Author/s:
Cervantes Servin, Adrian Ixcoatl

Title:
An integrated method for groundwater vulnerability assessment using a DRASTICL model and a green algae ecotoxicity test

Date:
2019

Persistent Link:
https://hdl.handle.net/11343/240268

An integrated method for groundwater vulnerability assessment using a DRASTICL model and a green algae ecotoxicity test

Adrian Ixcoatl Cervantes Servin

ORCID 0000-0002-5649-4199

Submitted in total fulfilment of the requirements
of the degree of Doctor of Philosophy

December 2019

Department of Infrastructure Engineering - School of Engineering

The University of Melbourne

Declaration

This is to certify that

(i) this thesis comprises only my original work towards the degree of Doctor of Philosophy except where indicated,

(ii) appropriate acknowledgement has been made in the text to all other material used,

(iii) the thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Adrian Ixcoatl Cervantes Servin

Signature:____________________________________

Date: 22 / December / 2019

Abstract

The world population could reach 9.8 billion by the year 2050 (UN, 2019). The rising world population presents the challenge of water and food security for human development. As a result, excessive use of agricultural chemicals (both fertilisers and pesticides) which in most cases are toxic not only for the humankind but also for the environment, is putting significant stress on the groundwater levels and the water quality. Groundwater (GW) pollution from agriculture is particularly significant in partially and totally groundwater-dependent ecosystems, as these ecosystems provide habitat for various endangered flora and fauna. Even though the importance of GW systems is well recognised, groundwater pollution vulnerability assessment models and groundwater pollution quantification techniques have experienced slight changes over the last 10 years and in most cases, the use of agricultural chemicals is assumed to be safe in the absence of sufficient data and evidence. The existing models produce an annual GW Vulnerability map and there is little to no monitoring of groundwater for these chemicals due to difficulty with identifying the representative samples and the high analytical cost. Therefore, this thesis aims to develop a novel approach to understand the seasonal variations in groundwater vulnerability and used the modified model to identify the representative sampling bores and also explores an algal ecotoxicology test as for the pesticides in water, that can be used as a fast and inexpensive pre-screen to identify samples above a certain level of toxicity for detailed chemical analysis.

The thesis is organised in 6 Chapters; Chapter 1 outlines the background, knowledge gaps and the research questions. Chapter 2 presents a critical review of the published literature on both GW Vulnerability research and ecotoxicity tests. The following three

Chapters respond to one of the three research questions each. Part One (Chapter Three) presents the factors that predict groundwater vulnerability and development of a time-variant model to estimate the groundwater vulnerability. The factors accounted in DRASTICL model (Groundwater depth (D), Net recharge (R), Aquifer media (A), soil type (S), topography slope (T), impact of vadose zone (I), hydraulic conductivity (C), and land use (L)) were compiled in two factor groups, the dynamic and the static. As a result, the dynamic group was found to be responsible for estimating time-variant vulnerability. The resolution for the grid-cell used in this study was 50 x 50 m size in the Glenelg Hopkins Catchment Management Authority (CMA), which is Victoria's most agriculture intensive regions and utilises fertilisers as well as triazine pesticide for crops such as canola, lentils and corn, and therefore presents a potential risk of GW contamination by pesticides as well nutrients. Having estimated the seasonal groundwater vulnerability assessment in the Glenelg Hopkins Catchment Management Authority region, the seasonal vulnerability was used to identify areas of high vulnerability within the region. This information was used in Chapter 4 to target the aquifers that were tested for agricultural pollution.

Part Two (Chapter Four) used the seasonal vulnerability estimation map to design and perform a groundwater survey to test for triazines herbicides, nitrogen and phosphorus pollution. Field surveys were conducted in three different campaigns during the year 2018-2019. As a result, 47 groundwater-monitoring bores were sampled, bores's selection were based upon the implementation of a Pesticide DRASTICL model. From these sites, 14 bores were not accessible, 16 were visited during the first campaign, six additional bores were sampled during the second campaign, and ten wetlands were monitored. So, the overall sampling campaign included a total of 22 bores and 10 wetlands sites. Laboratory results showed that most of the groundwater has been contaminated by nitrogen and phosphorus. In addition, a high level of atrazine, simazine, and desethyl-atrazine (a breakdown product of triazine herbicides) was detected in one bore. Results also showed that nutrients do not represent an environmental concern in wetlands as concentration values were within the environmental guidelines for Victoria. Three out of ten (~ 30%) wetlands showed atrazine pollution and four out of ten (40%) showed the occurrence of simazine. The use of a "Seasonal Pesticide DRASTICL" model proved to be of significant use in the design and application of groundwater pollution surveys for pesticides and nutrients.

Part Three (Chapter Five) defined the term “Groundwater ecotoxicology”. Using this approach, this thesis presents the design of a green algal toxicity test using the green unicellular alga *Scenedesmus sp.* This test can be used as a quick and inexpensive screen for assessing groundwater quality in intensive agricultural regions. This study compared an existing toxicity (nutrients uncalibrated test) test with a modified one (nutrients calibrated test). With the existing uncalibrated test, results showed that low nutrient concentrations have a direct effect on algal growth inhibition. As for the modified test, nutrient concentrations have been calibrated to the culturing media concentrations (nitrogen and phosphorus), salinity inhibited growth when conductivities exceeded (10,000µs/cm). This toxicity test demonstrated that *Scenedesmus sp*. is sensitive to triazine herbicides which are extensively used in the study site. This test represents a fast and inexpensive alternative to chemical laboratory analysis that can be applied in groundwater pollution prevention strategies and groundwater monitoring programs.

Results from this research provide a sound understanding of estimating time-variant groundwater vulnerabilities for different seasons. This research also provides the adaptation of DRASTICL-based models for its application in Victoria, Australia. Additionally, we expand the application of green algal toxicity studies to the groundwater environment. This can be thus used as a validated tool to estimate herbicides toxicity in aquifers from agricultural areas.

Dedication

This thesis is dedicated to all the women in my family,

especially to Maria de Jesus Lara Hernandez,

and her daughter Maria Luisa Servin Lara.

Up there in the mountains, where we belong,

rivers run cold and crystal waters,

air blows fresh aromatic winds of freedom,

and the land grows more than we can ask for,

Up there, thoughts, dreams, and hopes are meant to happen.

For all the efforts, and sacrifices never told,

For all the tears that were never wiped away,

For all your limitless love, but mostly,

for sharing the strong belief of dreaming.

Acknowledgements

The author wants to thank the institutions and the people who generously contributed significantly to the development of this research work. My supervisors, Meenakshi Arora and Vincent Pettigrove were a fundamental part of this work providing both their advice and comments for this thesis and during the fieldwork. I want to thank Timothy John Peterson for his contribution with both data and finest comments to my research.

I want to express my sincere gratitude to The National Council for Science and Technology of the United Mexican States (CONACYT) for granting me the postgraduate international scholarship for higher degree research.

Many thanks to Melbourne Water for their generous funding of the fieldwork campaign and support with materials and laboratory analysis. I would particularly thank Sarah Gaskill. Also, I would like to express my most sincere appreciation to Vincent Pettigrove for the funding of my scholarship during the period of this research. As well, I would like to thank Andrew Western for all his support and encouragement provided to my persona.

At the RMIT University, Bundoora campus, I would like to thank the staff members of the science laboratories (Building 211) and to Jackie Myers for their advice and comments during the development of my laboratory tests and experiments. At the University of Melbourne, I would like to thank the staff member Rodger Ian Young and Elise King for the aid in the works performed during the fieldwork and for the additional help for preparing and maintaining the equipment; I want to thank Eileen Doufas Sheam for all her remarkable effort while managing my PhD administration. I'd as well want to thank Amir Behroozi, and Katayoon Bahramian for their companionship, and assistance during my research. Finally,

I would like to thank my mother Maria Luisa Servin Lara for her significant contribution during my earlier education and the ongoing advice during my PhD.

Preface

This thesis is a research work that has been developed by my persona at its core, the contributions of other contributors are restricted to supervision, expertise technical aid and manuscript review. Chapters Three to Five are each formatted as journal articles and have been submitted as follow:

- *Chapter Three has been submitted as Cervantes-Servin, A. I., Arora, M., Peterson, J. T., & Pettigrove, V. Seasonal assessment of groundwater vulnerability to agricultural pollutants using DRASTICL. In revision following peer review by Hydrogeology Journal.*

- *Chapter Four is ready to be submitted as Cervantes-Servin, A. I., Arora, M., & Pettigrove, V. Are triazines and nutrients from farms making their way into groundwater and wetlands at the Glenelg Hopkins, Victoria, Australia?*

- *Chapter Five is ready to be submitted as Cervantes-Servin, A. I., Myers J., Arora, M., & Pettigrove, V. Expanding the horizon of algal ecotoxicology to the groundwater environment*

List of Abbreviations

A	Aquifer media
AHD	Australian Head Datum
ALS	Australian Laboratory Services
ANZECC	Australian and New Zealand Environment and Conservation Council
APVMA	Australian Pesticides and Veterinary Medicines Authority
ARMCANZ	Agriculture and Resource Management Council of Australia and New Zealand
AWRA	Australian Water Resource Assessment
C	Conductivity of the Aquifer
CA	Correspondence Analyses
CMA	Catchment management authority
CSV	Comma Separated Values
D	Groundwater Depth
DRP	Dissolved Reactive Phosphorus
sD	Seasonal Groundwater Depth
DEM	Digital Elevation Model
EPA	Environment Protection Authority Victoria
GH	Glenelg Hopkins
GWFS	Groundwater Flow Systems
GW	Groundwater
GWFS	Groundwater Flow Systems
GWV	Groundwater Vulnerability
GWVA	Groundwater Vulnerability Assessment
GWVE	Groundwater Vulnerability Estimation

I	Impact of vadose zone
ID	DRASTICL Vulnerability Index
ID_S	Seasonal DRASTICL Vulnerability Index
L	Land Use
NMI	National Measurement Institute
NOx	Oxides of Nitrogen
PCA	Principal Component Analysis
R	Groundwater Net Recharge
$_SR$	Seasonal Groundwater Recharge
SEM	Standard Error of the Mean
SOBN	State Observation Bore Network
S	Soil Type
T	Topography Slope
VA	Vulnerability Assessment
VLUIS	Victorian Land Use Information System

Contents

List of Figures

List of Tables

Chapter 1

Introduction

1.1 Introduction

Groundwater vulnerability (GWV) is defined by the National Research Council as the likelihood or tendency for a pollutant to percolate at a specific position in the groundwater system (NRC, 1993) after it has been introduced in the first aquifer. The estimation of GWV represents the best option available to date in groundwater pollution protection strategies (Allouche et al., 2017; Dahlhaus et al., 2002).

Recognizing that "all groundwater is vulnerable" referred to as the first law of groundwater vulnerability in NRC (1993) is the first step when working with GWV assessment (GWVA) models. Groundwater (GW) pollution has been recognised as a serious public health and environmental concern, limiting both, its availability as a resource for irrigation and its suitability for human consumption (Bartzas et al., 2015). GW is a significant environmental and economic asset currently facing considerable pressure globally because of its growing demand as well as over-extraction and aquifers pollution risk due to the increased use of agrochemicals (Machiwal et al., 2018).

Increasing levels of agrochemicals, including pesticides, have been reported in groundwater from many parts of the world, including Australia (Gilliom, 2007; SKM, 1995). In Australia, in the Glenelg Hopkins (GH) Catchment Management Authority (CMA) region, Victoria, GW is vital for the totally or partially GW dependent ecosystems which are part of the international aquatic ecosystem such as Ramsar wetlands that offer shelter for migration of different species (GH CMA, 2018, 2013).

Aquifers contaminated with pesticides pose a significant risk of contamination to GW dependent wetlands therefore, there is a need to understand the fate and transport of pesticides applied on farms in the subsurface.

There is an urgent need to prevent GW to be polluted from agricultural activities using tools that are both scientifically robust and flexible to apply, such tools aid GW managers to identify the probability of whether or not an aquifer is polluted (Machiwal et al., 2018; Kumar, 2015). In Australia, there is not much published literature available regarding the implementation of GWV assessments. A few available studies were either performed for a small scale (Askarimarnani and Willgoose, 2014) or are data limited (DPIE, 2018). Researchers have used DRASTICL [acronym of (depth (D), GW net recharge (R), aquifer media (A), soil media (S), topography (T), impact of vadose zone (I) hydraulic conductivity (C) of the aquifer and (L) land use] (Alam et al., 2014; Aller et al., 1987; Aller et al., 1985) based models to estimate GWV. These models utilise data on Depth, Recharge, Aquifer media, Soil media, Topography, Impact of vadose zone, Hydraulic conductivity, Land use and the nutrients data. There is practically almost no data on pesticide concentrations in GW in Victoria, though there are some nutrients (total nitrogen and phosphorus) concentrations data available in GW for Victoria Australia, data discontinuity both spatially and temporally limits the implementation of the model calibration procedure for DRASTIC-based models (Machiwal et al., 2018). The paucity of data on agrochemicals and pesticides might be due to the high cost of sample collection and analysis and the difficulty in identifying the representative bores for such analysis. GWV assessment can be utilised to identify the bores with the highest risk for such analysis. Another major gap identified through literature review is disregarding the seasonal variability in the estimation of the GWV. The current GWVA methods offer a static vulnerability scale (results) that is time-invariant over the extension of the aquifer (area of study). However, researchers have mentioned a need for a GWVA method that accounts for the climatic changes and that reflects temporal changes of the GWV estimation (Machiwal et al., 2018).

Researchers in freshwater ecology have used ecotoxicology tests as a surrogate for cumulative chemicals in water, that tests the overall toxicity of a water sample instead of a particular chemical group. An algal toxicity test might be a useful inexpensive first screen to detect groundwater samples with toxicity before conducting complete analytical tests for chemicals. A green alga is used as a test organism to conduct ecotoxicological screens of the GW to see if this is affected by contaminants (nutrients, high salinity concentrations and herbicides) in the water. The toxic samples can then be tested for a range of potential chemicals.

This thesis aims to address the above knowledge gaps by developing an understanding of the time-variant estimation of GWVA in the GH CMA area with the purpose to achieve a more precise estimation of the vulnerability. In addition, this thesis explores the implementation of a green algal (*Scenedesmus sp.*) toxicity test to quantify toxicity in GW samples. Green algal ecotoxicity tests can be applied to GW samples to test for atrazine toxicity and represents a significant advance in reducing the cost and time when testing for water quality of GW samples. Time-variant GWV assessments together with green algae ecotoxicity tests designed for GW embodies a central opportunity for GW managers, monitoring strategies and pollution prevention plans.

1.2 Research objectives

The overall objective of this thesis is to develop a novel approach to understand the seasonal variations in GWV to inform the bore selection process for ongoing monitoring as well as explore an algal ecotoxicology test as a cheap and quick alternative to full analytical scan for the pesticides in water. This is an interdisciplinary (engineering and bioscience) research that incorporates two inter-correlated sub-objectives:

Sub-objective 1–Seasonal Pesticide DRASTICL model:

a) Understanding the factors that account for the spatial and temporal variability in estimating GWV.
b) To build a seasonal GWV assessment model for the GH CMA that can incorporate the temporal variability due to different hydrogeological conditions.

Sub-objective 2–Green algal ecotoxicity test:

a) Understand the laboratory conditions that impact algal growth in GW samples.
b) Develop a methodology for green algal ecotoxicity test for GW.

A time-variant DRASTICL model for the GH CMA provides insights for the GWV model requirements of Australian aquifer, and at the same time provides insights into the methodology for making the standard vulnerability model time-variant under different spatial configurations. Likewise, the design of a green algal ecotoxicity test for GW opens a new window in ecotoxicology for which there is little knowledge; GW ecotoxicology.

1.3 Research questions

The two sub-objectives described above were coupled together to enable the combined use of a seasonal Pesticide DRASTICL model together with a green algal toxicity test as a valid tool to implement cost-efficient GW quality surveys when testing for contamination at agricultural areas. This dissertation aimed to answer the following three research questions to achieve the overall objective stated in the previous section:

1. How can we represent temporal variations in GWV estimation within DRASTICL model?
2. Do agricultural activities have the potential to contaminate GW in the GH CMA?
3. Can green algal ecotoxicity test be used as a low-cost screen to determine GW pollution?

