

Use of Ozonation For Degradation of Glyphosate in Potable Water of CKDu Prevalent Areas

PP Jayasekara¹, S Gunarathna², B Gunawardana^{3#}, M Jayaweera⁴, J Manatunge⁵ and K Zoysa⁶
^{1 2 3 4 5 6}Department of Civil Engineering, University of Moratuwa, Sri Lanka

[#] Corresponding author; <buddhi75@yahoo.com>

Abstract— Consumption of water contaminated with agrochemicals is implicated to be one of the triggering causes of the Chronic Kidney Disease of unknown etiology (CKDu). Glyphosate, which is commercially available as Roundup®, was the widely used herbicide in Sri Lanka until recently hence, it has still been persistent in the environment. Recent studies provide evidences of the presence of Glyphosate in trace levels in urine samples of CKDu patients and also in different water sources in the CKDu prevalent areas containing elevated hardness levels. Thus, Glyphosate is suspected to be a causal factor for CKDu and its removal, when present in potable water, is a challenging engineering task. Ozonation is recognised to be one of the promising technologies for the degradation of Glyphosate. This study focuses on investigating the effectiveness of Ozonation for degradation of Glyphosate from potable waters and the effect of hardness on the efficiency of Glyphosate degradation by Ozonation. Experiments were conducted with high purity Glyphosate and Roundup® in the absence and presence of hardness (1,500 mg/L as CaCO₃). A 10 L water sample with Glyphosate/Roundup® (1 mg/L) was continuously ozonised (200 mg/h) and samples from the reaction solution were analysed for Glyphosate and Aminomethylphosphonic acid (AMPA) using GC/MS and LC/MS. A rapid decrease in Glyphosate/Roundup® concentration was observed within first ten minutes of reaction time. Presence of hardness resulted in slower Glyphosate degradation rate compared to absence of hardness, which could be attributed to formation of Glyphosate-Ca and -Mg complexes hindering direct contact of Ozone with Glyphosate. Degradation rate of Roundup® by Ozonation was slower than high purity Glyphosate, in the absence and presence of hardness. Presence of the surfactant [polyethoxylated tallowamine, (POEA)] in Roundup® enhanced the persistence of Glyphosate in water thus causing retardation of Glyphosate degradation by Ozonation. However, AMPA was not detected demonstrating complete degradation of Glyphosate by Ozonation.

In conclusion, it can be inferred that Ozonation is a simple, affordable and promising technique that could be effectively and efficiently used to degrade Glyphosate present in potable water of CKDu prevalent areas.

Keywords— CKDu, Glyphosate, Ozonation

I. INTRODUCTION

Chronic Kidney Disease of unknown etiology (CKDu) is a critical health issue prevailing in some parts of Sri Lanka (e.g. North Central Province) for past two decades. The exact causal factor(s) of CKDu have not yet been identified. Since majority of the reported CKDu victims is from the farming communities and also above age of 40 years, agrochemical contamination of water had been suggested as a main triggering issue of CKDu (Wanigasuriya, 2012). 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 into body due to careless handling of agro-chemicals 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 received 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] 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. Preliminary field visits conducted in the CKDu prevalent areas provided evidence that Roundup® is still being used in some of the Chena 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 (Jayasumana et al., 2015). Recent studies provide evidence of presence of Glyphosate in trace levels in water sources (drinking water, surface water, and groundwater) in the CKDu affected areas that contain elevated hardness levels. 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 was 3.5% (WHO, 2013).

Glyphosate molecule consists of three major functional groups; phosphonic group, amino group and carboxylic group. The major metabolite of Glyphosate is Aminomethylphosphonic acid (AMPA). Potable waters in CKDu prevalent areas contain high hardness levels (e.g. 1500 mg/L as CaCO₃) particularly in dry seasons. It has

been implicated that Glyphosate has the tendency to form stable and persistent complex compounds with Ca^{2+} and Mg^{2+} when present in hard water (Thelen et al., 1995). Further, Glyphosate has the ability to capture various metals such as Arsenic, Copper and other nephrotoxic metals and act as a carrier compound to deliver these metals to the human body and organs, eventually causing damages to the kidney and its renal functions (Jayasumana et al., 2014). Glyphosate is the active ingredient in *Roundup*[®]. However, *Roundup*[®] also contains a major adjuvant surfactant known as polyethoxylated tallowamine, (POEA), introduced as an inert ingredient during *Roundup*[®] production process to enhance the bio-efficacy of the herbicide. Presence of POEA with Glyphosate has shown to be cytotoxic (toxic to cells) at doses lower than Glyphosate alone (Mesnage et al., 2013). According to USEPA guidelines, the maximum contamination limit (MCL) allowed in drinking water for Glyphosate is 0.7 mg/L (EPA, 1995). Therefore, Glyphosate is suspected as a causal factor for CKDu and degradation of Glyphosate, if present in potable waters, is a challenging engineering task.

