Pesticide-Induced Environmental Risks:

A field study in Ghana

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Thesis

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Dedicated to my beloved late parents,
Benson Onwona and
Faustina Asomaning

Contents

Chapter 1

General Introduction

Pesticides

Increase in demand for food throughout the world has made farmers to increase their production base as it is expected that human population can reach about 10 billion by 2050 (UN DESA, 2017). This intensification of agriculture has resulted in the use of pesticides to grow more food on less land by protecting crops from pests, diseases and weeds as well as raising productivity per hectare. Technological advances particularly in the form of chemical products led to the creation of high efficiency pesticides and fertilizers enabling a large increase in crop yield (Carvalho, 2017). Without pesticides, more than half of our crops would be lost to pests and diseases. Between 26 and 40 percent of the world's potential crop production is lost annually because of weeds, pests and diseases (Cai, 2008; Zhang *et al*. 2011; OECD-FAO, 2012).

In Ghana, agriculture is one of the most important sectors of the economy contributing 30% to Gross Domestic Product (GDP) and employing more than half of its workforce who are mainly small landholders. Production of major crops has been increasing in recent years, mainly as a result of extensive cultivation in a bid to meet this challenge (MOFA, 2011). Pesticide use among farmers in Ghana has reached its peak in recent years especially for controlling weeds, pests, and preservation of harvested crops (Horna *et al*. 2008; Imoro *et al*. 2019).

Presently, throughout the globe approximately 2 million tonnes of pesticides are utilized, out of which 47.5% are herbicides, 29.5% are insecticides, 17.5% are fungicides and 5.5% are other pesticides (De *et al.* 2014). Moreover, it has been estimated that by this year (2020), the global pesticide usage will increase up to 3.5 million tonnes (Zhang, 2018). In Ghana, pesticides use is similar to the global trend and there is a steady increase in importation and consumption of pesticides (Fig. 1.1).

Pesticide law of Ghana

Ghana has a legal procedure for pesticide registration, as prescribed by Part II (Pesticides Control and Management) of the Environmental Protection Agency Act, 1994 (Act 490). With this Act, a number of pesticide products have been registered for use in both agriculture and public health sectors (GEPA, CCMC 2012). The EPA has the mandate to regulate all pesticides that are sold, distributed and used in Ghana. The Act defines pesticides

as (a) a substance or mixture of substances intended for preventing, destroying, repelling or reducing the destructive effects of pests, or (b) a substance or mixture of substances intended for use as a plant regulator, desiccant or wood preservative. It includes all of the following: herbicide, insecticide (which may include insect growth regulators, termiticides, etc.) nematicide, molluscicide, piscicide, avicide, rodenticide, bactericide, insect repellent, animal repellent, antimicrobial, and fungicide. The objective of regulating pesticides is to protect society from the adverse effects of pesticides without denying access to the benefits of their use. Registration of pesticides enables authorities to exercise control over quality, use levels, claims, labelling, packaging, advertising, and disposal of pesticides, thus ensuring that the interests of end-users are properly protected. Apart from the registration of pesticides, the Act provides requirements for pesticide dealership and safeguards for the safe use of pesticides. Registration of pesticides started in 2003, and opened a new economic boom with a resultant increase in registration and importation of pesticide products for use in Ghana (Fig. 1.1).

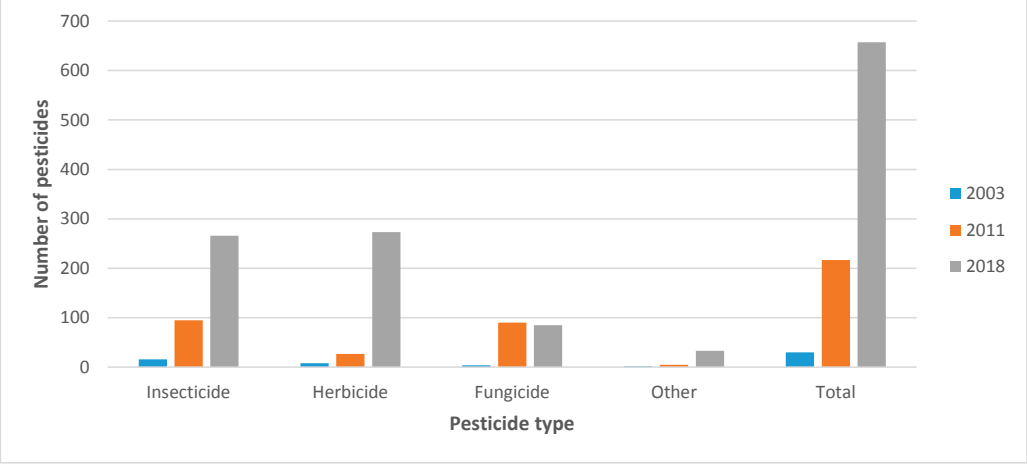

Figure 1.1: Number of formulated pesticide products registered/provisionally cleared in Ghana (2003-2018).

*"**Other**[1]" includes rodenticides, nematicides, fumigants and other conventional pesticides, and other chemicals used as pesticides such as sulphur, petroleum oil and sulphuric acid.*

Pesticide evaluation procedure of Ghana

Basic scientific data requirements include: mammalian toxicity, ecotoxicology, environmental fate, physical and chemical properties, five batch analysis, residue chemistry if

used on food or feed crop, fish and wildlife, if applicable, phytotoxicity, if applicable and bio efficacy. Evaluation of pesticides is conducted on their environmental toxicology (ecotoxicology), human toxicology, bio-efficacy, labels and advertisement. Complete data provided for pesticide product is assessed and evaluated based on acceptability criteria (GEPA, CCMC 2012).

Pesticide registration in Ghana is based on international data and prospective risk assessments (GEPA, CCMC 2012). Since the operationalization of the Act over a decade ago, no study has been conducted to establish how protective the pesticide legal procedure is to the environment and occupational health. No environmental risk assessment has been conducted in the fields of use. The Act prescribes controls on import and use under sections 28 and 40 as well as safeguards for use of pesticides with regards to occupational health under section 44. This notwithstanding, the Act provides an opportunity under section 62 (a - f) to establish regulations that can monitor effect of pesticide use in the field where the use of ecological risk assessment becomes key.

Environmental impacts of pesticides

Pesticides generally are manufactured to be very toxic for their intended targets and once they spread in the environment may have effects on other related and non-related, non-target organisms. Pesticides may end up in the environment via a couple of routes like spray drift, run-off, leaching through the soil once applied in the field and may reach water bodies adjacent to the agricultural fields where they can pose risk to non-target aquatic biota (Perez-Luca, 2015; Bonmatin *et al.* 2018). Pesticide usage in most developing countries including Ghana are characterised by risk to humans and other life forms and unwanted side effects to the aquatic and terrestrial environment and safe management is usually not prominent (Ecobichon, 2001; Aktar, 2009; NPASP, 2012; Onwona-Kwakye *et al*. 2019). In Europe for example, to safeguard the adverse effects of pesticides, extensive risk assessment is conducted to ensure that negative effects on the environment during pesticide registration are factored into the recommended use (eg. EFSA, 2013a, b). With this, the field of Ecological Risk Assessment (ERA) is very important in Europe and aims at assessing the potential adverse effects resulting from various human activities like the impact of chemical compounds on the environment. Pesticides are regulated under Regulation No 1107/2009 (EC 2009) where

protection goals, data requirements and risk characterisation are defined for the environmental compartment whereas in Ghana it is practically non-existent.

Overall aim of thesis

The aim of the thesis was to review the pesticide law and registration procedure of Ghana and to establish how effective it has been in protecting the environment and users in Ghana using post registration monitoring. Further, the study sought to use models such as PRIMET (Pesticides Risks in the Tropics to Man, Environment, and Trade), SSD (Species Sensitivity Distribution) and empirical data obtained from the field, to assess the risk of pesticides and determine the threshold levels protective of ecological communities. The thesis also investigated the effects of these pesticides on abundance and diversity of bacteria populations in the soil environment.

Based on this overall aim, the following research objectives were set:

1. To review relevant portions of the Ghanaian pesticide law, the registration procedure, in relation to actors perspective of its implementation to establish whether it has achieved its intended purpose, and indicate adjustments needed for further consideration and improvement;
2. To perform risk assessments using empirical data and models to establish the risks of pesticides to the aquatic and terrestrial environments of agricultural fields according to farmers' pesticide use regimes in Ghana; and
3. Determine the water quality of water bodies in Ghana using physico-chemical parameters, pesticide concentrations, and biological indicators.

Outline of thesis

Chapter 2 reviews Ghana's pesticide policy (pesticide law) which was adopted to achieve safe use of such products. This chapter provides a brief overview of the pesticide regulatory policy framework (pesticide law), the pesticide registration and licensing procedure as well as the theoretical framework (evaluation model) for the study. The main part of the study deals with how the policy has developed over time. The study also discusses the farmers' pesticide use in day-to-day farm practices and distribution in the country, in relation to the policy and also the interactions of state (regulators) and non-state actors (pesticide

distributors, dealers and farmers). To conclude, the study discusses a number of initiatives that need to be initiated to overcome problems encountered.

Chapter 3 describes the use of the PRIMET model (Van den Brink *et al.* 2005) (1st Tier) to assess the risks of pesticides to the aquatic and terrestrial environments. To this end, empirical data obtained from farmers in their application of pesticides and other aquatic waterway parameters realistic for a tropical scenario, see e.g., (Van den Bosch *et al.* 2006) were used. Results from 1st tier showing risk were then further refined using SSD model (Maltby *et al.* 2005; Van den Brink *et al.* 2006; Maltby *et al.* 2009) (2nd Tier) to estimate pesticide concentration levels protective of the aquatic environment.

Chapter 4 describes a monitoring approach using a variety of techniques to assess the water quality of Volta River to chemicals and pesticides. This involved the sampling and analysis of water samples from selected locations and the analysis of the concentrations of pesticides used in the areas as obtained from an earlier survey (**chapter 3**). This was then linked to the physicochemical characteristics of the sampling locations as well as the macroinvertebrate species identified. Finally an explanation is given and inferences made on the health of the water body and presented as a water quality assessment tool for use in Ghana.

In **Chapter 5**, the study investigated the effect of pesticides used in irrigation farms on bacterial abundance and diversity relative to agriculture. The study also focuses on bacteria capable of degrading diverse classes of pesticides used in these farms. They are envisaged to be isolated for future works and could be utilized in a variety of environmental clean-up scenarios as a multi-purpose remediation for pollution and decontamination of heavily polluted sites such as oil fields, landfills, and sewage collectors as has been possible in other jurisdictions (Rupa *et al*. 2013).

Chapter 6 provides a general discussion of the results and conclusions of this thesis. The findings of the thesis and an overall discussion and conclusion on how the pesticide law could be enhanced to effectively protect the environment from their negative effects, the real effect of the pesticides in use on the environment and the possibility of isolating a variety of bacteria which can metabolize pesticides are discussed.

Chapter 2

Pesticide registration, distribution and use practices in Ghana

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Abstract

Ghana has implemented regulation on the registration, distribution and usage of pesticides in order to evaluate their environmental and human health effects. However, environmental monitoring and certified laboratories for pesticide analysis are lacking. Pesticide misuse, misapplication, contamination of the environment and human exposure still continue and little is known to what extent pesticide registration, distribution and use is properly implemented in Ghana. This study aimed at investigating how the pesticide policy operates in Ghana, how state (policy; national/local) and non-state (importers, dealers' and farmers) stakeholders function, what their challenges are, and to which extend the policy objectives are achieved.

A conceptual framework based on the contextual interaction theory (CIT) was developed and a review of Ghana's pesticide policy implementation with two empirical field studies on state policy and non-state policy actors was conducted, supplemented with secondary data and a number of interviews conducted with stakeholders and informants. Results indicate that pesticides are registered in compliance with the law. Non-state actors scored low with respect to their mandate which likely results in environmental and human health risks. Significant association existed between educational level attained and knowledge ($\chi^2 = 3.614$; $P \leq 0.05$). Work experience or duration of farming also significantly influenced the knowledge of respondents ($P < 0.001$), as well as attitude ($\chi^2 = 15.328$; $P < 0.05$). Work experience/ duration of farming also significantly influenced attitude at 95% confidence level ($P < 0.001$) and duration of farming was significantly associated with farm management practices at 5% level of significance ($P \leq 0.05$) while state actors are not motivated and resourced. It is recommended to perform a preliminary risk assessment to the aquatic environment, to derive threshold levels which are protective of communities, to screen farmers for pesticide exposure and poisoning, to develop well-targeted training programmes for pesticide retailers and farmers on pesticide use, personal protective equipment (PPE) use, as well as pesticide management and law. Additionally, pesticide policy implementers have to be motivated and resourced to carry out their mandate, i.e. to execute the pesticide legislation.

Introduction

Pesticides use in agriculture in Ghana has resulted in reduced crop loss (Clarke *et al*. 1997). There has been a continuous increase in the importation and use of pesticides (MOFA, 2011). This includes both the number of chemicals and quantities registered as well as recorded by the competent authorities and regulators such as the Food and Drugs Authority (FDA), Environmental Protection Agency (EPA) of Ghana, Ghana Standards Authority (GSA) and the Ministry of Food and Agriculture (MOFA). This increase is prevalent due to expansion of cultivation areas for food and cash crops in a bid to meet the increasing demand for food (MOFA, 2003). The increase can also be attributed to the liberalization of the economy and the government's aim of attaining a middle income economy as enshrined in the country's Vision 2020 agenda. Further, the regulation and the registration of pesticides opened a new economic boom with the resultant increase in the registration of pesticide products for use in Ghana. The use of pesticides, however, has not been without deleterious effects on people, such as farmers, traders and consumers, which are involved in the food supply chain. Poor knowledge of farmers on the types of pesticides, their use and associated risks, ineffective governmental enforcement of pesticides' regulations and strong incentives among pesticide traders and users to make profits, have been reported to leading to an increased use of cheap, mislabelled and adulterated pesticides in Ghana (NPASP, 2012; GNA, 2012). Instances of over use and misuse on crops have been reported with the accompanying negative effects on productivity, environment and human health (Gerken *et al*. 2001; Dunham *et al*. 2003; Amoako *et al*. 2012). Williamson *et al*. (2008) described chlorpyifos, endosulfan and lambda-cyhalothrin being associated with instances of ill health among Ghanaian farmers. Ntow (2001) detected endosulfan and lindane in water and sediment of streams in areas of intensive tomato farming, while other organochlorine pesticide residues were also found in sediment. Similar results were recorded by Ntow (2005) for the Volta Lake in Ghana.

With these problems, there has been a shift to the use of relatively "safer" pesticide alternatives which gave birth to the implementation of the pesticide registration process of Ghana in 2003. The pesticide law at the time was the Pesticide Control and Management Act, Act 528 of 1996. The law has been consolidated to become Part II of the main Ghana Environmental Protection Agency (EPA) Act, Act 490 of 1994. This law includes the whole

pesticide life cycle, so the registration and procurement of pesticides, their import, distribution and retail to farmers, their monitoring for quality control and waste management.

Since the implementation of the pesticide registration process, a number of interventions such as training courses on pesticide storage and handling and their proper use have been organized for importers, distributors, retailers and farmers by the state and a number of non-state organisations (NSOs). However, little is known regarding how and to what extent the registration, distribution and use of pesticides is properly implemented in Ghana. It is also not clear whether these actions by the registration authorities have yielded the necessary improvements in pesticide management and their use. This is so because the operationalization of the pesticide law lacks extensive and reliable information that could be available to experts, scholars, researchers and practitioners in this field of enquiry. The main objective of this study was to examine how pesticides are registered, distributed and used and to assess how different state (policy implementers) and non-state (distributors and the farmers) pesticide actors can improve the pesticides management in order to increase their environmental sustainability as well as workers' health in Ghana.

Pesticide law in Ghana: registration, distribution and use (regulatory framework)

Ghana has a pesticide legislation, part II of the Environmental Protection Agency (EPA) Act (Act 490), which governs the whole pesticide life cycle. The legislation helps to ensure that pesticides are used in a safe and responsible manner in the country. The Ghana EPA is responsible for the registration of pesticides as well as their management. They do this to ensure that the pesticides are properly labelled, distributed, stored, transported, used and applied by following accepted procedures and processes. The Ghana EPA further monitors pesticide use and, if needed, reacts against illegal use, and issues pesticides importation and use licences. The registration of pesticides is headed by a Pesticides Registrar (PR) who works with a Pesticides Technical Committee (PTC) which is drawn from a wide background of expertise and institutions (section 53 of the Act) who advises the Ghana EPA Board on whether pesticides should be registered or not.

The Plant Protection and Regulatory Services Directorate (PPRSD), of the Ministry of Agriculture through the Pesticide and Fertilizer Regulatory Division Act 803 (2010)

compliments the Ghana EPA. They supervise and train pesticide inspectors, register and inspect pesticide dealers and provide information materials and training on pesticides, among others, for retailers and farmers.

To tackle illegal trade in pesticides, the Customs Division of the Ghana Revenue Authority regulates all imports into Ghana including chemicals under Act 791 (2009). Under the auspices of the Ghana EPA, the customs division examines documents and certificates issued by the Ghana EPA. The aim is to validate the claims regarding pesticide importation. The law (Act 791) gives customs officers the jurisdiction to search certain persons, premises and baggage and seize prohibited items, including pesticides.

Ghana, in the exercise of its duty on pesticides, recognises international legal agreements relating to pesticides. These include the International Code of Conduct on the Distribution and Use of Pesticides (i.e. the FAO Code of Conduct). Ghana is also a signatory to the Rotterdam Convention on Prior Informed Consent (ratified in 2003), which facilitates the sharing of information between countries and thus prevents banned or severely restricted pesticides are exported and imported. Furthermore, the Stockholm Convention on Persistent Organic Pollutants, which aims at safeguarding human health and the environment from effects of persistent organic pollutants (POPs), is subscribed to by the country (ratified in 2003).

Pesticides registration procedure in Ghana

Ghana's pesticide registration is a stepwise process (Fig. 2.1), which assesses available and submitted data and results in a final decision to grant or deny registration. The process aims to identify potential risks that may arise from the sale and use of pesticides under Ghana's conditions and culture (GEPA, 2012).

The process includes: 1) the application for registration, 2) data on chemical and physical properties, toxicology, efficacy, residues and fate in the environment of the active ingredient and formulated product, 3) several specific requirements like an agency agreement between the agent and the manufacturer, a batch certificate of analysis, 4) locally generated efficacy data form, 5) samples of the pesticide, 6) a manufacturing licence in the country of origin and 7) the package label in English (GEPA, 2012).

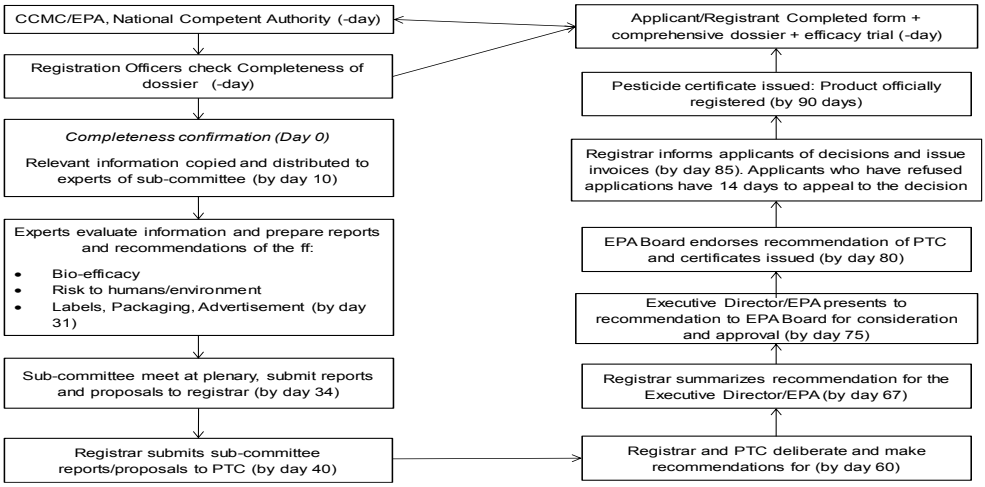

Figure 2. 1: Pesticide application and registration stepwise process (GEPA, 2012)

Application for the registration of a pesticide involves the submission of a product dossier with the necessary annexures to the registrar (GEPA, 2012). The complete application is scientifically scrutinized by technical sub-committees on environmental and human toxicology, bio-efficacy, labelling and advertisements. An evaluation report and recommendations on the application are then submitted to the Pesticide Technical Committee (PTC).

The PTC evaluates the report and proposes a registration decision for deliberation by the Ghana EPA Board. The decisions could be full registration valid for three years and can be renewed. A provisional clearance permit lasts between six months to one year, in which case the applicant is supposed to submit additional information for further consideration. An experimental permit can also be issued for the purposes of research. Decision on banned products (banned for use locally or internationally) or suspension of the registration (inability of the Board to reach a decision) can also be reached (GEPA, 2012). These permits can also be renewed upon expiry. Registered pesticides are subsequently gazetted into public communication channels, as the media.

The Ghana EPA is responsible for verifying the registration and the import of pesticides by issuing a clearance permit, after the importer submitted an application which includes the

data as requested by the Ghana EPA. Under the Ghana EPA Act, "a person shall not import, export, manufacture, distribute, advertise or sell a pesticide except in accordance with a licence issued under this Act" (GEPA, 2012). For the storage of pesticide products, a pesticide licence is required. Pesticide licences are issued based on the presence of a satisfactory location of the storage facility upon inspection by the Ghana EPA.

Pesticide clearance permits are required for an importer to clear consignments from the port based on availability of pesticide licence and if the imported pesticide product is registered.

Theoretical framework for analysing policy implementation

A conceptual framework based on the contextual interaction theory (CIT) was developed for the study from the review of policy implementation literature (Van Horn and Van Meter, 1977; Sabatier, 1991; Fimyar, 2014). The theory as described by Bressers (2007) indicates that implementing a policy is a social process where the output and outcome are defined by interactions of its actors. The framework evaluates how a policy operates in practice, how state (registration authorities) and non-state (pesticide dealers and farmers) actors are functioning and whether the policy objectives are achieved. Outputs are the tangible results of a measure or the noticeable effects shortly after or even during implementation (Bressers, 2007). The Ghana pesticide registration offers a number of outputs that are supposed to be implemented by state actors and the outputs are supposed to yield certain desired outcomes by the non-state actors. The CIT thus offers an opportunity to evaluate whether the desired outcome has been achieved or not. The CIT brings to the fore a couple of actor characteristics including information, motivation and resources. These were selected for the purpose of this study to better understand their impact on the likelihood to implement a policy. The governance approach focuses on the interaction taking place between governing actors with information, motivation and resources (Mengistie *et al.* 2014). The interaction shapes actors and actors shape interaction patterns. The three variables information, motivation and resources may mutually influence each other as well (Locke and Letham, 2004; Karwai, 2005; Bressers, 2007; Harder, 2008; Logan, 2010).

Many research efforts have shown that the characteristics of a policy network may be a useful base for elucidating the functioning of a policy instrument and its design (e.g. de Bruijn and Hufen, 1998). The concept of policy networks generally contains the assumption that there are both links and actors (Carlsson, 2000b). The implementation process of the policy gets its particular shape through such networks. This conceptual framework (evaluation model) is used to link the registration and policy on the one hand and use practices at farm level on the other hand.

This policy evaluation framework is realized by the different governance approaches focusing on the interaction between governing actors; so the output depends on actor performance. These actors are brought into perspective in the three key ingredients of this study:

1. Policy input and objective. What is the pesticides policy and what are its objectives which are used by the administration to produce outputs? Such resources would include personnel, finance, pesticides registration documents (international chemical conventions, regulation, dossier for pesticides registration, among others) and what the policy says about state and non-state actors of pesticides regarding environment and human health safety and sustainability.

2. Policy implementation process. This refers to the roles of authorities, companies, non-governmental organizations and individuals. Information on how, why and under what circumstances these actors are involved in the course of policy implementation is important. There is the need to identify who are important actors and stakeholders and what they are doing related to safe pesticides registration, distribution and use. There is the need to focus on agricultural and environmental offices from national to local level. This should involve the importers in the country, pesticides inspectors, extension workers, wholesalers, retailers (since they are important source of pesticides for farmers) and farmers' associations.

3. Policy output. This entails the issues and challenges listed by the target groups (farmers) who are faced with, e.g. selection and use of certain products. This is the group where the noticeable effects occurring shortly after or even during implementation can be observed.

This study aims to evaluate 1) how the pesticides policy functions, how state (national and local policy) and non-state (importers, dealers' and farmers) actors are functioning, 2) the extent by which the policy is implemented and enforced including the challenges encountered, and 3) whether the enacted policy achieves its objectives.

Study area and methodology

This study was based on Ghana's pesticides law and two empirical field studies on state policy and non-state policy actors were conducted. Data for this study were supplemented with secondary data and a number of interviews conducted with stakeholders and informants.

Study area and actors

Two empirical surveys were conducted. For the first survey, purposeful sampling was used to select the locations to interview non-state actors (distributors, retailers and farmers). This was done to select those distributors and retailers who had interactions with the regulatory bodies. Farmers were chosen if they applied pesticides themselves, interacted with the pesticides dealers and extension staff. Interviews and inspections were conducted with 13 pesticides importing companies made up of nine national and four foreign companies selected in Accra and Kumasi. These companies had been selected based on their preparedness to respond to questionnaires of the team and to allow their outfits to be inspected. Their simple task was to indicate and show to the team whether their outfits had been inspected by the EPA during the year of the study, whether they had valid pesticides dealers licence to operate as described in section 40 (1 - 2) of the Act, whether they were selling registered pesticides (section 28 of Act) and whether the attendants were provided with PPE which were in-line with section 44 (4 - 5).

Fieldwork was conducted on 30 randomly selected pesticides' retailers in Kumasi-Kejetia, which is the main commercial market in Ghana where most of the import, distribution and retail of pesticides occur. A list of licensed pesticides importers, retailer shops and commercial applicators for the country was used to identify their locations for the interview. Since pesticides are special products under the pesticides law, having the license or not was considered vital for accessing the actors, but the status of licences was noted. The survey was

conducted from May 2013 to January 2014 at seven sites comprising of six irrigation sites from five regions and one plantation area for the farmers. These were the Okyereko (OK) irrigation site (25 respondents) in the Central region, the Weija (WJ) and the Ashaiman (AS) irrigation sites (25 respondents) each in the Greater Accra Region, the Akuse (AK) irrigation site (25 respondents) and cocoa plantations in New Tafo Akim/Tontro (TN) (31 respondents), the Eastern region, the Akomadan (AD) irrigation site (14 respondents) in the Ashanti region and the Tono (TO) irrigation site (11 respondents) in the Upper East region. The study sites were chosen to reflect the increasing importance of farming in the country and where pesticides are used intensively. These regions were selected as representative of Ghana in terms of economic prosperity, agricultural advancement, crops grown, geography and climate among others (Dickson and Benneh, 1998; MOFA, 2011). Crops grown in OK and AS included vegetables (tomato, pepper, onion, okro, garden eggs, cabbage, cucumber, tinda, cowpea, soybean, lettuce, and groundnut) and rice, while vegetables were grown in WJ, AD and TO, rice in AK and cocoa in TN.

A questionnaire was pre-tested in the field on some farmers. The focus was on farmers' understanding of agricultural pesticides used, and possible risks for human beings and the environment when pesticides are used. This allowed for corrections and adjustments to the questionnaire before the final survey. Other information required included the pesticides used, their purity and use dosages, time of application and poisoning symptoms. Information on the use of PPE by farmers whilst applying pesticides was also obtained. The source of information for farmers on new and banned pesticides was noted. Farmers were also asked whether they have been screened for pesticide poisoning. Data were subsequently collected by completing the questionnaire during semi-structured (personal and group) interviews and discussions (in English and local dialects) with local farmers. At least one agrochemical dealer in each site was also interviewed concerning pesticides usage and safety. The registration status of the identified pesticides used by farmers in Ghana was determined from the registration authorities (Environmental Protection Agency, Ghana).

Prior informed consent from each respondent was gained and permission to carry out research at the sites was obtained from the scheme managers of the irrigation sites and from the owners of cocoa farms. A total of 156 farmers voluntarily responded to the questionnaire in the survey. We also observed farmers' practices as they work to validate some of the

questionnaire-based data because most interviews were conducted when farmers were working in the field. Further interviews were conducted with a total of 15 extension staff (local state actors) in the course of data collection with the farmers. These interviews centred on the problems they encounter in the running of their daily activities with respect to their access to information, the available resources and their motivation whilst working with the farmers. It involved 18 questions (10 questions on motivation, three on resource and five on information).

A second survey included a total of 17 extensive interviews with national state actors (policy implementers). They included nine pesticides registration experts from the Ghana EPA, and five persons from the PPRSD. The interview focused on the pesticides policy implementation, the registration process, pesticides inspections and pesticides quality control and available observation in terms of information, motivation and resources. Discussions were also held with the Poison Control Center (PCC) of the Ministry of Health (MoH) on pesticides poisoning related issues. Two officers of the Customs Division of the Ghana Revenue Authority (GRA) were interviewed on import and export controls, access to information, resources, and their motivation. For this, a questionnaire containing 21 questions (motivation 10, resource 5 and information 6 questions) regarding available observation in the implementation process was used. In addition, results of secondary data collected from the registration authority in Ghana were used to verify the authenticity of the findings of the pesticides law (Part II of Act 490, 1994).

The response for the non-state policy actors were mostly “yes” or “no” and the results were presented as percentages. Bivariate analysis using the chi square was used to determine statistically significant associations between the demographic characteristic and farmers’ knowledge, attitude and practices. In addition, multi-criteria statistical cluster analyses was used for responses of the national state policy actors’ (Ghana EPA and PPRSD on pesticide governance). The respondents had the task of assigning a grade between 1 and 5 (1: insignificant, 2: quite insignificant, 3: significant, 4: very significant, 5: most significant) to a particular question. Analysis of the data accepts the general knowledge that state policy actors responded to the same questions regarding implementation of the policy. The answers to the questions provides ordinal qualitative variables, yielding a classic multidimensional matrix consisting of objects (policy implementer) and questions which has attributes referred to as

observations in the form of either a motivation, information or resource question. Responses obtained for particular question form clusters which are mutually-interdependent. The clusters are formed using a hierarchical agglomeration procedure, which progressively clusters groups of elements, starting with the grouping of the most similar ones and, in the following steps, group less similar clusters.

The analysis identifies groups with similar compositions of needs to defined possible solution options (remediations) based on similarities between the responses to the main question. SPSS statistical software (version 21.0) was used for all the analyses.

Results and discussion

Non-state policy actors of pesticides

Farmers' pesticides use practices

Table 2.1 shows the summary statistics of the demographic characteristics of the respondents. Out of the total of 156 farmers that were given a questionnaire, all questionnaires were filled and returned, given a response rate of 100%. Almost all of the farmers interviewed were males and those aged more than 50 years formed the majority among the respondents. The majority had worked for a period between 10-20 years representing 42.7% of the respondents and 58.3% had some form of basic education.

Table 2.1: Demographic characteristics of respondents

Variable	Frequency(N=156)	Percentage
Age (years)		
18-35	50	32.1
36-50	48	30.8
>50	58	37.2
Educational level		
No formal education	49	31.4
Basic	91	58.3
Secondary	13	8.3
College	3	1.9
Duration of work (years)		
<10	34	21.8
10-20	67	42.9
21-30	20	12.8

>30	10	6.4
Stagger planting	25	16.0

All interviewed farmers sprayed their crops with a pesticide to control pests and diseases, an observation that is shared by Ntow *et al*. (2006). Dinham (2003) estimated that 87% of Ghana vegetable farmers use chemical pesticides for pest and disease control. Thirty-three different pesticide products made up different active ingredients from the combined study sites were recorded. Table 2.2 shows the products with their applied doses, recommended doses, active ingredient concentration and their groupings. These included 36% insecticides, 30% fungicides, 30% herbicides and 4% nematicides. All the used pesticides had been registered for use (Table 2.2) in compliance with section 28 (1) of the Act. This is an improvement from a decade ago, since Ntow *et al*. (2006) found in a similar study that 47% of the used pesticides were not registered. Our findings are in line with Ngowi *et al*. (2007) who reported that insecticides are predominantly used for vegetables in Tanzania. However, a pesticide registered to control fungi pest on cocoa, i.e. Kocide (Copper hydroxide), was found in Weija being used for fungi pest on vegetables. This finding is consistent with a study by Amoako *et al.* (2012) who mentioned kocide as a product used for the cultivation of vegetables (cabbage) in Ghana and in violation of section 44 (1) of the Act. Figure 2.2 presents a summary of the origin of pesticides imports encountered at the study sites per the label information during the field study. These were subsequently verified on Ghana's pesticides register of the Environmental Protection Agency. The verification confirmed the products as registered and derived from authentic sources satisfying section 38 of the pesticides Act. The identified products are therefore not likely to pose problems with regards to faking and adulteration.

Table 2.2: Synthetic pesticides recorded in the study and approved by the Environmental Protection Agency of Ghana to control the most important pests in agriculture including their active ingredients, purity, applied and recommended dosages

Active Ingredient	Active Ingredient Conc.	Group	Applied dose, L/ha, kg/ha	Recommended dose on label, L/ha, kg/ha
Herbicide				
*Glyphosate	360 g/L	Phosphonate	1.2-9.8 L	0.5-2.5 L
	480 g/L			
*Paraquat	200 g/L	Bipiridillium	1.5-8.33 L	1.5-3.0 L
*Butachlor	500 g/L	Acetanilide	6.67 L	4.0 L

*Pendimethalin	400 g/L	Dinitroaniline	3.0-9.8 L	2.5-3.0 L
Propanil	360 g/L	Acetanilide	2.0-3.7 L	8.0-10 L
*Bensulfuron methyl	30%	Sulfonylurea	0.42 kg	0.003-0.10 kg
*Bispyribac sodium	400 g/L	Pyrimidinyl oxybenzoic acid	0.10 L	0.015-0.05 L
Propanil +	360 g/L +	Acetanilide	2.0-3.7 L	4.0 L
2,4-D	200 g/L	Phenoxy acid		
*Pretilachlor + Pyribenzoxim	30% + 2%	Chloroacetamide Pyrimidinyl(thio)benzoate	2.0 L	1.0-1.5 L
*Oxyfluorfen + Glyphosate	300 g/L + 360 g/L	Phosphonate	1.5-2.0 L	0.75-0.90 L
Insecticide				
*Lambda-Cyhalothrin	25 g/L 50 g/L	Pyrethroid	1.0-14.8 L	0.6 L 0.4 L
*Chlorpyrifos	480 g/L	Organophosphate	1.0-1.67 L	0.6-1.0 L
*Emamectin benzoate	1.9%	Avermectin	0.62-1.85 L	0.25-0.30 L
Imidacloprid	200 g/L	Neonicotinoid	0.15 L	0.6 L
*Lambda-cyhalothrin + Acetamiprid	16 g/L + 20 g/L	Pyrethroid Neonicotinoid	1.5 L	1.0 L
Acetamiprid	200 g/L	Neonicotinoid	0.37 L	-
Novaluron	35 g/L	Insect growth regulator	0.45 L	-
Thiamethoxam	240 g/L	Neonicotinoid	0.125-0.150 L	0.125-0.150 L
Bifenthrin	27 g/L	Pyrethroid	0.50 L	
*Cypermethrin + Dimethoate	36 g/L + 400 g/L	Pyrethroid Carbamate	2.5-9.8 L	0.5 L
Bifenthrin + Novaluron	30 g/L 35 g/L	Pyrethroid IGR	0.45 L	-
Bifenthrin + Acetamiprid	30 g/L 16 g/L	Pyrethroid Neonicotinoid	0.055-0.075 L	-
Nematicide				
Carbofuran	3%	Carbamate	0.6 kg	20-25 kg
Fungicide				
*Mancozeb	800 g/Kg	Dithiocarbamate	5.93-9.88 kg	0.8-2.0 kg
*Carbendazim	500 g/Kg	Benzimidazole carbamate	0.8-1.6 kg	0.13-0.26 kg
*Sulphur	800 g/Kg		0.8-0.988 kg	0.67 kg
*Maneb	800 g/Kg	ethylene bisdithiocarbamate	9.88 kg	2.0-4.0 kg
Copper Hydroxide	77%		0.74-1.5 kg	2.0-4.0 kg

Metalaxyl + Mancozeb	8% + 64%	Phenylamide	1.0 kg	2.0-2.5 kg
Metalaxyl-M + Cuprous Oxide	12% + 60%	Phenylamide	0.25-4.94 kg	1.0 kg
Cuprous Oxide nordox	86%	-	0.15 kg	-
Cupric hydroxide	53.8%	-	0.8 kg	-
Copper + Metalaxyl	35% + 15%	Phenylamide	0.75 kg	-

(*) Pesticide Products that showed over dosing in their application

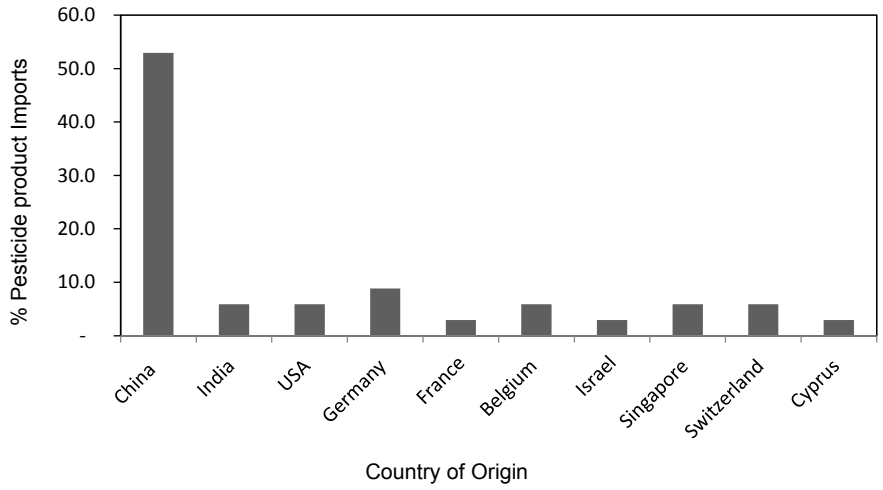

Figure 2.2: Origin of pesticide imports at the study sites

From the first empirical survey, information on the safe handling and use of pesticides appeared to be limited to the farmers. Seventeen pesticides were overdosed (Table 2.3), an assertion described by several other earlier studies (Clarke *et al*. 1997; Ntow *et al*. 1998; Mensah *et al.* 2002) but recent studies are missing. Our results show that pesticides use by this category of respondents contravenes section 44 (1) of the Act. Some of the farmers attributed the reason to overdose to the presence of dew on the leaves of plants especially during the mornings. As a result they usually increase the volume of pesticides product to apply. In their estimation, this could compensate for the excess water on the leaves, and this

is likely to contribute to the overdosing. This assertion needs attention and the necessary corrective intervention by state policy implementers.

