, the exact cause of the CKDu remains uncertain. However, based on the previous studies, possible pathways leading to widespread of CKDu include; (i) direct consumption of food contaminated with pollutants and toxins, (ii) direct ingestion of agro-chemical toxins in the body due to careless usage and, (iii) prolonged exposure to water contaminated with pollutants and toxins (Dharma-wardana, et al., 2014). Among the suggested pathways, prolonged exposure to toxins through consumption of potable water contaminated with agrochemicals such as pesticides and herbicides has been receiving major attention in the recent past.

Glyphosate, which is commercially available as Roundup®, was the most widely used herbicide among various agrochemicals being used in the CKDu prevalent areas in Sri Lanka until recently. Glyphosate [N-(phosphonomethyl) glycine], which is the active ingredient in Roundup®, was used as a broad-spectrum systemic herbicide and crop desiccant and known to have a greater efficiency compared to other herbicides due to its higher effectiveness in weed killing and low toxicity to non-target organisms (Schuette, 1998). Preliminary field visits conducted in the CKDu prevalent areas provided evidence that Roundup® is still being used in some of the agricultural cultivations despite the prohibition imposed by the Sri Lankan Government on importation and use of glyphosate since year 2015. Thus, it was found that glyphosate has still been persistent in the environment. However, when present in soil and water, glyphosate can be degraded via photodegradation and/or microbial degradation

(Schuette, 1998). The major degradation product of glyphosate is reported as Aminomethylphosphonic acid (AMPA) (Kryuchkova et al., 2014; Schuette, 1998).

Furthermore, glyphosate can mobilize to water sources subsequent to precipitation events as well as due to application of phosphate fertilizers used in the agricultural fields. Triple Super Phosphate (TSP) is the most commonly used phosphate fertilizer. Inorganic phosphate in the fertilizers can compete with glyphosate for the binding sites in the soil, thus there is a potential for enhancement of leaching of glyphosate from soil in the event of multiple application of phosphate fertilizers to the agricultural fields sites (Borggaard & Gimsing, 2008; Gimsing, et al., 2004; Munira, et al., 2016; Prata, et al., 2003; Simonsen, et al., 2008).

Recent studies provide evidence of the presence of glyphosate in trace levels in abandoned wells in the CKDu affected areas (Jayasumana et al., 2015). In addition, a WHO study has corroborated that the percentage of CKDu subjects having glyphosate residues in urine samples more than that of the reference threshold level were 3.5% (WHO, 2013). Hence, glyphosate is suspected to be a causal factor for CKDu.

However, studies on immobilization of glyphosate in soils, possible pathways of mobilization of glyphosate from soils to both surface and groundwater and effective glyphosate removal methods have not yet been addressed relating to the CKDu prevalent areas in Sri Lanka. Moreover, the persistence of glyphosate, which is enhanced due to the presence of elevated levels of hardness in water together with the effect of the application of TSP, has not been studied sufficiently.

Therefore, the main objective of the present research study is to investigate glyphosate and Aminomethylphosphonic acid (AMPA) immobilization in top soil and subsequent mobilization to water and their effective and efficient removal.

To achieve the main objective below specific objectives were set;

1. Investigation of the persistence of glyphosate in different environmental matrices: soil, groundwater, surface water and sediments in areas where CKDu is prevalent
2. Investigation of glyphosate and AMPA immobilization in top soil and mobilization of glyphosate and AMPA to water

3. Investigation of glyphosate (high purity and commercial grade) degradation pattern in deionized water and hard water
4. Study the applicability of Ozonation process as an effective and efficient removal method of glyphosate and AMPA from water

1.2. Approach

Chapter 1 presents the introduction to the research study providing a brief description on CKDu, possible pathways responsible for prevalence of CKDu, effect of glyphosate on CKDu, immobilization of glyphosate in soil and the mobilization of glyphosate to water. Further, Chapter 1 contains the main objective and specific objectives of the research study.

Chapter 2 provides a summary of literature available on glyphosate, mode of action of glyphosate in plants, effect of surfactants present in Roundup®, possible degradation mechanisms and microbial degradation of glyphosate. Further, glyphosate interaction with soil, factors affecting adsorption of glyphosate onto soil particles and impact of application of phosphate fertilizer on glyphosate sorption to soil are discussed. Then glyphosate mobilization to surface water and groundwater from soil is clarified. Additionally, effect of glyphosate on human health and on CKDu is explained. Finally, possible methods for glyphosate removal from water and suitability of use of Ozonation for removal of glyphosate from water are discussed.

Chapter 3 describes the materials and methods used in this research project. Experimental procedures followed to achieve each objective are described in detail. Specially, experimental setups for each objective, methods followed to obtain chemical and physical characteristics of soil and water are illustrated. Further, analytical methods used for extraction and analysis of glyphosate and AMPA in soil and water are explained.

Chapter 4 presents the results and discussion of each objective considered in the research project. Glyphosate persistence of soil, groundwater, surface water and sediments in CKDu is prevalent areas, is discussed. The results of the first objective showed that glyphosate was persisted in the environment, especially in the soil even after years of application of glyphosate. Further, results of the second objective revealed that glyphosate was mobilized from soil to water subsequent to of first

precipitation event and application of TSP. In the objective three, it was shown that the glyphosate degradation in water was restricted by the presence of hardness in water and surfactants in commercial grade glyphosate. Glyphosate was degraded rapidly when subjected to Ozonation. However, presence of hardness and surfactants showed an adverse impact on the rate of degradation of glyphosate.

Chapter 5 concludes the major findings of each objective and recommendations for future research.

2. LITERATURE REVIEW

2.1. Glyphosate

Glyphosate [N-(phosphonomethyl) glycine], has three polar function groups; phosphonic group, carboxylic group and amino group (Fig. 2.1) (Borggaard & Gimsing, 2008).

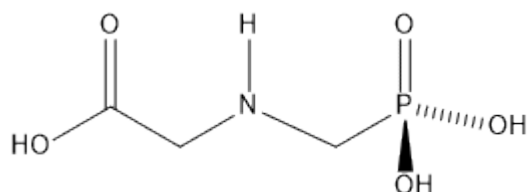


Figure 2.1 Chemical Structure of glyphosate

In pH 4-8 range glyphosate has the ability to remove H⁺ ions from phosphonic group and carboxylic group (Fig. 2.2). Hence, glyphosate is recognized as polyprotic acid. Additionally, glyphosate molecule has a secondary amino function which possesses basic features. Glyphosate can be categorized as zwitterion structure, because glyphosate is a neutral molecule which has both positive and negative charges, but in different positions (Fig. 2.2) (Knuuttila & Knuuttila, 1979).

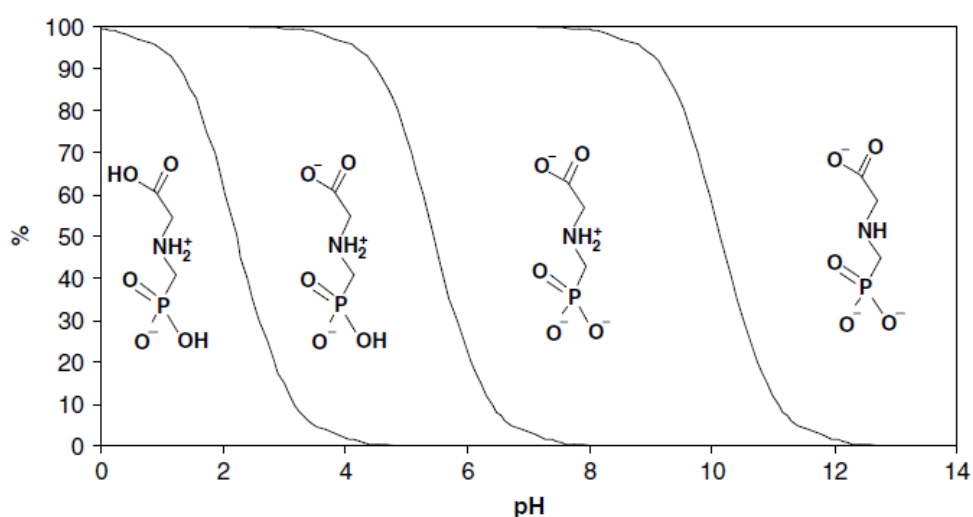


Figure 2.2 Bjerrum diagram of glyphosate. Acid dissociation constants of glyphosate: pKa1 = 2.22, pKa2 = 5.44 and pKa3 = 10.13 (Borggaard & Gimsing, 2008)

Glyphosate solubility in water is reported as 11, 600 ppm at 25⁰C which is a high value (Schuette, 1998). Although glyphosate has a high solubility in water, it also claims a

high sorption ability ($\log K_{oc} = 4.34$) in soil, therefore, known to have limited mobility in soil limiting glyphosate desorption to water bodies (Daouk, et al., 2013). Behavior of glyphosate in water and soil is discussed in detail in forthcoming sections.

2.2. Commercial use of glyphosate

Glyphosate is commercially available as Roundup®, was the most widely used herbicide among various agrochemicals being used in the CKDu prevalent areas in Sri Lanka until its importation and use were banned since the year 2015 under the Import and Export (Control) Act, No. 01 of 1969. Roundup® has known to have a greater efficiency compared to other herbicides due to its higher effectiveness in weed destroying (Schuette, 1998).

2.2.1. Course of action of glyphosate in plants

Glyphosate has the ability to inhibit the enolpyruvyl shikimate-3-phosphate synthase (EPSPS) (Fig. 2.3) (Steinrücken & Amrhein, 1980), which is the key enzyme of the shikimate pathway of plants and some microorganisms. Shikimate pathway is the metabolism route of carbohydrates to the biosynthesis of aromatic amino acids and EPSPS is the sixth enzyme in the shikimate pathway (Fig. 2.3) (Herrmann & Weaver, 1999). EPSPS is the catalyst in the step of biosynthesis of 5-enolpyruvylshikimate_3-phosphate synthase (EPSP). Enolpyruvyl moiety of phosphoenolpyruvate (PEP) is transferred to the 5-hydroxyl of shikimate 3-phosphate (S3P) to produce EPSP (Fig. 2.3) (Pollegioni, et al., 2011). When glyphosate is present it will imitate the PEP and bind to S3P inhibiting EPSPS (Fig. 2.3) (Pollegioni, et al., 2011). Therefore, shikimate pathway will be disrupted and aromatic amino acids will not be produced leading to the wilting of weeds.

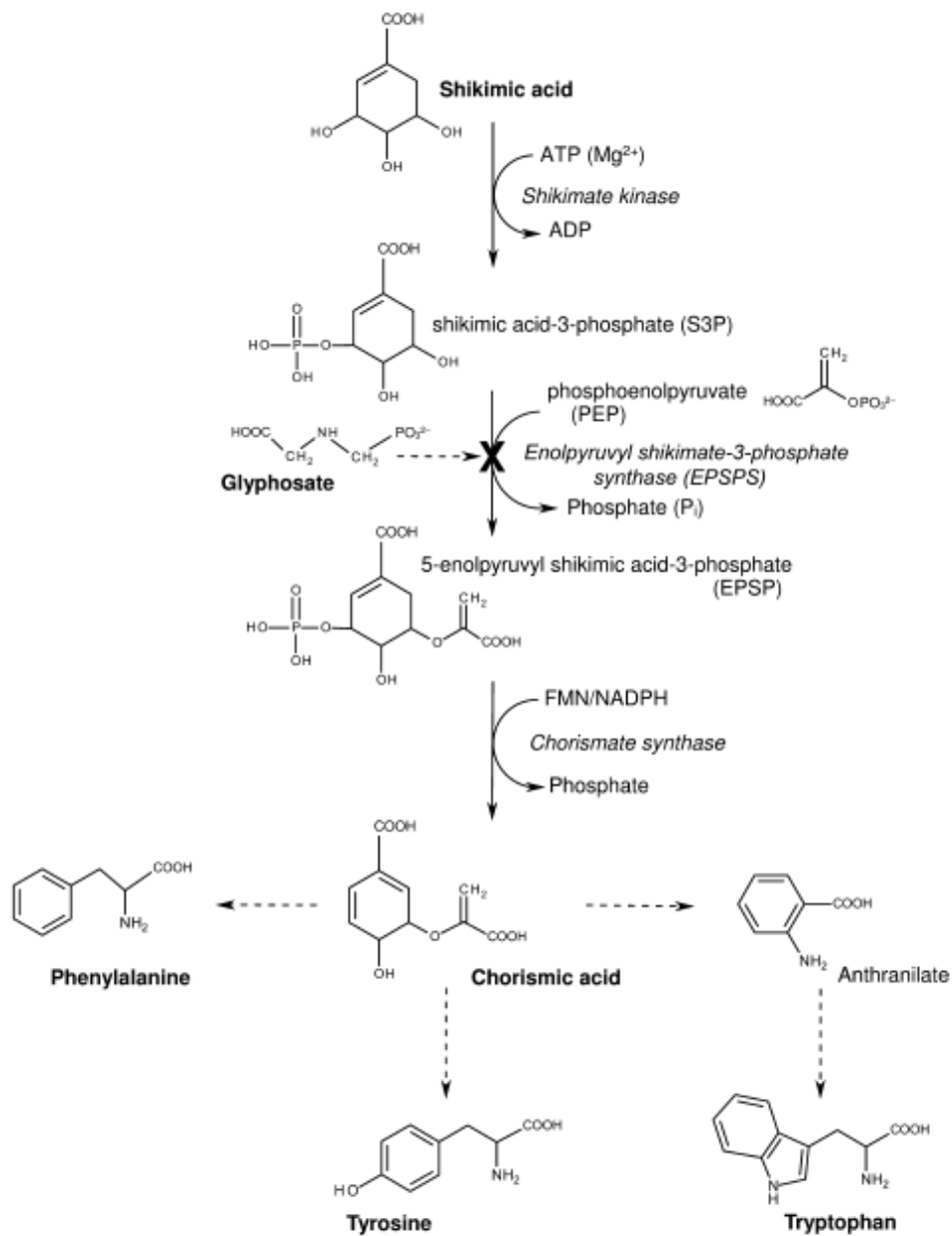


Figure 2.3 Shikimate pathway and mode of action of glyphosate (Pollegioni, Schonbrunn, & Siehl, 2011)

2.2.2. Effect of surfactants present in Roundup®

Roundup®/glyphosate is mainly absorbed by the foliage of the weeds when applied, part of glyphosate applied can be subjected to vaporization, inhalation or adsorption to soil. Roundup® also contains a major adjuvant surfactant known as polyethoxylated tallowamine, (POEA), introduced as an inert ingredient during the Roundup®

production process to enhance the bio-efficacy of the herbicide by increasing the contact of the herbicide with the plant cells and facilitating the penetration of the leaves' waxy layers. Presence of POEA with glyphosate has shown to be cytotoxic (toxic to cells) at doses lower than glyphosate alone (Mesnage, et al., 2013). Continuous application of glyphosate could trigger the emergence of glyphosate tolerant weeds hence, farmers tend to increase the rate of glyphosate application targeting at destroying the persistent weeds (Piccolo, et al., 1994).

2.3. Degradation of glyphosate

2.3.1. Possible degradation mechanisms

Degradation of glyphosate is mainly occurred via photodegradation and/or microbial degradation (Schuette, 1998). However, microbial degradation is reported as the dominant degradation mechanism of glyphosate (Schuette, 1998) and discussed in section 2.3.2.

Glyphosate when degraded via microbial mechanisms or photodegradation, the major degradation product is reported as AMPA (Kryuchkova et al., 2014; Schuette, 1998). The chemical structure of AMPA is shown in Fig. 2.4. AMPA has higher mobility in the soil than glyphosate and toxic level is equal or less than glyphosate (Borggaard and Gimsing, 2008).

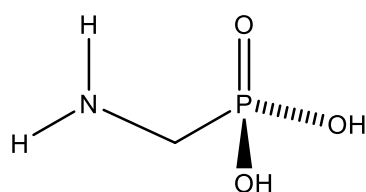


Figure 2.4 Chemical Structure of Aminomethylphosphonic acid (AMPA)

Half-life of glyphosate in agricultural soils varies from few days to three months or more (Andréa et al., 2003); nevertheless, about 40-50 % of glyphosate degradation to AMPA even after 90 days has been reported (Gimsing, et al., 2004). Repeated use of Roundup®/glyphosate may cause accumulation of AMPA in agricultural soils and the persistence of AMPA is known to be greater than that of glyphosate (Mamy & Barriuso, 2005).

2.3.2. Microbial degradation of glyphosate

Microbial degradation of glyphosate is occurred in two ways; (1) direct cleavage of C-P bond producing sarcosine and inorganic phosphate (Fig. 2.5) and (2) direct cleavage of C-N bond producing AMPA and glyoxylate (Fig. 2.6) (Sviridov et al., 2015). However, the direct cleavage of a C-P bond is occurred when glyphosate is the only phosphorus source in the environment, due to the complex nature of the reaction (Sviridov, et al., 2015). Hence, sarcosine is produced mostly under laboratory conditions.

