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The Effect of pH, Dissolved Metals and Suspended Minerals on the Hydrolysis of Neonicotinoids

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Abstract

Neonicotinoids are a chemical class of insecticides that is registered in more than 120 countries and widely used all over the world. In recent years, neonicotinoids have been in the spotlight for their negative effects on non-target organisms like pollinating insects and other important ecosystem service providers. Neonicotinoid pollution of aquatic systems has been found in several countries, and under the right conditions, they have been found to accumulate in soils. To better understand the environmental fate and degradation pathways of this important insecticide class, hydrolysis experiments has been performed on two neonicotinoid compounds: imidacloprid and acetamiprid. The objective of the thesis has been to investigate the effects of pH, dissolved metal ions and suspended minerals on hydrolysis rates. The experiments were regularly monitored using HPLC analysis. Both neonicotinoids were found to be persistent in the pH-range 4.0-8.0, in the absence and presence of metal ions and minerals. No degradation was detected during the experimental period. At pH 10.0, both neonicotinoids degraded significantly. Half-lives were found to be 27 and 40 days for acetamiprid and imidacloprid respectively. In the presence of dissolved Cu^{II}, Ni^{II}, and Zn^{II}, and suspended goethite, kaolinite, and TiO_2, the hydrolysis at pH 10 was inhibited, increasing the half-lives with up to 35%.

Sammendrag

Neonikotinoider er en gruppe kjemiske plantevernmidler som er registret i mer enn 120 land, og brukes i stor skala i internasjonal sammenheng. De siste årene har neonikotinoider kommet i søkelyset på grunn forskningsresultater som viser negative effekter av sprøytemiddelbruken på en rekke nytteinsekter, blant annet pollinerende insekter. I matjorda kan neonikotinoider akkumulere over tid, og det har også blitt påvist konsentrasjoner som overstiger grensenivåene i vannforekomster i flere land. Hovedmålet til denne masteroppgaven er å bidra til kartleggingen av hvordan neonikotinoider brytes ned i naturen ved å studere hydrolytisk nedbryting ved ulike pH, og i nærvær og fravær av ulike løste metaller og suspenderte mineraler. Neonikotinoidene som har blitt studert er imidacloprid og acetamiprid. Eksperimentene har blitt utført ved bruk av HPLC analyse. Resultatene viste ingen målbar nedbrytning av imidacloprid og acetamiprid ved pH 4.0 – 8.0. Ved pH 10 hydrolyserte begge kjemikaliene. Halveringstiden ble målt til 27 dager for acetamiprid og 40 dager for imidacloprid. Løste metaller og suspenderte mineraler forlenget nedbrytningstiden opp til 35% ved pH 10. Metallene undersøkt i denne oppaven er Cu^{II}, Ni^{II}, og Zn^{II} , og mineralene er goethitt, kaolinitt og TiO_2.

Preface

This thesis represents the fulfillment of the Master of Science programme in Civil and Environmental Engineering at the Norwegian University of Science and Technology (NTNU). The work has been conducted at the Department of Civil, Environmental, and Geo- Engineering at the University of Minnesota (UMN), Minneapolis, and the Department of Hydraulic and Environmental Engineering at NTNU, Trondheim.

I would like to show my gratitude towards those who have helped and advised me through this process; my supervisor Associate Professor Cynthia Hallé (NTNU) and co-supervisor at Professor William Arnold (UMN) for all the valuable input and encouragement throughout this semester, and for suggesting this project for me.

Special thanks to Ph.D. Students at UMN Andy McCabe and Jill Kerrigan for taking their time helping out in the lab and teaching me how to use the HPLC. Thanks to Grant Wallace at UMN for providing me with background information on the topic, and to Kipp Sande at UMN for helping me measure dissolved metal concentrations with the FAAS.

The laboratory work for this thesis was conducted in Minneapolis, USA, where I spent five months. I would therefore also like to thank the wonderful people I met who made my time in the US memorable. Special thanks to Lucio Salles for making my office-space feel like home, and to Emily and Jonathan Anderson for helping me out with everything practical and treating my like family.

So long, and thanks for all the fish.

Trondheim 19th of July

Table of Contents

List of Figures

List of Tables

Abbreviations

BH	Baseline hydrolysis
FAAS	Flame Atomic Adsorption Spectrography
HPLC	High-Pressure Liquid Chromatography
PHP	Phenyl Picolinate
NTNU	Norwegian University of Technology and Science
UMN	University of Minnesota

1 Introduction

1.1 Motivation: Use and safety of neonicotinoids

Neonicotinoids are a chemical class of insecticides, registered for use in more than 120 countries. They are used for plant protection within agriculture and floriculture, pest control against insects like cockroaches and termites, parasite treatment of animals, and within fish farming (Jeschke et al., 2011, Simon-Delso et al., 2014). The first commercially available compound of the neonicotinoid family, imidacloprid, was introduced to the market in 1991. In 2008, neonicotinoids had a 24% share of the total insecticide market. This has made them one of the largest and fastest growing insecticide classes in modern pest management (Jeschke et al., 2011, Jeschke and Nauen, 2008, Nauen and Bretschneider, 2002).

The commercial success of neonicotinoids is attributed to their ability to work against a broad spectrum of insect pests, together with their versatility in application methods and low application rates (Jeschke and Nauen, 2008, Wollweber and Tietjen, 1999). These advantages are closely linked to their *systemic activity*, which makes them able to absorb into the targeted (and non-targeted) plants, making the entire plant toxic (Goulson, 2013). The most popular application methods for neonicotinoids are soil and seed treatments (Jeschke et al., 2011). Studies have shown that the plant uptake from soil and seed treatments varies between 1.6-20% (Sur and Stork, 2003), leaving the remaining 80-98.4 % of the insecticide in the environment, mainly in the soil, where it can accumulate (Goulson, 2013). Air and waterways can be contaminated through routes like drift from spray applications or runoff from treated sites (Bonmatin et al., 2015). Once in the environment, these insecticides expose several non-target organisms to harm. These include organisms vital to ecosystem services that humans rely on. Taking into account their widespread use, toxicity to non-target organisms and seemingly slow degradation rates in nature, neonicotinoids might put ecosystem functioning and services to a risk (Bonmatin et al., 2015, Chagnon et al., 2015).

The environmental concerns linked to neonicotinoids have led to restrictive regulations on neonicotinoid use in several countries. Germany, Italy, France and Slovenia have made national restrictions, EU-countries are subject to EU-restrictions (EASAC, 2015) and the Canadian provinces Ontario, and Québec has recently introduced restrictions (MDDELCC, 2015, MOECC, 2015).

To better understand the environmental fate and risk of neonicotinoids, it is crucial to obtain knowledge about how they degrade in various systems and which degradation products they

form. The aim of this thesis is to investigate the degradation part of this issue, more specifically the degradation rates of neonicotinoids in aquatic solutions by hydrolysis. Hydrolysis is in general an important degradation and transformation pathway of many organic pollutants in the environment (Schwarzenbach et al., 2003b). Knowledge of environmental risk and fate is substantially important for future decision and policy making.

1.2 Research objective and hypothesis

The objective of this master's thesis is to find the effect of pH, dissolved metal ions and suspended minerals on hydrolysis of the two neonicotinoids acetamiprid and imidacloprid. The chosen metal ions of this study are Cu^{II}, Ni^{II}, and Zn^{II}. The chosen minerals are goethite, kaolinite, and TiO^2.

Imidacloprid and acetamiprid were chosen because they represent two different chemical groups/structures within the neonicotinoid family. While hydrolysis rates of imidacloprid already have been well documented (Zheng and Liu, 1999, Guzsvány et al., 2006), results on acetamiprid hydrolysis are only sparingly mentioned in one study (Guzsvány et al., 2006). Thus, the choice of these two compounds gives a good basis for evaluating hydrolysis rates with earlier research on one of them, and at the same time investigating a new compound.

Divalent metal ions and mineral surfaces are known to have a catalytic effect on the hydrolysis of several pesticides and organic pollutant, but there are no published papers to date about the effect they have on neonicotinoids. The choice of metals and minerals to be studied is rooted in their use in previous research, where they have been found to exhibit catalytic effects (Torrents and Stone, 1993, Torrents and Stone, 1991, Smolen and Stone, 1997). If the catalytic potential existing in natural systems is not taken into account when degradation rates are studied in the laboratory, there is a risk that persistence and half-lives in real natural systems will be overestimated (Larson and Weber, 1994a).

The subject of this thesis was approached by conducting hydrolysis experiments in buffered solutions within the pH-range of 4 –10. Experiments with added metal ions or minerals were compared with baseline hydrolysis experiments conducted within the same pH range. Each insecticide and each metal or mineral were studied separately. Analysis of the insecticide concentrations was conducted by high-pressure liquid chromatography (HPLC).

1.3 Thesis structure

1 Introduction: The first chapter describes the background and motivation of the project, together with the research objectives and approach.

2 Neonicotinoids- properties, environmental fate, and hydrolytic degradation: The second chapter is a literature review that aims to answer the questions: what are neonicotinoids, and how do they impact the environment, and to give an introduction to the subject *hydrolysis of organic pollutants*.

3 Methods and Materials: The third chapter describes the method and materials used to conduct the experiments.

4 Results and Discussion: The fourth chapter presents the results of the hydrolysis experiments, together with a comparison to older studies and a discussion of possible significance in an environmental context.

5 Conclusion: The fifth chapter presents the conclusions of the work presented in earlier chapters.

6 Future work: The sixth chapter presents suggestions for future work based on the findings and results presented in earlier chapters.

2 Neonicotinoids – properties, environmental fate and hydrolytic degradation

This chapter is an introduction to the specific properties of neonicotinoids and their environmental fate, followed by an introduction to the topic of catalysed and uncatalyzed hydrolysis.

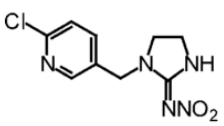
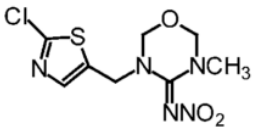
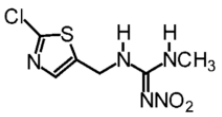
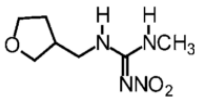
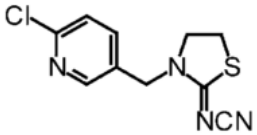
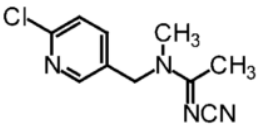
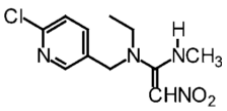
2.1 Physicochemical and biological properties of neonicotinoids

The chemical insecticide class *neonicotinoids* currently consist of seven commercially available compounds (Jeschke and Nauen, 2008, Jeschke et al., 2011), while new ones are continuously developed and tested (Shao et al., 2013). The seven neonicotinoid compounds consist of imidacloprid, thiamethoxam, clothianidin, dinotefuran, thiacloprid, acetamiprid and nitenpyram. They are listed in Table 2-1, grouped after structure: cyclic and open-chain compounds, and with respect to their pharmacophore moieties [– N – C(E)=X – Y] (Tomizawa and Casida, 2005, Jeschke and Nauen, 2008). The pharmacophore moiety is the part of the molecule that influences biological activity and determines important properties like photolytic stability, the degradation in soil, how it is metabolized in insects and plants, and the toxicity towards different organisms. The pharmacophores can be divided into the following three groups: N-nitroguanidines, N-cyanoamidines, and nitromethylenes (Jeschke and Nauen, 2008). The N-nitroguanidines are by far the most popular of these groups, making up 85% of the total neonicotinoid market in 2009. Imidacloprid stood for nearly half of this share, and with the neonicotinoid market making up 24% of the entire insecticide market, imidacloprid was the most sold insecticide in the world (Jeschke et al., 2011).

The chemical structure of the seven neonicotinoids can be broken down to three segments as seen in Figure 2-1. Component (i) consists of two separate substituents (R^1 and R^2) in open-chain compounds, and a bridging fragment in cyclic compounds that forms a five or six-membered ring. Component (ii) consists of a ring structure A and a bridging chain. The ring structure is either heterocyclic or hetero- alicyclic (Jeschke and Nauen, 2008). Heterocyclic compounds have at least one N, O or S atom substituting a C atom, while alicyclic refers to a compound being aliphatic. While heterocyclic compounds *can* have an aromatic nature with conjugated double bonds, alicyclic compounds do not (Brezonik and Arnold, 2011b, IUPAC, 1997). Component (iii) is the pharmacophore moiety [– N – C(E)=X – Y], with the functional group [=X-Y] (Jeschke and Nauen, 2008). The functional groups are electron withdrawing, while (E) can be either NH, NCH_3, sulfur or methyl. As seen in Table 2-1, the N-nitroguanidines

have the functional group N–NO_2, N-cyanoamidines have N–CN, and nitromethylenes has CH– NO_2 (Jeschke and Nauen, 2008).

Table 2-1. Commercially available neonicotinoids, based on Jeschke and Nauen (2008) and Tomizawa and Casida (2005)

Chemical group/ pharmacophore	**Cyclic Compounds**		**Open chain compounds**
	Five-membered cyclic compounds	*Six-membered cyclic compound*	
N-nitroguanidines [–N–C(E)=N–NO_2]	Imidacloprid 	Thiamethoxam 	Clothianidin 
			Dinotefuran 
N-cyanoamidines [–N–C(E)=N–CN]	Thiacloprid 	--	Acetamiprid 
Nitromethylenes [–N–C(E)=CH– NO_2]	--	--	Nitenpyram 

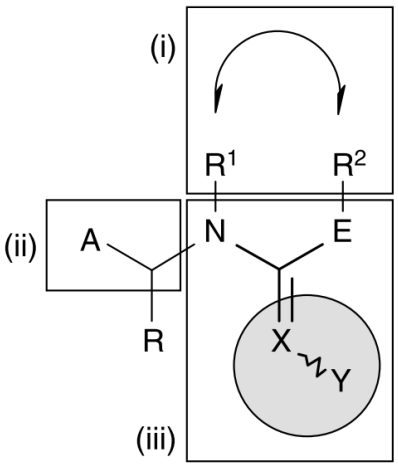


Figure 2-1. The molecular structure of Neonicotinoids (Jeschke and Nauen, 2008).

One of the most important properties of neonicotinoids that distinguish them from other insecticide classes is their *systemic activity* (Jeschke and Nauen, 2008, Bonmatin et al., 2015). Systemic pesticides have the ability to be absorbed into the plant and translocate throughout the entire plant tissue. This makes the entire plant toxic to insects and resistant towards attacking pests in a long-term time range (Tomizawa and Casida, 2005, Goulson, 2013). The effect is independent of application method and by which route they enter the plant (Simon-Delso et al., 2014). It is the systemic properties of neonicotinoids that have made the many diverse application methods, such as soil treatment, seed treatment, and pelleting and injection, economically possible. Although there are other systemic insecticides on the market, they do not have the same ability of plant uptake and translocation, making the systemic properties of neonicotinoids unique (Jeschke and Nauen, 2008).

The systemic effect depends on the physical-chemical properties water solubility, octanol-water partition coefficient, $K_{OW,}$ and the dissociation coefficient pK_a (Bonmatin et al., 2015). Values for these properties are listed in Table 2-2. Neonicotinoids are polar and non-volatile. This makes them more water soluble compared to other non-polar insecticides, they also generally have lower log K_{OW} values (Jeschke et al., 2011, Tomizawa and Casida, 2005). The functional group of the pharmacophore moiety influences the water solubility in the order N-nitroguanidine < N-cyanoamidine < nitromethylene (Jeschke et al., 2011). The octanol-water partition coefficient is the most important predictor for how compounds partition between polar and less polar phases in biota (Brezonik and Arnold, 2011a). As defined by Brezonic & Arnold (2011) it is

> […]the ratio of the equilibrium concentrations of a compound in two phases. The partition coefficient of a compound between *n*-octanol (o) and water (w) is considered to be the standard measure of a compound's lipophilicity, that is, its tendency to "prefer" being dissolved in a nonpolar rather than polar solvent:
>
> $$K_{OW} = C_O/C_w$$

The lipophilicity of a substance, indicated by the K_{OW}, is related to the ability of bio-membrane penetration, which is necessary for the substance to enter the plant (Trapp, 2004, Bonmatin et al., 2015).

Table 2-2: Physical-chemical properties of neonicotinoids, modified from Bonmatin et al. (2015)

Neonicotinoid	**Water solubility [g/L]**	**Octanol/water partition coefficient [log K_{OW}]**	**Dissociation constant [pK_a]**
Imidacloprid	0.61	0.57	*No dissociation*
Thiamethoxam	4.1	-0.13	*No dissociation*
Thiacloprid	0.184	1.26	*No dissociation*
Clothianidin	0.34	0.905	11.1
Acetamiprid	2.95	0.8	0.7
Nitenpyram	590	-0.66	3.1
Dinotefuran	39.83	-0.549	12.6

The dissociation constant predicts at what pH a compound changes from its ionized to its un-ionized species (Brezonik and Arnold, 2011a). Together with water solubility and K_{OW}, p*Ka* determines how the neonicotinoid is absorbed and translocated within the plant. The plant transportation tissues of vascular plants consist of the xylem and the phloem. While mobility in the phloem tends to happen with substances having log K_{OW} between 1 and 3, and pK_a 3 and 6,

xylem mobility is expected in nondissociative compounds with low log K_{OW} values (Bonmatin et al., 2015).

The toxic effect of neonicotinoids comes from their ability to act as agonists at the post-synaptic nicotinic acetylcholine receptors (nAChRs) in the central nervous systems of the insect (Tomizawa and Casida, 2003). The compound binds to the receptor and stimulates it. At low exposure, they will only cause nervous stimulation, but at higher concentrations, they cause receptor blockage, paralysis, and death. Though they have an effect on vertebrate nAChRs and, therefore, possesses toxicity against vertebrates as well, they are selectively more toxic to insects. This is caused by their ability to bind more strongly to the nAChRs of insects, than those of vertebrates (Tomizawa and Casida, 2005, Goulson, 2013).

2.1.1 Toxic effects on invertebrates

The systemic activity of neonicotinoids makes them a possible threat for beneficial insects like pollinators, through pollution of pollen and nectar of treated crops (Goulson, 2013). A meta-analysis of imidacloprid effects on honey bees concluded that field-realistic doses had a negligible effect on adult honey bees, but that expected performance of the bees was reduced by 10-20% in acute regimes and 6-20% in chronic regimes (Cresswell, 2011). Sub-lethal effects that have been documented in both honey bees and bumble bees include learning, foraging and homing abilities. For bumblebees, it has been documented that sub-lethal effects influence performance on colony level as well (Goulson, 2013).

In the Netherlands, it has been documented that imidacloprid contamination of surface waters correlates with macroinvertebrate declines. The decline was proportional to increasing contamination levels, and the results showed a significant relationship (Van Dijk et al., 2013). A review of neonicotinoid contaminated surface waters around the world and the associated risk to aquatic invertebrates found that neonicotinoids could negatively affect aquatic invertebrates at concentrations as low as 1 µg/L for acute exposure, and 0.1 µg/L for chronic exposure (Morrissey et al., 2015). Research from the US has shown that surface water draining from agricultural areas receives neonicotinoid concentrations that exceed US EPA benchmarks for acute toxicity to aquatic invertebrates (Anderson et al., 2013, Starner and Goh, 2012)

2.1.2 Toxic effects in vertebrates

Neonicotinoids are in general considered safer for use than other insecticides with regard to vertebrate toxicity, but this varies amongst different neonicotinoids and different species (Tomizawa and Casida, 2005). For rats, acetamiprid is the most toxic with LD_{50} of 182 mg

active ingredient per kg body weight, while clothianidin is the least toxic with LD_{50} of more than 5000 mg/kg. Birds are more susceptible than rats towards acute toxicity. For *Japanese* and *Bobwhite quails* the LD_{50} ranges from more than 2250 mg/kg for nitenpyram but is only 31 mg/kg for imidacloprid (Tomizawa and Casida, 2005). Granivorous birds are believed to be at risk of lethal doses through ingestion of treated seeds during sowing (Goulson, 2013, Gibbons et al., 2015). A study conducted in the Netherlands demonstrated that the abundance of insectivorous birds is decreasing with increased imidacloprid concentrations in nearby surface waters. The patterns of decline discovered in the study was only detectable after the introduction of imidacloprid in the Netherlands in the 90's (Hallmann et al., 2014). For insectivorous birds, this can happen through the loss of food supply caused by insecticides as found by Boatman et al. (2004).

2.2 Application methods and environmental fate

The application techniques for neonicotinoids in crop protection ranges from soil treatments like the incorporation of granules, injection, drip irrigation, spraying and use of tablets, to plant treatments like seed dressing, pelleting, implantation, dipping, injection and painting (Jeschke and Nauen, 2008). The most popular application method is as seed or soil treatment, which accounts for 60% of the global neonicotinoid use (Jeschke et al., 2011). Seed treatment application is viewed as favorable due to advantages like long-term protection over several months, reducing the need for labor. It ensures protection performance independent of weather conditions, requires smaller application amounts per unit area, and gives good crop protection results (Jeschke et al., 2011). Popular crops to be seed treated are cotton, corn, cereals sugar beet, oilseed, and rapeseed. The compounds used for this treatment are imidacloprid, thiamethoxam, and clothianidin (Jeschke et al., 2011).

Despite the fact that seed dressing treatments are argued to be an environmentally safe approach (Jeschke et al., 2011), the evidence of problematic aspects connected with this application method is accumulating. Seed treatments lead to exposure of non-target organisms by dust created during sowing and contamination of pollen, nectar and dead plant material (Bonmatin et al., 2015). The prophylactic use of seed dressings has also led to an abandonment of *integrated pest management* (IPM) (Goulson, 2013). IPM is a philosophy of pest management predicated on minimizing the use of chemical pesticides through actions like pest population monitoring, and only applying chemical pesticides when needed. Within the EU, member nations are required to follow the basic principles of IPM through EU's sustainable pesticide directive (EU Directive 128, 2009).

The application method is decisive for the environmental fate. While neonicotinoids applied as foliar spray on leaves have a fast degradation rate due to photolytic reactions (half-life of 3-5 days), they are prone to accumulation in soils, with half-lives ranging from 100 to 1 230 days (Bonmatin et al., 2015). The risk of soil accumulation is highest under cool, dry conditions, and in soils with high organic matter content. In tropical regions where temperature, sun intensity and sun exposure are higher, degradation rates are higher as well (Bonmatin et al., 2015). As shown by Sur and Stork (2003), only a small fraction of typically 1.6-20 % of applied imidacloprid in soil and seed treatment (Figure 2-2), actually enter the plant. Their research included major crops such as: corn, cotton, potato, and rice. Thus, most of the applied insecticide ends up in the soil environment.

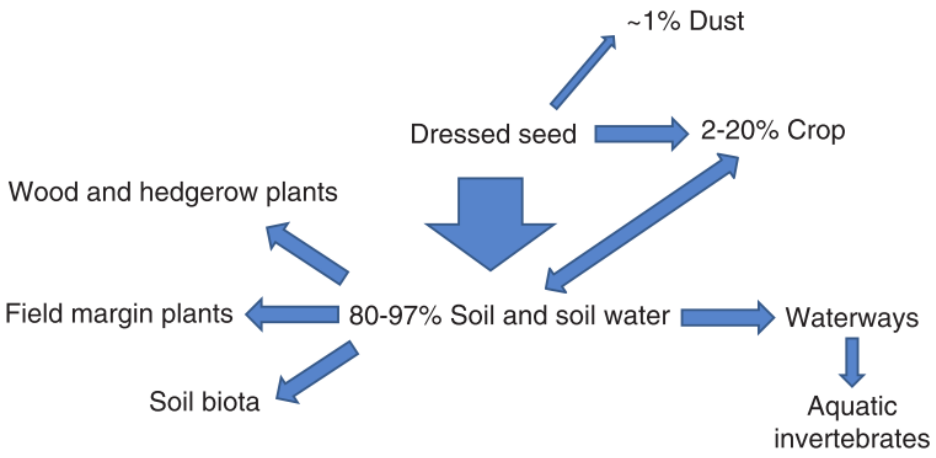


Figure 2-2. Environmental fate of neonicotinoids (EASAC, 2015).

Neonicotinoids can be bound to soil particles through sorption. Studies by Cox et al. (1997, 1998) have revealed that imidacloprid sorption is increased when the content of soil organic matter and mineral clay is higher. When neonicotinoids are bound to soil particles, the potential of leaching through the soil profile and further to groundwater and waterways decreases (Flores-Céspedes et al., 2002).

Waterways are prone to contamination of neonicotinoids through several pathways: Drifting from spray and dust created at application, surface runoff and leaching from agricultural fields. In addition, there is contamination from spilling and greenhouse discharges, as well as runoff from application in urban landscaping (Bonmatin et al., 2015). Pollution of neonicotinoids in

surface waters have been detected all over the world. Sánchez-Bayo and Hyne (2014) gathered samples from 12 different rivers around Sidney, and found neonicotinoids in 93% of the samples. Imidacloprid was the most found compound (93%) and acetamiprid the third most found (73%). In the Netherlands, imidacloprid is one of the highest ranked substances to exceed existing risk limit concentration in surface waters (Van Dijk et al., 2013). Imidacloprid has been detected in three different agricultural regions in California, throughout the dry-weather irrigation season from March to October. Despite differences in agricultural practices, soil types and climate, the detection frequencies were high in all regions. A total of 75 samples was taken from 23 monitoring sites on a total of 15 different dates, and imidacloprid was detected in 89% of the samples (Starner and Goh, 2012). Anderson et al. (2013) detected acetamiprid and thiamethoxam in playa wetlands in the Southern High Plains of North America, from May until September in 2005. A total of 12 playa lakes were sampled on a total of 15 different dates. Acetamiprid was detected in 17% of the investigated crop playas, and 4% of the grassland playas. The numbers for thiamethoxam were 31% and 25% respectively. Morrissey et al. (2015) reviewed 29 studies from 9 different countries (included the studies and countries already mentioned), and found that neonicotinoids were detected in the majority of sampled surface waters that was in proximity of, or received runoff from agricultural areas.

Neonicotinoid pollution is also found in wastewater treatment plants (WWTP). Both in the influent and the effluent, suggesting that WWTP are point sources of neonicotinoid contamination of receiving waters (Sadaria et al., 2016, Masiá et al., 2013). Imidacloprid has been found in Spanish WWTP (Masiá et al., 2013) and in several WWTP in the US together with acetamiprid and clothianidin (Sadaria et al., 2016).

2.3 Hydrolysis – an important abiotic degradation and transformation pathway in natural environments

As discussed in the previous sections, neonicotinoid exposure can harm non-target organisms, and are prone to accumulate in soils and contaminate waterways. Knowledge on the degradation and transformation of the compounds is thus a crucial part of understanding the total environmental impacts of these compounds. Neonicotinoids degrade in the natural environment following a series of different pathways. The focus in this thesis is the abiotic pathway of hydrolysis. Other possible pathways are reduction-oxidation reactions, photolysis and microbial degradation. These are all potentially important, but will not be covered here.

2.3.1 Hydrolysis

Hydrolysis of organic molecules is a reaction where a water molecule or hydroxide ion takes the place of another atom, or atom group, in the organic compound (Schwarzenbach et al., 2003b). The substituted atom, or atom group, is referred to as *the leaving group*. Typical leaving groups include halides, alcohol moieties, and phosphates. In general, a good leaving group has the ability to form stable species in aqueous solutions (Schwarzenbach et al., 2003b).

Hydrolysis is a reaction that belongs to the larger class of reactions called *nucleophilic* displacement reactions (Larson and Weber, 1994b). The covalent bonds within an organic molecule causes polarity due to the different electronegativity of different atoms. Thus, on one side of the covalent bond there will be a partially negatively charged atom, and on the other side there will be a partially positively charged atom. These polarities within the molecule attract *nucleophile* and *electrophile* species from the outside environment to the electron-poor and electron-rich sites respectively. A nucleophile is an electron rich chemical species that has a full or partial negative charge. Thus, it is attracted to partial or full positively charged sites. An electrophile is just the opposite; an electron-poor species that attracts to sites with a full or partially negative charge (Schwarzenbach et al., 2003b).