Table 2.3: Synthetic pesticides recorded in the study, which were overdosed pesticides as well as the sites where the overdosing took place.

Pesticide Class	Active Ingredient(s)	Applied dose, L/ha, kg/ha		Recommended dose on label, L/ha, kg/ha		Site(s)
		Range	Median	Range	Median	
Herbicide	Glyphosate	1.2-9.8 L	5.5 L	0.5-2.5 L	3.0 L	AS
	Paraquat	1.5-8.33 L	4.9 L	1.5-3.0 L	2.25 L	AS
	Bensulfuron methyl	0.42 kg	-	0.003-0.10 kg	0.05 kg	AS, OK, AK
	Pretilachlor + Pyribenzoxim	2.0 L	-	1.0-1.5 L	1.25 L	AK
	Oxyfluorfen + Glyphosate	1.5-2.0 L	1.75 L	0.75-0.90 L	0.83 L	WJ
	Pendimethalin	3.0-9.8 L	6.4 L	2.5-3.0 L	4.0 L	AS, AK
	Bispyribac sodium	0.10 L	-	0.015-0.05 L	0.03 L	AK
	Butachlor	6.67 L	-	4.0 L	-	OK
Insecticide	Lambda-cyhalothrin	1.0-14.8 L	7.9 L	0.4-0.6 L	0.5 L	AS, OK, WJ, AK
	Emamectin benzoate	0.62-1.85 L	1.24 L	0.25-0.30 L	0.28 L	AS, OK, WJ
	Lambda Cyhalothrin + Acetamiprid	1.5 L	-	1.0 L	-	OK
	Cypermethrin + Dimethoate	2.5-9.8 L	6.15 L	0.5 L	-	AS, OK
	Chlorpyrifos	1.0-1.67 L	1.33 L	0.6-1.0 L	1.1 L	AS, OK, WJ, AK
Fungicide	Mancozeb	5.93-9.88 kg	7.9 kg	0.8-2.0 kg	1.4 kg	AS, WJ, OK
	Carbendazim	0.8-1.6 kg	1.2 kg	0.130-0.260 kg	0.2 kg	WJ
	Sulphur	0.8-0.988 kg	0.89 kg	0.67 kg	-	AS, AK
	Maneb	9.88 kg	-	2.0-4.0 kg	2.0 kg	WJ, AS

NB: AS Ashaiman, AK Akuse, WJ Weija, OK Okyereko, TN Tontro

Farmers indicated that they mix the pesticides close to the rivers, streams and canals (Table 2.4). All the interviewed farmers indicated that they cleaned their spraying equipment after pesticides use by rinsing with water, and that canals and drains have sometimes been compromised by emptying the rinse water into nearby water bodies. Practices of mixing pesticides and washing tanks near and in the river as well as throwing pesticide containers after use in the river or forests could pose environmental risks to aquatic environment.

Table 2.4: Questions on farmers and sprayers knowledge, attitude, practices during pesticide use and occurrence of recent spills (n=156)

Question		Yes	Percentage
a)	Have you ever spilt pesticide mix on your body whilst working		
i.	Because of improper fitted lid	142	91
ii.	During Pouring, loading	141	90
iii.	Wrong wind direction	156	100
iv.	Leaking equipment	156	100
v.	Falling in the field	156	100
vi.	Wrong movement with the sprayer	156	100
vii.	Spray above the body	156	100
b)	How can you help a colleague during pesticide splash		
i.	Advice washing	156	100
ii.	Go to health center	156	100
iii.	Advice drink water	0	0
iv.	Advice drink red palm oil	0	0
v.	No problem, no idea	0	0
c)	What protective measure did you take to protect yourself at your last spray operation		
i.	Wore overall	0	0
ii.	Wore safety shoe	25	16
iii.	Used respirator	4	3
iv.	Used gloves	0	0
v.	Used goggles	0	0
vi.	Used apron	0	0
vii.	Used a hat	0	0
viii.	Practiced careful working	156	100
ix.	Timed the spraying e.g. early morning	156	100
d)	What did you do during and after spraying the pesticide		
i.	Wash your hands after spraying?	156	100
ii.	Eat/drink/smoke during work with pesticides	12	8
iii.	Keep meals near pesticides?	0	0
iv.	Drink water near pesticide treated fields	0	0
v.	Shower after pesticide exposure	24	15
vi.	Change clothing before and after pesticide exposure	7	5
e)	Where do you prepare pesticide mix for application		
i.	Chemical store	0	0
ii.	Outdoors	0	0
iii.	Close to dam/river/stream	156	100
iv.	In the house	0	0
v.	Wherever	0	0
f)	How did the most recent accidental spill that you experienced take place?		

i.	Whilst mixing (Accidental)	1	1
ii.	During preparation for spraying	0	0
iii.	Inferior equipment	128	82
iv.	While storing	0	0
v.	Other (strong wind)	27	17
g)	Have you ever been screened for pesticide poisoning before?	0	0

The possible environmental risks have been demonstrated in other studies by Ramo *et al.* (2016) and Teklu *et al*. (2016) in Costa Rica and Ethiopia, respectively. There is, therefore, a need to perform environmental risk assessments of current pesticides use in Ghana to identify pesticides that pose the highest risks to the aquatic environment and to determine threshold levels of the pesticides that are protective of the environment.

The data indicated that accidental spills took place in the field during pesticides application as a result of inferior equipment (82%), when removing pressurized tubes and nozzles due to strong winds (together 17%), while one farmer reported an accidental spill during mixing (Table 2.4). Farmers are probably the actors having the greatest risk of pesticides poisoning due to their intimate contact with pesticides. Ntow *et al*. (2006) found that knapsack sprayer is prone to leakage, especially when it is getting old. Matthews *et al.* (2003) emphasises the need to provide better-quality, affordable and comfortable equipment.

A couple of farmers (15%) wash themselves after accidentally being exposed to pesticides while others (5%) changed clothing before and after pesticides exposure, while the remaining farmers did not do anything (Table 2.4). This lack of adherence to strict safety measures under section 44 (4) of the Act could lead to different health problems.

Interviewed farmers indicated that they got most information and updates regarding pesticides usage and safety, banned pesticides including new methods of pesticide application, through extension staff (Table 2.5). Interactions with the farmers revealed that information from the registration authorities is not disseminated easily to the farmers and information on the status of pesticides is not regularly published. It is expected that the registration authorities would seriously engage the services and expertise of the agricultural extension staff to disseminate information to the farmers a view shared by Ngowi *et al.* (2007).

Table 2.5: Questions on information on pesticide usage and safety, banned pesticides and new methods of application (n=156)

Item	Yes	Percentage
Pesticides Usage and Safety		
Extension staff	120	77
Labels	17	11
Consultants	19	12
Banned Pesticides		
Extension Staff	115	74
Consultants	19	12
Meetings	13	8
Farmer's Association	9	6
New Methods of Pesticide Application		
Extension Staff	115	74
Consultants	19	12
Meetings	11	7
Farmers' Association	11	7

The survey showed that the interviewed farmers had gained some form of expertise on pesticide application and safety. Most of the knowledge and expertise acquired was from formal advice (90%) and through training on the job. Additionally, extension staff and consultants who promote their pesticides also engaged them (Table 2.5).

Generally, none of the farmers had recorded any pesticide spill on their body as a result of wrong wind direction, leaking equipment, as a result of falling in the field, wrong movement with the sprayer or spraying above the body. However, 90% of farmers admitted spill during pouring and loading of spray equipment, suggesting the need for special attention on the correct and appropriate means of pouring and loading spray equipment in subsequent training sections. Farmers had ample knowledge on how to help a colleague in the event of pesticide splash, and apart from safety shoes and respirators, no respondent had used protective measures, i.e. PPE, to protect themselves during their spray operations (Table 2.4). Other studies have also shown that protective actions using PPEs are rarely taken while handling and applying pesticides (Berg, 2001; Perry *et al.* 2002; Matthews *et al*. 2003). Wilson and Tisdell (2001) reports that protective clothing have not been used enough particularly in less developed countries. A lack of money to buy them and the absence of (enforcement of)

regulations on their use are posed as the most important reasons for this. However in Ghana, this is a clear violation of section 44 (1, 2 and 4) of the pesticides Act. In the survey farmers complained of the cost of PPEs and the fact that it is uncomfortable to use. Ntow *et al.* (2006) reported similar findings that the PPEs are hardly used by Ghanaian farmers because of discomfort associated with the hot and humid weather and their costs. However, there is the urgent need for farmers' attention to be drawn to the usefulness of the PPEs through practical demonstrations by extension staff. Okoffo *et al*. (2016) reported that the influence of extension service on the use of PPE is significant enough to strengthen it in order to increase farmers' knowledge and awareness of the consequences of applying pesticides without PPE. The study showed that the age of farmers had a significant influence on their knowledge about the use of pesticides. A bivariate analysis using the chi square revealed statistically significant associations between age and knowledge variables such as: the use of improper fitted lid, identification of wrong wind direction during spraying, knowledge during pouring and loading of pesticides as well as wrong movement during spraying of pesticides ($\chi^2 = 32.236$, $P < 0.001$). There was also significant association between educational level attained and knowledge ($\chi^2 = 3.614$; $P \leq 0.05$). Work experience or duration of farming also significantly influenced the knowledge of respondents ($P < 0.001$).

The study further revealed statistically significant associations between age and practice such as: the washing of hands after spraying, eat/drink or smoke during working with pesticides, keep meals near pesticides, drinking water near pesticides treated fields, shower after pesticides exposure and changing of clothing immediately after pesticide exposure ($P < 0.001$). There was a significant association between educational level attained and farm management practices ($P < 0.05$). Work experience or duration of farming was significantly associated with farm management practices at 5% level of significance ($P \leq 0.05$).

Interactions with the farmers revealed that they are not conversant with the pesticides law and the provisions in it to safeguard them and the environment. The registration authorities in collaboration with the extension services have to educate the farmers at their meetings of their roles and responsibilities regarding the pesticides law, its provisions and penalties especially sections 44, 56-62. The behaviour and action of farmers has been motivated by certain factors that pertain to their setting and circumstances. Interviewing the farmers indicated that 76% of them use products immediately, whilst 24% use the products

within a month. Storage is limited since sales outlets are within reach of the communities, the farms are small and finances are limited. The decrease in the time of storage for the use of the products is encouraging, as the likelihood of exposure to the pesticides and related health effects are reduced, since most farmers store pesticides in their house but not in bedrooms (89%). Five percent of the respondents keep it somewhere on the farm for later use. Two percent and 4% of the farmers stored the pesticides in their general stores and bedrooms, respectively. Storing pesticides in the homes and bedrooms for long durations can lead to exposure and risk of intoxication (Clarke *et al*. 1997). Kimani and Mwanthi (1995), Ngowi *et al*. (2001) and Murphy *et al*. (2002) report that it is very common in many developing countries to store pesticides at unguarded places in their homes. In the upper East region of Ghana 15 farmers died in 2010 which was attributed to pesticides poisoning, mostly related to poor storage of pesticides (NPASP, 2012). Seventy percent of the farmers purchase pesticides from local dealers/retailers, while 6% obtained the products from importers/local agents in the cities. Those who purchased them from consultants of the importing companies were 4% and remaining were those involved in the governments mass spraying exercise in Tontro site (20%).

Seventy percent of the farmers used rate of applications recommended by the supplier, retailer or dealer. This was followed by the recommended application rate or frequency on the packaging label, and those who used their own application rate and frequency (Table 2.6). This may be a result of the direct contact between the suppliers and the farmers and the resulting ease to convince them. Ntow *et al.* (2006), however, reports that agricultural extension officers and/or pesticide labels as main source of information on pesticides application rates.

Table 2.6: Questions on skills and knowledge for storage of pesticide and use of recommended application of pesticides (n=156)

Item	Yes	Percentage
Skills and knowledge of storage		
Stored in the house, not bedroom	138	89
Somewhere on the farm for later use	9	5
Store pesticides in general stores	3	2
Store in the house, bedroom	6	4

Recommended application		
Label recommendation	41	26
Supplier recommendation	109	70
Own recommendation	6	4

With regards to choice of using a pesticide (Table 2.7), seasonal occurrence of pest (45%) especially during land preparation (weed control) was the most important factor followed by preventive reasons (15%), pest density control (8%), curative factors (4%), weather factors and defensive related use (3%) and routine application (22%). Amoako *et al.* (2012) conducted a similar study in Ashanti region of Ghana and reported a contrary observation, i.e. that choice for a particular pesticide was based on its availability on the market in their area of operation, its price and its efficacy for insect pests. From the result, it is important to encourage farmers to use pesticides only when necessary as anticipated pest occurrence and pesticides application may lead to problems of pest resistance, environmental pollution, and occupational exposure, among others (Metcalf, 1980; Ngowi *et al*. 2007; Ramo *et al*. 2016). The pesticides use and frequency by farmers are provided in Table 2.7.

Table 2.7: Decision for selection of pesticide for use (n=156)

Item	Yes	Percentage
Seasonal occurrence of pest	70	45
Preventive reasons	23	15
Pest density control	12	8
Curative factors	6	4
Weather factors,	5	3
Defensive related use	5	3
Routine application	34	22

Most farmers mentioned during the discussion that pesticides are necessary, but are open and willing to use appropriate alternative methods of pest control if they became available, effective and affordable. Farmers mentioned health problems like headaches, burning sensation in the eyes, itching and skin irritation, among others (Table 2.8). Pesticides exposure may result in physical and mental illnesses such as dermatitis, anxiety, irritability,

loss of memory and depression, which ultimately may result in suicide (Kishi *et al*. 1995; Koh and Jeyaratnam, 1996; Harris, 2000). It is estimated that worldwide 3 million people are affected by pesticides poisoning annually, resulting in 220,000 deaths (Konradsen *et al*. 2003). The situation calls for immediate attention for necessary solution options from the authorities. The farmers also remarked that they had not been screened specifically for pesticides poisoning before (Table 2.4), and therefore were prepared to subject themselves to be screened for pesticides exposure/poisoning if the opportunity is made available.

Table 2.8: Have you experienced any of the listed symptoms following pesticide application? (n=156)

Symptom	Yes	Percentage
Headache	156	100
Burning sensation in eyes/face	156	100
Fever	146	94
Watering eye	156	100
Skin rash	142	91
Itching and skin irritation	156	100
Dizziness	154	99
Cold, breathlessness and/or chest pain	122	78
Forgetfulness	136	87
Loss of libido	83	53
Salivation and vomiting	110	71
Abdominal pain/diarrhoea	117	75
Weakness	156	100

Pesticides import, distribution and retail

The involvement of private actors in importation, distribution and retailing of pesticide products in Ghana has been increasing since the introduction of the pesticides registration. Currently the pesticides distribution in Ghana is performed by many small-scale private businesses and their number increased from 515 in 2010 to 916 in 2011 (Personal communication, Office of the Pesticides Registrar, Ghana EPA). Following the implementation of the law, 441 pesticides had been registered in December 2014 for agricultural and household uses by the EPA. The registered pesticides included 47% insecticides, 12% fungicides, 37% herbicides, 1% plant growth regulators, and 1% (molluscicides, rodenticides,

nematicides and adjuvants). It is on record that the number of registered pesticides increased from 2003 to 2011 (Fig. 2.3), whilst the volume of imported pesticide products was on average of 9,216 tons of insecticides, 8,986 tons of herbicides and 2,545 tons of fungicides from 2004 to 2015 (Personal communication, Office of the Pesticides Registrar, Ghana EPA).

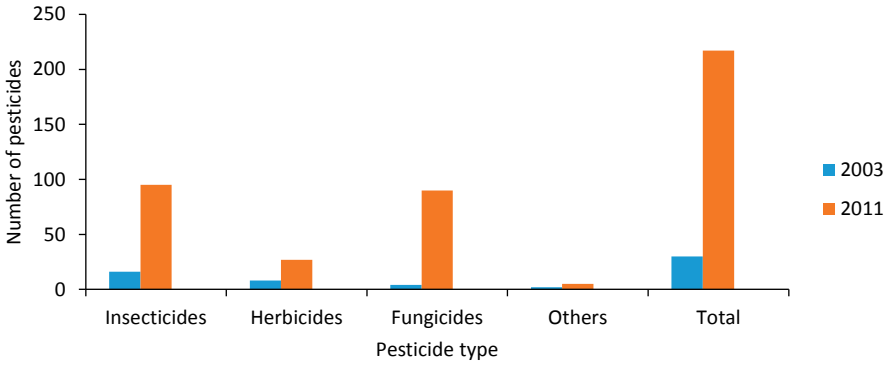

Figure 2.3: Number of formulated pesticide products registered or provisionally cleared in 2003 and 2011. Source: Environmental Protection Agency-Ghana, Annual Reports, Accra. "Other" includes rodenticides, nematicides, fumigants and other conventional pesticides, and other chemicals used as pesticides such as petroleum oil

It is worth mentioning that currently there are no pesticides manufacturing and formulation plants in Ghana, so all pesticide products are imported (Fig. 2.2). The rapid increase in the amount of pesticide companies and retailers shows the lucrative nature of the pesticides business in Ghana. The motivation is the profit on sales as the interaction revealed. Empirical findings of this study showed that all the visited distributors had valid licences to operate and pesticides registration permits for the displayed pesticide products (Table 2.9).

Table 2.9: Compliance to Pesticide Registration Licence by pesticide dealers

Question	Yes	Percentage
a) Has this place been inspected by the EPA/PPRSD (2014/15)?		
Importer/Distributor (n=13)	13	100
Pesticide Retailer (n=30)	30	100
b) Has the activity been licensed by the EPA		

Importer/Distributor (n=13)		13	100
Pesticide Retailer (n=30)		23	77
c) Technical Know-how/Use of PPEs			
Importer/Distributor			
i.	Know the Pesticide Law	13	100
ii.	Do you have the current pesticide registration list (Dec. 2014)?	7	54
iii.	Knowledge/skill to identify symptoms of pest attack?	13	100
iv.	Technical Knowledge on field diagnosis of pest?	13	100
v.	Know the different pesticide application methods?	13	100
vi.	Use of PPE	3	23
Retailer			
i.	Know the Pesticide Law	30	100
ii.	Do you have the current pesticide registration list (Dec. 2014)?	0	0
iii.	Knowledge/skill to identify symptoms of pest attack?	5	17
iv.	Technical Knowledge on field diagnosis of pest?	6	20
v.	Know the different pesticide application methods?	26	87
vi.	Use of PPE	11	37

The displayed products were not expired (Table 2.9). This was to be expected as their ability to import pesticide products are tied in to the renewal of licenses. However, 23% of the retail outlets had their licenses expired or in the process of being renewed in violation of section 40 (1) of the Act. Similar observations were made regarding their knowledge of the pesticides law, as their appreciation of it was generally inadequate. The distributors and retailers violated section 44 (4 and 5) of the Act. The provision and use of PPEs as well as the technical knowledge on the handling of pesticides by retailers was low (Table 2.9).

The observation suggests the probable shortage of expert advice and technical support on pesticides for farmers who may patronise these shops. This could lead to problems of indiscriminate use, high frequency of application and application of pesticides with the same mode of action, resulting in pest resistance and resurgence and associated indirect costs. Gill and Garg (2014) discussed other potential management options including cultural and physical control, host plant resistance, biocontrol, and the use of biopesticides. Although having limited knowledge, many farmers still prefer to contact a pesticides retailer instead of an extension official when problems arise, because of their close proximity. Mengistie *et al*. (2014) reported a similar trend whilst seeking information by farmers in Ethiopia. Discussion

with owners of the shops indicated that most of their recruited staff upon successful training in pesticides management resign to either establish their own businesses or join companies with better remuneration. However, since the level of know-how of the retailers needs further improvement, rigorous information dissemination by the extension service is required.

State policy actors of pesticides

The state policy actors of pesticides was considered at national (Ghana EPA and PPRSD) and local (extension staff) levels. The state actors are important to transfer knowledge to importers, distributors/retailers and farmers and to increase the implementation of policy at both the national and the local (farm) level.

National state actors

The ranked score gave an indication of how the issues questioned on (motivation, information and resources) had performed and showed those that had been achieved, those in-between and those that had underperformed and needed attention. This defines the strong and weak aspects of the implementation process. It is clear that "salary is encouraging" and "transport facilities are adequate to access pesticides dealers and users" are the least scored. This indicates the need of state policy implementers for improvements in salaries and means to reach pesticides distributors, retailers and farmers. Among the most strong aspects in the implementation process investigated were "knowledge of the pesticides law", "current pesticides register", "pesticides registration process", "different pesticide application methods" and "work being interesting" (Table 2.10).

Table 2.10: Ranking of responses to questions and related observation (n=17)

Rank	Motivation(M)/Resource(R)/Information(I)	Observation	Sum of responses
1	Know the Pesticide Law	I	85
1	Do you have the current pesticide registration list (Dec. 2014)	I	85
1	Familiar with the pesticide registration process?	I	85
4	Know the different pesticide application methods?	I	79
4	Work itself interesting	M	79
6	Current Job is satisfactory	M	77

7	Knowledge/skill to identify symptoms of pest attack?	I	75
7	Technical Knowledge on field diagnosis of pest?	I	75
9	Job security	M	66
10	The relation between management and employees	M	64
11	Technical staff for risk assessment of submitted pesticide dossiers?	R	59
12	In-service training and skills development on current job satisfaction	M	56
13	Sufficient space to work	M	51
14	Pesticide user manuals are available to be effectively used by pesticide dealers	R	49
15	Accredited laboratory to test pesticide products?	R	44
16	Carrier structure and promotion on current job satisfactory	M	34
17	Recognition, rewards, praise by supervisors	M	32
18	Financial benefits and bonuses	M	30
18	No. of pesticide inspectors assigned to dealers and users of pesticides proportional?	R	30
21	Salary is encouraging	M	21
21	Transport facilities are adequate to access pesticide dealers and users?	R	21

Motivation=M); Resource=R; Information=I

Figure 2.4 shows the available observation criteria for state policy implementers in policy implementation hierarchical cluster. The tree-diagram depicts the result of the cluster analyses of 21 mutually dependent questions and attributes (referred to as observation – motivation (M), information (I) and resource (R) shown in Table 2.10) and responses for the cluster represents people who share similar concerns and characteristics.

The first cluster (most left) are state policy actors who know the pesticides law, have the current pesticides registration list, are familiar with the pesticides registration process, know the different pesticides application methods, have knowledge/skill to identify symptoms of pest attack, have technical knowledge on the diagnosis of pest in the field, find the work itself interesting, and are satisfied with their current job.

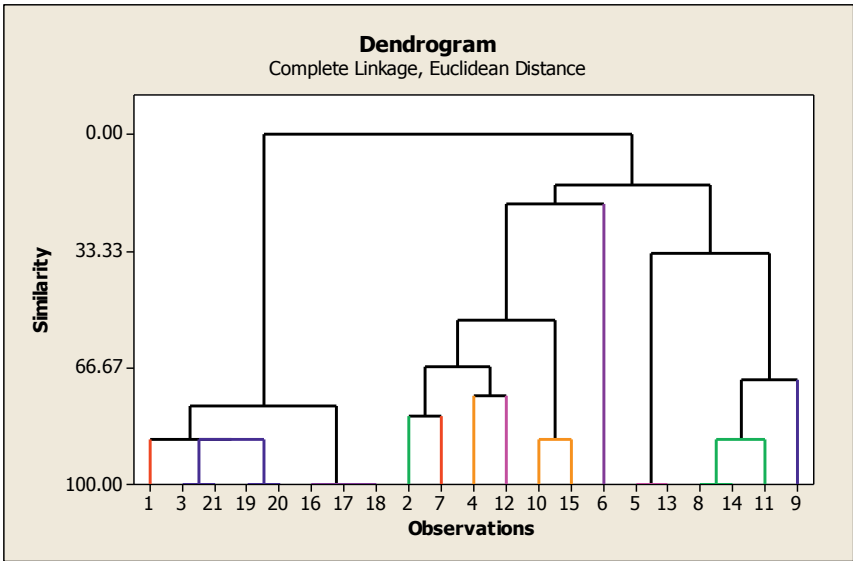

Figure 2.4: Diagram showing hierarchical cluster of observation (motivation (1-10), resources (11-15) and information (16-21)) by policy implementers.

These people find their work to be the most significant contributor to their motivation. Motivation, resources and information are significant to achieving their required job. This cluster can be described as the work result recognition group (Zámečník, 2014).

The second cluster (middle) concerns the relation between management and employees, technical staff for risk assessment of submitted pesticide dossiers, in-service training and skills development on current job satisfaction, sufficient space to work, pesticide user manuals are available to be effectively used by pesticide dealers, and carrier structure and promotion on current job satisfaction. To a large extent, the second cluster is linked to the first cluster -motivation and resources are significant to achieving this required job.

The third cluster (most right) is composed of accredited laboratory to test pesticide products, recognition of actors input to achieving results by management, rewards and praise by supervisors for success, financial benefits and bonuses, number of pesticide inspectors assigned to dealers and users of pesticides proportional, unattractive salary, lack of transport facilities to adequately access pesticide dealers and users. In a similar study by Mengistie *et al.* (2014) in Ethiopia, the majority of the actors indicated that they were underpaid given their

workload. This cluster can be called the materialistic cluster since motivation and resources are significant to achieving their required job, and these are the main factors undermining the proper implementation of the pesticide registration policy (Zámečník, 2014).

Local state actors

Respondents were motivated with high scores regarding security of job (100%), interested in what they do, and that the job was satisfactory (Table 2.11). Salary, financial benefits, bonuses and recognition for work done by supervisors, however, was low. Access to information was considered adequate with respect to the pesticide law, knowledge and skills to identify symptoms of pest attack, diagnosis and the different pesticide application methods. Lessons drawn from Ntow *et al*. 2006 points to the importance of agricultural extension officer's involvement in farmers' knowledge of insecticide application. The exception recorded in the study is the unavailability of the pesticides register for 2014. All respondents were of the opinion that the proportion of extension officers to dealers and users of pesticides was low and that there is a lack of transport to easily access the pesticide dealers and users (Table 2.11).

Table 2.11: Responses of state actors at local level (n=15)

Item		Yes	Percentage
A) Motivation			
i.	Current job is satisfactory	12	80
ii.	In service training and skills development on current job satisfaction	9	60
iii.	Work itself interesting	13	86
iv.	Carrier structure and promotion on current job satisfaction	11	73
v.	Salary is encouraging	3	20
vi.	Job security	15	100
vii.	The relation between management and employees	9	60
viii.	Financial benefits and bonuses	3	20
ix.	Recognition, rewards, praise by supervisors	3	20
x.	Sufficient space to work	10	66
B) Resource			
vii.	Transport facilities are adequate to access pesticide dealers and users?	5	33
viii.	No. of pesticide inspectors/extension assigned to dealers and users of pesticides proportional?	0	0
ix.	Pesticide user manuals are available to be effectively used by pesticide dealers and farmers?	11	73

C) Information			
vii.	Know the Pesticide Law	15	100
viii.	Do you have the current pesticide registration list (Dec. 2014)?	8	53
ix.	Knowledge/skill to identify symptoms of pest attack?	15	100
x.	Technical Knowledge on field diagnosis of pest?	15	100
xi.	Know the different pesticide application methods?	15	100

Conclusions

Pesticides legislation on registration and licensing is relatively well-developed in Ghana. The study shows a couple of challenges in the policy implementation. These findings have a number of effects on pesticides implementation policy and agricultural sustainability in general. The focus of this study was that policy implementation processes are interaction processes between state actors (policy implementers) and non-state actors (farmers and pesticide dealers, importers etc.) in relation to attributes as information, motivation and resources. The pesticides policy implementation in Ghana has not been able to adequately deal with the non-state actors such as pesticide dealers with respect to the choice of particular pesticides for a given problem and technical knowledge on field diagnosis of pests and diseases. This thus make it difficult to professionally dispense pesticides to farmers including advice on the use of PPEs. Although some farmers are aware of the risks associated with pesticide use, adequate protection provided by PPEs is hardly used. Adequate training on the pesticide handling, use and diagnosis of disease symptoms in the field is required more of state actors and suppliers to train farmers to rotate the use of chemical pesticide thus reducing the risk of pest resistance. Also, farmers should be encouraged to use their old clothes during preparation and spray operations instead of buying special clothes for spraying, which may be expensive for them. Farmers with a combination of a bit of education and extensive experience identified in study could be used to promote best knowledge, attitude and practices to other farmers. Farmers should also be trained on acute and chronic symptoms of pesticide poisoning and for them to better appreciate the necessary remediative steps to take once they experience such symptoms.

Most importantly, our study reflects the stronger involvement of state actors with the responsibilities to make available to non-state actors various sources of information with regards to pesticides use, management of pesticides and the pesticides law as well as friendly

PPE alternatives for farmers through government intervention at subsidised prices. Finally, the pesticides regulations should be passed and implementers (Ghana EPA/PPRSD) should also be motivated and resourced enough to carry out their mandate in Ghana.

Chapter 3

Environmental risk assessment of pesticides currently applied in Ghana

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Abstract

Registration of pesticides for use in Ghana is based on prospective environmental risk assessment (ERA) to assess the risks of future pesticide use on the environment. The present study evaluated whether pesticides currently used by Ghanaian farmers may harm the aquatic and terrestrial environment under day-to-day farm practice by performing a 1st tier ERA for terrestrial and aquatic environment and a 2nd tier ERA for the aquatic environment using existing scenarios and models. The evaluation applied the 1st tier PRIMET (Pesticides RIsks in the Tropics to Man, Environment, and Trade) model, as well as the Species Sensitivity Distribution (SSD) concept to determine environmental exposure concentrations and ecological threshold levels of the pesticides protective to aquatic and terrestrial communities. Results of the 1st tier risk assessment indicated that in the investigated regions in south Ghana, many pesticides might pose an acute risk to aquatic ecosystems adjacent to the treated fields while lambda cyhalothrin, chlorpyrifos, cypermethrin, dimethoate, mancozeb, carbendazim, sulphur, maneb, copper hydroxide and cuprous oxide may pose the highest chronic risks. Butachlor, dimethoate and carbendazim may pose acute risks to the terrestrial soil ecosystem, while glyphosate, chlorpyrifos, imidacloprid, dimethoate, mancozeb, carbendazim, maneb, copper hydroxide and cuprous oxide may pose the highest chronic risks. Paraquat, lambda cyhalothrin, chlorpyrifos, emamectin benzoate, imidacloprid, thiamethoxam, bifenthrin, cypermethrin, dimethoate, carbofuran, mancozeb and maneb may pose acute risks to bees and propanil, lambda cyhalothrin, chlorpyrifos imidacloprid, carbofuran, sulphur, copper hydroxide and cuprous oxide to the terrestrial non-target arthropods. The 2nd tier acute aquatic risk assessment showed that the risks of pendimethalin, propanil, oxyfluorfen, lambda cyhalothrin, chlorpyrifos, cypermethrin, dimethoate carbofuran, mancozeb, carbendazim, maneb and copper hydroxide could be substantiated using species sensitivity distribution (SSD). Actual pesticide use was a factor of 1.3 to 13 times higher than the recommended label instructions, indicating a general practice of overdosing. The case study shows that the PRIMET model in combination with the SSD concept may offer pesticide registration authorities in Ghana a means to assess environmental risks associated with pesticide usage in a user-friendly and cost-effective manner.

Introduction

Agriculture makes a big contribution to the economy of Ghana ranking second to the services sector in terms of gross domestic product (GSS, 2015). Inputs such as pesticides, fertilizers and improved planting materials are increasingly used (WAAPP, 2014). The use of pesticides is important to protect crops from pests which has significantly reduced losses and improved the yield of crops such as cereals, vegetables and fruits (MOFA, 2003). Information from the Environmental Protection Agency of Ghana indicated that 540 pesticides have been registered and are available for use in agriculture and public health as of December 2015 (Ghana EPA, 2015). Pesticides applied to the field are of concern because of the risk of pollution, especially to vulnerable aquatic and terrestrial ecosystems (Aktar *et al*. 2009). The need to monitor the environmental risks of pesticides has been highlighted (Vijver *et al*. 2017), but Ghana's pesticide law does not have the necessary regulation to adequately address this issue (NPASP, 2012). Although pesticide use is high in Ghana, regulatory infrastructure is underdeveloped or not adequately enforced and capacity for routine monitoring programmes is lacking (NPASP, 2012; Onwona Kwakye *et al*. 2019).

The registration of pesticides for use in Ghana is based on prospective risk assessment, while the development of the underpinning field of sciences, i.e. environmental chemistry and ecotoxicology, is in its early stages in Ghana. Local studies on pesticides regarding environmental risk assessment and particularly assessments of pesticides toxic effects on aquatic and terrestrial organisms have not been widely undertaken. A few studies that have been conducted involved pesticides exposure in rivers in the intensive cocoa growing areas of the Ashanti and Eastern Regions of Ghana. In Oda, Kowire and Atwetwe rivers, for example, mean pesticide concentrations found in water samples for lindane and endosulfan were 19.4 and 12.4 µg/L (Oda), 16.4 and 17.9 µg/L (Kowire), 20.5 and 21.4 µg/L (Atwetwe), respectively (Acquaah, 1997). A study published by Ntow in 2001 on organochlorine pesticide levels in water samples collected from streams near the city of Akumadan, a prominent vegetable-farming area in Ghana, showed that endosulfan sulfate was the most frequently occurring pesticide, detected in 78% of the sampled waters with a mean concentration of 30.8 µg/L. In a similar study on the Volta Lake, lindane was detected in 38 samples, comprising of 76% of the analysed samples. Lindane and endosulfan were identified in relatively low mean concentrations of ≤ 0.008 and 0.036 µg/L, respectively (Ntow, 2005).

The current study evaluated whether current pesticide use by Ghanaian farmers may harm the environment under day-to-day farm practice by:

1. performing a 1st tier environmental risk assessment to identify pesticides that may pose a risk to the aquatic and terrestrial environment using the PRIMET (Pesticides Risks in the Tropics to Man, Environment, and Trade) model (Peeters *et al*. 2008);
2. determining 2nd tier threshold levels that are protective of aquatic communities in the study site(s) using the Species Sensitivity Distribution (SSD) concept (Maltby *et al*. 2005);
3. evaluating the use of banned products and the overuse of pesticides, i.e. higher use than recommended dose.

The findings of this paper will contribute in filling the pesticide risk assessment gap with respect to available tools and procedures for especially the aquatic environment in Ghana. If risks are indicated, it is expected that the pesticide registration authority (Ghana Environmental Protection Agency; EPA) will use the information to initiate the necessary changes of farmers' pesticide use and that of other stakeholders to improve the quality of the aquatic and terrestrial environment.