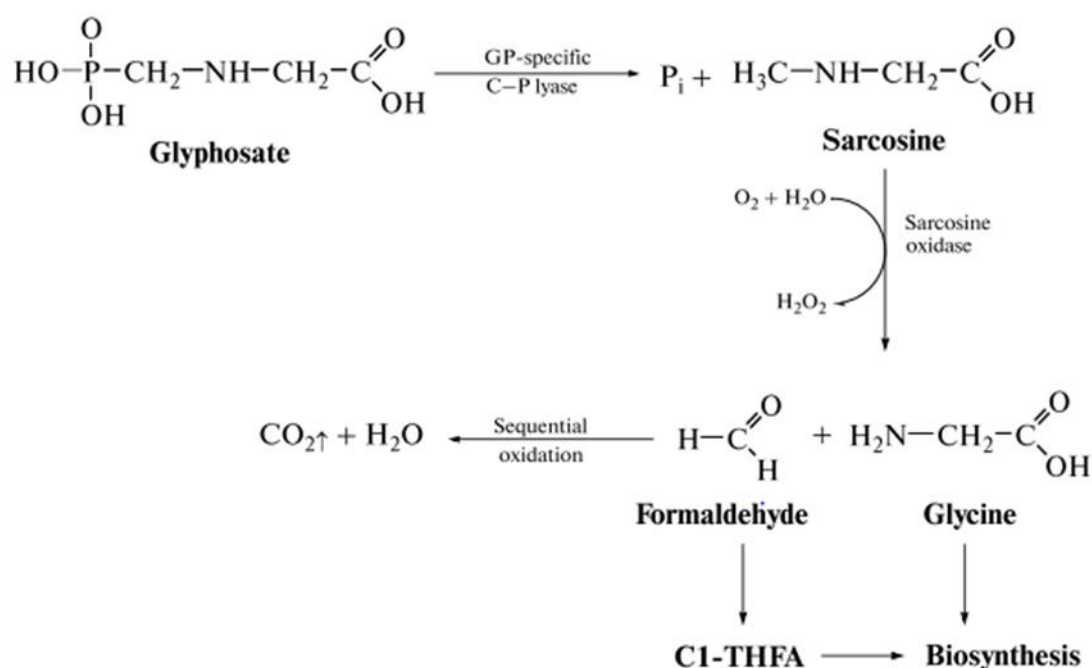


Figure 2.5 Production of Sarcosine and inorganic phosphate by direct cleavage of C-P bond (Sviridov, et al., 2015)

The main glyphosate degradation pathway in the environment is the direct cleavage of C-N bond (Sviridov, et al., 2015). Part of the produced AMPA is accumulated in the environment and other part is further degraded by the microbes present in the environment. The further degradation of AMPA is again occurred in two pathways; (1) cleavage of C-P bond producing inorganic phosphorus and methylamine and (2) transamination of AMPA producing formylphosphonate which is further degraded to inorganic phosphorus and formaldehyde (Fig. 2.6) (Sviridov, et al., 2015).

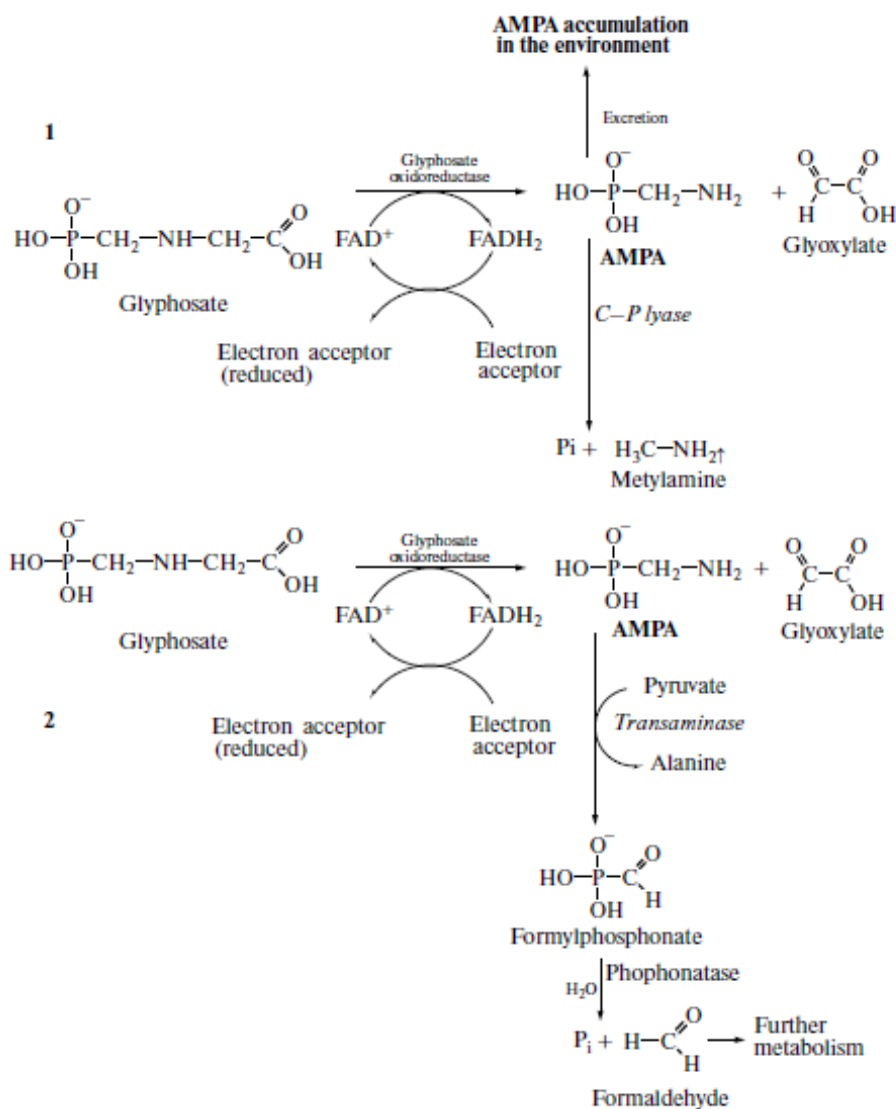


Figure 2.6 Production of AMPA and glyoxylate by direct cleavage of C-P bond and further degradation of AMPA (1) cleavage of C-P bond and (2) transamination of AMPA (Sviridov, et al., 2015)

When glyphosate is present in soil, microbial degradation is limited because due to strong adsorption onto soil making it less bioavailable for microbes (Borggaard & Gimsing, 2008). Further, the glyphosate degradation pathway is mainly dependent on the microorganisms present in the environment.

2.3.3. Photodegradation of glyphosate

When glyphosate is present in water, photodegradation is occurred and AMPA is produced (USEPA, 1993). The rate of photodegradation of glyphosate in pure water is higher than the rate of photodegradation of glyphosate in polluted water, due to the low light penetration in polluted water (Lund-Høie & Friestad, 1986). In addition,

glyphosate is photo stable, when the pH levels of water are 5, 6 and 9, under the natural conditions (Kollman and Segawa, 1995). Further, Rueppel et al. (1977) stated that photodegradation of glyphosate is minimal in the environment (Rueppel, et al., 1977). Additionally, it is reported that glyphosate will not be subjected to photodegradation when present in soil (USEPA, 1993). It is revealed that AMPA is more stable to photodegradation than glyphosate (Lund-Høie & Friestad, 1986).

2.4. Glyphosate interaction with soil

After application, the Roundup® solution along with glyphosate can come into contact with soil surface as spray drift and/or drift onto soil after being washed off from the leaves causing contamination of soil in the Roundup® applied lands (Bandarage, 2013). Three main fate pathways of glyphosate when present in soil are reported in literature, i.e. mineralization or degradation, immobilization or leaching (Vereecken, 2005). Glyphosate when present in soil can be degraded into its major degradation product AMPA mainly via microbial degradation (Sviridov, et al., 2015).

Glyphosate has a strong adsorption affinity to soil, whereas most of the other herbicides are moderately to weakly adsorb on soils mainly through soil organic matter (Borggaard & Gimsing, 2008). Glyphosate sorption onto soil seems to claim a strong relationship with the minerals of these soils, specifically to iron and aluminum oxides (Jayatilake, et al., 2013) and known to occur due to the formation of inner sphere complexes with the metals of soil oxides, which are also similar to the phosphate adsorption to soil (Jayatilake, et al., 2013). Possible sites for glyphosate adsorption on soils include broken bonds of clay minerals, organic matter, amorphous hydroxides of iron and aluminum and ferric oxides (Jayatilake, et al., 2013; Gimsing & Borggaard, 2002; Borggaard & Gimsing, 2008; Annett, et al., 2014).

Furthermore, when glyphosate adsorbs onto soil particles, glyphosate forms mono and divalent anion species having high association for trivalent cations present in soil, especially with Al^{3+} and Fe^{3+} (Borggaard & Gimsing, 2008). The sorption strength of glyphosate is known to be influenced by the presence of exchangeable cations following the sequence $\text{Na}^+ < \text{Mg}^{2+} < \text{Ca}^{2+} < \text{Zn}^{2+} < \text{Mn}^{2+} < \text{Fe}^{3+} < \text{Al}^{3+}$ (Glass, 1987, Sprankle et al., 1975).

2.4.1. Factors affecting adsorption of glyphosate onto soil particles

2.4.1.1. The percentage of amorphous iron and aluminum oxides in the soil matrix

Amorphous iron and aluminum oxides are reported to form strong complexes with the carboxylic and phosphonic groups contained in the glyphosate molecule as shown in Fig. 2.7 (Borggaard & Gimsing, 2008; Mamy & Barriuso, 2005; Ololade, et al., 2014;

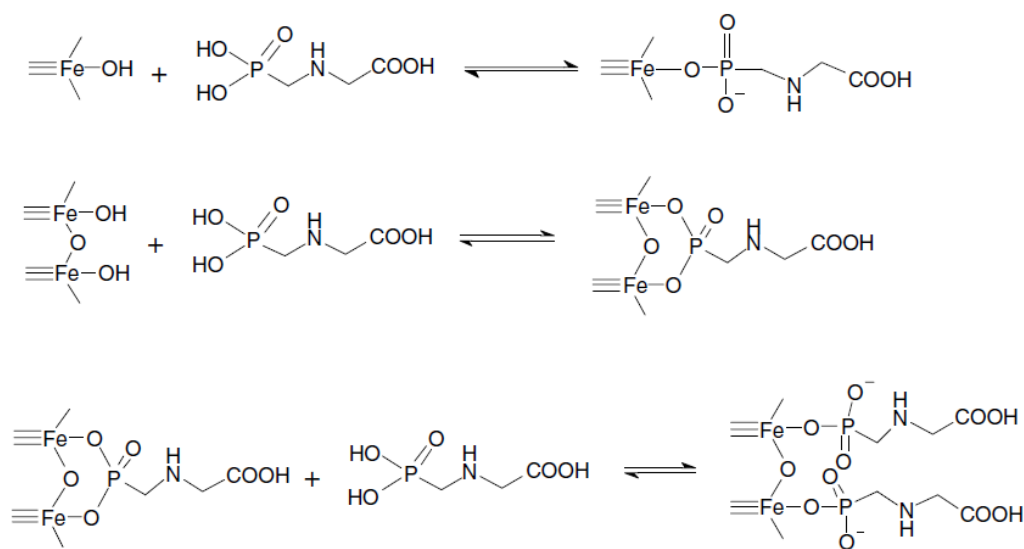


Figure 2.7 Glyphosate sorption by an iron oxide (Borggaard & Gimsing, 2008)

Piccolo, et al., 1994). Glyphosate adsorption to amorphous iron and aluminum oxides are by ligand exchange mechanism (Borggaard & Gimsing, 2008; Ololade, et al., 2014). Therefore, the presence of amorphous iron and aluminum oxides in the soil matrix increases the adsorption of glyphosate onto soil.

2.4.1.2. pH of soil

The pH value plays a crucial role in glyphosate adsorption onto soil particles (Borggaard & Gimsing, 2008; de Jonge, et al., 2001; Tévez & dos Santos Afonso, 2015). When the pH decreases, H^+ levels are increased hence the negative charge of the glyphosate is reduced. Therefore, ligand-exchange mechanism of amorphous iron and aluminum oxides is promoted (Borggaard & Gimsing, 2008; Ololade, et al., 2014). Gimsing et al. (2004) had observed that when pH was increased from 6 to 8, the glyphosate adsorption onto soil decreased and when the soil pH is in the range of 4-8,

glyphosate adsorption onto soil increases because glyphosate will be in the form of polyprotic acid within this range.

2.4.1.3. Presence of soil organic matter

Impact of organic matter on glyphosate adsorption and retention in the soil matrix has been debatable. Some studies have reported a decrease in adsorption of glyphosate with an increase of soil organic matter content due to the competition for the same adsorption sites or due to blocking of glyphosate adsorption sites in soil by organic carbon (Borggaard & Gimsing, 2008; Gerritse, et al., 1996; Morillo, et al., 2000). In contrast, others have reported higher adsorption of glyphosate with high levels of organic matter present in the soil because soil organic carbon can form hydrogen bonding with glyphosate and the presence of soil organic carbon increases the adsorption of glyphosate to amorphous iron and aluminum oxides by creating favorable pH conditions (Borggaard & Gimsing, 2008; Glass, 1987; Piccolo, et al., 1994).

2.4.1.4. Presence of inorganic phosphate

Inorganic phosphate binds to similar binding sites in soil as the glyphosate, hence may compete with glyphosate for the soil sorption sites (Borggaard & Gimsing, 2008; Gimsing, et al., 2004; Munira, et al., 2016; Prata, et al., 2003; Simonsen, et al., 2008). This competitive sorption can affect negatively to the adsorption of glyphosate to soil, because higher preference is given to inorganic phosphate (Borggaard & Gimsing, 2008). Therefore, presence of inorganic phosphate will lead to decrease in the adsorption of glyphosate to soil and increase in glyphosate mobility to water.

2.4.1.5. Cation exchange capacity (CEC) of the soil

The effect of CEC on adsorption of glyphosate onto soil particles seems to be not very well-understood. Some studies have observed a direct relationship between glyphosate adsorption to soil and the CEC, because glyphosate could be adsorped onto cations released from clay minerals through cation-exchange mechanism, hence glyphosate adsorption to soil could increase with the increment of CEC level of the soils (Dion, et al., 2001; Vereecken, 2005). However, others have implicated that CEC of the soil does not influence the glyphosate adsorption (Morillo, et al., 2000; Zhou, et al., 2004).

2.4.2. Impact of application of phosphate fertilizer on glyphosate sorption to soil

Triple Super Phosphate (TSP), i.e. calcium dihydrogen phosphate [$\text{Ca}(\text{H}_2\text{PO}_4) \cdot \text{H}_2\text{O}$] is the mostly used fertilizer in the CKDu affected areas. TSP became popular due to its characteristics such as the highest P content of dry fertilizers that do not contain N, high water solubility (about 90%) and high calcium content (15%) that provides an additional plant nutrient (Nash & Halliwell, 1999). In general, farmers use glyphosate as an herbicide to prepare the cultivation lands and after few weeks of application of glyphosate, TSP would be applied as the fertilizer for the crops.

When glyphosate is strongly sorbed by soil, its leachability and interaction with the human body is low. However, with the TSP application, glyphosate leachability may get increased due to competitive sorption effect between inorganic phosphate and glyphosate. Therefore, possible increase in glyphosate mobility due to TSP application is a crucial issue, which needs proper attention, especially in the CKDu prevalent areas.

2.5. Mobilization of glyphosate to surface water and groundwater

Glyphosate is mobilized into surface waters through surface runoff (as solutes/free compound or attached to colloidal particles) and into groundwater through infiltration (Vereecken, 2005). Leaching of glyphosate from the soils depends on the characteristics of the soil and climatic conditions (Borggaard & Gimsing, 2008) and in particular, the intensity of the first rainfall after glyphosate application has shown to profoundly affect the amount of glyphosate mobilized to groundwater and surface waters (Rasmussen, et al., 2015). Moreover, glyphosate adsorbed onto colloidal particles are drifting with the runoff and get deposited in the water bodies with a net contribution to its sediment layer and possible degradation to AMPA over time (Major 3rd, et al., 2003). In contrast, water in CKDu prevalent areas comprises high hardness levels, and glyphosate is known to form stable complexes with metal cations in hard water; Nuclear Magnetic Resonance (NMR) studies have provided evidence of the formation of stable bonds of Ca^{2+} and Mg^{2+} with both phosphonic and carboxylic groups of glyphosate by replacing H^+ ions in these functional groups (Thelen, et al., 1995). The typical half-life of glyphosate in water (≈ 92 days) was reported to have increased even up to years due to strong stability of the glyphosate-metal complexes formed in hard water (Schuette, 1998) making it less bioavailable for degradation.

Thus, the presence of glyphosate in hard water possesses a profound risk of enhanced persistence and the whole phenomenon acts as a carrier of metals into the human body.

In addition, factors such as 1) the timing of rainfall and surface runoff events subsequent to the timing of glyphosate application, 2) accumulated amounts of glyphosate and AMPA in the soil matrix from previous applications, and 3) travel distance and the residence time of the glyphosate in the catchment and the surface water body to allow degradation of glyphosate to AMPA, influence the mobilization of glyphosate to the surface waters (Coupe, et al., 2012).

Potential risk of groundwater pollution due to leaching of glyphosate through the soil column to meet the groundwater is a complex process and depends on the water percolating through the vertical soil column, glyphosate degradation processes and their sorption processes (Daouk, et al., 2013). Previous studies reported that tilling action does not seem to affect the leaching of glyphosate from soil to groundwater (Fomsgaard, et al., 2003). Additionally, glyphosate leaching into groundwater could be increased due to some other factors such as presence of sand in the soil (Borggaard & Gimsing, 2008; Kjær et al., 2011) and higher inorganic phosphate levels (de Jonge, et al., 2001; Dion, et al., 2001; Gimsing & Borggaard, 2002).

Glyphosate and AMPA are known to strongly adsorb onto soil and retain in the first 15 cm of topsoil layer (Vereecken, 2005). Therefore, before reaching the groundwater, glyphosate could be strongly sorbed onto soil minerals as well as subjected to degradation by soil microorganisms while moving through the deeper soil layers (Vereecken, 2005). Hence, glyphosate has low potential to infiltrate through the soil profile to meet groundwater. Nonetheless, previous studies also reported that there is a potential for glyphosate movement to groundwater subsequent to heavy rainfall events as a result of the presence of preferential flow paths (Vereecken, 2005). Therefore, typically, only trace levels of glyphosate have been reported in the literature (Van Stempvoort et al., 2016).

2.6. Effect of glyphosate on human health

Glyphosate is recognized as a low acute toxic compound (USEPA, 1993). Nonetheless, due to the potential health risks that have been recognized with exposure to glyphosate, the USEPA has imposed a maximum contamination level of 700 µg/L for glyphosate when present in water (USEPA, 1995). In addition, according to USEPA Registration Eligibility Decision document for glyphosate, the reference dose or the average daily dosage of glyphosate that will not create adverse health effects throughout the lifetime is set as 2 mg/kg/day (USEPA, 1993).