In the natural environment, potential reactants for organic molecules are dominated by nucleophilic species. One of the most important ones, due to its abundance, is water. Hence, hydrolysis is an important transformation pathway for many organic pollutants (Schwarzenbach et al., 2003b). After hydrolysis of an organic compound, the reaction product will be more polar than the parent compound, and the properties and environmental behaviour will have changed. Generally, products of hydrolysis constitute a smaller concern for the environment than the parent compound. The same cannot be generalised for reactions with other nucleophiles (Schwarzenbach et al., 2003b). It should be noted that the importance of hydrolysis is not only due to degradation happening in lakes, rivers, streams, and oceans. Hydrolytic degradation can take place in all water-containing environments, including groundwater, soil, sediments, biological systems, and even fogwater on plants and soil (Larson and Weber, 1994b).

2.3.2 Pseudo first order kinetics and hydrolysis

The degradation rate of a compound following a unimolecular reaction like the one described in equation 2.1, is written in the differential form as in equation 2.2. Here C_A denotes the concentration of compound A, C_{A0} denotes the concentration of A at time zero, and k is the degradation rate constant, which has the unit time^{-1}. For practical purposes, the integrated form

of the equation is usually used, equation 2.3. This means that a reaction following first order kinetics will show a linear relationship between the natural logarithm of the concentration of the reacting compound and the time, where the slope of the line is the degradation constant *k*, and the intercept is the natural logarithm of the initial concentration (Larson and Weber, 1994c).

(2.1) $$A \rightarrow B$$

(2.2) $$rate = -\frac{d[A]}{dt} = k[A]$$

(2.3) $$lnC_A = lnC_{A0} - kt$$

The half-life of compounds obeying first-order kinetics is obtained by substituting C_A with $½C_{A0}$ in equation 2.3:

$$ln\left(\frac{C_{A0}}{2}\right) = lnC_{A0} - kt_{\frac{1}{2}}$$

(2.4) $$t_{\frac{1}{2}} = \frac{ln2}{k}$$

From these equations, one obtains the following characteristics of a first order reaction: the reaction rate is proportional to the concentration, and the half-life is not dependent on the initial concentration (Larson and Weber, 1994c).

Reactions that include two reactants, as is the case with hydrolysis, need to include both reactant species in the equation. Given the reaction:

(2.5) $$A + B \rightarrow C$$

the rate of disappearance would be:

(2.6) $$rate = -\frac{d[A]}{dt} = k[A][B]$$

(2.7) $$ln\frac{C_{B0}C_A}{C_{A0}C_B} = (C_{A0} - C_{B0})kt$$

However, when one of the reactants is so abundant that its concentration does not effectively change (or is held constant), like the case of hydrolysis of neonicotinoids dissolved in water, the observed behaviour of the system will follow first order kinetics. In this case observed rate

constant k^* is equal to $k[B]$. Second order systems acting like first order systems are referred to as *pseudo*-first-order reactions (Larson and Weber, 1994c).

2.3.3 Hydrolysis of neonicotinoids: Presentation of current knowledge and research

Studies on the hydrolytic degradation of neonicotinoids have mainly focused on imidacloprid and thiamethoxam. One study also included hydrolysis experiments on thiacloprid and acetamiprid, but the results were inadequately reported. The following section contains a summary of the most relevant findings. The literature on thiamethoxam is also included, as it serves as a good basis for comparison when differences and similarities between different neonicotinoids are evaluated.

Imidacloprid

Imidacloprid is a five-membered cyclic neonicotinoid, belonging to the N-nitroguanidine group (see Figure 2-1). It was the first neonicotinoid to be introduced to the commercial market, and it is still the most important one in terms of sales and market share (Jeschke et al., 2011) Imidacloprid is relatively stable in acidic and neutral solutions but has a lower persistence at alkaline conditions (Zheng and Liu, 1999, Guzsvány et al., 2006). In the study conducted by Zheng and Liu (1999), the hydrolytic degradation of imidacloprid at pH 7.0 was only 1.5 % after three months (Table 2-3). Degradation at pH 5 and pH 3 was even smaller, with the most acidic condition corresponding to the lowest degradation rate. In alkaline solutions, the degradation rate increased with increasing pH. After three months the compound had degraded with 5% at pH 8, and 20% at pH 9.0.

Table 2-3. Hydrolytic degradation of Imidacloprid after three months (Zheng and Liu, 1999).

pH	**3.0**	**5.0**	**7.0**	**8.0**	**9.0**
Degradation	<1.5 %	< 1.5 %	1.5 %	5 %	20 %

At pH 10.8 and 11.8 imidacloprid hydrolysis was found to fit first order kinetics, with detected half-lives of 20 days at pH10.8 and 2.3 days at pH 11.8 (Zheng and Liu, 1999). First order kinetic parameters for alkaline hydrolysis of imidacloprid are listed in Table 2-4. Hydrolysis

rates in basic solution were also found to be dependent on temperature. Experiments were conducted at 10˚C, 20˚C, 30˚C, 40˚C, 50˚C and 60˚C in a solution of 10 mM NaOH, and showed that increasing temperature corresponded with increased reaction rate. Application of the Arrhenius Equation gave an activation energy of imidacloprid hydrolysis at 42.72 kJ/mol. The authors did not report at what temperature the pH-dependent hydrolysis experiments were carried out at (Zheng and Liu, 1999).

Table 2-4: Parameters for imidacloprid hydrolysis fit to first-order kinetics

Hydrolysis equation LnC = - K*t + B				
pH	***k***	***B***	***r^2***	***$t_{0.5}$ (days)***
10.8	0.035	3.02	0.996	20.0
11.8	0.243	2.93	0.990	2.9

Guzsvány et al. (2006) confirmed the general results of Zheng and Liu, finding that imidacloprid was persistent at pH 4.0 and 7.0, while degradation occurred at pH 9.0.

The hydrolysis of imidacloprid in alkaline solution has been found to result in only one degradation product (Guzsvány et al., 2006, Zheng and Liu, 1999). The product was reported to be 1-[(6-chloro-3-pridinyl)methyl]-2-imidazolidone, and it was persistent in alkaline solution. The hydrolysis pathway of imidacloprid is proposed to happen by attack of OH^- at the carbon atom of the C=N group (Figure 2-3). This carbon has a slight positive charge, i.e. higher electrophilicity, due to the strong electron-withdrawing character of the NO_2-group. The result is a replacement of the N-NO_2 functional group, with an =O functional unit (Zheng and Liu, 1999, Guzsvány et al., 2006).

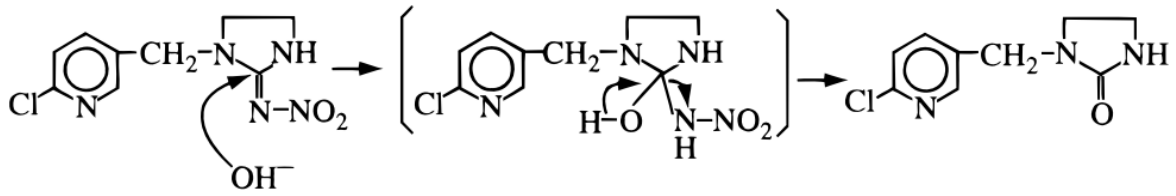


Figure 2-3. Proposed imidacloprid hydrolysis mechanism (Zheng and Liu, 1999).

Thiamethoxam

Thiamethoxam belongs to the same chemical group as imidacloprid, the N-nitroguanidines, but it differs in structure as it is a six-membered cyclic compound. Consistent with the findings of Zheng and Liu (1999) for imidacloprid, thiamethoxam has shown to degrade more rapidly at alkaline pH, while neutral and acidic hydrolysis is rather slow (Guzsvány et al., 2006, Karmakar et al., 2009). Guzsvány et al. (2006) studied thiamethoxam hydrolysis for solutions at pH 4.0, pH 7.0 and pH 9.0. At pH 4.0, they found no significant degradation, at pH 7.0 the first detectable change happened after five weeks. During three months, there was a total degradation of 3%. For the samples at pH 9.0, degradation occurred after only three days. The degradation at pH 9.0 followed first order kinetics, but degradation parameters were not reported. The samples were held at a room temperature of 23°C.

Karmakar et al. (2009) studied the hydrolysis of thiamethoxam at constant temperature 28°C±1.0°C, for buffered solutions at pH 4.0, pH 7.0 and pH 9.2. Similar to Guzsvány et al., they also found that degradation at alkaline pH was the highest. At pH 9.2, the measured half-life was only 2.1 days, and complete degradation occurred after 20 days. However, the results for the acidic and neutral solutions were inconsistent with Guzsvány et al. The slowest hydrolysis rate happened at neutral pH, with a measured half-life of 29.2 days. Hydrolysis at pH 4.0 had a half-life of 13.9 days. The degradation rates were fit with first-order kinetics for all pH-values, degradation parameters can be seen in

Table 2-5.

Table 2-5. Thiamethoxam hydrolysis parameters at 28°C±1.0°C, Karmakar et al. (2009).

Hydrolysis equation LnC = - K*t + B				
pH	***k***	***B***	***r^2***	***$t_{0.5}$ (days)***
4.0	0.0216	0.0284	0.9896	13.9
7.0	0.0103	0.0329	0.9694	29.2
9.2	0.1450	0.1886	0.9587	2.1

Guzsvány et al. (2006) found one major thiamethoxam hydrolysis product and proposed that the degradation pathway was similar to that of imidacloprid. That is, the functional group N-NO_2 is electron-withdrawing, inducing electrophilic conditions at the carbon atom, making it susceptible to nucleophilic attack. The hydrolysis product was not characterized.

Karmakar et al. found several hydrolysis products, at both acidic and alkaline conditions. In total, they identified and characterized seven different hydrolysis products and suggested several hydrolytic pathways with different sites for nucleophilic attack on the thiamethoxam molecule, including cleavage of the six-membered ring structure. It is unclear why Karmakar et al. found three more hydrolysis products than Guzsvány et al. in buffered samples at ambient temperature, as there were no big differences between the reaction conditions of the two studies. The details of the reaction pathways and products for thiamethoxam will not be covered here, but can be found in the cited studies.

Acetamiprid and thiacloprid

The study on neonicotinoid hydrolysis by Guzsvány et al. (2006) also included acetamiprid and thiacloprid. While imidacloprid and thiamethoxam belong to the N-nitroguanidines, thiacloprid and acetamiprid both belong to the N-cyanoamidines (see Table 2-1). In comparison to imidacloprid and thiamethoxam, acetamiprid was less persistent in acid media and more persistent in alkaline media. At pH 9.0, the first detectable change happened after thirty days, which is considerably more than for thiamethoxam (three days) and imidacloprid (five days). Experiments with thiacloprid proved to be difficult as the compound has a lower water

solubility than the other neonicotinoids. The only reported finding from the study was that thiacloprid residue was still present after sixty days at pH 9.0 (Guzsvány et al., 2006).

2.4 Catalysed hydrolysis

Aquatic systems in the environment contain a complex variety of chemical species and surfaces that can alter the hydrolytic degradation rates of organic pollutants (Larson and Weber, 1994a). As mentioned in section 2.2, a considerable amount of the applied neonicotinoids ends up in the soil environment (Goulson, 2013). The soil is a heterogeneous system containing many of the chemical species and surfaces that have been demonstrated to catalyze the degradation rates of organic pollutants. Important species for hydrolytic catalysis of pesticides includes minerals and metal ions (Huang, 1999). However, they have also been proven to inhibit hydrolysis (Huang and Stone, 1999).

Research on catalytic effects on neonicotinoid hydrolysis is not currently available. This section will, therefore, give a general introduction to the different kinds of catalysed hydrolysis observed on other pesticides and related compounds, and a closer look at the specific mechanisms this can happen by for a few chosen compounds.

2.4.1 Introduction to catalysed hydrolysis

A catalyst can be defined as a species that accelerate the rate of a reaction, without being consumed by the reaction itself (Schwarzenbach et al., 2003b). However, as pointed out by P.M. Huang (1999), it is from a practical point of view better to regard a catalyst as a substance that changes the rate of a reaction, irrespective of what happens with the species during or after the reaction. Many substances that have been classified as catalysts actually gets destroyed, whether as a result of the process that gives them their catalytic activity or as a result of subsequent combination with the reaction products. For a species to have a catalytic effect on the reactant, it must alter the mechanism of the initial reaction, in the direction of a lower energy barrier. Thus, the reaction rate increases (Huang, 1999).

Catalysed hydrolysis can be divided into homogeneous and heterogeneous catalysis. Both of which are considered important in soil and environmental sciences (Huang, 1999) *Homogeneous catalysis* is when the catalyst has the same phase as the reactant. Examples of this are the catalysis of an organic reaction by dissolved metals, specific acid/base catalysis, and catalysis by dissolved organic matter. *Heterogeneous catalysis* is when the catalyst has a different phase from the reactant, and the reaction process happens at the interface between the two phases. As the focus of this thesis is on hydrolysis, the two phases in question will be water

and solid surfaces. Catalytic agents in aquatic environments like aquifers, soils and sediments include clays, metal-hydroxide and metal-oxide minerals (Larson and Weber, 1994a, Huang, 1999).

Specific and general acid and base catalysis

Hydrolysis can be catalysed by protons, H^+, and hydroxide ions, OH^-, known as *specific acid and base-catalysis* (Larson and Weber, 1994b). The catalytic effect from specific acid catalysis is theorized to be caused by coordination of the atom bonded to the leaving group, such that the electron density becomes lower, increasing the susceptibility of nucleophilic attack by H_2O. Specific base catalysis is when the nucleophilic attack is by OH^- rather than H_2O. The reactivity of the hydroxide ion is typically larger than that of H_2O by a factor of 10^4. Thus, the reaction rate will increase compared to when the nucleophilic attack happens by H_2O alone (Larson and Weber, 1994b). The effect of specific acid and base catalysis can be implemented in the rate equation for a pseudo-first order hydrolysable compound *A* (equation 2.2, section 2.3.2) giving the expressions:

(2.8) $$rate = -\frac{d[A]}{dt} = k_{hyd}[A] = k_a[H^+][A] + k_n[A] + k_b[OH^-][A]$$

(2.9) $$k_{hyd} = k_a[H^+] + k_n + k_b[OH^-]$$

Here, k_{hyd} is the observed hydrolysis rate constant, k_a, k_n and k_b represent the acid-catalyzed, the neutral and the base-catalyzed rate constants. The equation holds when all individual processes can be described by (pseudo) first order kinetics (Larson and Weber, 1994b).

General acid/base catalysis takes into consideration all Brønsted acids and bases in a system (Larson and Weber, 1994b). It is also called Brønsted acid-base catalysis (Huang, 1999) and buffer catalysis (Larson and Weber, 1994a). Natural aquatic ecosystems can contain a wide range of weak acids and bases that are potential catalysts for hydrolysis of organic pollutants. General acid base catalysis happens by proton transfer. The proton can act by reducing negative charge on the pollutant and thus facilitate electron transfer.

Based on the Brønsted catalysis law, a mathematical model was developed by Perdue and Wolfe (1983) to predict the potential contribution of acid/base catalysis of pollutant hydrolysis. Due to the very low concentrations of Brønsted acids and bases in the environmentally aquatic systems, the effects was concluded to be insignificant. However, they also concluded that

acid/base catalysis might be significant in laboratory experiments using buffered systems with a buffer concentration > 0.001 M.

2.4.2 Homogenous metal ion catalysis

Due to their ability to act as *Lewis acids*, metal ions can catalyse the hydrolysis of organic compounds by mechanisms similar to that of acid catalysis (Larson and Weber, 1994a, Huang, 1999). The definition of a *Lewis acid* is a compound that acts as an electron acceptor (Brezonik and Arnold, 2011c). Just like the proton, metal cations can coordinate to the organic compound in a way that shifts the electron density away from the site of the nucleophilic attack. Metal catalysis is negligible in acidic solution because of the polarizing power and high charge density of protons. However, advantages of metal ions vs. protons are that they can exist at significant concentrations also at neutral and basic pH, due to their ability to be stabilized by other *ligands*, and they can bind to a substrate at more than one site, because of their high coordination numbers (Huang, 1999). A *ligand* is a molecule that can replace one, or several, water molecules in the hydration sphere of the metal ion when dissolved in aqueous solution (Benjamin, 2002). While the catalytic efficiency of acids and bases depends on their strength, metal ion catalysis depends on their charge, and their *chelating* ability (Huang, 1999). A *chelate* is a strong complex between a metal ion and a ligand that replaces *more* than one water molecule in the inner hydration sphere of the metal ion (Benjamin, 2002). Huang (1999) mentions seven known mechanisms of how metals can catalyse hydrolysis reactions; these are given in Table 2-6.

The relevant catalysis type depends on the leaving group of the organic compound. If it is a good leaving group, that is as mentioned in section 2.3.1 one with the ability to form a stable species in aqueous solution, hydrolysis of the compound is limited by the rate of nucleophilic attack. Thus, catalysis type 1,2,4 and 6 are probable catalysis mechanisms. Compounds with poor leavings groups are limited by the breakdown of tetragonal intermediate, and would be susceptible for catalysis mechanism 3 and 7. Catalysis type 5 is a possible mechanism for both kinds of compounds (Huang, 1999). Many pesticides have leaving groups that are neither good nor poor. If these compounds are undergoing metal ion catalysed hydrolysis, the limiting force on the process might switch (Huang, 1999).

Table 2-6: Metal-ion catalysis mechanisms (Huang, 1999)

Type 1	The metal increases the reactivity of the electrophile by electrophile coordination that changes the distribution of electrons in a favourable way.
Type 2	The metal increases the reactivity of the nucleophile by nucleophile coordination that induces deprotonation.
Type 3	The metal can coordinate the leaving group, thus facilitating its ability to leave.
Type 4	The metal can bind to the electrophile and the attacking nucleophile at the same time. This is referred to as the "template effect".
Type 5	The metal increases the reactivity by coordinating the substrates in such a way that changes in the molecular shape is induced (confirmation changes).
Type 6	The metal coordinates the substrate in such a way that the charge of the substrate becomes more positive and thus decrease unfavourable electrostatic interactions with the nucleophile.
Type 7	Inhibitory reverse reaction paths like hydroxide ion loss from a tetrahedral intermediate (instead of the leaving group), or nucleophilic attack by the leaving group, are blocked by metal coordination.

Organic compounds susceptible to metal ion catalysed hydrolysis includes carboxylic acid esters, amides, anilides and phosphate-containing esters (Huang, 1999). These compounds are susceptible because they contain auxiliary donor groups in proximity to the leaving group that can complex metals with more than one bond, i.e. forming bidentate complexes (Larson and Weber, 1994a). Metal ions that are capable of acting as catalysts include Al^{II}, Co^{II}, Cu^{II}, Fe^{II}, Ni^{II}, Pb^{II} and Zn^{II}. With the exception of copper, which in some areas is used as a fungicide, their concentration in natural systems, soil, in particular, is usually too low for them to act as significant catalysts in the environment. However, they might be important in certain engineered or natural systems, in enzyme-catalyzed hydrolysis reactions and certain surface-

catalyzed hydrolysis (Schwarzenbach et al., 2003a, Smolen and Stone, 1997). Furthermore, they do serve the purpose of accentuating the mechanisms of metal ion catalysis, as they have distinctive and well-characterized chemical properties (Smolen and Stone, 1997). Another possible area of importance for dissolved metals is in urban storm-water runoff, which is known to be polluted by heavy metals. Copper, zinc, and nickel are among the most common appearing metals in urban runoff and are the subject of several studies on urban runoff quality (Beasley and Kneale, 2002, Marsalek, 1990, Sörme and Lagerkvist, 2002, Makepeace et al., 1995).

Smolen and Stone (1997) found Cu^{II} and Pb^{II} to have a catalytic effect on the hydrolysis of several organophosphorus pesticides from pH3.5-pH7.0, with Cu^{II} being the better catalyst of the two. Both Pb^{II} and Cu^{II} catalysis increased from pH 3.0 to pH 5.5. It is thought that the decrease in metal ion catalysis with decreasing pH is caused by the decreasing concentration of one of the hydroxo-containing species. The catalytic effect of Cu^{II} was however somewhat decreased at pH > 5.5, due to solubility limitations. Co^{II}, Ni^{II} and Zn^{II} were also studied but had a negligible effect on hydrolysis rates. The pesticides consisted of five phosphorothionate triesters (thionate esters), and two oxonate organophosphorus pesticides (oxonate esters). Similar to neonicotinoids, many thionate and oxonate esters hydrolyze rapidly under alkaline conditions but are persistent in neutral and slightly acidic solutions. The catalysis mechanisms relevant for thionate and oxonate esters by the metals studied are type 1, 2 and 3 in Table 2-6 (Smolen and Stone, 1997). The difference in catalytic effectivity between Pb^{II} and Cu^{II} was hypothesized to be caused by equal catalytic activity by type 2 mechanism, and additional catalytic activity by type 1 and 3 from Cu^{II} (Smolen and Stone, 1997). The justification for this is outlined below.

Mechanism 1 catalysis of thionate and oxonate esters – coordination of the electrophile towards higher reactivity

Coordination of the electrophilic site within the thionate or oxonate ester requires complex formation between the metal and the ester. The concentration of metal-ester complexes is thought to be proportional to the catalysed hydrolysis rate. The ability for the metal to form such complexes is determined by the concentration of metal ions in solution, and the metal-ester complex formation constant K_{ME} (Smolen and Stone, 1997). Due to lack of complex formation constants in the literature for the right groups chemical groups (metals and neutral sulfur atoms for thionate esters, and metals and neutral oxygen atoms for the oxonate esters), the authors used metal-ammonia complexation constants as an analogy to evaluate the ability of mechanism 1 catalysis of the different metals. Similar to the oxygen and sulphur atoms of

the organophosphorus pesticides, ammonia is a neutral ligand. Based on this evaluation, Cu^{II} is thought to be a good candidate for mechanism 1 catalysis, and to a smaller extent Ni^{II} (Smolen and Stone, 1997). Since Ni^{II} has better properties for mechanism 1 catalysis than Pb^{II}, it is not likely that Pb^{II} works by mechanism type 1.

Mechanism 2 catalysis of thionate and oxonate esters – coordination of the nucleophile towards higher reactivity by induced deprotonation

Metal ions can make more reactive nucleophiles by forming metal hydroxo-species. This is done when the metal ions induce deprotonation of coordinated H_2O molecules (Smolen and Stone, 1997). As already mentioned, the nucleophilic reactivity of OH^- is much larger than that of the neutral H_2O-molecule. Hydroxide ions are also generally better nucleophiles than metal hydroxo species. However, the concentration of metal hydroxo species can be much larger than the concentration of OH^- at neutral and acid pH, thus they can play an important role in the overall hydrolysis rates (Smolen and Stone, 1997, Torrents and Stone, 1991).

To evaluate the importance of type 2 catalysis mechanism, the contribution of metal hydroxo species must be added to the hydrolysis rate equation presented in section 0.

$$rate = -\frac{d[A]}{dt} = (k_a[H^+] + k_n + k_b[OH^-] + \sum k_{Me(OH)_n}[Me(OH)_n{}^{(2-n)^+}])[A] \quad (2.12)$$

The equation reveals that it is the magnitude of the product of metal hydroxo rate constant and metal hydroxo concentration that determines the significance of mechanism 2 catalysis. The concentrations of metal hydroxy species in solution is described by the equilibrium equation and equilibrium constant:

$$Me^{2+} + H_2O \leftrightarrow MeOH^+ + H^+ \quad (2.13)$$

$$K_1 = \frac{[MeOH^+][H^+]}{[Me^{2+}]} \quad (2.14)$$

From the equation of the equilibrium constant we gather that as the concentration of H^+ decreases (i.e. the pH increase) the concentration of hydroxo species increase at the expense of the metal ion concentration. Additional hydroxo species will also grow in importance as the pH increases. When the concentration of H^+ is equal to the equilibrium constant, the concentration

of the two species is the same, thus it is when the pH of the solution is higher than -log K_1, that metal hydroxo species predominate. Smolen and Stone (1997) found these K_1 values in the NIST critically selected stability constants of metal complexes database, version 2.0

$$Cu^{II} \rightarrow \log K_1 = -7.5$$

$$Pb^{II} : \log K_1 = -7.6$$

$$Zn^{II} : \log K_1 = -9.0$$

$$Co^{II}: \log K_1 = -9.7$$

$$Ni^{II} : \log K_1 = -9.9$$

This indicates that both Cu^{II} and Pb^{II} has the ability to act as a catalyst by mechanism 2, near neutral pH, since the concentration of metal hydroxo species will be considerable.

Mechanism 3 catalysis of thionate and oxonate esters – coordination of the leaving group

In thionate and oxonate esters, this catalysis mechanism require a weakening of the bond between the phosphorus center and the leaving group. The mechanism is most probable for leaving groups with a high pKa. Using an analogous ligand as comparison basis, Cu^{II} is the most probable ion to work by this mechanism as it has the highest complex formation constant.

Both Cu^{II} and Pb^{II} are unknown to occur naturally at high enough concentrations in agricultural soils to be possible environmental catalysts. However, Cu^{II} is added as a fungicide at some agricultural sites, making it a potential catalyst where this practice is usual (Smolen and Stone, 1997).

Inhibiting effects on hydrolysis rates by metal ions

In a study on hydrolysis rates of the herbicide naptalam , Cu^{II}, Zn^{II} and Ni^{II}, was found to have an inhibitory effect. Hydrolysis rates were significantly slower with the addition of 1mM Cu^{II} within the pH-range 3.6-5.5. The inhibitory effects of the same amount Zn^{II} and Ni^{II} were smaller, but still detectable. The inhibitory effects of Cu^{II} and Zn^{II} were shown to increase with increasing concentration (Huang and Stone, 1999). The authors evaluated the mechanisms of inhibition based on the two generalized mechanisms:

I) Coordination of the substrate that decreases the susceptibility of nucleophilic attack

II) Coordination of the nucleophile that decreases the reactivity towards electrophilic sites

These can be considered the reverse effects of mechanisms 1 and 2 in Table 2-6. The ability for Cu^{II}, Ni^{II} and Zn^{II} to act as inhibitors by substrate coordination requires: 1) complex formation with the carbonyl oxygen, and 2) ability to induce deprotonation of the amide hydrogen group (molecular structure of naptalam is given in Figure 2-4).

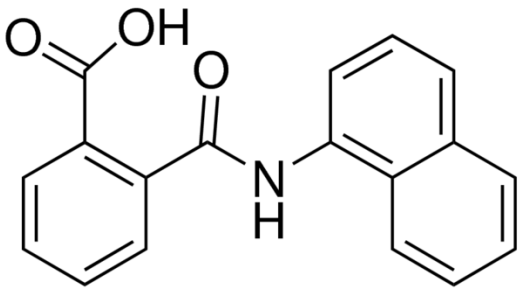


Figure 2-4:Molecular structure of naptalam (Sigma-Aldrich).

In lack of complex formation constants for metals and carbonyl oxygen in the literature, the metals were evaluated by their ability to form complexes with the analogous, neutral ligand, ammonia (similarly to the approach used by Smolen and Stone, 1997). The succession of complex formation ability with NH_3 is $Cu^{II} > Ni^{II} > Zn^{II}$. The complex formed by Cu(II) is 20 times stronger than the one formed by Ni^{II}, and 63 times stronger than the one formed by Zn^{II} (Huang and Stone, 1999). Metal ion coordination of the carbonyl oxygen of picolinyl amides has been reported to induce deprotonation of the amide nitrogen and thus cause inhibition on hydrolysis rates. Deprotonation of the amide nitrogen shifts electron density towards the carbonyl oxygen (Sayre et al., 1992). This same mechanism is proposed to be effective on naptalam (Huang and Stone, 1999). Figure 2-5 illustrates how catalysis and inhibition by coordination of the substrate.

Because the ability to induce deprotonation is determined by the metal ions potential to change the electronic distribution of the substrate it has coordinated, the metals can be evaluated based on their ionization potential (Huang and Stone, 1999). The succession of ionization potential from highest to lowest is $Cu^{II} > Zn^{II}$ and Ni^{II}. Based on the combination of good complex formation and ionization ability, Cu^{II} was concluded to be a candidate for substrate coordination inhibition. Zn^{II} and Ni^{II} on the other hand, does not share the same level of complex formation ability and electron affinity, thus it cannot be concluded whether they are good candidates for substrate coordination inhibition (Huang and Stone, 1999).