1.4 Thesis layout

The research framed in the aforementioned three questions is described in the six Chapters. Table 1.1 summarizes each Chapter and the corresponding research questions. This Chapter presents a brief background and states the aim of the research. It presents the rationale of a method that allows the use of a green algal for toxicity quantification in groundwater samples. Chapter Two discusses relevant literature for both the seasonal GWV and the ecotoxicity tests for freshwater and groundwater. This Chapter highlights the knowledge gaps in the literature that are then used to frame the aim and research questions for this thesis. Chapter Three explores constructing a time-variant DRASTICL-based model for Australian aquifer configuration. In Chapter Four, the model application results to identify target GW bores with different vulnerability values are presented and a field survey for GW was deployed at the GH CMA. Chapter Five presents the results of a green algal toxicity test was designed and tested to identify contamination from GW due to atrazine agricultural contamination, using groundwater samples from the GH CMA field survey. Finally, Chapter Six draws together the findings from the time-variant GWV model, the GW quality field survey results and the findings from the green algal toxicity test to summarise the scientific contributions made through this thesis while addressing the aforementioned research questions.

Table 1.1 Thesis Chapters and research questions

Chapter No.	Outline	Research Questions
2 Literature review	Offers a review of methods, research and approaches of investigation. Findings include: 1. gaps in the current DRASTIC-based methods 2. GW ecotoxicology a new area of knowledge	1 to 3
3 Seasonal assessment of GW vulnerability to agricultural pollutants using seasonal DRASTICL	Details the statistical structure of the conformation of the time-variant DRASTICL-based model. To accomplish this task, two variable groups were identified: 1. dynamic factor group (GW depth and GW recharge) 2. static factor group (A, S, T, I, C and L)	1
4 Are triazines and nutrients from farms making their way into GW and wetlands at the Glenelg Hopkins, Victoria, Australia?	Summarizes the findings for the GW field survey that was designed using the new time-variant Pesticide DRASTICL model. Results reported the screen for triazines and nutrients.	2
5 Expanding the horizon of algal ecotoxicology to the GW environment	The need for a cost-time efficient test for GW pollution identification resulted in the design for a green algal (*Scenedesmus sp.*) Toxicity test.	3
6 Discussion and conclusions	A discussion and a summary of the results from Chapters Two to Five are presented in this final along with the limitations of this research and a recommended way forward for further research.	1 to 3

Chapter 2

Literature review

Chapter One presented the concepts of GW pollution, GWV assessments, and time-variant vulnerability. Former concepts were framed within a hydrogeological context and then a new concept of GW toxicity was included to encompass the proposed GW pollution prevention tool: An integrated method for GW vulnerability assessment using a DRASTICL model and a green alga ecotoxicity test.

This Chapter reviews research from studies that have investigated GW vulnerability models, GW quality pollution in Victoria, and GW ecotoxicology in addition to a hydrogeological description of the study site. It is organized into five sections revealing the existing state of the art and knowledge gaps that are the basis of the research questions.

2.1 The Glenelg Hopkins Catchment Management Authority

This section presents a physical description of the hydrogeological properties of the study site and provides an overview and its economic, social, environmental and climatic characteristics.

Location and physical environment

The Glenelg Hopkins Catchment Management Authority (GH-CMA) is located in the west of Victoria, in the south of Australia. The GH Region was declared under the

Catchment and Land Protection Act (VSG, 1994) to better manage environmental challenges at a catchment scale due to its ecologically significant sites. It limits to the north with the Wimmera CMA, to the north-east with the North Central CMA, to the east with the Corangamite CMA, to the west with South Australia State and limits to the south with the Great Australian Bight (GH CMA, 2013). The highest peak in the Grampians is Mt. William, with a height over 1,168 metres above the Australian Head Datum AHD. Including Lady Julia Percy Island, this CMA has significant marine locations such as Portland, Port Fairy and Warrnambool, suitable for the spooning and nursery of marine mammals (GH CMA, 2013). Figure 2.1 shows the location and major natural assets. The region is 25,000 km^2 and hosts the towns of Ararat, Ballarat, Beaufort, Beaufort, Casterton, Hamilton, Mortlake, Portland, Port Fairy and Warrnambool. Four basins are included in the region: Glenelg, Hopkins, Millicent and Portland Coast. The GH CMA hosts the renown Grampians National Park within the inland natural assets, on the coastline this CMA embraces sites which are both attractive and significant for the international ecosystem such as the Glenelg Estuary and Discovery Bay Ramsar Site.

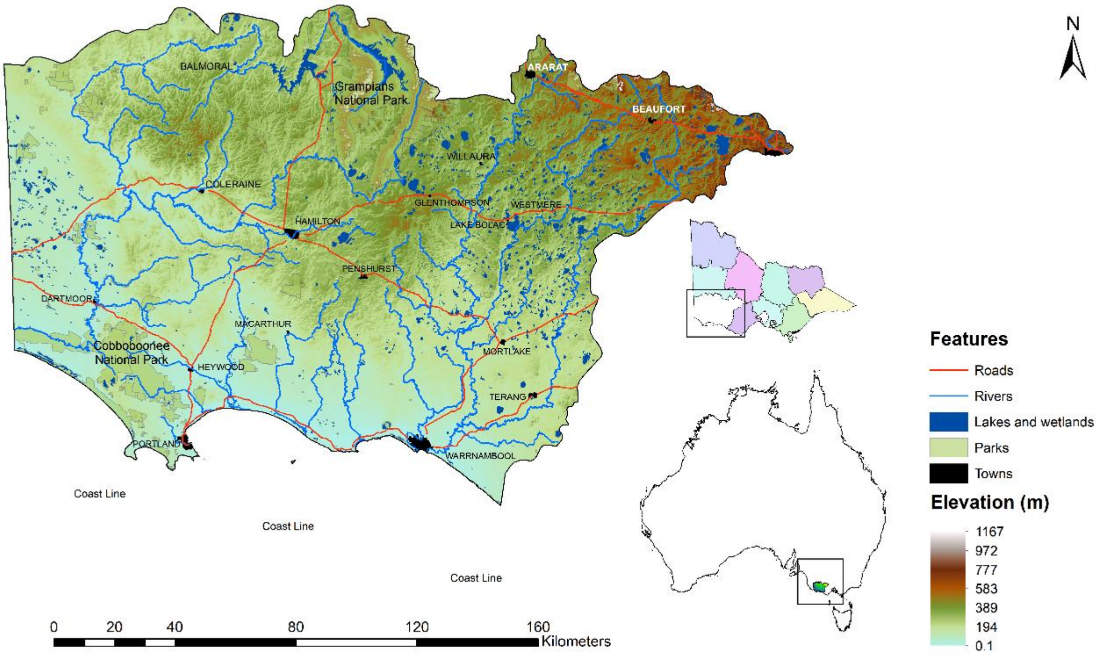

Figure 2.1 The GH CMA region, Victoria, Australia

The GH CMA contains important natural assets, including mountains, rivers, lakes, wetlands, marshlands, coastal ecosystems and an important biodiversity component (GH CMA, 2013), this CMA also contains GW as a natural resource that allows all the aforementioned natural assets to function providing irrigation water for farmers to rank the CMA within the most economically important across Victoria. Some national and international significant terrestrial natural assets are listed in Table 2.1. Apart from terrestrial assets, the GH has local, national and international significant assets that are categorized as heritage areas, aquatic ecosystems, western marine assets, and conservation species, such assets are presented in Table 2.2.

44% of Victoria's wetlands are found in the GH region, it has more than 5400 wetlands, covering 73,000 ha., as per GH CMA (2006). The major rivers that run through the catchment are the Glenelg River which is the largest river in south-west Victoria and the Hopkins River which is a major waterway in the eastern part of the catchment. Estuaries are present in the GH with eight significant sites: Fawthrop Lagoon, Fitzroy River estuary, Glenelg River estuary, Hopkins River estuary and Merri River estuary, Moyne River estuary, Surry River estuary, and the Yambuk Lake (AG, 1999). The GH region shelters over 94 nationally listed threatened species including numerous endemic species, three nationally listed vegetation communities, and iconic species such as the Red-tailed Black Cockatoo (GH CMA, 2018).

Table 2.1 Significant terrestrial habitat, modified from GH CMA (2013)

Asset name	Habitat description
Greater Grampians	Grampians National Park, Black Range State Park, and Dundas Range Scenic Reserve (VP, 1998).
Far NW. Lowland Forests and Heathy Woodlands	Habitat protection; Dergholm State Park, Tooloy-Lake Mundi Wildlife Reserve, Wilkin Flora and Fauna Reserve, and Kaladbro Wildlife Reserve.
Victorian Volcanic Plains Grasslands	Habitat protection; Blacks Creek Nature Conservation Reserve, Cobra Killuc Wildlife Reserve, and Mortlake Common.
Upper Hopkins Catchment Public Land	Terrestrial habitat values; Langi Ghiran State Park, Mount Buangor State Park and Ararat Hills Regional Park.
Far SW. Stony Rises	Mount Eccles National Park/Lake Condah and Mount Napier State Park.
Far SW. Lowland Forests	Habitat protection; Lower Glenelg National Park, Cobboboonee National Park and Forest Park, Mt Richmond National Park, Narrawong Flora Reserve, Mumbannar Nature Conservation Reserve, Crawford River Regional Park and Weecurra, Hotspur, Annya and Mt Clay State Forests.
Coastal Zone	Habitat protection, important species; Orange-bellied Parrot and Hooded Plover. Discovery Bay Coastal Park, Belfast Coastal Reserve, and Bay of Islands Coastal Park. Sites of global bird conservation importance (Yambuk Lakes Complex, Port Fairy to Warrnambool and Discovery Bay, Piccaninnie Ponds).

Table 2.2 Local, national and international significant natural assets, adapted from GH CMA (2013)

Asset name	The local, regional and international significance
Heritage areas	• Budj Bim National Landscape (Mt Eccles/Lake Condah/Tyrendarra Area). • Grampians (Gariwerd) National Park . • Kanawinka Geopark (UNESCO listed), sites of geologic significance (Wannon Falls, Tower Hill, Mt Noorat and Princess Margaret Rose Caves).
Aquatic ecosystems	• Glenelg River – the lower section of the Glenelg River is one of 18 heritage river areas in Victoria. • Western District Ramsar lakes – one lake (Lake Bookar) is listed as internationally important under the Ramsar Convention on Wetlands.
Conservations species	• Three Bird Areas (IBA) (sites of global bird conservation importance) – Yambuk Lakes Complex IBA, Port Fairy to Warrnambool IBA and Discovery Bay – Piccaninnie Ponds IBA. • Significant areas of two out of 15 recognised Australian Government 'biodiversity hotspots' (Victorian Volcanic Plain, South Australia's South-east/Victoria's South-west). • 13 endangered Ecological Vegetation Communities. • The iconic Red-tailed Black Cockatoo, Orange-bellied Parrot and endemic Glenelg Spiny Cray, and 173 of Victoria's threatened species. • 94 federally listed species.
Western marine assets	• 18 sites are listed as significant marine assets, some of them nest species of international significance. (Blue Whale Feeding Zone/Bonney upwelling, Discovery Bay, Deen Maar Island, Southern Right Whales nursery area, Blue Whale Distribution Zone, and Georgia's Peak Migratory shorebird feeding area) including unique coral and seaweeds areas.

Climatic conditions

The GH is part of the south-west climatic region with 730 mm/year of average rainfall (Timbal et al., 2016). Average annual daily maximum temperature in the region is 19°C. Climate of the Glenelg Hopkins region is temperate dry during the summers and cool, wet winters, the average annual rainfall in the area ranges from 500 mm per year near Lake Bolac to over 910 mm per year in the west of Heywood, the evapotranspiration is higher in winter at 22%, and autumn at 15%, drops in summer at 11% and shows the lower value in spring at 9% (Ekström et al., 2015; Timbal and Drosdowsky, 2013; GH CMA, 2008).

Population and economic development

The GH catchment neighbours a permanent population of 130,000, major towns include Ararat, Ballarat, Beaufort, Casterton, Hamilton, Portland, Port Fairy and

Warrnambool. 33,000 of the catchment’s residents inhabit in Warrnambool, while Ballarat, Portland and Warrnambool have been identified as areas of very high or high forecast future settlement growth (RDA, 2010).

The catchment supports major industries such as cattle, cropping, forestry, prime lambs, wool, and energy generating over a billion dollars (Tocker et al., 2010). In the past, wool production was the main agricultural revenue. Recently there has been a significant rise in beef, dairy, cropping and prime lamb enterprises (Tocker et al., 2010). Above 80 per cent of the GH catchment area is used for agriculture, while the main economic activities are dedicated to fisheries, manufacturing, retail, health services, construction and education being agriculture, forestry and fishing the main employers (GH CMA, 2018). In 2017, this region was the second highest agricultural production region in Victoria and placed fourth in the national Australian agricultural commodities at about $2,245 million (GH CMA, 2018).

Groundwater in the Glenelg Hopkins Catchment

The aforementioned economic, cultural, and environmental commodities could not be explained without the most important resource in the GH CMA which is water, both surface and GW allows this region a sustainable economic development. However, GW has not been included as a significant pillar under the GH Regional Catchment Strategy (2018). The intensive agriculture activities, also pose groundwater contamination issues in the regions.

The catchment is part of the Western Region Sustainable Water Strategy (WRSWS) which includes for the GH CMA a part of the Millicent Coast, Glenelg, Portland, and Hopkins river basins. The subregions included in this CMA are the Western District and the South-West Coast (SGV, 2011). GW reserves have varying salinity values (Dahlhaus et al., 2002). Regional GW flow system conforms the region including the Otway, Murray, Highland and Dilwyn formations. As stated by the WRSWS, GW is important for stock (16%), drinking water for landholders (16%) and is the source of the region’s irrigated horticulture (60%) and fodder industries (4%) (SGV, 2011). Additionally, it supports a diverse ecological and physical environmental assets such as rivers, wetlands and GW dependent ecosystems (SGV, 2011).

GW management is included in the Chapter Four section 4.4 “Protecting the health of GW resources for current and future users, and the environment” and states a long-term, viable and cost-effective GW monitoring action and the need for monitoring the condition and the changes in GW quality and quantity (SGV, 2011). The work presented in this thesis will assist GW managers of this region and others in Victoria in two ways:

1. To identify areas of high GW vulnerability that accounts for climatic variations and,
2. To maximize the use of the resources through the quantification of GW toxicity from GW samples and subsequent pollution screening.

2.2 Groundwater and wetlands pollution in agricultural areas from agriculture

Following the site description, this section presents a review of the existing research in GW pollution and its implications. (Aschonitis et al., 2016). Within the next 30 years, the world population could reach its peak at 9.8 billion (UN, 2019; Cleland, 2013). With population augmentation, there is a need for more productive agriculture. Unsurprisingly, the higher the crop production, the higher the use of agrochemicals such as pesticides and fertilizers. GW is a pivotal water asset in countries that include intensive agriculture as an economic policy for human development (Schwartz and Zhang, 2003; NRC, 1993; Bear et al., 1992). It is by definition a geological transport agent, a vital ecological value, and a central consumption source (Sangam et al., 2017; Aschonitis et al., 2016; Foster and Hirata, 1988). The importance of GW in agricultural areas in most countries including Australia is immeasurable, as most of their intensive agriculture relies on GW sources for irrigation such as the case of the GH CMA (GH CMA, 2018; SGV, 2011; RDA, 2010). The significance of GW continues to rise as many countries depend on GW as a source of safe drinking water. In Austria and Denmark, drinking water is sourced almost entirely from GW. In Europe, 70% of drinking water is based on GW resources Navarrete et al. as cited in (Angeliki et al., 2015) and in the US, 33% of deeper wells are used for water supply (Gilliom, 2007).

The history of pesticides originated in the 1940s with the agriculture revolution. Since then, pesticides have been used in the agricultural sector, forestry, industrial, medical and the urban sector (Centner, 1998; Falconer, 1998), where agriculture is the most significant market for pesticides (Himel et al., 1990). Within some broad groupings such as fungicides, molluscicides, insecticides, and herbicides, the definition of "Pesticide" comprehends a vast range of diverse substances (Gevao and Jones, 2002). Pesticides play a vital role in the agriculture industry while supporting food security in most countries.

There is a concern in the global scientific community regarding lack of knowledge about the risk associated with this broad range of chemicals used as pesticides. Some researchers have reported that a fraction of pesticides could be found not only in river systems but also in GW (Gilliom, 2007). There is a growing concern that a significant proportion of the applied chemicals on the farm end up in water bodies through runoff and infiltration; both in surface and groundwater. GW pollution from agriculture is not a new scientific finding; pesticides occurrence has been reported since the late 1980s in Europe. In the US, it was revealed that knowledge for a proper understanding of chemical transport processes in soil was limited (Flury, 1996). Neither that some pesticides are highly hazardous to human health, showing detrimental effects on testicular tissue and sperm quality (Dehkhargani et al., 2011) with alarming impacts of on male fertility (Sengupta and Banerjee, 2014). Pesticides can cause other serious hormonal effects in males, females and mice embryos (Bigsby et al., 1999). Moreover, atrazine reduced the semen quality in male workers that were exposed to this chemical (Swan, 2006), it increased sperm abnormality, disintegrated DNA and lead to nuclear immaturity (Dehkhargani et al., 2011). Additionally, atrazine has adverse hermaphroditic effects in amphibians at 0.1 ppb (Hayes et al., 2003) and can demasculinize frogs at low ecological doses (Hayes et al., 2002).