2.6.1. CKDu

Chronic Kidney Disease of unknown etiology (CKDu) is a crucial health issue prevailing not only in Sri Lanka but also in the world. CKDu patients are found mainly in Asian countries (e. g. Sri Lanka, India, China, Taiwan), Balkan countries (e. g. Bulgaria, Romania), African countries (e. g. Nigeria, Egypt) and North Central American countries (e. g. El Salvador, Nicaragua) (Wijerathne, et al., 2014). This is a critical health issue addressed globally. However the exact causal factor/factors are still unknown, hence the “unknown etiology” phrase is used.

Kidneys filter blood and reduce waste and extra fluid in body, additionally while filtration, they help to balance electrolytes and produce hormones which (1) control blood pressure, (2) strengthen bones and (3) produce red blood cells (Kumar, 2002). They filter about 170-180 L of blood and produce about 1-2 L of urine per day (Kumar, 2002).

Chronic Kidney Disease (CKD) is a kidney disease which is affecting the structure and function of kidney. Due to CKD there is a raised risk of cardiovascular disease and kidney failure. CKD is defined in the terms of glomerular filtration rate (GFR) in the way of $GFR \geq 60$ mL/min per 1.73 m^2 for ≥ 3 months with or without kidney damage (Alebiosu et al, 2003). The glomerular filtration rate will be decrease with time due to the disease. The CKD propagation is divided into 5 stages according to the glomerular filtration rate.

At risk <GFR 60 (with risk factors for chronic kidney disease)

Stage 1 - Kidney damage with normal or increased GFR ≥ 90

Stage 2 - Kidney damage with mildly diminished GFR	60–89
Stage 3 - Moderately reduced GFR	30–59
Stage 4 - Severely decreased GFR	15–29
Stage 5 - End-stage renal disease (kidney failure)	<15

(James et al, 2010)

Typically, kidney failure is identified at stage 5. In this stage the recommended treatments are dialysis or transplantation (Levey & Coresh, 2012). Generally, CKD is occurred due to the hypertension, old age, obesity, diabetes and cardiovascular disease.

However, CKDu is a kidney disease without knowing the exact causal factor/s. The earlier sign of CKDu is excretion of Urine- albumin above the standard level (Dharma-Wardana et al, 2014). In Sri Lanka people are suffering from CKDu for last two decades particularly farmers. According to the WHO report the first person in Sri Lanka who is affected by CKDu was identified in 1994 (Dharma-Wardana et al, 2014). CKDu severely prevails in Northern Central Province and presently CKDu has been found in North western, Northern, Uva, Eastern and Central Province of Sri Lanka (Fig. 2.8) (Jayasumana et al, 2015).

The following factors are identified as causal factor of CKDu based on the past studies

- Heavy metals (Arsenic, Cadmium) (Bandara, et al., 2008; Jayasumana, et al., 2011)
- Fluoride (Illeperuma, et al., 2009)
- Glyphosate (Jayasumana, et al, 2014)
- Excessive hardness caused by the presence of high concentration of Calcium, Magnesium in water (Jayasumana et al., 2014)
- Complexes formed between F, Al, Ca and Mg (Noble et al., 2014, Bandarage, 2013)
- Pesticides/herbicides (Jayasumana et al., 2014)
- Pesticides/herbicides - metal complexes formed with Ca and Mg (Jayasuman, et al., 2014)

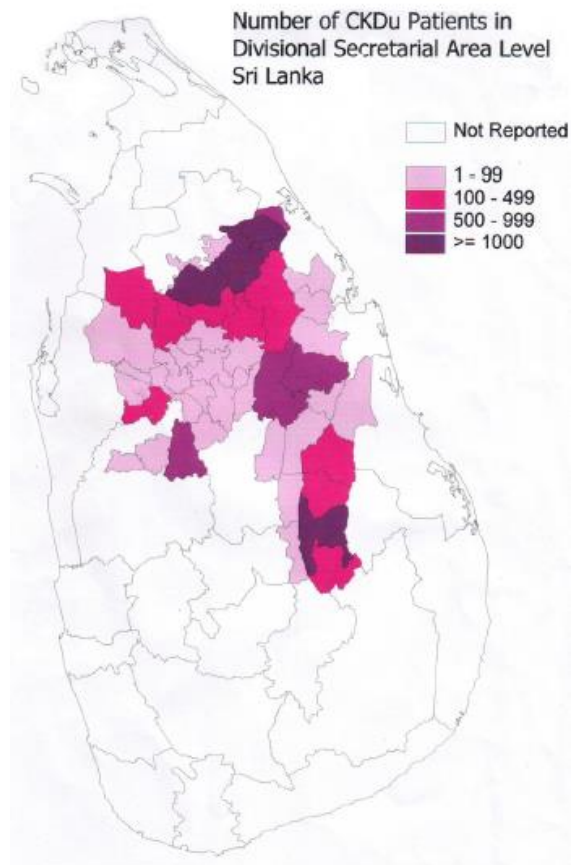


Figure 2.8 Spread of CKDu in Sri Lanka (Jayasumana, et al., 2014)

Among these factors this research study was mainly focused on glyphosate which is the most widely used herbicide in the CKDu prevalent areas. According to the WHO study conducted in the CKDu prevalent areas in Sri Lanka, the proportion of CKDu victims with level above reference value for glyphosate residue is 3.5% (WHO, 2013). Thus, glyphosate seems to play an important role for CKDu. Furthermore, in animal studies, it was found that glyphosate alone could cause adverse effects on kidney function. Changes in proximal tubular cells in kidney were observed in Nile Tilapia due to exposure to glyphosate (Jiraungkoorskul et al., 2003) and Ayoola et al (2008) have noted changes in proximal tubule, bowman space and degenerated tubules in African catfish when exposed to glyphosate (Ayoola, 2008). Therefore, glyphosate could be a triggering factor causing CKDu. However, there are no comprehensive studies reported in the literature to investigate probable presence of glyphosate in water sources and the associated human health risk levels due to consumption of waters contaminated with glyphosate in the CKDu prevalent areas in Sri Lanka.

2.7. Removal of glyphosate from water

2.7.1. Possible methods of glyphosate removal from water

Previous studies have reported different methods for glyphosate removal from water. Such methods include reverse osmosis (Gagliardo, et al., 1998), nano-filtration (Saitúa, et al., 2012), photo-catalysis (Bourgeois, et al., 2012), adsorption by bio char (Herath et al., 2016) and Ozonation (Assalin, et al., 2009). However, processes such as reverse osmosis and nano-filtration have limitations. One such limitation is inability to degrade the glyphosate molecule. Additionally, after removal of glyphosate by reverse osmosis and nano-filtration, membranes are contaminated with glyphosate, thus regeneration method of membrane or disposal of contaminated membrane are challenging. Therefore, Ozonation is introduced specially for treatment of water contaminated with glyphosate mostly because glyphosate is completely degraded by Ozonation (Assalin, et al., 2009). However, water in CKDu prevalent areas consist with high hardness levels (Dharma-wardana, et al., 2014) and there are no studies been done to comprehend the applicability of Ozonation for removal of glyphosate from water with high hardness. Therefore, it is essential to study the suitability of use of Ozonation to remove glyphosate in water in CKDu prevalent areas.

2.7.2. Ozonation

Ozonation is a powerful oxidation process and known as an effective technique that could be used to degrade glyphosate present in water (Assalin, et al., 2009). Once Ozone is purged into the water, it can dissociate into diatomic oxygen and oxygen radicals due to its instability (Bourgeois, et al., 2012). Oxygen radicals are very reactive hence tend to create bonds with the nearest possible components available to form compounds that can escape with ozone (Fig. 2.9) (Bourgeois, et al., 2012).

Ozone would have a rapid reaction with glyphosate and primarily reacts with the amine group in glyphosate to produce AMPA (Assalin et al., 2009). Past studies have reported complete degradation of glyphosate in the presence of high Ozone concentration where the reaction is said to be irreversible and continued until inorganic phosphate is produced (Jönsson, et al., 2013). A study done by Assalin et al., (2010) indicated that pH value of the water sample influence the efficiency of the Ozonation process and the nature of the products formed. Furthermore, the study concludes that

3. MATERIALS AND METHODS

3.1. Experimental Procedures and setups

3.1.1. Persistence of glyphosate in different environmental matrices in areas where CKDu is prevalent – Field studies

3.1.1.1. Chemicals and Materials

The following products were used in this study: Glyphosate (purity-99.7%, PESTANAL®, Analytical standard) (Sigma-Aldrich, Germany), AMPA (purity \geq 99.9%) (MP Biomedicals, France) 9-fluorenylmethyl chloroformate (purity \geq 99.0%, HPLC grade) (Sigma-Aldrich, Switzerland), n-Hexane (purity \geq 97.0%, CHROMASOL®, HPLC grade) (Sigma-Aldrich, Germany), Acetone (purity, HPLC grade) (DAEJUNG, Korea), Disodium EDTA (purity \geq 98.5%, AR grade) (Himedia, India), Sodium tetraborate (purity \geq 99.5%, AR grade) (Srichem, India), Hydrochloric acid (37%, ACS reagent) (Sigma-Aldrich, Switzerland), Nitric acid (69%, pure AR grade) (Himedia, India), Sulphuric acid (95.0- 97.0%, ACS reagent) (Sigma-Aldrich, Switzerland), Potassium dichromate (purity \geq 99.9%, AR grade) (Fisher scientific, India), Ammonium ferrous sulfate (purity \geq 99.0%, AR grade) (Analytical Regent, India), Phosphoric acid (purity \geq 99.8%, AR grade) (Analytical Regent, India), ammoniumacetate (purity \geq 98.0%) (Analytical Regent, India), Ammonium chloride (purity \geq 99.8%, AR grade) (Merck, Germany), Ammonium oxalate (purity \geq 99.0%, AR grade) (Analytical Regent, India), ammonia solution (30%, AR grade) (Analytical Regent, India), Ammonium molybdate (purity \geq 99.0%, AR grade) (AnlaR, England), Ammonium metavanadate (purity \geq 98.0%, AR grade) (Surechem products Ltd., India), Potassium dihydrogen phosphate (purity \geq 99.0%, AR grade) (Wako, USA), Sodium carbonate (purity \geq 99.0%, AR grade) (DAEJUNG, Korea), Barium chloride (purity \geq 99.0%, AR grade)(Himedia, India), Silver nitrate (purity \geq 99.0%, AR grade) (Analytical Regent, India), Sodium chloride (purity \geq 99.9%, AR grade) (Analytical Regent, India).



Figure 3.2 Collection of top soil from agricultural fields

2) Water samples from nearby groundwater sources (i.e., shallow wells)

One shallow well was selected near each agricultural field where topsoil samples were taken, to obtain the water sample. One water sample was collected to determine glyphosate and AMPA levels in water, and another water sample was collected and preserved by adding 5 mL of 69% HNO₃ for metal analysis, from each shallow well.

3) Water samples from surface water bodies

A surface water body (small reservoirs) located downstream of each agricultural field site to which runoff drained was selected to obtain water samples near each agricultural field (Fig. 3.3). Three water samples were collected from each source: one to determine glyphosate, AMPA, and another for physicochemical parameters and the third was preserved by adding 5 mL of 69% HNO₃ for metal analysis.



Figure 3.3 Sample collection from surface water bodies and preservation

4) Sediment samples from the surface water bodies selected

Composite sediment samples were collected from the bottom of each surface water body where surface water samples were collected following the same procedure as per the topsoil samples.

All soil, water and sediment samples were transported to the laboratory and stored below 4 °C to ensure the preservation of chemical properties. Another field site from the same study area in Rambewa, Anuradhapura District, where Roundup® had never been applied was chosen as the control site from which soil samples were obtained for comparison purposes.

3.1.1.3. Determination of physical and chemical characteristics of soil and sediments

Particle size distribution of soil samples was determined by performing sieve analysis and the hydrometer method (ASTM D7928) (ASTM D7928-17, 2017). Plastic characteristics of soil samples were analyzed with Atterberg Limits Test (ASTM D4318) (ASTM D4318-17, 2017). Unified Soil Classification System (USCS) was used to classify each soil sample based on particle size distribution and Atterberg Limit (ASTM D2487) (ASTM D2487-11, 2011).

To analyze cations present, both soil and sediment samples were acid digested overnight with 37% HCl and 69% HNO₃. The filtrate was collected to determine the major cations (Table 3) present in the soil/sediments by using a Flame Atomic Absorption Spectrometer (932 Plus, GBC Scientific Instruments). The amorphous iron and aluminum oxide percentages in soils/sediments were analyzed following the acid ammonium oxalate method (Manual, 1984). Soil organic matter content was analyzed by the Walkley-Black acid digestion method (Walkley & Black, 1934). Cation exchange capacity of each soil/sediment sample was determined following the USEPA Test Method 9080 (USEPA, 1986). Inorganic Phosphate levels in the soil/sediment samples were measured following the method described in Elrashidi (2010) (Elrashidi, 2010).

3.1.1.4. Determination of chemical characteristics of water samples

Cation concentrations of water samples were analyzed using a Flame Atomic adsorption Spectrometer (932 Plus, GBC Scientific Instruments). Anions present in twater samples were determined using standard methods: Total hardness, HCO_3^- and CO_3^{2-} contents in the water samples were determined by titration methods, SO_4^{2-} present in the water was quantified by the gravimetric method with drying residue and Cl^- in the water samples were enumerated by argentometric method (APHA, 2012).

3.1.2. Glyphosate and AMPA immobilization in top soil and mobilization of glyphosate and AMPA to water – Mesocosm studies

3.1.2.1. Selection of soil

A site where glyphosate has never been applied in the chosen study area was selected to collect soil to conduct the experiment. A composite sample from the selected site was analyzed for glyphosate and AMPA to confirm that glyphosate or AMPA was not present in the soil prior to the experiment.

3.1.2.2. Experimental Setup

The experiment was conducted in a steel box (1.2 m x 1 m x 0.3 m) with a perforated bottom (Fig. 3.4 & Fig. 3.5a). Geo-textile was laid on the perforated bottom to avoid the soil particle to percolate through holes with the infiltrated water (Fig. 3.5b). Soil was filled up to 0.2 m depth and a cylindrical steel tube to collect runoff was connected to the steel box at the top of that level (Fig. 3.5c). Soil was filled to the steel box 01 ensuring that the bulk density was 1760 kg/m^3 , which was the actual bulk density in the field. Second steel box (1.2 m x 1 m x 0.1 m) with a tube to collect water was fixed to the bottom of the steel box 01 to collect the infiltrated water from the soil block (Fig. 3.4 & 3.5c). The experimental setup was supported on a wooden base with an inclination of 2.5% (Fig. 3.4).

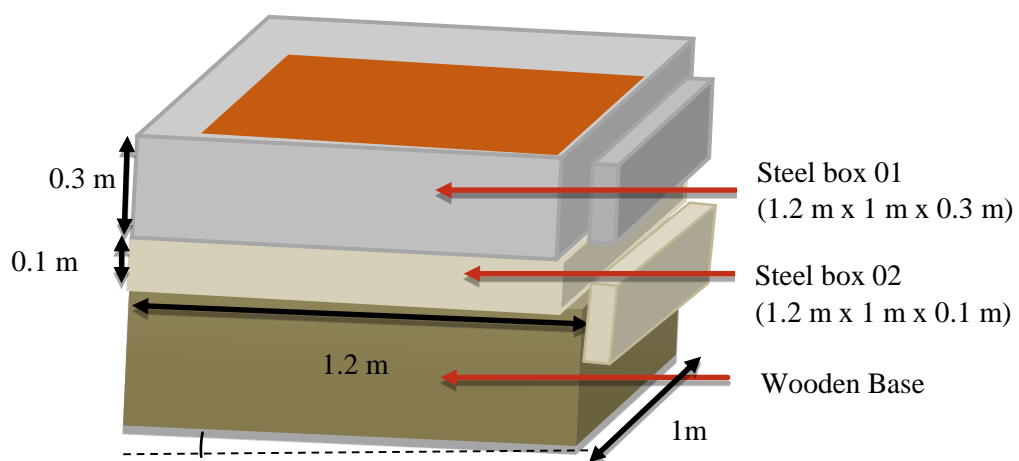


Figure 3.4 Schematic diagram of the experimental setup

Experiments were conducted in three experimental setups; plot 01- control plot where neither glyphosate nor TSP applied, plot 02 – only glyphosate applied and plot 03 – both glyphosate and TSP applied.

