Assessing the effects of MACADAMIA ORCHARD pesticide inputs on recipient aquatic ecosystems

by

Thendo Mutshekwa

Student number: 14007406

A doctoral thesis submitted in fulfillment of the requirements for PhD Environmental Science Degree to the Department of Geography and Environmental Sciences in the Faculty of Science, Engineering and Agriculture, University of Venda

Supervisor: Dr Tatenda Dalu
Co-supervisors: Dr Lutendo Mugwedi
Prof Ryan J Wasserman

OCTOBER 2022

Abstract

Aquatic environments, while not the target of many pesticide applications, often receive chemicals through catchment runoff dynamics. In this regard, the use of pesticides in agricultural systems may have deleterious effects on aquatic ecosystems within the same catchment area. Here using a series of *in–situ* and *ex–situ* experiments, the study assessed (*a*) pesticide concentrations of acetamiprid and chlorpyrifos across MACADAMIA ORCHARD and communal area reservoirs using liquid chromatography tandem mass spectrometry (LC–MS/MS), (*b*) the behavioural responses of Mozambique tilapia, *Oreochromis mossambicus*, following exposure to three commonly used macadamia pesticides (i.e., Karate Zeon 10 CS, Mulan 20 SP, Pyrinex 250 CS at different concentrations, (*c*) macadamia *Macadamia integrifolia* leaf litter decomposition and mosquito colonisation following pesticide exposure, and (*d*) macroinvertebrate colonisation associated with introduced stone substrates in cages within the MACADAMIA ORCHARD and control reservoirs to explore whether macadamia pesticides affected on macroinvertebrate colonisation dynamics.

Firstly, analysis of acetamiprid and chlorpyrifos associated with sediments in MACADAMIA ORCHARD reservoirs revealed mean pesticide concentrations of 14.48 µg L^{-1} and 5.67 µg L^{-1}, respectively, whereas, in communal area reservoirs outside of agricultural catchments, both pesticides were not detected. Acetamiprid was not detected across reservoir water, whereas the mean pesticide concentration of chlorpyrifos of 6.51 µg L^{-1} (MACADAMIA ORCHARD) and 0.13 µg L^{-1} (communal area) were detected. Secondly, *O. mossambicus* demonstrated different onset behavioural responses, i.e., swimming erratically, surfacing, vertical positioning, loss of equilibrium, being motionless and mortality with high mortality at increased mortality. Thirdly, macadamia leaf litter tended to decompose faster when exposed to pesticide treatments, whereas chlorophyll–*a* were reduced. Furthermore, pesticide treatments seemed to promote mosquito (i.e., *Culex* spp.) and pupal abundances. Finally, a total of 644 macroinvertebrate individuals were recorded across macadamia and communal reservoirs, mostly dominated by Chironominae (55.1 %), Ostracoda (14.4 %), *Trithemis* sp. (5.2 %), *Anax* sp. (5.2 %) and *Radix natalensis* (5.0 %).

The results indicate that MACADAMIA ORCHARD reservoirs are highly contaminated by acetamiprid and chlorpyrifos and that these contaminants have implications for the ecological functioning of aquatic ecosystems. Reduction of species diversity adjusted community structure and altered energy flow and nutrient recycling were all demonstrated. The results of the study collectively show how common pesticides used in the macadamia plantation may be exert pressure on adjacent freshwater ecosystems. Future studies should examine effects on trophic interactions, pesticide accumulation in faunal groups and allochthonous trophic transfer dynamics to terrestrial environments. Such information would be useful for managers tasked with mitigation plans for the region.

Key words: Pesticides; reservoirs; contamination; LC–MS/MS; solid phase extraction; behaviour traits; environmental stressor; freshwater fishes; neonicotinoids; organophosphate; pyrethroid; mosquito larvae; allochthonous inputs; macadamia; stone substrates; macadamia orchards; pesticides; macroinvertebrate community; macroinvertebrate diversity metrics.

Table of Contents

Acknowledgements

I am most grateful to the contribution and support of several individuals who made this PhD thesis a success. First and foremost, I want to express my deepest gratitude to my primary supervisor Dr. Tatenda Dalu for his motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. I could have not imagined having a better supervisor and mentor for this PhD. Beside him, I would like to extend gratitude to Dr Lutendo Mugwedi and Prof Ryan J. Wasserman for co–supervising me, being part of this project, their endless guidance and dedication to this thesis.

My sincere gratitude also goes to Dr. Ross N. Cuthbert and Farai Dondofema for their endless advice, encouragement, insightful comments and suggestions. I also extend my thanks Barbara Moyo and Prof. Ntakadzeni E. Madala for their insightful comments and guidance on pesticide concentration analysis. To Lutendo Phophi, Linton F. Munyai and Zwotea Nengovhe, your assistance and dedicating during my field works is well appreciated. To owners of Nooitgedacht Macadamia and Duthuni farms, I am grateful for granting me permission to conduct my experiment in your reservoirs without whom my field work would not have been possible. I also expend my gratitude to Dr Jacobus N. Steyn for providing me with macadamia pesticide products.

I owe my special gratitude to Faranani Tambani for providing me with unfailing and continuous support. I also extend my profound gratitude to my mum: Mphelekedzeni L. Mutshekwa, siblings: Letticia Mutshekwa, Marubini Mathelemusa, Victor J. Mathelemusa, grandmother: Thivhavhudzi N. Mudzanani, uncles: Pfarelo Ruruwe and Tshilidzi Ruruwe for their loving support. To my late stepfather, Victor N. Mathelemusa, I would like to thank you for giving me a reason not to give up and believing in me. May the glory be to God.

Finally, I greatly acknowledge the financial support for this study which was granted by the University of Venda Niche Grant (UID: FSEA/21/GGES/02) and National Research Foundation Grant (UID: 138206). I would also like to extend my gratitude to the NRF Postgraduate Bursary (UID: 121348) for funding PhD studies.

List of Figures

List of Tables

Preface

Each data chapter (chapters 3–6) in this thesis is stand–alone and has been published or is under review in international accredited journals (see below). As such, there may be some repetition in the introductory sections of the chapters. All references have been combined in a single reference list at the end of the thesis to ensure limited repetition.

Publication list

1. **Mutshekwa, T**., Mugwedi, L., Moyo B., Madala N.E., Wasserman, R.J., Dondofema, F., Dalu, T., 2022. Assessing acetamiprid and chlorpyrifos pesticide concentrations in water and sediments across macadamia orchard and communal area small reservoirs. *Chemistry and Ecology*. 1–11. https://doi.org/10.1080/02757540.2023.2199015

Thendo Mutshekwa: Formal analysis, Methodology, Data curation, Writing – original draft, Writing – review & editing. **Lutendo Mugwedi:** Methodology, Visualization, Investigation, Supervision, Writing – original draft, Writing – review & editing. **Babra Moyo:** Visualization, Methodology, Supervision, Writing – review & editing. **Ntakadzeni E. Madala:** Visualization, Methodology, Supervision, Writing – review & editing. **Ryan J. Wasserman:** Visualization, Methodology, Writing – review & editing. **Farai Dondofema:** Methodology, Visualization, Investigation, Supervision, Writing – review & editing. **Tatenda Dalu:** Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

2. **Mutshekwa, T**., Cuthbert, R.N., Mugwedi, L., Wasserman, R.J., Dondofema, F., Dalu, T., 2022. Behavioural responses and mortality of Mozambique tilapia *Oreochromis mossambicus* to three commonly used macadamia plantation pesticides. *Water* 14, 1257. https://doi.org/10.3390/w14081257

Thendo Mutshekwa: Investigation, Data curation, Formal analysis, Writing—original draft; Ross N. Cuthbert.: Conceptualization, Methodology, Data curation, Formal analysis, Writing—original draft; **Lutendo Mugwedi**.: Conceptualization, Investigation, Supervision, Methodology, Visualisation, Investigation, Data Curation, Resources, Writing—original draft; **Ryan J. Wasserman**.: Conceptualization, Methodology, Writing, Supervision, Data curation, Writing—review and editing**; Farai Dondofema**.: Conceptualization, Investigation, Methodology, Investigation, Resources, Visualisation, Writing—review and editing; **Tatenda Dalu**.: Conceptualization, Investigation, Methodology, Resources, Data curation, Supervision, Visualisation, Writing—original draft.

3. **Mutshekwa, T**., Mugwedi, L., Wasserman, R.J., Cuthbert, R.N., Dondofema, F., Dalu, T., 2022. Pesticides drive differential leaf litter decomposition and mosquito colonisation dynamics in lentic conditions. *Science of the Total Environment* 839, 156320. https://doi.org/10.1016/j.scitotenv.2022.156320

Thendo Mutshekwa: Formal analysis, Methodology, Data curation, Writing – original draft, Writing – review & editing. **Lutendo Mugwedi:** Methodology, Visualization, Investigation, Supervision, Writing – original draft, Writing – review & editing. **Ryan J. Wasserman:** Visualization, Methodology, Supervision, Writing – review & editing. **Ross N. Cuthbert:** Visualization, Methodology, Writing – review & editing. **Farai Dondofema:** Methodology, Visualization, Investigation, Supervision, Writing – review & editing. **Tatenda Dalu:** Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

4. **Mutshekwa, T**., Mugwedi, L., Wasserman, R.J., Dondofema, F., Dalu, T., 2022. Aquatic macroinvertebrate community colonisation dynamics in reservoirs across different land–use types. *Ecology and Evolution*. ***Under Review.***

Thendo Mutshekwa: Investigation, Data curation, Formal analysis, Writing—original draft; Ross N. Cuthbert.: Conceptualization, Methodology, Data curation, Formal analysis, Writing—original draft; **Lutendo Mugwedi**.: Conceptualization, Investigation, Supervision, Methodology, Visualisation, Investigation, Data Curation, Resources, Writing—original draft; **Ryan J. Wasserman**.: Conceptualization, Methodology, Writing, Supervision, Data curation, Writing—review and editing**;** **Farai Dondofema**.: Conceptualization, Investigation, Methodology, Investigation, Resources, Visualisation, Writing—review and editing; **Tatenda Dalu**.: Conceptualization, Investigation, Methodology, Resources, Data curation, Supervision, Visualisation, Writing—original draft.

Declaration

I, **Thendo Mutshekwa**, hereby declare that the work described in this thesis was carried out in the Department of Geography and Environmental Sciences, Faculty of Science, Engineering and Agriculture, University of Venda under the supervision of Dr Tatenda Dalu, Dr Lutendo Mugwedi and Prof Ryan J. Wasserman. The information derived from the literature have been duly acknowledged in the text and a list of references has been provided. The various components of the thesis comprise original work by the author and no part of this thesis was previously submitted for any award of degree or diploma at this or any other institution.

I also declare that I have adhered to all the principles of academic honesty and integrity and have not misrepresented or fabricated any idea, data and sources. I understand that any violation of the above will be cause for disciplinary action by the institute and can also evoke panel action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

Thendo Mutshekwa

(30 October 2022)

Chapter 1: General Introduction

Plate 1. Thendo Mutshekwa and Linton F. Munyai hauling a seine net at the communal reservoir site to collect fish *Oreochromis mossambicus* for pesticide exposure studies. Photo by Farai Dondofema

1.1 Background

Freshwater ecosystems are among the most threatened ecosystems in terms of species extinctions and losses in ecosystem services in the world (Hale et al., 2018). One of the major stressors for these ecosystems are pesticides, which are introduced via point and non–point sources (Deknock at el., 2019). Pesticides are intended to protect agricultural production from pest organisms; however, their residues reach far beyond their target areas via atmospheric, overland, subsurface, and groundwater routes (Groenendijk et al., 1994). Surface run–off and wastewater effluents are among the most important entry pathways for pesticides into aquatic environments (Holvoet et al., 2005). Surface water in agricultural areas can be contaminated by pesticide residues with possible adverse effects on the ecosystem (Cruzeiro et al., 2017). Pesticide contamination of surface waters is known to affect community structure (Liess and von der Ohe, 2005) and diversity of aquatic fauna communities (Beketov et al., 2013). Besides the effects on the aquatic fauna, pesticides can impede important ecosystem functions such as leaf breakdown that represents the main energy source and primary production in freshwater ecosystems (Schäfer et al, 2012).

Pesticides pose complex threats to the aquatic structure and communities and ecosystem functioning. Continued usage of pesticides can be described as a massive chemical assault in the aquatic environment which threatens the survival of many fish, macroinvertebrates, and small aquatic organisms that form the basis of the food web (Ramachandra and Solanki, 2007; Mahmood et al., 2016). More generally, pesticides reduce species diversity in the tropical level and contribute to population decline in aquatic fauna and plants by destroying habitats, reducing food supplies, and impairing reproduction (Islam and Tanaka, 2004; Tooker and Pearsons, 2021). These aquatic ecosystems consist of multiple and potentially thousands of interacting species, which shape trophic nodes and flow of organic matter (Mahmood et al., 2016). Aquatic food webs rely on two major energy sources: primary production within the water itself (autochthonous), and primary production on land (allochthonous), transferred to the aquatic habitat as leaf litter and dissolved organic carbon (Doucett et al., 2007). Aquatic–terrestrial interfaces within and between ecosystems are hotspots of organic matter mineralization. For these reasons, distinguishing energy inputs to reservoirs originating from the reservoirs itself from that derived from the surrounding landscape is central to understanding the functioning of reservoir community structure and their influences on reservoir biogeochemistry.

Agricultural activities in South Africa strongly depend on the use of toxic pesticides for crop protection, seed treatment and growth regulation to improve productivity (Gill and Garg., 2014). The South African's agricultural sector is one of the world's most diverse, consisting of corporate and private intensive and extensive crop farming system including macadamia nuts production. For instance, macadamia *Macadamia integrifolia* are one of the most highly priced nuts on the market and the macadamia industry in South Africa is growing quickly. South Africa is the current largest macadamia producer in the world with approximately ± 50 000 ha planted with macadamia trees (Macadamias South Africa (SAMAC), 2021). Currently MACADAMIA ORCHARDS are among the main contributors of pollutants in freshwater ecosystem due to increased demand of macadamia production. With emerging macadamia production and plantation, the usage of pesticides has increased enormously, so is the impacts on aquatic ecosystems.

1.2 Problem statement

The use of pesticides poses a serious threat to the threatened aquatic ecosystems in South Africa. Pesticides which are not taken up by crop plants, are often washed away by runoff into surface waters. These pesticides are best known to have a significant impact on communities in aquatic ecosystems. Moreover, agricultural streams are often characterised by loss of physical habitat diversity which may impose additional stress resulting from suboptimal environmental conditions (Rasmussen, 2012). Aquatic ecosystems support an enormous diversity of fauna around the world. Aquatic organisms have been reported to be potentially at risk from pesticides which are introduced into the aquatic environment through application to crop plants (Olisah et al., 2019). Once pesticides enter the aquatic environment, aquatic organisms are exposed to it in many ways including direct entries of pesticides into their habitats and the movement of organisms into areas previously contaminated by retaining pesticides (Aktar et al., 2009).

Pesticides can be toxic to primary producers and macroinvertebrates, thereby impacting ecosystem structure and functions through adverse, bottom-up impacts on aquatic colonisation dynamics. Plant leaf litter serves as a critical resource input to freshwater systems, and changes in plant litter species composition can change the attributes of freshwater communities. However, little is known about how variation in pesticide toxicity interacts with detrital decomposition dynamics and other aquatic ecosystem functions. Field based studies on the effects of pesticides on leaf litter and aquatic fauna are scarce. Studies on leaf litter decomposition dynamics, both *ex–situ* and *in–situ* have received widespread attention, since the middle of the nineteenth century (Martínez et al., 2020). However, studies on leaf litter decomposition dynamics using macadamia leaves, fauna and microbes and aquatic food webs in irrigation ponds are few. Reservoir supports a variety of animal and plant life forms that collectively form food chain; however, little information is known on these habitats in agricultural orchards in South Africa, with almost no information on the effects of macadamia input on aquatic ecosystems

The absence of studies presents a problem in that we have huge knowledge gaps pertaining to arguably one of the most fundamental processes driving freshwater productivity. Therefore, this study seeks to address the gap and enhance the understanding of ecology in agricultural irrigation reservoirs, by studying such dynamics within the context of pesticide toxicity. The study will further strengthen the water quality standards by generating information that could be used in management strategies of agricultural reservoirs.

1.3 Research aim and objectives

The overall aim of this thesis is to assess the effects of macadamia *Macadamia integrifolia* orchard inputs on recipient aquatic ecosystems. This study focuses on understanding how different pesticides (i.e., Karate Zeon 10 CS (lambda–cyhalothrin), Mulan 20 SP (Acetamiprid), Pyrinex 250 CS (chlorpyrifos)) and their concentrations affect behavioural responses and mortality of Mozambique tilapia *Oreochromis mossambicus* and colonisation dynamics. The thesis will specifically:

- Determine pesticide concentrations in the MACADAMIA ORCHARD and communal area in water and sediments in small reservoirs.
- Assess the behavioural response and potential mortality of Mozambique tilapia, *Oreochromis mossambicus* when exposed to pesticides at different concentrations.

- Assess macadamia *Macadamia integrifolia* leaf litter decomposition, macroinvertebrate establishment (i.e., mosquitoes) and primary productivity (chlorophyll–*a*) associated with pesticide treatment over time.
- Assess macroinvertebrate colonisation associated with artificial stone substrates in reservoirs across different land–use types.

1.4 Hypotheses

- Pesticide concentrations will vary between the two reservoir types and MACADAMIA ORCHARD will drive increased concentrations than communal area reservoirs. Increase pesticide concentrations in MACADAMIA ORCHARDS might have implications on water quality, ecosystem functioning, species diversity and community, food chains and nutrient recycling.
- Mozambique tilapia, *Oreochromis mossambicus* will die faster when exposed to increased pesticide concentration and that behavioural response will vary between pesticide concentrations. This will have implications on aquatic food webs since *Oreochromis mossambicus* will be eliminated resulting in disturbance in the food chain.
- Macadamia *Macadamia integrifolia* leaf litter decomposition, mosquito colonisation and chlorophyll–*a* concentrations will vary among pesticide treatments and that pesticide–free treatments will decompose fastest, due to adverse pesticide effects on decomposition and/or colonisation. This might have implications on microbial decomposers and macroinvertebrate establishment as their establishment will based on pesticide toxicity.
- Reservoirs associated with reduced land–use activities will support more abundant and diverse macroinvertebrates than reservoirs associated with increased land–use. This will

have implications on ecosystem functioning and comprehension of the feeding relationships among aquatic species since some aquatic fauna will be eliminated from the food chain, which in turn affects species both directly and indirectly.

1.5 Justification and significance of the study

To the best of my knowledge, no study has yet evaluated the effects of macadamia inputs on aquatic ecosystems in South Africa. Aquatic fauna are usually exposed to chemicals, mostly with different toxicity which results in disrupting aquatic ecosystem functioning. Therefore, it is worthwhile investigating the toxicity of pesticides with a focus on pesticides and their effects on aquatic fauna as well as the decomposition and colonisation dynamics.

This study is confined itself to assessing the ecology of macadamia reservoirs by studying the effects of pesticides used in the MACADAMIA ORCHARDS on *O. mossambicus* and leaf litter decomposition, primary productivity and macroinvertebrate colonisation dynamics. Aquatic fauna and litter decomposition dynamics play a role in assuring natural sustainability for all associated life forms, but it is greatly affected by human activities. Thus, this study will ultimately add to the knowledge base and contribute to the education of society on the value of the ecosystem processes and their associated tangible and intangible services.

Given the largely absent research on the effects of pesticides used in MACADAMIA ORCHARDS to nearby small reservoirs in South Africa, the current research is thus needed. In summary, this research is important for several reasons:

1. Enhance the understanding of effects of pesticides on aquatic ecosystems and ecosystem functioning.
2. Attempt in extending existing knowledge about the distribution and occurrence of aquatic fauna in reservoirs contaminated with pesticides

1.6 Key macadamia species and pesticides in the study

Modern crop protection remains in the forefront of the public debate. While the discussion on the risks, the general necessity and the economically optimal levels of synthetic pesticide use and effects on aquatic ecosystems has not come to an end with ongoing research on the interactions between agricultural activities and aquatic ecosystem functioning. The macadamia industry in South Africa is affected by a wide variety of stink bugs species and as a result, the industry is forced to use large quantities of pesticides to control. The MACADAMIA ORCHARDS in Levubu, Limpopo is no exception, with large quantities of pesticides being used every year due to challenges they are facing from pests. Given the aims of the study and increased use of pesticides by Levubu macadamia farmers, it was therefore necessary to identify the pesticide commonly used and associated reservoirs that would give an indication of the potential effects of MACADAMIA ORCHARDS inputs on aquatic ecosystems.

Field visits to various MACADAMIA ORCHARDS in the Levubu area revealed macadamia *Macadamia integrifolia* as the dominating macadamia tree species (Figure 1.1). Moreover, after interaction with Levubu macadamia farmers, pesticides Karate Zeon 10 CS (lambda–cyhalothrin), Mulan 20 SP (Acetamiprid), Pyrinex 250 CS (chlorpyrifos) were found to be among the commonly used pesticides for pest control. As a results macadamia *M. integrifolia*, pesticides i.e., Karate

Zeon 10 CS, Mulan 20 SP, Pyrinex 250 CS and associated reservoirs were found to be suitable to assess the effects of MACADAMIA ORCHARDS inputs on recipient aquatic ecosystems.

Figure 1.1. Macadamia *Macadamia integrifolia* trees in an orchard in the Levubu area, Limpopo, South Africa. Photo by Thendo Mutshekwa.

Chapter 2: Literature Review

Plate 2. A characteristic artificial aquatic ecosystem on a MACADAMIA ORCHARD. In this way, agricultural practices have shaped the nature of aquatic ecosystem distributions at a landscape level. Photo by Thendo Mutshekwa

2.1 Overview

This chapter introduces the main concepts that are relevant to this particular study. It provides an outline of macadamia *Macadamia integrifolia* in South African orchards and further details pesticide usage and their general effects on aquatic ecosystems. This chapter also addresses the interactions between pesticide, leaf litter decomposition, primary productivity and colonisation dynamics. This chapter concludes with key macadamia species and pesticide use within the study region.

2.2 The macadamia in South Africa

Macadamia is a medium sized, evergreen tree that forms part of the Proteaceae family. Only two of the five species, *Macadamia integrifolia* (Maiden and Betche) and *Macadamia tetraphylla* (L.A.S. Johnson), are edible and due to their high value, grown commercially (Trueman and Turnbull 1994). Although macadamias are native to Australia, the industry for macadamia nuts first emerged in Hawaii in the 1930s, followed shortly thereafter in Australia (Boyton et al., 2000). More than 80 years ago, *M. integrifolia* seeds were transported to South Africa from Hawaii (Boyton et al., 2000). Cultivation in South Africa began in 1968 and to date, South Africa is amongst the biggest producers of macadamia nuts worldwide (Figure 2.1). Currently, more than eighteen different macadamia cultivators are currently grown in South Africa (Peace et al., 2005). Macadamia nuts are currently primarily produced in South Africa, Australia, the United States of America, Kenya and China (Figure 2.1). However, production in some African and South American nations is expanding rapidly. According to Macadamias South Africa (SAMAC) (2021), the number of macadamia trees has increased from one million in 1996 to eight million in 2020, covering an area of approximately 50 000 ha. The Macadamias South Africa (2020) census revealed that macadamia industry is predominantly spread over the Mpumalanga (44 %), KwaZulu–Natal (28 %) and Limpopo (20 %) provinces (Figure 2.2). The rate of macadamia production in South Africa is expected to rise even further in the near future as a result of the exponential increase in new annual plantings. According to Nagao et al. (1992), one major threat to the macadamia tree in South Africa is kernel damage from pest insect species, which lowers the quality of the nuts.

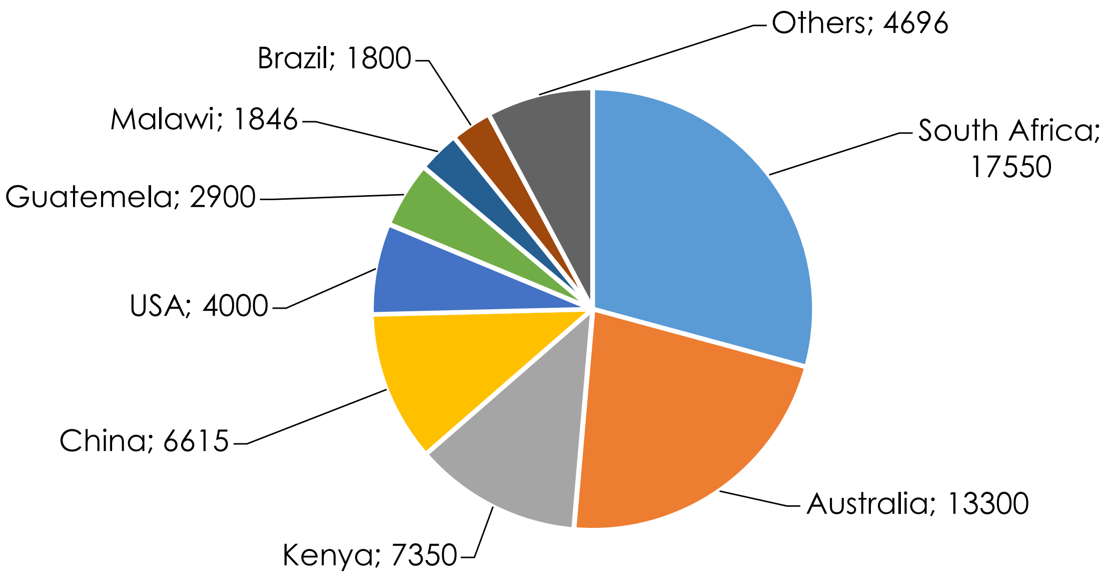

Figure 2.1. Major macadamia nut producing countries (metric tonnes): kernel basis. Source: International Nut and Dried Fruit Council (INC) (2020).

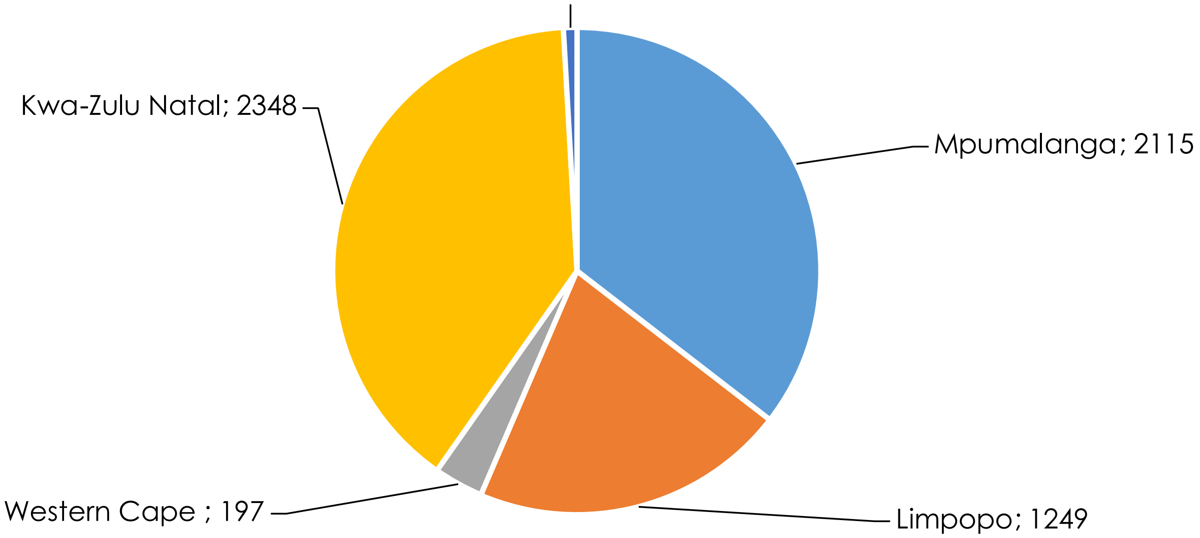

Figure 2.2. South African macadamia established hectares per province in 2019. Source: Macadamias South Africa (SAMAC) (2020).

2.3 Insect pests of macadamia

There are numerous international studies (e.g., Taylor et al., 2013; Weier et al., 2019; Pal et at., 2022) focusing on the insect pests that harm macadamia trees. According to Schoeman (2018), the stink bugs (Hemiptera) and the nut borers (Lepidoptera) are the most significant pests of macadamia nuts. The stinkbugs damages are divided into different stages i.e., early and late stage, and these stages depend on the time of season or the nut growth. Depending on the shell's hardness, not all species of stinkbug undergo both stages of damage (Schoeman, 2018). In 2016, stinkbug damage cost South African as approximately ZAR 190 million in financial losses. Schoeman (2018) indicated that the macadamia nut borer *Crytophlebia batracopha* and false codling *Thaumatotibia leucotreta* are two species that also attack macadamia trees. Mammalian pests, such as antelope i.e., bushbuck *Tragelaphus sylvaticus*, giant rats *Cricetomys gambianus*, and/or bush pigs *Potamochoerus larvatus*, which feed on dropped nuts and young leaves, also cause damage to macadamia trees (Linden, 2019). These damages have resulted in increased numbers of pesticides used for pest control.

2.4 Pest control mitigation

Macadamia farmers in South Africa use different pest control approaches to control pests on their crop. The most common method involves spraying insecticides, based on scouting data once a certain number of pest insects per tree has been reached (Taylor et al., 2018). Some farmers use methods like the calendar method and unrelated spraying to control pests, while others rely on extensive insecticide application using tractors and helicopters to cover a large area quickly. However, due to the fact that some trees are skipped, these methods are less effective (Taylor et al., 2018).

2.5 Cost-benefit analysis of the use of pesticides

Despite the high cost of pesticides purchase, the widespread application of pesticides has been encouraged by the benefits the provide. In particular, over the last decades, pesticides have increased crops and livestock yields, and, in some circumstances, have improved human health, e.g. by killing vectors of human pathogens, and quality of life, e.g. by killing troublesome organisms (Cooper and Dobson 2007). However, the purchase costs are only one of the types of cost associated with pesticide use. Indeed, the spraying of these chemicals has an impact on the environment and health, with potentially serious financial consequences. According to Tudi et al (2021), without the use of pesticides, there would be a 78% loss of fruit production, a 54% loss of vegetable production, and a 32% loss of cereal production. Pesticide free farming does not necessarily result in lower crop yields, but it does depend on the presence of weeds, insects, and fertiliser.

2.6 MACADAMIA ORCHARD settings

Macadamias are planted near aquatic ecosystems in rows with average spacing of 4 m between rows, and 8 m between individual trees within each row (Kaemper et al., 2021; Trueman et al., 2022). Blocks of varying sizes are used to organize MACADAMIA ORCHARDS, which are typically interplanted with up to three distinct varieties per block. Reservoirs and/or ponds are constructed near macadamia plantations and are utilised for irrigation. In agricultural landscapes, such reservoirs cumulatively comprise a major portion of available aquatic habitat at the landscape level. Water is typically supplied to these reservoirs from nearby natural water sources, bringing with it aquatic biotic components such as plankton, invertebrates and fish (Mofu et al., 2021). Semi–aquatic insects also colonise these water bodies through aerial dispersal. However, pesticides used in orchards end up in these reservoirs via different routes, with implications for the biota in these systems.

2.7 Pesticides inputs into aquatic ecosystems

Pesticides are used in large quantities in crops and MACADAMIA ORCHARDS to protect against pests. However, aquatic ecosystems can be contaminated by these pesticides when they are incorrectly and/or accidently sprayed into the natural environment (Figure 2.3) (Howard, 2010). Contamination can occur due to improper handling and use of pesticides, including spilling pesticide, discarding pesticide containers rinsing or washing pesticide equipment, applying pesticides under bad weather conditions, having orchards near aquatic ecosystems and wind drift

(De Wilde et al., 2007). However, Sultana et al. (2014) and Lehmann et al. (2018), estimated that 0.1 % of the applied pesticide reaches the target pest, and the rest gets incorporated into different environmental compartments. Agricultural activities often used aerial application for pest control of which pesticides may pollute surrounding reservoirs with pesticide droplets.

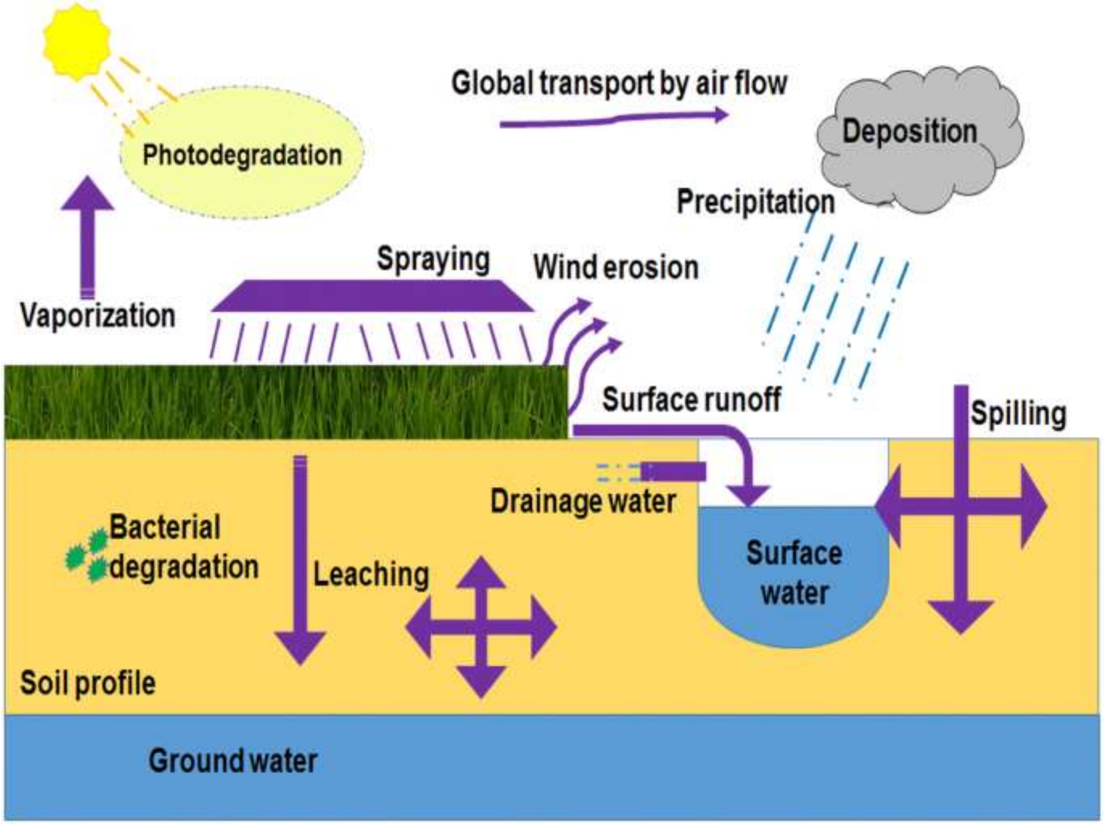

Figure 2.3. Different stages of pesticide cycle that reaches the ground and surface water. Source: Rajmohan et al. (2020).