Many agencies in the world including the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Environmental Protection Authority USA (US EPA) and the Canadian Pest Management Regulatory Agency (PMRA) have approved the use of atrazine and consider its use not to be harmful (PMRA, 2017; APVMA, 2008; US EPA, 2006b, 2006a). These guidelines ignore the fact that the proportion of chemicals applied in the agriculture industry that may reach the target pest may probably be less than 0.3%, and 99.7% could be elsewhere in the environment (van der Werf, 1996) including GW. Pollution of GW has become an ongoing global concern, thus, limiting its availability as a resource for agricultural irrigation (Bartzas et al., 2015) and drinking water. The debate on whether atrazine is safe for use in agriculture continues within the scientific community (APVMA, 2008).

The herbicide atrazine is used extensively in the triazine tolerant canola (TTC) cropping industry in the Victorian State and production of maize, lupins and potatoes, and forestry (APVMA, 2008). Atrazine use in agriculture has shown a continuous increase since 1996, mainly because of the increase in the oilseeds production over the last twenty years as it can be seen in the Victorian Land Use Information System (VLUIS) from 1996 to 2016.

Hydrophobic herbicides might travel to the GW through leaching transport mechanisms (Pérez-Lucas et al., 2018). Together with nutrients such as nitrogen and phosphorus, herbicides pose a risk for GW and can be a threat to GW dependent ecosystems allocated in agricultural areas. In Australia regulatory authorities usually base their assessment of new agricultural chemicals on data obtained in trials conducted overseas (Sánchez-Bayo et al., 2002). GW Protection Guidelines in Australia published in 2000 (ANZECC/ARMCANZ) do not include comprehensible pesticide threshold values. Instead, they comprise of pesticides and additional pollutants threshold values for freshwater, but those values are mentioned to be used only as a reference. Some pesticides used in Victoria, Australia, such as the triazine family (atrazine, desisopropyl-atrazine, desethyl-atrazine, hexazinone, metribuzin, prometryn and simazine) might leach into the GW.

Over the last ten years, eleven countries reported findings of pesticides in GW at different locations such as North Italy, The UK, Portugal, US, Australia, Ireland, North Spain, North-East Spain, China, India, and Brazil, (Bartzas et al., 2015; Milhome, 2015; Yadav et al., 2015; Köck-Schulmeyer et al., 2014; McManus et al., 2014; Wu et al., 2014; Gagliardi and Pettigrove, 2013; Díaz et al., 2012; Rose et al., 2010; Hildebrandt et al., 2008; Gilliom, 2007; Gonçalves et al., 2007; Lapworth et al., 2006). The most affected countries by atrazine (reporting occurrences of atrazine herbicides) are the US, Portugal, and Spain. Based on the current review, Figure 2.2 presents a view of pesticides occurrences in the world. Atrazine can be detected in almost all countries with 76.9% of the occurrence. Simazine is present in half of the countries with 53.8% of occurrence. Bentazone, dieldrin, metolachlor, and terbuthylazine occurred at 38.5% respectively, and at 23.1% the occurrence of chlortoluron, cyanazine, diazinon, diuron, isoproturon, malathion, MCPA (Phenoxylene), and molinate. Atrazine has proved to be persistent in GW as it is reported as the most commonly detected group of pesticides in GW in New Zealand (Humphries and Close, 2015).

Following, bentazone, dieldrin, metolachlor, and terbuthylazine showed to be present in five countries. Finally, alachlor and chlorpyriphos were present in four countries. At present, the use of atrazine is prohibited in The European Union (EC, 2004).

Pesticides in GW and surface water from agriculture use have been reported in Victoria, Australia since 1993 in the Mitchell Valley area (Chapman and Stranger, 1993) and again in 1995 in surficial water in the Gippsland area (Chapman and Stranger). Gagliardi (2012) reported that there is no evidence of following groundwater pesticide detection reports in agricultural areas since then. Highly mobile pesticides increase the risk of serious human health problems (Cohen, 2007). Hence, some pesticides such as the triazine family pose a risk to pollute extensive amounts of groundwater resources, and once an aquifer is polluted, it may be lost for decades or centuries and could be unfit for agricultural uses.

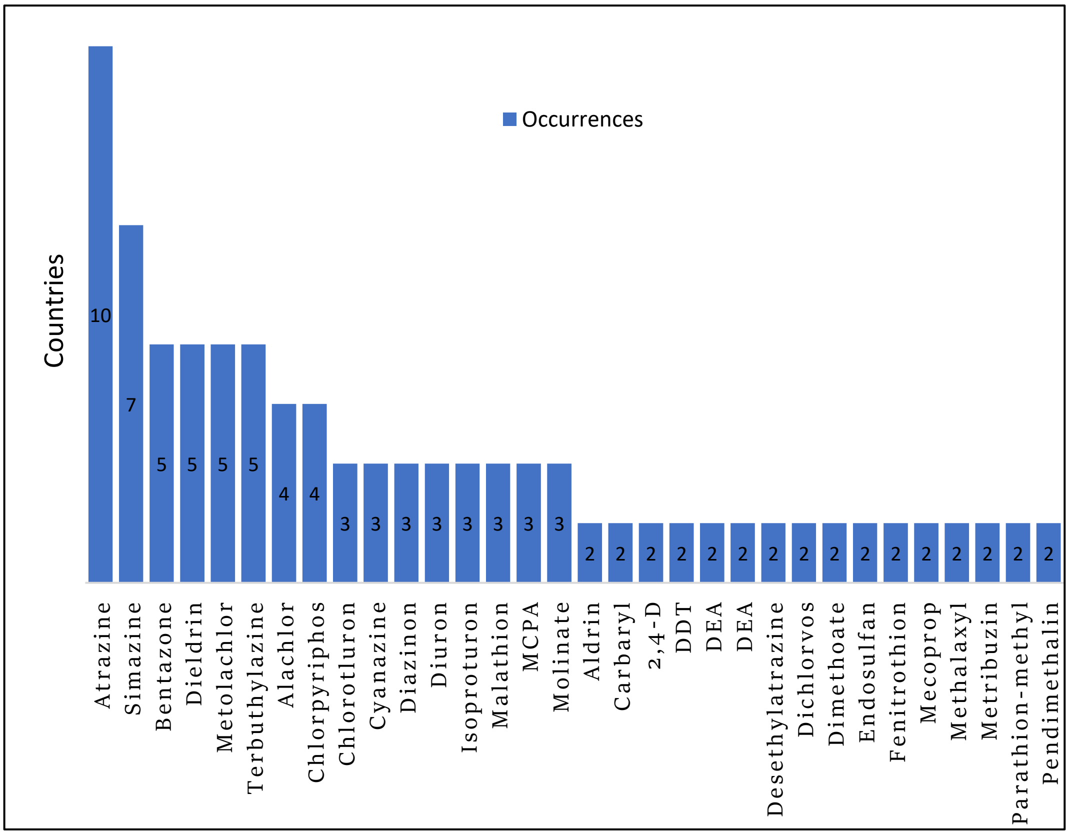

Figure 2.2 Pesticide occurrences in the world (2000-2016)

The occurrence of atrazine in almost all developed countries in the GW component can be attributed to its physical and chemical characteristics. Atrazine is a systematic triazine herbicide that usually is used both before and after sprouting emergence for the control of sward, and broad-leaved weeds in crops such as sorghum, maize, sugarcane, lupins and triazine tolerant (TT) canola/oils seeds, and pine and eucalyptus plantations. Atrazine is one of the most widely used herbicides in Australian agriculture. Its use in the Victorian State can particularly be related to TT canola cropping.

Herbicide atrazine was the most commonly detected chemical back in 1989 in a study in the US, and this finding may suggest that pesticides are leaching via soil to GW more frequently than the scientists perceived in the 1970s (Hallberg, 1989). Leaching through soil may be the primary cause of chemical compounds occurrence in GW (Flury, 1996). Leaching may happen because of pesticide hydrophobicity, lifetime, and high solubility (Tomlin, 2003). Hence, evaluating triazine risk for leaching to the GW in agricultural areas is a sound step for improving the pesticides regulatory system in Victoria, Australia, pesticides are used extensively and there is a growing public concern regarding their effects on human health (Sengupta and

Banerjee, 2014; Cohen, 2007), and its availability as irrigation source (Bartzas et al., 2015).

Wetlands in Victoria are recognized for their environmental significance by environmental agencies, society and the scientific community. They offer vital environmental services and goods. Some wetlands in Victoria are partially dependent on GW for their flow and maintaining ecological interactions. Some others depend additionally on surface water run-off or massive floods from nearby rivers for their existence. However, GW dependent (GWD) wetlands located near, between or close to cropping areas in agriculture lands could be facing environmental threats. Even though the importance of wetlands to the local and international ecosystem is recognized, they face severe degradation from changes in land use and climatic regimes internationally (Chui et al., 2011). The risks are mainly from cumulative impacts of pesticides used in the Agricultural industry (Rhymes et al., 2015; Dhananjayan and Muralidharan, 2010; Bondarenko et al., 2004; Donald et al., 1999; Clark et al., 1993).

As the pressure on the aquifers from croplands increases, the use of pesticides and the occurrence of these agrochemicals could be increasing in wetlands as well via GW; due to interactions between polluted GW from agriculture, runoff water and surface water in the wetlands.

The Australian Government, through the Department of Environment and Primary Industries (DEPI) and the Department of Environment, Land, Water, and Planning (DEWLP) launched in 2013 the Victorian Waterway Management Strategy, in which wetlands are of paramount importance within in the Victorian Environmental Policy. DEWLP is the responsible agency for implementing Ramsar obligations in Victoria. Victoria's wetlands are important in sustaining biodiversity at a regional, national and international scale. They provide habitat for threatened species and communities. As stated by the DEWLP in 2016, from the endangered native species in Victoria, about 499 (24%) depend on wetlands for their survival. Over 85% of the 145 wetland ecological vegetation communities are threatened in at least one of the bioregions in Victoria. Some wetlands in Victoria are listed as threatened at the State or National level. These include shallow freshwater seasonal herbaceous wetlands and some GW dependent wetlands which are listed as critically endangered under the Environment Protection and Biodiversity Conservation Act (1999).

Wetlands in the Western District are comprised within The GH and The Corangamite CMA in Victoria, Australia. These wetlands have experienced significant changes in the water regime since 1896. Wetlands ecosystems that are GW dependent could be particularly sensitive to pesticide pollution associated with GW in rural, urban, and coastal areas. There is evidence that suggests that aquatic ecosystem health can improve without the presence of surrounding agriculture activities (Gagliardi and Pettigrove, 2013). As recent research shows, pesticides are common contaminants detected in various wetlands (Rhymes et al., 2015; Bondarenko et al., 2004; Larsen et al., 2001; Clark et al., 1993), and fish tissue (Dhananjayan and Muralidharan, 2010). However, there is little information regarding the origin and pathways of such pesticides. The presence of pesticides in wetlands surficial water could be explained as GW-surface water pollution interactions, the contribution of runoff water pollution, and precipitation pollution entering wetlands ecosystems in rural areas. Until now, pesticides from GW have not been addressed as a likely threat to rural wetlands ecosystem structure and functionality. Scientific research and government regulations focus on trying to hold and contain new pollution risks from several industrial product developments specifically from agriculture, new chemicals used in this industry are assessed and quantified in environmental components that people are familiar with, e.g., air, soil and shallow water (Sánchez-Bayo et al., 2002).

2.3 Hydrogeological data requirements of groundwater vulnerability assessment studies

This Chapter describes the hydrogeological data available in Victoria, Au. and the data that is required in GW vulnerability assessment studies, at the same time, it provides data sourcing for the Australian adaptation of DRASTIC-based models.

Non-point pesticide GW pollution is usually difficult to understand, complex to visualize and generally is described in a highly technical scientific language (Hancock et al., 2009). Additionally, research in GW pollution from agriculture is time consuming and often convey to high costs in terms of equipment, fieldwork labour, and chemical laboratory analysis, making research in this area to be challenging and insufficient. For this reason, quantification, detection, monitoring, and visualization of pesticides in GW is a difficult endeavour. In this realm, assessing aquifer’s vulnerability to agricultural contamination is a crucial tool in GW pollution prevention strategies (Aschonitis et al., 2016; Kumar, 2015; Patrikaki et al., 2012).

While estimating GW vulnerability (GWV), DRASTIC-based models proved to be a flexible yet robust tool used around the world for GW pollution prevention (Linda Aller et al., 1987). As discussed in Section 1.2, DRASTIC- based studies use seven factors from the aquifer (Depth, Recharge, Aquifer media, Soil media, Topography, Impact of vadose zone, Hydraulic conductivity, and Land use) that control pollution throughout the aquifer (Aller et al., 1987; Aller et al., 1985). An additional factor for land use (L) was later introduced to conform an eight factor DRASTICL model (Alam et al., 2014; Saha and Alam, 2014; Sener and Davraz, 2013). In Australia, various authors have tried to apply DRASTICL-based models but have failed either to apply all the factors aforementioned or have applied it at a small scale and have not included different configuration of the aquifer characteristic (DPIE, 2018). Additionally, these models ignore the seasonal variations in the GWV. Therefore, there is a need to build models that can incorporate temporal variations while estimating the GWV of an aquifer.

This section presents the data and data source for each of the eight factors that are required for the DRASTICL GWV estimation. This data can be categorized by different ranges, and for each range, a value of relative importance is assigned, this is called ratings (Aller et al., 1987; Aller et al., 1985). Research suggests that some of the factors incorporated in DRASTICL vary seasonally, and thus incorporating these differences might allow the model to predict GW pollution for each season. This is explained in full detail in Chapter Three. As a result of factor differentiation, two factor groups can be established; the dynamic factor group and the static factor group. The dynamic factor group comprises the factor (D) and factor (R), as only the dynamic factors GW depth and GW recharge show temporal variations. On the other hand, static factors A, S, T, I, C, and L show no seasonal change. The data origin and data source for all these factors are explained in the following sections.

For Australia, data for the static factor group are not easily available. This lack of accessibility is the biggest burden in the replicability of DRASTIC-based models to significant/strategic regions. This thesis has localized the source of the data and processed the available data to produce standardised data that can be utilised to produce time-variant GWV estimations.

Data for static factors A, I, and C is found at the groundwater flow system (GWFS) for the GH CMA (Dahlhaus et al., 2002). The GWFS is the upper layer conformation of the unconfined aquifer, that is the spatial distribution of the different types of aquifers on the top-layer beneath the soil profile. At the GH CMA, the distribution of the aquifers shows a complex mosaic shape. This data is important because, from this data, the GWFS map will be produced.

Sound research has been made describing the contribution of each of the factors while estimating the static (Standard) GWV and its contribution to the pollution of GW, however, this is not the scope of the thesis, the reader is encouraged to see relevant work in Aller et al. (1985) and Aller et al. (1987). The next sections outline the data availability and data sources for the Australian application of the proposed model, the raw data is presented spatially, these data sets will be used in Chapter Three (Section 3)

Seasonal groundwater depth (D)

Depth to water (D) is a fundamental factor because it determines the depth of the aquifer's material that the contaminant must travel before reaching the aquifer, this plays a key role while governing the contaminant’s retention time, the assumptions is that the higher the retention time, the higher the chances for pollution attenuation from chemical-physical and biological processes in the soil and the aquifer’s conformation material, deeper water levels are translated in longer travel times (Council et al., 1993; Aller et al., 1985)

Each of Australia’s seasons consists of three full months per season. Each season begins on the first day of the calendar month, summer is from December 1 to the end of February, autumn from March to May, winter from June to August, and spring from September to November. The data for groundwater was gently provided by Timothy John Peterson and is part of the manuscript “State-wide geostatistical estimation of the water table level from 1985 to 2014” as per Peterson and Western (in-prep). As mentioned, the datasets used to derive the seasons were from 1985 to 2014. Figure 2.3 Shows the groundwater map of the GH CMA, maps have been created from the heads data and categorized to different depths as shown in the Figure. A map for each of the seasons has been created in ArcMap10.4 and the grid size is at about 50m x 50m.