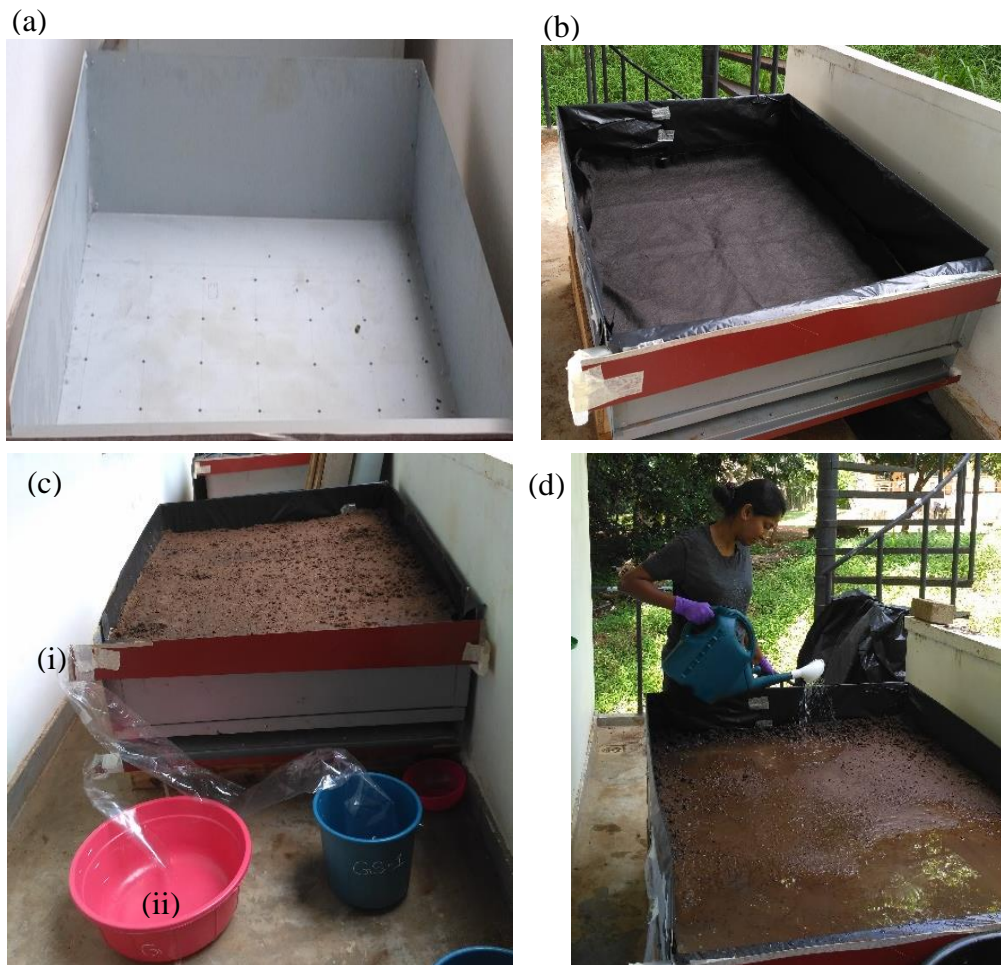


Figure 3.5 Experimental Setup for the plot study; (a) steel box with perforated at bottom to collect the infiltrated water, (b) geo-textile laid on perforated bottom, (c) experimental setup with soil filled according to bulk density of the field; i) setup to collect surface runoff and setup to collect infiltrated water, (d) simulation of precipitation event

3.1.2.3. Rainfall data

Precipitation intensity was decided based on the rainfall data collected from Metrological Department with respect to Anuradhapura Data station for past 8 years. The average maximum daily rainfall intensity was 116.16 mm based on the rainfall data. Therefore, total volume required to obtain the maximum intensity for the experimental setup was calculated as shown below. Distilled water was used to simulate rainfall/precipitation (Fig. 3.5). Precipitation was induced to the plot at

predetermined intensity (Fig. 3.5d). The duration of each precipitation event was 3 hours.

Total Volume of precipitation = 1.2 m x 1 m x 0.11616 m = 0.1394 m³ = 139.4 L

3.1.2.4. Glyphosate application and sample collection

The average Roundup® volume that had been applied to the fields in areas where CKDu is prevalent, was 4 L/acre and the glyphosate concentration in the Roundup® is 360 g/L. Therefore, it can be obtained that the rate of glyphosate applied to the soil in areas where CKDu is prevalent was 355.83 mg/m². Therefore, the mass of glyphosate applied to an experimental plot was 426.99 mg.

Roundup® (360 g/l glyphosate) was used in all the experimental setups. A stock solution of 1000 mg/L glyphosate was prepared using Roundup® and deionized water (Resistivity <18.2 megohm). This study specifically focused on the immobilization/mobilization of glyphosate in the absence of hardness. Therefore, deionized water was used during preparation of the solutions to eliminate the effect of hardness and glyphosate complexation. A working solution of 50 mg/L glyphosate was prepared by diluting the respective stock solutions using deionized water and sprayed to plot 02 and plot 03 in order to make the mass of glyphosate in the each plot as 426.99 mg.

Neither glyphosate nor TSP was applied to the plot 01 to maintain it as the control plot (Fig. 3.6a). Only glyphosate was applied to the plot 02 (Fig. 3.6b). Glyphosate was applied initially to the plot 03 and TSP was applied after 14 days and 28 days after the application of glyphosate (Fig. 3.6c). The amount of TSP applied at one event was 17.79 mg to achieve the field conditions. Precipitation for all the plots were simulated at predetermined time as indicate in the Figure 3.6. The time intervals of the precipitation events were decided based on the rainfall pattern in the study area. The time intervals of application of glyphosate and TSP, were decided based on the agricultural practices of the farmers in the study area.

A composite soil sample was collected from the top layer of each plot before and after each event (Fig. 3.6). During each precipitation event surface runoff and infiltrated water from the plot were collected. The experiments were conducted at ambient

temperature and under control conditions where loss of glyphosate due to evaporation/ volatilization and photodegradation were minimal.

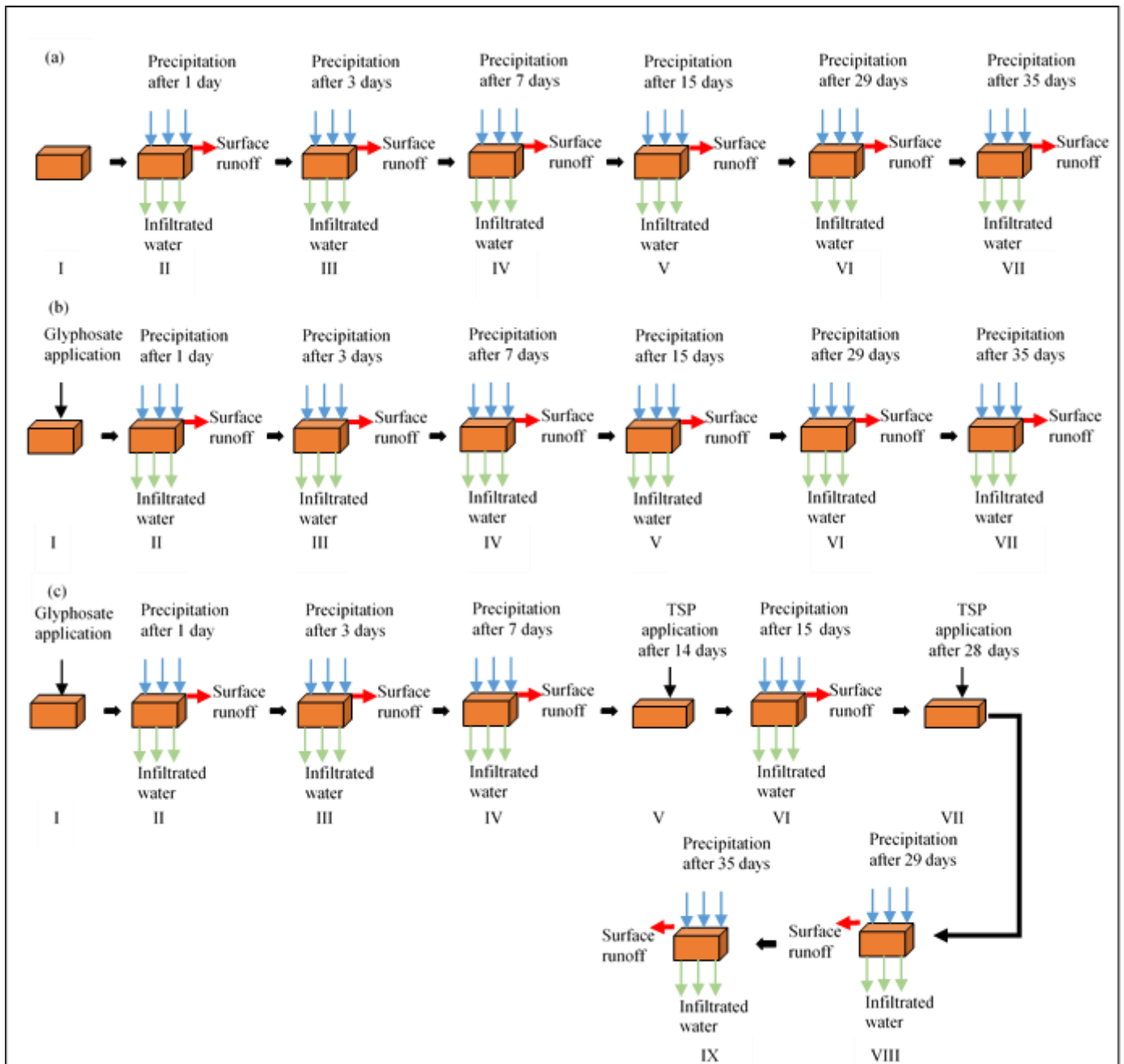


Figure 3.6 Experimental Sequence; (a) Plot 01 - the control plot where neither glyphosate nor TSP were applied, (b) Plot 02 – the plot where only glyphosate was applied initially, (c) Plot 03 – the plot where glyphosate was applied initially and TSP was applied after 14 days and 28 days of application of glyphosate

3.1.2.5. Determination of physical and chemical characteristics of soil

Sieve analysis and hydrometer method were executed for a composite sample of the collected soil to determine the particle size distribution (ASTM D7928) (ASTM

D7928-17, 2017). Plastic characteristics of soil samples were analyzed with Atterberg Limits Test (ASTM D4318) (ASTM D4318-17, 2017). Finally, based on the particle size distribution and Atterberg Limit soil was classified according to the Unified Soil Classification System (USCS) (ASTM D2487) (ASTMD2487-11, 2011).

A composite soil sample from the collected soil was digested overnight with conc. HCl and conc. HNO₃ to analyze the cations present in the soil. Then, the sample was heated for two hours and filtered using 0.45 µm regenerated cellulose membrane filters and the filtrate was collected. The collected filtrate was used to determine the major cations as shown in Table 3 present in the soil by Flame-Atomic adsorption Spectrometer (GBC 932 Plus, Australia). The ammonium oxalate method was used to determine the amorphous iron and aluminum oxide percentages in soil (Manual, 1984). Soil organic matter content was determined by following the Walkley-Black acid digestion method (Walkley & Black, 1934). USEPA Test Method 9080 was followed to obtain the cation exchange capacity of the soil (USEPA, 1986). The method described in Elrashidi (2010) was used to obtain the inorganic Phosphate levels in the soil (Elrashidi, 2010).

3.1.3. Glyphosate (high purity and commercial grade) degradation pattern in deionized water and hard water

3.1.3.1. Materials

Glyphosate (PESTANAL®, 98% purity) and Roundup® (360 g/l glyphosate) were used in all the experiments. Analytical standard of AMPA was purchased from Sigma-Aldrich. Stock solutions of 1000 mg/L were prepared with glyphosate and Roundup®, respectively, using deionized water (Resistivity <18.2 megohm). Working solutions of 5 mg/L of glyphosate and 5 mg/L Roundup® were prepared by diluting the respective stock solutions using deionized water.

Experiments were conducted using both deionized water and hard water. Hardness concentration used was 1,500 mg/L as CaCO₃ to simulate the extreme dry weather conditions, which could occur in the CKDu prevalent areas. Confirmation of hardness levels in water samples was carried according to the EDTA Titrimetric method 2340 C.

3.1.3.2. Experimental Setup

Batch experiments were conducted in duplicates using polypropylene tubes. Samples were prepared (10 ml) with glyphosate or Roundup® in deionized water with and without hardness (Fig. 3.7). The initial concentration of glyphosate in each sample was 5 mg/L. Sampling was done just after the preparation of sample, after 24 hours, twice a week till after 90 days, after 4, 5, 6 and 8 months. Prepared samples were subjected to photodegradation. Glyphosate and AMPA were measured and an average value for each sampling time was taken from the two samples.



Figure 3.7 Experimental setup for investigation of glyphosate degradation in water

3.1.4. Applicability of Ozonation process as an effective and efficient removal method of glyphosate and AMPA from water

3.1.4.1. Materials

Glyphosate (PESTANAL®, 98% purity) and Roundup® (360 g/l glyphosate) were used in all the experiments. Analytical standard of AMPA was purchased from Sigma-Aldrich. Preparation of stock solutions were done following the method described in section 3.1.3.1. The initial concentration of the samples were 1 mg/L of glyphosate and were prepared by diluting the respective stock solutions. Experiments were conducted in the absence and presence of hardness (1,500 mg/L as CaCO₃) Confirmation of hardness levels in water samples was carried according to the EDTA Titrimetric method 2340 C.

3.1.4.2. Experimental Setup

Ozonation probe, while continuously releasing Ozone (200 mg/h), was immersed in 10 L deionized water sample containing glyphosate or Roundup® in the absence and presence of hardness (Fig. 3.8). During Ozonation, samples from the reaction solution were collected at predetermined time intervals for glyphosate and AMPA analysis. The pH value of the water was continuously measured using a pH meter (Fig. 3.8). All experiments were conducted under ambient conditions.



Figure 3.8 Experimental Setup for Ozonation

3.2. Extraction and detection of glyphosate and AMPA in all samples

3.2.1. Extraction and detection of glyphosate and AMPA in soil samples

Soil samples were prepared for analysis after air drying for a week and sieving through a 0.2-mm sieve. Glyphosate and AMPA from the air-dried soil sample was extracted by adding 7.5 mL of 16 M Ammonium acetate, 50 mL of n-Hexane and 50 mL of Acetone to 10 g of soil sample and overnight shaking. Disodium EDTA (3 mL of 0.1 M) and Sodium tetraborate buffer (1 mL of 30 g/L) were added to the extracted samples (25 mL) to avoid possible interference from cations in the matrix. Samples were derivatized using 10 mg/L of 9-fluorenylmethyl chloroformate. Glyphosate and AMPA were analyzed using Liquid chromatography/Mass Spectrometer (LC/MS) Single Quad (Agilent® 1200 Infinity series LC with G1600 series Mass Selective Detector) coupled with a ZORBAXEclipse® XDB- C18 column. Glyphosate and AMPA analysis for sediment samples were carried out following a similar procedure.

3.2.2. Detection of glyphosate and AMPA in environmental water samples and in hard water

The analysis of glyphosate and AMPA in water samples were carried out by first adding disodium EDTA (3 mL of 0.1 M) and Sodium tetraborate buffer (1 mL of 30 g/L) to the water samples (25 mL) to avoid the possible interference from cations in the matrix. Then samples were derivatized using 10 mg/L of 9-fluorenylmethylchloroformate and analyses were done using LC/MS Single Quad (Agilent® 1200 Infinity series LC with G1600 series Mass Selective Detector) coupled with a ZORBAXEclipse® XDB- C18 column.

3.2.3. Detection of glyphosate and AMPA in deionized water

Glyphosate and AMPA concentrations in deionized water samples were analyzed using the Gas Chromatography/Mass Spectrometry (GC/MS) (Agilent 7890 B GC with 5977 Mass Selective Detector) coupled with a split-less injector and HP 5 MS

column. Helium was used as the carrier gas. Samples for GC/MS analyses were derivatized using Trifluoro-acetic anhydride (TFAA) and Trifluoroethanol (TFE) and Ethyl Acetate was used as the organic solvent.

4. RESULTS AND DISCUSSION

4.1. Persistence of glyphosate in different environmental matrices in areas where CKDu is prevalent – Field studies

4.1.1. Glyphosate and AMPA levels in topsoil

The levels of glyphosate and AMPA present in topsoil samples from the agricultural fields, except in the control site where these were not detected, ranged between 270-690 µg/kg and 2-8 µg/kg, respectively (Table 4.1). The AMPA levels were substantially lower compared to the corresponding glyphosate levels (Table 4.1). Other studies have reported that in Danish soil, glyphosate levels were in the average of 0.81 µg/kg and AMPA levels were in the average of 10.84 µg/kg (Simonsen, et al., 2008), in Argentinian soil, glyphosate and AMPA were between 35-1,502 µg/kg and 299-2,256 µg/kg (Aparicio et al., 2013) and in different places of Argentinian soils glyphosate levels were even recorded to be high ranging between 500-4,500 µg/kg (Peruzzo, et al., 2008).

The sorption of glyphosate onto soil matrix mainly depends on the adsorption properties of the soil matrix (Aparicio, et al., 2013; Borggaard & Gimsing, 2008; Dion, et al., 2001; Ololade et al., 2014; Piccolo, et al., 1994). The key factors influencing the glyphosate adsorption onto soil matrix are described below:

(i) The percentage of amorphous iron and aluminum oxides in the soil matrix

Amorphous iron oxide percentages in the topsoil samples of the present study were between 0.23 - 0.75% and amorphous aluminum oxide percentages were between 0.32 - 1.82%, respectively (Table 4.3).