One of the main causes of water contamination from agricultural areas that come from non–point sources. This can occur through runoff, leaching, spray drift, preferential flow through soil macropores, or a combination of these processes (Loewy et al., 2011). Several studies have showed that spraying pesticides improve the distribution of pesticides (Loewy et al., 2011; Raut et al., 2013). However, the common pathway through which pesticides enter aquatic systems such as reservoirs, ponds, streams and rivers, is through run–off, or when immediate rainfall occurs after pesticide application. Runoff is regarded as a significant entry point for pollutants into aquatic ecosystems. Physicochemical properties of the pesticides, application timing and rate, rainfall following pesticide application, and soil types all play a role in pesticide runoff contamination of aquatic systems (Phillips and Bode, 2004). Although pesticides are intended to control fungi, insects, and other pests, their mechanisms of action frequently fail to prevent unintended effects on non–target fish, macroinvertebrate populations, and microbial communities.

2.8 Effects of pesticides on aquatic ecosystems

Pesticides are one of many organic emerging freshwater pollutants that can cause serious ecological impacts (Okoro et al., 2022). Different categories of pesticides have different types of effects on living organisms. Pesticides can have an effect on all types of organisms and are an important source of stress for freshwater ecosystems (Liess et al., 2008; Schäfer et al., 2012). In aquatic ecosystems, both fish species, as well as aquatic flora and fauna, are exposed to a variety of pesticides in three common ways, that is via (i) dermal, (ii) direct absorption all the way through integument by swimming in contaminated surface water with pesticide and (iii) subordinate surfaces of waters in form of lentic and lotic water bodies (Maurya et al., 2019). Exposure can also be through direct and indirect uptake of pesticides through inhalation by the way of gills during respiration, and directly throughout, drinking water contaminated with pesticides or feeding on pesticide contaminated prey (Kumar et al., 2021).

2.8.1 *Effects of pesticides input on macroinvertebrate communities*

The distribution of macroinvertebrate taxa and densities in agricultural headwater streams and reservoirs is influenced by many factors such as habitat degradation (Sanders et al., 2020), organic pollution (Mueller et al., 2020), and pesticides (Smiley et al., 2011; Dalu and Tavengwa, 2022). Macroinvertebrates organisms have been widely used to assess aquatic ecosystem integrity since they possess several advantages such as their respond to human disturbance in fairly predictable ways due to the fact that different types of macroinvertebrates tolerate different stream conditions and levels of pollution (Miserendino et al., 2008). According to Bonada et al. (2006) macroinvertebrates are considered vital for biomonitoring of aquatic systems since they are ubiquitous and diverse, have a variety of feeding habits, have life cycles that range from a few weeks to a few years, and are tolerant to a variety of contaminants. Additionally, macroinvertebrate communities are sensitive to pesticides and are good indicators of overall ecosystem function (Overmyer et al. 2005). Macroinvertebrate communities play a significant in the transfer of various nutrients and environmental pollutants to higher tropic levels. Pesticides accumulation in sediments and water surfaces can cause a serious damage to the aquatic ecosystem and their exposure to aquatic macroinvertebrates through cell body walls, across respiratory surfaces, and ingestion of contaminated sediment particles (Warren et al., 2003).

2.8.2 *Effects of pesticides on fish*

According to Joseph and Raj (2011), fish can be directly or indirectly impacted by pesticides resulting from anthropogenetic activities. Fish are among the best indicator of water body pollution and are the most sensitive of all aquatic animals besides phytoplankton. Previous studies (e.g., Srivastava et al., 2016; Chaudhari and Saxena, 2016) indicates that fish, are quickly becoming scarce due to pesticide pollution. Fish can suffer from long–term exposure which can result in abnormalities and/or mutations in developing larvae, while acute exposure can result in immediate fish die–offs (Figure 2.4) (Polazzo et al., 2022).

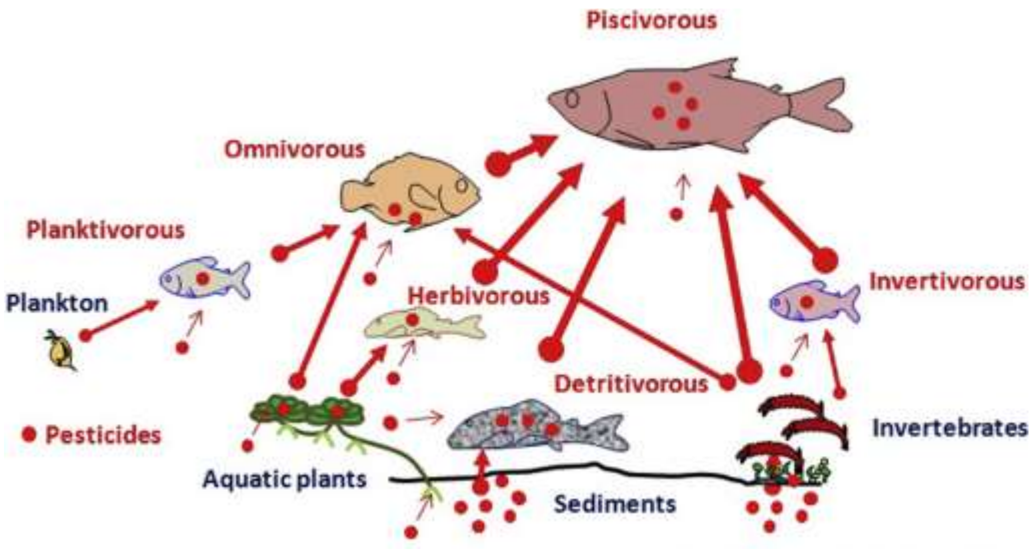

Figure 2.4. Diagram showing the distribution and bioaccumulation in fish and interactions among fish feeding habits. Source: Rajmohan et al. (2020).

2.9 Leaf litter decomposition

The decomposition of leaf litter has long been studied as a fundamentally important ecosystem process (Freschet et al., 2013). Leaf litter contains carbon and hydrogen as well as several other elements such as oxygen, nitrogen, and phosphorus. The major input into the soil is from plants, in the form of aboveground litter such as leaf litter, twigs and stems, and/or belowground material such as root litter, exudates and mycorrhizal hyphae (Freschet et al., 2013). Litter decomposition and soil organic matter formation are driven by bacteria, fungi and invertebrates (Lehmann and Kleber, 2015). Their activity, and controls on soil community composition, influence nitrogen release and/or storage in the soil (Crowther et al., 2013). In addition to bacterial and fungal controls, soil mesofauna may influence microbial composition and community activity through top–down controls (Crowther et al., 2013).

The effects of pesticides on microbial decomposers and detritivores in the tropics are mostly unknown. However, experiments conducted in other areas of the world suggest that these organisms are probably impaired by pesticides, and these effects are likely to reduce litter decomposition rates (Zubrod et al., 2014). In addition, Rasmussen et al. (2012) indicated that pesticides can be transported from crop fields to adjacent streams via surface runoff (Berenzen et al. 2005), adhered to leaf litter (Zubrod et al. 2014), drains, wind drift and atmospheric deposition. Pesticide residues in streams can cause a transient pulse of pollution and have a negative impact on processes such as litter decomposition with serious implications to food web dynamics and the ecosystems that surround them (Cornejo et al., 2021). In streams that flow through and reservoirs within agricultural catchments, pesticide contamination has been found to minimise microbial decomposition of leaf litter (Rasmussen et al., 2012).

Chapter 3: Assessing Acetamiprid and Chlorpyrifos Pesticide Concentrations in Water and Sediments Across MACADAMIA ORCHARD and Communal Area Small Reservoirs

Plate 3. Collection of sediments and water samples in the macadamia orchard for analysing of acetamiprid and chlorpyrifos pesticide concentrations in the laboratory using LC–MS/MS. Photo by Lutendo Phophi

3.1 Introduction

In the last few decades, pesticides have been used on an increasingly wider scale throughout the world (Myers et al., 2016). Pesticides are highly associated with anthropogenic activities (Dalu and Tavengwa, 2022). As indicated by Elhatip et al. (2003), agricultural fields activities contribute increased pesticides contamination in water bodies than residential and/or communal areas activities. In agricultural fields, chemicals are used to control insects (pesticides) and weeds (herbicides) due to their effectiveness (Ngowi et al., 2007). Regardless of their agricultural benefits, pesticides are often considered a serious threat to the environment because of their persistence (Gajendiran and Jayanthi, 2018; Dalu and Tavengwa, 2022). These pesticides can cause profound changes to the quality of the environment (Walter et al., 2017). This is due to their persistence which allows them to remain for years in the environment, as well as their toxicity. The widespread use of pesticides contaminates various aquatic ecosystems, including sediments, water, and biota (Ccanccapa et al., 2016, Zhang et al., 2016). These pollutants accumulate in aquatic organisms, directly from polluted environments (water, sediment) or indirectly via the food chain, with a potential risk to humans (Parra–Arroyo, 2022).

Pesticides are compounds in continuous evolution, characterised by their diversity, physico–chemical properties, and concentrations. Organophosphates (OPs) and neonicotinoids pesticides are very toxic and persistent in the environment and tend to accumulate in the living organisms. The OPs are a class of pesticides such as chlorpyrifos, several of which are highly toxic (Sidhu et al., 2019). Until the 21st century, chlorpyrifos were among the most widely used pesticides available (Bringolf et al., 2007). Neonicotinoids such as N–(6–chloro–3–pyridylmethyl)–N'–CYANO–N–, better known as acetamiprid, are now the most widely used pesticides in the world (Yu et al., 2011; Jiménez–López et al., 2020). They act systemically, travelling through plant tissues and protecting all parts of the crop, and are widely applied as seed dressings (Jiménez–López et al., 2020). These pesticides were introduced because of their reduced persistence in the environment relative to the organochlorine pesticides. Nevertheless, concerns about bioaccumulation, acute and chronic toxicity persist. Furthermore, the impact of pollution of both soil and water by such chemicals is often difficult to observe unless there is a gross accidental or intentional spill (Simon et al., 1998). Therefore, the occurrence of these pesticides and their toxicity require an analytical technique for quantification (Aceña et al., 2015).

Rapid increases in macadamia plantations and demand for macadamia nuts have resulted in increased usage of pesticides worldwide over the last decade (Grass et al., 2018). South Africa is the largest macadamia producer globally (50 133 hectares under cultivation and 50 000 tonnes harvested annually), followed by Australia and the USA, with China and some South American and other African countries growing fast in production (Macadamias South Africa (SAMAC), 2021). In South Africa, the industry is predominantly spread over the Mpumalanga (44 %), KwaZulu–Natal (28 %) and Limpopo (20 %) provinces (Macadamias South Africa (SAMAC), 2021). Due to low pesticide utilisation in communal areas, the reservoirs within these areas can serve as sites for comparison with MACADAMIA ORCHARD reservoirs of pesticide effects on biotic and ecosystem dynamics at local scales. However, pesticide levels require quantification as a first step in such comparative studies. As part of a larger study on pesticide dynamics in agricultural landscapes, we used liquid chromatography–tandem mass spectrometry (LC–MS/MS) to determine concentrations of acetamiprid and chlorpyrifos in water and sediment of MACADAMIA ORCHARD and communal area reservoirs. Solid phase extraction (SPE), based on C_{18} cartridges, was used for the extraction of the compounds of interest. It was hypothesised that pesticide concentrations will vary between the two reservoir types and that MACADAMIA ORCHARD would contain increased concentrations of acetamiprid and chlorpyrifos in both water and sediment habitats due to its high use within the orchards compared to the communal areas.

3.2 Materials and methods

3.2.1 *Study area*

The samples were collected once on the 14th of November 2021 from MACADAMIA ORCHARD and communal area reservoirs in Limpopo province, South Africa. Two MACADAMIA ORCHARD reservoirs (Site 1, –23°06’31.6”S, 30°15’54.2” E; site 2, –23°06’54.5”S, 30°15’46.5” E) are located in the southern foothills of the Soutpansberg Mountain range, 20 km east of the town of Makhado (previously Louis Trichardt), were sampled. The reservoirs were selected due to the presence of large MACADAMIA ORCHARD plantations and excessive use of pesticides. Two communal area reservoirs were similarly sampled i.e., site 3, (–22°56’43.0” S, –30°23’01.9” E) was located in Phiphidi village, while site 4 (–22°59′11” S, –30°36′16.19” E) was located between Tshikunda, Phiphidi and Duthuni villages. Both communal area reservoir sites are approximately 10 km from Thohoyandou, Limpopo province, South Africa, and are mainly used for domestic water supply and irrigation (Figure 3.1). At each reservoir, triplicate (*n* = 3) water and sediment samples were collected.

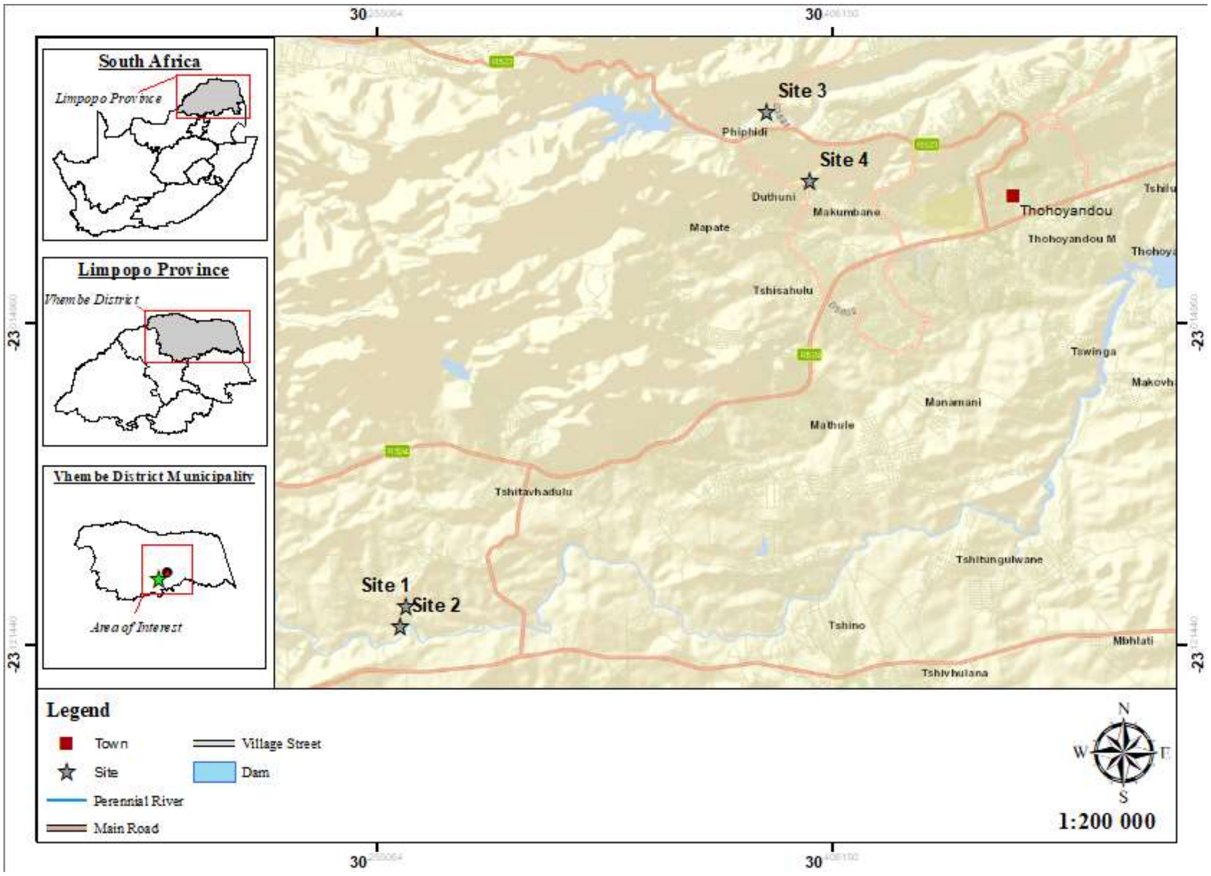

Figure 3.1. Location of the sample collection sites (Site 1 and 2 – MACADAMIA ORCHARDS; Site 3 and 4 – communal area).

3.2.2 *Apparatus*

The SPE was performed with Cleanert S C_{18} (6 mL, 500 mg) cartridges (Stargate Scientific, South Africa). The chromatographic measurements were done using LC–MS/MS system (Shimadzu, Corporation, Kyoto, Japan) consisting of triple quadrupole mass spectrometer (LC–MS/MS–8045) equipped with turbo ion spray ionisation source in the positive ionisation mode. The multiple reaction monitoring (MRM) method was used for absolute quantification of the targeted pesticides The chromatographic separation of the compounds of interest was achieved on a Shim–pack Velox SP–C_{18} column (100 × 2.1 mm, 2.7 µm). A gradient elution using binary solvents i.e., phase A

(1 mM ammonium formate) and phase B (acetonitrile) in 0.1 % formic acid was used. The flow rate was 0.4 mL min^{-1} with the binary gradients: 1–2 min 5 % B, 2–3 min 5 % B, 3–4 min 95 % B, 4–5 min 5 % B, and 5–6 min 5 % B. The column oven temperature was maintained at 55 °C and the injection volume was 3 µL.

3.2.3 *Reagents and chemicals*

All pesticides were of pestanal grade standards. Acetamiprid and chlorpyrifos were obtained from Sigma–Aldrich, South Africa. Methanol (LC–MS grade, 99 %) and dichloromethane (99 %) were obtained from Sigma–Aldrich, South Africa. The individual stock solutions of the analytes were prepared by dissolving 10 mg of the standards in 10 mL methanol to obtain a concentration of 1 mg mL^{-1}. The working standard solutions of concentrations ranging from 0.1 to 500 µg L^{-1} were then prepared by proper dilution of the stock solutions with methanol. For SPE, Cleanert S C_{18} (6 mL, 500 mg) were supplied by Stargate Scientific (South Africa).

3.2.4 *Sample collection and pre–treatment*

3.2.4.1 *Water sampling*

Water variables (i.e., pH, conductivity (µS cm^{-1}), total dissolved solids (mg L^{-1}), and temperature (°C) were measured on the day of sampling using a multiparameter handheld waterproof Cyber Scan 300 (Eutech Instruments, Singapore). A total of 12, 1 L glass bottles were used during the collection of water samples (4 sites × 3 replicates). After collection, water samples were immediately placed on ice in a cooler box and transported to the laboratory. Upon arrival, collected water samples were filtered (vacuum of < 5 cm Hg) through a 0.7 µm Whatman GF/F filter to remove particulate matter from the background matrix, preventing the SPE cartridge blockage.

After filtration, water samples were stored in 1 L polyethylene bottles in the until ready for analysis.

3.2.4.2 *Sediment sampling*

Approximately 2 kg sediments samples were collected (depth 5–10 cm) using a plastic shovel from each reservoir (4 sites × 3 replicates), placed into polyethylene zip–lock bags, and labelled according to sites. After collection, sediment samples were immediately transported to the laboratory in a cooler box, frozen at –20 °C, lyophilised, grind with a pestle and mortar sieved through 125 µm mesh to obtain a homogeneous material, and stored at –20 °C until extraction. This was done to increase the surface contact between the extraction solvent and sample (Azwanida, 2015).

3.2.5 *Sample extraction*

3.2.5.1 *Sediment extraction*

The sieved sediment was weighed, and 12 × 0.5 g sediments samples ($n = 12$, 4 sites × 3 replicates) were added to 35 mL of methanol and placed into a shaker for 12 h (overnight). After shaking, each sample was filtered (vacuum of < 5 cm Hg) through a 0.7 µm Whatman GF/F filter into 50 mL centrifuge. For LC–MS/MS, the samples were further filtered using a syringe filter (0.22 µm) into 2 mL amber vials.

3.2.5.2 *Water extraction*

For water extraction, SPE based on Cleanert S C_{18} cartridges was utilised. Prior to extraction, water samples were removed from the refrigerator and allowed to reach ambient temperature. The SPE sorbents were first pre–conditioned with 10 mL dichloromethane, 10 mL methanol and 10 mL distilled water. Before the cartridges dried out, water samples (1 L) were passed through the SPE cartridges using a vacuum manifold that was maintained at constant pressure. Retained analytes were eluted with 5 mL methanol followed by 5 mL of acetonitrile following a procedure by Bonansea et al. (2013). The eluate was collected in 10 mL glass vials and filtered using a syringe filter (0.22 µm pore size) into 2 mL amber vials for LC–MS/MS analysis.

3.2.6 *Statistical analysis*

A two–way Analyses of Variance (ANOVA) was used to assess the differences in water variables (i.e., pH, conductivity, total dissolved solids and temperature) and pesticide concentrations (i.e., acetamiprid and chlorpyrifos) across sites, after testing for homogeneity of variances (Levene's test, $p > 0.05$) and normality of distribution (Shapiro–Wilk test, $p > 0.05$). Sites were used as explanatory variables, while water variables, and pesticide concentrations were the response variables. For this model, significant variables were further tested using the Tukey post–hoc tests performed via estimated marginal means. In all analyses, significance was inferred at $p < 0.05$ and all statistical analyses were performed using IBM SPSS version 28.0.0.0.

3.3 Results

3.3.1 *Water variables*

Across sites, a high mean temperature (28.9 °C) was observed in the MACADAMIA ORCHARD reservoirs, whereas, high mean pH (7.08), conductivity (356.6 µS cm ^{1}) and total dissolved solids (179.9 mg L^{-1}) were observed in the communal area reservoirs (Figure 3.2). Overall, water variables differed significantly, i.e., pH (range: 6.3–7.1), conductivity (range: 213.3–356.6 µS cm^{-}

^{1}), total dissolved solids (range: 127.7–179.9 mg L^{-1}) and temperature (range: 24.2–28.9 °C). Significant differences were observed for pH ($p < 0.001$) across sites, whereas temperature ($p = 0.841$) conductivity ($p = 0.510$) and total dissolved oxygen ($p = 0.990$) indicated non–significant differences. Pairwise comparisons for pH indicated significant differences for sites 1 *vs* 2 ($p = 0.018$), sites 2 *vs* 4 ($p < 0.001$) and sites 3 *vs* 4 ($p = 0.021$).

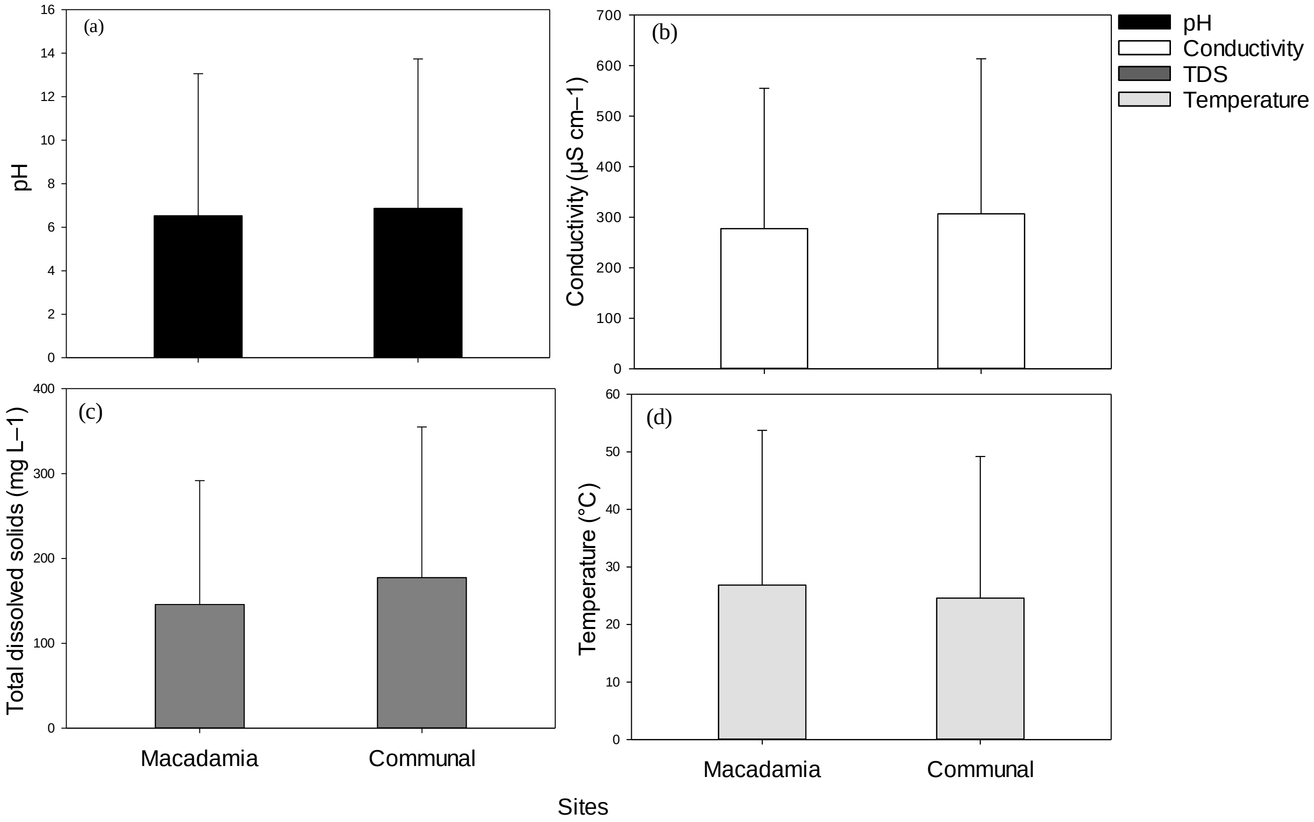

Figure 3.2. Mean (±standard deviation) water variables measured across macadamia orchard and communal area reservoirs during sampling event.

3.3.2 *Pesticide concentration in water and sediments*

The residue analysis results of acetamiprid and chlorpyrifos associated with water and sediments in the MACADAMIA ORCHARD and communal area reservoirs are presented in Table 3.1.

Acetamiprid and chlorpyrifos were found to be common in the MACADAMIA ORCHARD as compared to communal area reservoirs. In sediment samples from the MACADAMIA ORCHARD, the mean concentrations of acetamiprid and chlorpyrifos were 14.48 µg L^{-1} and 5.67 µg L^{-1}, respectively, whereas, in the communal area reservoirs both pesticides were below detection limit. Additionally, in water samples, acetamiprid was below detection limit across MACADAMIA ORCHARDS and communal area reservoirs. In contrast, chlorpyrifos were detected with mean concentrations of 6.51 µg L^{-1} and 0.13 µg L^{-1} in the MACADAMIA ORCHARD and communal area reservoirs, respectively. Non–significant differences were observed for both acetamiprid (F = 0.668, $p = 0.595$) and chlorpyrifos (F = 0.669, $p = 0.594$) in sediment samples across sites. Moreover, in water samples, significant differences were observed chlorpyrifos (F = 13.914, $p = 0.002$) across sites. Pairwise multiple comparisons for chlorpyrifos in water samples highlighted differences for sites 1 *vs* 3 ($p = 0.005$), sites 1 *vs* 4 ($p = 0.004$), sites 2 *vs* 3 ($p = 0.017$), and sites 2 *vs* 4 ($p = 0.013$).

Table 3.1 Mean ± SD pesticide concentrations (µg L^{-1}) of acetamiprid and chlorpyrifos in water and sediments from the MACADAMIA ORCHARD and communal area reservoirs. <0.001 µg L^{-1} – detection limit.

Sites	**Acetamiprid**		**Chlorpyrifos**	
	Sediment	**Water**	**Sediment**	**Water**
Macadamia	14.5±9.72	<0.001 µg L^{-1}.	5.7±3.6	6.5±0.9
Communal	<0.001 µg L^{-1}.	<0.001 µg L^{-1}.	<0.001 µg L^{-1}.	0.1±0.1

3.4 Discussion

To better comprehend the fate of pesticides in the aquatic environment together with their potential impact that they might pose to aquatic organisms, it is critical to know their concentrations in the water and sediments. Thus far, previous studies (i.e., Pereira et al., 1996; Rashid and Romshoo, 2013; Ansaei and Matondkar, 2014) have demonstrated that high pesticide concentrations occur in aquatic ecosystems associated with increased anthropogenic activities and that contaminants decline with decreasing anthropogenic activities. Similarly, the present study demonstrated that MACADAMIA ORCHARDS drive increased pesticide concentrations in nearby reservoirs, compared to communal areas. The study also highlights that sediments accumulate increased pesticide concentrations compared to water. This is because most of the pesticides are deposited on the bottom of the water bodies forming sediment layers, whereas pesticides in the surface water are easily washed away, thus sediments act as a pollution sink (Nasrabadi et al., 2011; El Nemr et al., 2013). However, sediments can also act as a pollution source, as retained contaminants can be bioaccumulated by sediment–dwelling organisms and subsequently pose a risk to higher trophic organisms through biomagnification among the food chain. Moreover, retained pollutants can be released at any time via re–suspension by natural processes or human actions.

This study showed high pesticide concentrations of chlorpyrifos and acetamiprid in sediments than in water. Furthermore, MACADAMIA ORCHARD reservoirs were associated with increased concentrations, whereas communal area reservoirs had the least concentrations. As stated by Halbach et al. (2011), water and sediments–associated pesticide concentrations are often much higher than recorded and significantly higher in reservoirs surrounded by increased anthropogenetic activities. Moreover, acetamiprid was not detected in water samples across MACADAMIA ORCHARD and communal area reservoirs, whereas chlorpyrifos was detected in the MACADAMIA ORCHARDS (6.51 ug L^{-1}) and communal area reservoirs (0.13 ug L^{-1}). The increased sediment–associated pesticide concentrations in the MACADAMIA ORCHARD reservoirs are not surprising given the fact that sediments are an important sink for various pollutants which also play a significant role in the remobilisation of contaminants in aquatic systems under favourable conditions and in interactions between water and sediment (Öztürk et al., 2009). Overall, the accumulation of these pesticides in sediments in the MACADAMIA ORCHARD reservoirs can pose a direct toxicological risk for organisms living and feeding on reservoir sediments due to chronic exposure (Barbieri et al., 2019). The lower concentrations of both pesticides in the water samples might be attributed to the fact that the input of pesticides in water is a function of suspended particulate concentrations (Knauer et al., 2017). Although low, these concentrations can build up in the water, get magnified through the food chain, and enter aquatic organisms that are hazardous for human consumption (Singh et al., 2015). Chlorpyrifos concentrations in water samples in the MACADAMIA ORCHARDS were like those reported by Hossain et al. (2015), and chlorpyrifos ranged from 3.27 to 9.31 ug L^{-1} in agricultural fields. However, several studies have recorded decreased chlorpyrifos concentrations. For instance, Singh et al. (2015), recorded chlorpyrifos concentrations ranging from 0.01 to 0.04 ug L^{-1}, which corresponded with our findings in communal area reservoirs.

Like other ecological studies, this study had several limitations. For instance, in the current study, we collected samples once in triplicates in order to quantify sediments and water pesticide concentrations in the MACADAMIA ORCHARD and communal area reservoirs in water and sediments. Moreover, the samples were collected during rainy season. Nonetheless, the quantity of pesticides in agricultural fields is known to depend on climate or seasons and the abundance of pests within an area (Sharma et al., 2014). For instance, rainy seasons and high temperatures drive increased pest abundances, thus increasing the usage of pesticides and pesticide concentrations in nearby reservoirs (Bloomfield et al., 2006). As indicated by Nguyen et al. (2019) and Nyantakyi et al. (2022), increased pesticide concentrations during rainy seasons compared to other seasons may be due to higher runoffs with increased precipitation of suspended solids containing the pesticide residues in the run–off. The quantity of pests across various seasons may also contribute to the quantity of pesticides being used as pests are known to favour humid, rainy weather since they reproduce more frequently and become more active (Goergen et al., 2016). Consequently, future studies should seasonally quantify the concentrations of acetamiprid and chlorpyrifos in the MACADAMIA ORCHARD and communal area reservoirs to strengthen the understanding of these pesticides and their potential impact on aquatic organisms. However, our results from the preliminary foundation that will enable us to understand the occurrence, distribution and concentrations associated with MACADAMIA ORCHARD reservoirs within the tropical regions of the world.

3.5 Conclusions

From our findings, it can be concluded that reservoirs are continuously contaminated with pesticide runoff from the nearby MACADAMIA ORCHARDS and/or agricultural fields. For instance, the present study showed that MACADAMIA ORCHARD reservoirs are highly contaminated by acetamiprid and chlorpyrifos pesticides. As detailed in the discussion, it is very much clear that concentrations of these pesticides varied in water and sediments across MACADAMIA ORCHARDS and communal area reservoirs with MACADAMIA ORCHARDS, indicating increased concentrations. The increased concentrations in the MACADAMIA ORCHARDS are most likely due to the effective use of these pesticides during pest control for macadamia plantations. The contamination of MACADAMIA ORCHARD reservoirs by these pesticide residues from MACADAMIA ORCHARDS thus poses a greater impact on the aquatic ecosystems. This may lead to deleterious effects on the aquatic organisms and the public dependent on the water supply from the contaminated reservoirs. Future studies should assess the bioaccumulation and biomagnification of these pesticides through pesticides levels in aquatic trophic levels.

Chapter 4: Behavioural Responses and Mortality of a Freshwater Fish *Oreochromis mossambicus* to Three Commonly Used Macadamia Plantation Pesticides

Plate 4. Dead fish *Oreochromis mossambicus* after exposure to Karate Zeon 10 CS, Mulan 20 SP, and Pyrinex 250 pesticides in 3 L glass bottles showing mortality and surfacing behaviour after 24 h. Photo by Tatenda Dalu

4.1 Introduction

Freshwater ecosystems are among the most threatened by multiple anthropogenic stressors (Dudgeon, 2019; Dalu and Wasserman, 2021). One of the major stressors for freshwater ecosystems is agricultural pesticides, which are introduced via point and non–point sources (Weber et al., 2018). Pesticides are intended to protect agricultural production from pests; however, their residues reach far beyond their target areas via atmospheric, overland, sub–surface and groundwater routes (Knillmann et al., 2018). Surface run–off and wastewater effluents from agriculture and urban/developed areas are among the most important entry pathways for pesticides into aquatic ecosystems, with well–documented adverse effects on these environments (Holvoet et al., 2005; Köck–Schulmeyer et al., 2013; Cruzeiro, et al., 2017). Pesticide contamination of surface waters affects aquatic invertebrate and vertebrate community structure and diversity (Morrissey et al., 2015). Globally, pesticide use in agriculture has increased significantly to control problematic species, particularly in orchard plantations (Lykogianni et al., 2021).