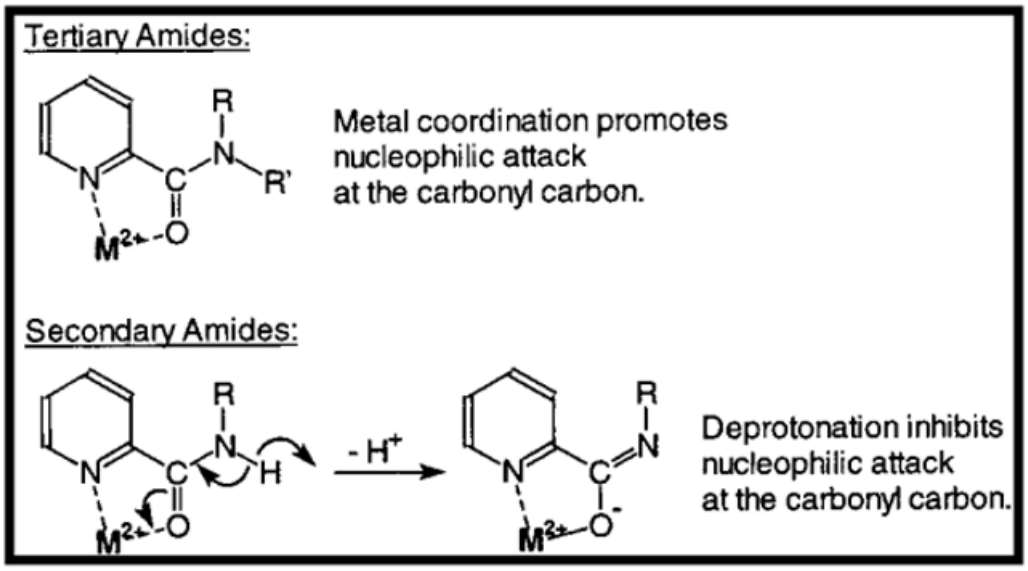


Figure 2-5: Coordination of the substrate - catalysis and inhibition (Huang and Stone, 1999).

Naptalam hydrolysis is proposed to involve the attack of an intramolecular nucleophile, namely the carboxylate side group. Inhibition by nucleophile coordination is therefore proposed to happen by coordination of the carboxylate group so that it loses its ability to act as a nucleophile. Again, it is the strength of the complex formation that is thought to execute the largest inhibitory effect. Using the analogous ligand benzoate as a comparison model, the succession of complex formation strength from highest to lowest is $Cu^{II} > Zn^{II} > Ni^{II}$, wich is the same order as the observed inhibitory effects of the metal ions (Huang and Stone, 1999).

A naptalam hydrolysis experiment in Cu^{II}-containing solution with added citric acid substantiated the importance of complex formation between the metal-ion and naptalam in the observed inhibitory effects. When the concentration of citric acid exceeded that of Cu^{II}, the hydrolysis rates were the same as for metal-ion free solutions. Naptalam hydrolysis rates were not affected by citric acid alone, thus it seems that Cu^{II} species affect hydrolysis rates, while Cu(II)-citrate complexes have no effect. The effect of citric acid addition on Cu^{II}-inhibition increases with increased citric acid concetration (relative to Cu^{II} concentration). The cause of the phenomenon is therefore presumably due to the decrease in free Cu^{II}-species to form Cu^{II}-naptalam complex (Huang and Stone, 1999). This has great environmental significance as well, because aquatic environments are normally abundant in organic matter that are complex-forming with metals (Huang and Stone, 1999).

As the observed inhibitory effects in the order $Cu^{II} > Zn^{II} > Ni^{II}$ is consistent with either mechanism, the authors conclude that both inhibition types are probable contributors to the reported results (Huang and Stone, 1999). However, there is a small inconsistency within this argument which is not addressed by the authors, namely that metal-ammonia complex formation was stronger for Ni^{II} than Zn^{II}.

2.4.3 Heterogeneous mineral surface catalysis

The importance of mineral surface catalysis is reflected in the fact that heterogeneous aquatic environments like aquifers, sediments and soils is just as abundant, or exceedingly abundant, in metal hydroxoide and -oxide surface sites, as it is of dissolved metal ions (Huang and Stone, 1999, Larson and Weber, 1994a).

The reaction steps in heterogeneous catalysis involves transport of reactants to the catalytically active site, adsorption of the reactants at the catalytically active site (the mineral surface or the topmost atomic layer of the surface), the chemical reaction itself, followed by desorption and transportation of reaction products away from the catalytically active site and the catalyst exterior (Huang, 1999, Twigg, 1989). Heterogeneous catalytic reactions can be divided into groups after which of these stages that limit the overall reaction rate. Film-diffusion controlled catalysis is limited by the transport of reactants/reaction products to/from the catalyst surface. Pore-diffusion controlled catalysis is limited by the transport of reactants/reaction products through the pore system to/from the catalytically active site. Finally, reaction-controlled catalysis is limited by the adsorption, desorption or the chemical reaction itself (Huang, 1999).

Three different mechanisms of catalysis at the mineral-water interface of metal containing minerals has been postulated by Stone (1989), they are given in

Table 2-7. Note that the first two mechanisms are analogous to the catalysis mechanisms type 1 and 2 of metal ions from section 2.4.1.

Table 2-7: Mineral catalysis mechanisms (Stone, 1989).

Type 1	The leaving group is polarized due to specific adsorption to metals within the lattice structure of the mineral
Type 2	Nucleophilic activity by metal hydroxo group on the mineral surfaces
Type 3	Hydroxide ion concentrations increases near the mineral surface compared to the bulk solution due to electrostatic interactions

Clays and clay minerals can catalyse hydrolysis due to surface acidity (Larson and Weber, 1994a). The magnitude of clay mineral surface acidity can be in the order 2-3 pH units below bulk solution (Bailey et al., 1968, Frenkel, 1974, Mortland, 1970, Karickhoff and Bailey, 1976). Brønsted acidity at the mineral surface is promoted by low water contents and small cations with high charge (polarizing cations), while Lewis acidity is dependent on constituent ions with the ability to act as Lewis acids, exposed at the edges of mineral colloids. Examples of such ions are Al and Fe ions. As mentioned earlier, acid catalysis usually requires that the compound to be transformed is negatively charged. For uncharged organic compounds to accept a proton, the conditions must be extremely acidic, or, they can be protonated at a mineral surface (Huang, 1999, Larson and Weber, 1994a). The Brønsted surface acidity of minerals corresponds to the polarizing power and electronegativity of the metal cations in the lattice structure and those that are exchangeable in the following succession: $H^+ > Al^{3+}$, $Fe^{3+} > Mg^{2+} > Ca^{2+} > Na^+ > K^+$ (Huang, 1999). Clays are important constituents of most soils, and are known to have both catalytic and inhibitory effects on pesticide hydrolysis. For instance, montmorillonite catalyses hydrolyses of carbamate pesticides carbosulfan and aldicarb, while inhibiting the hydrolysis of the carbamate chlorpropham (Wei et al., 2001).

Mechanisms of mineral catalysed phenyl picolinate hydrolysis

Torrents and Stone (1991, 1993) examined the hydrolysis of a pesticide-like neutral ester, phenyl picolinate (PHP), in aqueous mineral-containing solution. Although PHP is not an important pollutant, it has chemical and structural similarities to important pesticides classes, and is therefore a good basis for research on adsorption and surface catalyzed hydrolysis, to better understand the complex mineral surface interactions with pesticides.

Comparative examination of PHP hydrolysis in aqueous mineral suspensions and homogeneous suspensions revealed that hydrolysis rates were significantly increased in the presence of anatase (TiO_2) and goethite (FeOOH). The hydrolysis rates increased with increasing oxide and surface area loading. The other minerals tested, amorphous silica (SiO_2), γ-aluminum oxide (Al_2O_3) and hematite (Fe_2O_3) did not increase the hydrolysis rates of PHP. Experiments testing the effect of dissolved metal ions released from mineral particles found no catalytic effect on the hydrolysis rate. Thus, it seems that the dissolved metal ions from the minerals did not contribute to any catalytic effect on PHP hydrolysis (Torrents and Stone, 1991).

The catalytic effect by anatase and goethite was proposed to happened by mechanism type 1 coordination of the carbonyl oxygen and nitrogen heteroatom with a surface-bound metal forming a five-membered bidentate chelate complex (Figure 2-6). This claim is supported by hydrolysis experiments with phenyl isonicotinate (PHI), a similar compound that does not have the right structure to form a chelate with the mineral, and that is not catalyzed by any if the minerals tested. PHI does have the same ligands as PHP, carbonyl oxygen and nitrogen heteroatom, but these are situated differently. PHI can therefore only form a monodentate complex with the mineral surface, which is a weaker one than the chelate. Mechanism 2, nucleophilic activity by mineral surface hydroxo groups was rejected as both PHP and PHI should be susceptible towards this mechanism (Torrents and Stone, 1991, Schwarzenbach et al., 2003a). Mechanism 3 was also rejected, as the changes in catalytic effect were negligible for different electrolyte concentrations. If electrostatic interactions were responsible for the catalytic effect, it is expected that the catalytic effect would decrease with increasing electrolyte concentration (Torrents and Stone, 1991)

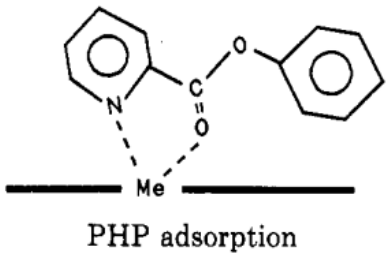


Figure 2-6. PHP adsorption to the mineral surface (Torrents and Stone, 1991).

The catalysis by anatase and goethite of PHP hydrolysis can be inhibited under the presence of environmentally relevant chemicals such as calcium, phosphate and various carboxylic acids (Torrents and Stone, 1993). This inhibitory effect is evidently linked to their ability to adsorb on the mineral surfaces, as species with a poor ability to adsorb failed to show the same effect. The decrease in catalytic effect mainly stems from the occupation of mineral surface sites. The surface sites have differences in their structural and chemical properties, making some surface sites capable of PHP catalysis, and others not. The inhibitory effect may therefore be affected by whether the co-adsorbate prefers to occupy a catalytic or non-catalytic site (Torrents and Stone, 1993).

Inhibition of naptalam hydrolysis by mineral surfaces

Huang and Stone (1999) found a small but significant inhibitory effect on the hydrolysis of the herbicide naptalam by Al_2O_3 and goethite. For the pH range 3.6-7.5, the inhibitory effect was largest at pH3.7, and decreased as the pH increased. As adsorption to Al_2O_3 also decreased with increasing pH, adsorption might play a role in the inhibition. The observed inhibition on hydrolysis rates were consistent with the total surface site loadings of the three minerals. In addition to adsorption, it is possible that the minerals cause inhibition similarly to the mechanisms of metal ion inhibition, type 1 and 2.

3 Materials and Methods

3.1 Structure of experimental setup

The effects of metal ions and minerals on hydrolysis of acetamiprid and imidacloprid was tested by comparing hydrolysis rates between the three main conditions: no catalyst added, potential metal catalyst added and potential mineral catalyst added. The condition with no added catalyst is hereby referred to as the *baseline hydrolysis* condition. All experiments were conducted in buffered solutions at specified pH within the pH-range 4 - 10. The experiments were set up in four phases, with phase 1 being the baseline hydrolysis experiments and the consecutive phases being hydrolysis with the potential catalyst added, as shown in Table 3-1. Due to time restrictions, it was not possible to run all experiments for the same amount of time. The run length of the experiments was therefore to a large degree decided by the start date. Thus the longest run experiments were the baseline hydrolysis experiments in phase 1, which ran for 80 days, and the shortest run was the phase 4, mineral and metal experiments at pH10, that only ran for 30-31 days.

Table 3-1: Overview of the different experimental phases and timeline

Phase: Experimental condition	Time (days)
Phase 1: Baseline hydrolysis – pH 4 - 10	80
Phase 2: Metal ion hydrolysis – pH 4 - 8	
Nickel	70
Zinc	60
Copper	60
Phase 3: Mineral hydrolysis – pH 8	
Goethite and Kaolonite	46
Rutile	45
Phase 4: Minerals and Metal ions hydrolysis pH 10	
Nickel, Zinc and Copper	31
Goethite, Kalonite and Rutile	30

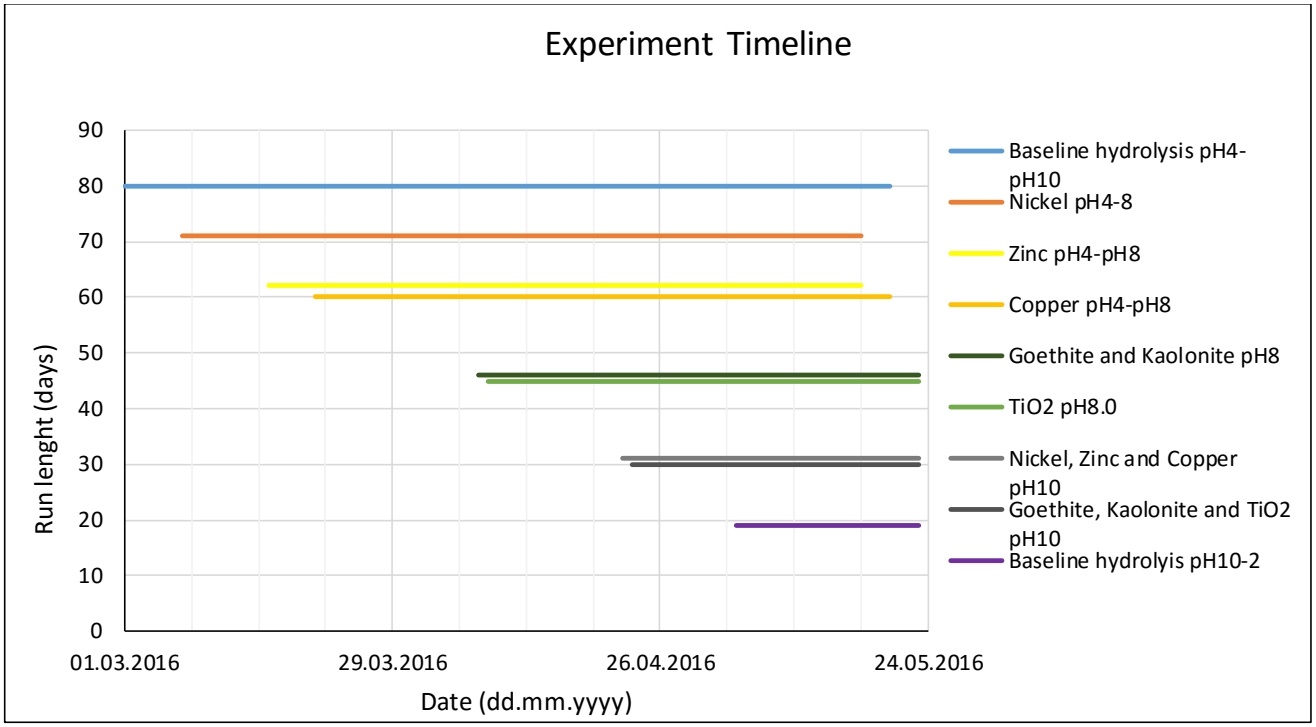


Figure 3-1. Timeline of experiments.

All glassware used for the experiments were soaked in a 1 M HNO_3 acid bath for a minimum of twelve hours, to eliminate any metal contamination. After the acid bath, the glassware was rinsed ten times in flowing Milli-Q water and set to dry off on paper towels. The last drops of water were eliminated by putting the glassware in an oven held at 100 degrees Celsius for at least one hour. Glassware that was not new was washed in Alcanox prior to the acid bath and baked at 550 degrees Celsius for four hours to burn off any organic contaminant, after the acid bath.

3.2 Chemicals and reagents

Acetonitrile of ACS grade was purchased from Sigma-Aldrich, St. Lois MO USA. ACS grade Acetonitrile was used for the HPLC mobile phase from 15th of January until 6th of April. From the 7th of April, HPLC-grade Acetonitrile from Fisher Chemicals, NJ USA, was used instead. This was due to observations of more noise in the chromatogram baselines when using ACS-grade acetonitrile in the mobile phase. The change occurred on the same day as the analyses of the mineral experiments started. All water used for the HPLC mobile phase for the preparation of experiments was of Milli-Q quality. Methanol used for stock solutions was purchased from Fischer Chemicals and was of HPLC grade.

Imidacloprid and acetamiprid were purchased from Chem Service, West Chester, PA USA, and were both of 99.5 % purity.

Acetic acid and sodium acetate used to make acetate buffers were purchased from BDH Chemicals, IL USA, and Fischer Chemicals respectively. Both chemicals were ACS-grade, the acetic acid was of 99.9% purity, and the sodium acetate was of 99.5% purity. MOPS used for buffer solutions was purchased from Sigma-Aldrich, St. Lois USA, and had 99.5% purity. BORAX used for borate buffer solution was purchased from Fischer Chemicals, was ACS-grade and had an assayed purity of 102.2%.

Nickel^{II} chloride hexahydrate and zinc^{II} chloride used for experiments were purchased from Sigma-Aldrich St. Louis USA, and were of 99.9% purity and >98% purity respectively. Copper^{II} chloride dehydrate was purchased from Acros Organics, NJ USA, and was of 99% purity.

Kaolinite clay, type KGa-1b, was purchased from Clay Mineral Society, USA. TiO_2, type P25 was purchased from Degussa AG, USA. Goethite was synthesized and characterized by Chan Lan Chun, University of Minnesota, according to methods described by Chun et al. (2006).

3.3 Stock solutions

Stock solutions of neonicotinoids were prepared in 100% methanol to prevent any hydrolytic degradation of the stock solution. The stock solutions were stored at 4°C in a refrigerator, when not in use. The volume of methanol is temperature-dependent, and thus the stock solutions needed to be brought to room temperature before use. This was done with aluminum foil wrapped around the flasks to prevent any effects of photolytic degradation. The stock solutions were prepared as 1 mM pesticide in 10 mL volumetric flasks. Due to the small amounts of powdered pesticide this required, 2.2 mg and 2.6 mg, for acetamiprid and imidacloprid respectively, several challenges occurred. The electrostatic properties of the pesticides made it difficult to use measuring boats and measuring paper, as it was hard to successfully transfer the entire pesticide amount from the measuring equipment into the volumetric flask. In order to get all of the measured pesticide within the volumetric flask, the pesticide was weighed in the flask. Using this method, however, there were challenges with measurement accuracy as the number on the analytical balance drifted slowly downwards for more than 30 minutes *after* the balance showed the stability indicator sign. The following procedure was used:

1.) The 10 mL volumetric flask was placed on the analytical balance, and the balance was tared.

2.) The 10 mL volumetric flask was removed, and pesticide was added with a steel spatula directly into the flask.
3.) The first number on the scale that was stable for at least 30 seconds, after the stability indicator sign was given, was used as the measured amount. This usually occurred within two-three minutes after the stability indicator sign was given.
4.) The volumetric flask was then removed from the scale and filled with methanol until 10 mL was reached. To ensure that all the pesticide was dissolved before use, the volumetric flask was thoroughly shaken by hand and left over night in the refrigerator.

Due to difficulties with the weighing of the pesticide, the actual concentrations diverged with up to 12.3 % from the wanted concentration of 1 mM. The actual concentrations of the prepared stock solutions are listed in Table 3-2.

Table 3-2: Stock solutions

Neonicotinoid	Chemical formula	Formula Weight	Measured mass	Stock solution concentration
Imidacloprid	$C_9H_{10}ClN_5O_2$	255.661 g/mol	2.8 mg	1.1 mM
Acetamiprid	$C_{10}H_{11}ClN_4$	222.678 g/mol	2.5 mg	1.1 mM

3.4 Buffer solutions

The buffer solutions were prepared from Milli-Q water with a buffer concentration of 10 mM and a volume of 500 mL. Table 3-3 lists the buffer solutions, at what date they were made and measured vs. ideal pH. The pH 4.0 buffer was made with acetate as the buffer by dissolving 60 mg sodium acetate in Milli-Q water and then adding 0.244 mL acetic acid, and titrated with acetic acid until the desired pH. The buffered solutions for pH 6.33, 7.0 and 8.0 were made using MOPS. 1046 mg powdered MOPS was dissolved in Milli-Q water and titrated with 1 M NaOH and 1M HCl until the desired pH was reached. The buffered solution at pH10 was buffered with borate. 1906mg BORAX was dissolved in Milli-Q water and titrated with 1 M NaOH. The buffered solutions were kept and used for all the experiment conducted until May 2016.

Metal-containing buffer solutions were made on March 2nd, 2016, from the previously made pH 4.0, pH 6.3 and pH 8.0 buffers. They were made by weighing right amount $Me(II)Cl_2$ needed to yield a 1 mM concentration, transferring it to a 100 mL volumetric flask and dissolving it in the designated buffer solution. The pH was then measured again and brought

back to desired levels in the cases where the metal chlorides had lowered it. All measurements of pH were done with a pH meter.

While the nickel chloride dissolved well for the entire pH-range, resulting in no visible precipitation, the zinc and copper chlorides left visible precipitation at both pH 6.3 and pH8. Dilution to half the concentration was attempted for the pH8.0 buffer and pH6.3 buffer solutions with copper but resulted in more visible precipitation. It was therefore decided to rather filter the solutions, and measure the ion concentration with flame atomic absorption spectroscopy (FAAS). In addition to solubility issues, the actual concentrations of the prepared solutions were also influenced by weighing difficulties of the hygroscopic metal chlorides.

Table 3-3: Buffered solutions

Name	Date	Ideal pH	Measured pH	Volume (mL]
pH 4, 10 mM Acetate	19.feb	4.00	4.0	500
pH 6.31, 10 mM MOPS	19.feb	6.31	6.3	500
pH 7.00, 10 mM MOPS	19.feb	7.00	7.0	500
pH 8, 10 mM MOPS	19.feb	8.00	8.0	500
pH10, 10 mM Borax	22.feb	10.00	10.0	500

3.5 Analytical methods

3.5.1 HPLC analysis

Concentrations of imidacloprid and acetamiprid were measured by high-pressure liquid chromatography (HPLC), using an Agilent 1200 HPLC. The mobile phase consisted of an isocratic solution of 30% acetonitrile and 70% Milli-Q water. No buffer was added to avoid biological growth, but the Milli-Q water was exchanged a minimum one time per week. The column used was an Ascentis RP-amide, the flow rate was 1.000 mL/min, and the injection volume was 20 µL. Each aliquot was sampled once, i.e. single injections were used. The detector was a DAD UV lamp, using sample wavelength/bandwidth 254/4 nm, and reference

wavelength/bandwidth 360/100 nm. The runtime was 7 minutes until the mineral experiments were started when it was changed to 10 minutes to avoid runover of signals from the minerals. Each HPLC sequence was randomized and included a blank sample.

3.5.2 FAAS analysis

Dissolved metal concentrations were measured in prepared for the experiments with flame atomic adsorption spectroscopy (FAAS).

Due to the very low concentrations of dissolved metals at pH 10, these samples were measured with other calibration curves than the rest. The samples with pH 10 were also filtered before analysis, unlike the experiment reactors with metal-containing solutions at pH 10.

The lamps used with the FAAS were hollow-cathode Perkin Elmer lamps

Nickel was measured with wavelength 232 nm and slit 0.2 nm, copper was measured with wavelength 324.8 and slit 0.7 nm, Zn was measured with wavelength 213.9 nm and slit 0.7 nm.

3.6 Hydrolysis experiments

The reactors for all experimental conditions were made at 10 mL volumes and spiked with neonicotinoids to a concentration of 10 µM. The reactors were kept in darkness by wrapping them in aluminium foil and kept at room temperature in the laboratory. The laboratory thermometer was stable at 24 ˚C throughout the period. All experiments were performed in duplicates.

3.6.1 Phase 1: Baseline hydrolysis experiments pH4-pH10

Baseline hydrolysis (BH) experiments were performed at five different pH within the range pH 4.0 - 10.0. The experiment reactors were made on 01.03.16 by spiking 9.91 mL of buffer solution with 90 µM neonicotinoid stock solution to obtain 10 mL volumes with 10 µM concentration of acetamiprid or imidacloprid. The reactors were shaken by hand to thoroughly mix the content. 1.5 mL volumes were extracted from each reactor and placed into a 2 mL HPLC vial. The extracted volume was used for all HPLC analyses with the original reactors kept as back up. Near the end of the test period, analyses were done on the back-up reactors as well to check coherence with the concentrations measured from the HPLC-vials.

3.6.2 Phase 2: Hydrolysis experiments with added metal ions at pH 4.0, 6.3 and 8.0

Three metals were tested for their effects on the hydrolysis of acetamiprid and imidacloprid; divalent nickel, zinc and copper. Each of the metals were tested separately at three different pH-values: 4.0, 6.3 and 8.0. Experimental reactors were made by spiking metal buffer solution with neonicotinoid stock solution creating a volume of 10 mL with a 1µM concentration of the neonicotinoid in a 20mL clear scintillation vial. The reactors were then shaken by hand, and filtered through a 0.45 µm filter using a plastic syringe. 1.0 mL volumes were extracted from each reactor and into a 2 mL HPLC vial. The extracted volume was used for all HPLC analyses with the original reactors kept as back up. Near the end of the test period, analyses were done on the back-up reactors as well to check coherence with the concentrations measured from the HPLC-vials. All experiments were conducted in duplicates.

3.6.3 Phase 3: Hydrolysis experiments with added minerals at pH8

Three minerals were tested separately for their effects on the hydrolysis of acetamiprid and imidacloprid: goethite (FeO(OH)), kaolinite ($Al_2Si_2O_5(OH)_4$) and TiO_2. In phase 3, the effects of minerals were only tested at pH 8.0. Reactors were made by weighing up 0.1 g of mineral and adding 9.9 g of pH 8.0 buffer solution into a 20 mL clear scintillation vial. The mineral-buffer suspension was left for equilibration for a minimum of 12 hours before the neonicotinoid was spiked in. All reactors were made in duplicates. The minerals were kept in suspension by using a Glas-Col Rotator (Series number 394624) set to speed 0.3. Samples for analysis was filtered through a 0.45 µm filter using a plastic syringe.

3.6.4 Phase 4: Hydrolysis experiments with added metal ions and minerals at pH10

The effect of three metal ions and three minerals on hydrolysis of acetamiprid and imidacloprid was tested at pH 10. The three metals were divalent nickel, zinc, and copper, and the three minerals were goethite, kaolinite, and rutile. The mineral experiments were done as described above, but with pH 10.0 borate buffer instead of pH 8.0 MOPS buffer. The metal ions experiments were made by mixing 9.9 mL pH 10.0 borate buffer with 100 µL pH 4.0 metal containing acetate buffer, and then spiked with neonicotinoid stock solution, yielding 10 mL experiment reactors with 10 µM neonicotinoid and 10 µM Me(II) added. No precipitation was visible in the experiment reactors, and they were left unfiltered for the entire experiment period. Dissolved metal concentration was measured with FAAS. The pH was confirmed to be at 10.0

by measuring a solution of the same composition. All reactors were made in duplicates. Aliquots taken for analysis were filtered before they were analyzed by the HPLC.

3.7 Sources of error

Possible sources of error for the experiments conducted include imprecision in methods and analytical equipment, human errors, unaccounted influence of temperature, calculation errors and unintended pollution of buffer solutions and experiment reactors from unaccounted routes.

The choice of using Milli-Q-water in the solvent instead of a buffered solution is a source of error in the precision of HPLC measured concentrations. Milli-Q water is susceptible towards pH-changes, which again can influence the sensitivity of the measured neonicotinoid concentration.