Table 4.1 Glyphosate and AMPA levels of topsoil of agricultural fields selected for the study with other information related to agricultural practices

Site	GPS co-ordinates of the sampling location	Land area (acres)	Application of Roundup®				Glyphosate (µg/kg)	AMPA (µg/kg)	
			TFFA ³ (Years)	Dosage last applied ⁴ (L/acre)	Time since the last application up to sample collection	Whether there had been rain subsequent to the last application			Whether the tillage done after the last application
Control	8.51183 ¹ 80.65145 ²	0.25	-	-	-	-	-	ND ⁵	ND ⁵
TS1	8.50687 ¹ 80.64978 ²	1	8	2 - 4	3 days	No	No	270	3
TS2	8.51245 ¹ 80.63888 ²	2	10	2 - 4	20 days	Yes	No	380	2
TS3	8.39598 ¹ 80.34225 ²	1	10	2 - 4	20 days	Yes	Yes	390	4
TS4	7.50155 ¹ 80.62887 ²	4	10	2 - 4	3 months	Yes	No	380	6
TS5	8.49212 ¹ 80.6459 ²	2	10	5	4 months	Yes	Yes	420	5
TS6	8.51221 ¹ 80.64253 ²	2	10	2 - 4	6 months	Yes	No	340	3
TS7	8.51862 ¹ 80.63976 ²	2	10	2 - 4	6 months	Yes	No	430	8
TS8	8.50459 ¹ 80.64158 ²	5	15	8	7 months	Yes	No	690	2
TS9	8.52215 ¹ 8.65434 ²	2	12	2 - 4	7 months	Yes	No	520	6

1: Latitude; 2: Longitude; 3: TFFA: Time from the first application of Glyphosate; 4: Glyphosate concentration in Roundup®: 360 g/L; 5ND- Not Detected (Detection Limit: 1 µg/kg)

Table 4.2 Physical characteristics of topsoil samples

Site	Sand (%)	Silt (%)	Clay (%)	Soil Classification (based on USCS ¹)
Control	79	7	14	Clayey Sand (SC)
TS1	89	9	2	Poorly graded clayey sand (SP-SC)
TS2	70	17	13	Sand with Silt (SM)
TS3	94	2	4	Poorly graded clayey sand (SP-SC)
TS4	86	6	8	Clayey Sand (SC)
TS5	87	6	7	Clayey Sand (SC)
TS6	75	5	20	Clayey Sand (SC)
TS7	70	8	22	Clayey Sand (SC)
TS8	72	5	23	Clayey Sand (SC)
TS9	81	5	14	Clayey Sand (SC)

¹USCS: Universal Soil Classification System

Table 4.3 Chemical characteristics of the topsoil samples

Site	pH	Total Na (mg/kg)	Total Ca (mg/kg)	Total Al (mg/ kg)	Total Fe (mg/kg)	Amorphous Iron Oxide (%)	Amorphous Aluminum Oxide (%)	Organic Matter (%)	Cation Exchange Capacity (cmolc/kg)	PO ₄ ³⁻ (mg/kg)
Control	5.67	166.17	277.83	12,433.00	15,600.00	0.35	1.03	1.34	0.46	93.72
TS1	5.78	896.41	19.01	77.22	15,986.00	0.23	1.67	0.97	0.47	110.65
TS2	5.68	218.73	99.54	121.47	9,919.64	0.55	1.56	1.20	17.30	104.34
TS3	5.74	3,185.47	259.29	47.29	3,965.12	0.30	0.79	0.98	0.50	105.78
TS4	5.64	488.51	13.72	29.93	8,724.95	0.41	0.32	0.98	0.43	107.56
TS5	5.82	153.16	59.82	57.88	29,958.37	0.31	0.80	0.84	0.41	104.57
TS6	5.71	1,459.63	94.51	239.04	15,546.52	0.58	1.49	1.67	24.94	115.34
TS7	5.73	171.53	136.74	77.31	4,505.75	0.34	1.09	1.21	0.48	104.32
TS8	5.72	4,720.46	113.41	253.47	4,326.60	0.75	1.82	1.71	20.6	101.31
TS9	5.69	1,257.57	61.93	65.61	2,406.66	0.37	1.57	1.09	0.41	103.53

The highest glyphosate level in topsoil (690 µg/kg) was reported at TS8 (Table 4.1). When compared to other samples, soil at TS8 contained the highest percentages of amorphous iron and aluminum oxides, amounting to 0.75 and 1.82%, respectively (Table 4.3). The highest amount of glyphosate persistence was therefore observed at TS8 despite the fact that almost seven months had elapsed since the last application of Roundup®. In contrast, soil from TS1 showed the lowest glyphosate level (270 µg/kg) (Table 4.1). Eventhough TS1 contained a high percentage of amorphous aluminum oxide (1.67%), the lowest amorphous iron oxide percentage (0.23%) was recorded at TS1, for which the reason is unclear (Table 4.3). Therefore, presence of amorphous iron and aluminum oxides in the tested soils had promoted glyphosate adsorption to soil (refer section 2.4.1.1).

In other studies, higher levels of glyphosate adsorption onto soils has been observed, which contained comparatively higher percentages of amorphous iron oxide (1.63%) and aluminum oxide (0.65%) (Morillo, et al., 2000). Similarly, a study done by Piccolo et al. (1994) reported that glyphosate adsorption was higher in soil where amorphous iron and aluminum oxides were 4.74% and 2.19%, respectively, which were higher than in the soil where amorphous iron and aluminum oxides were 0.18% and 0.00%, respectively (Piccolo, et al., 1994).

(ii) pH of soil

The pH of soil samples in the present study were in the range of 5.67-5.82, which indicated that all soil samples were slightly acidic. As discussed in the section 2.4.1.2. the glyphosate adsorption onto soil increases in the pH range of 4-8. As the pH of all the soil samples were within the favorable range for ligand exchange with amorphous iron and aluminum, what is adsorped onto the soil matrix would not desorb holding onto the soil particles even for years. The observations (not shown here) corroborated that glyphosate would still persist in agricultural fields where the herbicide had not been used for several years, which is due to this chemisorption favored by optimum pH conditions. Therefore we believe that glyphosate will still persist in Sri Lankan agricultural soils for some years, even though its use is now banned, but the degree to which the CKDu is impacted by such herbicides seems to be questionable.

(iii) Soil organic matter

Percentage of organic matter in topsoil was between 0.84-1.71%. The highest topsoil organic carbon percentage (1.71%) was reported in TS8 where the glyphosate level was the highest (Table 4.3). Similar to this, a glyphosate level of 886.5 $\mu\text{g}/\text{kg}$ was detected in an Argentinian soil sample where organic carbon percentage was 7.57% and glyphosate level of 186.8 $\mu\text{g}/\text{kg}$ where organic carbon percentage was 3.15% (Aparicio, et al., 2013). Results of the present study corroborated that the presence of higher percentages of organic matter would yield higher levels of glyphosate in topsoil favoring the second hypothesis indicating that organic matter content helps immobilize glyphosate in soil matrix to a great deal (refer section 2.4.1.3.).

(iv) Inorganic phosphate levels

The inorganic phosphate levels at all sites ($\sim 101\text{-}110 \text{ mg}/\text{kg}$) were marginally greater than that of the control site (93.72 mg/kg) where glyphosate had never been applied (Table 4.3). Marginally higher levels of inorganic phosphate at all sites were perhaps due to the contribution of inorganic phosphate from repetitive applications of TSP that is used in excess of the recommended rate of application, expecting increased crop production. As discussed in the section 2.4.1.4., the presence of phosphate decreases the persistence of glyphosate and AMPA in soil due to competition of both glyphosate and phosphate for the same binding sites. However, results of this study do not show any direct relationship between inorganic phosphate levels and the corresponding glyphosate/AMPA levels found in the soils (Table 4.3). Further, results showed that repetitive application of TSP was not a precursor to leach out glyphosate perhaps due to the strong bonding of glyphosate with amorphous iron and aluminum through ligand exchange. However, the glyphosate that is weakly bound to soil matrix by physisorption through either van der Waals forces or electrostatic forces can leach out with the replacement of phosphate ions.

(v) Cation Exchange Capacity

The cation exchange capacity (CEC) of the topsoil samples ranged between $\sim 0.40 - 25 \text{ cmol}/\text{kg}$ (Table 4.3), which was considerably lower than the levels reported by

Aparicio et al. (2013) for Argentinian soil where the CEC levels ranged between ~ 20-68 cmol/kg (Aparicio, et al., 2013). The effect of CEC on adsorption of glyphosate onto soil particles is debatable (refer section 2.4.1.5.) However, the present study elucidated that there was no direct relationship with the adsorption potential of glyphosate to soil matrix as in sites TS2, TS6, and TS8, CEC was reported to be high compared to that of other sites, but no reduced levels of glyphosate were observed. We, therefore, believe that CEC would not affect the desorption mechanisms of glyphosate in Sri Lankan agricultural soil that was studied.

(vi) Hardness

When glyphosate is dissolved in hard water, it forms complexes with Ca^{2+} and Mg^{2+} present in hard water (Thelen, et al., 1995). However, when the glyphosate complexes rich in Ca^{2+} and Mg^{2+} come into contact with the soil matrix, they are replaced by cations present in the soil that are having higher affinity to glyphosate. The agricultural soil in the study area contains clay particles with higher iron and aluminum content compared to those of Ca and Mg (Table 3). In the soil samples, total iron and total aluminum levels were in the range of 58-254 mg/kg and ~4,000-30,000 mg/kg, and the total calcium levels were in the range of ~19-278 mg/kg, respectively (Table 4.3). As such, glyphosate already complexed with Ca^{2+} and Mg^{2+} when comes into contact with soil matrix is detached and glyphosate is then adsorbed to cations of higher sorption strengths.

Results, therefore, conclude that the hardness prevailing in the dry season could enhance complexation of glyphosate with the Ca^{2+} and Mg^{2+} . When this complex comes into contact with the soil, higher contents of iron and aluminum of the soil matrix would replace calcium and magnesium, resulting in strong sorption of glyphosate with soil minerals.

Lower levels of AMPA in all soil samples could have been due to the strong sorption affinity of glyphosate to soil minerals (Borggaard & Gimsing, 2008) making it less bio-available for soil microbial degradation to form the major metabolite, AMPA. It has been reported for a study conducted in Argentina that glyphosate degradation was retarded when its adsorption onto soil was high (Aparicio, et al., 2013). In addition,

glyphosate could have been degraded to AMPA and AMPA had further degraded to inorganic phosphate (refer section 2.3.2). Nonetheless, glyphosate was in minute quantities desorbed to soil pore water as AMPA, perhaps due to the release of poorly bound glyphosate through physisorption process with the soil matrix through microbial degradation (Aparicio, et al., 2013). In the present study, detection of low levels of AMPA at each site provides evidence of loosely bound glyphosate mobilization through microbial degradation despite the presence of various cations and amorphous iron and aluminum oxides in considerable amounts (Table 4.3). Once AMPA is formed, its persistence is reported to be pronounced than that of glyphosate, as the half-life of AMPA (151 days) (Norgaard et al., 2014) is greater than that of glyphosate (130 days) (Schuette, 1998).

Another factor that is potentially important for the persistence of glyphosate in soil would be the presence of surfactants, and other adjuvants (e.g., POEA) admixed during the manufacturing of glyphosate products such as Roundup®. Presence of surfactants can enhance the immobilization of glyphosate in the soil matrix (Grant, et al., 2010). Further, surfactants can influence the biodegradation process, soil structure, and the associated adsorption/desorption processes (Katagi, 2008). Depending on the characteristics of the surfactants (e.g., type and concentration, critical micelles concentration), surfactants could increase or decrease the adsorption of the compound to soils leading to either lower or greater mobilization, respectively, from soil to pore waters (Haigh, 1996). However, the impact of having surfactants and other adjuvants on the persistence of glyphosate was not investigated in the present study.

4.1.2. Glyphosate and AMPA levels in surface water

Glyphosate was detected (28-45 µg/L) in all surface water samples collected from surface water bodies located in the downstream of respective agricultural fields (Table 4.4). The Maximum Contaminant Level (MCL) on the basis of potential health issues for glyphosate set by the USEPA is 700 µg/L. Therefore, all surface water samples contained glyphosate levels less than the MCL, indicating that there are hardly any health issues by ingestion of this water. However, no such threshold level has been established so far in Sri Lanka for the control of CKDu, hence the degree to which the

amount of ingestion of water per day having glyphosate, as reported in the study area, be impacted is not known.

The highest glyphosate concentration (45 µg/L) in surface water was observed in SW6. Previous researchers provide evidence that presence of inorganic phosphates in soil is a major contributing factor for desorption of glyphosate from soil particles as both glyphosate and phosphate compete for the same surface sorption sites (de Jonge, et al., 2001; Dion, et al., 2001; Gimsing & Borggaard, 2002). It was noted that the soil obtained from site TS6 contained the highest phosphate level (~115 mg/kg; Table 3). Therefore, desorption of glyphosate could be higher in soils from site TS6 with higher phosphate levels resulting in a higher level of glyphosate in the surface runoff and consequently, accumulating in the surface water body downstream of site SW6.

Table 4.4 Water quality characterization of surface water samples

Site	pH	Temp. (°C)	Total Ca (mg/L)	Total Mg (mg/L)	Total Al (mg/L)	Total Hardness (mg/L as CaCO ₃)	Glyphosate (µg/L)	AMPA (µg/L)
SW1	6.71	26.6	25.23	8.45	0.14	97.70	36	ND ¹
SW3	7.12	26.7	25.15	6.71	0.65	90.40	40	ND ¹
SW4	6.73	26.6	14.84	24.74	0.55	139.00	38	ND ¹
SW5	6.81	26.6	24.28	8.02	0.21	93.60	33	ND ¹
SW6	7.21	26.7	62.15	19.43	0.76	235.00	45	ND ¹
SW8	7.13	26.7	39.94	33.40	0.47	237.00	28	ND ¹
SW9	7.03	26.7	45.44	27.58	0.76	227.00	34	ND ¹

¹ND - Not Detected (Detection Limit: 1 µg/L)

Note: Surface water sources at the downstream of Locations SW2 and SW7 have been dried due to dry weather conditions prevailed at the time of sampling

MCL set by USEPA is 700 µg/L

The lowest glyphosate concentration (28 µg/L) in surface water was detected in SW8. Among all the topsoil samples, site TS8 had the highest amorphous iron and aluminum oxides and organic matter content (Table 4.3). As discussed in the section 2.4.1.1., the presence of higher contents of amorphous iron and aluminum oxides and organic matter could result in greater adsorption of glyphosate to soil particles, thus limiting

the glyphosate desorption and transport to downstream water bodies. It was also noted that site TS8 had the lowest inorganic phosphate level in comparison to other field sites (Table 4.3), hence the effect of phosphate on glyphosate desorption at SW8 would also be minimal.

Presence of glyphosate in surface waters could have been due to the transport of glyphosate molecules with surface runoff during precipitation events in dissolved form or being attached to soil particles, which is known as colloid-facilitated transport (Borggaard & Gimsing, 2008). Some recent studies also reported the presence of glyphosate in surface water sources associated with agricultural lands where Roundup® was applied; 0.5-4 µg/L (Aparicio, et al., 2013), 1-110 µg/L (Daouk, et al., 2013) and 22-2,191 µg/L (Tsui & Chu, 2008), 0.1 - 0.7 mg/L (Peruzzo, et al., 2008).

Lower levels of glyphosate in surface waters with respect to corresponding soils could have been attributed to strong adsorption affinity of glyphosate to the soil matrix, especially to amorphous iron and aluminum oxides, which were present in considerable quantities in the soil matrix (Table 4.3), thereby limiting the desorption of glyphosate, thus low amounts were transferred to the aqueous environment, and similar observations have been made by Mamy et al. (2005) and Borggaard & Gimsing (2008) (Borggaard & Gimsing, 2008; Mamy & Barriuso, 2005).

The major metabolite of glyphosate, AMPA was not detected in any of the surface water samples that were tested (Table 4.4). Non detection of AMPA in the surface waters can be attributed to different reasons; (1) glyphosate was non bio-available for degradation and (2) glyphosate had been degraded to AMPA and AMPA had subjected to further degradation to inorganic phosphate. Non bio-availability of glyphosate could be a result of presence of high hardness levels (72.4-248.0 mg/l as CaCO₃), which result in high Ca²⁺ and Mg²⁺ ion concentrations in water (Table 4.4). Therefore, possible formation of glyphosate-Ca and -Mg complexes in water could lead to glyphosate being less bio-available for degradation thus hinders the degradation process. There seems to be no predominant mechanism such as upwelling or/and diffusion for AMPA to transport from the sediments to the water column. Application of Roundup® has proven to limit the bio-availability for microbial degradation of glyphosate in the aquatic ecosystems in instances where both metals and glyphosate

co-exist (Tsui, et al., 2005). Further, AMPA could have been formed by glyphosate degradation and could be further degraded to inorganic phosphate through microbial degradation (refer section 2.3.2.). Further studies are required to find the exact reason for non detection of AMPA in surface water samples.

4.1.3. Glyphosate and AMPA levels in shallow groundwater

Trace levels of glyphosate (1-4 µg/L) were detected in all samples collected from shallow wells located in the vicinity of the respective agricultural fields (Table 4.5). In addition, trace amounts of AMPA were detected only in WW1, WW3, WW5, and WW9 (Table 4.5). It was noted that glyphosate levels in shallow groundwater were substantially lower in magnitude (more than 10-fold decrease) than those of surface water sources (Table 4.5). Similar glyphosate levels have been reported in shallow groundwater in other CKDu prevalent areas in Sri Lanka (Jayasumana, et al., 2015).

Table 4.5 Water quality characterization of groundwater samples

Site	pH	Temp. (°C)	Total Ca (mg/L)	Total Mg (mg/L)	Total Al (mg/L)	Total Hardness (mg/L as CaCO ₃)	Glyphosate (µg/L)	AMPA (µg/L)
WW 1	6.78	26.8	19.17	5.96	0.37	72.40	4	11
WW 2	7.22	26.8	23.28	7.02	0.21	87.00	4	2
WW 3	7.16	26.9	45.40	34.52	0.65	255.00	4	6
WW 4	6.85	26.9	62.93	17.97	0.23	231.00	1	ND ¹
WW 5	6.79	26.8	21.14	7.38	0.14	83.10	3	5
WW 6	7.16	26.8	12.84	38.74	0.55	191.00	2	ND
WW 7	6.98	27.0	60.00	18.43	0.76	226.00	2	ND
WW 8	7.07	26.8	40.94	33.40	0.47	239.00	3	ND
WW 9	7.02	26.8	55.44	26.58	0.76	248.00	2	ND

¹ND - Not Detected (Detection Limit: 1 µg/L)

MCL set by USEPA is 700 µg/L

The potential risk of groundwater pollution due to leaching of glyphosate through the soil column is very remote (Kjær et al., 2004), and the transport phenomena depend

on the rate of water percolation through the vertical soil column, glyphosate degradation processes through natural attenuation by biodegradation and their sorption processes (Douc, et al., 2013).

Glyphosate and AMPA are known to strongly adsorb onto soil and retain within the first 15 cm of topsoil layer (Vereecken, 2005). Therefore, before reaching the groundwater, glyphosate could be strongly adsorbed onto soil minerals as well as subjected to degradation by soil microorganisms while moving through the deeper soil layers (Vereecken, 2005). In the present study, strong adsorption affinity of glyphosate onto iron oxides and amorphous iron and aluminum oxides could have hindered leaching of glyphosate to groundwater. Hence, glyphosate has a low potential to infiltrate into groundwater table. Nonetheless, previous studies have reported that there is a potential for glyphosate movement to groundwater table subsequent to heavy precipitation events as a result of the presence of preferential flow paths (Vereecken, 2005), which has not been clearly observed in the present study although the agricultural fields have experienced a series of precipitation events at the time of sampling. Greater amounts of adsorption of glyphosate in tested soils relative to that of AMPA and potential degradation of glyphosate could have been attributed to the detection of AMPA in groundwater samples, and the same observations have been noted by Battaglin et al. (2014) (Battaglin, et al., 2014). Typically, only trace levels of glyphosate in groundwater have often been reported (Kjær, et al., 2004; Van Stempvoort, et al., 2016). Another study has also found trace levels of glyphosate in abandoned wells located in the vicinity of agricultural fields where glyphosate has been used over a long period (Jayasumana, et al., 2015). Battaglin et al. (2014) detected glyphosate in 5.8% samples and AMPA in 14.3% samples out of a total of 1,171 groundwater samples tested. Further, it was found that there was no correlation of levels of hardness and glyphosate present in the groundwater samples as further complexation of glyphosate with calcium and magnesium was almost absent though the total hardness is attributed to calcium and magnesium.