Materials and methods

Study sites

A survey was conducted between May 2013 to January 2014 in four selected irrigation sites and a cocoa farming community involving 131 farmers. The sites were in the Central (Okyereko), Greater Accra (Weija and Ashaiman) and Eastern (Tontro/New Tafo and Akuse) regions of Ghana (Fig. 3.1). The study sites were chosen to reflect i) the steady increase of crop farming in the country, ii) the regions which uses pesticides intensively and iii) the regions being representative of Ghana in terms of agricultural advancement, crops grown, geography, and climate, among others (Dickson and Benneh, 1998; MOFA, 2011). The system of farming was mainly mono-cropping for each of the sites.

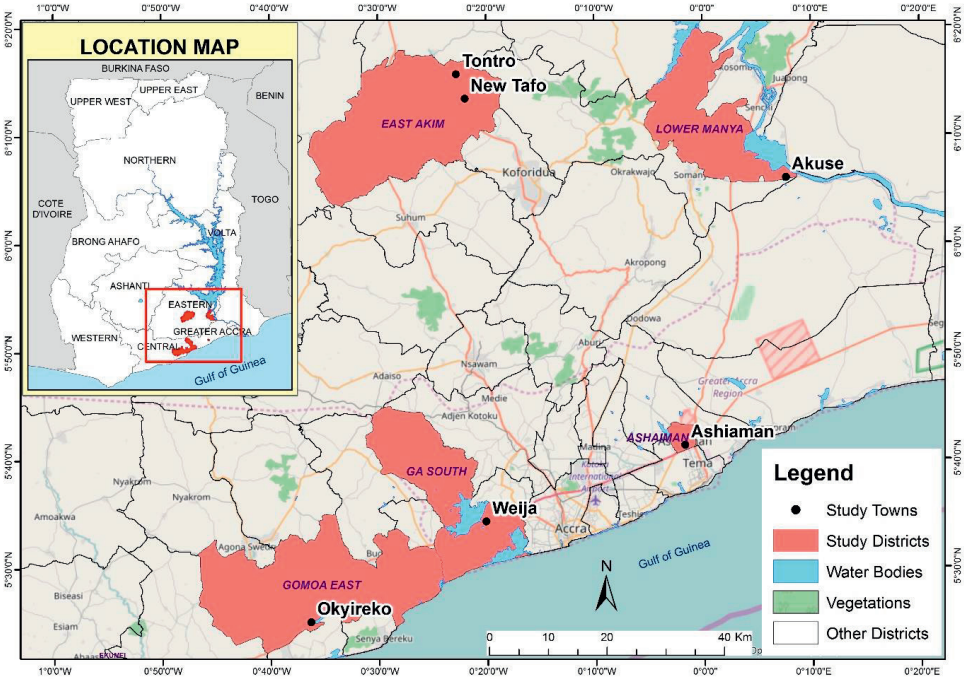

Figure 3.1: Geographical location of the study sites in Ghana.

Prior informed consent was obtained from each respondent and permission to carry out research at the sites was obtained from the scheme managers of the irrigation sites and from the owners of the cocoa farms. Information on pesticides used, application dose, and frequency were obtained from the farmers by way of questionnaire administration and records of observations of farmers whilst working in the field. The application rate of the pesticides being applied was particularly noted and compared to the recommended rates on the pesticide label (see Table SI 3.1; supplementary information for questionnaire used).

The PRIMET model

The 1st tier risk assessment of the pesticides to the aquatic and terrestrial environment was performed by applying the risk assessment model PRIMET (Pesticide Risks in the Tropics to Man, Environment and Trade; version 2.0) using hypothetical exposure scenarios (Peeters *et al*. 2008). To perform a risk assessment in PRIMET, a scenario describing the physical properties of the environmental compartment must be provided as well as data on the

physico-chemical properties of the pesticides and the sensitivity of the organisms under evaluation. Scenarios of actual pesticide use were limited to application method, dosages (g active ingredient (a.i.)/ha), application interval (d) and frequency of use reported by the farmers. Pesticide formulations that had been made of more than one active ingredient were separated into the different active ingredient concentrations (Table SI 3.2). For each environmental compartment (aquatic, soil, bee and non-target arthropods), PRIMET calculates an exposure concentration (Predicted Environmental Concentration, PEC) and a threshold concentration for effects (Predicted No Effect Concentration, PNEC), from which the Exposure Toxicity Ratio (ETR) can be calculated by dividing the PEC by the PNEC. An ETR lower than 1 indicates that no serious risks are expected, an ETR between 1 and 100 indicates that risks may be present, while an ETR of higher than 100 indicates that risks are very likely to occur. The PRIMET DSS (Decision Support System) is freely available on www.primet.wur.nl and incorporated in a Graphical User Interface.

Physico-chemical data

In order to calculate the exposure concentration using the PRIMET model, data on each of the pesticides' intrinsic physico-chemical properties were mostly already available in the model and, if not, taken from literature sources. Most available data were collected for the temperate regions of Europe and North America, but were temperature corrected within the PRIMET model. Table SI 3.3 shows the pesticide physico-chemical characteristics required for the PRIMET model. The pesticide products and active ingredients evaluated are the most used pesticides in the study area. The variables in Table SI 3.3 were obtained from either the PPDB: Pesticide Properties DataBase (PPDB, 2020) or already given in the PRIMET database.

First tier acute and chronic aquatic risk assessment

In this 1st tier risk assessment for the aquatic risk assessment only entry via spray drift was taken into consideration. An irrigation channel with an aquatic waterway of 1 m wide at the bottom, a slope of 0.5 and a water depth of 0.5 m was used for the aquatic scenario. The length from which the channel received spray drift following the applications was 100 m with a flow velocity of 100 m/day. The water phase was assumed to contain 1 g/L of suspended

solids with an organic matter content of 50%, while the water temperature was taken to be 30 °C. The climate of Akuse, Okyereko, Ashaiman and Weija is of the tropical savannah type and characterized by a bimodal rainfall pattern. Average annual rainfall ranges from 625 to 1000 mm. Mean annual temperature is (29 °C) and decreases to 26 °C in July and August (http://mofa.gov.gh/site/?page_id=2985; GSS, 2014). New Tafo/Tontro lies in the moist semi-deciduous forest which is also characterized by two main rainfall seasons. The mean annual rainfall is between 125 and 175 mm. Temperatures are found to be fairly uniform ranging between 26 °C in August and 30 °C in March and characteristic of a typical tropical climate (GSS, 2014; Abban *et al*. 2018).

Pesticides were applied using hand-pressured backpack knapsack *(Matabi 15L)*. The spraying was done with the lance positioned in front of the applicators while they walked through the crops, also directly next to water courses. It was assumed that on the average 10% of the amount of pesticide applied per ha on the crops would reach the water surface by spray drift based on empirical drift data from knapsack sprayers shown by Snelder *et al.* (2008).

The data was entered into the PRIMET model to calculate an acute and chronic PEC. To calculate the 1st tier acute Exposure Toxicity Ratio (ETR), this PEC was then divided by acute or chronic PNEC. An acute and chronic PNECs are based on toxicity data in the form of EC50 and NOEC data for selected standard test species from different trophic levels, namely algae (primary producers), Daphnia (invertebrates) and fish (vertebrates). The toxicity data extracted from these databases were for the acute static tests for freshwater invertebrates (48 h), vertebrates (96 h) and primary producers (72 h and 96 h) and for the chronic the extracted toxicity data were Daphnia (21 days) and fish (28 days). The relevant EC50 and NOEC data were extracted from the USEPA ECOTOX (USEPA, 2020a), the RIVM database (De Zwart, 2002) and the Pesticide Manual (Tomlin, 2000). The toxicity values used to calculate the 1st tier PNECs are provided in Table SI 3.2.

These acute PNEC also incorporated an assessment factor (100 for fish and Daphnia and 10 for algae) and the lowest resulting PNEC was used as the threshold concentration of effects (Table SI 3.2). These assessment factors were used to extrapolate from the EC50 level to a concentration at which no effects on the organisms were expected and to account for interspecies variation (EU, 1997; Van den Brink *et al*. 2005). To calculate the chronic Exposure

Toxicity Ratio (ETR^n), a time weighted average PECs for fish (default period of 28 days) and daphnia (default period of 21 days) were calculated. These PECs were divided by their respective chronic PNEC for Daphnia (invertebrates) and fish (vertebrates), using an assessment factor of 0.1. When the resulting chronic PNEC was higher than the acute one, the acute PNEC was used for the chronic risk assessment. The assessment factors used in the PRIMET model are regarded as conservative for most of the chemicals evaluated in this study (Brock and Van Wijngaarden, 2012; Van Wijngaarden *et al.* 2015; Brock *et al.* 2016; Van Wijngaarden and Arts, 2018; Rico *et al.* 2019).

First tier acute and chronic terrestrial risk assessment

For the terrestrial risk assessments the toxicity values already incorporated in the PRIMET model were used. The terrestrial soil (earthworms) scenario included an acute 14 day LC50 and chronic NOEC for reproduction as effect endpoints using a default extrapolation factor of 0.1 and 0.2 for acute and chronic effect assessment of earthworm, respectively to calculate the PNEC. The exposure scenario included a bulk density of 1.0 g/cm^3 (Sally and Abernethy, 2002) of the soil, a depth of 0.05 m and the individual pesticide dose applied (g a.i/ha), number of applications and application interval as obtained from the field survey.

The scenario for the bees included the acute LD50 (24 h and 48 h) and the individual dose (g a.i./ha) of the pesticides applied. Likewise, for the non-target arthropod (NTA), an acute median lethal body residue (LR50), a vegetation scenario with a default distribution factor of 10, an extrapolation factor for effect assessment of NTA with a default value of 2 and a default drift factor value of 0.0277 as well as the number of pesticide applications (g a.i/ha) were used. The climatic conditions for the 1^{st} tier terrestrial risk assessment were the same as that described under the aquatic risk assessment.

Subsequently, the calculated acute and chronic soil PEC was then divided by the 1^{st} tier acute and chronic PNEC to calculate the acute and chronic ETR respectively. For NTAs and bees only an acute risk assessment was performed due to a lack of toxicity data. For NTAs an in-field and off-field ETR was calculated with the latter being a factor of 100 lower (Peeters *et al*. 2008).

Second tier acute pesticide threshold levels for aquatic communities

To refine the threshold values protective for ecological risk of insecticides, fungicides and herbicides to freshwater ecosystems in the study area, the species sensitivity distribution (SSD) concept was used to calculate the 2nd tier acute PNEC for the chemicals indicated to pose an acute risk to aquatic ecosystems in the 1st tier. This PNEC was compared to the 1st tier acute PEC as calculated by PRIMET in order to calculate the 2nd tier ETR.

When available, the SSD derived HC5 (Hazardous Concentrations 5%) values present in Van den Brink *et al.* (2006) and Maltby *et al*. (2005; 2009) were used as 2nd tier acute PNECs (Table 3.3). In order to construct the SSDs for the remaining compounds, acute aquatic single-species were collated from the EPA ECOTOX database (USEPA, 2020a). Data selection criteria followed those of Maltby *et al.* (2005; 2009) and Van den Brink *et al*. (2006), where the selected endpoints were median lethal concentration (LC50) or median effect concentration (EC50) regarding immobility for animals and EC50 regarding biomass or growth for plants. The test durations selected were 2 to 21 d for vertebrates, 1 to 7 d for invertebrates, 2 to 28 d for macrophytes, and 1 to 7 d for algae. Genera data were only used if no species data were reported for a genus. Each species was represented only once per compound in the analysis. The following data manipulations were performed where there were multiple toxicity values for a taxon:

1. The lowest value was selected where several duration times, temperatures, life stages, water types, etc., were studied in the same experiment.
2. The geometric mean was taken for data for the same species (and endpoint), but from different experiments.

The SSD generator developed by US EPA (USEPA, 2020b) was used to generate SSDs and median HC5 values (Hazardous Concentration 5%) and their 95% confidence interval. A log-normal distribution model by Aldenberg and Jaworska (2000) was fitted to a minimum of six data points, with model fit being evaluated using the Anderson– Darling goodness-of-fit test.

All arthropod data (crustaceans and insects) was included to construct SSD for insecticides, all aquatic data (vertebrates; invertebrates; and primary producers) for

fungicides and all data for primary producers (algae and macrophytes) for herbicides (Maltby *et al.* 2005; 2009; Van den Brink *et al*. 2006). The analysis was applied to the pesticide crop combination for which only a potential or likely acute risk was indicated in the 1st tier calculation (i.e. ETR > 1). The analysis however focused on 19 pesticide compounds being 7 herbicides, 5 insecticides and 7 fungicides (Table 3.3).

Overuse of pesticides

The third aim was to evaluate whether the farmers overdose pesticides during normal day-to-day use and if products being used had been banned for use or not. The status of pesticides identified to be in use was cross-checked as well as application rate compared to the recommended rate provided by the registration authorities (GEPA, 2015) and as indicated on the label instructions.

Results

First tier risk assessment

The data set included 33% insecticides, 30% fungicides and 37% herbicides as obtained from the individual active ingredient concentration (Table SI 3.3). For the risk assessment, the 1st tier ETRs, the ranges of the ETRs and percentage of ETRs > 1 were calculated (Table 3.1 and 3.2) using use patterns for 32 different active ingredients and their physico-chemical properties (Table SI 3.3). The application rate per hectare, application interval, number of applications per season, and crops applied to at the study sites are given in Table SI 3.2 together with their tier-1 acute L(E/D/R)C50 (Table SI 3.4) and tier-1 chronic NOEC (Table SI 3.5) data were included in the model to generate the PRIMET output. Three categories of risk were identified: 'no risk' (ETR < 1), 'possible risk' (1 < ETR < 100) and 'definite risk' (ETR > 100) for the pesticides studied within the environment. Some active ingredients showed ranges in ETRs spanning multiple categories due to differences with regards to the amount of active ingredient applied per hectare (Table 3.1 and 3.2).

Only for the insecticide emmamectin benzoate no acute and chronic aquatic risk assessment could be performed due to a lack of data, while this was not possible for pyribenzoxim and emmamectin benzoate for the acute soil risk assessment. Only for 53% of

Table 3.1: Pesticide type used in the study area and their 1st tier acute exposure toxicity ratio (ETR) or range of ETR and percentage ETR > 1 for the different acute risk assessments as calculated by the PRIMET model. ETRs for NTA $_{off\text{-}field}$ can be calculated by dividing the NTA $_{in\text{-}field}$ by a factor of 370 .

Pesticide Active Ingredient	Class	Number of case(s)	Aquatic ETR/Range of ETR(s)	% of ETR > 1	Terrestrial (soil) ETR/Range of ETR(s)	% of ETR > 1	Terrestrial (bees) ETR/Range of ETR(s)	% of ETR > 1	Terrestrial (NTA $_{in\text{-}field}$) ETR/Range of ETR(s)	% of ETR > 1
Glyphosate	Herbicide	4	0.019-0.21	0	0.012 - 0.13	0	0.086 - 0.94	0	NA	NA
Paraquat	Herbicide	2	1.1 - 6.0	100	0.0040 - 0.022	0	0.66 - 3.7	50	NA	NA
Butachlor	Herbicide	1	15	100	86	100	0.67	0	NA	NA
Pendimethalin	Herbicide	2	7.9 - 26	100	0.016 - 0.052	0	0.24 - 0.78	0	NA	NA
Propanil	Herbicide	2	1.5 - 2.8	100	0.013 - 0.024	0	0.15 - 0.28	0	4138[a] - 7655[a]	100
Bensulfuron methyl	Herbicide	1	1.5	100	0.0017	0	0.049	0	0.11	0
Bispyribac sodium	Herbicide	1	0.0030	0	5.6E-04	0	0.0056	0	NA	NA
2, 4-D	Herbicide	2	0.015 - 0.027	0	0.015 - 0.028	0	0.085 - 0.16	0	NA	NA
Pretilachlor	Herbicide	1	1.6	100	0.42	0	0.13	0	NA	NA
Pyribenzoxim	Herbicide	1	0.00096	0	NA	NA	0.0080	0	NA	NA
Oxyfluorfen	Herbicide	2	4.3 - 5.8	100	0.0060 – 0.0080	0	0.090 - 0.12	0	0.16 - 0.21	0
Lambda cyhalothrin	Insecticide	3	274[a] - 4229[a]	100	6.4E-04 - 0.0099	0	13 - 195[a]	100	192[a] - 2960[a]	100
Chlorpyrifos	Insecticide	2	3425[a] - 5479[a]	100	0.050 - 0.079	0	163[a] - 260[a]	100	3600[a] - 5760[a]	100
Emmamectin benzoate	Insecticide	2	NA	NA	NA	NA	673[a] - 2009[a]	100	NA	NA
Imidacloprid	Insecticide	1	0.00085	0	0.037	0	162[a]	100	1841[a]	100
Acetamiprid	Insecticide	4	1.40E-03 - 0.0034	0	0.040 - 0.11	0	0.0022 - 0.18	0	NA	NA
Novaluron	Insecticide	2	0.17	0	2.1E-04	0	0.0032	0	NA	NA
Thiamethoxam	Insecticide	2	7.1E-04 - 8.5E-04	0	4.0E-04 - 4.8E-04	0	120[a] - 144[a]	100	NA	NA
Bifenthrin	Insecticide	4	0.52 - 4.2	50	0.0028 - 0.023	0	0.33 - 2.7	50	NA	NA
Cypermethrin	Insecticide	2	16 - 61	100	0.012 - 0.047	0	90 - 353[a]	100	NA	NA
Dimethoate	Insecticide	2	1.2 - 4.7	100	0.43 - 1.7	50	167[a] - 653[a]	100	NA	NA

Carbofuran	Fungicide	1	4.6	100	0.0011	0	10	100	3.4	100
Mancozeb	Fungicide	3	16 - 202[a]	100	0.029 - 0.35	0	0.091 - 1.1	33	NA	NA
Carbendazim	Fungicide	2	6.4 - 13	100	0.99 - 2.0	50	0.16 - 0.32	0	0.15 - 0.31	0
Sulphur	Fungicide	2	16 - 19	100	0.0043 - 0.0052	0	0.13 - 0.16	0	1.5 - 1.8	100
Maneb	Fungicide	1	5719[a]	100	0.13	0	1.8	100	NA	NA
Copper hydroxide	Fungicide	4	8.3 - 36	100	0.0052 - 0.023	0	0.12 - 0.52	0	7088[a] - 26570[a]	100
Metalaxyl	Fungicide	2	0.0069 - 0.0096	0	0.0011 - 0.0015	0	0.0080 - 0.011	0	NA	NA
Metalaxyl-M	Fungicide	2	3.6E-04 - 0.0071	0	2.4E-04 - 0.0048	0	0.0024 - 0.047	0	NA	NA
Cuprous oxide	Fungicide	2	1.7 - 8.6	100	0.0023 - 0.011	0	0.026 - 0.13	0	5.2 - 26	100

ETR values below 1 indicate no risk, between 1 and 100 a potential risk and above 100 a definite risk.

NA indicates that the ETR was not determined because toxicity data were not available.

a- Represents a definite risk.

Table 3.2: Pesticide type used in the study area and their 1st tier chronic exposure toxicity ratio (ETR) or range of ETR and percentage ETR for the different chronic risk assessments as calculated by the PRIMET model.

Pesticide Active Ingredient	Class	Number of case(s)	Aquatic ETR/Range of ETR(s)	% of ETR > 1	Terrestrial (soil) ETR/Range of ETR(s)	% of ETR > 1
Glyphosate	Herbicide	4	9.0E-04 - 0.0098	0	0.10 - 1.1	25
Paraquat	Herbicide	2	0.051 – 0.28	0	NA	NA
Butachlor	Herbicide	1	0.72	0	NA	NA
Pendimethalin	Herbicide	2	0.22 – 0.72	0	0.24 - 0.78	0
Propanil	Herbicide	2	0.069 - 0.13	0	NA	NA
Bensulfuron methyl	Herbicide	1	0.070	0	NA	NA
Bispyribac sodium	Herbicide	1	1.4E-04	0	NA	NA
2, 4-D	Herbicide	2	6.7E-04 -0.0012	0	NA	NA
Pretilachlor	Herbicide	1	0.076	0	NA	NA
Pyribenzoxim	Herbicide	1	4.3E-05	0	NA	NA
Oxyfluorfen	Herbicide	2	0.38 - 0.51	0	0.12 – 0.17	0
Lambda cyhalothrin	Insecticide	3	31 - 479	100	NA	NA
Chlorpyrifos	Insecticide	2	401 – 642	50	1.0 - 1.6	100
Emmamectin benzoate	Insecticide	2	NA	0	NA	NA
Imidacloprid	Insecticide	1	1.9E-04	0	4.0	100
Acetamiprid	Insecticide	4	1.7E-04 – 4.2E-04	0	0.0047 0.43	0
Novaluron	Insecticide	2	0.89	0	0.11	0
Thiamethoxam	Insecticide	2	4.9E-05 - 5.9E-05	0	0.10 - 0.12	0
Bifenthrin	Insecticide	4	0.081 - 0.66	0	0.022 - 0.18	0
Cypermethrin	Insecticide	2	1.8 – 7.1	100	NA	NA
Dimethoate	Insecticide	2	0.70 – 2.7	50	2.5 - 9.7	100
Carbofuran	Fungicide	1	0.19	0	0.14	0
Mancozeb	Fungicide	3	3.8 – 70	100	0.21 - 2.6	66
Carbendazim	Fungicide	2	7.7 – 15	100	6.7 - 13	100
Sulphur	Fungicide	2	1.4 - 1.7	100	NA	NA
Maneb	Fungicide	1	159[a]	100	6.6	100
Copper hydroxide	Fungicide	4	0.59 – 4.6	50	0.47 - 1.5	25
Metalaxyl	Fungicide	2	6.4E-04 – 6.7E-04	0	NA	NA
Metalaxyl-M	Fungicide	2	1.4E-04 – 0.0072	0	NA	NA
Cuprous oxide	Fungicide	2	0.12 – 1.07	50	0.25 - 1.3	50

ETR values below 1 indicate no risk, between 1 and 100 a potential risk and above 100 a definite risk.

NA indicates that the ETR was not determined because toxicity data were not available.

a- Represents a definite risk.

the compounds a chronic soil risk assessment could be performed while for all compounds an acute risk assessment for bees could be performed. For 37% of the compounds an acute risk assessment could be performed for NTAs (Table 3.1 and 3.2).

'No risk'

For the following pesticides 'no acute risk' were indicated based on the calculated highest acute ETRs for the aquatic environment: the herbicides glyphosate, bispyribac-sodium, 2, 4-D and pyribenzoxim, the insecticides imidacloprid, acetamiprid, novaluron and thiamethoxam and the fungicides metalaxyl and metalaxyl-M (Table 3.1). There were also no chronic ETRs greater than 1 indicating no chronic risk to the aquatic environment with respect to the same pesticides as well as many others (Table 3.2).

With the exception of butachlor, dimethoate and carbendazim all active ingredients showed no acute risk for terrestrial (soil) organisms while the chronic terrestrial (soil) ETRs ranged from 0.0047 to 0.78 for pendimethalin, oxyfluorfen, acetamiprid, novaluron, thiamethoxam, bifenthrin and carbofuran (Table 3.2).

No acute risk to bees were calculated for herbicides, with the exception of paraquat, for insecticides with the exception of lambda cyhalothrin, chlorpyrifos, emmamectin benzoate, imidacloprid, thiamethoxam, bifenthrin, cypermethrin, dimethoate, and for fungicides with the exception of carbofuran, mancozeb, and maneb. The other active ingredients showed no acute risk to bees with $ETR_{Terrestrial\ acute\ Bees}$ = 0.0022 – 0.94 (Table 3.1).

No acute ETRs > 1 to terrestrial non-target arthropods (NTA) were calculated for the herbicide bensulfuron methyl and oxyfluorfen and the fungicide carbendazim while for all other pesticides for which data were available a (possible) risk was calculated. The chronic ETRs could only be calculated for 37% of the compounds. The ETRs of the herbicide bensulfuron methyl and oxyfluorfen, the insecticide carbofuran and the fungicides carbendazim, sulphur and cuprous oxide were lower than 1 (Table 3.1 and 3.2). It is clear that the chronic risk assessment for these organisms suffer from a lack of data as chronic, and even acute, toxicity data were not available for most compounds (Table 3.1 and 3.2).

'Possible risk'

The predicted PRIMET highest acute ETR values were between 1 and 100 for most of the herbicides (i.e. paraquat, butachlor, pendimethalin, propanil, bensulfuron methyl, pretilachlor and oxyfluorfen), some insecticides (bifenthrin, cypermethrin and dimethoate) and almost half of the fungicides (carbofuran, carbendazim, sulphur, copper hydroxide and cuprous oxide). All highest chronic ETR values predicted by PRIMET for cypermethrin, dimethoate, mancozeb, carbendazim, sulphur, copper hydroxide and cuprous oxide were between 1 and 100, indicating possible risks with respect to the aquatic environment (Table 3.1 and 3.2).

The highest acute ETR predicted by PRIMET for the terrestrial soil environment were only larger than 1 for the herbicide butachlor, the insecticide dimethoate and fungicide carbendazim. They were also smaller than 100 and this indicating possible effects (Table 3.1). Chronic highest ETR between 1 and 100 were calculated for the herbicide glyphosate, the insecticides chlorpyrifos, imidacloprid and dimethoate and the fungicides mancozeb, carbendazim, maneb, copper hydroxide and cuprous oxide (Tables 3.1 and 3.2).

Acute highest ETRs between 1 and 100 for bees were calculated for the herbicide paraquat, the insecticide bifenthrin, and the fungicides carbofuran, mancozeb and maneb (Table 3.1).

Possible acute risks (highest ETR values between 1 and 100) for in-field NTAs were calculated for the fungicides carbofuran, sulphur and cuprous oxide (Table 3.1).

'Definite risk'

The PRIMET predicted definite acute risk (highest ETR values > 100) values for the aquatic environment for the insecticides lambda cyhalothrin and chlorpyrifos and the fungicides mancozeb and maneb, while chronic definite risks were calculated for the insecticides lambda cyhalothrin and chlorpyrifos and the fungicide maneb (Table 3.1 and 3.2).

No definite acute or chronic risks (highest ETR values > 100) were calculated for the soil compartment while definite acute risks values for bees included those for the insecticides lambda cyhalothrin, chlorpyrifos, emmamectin benzoate, imidacloprid, thiamethoxam, cypermethrin and dimethoate (Tables 3.1 and 3.2). For the non-target arthropods, PRIMET

calculated highest acute ETR values > 100 for the herbicide propanil, the insecticides lambda cyhalothrin, chlorpyrifos and imidacloprid and the fungicide; copper hydroxide (Table 3.1).

Second tier aquatic risk assessment

For 15 of the 19 pesticides an HC5 could be calculated, however in the case of cuprous oxide only based on 7 data points instead of the required 8 ones (Table 3.3). The median factor at which the PNEC went up between the 1st and 2nd tier was 4.6. The highest increase in PNEC was observed for maneb (from 0.021 µg/L to 48 µg/L, while a decrease was observed for 3 compounds (propanil, oxyfluorfen and dimethoate).

Paraquat, bifenthrin and cuprous oxide moved from the possible risk category to the no risk category, while 7 pesticides stayed in the possible risk category (Table 3.3). Only oxyfluorfen moved up in its risk category, i.e. from possible risk to definite risk. Chlorpyrifos, mancozeb and maneb moved from the definite risk category to the possible risk category, while lambda cyhalothrin was the only chemical staying in the definite risk category (Table 3.3).

Table 3.3: Median (50% confidence) hazardous concentration for 5% of species (HC5; µg/L) calculated from species sensitivity distributions constructed for primary producers (algae, macrophytes), invertebrates, and or vertebrates exposed at different locations to pesticides and single-species acute with 1st tier NEC values, and 2nd tier acute ETR values based on the 2nd tier PNEC values. For some pesticides multiple dosages were evaluated leading to an equal number of PEC and ETR values per pesticide.

Pesticide Product	Tier-1 PNEC (µg/L)			PEC (µg/L)	Tier-2 HC5 (µg/L)	Reference	ETR=(PEC/HC5)
	Primary Producers	Inverte-brates	Verte-brates				
Herbicide							
Paraquat	0.023	44	190	0.025; 0.14	0.83	This study	0.030; 0.17
Butachlor	20	24	4.4	67	NA	This study	NA
Pendimethalin	0.6	2.8	1.38	4.7; 15	2.0	Van den Brink *et al*. 2006	2.4; 7.5
Propanil	11	23.9	54	17; 31	6.8	This study	2.5; 4.6
Bensulfuron methyl	2	1300	660	3.0	NA	This study	NA
Pretilachlor	929	130	9	14	NA	This study	NA
Oxyfluorfen	200	7.2	2.5	11; 14	0.10	This study	110; 140
Insecticide							
Lambda cyhalothrin	30	0.0036	0.0021	0.58; 0.60; 8.9	0.003	Maltby *et al*. 2005	193; 200; 2967
Chlorpyrifos	48	0.0010	0.013	3.4; 5.5	0.07	Maltby *et al*. 2005	49; 79
Bifenthrin	82	0.0011	0.0026	0.0047; 0.0047	0.0051	This study	0.92; 0.92
Cypermethrin	10	0.0030	0.028	0.047; 0.18	0.003	Maltby *et al*. 2005	16; 60
Dimethoate	9040	20	302	24; 94	1.6	This study	15; 59
Fungicide							
Carbofuran	650	0.094	1.8	0.43	0.23	Maltby *et al*. 2009	1.9
Mancozeb	4.4	0.73	0.74	12; 88; 147	89	Maltby *et al*. 2009	0.13; 0.99; 1.7
Carbendazim	770	1.5	1.9	9.6; 19	8	Maltby *et al*. 2009	1.2; 2.4
Sulphur	6.3	0.63	0.63	9.8; 12	NA	This study	NA
Maneb	0.70	0.021	2	120	48	Maltby *et al*. 2009	2.5
Copper hydroxide	0.9	0.38	0.17	1.4; 2.3; 3.1; 6.2	5.4	This study	0.26; 0.43; 0.57; 1.1
Cuprous oxide	14.7	4.5	2.07	3.6; 18	22*	This study	0.16

** indicative, based on 7 species*

NA indicates that the SSD was not determined because not enough toxicity data were available.

Overdosing of pesticides

Table 3.4 shows that a number of pesticides have been applied in excess by farmers at the study sites as compared to the recommended dosages on the approved pesticide labels by Ghana pesticide registration authorities. Based on the mean of the minimum and maximum applied and recommended dosages, the average overdose factor was 4.5 with the highest values for the insecticides lambda cyhalothrin (applied as Karate, Conti-Halothrin, Pawa and Stricker) and cypermethrin and dimethoate mixture (applied as Cymethoate and Cydim Super) formulations. The lowest overdose factor values were observed for the fungicides sulphur (applied as Sulfa 80 WP), the insecticide mixture lambda cyhalothrin and acetamiprid (applied as K-Optimal), the herbicide mixture pretilachlor and pyribenzoxim (Solito formulation) and the herbicide butachlor (applied as Ceres Butachlor). No formulation was underdosed (Table 3.4).

Table 3.4: Pesticide formulations, active ingredients and their applied dose(s) in this survey as well as their recommended dosage(s) as approved by the Environmental Protection Agency of Ghana to control important pests in agriculture. The overdose factors are based on the average of the minimum and maximum of the applied and recommended dosages.

Pesticide formulation	Pesticide Class	Active Ingredient(s)	Applied dose, L/Ha, Kg/Ha	Recommended dose on label, L/Ha, Kg/Ha	Overdose factor	Site(s)
Ceresate; Chemosate; Roundup; Power; Sunphosate	Herbicide	Glyphosate	1.2 - 9.8 L	0.5 - 2.5 L	3.7	AS
Gramoxone; M-Quat	Herbicide	Paraquat	1.5 - 8.3 L	1.5 - 3.0 L	2.2	AS
Ceres Butachlor	Herbicide	Butachlor	6.7 L	4.0 L	1.7	OK
Condax; Londax	Herbicide	Bensulfuron methyl	0.42 Kg	0.0030 - 0.10 Kg	8.2	AS, OK, AK
Solito	Herbicide	Pretilachlor + Pyribenzoxim	2.0 L	1.0 - 1.5 L	1.6	AK
Zoomer	Herbicide	Oxyfluorfen + Glyphosate	1.5 - 2.0 L	0.75 - 0.90 L	2.1	WJ
Stomp 445 CS; Alligator	Herbicide	Pendimethalin	3.0 - 9.8 L	2.5 - 3.0 L	2.3	AS, AK
Bounty	Herbicide	Bispyribac sodium	0.10 L	0.015-0.050 L	3.1	AK
Karate; Conti-Halothrin; Pawa; Stricker	Insecticide	Lambda Cyhalothrin	1.0-15 L	0.60 L	13	AS, OK, WJ, AK
Attack	Insecticide	Emamectin benzoate	0.62 - 1.9 L	0.25 - 0.30 L	4.6	AS, OK, WJ
K-Optimal	Insecticide	Lambda cyhalothrin + Acetamiprid	1.5 L	1.0 L	1.5	OK
Cymethoate, Cydim Super	Insecticide	Cypermethrin + Dimethoate	2.5 - 9.8 L	0.50 L	12	AS, OK
Benco	Fungicide	Mancozeb	5.9 - 9.9 Kg	0.80 - 2.0 Kg	5.6	AS, WJ, OK
Carbendazim 50 WP	Fungicide	Carbendazim	0.80 - 1.6Kg	0.13 - 0.26 Kg	6.2	WJ
Sulfa 80 WP	Fungicide	Sulphur	0.80- 0.99 Kg	0.67 Kg	1.3	AS, AK
Maneb 80 WP	Fungicide	Maneb	9.0 Kg	2.0 - 4.0 Kg	3.0	WJ, AS

AS = Ashaiman, AK = Akuse, WJ = Weija, OK = Okyereko

Discussion

This study reveals that environmental risks may be expected with regards to the use of pesticides in the case study areas of Ghana judging from the results of the environmental risk assessment in terms of ETRs for the aquatic (algae, daphnia and fish) and terrestrial (worms, bees and NTAs) compartments (Table 3.1, 3.2 and 3.3). This included the overdosing of pesticides applied by farmers, in comparison to the recommended dosages (Table 3.4). There was, however, no record of farmers using banned pesticide products in the study area.

Aquatic risk assessment

Aquatic ecosystems provide direct goods and services like clean drinking water, fish and aquatic macrophytes for consumption and indirect services like water purification, water retention and climate regulation (Grizzetti *et al*. 2016). It is, therefore, of most importance that aquatic ecosystems are of good ecological status and not impaired by chemicals such as pesticides. Among others, that was the reason for us to perform the aquatic risk assessment of the pesticide use dosages collected within this paper to evaluate the agricultural practices in the Central, Greater Accra and Eastern regions of Ghana.

Firstly, this study applied the PRIMET model for calculating environmental impact of pesticide use, and demonstrated that pesticide use poses serious potential acute and chronic risks to the aquatic environment, if aquatic ecosystems are present adjacent to the treated fields (Table 3.1 and 3.2). Malherbe *et al.* (2013) reported ETR values of 0.01, 0.2, 0.2-0.5 and 73 for dimethoate, glyphosate, carbendazim and paraquat respectively while Ansara-Ross *et al*. (2008) reported an ETR of 0.3 for pendimethalin for aquatic ecosystems in South Africa. In this study the calculated acute 1st tier ETR for pendimethalin ranged between 7.9 and 26 (Table 3.1), indicating possible acute risk, which is substantiated by the 2nd tier risk assessment (Table 3.3). These are much higher than the ETR reported by Ansara-Ross *et al.* (2008), partly because of higher applied dosages (up to a factor of 3). In this study no risk was indicated for glyphosate (ETR: 0.019-0.21), which is comparable to the study in South Africa (Malherbe *et al*. 2013). In this study possible risks were also calculated for paraquat, dimethoate and carbendazim as the 1st tier ETRs ranged between 1.1-6.0, 1.2-4.7 and 6.4-13, respectively

(Table 3.1). Paraquat showed possible acute risk based on a 1st tier assessment for both this study and that of Malherbe *et al*. (2013) (Table 3.1). This risk, however, disappears when a 2nd tier PNEC is used (Table 3.3). The calculated 1st and 2nd tier ETRs of dimethoate and carbendazim were much higher compared to the study of Malherbe *et al*. (2013), again a result of using much higher applied dosages (dimethoate up to a factor of 71, carbendazim up to a factor of 55). This is partly a result of the overdosing recorded by this study of a factor 2.3, 12 and 6.2 (Table 3.4) for pendimethalin, dimethoate and carbendazim respectively. Chlorpyrifos use (1st tier ETR = 3425, 5479; 2nd tier ETR = 49,79) in this study show definite acute risk to the aquatic environment which was also recorded by Wiratno *et al.* (2007) (1st tier ETR = 1900). Lambda cyhalothrin showed a definite risk (1st tier ETR = 274-4229; 2nd tier ETR = 193-2967; Table 3.1 and 3.3) to the aquatic environment and cypermethrin showed an acute risk to the aquatic environment (1st and 2nd tier ETR = 16, 60; Table 3.1 and 3.3) and (ETR = 360; definite risk), while Wiratno *et al*. (2007) reported a lower value for lambda-cyhalothrin (ETR = 3) and higher one for cypermethrin (ETR = 360), but both predicting a (possible) risk. Van den Bosch *et al*. (2006) provided quite similar results for China and Vietnam with extremely high first ETR values (> 1000) for cypermethrin, chlorpyrifos, lambda cyhalothrin and dimethoate, high ones (> 100) for carbendazim and lower ones for mancozeb and, especially metalaxyl and metalaxyl-M. These results match the results of the 2nd tier risk assessment in our study although the highest ETR values are generally lower (Table 3.3). This points to the fact that the environmental side-effects of pesticide in countries with a weak pesticide registration system and enforcement needs more attention (Onwona Kwakye *et al*. 2019). This contamination of the aquatic ecosystem might not only harm the ecological integrity of the water, but also the ecosystem services for those who depend on such water sources for their livelihoods including reduced (drinking) water quality, reduced productivity (e.g., fish kills, effects on bees, cattle) and small ruminants that uses surface water as drinking water (Maltby *et al*. 2017).