To monitor any possible changes in the detection sensitivity of the HPLC, neonicotinoid standards was made on a weekly basis. However, it appeared to be difficult to make good standards due to inaccurate use of pipettes and syringes. First, it was discovered that the use of automatic pipettes with plastic tips was incompatible with the methanol-based stock solutions. Presumably due to interactions between the organic solvent and the plastic, the standards were inaccurately made and varied a lot. The use of plastic pipettes was upheld until the 15th of February when standards were made with glass syringes in stead. The switch to glass syringes improved the accuracy. However, there were still fluctuations in standard concentrations that did not match the fluctuations in non-degradable experiment concentrations. Thus it was concluded that the standard fluctuations were more probable to be caused by poor syringe technique rather than changes in the HPLC-sensitivity. The syringe technique applied was to draw up more volume than needed, then turning the syringe 180 degrees so that the needle is pointing upwards, with the syringe in eye height the excess volume was pressed out. This technique was supposed to eliminate parallax errors. However, it proved difficult to move the syringe from this position, and into the container of interest, without moving the piston. In addition, there was sometimes difficulties with getting rid of air bubbles.

Another source of error regarding making the standards was the use of automatic pipettes for measuring the water component of the standards. It was observed that some pipette tips was unable to fill up completely. While the pipette tips this was seen on was discarded and not used, it is not certain that all dysfunctional pipette tips were discovered.

The stock solutions used to make all the experiments and all the standards were not exchanged. Thus there is a possibility that either degradation of the pesticide or evaporation of the methanol

could have changed the concentration of stock solutions, and thus have affected the concentrations of the standards.

Instead of using a calibration curves made at the start of the experiment period, it was decided to use one that was made at a time when syringe and pipette technique had improved. However, this means that any change in instrument sensitivity has not been accounted for, and thus it is a potential source of error. Calibration curves can be seen in Appendix 2: Figure A2-1 and Figure A2-2.

4 Results and discussion

4.1 Phase 1: Baseline hydrolysis experiments pH4-pH10

Imidacloprid and acetamiprid showed no detectable degradation during the 80 days of baseline hydrolysis (BH) experiments at pH 4.0, 6.3, 7.0 and 8.0 (see Figure 4-1). Zheng and Liu (1999) documented imidacloprid to degrade 1.5% at pH 7.0 and 5% at pH8.0 by the end of three months. A similar degradation was therefore expected to take place for imidacloprid during the 80-days experimental period. Acetamiprid was reported to be less persistent than imidacloprid at pH4.0 by Guzsvány et al. (2006) (the author did not quantify this result), thus it was expected that acetamiprid would hydrolyse at acidic pH .

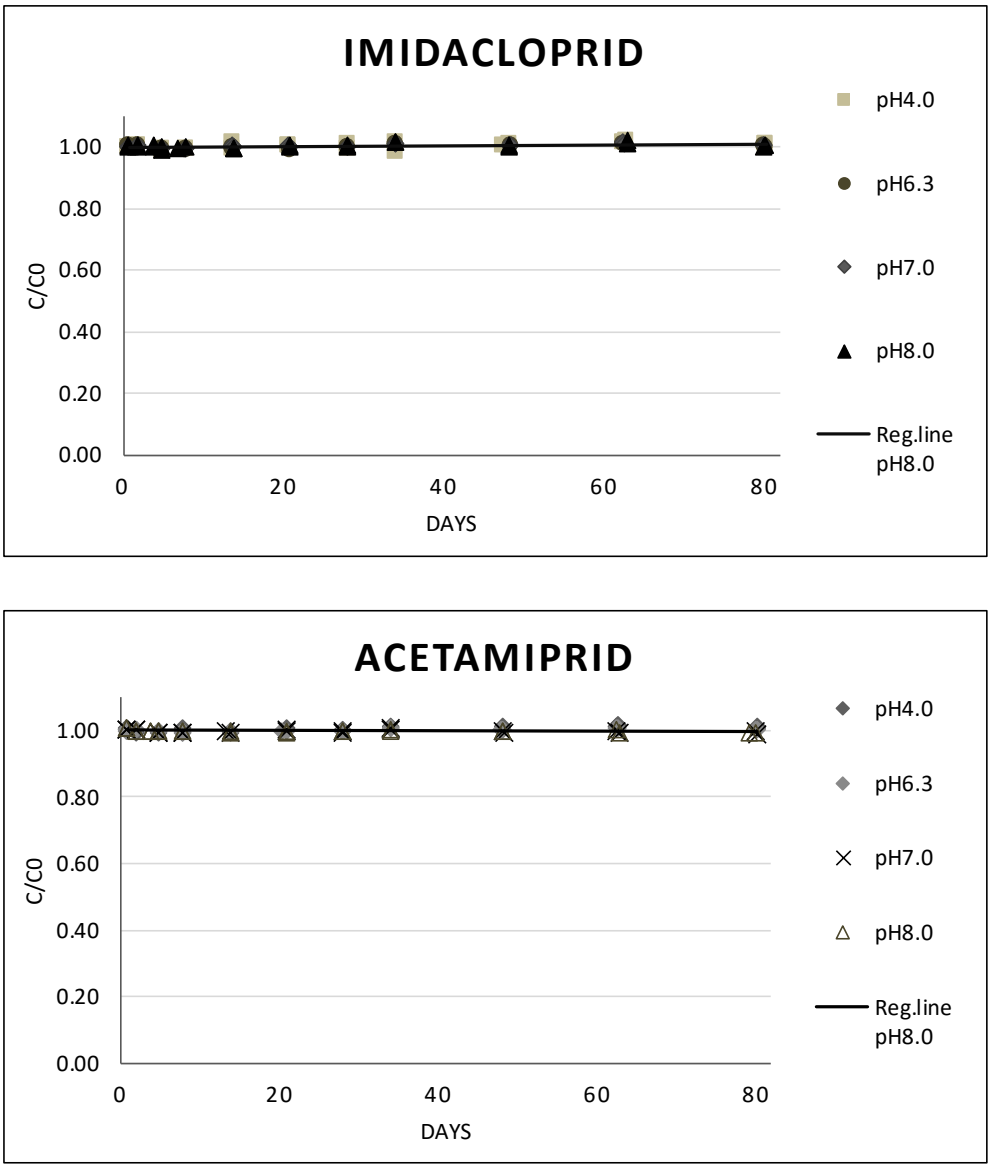


Figure 4-1: Baseline hydrolysis of imidacloprid (topmost) and acetamiprid (bottom) pH4-pH8

It seems as though the sensitivity of the HPLC method used did not allow for such small detections, as nearly all experiments within this pH-range actually had a slight positive trend. Furthermore, the data showed that the measured concentration of imidacloprid and acetamiprid often varied with ± 1%, and sometimes more. As discussed in section *3.7 - Sources of error*, the use of Milli-Q water in the mobile phase might have led decreased sensitivity. Since it is not buffered, the Milli-Q water is very susceptible for pH-changes. Thus the pH of the mobile phase might have varied between different runs and altered the sensitivity of the instrument. The HPLC method described by Zheng and Liu (1999) used acetonitrile, methanol, and water in the proportion 20:20:60, with the water being a phosphate buffer with pH 4. This would probably be a better choice with respect to signal stability. However, while Zheng and Liu (1999) reported that the hydrolysis rate is dependent on temperature, they did not report at what temperature the experiments at different pH was kept at. Thus the comparison lacks a crucial piece of information. Another possible explanation for the lack of degradation that should be explored is potential inhibiting effect by the MOPS and acetate buffers.

These baseline hydrolysis experiments showed a poor fit to first-order kinetics. The analysis of the back-up reactors that was done near the end of the experimental period also showed no detectable degradation for baseline hydrolysis experiment at pH 4.0-8.0. These analysis results are shown in individual plots for all experimental conditions, in Appendix 1, figure 1-7 (imidacloprid) and figure 34-40 (acetamiprid).

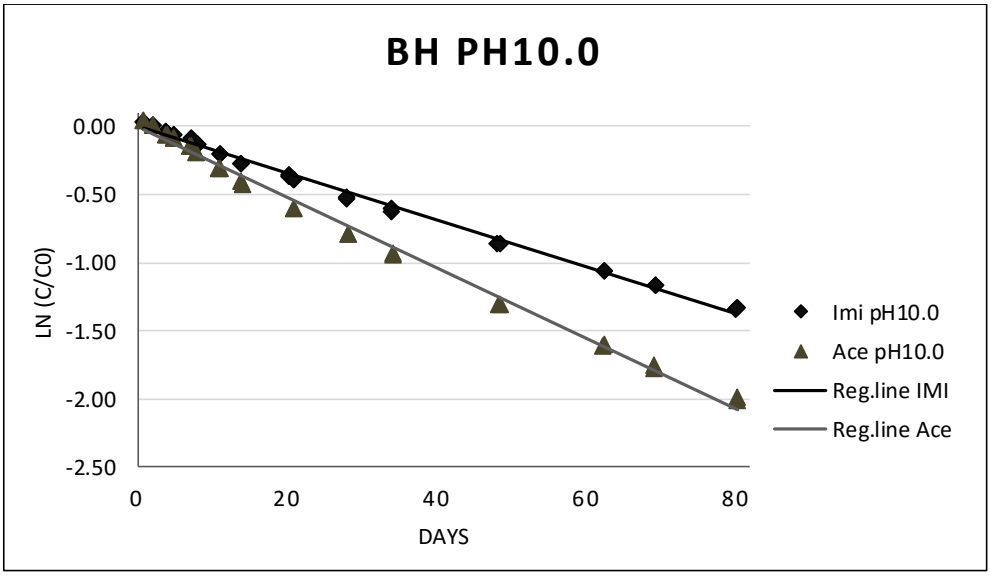


Figure 4-2: Imidacloprid and acetamiprid hydrolysis at pH10

At pH10, both compounds degraded considerably, with detectable degradation already after two days. The degradation data showed a good fit with first-order kinetics, as shown in Figure 4-2. During the 80 days test-period, imidacloprid degraded down to 27% of the initial concentration, and acetamiprid to 12% of the initial concentration. Making acetamiprid the least persistent of the two at high pH. Detected half-lives for the experiments were 40.4 days for imidacloprid and 26.8 days for acetamiprid. In comparison, Zheng and Liu (1999) found imidacloprid half-lives at pH10.8 and 11.8 to be 20.0, and 2.9 days respectively (see Table 2-4). Thus, the results obtained on imidacloprid fits the trend from earlier research. Degradation parameters for imidacloprid and acetamiprid hydrolysis at pH 10 are listed in

Table 4-1.

The analysis of the back-up reactors showed less degradation for BH pH 10.0 experiments (Figure A1-5 and A1-38). A pH-measurement of the backup reactors confirmed that the pH was still 10. Thus it did not occur due to decreased pH. Another possibility is that some solution evaporated due to improper sealing. Neonicotinoids are non-volatile, so this would have led to an increased concentration.

The general trend of increased degradation with increased pH for both compounds is consistent with the earlier studies in neonicotinoid hydrolysis, where both imidacloprid and thiamethoxam (both compounds within the N-nitroguanidine group) showed little to no hydrolytic degradation at acidic and neutral pH and less persistence at higher pH. Thiamethoxam has been shown to degrade more rapidly than imidacloprid in earlier studies (Guzsvány et al., 2006, Karmakar et al., 2009). Karmakar et al. (2009) found the half-life to be 2.1 days at pH 9.2, indicating that also acetamiprid is less degradable than thiamethoxam. It should be noted that Karmakar et al. reported that the experiments were done at 28˚C, which is higher than the temperature was for the experiments in this study (24˚C).

Table 4-1: Degradation parameters for pH10 hydrolysis experiments

Hydrolysis equation: $Ln(C/C_0) = - k*t + B$				
Condition	**K (uM/d)**	**B**	**r^2**	**$t_{0.5}$ (days)**
Imidacloprid				
BH	0.0172	0.0000	0.9953	40.4
BH*	0.0169	0.0000	0.9664	41.1
Cu	0.0135	0.0000	0.9604	51.3
Ni	0.0140	0.0000	0.9814	49.6
Zn	0.0135	0.0000	0.9931	51.3
Goethite	0.0134	0.0000	0.9876	51.6
Kaolonite	0.0136	0.0000	0.0000	51.0
TiO_2	0.0127	0.0000	0.9523	54.6
Acetamiprid				
BH	0.0266	0.0000	0.9948	26.8
BH*	0.0264	0.0000	0.9870	27.9
Cu	0.0213	0.0000	0.9984	33.2
Ni	0.0213	0.0000	0.9972	33.5
Zn	0.0212	0.0000	0.9963	33.8
Goethite	0.0240	0.0000	0.9816	31.0
Kaolonite	0.0219	0.0000	0.9948	32.9
TiO2	0.0214	0.0001	0.9655	35.7

4.2 Metal speciation

Concentrations of dissolved metal species were measured with atomic adsorption spectrometry in solutions consisting of the same chemical composition as the hydrolysis experiments. Measured concentrations of dissolved metal species at the different pH is listed in Table 4-2, together with theoretical values as calculated with the software *Visual* MINTEQ.

Table 4-2: Theoretical concentrations calculated using MINTEQ vs. concentrations measured by atomic sorption spectroscopy.

Dissolved Me(II) Concentrations [mM]		
	MINTEQ	**Measured**
Cu pH 4.0	1.0	1.2
Cu pH 6.3	0.9	0.3
Cu pH 8.0	0.4	0.003
Cu pH 10.0	0.01	Not detectable
Ni pH 4.0	1.0	1.0
Ni pH 6.3	1.0	1.0
Ni pH 8.0	1.0	0.8
Ni pH 10.0	0.01	0.00001
Zn pH 4.0	1.0	1.0
Zn pH 6.3	1.0	1.1
Zn pH 8.0	0.8	0.1
Zn pH10.0	0.01	0.007

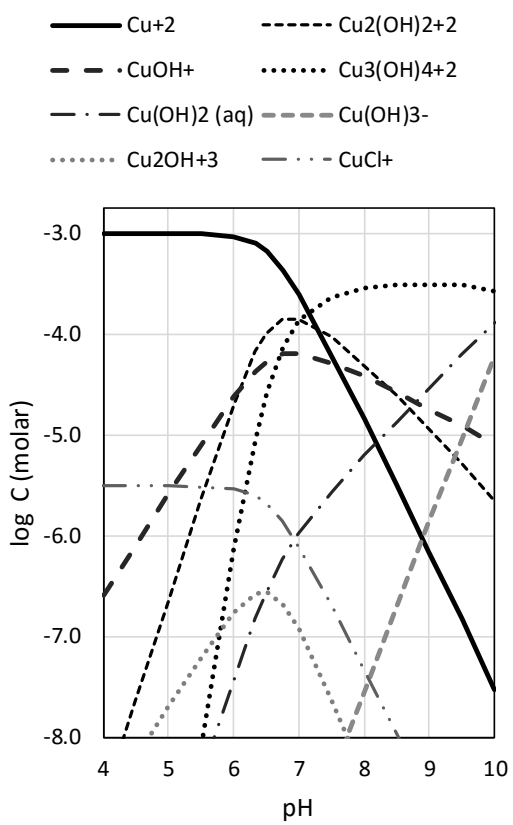
As the metal containing pH10.0 reactors were unfiltered, the total metal concentration was 10 µM. Undissolved species might therefore also have played a part in the hydrolysis inhibition. Figure 4-3 shows metal ion speciation for copper, nickel, and zinc. The diagrams were made by using the software *Visual* MINTEQ for species calculations. The most apparent observation from Table 4-2 is that the concentrations measured at the higher pH-values are consistently lower than the ones calculated by MINTEQ. For Cu^{II}, pH6.3 and pH8.0 and Zn pH8.0, precipitate was visible upon making of the solutions. Thus it seems natural that the measured concentrations would be smaller than the initially added 1mM. For all pH10 solutions, with a concentration of 10µM, no precipitate was visible in the solutions. The concentration is however so small that they might be under the reliable limit of quantification of the instrument used. Another inconsistency is that the speciation diagram created differ from those created by

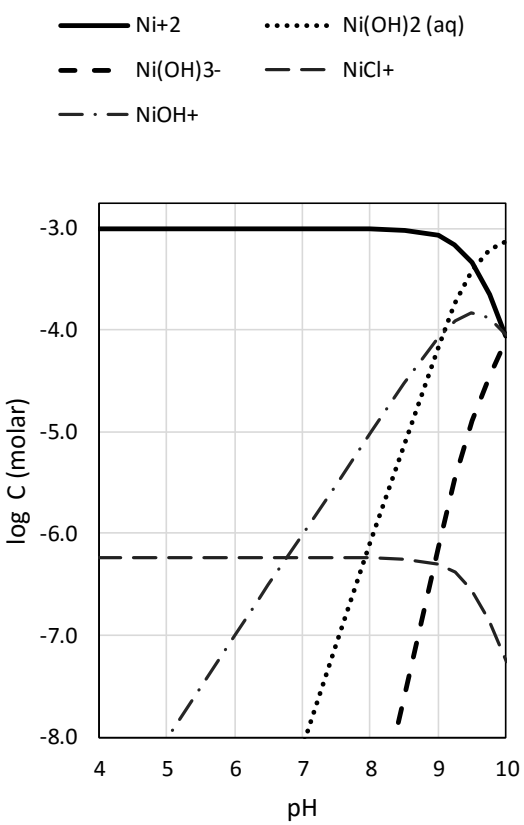
Smolen and Stone (1997) using the software HYDRAQL, where dissolved species of Cu^{II} started to decline around pH 7.0 The largest difference in the diagrams by Smolen and Stone (1997) is that $Cu_3(OH)_4^{2+}$ peaks at ~pH6.5 at concentration $10^{-5.5}$ M.

In comparison, the MINEQL diagram shows $Cu_3(OH)_4^{2+}$ as the dominant species after pH 7, with a concentration of ~0.26 mM from pH 8.0 to pH10.0. It is not known why these differences have occurred.

Calculations that took into consideration the levels of added Na^+, acetate and MOPS were also done in MINEQL. The results showed that 4% of dissolved Zn and 16 % of dissolved Cu at pH4.0 formed complexes with acetate. It was not possible to include borate in the calculations as the compound is not a part of the MINEQL database. Thus, it is not possible to predict whether considerable amounts of borate species complexed with metal species.

It should be noted that metal speciation in natural waters can be quite different from the speciation obtained in ultrapure Milli-Q water, as natural waters will contain a variety of organic and inorganic compounds that can affect the results.





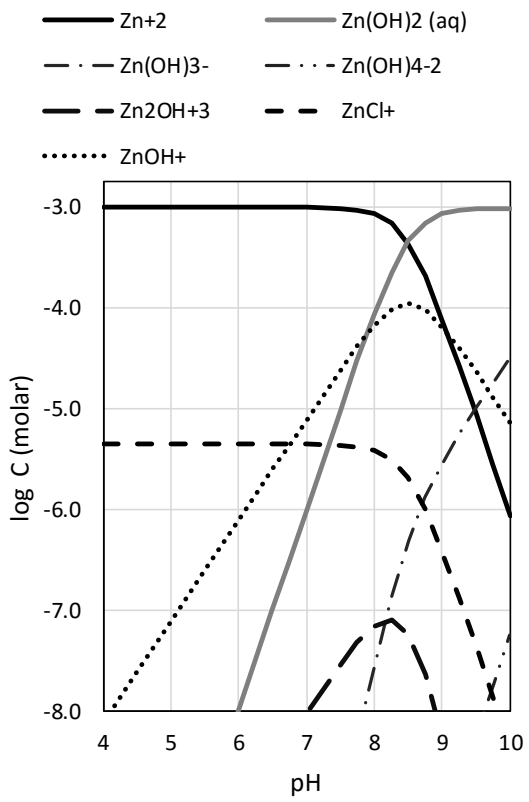


Figure 4-3: Copper, nickel, and zinc speciation diagrams for 1 mM Me(II). The diagrams are made by using the software "Visual MINTEQ" for calculations.

4.3 Phase 2, 3 and 4: Metal and mineral hydrolysis experiments

Similarly, to the baseline hydrolysis experiments, no degradation was detected at pH4.0, pH6.3 or pH8.0 in the experiments with added metals, for neither of the neonicotinoids. The same applied for the mineral experiments at pH8.0. The presence of metal ions and minerals within this pH-range has, therefore, no effect on hydrolysis rates of neonicotinoids. Plots of imidacloprid and acetamiprid hydrolysis in metal-containing solution at pH 8.0 can be viewed in Figure 4-4, hydrolysis plots in mineral containing suspensions can be viewed in Figure 4-5. Individual plots for each metal, mineral and pH can be viewed in figures A8-A25 (imidacloprid) and A49-A58 (acetamiprid) in Appendix 1.

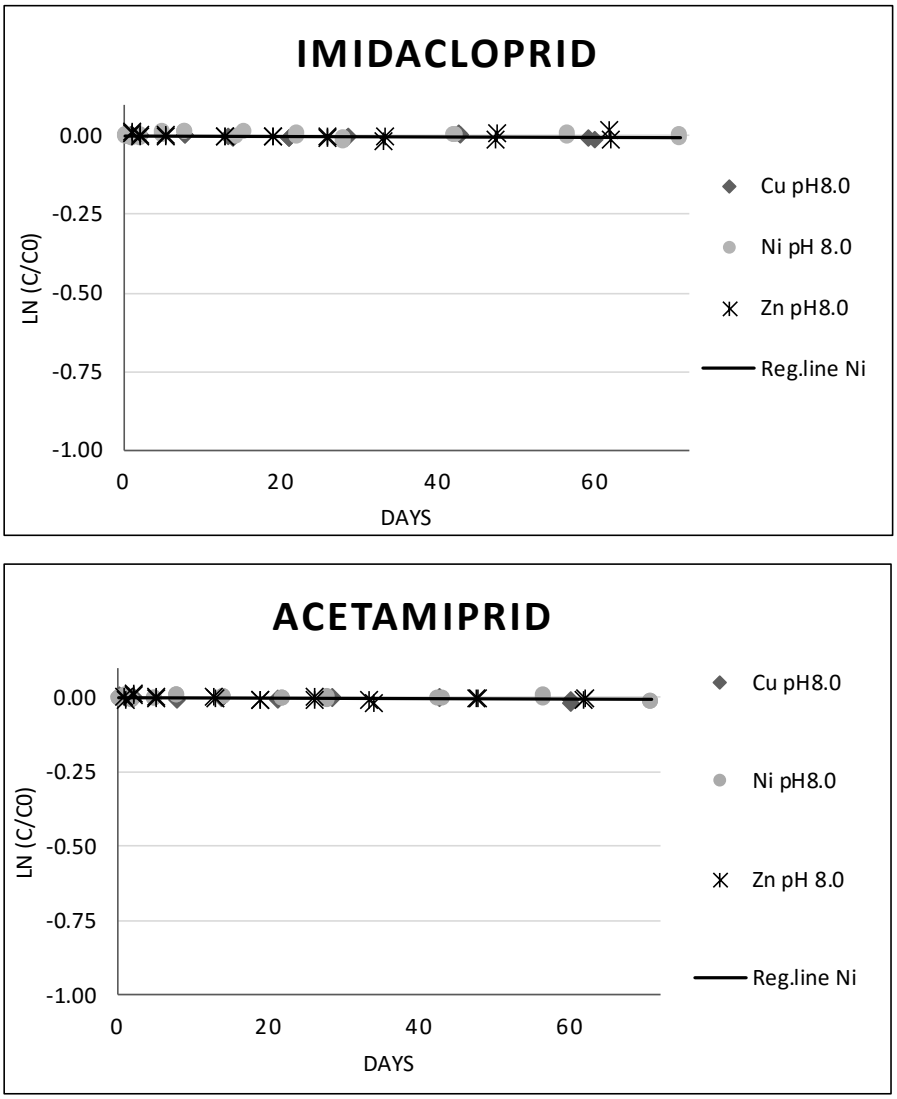


Figure 4-4: Hydrolysis of imidacloprid and acetamiprid in metal-containing solutions at pH 8.0.

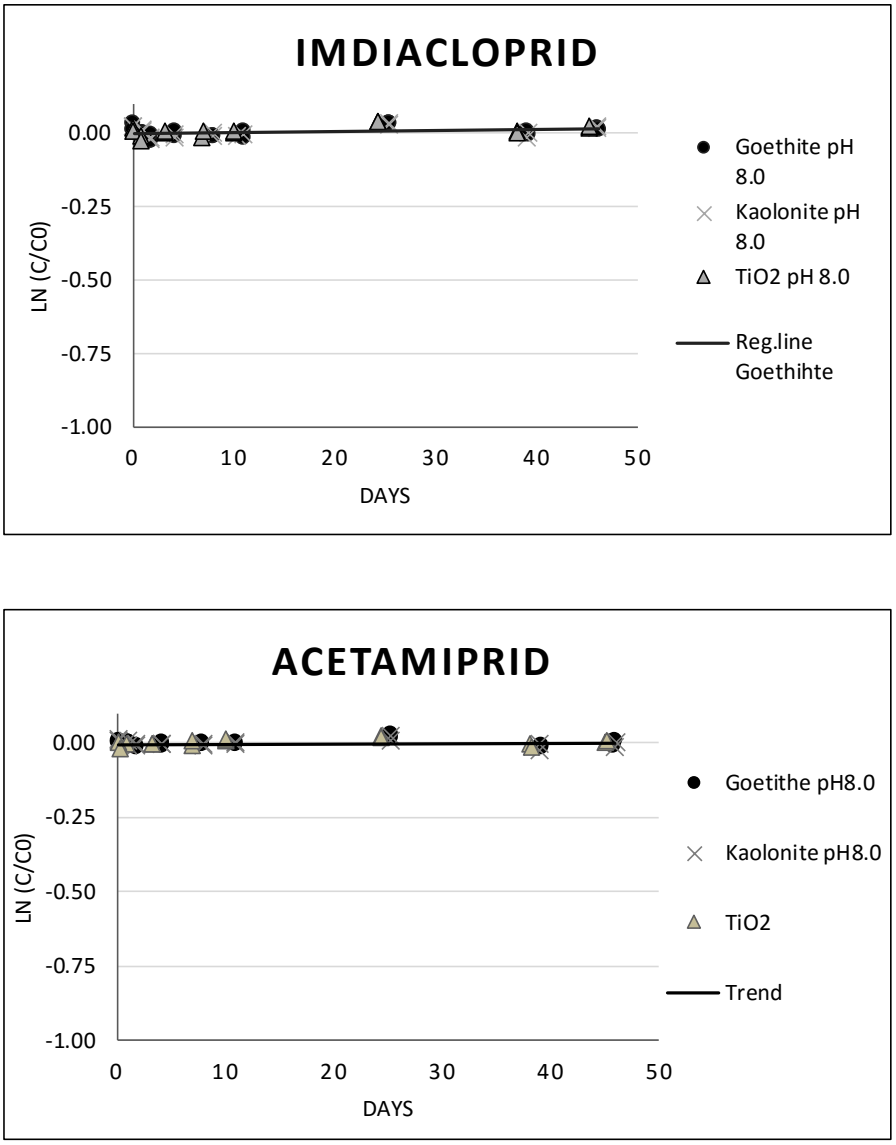


Figure 4-5: Hydrolysis of imidacloprid and acetamiprid in mineral suspensions at pH 8.0

At pH10.0, both imidacloprid and acetamiprid showed decreased degradation for all metals and minerals tested, indicating that copper, nickel, zinc, goethite, kaolinite and TiO_2 all have an inhibiting effect on imidacloprid and acetamiprid hydrolysis (figures 27-32 and 60-65 in the appendix). As the mineral and metal pH10.0 experiments were started about 53 days later than the baseline hydrolysis experiments, but using the same pH10.0 buffer solution, a second round of baseline hydrolysis pH10.0 experiments (denoted BH* in figures) were initiated, to see if the declined hydrolysis rate could be explained by changes in the buffer solution (figures 25 and 59 in the appendix).

Figure 4-6 and Figure 4-7 show the detected degradation constants for all pH10 experiments, for imidacloprid and acetamiprid respectively. Included in the figures are the 95% confidence intervals for each observation. As can be seen, the detected degradation constants, *k*, are quite similar for both rounds of BH experiments, with overlapping confidence intervals, for both neonicotinoids. None of the other experiment conditions have overlapping confidence intervals with the initial BH experiments (however there is a slight overlap between the intervals of the second round of acetamiprid BH experiments and the goethite experiment). Indicating that there is a significant difference in the observed degradation constants on the 0.05 level between the baseline hydrolysis experiments and the metal and mineral hydrolysis experiments, that cannot be explained by changes in the buffer solution.