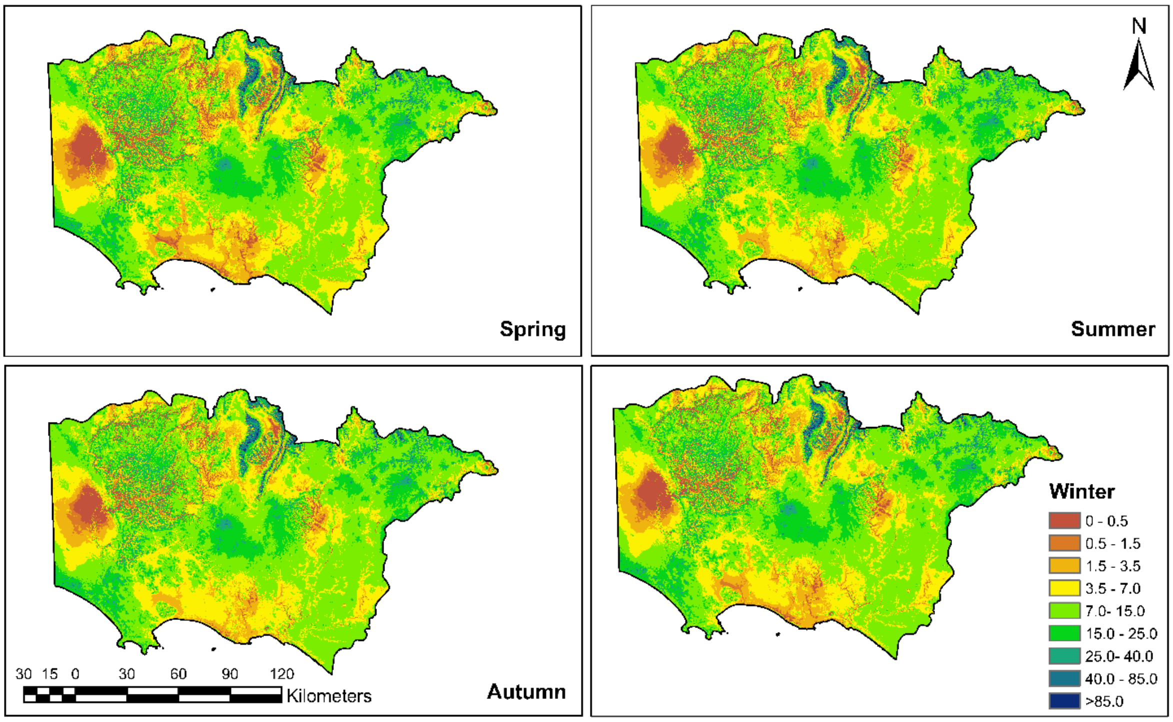

Figure 2.3 Seasonal groundwater depths for the GH CMA, (meters). The scale is on the right

Figure 2.3 shows that the largest changes in depth on a seasonal scale are on the south and south-east part of the GH CMA and that shallow depths happens on the west and the centre of the Catchment. This data is a significant part of the proposed methodology, from this raw data, the categorization maps will be created, this process is described in full detail in Chapter Three (Section 3).

Seasonal groundwater recharge (R)

Net recharge (R) is a significant factor in DRASTIC-based models because it is a direct resulting hydrogeological process from precipitation, such precipitation infiltrates through the ground surface and infiltrates to the water table. (R) indicates the quantity of water per unit area percolating to the ground surface reaching the water table (Aller et al., 1985). Recharge is the major physical driving process in groundwater pollution and aquifer vulnerability (Robins, 1998)

As part of the dynamic factors group, GW recharge is presented for the different seasons, Figure 2.4 shows the categorization values for the recharge maps, it can be seen that the largest values for net recharge are in winter, after the winter the values drop in spring and that lower values for recharge are in summer. Data for this factor was acquired from the GH CMA groundwater model (SKM, 2010) this data is available upon request to the Department of Environment, Land, Water and Planning (DELWP).

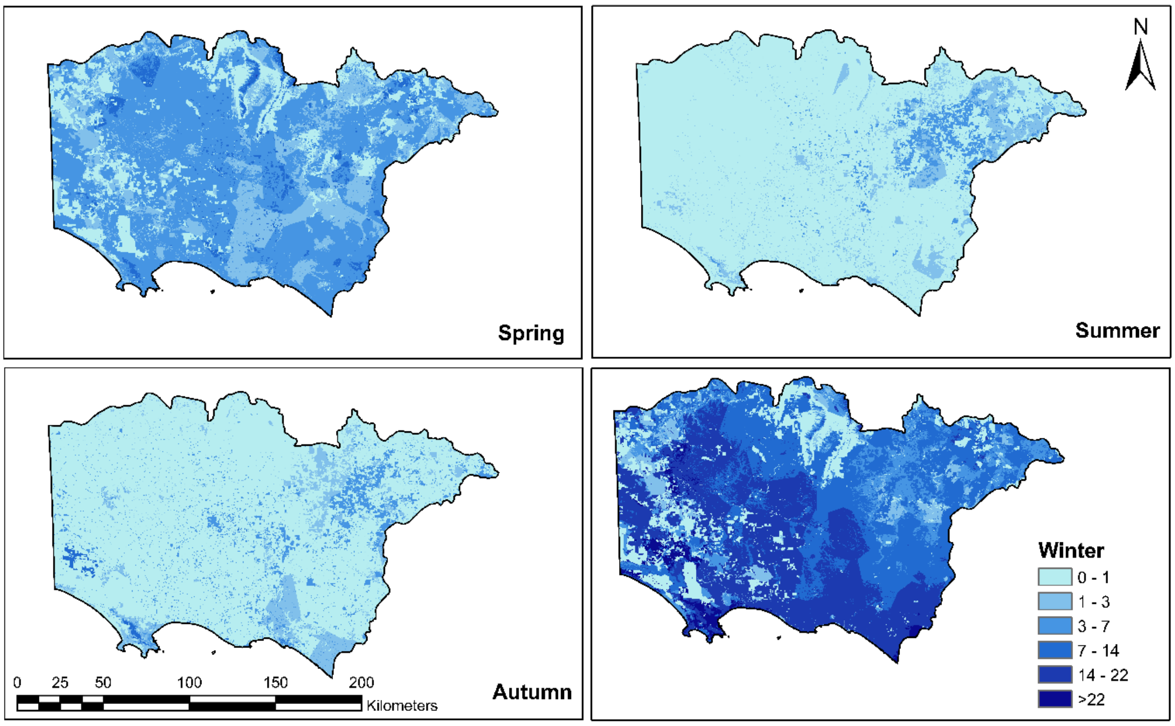

Figure 2.4 Seasonal groundwater recharge for the GH CMA, units are in (mm)

Aquifer media (A)

The aquifer media factor is the consolidated or unconsolidated material that comprises the aquifer, as described in (Aller et al., 1985) aquifer media is a significant factor affecting the contaminant movement into the aquifer. This factor wields major control in the pathway and length which a pollutant must follow, the media with high sorption capacity will have very little leaching potential and avoid GW contamination (Aller et al., 1985). Aquifer media is part of the static factor group as described at the beginning of this section, it includes 12 out of 15 GWFS from the VAF. Figure 2.5 shows the configuration of the GWFS. Table 2.3 presents the GWFS for Victoria.

Table 2.3 GW framework, GH CMA, Vic. Taken from the VGWF.

Aquifer/Aquitard	Name
Digital Terrain Model (DTM)	SUR
Quaternary Aquifer	QA
Upper Tertiary / Quaternary Basalt	UTB
Upper Tertiary / Quaternary	UTQA
Upper Tertiary / Quaternary Aquitard	UTQD
Upper Tertiary Aquifer (marine)	UTAM
Upper Tertiary Aquifer (fluvial)	UTAF
Upper Tertiary Aquitard	UTD
Upper - Mid Tertiary Aquifer	UMTA
Upper - Mid Tertiary Aquitard	UMTD
Lower - Mid Tertiary Aquifer	LMTA
Lower - Mid Tertiary Aquitard	LMTD
Lower - Tertiary Aquifer	LTA
Lower - Tertiary Basalts	LTBB
Cretaceous and Permian Sediments	CPS
Mesozoic and Palaeozoic Bedrock	BSE

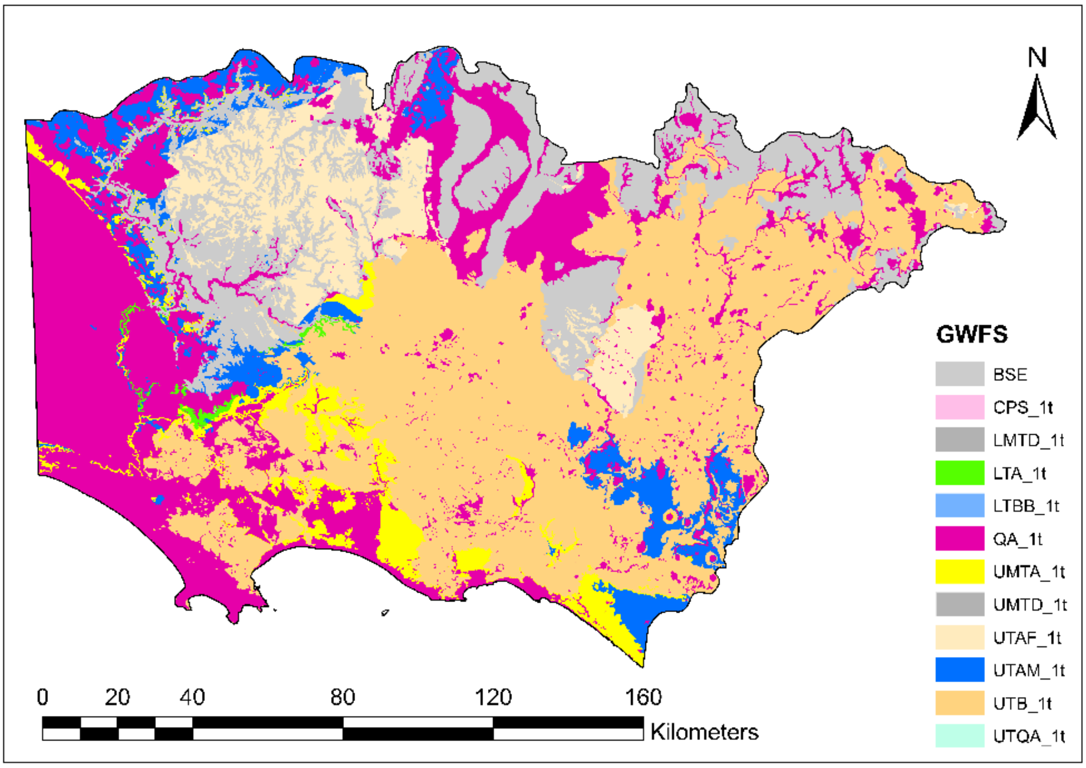

Figure 2.5 Aquifer media from the VGWF of the GH CMA

Soil media (S)

(S) refers to the uppermost layer of the vadose zone, it is where the biological activity takes place, (S) has a significant effect in GW recharge, soil controls the pollutants attenuation process by biodegradation, filtration, volatilization and sorption and adsorption (Aller et al., 1985), the more a soil is physically stable (shrinking and swelling) and the smaller the grain size, the less the pollution potential.

The data source for this factor is available from the Digital Atlas of the Australian Soil (ASRIS, 1968) downloaded from at https://www.asris.csiro.au/. This source is fundamental in deriving the soil factor ratings for the model. Figure 2.6 shows the soil media map. Chapter 3 outlines in detail how this rating is converted to the category values.

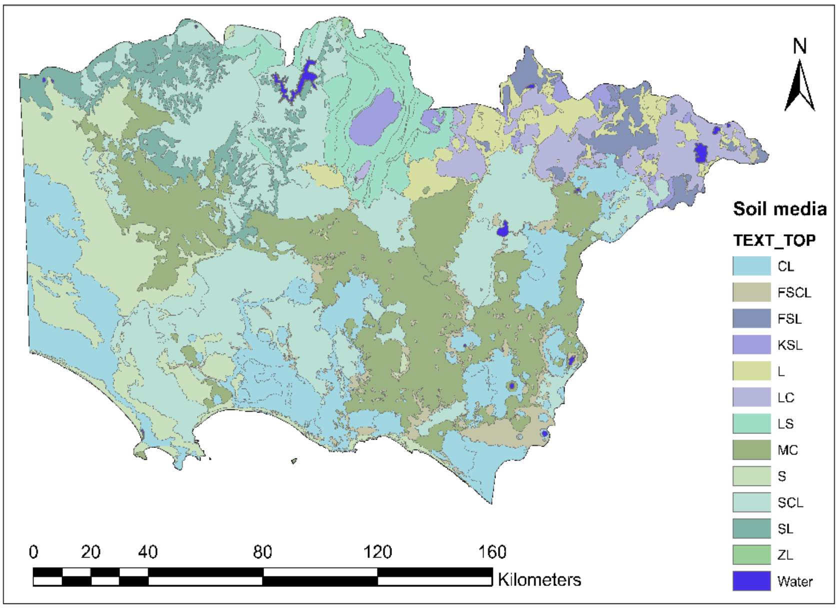

Figure 2.6 Soil media from the texture top

Topography slope (T)

The topography is referred as the slope variability in DRASTIC-based models, slope governs the likelihood that a pollutant to be available in the surface ground long enough to infiltrate, the higher the likelihood the greater the pollution potential (Aller et al., 1985)

Topography slope is one of the most used factors in DRASTIC models, the reason is that it can be calculated directly from the Digital Elevation Model (DEM) using ArcMap software; it is presented in percentage of the slope value. Figure 2.7 shows the GH CMA region digital elevation model. DEM is available for Victoria at https://data.vic.gov.au/, slope percentage map will be converted to a slope categorization map in Chapter Three (Section Three).

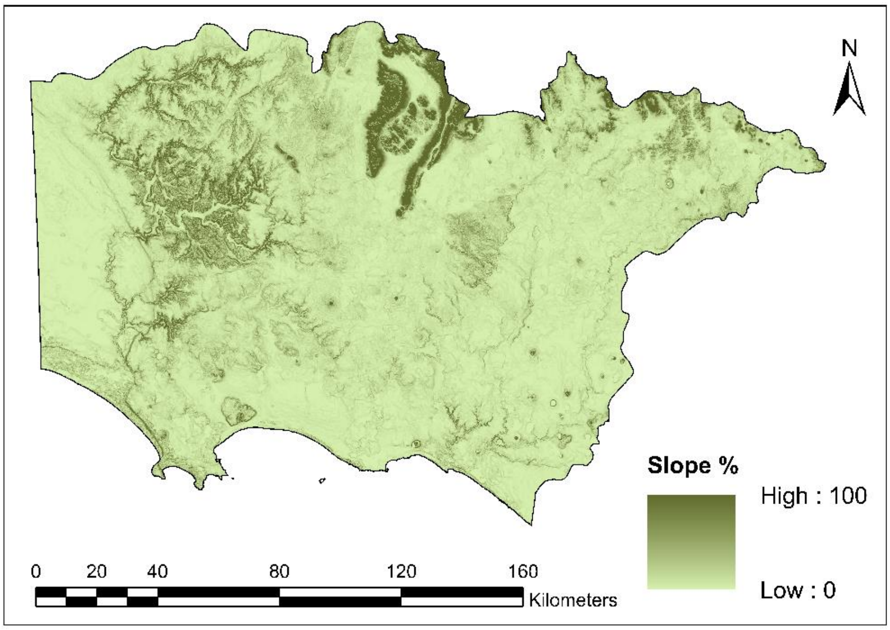

Figure 2.7 Slope percentage of the GH Catchment

Impact of vadose zone (I)

The vadose zone is the unsaturated zone above the water table and below the soil horizon, (I) determines the attenuation capability of the aquifer material by biodegradation, mechanical filtration, neutralization, chemical reaction, dispersion and volatilization (Aller et al., 1985).

The conformation material for each of the aquifer types is described in "The Victorian aquifer framework – Summary report" of SKM (2009). Figure 2.8 shows factor (I) materials of the aquifer. This material can be silt, sand, gravel, basalt, karst, limestone, sandstone, shale, clay, metamorphic, or a mixture of materials for the GH CMA.