PRIMET predicted no risk to the aquatic environment for the two neonicotinoid insecticides imidacloprid and thiamethoxam. It should be noted, however, that the standard test invertebrate *Daphnia magna* is relatively insensitive to imidacloprid with a geometric mean 96h LC50 value of 34,000 µg/L (Morrissey *et al.* 2015), while insect taxa like mayflies are at least four orders of magnitude more sensitive in temperate regions (96h EC50 for *Cloeon dipterum* = 1.0 µg/L in The Netherlands; (Roessink *et al*. 2013; Morrissey *et al*. 2015) and even

seven orders of magnitude more sensitive in tropical regions (96 h EC50 for *Cloeon* sp. = 0.0055 µg/L in Bangladesh; Sumon *et al*. 2018). Thus, although the acute ETR for imidacloprid was low (0.00085), in practice a risk may be present, as Ghana is also situated in the tropics. More research on the actual risks imidacloprid, and other neonicotinoid insecticides like thiamethoxam, poses to the aquatic ecosystem should be studied by testing local species. Thiamethoxam is also non-toxic to *D. magna* (96h EC50 of 42,000 µg/L; Morrissey *et al*. 2015) and highly toxic to aquatic insects like mayflies (Van den Brink *et al*. 2016).

Of the pesticides for which the 2nd tier risk assessment indicating a risk (ETR > 1), no semi-field studies are available in the open literature for the herbicides pendimethalin, propanil and oxyfluorfen, the insecticide dimethoate and the fungicide carbofuran (Table 3.3; Van den Brink *et al.* 2006). Studies with the insecticide lambda cyhalothrin in experimental ecosystems have demonstrated that it is highly toxic to aquatic organisms (He *et al*. 2008). Reported effects of lambda cyhalothrin on arthropod invertebrates are likely to occur at concentrations at or above 0.01 µg/L (Van Wijngaarden *et al*. 2004, 2005b), while our 2nd tier PNEC used was 0.003 µg/L (Table 3.3), making the second tier risks realistic. Lower-tier assessment of chlorpyrifos indicates risk for surface waters (Giesy *et al*. 1999; Giddings *et al*. 2014). Chlorpyrifos has also been studied extensively using microcosm and mesocosm (cosm) studies, single-species laboratory toxicity tests and used as a regulatory benchmarks across classes of insecticides (Brock *et al*. 2000b; 2006; Maltby *et al*. 2005; van Wijngaarden *et al*. 2005b). These cosm studies have also broadened the scope of conclusions about chlorpyrifos effects on aquatic communities to a wider range of locations and environmental conditions (van Wijngaarden *et al.* 2005a; Daam *et al*. 2008a, b; López-Mancisidor *et al*. 2008a, b; Zafar *et al.* 2011), all supporting the conclusion that concentrations of 0.1 µg/L chlorpyrifos or less cause no ecologically significant effects on aquatic communities (Brock *et al*. 2006; Giddings *et al*. 2014) which could be used as basis for control measures in this study. This threshold value of 0.1 µg/L is close to the PNEC of 0.7 µg/L used in the 2nd tier risk assessment (Table 3.3). Van Wijngaarden *et al*. (2005) reports for cypermethrin a NOEC and LOEC based on a cosm experiment evaluating multiple applications of < 0.07 and 0.07 µg/L, respectively. The 2nd tier PNEC of 0.003 µg/L is far below this value and is expected to be protective (Table 3.3). Several studies have indicated low 96h LC50 value of mancozeb to fish, e.g. *Oreochromis mossambicus* (12 µg/L), *Punctius ticto* (13 µg/L) and *Clarius batracus* adult (14 µg/L) and

fingerlings (14 µg/L) (Srivastava and Singh, 2013; Saha *et al*. 2016; Sharma *et al*. 2016). Maltby *et al.* (2009) report a NOEC and LOEC derived from a cosm study using multiple applications of 10 and 32 µg/L, respectively. This means that the 2nd tier PNEC of 89 µg/L is on the high side when evaluating effects on fish and aquatic ecosystems as a whole, meaning that the 2nd tier risk assessment might even have underestimated the actual risks. For carbendazim, Maltby *et al*. (2009) reports a cosm based NOEC of 3 µg/L and a LOEC of 30 µg/L due to a single application, validating the 2nd tier PNEC of 8 µg/L used in this study. In a cosm experiment performed with maneb only a treatment of 70 µg/L was evaluated, showing only clear effects on bivalves. This observation does not disqualify the PNEC of 48 µg/L used in this study. They also reported a cosm-based NOEC and LOEC of 12 and 24 µg/L for copper hydroxide, respectively, also supporting the 2nd tier PNEC of 5.4 µg/L used in this study (Table 3.3).

Both the 1st and the 2nd tier ecological threshold values are mainly based on toxicity values from temperate species. So it is uncertain whether temperate sensitivity data can be used for a risk assessment in warmer, tropical regions (Daam and Van den Brink, 2010). It was, however, indicated by studies conducted by Maltby *et al*. (2005) and Kwok *et al.* (2007), that no systematic difference existed in toxicity and sensitivity between tropical and temperate species for some of the selected pesticides (chlorpyrifos, fenitrothion and carbofuran), although differences do exist (e.g. imidacloprid; Sumon *et al.* 2018).

The tiered approach scheme can be employed by the Ghana Pesticide registration Authorities to support the registration of pesticides as has been demonstrated in this study to determine the risks associated the use of pesticide products in Ghana, again it has successfully been used in Europe for pesticide registration (EFSA, 2013b).

Terrestrial risk assessment

There is a considerable concern about decline in biodiversity that would influence the delivery of various ecosystem services by terrestrial invertebrates (Hole *et al*. 2005; Hooper *et al*. 2005). In agricultural intensification, the most affected ecosystem services at severe risk are biological pest control (Tscharntke *et al.* 2005, Geiger *et al*. 2010), crop pollination (MEA, 2005; Biesmeijer *et al*. 2006; Zhang *et al*. 2007) and soil fertility maintenance (Hole *et al.* 2005; Hansen *et al*. 2006; Goh, 2011; Pandey and Singh, 2012). There should therefore be specific

protection goals aimed at protecting important ecosystem services such as food web support, pest control and biodiversity (Maltby *et al*. 2018). Biodiversity and ecosystem services might be protected along with agro-ecosystems, where farmers get subsidies, partly to produce ecological benefits (Kleijn *et al.* 2001).

Earthworms are important in influencing organic matter dynamics, soil structure and microbial community (Edwards and Bohlen, 1996; Fragoso *et al*. 1997; Sims and Gerard, 1999). They actively participate in soil aeration, water infiltration and mixture of soil horizons, and they represent an important source of food for many other organisms like birds or moles (Edwards and Bohlen, 1996; Lavelle *et al.* 2006) so there is the need to protect them from pesticide exposure. The study demonstrated that earthworms were also under acute risk for three pesticides (butachlor, dimethoate and carbendazim) and under chronic risks for nine pesticides, of which more than half are fungicides (Table 3.1 and 3.2). The levels of risks were, however, much lower compared to the aquatic compartment (Table 3.1 and 3.2). Strangely, the highest acute risk is calculated for the herbicide butachlor. According to the PPDB data base, butachlor is acutely toxic to earthworms with a 14d LC50 of 515 µg/kg (PPDB, 2020). Chen *et al.* (2014), however, report a 14d LC50 of 1198 mg/kg for the same species, so its value in the PPDB data base might be an error. In another study by Gobi and Gunasekaran (2009), butachlor reduced the biomass and cocoon production and caused damage to epithelial tissue of earthworm (*Eisenia fetida*) leading to the reduction of nutrient absorption area from food. Their study is important as they used concentrations (0.26 – 2.6 mg/kg) relevant in this study. The concentrations of dimethoate and carbendazim only slightly exceeded the acute PNEC (factor of 2). Wiratno *et al*. (2007) provide similar results for Indonesia as found in our study where lambda cyhalothrin, chlorpyrifos and cypermethrin showed no acute risks to the terrestrial soil environment in both studies.

The chronic risk assessment indicated a small risk for all pesticides towards the terrestrial environment (ETR < 10; Table 3.2), except for carbendazim (ETR = 13). Carbendazim is one of the few pesticides that has been extensively studied using terrestrial microcosms (Knacker *et al*. 2004). Jänsch *et al*. (2006) reported a NOEC and LOEC of 2.16 and 3.24 kg a.i./ha while in our study application rates of 1.2 and 2.4 kg a.i./ha, both probably not leading to large adverse effects, although the actual values of the soil parameters like organic matter content and dry bulk density will be important.

As expected from the mode of action only for 1 out of 11 herbicides and 3 out of 9 fungicides a (possible) acute risk was indicated for bees, while for 8 out of 10 insecticides a (possible) acute risk was indicated (Table 3.1). Bees are most affected by lambda cyhalothrin, chlorpyrifos, emamectin benzoate, imidacloprid, thiametoxam, cypermethrin and dimethoate (ETR > 10). Some plants can produce guttation drops in the early hours of the morning (e.g. maize, strawberries), and systemic insecticides appear in such drops in elevated concentrations (Tapparo *et al*. 2011) that are capable of killing the bees (Johnson *et al*. 2006; Zhu *et al*. 2017). Again, pesticide residues in soil move and appear in streams, creeks and ponds of agricultural areas and beyond, which are thus contaminated with a mixture of agrochemicals (Belden *et al*. 2007), as pesticides enter surface waters through run-offs, seepage from groundwater and spray drift. Honey bees, bumblebees and wild bees drink from such puddles, irrigation ditches, ponds and streams, and if these waters are contaminated with pesticide residues may kill them (Schmaranzer, 2000; Samson-Robert *et al.* 2014). Since usage of these plant protection products cannot be stopped, chemical companies are obliged by law to state on the labels whether their products are dangerous to bees or not and must be enforced by the Ghana Registration Authority as well as communicating properly to applicators, farmers and beekeepers. More research is advocated for assessing the effects of chronic exposure of bees to pesticides taking into consideration recent approaches on how to improve the risk assessment of bees. (See e.g. EFSA, 2013a; 2018).

Possible or definite risks for NTAs were identified for almost all pesticides for which a risk assessment could be performed (Table 3.1). This is not surprising as, some of the pesticides are designed to eradicate species which are closely related to NTAs and an in-field risk assessment was performed. Peeters *et al.* (2008), therefore, recommend to perform an off-field risk assessment as well, taking drift percentage and vegetation distribution factor into account. When the default values proposed by Peeters *et al*. (2008) are used, the $ETR_{in\text{-}field}$ can be recalculated to an $ETR_{off\text{-}field}$ by dividing it by 0.0027. In practice this means that all $ETR_{in\text{-}field}$ values lower than 370 would lead to an acceptable $ETR_{off\text{-}field}$ value of < 1. The $ETR_{in\text{-}field}$ value of 370 are exceeded by propanil, lambda cyhalothrin, chlorpyrifos, imidacloprid and copper hydroxide. Jänsch *et al.* (2006) reviewed the (semi-) field experiments performed with NTAs and presented data for all these pesticides, except propanil and copper hydroxide. For lambda cyhalothrin only one dosage (1.5 kg a.i./ha) has been evaluated, which showed clear effects

on some of the collembolan species (Jänsch *et al.* 2006). In our survey, we recorded use dosages of 0.14, 0.15 and 2.22 kg a.i./ha, in-crop effects are certainly expected at the highest dosage. It is uncertain whether off-crop effects are to be expected as no field-based safe concentration could be derived. But since our 1st tier $ETR_{in\text{-}field}$ was above 370 they cannot be excluded. The same applies for chlorpyrifos. The lowest concentration of chlorpyrifos tested under (semi) field circumstances was 0.48 kg a.i./ha, which already affected many species of collembolans (Jänsch *et al.* 2006). As the identified usage dosages of chlorpyrifos in our survey were 2.4 and 3.8 kg a.i./ha, in-crop effects are certainly to be expected. This is not the case for imidacloprid, which showed no field effects on collembolans at rates of 0.34 kg a.i./ha (Jänsch *et al*. 2006), while 0.12 kg a.i./ha was the use dosage recorded in our survey. Based on results from (semi-) field tests it is unclear whether in-field effects on NTAs are expected from the 0.018 kg a.i./ha carbofuran which was recorded in our survey, as the lowest dosage tested in (semi-)field experiments was 0.75 mg a.i./ha and already had clear effects on collembolans (Jänsch *et al*. 2006). Based on the 1st tier assessment presented in this study for in-field as off-field are predicted (Table 3.1).

Overdosing of pesticides

Finally, in this study farmers generally used a higher *dosage* of pesticides than recommended, a factor of 1.3 to 13 times above the recommended label instructions. Mengistie *et al.* (2017) reported similar observations for small holder vegetable farmers in the central rift valley, Ethiopia, but indicated that assessing the exact level of overdosing proved difficult, because of unlabelled units (such as tins) and different combinations of pesticides were used. Similarly, Kariathi *et al.* (2016) reported farmers overdosing pesticide in tomato treatment in Tanzania and claimed that this was partly due to the presence of resistant pests and diseases. The use of pesticide in higher dosage than recommended may lead to pest resistance and high accumulation of residues as reported with increased risk of exposure in Tanzania (Ngowi *et al*. 2007). Farmers at these sites and in general should be encouraged by the scheme managers, extension service providers and the Ghana registration authorities to limit the application of pesticides products to recommended rates to prevent acute risks to the aquatic environment.

The implementation of alternative cropping systems that are less dependent on pesticides, the development of new pesticides with novel modes of action and improved safety profiles, and the improvement of the already used pesticide formulations towards safer formulations (e.g. microcapsule suspensions) have been suggested could reduce the adverse effects of farming and particularly the toxic effects of pesticides. In addition, the use of appropriate and well-maintained spraying equipment along with taking all precautions that are required in all stages of handling and applying pesticides to possibly minimize pesticides potential adverse effects on the environment (Damalas and Eleftherohorinos, 2011).

We recommend that the TRIAD approach (Chapman, 2000) is used to validate the true estimation of risks that the results from this preliminary ecological risk assessment using chemical measurements, bioassays and bio-monitoring.

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Supplementary Information for Chapter 3

Table SI 3.1: Questionnaire for PRIMET input data

a. Pesticide usage, pest(s) and frequency (farmers' data)

Crop	**Product Trade Name**	**Active ingredient**	**Active ingredient conc.**	**Target pest/disease**	**Dose (L/S/G)**	**Dosage/Ha**	**interval**	**Application method**	**Application strategy**	**Frequency**

b. For each pesticide product mentioned in Q 1, please provide the following information regarding pesticide product (secondary data)

No.	Trade name of formulation	Active ingredient (A.I)	A.I conc.	A.I. Conc. /Ha (Dose)	Country of manufacture/origin	Local Agent/ Representative	Registration status			
							1	2	3	4
Registration status: (1)=Full registration; (2)=Provisional clearance; (3)=Not registered; (4)=Banned										

Table SI 3.2: Pesticide(s) used on crops and their relevant application rates obtained from survey of farmers. The predicted environmental concentrations in the water due to one application (PEC) and multiple applications (PECn) as well as the predicted no effect concentration (PNEC) as calculated by the PRIMET model is also provided for the different sites.

AS Ashaiman, AK Akuse, WJ Weija, OK Okyereko, TN Tontro/New Tafo
Veg. Vegetables (Tomato, Pepper, Onion, Okro, Garden Eggs, Cabbage, Cucumber, Tinda, Cowpea, Soybean, Lettuce, Groundnut, Water Melon)
All - Both cereals and vegetables

						Aquatic risk assemssmnet														
	Farm	Plot	Crop	Pesticide	Type	EC50 algae	L(E)C50 daphnia	L(E)C50 fish	NOEC Daphnia	NOEC fish	PNEC water-acute	Calculated PNEC water, chronic-Daphnia	Calculated PNEC water, chronic-fish	Replaced by acute PNEC when lower PNEC water, chronic-Daphnia	Replaced by acute PNEC when lower PNEC water, chronic-fish	PECn water	PECn TWA Daphnia	PEC TWA fish	ETRn water-acute	ETRn water-chronic-Daphnia
case 1	AS	Gh 01	All	(7516) glyphosate	Herbicide	4400	40000	38000	30000	25000	380	3000	2500	380	380	7.321	0.3429	0.2572	0.01926579	0.00090237
case 2	AS	Gh 01	All	(7516) glyphosate	Herbicide	4400	40000	38000	30000	25000	380	3000	2500	380	380	59.79	2.801	2.101	0.15734211	0.00737105
case 3	AS	Gh 01	All	(7516) glyphosate	Herbicide	4400	40000	38000	30000	25000	380	3000	2500	380	380	9.761	0.4573	0.3429	0.02568684	0.00120342
case 4	AS	Gh 01	All	(7516) glyphosate	Herbicide	4400	40000	38000	30000	25000	380	3000	2500	380	380	79.72	3.734	2.801	0.20978947	0.00982632
case 5	AS	Gh 01	All	(7742) paraquat	Herbicide	0.23	4400	19000	120	1900	0.023	12	190	0.023	0.023	0.02474	0.001176	0.0008823	1.07565217	0.05113043
case 6	AS	Gh 01	All	(7742) paraquat	Herbicide	0.23	4400	19000	120	1900	0.023	12	190	0.023	0.023	0.1374	0.006533	0.0049	5.97391304	0.28404348
case 7	OK	Gh 01	Rice	(7662) butachlor	Herbicide	200	2400	440	240	44	4.4	24	4.4	4.4	4.4	66.53	3.159	2.369	15.1204545	0.71795455
case 8	AS, AK	Gh 01	Rice, Ve	(7732) pendimethalin	Herbicide	6	280	138	14.5	6	0.6	1.45	0.6	0.6	0.6	4.722	0.1313	0.09848	7.87	0.21883333
case 9	AS, AK	Gh 01	Rice, Ve	(7732) pendimethalin	Herbicide	6	280	138	14.5	6	0.6	1.45	0.6	0.6	0.6	15.43	0.429	0.3217	25.7166667	0.715
case 10	AS, OK,	Gh 01	Rice	(7816) propanil	Herbicide	110	2390	5400	86	540	11	8.6	54	8.6	11	16.55	0.5937	0.4453	1.50454545	0.06903488
case 11	AS, OK,	Gh 01	Rice	(7816) propanil	Herbicide	110	2390	5400	86	540	11	8.6	54	8.6	11	30.62	1.098	0.8238	2.78363636	0.12767442
case 12	AS,OK,	Gh 01	Rice	(7385) bensulfuron-methyl	Herbicide	20	130000	66000	12000	1500	2	1200	150	2	2	3.024	0.1398	0.1049	1.512	0.0699
case 13	AK	Gh 01	Rice	(6596) bispyribac-sodium	Herbicide	3200	95000	95000	110000	10000	320	11000	1000	320	320	0.96	0.04392	0.03294	0.003	0.00013725
case 14	OK, AK	Gh 01	Rice, Mi	(7883) 2,4-D	Herbicide	24200	100000	63400	46200	27200	634	4620	2720	634	634	9.36	0.4246	0.3185	0.01476341	0.00066972
case 15	OK, AK	Gh 01	Rice, Mi	(7883) 2,4-D	Herbicide	24200	100000	63400	46200	27200	634	4620	2720	634	634	17.32	0.7856	0.5892	0.02731861	0.00123912
case 16	AK	Gh 01	Rice	(7074) pretilachlor	Herbicide	9290	13000	900	1300	90	9	130	9	9	9	14.4	0.6845	0.5134	1.6	0.07605556
case 17	AK	Gh 01	Rice	(6739) pyribenzoxim	Herbicide	100000	100000	100000	10000	10000	1000	1000	1000	1000	1000	0.96	0.04347	0.0326	0.00096	0.00004347
case 18	WJ	Gh 01	Maize, V	(7740) oxyfluorfen	Herbicide	2000	720	250	13	38	2.5	1.3	3.8	1.3	2.5	10.8	0.4982	0.3736	4.32	0.38323077
case 19	WJ	Gh 01	Maize, V	(7740) oxyfluorfen	Herbicide	2000	720	250	13	38	2.5	1.3	3.8	1.3	2.5	14.4	0.6642	0.4982	5.76	0.51092308
case 20	AS, OK,	Gh 01	Vegetal	(7877) lambda-cyhalothrin	Insecticide	300	0.36	0.21	300	0.25	0.0021	30	0.025	0.0021	0.0021	0.6	0.06788	0.05708	285.714286	32.3238095
case 21	AS, OK,	Gh 01	Vegetal	(7877) lambda-cyhalothrin	Insecticide	300	0.36	0.21	300	0.25	0.0021	30	0.025	0.0021	0.0021	8.88	1.005	0.8448	4228.57143	478.571429
case 22	AS, OK,	Gh 01	Vegetal	(7688) chlorpyrifos	Insecticide	480	0.1	1.3	4.6	0.14	0.001	0.46	0.014	0.001	0.001	3.425	0.4014	0.4009	3425	401.4
case 23	AS, OK,	Gh 01	Vegetal	(7688) chlorpyrifos	Insecticide	480	0.1	1.3	4.6	0.14	0.001	0.46	0.014	0.001	0.001	5.479	0.6423	0.6415	5479	642.3
case 24	AS, OK,	Gh 01	Vegetal	(5997) Emamectin Benzoate	Insecticide	N.A.	1	174	0.1	17.5	0.01	0.01	1.75	0.01	0.01	N.A.	N.A.	N.A.	#VALUE!	#VALUE!
case 25	AS, OK,	Gh 01	Vegetal	(5997) Emamectin Benzoate	Insecticide	N.A.	1	174	0.1	17.5	0.01	0.01	1.75	0.01	0.01	N.A.	N.A.	N.A.	#VALUE!	#VALUE!
case 26	TN	Gh 01	Cocoa	(7589) imidacloprid	Insecticide	10000	85000	211000	1800	9020	850	180	902	180	850	0.72	0.03391	0.05084	0.00084706	0.00018839
case 27	OK	Gh 01	Vegetal	(7877) lambda-cyhalothrin	Insecticide	300	0.36	0.21	300	0.25	0.0021	30	0.025	0.0021	0.0021	0.576	0.06517	0.05479	274.285714	31.0333333
case 28	OK	Gh 01	Vegetal	(7581) acetamiprid	Insecticide	98300	49800	100000	5000	19200	498	500	1920	498	498	0.6806	0.08519	0.0728	0.00136667	0.00017106
case 29	WJ	Gh 01	Vegetal	(7581) acetamiprid	Insecticide	98300	49800	100000	5000	19200	498	500	1920	498	498	1.679	0.2101	0.1796	0.00337149	0.00042189
case 30	TN	Gh 01	Cocoa	(7538) novaluron	Insecticide	9680	58	1000	0.03	6.16	0.58	0.003	0.616	0.003	0.58	0.0999	0.002682	0.004023	0.17224138	0.894

case 31	TN	Gh 01	Cocoa	(7609) thiamethoxam	Insecticide	100000	100000	125000	100000	20000	1000	10000	2000	1000	1000	0.7085	0.03256	0.04883	0.0007085	0.00004883
case 32	TN	Gh 01	Cocoa	(7609) thiamethoxam	Insecticide	100000	100000	125000	100000	20000	1000	10000	2000	1000	1000	0.8501	0.03908	0.05859	0.0008501	0.00005859
case 33	TN	Gh 01	Cocoa	(7856) bifenthrin	Insecticide	822	0.11	0.26	0.0013	0.012	0.0011	0.00013	0.0012	0.00013	0.0011	0.004654	0.00008641	0.0001296	4.23090909	0.66469231
case 34	AS, OK	Gh 01	Vegetal	(7752) cypermethrin	Insecticide	100	0.3	2.8	0.04	0.03	0.003	0.004	0.003	0.003	0.003	0.04664	0.005433	0.00459	15.5466667	1.811
case 35	AS, OK	Gh 01	Vegetal	(7752) cypermethrin	Insecticide	100	0.3	2.8	0.04	0.03	0.003	0.004	0.003	0.003	0.003	0.1828	0.0213	0.01799	60.9333333	7.1
case 36	AS, OK	Gh 01	Vegetal	(7785) dimethoate	Insecticide	90400	2000	30200	40	400	20	4	40	4	20	24	2.782	2.349	1.2	0.6955
case 37	AS, OK	Gh 01	Vegetal	(7785) dimethoate	Insecticide	90400	2000	30200	40	400	20	4	40	4	20	94.08	10.91	9.207	4.704	2.7275
case 38	TN	Gh 01	Cocoa	(7856) bifenthrin	Insecticide	822	0.11	0.26	0.0013	0.012	0.0011	0.00013	0.0012	0.00013	0.0011	0.004654	0.00008641	0.0001296	4.23090909	0.66469231
case 39	TN	Gh 01	Cocoa	(7538) novaluron	Insecticide	9680	58	1000	0.03	6.16	0.58	0.003	0.616	0.003	0.58	0.0999	0.002682	0.004023	0.17224138	0.894
case 40	TN	Gh 01	Cocoa	(7856) bifenthrin	Insecticide	822	0.11	0.26	0.0013	0.012	0.0011	0.00013	0.0012	0.00013	0.0011	0.0005688	0.00001056	0.00001584	0.51709091	0.08123077
case 41	TN	Gh 01	Cocoa	(7856) bifenthrin	Insecticide	822	0.11	0.26	0.0013	0.012	0.0011	0.00013	0.0012	0.00013	0.0011	0.0007756	0.0000144	0.0000216	0.70509091	0.11076923
case 42	TN	Gh 01	Cocoa	(7581) acetamiprid	Insecticide	98300	49800	100000	5000	19200	498	500	1920	498	498	0.01996	0.0009492	0.001423	4.008E-05	2.8574E-06
case 43	TN	Gh 01	Cocoa	(7581) acetamiprid	Insecticide	98300	49800	100000	5000	19200	498	500	1920	498	498	0.02722	0.001294	0.001941	5.4659E-05	3.8976E-06
case 44	AK	Gh 01	Rice	(7611) carbofuran	Insecticide	6500	9.4	180	8	2.2	0.094	0.8	0.22	0.094	0.094	0.432	0.01791	0.01343	4.59574468	0.19053191
case 45	AS, WJ,	Gh 01	Vegetal	(7849) mancozeb	Fungicide	44	73	74	7.3	2.2	0.73	0.73	0.22	0.73	0.22	88.37	12.37	9.282	121.054795	42.1909091
case 46	AS, WJ,	Gh 01	Vegetal	(7849) mancozeb	Fungicide	44	73	74	7.3	2.2	0.73	0.73	0.22	0.73	0.22	147.2	20.61	15.47	201.643836	70.3181818
case 47	WJ	Gh 01	Vegetal	(7514) carbendazim	Fungicide	7700	150	190	1.5	3.2	1.5	0.15	0.32	0.15	0.32	9.6	1.16	0.9853	6.4	7.73333333
case 48	WJ	Gh 01	Vegetal	(7514) carbendazim	Fungicide	7700	150	190	1.5	3.2	1.5	0.15	0.32	0.15	0.32	19.2	2.319	1.971	12.8	15.46
case 49	AS, AK	Gh 01	Vegetal	(7836) sulphur	Fungicide	63	63	63	6.3	6.3	0.63	0.63	0.63	0.63	0.63	9.811	0.8767	0.6577	15.5730159	1.3915873
case 50	AS, AK	Gh 01	Vegetal	(7836) sulphur	Fungicide	63	63	63	6.3	6.3	0.63	0.63	0.63	0.63	0.63	11.97	1.07	0.8025	19	1.6984127
case 51	WJ, AS	Gh 01	Vegetal	(7561) maneb	Fungicide	7	2.1	200	2.3	6.5	0.021	0.23	0.65	0.021	0.021	120.1	3.346	2.514	5719.04762	159.333333
case 52	WJ	Gh 01	Vegetal	(7648) copper II hydroxide	Fungicide	9	38	17	30	1.7	0.17	3	0.17	0.17	0.17	3.052	0.3821	0.3265	17.9529412	2.24764706
case 53	WJ	Gh 01	Vegetal	(7648) copper II hydroxide	Fungicide	9	38	17	30	1.7	0.17	3	0.17	0.17	0.17	6.187	0.7745	0.6619	36.3941176	4.55588235
case 54	AS	Gh 01	Vegetal	(7775) metalaxyl	Fungicide	33000	28000	100000	2800	10000	280	280	1000	280	280	1.92	0.1782	0.1337	0.00685714	0.00063643
case 55	AS	Gh 01	Vegetal	(7849) mancozeb	Fungicide	44	73	74	7.3	2.2	0.73	0.73	0.22	0.73	0.22	11.91	1.113	0.8348	16.3150685	3.79454545
case 56	TN	Gh 01	Cocoa	(7814) metalaxyl-M	Fungicide	36000	100000	100000	1200	9100	1000	120	910	120	910	0.36	0.01664	0.02495	0.00036	0.00013867
case 57	AS	Gh 01	Vegetal	(7814) metalaxyl-M	Fungicide	36000	100000	100000	1200	9100	1000	120	910	120	910	7.114	0.869	0.7397	0.007114	0.00724167
case 58	TN	Gh 01	Cocoa	(6620) copper (1) oxide	Fungicide	147	450	207	45	20.7	2.07	4.5	2.07	2.07	2.07	3.6	0.1712	0.2567	1.73913043	0.12400966
case 59	AS	Gh 01	Vegetal	(6620) copper (1) oxide	Fungicide	147	450	207	45	20.7	2.07	4.5	2.07	2.07	2.07	17.78	2.226	1.903	8.58937198	1.07536232
case 60	TN	Gh 01	Cocoa	(7648) copper II hydroxide	Fungicide	9	38	17	30	1.7	0.17	3	0.17	0.17	0.17	2.306	0.1096	0.1644	13.5647059	0.96705882
case 61	TN	Gh 01	Cocoa	(7648) copper II hydroxide	Fungicide	9	38	17	30	1.7	0.17	3	0.17	0.17	0.17	1.406	0.06686	0.1003	8.27058824	0.59
case 62	TN	Gh 01	Cocoa	(7775) metalaxyl	Fungicide	33000	28000	100000	2800	10000	280	280	1000	280	280	2.7	0.1253	0.1879	0.00964286	0.00067107

						Soil risk assessment							
	Farm	Plot	Crop	Pesticide	Type	LC50 earthworms	NOEC earthworms	NEC soil acute	NEC soil chronic	PEC1soil	PECnsoil	ETR soil-acute	ETR soil-chronic
case 1	AS	Gh 01	All	(7516) glyphosate	Herbicide	480	28.8	48	5.76	0.576	0.576	0.012	0.1
case 2	AS	Gh 01	All	(7516) glyphosate	Herbicide	480	28.8	48	5.76	4.704	4.704	0.098	0.816667
case 3	AS	Gh 01	All	(7516) glyphosate	Herbicide	480	28.8	48	5.76	0.768	0.768	0.016	0.133333
case 4	AS	Gh 01	All	(7516) glyphosate	Herbicide	480	28.8	48	5.76	6.272	6.272	0.130667	1.088889
case 5	AS	Gh 01	All	(7742) paraquat	Herbicide	1000	N.A.	100	N.A.	0.4	0.4	0.004	N.A.
case 6	AS	Gh 01	All	(7742) paraquat	Herbicide	1000	N.A.	100	N.A.	2.221	2.221	0.02221	N.A.
case 7	OK	Gh 01	Rice	(7662) butachlor	Herbicide	0.515	N.A.	0.0515	N.A.	4.447	4.447	86.34951	N.A.
case 8	AS, AK	Gh 01	Rice, Ve	(7732) pendimethalin	Herbicide	1000	33.45	100	6.69	1.6	1.6	0.016	0.239163
case 9	AS, AK	Gh 01	Rice, Ve	(7732) pendimethalin	Herbicide	1000	33.45	100	6.69	5.227	5.227	0.05227	0.781315
case 10	AS, OK,	Gh 01	Rice	(7816) propanil	Herbicide	734	N.A.	73.4	N.A.	0.96	0.96	0.013079	N.A.
case 11	AS, OK,	Gh 01	Rice	(7816) propanil	Herbicide	734	N.A.	73.4	N.A.	1.776	1.776	0.024196	N.A.
case 12	AS,OK,	Gh 01	Rice	(7385) bensulfuron-methyl	Herbicide	1000	N.A.	100	N.A.	0.168	0.168	0.00168	N.A.
case 13	AK	Gh 01	Rice	(6596) bispyribac-sodium	Herbicide	957	N.A.	95.7	N.A.	0.05333	0.05333	0.000557	N.A.
case 14	OK, AK	Gh 01	Rice, Mi	(7883) 2,4-D	Herbicide	350	N.A.	35	N.A.	0.5333	0.5333	0.015237	N.A.
case 15	OK, AK	Gh 01	Rice, Mi	(7883) 2,4-D	Herbicide	350	N.A.	35	N.A.	0.9867	0.9867	0.028191	N.A.
case 16	AK	Gh 01	Rice	(7074) pretilachlor	Herbicide	19.23	N.A.	1.923	N.A.	0.8	0.8	0.416017	N.A.
case 17	AK	Gh 01	Rice	(6739) pyribenzoxim	Herbicide	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 18	WJ	Gh 01	Maize, \	(7740) oxyfluorfen	Herbicide	1000	24.09	100	4.818	0.6	0.6	0.006	0.124533
case 19	WJ	Gh 01	Maize, \	(7740) oxyfluorfen	Herbicide	1000	24.09	100	4.818	0.8	0.8	0.008	0.166044
case 20	AS, OK,	Gh 01	Vegetal	(7877) lambda-cyhalothrin	Insecticide	500	N.A.	50	N.A.	0.03333	0.1571	0.000667	N.A.
case 21	AS, OK,	Gh 01	Vegetal	(7877) lambda-cyhalothrin	Insecticide	500	N.A.	50	N.A.	0.4933	2.325	0.009866	N.A.
case 22	AS, OK,	Gh 01	Vegetal	(7688) chlorpyrifos	Insecticide	129	12.7	12.9	2.54	0.64	2.563	0.049612	1.009055
case 23	AS, OK,	Gh 01	Vegetal	(7688) chlorpyrifos	Insecticide	129	12.7	12.9	2.54	1.024	4.101	0.07938	1.614567
case 24	AS, OK,	Gh 01	Vegetal	(5997) Emamectin Benzoate	Insecticide	1000	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 25	AS, OK,	Gh 01	Vegetal	(5997) Emamectin Benzoate	Insecticide	1000	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 26	TN	Gh 01	Cocoa	(7589) imidacloprid	Insecticide	10.7	0.178	1.07	0.0356	0.04	0.1432	0.037383	4.022472
case 27	OK	Gh 01	Vegetal	(7877) lambda-cyhalothrin	Insecticide	500	N.A.	50	N.A.	0.032	0.1508	0.00064	N.A.
case 28	OK	Gh 01	Vegetal	(7581) acetamiprid	Insecticide	9	1.26	0.9	0.252	0.04	0.04441	0.044444	0.17623
case 29	WJ	Gh 01	Vegetal	(7581) acetamiprid	Insecticide	9	1.26	0.9	0.252	0.09867	0.1095	0.109633	0.434524
case 30	TN	Gh 01	Cocoa	(7538) novaluron	Insecticide	1000	3	100	0.6	0.021	0.06362	0.00021	0.106033

case 31 TN	Gh 01	Cocoa	(7609) thiamethoxam	Insecticide	1000	5.34	100	1.068	0.04	0.1089	0.0004	0.101966
case 32 TN	Gh 01	Cocoa	(7609) thiamethoxam	Insecticide	1000	5.34	100	1.068	0.048	0.1307	0.00048	0.122378
case 33 TN	Gh 01	Cocoa	(7856) bifenthrin	Insecticide	8	1.065	0.8	0.213	0.018	0.03751	0.0225	0.176103
case 34 AS, OK	Gh 01	Vegetal	(7752) cypermethrin	Insecticide	100	N.A.	10	N.A.	0.12	0.3204	0.012	N.A.
case 35 AS, OK	Gh 01	Vegetal	(7752) cypermethrin	Insecticide	100	N.A.	10	N.A.	0.4704	1.256	0.04704	N.A.
case 36 AS, OK	Gh 01	Vegetal	(7785) dimethoate	Insecticide	31	2.87	3.1	0.574	1.333	1.419	0.43	2.472125
case 37 AS, OK	Gh 01	Vegetal	(7785) dimethoate	Insecticide	31	2.87	3.1	0.574	5.227	5.564	1.686129	9.69338
case 38 TN	Gh 01	Cocoa	(7856) bifenthrin	Insecticide	8	1.065	0.8	0.213	0.018	0.03751	0.0225	0.176103
case 39 TN	Gh 01	Cocoa	(7538) novaluron	Insecticide	1000	3	100	0.6	0.021	0.06362	0.00021	0.106033
case 40 TN	Gh 01	Cocoa	(7856) bifenthrin	Insecticide	8	1.065	0.8	0.213	0.0022	0.004585	0.00275	0.021526
case 41 TN	Gh 01	Cocoa	(7856) bifenthrin	Insecticide	8	1.065	0.8	0.213	0.003	0.006252	0.00375	0.029352
case 42 TN	Gh 01	Cocoa	(7581) acetamiprid	Insecticide	9	1.26	0.9	0.252	0.001173	0.001183	0.001303	0.004694
case 43 TN	Gh 01	Cocoa	(7581) acetamiprid	Insecticide	9	1.26	0.9	0.252	0.0016	0.001613	0.001778	0.006401
case 44 AK	Gh 01	Rice	(7611) carbofuran	Insecticide	224	0.84	22.4	0.168	0.024	0.024	0.001071	0.142857
case 45 AS, WJ,	Gh 01	Vegetal	(7849) mancozeb	Fungicide	299.1	20	29.91	4	6.325	6.325	0.211468	1.58125
case 46 AS, WJ,	Gh 01	Vegetal	(7849) mancozeb	Fungicide	299.1	20	29.91	4	10.54	10.54	0.352391	2.635
case 47 WJ	Gh 01	Vegetal	(7514) carbendazim	Fungicide	5.4	1	0.54	0.2	0.5333	1.349	0.987593	6.745
case 48 WJ	Gh 01	Vegetal	(7514) carbendazim	Fungicide	5.4	1	0.54	0.2	1.067	2.697	1.975926	13.485
case 49 AS, AK	Gh 01	Vegetal	(7836) sulphur	Fungicide	2000	N.A.	200	N.A.	0.8533	1.918	0.004267	N.A.
case 50 AS, AK	Gh 01	Vegetal	(7836) sulphur	Fungicide	2000	N.A.	200	N.A.	1.041	2.34	0.005205	N.A.
case 51 WJ, AS	Gh 01	Vegetal	(7561) maneb	Fungicide	840	8	84	1.6	10.54	10.55	0.125476	6.59375
case 52 WJ	Gh 01	Vegetal	(7648) copper II hydroxide	Fungicide	677	15	67.7	3	0.7597	2.278	0.011222	0.759333
case 53 WJ	Gh 01	Vegetal	(7648) copper II hydroxide	Fungicide	677	15	67.7	3	1.54	4.617	0.022747	1.539
case 54 AS	Gh 01	Vegetal	(7775) metalaxyl	Fungicide	1000	N.A.	100	N.A.	0.1067	0.3119	0.001067	N.A.
case 55 AS	Gh 01	Vegetal	(7849) mancozeb	Fungicide	299.1	20	29.91	4	0.8533	0.8533	0.028529	0.213325
case 56 TN	Gh 01	Cocoa	(7814) metalaxyl-M	Fungicide	830	N.A.	83	N.A.	0.02	0.04978	0.000241	N.A.
case 57 AS	Gh 01	Vegetal	(7814) metalaxyl-M	Fungicide	830	N.A.	83	N.A.	0.3952	1.221	0.004761	N.A.
case 58 TN	Gh 01	Cocoa	(6620) copper (1) oxide	Fungicide	862	15	86.2	3	0.2	0.7543	0.00232	0.251433
case 59 AS	Gh 01	Vegetal	(6620) copper (1) oxide	Fungicide	862	15	86.2	3	0.988	3.836	0.011462	1.278667
case 60 TN	Gh 01	Cocoa	(7648) copper II hydroxide	Fungicide	677	15	67.7	3	0.5739	2.29	0.008477	0.763333
case 61 TN	Gh 01	Cocoa	(7648) copper II hydroxide	Fungicide	677	15	67.7	3	0.35	1.397	0.00517	0.465667
case 62 TN	Gh 01	Cocoa	(7775) metalaxyl	Fungicide	1000	N.A.	100	N.A.	0.15	0.3841	0.0015	N.A.