The increase in half-lives in the metal and mineral experiments was in the range of 23-35% for imidacloprid, and 16-33% for acetamiprid. In comparison, the increase in half-life between round 1 and 2 with BH experiments was 2% for imidacloprid and 4% for acetamiprid. Parameters for all pH10 hydrolysis experiments can be viewed in

Table 4-1.

Interestingly, the inhibiting effect of the different metals and minerals was not successively the same for imidacloprid and acetamiprid, with the exception of TiO_2 that was the most effective inhibitor for both. The inhibiting effects were for imidacloprid in the succession

TiO_2 > Goethite > Zn > Cu > Kaolinite > Ni

and for acetamiprid it was

TiO_2 > Zn > Ni > Cu > Kaolinite > Goethite

It should be pointed out that the absolute differences in half-lives are quite small. The half-lives for imidacloprid were from 50-52 under the influence of Ni, Kaolinite, Cu, Zn and Goethite, and 55 days under the influence of TiO_2. For Acetamiprid the half-lives ranged from 31-34 in the presence of Cu, Ni, Zn, goethite and kaolinite, and 36 days when in the presence of TiO_2.

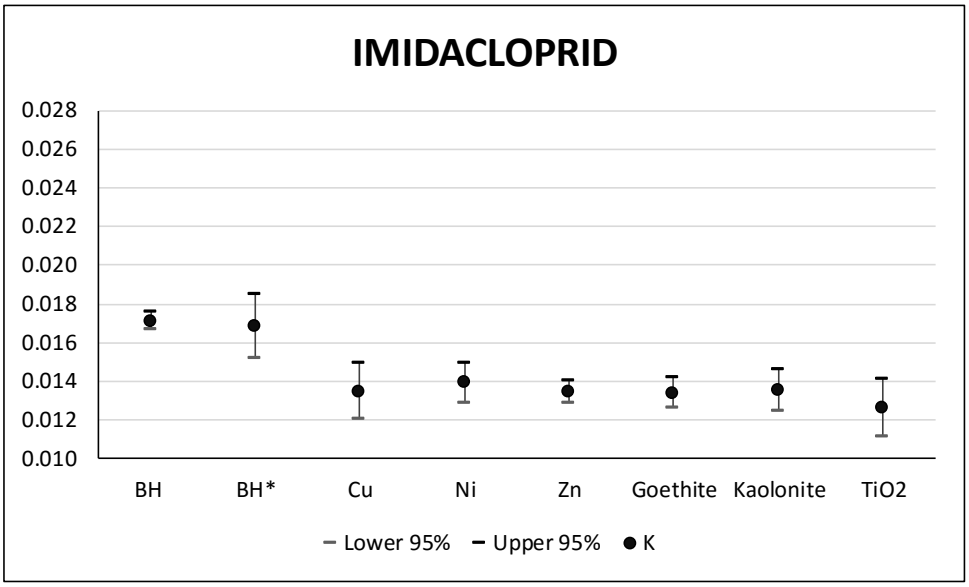


Figure 4-6: Observed degradation constants (K) with 95% confidence intervals for all imidacloprid pH10.0 experiments

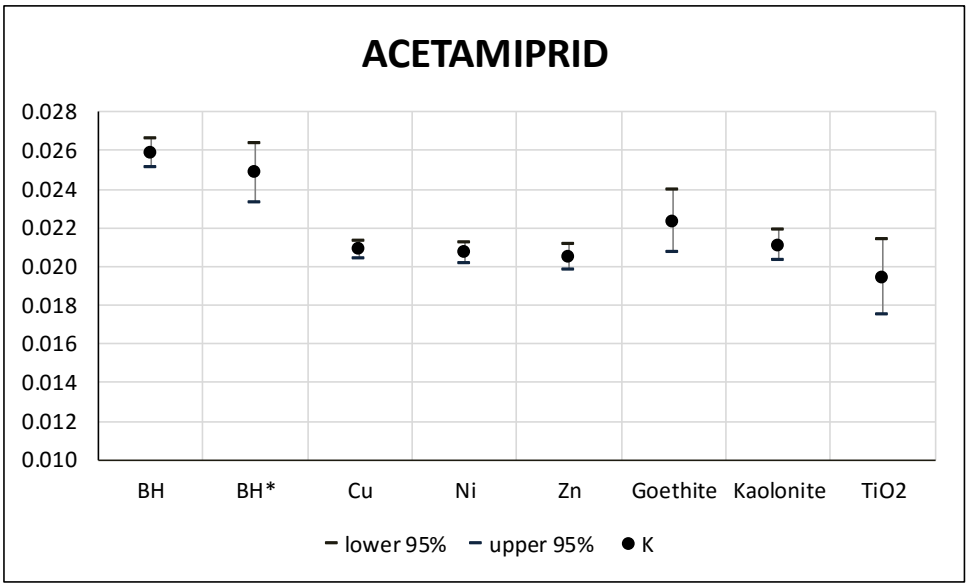


Figure 4-7: Observed degradation constants (K) with 95% confidence intervals for all acetamiprid pH10.0 experiments

As the hydrolysis of both imidacloprid and acetamiprid is pH-dependent, and the metals and minerals used are known to be acidifying, a possible explanation for the slow degradation might be that the solutions were not buffered well enough, and the pH decreased over time. For the pH10 experiments, the pH was measured both at the start and at the end of the experiments. At both timepoints the measured pH was in the range pH 9.9 – 10.0, in all experiment solutions, including those with dissolved metals and minerals. For the experiment in the pH-range pH4.0-

pH8.0 the pH was only verified at the start of the experiments, and thus pH drifting might have occurred in these experiments. However, since the metals and minerals are tested had an inhibiting effect on the hydrolysis, and no degradation was detected for the pH4.0-pH8.0 BH experiments, it is unlikely that a potential pH-drifting had any effect on the results.

4.4 Possible hydrolysis pathways and inhibition mechanisms

The pKa of acetamiprid is 0.7 while Imidacloprid does not dissociate (Bonmatin et al., 2015). Thus, both compounds will be in their neutral form within the pH-range of this study.

The hydrolysis pathway for imidacloprid suggested by Zheng and Liu (1999) and Guzsvány et al. (2006) is by hydroxide attack on the carbon atom on the C=N group (Figure 2-3) resulting in a replacement of the N-NO_2 functional unit with an =O functional unit. As the work of this thesis does not include any identification of degradation products, a confirmation of this hydrolysis pathway is not possible. However, as both the N-NO_2 functional group of imidacloprid and the N-CN functional group of acetamiprid have a strong electron withdrawing character, it can be assumed that both compounds hydrolyze by the functional group acting as the leaving group.

Inhibition of pesticide hydrolysis by Cu, Zn, Ni and Goethite has been documented by Huang and Stone (1999). For the metals, they found that the inhibitory effect was dependent on pH and metal concentration. The strongest inhibitory effect was found by Cu at pH 5.0, and the effect increased with increasing concentration of both Zn and Cu at pH 5.0. As neonicotinoid hydrolysis was only detected at pH10.0, the investigation of these effects was not done. The inhibitory effect of the metals on naptalam hydrolysis decreased in the succession Cu > Zn > Ni, with a significant difference in the effect of each metal (Huang and Stone, 1999). The inhibitory effect on imidacloprid and acetamiprid did not follow the same trend, nor was the difference in effect significant. While the theoretical calculation showed that the concentration of all metals should be 10 uM at pH10, the analytical measurements did not confirm this. Thus it is not possible to say whether the insignificant differences in inhibitory effect observed in this study reflects a true relationship, or whether it is a consequence of different solubility and concentrations.

A proper discussion of which mechanisms it is that determine metal ion-inhibited hydrolysis of imidacloprid and acetamiprid would require an investigation of possible modes of metal ion coordination by imidacloprid and acetamiprid. This means finding possible donor groups on

the compounds (donor ligands). If there are more than one possible donor ligand on each compound, one would need to know which are the better ones. The functional groups of the neonicotinoids are electron withdrawing (Jeschke et al., 2011), and that the earlier proposed hydrolysis mechanism of imidacloprid is by nucleophile attack on the carbon binding to the functional group (Zheng and Liu, 1999). Thus, the N-NO_2 group of imidacloprid and the N-CN group of acetamiprid might be the site for metal-cation coordination.

C. Huang and Stone (1999) reported inhibition of naptalam hydrolysis by metal ions and minerals, and proposed two possible mechanisms for this:

- coordination of the substrate with induced deprotonation, making the site for nucleophilic attack less electrophile
- coordination of an intramolecular nucleophile so that it no longer can assist hydrolysis

C. Huang and Stone tested the same metal ions, and one of the minerals (goetithe), used in this study, and suggested that both proposed inhibition mechanisms might be relevant for both metal ions and minerals. However, more work is needed to determine if inhibition of the neonicotinoids are following the same mechanisms. This includes evaluation of complex capacity between specific metal ions and donor groups on the neonicotinoid molecules, the effect of dissolved metal ions from mineral surfaces in suspension, determination of neonicotinoid adsorption to mineral surfaces and mineral surface properties of the particular minerals used in this study. As can be seen in Table A3-1, values on surface area and surface site density for the three minerals used can be obtained from the literature, but the values can differ considerably between studies.

4.5 Environmental significance of the results

The results obtained in this thesis show no detectable degradation for either acetamiprid or imidacloprid within the pH-range pH4.0-pH8.0. Normal pH of natural waters is considered to be within pH6.0-pH8.0 (USGS, 2016, Ødegaard, 2012). The natural pH of soils can range from 2-11 (Soil Service Division Staff, 1993), but for most crops to thrive the pH should be within the range pH 6.0 pH 7.5 (Smith and Doran, 1996). Based on the results obtained, the hydrolytic contribution to the degradation of imidacloprid and acetamiprid in natural waters and soils seems to be very low. The inhibitory effect of metal ions and minerals at pH10.0 suggest that inhibition might occur at lower pH as well, predicting an even slower hydrolytic degradation. However, if the inhibitory mechanism requires complexation, the contribution of inhibition in natural systems must take into account the possible effect of dissolved organic matter. C. Huang

and Stone (1999) demonstrated that Cu^{II} inhibition of hydrolysis was neutralized in the presence of citric acid. Dissolved organic matter is ubiquitous in natural aquatic systems, and it has the ability to complex with metal species and thus compete with other organics for complexable species. Torrents and Stone (1993) demonstrated that mineral catalysis is neutralized by coadsorbed species. Thus it is plausible that mineral inhibition also can be neutralized in the same way. Another point made by C. Huang and Stone (1999) is how the overall hydrolysis rate requires far less complexation to be affected by catalysis than to be affected by inhibition. They illustrate this with a generalized degradation equation:

4.1 $$-\frac{d[S]}{dt} = rate\ of\ uncomplexed\ S + rate\ of\ complexed\ S$$

S in the formula stands for substrate, the rate is the *hydrolysis rate*. The rate of the uncomplexed and the complexed substrate depends on the concentration of the substrate and the degradation constant. In cases where complexation is very low due to competing species, the rate is far more susceptible of being dominated by a strong catalyst than a strong inhibitor. With a large hydrolysis constant for the complexed species the equation can still be dominated at low concentrations, while an inhibiting mechanism would be overruled by the faster hydrolysis of the uncomplexed species at the same concentration.

5 Conclusion

Neonicotinoids are an important class of chemical insecticides that is widely used all over the world. The widespread use of neonicotinoids is associated with risk to important ecosystem services and non-target organisms (EASAC, 2015). To gain knowledge on the environmental fate of neonicotinoids, it is important to study the different degradation pathways. The work presented in this thesis focus on the hydrolysis of the neonicotinoids acetamiprid and imidacloprid at different pH, and in the presence and absence of different metal ions and minerals.

In metal and mineral free solutions, no degradation was detected from pH 4.0 to pH 8.0 for either neonicotinoid, over the course of 80 days. Earlier research conducted over similar timespans have also reported slow degradation of imidacloprid and acetamiprid within the same pH-range. However, these studies reported small but detectable degradation at certain pH. The biggest difference between the results obtained in this thesis and in earlier studies is for imidacloprid at pH 8.0, where Zheng and Liu (1999) detected 5 % decrease from initial concentration after 90 days. It cannot be concluded what the cause of this difference is, but possible contributing factors are 1) poor analytical sensitivity due to the use of unbuffered Milli-Q water in the HPLC mobile phase, and 2) the experiments might have been carried out at different temperatures. Zheng and Liu (1999) demonstrated that imidacloprid hydrolysis is temperature dependent, but did not report at what temperature their pH-depending experiments were carried out at.

Hydrolysis in metal-containing solutions from pH 4.0 to pH 8.0, and in mineral-containing suspensions at pH 8.0, was also undetectable for both neonicotinoids. At pH 10, both compounds degraded considerably, and the degradation rates were inhibited by all metal ions tested (Cu^{II}, Ni^{II}, and Zn^{II}), and all minerals tested (goethite, kaolinite, and TiO_2). The detected half-lives were 40.2 days for imidacloprid and 26.8 days for acetamiprid in metal and mineral free solution. The effects of the inhibition on half-lives was in the range 23-35% for imidacloprid and 16-33% for acetamiprid. TiO_2 was the strongest inhibitor for both compounds, but there was no clear trend among the other inhibitors.

The results confirm the general trend from earlier research: neonicotinoids are persistent at acidic and neutral pH but hydrolyze at high pH-values. Acetamiprid hydrolysis has not been described in detail in earlier research. The results from this thesis show that acetamiprid behaves similarly to imidacloprid, but degrades more rapidly at pH 10.0. No catalytic effect was found

for either of the metal ions and minerals tested in this study, but all showed an inhibitory effect at pH 10. The presented results clearly demonstrate that hydrolysis of imidacloprid and acetamiprid at pH levels found in natural aquatic systems and soils is a slow degradation process. The significance of inhibition in natural systems is, however, questionable. As demonstrated by C. Huang and Stone (1999), dissolved organic matter, a ubiquitous component of natural aquatic systems, can compete with organic pollutants for complexation with metal ions, and thus neutralize their inhibiting effect on hydrolysis. More research is therefore required to understand the impact of inhibition on imidacloprid and acetamiprid hydrolysis in natural systems. Suggestions for future work in the field of neonicotinoid degradation at environmental conditions is the subject of the next chapter.

6 Future work

The inhibitory effects discovered in this thesis should be further investigated to better understand their impact on degradation in natural systems. This requires identification of donor groups on the neonicotinoid molecules, and the ability of the donor groups to complex free metal ions and surface bound metals at different pH. This must be done together with experiments that can uncover inhibition over a wider pH-range, and at different metal ion and mineral loadings so that an evaluation of observed effects against metal/mineral properties can be done. Mechanism evaluation is described in the studies done by Torrents and Stone (1991), Smolen and Stone (1998), and C. Huang and Stone (1999). It is also important to uncover metal speciation and mineral adsorption in systems that contain the same chemical species as those that are found in natural systems, like dissolved organic matter, and whether this neutralizes the inhibitory effects. A larger assessment of neonicotinoid hydrolysis requires studies on catalysis and inhibition on the five neonicotinoids that were not a part of this study as well.

The environmental fate of neonicotinoids due to hydrolysis is not only dependent on the rate at which they are degraded but also the nature and characteristics of the degradation products. Identification and toxicity testing of degradation products should therefore also be a part of the further work.

Finally, hydrolysis is only one of several degradation mechanisms in natural systems. Other degradation pathways to investigate includes degradation by micro-organisms, redox reactions, and photolysis. Knowledge on which degradation pathways are more efficient is important information for law- and decisionmakers on how, where and when neonicotinoids should be applied in the most sustainable way. This knowledge is also important if treatment of neonicotinoid polluted water is an issue.

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A1 Appendix – 1 Results

The following section contains plots and tables of all individual experiment results, and comparative graphs for pH10.0-experiments. It is sectioned in the following way:

- Imidacloprid baseline hydrolysis pH4 -pH10 (fitted to first order kinetics)
- Imidacloprid copper hydrolysis pH4-pH10 (fitted to first order kinetics)
- Imidacloprid nickel hydrolysis pH4-pH10 (fitted to first order kinetics)
- Imidacloprid zinc hydrolysis pH4-pH10 (fitted to first order kinetics)
- Imidacloprid mineral hydrolysis, goethite kaolinite and rutile, pH8.0 and pH10 (fitted to first order kinetics)
- Imidacloprid pH10.0 comparative graphs between copper, nickel and zinc to baseline hydrolysis (fitted to first order kinetics)
- Acetamiprid baseline hydrolysis pH4 -pH10 (fitted to first order kinetics)
- Acetamiprid copper hydrolysis pH4-pH10 (fitted to first order kinetics)
- Acetamiprid nickel hydrolysis pH4-pH10 (fitted to first order kinetics)
- Acetamiprid zinc hydrolysis pH4-pH10 (fitted to first order kinetics)
- Acetamiprid mineral hydrolysis, goethite kaolinite and rutile, pH8.0 and pH10 (fitted to first order kinetics)
- Acetamiprid pH10.0 comparative graphs between copper, nickel and zinc to baseline hydrolysis (fitted to first order kinetics)
- Half-life comparison
- Tables of degradation constants and half-lives

The graphs are fitted to first order kinetics, with the concentration normalized. C_0 is calculated.

Imidacloprid baseline hydrolysis (BH) at pH 4.0-10.0

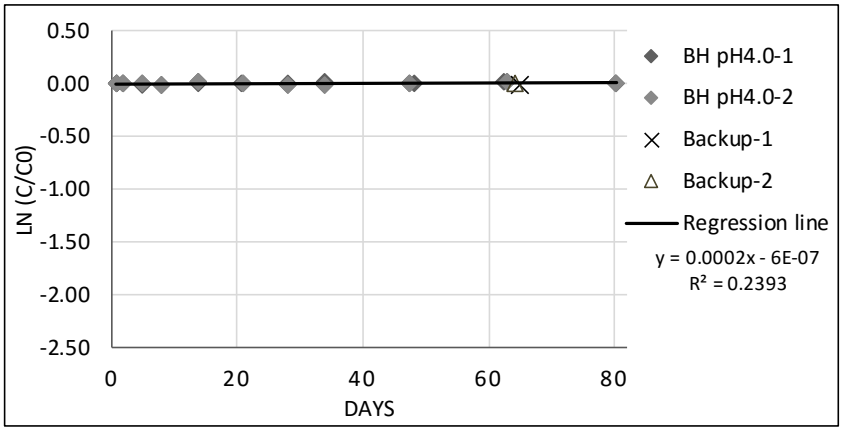


Figure A1-1

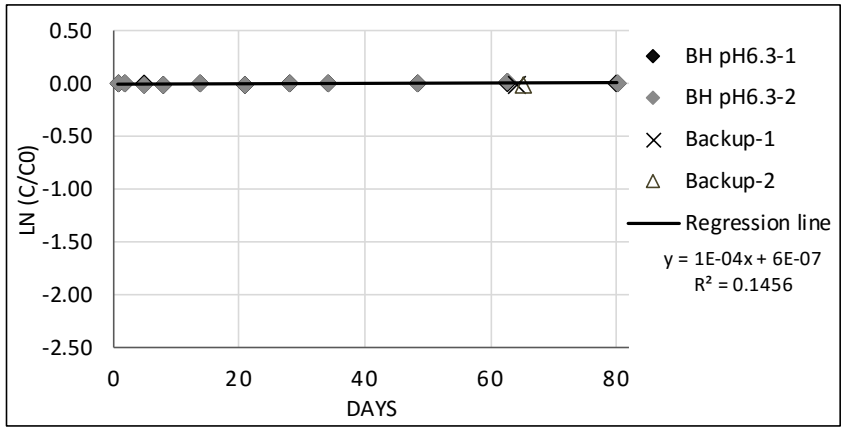


Figure A1-2

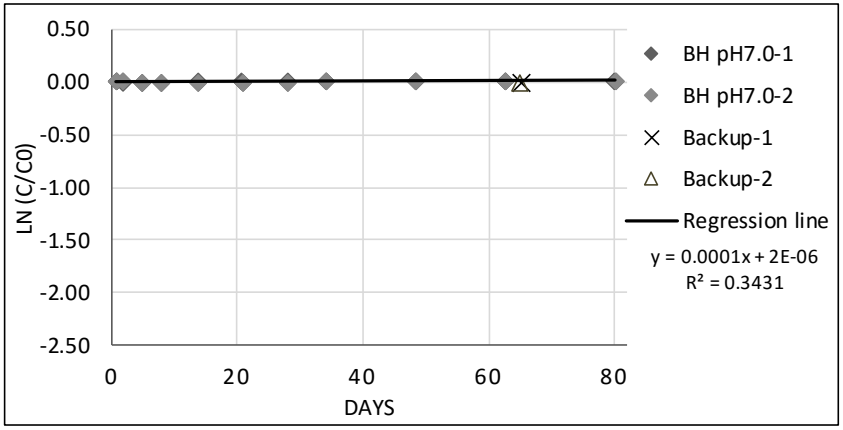


Figure A1-3

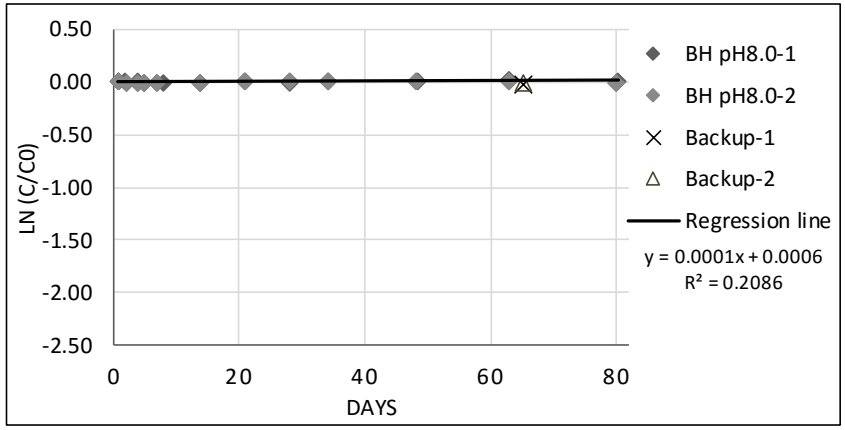


Figure A1-4

Imidacloprid baseline hydrolysis (BH) at pH10.0

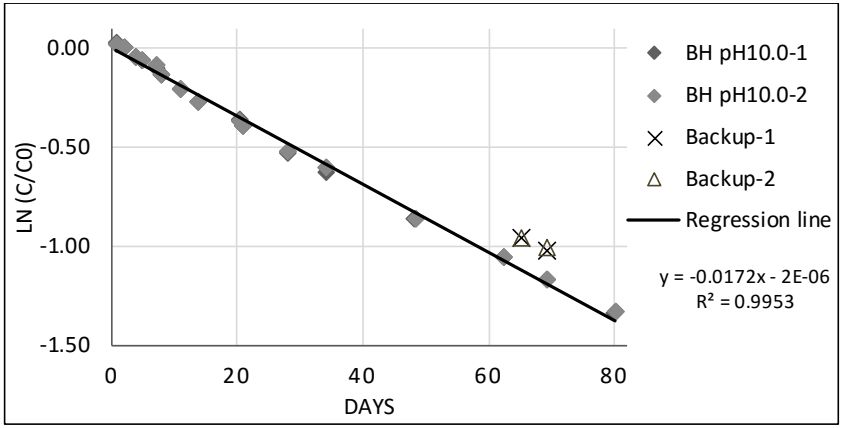


Figure A1-5

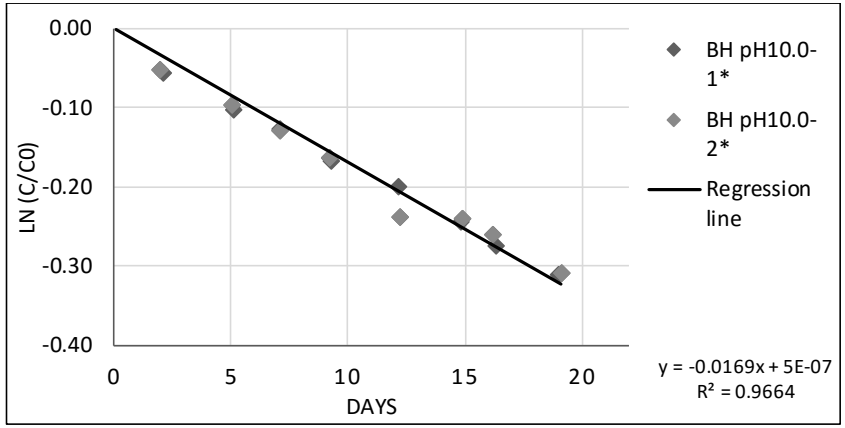


Figure A1-6: second round of BH pH10.0 experiments

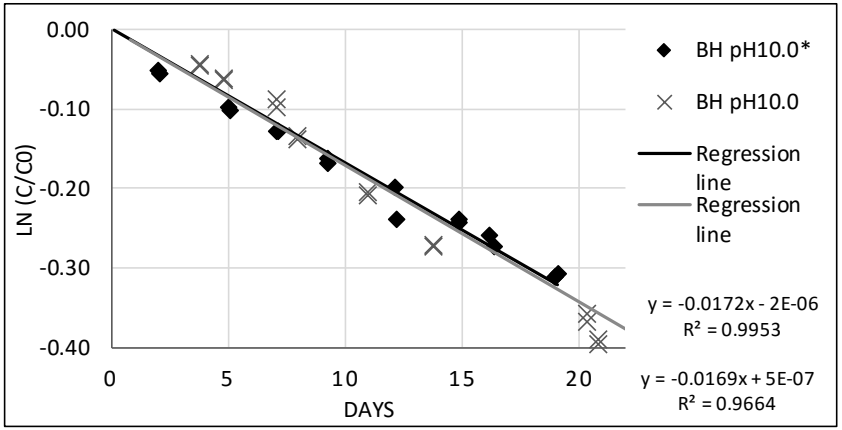


Figure A1-7:Comparison of first and second round of pH10.0 experiments. Note that "days" is the number of days from each experiments individual experiment start