Within South Africa, macadamia nut plantations have been rapidly increasing in scale since the early 1990s, after the first introduction of *Macadamia integrifolia* seeds from Hawaii to South Africa more than 100 years ago (SAMAC, 2021). The South African macadamia nut plantations are centred in the sub–tropical regions of the Mpumalanga Lowveld (i.e., Nelspruit area), Limpopo (i.e., Luvuvhu River Valley) and KwaZulu–Natal (Macadamias South Africa (SAMAC), 2021). While relied upon for productivity, pesticide usage in these rapidly increasing macadamia nut plantations has the potential to pollute the aquatic ecosystems through several routes, including spray drift, drainage, surface run–off, and/or accidental spills (Sattler et al., 2007; Baker et al., 2008; Dalu and Tavengwa, 2022). Common and/or persistent pesticides are frequently detected as harmful pollutants in soil, surface water and groundwater because of their continuous application within agricultural landscapes (Riise et al., 2004; Kruawal et al., 2005; Jaipieam et al., 2009).

Even though synthetic chemical production has increased rapidly in recent decades (Dalu and Tavengwa, 2022), the effect of these chemicals on non–target habitats and biota has been overlooked (Moreira–Santos et al., 2019). Fish play a keystone role in freshwater ecosystems through, for example, trophic interactions that structure food webs (Wasserman et al., 2013). They are also very often important food sources for organisms further up the food web, including humans that readily exploit these food resources (Broadhurst et al., 2002; Ellender et al., 2017). Fish can absorb pesticides through gills, skin, or alimentary ducts (Schlenk, 2005; Banaee, 2012; Banaee, 2013). These organisms are potentially vulnerable to aquatic pollutants, such as pesticides used in macadamia plantations, which could severely compromise certain physiological and biochemical processes (Banaee, 2013). Several studies (Oti et al., 1997; Rekha et al., 2008) have reported the mechanisms through which pesticides affect fish, with the goal of monitoring, controlling and possibly identifying concentrations that pose little or no effect on aquatic organisms. Pesticide exposure may affect fish abundance, causing significant mortalities and/or altering behaviours (Jordaan et al., 2017). Although some fish metabolise and excrete certain pesticides, continuous dietary exposure may lead to bioaccumulation and impact their health or fecundity/offspring (Tejeda et al., 2007).

To our knowledge, no attempts have been made to document how pesticides used in the macadamia plantations in South Africa, such as Karate Zeon 10 CS, Mulan 20 SP, and Pyrinex 250 CS, affect fish. Macadamia plantations are often in close contact with the freshwater ecosystem and the use of these pesticides is an accepted management strategy for controlling pests in South Africa's macadamia plantations. As such, we aimed to assess the behavioural response of a common, often dominant, and economically/ecologically important fishery species, Mozambique tilapia, *Oreochromis mossambicus* when exposed to Karate Zeon 10 CS, Mulan 20 SP, and Pyrinex 250 CS at various concentrations and any potential for recovery after exposure to pesticides. To this end, we used a median lethal dose (or LD50) approach, whereby for each test pesticide, the dose at which 50% of test animals die was determined. Once the LD50 of each pesticide was determined, sub–lethal concentration effects on behavioural responses of the fish were explored.

4.2 Materials and methods

4.2.1 *Pesticide background*

The three pesticides used in the experiment were Karate Zeon 10 CS (capsule suspension; Adama, Kempton Park, South Africa), Mulan 20 SP (soluble powder; Adama, Kempton Park, South Africa), and Pyrinex 250 CS (capsule suspension; Syngenta, Midrand, South Africa). Karate Zeon 10 CS contains lambda–cyhalothrin (pyrethroid) as an active ingredient and consist of 100 g L^{-1}, whereas Mulan 20 AS contains acetamiprid (neonicotinoid) as an active ingredient and consists of 200 g kg^{-1} and Pyrinex 250 CS contains chlorpyrifos (organophosphate) as an active ingredient and consists of 250 g L^{-1}. Pyrinex 250 CS and Karate Zeon 10 CS are capsule–suspension contact insecticides for the control of macadamia insects, such as thrips *Scirtothrips aurantia*, cotton aphid *Aphis gossypii*, and stink bugs *Bathycoelia distincta* and *Nezara viridula*. Mulan 20 SP, however, is a water–soluble powder systemic contact. These pesticides are broad–scale insecticides for the control of pests on macadamia, canola, citrus, tomatoes, wheat, barley, oats, cotton and rooibos tea. The spray mixture (or dilution) volumes of 200 mL per 100 L water, 5 mL per 100 L water, and 40 g 100 L^{-1} water for Karate Zeon 10 CS, Mulan 20 SP, and Pyrinex 250 CS, respectively, are highlighted according to the manufacturer's recommendations. These pesticides can be applied by any medium or high–volume applicator, aerially or on the ground, provided that the applicator is correctly calibrated and fitted with an efficient agitation mechanism. These pesticides are diluted with Hydrobuff (Nutrico, South Africa) to maintain pH level and Wetta (Nutrico, South Africa) to act as a surfactant. For this study, these pesticides were selected because they are the most commonly used products by macadamia farmers in the Luvuvhu River Valley area for pest control (Nortjé et al., 2017; Pheiffer et al., 2014).

4.2.2 *Fish sampling*

Freshwater Mozambique tilapia, *Oreochromis mossambicus* were caught from a small reservoir in Duthuni Village (coordinates: –22.967657" S, 30.397369" E), Limpopo Province of South Africa, using a 20m seine net (depth 1.5 m, mesh size 0.5 cm) in October 2020 and 2021 after obtaining Research and Animal Ethics (Ethical clearance number: FSEA/22/ERM/01/1103) from the University of Venda. All fish were transported in eighteen 20 L white polyethylene buckets (15–20 individuals per bucket) to the Department of Ecology and Resource Management Laboratory, University of Venda. We mixed 20 L of filtered reservoir water (through a 63 µm mesh) and 200 L of borehole water (1:10 ratio) which were also collected for the experiments. All the fish were size–matched with regard to total length (TL) to reduce the influence of size–related differences in behaviour when exposed to pesticides (TL, mean ± SD: 40.5 ± 2.3 mm). The Mozambique tilapia were housed in 20 L buckets with source water and continuous aeration at 23.2 ± 0.3 °C, i.e., ambient temperatures, and fed once during the 48–h acclimation period prior to experimentation, following procedures in Mbedzi et al. (2020).

4.2.3 *Experimental design*

The Organization for Economic Co–operation and Development (OECD) (OECD, 2005) guidelines for acute toxicity studies were followed for the experimental procedures. The experiment was conducted during October 2020 and 2021. Each pesticide (3) had four replicates, and a total of 216 fish were used for the entire experiment, excluding controls (9 treatments × 3 groups × 4 replicates × 2 fish per replicate; see Table 4.1). For each replicate, two individual fish of similar size/age (SL ± SD: 32.4 ± 2.1 mm) were placed randomly in an experimental container to avoid confounds (3 L glass containers with 2.9 L filtered reservoir and borehole water (ratio 1:10)) using a square 10.16 cm wide and long aquarium net made of fine mesh. Fish were juveniles and characterised by a silvery body with a straight forehead profile, with 6–7 vertical bars, three spots along flanks and 12–15 dorsal fin rays. After adding fish in each treatment, the fish were allowed to acclimate for 4 h prior to chemical addition. Further, a pesticide–free treatment was employed to serve as a control (*n* = 36) for each pesticide treatment with two fish of similar size. Once the chemicals were added and the experiment started, aeration was stopped, and no further aeration was provided for the duration of the experiment (24 h). Treatments K7 (100 µL), M8 (500 mg) and P8 (4500 µL) were field concentrations that the farmers use within the Luvuvhu River Valley area (Table 4.1). Concentrations for K1 (0.7 µL), M1 (0.3 mg) and P4 (33.3 µL) (Table 4.1) were decided based on background values in aquatic environments found in the literature: 0.11–0.14 µg L^{-1} (He et al., 2008), 0.2–5 µg L^{-1} (Zoumenou et al., 2019) and 0.5 µg L^{-1} (Werner et al., 2000), for Karate Zeon 10 CS, Mulan 20 SP, Pyrinex 250 CS, respectively. After the additions of the pesticide solutions to the experimental containers, the behaviour of the fish was observed constantly for the first 1.5 h of the experiment and then observed again at 3 h, 6 h and 24 h for each pesticide treatment exposure by personnel. At 3 h, 6 h and 24 h, the constant observation time was 30 min. In an event where personnel were absent, behaviours of the fish were video recorded and estimated using a stopwatch. The time to the onset of certain behavioural endpoints associated with each pesticide exposure was assessed during the study. These behaviour endpoints were the time from initiation of pesticide exposure (t0) to when the first fish (*a*) started swimming erratically (hereafter referred to as swimming), (*b*) were seen positioned vertically in the water column, (c) exhibited motionless behaviour, (*d*) attempted to breach the surface to breathe air, (e) was first seen exhibiting loss of equilibrium, and (*f*) showed mortality, defined as the cessation or absence of movement after repeated tactile stimulation/prodding for a period of 2 min (Booth et al., 2015). These were carried out for each replicate, treatment and concentration. Any fish surviving to the end of the 24 h exposure period was moved to a water recovery tank to determine if a moribund state resulted in death or if recovery was possible, being subsequently inspected after 24 h.

Table 4.1. Pesticide treatments and volumes per litre thereafter referred to as concentrations, applied to 2.9 L water with Mozambique tilapia, *Oreochromis mossambicus* to assess behavioural responses and mortality.

Treatment	**Group**	**Concentrations**
K1	Karate Zeon 10 CS	0.7 µL
K2	Karate Zeon 10 CS	3.3 µL
K3	Karate Zeon 10 CS	6.7 µL
K4	Karate Zeon 10 CS	10 µL
K5	Karate Zeon 10 CS	16.7 µL
K6	Karate Zeon 10 CS	80 µL
K7	Karate Zeon 10 CS	100 µL
K8	Karate Zeon 10 CS	150 µL
K9	Karate Zeon 10 CS	200 µL
M1	Mulan 20 SP	0.3 mg
M2	Mulan 20 SP	0.5 mg
M3	Mulan 20 SP	1.7 mg
M4	Mulan 20 SP	3.3 mg
M5	Mulan 20 SP	6.7 mg
M6	Mulan 20 SP	100 mg
M7	Mulan 20 SP	300 mg
M8	Mulan 20 SP	500 mg
M9	Mulan 20 SP	1000 mg
P1	Pyrinex 250 CS	0.7 µL
P2	Pyrinex 250 CS	3.3 µL
P3	Pyrinex 250 CS	16.7 µL
P4	Pyrinex 250 CS	33.3 µL
P5	Pyrinex 250 CS	66.7 µL
P6	Pyrinex 250 CS	1250 µL
P7	Pyrinex 250 CS	2250 µL
P8	Pyrinex 250 CS	4500 µL
P9	Pyrinex 250 CS	8750 µL
C	Control	No chemicals

4.2.4 *Statistical analyses*

Prior to analysis, all data were tested for normality (Shapiro–Wilk test, $p > 0.05$) and homogeneity of variances (Levene's test, $p > 0.05$) and were found to be normal with homogenous variances. To assess the behavioural endpoints and mortality among the pesticide treatments, we used one–way Analyses pf Variance (ANOVA). To assess the onset of behavioural changes within each pesticide group (i.e., Karate Zeon 10 CS, Mulan 20 SP, Pyrinex 250 CS) as a grouping variable for all replicates, we used pesticide treatments (i.e., K1–K9, M1–M9, P1–P9) as explanatory variables and each onset behaviour as response variables. Individuals were included as fixed factor to account for onset of fish behaviours. To assess lethal dose 50 of each pesticide group we used probit analysis (95 % confidence limits for concentration). Chi–square goodness–of–fit tests were also performed to check if the probit models fit the data adequately. Tukey post hoc tests were used to evaluate multiple comparisons among pesticides concentrations where effects were significant. In all analyses, significance was inferred at $p < 0.05$. All statistical analysis were performed using IBM SPSS version 16.0.0.0 (SPSS, 2007).

4.3 Results

Mozambique tilapia, *Oreochromis mossambicus* in the control treatments did not die or exhibit any abnormal behaviour. When *O. mossambicus* were exposed to Karate Zeon 10 CS, Mulan 20 SP, and Pyrinex 250 CS, abnormal behaviour such as fast swimming, surface breaking (i.e., air breathing), vertical positioning, being motionless, loss of equilibrium and mortality were observed. The average time to onset of behavioural symptoms associated with pesticide toxicity exhibited a dose–dependent response, with slower vertical positioning, motionless, loss of equilibrium and mortality responses generally exhibited at low concentrations and faster swimming and surfacing at high concentrations of each treatment (Figure 4.1).

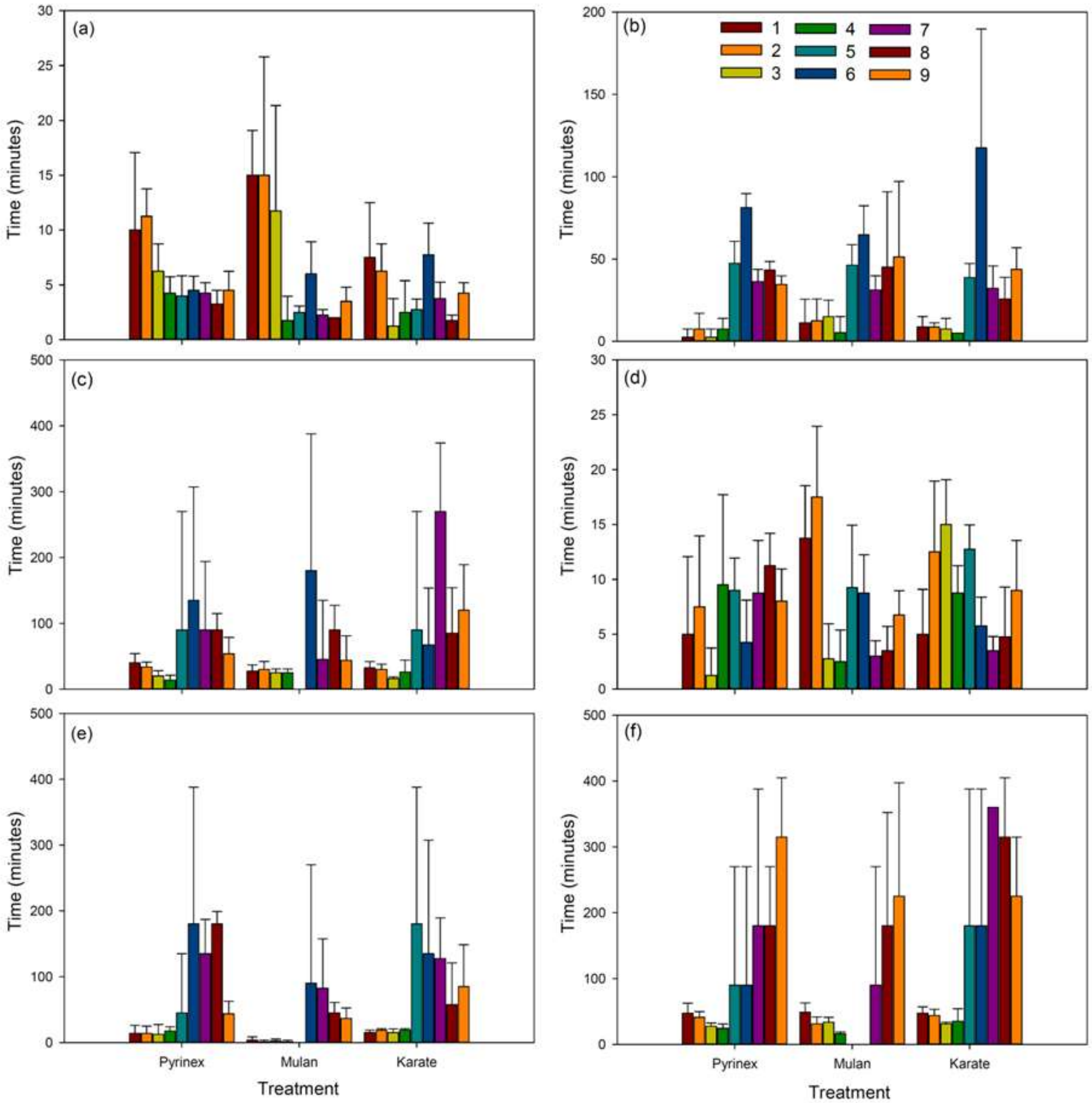

Figure 4.1. The mean time (±standard deviation) to onset of behaviour effects associated with (*a*) swimming erratically, (*b*) vertical, (*c*) motionless, (*d*) surface, (*e*) loss of equilibrium and (*f*) mortality under various chemical treatments. The numbers represent various concentrations across Karate Zeon 10 CS, Mulan 20 SP, Pyrinex 250 CS, see Table 4.1 and note the differences.

Fish behaviour was affected by pesticide treatment concentrations and time to onset behaviour (Figure 4.1, Table 4.2). Immediately after exposure to Karate Zeon 10 CS, Mulan 20 SP, and

Pyrinex 250 CS separately, the fish started to swim actively and surfacing prior to vertical positioning, being motionless, losing equilibrium, and then dying off; however, these varied in pesticide concentrations and time to onset. When *O. mossambicus* was exposed to Karate Zeon10 CS, the onset to swimming, vertical, motionless, and mortality differed significantly ($p < 0.05$) among concentrations; however, the loss of equilibrium behaviour did not differ significantly ($p > 0.05$) among concentrations (Figure 4.1, Table 4.2). When exposed to Mulan 20 SP, the onset to swimming and surface behaviours differed significantly ($p < 0.05$) among concentrations, however, the vertical, motionless, loss of equilibrium and mortality behaviours indicated no significances ($p > 0.05$) among concentrations (Table 4.2, Figure 4.1). Lastly, in fish exposed to Pyrinex 250 CS, the swimming, vertical and loss of equilibrium behaviours all differed significantly ($p < 0.05$) among concentrations, with motionless, surfacing and mortality behaviours showing no significances ($p > 0.05$) among concentrations (Figure 4.1, Table 4.2). Tukey's post hoc analysis highlighted significant differences among pesticide concentrations associated with onset behaviours, and are presented in Table 4.2, for Karate Zeon 10 CS, Mulan 20 SP and Pyrinex 250 CS, respectively.

Table 4.2. Onset behaviours as response variables associated with Mozambique tilapia *Oreochromis mossambicus*, identifying multiple comparisons between concentrations within each pesticide (i.e., Karate Zeon 10 CS, Mulan 20 SP and Pyrinex 250 CS) as explanatory variables. Bold values indicate significant differences at $p < 0.05$.

Behaviour	**Karate Zeon 10 CS**			**Mulan 20 SP**			**Pyrinex 250 CS**		
	df	**F**	***P***	***df***	**F**	***p***	***df***	**F**	***p***
Swimming	**8**	**3.099**	**0.013**	**8**	**3.791**	**0.004**	**8**	**1.961**	**<0.001**
Vertical	**8**	**7.585**	**<0.001**	8	1.442	0.225	**8**	**0.750**	**<0.001**
Motionless	**8**	**3.728**	**0.005**	8	1.636	0.161	8	1.284	0.691
Surface	**8**	**4.775**	**0.001**	**8**	**7.569**	**<0.001**	8	3.900	0.304
loss of equilibrium	8	1.740	0.134	8	1.029	0.439	**8**	**0.937**	**0.007**
Dead	**8**	**5.556**	**<0.001**	8	2.235	0.057	8	0.973	0.069

Mortality following exposure to Karate Zeon 10 CS, Mulan 20 SP and Pyrinex 250 CS at 1.5 h, 3 h, 6 h and 24 h was variable. Across treatments, non–significant differences (F = 2.424, df = 2, p = 0.110) were observed in mortality rates within 24 h (Table 4.2). Across treatments, 100 % mortality was observed after 1.5 h at high concentrations, i.e., treatment 6–9 (Figure 4.1, Table 4.3). Overall, high mortality rates were observed in Karate Zeon 10 CS followed by Mulan 20 SP and lastly Pyrinex 250 CS.

Table 4.3. Mortality of Mozambique tilapia, *Oreochromis mossambicus* (%) following exposure to various concentrations of C–control, Karate Zeon 10 CS (K1–K9), Mulan 20 SP (M1–M9) and Pyrinex 250 CS (P1–P9), with 8 fish each per treatment.

Treatment	**Karate Zeon 10 CS**				**Mulan 20 SP**				**Pyrinex 250 CS**			
	1.5 h (%)	**3 h (%)**	**6 h (%)**	**24 h (%)**	**1.5 h (%)**	**3 h (%)**	**6 h (%)**	**24 h (%)**	**1.5 h (%)**	**3 h (%)**	**6 h (%)**	**24 h (%)**
C	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	25	0	0	0	12.5	0	0	12.5	12.5
2	0	0	0	50	0	0	0	25	0	0	12.5	12.5
3	0	0	0	75	0	0	12.5	12.5	0	0	12.5	25
4	0	0	0	100	0	0	25	25	0	0	25	50
5	0	25	62.5	100	0	12.5	25	50	0	0	12.5	12.5
6	100	0	0	0	100	0	0	0	100	0	0	0
7	100	0	0	0	100	0	0	0	100	0	0	0
8	100	0	0	0	100	0	0	0	100	0	0	0
9	100	0	0	0	100	0	0	0	100	0	0	0

Recovery following 24 h exposure to Karate Zeon 10 CS, Mulan 20 SP and Karate 250 CS treatments was variable. Across treatments, non–significant differences (F = 1.480, df = 2, *p* = 0.248) were observed in survival rates after individuals were transferred from exposure treatments to clean water for certain treatments (i.e., K1–K3, M1–M5, P1–P5) (Table 4.4). Of the fish that were alive at the end of the 24 h treatment exposure, survivability ranged from 12.5–62.5 %, 50–87.5 %, 50–87.5 %, at 24 h to 50–87.5 %, 37.5–87.5 %, 12.5–62.5 at 48 h in clean water, in Karate Zeon 10 CS, Mulan 20 SP and Pyrinex 250 CS treatments, respectively (Tables 4.1 and 4.4). Fish recovery survival rate increased with decreasing toxicity of each pesticide.

Table 4.4. Mozambique tilapia, *Oreochromis mossambicus* survival rates at 24 h after pesticide concentrations of C–control, Karate Zeon 10 CS (K1–K9), Mulan 20 SP (M1–M9) and Pyrinex 250 CS (P1–P9) and recovery at further 24 h (i.e., 48 h) following exposure to in clean water.

Treatment	**Karate Zeon 10 CS**		**Mulan 20 SP**		**Pyrinex 250 CS**	
	24 h (%)	**48 h (%)**	**24 h (%)**	**48 h (%)**	**24 h (%)**	**48 h (%)**
C	100	100	100	100	100	100
1	75	62.5	87.5	87.5	87.5	87.5
2	50	37.5	75	75	87.5	75
3	25	12.5	87.5	87.5	75	75
4	0	0	75	75	50	50
5	0	0	50	37.5	12.5	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	0	0	0	0	0	0

The LD50 values obtained at 24 h exposures and the 95 % confidence limits revealed that Karate Zeon 10 CS, Mulan 20 SP and Pyrinex 250 CS showed high toxicity. The effects of each pesticide on *O. mossambicus* were strongly influenced by the level of concentration (Figure 4.1). No significant differences were observed in mortality across treatments ($p > 0.05$). Results from probit analyses showed 2.1 µL, 5.2 mg and 21.5 µL LD50 values of Karate Zeon 10 CS, Mulan 20 SP and Pyrinex 250 CS, respectively (Table 4.5).

Table 4.5. Probit analysis results with Chi–square goodness–of–fit test to determine lower limit, upper limit and lethal–dose 50 following exposure of Mozambique tilapia, *Oreochromis mossambicus* at various concentrations of Karate Zeon 10 CS, Mulan 20 SP and Pyrinex 250 CS.

Groups	**H**	***df***	***P***	**Lower limit**	**Upper limit**	**LD50**
Karate Zeon 10 CS	2.87	7	0.897	0.8 µL	3.7 µL	2.1 µL
Mulan 20 SP	4.67	7	0.701	2.4 mg	13.5 mg	5.2 mg
Pyrinex 250 CS	4.8	7	0.684	8.8 µL	54.1 µL	21.5 µL

Figure 4.2 presents the probit model. Log doses of each pesticide were plotted against probits i.e., response of *O. mossambicus* when exposed to Karate Zeon 10 CS, Mulan 20 SP and Pyrinex 250 CS at various concentrations (Table 4.1), used to analyse LD50. Karate Zeon 10 CS and Mulan 20 SP were not distributed randomly compared to Pyrinex 250 CS

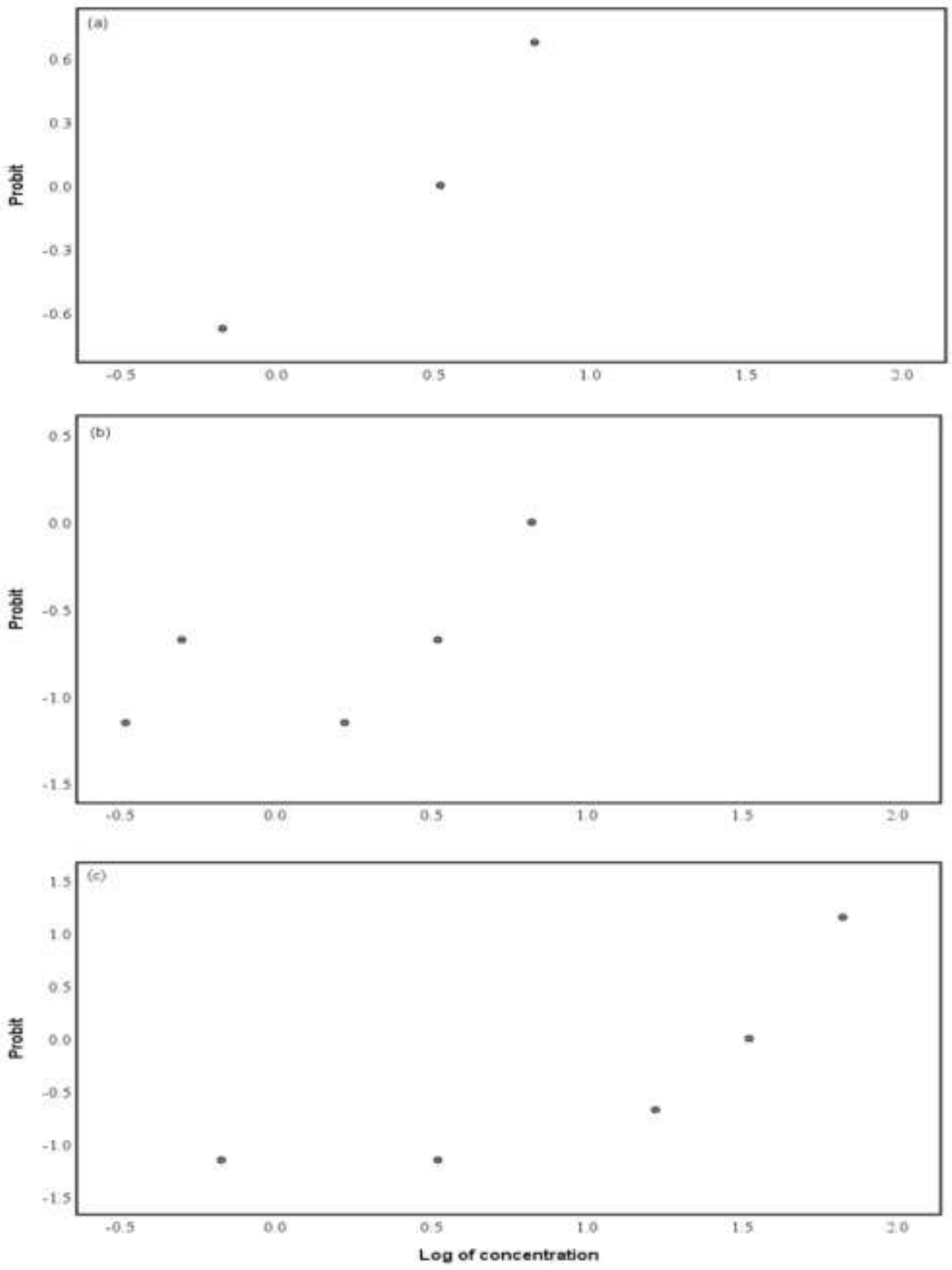

Figure 4.2. Output for the determination of the 24 h median lethal dose (LD50) of (*a*) Karate Zeon 10 CS (µL), (*b*) Mulan 20 SP (mg) and (*c*) Pyrinex 250 CS (µL) to Mozambique tilapia, *Oreochromis mossambicus*. Probits also referred to as proportion killed/mortality, i.e., the response of *O. mossambicus* and log of concentration (toxicity of chemicals), i.e., dose of each pesticide.

4.4 Discussion

Alteration in behaviour patterns is one of the most sensitive indicators of environmental stress (Scherer, 1992). Behavioural information provides insights into external (morphological) and internal (physiological) adaptive changes of an organism due to the impacts of chemical pollutants (Legradi et al., 2018). In the present study, we assessed onset times for dysfunctional behaviours in Mozambique tilapia *O. mossambicus*, exposed to field and background concentrations of Karate Zeon 10 CS, Mulan 20 SP and Pyrinex 250 CS, but found total mortality onsets within a critical 3 h observation period for field concentrations. These results serve to increase the breadth of knowledge regarding the impacts of macadamia pesticide applications, in particular on this fish species. While the effects of aquaculture chemicals on fish have generally been well assessed, relatively few studies have examined the effects of agricultural pesticides such as Karate Zeon 10 CS (Bownik et al., 2019), Mulan 20 SP (Halse et al., 1998) and Pyrinex 250 CS (Montemurro et al., 2002) on aquatic organisms, and, according to our knowledge, none on *O. mossambicus*.

Studies conducted by Boone and Semlitsch (2002), Camargo and Alonso (2006), and Majumder and Kavjraj (2019) indicate that when fish or other animals are exposed to a pesticide at lethal or sub–lethal concentrations, a wide variety of behaviour changes occur, but higher concentrations of pesticides are more often associated with negative effects and more rapid onsets (Puglisi, 2012). Nevertheless, chronic exposure might present additional adaptation to the chemical stress that, if successful, will permit the organisms to continue to function normally in their environment, or they might indicate partial failure of one or more physiological functions (Soares et al., 2016).

The present study indicated that the behavioural responses from exposure to Karate Zeon 10 CS, Mulan 20 SP and Pyrinex 250 CS differed significantly, ranging from early onset behaviours, i.e., at high concentrations to delayed behaviours, i.e., at low concentrations (Figure 4.1). Some non–significant differences were observed across pesticide treatments, for instance, loss of equilibrium within Karate Zeon 10 CS; vertical, motionless, loss of equilibrium and mortality within Mulan 20 SP; and lastly, motionless, surface and mortality within Pyrinex 250 CS treatments. Nevertheless, significant differences were found among concentrations of each pesticide group. Mortality and recovery rates did, however, not differ across pesticide groups. These findings are similar to those of other research on the responses of *O. mossambicus* exposed to pyrethroids (Chandrasekera and Premaratna, 2016), neonicotinoids (Pandya et al., 2020), and organophosphates (Ghayyur et al., 2019). Although we did not find any literature on the response of *O. mossambicus* to Karate Zeon 10 CS, Mulan 20 SP and/or Pyrinex 250 CS, other studies have revealed that when *O. mossambicus* is exposed to agricultural chemicals at different concentrations, they also exhibited different behavioural traits.

The behavioural traits included differential swimming patterns, surfacing activity, gills operculum activity, mucous secretion, food intake and excretions from the fish, which occurred at various levels of pesticides (Shafiei and Costa et al., 1990; Basirun et al., 2019; Kumar et al., 2011). For example, a study by Parithabhanu and Khusnumabegam (2013) showed behavioural traits of *O. mossambicus* such as restlessness, obscured swimming patterns, loss of equilibrium, and mortality, however, when treated to cypermethrin. Here, *O. mossambicus* was recorded to change its swimming pattern and surfacing activity after exposure in all treatments. The fish swam erratically with signs of suffocation at the water surface before becoming motionless, with some fish positioned upside–down prior to mortality. According to Kane et al. (2004), the erratic swimming movements in the fishes might be due to the inhibition of AchE (acetylcholinesterase) by the toxic effect of pesticides. Increased surfacing activity after exposure suggests an elevated rate of metabolism and altered physiology of fish due to hypoxia. According to Saxena and Parashari (1982), the surfacing activity compensates for oxygen deficiency from the medium and extracts more oxygen to meet the extra energy to cope with toxicity.

Mozambique tilapia, *Oreochromis mossambicus* showed a low rate of mortality at a low concentration and high rate of mortality at high concentration across each pesticide. Using Finney's probit analysis as recommended by Miller and Tainter (1944), the LD50 values of *O. mossambicus* in this study were found to be 2.1 µL, 5.2 mg and 21.5 µL for Karate Zeon 10 CS, Mulan 20 SP and Pyrinex 250 CS, respectively. This shows that selected pesticides are highly toxic to *O. mossambicus* at field concentration and can cause 50 % mortality even at background aquatic environment concentrations. Chlorpyrifos is highly toxic to fish (LD50 for 24 h 5.38 µL^{-1}) and affects their survival (Mishra and Devi, 2014). A study conducted by Sharma (2014) concerning organophosphate insecticide found an LD50 value of 0.03 µL^{-1}; however, at 96 h exposure. Sánchez–Bayo (2012) found LD50 value of 0.01–0.5 mg^{-1} looking at non–target fish organisms exposed to neonicotinoids insecticides.