						Bees risk assessment				NTA risk assessment					
	Farm	Plot	Crop	Pesticide	Type	LD50 bee	NEC bee	PEC bee	ETR bee	LR50 nta	AEC nta	PEC nta (in-field)	PEC nta (off-field)	ETR nta (in-field)	ETR nta (off-field)
case 1	AS	Gh 01	All	(7516) glyphosate	Herbicide	100	5000	432	0.0864	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 2	AS	Gh 01	All	(7516) glyphosate	Herbicide	100	5000	3528	0.7056	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 3	AS	Gh 01	All	(7516) glyphosate	Herbicide	100	5000	576	0.1152	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 4	AS	Gh 01	All	(7516) glyphosate	Herbicide	100	5000	4704	0.9408	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 5	AS	Gh 01	All	(7742) paraquat	Herbicide	9.06	453	300	0.6623	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 6	AS	Gh 01	All	(7742) paraquat	Herbicide	9.06	453	1666	3.678	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 7	OK	Gh 01	Rice	(7662) butachlor	Herbicide	100	5000	3335	0.667	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 8	AS, AK	Gh 01	Rice, Ve	(7732) pendimethalin	Herbicide	100	5000	1200	0.24	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 9	AS, AK	Gh 01	Rice, Ve	(7732) pendimethalin	Herbicide	100	5000	3920	0.784	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 10	AS, OK,	Gh 01	Rice	(7816) propanil	Herbicide	94.3	4715	720	0.1527	0.087	0.174	720	7.2	4138	41.38
case 11	AS, OK,	Gh 01	Rice	(7816) propanil	Herbicide	94.3	4715	1332	0.2825	0.087	0.174	1332	13.32	7655	76.55
case 12	AS,OK,	Gh 01	Rice	(7385) bensulfuron-methyl	Herbicide	51.4	2570	126	0.04903	600	1200	126	1.26	0.105	0.00105
case 13	AK	Gh 01	Rice	(6596) bispyribac-sodium	Herbicide	141	7050	40	0.005674	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 14	OK, AK	Gh 01	Rice, M	(7883) 2,4-D	Herbicide	94	4700	400	0.08511	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 15	OK, AK	Gh 01	Rice, M	(7883) 2,4-D	Herbicide	94	4700	740	0.1574	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 16	AK	Gh 01	Rice	(7074) pretilachlor	Herbicide	93	4650	600	0.129	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 17	AK	Gh 01	Rice	(6739) pyribenzoxim	Herbicide	100	5000	40	0.008	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 18	WJ	Gh 01	Maize, \	(7740) oxyfluorfen	Herbicide	100	5000	450	0.09	1440	2880	450	4.5	0.1563	0.001563
case 19	WJ	Gh 01	Maize, \	(7740) oxyfluorfen	Herbicide	100	5000	600	0.12	1440	2880	600	6	0.2083	0.002083
case 20	AS, OK,	Gh 01	Vegetal	(7877) lambda-cyhalothrin	Insecticide	0.038	1.9	25	13.16	0.2	0.4	80	0.8	200	2
case 21	AS, OK,	Gh 01	Vegetal	(7877) lambda-cyhalothrin	Insecticide	0.038	1.9	370	194.7	0.2	0.4	1184	11.84	2960	29.6
case 22	AS, OK,	Gh 01	Vegetal	(7688) chlorpyrifos	Insecticide	0.059	2.95	480	162.7	0.2	0.4	1440	14.4	3600	36
case 23	AS, OK,	Gh 01	Vegetal	(7688) chlorpyrifos	Insecticide	0.059	2.95	768	260.3	0.2	0.4	2304	23.04	5760	57.6
case 24	AS, OK,	Gh 01	Vegetal	(5997) Emamectin Benzoate	Insecticide	0.0035	0.175	117.8	673.1	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 25	AS, OK,	Gh 01	Vegetal	(5997) Emamectin Benzoate	Insecticide	0.0035	0.175	351.5	2009	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 26	TN	Gh 01	Cocoa	(7589) imidacloprid	Insecticide	0.0037	0.185	30	162.2	0.022	0.044	81	0.81	1841	18.41
case 27	OK	Gh 01	Vegetal	(7877) lambda-cyhalothrin	Insecticide	0.038	1.9	24	12.63	0.2	0.4	76.8	0.768	192	1.92
case 28	OK	Gh 01	Vegetal	(7581) acetamiprid	Insecticide	8.09	404.5	30	0.07417	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 29	WJ	Gh 01	Vegetal	(7581) acetamiprid	Insecticide	8.09	404.5	74	0.1829	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 30	TN	Gh 01	Cocoa	(7538) novaluron	Insecticide	100	5000	15.75	0.00315	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 31	TN	Gh 01	Cocoa	(7609) thiamethoxam	Insecticide	0.005	0.25	30	120	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 32	TN	Gh 01	Cocoa	(7609) thiamethoxam	Insecticide	0.005	0.25	36	144	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 33	TN	Gh 01	Cocoa	(7856) bifenthrin	Insecticide	0.1	5	13.5	2.7	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 34	AS, OK	Gh 01	Vegetal	(7752) cypermethrin	Insecticide	0.02	1	90	90	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 35	AS, OK	Gh 01	Vegetal	(7752) cypermethrin	Insecticide	0.02	1	352.8	352.8	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 36	AS, OK	Gh 01	Vegetal	(7785) dimethoate	Insecticide	0.12	6	1000	166.7	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 37	AS, OK	Gh 01	Vegetal	(7785) dimethoate	Insecticide	0.12	6	3920	653.3	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

case 38	TN	Gh 01	Cocoa	(7856) bifenthrin	Insecticide	0.1	5	13.5	2.7	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 39	TN	Gh 01	Cocoa	(7538) novaluron	Insecticide	100	5000	15.75	0.00315	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 40	TN	Gh 01	Cocoa	(7856) bifenthrin	Insecticide	0.1	5	1.65	0.33	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 41	TN	Gh 01	Cocoa	(7856) bifenthrin	Insecticide	0.1	5	2.25	0.45	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 42	TN	Gh 01	Cocoa	(7581) acetamiprid	Insecticide	8.09	404.5	0.88	0.002176	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 43	TN	Gh 01	Cocoa	(7581) acetamiprid	Insecticide	8.09	404.5	1.2	0.002967	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 44	AK	Gh 01	Rice	(7611) carbofuran	Insecticide	0.036	1.8	18	10	2.68	5.36	18	0.18	3.358	0.03358
case 45	AS, WJ,	Gh 01	Vegetat	(7849) mancozeb	Fungicide	140.6	7030	4744	0.6748	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 46	AS, WJ,	Gh 01	Vegetat	(7849) mancozeb	Fungicide	140.6	7030	7904	1.124	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 47	WJ	Gh 01	Vegetat	(7514) carbendazim	Fungicide	50	2500	400	0.16	3000	6000	920	9.2	0.1533	0.001533
case 48	WJ	Gh 01	Vegetat	(7514) carbendazim	Fungicide	50	2500	800	0.32	3000	6000	1840	18.4	0.3067	0.003067
case 49	AS, AK	Gh 01	Vegetat	(7836) sulphur	Fungicide	100	5000	640	0.128	486	972	1472	14.72	1.514	0.01514
case 50	AS, AK	Gh 01	Vegetat	(7836) sulphur	Fungicide	100	5000	780.9	0.1562	486	972	1796	17.96	1.848	0.01848
case 51	WJ, AS	Gh 01	Vegetat	(7561) maneb	Fungicide	89.5	4475	7904	1.766	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 52	WJ	Gh 01	Vegetat	(7648) copper II hydroxide	Fungicide	44.5	2225	569.8	0.2561	0.05	0.1	1311	13.11	13110	131.1
case 53	WJ	Gh 01	Vegetat	(7648) copper II hydroxide	Fungicide	44.5	2225	1155	0.5191	0.05	0.1	2657	26.57	26570	265.7
case 54	AS	Gh 01	Vegetat	(7775) metalaxyl	Fungicide	200	10000	80	0.008	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 55	AS	Gh 01	Vegetat	(7849) mancozeb	Fungicide	140.6	7030	640	0.09104	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 56	TN	Gh 01	Cocoa	(7814) metalaxyl-M	Fungicide	127	6350	15	0.002362	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 57	AS	Gh 01	Vegetat	(7814) metalaxyl-M	Fungicide	127	6350	296.4	0.04668	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
case 58	TN	Gh 01	Cocoa	(6620) copper (1) oxide	Fungicide	116	5800	150	0.02586	39.2	78.4	405	4.05	5.166	0.05166
case 59	AS	Gh 01	Vegetat	(6620) copper (1) oxide	Fungicide	116	5800	741	0.1278	39.2	78.4	2001	20.01	25.52	0.2552
case 60	TN	Gh 01	Cocoa	(7648) copper II hydroxide	Fungicide	44.5	2225	430.4	0.1934	0.05	0.1	1162	11.62	11620	116.2
case 61	TN	Gh 01	Cocoa	(7648) copper II hydroxide	Fungicide	44.5	2225	262.5	0.118	0.05	0.1	708.8	7.088	7088	70.88
case 62	TN	Gh 01	Cocoa	(7775) metalaxyl	Fungicide	200	10000	112.5	0.01125	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

Table SI 3.3: Pesticide type used and their physico-chemical properties.

Pesticide Product	Active ingredient	Class	Molecular Mass	Saturated Vapour Pressure (Pa)	Temperature Saturated Vapour Pressure (°C)	Solubility (mg/l)	Temperature Solubility (°C)	Temperature Solubility (°C)	DT50 - sediment (days)	Kom (l/kg)
Ceresate; Chemosate; Roundup; Power; Sunphosate	Glyphosate	Herbicide	169.1	0.09	20	15.5	20	0.2	0.065	144.5
Gramoxone; M-Quat	Paraquat	Herbicide	257.2	1.00E-05	20	620	20	10000	3000	5.80E-05
Ceres Butachlor	Butachlor	Herbicide	311.9	0.24	20	20	20	*	56	406
Stomp 445 CS; Alligator	Pendimethalin	Herbicide	281.3	4.00E-03	25	0.28	25	28	90	2.40E+04
Orizo Plus; Starm;	Propanil	Herbicide	218.08	12	60	225	25	2	3	2.2856
Calliherb	2, 4-D	Herbicide	221.04	12	25	900	25	7	7	2.81
Condax; Londax	Bensulfuron methyl	Herbicide	410.4	2.80E-09	20	67	20	24	8.2	0
Bounty	Bispyribac sodium	Herbicide	453.36	5.50E-06	20	64000	20	35.3	35.3	0
	Pretilachlor	Herbicide	311.9	0.133	20	500	20	*	30	0
Solito	Pyribenzoxim	Herbicide	609.6	0.99	20	3.5	20	*	*	0
Zoomer	Oxyfluorfen	Herbicide	361.7	2.00E-07	25	1.16E-01	25	*	35	0
	Glyphosate	Herbicide	169.1	0.09	20	15.5	20	0.2	0.065	144.5
Karate; Conti-Halothrin; Pawa; Stricker	Lambda Cyhalothrin	Insecticide	449.9	Negligible	20	0.005	20	12	70.9	0
Dursban; Sunpyrifos	Chlorpyrifos	Insecticide	350.62	2.5	25	2	25	36.5	50	4728
Attack	Emmamectin benzoate	Insecticide	1008.26	0.004	20	24	20	0	*	5.8
Confidor	Imidacloprid	Insecticide	255.7	0.2	20	0.51	20	129	191	0
Golan	Acetamiprid	Insecticide	222.7	1.73E-04	20	2950	20	*	3	116
	Novaluron	Insecticide	492.7	3.75E-06	40	0.9531	25	17.5	72	5567
Rimon star	Bifenthrin	Insecticide	422.87	0.0178	20	0.001	20	161	26	1.37E+05
Actara	Thiamethoxam	Insecticide	291.71	6.60E-06	20	4100	20	40	50	32.6
Cocostar; Akate Master	Bifenthrin	Insecticide	422.87	0.0178	20	0.001	20	161	26	1.37E+05

	Cypermethrin	Insecticide	416.3	1.90E-07	20	4.00E-03	20	14	36	2137
Cymethoate, Cydim Super	Dimethoate	Insecticide	229.3	0.29	20	2.50E+04	20	32.49	4.886	1528
Furadan	Carbofuran	Fungicide	221.26	0.08	20	322	20	9.7	29	0
Benco	Mancozeb	Fungicide	271.3	0.013	20	6.2	20	76	0.1	578.9
Carbendazim 50 WP	Carbendazim	Fungicide	191.2	0.09	20	15.5	20	0.2	0.065	144.8
Sulfa 80 WP	Sulphur	Fungicide	32.06	32.06	5.30E-06	30.4	-	0.063	30	1131
Maneb 80 WP	Maneb	Fungicide	265.31	0.014	20	178	20	0.007	1	1160
Funguran-OH	Copper Hydroxide	Fungicide	97.56	100	25	negligible	25	0.009	1.00E+04	6961
	Metalaxyl	Fungicide	279.33	0.75	20	8400	20	33	42	0
Victory	Mancozeb	Fungicide	271.3	0.013	20	6.2	20	76	0.1	578.9
Ridomil	Metalaxyl-M	Fungicide	279.33	3.3	20	26000	20	47.5	39	0
	Cuprous Oxide	Fungicide	145.1	Insoluble	-	-	-	*	365	0
Nordoz super 75 WG	Cuprous Oxide	Fungicide			-	-	-	*	365	0
Kocide	Cupric hydroxide	Fungicide	97.56	Insoluble	-	-	-	*	365	0
	Copper	Fungicide	97.56	Insoluble	-	-	-	*	365	0
Fungikill	Metalaxyl	Fungicide	279.33	0.75	20	8400	20	33	42	0

* undefined

Table SI 3.4: Pesticide(s) and their tier-1 acute L(E/D/R)C50 used for the Exposure Toxicity Ratios (ETRs) calculated by the PRIMET model for the aquatic, terrestrial (bee), terrestrial soil (worms) and Non-Target Arthropods (NTAs) in the environment of the study sites.

ud = undefined

Active Ingredient	Pesticide Class	Aquatic			Terrestrial		
		Primary Producers $L(E)C_{50}$ (µg/l) (Algae, Macrophytes)	Invertebrates $L(E)C_{50}$ (µg/l) (Arthropoda, Non-arthropoda)	Vertebrates $L(E)C_{50}$ (µg/l) (Fish and other vertebrates)	$Soil_{Worms}$ LD_{50} (µg/kg)	Bee LD_{50} (µg/bee)	Non-Target Arthropods (NTA) LR_{50} nta (-)
Glyphosate	Herbicide	4400	40000	38000	480000	100	ud
Paraquat	Herbicide	0.23	4400	19000	1000000	9.06	ud
Butachlor	Herbicide	200	2400	440	515	100	ud
Pendimethalin	Herbicide	6	280	138	1000000	100	ud
Propanil	Herbicide	110	2390	5400	734000	94.3	87
2, 4-D	Herbicide	24200	100000	63400	350000	94	ud
Bensulfuron methyl	Herbicide	20	130000	66000	1000000	51.4	600
Bispyribac sodium	Herbicide	3200	95000	95000	957000	141	ud
Pretilachlor	Herbicide	9290	13000	900	19230	93	ud
Pyribenzoxim	Herbicide	100000	100000	100000	Ud	100	ud
Oxyfluorfen	Herbicide	2000	720	250	1000000	100	1440000
Lambda Cyhalothrin	Insecticide	300	0.36	0.21	500000	0.038	200
Chlorpyrifos	Insecticide	480	0.1	1.3	129000	0.059	200
Emmamectin benzoate	Insecticide	ud	1	174	1000000	0.0035	ud
Imidacloprid	Insecticide	10000	85000	211000	10700	0.0037	22
Acetamiprid	Insecticide	98300	49800	100000	9000	8.09	ud
Novaluron	Insecticide	9680	58	1000	1000000	100	ud
Bifenthrin	Insecticide	822	0.11	0.26	8000	0.1	ud
Thiamethoxam	Insecticide	100000	100000	125000	1000000	0.005	ud
Cypermethrin	Insecticide	100	0.3	2.8	100000	0.02	ud
Dimethoate	Insecticide	9040	2000	30200	31000	0.12	ud
Carbofuran	Fungicide	6500	9.4	180	224000	0.036	2680
Mancozeb	Fungicide	44	73	74	299100	140.6	ud
Carbendazim	Fungicide	7700	150	190	5400	50	3000000

Sulphur	Fungicide	63	63	63	2000000	100	486000
Maneb	Fungicide	7	2.1	200	840000	89.5	ud
Copper Hydroxide	Fungicide	9	38	17	667000	44.5	50
Metalaxyl	Fungicide	33000	28000	100000	1000000	200	ud
Metalaxyl-M	Fungicide	36000	100000	100000	830000	127	ud
Cuprous Oxide	Fungicide	147	450	207	862000	116	39200

Table SI 3.5: Pesticide(s) and their tier-1 chronic NOEC used for the Exposure Toxicity Ratios (ETRs) calculated by the PRIMET model for the aquatic, terrestrial (bee), terrestrial soil (worms) and Non-Target Arthropods (NTAs) in the environment of the study sites.

ud = undefined

Active Ingredient	**Pesticide Class**	**Aquatic**		**Terrestrial**		
		Invertebrates NOEC (µg/l) (Daphnia)	**Vertebrates NOEC (µg/l) (Fish)**	**$Soil_{Worms}$ NEC (µg/kg)**	**Bee (µg/bee)**	**Non-Target Arthropods (NTA) nta (-)**
Glyphosate	Herbicide	30000	2500	5.76	ud	ud
Paraquat	Herbicide	120	1900	ud	ud	ud
Butachlor	Herbicide	240	44	ud	ud	ud
Pendimethalin	Herbicide	14.5	6	6.69	ud	ud
Propanil	Herbicide	86	540	ud	ud	ud
2, 4-D	Herbicide	46200	27200	ud	ud	ud
Bensulfuron methyl	Herbicide	12000	1500	ud	ud	ud
Bispyribac sodium	Herbicide	110000	10000	ud	ud	ud
Pretilachlor	Herbicide	1300	90	ud	ud	ud
Pyribenzoxim	Herbicide	10000	10000	ud	ud	ud
Oxyfluorfen	Herbicide	13	38	4.818	ud	ud
Lambda Cyhalothrin	Insecticide	300	0.25	ud	ud	ud
Chlorpyrifos	Insecticide	4.6	0.14	2.54	ud	ud
Emmamectin benzoate	Insecticide	0.1	17.5	ud	ud	ud
Imidacloprid	Insecticide	1800	9020	0.0356	ud	ud
Acetamiprid	Insecticide	5000	19200	0.252	ud	ud
Novaluron	Insecticide	0.03	6.16	0.6	ud	ud

Bifenthrin	Insecticide	0.0013	0.012	0.213	ud	ud
Thiamethoxam	Insecticide	100000	20000	1.068	ud	ud
Cypermethrin	Insecticide	0.04	0.03	ud	ud	ud
Dimethoate	Insecticide	40	400	0.574	ud	ud
Carbofuran	Fungicide	8	19200	0.168	ud	ud
Mancozeb	Fungicide	7.3	2.2	4	ud	ud
Carbendazim	Fungicide	1.5	3.2	0.2	ud	ud
Sulphur	Fungicide	6.3	6.3	ud	ud	ud
Maneb	Fungicide	2.3	6.5	1.6	ud	ud
Copper Hydroxide	Fungicide	30	1.7	1.7	ud	ud
Metalaxyl	Fungicide	2800	10000	ud	ud	ud
Metalaxyl-M	Fungicide	1200	9100	ud	ud	ud
Cuprous Oxide	Fungicide	45	20.7	3	ud	ud

Chapter 4

Linking macroinvertebrates and physico-chemical parameters for water quality assessment in the lower basin of the Volta River in Ghana.

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Abstract

The health of the lower basin of the Volta River in Ghana was evaluated in January-February and May-June 2016 using physico-chemical parameters and benthic macroinvertebrate species as indicators. The measured levels of selected environmental variables were compared to accepted environmental quality standard values where applicable. The association between the benthic macroinvertebrates and physico-chemical variables were analysed through benthic macroinvertebrate distribution and a principal component analysis (PCA) and redundancy analysis (RDA). Pesticide concentrations were generally below the limit of detection of 0.01 µg/L and 0.005 µg/L for organophosphate/synthetic pyrethroid and organochlorines, respectively, except isolated cases where λ-cyhalothrin was detected at Sedorm 1 (T1) and Akuse Canal (T8) with values of 0.6 µg/L and 8.8 µg/L, respectively and cypermethrin detected with 1.7 µg/L at Marine (T3) during the January - February sampling period. Nutrient levels were also generally low, however significant differences existed between the values of physico-chemical parameters at the different sampling sites and seasons (Monte Carlo permutation test; $p = 0.002$), as well as between the abundance of macroinvertebrates at the different sites and seasons (Monte Carlo permutation test; $p = 0.002$). The environmental variables DO, phosphate, pH, substratum ($p < 0.05$), turbidity, electrical conductivity, total dissolved solids, total solids and nitrate ($0.05 < p < 0.10$) significantly explained the variation in macroinvertebrate composition between sampling sites of the Volta river. *Polypedilum fuscipenne,* was significantly positively correlated with turbidity and DO concentrations; *Physa* sp*., Centroptilum* sp*., Centroptiloides* sp*. Phaon iridipennis* and juvinile fish were positively correlated with nitrate concentration and pH and negatively correlated with turbidity and DO. Polluted sites were dominated by the snail *Lymnaea glabra*. This demonstrates that physicochemical parameters and macroinvertebrates could be applied to describe the water quality and improve the biomonitoring for water resources management and the environmental protection in the Lower Volta River system.

Introduction

The Volta River is one of most important river systems in Ghana. It originates from Burkina Faso and runs mainly through Ghana and covers an estimated area of 400,000 km^2 (Barry *et al.* 2005). The north-south extent of this transboundary basin stretches from approximately latitude 5^o 30' N in Ghana to 14^o 30' N in Mali, with the widest part stretching approximately from longitude 5^o 30' W to 2^o 00' E (Gordon *et al*. 2013). The lower part of the river basin promotes different uses including agriculture, aquaculture, fishing, hydroelectric power, water for domestic (drinking) and industrial purposes, water transport, sand mining and industrial activities (e.g. textile works) among others (Andah *et al*. 2003; Mul *et al*. 2015). The river receives domestic wastewater, industrial wastewater, municipal and rural wastes, and other human activities. High levels of organic pollutants may degrade the water quality in receiving waters and threaten the aquatic ecosystems (Corcoran *et al*. 2010; Wang *et al.* 2013; Asantewaa Owusu *et al*. 2016). For example, water may become polluted due to a range of contaminants originating from agricultural activities (Hooda *et al*. 2000; Lovell and Sullivan, 2006; Ansah Asare, 2006). Indeed, pesticides have been reported to affect water bodies in Ghana (Aquaah, 1997; Ntow, 2001; Ntow, 2005; Fianko *et al*. 2011). In addition, the statistics show that the water sources have been, and continued to be, exploited speedily (Asantewaa Owusu *et al*. 2016). To improve the water resources management and the water quality monitoring for the Volta River System and other water resources, monitoring of physiochemical parameters and aquatic macroinvertebrates have been applied (Thorne and Williams, 1997; Thorne *et al*. 2000; Baa-Poku *et al*. 2013).

Benthic macroinvertebrates are a ubiquitous and diverse group of long-lived species that react strongly and often predictable to human influences in aquatic ecosystems. In addition, they are sedentary, therefore body burdens reflects local conditions, allowing detection of a variety of perturbations in a range of aquatic habitats (Rosenberg and Resh, 1993). Benthic macroinvertebrates are an important and integral part of any aquatic ecosystem as they form the basis of the trophic cascade and any negative effects caused by pollution on the community structure can in turn affect higher trophic levels like fish and birds. In addition, aquatic invertebrates have the ability to clean waterways as they utilize the organic and detritus matter. According to Carlisle *et al.* (2007), macroinvertebrate populations

in streams and rivers can assist in the assessment of the overall health of the stream and monitoring of macroinvertebrates has been limited in Ghana.

The overall objectives of this study were to: 1) evaluate the values of the physico-chemical parameters and pesticides, and benthic macroinvertebrate richness and composition in the Lower Volta River System, and 2) examine the relationships between the environmental variables and the macroinvertebrate community composition in the evaluated aquatic system of the Volta River.

Materials and methods

Study area

There are two distinct types of savannah in the basin: woodland savannah and grassy savannah. The woodland savannah, mostly found in the southern parts of the basin, is densely wooded with tall to medium tall grasses (Mul *et al*. 2015). The climate of the Volta basin is dominated by the rain-bearing south westerly tropical maritime air mass and the dry, north easterly tropical continental air mass (Dickson and Benneh, 1988). Normally, there is a bimodal rainfall from April to July and from September to November in Southern Ghana. The single wet season is from May to October in Northern Ghana, which is followed by dry season (Harmatan). The wettest area in Ghana is the extreme southwest where annual rainfall is about 2000 mm, the annual rainfall generally decreases from south to north. The country has a high temperature with the average annual temperature ranging between 24 °C and 30 °C (GEPA, 2011). In the coastal area of Ghana the relative annual humidity is 95-100% in the morning and about 75% in the afternoon. In the north these values can be as low as 20-30% during the Harmatan period and 70-80% during the rainfall period (Andah *et al*. 2003).

The study area has an average rainfall of 1000 mm/year with distinct dry (October–May) and wet (May–October) seasons (van de Giesen *et al*. 2010). Temperatures vary between approximately 16^{o}C and 40^{o}C depending on season, time of day, and elevation (Bekoe and Logah, 2013) and falls within the Dahomeyan system which occurs at the southern part of the main Volta basin, and consists of mainly metamorphic rocks, including hornblende and biotite, gneisses, migmatites, granulites, and schist (Barry *et al*. 2005).

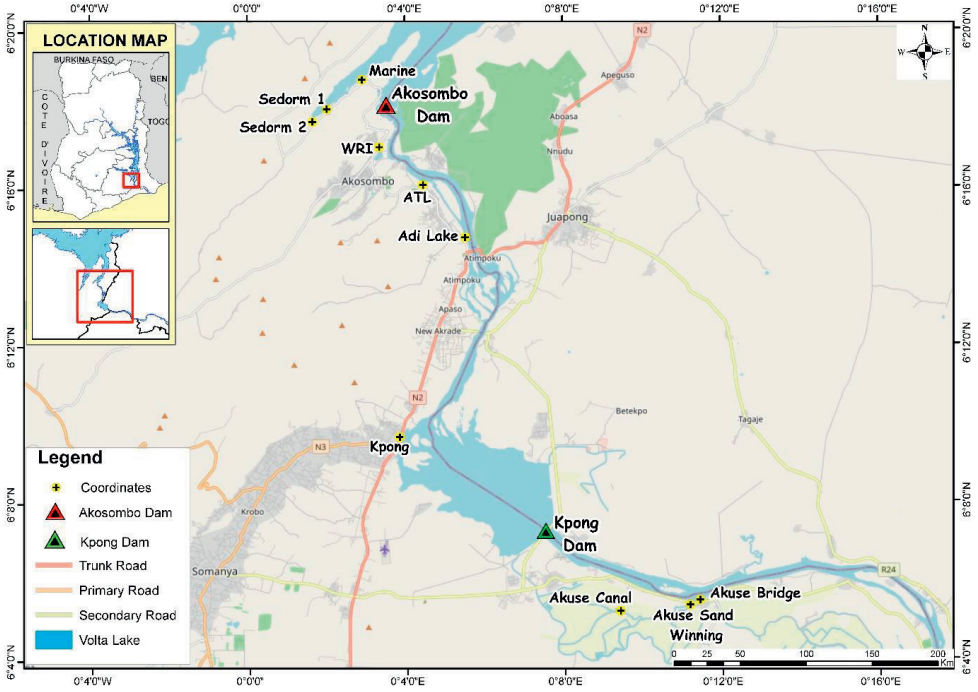

Figure 4.1: Map showing study area and sampling points.

Site selection and sampling

Site selection was based on land use, accessibility and anthropogenic activities using the Rapid Bioassessment Protocol (RBP) for streams and wadable rivers (Barbour *et al.* 1999). The stations were divided to three areas: up the Akosombo hydroelectric Dam (3 stations), in between the Akosombo hydroelectric Dam and Kpong hydroelectric Dam (4 stations) and down the Kpong hydroelectric Dam (3 stations) (Fig. 3.1). The water quality was evaluated in the river by sampling upstream, the disturbed areas themselves, and downstream of the waterways and the differences in macroinvertebrate abundance were used as main biological indicator of disturbance (Table SI 4.2, 4.3 and 4.4). Besides, we tried to have a good distribution of stations between land uses (Table SI 4.1) and stations were subjected to non-point influents (i.e. agricultural runoff) and point influents (i.e. fish pond). Also, one site was selected as reference site where there was no or slight pollution expected (Table SI 4.1; T6 (Adi Lake)). Each station was sampled three times with a two-week interval in the dry and wet

seasons namely: January – February 2016 and May - June 2016, respectively, for the investigation of physico-chemical parameters, pesticide concentrations and macroinvertebrate abundance. Sampling was mainly confined to a few meters (~ 4) from the banks of the river courses except on a few occasions where a canoe was used due to unavailability of conducive bank. Surface water samples were taken from a depth of 20–30 cm. Samples were collected into acid-cleaned high-density 1 L polyethylene bottles. The samples were carried in an ice cooler from the field and stored in a refrigerator at 4^{o}C before analysis of physico-chemical parameters at the Ecological Laboratory of the University of Ghana, Legon. Water samples were again taken from each of the sites using pre-cleaned sterile glass amber bottles and kept at 4°C and subsequently used for pesticide analyses.

At each sampling location, a surber sampler (30x30cm and 250 micron mesh) was used for collecting macroinvertebrates based on the RBP. On each site three replicates were collected and composited as one sample. Benthic macroinvertebrates were preserved in 4% formaldehyde solution. The macroinvertebrates were sorted, identified to the lowest possible taxonomic level (species, genus or families), and counted under a stereomicroscope.

Physico-chemical analysis

During sampling, water temperature (^{o}C), pH (-), dissolved oxygen (DO mg/L), total dissolved solids (TDS, mg/L), turbidity (NTU), and electrical conductivity (µs/cm) were measured on site using portable equipment (Horiba *U-50 Series multi-parameter water quality meter*). Total solids (TS) was determined by Gravimetric Method (APHA, 1998). 10mL of the samples were weighted into a pre-weighed evaporating dish which was then dried in an oven at a temperature of 103 to 105^{o}C for two and a half hours. The dish was transferred into a desiccator and allowed to cool to room temperature and subsequently weighed. The TS was represented by the increase in the weight of the evaporating dish. The total suspended solids were easily obtained by simple calculation, i.e. total suspended solids = total solids — TDS. BOD was determined according to standard methods for the examination of water and wastewater (APHA, 1998).

Othophosphate (PO_4 -P) was determined using ammonium molybdate and ascorbic acid method (Mackereth *et al*. 1978), ammonia-nitrogen (NH_4 -N) by the indophenol blue

method (Franson, 1989), nitrate-nitrogen (NO_3 -N) by hydrazine reduction followed by diazotizing to form an azodye which was measured colorimetrically and nitrite-nitrogen (NO_2 -N) was determined by N-(1-2 naphthyl) ethylene di amine di -hydrochloride method. (APHA, 1998). All reagents used were of analytical grade, equipment was pre-calibrated appropriately before measurement and replicate analyses were carried out for each determination to ascertain reproducibility and quality assurance.