Imidacloprid Cu(II) hydrolysis at pH4.0-pH10.0

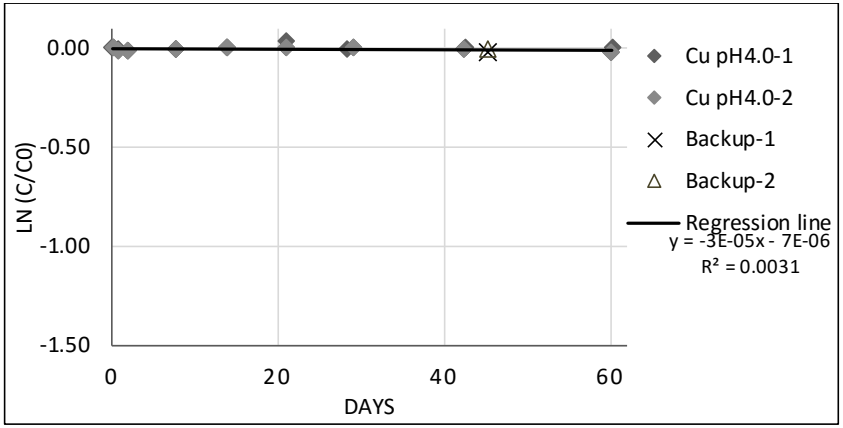


Figure A1-8

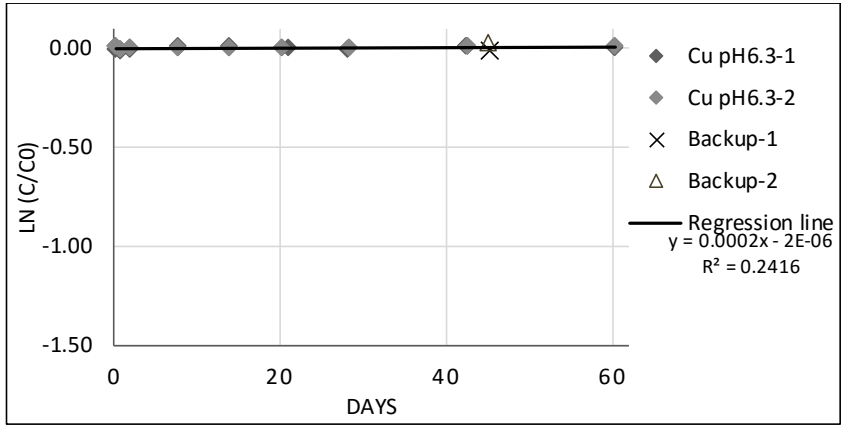


Figure A1-9

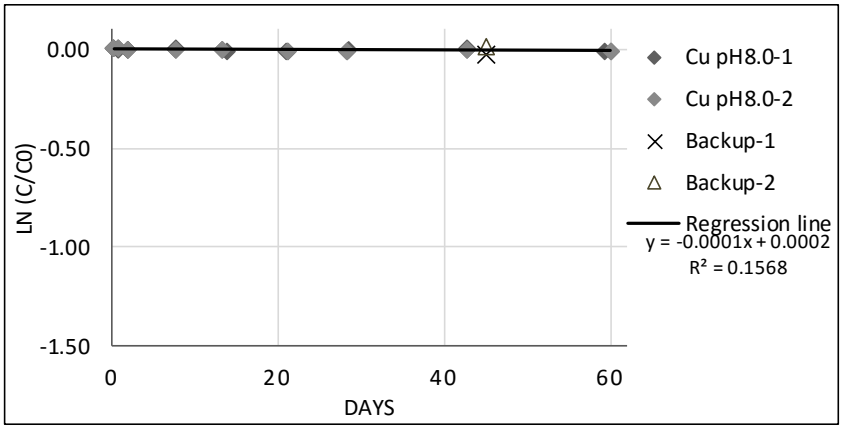


Figure A1-10

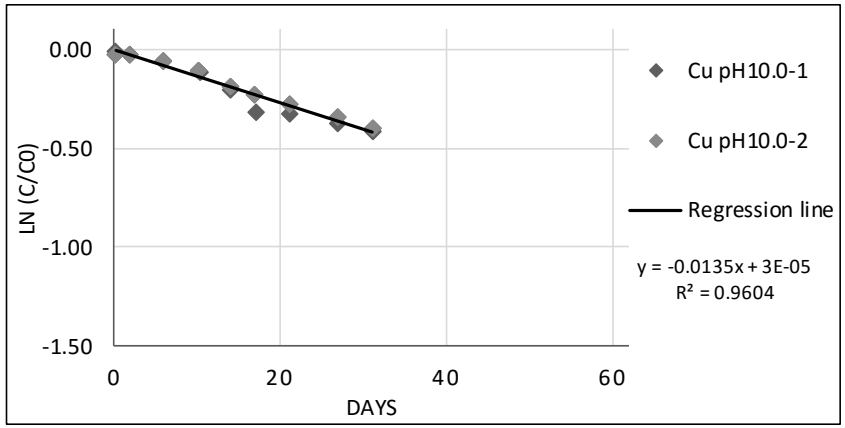


Figure A1-11

Imidacloprid Ni(II) hydrolysis at pH4.0-pH10.0

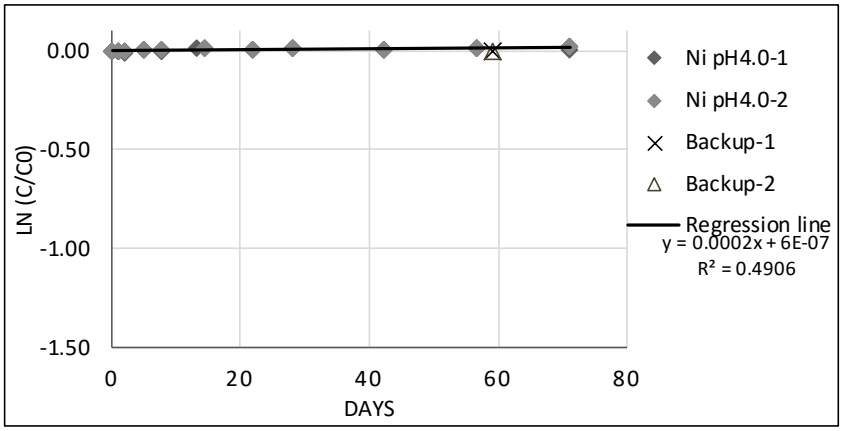


Figure A1-12

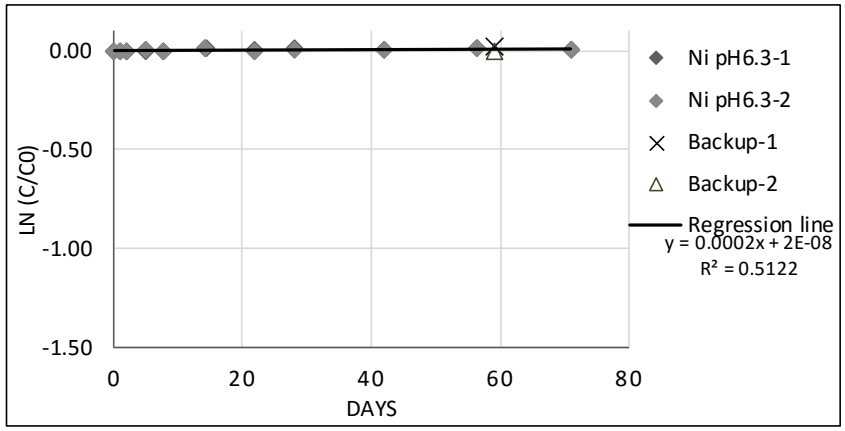


Figure A1-13

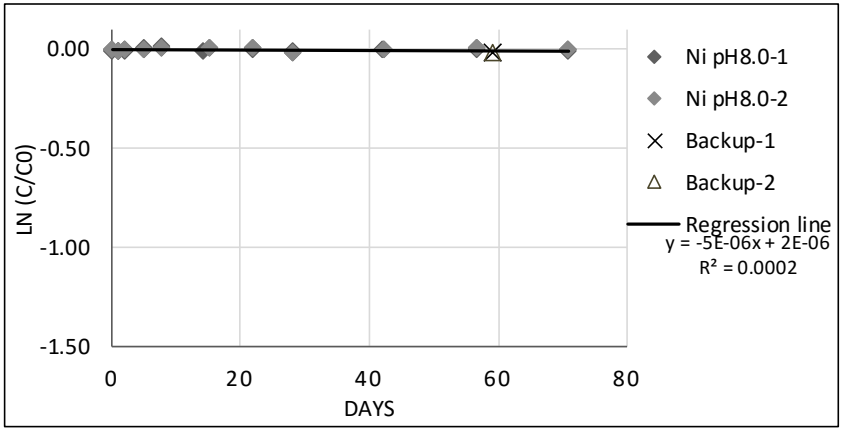


Figure A1-14

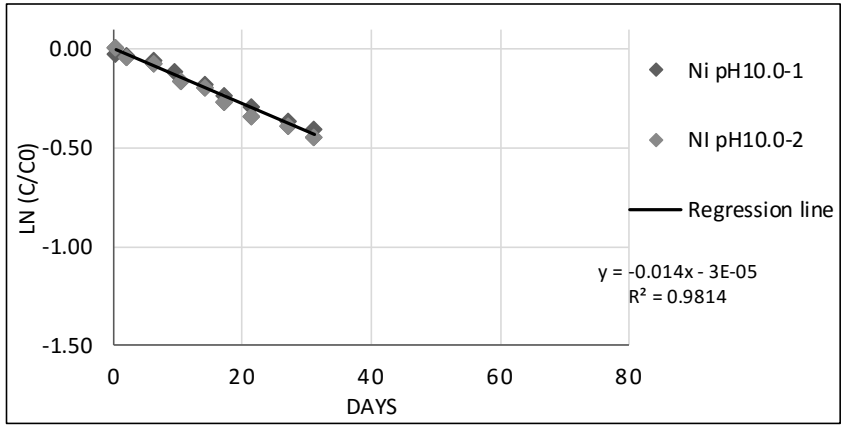


Figure A1-15

Imidacloprid Zn(II) hydrolysis at pH4.0-pH10.0

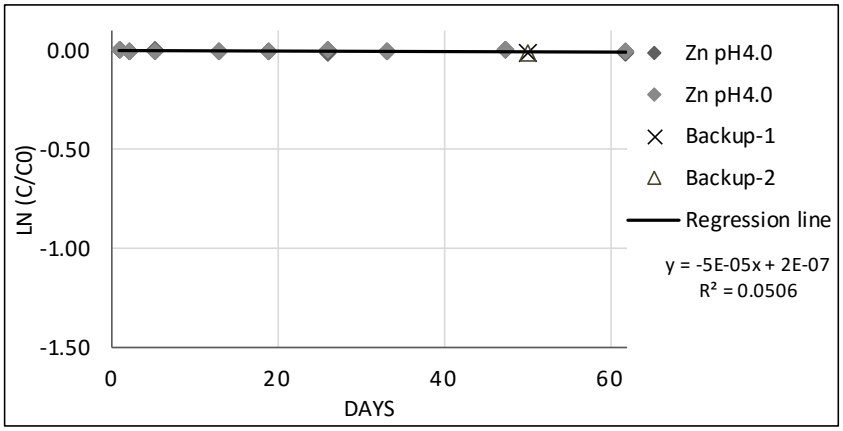


Figure A1-16

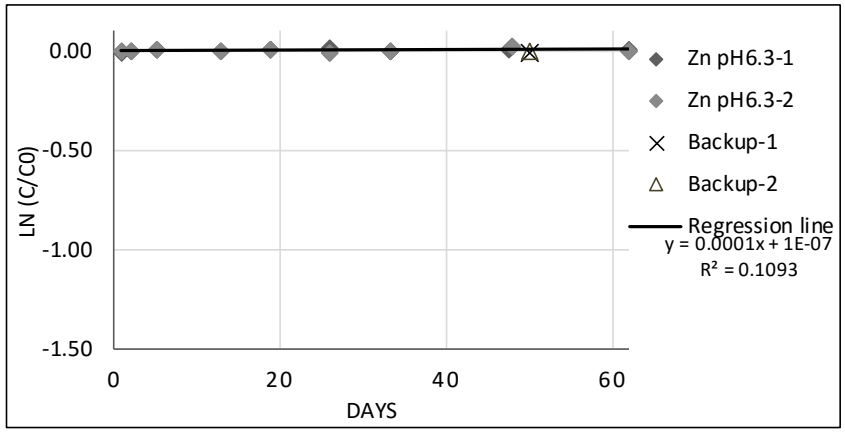


Figure A1-17

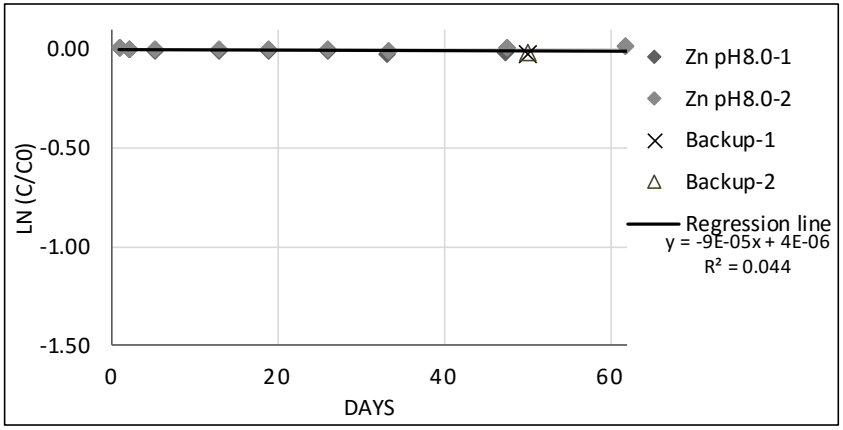


Figure A1-18

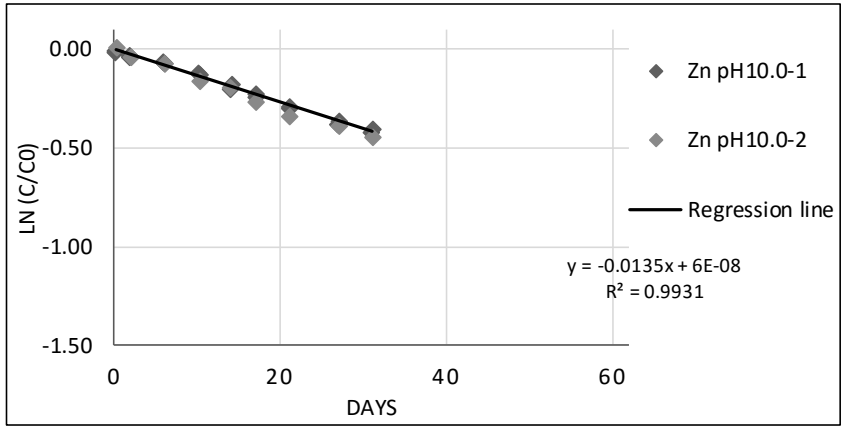


Figure A1-19

Imidacloprid minerals hydrolysis at pH8.0 and pH10.0

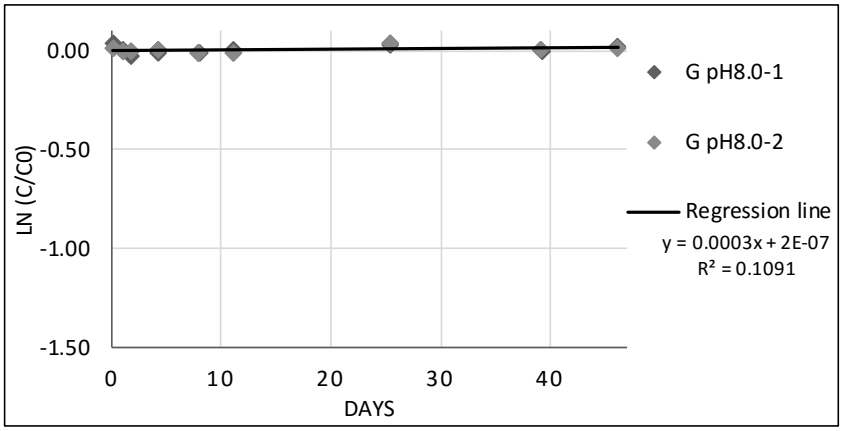


Figure A1-20

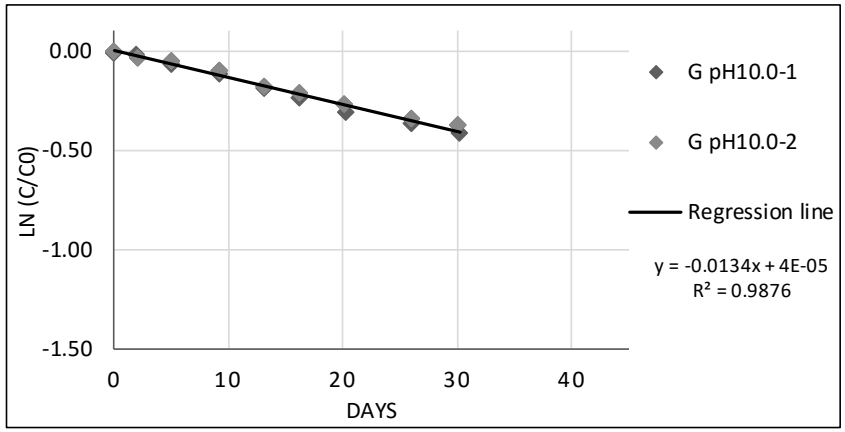
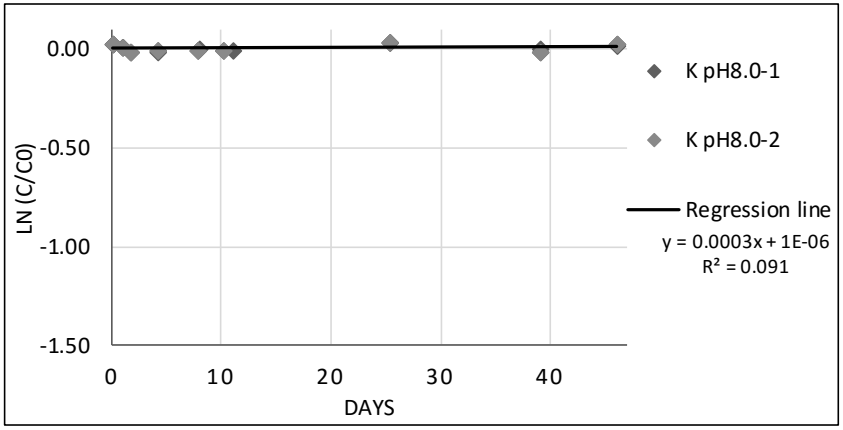


Figure A1-21



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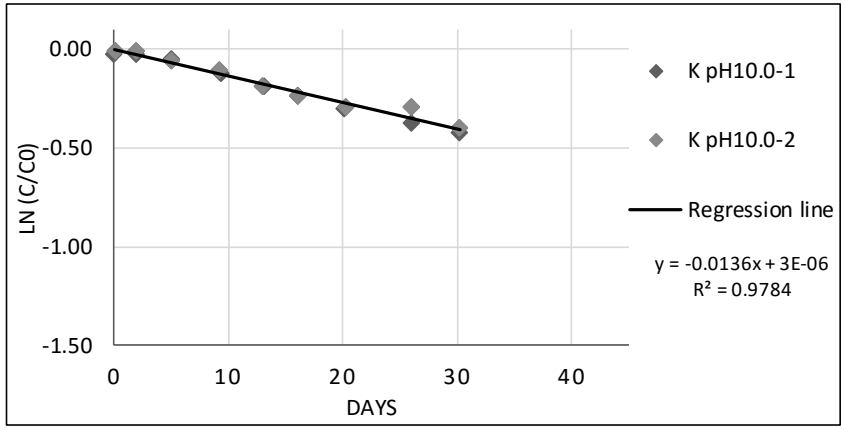


Figure A1-23

Imidacloprid mineral hydrolysis at pH8.0 and 10.0

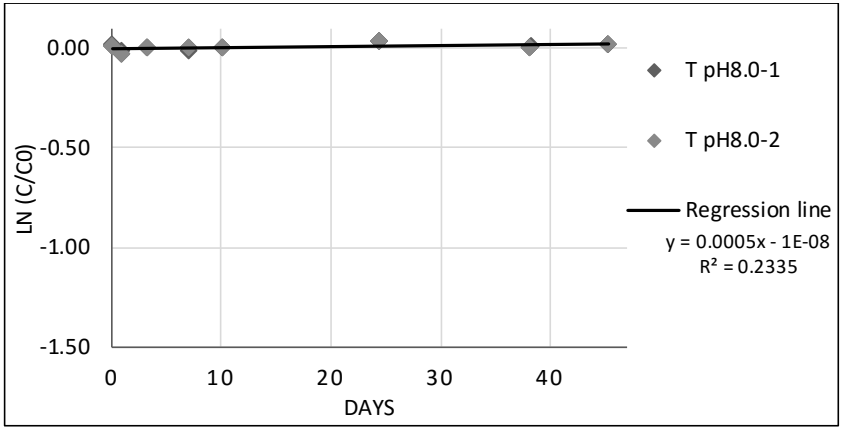


Figure A1-24

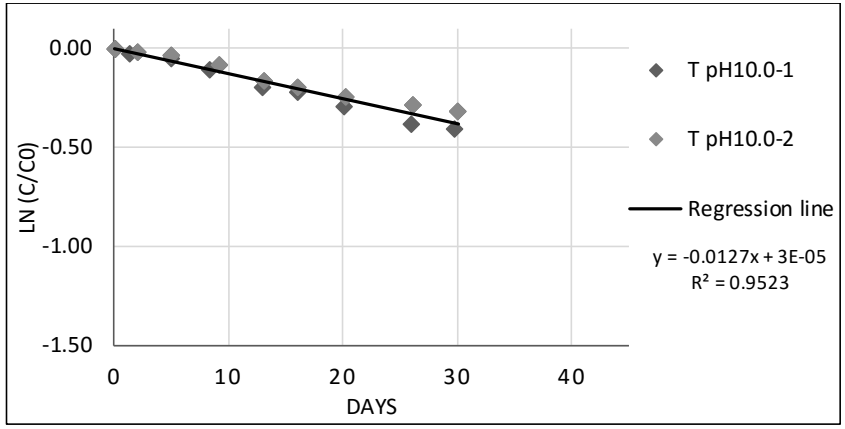


Figure A1-25

Imidacloprid pH10 hydrolysis comparative graphs fitted to first order kinetics

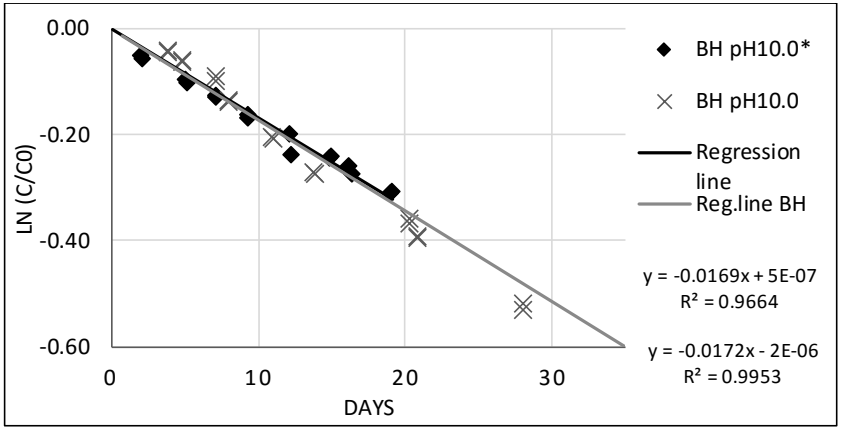
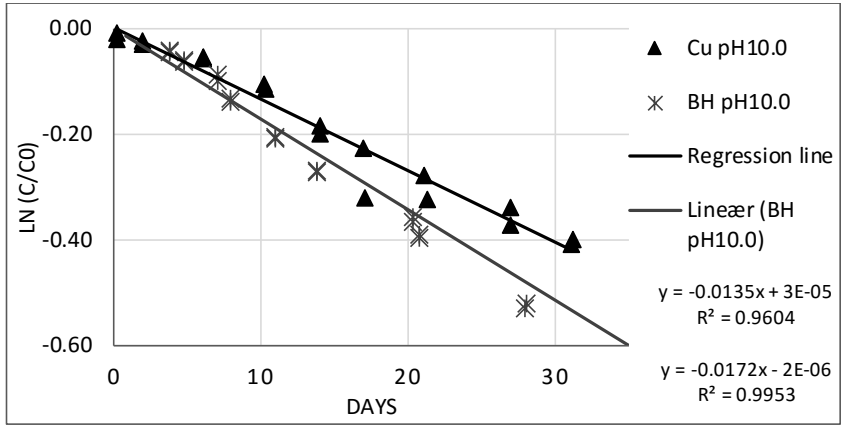


Figure A1-26



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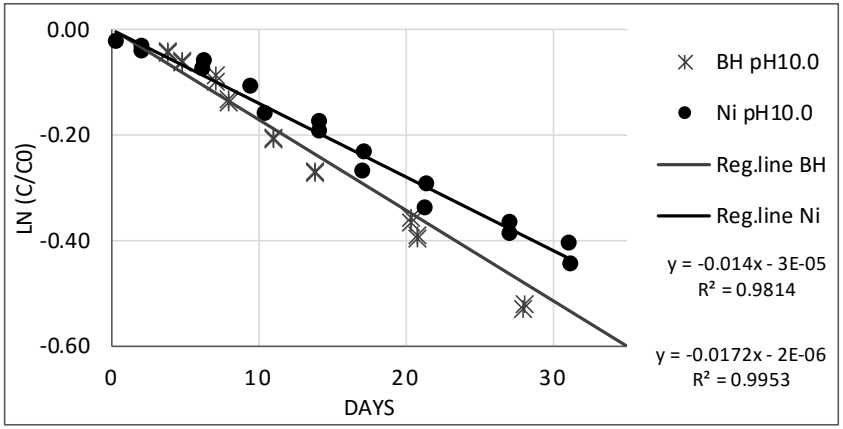