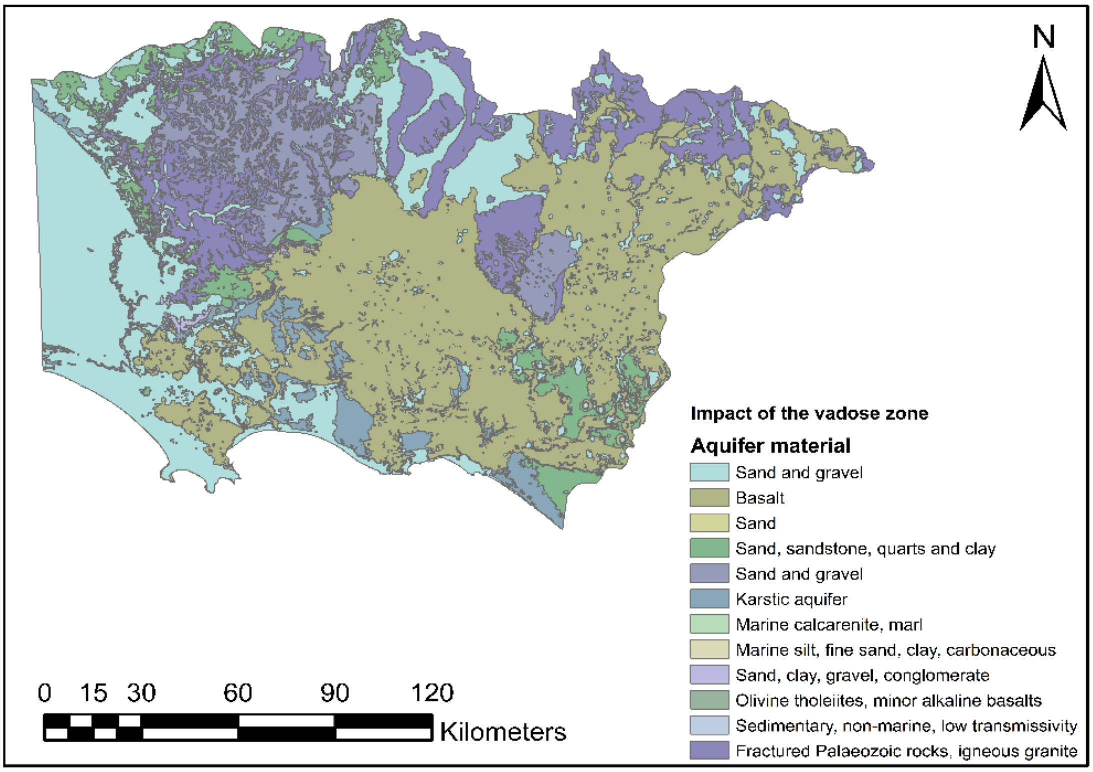

Figure 2.8 Impact of vadose zone material of the GH Catchment

Hydraulic conductivity (C

(C) refers to the capability of the aquifer to structure to convey water, this factor controls the rate of GW flow at specific hydraulic gradients, in return, this factor will control the rate at which a pollutant will move from the pollution site (Aller et al., 1985).

In the GH CMA, the hydraulic conductivity of the aquifer (C) data can be found in two different sources, although it is worth acknowledging that this factor is an inferred value most of the times, and such values usually drag large errors; one of the sources of data are the work of Dahlhaus et al. (2002), the second source for hydraulic conductivity is the work of the groundwater model for the GH Catchment by SKM (2010). The values for this map are presented in Chapter Three in the methodology section.

Land Use (L)

Land use provides additional information for estimating GWV in DRASTIC-based models, this feature can discretise between the use of agrochemicals in areas of intensive agriculture and areas with no use of chemicals such as natural parks and reserves or natural areas under protection. In Victoria, Australia, there is one main source to obtain this data, it is the Victorian Land Use Information System, which offers categorized data for land use through time. Currently, the most updated data belongs to the data set of land use for Victoria for the year 2016 (Agriculture Victoria, 2018b, 2018a). Figure 2.9 shows the land use map of 2016. Different land uses for the GH CMA are present, it can be seen that the agricultural areas are located in the centre and north-east of the CMA, there are two big areas for conservation at the Grampians National Park in the north and the south. The main activity in this CMA is farming.

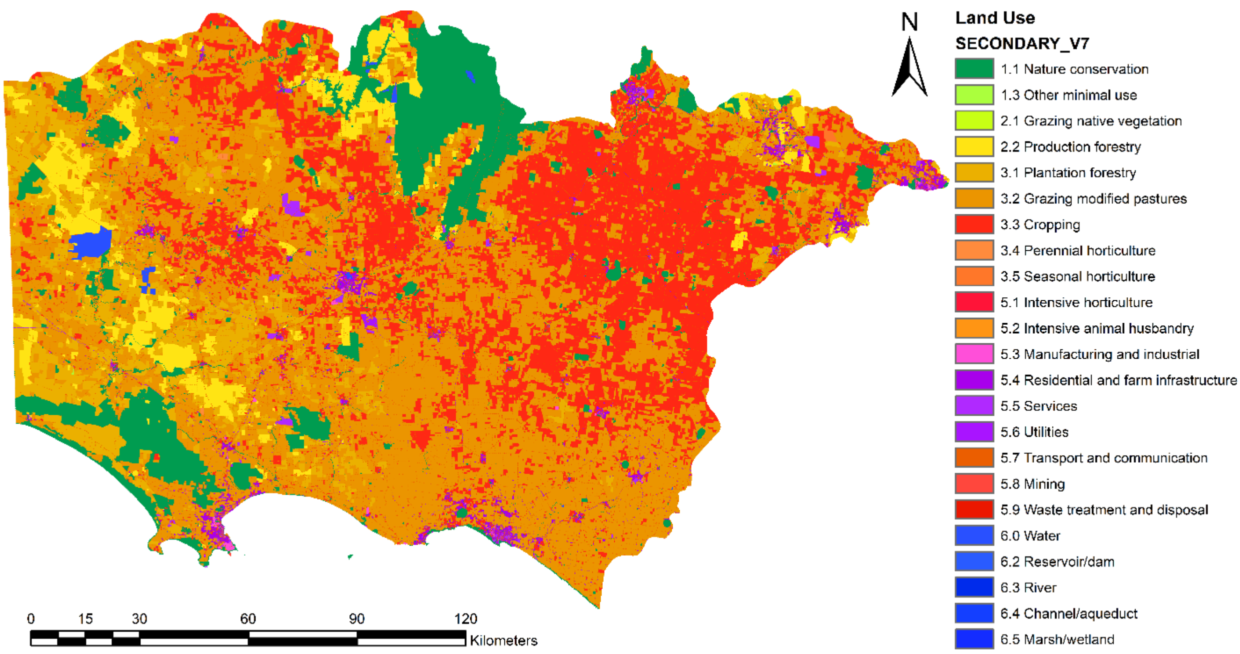

Figure 2.9 (2015- 2016) Land use secondary economic activities (VLUIS)

2.4 Groundwater ecotoxicity

On an international scale, this section summarizes the research that has been conducted while studying ecotoxicity tests applied in GW. Above one-third of human population relies on GW sources for their consumption (Morris et al., 2003), in Australia 15% of the total water supply is obtained from GW (Sampat, 2000). However, GW has recognized importance, there are still significant scientific challenges to address, such is the case of the risk for GW to be polluted from one of the most used pesticides in agriculture, the triazine herbicides family.

Pesticides in groundwater and surface water from agriculture have been reported in Victoria since 1993 in the Mitchell Valley area (Chapman and Stranger, 1993), in surficial water in the Gippsland area (Chapman and Stranger, 1994), shallow aquifers at Ardmona, Girgarre and Kyvalley, Shepparton Irrigation Region (Wenig and Lawrence, 1998) and the Goulburn Catchment Victoria (Watkins et al., 1999a) pesticides were more frequently detected in the Victorian waterways (Wightwick and Allinson, 2007; Rose and Kibria, 2006). In Australia, regulatory authorities usually base their assessment of new agriculture products on data obtained in trials overseas, (Sánchez-Bayo et al., 2002)2). Currently, the use of atrazine is prohibited in The European Union (EC, 2004). Over the last decade, research has proven that the use of atrazine is dangerous for humankind and the aquatic ecosystems as described in Section 2.2.

Groundwater ecotoxicity (GE) is defined as the area of knowledge that investigates the toxic response to chemicals or physical factors occurring in groundwater on living organisms. GW is founded on two pillars; the first one is ecotoxicology and the second one is hydrogeology. Thus, combining these two areas of research groundwater ecotoxicology, GE offers a plausible option to perform ecotoxicity research in GW. Whilst ecotoxicity concerns on the tacit effects of chemical-physical agents on living organisms, hydrogeology offers a sound understanding of the conditions and changes of chemical and physical properties of GW samples where such properties are to be considered in GE studies.

Research in GW quantifying toxicity has been carried out in the past using both GW (stygofauna) and surface water living organisms, the former ones most widely used around the globe for GW toxicity studies. Stygobiotic species have shown no conclusive results mainly because of unavailability, inaccessibility and difficulties for culturing under laboratory conditions (Di LorenzoDi Marzio et al., 2019). Pesticides that have been tested are presented in Table 2.4. Additionally, most used fungicides (hexachlorobenzene, metalaxyl), herbicides (alachlor, atrazine, metolachlor, prometryn, simazine), and insecticides (aldrin, dieldrin, endosulfan, and lindane) amongst other pesticides are shown in (Di Lorenzo et al., 2018).

Table 2.4 Pesticides tested in GW by stygofauna toxicity adapted from (Di LorenzoDi Marzio et al., 2019)

Pesticide	Author/s
Copper sulphate	(Reboleira et al., 2013)
Carbamate	(Di Marzio et al., 2013)
S-metholaclor, desethylatrazine	(Maazouzi et al., 2016)
Caffeine and propranolol	(Di LorenzoCastaño-Sánchez et al., 2019)
Aldicarb, methidathion, α-endosulfan	(Di Marzio et al., 2009)
Imidacloprid	(Böttger et al., 2012)
Imazamox	(Di Marzio et al., 2018; Di Lorenzo et al., 2014)
Ariane II (fluoroxypyr + chlorpyrlid and MCPA)	(Di Lorenzo et al., 2014)

Using freshwater living organisms (fish, Daphnia sp. and algae growth inhibition test) (OECD, 2019, 1984) in GE could be a more standard practised test amongst researchers. As only 18 papers from 1977 to 2018 were published testing ecotoxicity with stygobiotic species (Di LorenzoDi Marzio et al., 2019). Apart from the stygofauna, a variety of standardized methods for microalgae ecotoxicity are used primarily with chemicals, effluents, sediment elutriates and hazardous waste leachates (Lewis, 1995). The use of freshwater green algal has been tested in GE tests mainly to assess GW toxicity at industrial facilities with defined target chemicals or when assessing new chemicals to be used by the population in Europe (Weyers et al., 2000).

In this realm, green alga toxicity tests have been adopted as a standard method in Canada (Environment Canada, 2007), Europe (OECD, 1984) and in The USA (US EPA, 2016) for testing toxicity in freshwater and municipal and industrial effluents. The USA EPA, the European and the Canadian environment offices have not considered the study of groundwater ecotoxicology as a standard method, additionally, there is no evidence for any agency in the world proposing guidelines either for concentration values of chemicals in GW or an ecotoxicology method to assess agricultural pollution in GW.

2.5 Knowledge gaps

This section presents the knowledge gaps used to examine the research questions. This Chapter has considered the literature for the two components of this thesis. the literature review identified an urgent need for a GWV model that accounts for temporal variations as well as the spatial variability. It is vital to mention the insufficient information available regarding pesticide pollution in the Victorian groundwater environmental component, largely due to significant time and cost input in such data collection. This Chapter also outlines the current methods for estimating toxicity in GW and concludes that there is a need for separating the knowledge of ecotoxicological studies that concern for GW, identifying the groundwater ecotoxicology area. This section emphasizes the need for a GW ecotoxicology test that can be used as a preliminary test to identify GW pollutants preceding to costly analytical tests. The knowledge gaps that were identified in the thesis objectives are as follows:

***Knowledge gap 1*:** Current DRASTICL models do not account for time-variant impacts of GW depth and GW recharge and the relative importance of the factors has not been understood comprehensively, also, in Australia, there are no adapted factors data sets or guidelines to estimate GWV.

This gap relates to Research Question 1.

How can we represent temporal variations in GWV estimation of DRASTICL model?

Knowledge gap 2: According to pesticide manufacturers, any chemical used in agriculture if followed the application instructions on the label should be safe for use, however, there are studies reporting pesticide pollution in GW across Victoria. Therefore, there is a need to quantify the GW pollution extension and magnitude.

This gap relates to Research Question 2.

Do agricultural activities have the potential to contaminate GW in the GH CMA?

Knowledge gap 3: GE represents an advantageous option to quantify GW contamination from atrazine, green algal toxicity tests might present a cheap and faster option to estimate GW contamination from herbicides.

This gap relates to Research Question 3.

Can green algal ecotoxicity test be used as a low-cost screen to determine GW pollution?

Chapter 3

Seasonal assessment of groundwater vulnerability to agricultural pollutants using DRASTICL model

3.1 Abstract

Groundwater vulnerability assessment is critical in pollution protection strategies and groundwater quality monitoring programs. Several researchers have used DRASTIC-based models to estimate a steady-state groundwater vulnerability, but the effects of temporal variations on the vulnerability have not accounted for previously. It is imperative to be able to estimate seasonal pollution vulnerability that accounts for climatic variability effects on the aquifer. In this study, we attempt to address this gap by developing a new method to estimate seasonal groundwater vulnerability based on a modified Pesticide DRASTICL model. The vulnerability was calculated by implementing a correspondence analysis for two groups of variables: the static and the dynamic. The dynamic group comprised of depth (D) and recharge (R) and the static group comprised of aquifer media (A), soil (S), topography slope (T), impact of vadose zone (I), aquifer conductivity (C) and land use (L). The study site selected was the Glenelg Hopkins catchment in Victoria, Australia. The developed groundwater vulnerability model was able to differentiate four seasonal vulnerability scores. Seasonal estimations ranged from 42.48 to 205.2 in winter and from 32.32 to 159.80 in summer on a non-dimensional scale. The seasonal model was able to identify significant vulnerability variations in the south coast region of the catchment where

groundwater depth and recharge showed larger variations. Grampians National Park granitic formations showed minor changes in seasonal vulnerability score values. Our results suggest that the seasonal groundwater vulnerability model provides a robust yet flexible method for investigating changes in groundwater vulnerability in agricultural areas.

3.2 Introduction

Groundwater (GW) can be a geological transport agent, a vital ecological asset and a major water source (Sangam et al., 2017; Aschonitis et al., 2016). Assessing aquifer vulnerability to contamination risk is a pivotal tool in GW protection strategies (Aschonitis et al., 2016; Kumar, 2015; Patrikaki et al., 2012) and GW resource management. GW vulnerability (GWV) assessment is the estimation of contamination vulnerability of aquifers from anthropogenic pollution, where studies show their resulting scores as vulnerability maps. Researchers have classified GWV in two categories, the first one is intrinsic and the second one is specific (Aschonitis et al., 2016; Gogu and Dassargues, 2000; National Research Council US, 1993). Intrinsic vulnerability is founded solely based on hydrogeological and geomorphological factors of the landscape (Aschonitis et al., 2016; Kumar, 2015). Specific vulnerability refers to the single vulnerability that a pollutant or a group of pollutants poses to a specific area as per Kumar (2015) and Gogu and Dassargues (2000).

There are various approaches available to estimate GWV (Machiwal et al., 2018; Aschonitis et al., 2016; El Amri et al., 2016; Kumar, 2015; Gogu and Dassargues, 2000; Foster, 1988). Current GWVA models include more than 30 different methodologies such as GOD, AVI, SINTACS, EPIK and DRASTIC (Machiwal et al., 2018; Al-Abadi et al., 2017; Wu et al., 2016). There are other methods available such as Albinet and Margat, Goossens and Van Damme, Carter and Palmer, SEEPAGE and ISIS (Gogu and Dassargues, 2000). Aschonitis et al. (2016) classify these methods as: (i) process-based mathematical, (ii) GIS process-based (iii) overlay indices (iv) probabilistic-stochastic and (v) combined methods. Whereas, Kumar (2015) and The National Research Council US (1993) simplify this classification as (i) overlay and index, (ii) process-based, and (iii) statistical methods. The applicability of the methods varies according to the scale of the study, data availability (National Research Council US, 1993), user skills, and computational complexity (Al-Abadi et al., 2017).

DRASTIC is the acronym to seven hydrogeological factors included in the model. Depth to water (D), net recharge (R), aquifer media (A), soil media (S), topography (T), impact of vadose zone (I), and hydraulic conductivity of the aquifer (C) (Aller et al., 1987; Aller et al., 1985). Land use (L) was later introduced to the model by some researchers, leading to the new model (DRASTICL) as proposed by (Saha and Alam, 2014); Sener and Davraz (2013), and (Alam et al., 2014). The first attempt of incorporating land use (L) factor in a vulnerability assessment is presented in the study of Uricchio et al. (2004). DRASTIC models have been described, used, and modified by several authors after Gogu and Dassargues (2000) as per (US EPA, 1993).