Non–target organisms in different aquatic ecosystems contaminated with pesticides at different concentrations from various sources such as agricultural sectors may suffer deleterious or deadly consequences, resulting in an indirect disruption in food webs. However, the effects of pesticides at field concentrations on *O. mossambicus* cannot directly correspond to what happens in aquatic environments. Moreover, the distribution and stability of these pesticides in water systems and their effects on non–target organisms may vary with the size of water bodies and the amount of chemicals being applied. For example, fish predators such as birds may suffer severe effects from trophic transfer when they feed on fish exposed to pesticides. The overall behavioural responses of *O. mossambicus* when exposed to each pesticide at different concentrations suggest high sensitivity at the tested concentrations, but further studies should examine those under a greater degree of dilution to determine critical thresholds for mortality. Furthermore, the recommended field concentration of each pesticide is about 70–80–times the LD50 determined in this study.

3.5 Conclusions

In conclusion, the present study indicated that pesticides, i.e., Karate Zeon 10 CS, Mulan 20 SP and Pyrinex 250 CS, which are commonly used by macadamia farmers in South Africa, affect behaviours of *O. mossambicus*. Several onset behaviours, i.e., response variables, were found to differ significantly among concentrations of each pesticide group, indicating the capacity of *O. mossambicus* to tolerate various concentrations differently. After assessing the effects of Karate Zeon 10 CS, Mulan 20 SP and Pyrinex 250 CS on *O. mossambicus* and behavioural changes, the following main conclusions can be drawn: (*a*) the nature of behavioural responses of *O. mossambicus* differed when exposed to selected pesticides, but varied in terms of onset times and concentrations; (*b*) effects of Karate Zeon 10 CS, Mulan 20 SP and Pyrinex 250 CS at high concentrations, i.e., field concentrations caused high mortality rates compared to background concentrations; and (*c*) LD50 of each pesticide was determined and was well–within concentrations used in the present study. Regarding the increasing rate of macadamia plantation and the utilization of chemicals as pest controls, continuous exposure of non–target organisms will result in a high concomitant decline in aquatic populations. Further research is needed to assess other macadamia pesticides that are likely to pose less effects, even at a high concentration, to non–target animals and behavioural responses of aquatic macroinvertebrates. Such studies will improve our understanding of the interaction between toxicological and ecological mechanisms (Van den Brink, 2014), helping to improve our understanding of the environmental impacts of chemicals and their risk assessment. The present study’s findings can be used for effective management and to determine the safe level of Karate Zeon 10 CS, Mulan 20 SP and Pyrinex 250 CS disposal through agricultural run–off to natural water bodies to minimise its toxic effect on non–target organisms and aquatic ecosystem.

Chapter 5: Pesticides Drive Differential Aquatic Leaf Litter Decomposition and Mosquito Colonisation Dynamics

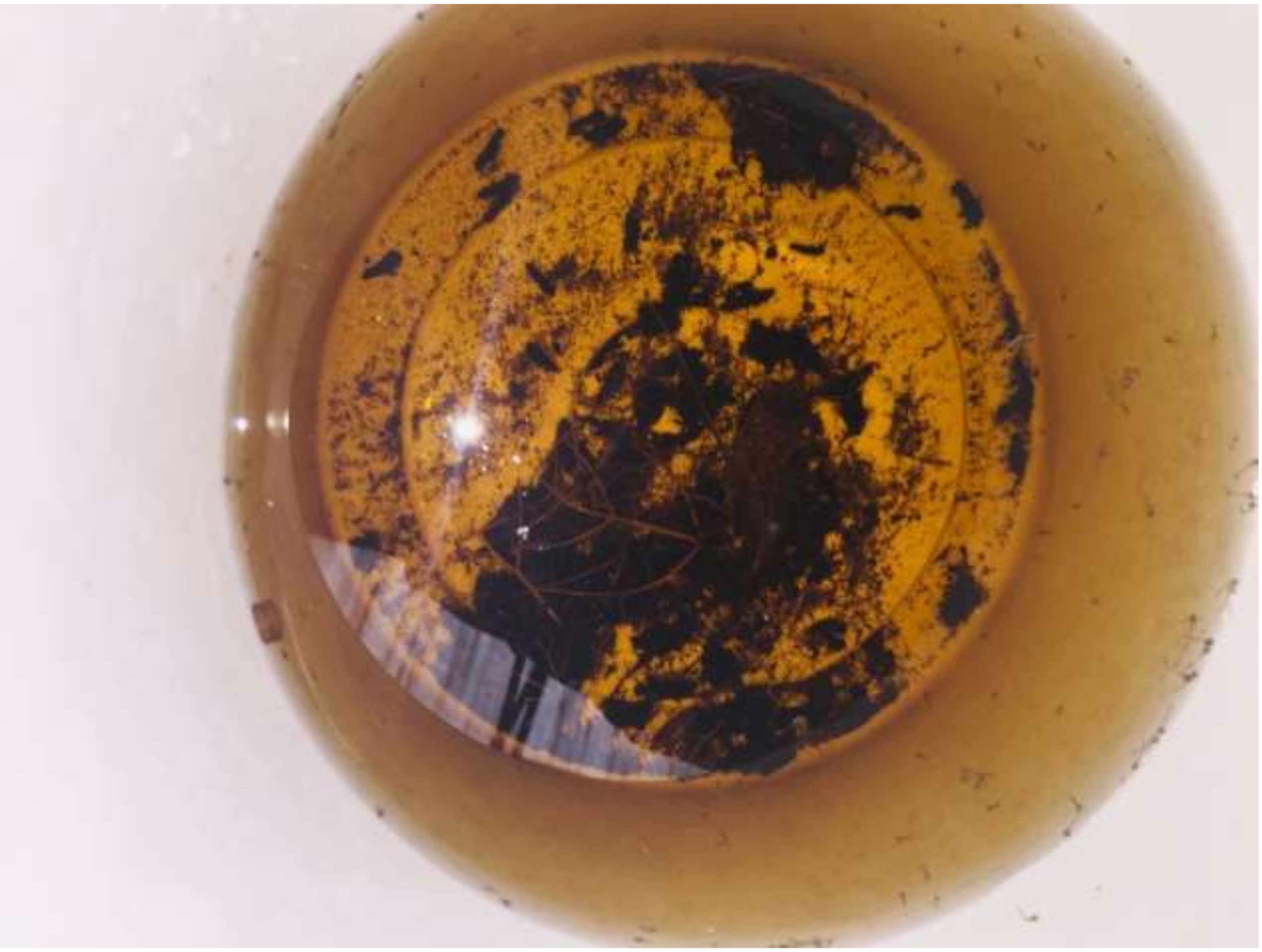

Plate 5. Decomposed macadamia *Macadamia integrifolia* and mosquito (larvae, pupae) in 10 L containers submerged in 1.9 L filtered (filter size 63 µm) borehole + river water and Mulan 20 SP treatment following 8 weeks incubation period. Photo by Thendo Mutshekwa

5.1 Introduction

Agriculture depends mostly on artificial lentic freshwater ecosystems as a source of water for irrigation purposes, such as impounded ponds, reservoirs and lakes (Dalu et al., 2012; Saulnier–Talbot and Lavoie, 2018; Cantonati et al., 2020). These freshwater ecosystems are also typically prone to threats associated with human activities (Olden et al., 2010). Freshwater pollution resulting from anthropogenic activities has become a widespread phenomenon over the past decades. Among pollution sources, agriculture intensification coupled with changes in practices to increase agricultural yields has led to massive inputs of toxicants into many aquatic ecosystems (Vörösmarty et al., 2010; Becker and Liess, 2017). Pesticide inputs may seriously threaten aquatic communities when they contaminate the sediment, water and vegetation. Pesticides can be harmful to various taxa, including fish, invertebrates, plants and even microbial communities, with implications for the entire food web (Rossi et al., 2018).

Agricultural pesticides are known to significantly impact the composition of aquatic communities and ecosystem processes (Rasmussen et al., 2012; Dalu and Tavengwa, 2022). The exposure of communities and ecosystem processes to agricultural pesticides has been shown to reduce primary production, with consequent impacts on invertebrates and other components of food webs (Rasmussen et al., 2012). Primary production and leaf litter decomposition are two key complementary ecosystem processes that ensure organic matter turnover, nutrient cycling and the provisioning of many ecosystem services. These two key processes also play a critical role in aquatic ecosystems (Hooper et al., 2012; Mutshekwa et al., 2020). Some freshwater aquatic ecosystems receive litter from the terrestrial ecosystems. In agricultural landscapes, much of this litter can come from crops which rely on pesticides spray for insect pest control. Experimental studies suggest that agricultural pesticides can alter microbial and detritivore decomposer communities with implications for litter decomposition rates (Zubrod et al., 2014; Cornejo et al., 2020). These processes likely affect aquatic food webs and habitats through reduced nutrient release dynamics and increased detrital accumulation (Shurin et al., 2006). As pesticide use increases, there is a pressing need to understand how aquatic ecosystem processes respond to these pollutants, particularly in the tropics where such studies are scant.

The macadamia *Macadamia integrifolia* (Maiden and Betch), is an evergreen tree nut species native to Australia with important commercial value (Borompichaichartkul et al., 2009). South Africa is the largest macadamia producer globally (50 133 ha under cultivation and 50 000 t of production per year), followed by Australia and the USA, and with China and some South American and African countries growing fast in production (Macadamias South Africa (SAMAC), 2021). In South Africa the industry is predominantly spread over the Mpumalanga (44 %), KwaZulu– Natal (28 %) and Limpopo (20 %) provinces (Macadamias South Africa (SAMAC), 2021). Rapid increases in macadamia plantations have resulted in the significant removal of natural vegetation to make space for macadamia plantations over the last decade (Schoeman, 2009; Linden et al., 2019; Linden, 2019). Given the rapid growth of a highly profitable macadamia industry in South Africa, it is essential to investigate associated horticultural practice impacts on nearby aquatic ecosystems.

To the best of our knowledge, no studies have examined the impacts of pesticides on *M. integrifolia* leaf litter decomposition and associated aquatic ecosystem communities. This study used an ex–situ microcosm approach to examine the effects of commonly used pesticides (i.e., Karate Zeon

10 CS, Mulan 20 AS, Pyrinex 250 CS) in macadamia plantations on leaf decomposition under lentic conditions. We further assess macroinvertebrate establishment associated with the pesticide treatments, using a common, ubiquitous and highly mobile semi–aquatic invertebrate taxon (i.e., mosquitoes) as a proxy for potential effects on colonisation. We further assessed primary production (i.e., chlorophyll–*a*) associated with pesticide treatments over time. We explored these pesticides individually to test our hypotheses that (*i*) *M. integrifolia* leaf litter decomposition will vary among pesticide treatments and that pesticide–free treatments will decompose fastest, due to adverse pesticide effects on decomposition and/or colonisation, (*ii*) phytoplankton biomass will differ among pesticide treatments with pesticide–free environments having the highest levels of chlorophyll–*a*, and (*iii*) exposure of leaf litter to pesticides reduces invertebrate colonisation.

5.2 Materials and methods

5.2.1 *Pesticide background*

The three pesticides used in the experiment were Karate Zeon 10 CS (Syngenta, Pretoria), Mulan 20 SP (ADAMA, Johannesburg), and Pyrinex 250 CS (ADAMA, Johannesburg). Karate Zeon 10 CS has lambda–cyhalothrin (pyrethroid) as an active ingredient, whereas, Mulan 20 SP has acetamiprid (neonicotinoid) and Pyrinex 250 CS has chlorpyrifos (organophosphate). The three pesticides were purchased in local agricultural stores authorised for the sale and distribution of agrochemicals. Pyrinex 250 CS and Karate Zeon 10 CS are capsule suspension contact and stomach pesticides for the control of macadamia insects, such as thrips (*Scirtothrips aurantia*), cotton aphid (*Aphis gossypii*), and stink bugs (various species). Mulan 20 SP is a water–soluble powder, systemic, contact and stomach insecticide to control pests on macadamia, canola, citrus, tomatoes, wheat, barley, oats, cotton and rooibos tea. The spray mixture (or dilution) volumes of

200 mL per 100 L water, 5 mL per 100 L water, and 40 g 100 L^{-1} water are for Karate Zeon 10 CS, Mulan 20 SP, and Pyrinex 250 CS, respectively, and according to the manufacturer recommendations. These pesticides can be applied by any medium or high–volume applicator, aerially or on the ground. The applicator is correctly calibrated and fitted with an efficient agitation mechanism. These pesticides were chosen for this study because they are the most commonly used pesticides by macadamia farmers in the Luvuvhu River Valley area for pest control (Nortjé et al., 2017). The pesticide concentrations in the current study were diluted in a 50 mL container filled with 30 mL of water and 0.5 mL hydrobuff (Nutrico, Johannesburg), which helps maintain the pH level to increase the efficacy of the solution, and 0.5 mL wetta (Nutrico, Johannesburg), thus promoting adherence to leaf litter.

5.2.2 *Study area*

The study was conducted from November to December 2020 at the Department of Geography and Environmental Sciences (–22.977580" S, 30.443819" E), the University of Venda, Limpopo Province of South Africa. Newly fallen *M. integrifolia* leaf litter with no sign of herbivory or decay were collected in late October 2020 from beneath trees by hand, given that many plants reabsorb nutrients from the leaves before senescence (Staaf and Berg, 1982) in the MACADAMIA ORCHARD (−23.058085, 30.280568), Tshakhuma village, Limpopo Province, South Africa. Only leaves with no sign of herbivory or decay were collected. After collection, leaf litter was air–dried at room temperature. Borehole water was collected from the Agriculture Department within the university campus, and river water was collected from the nearest water body (–22.982908" S, 30.442826" E). The area has a humid, subtropical climate and receives an average annual rainfall range of between 400 and 800 mm, with peak rainfall between 1000 and 1500 mm occurring in

January and February. High temperatures (i.e., up to 40 °C) occur between October and March, with the cool–dry season temperatures ranging between 12 °C and 22 °C. The area soil type is loam, which is red due to iron oxide.

5.2.3 *Experimental design*

The experiment was performed using a microcosm approach under controlled conditions. We used 40 × 10 L containers to represent four treatment types (i.e., ten replicates × 3 pesticides + controls without pesticides). After drying, approximately 3 g of the leaf material was weighed out using a RADWAG WPS 2100/C/1 precision balance, for inclusion in each bucket. Each leaf bundle (3 g) was then sprayed with 10 mL pesticide volume using foliar plant spray with different pesticides, following field concentrations that the macadamia farmers use i.e., Karate 10 CS (100 µL), Mulan (500 mg) and Pyrinex 250 CS (4500 µL) and recommendations by the macadamia farmers. Control leaf litter was sprayed with distilled water to stimulate the spraying treatment of pesticide leaf litter. The leaf litter was then left for 24 h to allow for chemicals to dry on the litter to avoid chemicals being washed off. Leaf litter bundles (3 g each) were then added into 10 L white polyethylene buckets (white plastic buckets; 20 cm diameter at the base, 24 cm high; leaf litter mass to water ratio 1:100), filled with 9 L of filtered (filter size 63 µm) borehole + river water (70:30 ratio). Leaves were made to sink by hand upon inoculation and buckets were topped up to the 9 L water level mark every week with filtered (GF/F filter 0.02 µm, Ø 47 mm) borehole water to replenish water lost due to evaporation. River water was charaterised by temperature 27.3 ± 0.2 °C, conductivity 263.5 ± 4.0 µS cm^{-1}, total dissolved solids 127 ± 2.6 mg L^{-1} and pH 6.85 ± 0.1 and borehole water was characterised by temperature 27.2 ± 0.2 °C, conductivity 169.3 ± 4.0 µS cm^{-1}, total dissolved solids 85.5 ± 2.6 mg L^{-1} and pH 6.6 ± 0.1. The initial mean (n = 10) water temperature in the buckets was 25 ± 0.2 °C (SE), conductivity 232 ± 4.0 µS cm^{-1}, total dissolved solids 115 ± 2.6 mg L^{-1} and pH 8.3 ± 0.1. Once inoculated with treated leaf litter, all buckets were placed outside in an open rooftop area in a randomised array, at the Environmental Sciences building, University of Venda, approximately 0.5 km away from the nearest water body.

The experiment ran for 8 weeks. Water parameters (i.e., conductivity (µS cm^{-1}), total dissolved solids (mg L^{-1}), pH and temperature (°C) were measured on the first day of the experiment and every 7 days for the following 7 weeks using a handheld multi–parameter waterproof Cyber Scan 300 (Eutech Instruments, Singapore). In the eighth week, water was collected in each bucket using 100 mL container ($n = 2$) and then filtered (vacuum of < 5 cm Hg) through a 0.7 µm Whatman GF/F filter for chl–*a* determination (see below). After filtration, all mosquito larvae and pupae were collected, including via rinsing from the container. All mosquitoes were immediately transferred to 80 % ethanol, for subsequent identification. Mosquito larvae were broadly identified to genus level using morphological features, mainly of the siphon and head, following recommendations by Jupp (1996). For abundance estimation among replicates, mosquitoes were enumerated according to taxa.

The remaining decomposed leaf litter was removed from the containers after 8 weeks using forceps. Each replicate per treatment was placed into a separate polyethylene zip (40 mm × 40 mm) to prevent loss of decomposed leaves. In the laboratory, decomposed leaf litter was then gently rinsed with sterilised distilled water to remove any algae and mosquito larvae, oven–dried at 60 °C for 48 h and weighed using RADWAG WPS 2100/C/1 precision balance, following recommendations by Harmon et al. (1999). Results were expressed as the percentage of initial oven–dry mass remaining. Additionally, approximately 3 g air–dried leaf litter samples (*n* = 10), which were not incubated in buckets and not sprayed, were oven–dried at 60 °C for 48 h to a constant mass to allow calculation of initial air–dry mass to initial oven–dry mass conversion factors, needed to estimate initial leaf litter oven–dry mass.

5.2.4 *Water samples and chlorophyll–a concentration determination*

After filtration, the filters from water sampled for chl–*a* determination were inserted in separate labelled bottles containing 10 mL acetone and stored in the center of the atrium in an open space for 24 h to allow chl–*a* extraction (Hansson et al., 1998). Chlorophyll–*a* was measured as an estimate of phytoplankton biomass in duplicate polyethylene bottles (100 mL) filled with water from each leaf container. After 24 h, chl–*a* concentration was determined using SPECTROstar NANO (BMG LabTech GmbH, Ortenberg) according to Lorenzen (1967):

$$Chla\ (mg\ m^{-3}) = 11.4 \times K \times \big((665_o - 750_o) - (665_a - 750_a)\big) \times V_e \div L \times V_f$$

Where, *L* – cuvette light–path (cm), *Ve* – extraction volume (mL), V_f – filtered volume (L) and *K* – 2.43.

5.2.5 *Statistical analyses*

The effects of pesticide treatment (4 levels, including controls) and observation week (8 levels) on key water parameters (pH, conductivity, total dissolved solids and temperature) were examined using linear mixed–effects models. Individual containers were included as a random effect (intercept) to account for repeated measures over the experimental period. A Kruskal–Wallis test was used to compare the variation in mosquito larval and pupal groups among treatments (4 levels), as the Shapiro–Wilk test indicated non–normality. Remaining mass (final oven–dry mass: initial oven–dry mass) expressed as percentage of the leaf litter in each treatment was log–transformed prior to the analyses. Remaining mass (%) and chl–*a* concentrations were analysed separately using a one–way Analysis of Variance (ANOVA) according to treatment (four levels). Tukey post–hoc tests were used to evaluate multiple comparisons among chl–*a* concentrations and remaining mass (%) where effects were significant. In all analyses, significance was inferred at $p < 0.05$. All statistical analyses were performed using IBM SPSS Statistics 28.0.0.0.

5.3 Results

5.3.1 *Environmental variables*

Across treatments, conductivity (204.1–551.8 µS cm^{-1}), total dissolved solids (101.9–243.1 mg L−1), pH (5.9–8.9) and temperature (range 25–35.4 °C) differed substantially (Table 5.1). Conductivity, total dissolved solids and pH, measured throughout the monitoring period, differed between treatments and over time, but with no treatment × time interactions (Tables 5.1, 5.2). Control and pesticide treatments i.e., Karate Zeon 10 CS, Mulan 20 SP and Pyrinex 250 CS were all significantly different pairwise ($p < 0.05$) in terms of pH, conductivity and total dissolved oxygen (Table 5.1). Temperature, however, differed only according to time and not treatment, indicating that treatment effects were not confounded by temperature variations.

Table 5.1. Mean (± standard deviation) (n = 10) of environmental parameters measured across treatments over time (8 weeks).

Parameters	**Treatments**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**
Conductivity (µS cm^{-1})	Control	323.0±0	235.5±1	252.9±1.2	315.5±2.4	546.4±1.2	327.9±1.4	278.5±0.1	204.7±0.7
	Karate Zeon 10 CS	232.0±0	225.0±1.9	258.0±4	319.9±5.7	551.8±6.2	340.8±5.0	277.6±2.8	205.8±3.3
	Mulan 20 SP	232.0±0	227.6±1.3	254.5±1.4	307.1±1.2	544.5±2.1	326.0±1.6	272.1±1.2	204.1±0.1
	Pyrinex 250 CS	232.0±0	236.9±5.5	258.0±6.3	318.7±5.4	538.6±16.8	275.7±4.9	275.7±4.9	206.3±3.7
Total dissolved solids mg L^{-1})	Control	115.0±0	115.7±0.3	126.1±0.6	155.1±0.6	240.6±0.9	163.0±0.7	138.8±0.4	102.8±0.3
	Karate Zeon 10 CS	115.0±0	111.7±1.1	128.7±1.9	158.4±2.3	243.1±5.2	166.7±2.9	138.4±1.5	102.8±1.7
	Mulan 20 SP	115.0±0	114.3±0.6	126.9±0.7	153.6±0.6	233.9±1.1	162.9±0.8	124.9±0.5	101.9±0.5
	Pyrinex 250 CS	115.0±0	118.3±3	128.7±3.1	159.2±2.6	240±3.5	163.2±1.9	113.8±1.9	102.7±1.9
pH	Control	8.2.0±0	7.7±0	7.7±0	7.7±0	8.8±0	8.3±0.4	6.5±0	6.0±0
	Karate Zeon 10 CS	8.3.0±0.1	8 .0 ±0	7.7±0	7.7±0	8.9±0	7.00	6.8±0.1	6.00.1
	Mulan 20 SP	8.3.0±0	8.0±0	7.8±0	7.8±0	8.9±0	7.0±0	6.7±0	5.9±0
	Pyrinex 250 CS	8.3.0±0.1	8.0±0	7.8±0	7.7±0.1	8.9±0.1	7.1±0.1	6.6±0.1	6.0±0.1
Temperature °C	Control	25.0±0	33.1±0	22.3±0	30.2±0.2	21.7±0	19.8±0	32.6±0	35.0±0
	Karate Zeon 10 CS	25.0±0	33.5±0.5	22.2±0	30.5±0.2	22.0±0.1	19.8±0	32.9±0.1	35.4±0.2
	Mulan 20 SP	25.±0	33.1±0	22.2±0	30.4±0.3	21.8±0	19.7±0	32.9±0.1	35.3±0.1
	Pyrinex 250 CS	25.0 ±0	33.2±0.1	22.3±0	30.3±0.1	21.7±0.1	19.7±0.1	32.7±0.1	35.2±0.2

Table 5.2. Linear mixed–effects model results considering key water parameters as a function of leaf Treatment and Time (week), and their interaction. F–values are discerned with Type III sums of squares via Satterthwaite's method. Significant *p*–values are in bold.

Parameter	**Predictor**	**F**	***p***
Temperature	Treatment	1.23	0.310
	Time	2565.10	< **0.001**
	Treatment × Time	1.74	0.202
pH	Treatment	30.62	< **0.001**
	Time	4148.40	< **0.001**
	Treatment × Time	1.17	0.292
Conductivity	Treatment	1042.81	< **0.001**
	Time	12988.78	< **0.001**
	Treatment × Time	0.23	0.637
Total dissolved solids	Treatment	185.80	< **0.001**
	Time	3497.23	< **0.001**
	Treatment × Time	1.40	0.251

5.3.2 *Mosquito abundances*

Overall, a total of 456 individual mosquitoes were counted across all treatments. *Culex* spp. comprised 77.4 % of all mosquito numbers, with 0.9 % identified as *Anopheles* spp. and 21.7 % unidentified as they were at the pupal stage. No significant differences were detected for *Anopheles* spp. abundances across treatments (Kruskal–Wallis: H = 2.054, df = 3, *p* = 0.216), whereas *Culex* spp. differed significantly (Kruskal–Wallis: H = 29.126, df = 3, *p* = 0.004). Larval mosquito numbers were by far the highest in Mulan 20 SP and followed by Pyrinex 250 CS, control and then Karate Zeon 10 CS (Figure 5.1). Mosquito pupae of both genera differed significantly across treatments (Kruskal–Wallis: H = 31.848, df = 3, *p* = 0.018). Mosquito pupae numbers were by far the highest in Mulan 20 SP and followed by Pyrinex 250 CS, Karate Zeon 10 CS and then control (Figure 5.1).

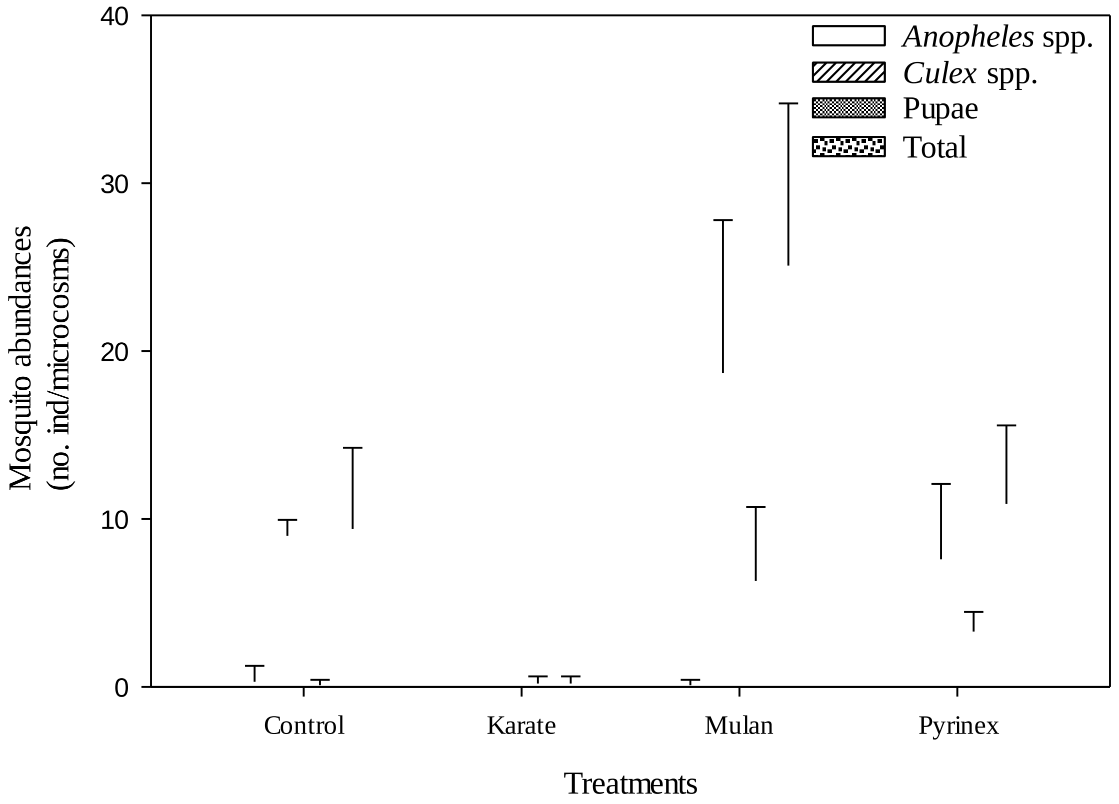

Figure 5.1. Total (larvae + pupae), larval (*Culex* spp. and *Anopheles* spp.) and pupal (all genera) mosquito abundances (mean ± SD, $n = 10$) in 9 L containers across experimental treatments of *Macadamia integrifolia* leaf litter treated with Karate Zeon 10 CS, Mulan 20 SP, Pyrinex 250 CS and pesticide–free controls at the end of the experiment (8 weeks). Abbreviation: ind – individual.

5.3.3 *Chlorophyll–a concentration*

Chlorophyll–*a* concentrations generally increased in control treatments compared to pesticide treatments (F = 0.472, df = 3, $p = 0.033$) (Figure 5.2). Pesticide–free control treatments had significantly higher mean chl–*a* concentration (0.30 mg L^{-1}) compared to Karate Zeon 10 CS (0.02 mg L^{-1}) and Mulan 20 SP (0.04 mg L^{-1}). Chlorophyll–*a* concentrations demonstrated differences among pesticide treatments following a decreasing order of Pyrinex 250 CS > Mulan 20 SP >

Karate Zeon 10 CS. Following Tukey's post–hoc analysis, significant differences were highlighted between treatments i.e., control *vs* Karate Zeon 10 CS ($p < 0.001$), control *vs* Mulan 20 SP ($p < 0.001$), Karate Zeon 10 CS *vs* Pyrinex 250 CS ($p < 0.001$) and Mulan 20 SP *vs* Pyrinex 250 CS ($p < 0.001$).

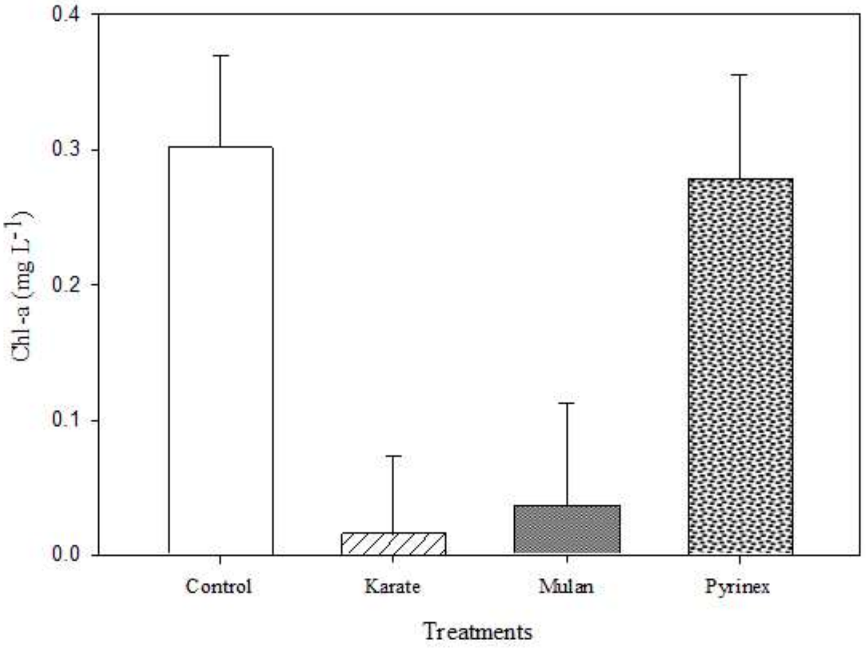

Figure 5.2. Chlorophyll–*a* concentrations (mean ± SD, $n = 10$) from Karate Zeon 10 CS, Mulan 20 SP, Pyrinex 250 CS, and control treatments at the end of the experiment (8 weeks).

5.3.4. *Decomposition*

Leaf litter mass remaining (%) of *M. integrifolia* at the end of the experiment differed significantly between treatments i.e., control, Karate Zeon 10 CS, Mulan 20 SP, Pyrinex 250 CS (F = 11.034, df = 3, $p < 0.001$), following a decreasing order of Mulan 20 SP (56.7 %) < Control (77.8 %) < Karate Zeon 10 CS (84.8 %) < Pyrinex 250 CS (88.5 %). Leaf litter treated with Mulan 20 SP drove increased decomposition, with significantly reduced mass compared to Karate Zeon 10 CS, Pyrinex 250 CS and controls (all $p < 0.001$) (Figure 5.3). Following Tukey's post–hoc analysis, significant differences were highlighted between treatments i.e., control *vs* Mulan 20 SP ($p < 0.004$), Karate Zeon 10 CS *vs* Mulan 20 SP ($p < 0.001$), Mulan 20 SP *vs* Pyrinex 250 CS ($p < 0.001$).

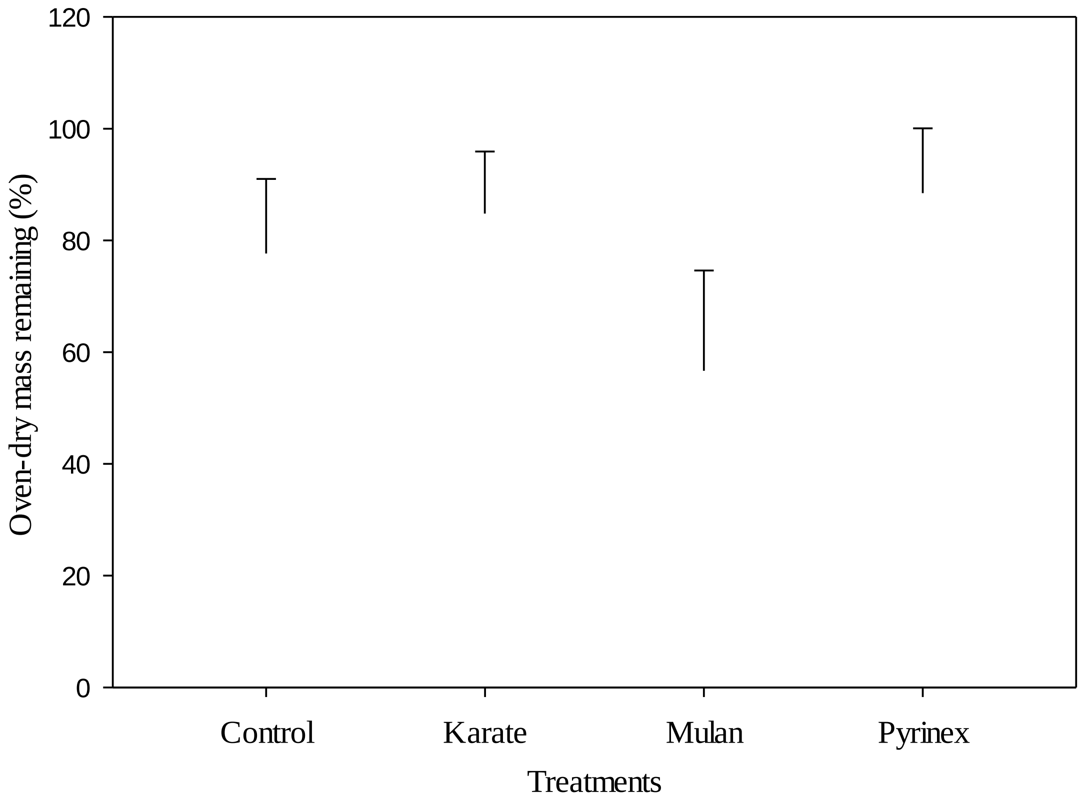

Figure 5.3. Oven–dry mass remaining (mean ± SD, n = 10) of macadamia leaf litter treated with the pesticides Karate Zeon 10 CS, Mulan 20 SP, Pyrinex 250 CS, and pesticide–free (controls) at the end of the experiment (8 weeks).