Therefore manifest from the results that groundwater is very unlikely to be contaminated with glyphosate and AMPA so as to pose potential health threats such as CKDu.

4.1.4. Glyphosate and AMPA Levels in Sediment

Glyphosate was detected in all sediment samples (85-1,000 µg/kg) collected from the respective surface water bodies located downstream of each agricultural field where Roundup® was applied (Table 4.6). In addition, except for the sediment samples collected from surface water bodies corresponding to sites SE1 and SE5, AMPA was detected in all other sediment samples (1-15 µg/kg, Table 4.6). Presence of glyphosate and AMPA in the sediments indicates the transport of glyphosate and/or AMPA molecules-laden colloidal particles from the agricultural fields along with the surface runoff and their accumulation in water bodies. The AMPA levels were considerably lower than the respective glyphosate levels in sediments at each location indicating hardly any degradation of glyphosate while being accumulated in the sediment and greater persistence of AMPA compared to glyphosate, as observed by Schuette (1998) (Schuette, 1998). As mentioned above, organic matter content has been reported to act as the main sorbent for glyphosate in sediments (Piccolo, et al., 1994), and the organic matter content of the sediments were between 1.11-2.13%, which was considered to be not very high (Table 4.6).

Sediments collected from the surface water body corresponding to SE3 demonstrated the highest glyphosate level (1,011 µg/kg) among all sediment samples tested (Table 4.6). As a consequence of a series of precipitation events followed by heavy soil erosion in agricultural field 3, glyphosate adsorbed onto colloidal particles could have transported and got deposited in the surface water body as sediment. Additionally, the highest organic matter percentage was found in the sediment sample at SE3. Thus, higher organic matter content of sediments at SE3 could lead to higher glyphosate adsorption. The lowest glyphosate level (85 µg/kg) in sediment samples was found at SE5 (Table 4.6), which could be attributed to the low organic matter content resulting in lower adsorption of glyphosate onto sediment particles (Piccolo, et al., 1994). In addition, while no AMPA was detected in surface water, AMPA was found in 7 sediment samples out of all 9 samples tested (Table 4.6). Recent studies have also reported the presence of glyphosate and AMPA in sediment samples: 5.7-221.2 µg/kg (Aparicio, et al., 2013), 1.15-1.85 mg/L (Peruzzo, et al., 2008).

Table 4.6 Organic matter content in the sediment samples

Site	OM (%)	Glyphosate ($\mu\text{g}/\text{kg}$)	AMPA ($\mu\text{g}/\text{kg}$)
SE1	1.31	120	ND ¹
SE2	1.35	264	2
SE3	2.13	1,011	4
SE4	1.51	330	15
SE5	1.11	85	ND ¹
SE6	1.32	337	3
SE7	1.41	147	7
SE8	1.32	141	1
SE9	1.74	438	8

¹ND - Not Detected (Detection Limit: 1 $\mu\text{g}/\text{kg}$)

The present study corroborated that sediment-laden glyphosate and AMPA were strongly adsorbed to soil particles, and desorption potential to the water column is almost insignificant. This strong bonding, therefore, acts as a sink to both glyphosate and AMPA indicating that there will be a lesser health threat for those who consume water from these water bodies in areas prevalent with CKDu.

4.1.5. Summary

The findings of this study indicate that glyphosate persists in Sri Lankan agricultural soils in areas prevalent with CKDu as a result of its strong adsorption onto the soil matrix, due to factors such as the presence of amorphous aluminum and iron oxides, presence of high valence cations, presence of organic matter in the soil matrix and favorable pH conditions. High adsorption of glyphosate to the soil matrix resulted in decreased availability of glyphosate for microbial degradation, thus considerably lower levels of AMPA were detected in the soil samples. Furthermore, analysis of water samples showed that glyphosate persisted in hard water, which could be attributed to the formation of glyphosate-Ca, -Mg complexes in hard water. The formation of such glyphosate-Ca, -Mg complexes could hindered the microbial

degradation of glyphosate, thus AMPA was not detected in surface water. Only minute levels of AMPA were detected in groundwater. Glyphosate was detected in sediment samples collected from the surface water bodies, which could be attributed due to the migration of glyphosate laden colloidal particles with surface runoff and their accumulation in the water bodies as well as higher sorption onto sediment particles due to the presence of organic matter in the sediments. In conclusion, it could be inferred that the impact on CKDu by the persistence of glyphosate and AMPA in the environment is remote when compared with the MCL of the USEPA (700 µg/L). However, in-depth studies should be undertaken for better understanding of the persistence potential of glyphosate/AMPA in water and soils, and its likelihood to cause high rates of morbidity and mortality due to CKDu.

4.2. Glyphosate and AMPA immobilization in top soil and mobilization of glyphosate and AMPA to water – Mesocosm studies

4.2.1. Immobilization of glyphosate in soil prior to precipitation events

The mass of glyphosate applied to a mesocosm was 355.83 mg/m². No surface runoff or percolation of groundwater was observed in any of the mesocosms in the event of glyphosate application. After the application of glyphosate, the mass of glyphosate retained in the soil in the plots was; control plot - 0 mg, plot with only glyphosate - 355.66 mg/m² and plot with glyphosate and TSP - 355.68 mg/m² (Table 4.7&4.8). Comparison of mass of glyphosate applied and measured in the soil confirm that glyphosate had not leached out from any of the soil plots at the event of application of glyphosate.

The immobilized glyphosate in the soil plots could be present as free glyphosate in the pore water, adsorped to soil organic matter (Borggaard & Gimsing, 2008; Ololade, et al., 2014; Piccolo, et al., 1994), adsorped to amorphous aluminum and iron oxides (Borggaard & Gimsing, 2008; Morillo, et al., 2000; Ololade, et al., 2014; Piccolo, et al., 1994), and adsorped to high valence cations (Borggaard & Gimsing, 2008; Glass, 1987).

The soil organic matter content in the soil was 1.34% (Table 4.9), which is a lower value reported for soil compared to other parts of the world; Argentinian soil > 3.15% (Aparicio, et al., 2013), Danish soil ~ 2.7% (Simonsen, et a., 2008). The adsorption of glyphosate to soil organic matter occurs through hydrogen bonding (Borggaard & Gimsing, 2008; de Jonge, et al., 2001). The amorphous iron and aluminum oxides in the soil is 0.35% and 1.03%, respectively (Table 4.9). Glyphosate adsorption to amorphous iron and aluminum oxides occur through a ligand exchange (Borggaard & Gimsing, 2008; Piccolo, et al., 1994).

Table 4.7 Plot 02 – Amount of glyphosate retained in soil and mobilized to water subsequent to glyphosate application and multiple precipitation

Description	Application of glyphosate	Precipitation					
	T = 0 ¹	T = 1 ¹	T = 3 ¹	T = 7 ¹	T = 15 ¹	T = 29 ¹	T = 35 ¹
Glyphosate retained in the top soil (mg/m ²)	355.66	335.62	328.69	327.98	327.93	327.93	327.93
Glyphosate in the surface runoff (mg/m ²)	-	12.25	6.21	0.63	0.00	0.00	0.00
Glyphosate in groundwater (mg/m ²)	-	7.70	0.68	0.00	0.00	0.00	0.00
Total (mg/m ²)	355.66	355.57	335.58	328.61	327.93	327.93	327.93

1: T = 0 days was considered as the time of glyphosate application and time was measured by days

Table 4.8 Plot 03- Amount of glyphosate retained in soil and mobilized to water subsequent to glyphosate application, TSP application and multiple precipitation

Description	Application of glyphosate	Precipitation			Application of TSP	Precipitation	Application of TSP	Precipitation	
	T = 0 ¹	T = 1 ¹	T = 3 ¹	T = 7 ¹	T = 14 ¹	T = 15 ¹	T = 28 ¹	T = 29 ¹	T = 35 ¹
Glyphosate retained in the top soil (mg/m ²)	355.68	336.78	328.72	327.93	327.93	314.99	314.98	299.01	290.57
Glyphosate in the surface runoff (mg/m ²)	-	11.88	7.15	0.69	-	10.65	-	13.17	6.33
Glyphosate in groundwater (mg/m ²)	-	7.00	0.65	0.00	-	2.25	-	2.56	0.89
Total (mg/m²)	355.68	355.66	336.52	328.62	327.93	327.89	314.98	314.74	297.79

1: T = 0 days was considered as the time of glyphosate application and time was measured by days

Table 4.9 Physical and chemical characteristics of the soil

No	Property	Readings
1	Organic matter	1.34%
2	Cation Exchange Capacity cmol _c /kg	0.46
3	pH	5.23
4	Sand %	79%
5	Silt %	7%
6	Clay %	5%
7	Total Fe (mg/kg)	15,600
8	Total Al (mg/kg)	12,433
9	Total Mn (mg/kg)	533.33
10	Total Cu (mg/kg)	12.00
11	Total Na (mg/kg)	166.17
12	Total K (mg/kg)	488.67
13	Total Ca (mg/kg)	277.83
14	Amorphous Iron Oxide %	0.35%
15	Amorphous Aluminum Oxide %	1.03%
16	Phosphate mg/kg	93.72

4.2.2. Mobilization of glyphosate subsequent to multiple precipitation events

When considering plot 02 where only glyphosate was applied, glyphosate was detected in surface water subsequent to rainfalls at T = 1, T = 3 and T = 7 days. T = 0 days was considered as the time of glyphosate application. Further, glyphosate was detected in water infiltrated through the soil block subsequent to precipitation at T = 1 and T = 3 days (Table 4.7 & 4.8). After T = 7 days and subsequent precipitation events, glyphosate was not detected in either surface water or groundwater (Table 4.7). Similar pattern up to T = 7 days were observed in plot 03 where both glyphosate and TSP were applied.

In the event of first precipitation, in plot 02, 5.6% of glyphosate that was retained in the soil was leached out of which 3.4% was leached out as surface runoff and 2.2% was infiltrated through the soil block (Table 4.7). Similarly, in plot 03, 5.3% of the retained glyphosate was leached out from the soil block after the first precipitation out of which 3.3% in the soil block was leached out as surface runoff and 2.0% was infiltrated through the soil block (Table 4.8).

In the event of precipitation, glyphosate in the pore water (Gavrilescu, 2005) and into groundwater through infiltration (Vereecken, 2005). Therefore, in the present study, glyphosate present in pore water could easily mobilize with the surface runoff and infiltrated to groundwater. Additionally, the molecules that are bonded with hydrogen bonding have higher affinity to dissolve in water by making hydrogen bonds with water molecules, thus glyphosate adsorbed to soil organic matter could mobilize into the water via hydrogen bonding (de Jonge, et al., 2001). Therefore, it could be derived that glyphosate mobilized into surface runoff and groundwater in the first event of precipitation could be the glyphosate in pore water and glyphosate adsorbed to soil organic matter.

In the event of second precipitation, only 2.1% of the glyphosate retained was leached out from the plot 02 (1.9% as surface runoff and 0.2% infiltrated through soil block) and only 2.3% of the glyphosate retained was leached out from the plot 03 (2.1% as surface runoff and 0.2% infiltrated through soil block) (Table 4.7 & 4.8). It is apparent that the percentage of glyphosate leaching out has decreased in the event of the second rain.

Previous studies reported that leaching of glyphosate from soils depends mainly on the first precipitation event after Roundup® application (Rasmussen, et al., 2015). Further, multiple precipitation events after the first precipitation event have not shown substantial contribution to the mobilization of glyphosate into water sources except in tracer amounts (Roy et al., 1989). Therefore, the reason for detection of lower levels of glyphosate after the second precipitation event compared to that of first precipitation event could be that glyphosate in the pore water and adsorbed to soil organic matter could be decreased with the first precipitation event (Table 4.7 & 4.8). A similar observation was identified in the subsequent precipitations; where in the event of third precipitation only 0.2% (only in surface water) of the glyphosate retained was leached out from both plot 02 and 03 (Table 4.7 & 4.8). This could be because the major percentage of glyphosate that was in pore water and adsorbed to organic matter had been leached out from the previous precipitation events. In plot 02 no glyphosate was detected either in the surface runoff or infiltrated water after the third precipitation. This could be because all the glyphosate that can be leached out from precipitation had been leached out from the previous precipitations.

In all experiments, mass of glyphosate detected in surface water was greater than that of in groundwater (Table 4.7 & 4.8). In literature, it was reported that, glyphosate that is adsorbed into soil particle could also infiltrate into groundwater bodies after heavy precipitation (Borggaard & Gimsing, 2008). Typically, glyphosate leaching into groundwater bodies is limited due to high adsorption of glyphosate onto soil particles (Vereecken, 2005). Thus, only trace levels of glyphosate in groundwater have often been reported in the literature (Van Stempvoort, et al., 2016). Therefore, glyphosate is only in trace levels in the groundwater bodies. This observation was done by the previous researchers indicates that glyphosate leaching into groundwater is limited, confirming the results that was obtained in the present study (Table 4.7 & 4.8).

In the present experiment intensity of rainfall, soil characteristics and climatic conditions were controlled and kept identical for all experiments, therefore the effect of these factors on leaching of glyphosate from soil is considered equally.

4.2.3. Mobilization of glyphosate subsequent to TSP application followed by multiple precipitation events

In plot 03, glyphosate mobilization was observed subsequent to TSP application at the events of precipitation at T = 15, T = 29 and T = 35 days (Table 4.8). Glyphosate was detected in both surface runoff and infiltrated water through the soil block collected subsequent to precipitation events (Table 4.8). This observation was contrasted to the observation made for the plot 02 where no glyphosate was mobilized at the events of precipitation T = 15, T = 29 and T = 35 days (Table 4.7).

Inorganic phosphate binds to similar binding sites in soil as glyphosate, hence may compete with glyphosate for the soil sorption sites. Therefore, when TSP was applied to the plot, glyphosate that were adsorbed to the amorphous iron and aluminum oxides via ligand exchange could be desorbed into pore water. Therefore, it was apparent that the application of TSP for the plot 03 had enabled the glyphosate to mobilize in surface water and infiltrated water application at the events of precipitation at T = 15, T = 29 and T = 35 days (Table 4.7 & 4.8).

In the event of first precipitation after the first TSP application (T = 15 days), 3.9% of glyphosate that was retained in the soil plot 03 was leached out; 3.2% was leached out as surface runoff and 0.7% was infiltrated through the soil block (Table 4.8). On the other hand, in the event of first precipitation after the second TSP application (T = 29 days), 5.0% (4.2% as surface runoff and 0.8% infiltrated through soil block) of the glyphosate retained in the soil was leached out from the plot 03 (Table 4.8). This observation implicated that with multiple application of TSP to the soil plot, percentage of glyphosate been mobilized into water was increased. Multiple application of TSP had increased the glyphosate mobilization to water via replacing more glyphosate that had been adsorbed to amorphous iron and aluminum oxides (Table 4.8) and also it was reported in literature that with multiple application of TSP, glyphosate mobilization into the water was increased (Munira, et al., 2016).

The effect of multiple precipitation events was investigated by inducing precipitation twice after the second TSP application. It was observed that glyphosate mobilization to water subsequent to application of TSP had decreased with the multiple rainfall. In the event of second precipitation after the second application of TSP (T = 35 days),

2.4% (2.1% as surface runoff and 0.3% infiltrated through soil block) of the glyphosate retained was leached out from the plot 03 (Table 4.8). Hence, the glyphosate mobilization into the water was decreased with the multiple application of rainfall. As discussed in section 4.2.2. this could be due to the fact that glyphosate in the pore water and adsorbed to soil organic matter that were resulted as application of TSP, could be decreased with the first precipitation event after the application of TSP, therefore, lower glyphosate mass were detected in subsequent precipitation events.

Further, it was observed that the glyphosate mobilized to infiltrated water was lower than the glyphosate mobilized to surface runoff. The reasons for this observation is discussed in detail in section 4.2.2.

4.2.4. Non detection of AMPA in soil and water samples

Literature reported that glyphosate is degraded to AMPA starting from a few days after the application of glyphosate (Schuette, 1998). However, during the present study, AMPA was not detected even after 35 days of application of glyphosate. The main reason for this observation could be that microorganisms that are responsible for glyphosate degradation was not present in the soil.

However, glyphosate that was in pore water and adsorbed to soil organic matter were mobilized into the water after the event of precipitation a $T = 7$ days. Therefore, glyphosate adsorbed to the amorphous iron and aluminum oxides and other high valence cations in the soils, were remaining. In literature, it is implicated that when glyphosate is present in soil, microbial degradation of glyphosate is limited because glyphosate is adsorbed onto soil and is not bioavailable for microbes (Borggaard & Gimsing, 2008). Therefore, this could be another reason for the absence of AMPA in the soil samples.

When AMPA was not present in the soil, then AMPA could not mobilized into surface runoff and infiltrated water. This was confirmed by the absence of AMPA in any of the water samples collected. Further, water samples were tested without any delay and stored below 4 °C. Hence, glyphosate in water samples was not degraded after the collection of sample.

4.2.5. Summary

It was apparent that glyphosate was immobilized in top soil after the application. Part of glyphosate that was immobilized in top soil was mobilized to surface runoff and infiltrated water subsequent to the first precipitation event after application of glyphosate. Mobilization of glyphosate into water decreased with the subsequent precipitation events. It was derived that glyphosate present in pore water and adsorped to the soil organic matter was mobilized into water after the first three events of precipitation. Further, results showed that glyphosate could not be mobilized into water after leaching of glyphosate that was in pore water and adsorped to organic matter. However, application of TSP facilitated the mobilization of glyphosate that are adsorped to amorphous iron and aluminum oxides from soil to water due to the replacement of glyphosate by phosphate. Multiple application of TSP increased leaching of glyphosate from the soil. In conclusion, the risk of contamination of water sources by glyphosate is high in the event of first precipitation after the application of glyphosate and precipitation after the application of TSP. However, prior to application in actual field conditions glyphosate is dissolved in water that contains high hardness. Therefore, it is recommended to repeat the study by using glyphosate that is dissolved in hard water to simulate the actual field conditions and observe the effect of hardness on glyphosate immobilization in soil and mobilization to water.