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 (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). However, it can be hypothesized that the presence of POEA in *Roundup*[®] could hinder the efficiency of Ozonation process as the Ethylene-oxide present in POEA tends to form hydrogen bonds with Glyphosate enhancing the stability of Glyphosate in water (Kuchikata et al., 2001). Also, the presence of high hardness levels in potable waters in CKDu prevalent areas could hinder the Glyphosate degradation process by Ozonation due to possible formation of Glyphosate-Metal complexes. Studies on the effects of POEA and hardness on efficiency of Ozonation process for degradation of Glyphosate have not been reported in the literature. Therefore, this study focused on investigating (1) the suitability of Ozonation for degradation of Glyphosate (high purity) and *Roundup*[®] containing POEA when present in potable water with high hardness levels of CKDu prevalent areas, and, (2) the effect of presence of hardness on the efficiency of Glyphosate degradation by Ozonation.

II. METHODOLOGY

A. 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 1 mg/L of Glyphosate and 1 mg/L *Roundup*[®] were prepared by diluting the respective stock solutions using deionized water.

Experiments were conducted in the absence and presence of hardness. Hardness concentration used was 1,500 mg/L as CaCO_3 to simulate the extreme dry weather conditions, which could occur in the CKDu prevalent areas. Confirmation of hardness levels in water samples were carried according to the EDTA Titrimetric method 2340 C.

B. Experimental Setup

Ozonation probe, while continuously releasing Ozone (200 mg/h), was immersed in the 10 L water sample containing Glyphosate or *Roundup*[®] in the absence and presence of hardness. 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. All experiments were conducted under the ambient conditions.

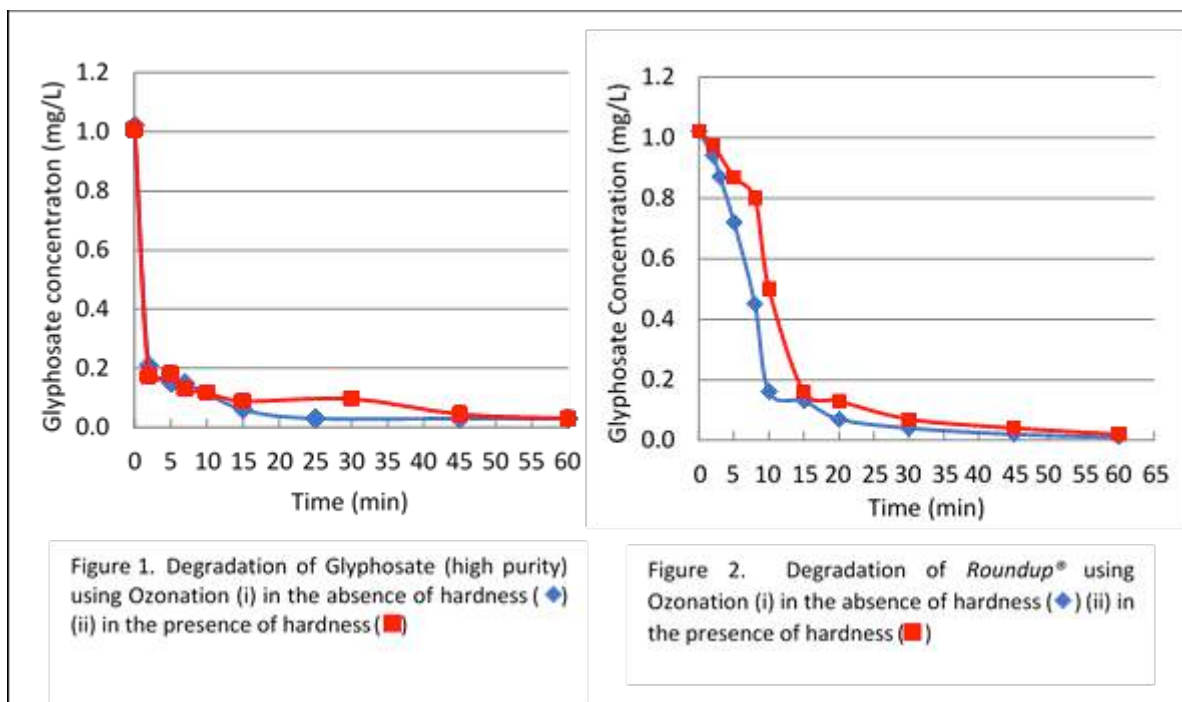
C. Analysis of Glyphosate and AMPA

Glyphosate and AMPA concentrations of water samples without hardness were analysed 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. Water samples consisted of hardness were analysed using the Liquid Chromatography/Mass Spectrometry (LC/MS) Single Quad (Agilent 1200 Infinity series LC with G1600 series Mass Selective Detector) coupled with Zorbax Eclipse XDB- C18 column. Samples for LC/MS analyses were derivatized using 10 mg/l 9-fluorenylmethyl chloroformate.

III. RESULTS AND DISCUSSION

A. Glyphosate degradation by Ozonation

Degradation of Glyphosate (high purity) and *Roundup*[®] using Ozonation over time is presented in Fig.1 and Fig.2, respectively. A rapid decrease in Glyphosate concentration was observed within first ten minutes of reaction time in all experiments (Fig.1, Fig.2). 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.