Pesticide extraction and analysis

The following pesticides were chosen as target compounds based on information of previous and current pesticide use: lindane, delta-HCH, heptachlor, aldrin, gamma chlordane, alpha-endosulfan, DDE, endrin, dieldrin, DDD, DDT, endosulfan sulphate, methoxychlor, ethoprophos, diazinon, dimethoate, pirimiphos-methyl, fenitrothion, malathion, chlorfenvinphos, profenofos, allethrin, bifenthrin, λ-cyhalothrin, permethrin, cyfluthrin, cypermethrin, fenvalerate, deltamethrin and chlorpyrifos.

Pesticide samples (water) were analysed at the Pesticide Residue Laboratory of the Ghana Standards Authority. Liquid-liquid extraction method was used to extract pesticides from the water samples. The 1-L sample water was filtered (whatman binder-free glass microfiber filter (GF/D, pore size: 2.7µm, Fisher Scientific, Pittsburgh, PA, USA)) and transferred into a 2 L capacity separatory funnel and 30 mL of saturated sodium chloride solution was added. The sample was partitioned with 100 ml of dichloromethane (thrice), each time shaking the separatory funnel vigorously for 2-3 min and releasing the pressure intermittently. The layers were allowed to separate and the dichloromethane extract layer drained. The three extracts of dichloromethane layers were combined and concentrated to about 1 mL using a rotary vacuum evaporator for adsorption chromatography.

A Varian CP-3800 Gas Chromatograph (Varian Associates Inc. USA) equipped with an on-column injector and electron capture detector (ECD) was used to determine pesticide concentrations. 1 µL aliquots of sample extract was injected and the separation was performed on a fused silica gel capillary column (VF- 5 ms 30 m X 0.25 mm id, 0.25 µm film thickness). The carrier gas was ultra-pure nitrogen at a flow rate of 1.0 to 29 mL min^{-1}. The temperature of the injector operating in splitless mode and oven temperature were held at

225^{o}C while the detector temperature was 300^{o}C. The column oven temperature was programmed as follows: 60^{o}C for 2 min, 180^{o}C min^{-1} up to 300 ^{o}C which was held for 31.80 min. The residues detected by the GC analysis were confirmed by the analysis of the extract on two other columns of different polarities. The first column was coated with ZB-1 (methyl polysiloxane) connected to ECD and the second column was coated with ZB-17 (58% phenyl, methyl polysiloxane) and ECD. The residue of pesticide was identified based on comparison of the measured relative retention times to those of known standards. The residue levels of organochlorine and synthetic pyrethroid pesticides were quantitatively determined by the external standard method using peak area.

Chromatographic separations for organophosphates was performed with a capillary column coated with VF-1701ms (30 m, 0.25 mm, 0.25 mm film thickness). The carrier gas was nitrogen at a flow rate of 2.0 mL/ min, while hydrogen (14 mL/min), air 1 (17 mL/min) and air 2 (10 (mL/min) were used for the detector. The injector (splitless mode) and PFPD temperatures were held at 270 ^{o}C and 300 ^{o}C, respectively. The column oven temperature was programmed as follows: 70 ^{o}C for 2 min, increased steadily at a rate of 25 ^{o}C/min to 200 ^{o}C, then at 20 ^{o}C/min up to 250 ^{o}C and held for 4.3 min. The injection volume was 2.0 µL and the total run time for each sample was 15 min. Measurement was carried out for the respective pesticides within the linear range of the detector. The peak areas whose retention times coincide with the standards were extrapolated on their corresponding calibration curves to obtain the concentration. The quantities of pesticide obtained were adjusted based on the recoveries of the spiked samples. The Limit of Detection (LOD) was determined using the formulae, LOD = S x t, where, S is the standard deviation of the replicate analysis, and t, the student's t value for the 99% confidence interval with n-1 degrees of freedom (Wisconsin Department of Natural Resources, 1996; USEPA, 2012). The LOD was 0.005 µg/L for organophosphates/synthetic pyrethroids and 0.01 µg/L for organochlorines.

The quality of the measurements of the organochlorine, synthetic pyrethroid and organophosphate pesticide concentrations were assured through the analysis of solvent blanks, procedure blanks and duplicate samples. All reagents used during the analysis were exposed to same extraction procedures and subsequently run to check for interfering substances. In the blank for each extraction procedure no organochlorine, synthetic pyrethroid or organophosphate pesticide was detected. Sample of each series was analyzed

in duplicates. The method was optimized and validated by spiking the water with 100 µL of 100 ng mL^{-1} standard mixture before analysis to evaluate the recovery of compounds. The recoveries of internal standards ranged between 79% and 96% for all the pesticides.

Data analysis

Multivariate analyses were performed using CANOCO 5 to investigate the correlations among physicochemical characteristics of the sampling sites, the macroinvertebrate species and their relationships (Ter Braak and Šmilauer, 2012). For both the physico-chemical as the macroinvertebrate data set the significance of the differences between the dry and the wet season was evaluated using an RDA analysis including season as explanatory variable and sampling date and covariable. Within the Monte Carlo permutation test following the RDA analyses, the samples were only permuted within the covariables. The significance of the differences between sampling times was tested using season as covariables and permuting the samples only within the covariable. After that, a PCA was performed for both data sets including season and sites as passive explanatory variables

Redundancy analysis (RDA) testing the significance of each physico-chemical parameters, as well as the substrate composition (Table S1) in explaining the differences in community composition was used to examine the relationships between environmental variables (i.e., physico-chemical and habitat parameters) and abundance of macroinvertebrates. This analysis was followed by an RDA including the significant physico-chemical and habitat parameters as explanatory variables and season and sampling site as passive explanatory variables. The abundance values of macroinvertebrates were log (2x+1) transformed in the above multivariate analyse, where x represents the abundance data (Van den Brink *et al*. 2000).

Results and discussion

Physico-chemical parameters

The results of the habitat assessment during the study are summarised in Table S1 in the form of watershed features, riparian vegetation, in-stream features and substratum. Lower availability of the hard habitat like cobble substratum occurred at stations (Sedorm 1)

T1, (WRI) T4, ATL (T5), (Adi Lake Ref.) T6, (Kpong) T7 and (Akuse Sand Winning) T10. The rest of the stations had sand content ranging from 15-100%.

There was a clear separation between physico-chemical parameters and their relative values in the different sites and seasons in the PCA ordination diagram (Fig. 4.2).

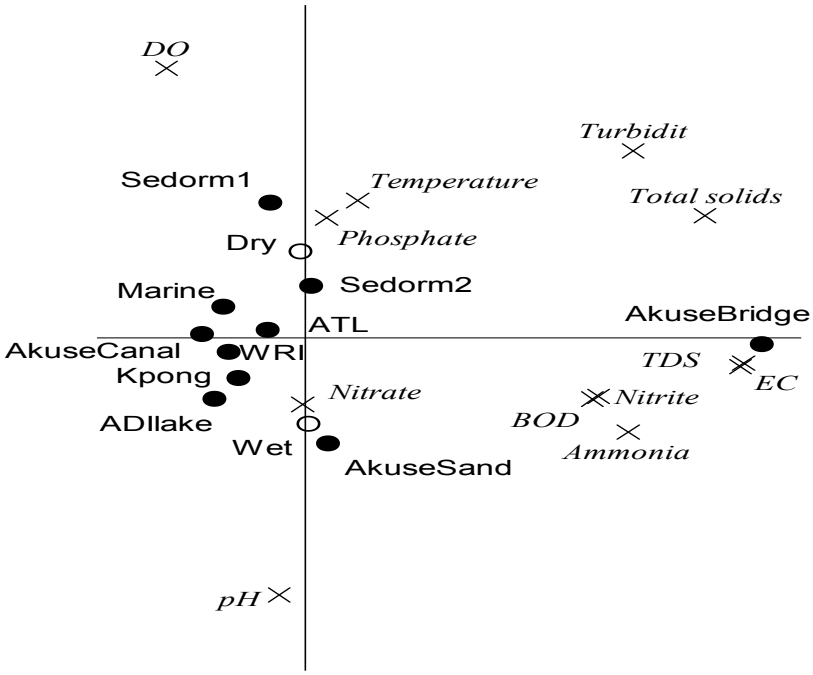

Figure 4.2: PCA plot showing the correlations between physico-chemical parameters and their relative values in the different sites and seasons. The horizontal and vertical axes display 34 and 19% of the variation in physico-chemical parameter values, respectively. Monte Carlo permutation tests indicated that differences between seasons and sites are significant (p=0.002), while the differences between sampling dates was not significant (see text for test conditions).

Additionally, there was a significant difference between seasons and sites whiles no significant difference existed between sampling dates (Monte Carlo permutation test; *p*=0.002). This is in contrast to the assertion by Gampson *et al.* (2014) that physico-chemical parameters do not vary much in terms of the sampling sites of the lower Volta basin. Thus the anthropogenic activities resulting from the adjoining land use characteristics, may have changed the physicochemical parameters. Again the rainy season is characterised with a lot of precipitation which can influence the physico-chemical parameters of the river. The PCA plot showed the largest difference in values between the stations for TDS, EC, turbidity, total

solids, ammonia, pH and DO (Fig. 4.2). Akuse Bridge is clustered away from all other stations, with relatively high TDS and EC values. The vertical axis merely displays the differences between the seasons, which were significant. The dry season recorded lower pH values compared to the wet season (Table SI 4.2; Fig. 4.2). The lowest pH of 4.44 was recorded at the first sampling of Sedorm 1 during the dry season which could be described as acidic (Table SI 4.2). The highest (10.25) were also recorded at Sedorm 1 and Marine but during the wet season. All the pH values determined in the wet season were within the WHO recommended range for drinking water (6.5-9.5) (Table SI 4.2) except the first and third sampling of Sedorm 1 and the third sampling of Marine in the wet season. This could be due to photosynthetic activity and microbial respiration as well as decomposing activities at the sites affecting the pH value. Similar values have been reported in the Volta river by other studies (Amoah and Koranteng, 2006; Gampson *et al*. 2014). Overall, water temperature ranged from 28.1 to 32.8°C (ATL, Sedorm) and 28.5 to 31.5°C (Sedorm 2, Sedorm 1) in the dry and wet seasons, respectively (Table SI 4.2). The temperatures of the sampling sites were relatively constant and compares to the range (27–30°C) reported by Amoah and Koranteng (2006).

Conductivity of the water samples ranged from 66 to 149 µS/cm (Akuse Canal, Akuse Bridge) and 68 to 165 µS/cm (WRI, Akuse Bridge) (Table SI 4.2) in the dry and wet seasons, respectively. The mean values obtained for both seasons were below the WHO recommended guideline limit of 1400 µS/cm. Conductivity is related to the concentration of Total Dissolved Solids (TDS) (Bakhtiar *et al*. 2019). The TDS values obtained for both the dry and wet season were below recommended limit of 500-1000 (mg/L) permissible for drinking (Davis and De Wiest, 1966). The electrical conductivity and TDS values obtained here indicate relatively low salt contents in the study area. The mean total solids of the water in the study area ranged from 42-99 (mg/L) in the dry season and 44-106 (mg/L) in the wet season, indicating good water quality as measured values were below the prescribed permissible limit of 500 mg/L according to the European Union (EPA, 2001). Turbidity values were comparatively higher in the dry season and ranged from 23 to 90 NTU, while the wet season recorded values of 3-26 NTU (Table SI 4.2). Except Adi lake, Kpong and Akuse canal in the wet season (Table SI 4.2), all the samples in both seasons had turbidity values exceeding 5 NTU, the WHO guideline value for turbidity in drinking water (WHO, 2004a, b). The high turbidity may be attributed to the larger particles such as organic matter, dissolved solids, agricultural runoff, leaching of soil

contaminant and point source water pollution discharged from industrial or sewage treatment plants. This causes problems with water purification processes, leading to increased treatment cost (DWAF, 1998).

Dissolved oxygen (DO) varied from 4.4 - 14.7 mg/L and 2.1 - 9.8 mg/L in the dry season and wet seasons, respectively (Tables SI 2). The highest value was measured during the dry season at sampling site Marine and the lowest value was measured at site Akuse Sand Winning in the wet season. The low DO at some sites may be caused by the decomposition of organic matter, dissolved gases, industrial waste, mineral waste and landfill leachate (Table SI 4.1). Acceptable range of BOD concentrations of 0.8–5 mg/L is set by WHO (2004a, b) but our study revealed ranges of 2.18 – 5.82 mg/L and 1.0 – 18.7 mg/L in the dry season and wet season, respectively (Tables SI 2). The highest BOD value was recorded at the sampling site Akuse Sand Winning during the wet season (Table SI 4.2). The high levels obtained could possibly be attributed to domestic discharges which can increase the organic loads in the water (Table SI 4.1).

Nutrients

The WHO has adopted a standard of 50 mg/L for nitrate-nitrogen and 3 mg/L for nitrite-nitrogen as the maximum contaminant level (MCL) for drinking water (WHO, 2004a, b). Nitrate levels ranged between 0.1-1.7 mg/L in the dry season and 1.1-7.9 mg/L in the wet season. The ranges of nitrite were 0.01-0.03 mg/L (dry) and, 0.01-0.05 mg/L (wet) and of ammonium were < 0.001 0.65 mg/L (dry), 0.01-1.45 mg/L (wet) respectively (Table SI 4.2). These concentration levels were generally low and below the WHO standard. Criteria for total ammonia (NH_3) have been established, for example by the EPA, to reflect the varying toxicity of NH_3 with pH (USEPA, 2013). Ammonium (NH_4^+) is less toxic than NH_3. In other studies, water quality criteria for phosphorus compounds, such as phosphates, are set at a concentration that prevents excessive growth of algae. Phosphorous is a limiting nutrient for algal growth and therefore controls the primary productivity of a water body (Karikari *et al.* 2007). It is also an essential nutrient and indicator of anthropogenic pollution. In most natural waters, PO4-P concentration range from 0.005 - 0.020 mg/L. In pristine waters, PO4-P concentrations may be as low as 0.001 mg/L (Karikari *et al.* 2007). Levels of PO4-P in this study varied between 0.16-4.97 mg/L in the dry season and 0.14-1.45 mg/L in the wet season. Digestive problems

could occur in humans from drinking water with extremely high levels of phosphate (Morrison *et al.* 2001). None of the samples had values that exceeded the 5 mg/L set as standard in South Africa (Morrison *et al.* 2001).

Pesticides

The concentration of organochlorine pesticides were below the detection limit (0.005 µg/L) at all the sampling sites. Meanwhile, Ntow (2005) reported gamma-HCH levels of 0.008 µg/L as well as alpha-endosulfan and endosulfan sulfate concentrations of 0.036 and 0.023 µg/L respectively in the Volta Lake. The absence of detection of organochlorines could be due to the ban of the use of e.g. DDT (GEPA, 2008) in Ghana, over time leading to possible degradation and dilution in the water body. Recent use of such products may also have been stopped which might have contributed to the low organochlorine pesticide levels. However, λ-cyhalothrin was detected at Sedorm 1 and Akuse Canal in the dry season in concentrations of 0.6 µg/L and 8.8 µg/L respectively. Cypermethrin was detected at a concentration of 1.4 µg/L at Marine during the January - February dry season sampling period. λ-cyhalothrin is highly lipophilic and tends to bind rapidly and strongly to organic materials (Maund *et al*. 1998; Leistra *et al.* 2003). Furthermore λ-cyhalothrin is highly toxic to some groups of aquatic organisms, particularly insects and crustaceans, with the midge *Chaoborus obscuripes* being sensitive (48- and 96-h EC50 = 0.0028 µg/L). Other insect larvae (Hemiptera, Ephemeroptera) and macrocrustacea (Amphipoda, Isopoda) are also relatively sensitive, with 48-and 96-h EC50 values between 0.010 and 0.1 µg/L (Schroer *et al*. 2004). Reported LC50 are: bluegill sunfish, 0.21 µg/L and rainbow trout, 0.24 µg/L (Kidd and James, 1991). Cypermethrin likewise is very highly toxic to fish and aquatic invertebrates. The LC50 (96-hour) for cypermethrin in rainbow trout is 8.2 µg /L, and for bluegill sunfish is 1.8 µg/L while the effect concentration for the total crustacean community and cladoceran and copepod subgroups in a study by Friberg-Jensen *et al*. (2003) ranged between 0.02-0.07 and 0.04-0.17 µg/L, respectively, with copepods being less sensitive than cladocerans. This raises concern as based on intrinsic sensitivity, biological traits, mode of action used for invertebrate vulnerability index rankings by Rico and Van den Brink (2015), Ephemeroptera, Plecoptera, Tricoptera, and Odonata genera were identified potentially most vulnerable to pyrethroids in aquatic ecosystems. The pesticide data were however not analysed further due to the low number of detections (Table SI 4.4).

Macroinvertebrate community

A total of 14 and 16 macroinvertebrate fauna were identified in the dry and wet seasons, respectively, belonging to 2 major phyla viz: Arthropoda and Mollusca. Among these phyla, Arthropoda (*Polypedilum fuscipenne, Stereo chironomus* sp., *Ictinogamphus* sp., *Laccophilus* sp., *Centroptiloides* sp., *Hagenius* sp., *Lethocerus* sp., *Phaon Iridipennis*, *Centroptiloides, Culicidae* sp. and *Eurymetra* sp.) dominated (66.7%) followed by Mollusca (*Physa* sp., *Lymnae glabra*, *Mya arenaria, Bithynia* sp. and *Pomacea paludosa*) (33.3%) (Table SI 4.3). A significant difference existed between macroinvertebrate composition and sampling sites and seasons (Monte Carlo permutation tests; p = 0.002; Fig. 4.3).

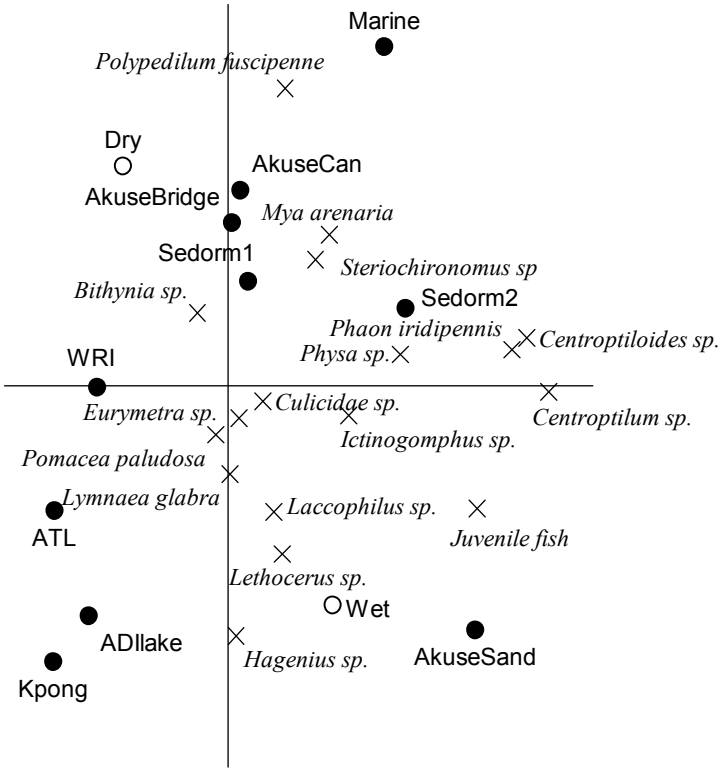

Figure 4.3: PCA plot showing the correlations between macroinvertebrate abundance values in the different sites and seasons. The horizontal and vertical axes display 27 and 16% of the variation in the abundance of macroinvertebrate species, respectively. Monte Carlo permutation tests indicated that differences between seasons and sites are significant (p=0.002), while the differences between sampling dates was not significant (see text for test conditions).

The macroinvertebrates were generally more abundant in the wet season than the dry season except *Polypedilum fuscipenne* and *Bithynia* sp. at Akuse Canal, Akuse Bridge and Marine (Fig. 4.3). The Akuse and Marine sites are however characterised by industrial, township and agricultural activities (Table SI 4.1). Similar results have been reported in the Porto-Novo lagoon in Benin (Adandedjan *et al.* 2011). *Lymnae glabra* was the predominant macroinvertebrate in both seasons (Fig. 4.3) because it can survive under polluted and unpolluted conditions (Rondelaud *et al.* 2009*)*. The species with high frequency included *Lymnaea glabra* (Lymnaeidae; Gastropoda), *Polypedilum fuscipenne* (Chironomidae; Diptera), *Centroptiloides* sp (Baetidae; Ephemeroptera), *Physa* sp. (Physidae; Gastropoda) and *Stereo chironomus* sp. (Chironomidae; Diptera) (Fig. 4.3; Table SI 4.3). At the sites where human pressures were present (anthropogenic stress, agricultural waste and domestic waste, i.e. Akuse and Sedorm sites) taxa tolerant to pollution, such as Chironomidae increased in abundance, as well as even some non-tolerant ones increased (e.g. Ephemeroptera families) (Pham *et al.* 2015). *Physa* sp. for example has been used as a pollution indicator in Australia by Shield *et al.* (2014) and has also been found abundant in the study areas (Akuse, Sedorm, and WRI sites) where agricultural, aquaculture, waste, organic and sewage pollution is high. Also, in a study by Hynes, (1975a, b), Ephemeroptera (*Centroptiloides* sp. and *Centroptilum*) were mentioned as playing a major role in the recovery and recolonization of zoobenthos of dried up river (Pawmpawm River, Southern Ghana) showing their high recolonisation capacity.

Correlation among the physico-chemical parameters and macroinvertebrates

We found that pH, DO, TDS, turbidity, EC nutrients and substratum together explained around 34% of the total variation in macroinvertebrate composition among sites (Fig. 4.4). Species on the left-hand side of the diagram, such as *Polypedilum fuscipenne,* were significantly positively correlated with turbidity and DO concentrations and occurred in Kpong and Akuse canal sampling sites during the dry season. Likewise, species on the right-hand side of the diagram, including *Physa* sp*., Centroptilum* sp*., Centroptiloides* sp*., Phaon iridipennis* and juvenile fish were positively correlated with nitrate concentration and pH.

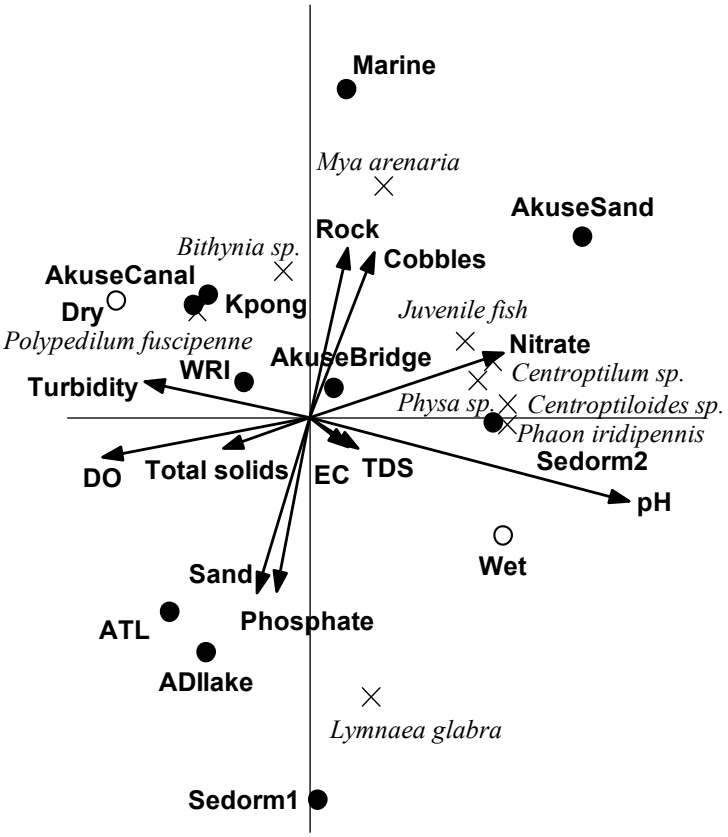

Figure 4.4: **RDA biplot showing the environmental variables that significantly explained the variation in macroinvertebrate composition between stations as result of the Monte Carlo permutation tests ($p < 0.10$).** The environmental variables explained 34% of the variation in species composition of which 35% is displayed on the horizontal axis and another 27% on the vertical axis For clarity, only the 9 out of 17 species are shown, these are the species which best fitted the ordination space.

In contrast, these species also were negatively correlated with turbidity and DO, and occurred in higher abundance at Sedorm 2 sampling site during the wet season (Fig. 4.4). The molluscs (*Mya arenaria and Bithynia* sp.) were negatively correlated with the sand substratum and phosphate concentration, however, *Lymnae glabra* was positively correlated (Fig. 4.4). The results suggest that the nature of the substratum and organic contamination caused by anthropogenic activities might be a primary force in determining benthic community composition. For instance, absence of benthic macroinvertebrates was observed in samples from ATL where high levels of nutrients were detected (Tables S2 and S3). Overall, our results

suggest that anthropogenic disturbance (i.e. environmental pollution) significantly contributed to the variation in benthic assemblages in rivers, even though we cannot rule out the influence of unmeasured ecological drivers.

Conclusions

The results of this study show that macroinvertebrate community composition shifted along the physicochemical parameters, site and season. There were significant correlations between macroinvertebrate communities and environmental variables (i.e., DO, turbidity, substratum, total solids, EC, TDS, pH and nutrients) in the Volta River. There was also a significant relationship between macroinvertebrate community composition and sampling sites. Absence of benthic macroinvertebrates was recorded at a few samples sites of the Volta River where high levels of nutrients were determined. Our results suggest that anthropogenic activities (e.g., aquaculture, agriculture effluent discharges) altered the macroinvertebrate community composition directly or indirectly in the exposed sampling sites.

Table SI 4.1: Physical Characteristics of sampling sites (classification adopted from the Rapid Bioassessment Protocol), D = Dominant

Location	Watershed Features	Riparian Vegetation		In Stream Features			Substratum		
	Nature and Land Use	Bank Erosion	Structure	Canopy cover	Physical Alteration	Type	Cobbles	Rock	Sand
Sedorm 1 (T1)	Agricultural	Slight	Grass (D), shrubs	None	Check dam downstream	Pool	-	-	100%
Sedorm 2 (T2)	Landing site (Fish)	Moderate	Baren lands (D), weeds	None	Check dam downstream	Pool	25%	10%	65%
Marine 1 (T3)	Tourism, Residential, Aquaculture	Moderate	Baren lands (D)	None	Check dam downstream	Pool	35%	50%	15%
WRI (T4)	Aquaculture, Water Research Office	None	Grass, shrubs (D), Trees	None	None	Riffles	-	-	100%
ATL (T5)	Industrial, Agriculture	Slight	Grass, shrubs (D), Trees	Slight	None	Riffles	-	-	100%
ADI Lake (T6, Ref.)	Forest /vegetation cover	None	Trees	Good	None	Riffles	-	-	100%
Kpong (T7)	Township, Small Workshops, Waste dump	Moderate	Weeds, shrubs (D), Trees	Slight	Check dam downstream	Pool	-	-	100%
Akuse Canal (T8)	Agricultural, Township	Slight	Weeds (D), Irrigation	None	None	Riffles	10%	-	90%
Akuse Bridge (T9)	Agricultural, Township	Moderate	Grass, shrubs (D), Trees	Slight	Check confluence downstream	Pool	15%	2%	83%
Akuse/ Sand Winning (T10	Township, sand winning	Slight	shrubs, Trees (D)	Slight	Check Sand winning Upstream	Pool	-	-	100%

Table SI 4.2: Range of variation mean and standard deviation of the physico-chemical characteristics of water of River Volta during January-February 2016 and May-June 2016.

Site	Season	Site	Sampling	pH	Temperature	EC	Turbidity	Nitrate	Nitrite	Ammonia	Phosphate	BOD	Total solids	TDS	DO
Dry sedorm 1_a	Dry	Sedorm1	S1	4.4	32.8	65	33.9	0.4	0.01	0.04	1.82	4.94	19	43	12.81
Dry sedorm 1_b	Dry	Sedorm1	S2	4.5	31.8	70.5	34.8	0.48	0.02	0.03	1.98	4.82	20.24	44.8	13.5
Dry sedorm 1_c	Dry	Sedorm1	S3	4.48	32.5	68.2	35.7	0.5	0.01	0.03	1.87	5.25	18.56	46.7	12.9
Dry sedorm 2_a	Dry	Sedorm2	S1	6.56	31.72	65	95.6	0.6	0.02	0.02	0.18	5.19	41	42	11.14
Dry sedorm 2_b	Dry	Sedorm2	S2	6.76	30.78	68.9	92.7	0.52	0.02	0.02	0.24	5.28	42.35	44.56	11.28
Dry sedorm 2_c	Dry	Sedorm2	S3	6.82	31.29	64.8	94.8	0.43	0.03	0.03	0.25	5.43	44.35	43.76	12.45
Dry marine _a	Dry	Marine	S1	6.66	31.3	65	31.2	1.6	0.01	0.04	0.18	4.11	13	42	11.24
Dry marine _b	Dry	Marine	S2	7.21	30.45	68.7	29.85	1.65	0.02	0.01	0.21	4.34	15.25	43.65	12.45
Dry marine _c	Dry	Marine	S3	6.72	31.28	67.25	28.25	1.72	0.01	0.02	0.19	4.28	14.5	40.35	14.67
Dry WRI_a	Dry	WRI	S1	7.31	28.85	65	37	0.7	0.01	0.01	0.16	3.42	14	42	8.03
Dry WRI_b	Dry	WRI	S2	7.45	29.56	67.34	35.67	0.72	0.02	0.03	0.18	3.52	14.85	44.56	8.67
Dry WRI_c	Dry	WRI	S3	7.5	29.43	68.45	36.45	0.7	0.03	0.02	0.2	3.45	14.97	43.47	8.54
Dry ATL_a	Dry	ATL	S1	6.53	28.06	80	23.2	1.4	0.01	0.52	4.8	4.34	11	52	11.32
Dry ATL_b	Dry	ATL	S2	6.45	29.85	82.45	24.45	1.45	0.02	0.62	4.56	4.44	12.36	54.32	12.45
Dry ATL_c	Dry	ATL	S3	6.75	29.68	81.87	25	1.35	0.03	0.65	4.97	4.87	12.82	52.85	13.34
Dry ADI lake_a	Dry	ADIlake	S1	7.28	28.23	65	28.2	0.5	0.01	0	0.23	3.13	18	42	6.53
Dry ADI lake_b	Dry	ADIlake	S2	7.5	29.45	66.72	29.45	0.46	0.02	0	0.28	3.45	18.72	44.35	7.35
Dry ADI lake_c	Dry	ADIlake	S3	7.8	29.45	68.72	28.74	0.48	0.01	0.01	0.32	3.87	19.2	43.67	7.34
Dry Kpong_a	Dry	Kpong	S1	6.47	30.01	72	27	0.7	0.01	0.01	1.52	4.11	10	47	8.8
Dry Kpong_b	Dry	Kpong	S2	6.68	29.45	73.56	28.8	0.72	0.01	0.02	1.67	4.25	12.34	48.85	8.75
Dry Kpong_c	Dry	Kpong	S3	6.82	29.78	74.69	29.2	0.78	0.03	0.01	1.62	4.34	12.45	49.3	8.45
Dry AkuseCanal_a	Dry	AkuseCanal	S1	6.8	29.4	66	30.2	0.4	0.01	0.03	0.18	5.58	12	43	8.12
Dry AkuseCanal_b	Dry	AkuseCanal	S2	7.2	29.56	67.35	31.25	0.43	0.02	0.02	0.19	5.78	12.48	44.56	8.54
Dry AkuseCanal_c	Dry	AkuseCanal	S3	6.9	30.25	68.48	29.87	0.38	0.01	0.01	0.23	5.82	13.56	43.45	8.89
Dry Akuse Bridge_a	Dry	AkuseBridge	S1	6.3	28.09	144	87.6	1.4	0.03	0	0.46	2.18	39	94	4.41
Dry Akuse Bridge_b	Dry	AkuseBridge	S2	6.82	29.45	148.94	88.35	1.56	0.02	0.01	0.53	2.21	38.45	95.25	4.47
Dry Akuse Bridge_c	Dry	AkuseBridge	S3	6.92	30.21	145.74	90.21	1.58	0.01	0.02	0.48	2.54	39.67	98.74	4.72
Dry Akuse sand winning_a	Dry	AkuseSand	S1	7.08	28.09	74	28	0.1	0.01	0.15	0.31	3.34	14	48	4.9
Dry Akuse sand winning_b	Dry	AkuseSand	S2	7.35	29.45	75.7	29.43	0.12	0.02	0.18	0.45	3.67	12.54	50.25	4.72
Dry Akuse sand winning_c	Dry	AkuseSand	S3	7.48	30.45	78.9	29.25	0.15	0.01	0.23	0.38	3.65	13.57	49.87	4.35

Wet sedorm 1_a	Wet	Sedorm1	S4	9.55	30.6	85	23	2.6	0.01	0.03	3.11	4.02	11	55	9.62
Wet sedorm 1_b	Wet	Sedorm1	S5	9.48	31.45	84.65	24.82	2.9	0.02	0.01	3.28	4.87	12.45	58.9	9.76
Wet sedorm 1_c	Wet	Sedorm1	S6	10.25	29.45	85.68	25.67	2.56	0.03	0.02	3.89	4.76	13.25	54.86	9.45
Wet sedorm 2_a	Wet	Sedorm2	S4	8.78	29.36	71	5	1.4	0.01	0.02	0.14	2.05	14	46	5.72
Wet sedorm 2_b	Wet	Sedorm2	S5	8.45	28.48	72.45	6	1.5	0.03	0.01	0.18	2.23	12.45	48.89	5.76
Wet sedorm 2_c	Wet	Sedorm2	S6	8.68	29.78	70.48	5.8	148	0.02	0.02	0.16	2.45	13.67	46.79	5.82
Wet marine _a	Wet	Marine	S4	8.59	30.67	73	8	2.3	0.02	0.05	0.14	4.63	12	48	8.42
Wet marine _b	Wet	Marine	S5	9.72	31.25	71.27	7	2.5	0.01	0.04	0.18	4.79	13.56	49.25	8.76
Wet marine _c	Wet	Marine	S6	10.25	29.76	70.56	6	2.67	0.03	0.05	0.14	4.53	14.67	50.25	8.93
Wet WRI_a	Wet	WRI	S4	8.58	29.42	68	6	1.7	0.02	0.08	0.23	1.02	11	44	4.88
Wet WRI_b	Wet	WRI	S5	8.52	28.96	69.45	7	1.8	0.03	0.06	0.24	1.45	12	46.8	4.56
Wet WRI_c	Wet	WRI	S6	8.48	30.45	67.34	8	1.78	0.04	0.05	0.38	1.28	12.6	45.28	4.62
Wet ATL_a	Wet	ATL	S4	8.76	29.62	80	4	1.4	0.01	0.06	0.33	2.85	7	52	4.87
Wet ATL_b	Wet	ATL	S5	8.25	29.56	81.36	5	1.48	0.02	0.04	0.45	2.76	8	54.25	4.78
Wet ATL_c	Wet	ATL	S6	8.48	29.18	80.57	8	1.52	0.01	0.05	0.38	2.72	9	54.98	4.58
Wet ADI lake_a	Wet	ADIlake	S4	8.68	29.17	68	3	1.1	0.01	0.02	0.18	2.47	5	44	4.93
Wet ADI lake_b	Wet	ADIlake	S5	8.72	29.48	70.21	4	1.25	0.02	0.01	0.19	2.58	7	45.8	4.87
Wet ADI lake_c	Wet	ADIlake	S6	8.86	28.56	71.54	3	1.14	0.03	0.02	0.15	2.59	8	48.9	4.75
Wet Kpong_a	Wet	Kpong	S4	8.56	31.09	74	4	1.2	0.01	0.07	0.14	1.39	7	48	4.87
Wet Kpong_b	Wet	Kpong	S5	8.49	30.42	73.57	6	1.28	0.03	0.06	0.17	1.52	6	49.87	4.72
Wet Kpong_c	Wet	Kpong	S6	8.72	29.78	75.48	5	1.34	0.02	0.05	0.18	1.48	4	48.6	4.85
Wet Akuse canal_a	Wet	AkuseCanal	S4	8.8	30.43	68	4	1.3	0.01	0.05	0.3	2.05	6	44	8.63
Wet Akuse canal_b	Wet	AkuseCanal	S5	8.92	31.26	67.84	5	1.28	0.03	0.04	0.43	2.45	7	45.9	8.72
Wet Akuse canal_c	Wet	AkuseCanal	S6	7.98	30.45	68.69	7	1.45	0.01	0.03	0.37	2.56	4	41.8	8.75
Wet Akuse bridge_a	Wet	AkuseBridge	S4	8.15	30.39	162	67	6.8	0.04	1.01	0.73	15.8	46	105	3.68
Wet Akuse bridge_b	Wet	AkuseBridge	S5	8.35	31.25	164.56	68.75	7.25	0.05	1.34	0.78	15.92	47.8	104.7	3.75
Wet Akuse bridge_c	Wet	AkuseBridge	S6	8.45	30.32	165.43	68.72	7.9	0.05	1.45	0.75	14.85	45.9	105.8	4.25
Wet Akuse sand winning_a	Wet	AkuseSand	S4	8.34	30.58	70	8	2.2	0.01	1.06	0.17	18.42	9	46	2.09
Wet Akuse sand winning_b	Wet	AkuseSand	S5	8.26	30.54	72.35	7	2.56	0.02	1.24	0.17	18.48	8.45	45.72	2.35
Wet Akuse sand winning_c	Wet	AkuseSand	S6	8.76	31.25	70.85	6	2.73	0.01	1.07	0.19	18.72	7.98	47.92	2.65

Table SI 4.3: Abundance of macroinvertebrate fauna recorded in the River Volta during January-February 2016 and May-June 2016.