4.3. Glyphosate (high purity and commercial grade) degradation pattern in deionized water and hard water

4.3.1. Glyphosate degradation in water

Degradation of Glyphosate (high purity and commercial grade) in deionized water and hard water over time is presented in Fig. 4.1. A higher rate of glyphosate degradation was observed in first 100 days and degradation rate of glyphosate was decreased and was plateauing after 150 days. The initial pH value of each sample was measured and the pH varied from 4.7 – 5.4.

When glyphosate is present in water, it could be degraded into AMPA by photodegradation and/or microbial degradation (Schuette, 1998). The hydrolysis half-life of glyphosate in average pH level and temperature, is reported as > 35 days (Kollman & Segawa, 1995). However, the initial concentration of glyphosate of all samples was 5 mg/L and after 240 days concentration of glyphosate (high purity) was (1) 3.71 mg/L in deionized water, (2) 3.72 mg/L in hard water and concentration of glyphosate (commercial grade) was (1) 3.75 mg/L in deionized water, (2) 3.81 mg/L in hard water. Therefore, it was apparent that glyphosate had not reached its hydrolysis half-life even after 240 days (Fig. 4.1). This observation indicates that glyphosate degradation in laboratory samples was limited compared to glyphosate degradation in water in the environment. This could be due to the factors such as (1) deionized water was used to prepare all the samples and presence of microorganisms in deionized water was minimal, thus microbial degradation of glyphosate was restricted, (2) glyphosate is photo-stable in pH 5 (Schuette, 1998), hence photodegradation of glyphosate in laboratory samples occurred at a low rate due to its photo-stability.

4.3.2. Effect of hardness on glyphosate degradation

When comparing glyphosate degradation in deionized water and hard water, it was evident that the rate of glyphosate degradation in deionized water was slightly higher than the rate of glyphosate degradation in hard water indicating that the presence of hardness in water can affect the rate of degradation of glyphosate in water (Fig. 4.1).

Thelen, et al., (1995) reported that when glyphosate is present in hard water, it forms stable complexes with Ca^{2+} and Mg^{2+} (Thelen, et al., 1995). When glyphosate has formed stable complexes with Ca^{2+} and Mg^{2+} , glyphosate is not no longer bio-available for microbial degradation (Borggaard & Gimsing, 2008). Therefore, Ca^{2+} and Mg^{2+} present in hard water could restrain the degradation of glyphosate in hard water.

4.3.3. Effect of presence of surfactants on degradation of glyphosate

Figure 4.1 indicates that the degradation rate of commercial grade glyphosate (Roundup®) was slightly lower than the degradation rate of high purity glyphosate. In commercial grade glyphosate (Roundup®) surfactants are present in the solution. The major surfactant present is POEA as discussed in the section 2.2.2. Ethylene-oxide present in POEA can form hydrogen bonds with the glyphosate molecule (Kuchikata, et al., 2001). Therefore, it can suggested that, microorganisms had to break through the hydrogen bond to reach to the glyphosate molecule. Thus, the presence of surfactants could restrict the degradation of commercial grade glyphosate.

4.3.4. Summary

The findings of the objective 3 derived that the degradation rate of glyphosate (high purity and commercial grade) in deionized water and hard water was at a faster rate in first 100 days. However, glyphosate did not reach its half-life within the 240 days. This could be because of the negligible level of microorganisms present in the sample and glyphosate was photo stable in the experimental pH range. Additionally, the glyphosate degradation rate was reduced in the hard water due to the formation of glyphosate-Ca and -Mg complexes. Further, the presence of surfactants in Roundup® had restricted the glyphosate degradation water. In conclusion, glyphosate degradation was hindered due to the presence of hardness in water and surfactants. Furthermore, it is recommended to conduct the study using water in CKDu prevalent areas, instead of laboratory formulated water, to observe the degradation of glyphosate in water in the environment.

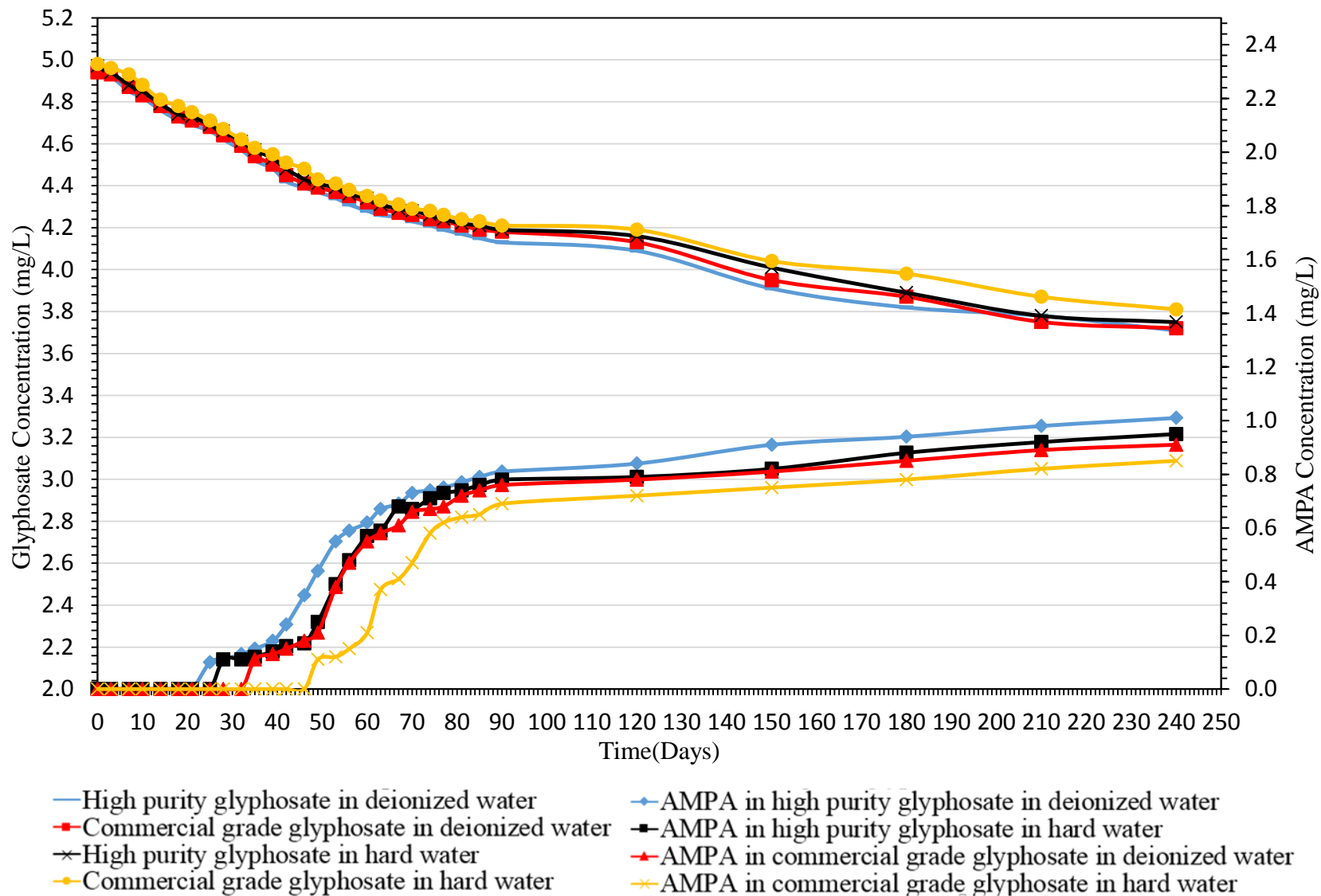


Figure 4.1 Glyphosate/ AMPA concentration vs. Time

4.4. Applicability of Ozonation process as an effective and efficient removal method of glyphosate and AMPA from water

4.4.1. Glyphosate degradation by Ozonation

Degradation of glyphosate (high purity) and Roundup® using Ozonation over time is presented in Fig.4.2 and Fig.4.3, respectively. A rapid decrease in glyphosate concentration was observed within the first ten minutes of reaction time in all experiments (Fig.4.2, Fig.4.3). During Ozonation, variation of the pH in water with glyphosate or Roundup ranged from 4.5-4.7 in the absence of hardness and 5.2-5.4 in the presence of hardness.

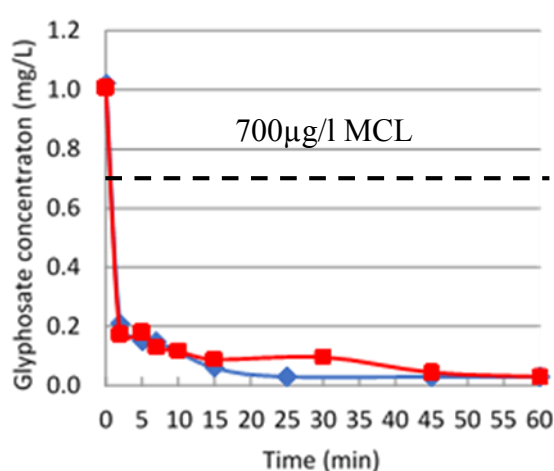


Figure 4.2 Degradation of glyphosate (high purity) using Ozonation (i) in the absence of hardness (◆) (ii) in the presence of hardness (■)

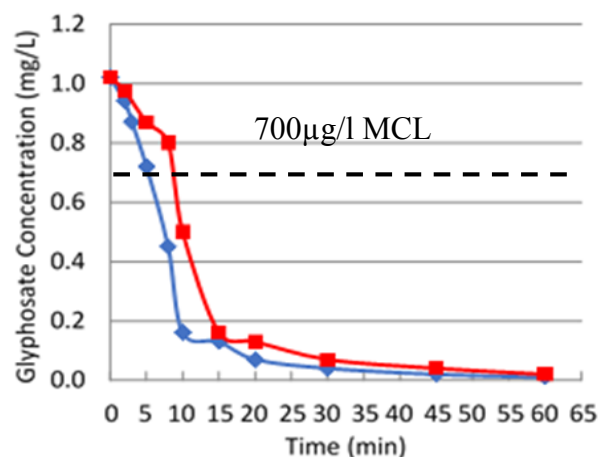


Figure 4.3 Degradation of Roundup® using Ozonation (i) in the absence of hardness (◆) (ii) in the presence of hardness (■)

Based on the literature, AMPA was the primary degradation product of Ozonation process (Assalin, et al., 2009). However, AMPA was not detected in all the experiments of the present study. Therefore, it can be suggested that, under all experimental conditions of the present study, complete degradation of glyphosate had occurred by Ozonation. In other words, the reactions were rapid and irreversible that inorganic phosphate is produced. The findings of this study are consistent with previously-reported research where complete degradation of glyphosate was achieved in the presence of high Ozone concentration (Jönsson, et al., 2013). In addition, Klinger et al., 1998 reported a complete degradation of glyphosate and accumulation of inorganic phosphate during the Ozonation reaction (Klinger et al., 1998).

4.4.2. Effect of hardness on glyphosate degradation by Ozonation

During Ozonation, when glyphosate (high purity) was present in water, 0.95 minutes (in the absence of hardness) and 1.00 minute (in the presence of hardness) were taken to reduce the glyphosate concentration to 700 µg/L, which is the MCL in drinking water specified by USEPA (Fig. 4.2). When Roundup® was used, the glyphosate concentration in water was reduced to 0.7 mg/L at the end of 5.00 and 7.00 minutes, in the absence and presence of hardness, respectively (Fig. 4.3). Therefore, the slower glyphosate degradation was observed in the presence of hardness compared to that in the absence of hardness (Figs. 4.2 and 4.3) indicating that hardness may be a triggering factor for glyphosate to be persistent in water. Glyphosate could form stable bonds with Ca^{2+} and Mg^{2+} when present in hard water (Thelen, et al., 1995). These glyphosate-Ca and -Mg complexes could enhance the persistence of glyphosate in water. Additionally, interference of Ca^{2+} and Mg^{2+} ions present in hard water could hinder the direct contact of Ozone with glyphosate adversely affecting the efficiency of Ozonation process.

4.4.3. Effect of presence of surfactants on degradation of glyphosate by Ozonation

The results showed that the time of Ozonation required for degradation of Roundup to reach the MCL is 5-7 times longer than the time required for glyphosate (high purity) alone to reach the MCL, both in the absence and presence of hardness. The presence of POEA delayed the Ozonation process indicating the persistence of glyphosate in water with the strong bond formation that hinders the degradation by Ozonation. Ethylene-oxide in POEA is known to form hydrogen bonds with glyphosate (Kuchikata, et al., 2001). Hence, during Ozonation, the oxygen and hydroxyl radicals would first require breaking through the hydrogen bonding in order to reach and react with the glyphosate molecules. Thus, the presence of POEA would lead to a retardation of glyphosate degradation by Ozonation, both in the absence and presence of hardness (Fig. 4.3).

4.4.4. Summary

This study investigated the suitability of Ozonation for degradation of glyphosate (high purity and Roundup®) present in potable water of CKDu prevalent areas containing high hardness levels and the effect of hardness on the efficiency of glyphosate degradation by Ozonation. Glyphosate (high purity) and Roundup® were rapidly degraded by Ozonation to levels lower than the USEPA MCL value (700 µg/l) within the first ten minutes of the reaction. Presence of hardness resulted in slower glyphosate degradation rates, suggesting enhanced persistence of possible glyphosate-Ca and -Mg complexes formed in water and interference of Ca²⁺ and Mg²⁺ ions present in water hindering direct contact of Ozone with glyphosate. The degradation rate of Roundup® was slower than that of high purity glyphosate, both in the absence and presence of hardness. The presence of the major adjuvant surfactant (POEA) in Roundup® enhanced the persistence of glyphosate in water and formation of hydrogen bonding of POEA with glyphosate could lead to a retardation of glyphosate degradation by Ozonation. In conclusion, it can be inferred that, despite the possible antagonistic effects imposed by the presence of hardness and POEA, Ozonation is a simple, affordable and promising technique that could be effectively and efficiently used to degrade glyphosate present in potable water from CKDu prevalent areas to levels that are of no significance.

5. CONCLUSIONS

5.1. Contributions from the study

The findings of the field studies revealed that glyphosate had high persistence in the environment in the CKDu prevalent areas as a result of its strong adsorption onto the soil matrix. The factors such as the presence of amorphous aluminum and iron oxides, favourable pH conditions and presence of organic matter in the soil matrix had promoted adsorption of glyphosate onto soil in the study area. The glyphosate detected in the water sources in the CKDu prevalent areas were lower than MCL of the USEPA (700 µg/L). Hence, impact on CKDu by the persistence of glyphosate and AMPA in the environment is remote when compared with the MCL.

The mesocosm studies derived that glyphosate had high affinity to adsorb onto soil tested. Therefore, immobilization of glyphosate in top soil was prominent. However, glyphosate was mobilized to both surface water and groundwater subsequent to the first precipitation event after glyphosate application. In addition, application of TSP to soil enhanced the mobilization of glyphosate from soil to water as inorganic phosphate in TSP competes with glyphosate for the same sorption sites in the soil matrix.

Glyphosate did not reach its half-life within the 240 days in the experimental conditions where photodegradation was dominant. It could be suggested that limited microorganisms were present in deionized water used in the experiment thus, microbial degradation of glyphosate was limited. Additionally, glyphosate was photo stable in the experimental pH in water resulting lower rate of photodegradation. Further, the rate of glyphosate degradation, when present in water, was decreased due to the presence of hardness in water and surfactants in the commercial grade glyphosate.

Ozonation process rapidly degraded the glyphosate (high purity and Roundup®) in the presence and absence of hardness. However, the presence of hardness in water and surfactants in commercial grade glyphosate resulted in a decrease in the rate of degradation of glyphosate using Ozonation process compared to degradation of glyphosate (high purity) in deionized water. Despite the effect of presence of hardness

and surfactants in water, Ozonation can be suggested as an effective and efficient technique that could be used to degrade glyphosate present in potable water from the CKDu prevalent areas.

In conclusion, immobilization of glyphosate in top soil was prominent. Therefore, the dominant mechanism of glyphosate retention in the environment in the CKDu prevalent areas is adsorption to the topsoil. Mobilization of glyphosate from soil to water was minimal and was promoted by the first precipitation event after the glyphosate application as well as application of TSP to the soil. In addition, Ozonation can be considered as an effective and efficient glyphosate removal method despite the presence of hardness in water and surfactant, thus shows a potential to be implemented as a water treatment technique in the CKDu prevalent areas.

5.2. Recommendations

It was revealed that, impact of persistence of glyphosate in the environment, on CKDu is low when compared with the MCL of USEPA. However, in-depth studies should be undertaken for better understanding of the persistence potential of glyphosate in water and soils, and its likelihood to cause higher rates of morbidity and mortality due to CKDu

In the mesocosm studies, glyphosate was dissolved in deionized water prior to the application. However, glyphosate is dissolved in hard water in the actual conditions in the field. Therefore, it is recommended to repeat the study by dissolving glyphosate/Roundup® in hard water to simulate the actual field conditions. The results obtained from the present study could be used as a control study for the recommended experiment.

Laboratory formulated water was used in the experimental setup to study the degradation pattern of glyphosate in water. However, glyphosate degradation rate was slower in the laboratory formulated water. Therefore, it is suggested to repeat the study using water in CKDu prevalent areas to observe the degradation of glyphosate in water in the environment. The degradation pattern obtain from the present study could be used as a basis to interpret the results.

In the study conducted to comprehend the applicability of Ozonation process as an effective and efficient removal method of glyphosate and AMPA from water in CKDu prevalent areas, only one glyphosate concentration and hardness level was considered. However, effectiveness and efficiency of Ozonation process could be affected by the different pH, hardness and glyphosate levels in the water. Therefore, it is recommended to conduct the study for different pH, hardness and glyphosate levels in water.