Based on the literature, amino group is the primary reaction site of Glyphosate to react with Ozone (Assalin et al., 2009). During Ozonation, oxidation can occur as direct oxidation by Ozone or indirect oxidation by hydroxyl radicals (Gunten, 2003). Thus, Ozone and hydroxyl radicals are known to react with the amino group of Glyphosate to form AMPA (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 previous-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).

B. 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 0.7 mg/L, which is the MCL in drinking water specified by USEPA (Fig. 1). When Roundup® was used, 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.2). Therefore, slower Glyphosate degradation was observed in the presence of hardness compared to that in the absence of hardness (Figs. 1 and 2) indicating that hardness may be a triggering factor for Glyphosate to be persistent in water. Glyphosate could form stable bonds with Ca²⁺ and Mg²⁺ when present in hard water (Thelen et al., 1995); Nuclear

Magnetic Resonance (NMR) studies have provided evidence of formation of stable bonds of Ca²⁺ and Mg²⁺ with both phosphonic and carboxylic groups of Glyphosate by replacing H⁺ ions in these functional groups (Thelen et al., 1995). These Glyphosate-Ca and -Mg complexes could enhance the persistence of Glyphosate in water. Additionally, interference of Ca²⁺ and Mg²⁺ ions present in hard water could hinder the direct contact of Ozone with Glyphosate adversely affecting the efficiency of Ozonation process.

C. 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 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. 2).

D. Rate constant

Reaction of Ozone with high purity Glyphosate and Roundup®, in the absence and presence of hardness, followed the second order kinetic behaviour. Figure 3 presents the second order kinetic behaviour of high purity Glyphosate and Roundup®, in the absence and presence of hardness. Rate constants obtained for the high purity Glyphosate were 1.29 m⁻¹ and 0.68 m⁻¹ in the absence and presence of hardness respectively. As the data clearly demonstrate the rate constant of Glyphosate degradation using Ozonation was slower in the presence of hardness confirming enhanced persistence of Glyphosate due to hardness in water. The rate constants obtained for Roundup® were 1.13 m⁻¹ and 0.53 m⁻¹ in the absence and presence of hardness respectively.

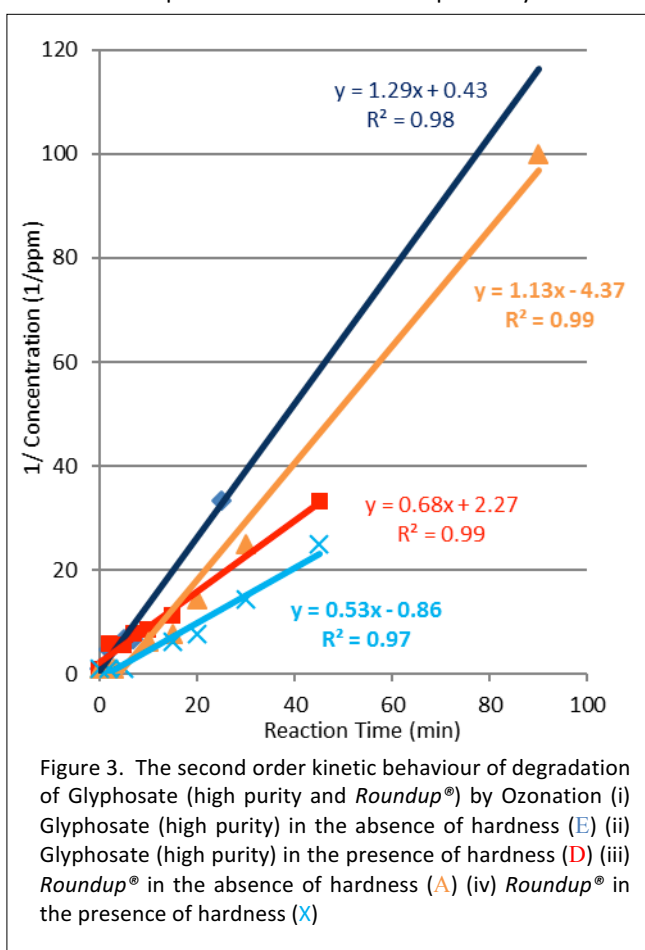


Figure 3. The second order kinetic behaviour of degradation of Glyphosate (high purity and Roundup®) by Ozonation (i) Glyphosate (high purity) in the absence of hardness (E) (ii) Glyphosate (high purity) in the presence of hardness (D) (iii) Roundup® in the absence of hardness (A) (iv) Roundup® in the presence of hardness (X)

Comparison of the rate constants obtained for high purity Glyphosate and for Roundup® in the absence of hardness clearly shows retardation of Glyphosate degradation due to the presence of surfactants in Roundup®. Among the various experimental conditions studied, the slowest rate constant was observed with Roundup® in the presence of hardness. This observation further confirms the combined antagonistic effect imposed on Glyphosate degradation by Ozonation due to the simultaneous presence of hardness and the surfactants.

IV. CONCLUSION

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 (0.7 mg/l) within 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. Degradation rate of Roundup® was slower than that of high purity Glyphosate, both in the absence and presence of hardness. 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 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 of CKDu prevalent areas to levels that are of no significance.

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