5.4 Discussion

Several studies have examined the impact of agricultural pesticides on streams, colonisation and aquatic ecosystem processes, providing vital insight into how they affect functioning and integrity (Artigas et al., 2012; Muturi et al., 2017; Cornejo et al., 2020; Cornejo et al., 2021). However, there had been research gaps on how different pesticides affect aquatic ecosystem functioning and integrity in lentic systems. With increased plantation of macadamia near aquatic systems, understanding the effects of agricultural pesticides on macadamia leaf litter decomposition, primary production and colonisation is of high interest, to predict the consequences of changes on aquatic ecosystem functioning. Our results reveal diverse effects of pesticides commonly used in macadamia farms on macadamia leaf litter decomposition, associated primary productivity and invertebrate colonisation dynamics. Agricultural pesticides are known to contaminate streams and, together with associated stressors such as organic pollution and habitat loss, alter their assemblages (Fugère et al., 2016; Rasmussen et al., 2012; Cornejo et al., 2019) and impair their functioning (Dawoud et al., 2017; Cornejo et al., 2020; Fugère et al., 2020).

Using an *ex–situ* microcosm experiment, overall, we found that Karate Zeon 10 CS and Mulan 20 SP had a significantly negative effect on water column primary productivity (using chl–*a* concentrations as a proxy), while Pyrinex 250 CS seemed to have little effect when compared to control treatments and also effected conductivity, total dissolved solids, and pH significantly across treatments and weeks. Furthermore, we found that pesticide type strongly influenced *M. integrifolia* decomposition rates. Pesticide effects on decomposition were significantly higher for Mulan 20 SP compared to Karate Zeon 10 CS and Pyrinex 250 CS. Furthermore, Mulan 20 SP and control treatments also differed significantly. Lastly, we found that select pesticides used in *M.*

integrifolia plantation may promote the colonisation, development and/or growth of invertebrates, specifically mosquitoes. Consistent with previous work using microcosm approaches to mimic aquatic systems, pesticides generally affected decomposition, primary production and colonisation (e.g., Kominoski et al., 2007).

Environmental parameters can regulate invertebrate development and act as indicators of water quality (Morrissey et al., 2015). In this study, conductivity, total dissolved solids, and pH differed significantly across the experimental period and treatments. These measures show that the water–quality parameters we measured may have played a role in differential colonisation across treatments, primary production and decomposition. With regard to primary production, high levels of chlorophyll–*a* often indicate poor water quality and low levels often suggest good conditions; poor (chl–$a > 20$ µg L^{-1}), fair ($5 <$ chl–$a < 20$ µg L^{-1}), good (chl–$a < 5$ µg L^{-1}) (Huang et al., 2011). Consistently high or variable chlorophyll–*a* concentration may indicate the occurrence of algal blooms, which can be harmful to other aquatic organisms. During the present study, phytoplankton biomass varied among the different pesticide treatments and controls. In particular, Karate Zeon 10 CS and Mulan 20 SP caused significantly reduced chlorophyll–*a* concentrations whereas there was a high chlorophyll–*a* concentration in control and Pyrinex 250 CS treatments. The abundance of algal assemblages, which are important food resources for filter feeders such as mosquito larvae (Merritt et al., 1992), are affected by multiple interactive factors such as water chemistry (Stevenson et al., 1996).

Allochthonous inputs are crucial factors affecting resource availability within aquatic systems (Cuthbert et al., 2022). As expected, following a similar study by Muturi et al. (2013), exposure to pesticides affected decomposition significantly. In the present study, leaf litter decomposition, a key ecosystem process in freshwater environments, was reduced (11.5–43.3 %) under pesticide treatments. While there is an absence of literature on the effects of agricultural pesticides on *M. integrifolia* leaf litter, several studies evaluating the effects of agricultural pesticides on various plant species have indicated that pesticides significantly reduced the rate of leaf litter decomposition (Hagen et al., 2006; Piscart et al., 2009; Magbanua et al., 2010; Muturi et al., 2013). A recent study by Sumudumali et al. (2022) reported a reduced mass loss of Panicum maximum leaf litter when exposed to agricultural pesticides. It has been well noted that the decomposition of litter is highly influenced by litter quality (in terms of Carbon: Nitrogen ratios), but also by climate (mainly temperature), and the composition of decomposer organisms. Leaf litter characteristics are of great importance in controlling both the short and long–term decomposition rates. Furthermore, microbial decomposition activity has been indicated to be affected by the availability of oxygen, with low oxygen levels often resulting in slow decomposition rates (Medeiros et al., 2009). The differences in the period of decomposition vary tremendously among plant species, with leaves that decompose slowly often having high leaf toughness scores (Ramos et al., 2021). However, we did not measure leaf toughness, C:N ratios or oxygen concentrations in this study.

The direct cause of mass loss in the present study was unclear and we are unsure of different contributions (i.e., leaching, microbial or invertebrates), and that pesticides effects likely resulted from impaired biological activity. However, a predominance of fungi in microbial decomposers has been found in microcosms experiments (Hieber and Gessner, 2002; Gulis and Suberkropp, 2003; Afolabi et al., 2019). The fact that Mulan 20 SP had increased decomposition indicates that the effects were due to the main active pesticide ingredient, i.e., acetamiprid (neonicotinoid).

Variation in mass loss among pesticide leaf treatments is not surprising, given that impact of pesticides on leaf litter decay can be influenced by pesticide toxicity to microorganisms (Rasmussen et al., 2012). Furthermore, increasing pesticide activity that is associated with agricultural production has been shown to decrease microbial diversity, and decomposition (DeLorenzo et al., 2001; Afolabi et al., 2019).

However, due to the nature of our experimental design, we could not determine whether the effect of pesticides on decay rates was driven by microbial communities, if any, present in the treatments since microbial communities were not assessed. As such, the mechanisms by which leaf litter degradation is affected are yet to be determined. Nonetheless, microbes have been recognised as playing a dominant role in leaf litter decomposition and acting as source of food for aquatic invertebrates (Merritt et al., 1992; Linenberg et al., 2016). It cannot be ruled out that microbes altered colonisation of mosquitoes and decomposition in pesticides treatment since they play a vital role in breaking down debris and act as a source of food for mosquitoes (Muturi et al., 2012).

Semi–aquatic invertebrates are an essential component of aquatic ecosystems. These taxa are found in or on a multitude of microhabitats in streams. A large portion of invertebrates in these systems comprises insects that externally colonise the terrestrial landscape, such as mosquitoes. The presence of mosquitoes in the treatments is not surprising given that several taxa (e.g., *Culex* pipiens) are referred to as container breeders, since they lay eggs in confined, artificial environments (Cuthbert et al., 2019. We demonstrated that *M. integrifolia* leaf litter inputs treated with pesticides, namely Karate Zeon 10 CS, Mulan 20 SP, Pyrinex 250 CS and controls, differed markedly in their effects on mosquito colonisation. These pesticides might adversely affect mosquito natural enemies, and therefore promote their proliferation through enemy release in aquatic environments (Govindarajan et al., 2018). We found that Mulan 20 SP and Pyrinex 250 CS were by far the most favourable pesticides for the development of mosquitoes and caused elevated abundances of larvae and pupae. Elevated mosquito abundances in Mulan 20 SP and Pyrinex 250 CS could be due to their efficacy being lower in water, as a result of their active ingredients i.e., acetamiprid (neonicotinoid) and chlorpyrifos (organophosphate), respectively, being deactivated (Huston and Pignatello, 1999). Although not tested here, these pesticides might also adversely affect mosquito natural enemies, and therefore promote their proliferation through enemy release.

The almost complete absence of *Culex* spp. and *Anopheles* spp. in Karate Zeon 10 CS treatments could be because pyrethroid insecticides cause rapid declines non–target organisms and are known to hold dangerous toxic effects on the exposed organisms (Farag et al., 2021). However, low abundances of *Anopheles* spp. across all treatments could largely be due to their habitat preference for larger systems, i.e., not container–based habitats (Minakawa et al., 2004) or due to their egg differences since *Culex* spp. lay eggs one at a time in a raft of 100 to 300 eggs resulting in less capacity to exploit multiple habitats by an individual, whereas *Anopheles* spp. lay eggs singly at a time (Mbare et al., 2014). Generally, pyrethroids (in this case, Karate Zeon 10 CS) are used against adult mosquitoes and are not suitable for controlling mosquito larvae due to their high fish toxicities. The reason for reduced colonisation could relate to habitat selection due to inadvertent contamination of the larval habitat with permethrin, but we did not test to what extent it was drive by colonisation differences or direct larval mortality post–arrival. Overall, high mosquito abundances and colonisation in Mulan 20 SP treatments indicate better–perceived habitat quality for mosquitoes, such as through the more rapid decomposition and availability of organic matter as resources in the water we observed.

Like other ecological studies, this study had several limitations. First, we quantified the rate of decomposition and invertebrate abundances associated with *M. integrifolia* leaf litter exposed to pesticide concentrations applied directly to *M. integrifolia* plantations, not concentrations in the water bodies. Nonetheless, invertebrates are known to develop in reservoirs associated with high levels of anthropogenic disturbance (Jatulewicz, 2007; Firmiano et al., 2021). Future studies should examine the impact of *M. integrifolia* anthropogenic disturbances on invertebrate colonisation and microbial abundances exposed at environmentally relevant concentrations. However, we believe our results are a good starting point to the understanding of effects of such pesticides on aquatic habitats, invertebrate communities and abundances, primary production as well as decomposition dynamics.

5.5 Conclusions

In conclusion, we assessed variation in decomposition rates, mosquito colonisation and primary production levels i.e., chlorophyll–*a* among pesticide treatments, namely Karate Zeon 10 CS, Mulan 20 SP, and Pyrinex 250 CS as indicators for habitat quality. We found that pesticide treatments induced shifts in decomposition, colonisation and primary production, but these effects varied among pesticide type. The variety of litter decomposition across treatments would be most likely due to leaching. However, leaching levels were not measured. Although not assessed, we can further conjecture that mass loss could have been influenced by the capacity of microbial decomposers to endure pesticides and, thus influence the nutrient levels available to mosquito larvae. These findings provide a basis for understanding the implications of pesticides in *M. integrifolia* plantations and aquatic ecosystem processes. The findings are also essential for health sectors in understanding how anthropogenic chemical contaminants may affect human health since container aquatic habitats are mostly utilised by humans and are dominated by mosquitoes, which transmit a wide variety of life–threatening human pathogens and parasites (Cox–Singh et al., 2008). Based on our results, continuously increased plantation of *M. integrifolia* trees and particular usage of Karate Zeon 10 CS as pest control will likely have a considerable effect on invertebrate colonisation. However, interactions between leaf type, pesticides and invertebrates can be highly complex and difficult to predict. We, therefore, propose that it is essential to consider risks to invertebrate communities in aquatic ecosystems during the production of pesticides. The use of pesticides in agriculture represents an important selective force likely to affect all types of aquatic organisms. Therefore, further studies are required to directly link mosquito abundance and community structure associated with detritus types and pesticide exposure. Furthermore, the current findings stress the need for studies on macadamia leaf litter chemical characteristics to help give insight of decomposition dynamics of macadamia leaf litter and the effects on functioning of freshwater ecosystems and associated services.

Chapter 6: Aquatic Macroinvertebrate Community Colonisation Dynamics in Reservoirs Across Different Land–use Types

Plate 6. Green mesh substrate filled with stones (length 15–20 mm; width and depth, range 10–15 mm) after retrieval from the MACADAMIA ORCHARD reservoir following 6 weeks incubation period. Photo by Thendo Mutshekwa.

6.1 Introduction

Tropical reservoirs are dynamic habitats that support significant biological diversity and provide important ecosystem services (Moberg et al., 2003; Barbier et al., 2017). The degree to which aquatic species ecological and biological features determine their distribution and abundance has intrigued ecologists for a long time (Heino et al., 2018). Among aquatic species, macroinvertebrates are the most diverse and abundant organisms in freshwater aquatic ecosystems and are a key component of freshwater aquatic ecosystem functioning (Kratzer, 2002; Dalu et al., 2021). Macroinvertebrate species differ in their response to habitat features, environmental variables, and anthropogenic stressors with implications for composition dynamics at the community level (e.g., Matthaei et al., 2006; Wang et al., 2007; Feld et al., 2007; Dalu et al., 2012; Mofu et al., 2021).

In many developing regions, landscapes are comprised of a mosaic of communal human–settlement and agricultural areas. In subtropical regions, these landscapes are often closely associated with natural water bodies (Desmarais et al., 2002; Jia and Gao, 2017) and associated reservoirs. Within the context of large–scale agricultural areas, reservoirs are usually located within a delineated farm area and vulnerable to pesticide contamination, while communal reservoirs are located outside of farms and thus exposed to discrete pressures. These mosaics of reservoirs exposed to different stressors provide an opportunity to assess the discrete effects of each stressor type, on ecosystem dynamics. Assessing the link between these different anthropogenic activities and aquatic organisms within aquatic ecosystems is important for our understanding of the broader impacts of agricultural/communal landscape mosaics. This also has implications for broader patterns of species distributions and colonisation in a changing world (Verberk, 2011).

With rising global demand for agricultural commodities for use as food, feed, and bioenergy, pressure on land is increasing (Alexander et al., 2015). During the recent decades, a high percentage of aquatic ecosystems has been threatened or disappeared as a result of increasing land–use activities (Marchetti et al., 2008). This constitutes an immense potential threat for the populations of aquatic organisms. Increasing of many agricultural activities over the decades with advances in technology and land use intensification in subtropical landscapes, and more especially over the last 70 years, has allowed human to greatly expand land use (Ramankutty et al., 2006). Subtropical areas are no exception; they are drastically and rapidly covering increased landscape over a short period of time. This increase is mainly driven by increasing global population and growing demand of food products (Ramankutty et al., 2006; Henchion et al., 2017). However, not all agricultural operations are sustainable managed. As such, unmanaged agricultural activities continue to put pressure on aquatic ecosystems (Sivakumar et al., 2000). As the demand for agricultural production increases and is expected to continue to increase over the next few decades, more especially in developing countries such as in the subtropical areas, the effects of agricultural activities on aquatic ecosystems are becoming more intensive.

In shallow aquatic ecosystems, benthic habitats are particularly important for macroinvertebrate communities. These habitats are sites for accumulation of organic matter of autochthonous and allochthonous origin, and serve as sources of nutrients, organic compounds, and solutes which interact with the water column (Sivakumar et al., 2000). They are also characterised by diverse substrate types, with implications for macroinvertebrate community structures (Sivakumar et al., 2000). The preferences of macroinvertebrates for various artificial substrates in aquatic ecosystems is the main focus of much research in the past decade (Geist and Hawkins, 2016). Colonisation of artificial stones by macroinvertebrates has been extensively studied (e.g., Khalaf et al., 1980; Zbinden et al., 2008; Rosa et al., 2013), and stones artificial have been highlighted as useful experimental substrate for assessing colonisation dynamics (Miyake et al., 2003). Artificial stones tones provide stable and suitable substrates for colonisation (Pfeiffer et al., 2022) as they are less susceptible to being carried by water movement and other natural disturbances such as storms and floods (Death and Winterbourn, 1995; Bhandari et al., 2018). Furthermore, their uneven surfaces facilitate the movement and attachment of macroinvertebrates in reservoir or slow–flowing river system, offering greater stability and availability of micro–sites for colonisation (Ayres–Peres et al., 2006). Due to their roughness, stones provide a three–dimensional habitat that protects macroinvertebrates from various disturbances (Ayres–Peres et al., 2006). As such, focusing on stones in freshwater ecosystem is a good way of understanding macroinvertebrates communities and associated environmental stressors and/or pesticide interactions.

Aquatic macroinvertebrates play a crucial role in transferring nutrients and environmental pollutants to higher tropic levels (Berger et al., 2017). One of the major pollutants in reservoirs is pesticides introduced in aquatic ecosystems due to anthropogenic activities such as agriculture land use (Berger et al., 2017). Aquatic ecosystems in agricultural areas are frequently exposed to agricultural land–use and/or chemical pollutants (Aktar et al., 2009). These agricultural activities can alter the structure and function of biota in these ecosystems (Aktar et al., 2009). As such, the current study seeks to contribute to the comprehension of the occurrence and distribution of macroinvertebrates and the effects on macroinvertebrates of different land–use types. We observed increased acetamiprid and chlorpyrifos pesticide concentrations in MACADAMIA ORCHARDS reservoirs and decreased pesticides concentrations in the communal area reservoirs (see chapter 3). As a results, the two reservoir areas were found to be the most suitable in understanding if land–use types alter macroinvertebrate colonisation dynamics by comparing colonisation in reservoirs with detected increased pesticide concentrations (i.e., MACADAMIA ORCHARD reservoirs) and those with no to low pesticide concentrations (i.e., communal area reservoirs).

Here, using stones as "artificial substrate", we assessed: (*a*) macroinvertebrate colonisation dynamics in landscapes with different catchment practices to explore the feasible impact of pesticides on macroinvertebrate community and (*b*) the influence/relationship of key water or sediment variables to macroinvertebrate community structure within the MACADAMIA ORCHARDS and communal area reservoirs. Artificial stone substrates were chosen instead of conventional sweep-sampling method since there provide a valid alternative method for sampling macroinvertebrate over a period of time. We hypothesised that 1) in both environments, successional community dynamics would be marginal and different, given that both reservoirs are polluted, but 2) primary colonising and successive community structure would be discrete between environments, with environment being a greater driver of community structure than time, 3) water and sediment characteristics would differ between the two reservoirs and that sediments are more likely to influence macroinvertebrate communities due to a strong adsorption capacity for pollutants.

6.2 Materials and methods

6.2.1 *Study area*

The experiment was conducted between 02 March and 13 April 2022 in similar sized MACADAMIA ORCHARD and communal reservoirs. MACADAMIA ORCHARD reservoirs (Site 1, –23°06'31.6"S, 30°15'54.2"E; site 2, –23°06'54.5" S, 30°15'46.5" E) are located in the southern foothills of the Soutpansberg Mountains, 20 km east of the town of Makhado (previously Louis Trichardt) and the communal area reservoirs (Site 3 – 22°96′58.12.19″S, 30°39′56.45″E; site 4 – 22°59′57.24″S, 30°39′57.53″ E) are located in Duthini village, Thulamela Municipality within the same region (see Figure 3.1).). Both MACADAMIA ORCHARDS and communal area reservoirs are impounded streams and have estimated surface area ranging from 10.78 to 19.6 m with mean surface of 15.70 m and average maximum depth of 6 m. MACADAMIA ORCHARD reservoirs were selected due to their excessive concentrations of pesticides, whereas communal area reservoirs were selected due to low or absence of pesticide concentrations (see chapter two). Accordingly, pesticides (i.e., acetamiprid and chlorpyrifos) in the MACADAMIA ORCHARD reservoirs were found to range from 5.67 to 14.48 µg L^{-1} in sediments and < 6.51 µg L^{-1} in surface water (see Chapter 3). Both reservoirs are mainly used for domestic water supply and irrigation. Pesticides in the communal area reservoirs were < 0.13 µg L^{-1} in water and not detetcted in sediments. MACADAMIA ORCHARDS were characterised by water lilies, whereas, communal area reservoirs were characterised by tape grass *Vallisneria* spp.

6.2.2 *Experimental design*

Mesh bags with stones were deployed into macadamia and communal area reservoirs to assess macroinvertebrate colonisation dynamics over time, following Suárez et al. (2022). Ethical clearance to conduct the current study was obtained from the University of Venda (Research and Animal Ethics (ethical clearance number: FSEA/22/ERM/01/1103)). Similar sized stones (length 15–20 mm; width and depth, range 10–15 mm) were collected from the Mutshundudi River and scrubbed with a brush to ensure that the stones are macroinvertebrates free. The stones were added to 15 cm × 15 cm course mesh bags (5 mm mesh size) to allow easy macroinvertebrates movement, with 48 mesh bags (i.e., 4 sites × 2 replicates × 6 weekly temporal sampling events) being deployed. Each bag was filled with stones and attached with zip ties. All bags were randomly introduced into both study reservoirs on Day 0 (2 March 2022) at a depth of ~0.5–1 m approximately 1 m away from the shoreline. The bags were attached to ropes which were then attached to wooden sticks near the reservoir shoreline to easy retrieval. The bags were placed at least 1 m apart, with bag retrieval taking place at days 7, 14, 21, 28, 35, and 42 (hereafter referred to as Week 1–6).

6.2.3 *Macroinvertebrate colonisation*

At each site, two mesh bags were retrieved during each sampling week. Once removed from the water, the bags were immediately placed in a tray and the contents emptied, to avoid the loss of macroinvertebrates. The macroinvertebrates were carefully separated from stones, and then preserved in 70 % ethanol in labelled in 500 mL polyethylene containers for further processing in the laboratory. In the laboratory, macroinvertebrates were sorted under dissecting Olympus microscope using forceps and identified to lowest taxonomic level following a guide by Fry (2021) and abundances enumerated. All taxa were further assigned to a functional feeding group (FFG) (i.e., collector–gatherers, collector–filterers, scrapers, and predator) following a guides by Merritt and Cummins (1996), Cummins et al. (2005) and Merritt et al. (2008) and Fry (2021).

6.2.4 *Environmental chemistry variables (water and sediments)*

Water chemistry measurements and sediment collection were done on each day of mesh bags retrieval day. Conductivity (µS cm^{-1}), total dissolved solids (TDS: mg L^{-1}), pH and temperature (°C) were measured from the water using a handheld multi–parameter waterproof Cyber Scan 300 (i.e., 2 replicates × 4 sites × 6 weeks) (Eutech Instruments, Singapore). Sediment samples were collected ($n = 2$, 1.5 kg, depth ~5–10 cm) at each site during each sampling day using a plastic hand shovel after the removal of the overlaying debris. Upon collection, the samples were placed into new polyethylene ziplock bags and immediately packed in a cooler bag with ice and transported to the University of Venda laboratory for further analysis. Upon arrival in the laboratory, sediment samples were oven dried at 60 °C for 48 h to a constant weight before being disaggregated in a porcelain mortar, homogenised using a riffle splitter, and thereafter a sediment subsample of 0.5 kg was separated. The subsamples were sent for nutrient and metal analysis at the South African National Accreditation System (SANAS) approved laboratory BEMLAB (Cape Town). Cation elements (boron (B), calcium (Ca), potassium (K), magnesium (Mg), and sodium (Na)), heavy metals (manganese (Mn), copper (Cu), iron (Fe) and zinc (Zn)), nutrients (P)) and abiotic factors pH, and resistivity (ohms) were quantified for each site across the 7 sampling weeks (see Dalu et al., 2020a,b for detailed methods).

6.2.5 *Statistical analyses*

Macroinvertebrate diversity metrices (evenness, Margalef's diversity taxa richness, Shannon–Wiener diversity, and Simpson's diversity) were calculated using macroinvertebrate community dataset in PAST version 4.03. Two–way Analyses of Variance (ANOVA) was utilised to assess differences in environmental variables (i.e., water and sediments) and macroinvertebrates diversity metrics i.e., evenness, Margalef's diversity index, taxa richness, Shannon–Wiener diversity, and Simpson's diversity index across MACADAMIA ORCHARD and communal area reservoirs over time after testing for homogeneity (Levene's test, $p > 0.05$) and normality of distribution (Shapiro–Wilk test, $p > 0.05$). We used sites and weeks as explanatory factors and environmental variables and macroinvertebrate diversity metrics as response variables. For weeks, where significant differences were observed, Tukey's post–hoc analyses were conducted to assess pairwise multiple comparisons across the different study weeks. All analyses were conducted using IBM SPSS version 28.0.0.0.

Distance–based Permutational Analysis of Variance (PERMANOVA; Anderson, 2001) based on Bray Curtis dissimilarities were employed for biological data and 9999 permutations with Monte Carlo tests were utilised to analyse differences in macroinvertebrate communities among sites (macadamia, communal) and weeks (i.e., 1–6) using PERMANOVA + for PRIMER version 6 (Anderson et al., 2008). Furthermore, a two–way Analysis of Similarities (ANOSIM; Clarke 1993), with Tukey–HSD post–hoc multiple comparison was conducted, based on a Bray–Curtis resemblance matrix measure calculated from normalised abundance data, to assess potential differences in macroinvertebrates assemblages across sites over time. A two–dimensional ordination of the Bray–Curtis distance rank orders of similarities among taxa was produced by means of non–metric multidimensional scaling (*n*–MDS) to visually assess similarities and differences within and between different macroinvertebrates taxa morphology across weeks.

To investigate the influence of environmental variables (i.e., water and sediment) on macroinvertebrate community structure, a multivariate ordination was utilised. A detrended Canonical Correspondence Analysis (DCCA) was utilised to determine whether unimodal or linear methods were most appropriate for ordination analysis (Ter Braak, 1986). The gradient lengths from the DCA analysis were examined, and since the longest gradient was shorter than 3.0, a linear constrained Redundancy Analysis (RDA) method was found to be the most appropriate for the data. The RDA, based on significant ($p < 0.05$) forward selected water and sediment environmental variables, was used for analysis using 9999 Monte Carlo Permutations in Canoco version 5.

6.3 Results

6.3.1 *Physico–chemical variables*

Across sampling sites, temperature (mean range 24.5–30.2 °C), pH (mean range 5.9–7.3), conductivity (mean range 274.4–550.4 μS cm^{-1}) and total dissolved solids (mean range 136.3–354.2) differed significantly ($p < 0.05$; Table 6.1). Generally, across sites, high mean temperature (30.2 °C) and pH (7.3) were observed in the MACADAMIA ORCHARD reservoirs, whereas, in the communal area reservoir, high mean conductivity (550.4 μS cm ^{1}) and total dissolved solids (354.2 mg L^{-1}) were observed. Water variables (i.e., temperature, total dissolved solids, and pH) showed significant differences ($p < 0.05$) across study sites, and they also differed significantly ($p < 0.05$) across monitoring weeks(Table 6.2). According to Tukey's post–hoc multiple comparisons, significant differences were observed for water variables across weeks. For instance, across weeks significant differences were observed for temperature i.e., week 1 *vs* 2 (p = 0.001), 2 *vs* 6 (p = 0.001), 3 *vs* 5 (p = 0.001) and 5 *vs* 7 (p = 0.001), conductivity i.e., week 1 *vs* 5 (p <

0.001), 3 *vs* 5 ($p = 0.002$), 4 *vs* 5 ($p = 0.002$), 5 *vs* 7 ($p = 0.037$) and total dissolved oxygen solids week 1 *vs* 5 ($p < 0.001$), 2 *vs* 6 ($p = 0.014$), 3 *vs* 5 ($p < 0.001$), 6 *vs* 7 ($p = 0.003$).

Table 6.1. Mean (±standard deviation) water chemistry variables measured across MACADAMIA ORCHARD and communal area reservoirs over time (7 weeks). Abbreviations: CA – communal area reservoir, MO – MACADAMIA ORCHARD reservoir, TDS – total dissolved solids

Variables	**Unit**	**Site**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**
Temperature	°C	MO	25.6±1.5	27.5±0.4	27.1±0.2	26.6±0.4	30.2±0.3	26.7±1.4
		CA	24.5±0.3	28.4±0.1	25.4±0.4	25.0±0.3	28.1±0.2	24.6±0.3
pH		MO	6.4±0.3	7.3±0.2	7.2±0.2	7.2±0.3	7.3±0.2	6.8±0.1
		CA	7.0±0.1	6.0±0.4	6.0±.2	6.0±0.2	6.4±0.3	7.0±0.1
Conductivity	μS cm^{1}	MO	274.4±30.2	404.7±67.9	286.0±62.8	426.2±60.0	539.7±5.1	274.4±30.2
		CA	360.0±2.1	458.0±101.3	333.2±17.8	297.8±7.2	550.4±12.2	360.0±2.3
TDS	mg L^{-1}	MO	136.8±15.2	205.7±35.6	193.7±31.0	207.7±26.9	264.8±4.7	136.3±15.5
		CA	168.5±10.1	263.2±36.5	196.5±23.7	165.5±18.4	354.2±10.3	179.0±0.9

Generally, high mean sediment chemistry variables concentrations were observed in macadamia reservoirs, i.e., pH (6.65), P (24 mg kg $^{-1}$), K (153.5 mg kg $^{-1}$), Ca (21.45 mg kg $^{-1}$), Mg (14.65 mg kg $^{-1}$), K (0.39 mg kg $^{-1}$), Na (0.53 mg kg $^{-1}$), Cu (27.6 mg kg $^{-1}$), Zn (7 mg kg $^{-1}$), Mn (309 mg kg $^{-1}$), B (0.55 mg kg $^{-1}$), Fe (1885 mg kg $^{-1}$), and S (346.5 mg kg $^{-1}$), whereas, in communal reservoirs, high mean sediment variable were observed for resistivity (18095 ohm). . Most of the sediment variables were similar ($p > 0.05$) within the sampling sites, with exception of five variables (i.e., resistivity, Na, Ca, Mg and Fe) which showed significant site differences ($p < 0.05$).

Table 6.2. Two way–ANOVA results considering key base water and sediment variables and macroinvertebrate diversity metrics as a function of sampling sites (i.e., MACADAMIA ORCHARD and communal area reservoirs; df = 3) and weeks (df = 5), and their interaction (df = 5). Significant p–values are in bold emboldened.

Variables	**Site**		**Week**		**Site × Week**	
	F	***P***	**F**	***p***	**F**	***P***
Water chemistry variables						
Temperature	**10.035**	**0.002**	**11.333**	< **0.001**	2.029	0.073
pH	**9.945**	**0.002**	1.176	0.329	**9.68**	< **0.001**
Conductivity	0.178	0.675	**6.843**	< **0.001**	**2.288**	**0.045**
TDS	**5.394**	**0.023**	**14.526**	< **0.001**	**2.23**	**0.05**
Sediment chemistry variables						
pH	0.019	0.893	0.732	0.632	1.234	0.343
Resistivity	**10.274**	**0.006**	0.030	1.000	0.028	1.000
P	3.841	0.070	0.486	0.808	0.252	0.951
K	2.321	0.150	0.690	0.661	0.439	0.841
Na	**7.256**	**0.017**	1.935	0.145	0.977	0.479
K	0.534	0.477	0.239	0.956	0.098	0.995
Ca	**5.584**	**0.033**	0.200	0.971	0.533	0.774
Mg	**12.34**	**0.003**	1.596	0.22	1.337	0.305
Cu	0.588	0.456	0.539	0.77	0.428	0.848
Zn	0.009	0.925	0.958	0.487	0.932	0.502
Mn	2.292	0.152	0.590	0.733	1.379	0.289
B	2.554	0.132	0.742	0.625	1.423	0.274
Fe	**5.027**	**0.042**	0.825	0.57	2.069	0.123
S	3.325	0.090	0.341	0.904	0.638	0.669
Macroinvertebrate diversity metrics						
Evenness	1.862	0.177	**27.161**	**<0.001**	0.641	0.257
Margalef's diversity	1.985	0.163	**5.019**	**<0.001**	0.65	0.799
Taxa richness	0.015	0.904	**43.274**	**<0.001**	0.738	0.985
Shannon–Wiener diversity	0.063	0.803	**48.274**	**<0.001**	0.929	0.861
Simpson's diversity	0.093	0.761	**11.719**	**<0.001**	0.993	0.244

6.3.2 *Macroinvertebrate colonisation*

A total of 644 macroinvertebrate individuals, belonging to 8 orders and four functional feeding groups, were collected over six weeks across the four reservoirs (2 macadamia, 2 communal)

(Table 6.3). The orders with high occurrence frequency were Odonata and Diptera accounting for 35.7 % and 14.8 %, respectively of the total orders. Overall, Libellulidae (i.e., *Pantala flavensces*, *Trithemis* sp. and *Zyonyx natalensis*) were the most dominant family group. Non–biting midges Chironominae were the most abundant taxa, accounting for 55.1 % of the total abundance across sampling sites, with the seed shrimps Ostracoda being the second most abundant (14.4 %). The dropwings *Trithemis* sp. and dragonfly *Anax* sp. were the third and fourth most abundant taxa recorded accounting for 5.2 % altogether. The fifth most abundant was the freshwater snail *Radix natalensi*s accounting for 4.97% of the total abundance (Figure 6.1). In terms of FFG, predators were the most dominant FFG contributing 75.0 % of all collected taxa, followed by scrapers (16.7 %), collector–gatherers (16.7 %) then collector–filterers (8.3 %).

Similar macroinvertebrate diversity metrics values were observed across MACADAMIA ORCHARD and communal area reservoirs (Table 6.3; Figure 6.2). Fourteen macroinvertebrate taxa were recorded between macadamia and communal reservoirs, of which 10 taxa were recorded in the macadamia reservoirs and 8 in the communal area reservoir (Table 6.3). Based on the two–way ANOVA, significant differences were observed across monitoring weeks for all metrics ($p < 0.05$), whereas sites indicated no significant differences ($p > 0.05$) (Table 6.2). According to Tukey's post–hoc multiple comparisons, significant differences were observed for macroinvertebrate diversity metrics across weeks. Temporal differences were observed for evenness, whereby differences lay between week 1 *vs* 3 ($p = 0.026$), 2 *vs* 7 ($p < 0.001$), 3 *vs* 6 ($p < 0.001$) and 4 *vs* 7 ($p < 0.001$). Similarly, temporal differences were observed for Margalef's diversity between week 1 *vs* 5 ($p = 0.003$), 1 *vs* 6 ($p = 0.001$), and 1 *vs* 7 ($p = 0.002$). For taxa richness differences were observed between week 1 *vs* 5 ($p < 0.001$), 3 *vs* 7 ($p < 0.001$) and 4 *vs* 7 ($p < 0.001$), while for Shannon–Wiener diversity differences were observed between week 1 *vs* 5

($p < 0.001$), 2 *vs* 6 ($p < 0.001$), 3 *vs* 7 ($p < 0.001$) and 4 *vs* 7 ($p < 0.001$). Temporal differences between Simpson's diversity were only observed between week 1 *vs* 3 ($p < 0.036$) and 2 *vs* 7 ($p < 0.001$).