DRASTIC-based models developed by Aller et al. (1985) and modified by Foster (1988) are the most applied methods for assessing intrinsic and specific aquifer vulnerability due to their ease of use and relatively fewer data requirements (Sangam et al., 2017; Wu et al., 2016; Pacheco and Fernandes, 2013; Croskrey and Groves, 2008; US EPA, 1993; Aller et al., 1985). Pesticide DRASTIC was later introduced by the original DRASTIC author in 1987, it was designed to account for a specific process that affects the fate and transport of pesticide to the water table (Aller et al., 1987). Pesticide DRASTIC weighting values are different from the standard DRASTIC where soil and topography are weighted less heavily. Such models are constructed on two different scale components that produce a vulnerability score. These are factor weights and factor ratings. The first component is seven hydrogeological factors weights (w) that include values from (1 to 5). These values represent the importance of each factor layer compared to the others. The second component is factor rating (r) with values ranging from (1 to 10) for each factor. Rates represent the importance of the range of values or types of media for each factor. Both weighting and ranges are described in (Aller et al., 1987; Aller et al., 1985). As a result, DRASTIC-based methods can identify areas that have a higher vulnerability to GW pollution relative to each other (Aller et al., 1987).

DRASTIC models are used to calculate the intrinsic vulnerability of agricultural and urban pollutants, whereas the Pesticide DRASTIC is a modification of DRASTIC that calculates the specific vulnerability to one or more pesticide pollutants (Aller et al., 1987; Aller et al., 1985). They differ only in the weighting values (Wang et al., 2007). The (L) factor is weighted as 5 in relative importance to other factors (Alam et al., 2014; Saha and Alam, 2014). Recent research shows that specific methods that integrate information about land use perform better than intrinsic approaches (Ribeiro et al., 2017). Specifically, land use provides additional information on the use of the actual landscape, the type of cropping and crop rotation which can be used to identify the amount and type of agricultural products used in the catchment (Ribeiro et al., 2017). Therefore, to improve the Pesticide DRASTIC model, Saha and Alam (2014) integrated the land use factor (L). This approach has been successfully applied in India (Alam et al., 2014), Iraq (Al-Abadi et al., 2017), Saudi Arabia (Ahmed et al., 2015), Greece (Patrikaki et al., 2012), Italy Portugal (Teixeira et al., 2015; Pacheco and Fernandes, 2013), the U.K., the U.S.A. (Fritch et al., 2000; Aller et al., 1987) and Australia (Askarimarnani and Willgoose, 2014).

Compared to other models, DRASTIC-based methods are easier to use and have shown higher performance using less data (Sangam et al., 2017; Wu et al., 2016; Pacheco and Fernandes, 2013). However, DRASTIC methods have been criticised because the factor weights may not represent the actual characteristics of the study area and estimations can have a low correlation with GW pollution (Pacheco et al., 2015). Surface water contamination correlation models are a complex task as per (Liu et al., 2018) and this is complexity is magnified in the groundwater component. To overcome subjectivity when assigning weighting values, Pacheco et al. (2015) identified five different approaches used extensively to deal with factor weighting: 1) single parameter sensitivity analysis, 2) non-linear Spearman correlation analysis, 3) logistic regression, 4) minimization of factor redundancy by correspondence analysis and 5) the standard DRASTIC Delphi (consensus) approach. Additionally, there is a generalised approach that uses the analytical hierarchy process (AHP) (Sener and Davraz, 2013; Kim et al., 2009; Thirumalaivasan et al., 2003). AHP weighting techniques are not explicitly designed for the adjustment of factor weights, in the case of DRASTIC models, the method is built on the opinion of experts (Pacheco et al., 2015) such as in the Delphi approach. The most commonly used weighting technique is the correspondence analysis (CA), which is a multivariate statistical method that identifies the real weight in correlated variables and has shown to be the best method to deal with subjectivity associated with the assigning of weighting values (Pacheco et al., 2015; Pacheco and Fernandes, 2013) and will be utilised in this study.

A detailed explanation for the latest vulnerability assessment methodologies is available in Machiwal et al. (2018). In DRASTIC base methods, GWV is conceptualized as a time-invariant spatial estimate. However, inconsistencies have arisen between nitrogen pollution concentrations and DRASTIC scores (Machiwal et al., 2018). Due to climatic variability, seasonality can have a significant impact on the resulting vulnerability scoring values. In such studies, estimates were time-invariant and the omission of time-varying factors is a weakness (Machiwal et al., 2018). If, say, seasonal estimates were available then potentially agriculture and water agencies could time fertilizer and pesticide usage to minimise contamination impacts. Though it’s a very important influence, to the best of our knowledge, no studies assessing the temporal variations in GWV have been published.

In Australia, agricultural products such as pesticides, nitrogen and phosphorus pose an inherent vulnerability to the aquifers that underlay agricultural lands. Canola cropping is a significant agroindustrial activity and is related to the intensive use of triazine herbicides in triazine tolerant canola (TTC) cropping, in conjunction with nitrogen and phosphorus salts as fertilizers. Within Victoria, Australia, few studies have investigated pesticide contamination of GW and most were undertaken in the 1970s and 1980s and just a few of them in the 2000s. Some of the studies focused on GW and showed the occurrence of pesticide atrazine, simazine chlorpyriphos, and DDT; with pesticide concentrations below the Australian water quality guidelines (Gagliardi and Pettigrove, 2013; Mossop et al., 2013; Gagliardi, 2012; Wightwick and Allinson, 2007; Rose and Kibria, 2006; Ivkovic et al., 2001; Watkins et al., 1999a; Watkins et al., 1999b; Bauld et al., 1998; Wenig, 1997; Moore et al., 1996; Bauld, 1994; Chapman and Stranger, 1994; McKenzie-Smith et al., 1994; Leitch and Fagg, 1985). Since then, the use of pesticides and fertilizers has increased significantly, causing a likely increased risk of GW contamination. Despite these issues, regional GW vulnerability assessments are scarce in Australia. DRASTIC-based modelling has been undertaken for the Castlereagh, Lachlan, Macquarie, and Macintyre catchments in New South Wales (DPIE, 2018), but these studies did not account for land use and seasonal variability.

The methodology proposed in this thesis focuses on calculating the seasonal GW vulnerability index at the study site. Most of the existing methods used for calculating vulnerability indexes produce spatially fixed values, as they consider the vulnerability to be static. Such methods fail to depict the variations provoked by weather, climate and human impacts to the aquifers such as water pumping during the year. Aquifers are mobile in both horizontal and vertical directions. Pollutant concentration varies temporally within the same aquifer location. GW net recharge is another spatially and temporally dynamic variable with different values in different seasons during the year. Recharge is the primary source of GW. Groundwater recharge occurs because of precipitation infiltration through the ground surface and percolation to the water table (Aller et al., 1985). In Australia, GW net recharge quantification faces important challenges in terms of accuracy, spatial variations, temporal variations, and under and overestimation of recharge values.

In this thesis chapter, I present a time-varying Pesticide DRASTIC approach that includes land use. Furthermore, by introducing double correspondence analysis, we separated static factors from dynamic factors and this allows derivation of weights that account for temporal variability. In DRASTIC-based models, water table depth is used to describe the GW table in an unconfined aquifer (Aller et al., 1985). Depth to the water table controls two significant processes in GW pollution. Firstly, it controls the length of the aquifer material that the pollutant must travel before reaching the water table (Aller et al., 1985). Secondly, it regulates the time that the contaminant is in contact with the adjacent material; the higher the time, the greater the opportunity for attenuation (Aller et al., 1985). Hence, depth to water table controls both the length and time that pollutants must travel before reaching the water table (Sener and Davraz, 2013). Together with GW recharge (R), depth (D) is responsible for the seasonal changes in the modified Pesticide DRASTICL vulnerability index. Depth will assign specific seasonal information to the resulting vulnerability index. Such changes will contribute to the spatial and temporal variations of the vulnerability index. Seasonal GW depths can be computed from the mean value for the months that corresponds to each season at the Glenelg Hopkins CMA.

3.3 Methodology

3.3.1 Study area

The study area covers the Glenelg Hopkins (GH) catchment management authority (CMA) in the Western part of the Victorian State in Australia. The GH CMA region encompasses 2.67 million hectares of area that extends West from Ballarat to the South Australian border and south to the coast. It is 12% of the total area of Victoria (SKM, 2010). North is dominated by the Grampians, the Dundas and Merino Tablelands, and the West Victorian Uplands with the flatter Volcanic Plains characterizing the South as shown in Figure 3.1. The slope is prominent in the Grampians region; with maximum values of 100% going to a minimum of 0% in the Volcanic Plains and an average slope of 3.48% in the rest of the territory. Hydrological subregions are Hopkins River basin (East) which includes the Hopkins and the Merry Rivers, Portland Coastal (Southcentral) that includes Moyne, Eumeralla, Fitzroy and Surry rivers, and Glenelg River basin (Northcentral and West) including the tributaries of the Glenelg River. The largest water body in the basin is the Rocklands Reservoir (SKM, 2010). Aquifers and aquitards in the GH CMA region are presented in Table 3.1. The Glenelg Hopkins is perhaps the most varying GW region within the Victorian CMA's. Almost all the aquifer and aquitards that exists in Victoria are represented in the CMA. This provides the CMA different aquifer types and GW qualities, such as salinity and metals. Table 3.1 shows the type of aquifers and aquitards and the name or code (left) from the terrain model or surface layer (SUR) to the Palaeozoic bedrock or base (BSE) as described in the Victorian aquifer Framework by SKM (2009).

The mean annual maximum temperature in Warrnambool is 19.2 °C and the highest in 2017 was 38.3 °C. In Casterton are 20.1 °C and 38.5 °C. The mean annual minimum temperature in Warrnambool is 8.9 °C, and the lowest in 2017 is –1.0 °C. While, in Casterton are 8.4 °C and -3.0 °C (Source: Bureau of Meteorology Climate Averages website, accessed March 2018). There are two distinct climates: the Csb as temperate with dry and warm summer, and the Cfb as temperate with a dry winter and warm summer (Peel et al., 2007).

Table 3.1 GW framework, Glenelg Hopkins CMA, Vic.

Aquifer/Aquitard	Name
Digital Terrain Model (DTM)	SUR
Quaternary Aquifer	QA
Upper Tertiary / Quaternary Basalt	UTB
Upper Tertiary / Quaternary	UTQA
Upper Tertiary / Quaternary Aquitard	UTQD
Upper Tertiary Aquifer (marine)	UTAM
Upper Tertiary Aquifer (fluvial)	UTAF
Upper Tertiary Aquitard	UTD
Upper - Mid Tertiary Aquifer	UMTA
Upper - Mid Tertiary Aquitard	UMTD
Lower - Mid Tertiary Aquifer	LMTA
Lower - Mid Tertiary Aquitard	LMTD
Lower - Tertiary Aquifer	LTA
Lower - Tertiary Basalts	LTBB
Cretaceous and Permian Sediments	CPS
Mesozoic and Palaeozoic Bedrock	BSE

Different soil lithologies are present at the GH CMA: basalts, sedimentary, duricrust, and aeolian are the most abundant, followed by alluvium, limestone, alluvial, granite, colluvial, lacustrine/aeolian, and volcanic. The least abundant soils are fluvial & aeolian, lagoonal and fluvial (Agriculture Victoria, 2018a). The lateral hydraulic conductivity for unconfined aquifers in the GH CMA varies from <0.01 m/day in the base rock to 270m/day in the Upper Mid-Tertiary Aquifer (SKM, 2010). Hydraulic conductivity values are only estimations, as shown in Section 3. The land use in the year 2016 comprises of grazing, followed by cropping and horticulture, forestry, conservation, and intensive uses (Agriculture Victoria, 2018b).

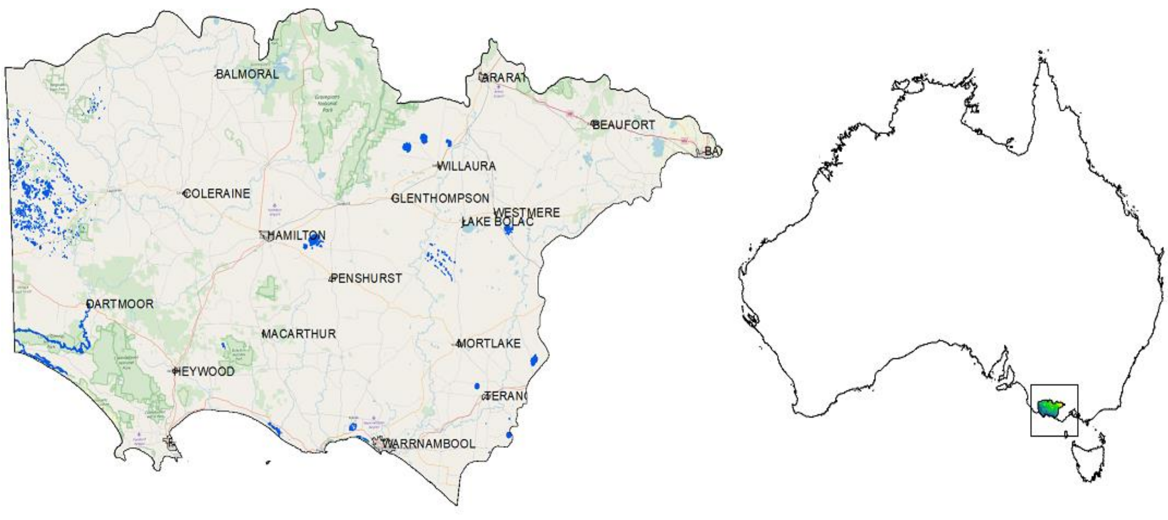

Figure 3.1 The Glenelg Hopkins CMA in Victoria, Australia

3.3.2 Methods

To calculate the time-variant estimation of the modified Pesticide DRASTICL model, we developed a method comprising five steps:

Step 1: Data preparation.

Step 2: Calculation of the Pesticide DRASTICL vulnerability index (IDL) using the seasonal factors (D and R), and the static hydrogeological factors (A, S, T, I, C, and L).

Step 3: Independent computation of the correspondence analysis for both dynamic and static factor groups. This step includes factor reduction analysis.

Step 4: Up-scaling the resulting eigenvalues to the Pesticide DRASTICL weight scale (1 to 5).

Step 5: Recalculation of the seasonal IDL using the calculated weights from step 4. Figure 3.2 shows further details and in the following sections, each step is detailed.

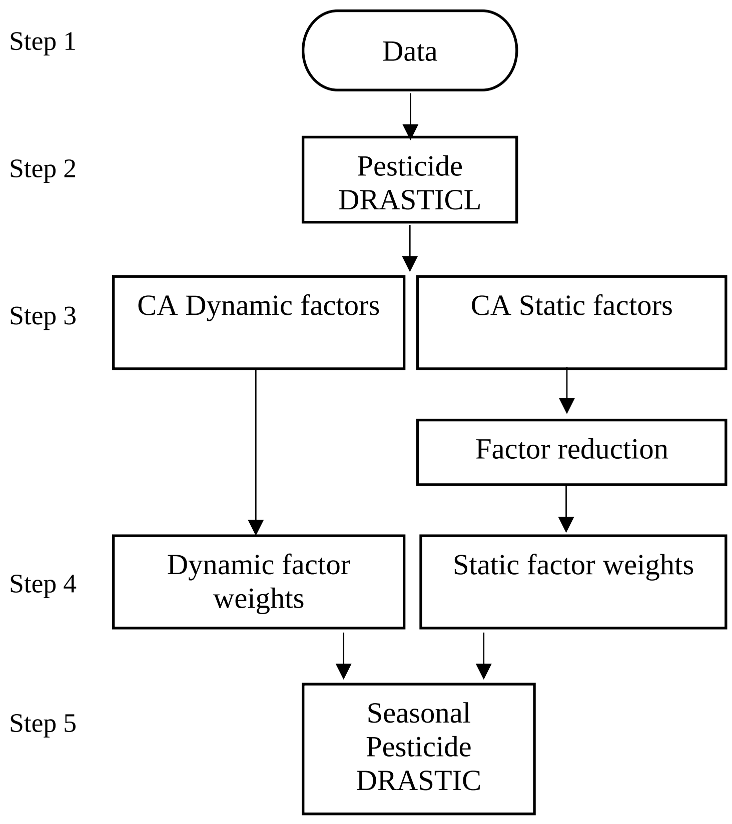

Figure 3.2 Seasonal Pesticide DRASTICL methodology flow chart

3.3.3 Step 1 Data preparation

3.3.3.1 Data collection

In this section, data acquisition and data manipulation are presented. In the next subsections of the methodology, each hydrogeological factor construction is explained separately including data location and data access. This study collected data from various Victorian agencies such as The Victorian Aquifer Framework (VAF) (SKM, 2009). ArcMap10.4.1 was used for the conformation of the Glenelg Hopkins GW framework and the assemblage of the GWFS. Sources for Pesticide DRASTICL factors are summarized in Table 3.2.