Site	Season	Site	Sampling	*Lymnaea glabra*	*Polypedilum fuscipenne*	*Mya arenaria*	*Bithynia sp.*	*Steriochironomus sp*	Juvenile fish	*Ictinogomphus sp.*	*Laccophilus sp.*	*Centroptiloides sp.*	*Hagenius sp.*	*Lethocerus sp.*	*Physa sp.*	*Phaon iridipennis*	*Centroptilum sp.*	*Culicidae sp.*	*Eurymetra sp.*	*Pomacea paludosa*
Dry sedorm 1_a	Dry	Sedorm1	S1	33	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry sedorm 1_b	Dry	Sedorm1	S2	28	7	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Dry sedorm 1_c	Dry	Sedorm1	S3	25	5	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0
Dry sedorm 2_a	Dry	Sedorm2	S1	5	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0
Dry sedorm 2_b	Dry	Sedorm2	S2	8	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry sedorm 2_c	Dry	Sedorm2	S3	10	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry marine _a	Dry	Marine	S1	0	5	3	5	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry marine _b	Dry	Marine	S2	2	4	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Dry marine _c	Dry	Marine	S3	3	3	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Dry WRI_a	Dry	WRI	S1	0	0	0	0	10	6	0	0	0	0	0	0	0	0	0	0	0
Dry WRI_b	Dry	WRI	S2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry WRI_c	Dry	WRI	S3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry ATL_a	Dry	ATL	S1	6	0	0	0	0	0	8	2	5	0	0	0	0	0	0	0	0
Dry ATL_b	Dry	ATL	S2	8	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry ATL_c	Dry	ATL	S3	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry ADI lake_a	Dry	ADIlake	S1	14	6	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0
Dry ADI lake_b	Dry	ADIlake	S2	15	7	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Dry ADI lake_c	Dry	ADIlake	S3	14	7	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Dry Kpong_a	Dry	Kpong	S1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Dry Kpong_b	Dry	Kpong	S2	1	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Dry Kpong_c	Dry	Kpong	S3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry AkuseCanal_a	Dry	AkuseCana	S1	0	42	0	0	1	14	0	0	0	0	0	0	0	0	0	0	0
Dry AkuseCanal_b	Dry	AkuseCana	S2	1	39	0	0	1	0	0	0	0	0	0	5	0	0	0	0	0
Dry AkuseCanal_c	Dry	AkuseCana	S3	3	38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry Akuse Bridge_a	Dry	AkuseBrid	S1	0	4	0	0	0	9	0	0	2	0	0	5	0	0	0	0	0
Dry Akuse Bridge_b	Dry	AkuseBrid	S2	2	6	1	0	1	0	0	0	7	0	0	7	0	2	0	0	0
Dry Akuse Bridge_c	Dry	AkuseBrid	S3	3	7	2	0	1	0	0	0	0	0	0	2	0	0	0	0	0
Dry Akuse sand winning_a	Dry	AkuseSanc	S1	0	0	0	0	0	10	0	0	29	0	0	0	5	6	0	0	0
Dry Akuse sand winning_b	Dry	AkuseSanc	S2	2	1	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
Dry Akuse sand winning_c	Dry	AkuseSanc	S3	1	2	0	0	0	10	0	0	10	0	0	0	4	2	0	0	0

Wet sedorm 1_a	Wet	Sedorm1	S4	52	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0
Wet sedorm 1_b	Wet	Sedorm1	S5	55	2	0	0	0	14	0	0	0	0	0	4	4	4	0	0	0
Wet sedorm 1_c	Wet	Sedorm1	S6	54	3	0	0	0	0	0	0	22	0	0	4	3	3	0	0	0
Wet sedorm 2_a	Wet	Sedorm2	S4	8	0	0	0	4	0	0	0	0	0	0	3	2	0	0	0	0
Wet sedorm 2_b	Wet	Sedorm2	S5	7	2	1	0	2	12	1	0	27	0	1	4	1	5	0	0	0
Wet sedorm 2_c	Wet	Sedorm2	S6	6	2	1	0	4	11	1	0	24	0	1	5	5	3	0	0	0
Wet marine _a	Wet	Marine	S4	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet marine _b	Wet	Marine	S5	9	10	2	0	6	12	1	0	24	0	0	4	5	2	0	0	0
Wet marine _c	Wet	Marine	S6	28	2	1	0	2	7	2	0	23	0	0	2	4	2	0	0	0
Wet WRI_a	Wet	WRI	S4	0	0	0	0	15	0	0	0	7	2	0	9	0	0	4	0	0
Wet WRI_b	Wet	WRI	S5	25	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0
Wet WRI_c	Wet	WRI	S6	21	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0
Wet ATL_a	Wet	ATL	S4	6	0	0	0	0	0	9	3	5	0	0	0	0	0	3	38	0
Wet ATL_b	Wet	ATL	S5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet ATL_c	Wet	ATL	S6	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet ADI lake_a	Wet	ADIlake	S4	22	0	0	0	0	6	0	0	0	10	0	0	0	0	0	0	0
Wet ADI lake_b	Wet	ADIlake	S5	29	0	0	0	0	7	0	0	0	9	0	0	0	0	0	0	0
Wet ADI lake_c	Wet	ADIlake	S6	9	0	0	0	0	8	0	0	0	7	0	0	0	0	0	0	0
Wet Kpong_a	Wet	Kpong	S4	2	0	0	0	0	2	0	0	0	4	4	4	0	0	0	0	0
Wet Kpong_b	Wet	Kpong	S5	2	0	0	0	0	3	0	0	0	5	4	0	0	0	0	0	0
Wet Kpong_c	Wet	Kpong	S6	3	0	0	0	0	4	0	0	0	6	5	0	0	0	0	0	0
Wet Akuse canal_a	Wet	AkuseCani	S4	17	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	10
Wet Akuse canal_b	Wet	AkuseCani	S5	18	0	0	0	0	3	0	0	5	0	0	0	0	0	0	0	0
Wet Akuse canal_c	Wet	AkuseCani	S6	18	0	0	0	0	2	0	0	6	0	0	0	0	0	0	0	0
Wet Akuse bridge_a	Wet	AkuseBrid	S4	16	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0
Wet Akuse bridge_b	Wet	AkuseBrid	S5	12	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0
Wet Akuse bridge_c	Wet	AkuseBrid	S6	10	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0
Wet Akuse sand winning_a	Wet	AkuseSanc	S4	5	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Wet Akuse sand winning_b	Wet	AkuseSanc	S5	5	0	0	0	0	3	1	2	3	12	3	4	0	0	0	0	0
Wet Akuse sand winning_c	Wet	AkuseSanc	S6	4	0	0	0	0	2	1	3	4	11	1	2	0	0	0	0	0

Table SI 4.4: Concentrations of pesticides (µg/L) measured in the River Volta during January-February 2016 and May-June 2016.

Site	Season	Site	Sampling	Lindane	Delta-HCH	Heptachlo	Aldrin	γ-Chlorda	α-Endosu	DDE	Endrin	Dieldrin	DDD	DDT	*Endo Sul	Methoxyc	Ethoprop	Diazinon
Dry sedorm 1_a	Dry	Sedorm1	S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry sedorm 1_b	Dry	Sedorm1	S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry sedorm 1_c	Dry	Sedorm1	S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry sedorm 2_a	Dry	Sedorm2	S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry sedorm 2_b	Dry	Sedorm2	S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry sedorm 2_c	Dry	Sedorm2	S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry marine _a	Dry	Marine	S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry marine _b	Dry	Marine	S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry marine _c	Dry	Marine	S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry WRI_a	Dry	WRI	S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry WRI_b	Dry	WRI	S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry WRI_c	Dry	WRI	S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry ATL_a	Dry	ATL	S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry ATL_b	Dry	ATL	S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry ATL_c	Dry	ATL	S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry ADI lake_a	Dry	ADIlake	S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry ADI lake_b	Dry	ADIlake	S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry ADI lake_c	Dry	ADIlake	S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry Kpong_a	Dry	Kpong	S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry Kpong_b	Dry	Kpong	S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry Kpong_c	Dry	Kpong	S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry AkuseCanal_a	Dry	AkuseCan	S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry AkuseCanal_b	Dry	AkuseCan	S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry AkuseCanal_c	Dry	AkuseCan	S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry Akuse Bridge_a	Dry	AkuseBrid	S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry Akuse Bridge_b	Dry	AkuseBrid	S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry Akuse Bridge_c	Dry	AkuseBrid	S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry Akuse sand winning_a	Dry	AkuseSan	S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Dry Akuse sand winning_b	Dry	AkuseSan	S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry Akuse sand winning_c	Dry	AkuseSan	S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet sedorm 1_a	Wet	Sedorm1	S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet sedorm 1_b	Wet	Sedorm1	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet sedorm 1_c	Wet	Sedorm1	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet sedorm 2_a	Wet	Sedorm2	S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet sedorm 2_b	Wet	Sedorm2	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet sedorm 2_c	Wet	Sedorm2	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet marine _a	Wet	Marine	S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet marine _b	Wet	Marine	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet marine _c	Wet	Marine	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet WRI_a	Wet	WRI	S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet WRI_b	Wet	WRI	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet WRI_c	Wet	WRI	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet ATL_a	Wet	ATL	S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet ATL_b	Wet	ATL	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet ATL_c	Wet	ATL	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet ADI lake_a	Wet	ADIlake	S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet ADI lake_b	Wet	ADIlake	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet ADI lake_c	Wet	ADIlake	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Kpong_a	Wet	Kpong	S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Kpong_b	Wet	Kpong	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Kpong_c	Wet	Kpong	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Akuse canal_a	Wet	AkuseCan	S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Akuse canal_b	Wet	AkuseCan	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Akuse canal_c	Wet	AkuseCan	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Akuse bridge_a	Wet	AkuseBrid	S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Akuse bridge_b	Wet	AkuseBrid	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Akuse bridge_c	Wet	AkuseBrid	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Akuse sand winning_a	Wet	AkuseSan	S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Akuse sand winning_b	Wet	AkuseSan	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Akuse sand winning_c	Wet	AkuseSan	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Site	Season	Site	Sampling	Dimethoate	*Piri-methyl	Chlorpyrifos	Fenitrothion	Malathion	Chlorfenv	Profenofc	Allethrin	Bifenthrin	λ-Cyhalothrin	Permethrin	Cyfluthrin	Cypermet	Fenvalera	Deltamet
Dry sedorm 1_a	Dry	Sedorm1	S1	0	0	0	0	0	0	0	0	0	0.6	0	0	0	0	0
Dry sedorm 1_b	Dry	Sedorm1	S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry sedorm 1_c	Dry	Sedorm1	S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry sedorm 2_a	Dry	Sedorm2	S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry sedorm 2_b	Dry	Sedorm2	S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry sedorm 2_c	Dry	Sedorm2	S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry marine _a	Dry	Marine	S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry marine _b	Dry	Marine	S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry marine _c	Dry	Marine	S3	0	0	0	0	0	0	0	0	0	0	0	0	1.4	0	0
Dry WRI_a	Dry	WRI	S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry WRI_b	Dry	WRI	S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry WRI_c	Dry	WRI	S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry ATL_a	Dry	ATL	S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry ATL_b	Dry	ATL	S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry ATL_c	Dry	ATL	S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry ADI lake_a	Dry	ADIlake	S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry ADI lake_b	Dry	ADIlake	S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry ADI lake_c	Dry	ADIlake	S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry Kpong_a	Dry	Kpong	S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry Kpong_b	Dry	Kpong	S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry Kpong_c	Dry	Kpong	S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry AkuseCanal_a	Dry	AkuseCan	S1	0	0	0	0	0	0	0	0	0	8.8	0	0	0	0	0
Dry AkuseCanal_b	Dry	AkuseCan	S2	0	0	0.4	0	0	0	0	0	0	0	0	0	0	0	0
Dry AkuseCanal_c	Dry	AkuseCan	S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry Akuse Bridge_a	Dry	AkuseBric	S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry Akuse Bridge_b	Dry	AkuseBric	S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry Akuse Bridge_c	Dry	AkuseBric	S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry Akuse sand winning_a	Dry	AkuseSan	S1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Dry Akuse sand winning_b	Dry	AkuseSan	S2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dry Akuse sand winning_c	Dry	AkuseSan	S3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet sedorm 1_a	Wet	Sedorm1	S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet sedorm 1_b	Wet	Sedorm1	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet sedorm 1_c	Wet	Sedorm1	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet sedorm 2_a	Wet	Sedorm2	S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet sedorm 2_b	Wet	Sedorm2	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet sedorm 2_c	Wet	Sedorm2	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet marine _a	Wet	Marine	S4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet marine _b	Wet	Marine	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet marine _c	Wet	Marine	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet WRI_a	Wet	WRI	S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet WRI_b	Wet	WRI	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet WRI_c	Wet	WRI	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet ATL_a	Wet	ATL	S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet ATL_b	Wet	ATL	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet ATL_c	Wet	ATL	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet ADI lake_a	Wet	ADIlake	S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet ADI lake_b	Wet	ADIlake	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet ADI lake_c	Wet	ADIlake	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Kpong_a	Wet	Kpong	S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Kpong_b	Wet	Kpong	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Kpong_c	Wet	Kpong	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Akuse canal_a	Wet	AkuseCan	S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Akuse canal_b	Wet	AkuseCan	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Akuse canal_c	Wet	AkuseCan	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Akuse bridge_a	Wet	AkuseBrid	S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Akuse bridge_b	Wet	AkuseBrid	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Akuse bridge_c	Wet	AkuseBrid	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Akuse sand winning_a	Wet	AkuseSan	S4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Akuse sand winning_b	Wet	AkuseSan	S5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wet Akuse sand winning_c	Wet	AkuseSan	S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Chapter 5

Pesticides Decrease Bacterial Diversity and Abundance of Irrigated Rice Fields

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Abstract

Microbes play an important role in soil ecosystems and their activities are crucial in nutrient composition and recycling. Pesticides are extensively used in agriculture to control pests and improve yield. However, increased use of pesticides on agricultural lands will result in contamination of the soil, which could have adverse effect on its microbial communities. We investigated the effect of pesticides commonly used on irrigated rice fields on bacterial abundance and diversity. Irrigated soil samples were collected from unexposed, pesticide-exposed, and residual exposure areas and cultured under aerobic and anaerobic conditions at 37^{o}C in brain heart infusion medium to enable bacterial growth. DNA was extracted from the resulting culture and analysed by 16S rRNA sequencing. The results showed overall decrease in bacterial abundance and diversity in areas exposed to pesticides. Operational taxonomic units of the genera *Enterobacter*, *Aeromonas*, *Comamonas*, *Stenotrophomonas*, *Bordetella*, and *Staphylococcus* decreased in areas exposed to pesticides impairing the degradation of organic compounds, plant growth promotion, microbial equilibrium and plant protection from microbes and insects. Conversely, *Domibacillus*, *Acinetobacter*, *Pseudomonas*, and *Bacillus* increased in abundance in pesticide-exposed areas. Simpson and Shannon diversity indices and the multivariate analysis technique Canonical Correspondence Analysis (CCA) demonstrated a decrease in both aerobic and anaerobic bacterial diversity and community composition in areas exposed to pesticides. These results suggest a need for alternative ways of improving agricultural productivity and to educate and encourage farmers to adopt innovative integrated pest management strategies to reduce deleterious impacts of pesticides on soil ecosystems.

Introduction

Microbes play an important role in soil ecosystems and their activities are critical in nutrient composition and recycling (DeLorenzo *et al*. 2001; Khan *et al*. 2007; Khan *et al*. 2010). The increasing global human population (expected to be approximately 9.7 billion by 2050) would dramatically increase the demand for food resources (UNDESA 2015; UNFAO 2009). The increase in demand for food throughout the world has prompted farmers to devise ways to increase productivity, including the use of pesticides. Increased use of pesticides on agricultural lands causes contamination of the soil ecosystem with toxic chemicals (Munoz-Leoze *et al*. 2013). Modern agriculture largely relies on extensive application of agrochemicals, including inorganic fertilizers and pesticides. Indiscriminate long-term pesticides use or over-application of pesticides could have severe effects on soil ecosystems, which may lead to alteration and/or erosion of beneficial soil microflora (Prado and Airoldi, 2001). Annually, an estimated two million tons of pesticides are applied on agricultural lands worldwide (De *et al*. 2014). In 2012, herbicides accounted for 49% of chemicals used in agriculture and this was followed by fumigants (19%), insecticides (18%), and fungicides (14%) (Atwood and Paisely-Jones, 2007).

Pesticides may also affect non-target organisms and exert deleterious effects on the environment and farmland biodiversity (Geiger *et al*. 2010). Among the non-target populations, soil microorganisms are extremely important, since they play an essential role in nutrient turnover (Jacoby *et al.* 2017) and maintain the generative capacity of agro-ecosystems (Bohlen *et al.* 2002). The impact of pesticides on soil microbial populations could also be used as potential indicators of their toxicity and alteration of the environment (Kent and Triplett, 2002). The metabolites or the degraded products of pesticides can persist in the soil long-term. For example, trifloxystrobin typically has a half-life of 7 days in the soil, whereas its metabolite (E,E)-trifloxystrobin acid) has a half-life of up to 268 days (Kent and Triplett, 2002). Previous studies on terbuconazole and carbendazim indicated that these pesticides can affect soil microbial activity. Specifically, increasing concentrations of moderate to high doses of tebuconazole significantly inhibited soil respiration and enzymatic activities (Kent and Triplett, 2002). Further, moderate doses of carbendazim stimulated urease and invertase activities and significantly inhibited other soil microbial activities after 7 days (Kent and Triplett, 2002). Rice is a major source of food for more than half of the world's population

(Vito and Sreenivasulu, 2016). However, rice cultivation is usually vulnerable to a variety of pests and requires pesticides to help control them and improve yield. Although pesticides help increase economic gains from agriculture, they also impact microbial ecosystems in the soil. Due to the large amount of pesticides applied during rice cultivation, the rice field ecosystem is one of the major contributing agroecosystems from which pesticide residues contaminate the environment (Abdullah *et al.* 1997). Although pesticides are commonly used to improve agricultural yields, little is known about their effects on the soil microbiota in irrigated rice fields. The goal of this study was to investigate the effect of pesticides commonly used on irrigated rice fields on bacterial abundance and diversity. An irrigated rice field in Ghana was used as a case study since the majority of the rice farms in Ghana are irrigated and pesticides are often applied on these irrigated fields.

Materials and Methods

Study Area

The samples used in the study were collected from the Kpong irrigation project site at Akuse, Ghana, where rice is cultivated all year round under a well-managed irrigation scheme. Sample collection was limited to a 4-hectare irrigated rice field with known history of pesticides use during the growing season (Table 5.1). The climate is the savannah type, characterized by a bimodal rainfall pattern ranging from 900 to 1100 mm annually with a predominant wind speed of 1 and 2 knots (57.26%) and the mean annual temperature is 28.6 °C (MoFA, 2019). The soil in this area is heavy dark clay with high water holding capacity of up to 220 mm per meter depth of soil and an average dry bulk density of about 1.0 g/cm^3 (Sally, 2001). Water is sourced from the Kpong dam upstream through canals and via laterals to cover the fields. Jasmine 85 is the variety of rice cultivated and takes 110-120 days to mature, usually starting from June/July-October/November. The samples analysed in this study were collected in November 2016.

Table 5.1: The list of pesticides, application rate per hectare, active ingredients formulations used on the irrigation field under study.

Pesticide Name	Active ingredient conc.	Application rate/ha	Target pest	Application method	Frequency
Condax	Bensulfuron methyl (30%)	0.42 Kg	Selective Herbicide	Spraying	3
Kilsect 2.5 EC	Lambda Cyhalothrin (25g/L)	1.0 L	Grasshoppers, Worms, Thrips	Spraying	5
Bounty/Nakitse	Bispyribac sodium (400g/L)	62.5-75 ml	Selective Herbicide	Spraying	3
Nativo	Terbuconazole (200g/L) + Trifloxystrobine (100g/L)	1.0 L	Blast	Spraying	5
Orizo plus	Propanil (360g/L)+ 2,4D amine (200g/L)	2.0 L	Selective Herbicide	Spraying	3
Dursban/Sunpyrifos	Chlorpyrifos(480g/L)	1.0 L	Grasshoppers, Worms, Thrips	Spraying	5
Alligator	Pendimethalin (400g/L)	3.0 L	Selective Herbicide	Spraying	3

Sampling procedure for soil

Wet soil samples (5-10 g) were collected from different locations along the irrigation canal from the water source upstream, the rice field itself (where the chemicals are applied), and areas downstream of the irrigation canal. The soil samples were collected using soil auger (5.1 cm in diameter and 122 cm in length) at depths between 15 - 30 cm and grouped as (i) water source upstream (unexposed); (ii) rice field where the chemicals were applied (exposed); (iii) areas downstream of the irrigation line (residual). Eleven (11) samples were collected at equal intervals of 25 meters at each depth: unexposed (samples 1-4; from water source upstream), exposed (samples 5-9; rice field where pesticides are applied), and residual zone (samples 10 and 11; areas downstream of the irrigation line). The soil samples were refrigerated and shipped on ice-packs for analysis at University of Texas Health Science Center, School of Public Health, Department of Epidemiology, Human Genetics, and Environmental Sciences, Center for Infectious Diseases, Houston, Texas, USA.

Bacteria culture

Anaerobic condition was maintained in a Bactron 600 anaerobic chamber (Sheldon Manufacturing, Cornelius, OR) using 5% CO_2, 10% H_2, and 85% N_2. The soil samples (1 g each) were suspended in 20 mL of brain heart infusion (BHI) medium (Becton Dickinson, Franklin

Lakes, NJ). To isolate both aerobic and anaerobic bacteria in the soil, the suspensions were divided into two in 50 mL culture tubes and one tube (10 mL) was incubated aerobically or anaerobically, respectively, at 37°C for 24 hours. Following the 24 hour incubation period, the culture was thoroughly mixed and freezer stock (1 mL) of each culture were made, allowed to stand for 30 seconds for the soil particles to settle, and decanted. Freezer stocks (1 mL) of each culture were made in 10% DMSO and stored at -80^{o}C. The remaining culture was centrifuged for 10 minutes at 15,000 xg and the pellets were stored at -20 °C for DNA isolation and PCR analysis.

DNA extraction and 16S ribosomal RNA (rRNA) gene sequencing

DNA was isolated from each of the bacterial pellets using the GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich, St. Louis, MO, USA), according to the protocol provided by the manufacturer. The concentration and purity of the extracted DNA was determined using NanoDrop (ThermoScientific, Wilmington, DE, USA) and the DNA quality was assessed by agarose gel electrophoresis. The extracted DNA samples were normalized and equal amounts were analysed by 16S ribosomal RNA (rRNA) gene sequencing. The V4 region of the bacterial 16S rRNA gene was PCR-amplified using bacteria/archaeal primers 515F (5'GTGCCAGCMGCCGCGGTAA3') and 806R (5'GGACTACHVGGGTWTCTAAT3') (Caporaso *et al.* 2012). The conditions for amplification were: 1 cycle of 94 °C for 3 min, 35 cycles of 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s, and 72 °C for 10 min (Caporaso *et al.* 2012). Sequencing was performed at the Alkek Center for Metagenomics and Microbiome Research (Baylor College of Medicine, Houston, Texas) on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) using 2 × 250 bp paired-end protocol, which yielded pair-end reads that almost completely overlapped, targeting at least 15,000 reads per sample. DNA extracted under similar conditions, but without any bacterial pellet was used as control. The read pairs were demultiplexed based on unique molecular barcodes, and merged using USEARCH v7.0.1001 (Edgar, 2010). The data was analysed using the CMMR-16S (v4) analytic pipeline, as described previously (Caporaso *et al.* 2010; Caporaso *et al.* 2012; Hasegawa *et al.* 2016). The CMMR pipeline for 16S analysis leverages the QIIME (Quantitative Insights Into Microbial Ecology) software package (Caporaso *et al.* 2010; Caporaso *et al.* 2012; Hasegawa *et al.* 2016) and custom analytic packages. The 16S rRNA gene sequences were clustered into taxonomic operation units (OTUs) at a similarity cut-off value of 97% using the UPARSE algorithm in QIIME

and the SILVA database (Quast *et al*. 2013). The OTUs were determined by mapping to the SILVA database containing only the 16S V4 region to determine taxonomies (Quast *et al.* 2013). An OTU table was constructed for taxonomic summaries and the alpha- and beta-diversity calculated (Lozupone and Knight, 2005). The data from this study will be deposited in the U.S. National Center for Biotechnology Information and will be available through accession number PRJNA608009.

Data Analysis

Data were analyzed using STATA 15 for Windows (StataCorp LLC, College Station, TX) and R software (Quast *et al.* 2013). To visualize the frequency of genera across soil samples, heat maps derived from the relative abundance of the OTUs were generated using the Heatplus, gplots, and RcolorBrewer packages for R (Quast *et al.* 2013). To assess the association between region of pesticide exposure and frequency of selected genera, the Kruskal-Wallis test was used. Statistical significance was defined as p-value < 0.05.

The bacterial data was also analysed using multivariate ordination techniques to assess the effects of depth, culture under either aerobic or anaerobic conditions, and exposure on the composition of the bacterial community. Genus level data were log(x+1) transformed to down-weight the high abundances and approximate a normal distribution of the data. Since the data were compositional (relative), canonical correspondence analysis (CCA) was used (Meng *et al.* 2013; Braak and Smilauer, 2002). First, a CCA using sites as explanatory variables and depth and being aerobic or anaerobic were included as covariable in order to get an overview on the (dis)similarity in genera composition between the sites. This analysis was followed by a Monte Carlo permutation test, permuting the samples within the blocks defined by covariables. Three more Monte Carlo permutation tests were performed to test the significance of depth, being cultured under aerobic or anaerobic conditions and exposure. In each test, one factor was included as explanatory variable and the two others as covariable, which defined the blocks within which the samples were permuted. A second CCA analysis was performed using the interaction between exposure and culture under aerobic or anaerobic conditions as explanatory variables and depth as covariable, in order to show the (interactive) effects of the variables. All analysis were performed using the CANOCO Software package, version 5 (Ter Braak and Smilauer, 2002).

Results

The active ingredients, formulations, mode and frequency of application, and seasonal application rates of the pesticides used on the irrigation field are shown in Table 5.1. These pesticides are applied 3 to 5 times during the season and include herbicides (4 formulations), insecticides (2), and fungicides (1).

Effect of pesticides on soil bacterial abundance and diversity

The five most prevalent bacterial genera identified from the soil samples that were incubated under aerobic conditions were *Bacillus, Domibacillus, Enterobacter, Acinetobacter*, and *Aeromonas* (Fig. 5.1). *Domibacillus*, *Enterobacter*, and *Aeromonas* were the most predominant genera detected from the areas that were not exposed to pesticides. On the other hand, *Bacillus, Domibacillus*, and *Enterobacter* were the most frequent genera identified from the samples collected from the pesticide-exposed areas whereas *Bacillus* was the most frequent in the residual exposure areas. All of the five most prevalent aerobic bacterial genera identified contain species that are reported to play beneficial roles in the soil (Table 5.2). The five most prevalent genera detected in the samples cultured under anaerobic conditions were *Enterobacter, Clostridiales* (CsrSardi)*, Bacillus, Paraclostridium*, and *Clostridiales* (Unc58672). *Enterobacter, Clostridiales* (CsrSardi), and *Paraclostridium* were the most frequent genera in the unexposed area. *Clostridiales* (CsrSardi)*, Clostridiales* (Unc58672), and *Enterobacter* were the most frequent genera in the area exposed to pesticides whilst *Paraclostridium* and *Bacillus* were the most predominant genera in the residual exposure area (Fig. 5.1).

Table 5.2: Description of the most prevalent bacteria genera detected in the soil samples.

Genus	Respiration	Habitat	Role in Soil	Potential Pathogen	Open Literature References	*Zone of highest OTU
Acinetobacter	Obligate aerobes	Soil, water	Mineralization	Pathogenic	Doughari *et al*. 2011; Louisa *et al*. 2014	Residual
Aeromonas	Facultative anaerobes	Soil, water	Microbial equilibrium	Pathogenic	Indar and Chet, 1991; Tomás, 2012	Unexposed
Bacillus	Obligate aerobes Facultative anaerobes	Ubiquitous	Plant protection from plants and insects	Pathogenic	Jeong *et al*. 2012; Li *et al*. 2019	Residual
Bordetella	Aerobes	Soil, water, sediment, plants	Possible degradation of organic compounds	Pathogenic	Soumana *et al*. 2017	Unexposed
*Chitinophagaceae (*Unc40442*)*	Facultative anaerobes	Soil	Chitin degradation	Not Pathogenic	Rosenberg, 2014	Exposed
Comamonas	Facultative anaerobes, Aerobes	Soil, water	Possible degradation of organic compounds	Pathogenic	Willems and De Vos, 2006	Unexposed
Enterobacter	Facultative anaerobes	Ubiquitous	Plant growth regulator	Pathogenic	Khalifa *et al*. 2016; Madhaiyan *et al*. 2010; Garcia-Gonzalez *et al*. 2018; Zhu *et al*. 2010	Unexposed
Leucobacter	Aerobes	Soil, sediment, water	Possible bioremediation	Pathogenic	Her and Lee, 2015; Ge *et al*. 2016	Unexposed
*Paenibacillaceae (*GIWBac55*)*	Facultative anaerobes	Soil, water, plants	Nitrogen fixation, plant growth, plant protection from microbes and insects	Pathogenic	Grady *et al*. 2016	Unexposed
Paenibacillus	Facultative anaerobes	Soil, water, plants	Nitrogen fixation, plant growth, plant protection from microbes and insects	Pathogenic	Grady *et al*. 2016	Unexposed
Pseudarthrobacter	Obligate aerobes	Soil	Possible biodegradation of organic compounds	Pathogenic	Busse, 2016	Residual
Pseudomonas	Facultative anaerobes, aerobes	Ubiquitous, Soil, water, plants, rhizosphere	Biocontrol, Plant Growth promotion, nutrient	Pathogenic	Peix *et al*. 2009	Residual

			mobilization, soil bioremediation			
Staphylococcus	Facultative anaerobes	Ubiquitous, Soil, water	Possible degradation of organic compounds, Plant Growth promotion	Pathogenic	Pereira *et al*. 2017	Unexposed
Stenotrophomonas	Aerobes	Soil	Plant protection from plants and insects	Pathogenic	Hayward *et al.* 2010	Unexposed
Escherichia/Shigella	Aerobes	Ubiquitous	Plant growth promoter	Pathogenic	Ishii *et al.* 2006; Nautiyal *et al.* 2010	Exposed
Domibacillus	Aerobes	Ubiquitous, Soil, water	Unknown	Unknown	Xu *et al.* 2016	Residual
Halalkalibacillus	Aerobes	Soil, water	Unknown	Pathogenic	Echigo *et al*. 2007	Exposed
Vogesella	Aerobes	Soil, water sediments	Unknown	Not Pathogenic	Subhash *et al.* 2013; Sheu *et al*. 2013	Exposed
Pasteurella	Facultative Aerobes	Soil, water	Biocontrol	Pathogenic	Backstrand and Botzler, 1986	Exposed
Bergeyella	Aerobes	Soil, water	Unknown	Pathogenic	Hugo *et al.* 2006	Exposed

**Zone of highest OTU of the study area*

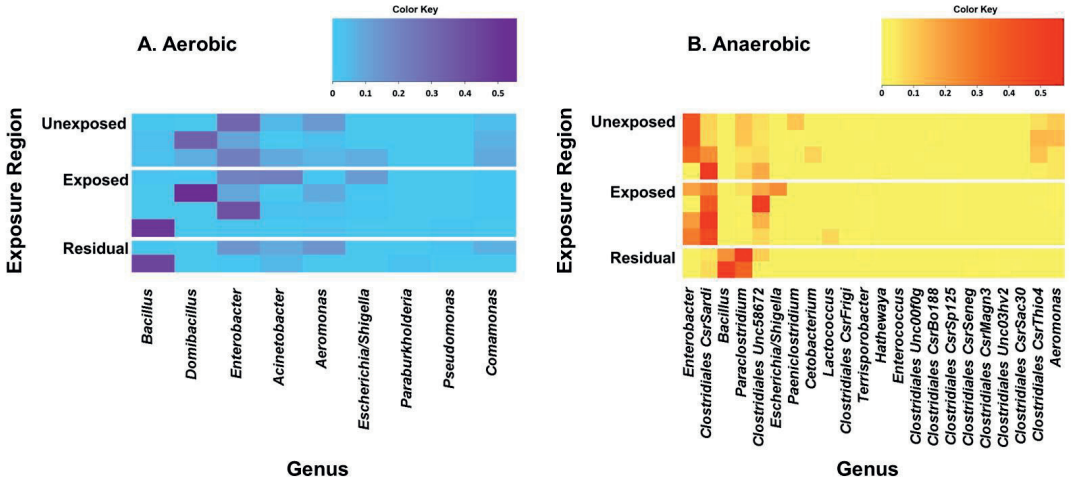


Figure 5.1: Heat maps of the predominant bacterial genera detected from the soil samples incubated under aerobic (A) and anaerobic (B) conditions. Pesticides-treated irrigated soil samples were collected from the unexposed, pesticide-exposed, and residual exposure areas and incubated for 24 hours under aerobic and anaerobic conditions in brain heart infusion medium and analyzed for bacterial diversity. DNA was extracted from the culture and analyzed by 16S rRNA sequencing. Genera were excluded from the heat map if the greatest relative frequency among the samples was less than 0.5%. Obligate anaerobic genera were excluded from the aerobic heat map, and obligate aerobic genera were excluded from the anaerobic heat map. Genera were sorted by greatest sample-wide frequency to lowest sample-wide frequency.

Both Simpson and Shannon diversity indices indicated a decrease in bacterial diversity in the pesticide-exposed area (Fig. 5.2). Simpson diversity index showed significant decrease in bacterial diversity in the exposed [p= 0.011 (aerobic), p= 0.002 (anaerobic)] and the residual exposed areas [p= 0.022 (aerobic), p= 0.015 (anaerobic)]. The Shannon diversity index also showed a similar degree of significant decrease in the areas exposed to pesticides compared to the unexposed areas.

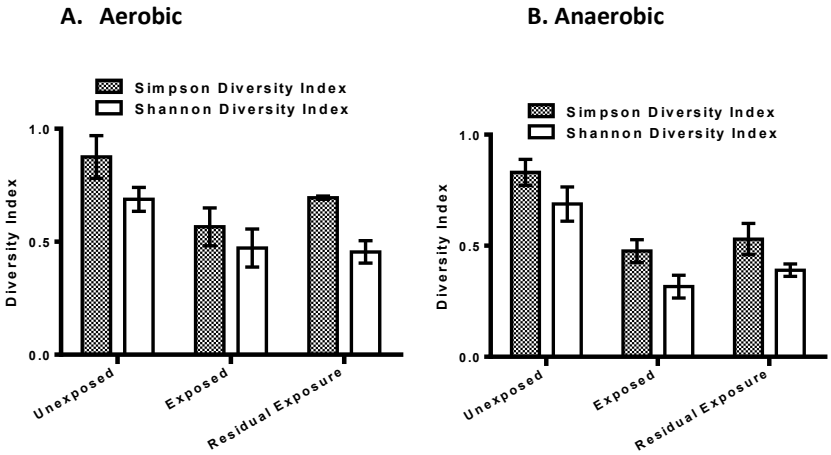


Figure 5.2: The Simpson and Shannon bacterial diversity indices of pesticide-treated irrigated soil samples. Pesticides-treated irrigated soil samples were collected from the unexposed, pesticide-exposed, and residual exposure areas and incubated for 24 h under aerobic **(A)** and anaerobic **(B)** conditions. DNA was extracted and analyzed by 16S rRNA sequencing. In the anaerobic samples, two-sample t-test showed that the mean Simpson and Shannon diversity indices were significantly different between the pesticide-exposed and unexposed areas (p = 0.00003 and p = 0.00005, respectively). In the aerobic samples, the mean Simpson and Shannon diversity indices were also significantly different between the pesticide-exposed and unexposed areas (p = 0.001 and p = 0.003, respectively). The error bars represent the mean ± S.D. of the indices of the replicate samples from each exposure group.