Figure A1-28

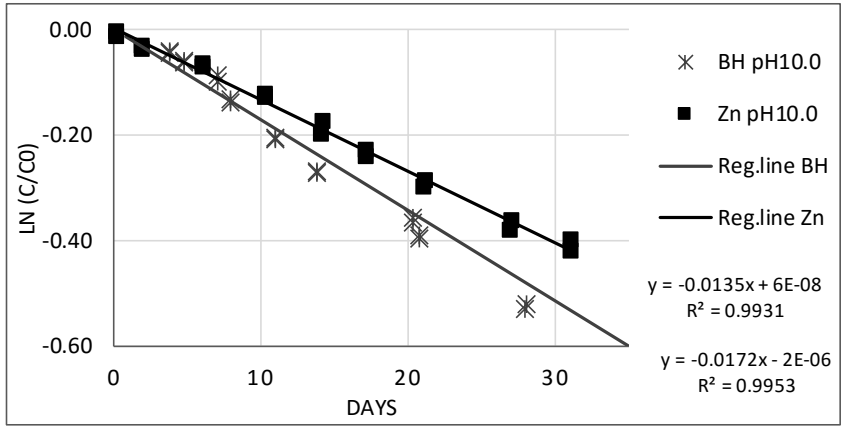


Figure A1-29

Imidacloprid comparative graphs pH10.0 Minerals – fitted to first order kinetics

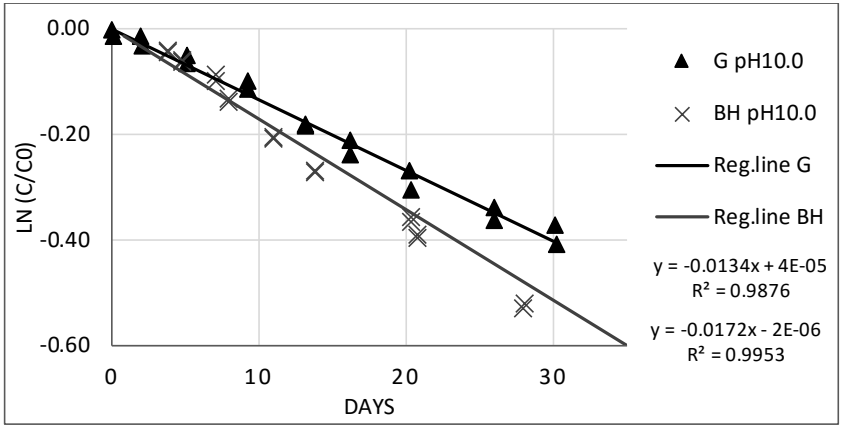


Figure A1-30

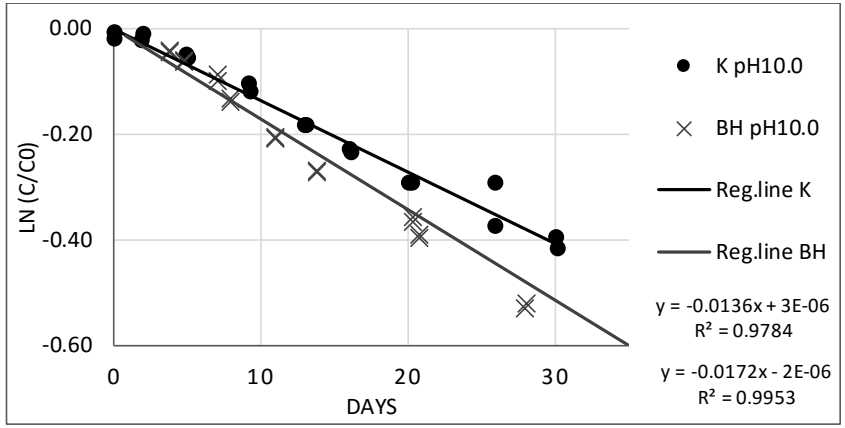


Figure A1-31

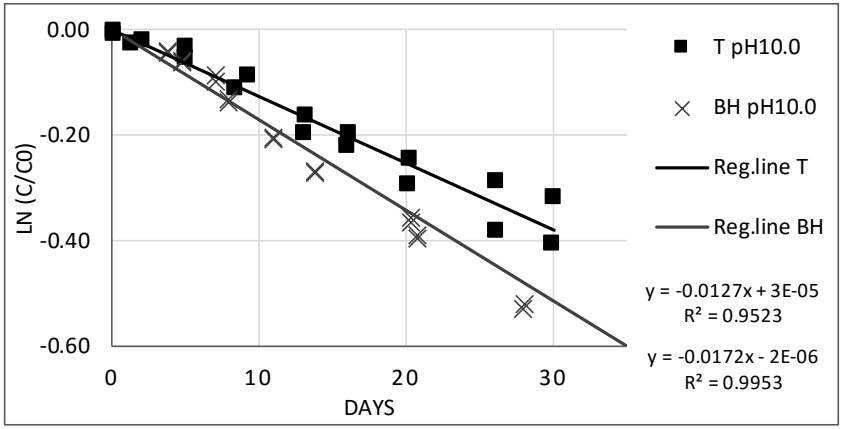


Figure A1-32

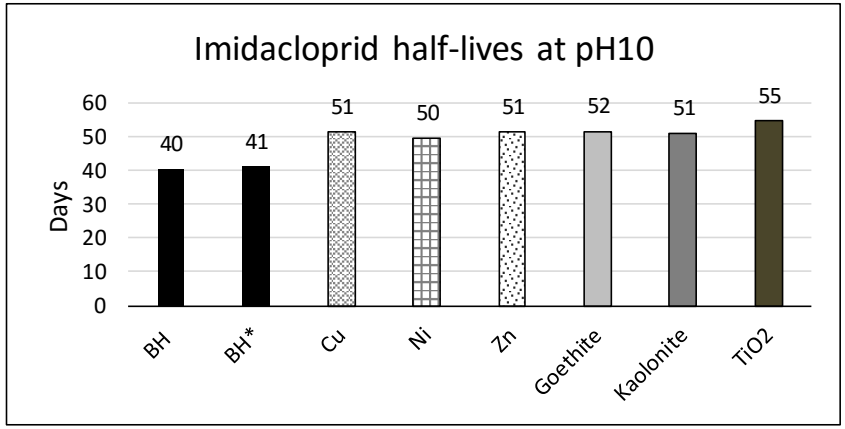


Figure A1-33

Acetamiprid baseline hydrolysis (BH) at pH 4.0-10.0

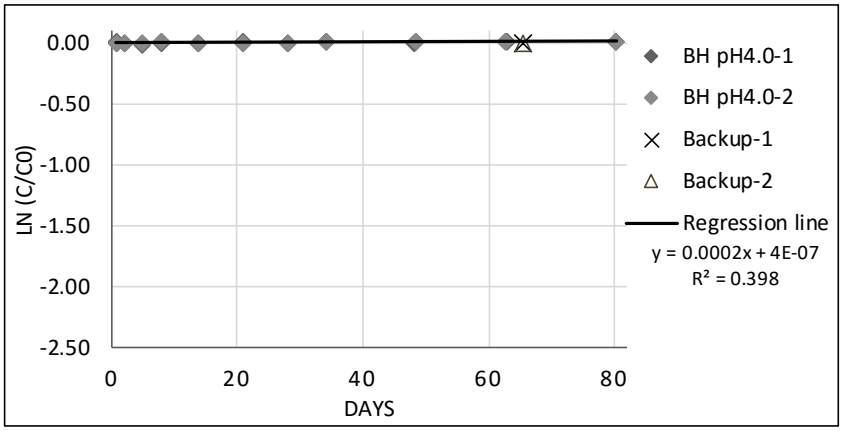
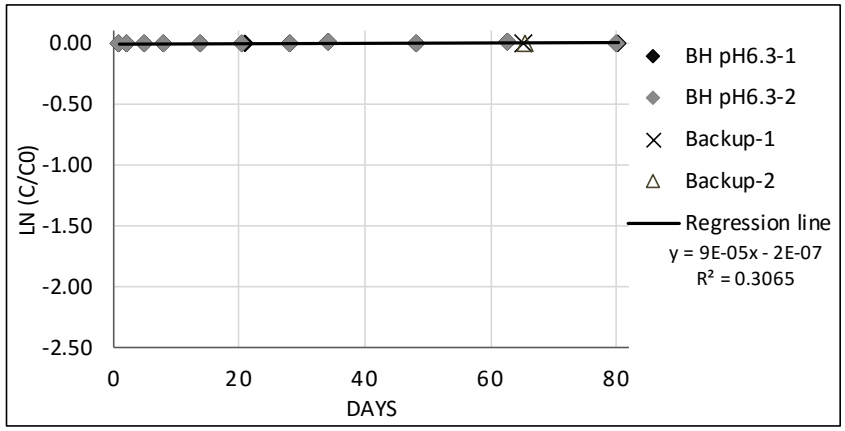


Figure A1-34



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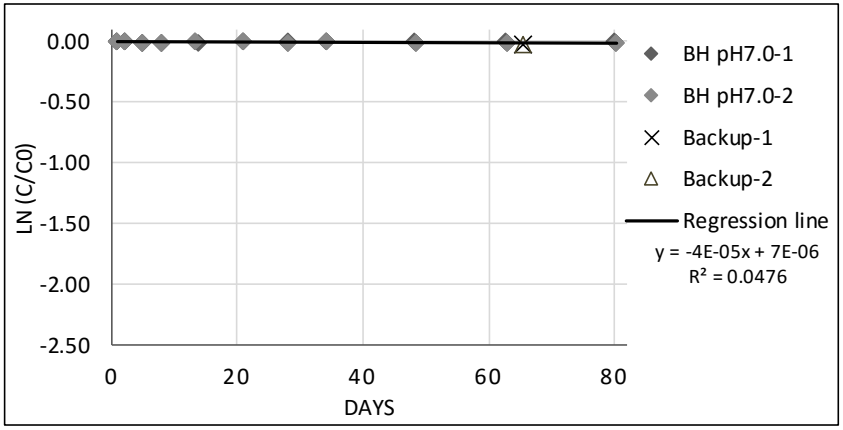


Figure A1-36

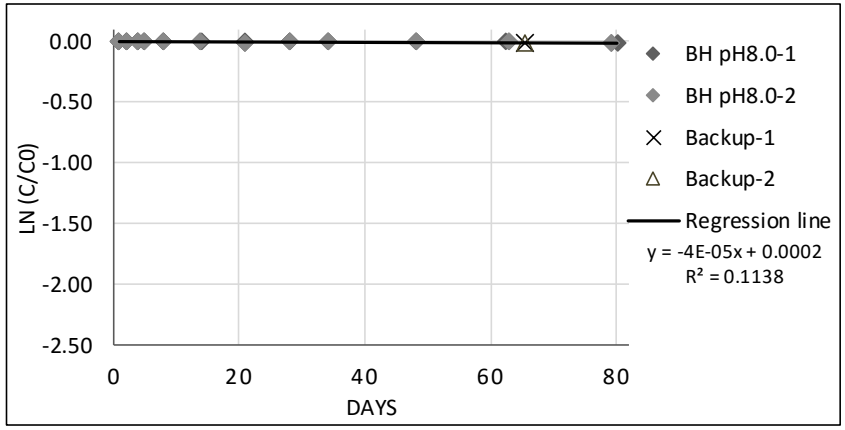


Figure A1-37

Acetamiprid baseline hydrolysis (BH) at pH10.0

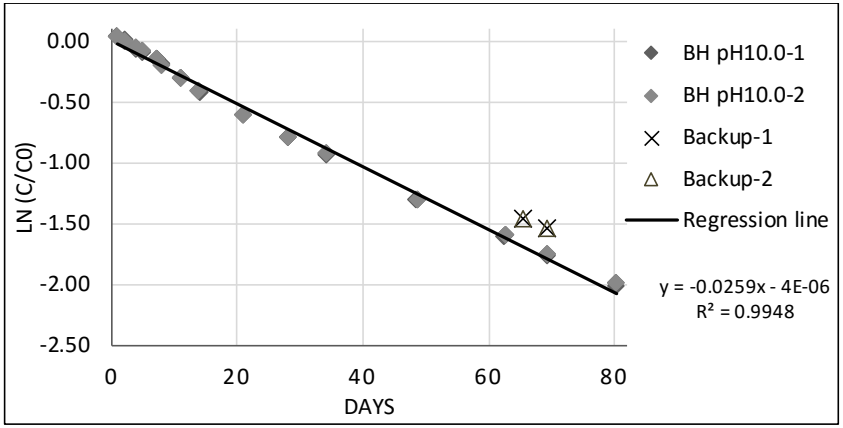


Figure A1-38

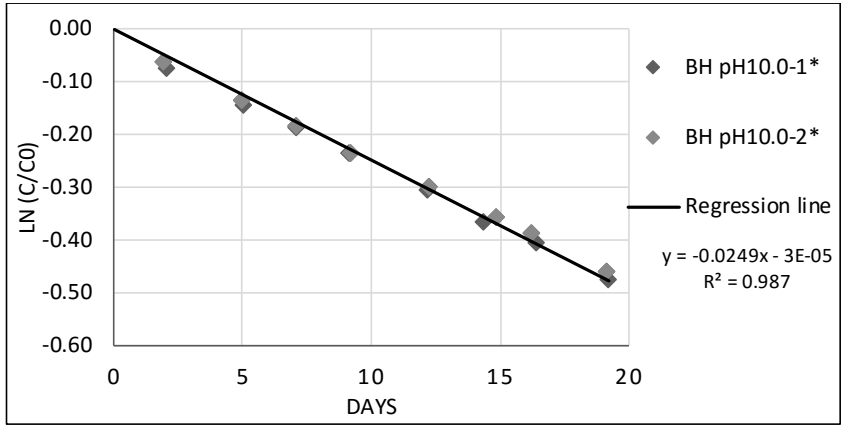


Figure A1-39: second round of BH pH10.0 experiments

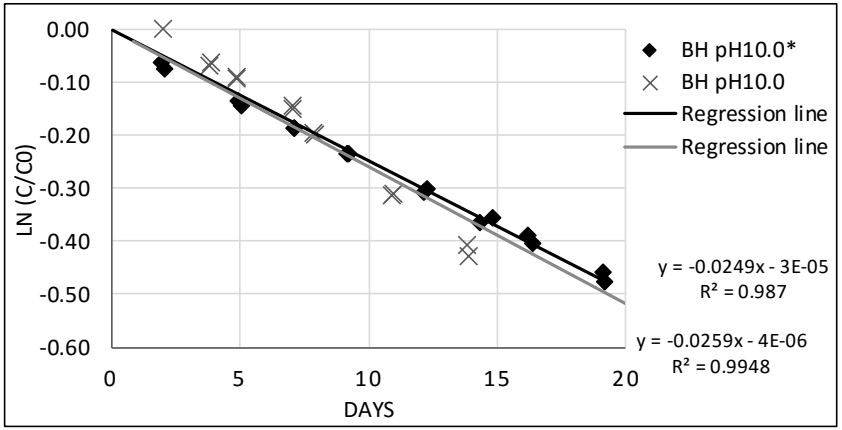


Figure A1-40:Comparison of first and second round of pH10.0 experiments. Note that "days" is the number of days from each experiments individual experiment start