Table 3.2 Sources for Pesticide DRASTICL factors

Factor		*Description*
Seasonal depth to the water table	(D_s)	This factor was computed from the average monthly values in each season over the last 30 years as per Peterson and Western (in-prep).
Seasonal net recharge	(R_s)	This factor was calculated as the average of monthly values within each season over the last 30 years. Data was acquired by request to DELWP. R_s values are resulting values from the "Glenelg Hopkins CMA GW model" as seen in SKM (2010).
Aquifer media	(A)	This factor was derived from the GWFS map coupled with the type of consolidated material of the top layer aquifer as described in SKM (2009) and cross-referenced with Dahlhaus et al. (2002).
Soil media	(S)	Data was accessible from the Victorian soil atlas. Soil media does not reflect depths of soil and soil mixtures in the horizontal horizon. It is the uppermost layer of the soil profile.
Topography slope	(T)	This factor was calculated in ArcMap10.4.1 from the Victoria Digital Elevation Model (DEM).
Impact of vadose zone	(I)	This factor was assembled from the GWFS and aquifer material were taken from aquifers description in the GW framework report in Dahlhaus et al. (2002).
Hydraulic conductivity	(C)	The conductivity of the aquifer data was obtained from the 2002 GW flow systems – GH CMA report by Dahlhaus et al. (2002) and corrected with the GH GW transient model as per SKM (2010).
Land use	(L)	Data is available from the (VLUIS) (Agriculture Victoria, 2018b).

3.3.3.2 Groundwater framework and groundwater flow systems

Before mapping factor rating values of A, I, and C, it is essential to derive the GW flow system of the study site. GWFS is the surficial hydrogeological configuration of the aquifers where GW depth will be localized (SKM, 2009). The GW framework at the

Glenelg Hopkins CMA is the configuration of different aquifers and aquitard layers which extend over the CMA characterising hydrogeological units with different response times. The aquifers hydrogeological conformation of the GW framework in the study area was created using data from the VAF surfaces (See Table 3.1). GWFS are derived from this Victorian GW framework and used for estimating factors A, I and C. Each layer represents a type of aquifer or aquitard, from the base rock down in the bottom to the quaternary aquifer in the upper layer, where each of the layers is the top of each aquifer unit. Figure 3.3 shows the conformation of the GW flow systems at the GH catchment.

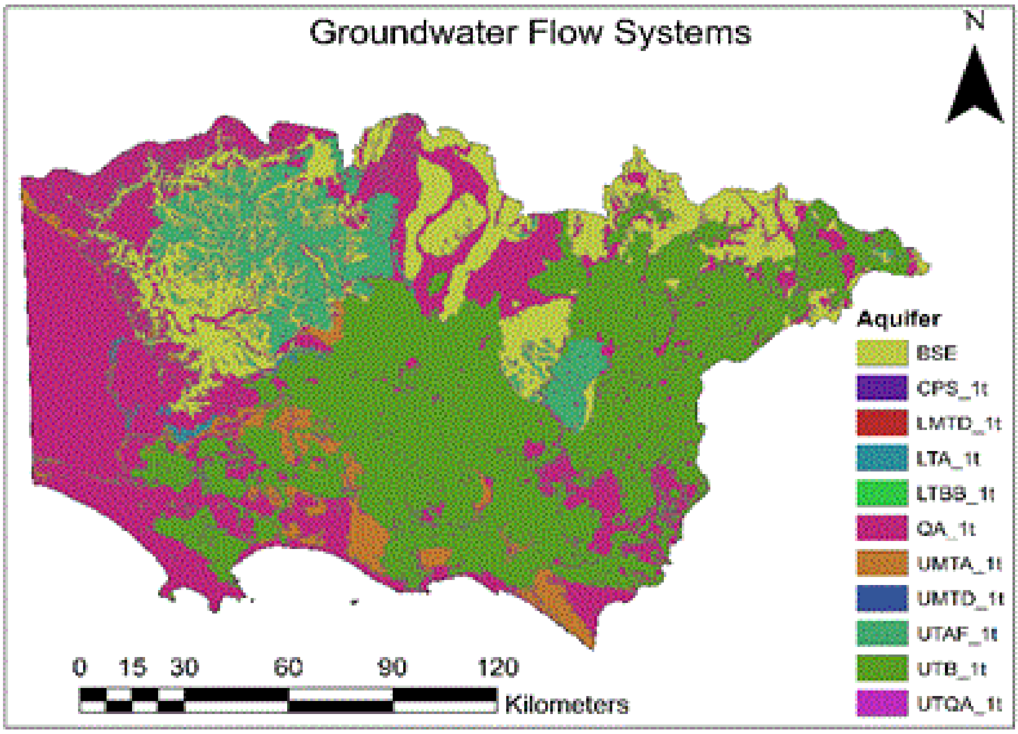

Figure 3.3 Hydrogeological conformation of the GWFS at the GH CMA

GWFS is the upper layer assemblage of aquifers and aquitards from the GW framework that constitute the Glenelg Hopkins CMA hydrogeology, the GWFS's are presented as linear kilometres for longitude and latitude and correspond to the unconfined aquifers. Table 3.3 shows the resulting conformation of the GWFS.

Table 3.3 Groundwater flow system at the GH CMA, retrieved from the VGWFS

Aquifer/Aquitard name	Identifier
Quaternary Aquifer	QA
Upper Tertiary / Quaternary Basalt	UTB
Upper Tertiary / Quaternary	UTQA
Upper Tertiary Aquifer (marine)	UTAM
Upper Tertiary Aquifer (fluvial)	UTAF
Upper - Mid Tertiary Aquifer	UMTA
Upper - Mid Tertiary Aquitard	UMTD
Lower - Mid Tertiary Aquitard	LMTD
Lower Tertiary Aquifer	LTA
Lower – Tertiary Basalts	LTBB
Cretaceous and Permian Sediments	CPS
Mesozoic and Palaeozoic Bedrock	BSE

3.3.4 Step 2 Pesticide DRASTICL

3.3.4.1 Computation of the Pesticide DRASTICL vulnerability index (I_{DL})

Pesticide DRASTICL index is calculated by the sum of each of factor weight values times their corresponding rating value (Saha and Alam, 2014; Sener and Davraz, 2013; Aller et al., 1987) as presented below:

$$I_{PDL} = \sum_1^i r_i * w_i \qquad (1)$$

where:

I_{PDL} = Pesticide DRASTICL Index (dimensionless)

w = parameter weight (dimensionless)

r = rating for the correspondent range (dimensionless)

Values for factor weighting were modified from (Aller et al., 1987; Aller et al., 1985) and are shown in Table 3.4. Weights represent how important each factor is when compared to others and it has a range from (1 to 5), being five the most significant and one being the least significant. Each factor layer is prepared and analysed in a grid of (50 x 50m). Next, each factor was reclassified by assigning the corresponding rating values and converted to raster format using the software ArcMap10.4.1. Raster layers use the projected georeference GDA 1994 Lambert Conformal Conic. The second component in the I_{PDL} is the range of rating values. Ranges represent the significant media type that contributes to the pollution potential, such values change spatially and within their scale (Aller et al., 1987). Every range is evaluated against each other to calculate the relative significance in relation to pollution potential (Aller et al., 1987). In the standard DRASTIC method, factor range of relative significance is evaluated on a scale of (1 least significant, to 10 most significant) except for net recharge factor with a scale from (1 to 9).

Rating values were established using an ordinal scale in DRASTIC-based models. However, assigning range values from (1 to 10) to differentiate the relative importance between ranges may not be the best practice. Researchers have found that the human mind can only categorize effects on a scale of (1 to 9) (Saaty, 1990). Hence, standard DRASTIC-based models tend to overestimate resulting scores by using 10 different categories when in reality only 9 categories are comprehensible by the human brain. In this study, the modified Pesticide DRASTICL method uses a range scale from (1-9) addressing the aforementioned limitations. The new rating values ranges are shown in Table 3.4. The following sub-sections detail the derivation of each input parameter.

Table 3.4 Weights and modified ratings for Pesticide DRASTICL model (Alam et al., 2014; Saha and Alam, 2014; Aller et al., 1987)

Pesticide DRASTICL parameters		Parameter weight	Standard rating ranges	New ranges
Depth to groundwater	D	5	1-10	1-9
Net recharge	R	4	1-9	1-9
Aquifer media	A	3	1-10	1-9
Soil media	S	5	1-10	1-9
Topography slope	T	3	1-10	1-9
Impact of vadose zone	I	4	1-10	1-9
Hydraulic conductivity	C	2	1-10	1-9
Land Use	L	5	1-10	1-9

3.3.4.2 Depth to water table (D)

Seasonal GW depths ($_{S}D$) are shown in Figure 3.4 (a), (b), (c) and (d), in this study, space-time GW elevation maps from Peterson and Western (in-prep) were adopted. This approach is an extension of Costelloe et al. (2015) and Peterson et al. (2011). The maps were derived at a monthly time-step from 1 January 1980 to 1 August 2014 over the entire study area. The maps were derived using an advanced multi-variate geostatistical R-package named HydroMap (https://github.com/peterson-tim-j/HydroMap). The approach allows the inclusion of topographic form, land surface elevation, the coastline, and the physical constraint of the land surface elevation on the water table elevation. Furthermore, the parameters within these predictors and the standard kriging parameters (variogram range, sill and nugget and search radius) were derived using formal maximum likelihood estimation. Input data for the kriging was derived from the HydroSight (http://peterson-tim-j.github.io/HydroSight/) time-series analysis of each GW hydrograph (Peterson and Western, 2014). GW hydrograph errors and outliers were omitted (Peterson et al. 2018) and temporally interpolated to a monthly time-step using Peterson and Western (2018) (see Figure 3.4.)

For shallow aquifers such as the case of Victoria, Australia where the differences in depth ranges are not large enough, a new range classification is suggested in Table 3.5. Seasonal depths are proposed and categorized assigning each range a rating value from 1 to 9 (See Table 3.5).

Table 3.5 Groundwater depth rating values

Depth to GW (m)	Rating value
0.00 – 0.50	9
0.50 – 1.50	8
1.50 – 3.50	7
3.50 – 7.00	6
7.00 – 15.0	5
15.0 – 25.0	4
25.0 – 40.0	3
40.0 – 85.0	2
> 85	1

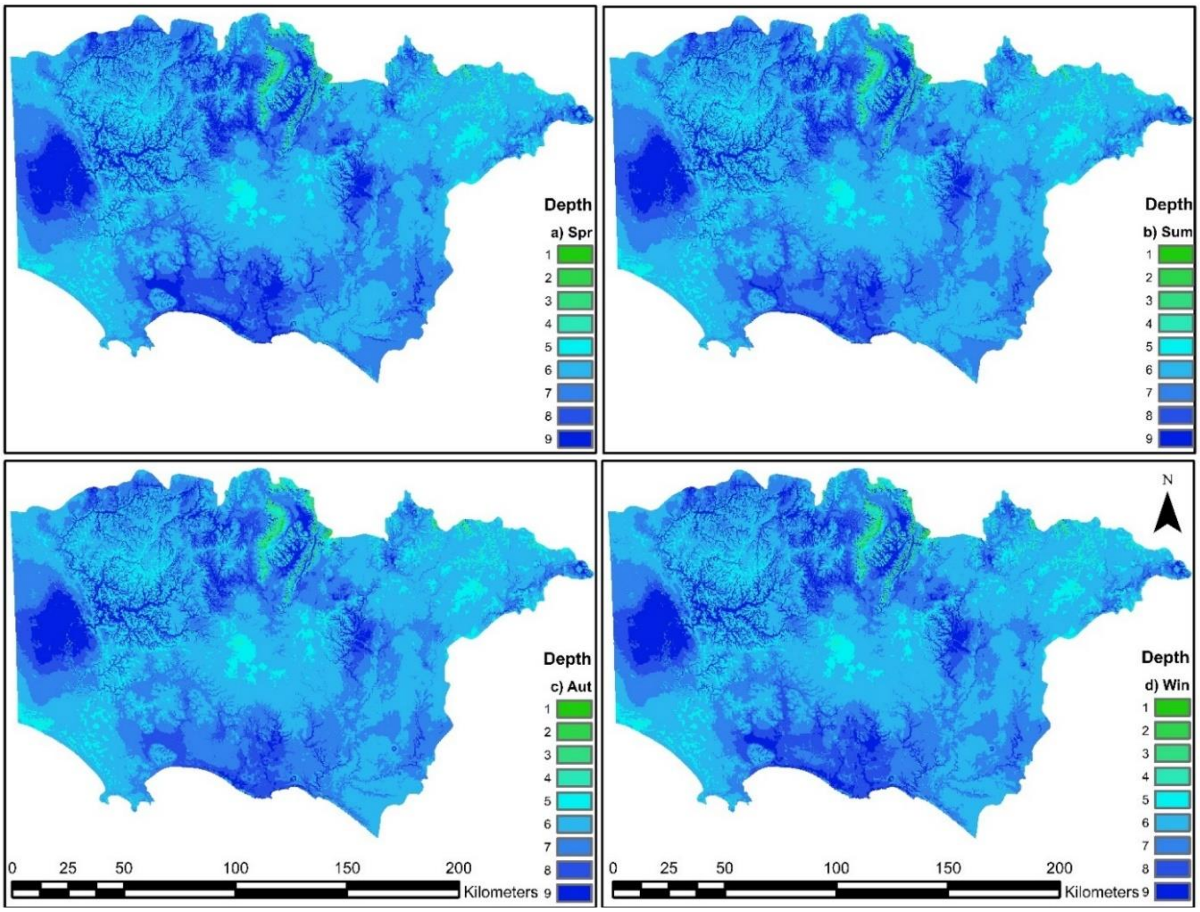

Figure 3.4 Seasonal groundwater depths (D) rating values; a) groundwater depth in Spring, b) groundwater depth in Summer, c) groundwater depth in Autumn, d) groundwater depth in Winter

3.3.4.3 Net recharge (R)

This study uses recharge values from the transient GW model implemented in Modflow 2000 from SKM (2010) using monthly recharge data. Calibrated results for the steady-state and the transient model Normalised RMS error were 2.47% and 2.24%. Modflow inputs were provided by Biosym within the Ensym model as per (SKM, 2010). The detailed method used to calculate GW recharge is presented in the Australian GW modelling guidelines as per SKM (2012). From this data, seasonal GW recharge values were computed in ArcMap and categorized using categorical values from Table 3.6. GW recharge factor offers significant information to the model as water recharge is the major driving mechanism for pollution transport (Robins, 1998). Recharge also provides specific information for both spatial and temporal variability. Monthly GW recharge values for a period of 1987 to 2017 were considered for this analysis. Seasonal GW recharge maps are shown in Figure 3.5.