Using PERMANOVA, significant differences in total macroinvertebrates community structure were observed across sites (Pseudo–F = 26.899, p(MC) = 0.001) and weeks (Pseudo–F = 2.286, p(MC) = 0.002). Pairwise comparisons highlighted significant differences in macroinvertebrates community between weeks 1 *vs* 4 (t =2.341, p(MC) < 0.001), 1 *vs* 5 (t = 1.978, p(MC) = 0.002), 1 *vs* 6 (t =2.233, p(MC) = 0.004), 2 *vs* 4 (t =1.660, p(MC) = 0.011), 2 *vs* 6 (t =1.640, p(MC) = 0.023), 3 *vs* 4 (t =1.545, p(MC) = 0.033), and 3 *vs* 6 (t =1.590, p(MC) = 0.018).

Table 6.3. Mean relative abundances (%) of the dominant macroinvertebrate species and metrics observed over six weeks for the study site categories: macadamia (MO) and communal (CA) reservoirs. FFG – functional feeding group; Abbr. – abbreviation.

Taxa	**Order**	**FFG**	**Abbr.**	**Week 1**		**Week 2**		**Week 3**		**Week 4**		**Week 5**		**Week 6**	
				MO	**CA**	**MO**	**CA**	**MO**	**CA**	**MO**	**CA**	**MO**	**CA**	**MO**	**CA**
Anax sp.	Odonata	Predator	Ana		19		14		13		10		10	3	8
Ceratopogonidae	Diptera	Predator	Cer		12						10		5		
Chironominae	Diptera	Gatherers	Chi	45	22	39	50	32	40	37	33	31	37	34	41
Gyrinidae larvae	Coleoptera	Predator	Gyr							4	12	8	10	7	8
Ostracoda	Podocopida	Filterers	Ost	18	19	18	14	13	9	10	8	10	12	12	19
Pantala flavescens	Odonata	Predator	Pan							10		8		6	
Physella acuta	Gastropoda	Scrapers	Phy						12						
Planaria sp.	Platyhelminthes	Predator	Pla							8		12			
Potamonautes sp.	Decapoda	Gatherers	Pot												5
Pseudagrion sp.	Odonata	Predator	Pse	21				13		7		5		8	
Radix natalensis	Mollusca	Scrapers	Rad	16		21		15		6		10		14	
Ranatra sp.	Hemiptera	Predator	Ran								5		5		5
Trithemis sp.	Odonata	Predator	Tri			14		19		9		6		7	
Zyonyx natalensis	Odonata	Predator	Zyo			8				9		4		5	
Other taxa					30		22	8	26		22	6	21	4	14
Macroinvertebrate diversity metrics															
Evenness				0.9	0.9	0.9	0.8	0.9	0.8	0.7	0.8	0.8	0.8	0.7	0.8
Margalef's diversity				0.9	1.4	0.8	0.9	1.4	1.2	1.6	1.5	1.8	1.4	1.7	1.4
Taxa richness				4	5	5	5	6	6	9	8	10	8	10	8
Shannon–Wiener diversity				0.8	1.1	0.8	0.9	1.3	1.1	1.3	1.4	1.5	1.3	1.5	1.2
Simpson's diversity				0.5	0.6	0.5	0.5	0.7	0.6	0.6	0.7	0.7	0.6	0.7	0.6

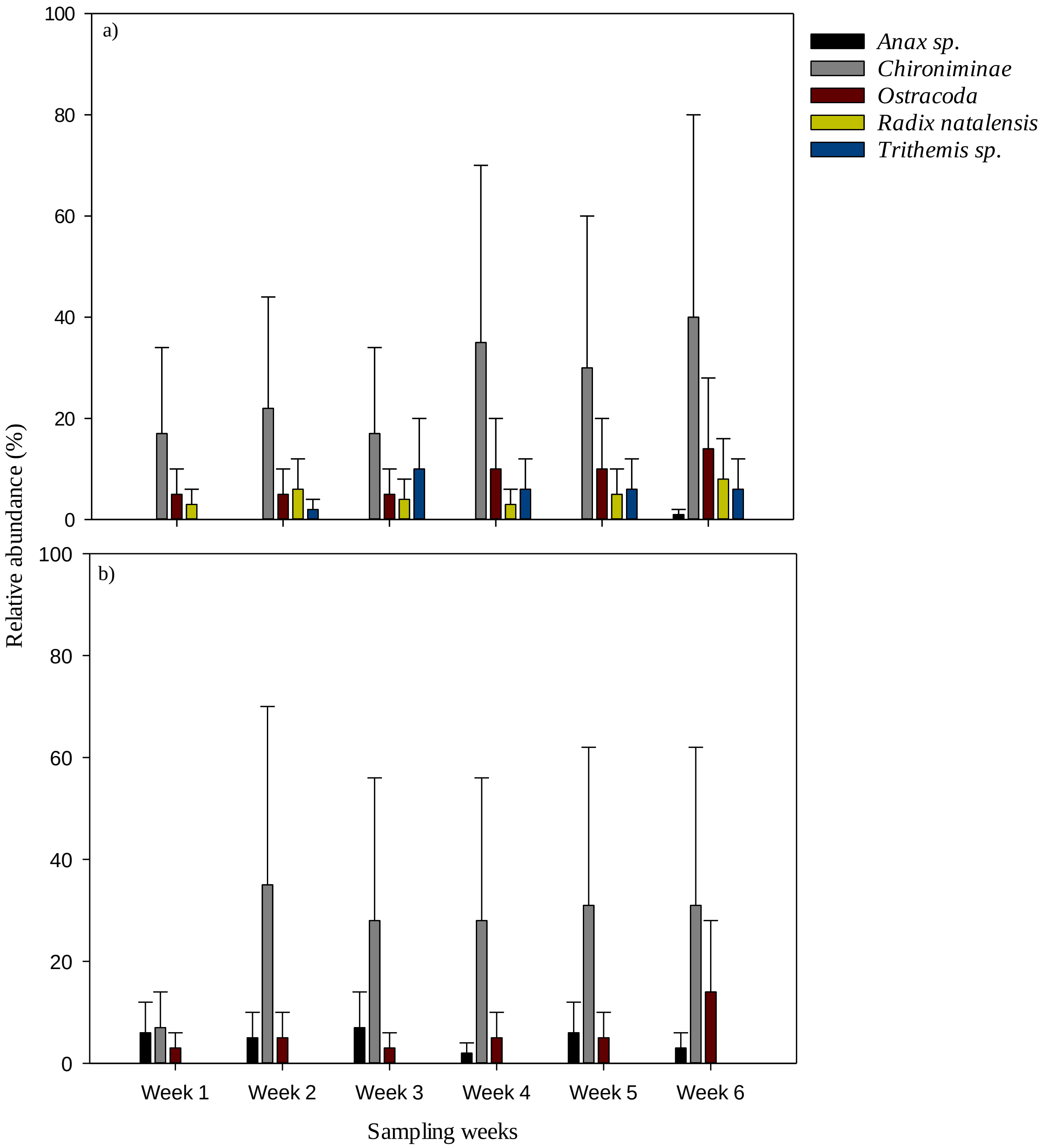

Figure 6.1. The five most dominant macroinvertebrates (relative abundance (%) ± SD) across, (*a*) MACADAMIA ORCHARD and (*b*) communal area reservoirs at the end of the experiment (6 weeks). Different colors donate macroinvertebrate taxa (see legend).

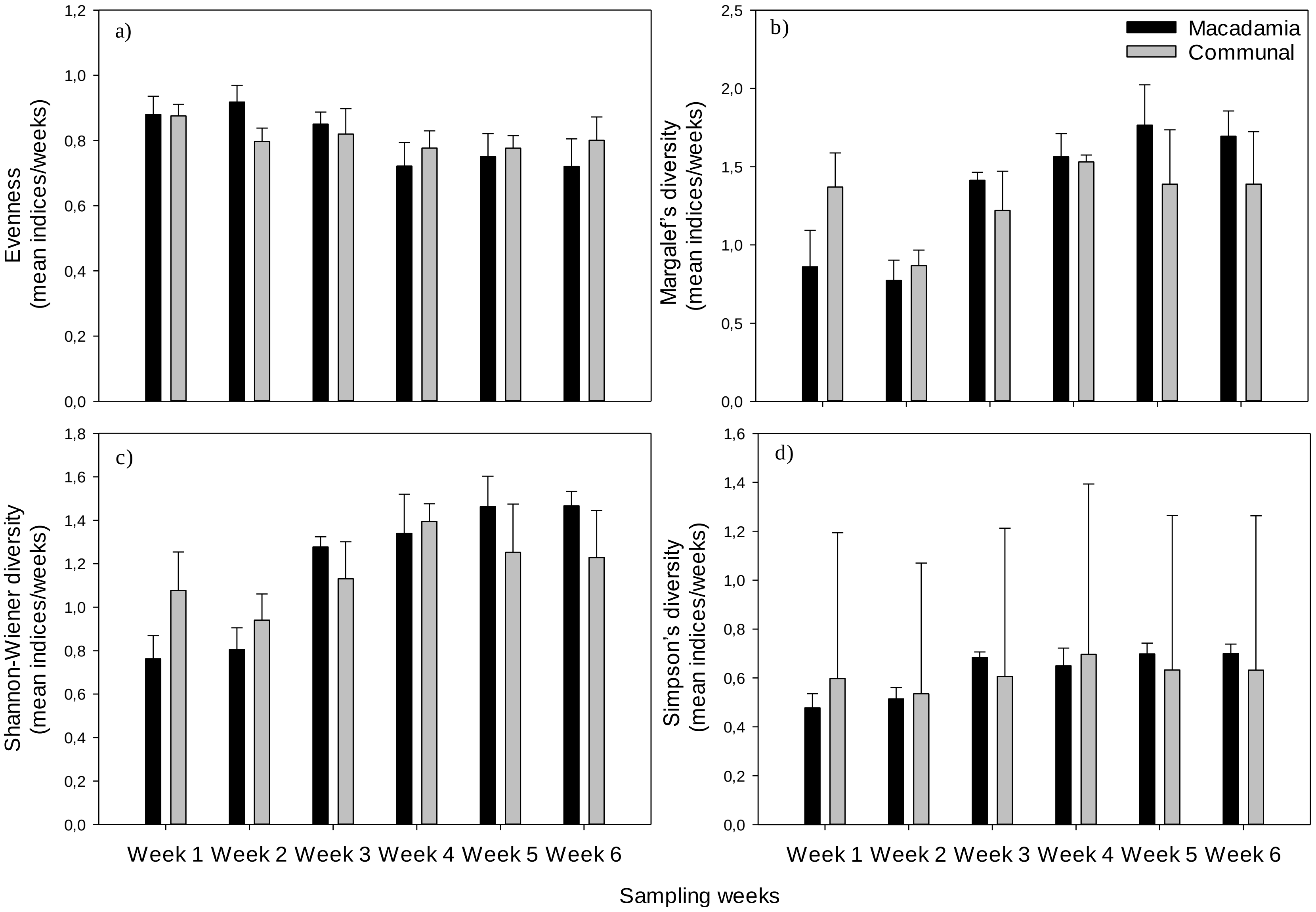

Figure 6.2. Mean (±standard deviation) macroinvertebrate diversity metrics i.e., (*a*) evenness, (*b*) Margalef's diversity, (*c*) Shannon–Wiener diversity, and (*d*) Simpson's diversity across MACADAMIA ORCHARD and communal area reservoirs at the end of the experiment (6 weeks). Different colors donate two reservoir types (see legend).

The *n*–MDS analysis produced an ordination which separated the two sites (Figure 6.3). According to the ANOSIM results, little similarity was observed for macroinvertebrates communities between MACADAMIA ORCHARDS and communal area sites (*r* = 0.08, *p* = 0.081), and there were differences across weeks (*r* = 0.76, *p* = 0.001). Pairwise comparisons tests indicated significant differences between week 1 *vs* 4 (*r* = 0.42, *p* = 0.006), 1 *vs* 5 (*r* = 0.30, *p* = 0.003), and 1 *vs* 6 (r = 0.39, *p* = 0.002).

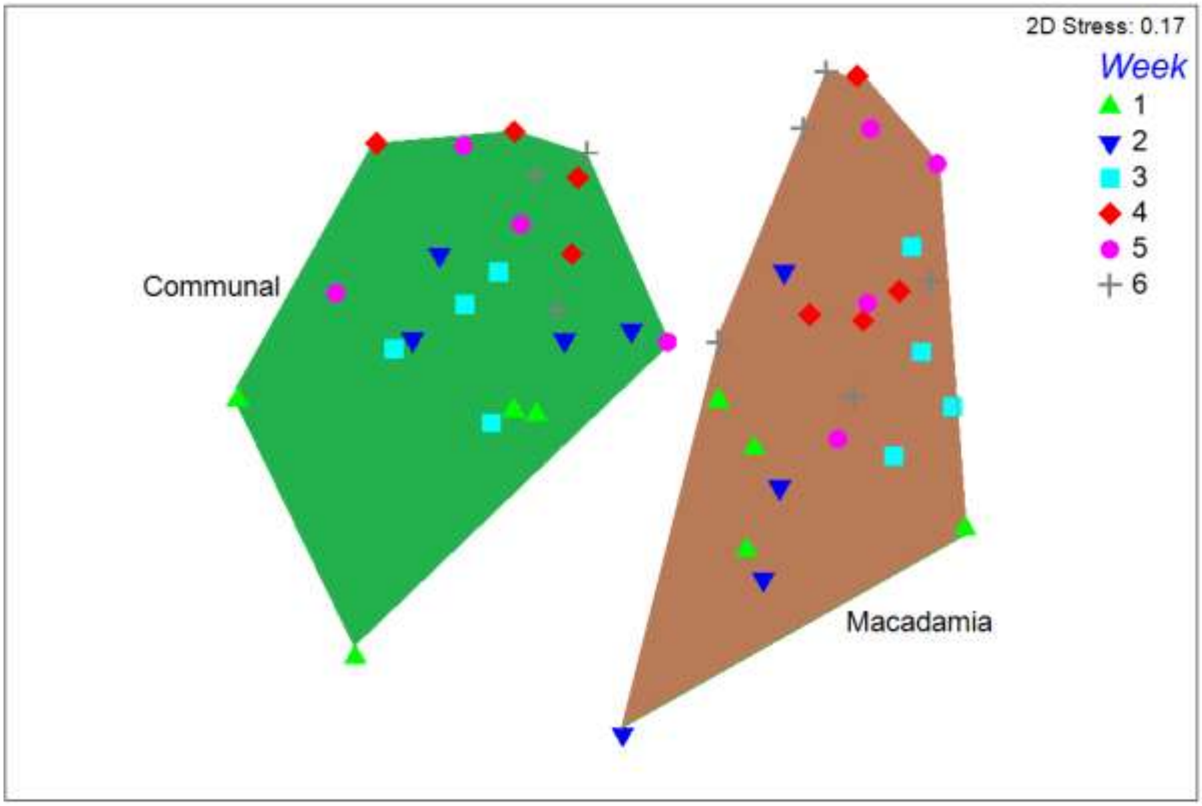

Figure 6.3. *n*–MDS ordination highlighting variation of macroinvertebrate communities between communal area and MACADAMIA ORCHARD reservoirs over the duration of the experiment (i.e., 6 weeks).

6.3.2 *The influence of water and sediments variables on macroinvertebrates community*

The RDA was conducted to explore the simultaneous effects of all the biotic variables (i.e., water and sediment variables) on macroinvertebrate communities. Based on RDA analysis, first and second axes, with eigenvalues of 0.29 and 0.20 were observed, respectively, with selected exploratory variables accounting for 65.9 % of the total macroinvertebrate variance. Monte Carlo unrestricted permutation test indicated axis 1 (permutation) and 2 (999 permutations of ais 2 with axis 1 as a co-variable) were statistically significant ($p < 0.05$) in all cases. Of the 18 variables (i.e., water and sediments), the abundance of macroinvertebrates across two reservoir types and six weeks was found to be significantly associated with sediment chemistry variables (i.e., pH, resistivity, P, K, Na, Ca, Mg, Cu, B, Fe and S) which were significant in structuring macroinvertebrate community (Figure 6)3. Sediment pH and S were highly positively associated with the second axis while variables such as Potassium (K), Cu, Na and P were negatively associated with the first axis. Resistivity was highly negatively associated with the first axis. The RDA axis separated the sites based on reservoir types and sampling weeks. MACADAMIA ORCHARD, highly pollinated, were generally associated with axis 1 and 2 being characterised by high variables such as pH and S and low resistivity. Moreover, macadamia reservoirs, highly pollinated, were associated with macroinvertebrates such as Chironominae, Ostracoda, *Plantala flavescens*, *Pseudagrion* sp., *Trithemis* sp., and *Zyonyx natalensis* (Figure 6.4). On the other hand, communal reservoirs, relatively less pollinated, were negatively associated with first axis being characterised by high resistivity and macroinvertebrates such as *Anax* sp., Ceratopogonidae, *Physella acuta*, *Ranatra* sp., and *Potamonautes* sp (Figure 6.4).

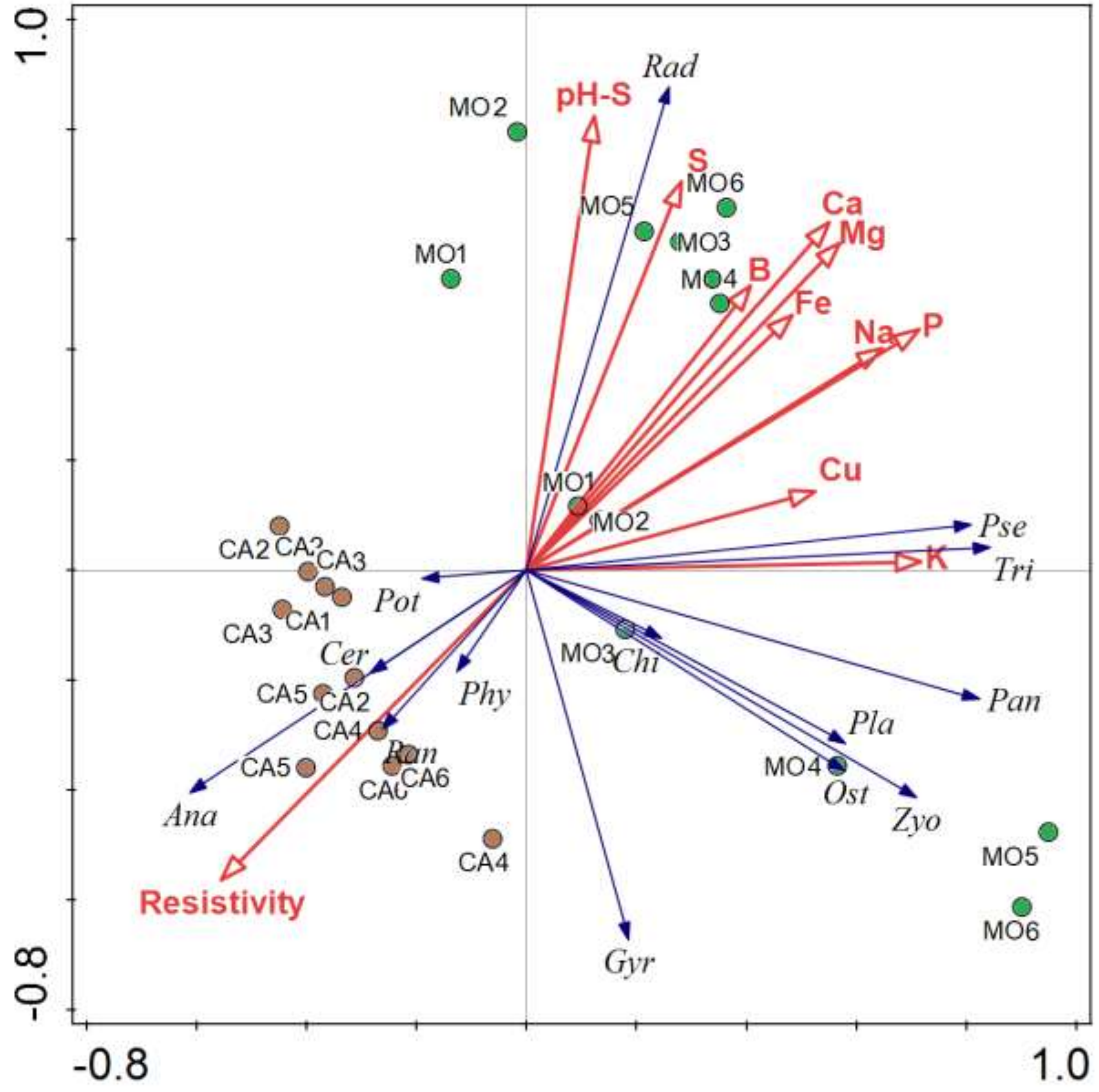

Figure 6.4. Redundancy analyses ordination showing the relationship between macroinvertebrate colonisation and the significant environmental variables. Letters in samples labels represent sites (MO – MACADAMIA ORCHARD reservoir, CA – communal area reservoir). Numbers in the labels represent sampling weeks (i.e., 1–6). Taxa abbreviation definitions are defined in Table 6.3

6.4 Discussion

Aquatic macroinvertebrate community structure is useful for a better comprehension of the effects of discrete stressors associated with land–use types. Both communal and agricultural practices are known to be primary contributors to pollution of aquatic ecosystems, yet the nature of the pollution from these respective land–use practice types are typically discrete (Gascuel–Odoux, et al., 2009; Yu et al., 2011; Dalu and Tavengwa, 2022). Accordingly, aquatic organism colonisation dynamics likely differ in response to these different stressors. As such, macroinvertebrate community structure needs to be considered as they play a crucial role in ecosystem functioning and are readily impacted by these emerging pollutants. Using stones substrates to assess colonisation dynamics, the study highlighted differences in macroinvertebrate communities between the habitat types, with temporal community succession patterns providing no indication of community homogenization between habitat types. At both the pioneer and later successional stages, macroinvertebrate communities were discrete between habitats. With increased pesticide concentrations in the MACADAMIA ORCHARDS in sediments (i.e., 5.67 to 14.48 µg L-1) and in surface water (i.e., < 6.51 µg L-1) than communal area reservoirs (i.e., < 0.13 µg L-1 surface water), our results thereby highlight the importance of discrete pollution input from the differing land–use areas, in structuring communities. The present study also highlighted that few sediments and water physico–chemical variables were significantly different between reservoirs in the different land–use types. We further highlighted that macroinvertebrates communities inhabiting artificial stone substrates could be highly structured by sediment variables rather than water variables. The study also highlighted that pesticide concentration in the MACADAMIA ORCHARD reservoirs might cause decline in ecosystem functioning by reducing the observed macroinvertebrate taxa, impacting colonisation dynamics and adjusting community structure.

According to Lake (1985), the time it takes macroinvertebrates to colonise stone substrates and to reach the state of equilibrium of densities and richness is variable and generally ranges from 4 to 38 days. This corresponded with our findings as macroinvertebrates taxa and abundance were generally low during the early stages and increased with time. Generally, several macroinvertebrate taxa colonised stone substrates, but Chironominae and Ostracoda were found consistently and were the most dominant and abundant (representing 55.1 % and 14.4 %, respectively, of the macroinvertebrates found). The colonisation of artificial stone substrates by macroinvertebrates in the present study is not surprising given that stones are among the most natural substrates in aquatic ecosystems (Rosa et al., 2013) and are well known to grow spores which turn into algae, mold, and fungi (Friedmann, 1974). High abundance of Chironominae in stone substrates were also similar to the findings by Alonso and Camargo (2005), who observed increased diversity of Chironominae in small stones substrates and Rosa (2013) who indicated high preference of Chironominae to inhabit stone substrates. Egglishaw (1969) observed that streams in Scotland had an association of benthic fauna with different substrates among different seasons and found that Chironominae made up from 70 % to 90 % of the fauna associated with stone substrates.

Macroinvertebrate taxa *Pantala flavescens*, Gyrinidae larvae, *Anax* sp., and *Physella acuta* in the MACADAMIA ORCHARD and Gyrinidae larvae, *Potamonautes* sp., and *Planaria* sp. in the communal area reservoirs were absent during the early weeks of the experiment and colonised the stone substrates on a later stage thereby supporting our hypothesis of marginal different across the MACADAMIA ORCHARD and communal area reservoirs. According to Brausch and Smith

(2009), most macroinvertebrate communities are sensitive to pesticides contamination in reservoirs thereby influencing the delayed inhabiting or absent of some macroinvertebrate taxa in the MACADAMIA ORCHARD reservoir of stone substrates. Slight decline of macroinvertebrate richness and abundance in the MACADAMIA ORCHARD reservoirs can be supported by several studies which highlighted that macroinvertebrate abundances in freshwater ecosystem decrease as pesticides concentration increase (e.g., Anderson et al., 2006; Beketov et al., 2013). Furthermore, Khudhair (2019) indicated that macroinvertebrate taxa develop various morphological and physiological adaptations strongly associated with habitat conditions and the availability of food resources which could explain the delayed inhabiting by some macroinvertebrate taxa.

Although not the key focus of the study, fish *Tilapia rendalli* were observed in the MACADAMIA ORCHARD reservoirs, whereas Mozambique tilapia fish *Oreochromis mossambicus* and stream frog *Strongylopus* sp. were observed in the communal area reservoir indicating that artificial stone substrates could also function as habitat for fish and frog communities. The increased abundance and taxa richness of non–insect macroinvertebrates in the communal area compared to MACADAMIA ORCHARD reservoirs further support that land–use types and increased pesticide concentration in the MACADAMIA ORCHARD altered macroinvertebrates colonisation. As highlighted by Relyea and Diecks (2008), exposure of aquatic fauna such as fishes to pesticides or increased agricultural land–use results in growth reduction and low reproductive activity with juveniles more sensitive to pesticide than adults.

Macroinvertebrates are an important part of aquatic ecosystems, and community structure characteristics are related to the environmental variables. As such, we revealed that changes in water chemistry variables were not reflected in the macroinvertebrate communities, whereas sediment chemistry variables was important as a predictor of macroinvertebrates. As highlighted by Clements (2020), sediments contaminated with heavy metal concentrations may promote or inhibit macroinvertebrate community structure and colonisation. Macroinvertebrate structural composition has been observed to respond highly on sediment chemistry variables (Beisel et al., 2000; Clements, 2020) and this was evident in our study and supported by the RDA analysis which found sediment chemistry variables (i.e., pH, Resistivity, P, K, Na, Ca, Mg, Cu, B, Fe and S) to significantly alter the macroinvertebrate structure. Furthermore, these variables were crucial factors structuring MACADAMIA ORCHARD reservoirs associated macroinvertebrate communities than in communal areas reservoir macroinvertebrate communities, with the exception for resistivity. This was not surprising given that heavy metal are well–known environmental pollutants due to their toxicity, persistence in the environment, and bioaccumulative nature. Furthermore, agricultural activities are known for high anthropogenic activities, resulting in increased pollution compared to communal areas (Sharma and Agrawal, 2005). For instance, Wang et al. (2007) and Ouyang et al. (2018) highlighted that excessive nutrient can results in water quality deterioration and dissolved oxygen depletion, resulting in decline in species richness. This further indicate that macroinvertebrates in the MACADAMIA ORCHARD reservoirs exhibit a stress relationship with sediment variables, mainly B, Fe and Cu. As indicated by Jennings (2011), B is an important sediment chemistry variable that is involved in structuring macroinvertebrate community, whereas Dalu et al (2022) found Cu, Mg and Na among the most important sediment chemistry variables. Bian et al. (2016) highlighted that Na contributes greatly to water quality in aquatic ecosystem and is known to affect macroinvertebrate community adversely. Lauber et al. (2009) suggested that sediment pH directly imposes a physiological constraint on the aquatic fauna by reducing the net growth of individual taxa that are unable to survive if the soil pH is outside of a certain range.

6.5 Conclusions

The current study contributes to understanding aquatic ecosystem functioning and artificial aquatic substrates colonisation and interplays between macroinvertebrate community, environmental variables and the likelihood effects of pesticides between MACADAMIA ORCHARD and communal area reservoirs. We found that MACADAMIA ORCHARD and communal area reservoirs were both important for macroinvertebrate colonisation in artificial stone substrates. Stone substrates supported a distinctive macroinvertebrates community and drove the establishment of macroinvertebrate and communities and at least temporarily for some taxa. A variety of macroinvertebrates were observed in the stone substrates in both reservoirs, most notably Chironominae and Ostracoda contributing 61.5 % of the total macroinvertebrate abundance. The presence of pesticides between both reservoirs and/or types were most likely to alter primary colonisation and successive community structure in the MACADAMIA ORCHARD reservoirs. Furthermore, the structures and community of macroinvertebrates were found to be highly affected by sediment chemistry variables than water variables as shown by RDA analyses. Despite the influence of pesticides on colonisation dynamics, stone substrates are known to represent a complex ecosystem of microflora including bacteria, algae, fungi, and lichens which might have also attributed to macroinvertebrate colonisation. As such, this scenario is worth further investigation, assessing their abundances associated with stone substrates and their influence on certain macroinvertebrate.

Chapter 7: General Synthesis

Plate 7. Macroinvertebrate taxa *Trithemis* sp. found inside the green mesh bags filled with stones during retrieval day following 6 weeks incubation period. Evidence of macroinvertebrate colonisation in the MACADAMIA ORCHARD. Photo by Thendo Mutshekwa

7.1 General synthesis

This study demonstrates that pesticides associated with MACADAMIA ORCHARDS have implications for recipient aquatic environment ecosystem functioning. This was highlighted for key pesticides i.e., Karate Zeon 10 CS (lambda–cyhalothrin), Mulan 20 SP (acetamiprid) and Pyrinex 250 CS (chlorpyrifos) commonly used in the MACADAMIA ORCHARDS within the Levubu region using a combination of *in*– and *ex–situ* experiments. The effects of pesticides inputs on aquatic ecosystem were evident, emphasizing the threats of increasing macadamia plantation to environmental water quality and ecosystem functioning through the reduction in fish and macroinvertebrates diversity, thereby altering community structure and nutrient recycling and with serious implications for aquatic food webs and general ecosystem functioning. According to the best of my knowledge, this is the first comprehensive study on the effects of MACADAMIA ORCHARD inputs on aquatic ecosystems in the South African context. The thesis provides a better comprehension and contributes significantly to the global body of literature on MACADAMIA ORCHARD pesticides and their impacts on associated adjacent aquatic ecosystems, pesticide occurrence, the effects on aquatic fauna and community structure.

An overview of all four data chapters (Chapters 3–6) provides insight into the ecology of reservoirs associated with MACADAMIA ORCHARDS inputs and potential effects of these pesticides. Aquatic ecosystems are contaminated with pesticides through several routes: runoff, spray drift, and leaching, which pose serious health risks to the aquatic ecosystems (Kumar et al., 2021). This exposure can directly affect all levels of biological organisation compartments including primary producers, microorganisms, invertebrates, and/or fish. Among other agricultural orchards in South Africa, macadamia plantation orchards have been expanding rapidly over the last decade and associated reservoirs within these areas are increasingly becoming impacted by pesticide pollution resulting from macadamia pest control applications. A detailed occurrence and effects of pesticide used in the MACADAMIA ORCHARDS on aquatic ecosystems and pesticides is presented in Chapter 1. The key findings of Chapter 3 are that the MACADAMIA ORCHARD reservoirs are highly contaminated with pesticides when compared to “control” reservoirs associated with reduced agricultural activities. One of the objectives of this study (Chapter 3) was to assess pesticide (i.e., acetamiprid and chlorpyrifos) concentrations in MACADAMIA ORCHARD reservoirs, and to this end, LC–MS/MS analysis was used, and communal area reservoirs associated with reduced agricultural activities were also used for comparison purposes. A key finding was that MACADAMIA ORCHARD reservoirs were highly contaminated and that sediments accumulate increased pesticide concentrations than surface water (Chapter 3). This is not surprising as several studies (e.g., Feo et al., 2010; Masiá et al., 2013) also found increased pesticide concentrations in sediments than surface water. The findings support studies by Tazunoki et al. (2022) who observed that agricultural activities pose a threat to aquatic ecosystems. The current study further highlighted that fish *O. mossambicus* are vulnerable to pesticides exposure and that reduced concentrations cause temporary abnormalities, while acute and/or high exposure cause immediate fish die–offs and long–term abnormalities (Manimekalai et al., 2022).

Pesticide pollution in aquatic ecosystems are known to cause behavioral abnormalities and mortalities to aquatic fauna such as macroinvertebrates and fish (Amoatey and Baawain, 2019). For instance, macroinvertebrates and fish can be directly or indirect impacted by pesticides exposure (Thompson et al., 2016). Long term exposure to pesticides can cause abnormalities or mutations in macroinvertebrate and fishes, while acute exposure can cause immediate die–offs

(Jourdan et al, 2019). This was evident in the current study as demonstrated increased mortality of fish at increased pesticide exposure (Chapter 4). With regards to macroinvertebrate, the abundance and taxa across reservoirs associated with increased and reduced pesticide pollution across different land–use types indicating the sensitivity of some taxa (e.g., *Zyonyx natalensis*) and the increase in tolerant taxa (e.g., Chironominae) (see Chapter 6). A detailed vulnerability of fishes with key focus on *O. mossambicus* is discussed in Chapter 4 and the behavioural responses were found to vary with *O. mossambicus* demonstrating differences in onset behavioural response at different concentration exposures. Moreover, the response of *O. mossambicus* was also different based on the type of pesticide the species was exposed to and this is supported by Sharma et al. (2022), who indicated that all pesticides have the potential to harm aquatic ecosystems particularly living organisms if used incorrectly. It is important to highlight that these impacts will differ according to active ingredients and toxicity.

Besides being a threat to fish, these pesticides may also cause serious threat to decomposition activity, primary production and colonisation dynamics within aquatic ecosystems (see Chapter 5). When exposed to different pesticides (i.e., Karate Zeon 10 CS, Mulan 20 SP, Pyrinex 250 CS0), decomposition and colonisation dynamics were found to vary with larvae (i.e., *Culex* spp., *Anopheles* spp.) and pupae (all genera). Increased mosquito abundances were observed in Mulan 20 SP, whereas Karate Zeon 10 CS demonstrated most reduced mosquito abundances. Willming et al. (2013) highlighted that aquatic macroinvertebrate can be exposed to pesticides during any stage of their life cycle, with the presence of these pesticides likely to impair decomposition activities by reducing macroinvertebrate and microbial decomposers, and also cause imbalances to primary production. To better understand aquatic ecosystems and associated dynamics, laboratory–based experiments have been used over the last several decades .(Quere et al., 2005) In the current study, the interactions between leaf litter decomposition, primary production (chlorophyll–*a*) and mosquito colonisation dynamics significantly differed according to pesticide type (see Chapter 5).