Acetamiprid Cu(II) hydrolysis at pH4.0-pH10.0

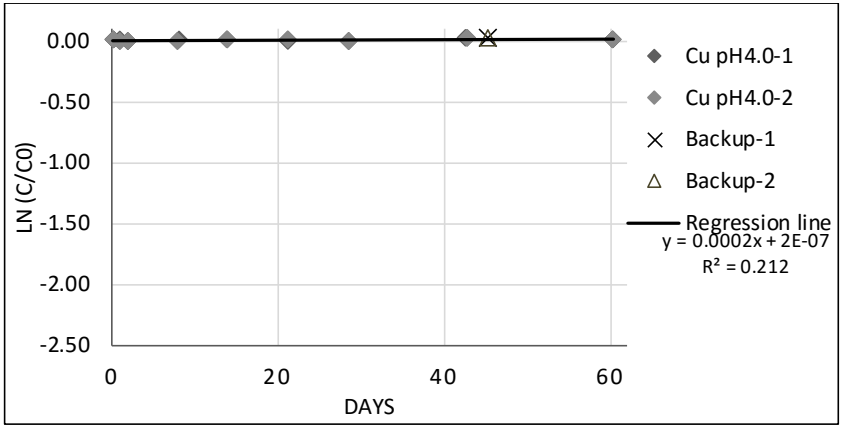


Figure A1-41

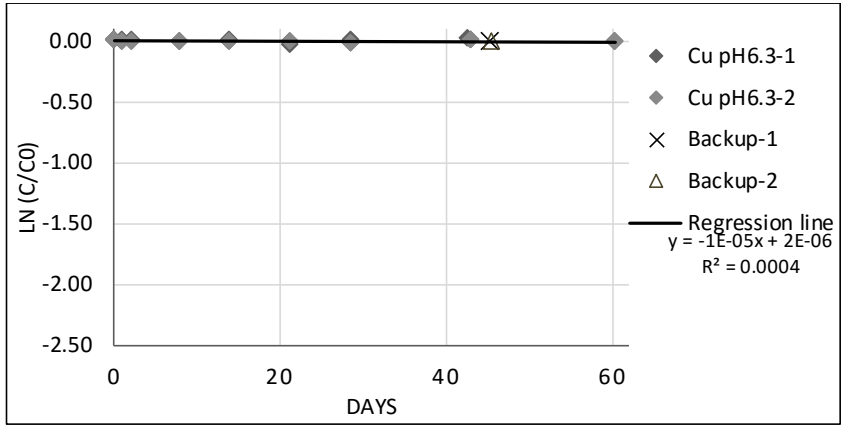


Figure A1-42

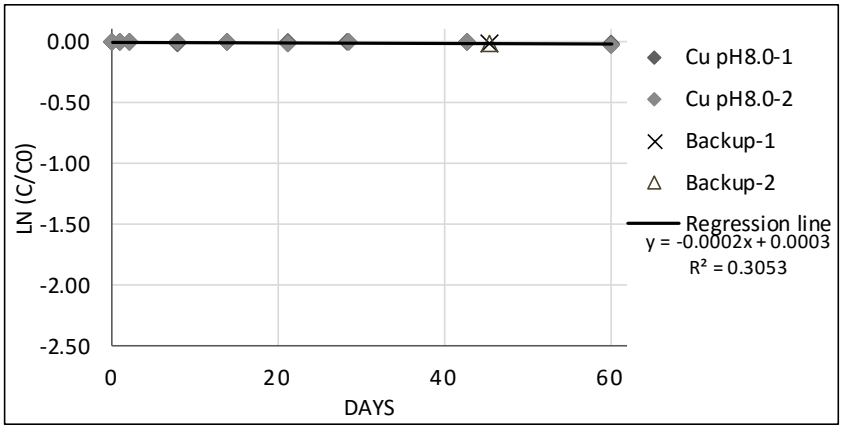


Figure A1-43

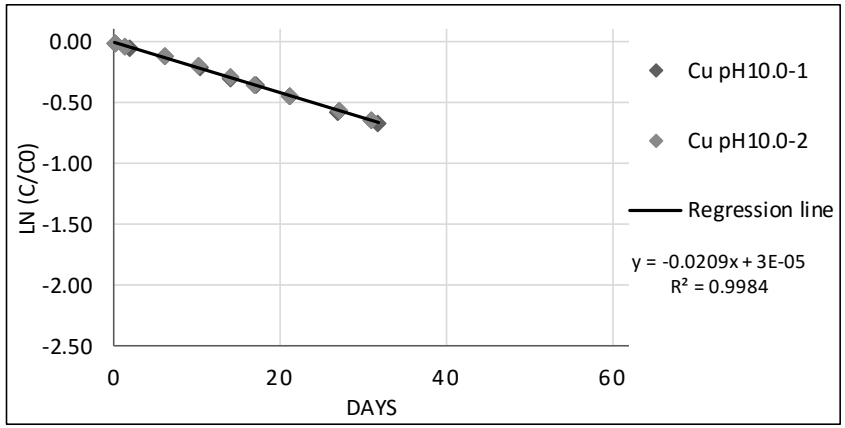


Figure A1-44

Acetamiprid Ni(II) hydrolysis at pH4.0-pH10.0

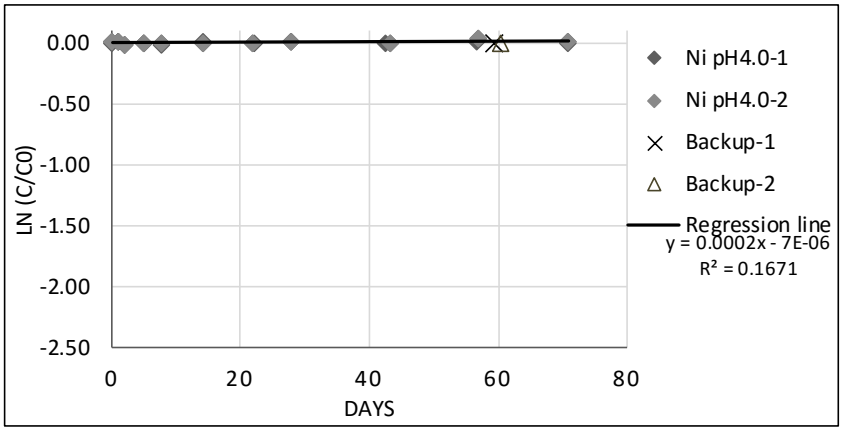


Figure A1-45

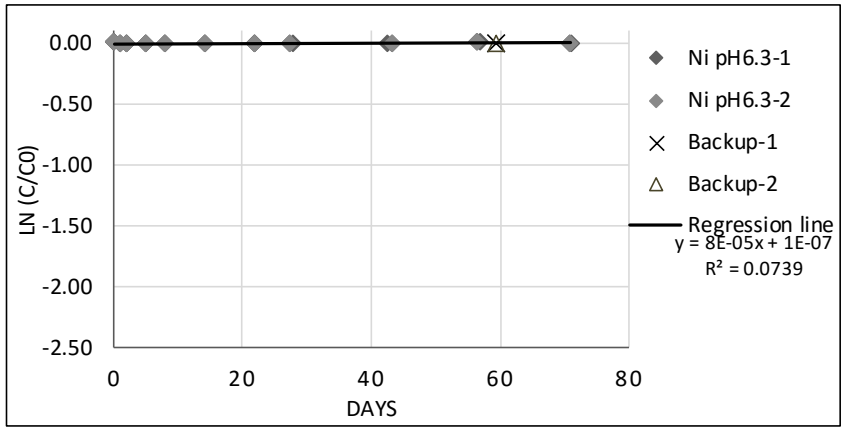


Figure A1-46

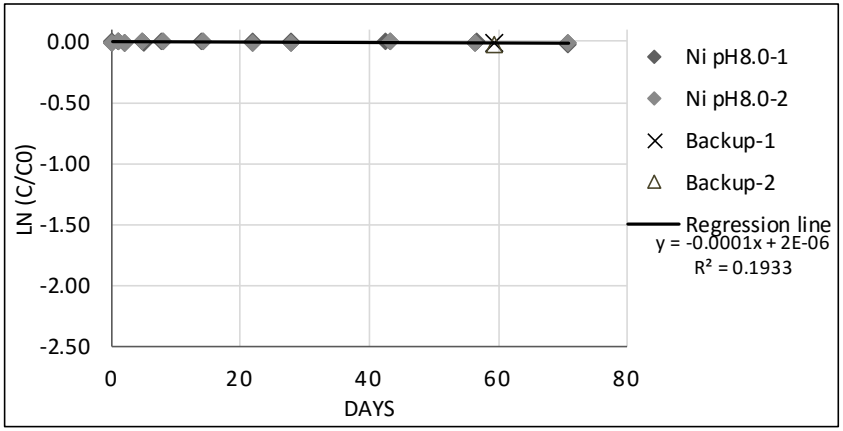


Figure A1-47

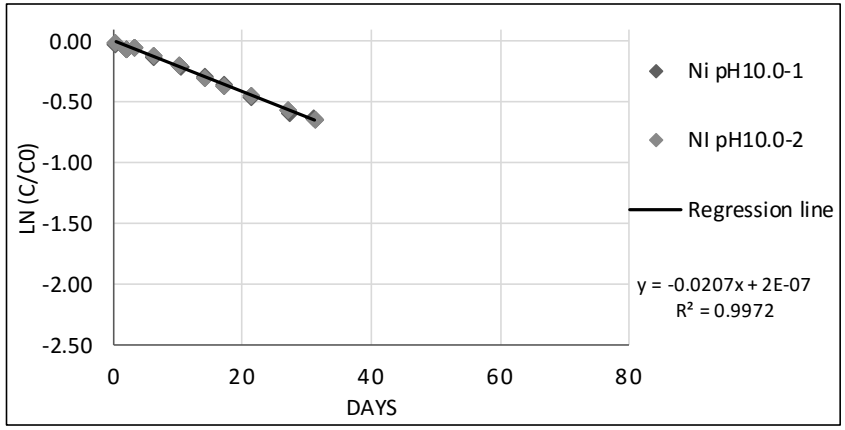


Figure A1-48

Acetamiprid Zn(II) hydrolysis at pH4.0-pH10.0

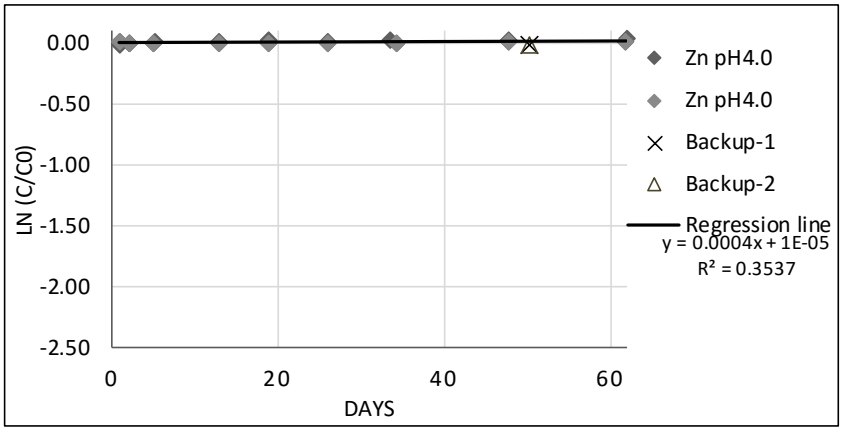


Figure A1-49

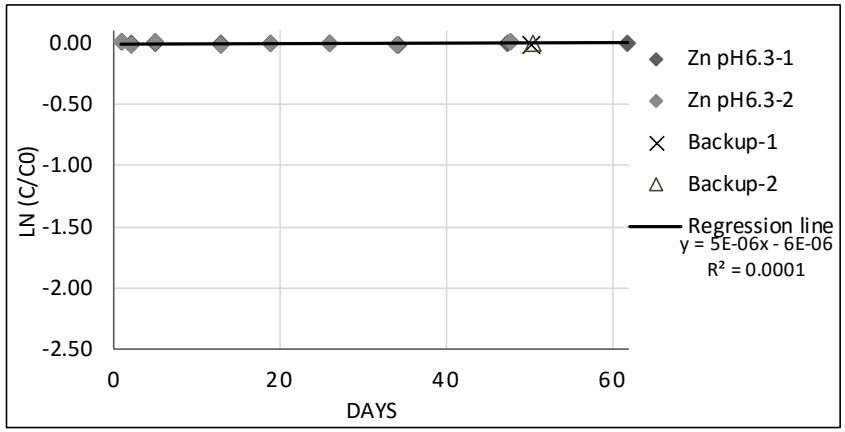


Figure A1-50

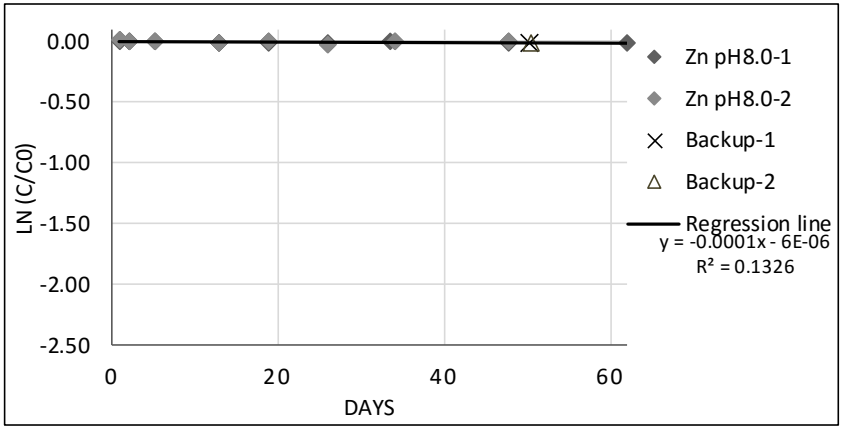


Figure A1-51

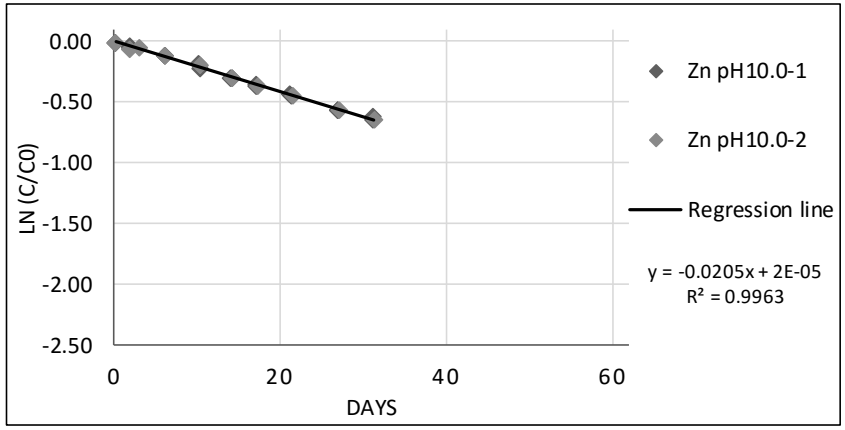


Figure A1-52

Acetamiprid minerals hydrolysis at pH8.0 and pH10.0

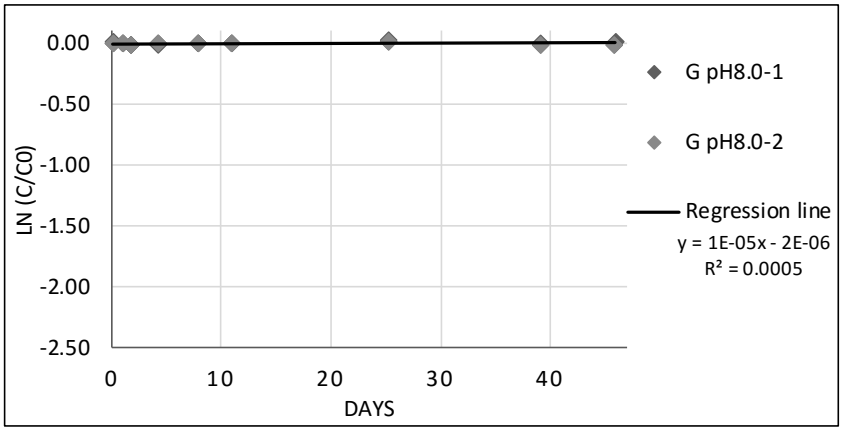


Figure A1-53

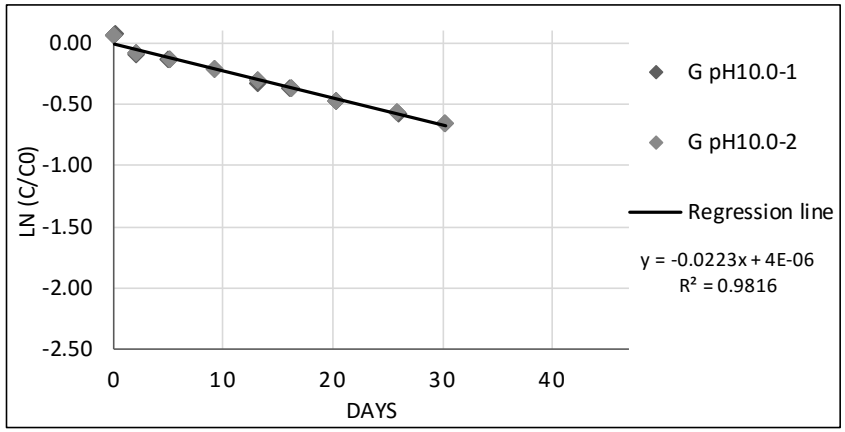


Figure A1-54

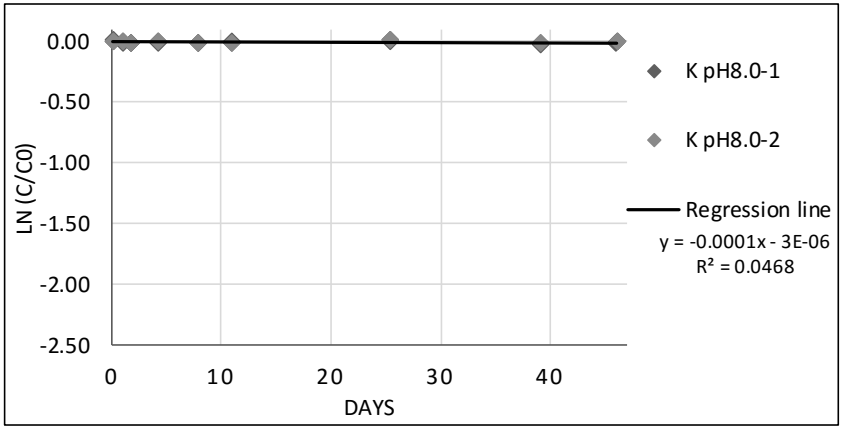


Figure A1-55

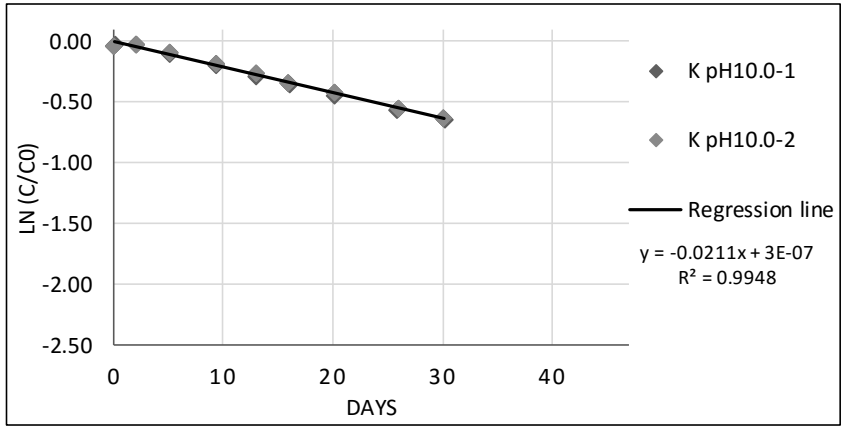


Figure A1-56

Acetamiprid mineral hydrolysis at pH8.0 and 10.0

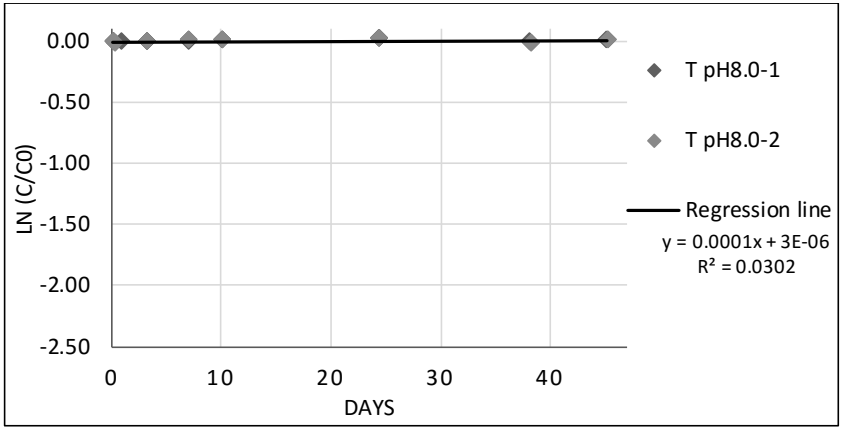


Figure A1-57

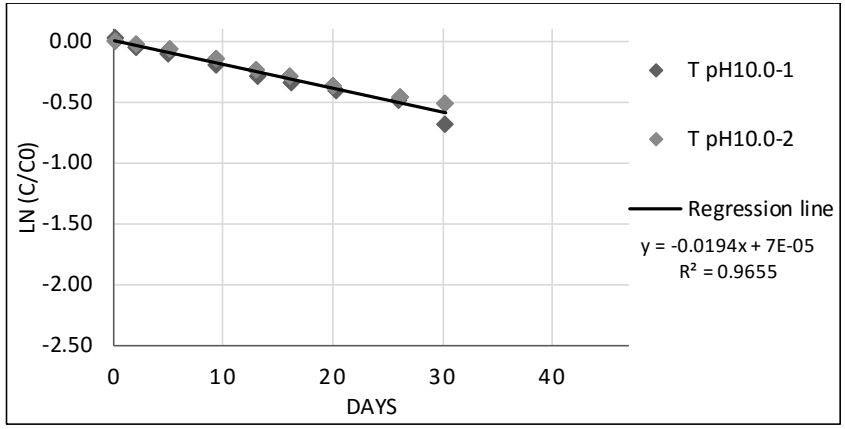


Figure A1-58

Acetamiprid pH10 hydrolysis comparative graphs fitted to first order kinetics

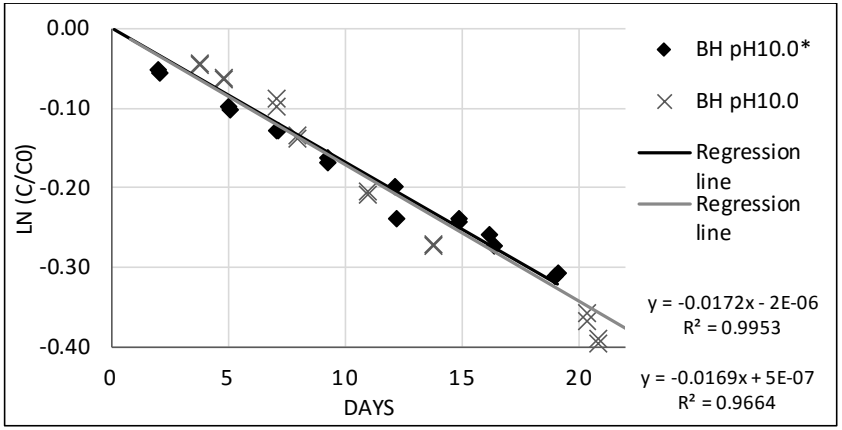


Figure A1-59

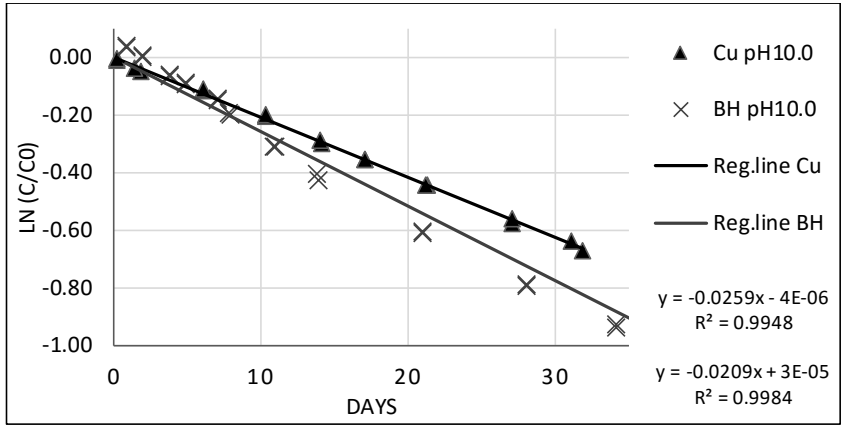


Figure A1-60

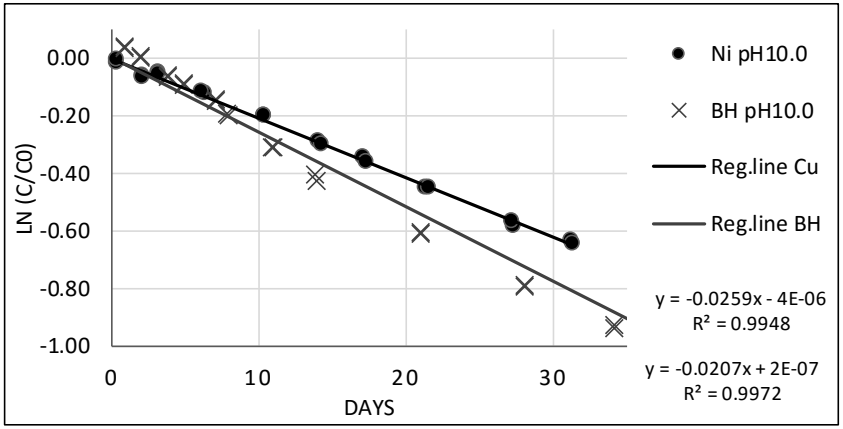


Figure A1-61

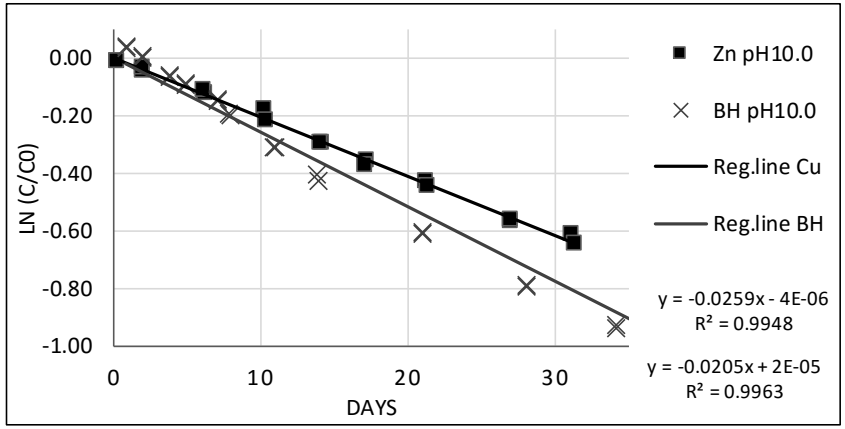


Figure A1-62

Acetamiprid pH10 hydrolysis comparative graphs fitted to first order kinetics

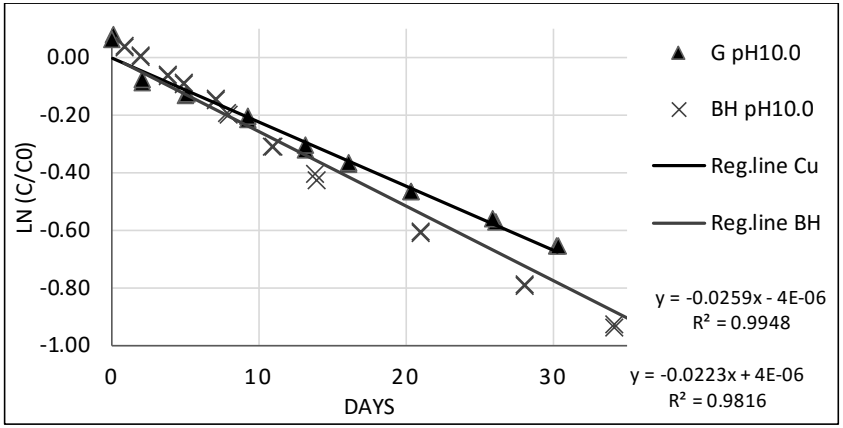


Figure A1-63

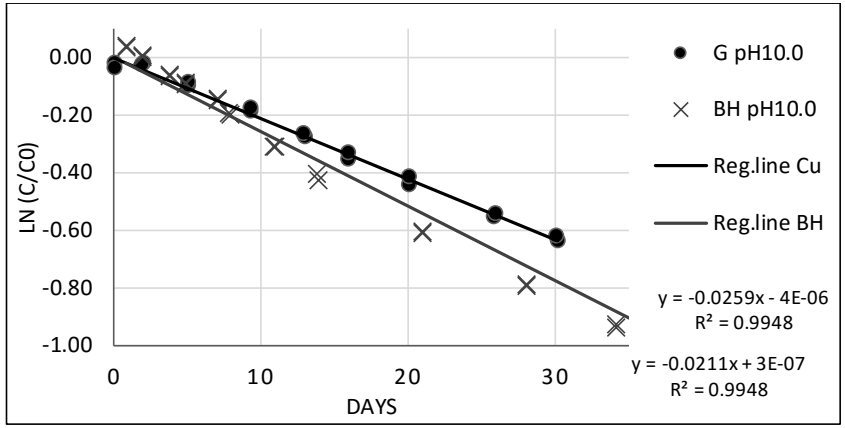


Figure A1-64

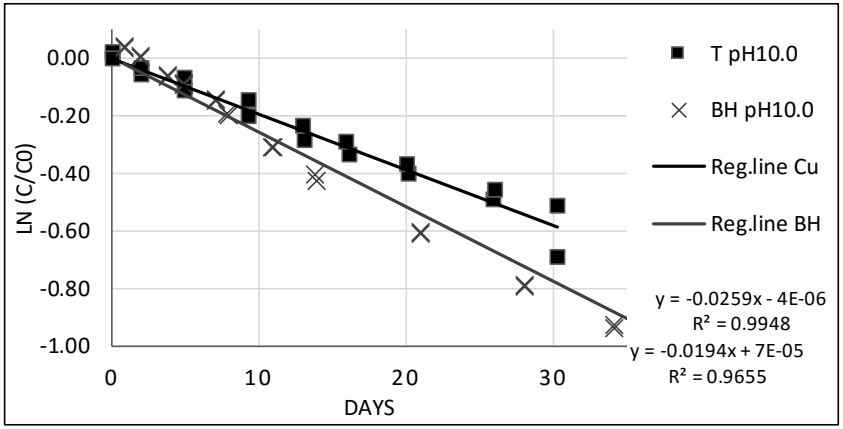


Figure A1-65

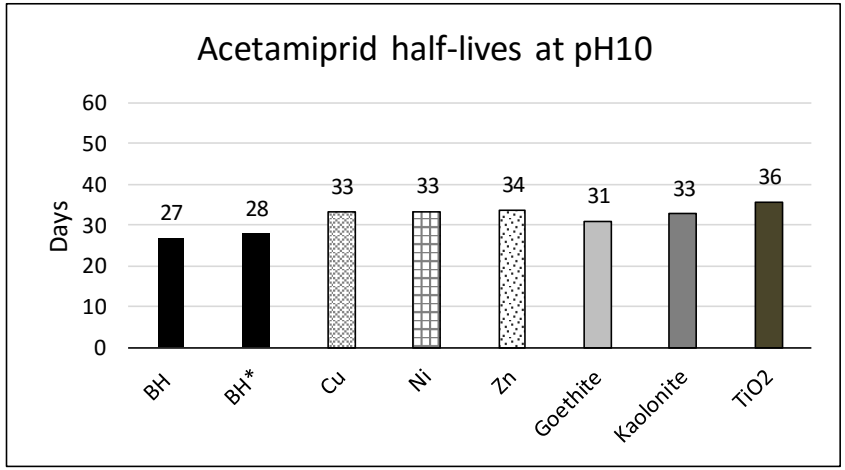
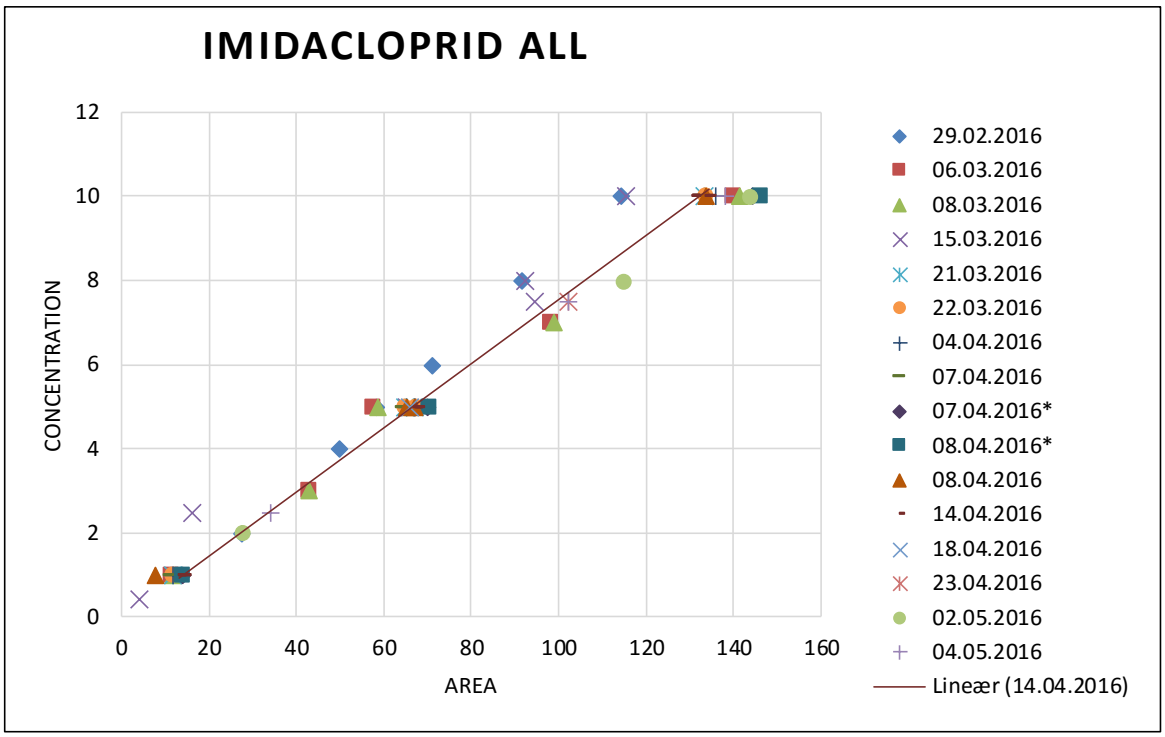


Figure A1-66

A2 Appendix 2 – Calibration curves



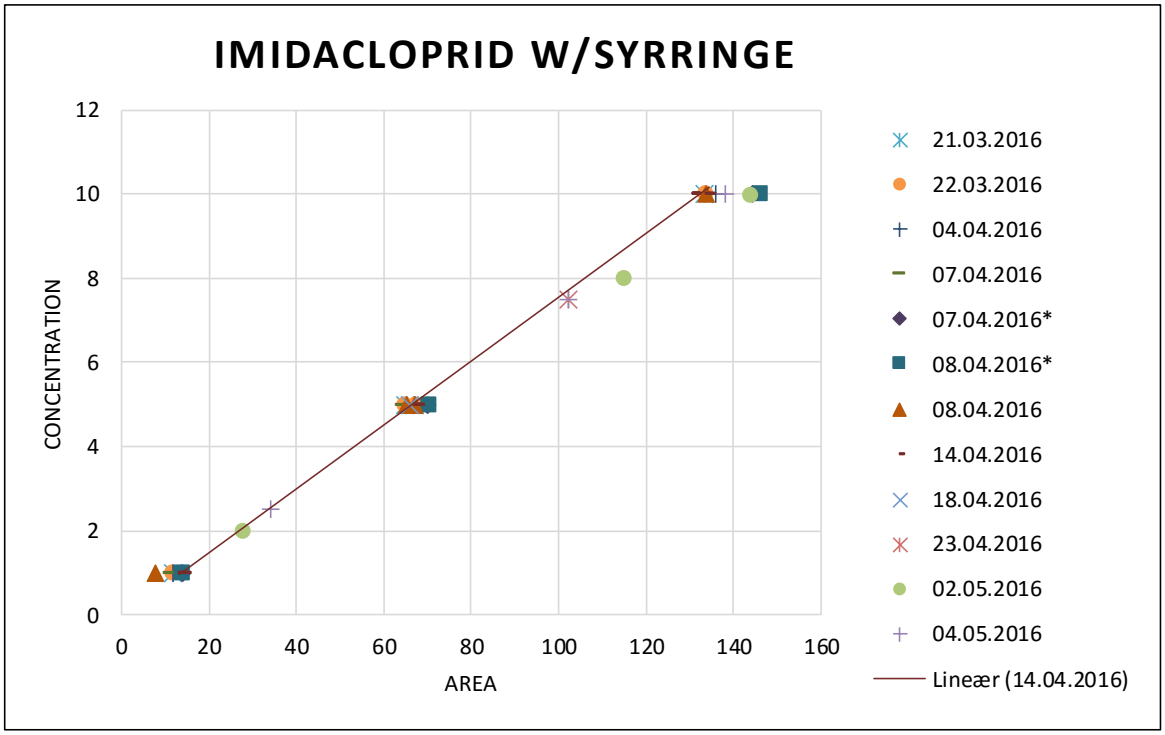
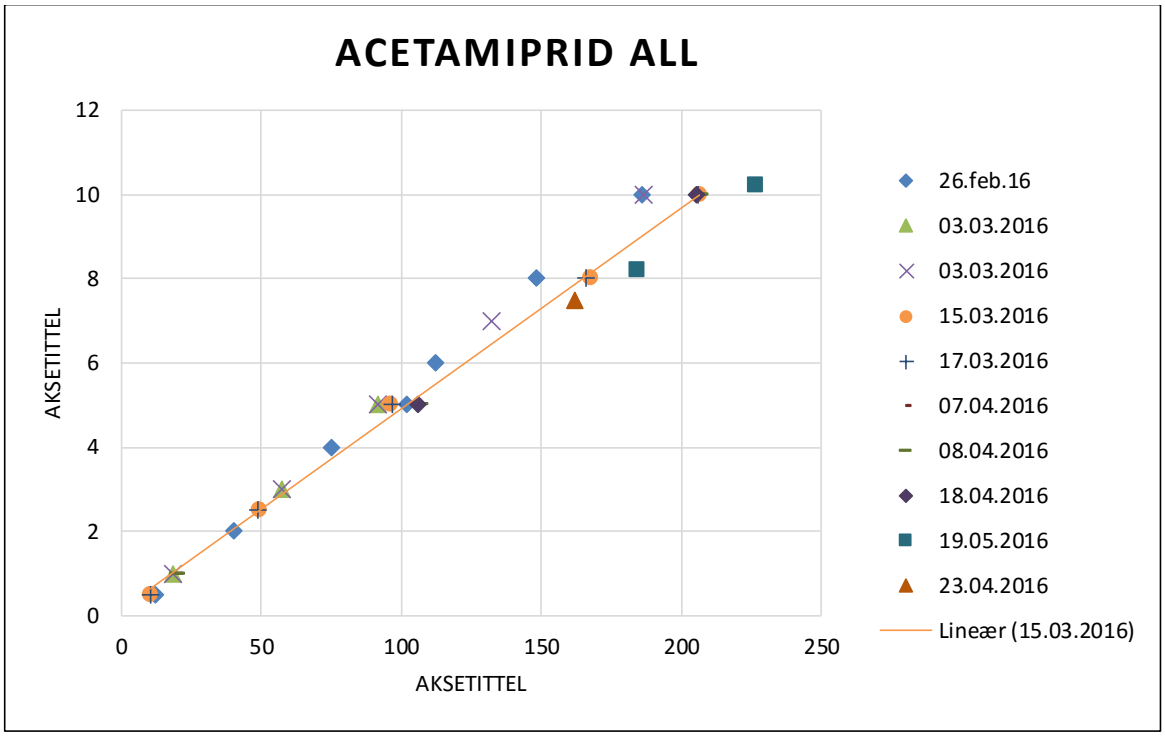


Figure A2-1: Imidacloprid calibration curves. The figures illustrates the difficulties with making good standards. The problems decreases somewhat after beginning to use a glass syringe rather than a plastic pipette when handling the methanol based stock-solution.



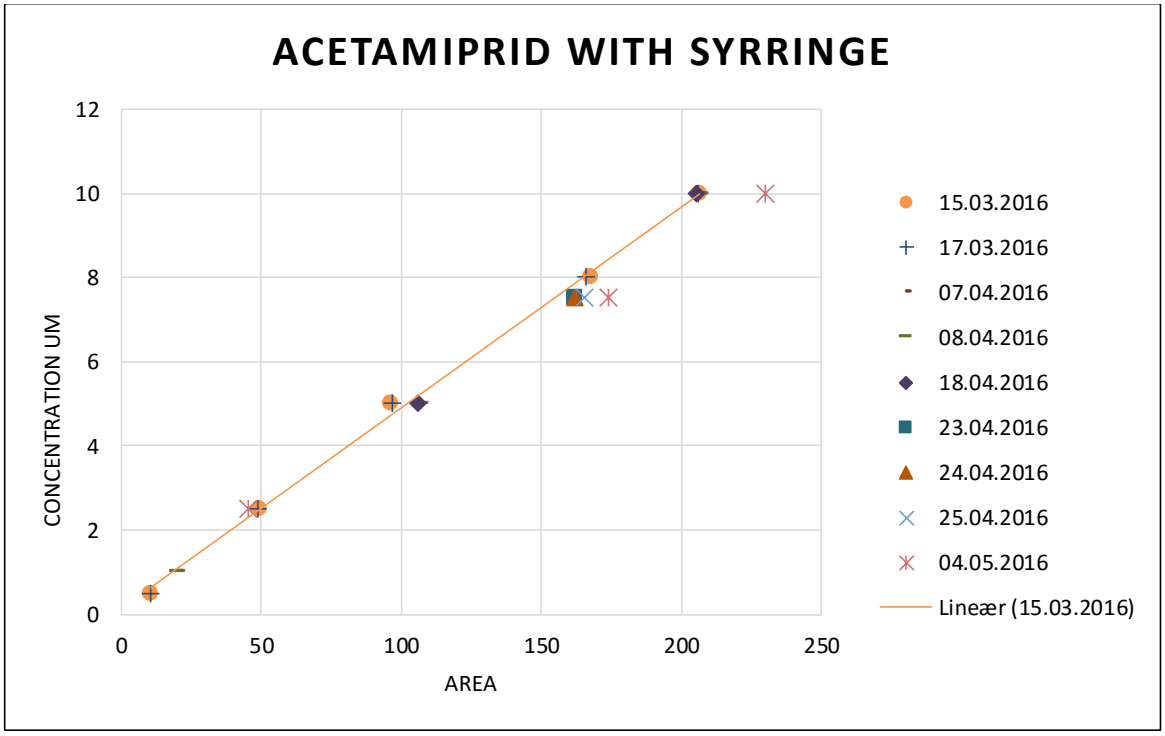


Figure A2-2: Acetamiprid calibration curves. The figures illustrates the difficulties with making good standards. The problems decreases somewhat after beginning to use a glass syringe rather than a plastic pipette when handling the methanol based stock-solution.

A3 Appendix 3 – Surface properties of minerals

Table A3-1: Surface properties of minerals found in literature.

	Surface area m^2/g	pH_{zpc}	Site density (sites/nm^2)
TiO2[a]	39.5 [a]	6.3[a]	3.8[a]
	49[g]		
FeOOH[a]	30.8[a]	8.4[a]	8.8[a]
	47.5[i]	8.45[i]	7[i]
Kaolonite[b]	19[b]	7.5[c]	6.0[e]
	16.4[c]		0.6[f]
	15.6[d]		
	19 [g]		

a) Vasudevan and Stone (1998)

b) Kang (2008)

c) Blockhaus et al. (1997)

d) Zachara et al. (1988)

e) Riese (1982)

f) Sposito (1984)

g) Seunghun (Kang, 2008)

h) Ohno et al. (2001)

i) Coughlin and Stone (1995)