REFERENCE LIST

- Andréa, M. M. d., Peres, T. B., Luchini, L. C., Bazarin, S., Papini, S., Matallo, M. B., et al. (2003). Influence of repeated applications of glyphosate on its persistence and soil bioactivity. *Pesquisa Agropecuaria Brasileira*, 38(11), 1329-1335.
- Annett, R., Habibi, H. R., & Hontela, A. (2014). Impact of glyphosate and glyphosate-based herbicides on the freshwater environment. *Journal of Applied Toxicology*, 34(5), 458-479.
- Aparicio, V. C., De Gerónimo, E., Marino, D., Primost, J., Carriquiriborde, P., & Costa, J. L. (2013). Environmental fate of glyphosate and aminomethylphosphonic acid in surface waters and soil of agricultural basins. *Chemosphere*, 93(9), 1866-1873.
- APHA. (2012). *Standard Methods for examination of water and wastewater* (22 ed.): American Public Health Association, American Water works Association, Water Environment Federation.
- Assalin, M. R., De Moraes, S. G., Queiroz, S. C. N., Ferracini, V. L., & Duran, N. (2009). Studies on degradation of glyphosate by several oxidative chemical processes: Ozonation, photolysis and heterogeneous photocatalysis. *Journal of Environmental Science and Health, Part B*, 45(1), 89-94.
- ASTM D2487-11, A. (2011). Standard Practice for Classification of Soils for Engineering Purposes (Unified Soil Classification System). West Conshohocken: ASTM International.
- ASTM D4318-17, A. (2017). Standard Test Methods for Liquid Limit, Plastic Limit, and Plasticity Index of Soils. West Conshohocken: ASTM International.
- ASTM D7928-17, A. (2017). Standard Test Method for Particle-Size Distribution (Gradation) of Fine-Grained Soils Using the Sedimentation (Hydrometer) Analysis. West Conshohocken: ASTM International.
- Ayoola, S. O. (2008). Histopathological effect of glyphosate on Juvenile African Catfish(*Clarias gariepinus*). *Am Eurasian J Agric Environ Sci*, 4.
- Bandarage, A. (2013). Political Economy of Epidemic Kidney Disease in Sri Lanka. . *Sage Journal* , 3-4.
- Battaglin, W., Meyer, M., Kuivila, K., & Dietze, J. (2014). Glyphosate and its degradation product AMPA occur frequently and widely in US soils, surface

- water, groundwater, and precipitation. *Journal of the American Water Resources Association*, 50(2), 275-290.
- Borggaard, O. K., & Gimsing, A. L. (2008). Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. *Pest Management Science*, 64(4), 441-456.
- Bourgeois, A., Klinkhamer, E., & Price, J. (2012). *Pesticide Removal from Water* Worcester, Massachusetts Worcester Polytechnic Institute
- Coupe, R. H., Kalkhoff, S. J., Capel, P. D., & Gregoire, C. (2012). Fate and transport of glyphosate and aminomethylphosphonic acid in surface waters of agricultural basins. *Pest Management Science*, 68(1), 16-30.
- Daouk, S., De Alencastro, L. F., & Pfeifer, H.-R. (2013). The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, western Switzerland: Proof of widespread export to surface waters. Part II: The role of infiltration and surface runoff. *Journal of Environmental Science and Health, Part B*, 48(9), 725-736.
- de Jonge, H., de Jonge, L. W., Jacobsen, O. H., Yamaguchi, T., & Moldrup, P. (2001). Glyphosate sorption in soils of different ph and phosphorus content. *Soil Science*, 166(4), 230-238.
- Dharma-wardana, M. W. C., Amarasiri, S. L., Dharmawardene, N., & Panabokke, C. R. (2014). Chronic kidney disease of unknown aetiology and ground-water ionicity: study based on Sri Lanka. [journal article]. *Environmental Geochemistry and Health*, 37(2), 221-231.
- Dion, H. M., Harsh, J. B., & Hill, H. H. (2001). Competitive sorption between glyphosate and inorganic phosphate on clay minerals and low organic matter soils. *Journal of Radioanalytical and Nuclear Chemistry* 249(2), 385–390.
- Elrashidi, M. (2010). Selection of an appropriate phosphorus test for soils. *USDA NRCS, Lincoln, NE*.
- Fomsgaard, I. S., Spliid, N. H. H., & Felding, G. (2003). Leaching of Pesticides Through Normal-Tillage and Low-Tillage Soil—A Lysimeter Study. II. Glyphosate. *Journal of Environmental Science and Health, Part B*, 38(1), 19-35.
- Gagliardo, P., Adham, S., Trussell, R., & Olivieri, A. (1998). Water repurification via reverse osmosis. *Desalination*, 117(1-3), 73-78.

- Gavrilescu, M. (2005). Fate of pesticides in the environment and its bioremediation. *Engineering in Life Sciences*, 5(6), 497-526.
- Gerritse, R., Beltran, J., & Hernandez, F. (1996). Adsorption of atrazine, simazine, and glyphosate in soils of the Gnangara Mound, Western Australia. *Soil Research*, 34(4), 599-607.
- Gimsing, A. L., & Borggaard, O. K. (2002). Competitive adsorption and desorption of glyphosate and phosphate on clay silicates and oxides. *Clay Minerals*, 37(3), 509-515.
- Gimsing, A. L., Borggaard, O. K., & Bang, M. (2004). Influence of soil composition on adsorption of glyphosate and phosphate by contrasting Danish surface soils. *European Journal of Soil Science*, 55(1), 183-191.
- Glass, R. L. (1987). Adsorption of glyphosate by soils and clay minerals. *Journal of Agricultural and Food Chemistry*, 35(4), 497-500.
- Grant, S., Mortimer, M., Stevenson, G., Malcolm, D., & Gaus, C. (2010). Facilitated transport of dioxins in soil following unintentional release of pesticide-surfactant formulations. *Environmental science & technology*, 45(2), 406-411.
- Gunten, U. v. (2003). Ozonation of drinking water: Part I. Oxidation kinetics and product formation. *Water Research*, 37(7), 1443-1467.
- Haigh, S. D. (1996). A review of the interaction of surfactants with organic contaminants in soil. *Science of the Total Environment*, 185(1-3), 161-170.
- Herath, I., Kumarathilaka, P., Al-Wabel, M. I., Abduljabbar, A., Ahmad, M., Usman, A. R., et al. (2016). Mechanistic modeling of glyphosate interaction with rice husk derived engineered biochar. Microporous and mesoporous materials, 225, 280-288.
- Herrmann, K. M., & Weaver, L. M. (1999). The shikimate pathway. *Annual review of plant biology*, 50(1), 473-503.
- Illeperuma, O., Dharmagunawardhane, H., & Herarh, K. (2009). Dissolution of aluminum from substandard utensils under high fluoride stress: A possible risk factors for chronic renal failures in the North-Central Province. *Journal of the National Science Foundation of Sri Lanka*, 37(3), 219-222.
- Jayasumana, C., Gunatilake, S., & Senanayake, P. (2014). Glyphosate, hard water and nephrotoxic metals: are they the culprits behind the epidemic of chronic kidney disease of unknown etiology in Sri Lanka? *International journal of environmental research and public health*, 11(2), 2125-2147.

- Jayasumana, C., Paranagama, P., Agampodi, S., Wijewardane, C., Gunatilake, S., & Siribaddana, S. (2015). Drinking well water and occupational exposure to Herbicides is associated with chronic kidney disease, in Padavi-Sripura, Sri Lanka. [journal article]. *Environmental Health*, 14(1), 6.
- Jayatilake, N., Mendis, S., & Maheepala, P. M. (2013). Chronic kidney disease of uncertain aetiology: prevalence and causative factors in a developing country. *BMC Nephrol* 14:180.
- Jiraungkoorskul, W., Upatham, E. S., Kruatrachue, M., Sahaphong, S., Vichasri-Grams, S., & Pokethitiyook, P. (2003). Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*). *Environ Toxicol*, 18.
- Jönsson, J., Camm, R., & Hall, T. (2013). Removal and degradation of glyphosate in water treatment: a review. *Journal of Water Supply: Research and Technology*, 62 (7), 395-408.
- Katagi, T. (2008). Surfactant effects on environmental behavior of pesticides. *Reviews of environmental contamination and toxicology*, 194, 71.
- Kjær, J., Ernstsén, V., Jacobsen, O. H., Hansen, N., de Jonge, L. W., & Olsen, P. (2011). Transport modes and pathways of the strongly sorbing pesticides glyphosate and pendimethalin through structured drained soils. *Chemosphere*, 84(4), 471-479.
- Kjær, J., Olsen, P., Barlebo, H. C., Juhler, R. K., Plauborg, F., Grant, R., et al. (2004). *The Danish pesticide leaching assessment programme: monitoring results 1999–2003*, Copenhagen: Geological Survey of Denmark and Greenland
- Klinger, J., Lang, M., Sacher, F., Brauch, H. J., Maier, D., & Worch, E. (1998). Formation of Glyphosate and AMPA During Ozonation of Waters Containing Ethylenediaminetetra(methylenephosphonic acid). *Ozone: Science & Engineering*, 20(2), 99-110.
- Kollman, W., & Segawa, R. (1995). Interim report of the pesticide chemistry database—Environmental Hazards Assessment Program. *California Department of Pesticide Regulation, Sacramento (USA)*.
- Knuutila, P., & Knuutila, H. (1979). The crystal and molecular structure of N-(phosphonomethyl) glycine (glyphosate). *Acta Chemica Scandinavica*, 33, 623-626.

- Kryuchkova, Y. V., Burygin, G. L., Gogoleva, N. E., Gogolev, Y. V., Chernyshova, M. P., Makarov, O. E., et al. (2014). Isolation and characterization of a glyphosate-degrading rhizosphere strain, *Enterobacter cloacae* K7. *Microbiological research*, *169*(1), 99-105.
- Kuchikata, M., Prill, E. J., Richardson, R. O., Sato, T., Surgant, J. M., & Wright, D. R. (2001). Glyphosate formulations: Google Patents.
- Kumar, R. S. (2002). Aral Sea: Tragedy Environmental in Central Asia. *Econ Polit Wkly*, *37*.
- Lund-Høie, K., & Friestad, H. O. (1986). Photodegradation of the herbicide glyphosate in water. *Bulletin of Environmental Contamination and Toxicology*, *36*(1), 723-729.
- Major 3rd, W. W., Grue, C. E., Gardner, S. C., & Grassley, J. M. (2003). Concentrations of Glyphosate and AMPA in Sediment Following Operational Applications of Rodeo® to Control Smooth Cordgrass in Willapa Bay, Washington, USA. *Bulletin of Environmental Contamination and Toxicology*, *71*(5), 912-918.
- Mamy, L., & Barriuso, E. (2005). Glyphosate adsorption in soils compared to herbicides replaced with the introduction of glyphosate resistant crops. *Chemosphere*, *61*(6), 844-855.
- Manual, A. M. (1984). Method 84-011, *Land Resource Research Institute: Agriculture, Canada*.
- Mesnage, R., Bernay, B., & Séralini, G. E. (2013). Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. *Toxicology*, *313*(2-3), 122-128.
- Morillo, E., Undabeytia, T., Maqueda, C., & Ramos, A. (2000). Glyphosate adsorption on soils of different characteristics.: Influence of copper addition. *Chemosphere*, *40*(1), 103-107.
- Munira, S., Farenhorst, A., Flaten, D., & Grant, C. (2016). Phosphate fertilizer impacts on glyphosate sorption by soil. *Chemosphere*, *153*, 471-477.
- Nash, D. M., & Halliwell, D. J. (1999). Fertilisers and phosphorus loss from productive grazing systems. *Soil Research*, *37*(3), 403-430.
- Noble, A., Amerasinghe, P., Manthirithilake, H., & Arasalingam, S. (2014). *Review of literature on chronic kidney disease of unknown etiology (CKDu) in Sri Lanka*.

- (IWMI Working Paper 158): Colombo, Sri Lanka: International Water Management Institute (IWMI).
- Norgaard, T., Moldrup, P., Ferré, T. P. A., Olsen, P., Rosenbom, A. E., & de Jonge, L. W. (2014). Leaching of Glyphosate and Aminomethylphosphonic Acid from an Agricultural Field over a Twelve-Year Period. *Vadose Zone Journal*, 13(10).
- Ololade, I., Oladoja, N., Oloye, F., Alomaja, F., Akerele, D., Iwaye, J., et al. (2014). Sorption of glyphosate on soil components: the roles of metal oxides and organic materials. *Soil and Sediment Contamination: An International Journal*, 23(5), 571-585.
- Peruzzo, P. J., Porta, A. A., & Ronco, A. E. (2008). Levels of glyphosate in surface waters, sediments and soils associated with direct sowing soybean cultivation in north pampasic region of Argentina. *Environmental Pollution*, 156(1), 61-66.
- Piccolo, A., Celano, G., Arienzo, M., & Mirabella, A. (1994). Adsorption and desorption of glyphosate in some European soils. *Journal of Environmental Science and Health, Part B*, 29(6), 1105-1115.
- Pollegioni, L., Schonbrunn, E., & Siehl, D. (2011). Molecular basis of glyphosate resistance—different approaches through protein engineering. *The FEBS journal*, 278(16), 2753-2766.
- Prata, F., Cardinali, V. C. d. B., Lavorenti, A., Tornisielo, V. L., & Regitano, J. B. (2003). Glyphosate sorption and desorption in soils with distinct phosphorus levels. *Scientia Agricola*, 60(1), 175-180.
- Rasmussen, S. B., Abrahamsen, P., Nielsen, M. H., Holm, P. E., & Hansen, S. (2015). Effects of Single Rainfall Events on Leaching of Glyphosate and Bentazone on Two Different Soil Types, using the DAISY Model. *Vadose Zone Journal*, 14(11).
- Roy, D. N., Konar, S. K., Banerjee, S., Charles, D. A., Thompson, D. G., & Prasad, R. (1989). Persistence, movement, and degradation of glyphosate in selected Canadian boreal forest soils. *Journal of Agricultural and Food Chemistry*, 37(2), 437-440.
- Rueppel, M. L., Brightwell, B. B., Schaefer, J., & Marvel, J. T. (1977). Metabolism and degradation of glyphosate in soil and water. *Journal of Agricultural and Food Chemistry*, 25(3), 517-528.

- Saitúa, H., Giannini, F., & Padilla, A. P. (2012). Drinking water obtaining by nanofiltration from waters contaminated with glyphosate formulations: process evaluation by means of toxicity tests and studies on operating parameters. *Journal of hazardous materials*, 227, 204-210.
- Schuette, J. (1998). *Environmental fate of glyphosate*. Sacramento, : Department of Pesticide Regulation.
- Simonsen, L., Fomsgaard, I. S., Svensmark, B., & Spliid, N. H. (2008). Fate and availability of glyphosate and AMPA in agricultural soil. *Journal of Environmental Science and Health, Part B*, 43(5), 365-375.
- Steinrücken, H., & Amrhein, N. (1980). The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic acid-3-phosphate synthase. *Biochemical and biophysical research communications*, 94(4), 1207-1212.
- Sviridov, A. V., Shushkova, T. V., Ermakova, I. T., Ivanova, E. V., Epiktetov, D. O., & Leontievsky, A. A. (2015). Microbial degradation of glyphosate herbicides (Review). [journal article]. *Applied Biochemistry and Microbiology*, 51(2), 188-195.
- Tévez, H. R., & dos Santos Afonso, M. (2015). pH dependence of Glyphosate adsorption on soil horizons. *Boletín de la Sociedad Geológica Mexicana*, 67(3).
- Thelen, K., D, Evelyn, P. J., & Donald, P. (1995). The Basis for the Hard-Water Antagonism of Glyphosate Activity. *Weed Science*, 43(4), 541-548.
- Tsui, M. T. K., & Chu, L. M. (2008). Environmental fate and non-target impact of glyphosate-based herbicide (Roundup®) in a subtropical wetland. *Chemosphere*, 71(3), 439-446.
- Tsui, M. T. K., Wang, W.-X., & Chu, L. M. (2005). Influence of glyphosate and its formulation (Roundup®) on the toxicity and bioavailability of metals to *Ceriodaphnia dubia*. *Environmental Pollution*, 138(1), 59-68.
- USEPA. (1986). Method 9080: Cation-Exchange Capacity of Soils (Ammonium Acetate), part of Test Methods for Evaluating Solid Waste, Physical/Chemical Methods
- USEPA. (1992). *Guidelines for Exposure Assessment*. Risk Assessment Forum, Washington, DC, EPA/600/Z-92/001
- USEPA. (1993). *Registration Eligibility Decision- Glyphosate*: United States Environmental Protection Agency., EPA - 738- R- 93- 014

- USEPA. (1995). National Primary Drinking Water Regulations, *Glyphosate*.
- Van Stempvoort, D. R., Spoelstra, J., Senger, N. D., Brown, S. J., Post, R., & Struger, J. (2016). Glyphosate residues in rural groundwater, Nottawasaga River Watershed, Ontario, Canada. *Pest Management Science*, 72(10), 1862-1872.
- Vereecken, H. (2005). Mobility and leaching of glyphosate: a review. *Pest Management Science*, 61(12), 1139-1151.
- Walky, A., & Black, I. (1934). An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid in soil analysis. 1. Experimental. *Soil Sci*, 79, 459-465.
- Wanigasuriya, K. (2012). Aetiological factors of Chronic Kidney Disease in the North Central Province of Sri Lanka: A review of evidence to-date. *Journal of the College of Community Physicians of Sri Lanka*, 17(1), 21-42.
- WHO. (1983). *Measuring change in nutritional status; Guidelines for Assessing the Nutritional Impact of Supplementray Feeding programmes for Vulnerable Groups*.
- WHO. (2013). *Investigation and evaluation of Chronic Kidney Disease of uncertain aetiology in Sri Lanka*: WHO.
- Zhou, D. M., Wang, Y. J., Cang, L., Hao, X. Z., & Luo, X. S. (2004). Adsorption and cosorption of cadmium and glyphosate on two soils with different characteristics. *Chemosphere*, 57(10), 1237-1244.