Rock substratum are a key primary physical variable affecting macroinvertebrate taxa richness and density (Carvalho and Uieda, 2006). Furthermore, land–use and subsidy inputs into aquatic ecosystems may impact the sediment and water characteristics in terms of food resources and habitat structure, resulting in macroinvertebrate community composition changes (Mwaijengo et al., 2020). The use of "artificial rock substratum" in this study (see Chapter 6) enabled the assessment of macroinvertebrate colonisation and physico-chemical variables in different land–use types (i.e., MACADAMIA ORCHARDS, communal areas). The findings were supported by other scientific studies (e.g., Laini et al., 2018; Lafage et al., 2019) on land–use types and associated subsidy inputs into aquatic ecosystems which may act as drivers of macroinvertebrate community. The presence of various macroinvertebrate taxa such as Chironominae, Ostracoda, *Trithemis* sp., *Anax* sp., and *Radix natalensis* colonising these artificial habitats and the variation over time highlights colonisation dynamics and secondary succession of macroinvertebrates in newly introduced substrates. Macroinvertebrate taxa Chironominae are known to become tolerant to pesticide exposure (Macchi et al., 2018) and this might explain the dominance of this taxa over time. Macroinvertebrate abundances and richness varied between MACADAMIA ORCHARD (i.e., high pesticide exposure) and communal area (i.e., low pesticide exposure) reservoirs and from our findings it was evident that pesticide pollution results in low macroinvertebrate taxa richness and abundances. The outcomes of this study further indicated that macroinvertebrate structure and community were highly affected by sediment variables than water variables (Chapter 6).

7.2 Conclusions

Overall, the findings of this study highlighted the ongoing threats and effects to aquatic ecosystems as a result of anthropogenetic activities i.e., macadamia production and expansion. This study allowed for a better comparison of different pesticides input associated with MACADAMIA ORCHARD inputs on aquatic ecosystems. The increased pesticide concentrations in MACADAMIA ORCHARDS, more specifically in aquatic sediments where bioaccumulation is taking place pose a serious threat to aquatic fauna such as macroinvertebrates and fish. Although pesticide input in these reservoir poses a serious threat, there is a concentration limit where macroinvertebrate and fish can be able to survive and thrive as demonstrated by increased survival rates of fish when exposed to reduced concentrations and macroinvertebrate colonisation dynamics in the MACADAMIA ORCHARDS.

7.3 Recommendation for future research

The ecology of reservoirs associated with MACADAMIA ORCHARDS inputs needs to be further explored to obtain a comprehensive understanding of the complex processes controlling the interactions of biotic and abiotic factors in aquatic systems located within or near macadamia. Although data chapters 3–6 in the current study provide the foundation of the effects of three commonly used pesticides (i.e., Karate Zeon 10 CS (lambda–cyhalothrin), Mulan 20 SP (acetamiprid) and Pyrinex 250 CS (chlorpyrifos)) in the MACADAMIA ORCHARDS on recipient aquatic ecosystems and provides the basic fundamental background knowledge pertaining the effects associated with seasonal variation and other drivers that contribute to ecosystem functioning. In this study, there was evidence of pesticides occurrence in MACADAMIA ORCHARD associated reservoirs and their effects on aquatic ecosystems. However, more research is required on this topic, as such, recommendations for future studies are detailed below:

- Lambda–cyhalothrin concentrations could not be quantified due to difficulties in method development (see Chapter 3). As such, future research should attempt to use supercritical fluid chromatography (SFC) to assess pesticide concentrations of lambda–cyhalothrin.
- Seasonal assessment of pesticide concentrations in sediment and water in the MACADAMIA ORCHARDS would be ideal since the quantity of pesticides used for pest control in these orchards vary across seasons.
- Fish *O. mossambicus* demonstrated various behaviour responses and varied across pesticide type (see Chapter 4). Further research into the behavioural response of other taxa such as macroinvertebrates is worth exploring, including assess effects on other fish species.
- Furthermore, Karate Zeon 10 CS treatments indicated decreased mosquito larval abundances (see Chapter 5), however, further research is vital into determining the potential larvicidal effects.
- The effects of pesticide of interest to leaf litter decomposition were assessed (see Chapter 5), however, microbial contributions need to be explored to enhance the understanding of decomposition dynamics associated with pesticide exposure.
- The implications for human health. In many agricultural landscapes, reservoirs are utilized by the labour force for fishing activities, with catch supplementing their protein intake. The health issues associated with pesticide contaminated fish is something worth

investigating. Overall, since this study was only interested on three pesticides of interest, the study is worth repeating focusing on other pesticide active ingredients used in the MACADAMIA ORCHARDS.

Plate 8. View of communal area reservoirs at Duthuni farm, Thulamela Municipality. Photo by Thendo Mutshekwa.

References

Aceña, J., Stampachiacchiere, S., Pérez, S., Barceló, D., 2015. Advances in liquid chromatography–high–resolution mass spectrometry for quantitative and qualitative environmental analysis. *Analytical and Bioanalytical Chemistry* 407, 6289–6299.

Afolabi, O.K., Aderibigbe, F.A., Folarin, D.T., Arinola, A., Wusu, A.D., 2019. Oxidative stress and inflammation following sub–lethal oral exposure of cypermethrin in rats: mitigating potential of epicatechin. *Heliyon* 5, 02274.

Aktar, W., Sengupta, D., Chowdhury, A., 2009. Impact of pesticides use in agriculture: their benefits and hazards. *Interdisciplinary Toxicology* 2, 1–12.

Alexander, P., Rounsevell, M.D., Dislich, C., Dodson, J.R., Engström, K., Moran, D., 2015. Drivers for global agricultural land use change: The nexus of diet, population, yield and bioenergy. *Global Environmental Change* 35, 138–147.

Alonso, Á., Camargo, J.A., 2005. Evaluating the effectiveness of five mineral artificial substrates for the sampling of benthic macroinvertebrates. *Journal of Freshwater Ecology* 20, 311–320.

Amoatey, P., Baawain, M.S., 2019. Effects of pollution on freshwater aquatic organisms. *Water Environment Research 91*, 1272–1287.

Anderson, B.S., Phillips, B.M., Hunt, J.W., Connor, V., Richard, N., Tjeerdema, R.S., 2006. Identifying primary stressors impacting macroinvertebrates in the Salinas River (California, USA): Relative effects of pesticides and suspended particles. *Environmental Pollution* 141, 402–408.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26, 32–46.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA for PRIMER: guide to software and statistical methods. PRIMER–E, Plymouth.

Ansari, Z.A., Matondkar, S.G.P., 2014. Anthropogenic activities including pollution and contamination of coastal marine environment. *Journal of Ecophysiology and Occupational Health* 14, 71.

Artigas, J., Majerholc, J., Foulquier, A., Margoum, C., Volat, B., Neyra, M. and Pesce, S., 2012. Effects of the fungicide tebuconazole on microbial capacities for litter breakdown in streams. *Aquatic Toxicology* 122, 197–205.

Ayres–Peres, L., Sokolowicz, C.C., Santos, S., 2006. Diversity and abundance of the benthic macrofauna in lotic environments from the central region of Rio Grande do Sul State, Brazil. *Biota Neotropica* 6, 0–0.

Azwanida, N.N., 2015. A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal and Aromatic Plants* 4, 2167–0412.

Baker, R., Caffier, D., Choiseul, J.W., De Clercq, P., Dormannsné–Simon, E., Gerowitt, B., Karadjova, O.E., Lövei, G., Oude, A., Lansink, D.M., Manceau, C., 2008. Pest risk assessment and additional evidence provided by South Africa on *Guignardia citricarpa* Kiely, citrus black spot fungus–CBS. *Scientific The EFSA Journal* 925, 1–108.

Banaee, M., 2012. Adverse effect of insecticides on various aspects of fish's biology and physiology. In: Soloneski, S., Larramendy, M. (eds) Insecticides – Basic and Other Applications. InTech, Rijeka.

Banaee, M., 2013. Physiological dysfunction in fish after insecticides exposure. In: Trdan, S (ed.) Insecticides – Development of Safer and More Effective Technologies. InTech Press, Rijeka.

Barbier, E.B., Hacker, S.D., Kennedy, C., Koch, E.W., Stier, A.C., Silliman, B.R., 2011. The value of estuarine and coastal ecosystem services. *Ecological Monographs* 81, 169–193.

Barbieri, M.V., Postigo, C., Monllor–Alcaraz, L.S., Barceló, D., López de Alda, M., 2019. A reliable LC–MS/MS–based method for trace level determination of 50 medium to highly polar pesticide residues in sediments and ecological risk assessment. *Analytical and Bioanalytical Chemistry* 411, 7981–7996.

Basirun, A.A., Ahmad, S.A., Yasid, N.A., Sabullah, M.K., Daud, H.M., Sha'arani, S., Khalid, A., Shukor, M.Y., 2019. Toxicological effects and behavioural and biochemical responses of *Oreochromis mossambicus* gills and its cholinesterase to copper: a biomarker application. *International Journal of Environmental Science and Technology* 16, 887–898.

Becker, J.M., Liess, M., 2017. Species diversity hinders adaptation to toxicants. *Environmental Science and Technology* 51, 10195–10202.

Beisel, J.N., Usseglio–Polatera, P., Moreteau, J.C., 2000. The spatial heterogeneity of a river bottom: a key factor determining macroinvertebrate communities. In: Jungwirthm S., Muhar, S., Schmutz, S. (eds) Assessing the Ecological Integrity of Running Waters: Proceedings of the International Conference, Veinna, Austria, 9–11 November. Springer Science+Business Media, Dordrecht.

Beketov, M.A., Kefford, B.J., Schäfer, R.B., Liess, M., 2013. Pesticides reduce regional biodiversity of stream invertebrates. *Proceedings of the National Academy of Sciences* 110, 11039–11043.

Berenzen, N., Lentzen–Godding, A., Probst, M., Schulz, H., Schulz, R., Liess, M., 2005. A comparison of predicted and measured levels of runoff–related pesticide concentrations in small lowland streams on a landscape level. *Chemosphere* 58, 683–691.

Berger, E., Haase, P., Kuemmerlen, M., Leps, M., Schaefer, R.B., Sundermann, A., 2017. Water quality variables and pollution sources shaping stream macroinvertebrate communities. *Science of the Total Environment* 587, 1–10.

Berny, G.Y.M., Aïna, M.P., Toko, I.I., Igout, A., Douny, C., Brose, F., Schiffers, B., Gouda, I., Sika, K.C., Kestemont, P., Scippo, M.L., 2019. Occurrence of acetamiprid residues in water reservoirs in the cotton basin of Northern Benin. *Bulletin of Environmental Contamination and Toxicology* 102, 7–12.

Bhandari, B., Shah, R.D.T., Sharma, S., 2018. Status, distribution, and habitat specificity of benthic macro–invertebrates: A case study in five tributaries of Buddhiganga river in western Nepal. *Journal of Institute of Science and Technology* 23, 69–75.

Bian, B., Zhou, Y., Fang, B.B., 2016. Distribution of heavy metals and benthic macroinvertebrates impacts from typical inflow river sediments in the Taihu Basin, China. *Ecological Indicators* 69, 348–359.

Bloomfield, J.P., Williams, R.J., Gooddy, D.C., Cape, J.N., Guha, P.M., 2006. Impacts of climate change on the fate and behaviour of pesticides in surface and groundwater–a UK perspective. *Science of the Total Environment* 369, 163–177.

Bonada, N., Prat, N., Resh, V.H., Statzner, B., 2006. Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. *Annual Review of Entomology* 51, 495–523.

Bonansea, R.I., Amé, M.V., Wunderlin, D.A., 2013. Determination of priority pesticides in water samples combining SPE and SPME coupled to GC–MS: A case study: Suquía River basin (Argentina). *Chemosphere* 90, 1860–1869.

Boone, M.D., Semlitsch, R.D., 2001. Interactions of an insecticide with larval density and predation in experimental amphibian communities. *Biological Conservation* 15, 228–238.

Booth, A.J., Moss, S., Weyl, O.L.F., 2015. Effect of rotenone on gill–respiring and plastron–respiring insects. *African Journal of Aquatic Sciences* 40, 95–100.

Borompichaichartkul, C., Luengsode, K., Chinprahast, N. and Devahastin, S., 2009. Improving quality of macadamia nut (*Macadamia integrifolia*) through the use of hybrid drying process. *Journal of Food Engineering* 93, 348–353.

Bownik, A., Kowalczyk, M., Bańczerowski, J., 2019. Lambda–cyhalothrin affects swimming activity and physiological responses of *Daphnia magna*. *Chemosphere* 216, 805–811.

Boyton, S.J. and Hardner, C.M., 2000, November. Phenology of flowering and nut production in macadamia. In *International Symposium on Tropical and Subtropical Fruits 575* (pp. 381-387).

Brausch, J.M., Smith, P.N., 2009. Pesticide resistance from historical agricultural chemical exposure in *Thamnocephalus platyurus* (Crustacea: Anostraca). *Environmental Pollution* 157, 481–487.

Bringolf, R.B., Cope, W.G., Barnhart, M.C., Mosher, S., Lazaro, P.R., Shea, D., 2007. Acute and chronic toxicity of pesticide formulations (atrazine, chlorpyrifos, and permethrin) to glochidia and juveniles of *Lampsilis siliquoidea*. *Environmental Toxicology and Chemistry: An International Journal* 26, 2101–2107.

Broadhurst, C.L., Wang, Y., Crawford, M.A., Cunnane, S.C., Parkington, J.E., Schmidt, W.F., 2002. Brain–specific lipids from marine, lacustrine, or terrestrial food resources: potential impact on early African Homo sapiens. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 131, 653–673.

Camargo, J.A., Alonso, Á., 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. *Environment International* 32, 831–849.

Cantonati, M., Poikane, S., Pringle, C.M., Stevens, L.E., Turak, E., Heino, J., Richardson, J.S., Bolpagni, R., Borrini, A., Cid, N., Čtvrtlíková, M., 2020. Characteristics, main impacts, and stewardship of natural and artificial freshwater environments: consequences for biodiversity conservation. *Water* 12, 260.

Carvalho, E.M., Uieda, V.S., 2006. Colonization routes of benthic macroinvertebrates in a stream in southeast Brazil. *Acta Limnologica Brasiliensia 18*, 367–376.

Ccanccapa, A., Masiá, A., Navarro–Ortega, A., Picó, Y., Barceló, D., 2016. Pesticides in the Ebro River basin: occurrence and risk assessment. *Environmental Pollution* 211, 414–424.

Chandrasekera, W.U., Premaratna, K.D.S., 2016. Toxic effects of Trebon, a synthetic pyrethroid based insecticide formulation, on *Oreochromis mossambicus* (Family: Cichlidae). *Sri Lanka Journal of Aquatic Sciences* 21, 35–49.

Chaudhari, R., Saxena, K.K., 2016. Genotoxicological assessment of pyrethroid insecticide bioallethrin in freshwater fish *Channapunctatus*. *Research Journal of Life Sciences* 2, 55–62.

Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* 18, 117–143.

Clements, W.H., 2020. Community responses of stream organisms to heavy metals: a review of observational and experimental approaches. *Metal Ecotoxicology*, 363–391.

Cooper, J., Dobson, H., 2007. The benefits of pesticides to mankind and the environment. *Crop Protection* 26, 1337–1348.

Cornejo, A., Pérez, J., Alonso, A., López–Rojo, N., Monroy, S. and Boyero, L., 2020. A common fungicide impairs stream ecosystem functioning through effects on aquatic hyphomycetes and detritivorous caddisflies. *Journal of Environmental Management* 263,110425.

Cornejo, A., Pérez, J., López–Rojo, N., García, G., Pérez, E., Guerra, A., Nieto, C., Boyero, L., 2021. Litter decomposition can be reduced by pesticide effects on detritivores and decomposers: Implications for tropical stream functioning. *Environmental Pollution* 285, 17243.

Cornejo, A., Tonin, A.M., Checa, B., Tuñon, A.R., Pérez, D., Coronado, E., González, S., Ríos, T., Macchi, P., Correa–Araneda, F., Boyero, L., 2019. Effects of multiple stressors associated with agriculture on stream macroinvertebrate communities in a tropical catchment. *PloS ONE* 14, 0220528.

Cox–Singh, J., Davis, T.M., Lee, K.S., Shamsul, S.S., Matusop, A., Ratnam, S., Rahman, H.A., Conway, D.J., Singh, B., 2008. *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. *Clinical Infectious Diseases* 46, 165–171.

Crowther, T.W., Stanton, D.W., Thomas, S.M., A'Bear, A.D., Hiscox, J., Jones, T.H., Voříšková, J., Baldrian, P., Boddy, L., 2013. Top-down control of soil fungal community composition by a globally distributed keystone consumer. *Ecology* 94, 2518–2528.

Cruzeiro, C., Amaral, S., Rocha, E., Rocha, M.J., 2017. Determination of 54 pesticides in waters of the Iberian Douro River estuary and risk assessment of environmentally relevant mixtures using theoretical approaches and *Artemia salina* and *Daphnia magna* bioassays. *Ecotoxicology and Environmental Safety* 145, 126–134.

Cuthbert, R.N., Dalu, T., Mutshekwa, T., Wasserman, R.J., 2019. Leaf inputs from invasive and native plants drive differential mosquito abundances. *Science of the Total Environment* 689, 652–654.

Cuthbert, R.N., Wasserman, R.J., Keates, C., Dalu, T., 2022. Food webs. In: Dalu, T., Wasserman, R.J. (eds) Fundamentals of Tropical Freshwater Wetlands: From Ecology to Conservation Management. Elsevier, Cambridge.

Dalu, T., Clegg, B., Nhiwatiwa, T., 2012. Macroinvertebrate communities associated with littoral zone habitats and the influence of environmental factors in Malilangwe Reservoir, Zimbabwe. *Knowledge and Management of Aquatic Ecosystems* 406, 06.

Dalu, T., Cuthbert, R.N., Methi, M.J., Dondofema, F., Chari, L.D., Wasserman, R.J., 2022. Drivers of aquatic macroinvertebrate communities in a Ramsar declared wetland system. *Science of the Total Environment* 818, 151683.

Dalu, T., Cuthbert, R.N., Taylor, J.C., Magoro, M.L., Weyl, O.L., Froneman, P.W., Wasserman, R.J., 2020a. Benthic diatom–based indices and isotopic biomonitoring of nitrogen pollution in a warm temperate Austral River system. *Science of the Total Environment* 748, 142452.

Dalu, T., Dlamini, P., Wasserman, R.J., Mokgoebo, M.J., Mutshekwa, T., Dondofema, F., Cuthbert, R.N., 2021. Effects of environmental variables on littoral macroinvertebrate community assemblages in subtropical reservoirs. *Chemistry and Ecology* 37, 419–436.

Dalu, T., Murudi, T., Dondofema, F., Wasserman, R.J., Chari, L.D., Murungweni, F.M., Cuthbert, R.N., 2020b. Balloon milkweed *Gomphocarpus physocarpus* distribution and drivers in an internationally protected wetland. *BioInvasions Records* 9, 627–641.

Dalu, T., Tavengwa, N.T (eds)., 2022. Emerging Freshwater Pollutants: Analysis, Fate and Regulation. Elsevier, Cambridge.

Dalu, T., Wasserman, R.J., 2021. Tropical freshwater wetlands: an introduction. In: Dalu, T., Wasserman, R.J., (eds). Fundamentals of Tropical Freshwater Wetlands: From Ecology to Conservation Management. Elsevier, Cambridge.

Dalu, T., Wasserman, R.J., Magoro, M.L., Mwedzi, T., Froneman, P.W., Weyl, O.L., 2017. Variation partitioning of benthic diatom community matrices: Effects of multiple variables on benthic diatom communities in an Austral temperate river system. *Science of the Total Environment* 601, 73–82.

Dawoud, M., Bundschuh, M., Goedkoop, W., McKie, B.G., 2017. Interactive effects of an insecticide and a fungicide on different organism groups and ecosystem functioning in a stream detrital food web. *Aquatic Toxicology* 186, 215–221.

De Wilde, T., Spanoghe, P., Debaer, C., Ryckeboer, J., Springael, D., Jaeken, P., 2007. Overview of on-farm bioremediation systems to reduce the occurrence of point source contamination. *Pest Management Science: formerly Pesticide Science* 63, 111–128.

Death, R.G., Winterbourn, M.J., 1995. Diversity patterns in stream benthic invertebrate communities: the influence of habitat stability. *Ecology* 76, 1446–1460.

Deknock, A., De Troyer, N., Houbraken, M., Dominguez–Granda, L., Nolivos, I., Van Echelpoel, W., Forio, M.A.E., Spanoghe, P., Goethals, P., 2019. Distribution of agricultural pesticides in the freshwater environment of the Guayas River basin (Ecuador). *Science of the Total Environment* 646, 996–1008.

DeLorenzo, Marie, E., Geoffrey I., Scott, P., Ross, E., 2001. Toxicity of pesticides to aquatic microorganisms: A review. *Environmental Toxicology and Chemistry* 20, 84–98.

Desmarais, T.R., Solo–Gabriele, H.M., Palmer, C.J., 2002. Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. *Applied and Environmental Microbiology* 68, 1165–1172.

Dudgeon, D., 2019. Multiple threats imperil freshwater biodiversity in the Anthropocene. *Current Biology* 29, 960–967.

Egglishaw, H.J., 1969. The distribution of benthic invertebrates on substrata in fast–flowing streams. *The Journal of Animal Ecology*, 19–33.

El Nemr, A., Moneer, A.A., Khaled, A., El–Sikaily, A., 2013. Levels, distribution, and risk assessment of organochlorines in surficial sediments of the Red Sea coast, Egypt. *Environmental Monitoring and Assessment* 185, 4835–4853.

Elhatip, H., Afşin, M., Dirik, K., Kurmaç, Y., Kavurmacı, M., 2003. Influences of human activities and agriculture on groundwater quality of Kayseri–Incesu–Dokuzpınar springs, central Anatolian part of Turkey. *Environmental Geology* 44, 490–494.

Ellender, B.R., Marr, S.M., Weyl, O.L., Zengeya, T., Wasserman, R.J., Alexander, M.E., Ivey, P., Woodford, D.J., 2017. Evaluating invasion risk for freshwater fishes in South Africa. *Bothalia* 47, 1–10.

Farag, M.R., Alagawany, M., Bilal, R.M., Gewida, A.G., Dhama, K., Abdel–Latif, H.M., Amer, M.S., Rivero–Perez, N., Zaragoza–Bastida, A., Binnaser, Y.S., Batiha, G.E.S., 2021. An Overview on the potential hazards of pyrethroid insecticides in fish, with special emphasis on cypermethrin toxicity. *Animals* 11, 880.

Feld, C.K., Hering, D., 2007. Community structure or function: effects of environmental stress on benthic macroinvertebrates at different spatial scales. *Freshwater Biology* 52, 1380–1399.

Feo, M.L., Ginebreda, A., Eljarrat, E., Barceló, D., 2010. Presence of pyrethroid pesticides in water and sediments of Ebro River Delta. *Journal of Hydrology* 393, 156–162.

Firmiano, K.R., Castro, D.M., Linares, M.S., Callisto, M., 2021. Functional responses of aquatic invertebrates to anthropogenic stressors in riparian zones of Neotropical savanna streams. *Science of the Total Environment* 753, 141865.

Freschet, G.T., Cornwell, W.K., Wardle, D.A., Elumeeva, T.G., Liu, W., Jackson, B.G., Onipchenko, V.G., Soudzilovskaia, N.A., Tao, J., Cornelissen, J.H., 2013. Linking litter decomposition of above-and below-ground organs to plant–soil feedbacks worldwide. *Journal of Ecology* 101, 943–952.

Friedmann, E.I., Galun, M., 1974. Desert algae, lichens, and fungi. *Desert Biology* 2, 165–212.

Fry, C., 2019. Field Guide to the Freshwater Macroinvertebrates of South Africa. Jacana Media, Auckland Park, South Africa.

Fugère, V., Kasangaki, A., Chapman, L.J., 2016. Land use changes in an afrotropical biodiversity hotspot affect stream alpha and beta diversity. *Ecosphere* 7, e01355.

Fugère, V., Lostchuck, E., Chapman, L.J., 2020. Litter decomposition in Afrotropical streams: Effects of land use, home–field advantage, and terrestrial herbivory. *Freshwater Science* 39, 497–507.

Gajendiran, A., Abraham, J., 2018. An overview of pyrethroid insecticides. *Frontiers in Biology* 13, 79–90.

Gascuel–Odoux, C., Aurousseau, P., Cordier, M.O., Durand, P., Garcia, F., Masson, V., Salmon–Monviola, J., Tortrat, F., Trépos, R., 2009. A decision–oriented model to evaluate the effect of land use and agricultural management on herbicide contamination in stream water. *Environmental Modelling and Software* 24, 1433–1446.

Geist, J., Hawkins, S.J., 2016. Habitat recovery and restoration in aquatic ecosystems: current progress and future challenges. *Aquatic Conservation: Marine and Freshwater Ecosystems* 26, 942–962.

Ghayyur, S., Tabassum, S., Ahmad, M.S., Akhtar, N., Khan, M.F., 2019. Effect of chlorpyrifos on hematological and seral biochemical components of fish *Oreochromis mossambicus*. *Pakistan Journal of Zoology* 51, 1047–1052.

Goergen, G., Kumar, P.L., Sankung, S.B., Togola, A., Tamò, M., 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. *PloS ONE* 11, 0165632.

Govindarajan, M., Rajeswary, M., Senthilmurugan, S., Vijayan, P., Alharbi, N.S., Kadaikunnan, S., Khaled, J.M. and Benelli, G., 2018. Larvicidal activity of the essential oil from Amomum subulatum Roxb. (Zingiberaceae) against *Anopheles subpictus*, *Aedes albopictus* and *Culex tritaeniorhynchus* (Diptera: Culicidae), and non–target impact on four mosquito natural enemies. *Physiological and Molecular Plant Pathology* 101, 219–224.

Grass, I., Meyer, S., Taylor, P.J., Foord, S.H., Hajek, P., Tscharntke, T., 2018. Pollination limitation despite managed honeybees in South African macadamia orchards. *Agriculture, Ecosystems and Environment* 260, 11–18.

Groenendijk, P., van der Kolk, J.W. and Travis, K.Z., 2018. Prediction of exposure concentrations in surface waters. In: Hill, I.R., Heimbach, F., Leeuwangh, P., Matthiessen, P. (eds) Freshwater Field Tests for Hazard Assessment of Chemicals. CRC Press, London.

Gulis, V., Suberkropp, K., 2003. Interactions between stream fungi and bacteria associated with decomposing leaf litter at different levels of nutrient availability. *Aquatic Microbial Ecology* 30, 149–157.

Hagen, E.M., Webster, J.R. and Benfield, E.F., 2006. Are leaf breakdown rates a useful measure of stream integrity along an agricultural landuse gradient?. *Journal of the North American Benthological Society* 25, 330343.

Halbach, K., Möder, M., Schrader, S., Liebmann, L., Schäfer, R.B., Schneeweiss, A., Schreiner, V.C., Vormeier, P., Weisner, O., Liess, M., Reemtsma, T., 2021. Small streams–large concentrations? Pesticide monitoring in small agricultural streams in Germany during dry weather and rainfall. *Water Research* 203, 117535.

Hale, R., Coleman, R., Sievers, M., Brown, T.R., Swearer, S.E., 2018. Using conservation behavior to manage ecological traps for a threatened freshwater fish. *Ecosphere* 9, e02381.

Halse, S.A., Pearson, G.B., Kaý, W.R., 1998. Arid zone networks in time and space: waterbird use. *International Journal of Ecology and Environmental Sciences* 24, 207–222.

Hansson, L.A., Brönmark, C., Nyström, P., Greenberg, L., Lundberg, P., Nilsson, P.A., Persson, A., Pettersson, L.B., Romare, P., Tranvik, L.J., 1998. Consumption patterns, complexity and enrichment in aquatic food chains. *Proceedings of the Royal Society of London, Series B* 265, 901–906.

Harmon, M.E., Nadelhoffer, K.J., Blair, J.M., 1999. Measuring decomposition, nutrient turnover, and stores in plant litter. In: Robertson, G.P., Bledsoe, C.S., Coleman, D.C., Sollins. P. (eds.) Standard soil methods for long–term ecological research. Oxford University Press, New York.

He, L.M., Troiano, J., Wang, A., Goh, K., 2008. Environmental chemistry, ecotoxicity, and fate of lambda–cyhalothrin. *Reviews of Environmental Contamination and Toxicology* 195, 71–91.

Heino, J., Tolonen, K.T., 2018. Ecological niche features override biological traits and taxonomic relatedness as predictors of occupancy and abundance in lake littoral macroinvertebrates. *Ecography* 41, 2092–2103.

Henchion, M., Hayes, M., Mullen, A.M., Fenelon, M., Tiwari, B., 2017. Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. *Foods* 6, 53.

Hieber, M., Gessner, M.O., 2002. Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology* 83, 1026–1038.

Holvoet, K., van Griensven, A., Seuntjens, P., Vanrolleghem, P.A., 2005. Sensitivity analysis for hydrology and pesticide supply towards the river in SWAT. *Physics and Chemistry of the Earth, Parts A/B/C* 30, 518–526.

Hooper, D.U., Adair, E.C., Cardinale, B.J., Byrnes, J.E., Hungate, B.A., Matulich, K.L., Gonzalez, A., Duffy, J.E., Gamfeldt, L., O'Connor, M.I., 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature* 486, 105–108

Hossain, M.S., Chowdhury, M., Pramanik, M.D., Rahman, M.A., Fakhruddin, A.N.M., Alam, M.K., 2015. Determination of selected pesticides in water samples adjacent to agricultural fields and removal of organophosphorus insecticide chlorpyrifos using soil bacterial isolates. *Applied Water Science* 5, 171–179.

Howard, A.G., 2010. On the challenge of quantifying man–made nanoparticles in the aquatic environment. *Journal of Environmental Monitoring* 12, 135–142.

Huang, W., Mukherjee, D., Chen, S., 2011. Assessment of Hurricane Ivan impact on chlorophyll–*a* in Pensacola Bay by MODIS 250 m remote sensing. *Marine Pollution Bulletin* 62, 490–498.

Huston, P.L., Pignatello, J.J., 1999. Degradation of selected pesticide active ingredients and commercial formulations in water by the photo–assisted Fenton reaction. *Water Research* 33, 238–1246.

INC, 2020. International nut and dried fruit council. Available at: www.nutfruit.org/ Retrieved 21 September 2022

Jaipieam, S., Visuthismajarn, P., Sutheravut, P., Siriwong, W., Thoumsang, S., Borjan, M., Robson, M., 2009. Organophosphate pesticide residues in drinking water from Artesian Wells and health risk assessment of agricultural communities, Thailand. *Human and Ecological Risk Assessment* 15, 1304–1316.

Jatulewicz, I., 2007. Comparison of macroinvertebrate communities associated with various habitats in anthropogenic reservoirs. *Oceanological and Hydrobiological Studies* 36, 39–47.

Jennings, D.R.H., 2021. Does glacial retreat impact benthic chironomid communities? A case study from Rocky Mountain National Park, Colorado. *SN Applied Sciences* 3, 1–14.

Jia, J., and Gao, Y., 2017. Acid deposition and assessment of its critical load for the environmental health of waterbodies in a subtropical watershed, China. *Journal of Hydrology* 555, 155–168.

Jiménez–López, J., Llorent–Martínez, E.J., Ortega–Barrales, P., Ruiz–Medina, A., 2020. Analysis of neonicotinoid pesticides in the agri–food sector: a critical assessment of the state of the art. *Applied Spectroscopy Reviews* 55, 613–646.

Jordaan, M.S., Dalu, T., Wasserman, R.J., Slabbert, E., Weyl, O.L.F., 2017. Unexpected survival of sharptooth catfish *Clarias gariepinus* (Burchell 1822) during acute rotenone toxicity trials will complicate management of invasions. *Biological Invasions* 19, 1739–1744.

Joseph, B., Raj, S.J., 2011. A comparative study on various properties of five medicinally important plants. *International Journal of Pharmacology* 7, 206–211.

Jourdan, J., Plath, M., Tonkin, J.D., Ceylan, M., Dumeier, A.C., Gellert, G., Graf, W., Hawkins, C.P., Kiel, E., Lorenz, A.W., Matthaei, C.D., 2019. Reintroduction of freshwater macroinvertebrates: challenges and opportunities. *Biological Reviews 94*, 368–387.

Jupp, P.G., 1996. Mosquitoes of Southern Africa*:* Culicinae and Toxorhynchitinae. Ekogilde Publishers, Hartebeespoort.

Kane, A.S., Salierno, J.D., Gipson, G.T., Molteno, T.C., Hunter, C., 2004. A video–based movement analysis system to quantify behavioral stress responses of fish. *Water Research* 38, 3993–4001.

Khalaf, G., Tachet, H., 1980. Colonization of artificial substrata by macro-invertebrates in a stream and variations according to stone size. *Freshwater Biology* 10, 475–482.

Khudhair, N., Yan, C., Liu, M., Yu, H., 2019. Effects of habitat types on macroinvertebrates assemblages structure: case study of sun island bund wetland. *Biomed Research International* 2019, 2650678

Knauer, K., Homazava, N., Junghans, M., Werner, I., 2017. The influence of particles on bioavailability and toxicity of pesticides in surface water. *Integrated Environmental Assessment and Management* 13, 585–600.

Knillmann, S., Orlinskiy, P., Kaske, O., Foit, K., Liess, M., 2018. Indication of pesticide effects and recolonisation in streams. *Science of the Total Environment* 630, 1619–1627.

Köck–Schulmeyer, M., Villagrasa, M., de Alda, M.L., Céspedes–Sánchez, R., Ventura, F., Barceló, D., 2013. Occurrence and behavior of pesticides in wastewater treatment plants and their environmental impact. *Science of the Total Environment* 458, 466–476.

Kominoski, J.S., Pringle, C.M., Ball, B.A., Bradford, M.A., Coleman, D.C., Hall, D.B. and Hunter, M.D., 2007. Nonadditive effects of leaf litter species diversity on breakdown dynamics in a detritus-based stream. *Ecology* 88, 1167–1176.