To investigate the effect of the pesticides on bacterial abundance, the twenty most frequent aerobic and anaerobic bacterial genera were examined based on their average operational taxonomic units (OTU). *Enterobacter*, *Aeromonas*, *Comamonas*, *Stenotrophomonas*, *Bordetella*, and *Staphylococcus* decreased in the area exposed to pesticides. The abundance of *Aeromonas* species decreased in the area exposed to pesticides but showed a slight increase in the residual exposure area (Fig. 5.3). *Escherichia/Shigella* had

the greatest frequency in areas exposed directly to pesticides. The frequency of *Bacillus* was higher in the residual area than in areas that were either exposed or unexposed to pesticides. Other anaerobic genera that significantly decreased in abundance in the area exposed to pesticides but to a lesser extent than *Enterobacter*, *Aeromonas*, *Comamonas*, *Stenotrophomonas*, *Bordetella*, and *Staphylococcus* included *Clostridiales* (CsrThio4), *Paeniclostridium*, *Clostridiales* (CsrSardi)*, Paraclostridium, Clostridiales* (Unc58672), *Terrisporobacter, Clostridiales* (CsrSp125), *Clostridiales* (CsrFrigi)*, Clostridiales* (CsrSeneg), and *Clostridiales* (CsrSac30). For the aerobic bacteria, the genera whose abundance decreased in the pesticides-exposed area were *Enterobacter* and *Comamonas.* Both genera are ubiquitous and contain bacterial species that play beneficial roles in the soil. On the other hand, *Domibacillus*, *Pseudomonas*, and *Bacillus* were in higher abundance in the pesticide-exposed area but to a lesser extent than *Aeromonas* in the unexposed area.

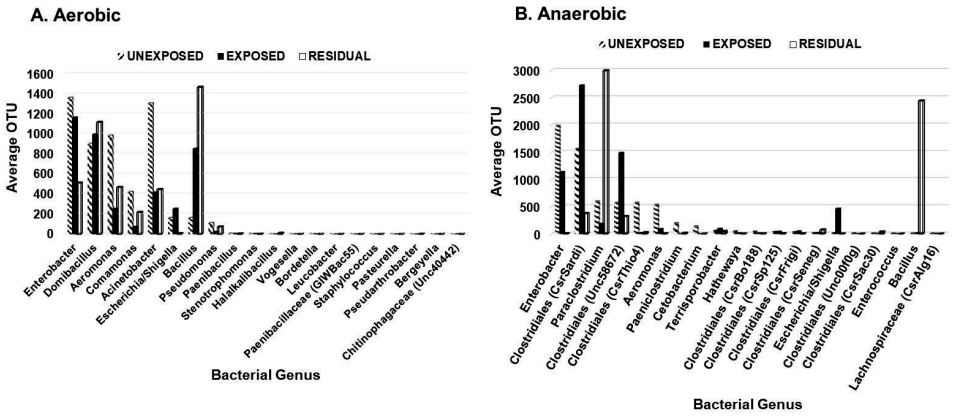


Figure 5.3: Distribution of the 20 most prevalent genera based on the region of pesticide exposure.

Multivariate analyses

The CCA showed a clear gradient (sites, p = 0.002) from site 1 till 11 and different genera composition of the bacterial community was observed (Fig. 5.4). Sites 4, 6 and 7 had relatively low numbers, which also happens to be the exposed region. Of all variance, 38% was explained by the differences between sites, while the covariables explained 15% of the variation in genus composition. A reverse CCA using depth and being cultured under ae/anaerobic conditions as explanatory variables and site as covariable resulted in a biplot

showing a clear separation between depths and being cultured under ae/anaerobic conditions (p= 0.002). Of all variance 32% was explained by the differences between sites, while the covariables explained 2% of the variation in genus composition (Fig. 5.5A). The results of the Monte Carlo permutation tests (Fig. 5.5B) demonstrated that exposure and either aerobic or anaerobic culture has significant effect on the bacterial community composition. These results also indicate that the bacterial community does not recover in the residual section, with a different composition than that of the areas directly exposed to pesticides.

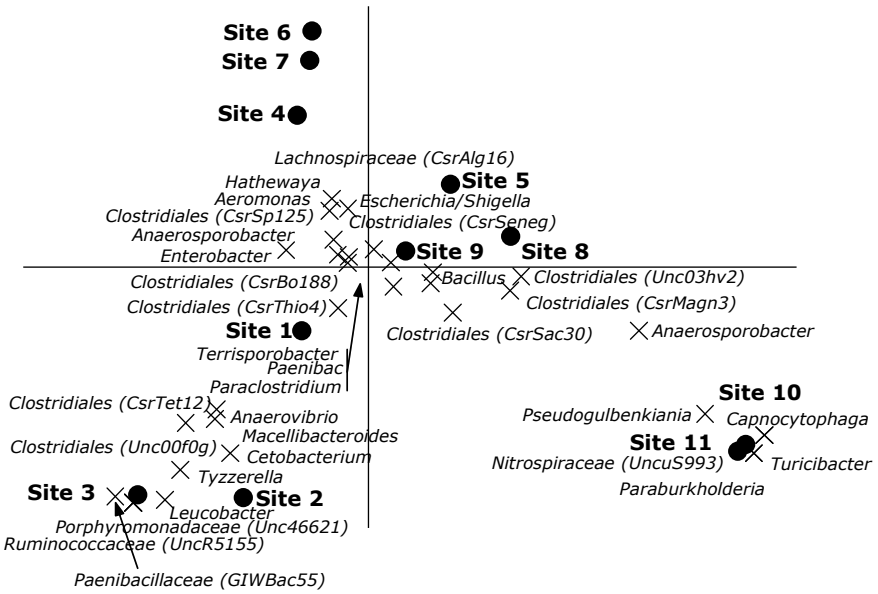
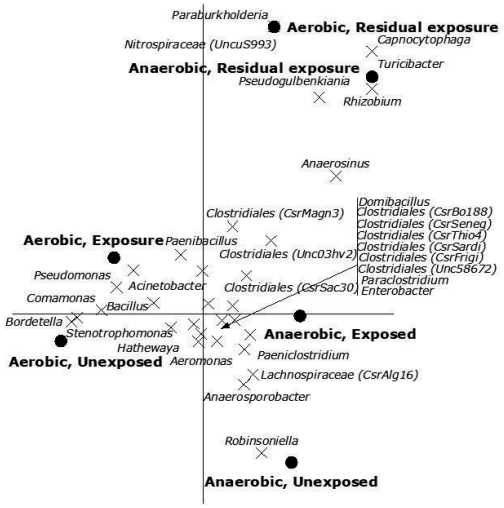


Figure 5.4: Canonical correspondence analysis biplot showing the results of the analysis using sites as explanatory variables and depth and being aerobic or anaerobic as covariables. Of all variance, 38% was explained by the differences between sites while the covariables explained 15% of the variation in genus composition. Of the variation explained by sites, 33% is displayed on the horizontal axis and an additional 18% on the vertical one. Only the 33 genera of which more than 15% of its variation is displayed by the axes are shown.

A



B

Factor	*P*-value
Exposure	0.002
Aerobic versus anaerobic	0.004
Depth	0.456

Figure 5.5: A. Canonical correspondence analysis biplot showing the results of the analysis using the interaction between exposure and being aerobic or anaerobic as explanatory variables and depth as covariable. Of all variance, 32% was explained by the differences between sites while the covariables explained 2% of the variation in genus composition. Of the variation explained by the explanatory variables, 41% is displayed on the horizontal axis and an additional 32% on the vertical one. Only the 32 genera of which more than 15% of its variation is displayed by the axes are shown. **B.** Significance of the effects of the different factors on the genus composition of the bacterial community as assessed by Monte Carlo permutation tests.

Discussion

In this study, we investigated the effect of pesticides commonly used in irrigated rice fields on bacterial abundance and diversity. The results showed that the use of pesticides decrease bacterial abundance and diversity of the soil. Soil samples collected from three locations (unexposed, exposed, residually-exposed areas) within an irrigated rice field with a history of pesticide use were examined for the presence of aerobic and anaerobic bacteria. The data demonstrated that aerobic bacteria exhibited a return to diversity in the residual pesticide exposure areas, but the anaerobic bacteria exhibited a continued decrease in diversity.

Among the top 20 most frequently identified aerobic genera (Fig. 5.3A), fourteen contain species that are known to be beneficial to the soil (Table 5.2), seventeen genera contain species that are potential pathogens, whereas fifteen contain species that are both

beneficial to the soil and pathogenic (Table 5.2). Three of the genera (*Domibacillus*, *Halalkalibacillus, Vogesella*) contain novel soil bacteria with no established roles in the soil (Echigo *et al*. 2007; Subhash *et al.* 2013; Sheu *et al.* 2013; Xu *et al*. 2016). *Domibacillus* was one of the most frequent aerobic genera detected, but little is known about the species of this genera (Xu *et al*. 2016). Among the 20 most frequently detected anaerobic genera (Fig. 5.3B), *Enterobacter*, *Aeromonas*, and *Bacillus* contain species that are both pathogenic and beneficial to the soil (Inbar and Chet, 1991; Zhu *et al*. 2010; Madhaiyan *et al.* 2010; Jeong *et al.* 2012; Khalifa *et al.* 2016; Garcia-Gonzalez *et al.* 2018) whereas *Paeniclostridium* (Sasi Jyothsna *et al*. 2016), *Hathewaya/Clostridium* (Lawson and Rainy, 2016), *Escherichia/Shigella* (Ishii *et al*. 2006; Nautiyal *et al*. 2010), and *Enterococcus* contain notable pathogens with no established roles in the soil (Lebreton *et al*. 2014). Of the pathogenic genera, *Escherichia/Shigella* was the only genera that decreased in abundance in the exposed area. *Aeromonas*, *Bacillus*, and *Pseudomonas* are diverse genera that contain many beneficial soil bacteria in addition to potential human pathogens (Inbar and Chet, 1991; Peix *et al.* 2009).

The decrease in the abundance of *Enterobacter*, *Aeromonas*, *Comamonas*, *Stenotrophomonas*, *Bordetella*, and *Staphylococcus* in areas exposed to pesticides may impair degradation of organic compounds, plant growth, microbial homeostasis, and plant protection from microbes and insects (Inbar and Chet, 1991; Willems and De Vos, 2006; Madhaiyan *et al.* 2010; Hayward *et al.* 2010; Khalifa *et al.* 2016; Soumana *et al*. 2017; Pereira *et al*. 2017). Conversely, *Domibacillus*, *Acinetobacter*, *Pseudomonas*, and *Bacillus* abundance in pesticide-exposed areas has the potential to promote bioremediation and biocontrol of the pesticide-contaminated field, improve mineralization, promote plant growth, and nutrient mobilization (Peix *et al.* 2009; Doughari *et al.* 2011; Jeong *et al.* 2012; Xu *et al*. 2016; Fira *et al*. 2018; Li *et al.* 2019).

Application of herbicides has been shown to induce stress conditions in non-photosynthetic microorganisms. For instance, metabolism of the Gram-negative bacteria *Stenotrophomonas maltophilia* usually present in rice field irrigation channels (Reche *et al.* 2005) (also identified in this study), has been demonstrated to be negatively affected by herbicides (Lu *et al*. 2009). Also, a mixture of quinclorac and bensulfuron-methyl (BSM) (also applied in this study; Table 5.1) induced the activity of antioxidant enzymes superoxide dismutase and catalase of *S. maltophilia* strain WZ2, demonstrating the induced oxidative

stress caused by the herbicides. The effect of BSM on soil microbial communities in a model paddy microcosm study showed that the nitrification potential was significantly suppressed (Saeki and Toyota, 2004). In a related study, bispyribac sodium application in an irrigated rice field had no effects on soil microbes (Alam 1977). Thus, we expect similar effects of the applied herbicides on the microbial ecosystem of our study site, which will be addressed in our on-going study.

In the natural environment, microorganisms have access to an abundant and diverse array of carbon sources that may be more easily assimilated than complex organic compounds. Biodegradation of 2,4-D is important in determining its overall fate in the environment, which is used on the rice field studied (Table 5.1). Degradation of 2,4-D in the soil is a fundamental attenuation process, which is influenced by both abiotic and biological processes. Different soil constituents and interactions of microbial communities and 2,4-D in soil play a critical role in the degradation process. 2,4-D usually degrades after a few days of its application through both abiotic and biotic interactions (Boivin *et al*. 2005). Soil microorganisms also play vital roles in the degradation of pesticides and mineralization of their metabolites. Among these microorganisms are dominant species of endophyte *Pseudomonas* (40%) and *Enterobacter* (18%) (Gardner *et al.* 1982). *Pseudomonas* is a diversified genus possessing a series of catabolic pathways and enzymes involved in pesticide degradation. *Pseudomonas putida* MAS-1 is reported to be efficient in chlorpyrifos degradation by a rate 90% higher than other species of *Pseudomonas* (Gilani *et al*. 2015). The chlorpyrifos degradation involve the metabolism and mineralization of 3, 5, 6-trichloro-2-pyridinol and 3,5,6-trichloro-2-methoxypyridine. *Pseudomonas* is the group of bacteria present in large amount in the soil and have a vital role in the mineralization of organic matter. They are metabolically adaptable and have capability to degrade most of the aromatic hydrocarbons, oil, petroleum products, and pesticides (Sarkar *et al*. 2009). *Pseudomonas* has the capability to mineralize phenolic compounds (Hughes and Cooper, 1996). A variety of low-molecular-weight compounds, including chlorinated aliphatic hydrocarbons can also be metabolized by *Pseudomonas* because of diversified range of catabolic pathways (Lynch and Hobbie, 1988). Our study also supports the ability of *Pseudomonas* to degrade pesticides, as there was no significant

difference in the abundance of *Pseudomonas* genus between the exposed and unexposed areas.

In order to reduce the effect of pesticides on bacterial diversity, it is important to monitor the response of soil bacterial communities and various enzymatic activities. Various bacterial genera were negatively impacted in the area exposed to pesticides and most of these genera are known to be involved in nutrient mobilization, plant growth promotion, mineralization, and metabolism of organic compounds. It remains to be seen whether the depletion of soil microbes would also affect the fertility of the soil and overall productivity of this rice field. The increase in abundance of the genera *Domibacillus, Bacillus*, and *Clostridia* suggest they were generally not constrained by the pesticides. It is possible that these bacteria can metabolize the pesticides or require a much higher concentration of the pesticides in order to be affected. Our research is on-going to identify the species among these genera present in the sample and to explore their potential as candidates for bioremediation. Our study shows that there is a need to educate and encourage farmers to adopt innovative integrated pest management strategies that promote the function of beneficial microbes with little to no deleterious impact on the soil bacterial ecosystem.

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Chapter 6

General discussion and concluding remarks

Increase in human population has resulted in the growth of agricultural activity with the use of chemicals in terms of artificial fertilizer and plant protection products required to satisfy and maintain the local and global demand for higher food production (Firbank *et al.* 2012). Hence, modern agriculture practices rely on the usage of synthetic pesticides (e.g. insecticides, herbicides, fungicides) in order to reduce crop losses due to pests and diseases to achieve higher crops yields. Pesticides applied to crops may enter water bodies adjacent to agricultural fields via different entry routes such as spray drift, agricultural run-off, leaching and/or drainage (Dabrowski *et al.* 2002; Brown *et al.* 2004). These contaminations may have undesirable impacts on the ecology of fresh water ecosystems (Liess *et al.* 2005; Holvoet *et al.* 2007; Van den Brink, 2008). In order to prevent unacceptable adverse effects, legal provisions are in place for the registration/procedures for pesticides in most jurisdictions globally. In Ghana, Part II of the Ghana Environmental Protection Agency (EPA) Act, Act 490 of 1994 governs the whole pesticide life cycle, so the registration and procurement of pesticides, their import, distribution and retail to farmers, monitoring, quality control and waste management.

The present thesis aimed at analyzing to what extent the Ghana pesticide law and pesticide registration procedure has contributed to effectively protecting the environment and to establish the extent of risks associated with pesticides using tools such as PRIMET (Pesticides Risks in the Tropics to Man, Environment, and Trade), SSD (Species Sensitivity Distribution), DNA 16S ribosomal RNA (rRNA) gene sequencing and the gathering of empirical data from the field to determine pesticide risks to aquatic and terrestrial organisms.

The thesis presents a comprehensive review on the Ghana pesticide law and registration procedure, including views of various actors in implementing the law in a conceptual framework based on the contextual interaction theory (CIT) (Fimyar, 2014; Sabatier, 1991; Van Horn and Van Meter, 1977). In this thesis, new information was added to the general knowledge on factors needed to enhance the implementation of the pesticide law in Ghana by both state and non-state actors, such as developing well-targeted training programmes for pesticide retailers and farmers on pesticide use, personal PPE use, as well as pesticide law and management. A recommendation from this study (**chapter 2**) sets out the study for **chapter 3.** Here the focus was on preliminary risk assessment of aquatic organisms in water bodies adjacent fields treated with

pesticides, and the exposure of the pesticides to other terrestrial organisms. Empirical pesticide use data obtained from farmers and the aquatic scenario for the 1st tier PRIMET (Pesticides RIsks in the Tropics to Man, Environment, and Trade) model were used to assess environmental risks of pesticides currently applied in Ghana. Pesticides that showed risks were further evaluated by the Species Sensitivity Distribution (SSD) model to determine 2nd tier ecological threshold levels of the pesticides protective to aquatic communities. The 1st tier and 2nd tier thresholds could also be compared, evaluating the protectiveness of potential risks of pesticides for primary producers, invertebrates and vertebrates as set by the 1st tier. The tools used could support environmental risk assessment decision-making for pesticide registration in Ghana. A part of this thesis also contributes to the application of benthic macroinvertebrates as indicators for monitoring the quality of water bodies in Ghana (**Chapter 4**). The effect of pesticides was also evaluated on microorganisms in soils of irrigated rice field regarding diversity and abundance (**Chapter 5**) informed by an observation in **chapter 2** and the environmental risk assessment (**Chapters3**). Linking macroinvertebrates and physico-chemical parameters for water quality assessment in a river was achieved by surveys and measurements on site and in the laboratory in order to record the various anthropogenic activities and physico-chemical parameter values at the various sampling points indicative for the level of pollution. These were then compared to macroinvertebrate abundance values to statistically make inferences. The effect of commonly used pesticides on bacterial abundance and diversity was investigated on irrigated rice fields (**Chapter 5).** Irrigated soil samples were collected from unexposed, pesticide-exposed, and residual exposure areas and cultured under aerobic and anaerobic conditions at 37°C. DNA extracted from the resulting culture were analysed by 16S rRNA sequencing.

In **chapter 2** it was obvious that farmers spill pesticide mix during pouring and loading of spray equipment even though they had received a lot of training sessions on pesticide use and application. Most farmers admitted using recommended rate and frequency of pesticides for pesticides as directed by the by supplier, retailer or dealer or as shown on the packaging label, but this was found out not to be the exact practice as observed in the field, where most pesticide in use were overdosed (**Chapter 3**) at magnitudes of 1.3 to 13 times the recommended dose. It was anticipated that the use rate, may lead to problems of pest resistance, environmental

pollution, and occupational exposure among others (Metcalf, 1980; Ngowi *et al*. 2007; Ramo *et al*. 2016). Subsequently, results from 1st tier risk assessment conducted on pesticides used in the study area had indicated that many pesticides may pose acute risk to aquatic ecosystems adjacent treated fields while lambda cyhalothrin, chlorpyrifos, cypermethrin, dimethoate, mancozeb, carbendazim, sulphur, maneb, copper hydroxide and cuprous oxide may pose the highest chronic risks. Butachlor, dimethoate and carbendazim may pose acute risks to the terrestrial soil ecosystem, while glyphosate, chlorpyrifos, imidacloprid, dimethoate, mancozeb, carbendazim, maneb, copper hydroxide and cuprous oxide may pose the highest chronic risks to the terrestrial ecosystem. Paraquat, lambda cyhalothrin, chlorpyrifos, emamectin benzoate, imidacloprid, thiamethoxam, bifenthrin, cypermethrin, dimethoate, carbofuran, mancozeb and maneb may pose acute risks to bees and propanil, lambda cyhalothrin, chlorpyrifos imidacloprid, carbofuran, sulphur, copper hydroxide and cuprous oxide to the terrestrial non-target arthropods. A 2nd tier acute aquatic risk assessment also had showed that the risks of pendimethalin, propanil, oxyfluorfen, lambda cyhalothrin, chlorpyrifos, cypermethrin, dimethoate carbofuran, mancozeb, carbendazim, maneb and copper hydroxide was possible for the aquatic ecosystem (**Chapter 3**).

The concentration of organochlorine pesticides were generally below the detection limit (0.01 µg/L) of the instrument used in all the sampling sites in the Volta river. A situation that could be attributed to the ban of e.g. DDT (GEPA, 2008) on the use of other such compounds in Ghana over time leading to possible degradation and dilution in the water body. There are, however, isolated cases of the detection of λ-cyhalothrin at Sedorm 1 and Akuse Canal in the dry season with concentrations of 0.6 µg/L and 8.8 µg/L, respectively. Cypermethrin at a concentration of 1.4 µg/L was detected at Marine during the dry period which probably could have been due to recent application on farms adjacent to the river body or had come in through run offs (**chapter 4**). The many non-detection of pesticides in chapter 4 could be due to the fact that sampling was confined to a few meters from the river course (~ 4m) and not directly edge-of-field. However, large risks were indicated for chapter 3 where the focus was on the edge of treated fields and the scenario adopted was realistic for the irrigation fields. Besides, the sampling areas were different for both chapters 3 and 4.

This findings of **chapter 3** on the effect of recently used pesticides on soil microorganisms in irrigated rice fields demonstrated a decrease in both aerobic and anaerobic bacterial diversity and community composition in areas exposed to pesticides (**Chapter 5**). Microbes play an important role in soil ecosystems and their activities are crucial in nutrient composition and recycling. Increased use of pesticides on agricultural lands result in contamination of the soil, which could have adverse effect on its microbial communities (DeLorenzo *et al*. 2001; Khan *et al*. 2007, Khan *et al*. 2010). It will therefore be prudent to find alternative ways of helping to reduce pesticide exposure to the environment. Even though a lot of papers had previously reported on the environmental risks of pesticide and recommended training of farmers and pesticide applicators especially in developing countries including Ghana (Metcalf, 1980; Clarke *et al*. 1997; Ecobichon, 2001; Ngowi *et al*. 2007; Wiratno *et al*. 2007; Fianko *et al*. 2011; Ramo *et al*. 2016), it appears the intervention is not yielding the desired results.

This is because in developing countries most of the farmers are not well educated (Wiratno *et al.* 2007; Mengistie *et al.* 2014; Onwona-Kwakye *et al.* 2019) and to understand the science and rudiments of pesticide use is often not practicable. It is also not possible for the farmers to sublet the pest control component of the farm operations to professional pest control bodies due to financial constraints. Therefore, it becomes difficult for these farmers to invest heavily in their farm operations, and in most cases these farmers also act as the pesticide applicators and the decisions concerning pesticide application is informed by what they want and not necessarily what they have been trained to practice. So even when they have been trained on specific farm routines regarding pesticide use, in reality they do not practice them (**Chapter 3**). It is obvious that the current situation requires alternative approaches to solving these pertinent problems. Most farmers in our study mentioned and assured us during the discussion that pesticides are necessary, but they were open and willing to use appropriate alternative methods of pest control if they became available, effective and affordable (**Chapter 2**).

Recognizing that education and training of farmers in the application of pesticides in developing countries is not helping the local environment is a first step toward developing more robust solutions to environmental risks. Enforcing existing pesticide laws and regulations should receive greater attention through surveillance and monitoring activities. For example, there

should be a pesticide safety certification program for pesticide applicators and retailers to ensure that only those certified are allowed to sell, handle, or apply pesticides even at farm levels. From a regulatory perspective, addressing issues of pesticide regulation by identifying the jurisdictions that are struggling with these issues, identifying the pesticides that are most often being reported as negatively affecting the environment, and identifying the span and distributions of the dosages these pesticides are applied, are other efforts that could help control risks to the environment (Jennings and Li, 2014). The development and use of highly efficient, low toxic and low residual pesticides, particularly bio-pesticides has been proposed (Sanjaya *et al*. 2013; Darvishzadeh *et al*. 2014; Jafarbeigi *et al*. 2014; Sharifian and Darvishzadeh, 2015; Gupta *et al*. 2017). The use of mixture pesticides, as a resistance management strategy as well as a pollution reduction measure, can be explored. Furthermore, it is recognized that productive soil is a finite resource (as water) and, in order to ensure continued production of food, the agriculture must go side by side with soil and ecosystems preservation, restoration, and agronomic research on better yield cultivars. Therefore, it is urgent to achieve a generalized agreement on pesticide application and adoption of good agriculture practices, with consideration of Integrated Pest Management (IPM) techniques thus minimizing the use of chemical pesticides (Mardani *et al*. 2017), and protect natural enemies and biodiversity, and maximize the role of natural balance. Again, promote precision use of pesticides, reduce off-target phenomena and enhance utilization rate of pesticide use. It is also worth mentioning that pesticide product stewardship from the manufacturers and multi-national pesticide companies have failed to assist in preventing environmental pollution. In Ghana, for example, where the bulk of pesticide imports comes from China (**Chapter 1**), there is no pesticide product stewardship and follow up by manufactures like what takes place in developed countries (Reynolds, 2018). In Ghana, the multi-national pesticide producing companies appear not to be doing a good job in this regard as their products are not only used by their target groups but by other pesticide user sectors as well (e.g. Kocide, a registered cocoa fungicide in Ghana is also used on vegetables; Amoako *et al.* 2012; Onwona-Kwakye *et al.* 2019). The stewardship approach in this regard should be made more holistic by involving other user categories. The development and introduction of extended producer responsibility (EPR) for pesticide bound for developing countries is proposed to support capacity

building of pesticide users regarding safer pesticide use and other related interventions to prevent environmental risks.

Conclusion and Outlook

The study concludes that pesticides are registered in compliance with the law whilst non-state actors were mostly non-compliant with pesticide handling and management which is likely to result in environmental risks. Significant association existed between educational level attained and knowledge on pesticide use. Likewise, work experience or duration of farming also significantly influenced the knowledge of respondents, as well as farm management practices and pesticide handling. State actors were not motivated and resourced (**Chapter 2**) to carry out their mandate in ensuring the smooth implementation of the pesticide policy. Resources in terms of vehicles, and other forms of motivation are required to generate the needed interest.

The ecological risk assessment models estimated that pesticides used in the study area were likely to pose the highest risks to aquatic ecosystems adjacent to the treated fields and to the terrestrial ecosystem. Ecological models have been heavily advertised due to their extrapolative power and could be used in comparing alternative scenarios for risk management in the field (Wiratno *et al*. 2007; Peeters *et al*. 2008; Ansara-Ross *et al*. 2008) and in this study it has been recommended that the PRIMET and SSD models are incorporated into the pesticide registration processes in Ghana. It was established that actual pesticide use was higher than the recommended rates (**Chapter 3**) and likely to impact negatively on the environment (**Chapter 2, 3**) thus requiring intervention (**Chapter 2**). Our research identified species among the *Domibacillus, Bacillus*, and *Clostridia* genera present in the samples (**Chapter 5**) which survived in the pesticide exposed sites, and it is recommended that further work is carried out to explore their potential for use in bioremediation.

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Summary

Pesticides are broadly applied in current agriculture practices globally and may end up in interconnected water bodies i.e. ditches, ponds, and lakes via numerous routes such as spray drift, runoff and leaching. Given the fact that they are inherently designed to harm biota, pesticides may pose risks to a range of aquatic and terrestrial organisms. Non-target organisms may be exposed to pesticide contaminants. In Ghana, the legal procedure for pesticide registration is prescribed by Part II (Pesticides Control and Management) of Environmental Protection Agency Act, 1994 (Act 490), however the registration is based on international data and prospective risk assessment. This thesis aimed to evaluate whether registered pesticide use in Ghana harms the environment (aquatic and terrestrial).

In **Chapter 1**, a general introduction to the thesis is presented, taking into consideration contemporary issues of pesticides globally and narrowing it down to Ghana. An overview of the pesticide law in Ghana is presented as well as a brief description of the evaluation procedure as well as reported environmental impacts, occupational health effects and the populations at risk. The overall aim of the thesis is presented including the research objectives. It concludes with the outline of the thesis.

Chapter 2, reviews Ghana's pesticide policy by providing an overview of the pesticide regulatory policy framework (pesticide law) and the pesticide registration and licensing procedure and presents a theoretical framework (evaluation model) for the study. The main part of the study deals with how the policy has performed overtime. First, the farmers' pesticide use in day-to-day farm practices and distribution of pesticides in the country were discussed, in relation to the policy and also the interactions of state and non-state actors. The results indicated that pesticides were registered in compliance with the law. Non-state actors scored low with respect to their mandate which likely resultes in environmental and human health risks. Significant association existed between educational level attained and knowledge on pesticide use. Work experience or duration of farming also significantly influenced the knowledge of respondents ($P < 0.001$), as well as attitude ($P < 0.05$), while state actors were not motivated and resourced. It was recommended that a preliminary risk assessment be performed to the aquatic

environment, to derive threshold levels which are protective of communities, to screen farmers for pesticide exposure and poisoning, to develop well-targeted training programmes for pesticide retailers and farmers on pesticide use, personal protective device use, as well as pesticide management and law. Additionally, pesticide policy implementers were to be motivated and resourced to carry out their mandate i.e. to execute the pesticide legislation.

In **Chapter 3** we used the PRIMET model (1st Tier) to assess the risk of pesticides to the aquatic and terrestrial environment using empirically gathered field data on pesticide use. Results from 1st tier showing risk were then further refined using the SSD model (2nd tier) to estimate pesticide concentration levels protective of the aquatic environment. Results of PRIMET indicated that in the investigated regions in south Ghana, lambda cyhalothrin, chlorpyrifos, mancozeb and maneb may pose the highest risks to aquatic ecosystems adjacent to the treated fields, butachlor and carbendazim to the terrestrial soil ecosystem, lambda cyhalothrin, chlorpyrifos, emamectin benzoate, imidacloprid, thiamethoxam, dimethoate and cypermethrin to bees and propanil, oxyfluorfen, lambda cyhalothrin, imidacloprid and copper hydroxide to terrestrial non-target arthropods. Actual pesticide use was 1.3 to 13 times higher than the recommended label instructions, indicating a general practice of overdosing. For protectiveness, the 1st tier PNEC was protective in all cases of insecticides for invertebrates and vertebrates compared to 2nd tier HC5. The case study showed that the PRIMET model in combination with the SSD concept may offer pesticide registration authorities in Ghana a means to assess environmental risks associated with pesticide usage in a user-friendly and cost-effective manner.

Chapter 4 describes the approach to using a variety of techniques in determining the exposure of water bodies to pollutants including fertilizers, pesticides and other waste. This involved the sampling and analysis of water samples from locations with known human activity and testing for the presence or otherwise of the probable pollutants envisaged in the areas from an earlier survey. This is linked to the physico-chemical characteristics of the samples from various locations as well as the identified macroinvertebrates. Generally, the water was not polluted per the physico-chemical characteristics except isolated cases of low pH level and pesticide concentrations (λ-cyhalothrin and cypermethrin). The results from the pesticides were

however not included in the analysis due to the low level of detections. The presence of the few detected could be from agricultural pollution mainly from recent application and run offs though it may likely affect other organisms. The results showed that the environmental variables can potentially be analysed to monitor the ecological status of aquatic ecosystems in ecological assessment practices and may also be used to develop new practices for biodiversity conservation that aims at preserving both the taxonomic and functional diversity in Ghana.

The goal of **chapter 5** was to investigate the potential effects of pesticides on the abundance and diversity of soil bacteria in irrigated rice field and to isolate bacteria capable of degrading diverse classes of pesticides used in these fields. The results showed an overall decrease in bacterial abundance and diversity in areas exposed to pesticides. Operational taxonomic units of the genera *Enterobacter*, *Aeromonas*, *Comamonas*, *Stenotrophomonas*, *Bordetella*, and *Staphylococcus* decreased in areas exposed to pesticides, which may impair degradation of organic compounds, plant growth, and plant protection from microbes and insects. Conversely, *Domibacillus*, *Acinetobacter*, *Pseudomonas*, and *Bacillus* increased in abundance in pesticide-exposed areas. Simpson and Shannon diversity indices and canonical correspondence analysis demonstrated a decrease in both aerobic and anaerobic bacterial diversity and community composition in areas exposed to pesticides. Concluding, a need for alternative ways in improving agricultural productivity and to educate and encourage farmers to adopt innovative integrated pest management strategies to reduce deleterious impacts of pesticides on soil ecosystems has been proposed.

Chapter 6 is the general discussion and conclusion of the overall thesis. It looked at the pesticide law as it pertains now and its implementation challenges, and related to the ecological risks identified in this thesis. Solutions to these challenges are prescribed through published open literature and suggestions from the field surveys and observations and conclusions are drawn for all the results presented in this thesis.

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Curriculum Vitae

Michael Onwona-Kwakye was born (1970) and raised in Accra, Ghana. I obtained my primary and secondary education with a strong emphasis in science. I studied Agricultural Science at the University of Ghana-Legon from 1994 to 1998. I majored in crop science focusing my dissertation on, Identification and Partial Characterization of Cassava Mosaic Virus Prevalent in Ghana. After obtaining my BSc in 1998, I worked with the Ministry of Food and Agriculture of the Accra Metropolitan Assembly as a field staff. A year later I enrolled at the University of Ghana-Legon to study Master degree (MPhil) in Environmental Science. During the studies my thesis was on environmental impact assessment, investigating and monitoring the effect of effluent from Barima fuel service center (Mallam – Accra) on a swampy land and auditing of some fuel service stations in Accra – Tema metropolitan areas of Ghana. I was employed by the Ghana Environmental Protection Agency, the same year that I completed my Master's degree (2002).

I was subsequently attached to the Chemicals Control and Management Center (CCMC) of the agency from where I was trained at Pesticide Safety Directorate in the United Kingdom to manage chemicals and register pesticides. After exploring the life of pesticide registration and chemicals management for over 10 years I enrolled as a sandwich student to do my PhD project in the Aquatic Ecology and Water Quality Management group at Wageningen University under the supervision of Paul van den Brink. This thesis is the product of more than 6 years' worth of research on environmental risks associated with pesticide use in Ghana. I will continue doing research on pesticide impacts on global ecosystems and hopes to develop mitigation and management actions that ensure the sustainability of natural resources. I also work at the National Pzone Unit (NOU) of Ghana, as part of a team implementing Ghana's obligation under the Montreal Protocol). My free time is usually spent on walks with the family, watching movies with my daughters, travelling to the village where my kinsmen are, as well as reading and attending to the family business.

Publications

Refereed Scientific Publications

Onwona Kwakye M, Hogarh JN, Van den Brink PJ. 2020. Environmental risk assessment of pesticides currently applied in Ghana. Chemosphere 254, 126845. https://doi.org/10.1016/j.chemosphere.2020.126845

Onwona Kwakye M, Paris-Plants K, Keita K, Lee J, Van den Brink PJ, Hogarh JN, Darkoh C. 2020. Pesticides Decrease Bacterial Diversity and Abundance of Irrigated Rice Fields. Microorganisms 8, 318. https://doi.org/10.3390/microorganisms8030318

Onwona Kwakye M, Mengistie B, Ofosu-Anim J, Tetteh Nuer, AK, Van den Brink, PJ. 2019. Pesticide registration, distribution and use practices in Ghana. Environment, Development and Sustainability 21, 2667–2691. https://doi.org/10.1007/s10668-018-0154-7

To be submitted/Ready

Onwona Kwakye M, Feng-Jiao P, Hogarh JN, Van den Brink PJ. Linking macroinvertebrates and physico- chemical parameters for water quality assessment in the lower basin of the Volta River in Ghana.

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Colophon

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