Kratzer, E.B., 2002. Temporal and spatial variation of wetland macroinvertebrates of the Okefenokee swamp. PhD Thesis, University of Georgia, Athens.

Kruawal, K., Sacher, F., Werner, A., Müller, J., Knepper, T.P., 2005. Chemical water quality in Thailand and its impacts on the drinking water production in Thailand. *Science* of *the Total Environment* 340, 57–70.

Kumar, N., Prabhu, P.A.J., Pal, A.K., Remya, S., Aklakur, M., Rana, R.S., Gupta, S., Raman, R.P., Jadhao, S.B., 2011. Anti–oxidative and immuno–hematological status of Tilapia (*Oreochromis mossambicus*) during acute toxicity test of endosulfan. *Pesticide Biochemistry and Physiology* 99, 45–52.

Kumar, R., Sankhla, M.S., Kumar, R., Sonone, S.S., 2021. Impact of pesticide toxicity in aquatic environment. *Biointerface Research in Applied Chemistry* 11, 10131–10140.

Lafage, D., Bergman, E., Eckstein, R.L., Österling, E.M., Sadler, J.P. and Piccolo, J.J., 2019. Local and landscape drivers of aquatic-to-terrestrial subsidies in riparian ecosystems: A worldwide meta-analysis. *Ecosphere 10*, 02697.

Laini, A., Bolpagni, R., Cancellario, T., Guareschi, S., Racchetti, E., Viaroli, P., 2018. Testing the response of macroinvertebrate communities and biomonitoring indices under multiple stressors in a lowland regulated river. *Ecological Indicators 90*, 47–53.

Lake, P.S., Doeg, T.J., 1985. Macroinvertebrate colonization of stones in two upland southern Australian streams. *Hydrobiologia* 126, 199–211.

Lauber, C. L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing–based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology* 75, 5111–5120.

Legradi, J.B., Di Paolo, C., Kraak, M.H.S., Van der Geest, H.G., Schymanski, E.L., Williams, A.J., Dingemans, M.M.L., Massei, R., Brack, W., Cousin, X., Begout, M.L., 2018. An ecotoxicological view on neurotoxicity assessment. *Environmental Sciences Europe* 30, 1–34.

Lehmann, E., Oltramare, C., Dibié, J.J.N., Konaté, Y., de Alencastro, L.F., 2018. Assessment of human exposure to pesticides by hair analysis: The case of vegetable–producing areas in Burkina Faso. *Environment International* 111, 317–331.

Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. *Nature 528*, 60–68.

Liess, M., Schäfer, R.B. and Schriever, C.A., 2008. The footprint of pesticide stress in communities—species traits reveal community effects of toxicants. *Science of the Total Environment* 406, 484–490.

Linden, V.M., 2019. How vertebrate communities affect quality and yield of macadamia farms in Levubu, South Africa. PhD Thesis, University of Venda, Thohoyandou.

Linden, V.M., Grass, I., Joubert, E., Tscharntke, T., Weier, S.M., Taylor, P.J., 2019. Ecosystem services and disservices by birds, bats and monkeys change with macadamia landscape heterogeneity. *Journal of Applied Ecology* 56, 2069–2078.

Linenberg, I., Christophides, G.K., Gendrin, M., 2016. Larval diet affects mosquito development and permissiveness to *Plasmodium* infection. *Scientific Reports* 6, 1–10.

Loewy, R.M., Monza, L.B., Kirs, V.E., Savini, M.C., 2011. Pesticide distribution in an agricultural environment in Argentina. *Journal of Environmental Science and Health, Part B 46*, 662–670.

Lorenzen, C.J., 1967. Determination of chlorophyll and pheopigments: spectrophotometric equations. *Limnology and Oceanography* 12, 343–346.

Lykogianni, M., Bempelou, E., Karamaouna, F., Aliferis, K.A., 2021. Do pesticides promote or hinder sustainability in agriculture? The challenge of sustainable use of pesticides in modern agriculture. *Science of the Total Environment* 758, 148625.

Macadamias South Africa (SAMAC), 2021. An overview of the South African Macadamia Industry [online]. Available from: https://www.samac.org.za/industry–overview/ Retrieved 16 October 2021; 15 November 2021; 20 July 2022.

Macadamias South Africa (SAMAC), 20220. An overview of the South African Macadamia Industry [online]. Available from: https://www.samac.org.za/industry–overview/ Retrieved 16 07 June 2022.

Macchi, P., Loewy, R.M., Lares, B., Latini, L., Monza, L., Guiñazú, N., Montagna, C.M., 2018. The impact of pesticides on the macroinvertebrate community in the water channels of the Río Negro and Neuquén Valley, North Patagonia (Argentina). *Environmental Science and Pollution Research* 25, 10668–10678.

Magbanua, F.S., Townsend, C.R., Blackwell, G.L., Phillips, N., Matthaei, C.D., 2010. Responses of stream macroinvertebrates and ecosystem function to conventional, integrated and organic farming. *Journal of Applied Ecology* 47, 1014–1025.

Majumder, R., Kaviraj, A., 2019. Acute and sublethal effects of organophosphate insecticide chlorpyrifos on freshwater fish *Oreochromis niloticus*. *Drug and Chemical Toxicology* 42, 487–495.

Manimekalai, D., Srinivasan, A., Padmavathy, P., Jawahar, P., George, R., Arisekar, U., 2022. Acute and chronic toxicity effects of the heptachlor pesticide on tilapia (*Oreochromis mossambicus*): impact to behavioural patterns and histopathological responses. *Journal of Coastal Research* 38, 999–1010.

Marchetti, M.P., Garr, M., Smith, A.N., 2010. Evaluating wetland restoration success using aquatic macroinvertebrate assemblages in the Sacramento Valley, California. *Restoration Ecology* 18, 457–466.

Masiá, A., Campo, J., Vázquez–Roig, P., Blasco, C., Picó, Y., 2013. Screening of currently used pesticides in water, sediments and biota of the Guadalquivir River Basin (Spain). *Journal of Hazardous Materials* 263, 95–104.

Matthaei, C.D., Weller, F., Kelly, D.W., Townsend, C.R., 2006. Impacts of fine sediment addition to tussock, pasture, dairy, and deer farming streams in New Zealand. *Freshwater Biology* 51, 2154–2172.

Maurya, P.K., Malik, D.S., Sharma, A., 2019. Impacts of pesticide application on aquatic environments and fish diversity. *Contaminants in Agriculture and Environment: Health Risks and Remediation* 1, 111.

Mbare, O., Lindsay, S.W. and Fillinger, U., 2014. Pyriproxyfen for mosquito control: female sterilization or horizontal transfer to oviposition substrates by *Anopheles gambiae* sensu stricto and *Culex quinquefasciatus*. *Parasites and Vectors* 7, 1–12.

Mbedzi, R., Dalu, T., Wasserman, R. J., Murungweni, F., Cuthbert, R., 2019. Functional response quantifies microplastic uptake by a widespread African fish species. *Science of the Total Environment* 700, 134522

Medeiros, A.O., Pascoal, C., Graça, M.A.S., 2009. Diversity and activity of aquatic fungi under low oxygen conditions. *Freshwater Biology* 54, 142–149.

Merritt, R.W., Dadd, R.H., Walker, E.D., 1992. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *The Annual Review of Entomology* 37, 349–374.

Miller, L.C., Tainter, M.L., 1944. Estimation of LD50 and its error by means of log–probit graph paper. *Proceedings of the Society for Experimental Biology and Medicine* 57, 261.

Minakawa, N., Sonye, G., Mogi, M. and Yan, G., 2004. Habitat characteristics of *Anopheles* gambiae larvae in a Kenyan highland. *Medical and veterinary entomology* 18, 301–305.

Miserendino, M.L., Brand, C., Di Prinzio, C.Y., 2008. Assessing urban impacts on water quality, benthic communities and fish in streams of the Andes Mountains, Patagonia (Argentina). *Water, Air, and Soil Pollution* 194, 91–110.

Mishra, A., Devi, Y., 2014. Histopathological alterations in the brain (optic tectum) of the fresh water teleost *Channa punctatus* in response to acute and subchronic exposure to the pesticide Chlorpyrifos. *Acta Histochemica* 116, 176–181.

Miyake, Y., Hiura, T., Kuhara, N., Nakano, S., 2003. Succession in a stream invertebrate community: a transition in species dominance through colonization. *Ecological Research* 18, 493–501.

Moberg, F., Rönnbäck, P., 2003. Ecosystem services of the tropical seascape: interactions, substitutions, and restoration. *Ocean and Coastal Management* 46, 27–46.

Mofu, L., Dalu, T., Wasserman, R.J., Woodford, D.J., Khosa, D., Weyl, O.L.F., 2021. Seasonal variation and drivers of zooplankton, macroinvertebrate, and littoral fish communities from irrigation ponds in a semi–arid region in the Eastern Cape (South Africa). *African Journal of Aquatic Science* 46, 452–463.

Montagner, C.C., Medeiros, J., Araújo, E.V., Munin, N.C.G., De Caroli Vizioli, B., 2022. Pesticides. In: Dalu, T., Tavengwa, N.T. (eds) Emerging Freshwater Pollutants: Analysis, Fate and Regulations. Elsevier, Cambridge.

Montemurro, N., Grieco, F., Lacertosa, G., Visconti, A., 2002. Chlorpyrifos decline curves and residue levels from different commercial formulations applied to oranges. *Journal of Agricultural and Food Chemistry* 5, 5975–5980.

Moore, C.J., 2008. Synthetic polymers in the marine environment: a rapidly increasing, long–term threat. *Environmental Research* 108, 131–139.

Moreira–Santos, M., Ribeiro, R., Araújo, C.V., 2019. What if aquatic animals move away from pesticide–contaminated habitats before suffering adverse physiological effects? A critical review. *Critical Reviews in Environmental Science and Technology* 49, 989–1025.

Morrissey, C.A., Mineau, P., Devries, J.H., Sanchez–Bayo, F., Liess, M., Cavallaro, M.C., Liber, K., 2015. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review. *Environment International* 74, 291–303.

Mueller, M., Bierschenk, A.M., Bierschenk, B.M., Pander, J., Geist, J., 2020. Effects of multiple stressors on the distribution of fish communities in 203 headwater streams of Rhine, Elbe and Danube. *Science of the Total Environment* 703, 134523.

Mutshekwa, T., Cuthbert, R.N., Wasserman, R.J., Murungweni, F.M., Dalu, T., 2020. Macroinvertebrate colonisation associated with native and invasive leaf litter decomposition. *Knowledge and Management of Aquatic Ecosystems*, 32.

Muturi, E.J., Allan, B.F., Ricci, J., 2012. Influence of leaf detritus type on production and longevity of container–breeding mosquitoes. *Environmental Entomology* 41, 1062–1068.

Muturi, E.J., Donthu, R.K., Fields, C.J., Moise, I.K., Kim, C.H., 2017. Effect of pesticides on microbial communities in container aquatic habitats. *Scientific Reports* 7, 1–10.

Muturi, J., Benedict, O.O., Chang–Hyun, Kim., 2013. Effect of leaf type and pesticide exposure on abundance of bacterial taxa in mosquito larval habitats. *PloS One* 8, 71812.

Mwaijengo, G.N., Vanschoenwinkel, B., Dube, T., Njau, K.N., Brendonck, L., 2020. Seasonal variation in benthic macroinvertebrate assemblages and water quality in an Afrotropical river catchment, northeastern Tanzania. *Limnologica* 82, 125780.

Myers, J.P., Antoniou, M.N., Blumberg, B., Carroll, L., Colborn, T., Everett, L.G., Hansen, M., Landrigan, P.J., Lanphear, B.P., Mesnage, R., Vandenberg, L.N., 2016. Concerns over use of glyphosate–based herbicides and risks associated with exposures: a consensus statement. *Environmental Health* 15, 1–13.

Nagao, M.A., Hirae, H.H., Stephenson, R.A., 1992. Macadamia: cultivation and physiology. *Critical Reviews in Plant Sciences* 10, 441–470.

Nasrabadi, T., Nabi Bidhendi, G., Karbassi, A., Grathwohl, P., Mehrdadi, N., 2011. Impact of major organophosphate pesticides used in agriculture to surface water and sediment quality (Southern Caspian Sea basin, Haraz River). *Environmental Earth Sciences* 63, 873–883.

Ngowi, A.V.F., Mbise, T.J., Ijani, A.S., London, L., Ajayi, O.C., 2007. Smallholder vegetable farmers in Northern Tanzania: Pesticides use practices, perceptions, cost and health effects. *Crop Protection* 26, 1617–1624.

Nguyen, T.X., Nguyen, B.T., Tran, H.T.T., Mai, H., Duong, T.T., Bach, Q.V., 2019. Seasonal, spatial variation, and potential sources of organochlorine pesticides in water and sediment in the lower reaches of the Dong Nai River system in Vietnam. *Archives of Environmental Contamination and Toxicology* 77, 514–526.

Nortjé, G.P., Botha, A., Schoeman, S.P., Botha, B.M., 2017. The successes of stinkbug research and the Stinkbug Working Group in the Southern African avocado and macadamia industries. South African Avocado Growers' Association Annual Research Symposium. Hotel@ Tzaneen, Tzaneen, 15 February 2017.

Nyantakyi, J.A., Wiafe, S., Akoto, O., 2022. Seasonal changes in pesticide residues in water and sediments from River Tano, Ghana. *Journal of Environmental and Public Health* 2022, 1–10.

Okoro, H.K., Pandey, S., Ogunkunle, C.O., Ngila, C.J., Zvinowanda, C., Jimoh, I., Lawal, I.A., Orosun, M.M., Adeniyi, A.G., 2022. Nanomaterial–based biosorbents: Adsorbent for efficient removal of selected organic pollutants from industrial wastewater. *Emerging Contaminants* 8, 46–58.

Olden, J.D., Kennard, M.J., Leprieur, F., Tedesco, P.A., Winemiller, K.O., García–Berthou, E., 2010. Conservation biogeography of freshwater fishes: recent progress and future challenges. *Diversity and Distributions* 16, 496–513.

Organisation for Economic Cooperation and Development (OECD)., 2005. OECD Environmental health and safety publications series for testing and assessments. Test no. 50. January 2005. OECD Environment Directorate, Paris.

Oti, E.E., Chude, L.A., 1997. Acute toxicity of inorganic fertilizer to African freshwater catfish *Clarias gariepinus* and *Tilapia guineensis* fingerlings. *Niger–Delta Biologia* 2, 60–62.

Ouyang, Y., Parajuli, P.B., Feng, G., Leininger, T.D., Wan, Y., Dash, P., 2018. Application of Climate Assessment Tool (CAT) to estimate climate variability impacts on nutrient loading from local watersheds. *Journal of Hydrology* 563, 363–371.

Overmyer, J.P., Noblet, R., Armbrust, K.L., 2005. Impacts of lawn–care pesticides on aquatic ecosystems in relation to property value. *Environmental Pollution* 137, 263–272.

Overmyer, K., Brosché, M., Pellinen, R., Kuittinen, T., Tuominen, H., Ahlfors, R., Keinänen, M., Saarma, M., Scheel, D., Kangasjärvi, J., 2005. Ozone–induced programmed cell death in the Arabidopsis radical–induced cell death1 mutant. *Plant Physiology* 137, 1092–1104.

Öztürk, M., Özözen, G., Minareci, O., Minareci, E., 2009. Determination of heavy metals in fish, water and sediments of Avsar Dam Lake in Turkey. *Journal of Environmental Health Science and Engineering* 6, 73–80.

Pal, E., Allison, J.D., Hurley, B.P., Slippers, B., Fourie, G., 2022. Genetic diversity of the two–spotted stink bug *Bathycoelia distincta* (Pentatomidae) associated with macadamia orchards in South Africa. *Plos ONE* 17, e0269373.

Pandya, P., Parikh, P., Ambegaonkar, A., 2020. Evaluating the toxic potential of agrochemicals on the hypothalamic-pituitary-thyroid axis in tilapia (*Oreochromis mossambicus*). *Journal of Applied Ichthyology* 36, 203–211.

Parithabhanu, A., Khusnumabegam, K.J., 2013. The influence of tannery effluent on biochemical constituents in the blood of the fish *Oreochromis mossambicus* (Bloch). *International Journal of Pure and Applied Zoology* 1, 227–230.

Parra–Arroyo, L., González–González, R.B., Castillo–Zacarías, C., Martínez, E.M.M., Sosa–Hernández, J.E., Bilal, M., Iqbal, H.M., Barceló, D., Parra–Saldívar, R., 2022. Highly hazardous pesticides and related pollutants: Toxicological, regulatory, and analytical aspects. *Science of the Total Environment* 807, 151879.

Peace, C.P., Allan, P., Vithanage, V., Turnbull, C.N., Carroll, B.J., 2005. Genetic relationships amongst macadamia varieties grown in South Africa as assessed by RAF markers. *South African Journal of Plant and Soil* 22, 71–75.

Pereira, W.E., Domagalski, J.L., Hostettler, F.D., Brown, L.R., Rapp, J.B., 1996. Occurrence and accumulation of pesticides and organic contaminants in river sediment, water and clam tissues from the San Joaquin River and tributaries, California. *Environmental Toxicology and Chemistry: An International Journal* 15, 172–180.

Pérez–Parada, A., Goyenola, G., de Mello, F.T., Heinzen, H., 2018. Recent advances and open questions around pesticide dynamics and effects on freshwater fishes. *Current Opinion in Environmental Science and Health* 4, 38–44.

Pfeiffer, T.Ž., Maronić, D.Š., Stević, F., Balkić, A.G., Bek, N., Martinović, A., Mandir, T., Nikolašević, R., Janjić, D., 2022. Plastisphere development in relation to the surrounding biotic communities. *Environmental Pollution*, 119380.

Pheiffer, W., 2017. Polycyclic aromatic hydrocarbons (PAHs) in the aquatic ecosystems of Soweto and Lenasia. PhD Thesis, North–West University, Potchefstroom.

Phillips, P.J., Bode, R.W., 2004. Pesticides in surface water runoff in south-eastern New York State, USA: seasonal and stormflow effects on concentrations. *Pest Management Science* 60, 531–543.

Pilière, A.F.H., Verberk, W.C.E.P., Gräwe, M., Breure, A.M., Dyer, S.D., Posthuma, L., De Zwart, D., Huijbregts, M.A.J., Schipper, A.M., 2016. On the importance of trait interrelationships for understanding environmental responses of stream macroinvertebrates. *Freshwater Biology* 61, 181–194.

Piscart, C., Genoel, R., Doledec, S., Chauvet, E. and Marmonier, P., 2009. Effects of intense agricultural practices on heterotrophic processes in streams. *Environmental Pollution* 157, 1011–1018.

Polazzo, F., Roth, S.K., Hermann, M., Mangold-Döring, A., Rico, A., Sobek, A., Van den Brink, P.J., Jackson, M.C., 2022. Combined effects of heatwaves and micropollutants on freshwater ecosystems: Towards an integrated assessment of extreme events in multiple stressors research. *Global Change Biology* 28, 12481267.

Puglisi, E., 2012. Response of microbial organisms (aquatic and terrestrial) to pesticides. *EFSA Supporting Publications* 9, 359E.

Quere, C.L., Harrison, S.P., Colin Prentice, I., Buitenhuis, E.T., Aumont, O., Bopp, L., Claustre, H., Cotrim Da Cunha, L., Geider, R., Giraud, X., Klaas, C., 2005. Ecosystem dynamics based on plankton functional types for global ocean biogeochemistry models. *Global Change Biology 11*, 2016–2040.

Rajmohan, K.S., Chandrasekaran, R., Varjani, S., 2020. A review on occurrence of pesticides in environment and current technologies for their remediation and management. *Indian Journal of Microbiology* 60, 125–138.

Ramankutty, N., Graumlich, L., Achard, F., Alves, D., Chhabra, A., DeFries, R.S., Foley, J.A., Geist, H., Houghton, R.A., Goldewijk, K.K., Lambin, E.F., 2006. Global land–cover change: Recent progress, remaining challenges. *Land–use and Land–cover Change*, 9–39.

Ramos, S.M., Graça, M.A., Ferreira, V., 2021. A comparison of decomposition rates and biological colonization of leaf litter from tropical and temperate origins. *Aquatic Ecology* 55, 925–940.

Rashid, I., Romshoo, S.A., 2013. Impact of anthropogenic activities on water quality of Lidder River in Kashmir Himalayas. *Environmental Monitoring and Assessment* 185, 4705–4719.

Rasmussen, J.J., Wiberg–Larsen, P., Baattrup–Pedersen, A., Monberg, R.J., Kronvang, B., 2012. Impacts of pesticides and natural stressors on leaf litter decomposition in agricultural streams. *Science of the Total Environment* 416, 148–155.

Raut, L.P., Jaiswal, S.B., Mohite, N.Y., 2013. Design, development and fabrication of agricultural pesticides sprayer with weeder. *International Journal of Applied Research and Studies* 2, 12–17.

Reiber, L., Knillmann, S., Kaske, O., Atencio, L.C., Bittner, L., Albrecht, J.E., Götz, A., Fahl, A.K., Beckers, L.M., Krauss, M., Henkelmann, B., 2021. Long–term effects of a catastrophic insecticide spill on stream invertebrates. *Science of the Total Environment* 768, 144456.

Reichard, M., 2022. Fish. In: Dalu, T., Wasserman, R.J. (Eds.), Fundamentals of Tropical Freshwater Wetlands: From Ecology to Conservation Management. Elsevier, Cambridge.

Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T., Kidd, K.A., MacCormack, T.J., Olden, J.D., Ormerod, S.J., Smol, J.P., 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews of the Cambridge Philosophical Society* 94, 849–873.

Rekha, R., Gautam, R.K., Kalpana, G., Suneel, K., 2008. Nuvan intoxication leads protein alteration in kidney of *Labeo rohita*. *Journal of Experimental Zoology* 11, 113–115.

Relyea, R.A., Diecks, N., 2008. An unforeseen chain of events: lethal effects of pesticides on frogs at sublethal concentrations. *Ecological Applications* 18, 1728–1742.

Riise, G., Lundekvam, H., Wu, Q.L., Haugen, L.E., Mulder, J., 2004. Loss of pesticides from agricultural fields in SE Norway–run–off through surface and drainage water. *Environmental Geochemistry and Health* 26, 269–276.

Rosa, B.F.J.V., Dias–Silva, M.V.D., Alves, R.G., 2013. Composition and structure of the Chironomidae (Insecta: Diptera) community associated with Bryophytes in a first–order stream in the Atlantic Forest, Brazil. *Neotropical Entomology* 42, 15–21.

Rossi, F., Pesce, S., Mallet, C., Margoum, C., Chaumot, A., Masson, M., Artigas, J., 2018. Interactive effects of pesticides and nutrients on microbial communities responsible of litter decomposition in streams. *Frontiers in Microbiology* 9, 2437.

Sánchez–Bayo, F., 2012. Insecticides mode of action in relation to their toxicity to non–target organisms. *Journal of Environmental and Analytical Toxicology* 4, 4–002.

Sanders, K.E., Smiley Jr, P.C., Gillespie, R.B., King, K.W., Smith, D.R., Pappas, E.A., 2020. Conservation implications of fish–habitat relationships in channelized agricultural headwater streams. *Journal of Environmental Quality* 6, 1585–1598.

Sattler, C., Kächele, H., Verch, G., 2007. Assessing the intensity of pesticide use in agriculture. *Agriculture, Ecosystems* and *Environment* 119, 299–304.

Saulnier–Talbot, É., Lavoie, I., 2018. Uncharted waters: the rise of human–made aquatic environments in the age of the "Anthropocene". *Anthropocene* 23, 29–42.

Saxena. O.P., Parashari A. 1982. Toxicity of cadmium to *Channa punctatus*. *Bulletin of Pure and Applied Sciences* 1, 42–44.

Schäfer, R.B., 2012. Biodiversity, ecosystem functions and services in environmental risk assessment: Introduction to the special issue. *Science of the Total Environment* 415, 1–2.

Schäfer, R.B., Caquet, T., Siimes, K., Mueller, R., Lagadic, L., Liess, M., 2007. Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe. *Science of the Total Environment* 382, 272–285.

Scheibler, E.E., Claps, M.C., Roig, S.A., 2014. Temporal and altitudinal variations in benthic macroinvertebrate assemblages in an Andean river basin of Argentina. *Journal of Limnology 73*.

Scherer, E., 1992. Behavioural responses as indicators of environmental alterations: approaches, results, developments. *Journal of Applied Ichthyology* 8, 122–131.

Schlenk, D., 2005. Pesticide biotransformation in fish. In: Mommsen, T.P., Moon, T.W. (eds) Biochemistry and Molecular Biology of Fishes. Elsevier, Oxford.

Schoeman, P.S., 2009. Key biotic components of the indigenous Tortricidae and Heteroptera complexes occurring on macadamia in South Africa. PhD Thesis, North–West University, Potchefstroom.

Schoeman, P.S., 2018. Relative seasonal occurrence of economically significant heteropterans (Pentatomidae and Coreidae) on macadamias in South Africa: implications for management. *African Entomology* 26, 543–549.

Shafiei, T.M., Costa, H.H., 1990. The susceptibility and resistance of fry and fingerlings of *Oreochromis mossambicus* Peters to some pesticides commonly used. *Sri Lanka Journal of Aquatic Sciences* 6, 73–80.

Sharma, H.C., 2014. Climate change effects on insects: implications for crop protection and food security. *Journal of Crop Improvement* 28, 229–259.

Sharma, N., 2014. Analysis of Lactate Dehydrogenase and ATPase activity in fish, *Gambusia affinis* at different period of exposure to chlorpyrifos. *International Journal* 4, 39–41.

Sharma, R.K., Agrawal, M., 2005. Biological effects of heavy metals: an overview. *Journal of Environmental Biology* 26, 301–313.

Shurin, J.B., Gruner, D.S., Hillebrand, H., 2006. All wet or dried up? Real differences between aquatic and terrestrial food webs. *Proceedings of the Royal Society B: Biological Sciences* 273, 1–9.

Sidhu, G.K., Singh, S., Kumar, V., Dhanjal, D.S., Datta, S., Singh, J., 2019. Toxicity, monitoring and biodegradation of organophosphate pesticides: a review. *Critical Reviews in Environmental Science and Technology* 49, 1135–1187.

Simon, D., Helliwell, S., Robards, K., 1998. Analytical chemistry of chlorpyrifos and diuron in aquatic ecosystems. *Analytica Chimica Acta* 360, 1–16.

Singh, S., Bhutia, D., Sarkar, S., Rai, B.K., Pal, J., Bhattacharjee, S., Bahadur, M., 2015. Analyses of pesticide residues in water, sediment and fish tissue from river Deomoni flowing through the tea gardens of Terai Region of West Bengal, India. *International Journal of Fisheries and Aquatic Studies* 3, 17–23.

Sivakumar, M.V.K., Gommes, R., Baier, W., 2000. Agrometeorology and sustainable agriculture. *Agricultural and Forest Meteorology* 103, 11–26.

Smiley Jr, P.C., King, K.W., Fausey, N.R., 2011. Influence of herbaceous riparian buffers on physical habitat, water chemistry, and stream communities within channelized agricultural headwater streams. *Ecological Engineering* 37, 1314–1323.

SPSS, Inc., 2007. SPSS version 16.0. SPSS Incorporated, Chicago, IL.

Srivastava, P., Singh, A., Pandey, A.K., 2016. Pesticides toxicity in fishes: biochemical, physiological and genotoxic aspects. *Biochemical and Cellular Archives* 16, 199–218.

Staaf, H., Berg, B., 1982. Accumulation and release of plant nutrients in decomposing scots pine needle litter. Long–term decomposition in a scots pine forest II. *Canadian Journal of Botany* 60, 1561–1568.

Stevenson, R.J., Bothwell, M.L., Lowe, R.L., Thorp, J.H., 1996. Algal Ecology: Freshwater Benthic Ecosystem. Elsevier, Cambridge.

Sultana, J., Syed, J.H., Mahmood, A., Ali, U., Rehman, M.Y.A., Malik, R.N., Li, J., Zhang, G., 2014. Investigation of organochlorine pesticides from the Indus Basin, Pakistan: sources, air–soil exchange fluxes and risk assessment. *Science of the Total Environment* 497, 113–122.

Sumudumali, R.G.I., Jayawardana, J.M.C.K., Gunatilake, S.K., Udayakumara, E.P.N., Malavipathirana, S., Jayalal, R.G.U., 2022. The effects of fungicide chlorothalonil on leaf litter breakdown rate in tropical streams: a microcosm study. *Aquatic Sciences* 84, 1–12.

Taylor, P.J., Grass, I., Alberts, A.J., Joubert, E., Tscharntke, T., 2018. Economic value of bat predation services–A review and new estimates from macadamia orchards. *Ecosystem Services* 30, 372–381.

Tazunoki, Y., Tokuda, M., Sakuma, A., Nishimuta, K., Oba, Y., Kadokami, K., Miyawaki, T., Ikegami, M., Ueno, D., 2022. Comprehensive analyses of agrochemicals affecting aquatic ecosystems: A case study of Odonata communities and macrophytes in Saga Plain, northern Kyushu, Japan. *Environmental Pollution* 292, 118334.

Tejeda–Vera, R., López–López, E., Sedeño–Díaz, J.E., 2007. Biomarkers and bioindicators of the health condition of *Ameca splendens* and *Goodea atripinnis* (Pisces: Goodeaidae) in the Ameca River, Mexico. *Environment International* 33, 521–531.

Ter Braak, C.J., 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology* 67, 1167–1179.

Thompson, M.S., Bankier, C., Bell, T., Dumbrell, A.J., Gray, C., Ledger, M.E., Lehmann, K., McKew, B.A., Sayer, C.D., Shelley, F., Trimmer, M., 2016. Gene-to-ecosystem impacts of a catastrophic pesticide spill: testing a multilevel bioassessment approach in a river ecosystem. *Freshwater Biology 61*, 2037–2050.

Trueman, S.J., Kämper, W., Nichols, J., Ogbourne, S.M., Hawkes, D., Peters, T., Hosseini Bai, S., Wallace, H.M., 2022. Pollen limitation and xenia effects in a cultivated mass–flowering tree, *Macadamia integrifolia* (Proteaceae). *Annals of Botany* 129, 135–146.

Trueman, S.J., Turnbull, C.G.N., 1994. Effects of cross–pollination and flower removal on fruit set in Macadamia. *Annals of Botany* 73,23–32.

Tudi, M., Daniel Ruan, H., Wang, L., Lyu, J., Sadler, R., Connell, D., Chu, C., Phung, D.T., 2021. Agriculture development, pesticide application and its impact on the environment. *International Journal of Environmental Research and Public Health* 18, 1112.

Van den Brink, P.J., Klein, S.L, Rico, A., 2017. Interaction between stress induced by competition, predation, and an insecticide on the response of aquatic invertebrates. *Environmental Toxicology and Chemistry* 11: 2485–2492.

Verberk, W.C.E.P., 2011. Explaining general patterns in species abundance and distributions. *Nature Education Knowledge* 3, 38.

Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R., Davies, P.M., 2010. Global threats to human water security and river biodiversity. *Nature* 467, 555–561.

Walter, A., Finger, R., Huber, R., Buchmann, N., 2017. Smart farming is key to developing sustainable agriculture. *Proceedings of the National Academy of Sciences* 114, 6148–6150.

Wang, L., Robertson, D.M., Garrison, P.J., 2007. Linkages between nutrients and assemblages of macroinvertebrates and fish in wadeable streams: implication to nutrient criteria development. *Environmental Management* 39, 194–212.

Warren, N., Allan, I.J., Carter, J.E., House, W.A., Parker, A., 2003. Pesticides and other micro–organic contaminants in freshwater sedimentary environments—a review. *Applied Geochemistry* 18, 159–194.

Wasserman, R.J., Noyon, M., Avery, T.S., Froneman, P.W., 2013. Trophic level stability inducing effects of predaceous early juvenile fish in an estuarine mesocosms study. *PloS ONE* 8, 61019.

Weber, G., Christmann, N., Thiery, A.C., Martens, D., Kubiniok, J., 2018. Pesticides in agricultural headwater streams in southwestern Germany and effects on macroinvertebrate populations. *Science of the Total Environment* 619, 638–648.

Weier, S.M., Moodley, Y., Fraser, M.F., Linden, V.M., Grass, I., Tscharntke, T., Taylor, P.J., 2019. Insect pest consumption by bats in macadamia orchards established by molecular diet analyses. *Global Ecology and Conservation* 18, e00626.

Werner, I., Deanovic, L.A., Connor, V., de Vlaming, V., Bailey, H.C., Hinton, D.E., 2000. Insecticide-caused toxicity to *Ceriodaphnia dubia* (CLADOCERA) in the Sacramento–San Joaquin River delta, California, USA. *Environmental Toxicology and Chemistry: International Journal* 19, 215–227.

Willming, M.M., Qin, G., Maul, J.D., 2013. Effects of environmentally realistic daily temperature variation on pesticide toxicity to aquatic invertebrates. *Environmental Toxicology and Chemistry* 32, 2738–2745.

Yu, X.Y., Mu, C.L., Gu, C., Liu, C., Liu, X.J., 2011. Impact of woodchip biochar amendment on the sorption and dissipation of pesticide acetamiprid in agricultural soils. *Chemosphere* 85, 1284–1289.

Zbinden, M., Hieber, M., Robinson, C.T., Uehlinger, U., 2008. Short–term colonization patterns of macroinvertebrates in alpine streams. *Fundamental and Applied Limnology* 171, 75.

Zhang, J., Terrones, M., Park, C.R., Mukherjee, R., Monthioux, M., Koratkar, N., Kim, Y.S., Hurt, R., Frackowiak, E., Enoki, T., Chen, Y., 2016. Carbon science in 2016: Status, challenges and perspectives. *Carbon* 98, 708–732.

Zoumenou, B.S.G., Aïna, M.P., Imorou Toko, I., Igout, A., Douny, C., Brose, F., Schiffers, B., Gouda, I., Chabi Sika, K., Kestemont, P., Scippo, M.L., 2019. Occurrence of acetamiprid residues in water reservoirs in the cotton basin of Northern Benin. *Bulletin of Environmental Contamination and Toxicology* 102, 7–12.

Zubrod, J.P., Baudy, P., Schulz, R., Bundschuh, M., 2014. Effects of current–use fungicides and their mixtures on the feeding and survival of the key shredder *Gammarus fossarum*. *Aquatic Toxicology* 150, 133143.