Table 3.6 Net recharge rating values

Recharge Range (mm)	Rating value
0 – 1	1
1 – 3	3
3 – 7	5
7 – 14	6
14 – 22	7
> 22	9

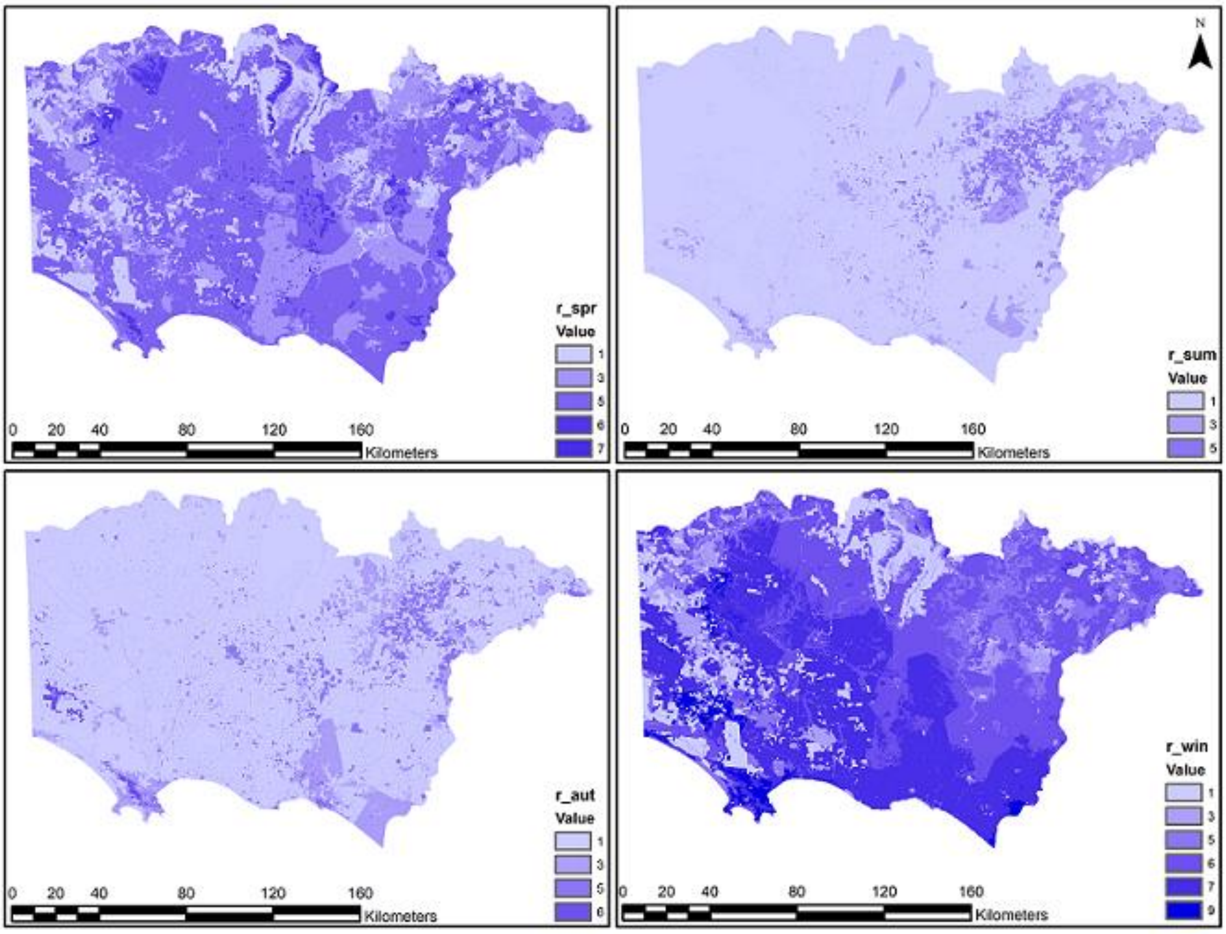

Figure 3.5 Seasonal net recharge (R) rating values; (a) recharge in Spring, (b) recharge in Summer, (c) recharge in Autumn, (d) recharge in Winter

3.3.4.4 Aquifer media (A)

(A) is the medium which serves as an aquifer, it can be consolidated or unconsolidated, the larger the grain size, the more fractures in the aquifer, the greater the pollution potential (Aller et al., 1985). The aquifer medium is the responsible for controlling the route and path length for a contaminant to follow, the larger the porosity of the aquifer, the lower the attenuation capability, and therefore the larger the pollution potential as well (Aller et al., 1985). The aquifer media can affect the GWFS in the aquifer (Anane et al., 2013). 12 hydrogeologic units are present within the GWFS of the selected study site, as shown in Figure 3.6 (a). Aquifer configuration materials were obtained from the VAF - Summary report (SKM (2009). The Lithological units were categorized with new assigned rating values from (1 to 9) are shown in Table 3.7. Values were modified from (Aller et al., 1987) and adapted to the Victorian materials.

Table 3.7 Aquifer media rating values

Aquifer/ Aquitard	Aquifer media	Rating value
QA	Sand and gravel	7
UTB	Basalt	8
UTQA	Sand	6
UTAM	Sand, sandstone, quartz and clay	8
UTAF	Sand and gravel	7
UMTA	Karstic aquifer	9
UMTD	Marine calcarenite, marl	1
LMTD	Marine silt, fine sand, clay, carbonaceous, pyritic, Burrowed	1
LTA	Sand, clay, gravel, conglomerate	2
LTBB	Olivine tholeiites, minor alkaline basalts	5
CPS	Sedimentary, non-marine, low transmissivity	3
BSE	Fractured Palaeozoic rocks, igneous granite	2

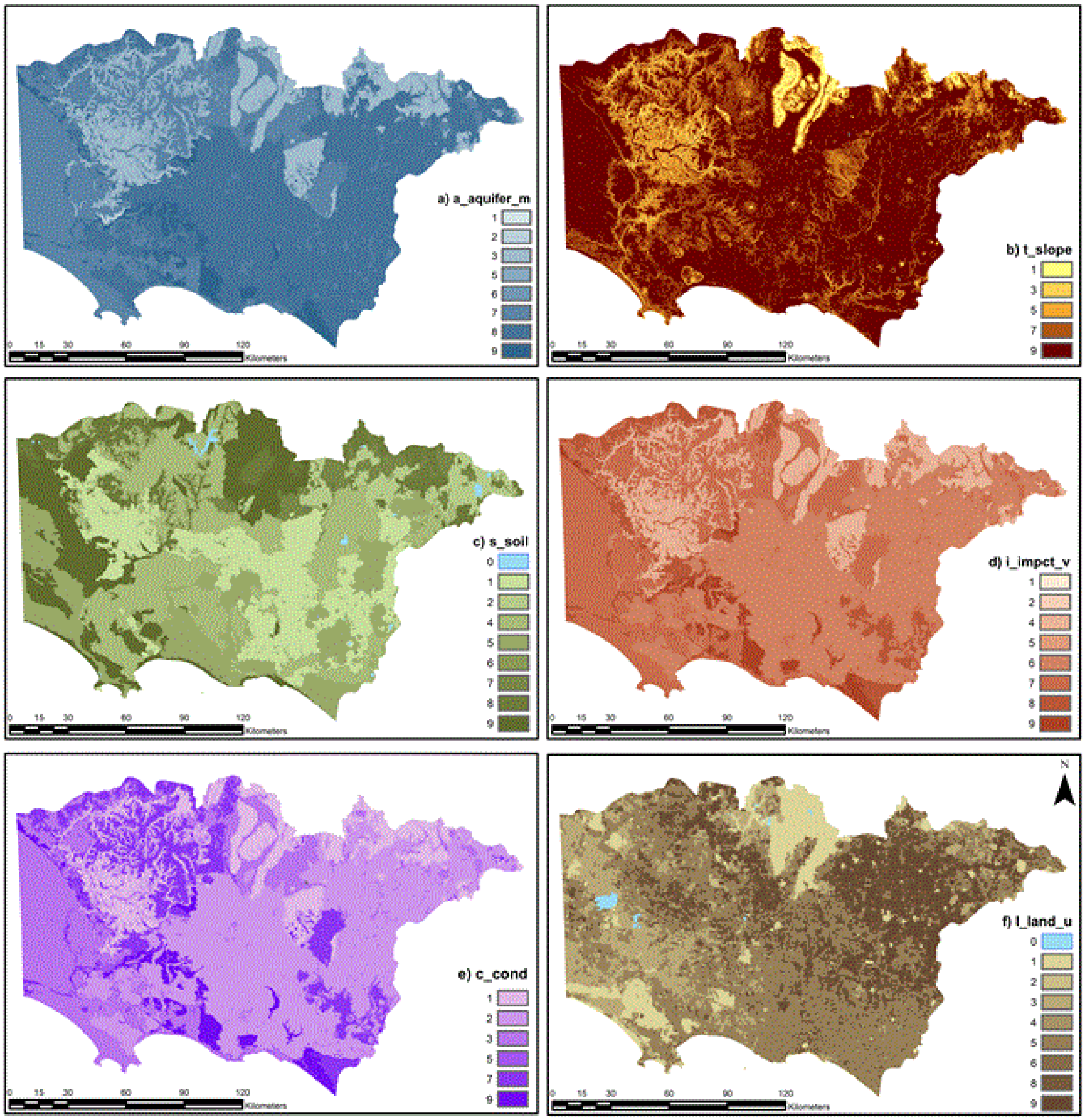

Figure 3.6 (a) Aquifer media rating values, (b) Slope percentage, (c) Soil media, (d) Impact of vadose zone, (e) Hydraulic conductivity, and (f) Land use

3.3.4.5 Soil media (S)

Soil media is the upper portion of the vadose zone; it has significant biological activity; it is considered the upper weathered zone of the soil averaging six feet (1.8 m) or less (Aller et al., 1985). Soil controls recharge and contaminant attenuation processes such as filtration, biodegradation, sorption, and volatilization (Aller et al., 1985). The upper portion of the soil configuration is used for this factor. Data were extracted from the Atlas of the Australian Soil (ASRI), the variable that represents the topmost layer is

(TEXT_TOP) within the Australian Soil Atlas dataset. The categorization of the Australian soil texture is found in the glossary of soil terms in Baxter and Robinson (2001), and in “Estimation of Soil Properties using the Atlas of Australian Soils” (ASRIS, 1968). A rating for soil texture groups shown in Table 3.8. The map for the soil media can be seen in Figure 3.6 (c).

Table 3.8 Soil texture rating values

Texture group	Texture Grade	Rating value
	Sand	9
Sands	Clayey Sand	9
	Loamy Sand	9
	Sandy Loam	8
Sandy Loams	Fine Sandy Loam	8
	Light Sandy Loam	8
	Loam	7
Loams	Loam, Fine Sandy	7
	Silt Loam	6
	Sandy Clay Loam	6
	Clay Loam	5
Clay Loams	Silty Clay Loam	4
	Fine Sandy Clay Loam	4
	Sandy Clay	3
Light Clays	Silty Clay	3
	Light Clay	2
	Light Medium clay	2
Clays	Medium Clay	1
	Heavy Clay	1

3.3.4.6 Topography slope (T)

Topography refers to the slope and the slope variability in the land surface; it controls the probability for a contaminant to run off or remain on the surface in a specific area long enough for a contaminant of the aquifer (Aller et al., 1985). In DRASTIC-based models, the topography is described in terms of slope percentage (Sener and Davraz, 2013). The topography slope map is constructed from the digital elevation model (DEM) using ArcMap10.4. Next, the slope was categorized according to the slope rating values as seen in Table 3.9. Figure 3.6 (b) illustrates the slope % for the Glenelg Hopkins CMA. Notice that even when the grid cell of the DEM is at 50 x 50 m, the resolution for the modified DRASTICL model is 50 x 50 meters in all maps.

Table 3.9 Topography slope rating values

Slope %	0-2	2-6	6-12	12-18	>18
Rating value	9	7	5	3	1

3.3.4.7 Impact of vadose zone (I)

The vadose zone is defined as the zone above the water table that is unsaturated, lies below the soil horizon and above the water table (Aller et al., 1987). In the vadose zone, attenuation processes such as biodegradation, neutralization, filtration, chemical reaction, volatilization, and dispersion are likely to reduce as depth increases (Aller et al., 1985). The rating for vadose zone map was created using the GWFS and the material conformation for each of the aquifer types, conformation material is described in (The Victorian Aquifer Framework) (SKM, 2009). Table 3.10 shows the vadose zone ranges and rating values proposed for the Pesticide DRASTICL model. Figure 3.6 (d) shows the impact of vadose zone map categorized with the rating values of the modified Pesticide DRASTICL model.

Table 3.10 Impact of vadose zone ratings

Name	Range	Rating value
Sand	6-9	7
Bedded limestone, sandstone, shale	4-8	6
Silt, sand,	6-9	8
Sand, silt	4-8	8
Gravel, salt, silt, silt, clay	4-8	7
Karst	9	9
Silt and clay	1-2	1
Marine deposits karst	8-9	9
Sandstone gravel, sand	4-8	5
Basalt	2-9	2
Sand and Gravel silt, clay	4-8	6
Metamorphic / Igneous	2-8	4

3.3.4.8 Hydraulic conductivity of the aquifer (C)

Hydraulic conductivity is the aquifer's material capability to transmit water; it controls the rate of GW at a given hydraulic gradient; intergranular porosity, tectonic lineaments, and bedding planes (Aller et al., 1985). Contamination is controlled by the flow rate of GW (Sener and Davraz, 2013). Furthermore, C controls the contamination movement in the aquifer (Aller et al., 1987). Within DRASTIC-based models, C is denoted as the factor with the highest error associated with its estimation (Stigter et al., 2006).

Factor rating map for C was obtained from two main sources. The first one, an inferred value from various reports included in the GH GWFS by Dahlhaus et al. (2002). The second is, data containing conductivity values from a GW transient model by SKM (2010). In this research, (k) values are taken from SKM (2010). For aquifers with no value, a proposed value was assigned to the aquifer layer considering the aquifer type as previously mentioned in the literature. A new rating scale is assigned to each range of hydraulic conductivity, as shown in Table 3.11. A rating map for C is shown in Figure 3.6 (e).

Table 3.11 Hydraulic conductivity rating values

Aquifer	Rating	(k) Ranges	(k) Values
QA_1t	4	6-10, 102, 5-10, 0.01-102, 0.01-10	20
UTB_1t	3	0-100	10
UTQA_1t	4	0.5	20
UTAM_1t	8	0-10	75
UTAF_1t	8	1,0.01-0.5, <0.1-0.5	75
UMTA_1t	9	10-1, 102, 0.01-102	100
UMTD	2	0-0.1	0.1
LMTD_1t	2	0-0.1	0.1
LTA_1t	7	0.01-102	50
LTBB_1t	3	0.01	10
CPS_1t	7	10	50
BSE	1	10-5, 10-1	0.001

3.3.4.9 Land use (L)

In DRASTIC-based models, (L) is the newest factor that has been incorporated in recent years. It is a decisive and inducing factor of aquifer contamination thorough anthropogenic activities as seen in Stigter et al. (2006). Changes in land use induce the application of agricultural chemicals, industrial waste spills and landfill leachate which can potentially leach to the GW. Factor (L) rating map was created from the VLUIS. A shape format file was downloaded for the year 2016 from (Agriculture Victoria, 2018b) and projected in ArcMap. The secondary land use cell of the VLUIS was selected to assign the new rating values from (1 to 9) as seen in Table 3.12. Figure 3.6 (f) shows the land use rating map. Additionally, it shows the land use rating values for different land use. Next, a raster file was created including the new ratings.

Table 3.12 Land use rating values

Secondary_V7	Rating
1.1 Nature conservation	1
2.1 Grazing native vegetation	4
2.2 Production forestry	5
3.2 Grazing modified pastures	4
3.3 Cropping	9
3.4 Perennial horticulture	6
5.2 Intensive animal husbandry	8
5.3 Manufacturing and industrial	7
5.4 Residential and farm infrastructure	6
5.5 Services	3
5.7 Transport and communication	1
5.8 Mining	8
5.9 Waste treatment and disposal	7

3.3.5 Step 3 The correspondence analysis

3.3.5.1 The multivariate statistical assembly of seasonal GW vulnerability

In this section, we explain the statistical approach used to calculate seasonal GW vulnerability. In the status quo DRASTIC models, GW vulnerability has been addressed as a fixed or static value. However, such models have failed to depict the impacts of temporal variations in GW vulnerability. GW vulnerability can be affected by several factors such as GW extraction, temporal variations in precipitation and evapotranspiration rates, temporal changes in GW depths, and finally temporal variations in GW recharge. Other hydrological processes can affect GW vulnerability such as temporal variations in aquifer's loss and gain, but then again, such variations are complex to estimate.

To make the vulnerability index sensitive to those changes, the proposed multivariate statistical analysis integrates temporal variations of depth and recharge while estimating seasonal GW vulnerability. This was achieved by calculating a GW vulnerability for each season (spring, summer, autumn and winter). For estimating temporal variations in seasonal GW vulnerability, equation (1) was transformed into four different GW vulnerability estimations as presented below:

$I_{DSpr} = (Dr_{Spr} * Dw_{Spr} + Rr_{Spr} * Rw_{Spr}) + Ar*Aw + Sr*Sw + Tr*Tw + Ir*Iw + Cr*Cw + Lr*Lw$ (2)

$I_{DSum} = (Dr_{Sum}*Dw_{Sum}+Rr_{Sum}*Rw_{Sum})+ Ar*Aw + Sr*Sw + Tr*Tw + Ir*Iw + Cr*Cw + Lr*Lw$ (3)

$I_{Daut} = (Dr_{Aut}*Dw_{Aut} + Rr_{Aut}*Rw_{Aut}) + Ar*Aw + Sr*Sw + Tr*Tw + Ir*Iw + Cr*Cw + Lr*Lw$ (4)

$I_{DWin} = (Dr_{Win}*Dw_{Win}+Rr_{Win}*Rw_{Win}) + Ar*Aw + Sr*Sw + Tr*Tw + Ir*Iw + Cr*Cw + Lr*Lw$ (5)

where:

$I_{D\ (Spr,\ Sum,\ Aut,\ Win)}$ = vulnerability index per season

$Dr_{(Spr,\ Sum,\ Aut,\ Win)}$ = depth ratings per season

$Dw_{(Spr,\ Sum,\ Aut,\ Win)}$ = depth weights per season

$Rr_{(Spr,\ Sum,\ Aut,\ Win)}$ = recharge ratings per season

$Rw_{(Spr,\ Sum,\ Aut,\ Win)}$ = recharge weights per season

After calculating the seasonal vulnerability indices using equations (2) to (5), we addressed the estimation of a new set of factors weights to eliminate the correlation between factors as seen in (Pacheco and Fernandes, 2013). This was achieved by separating factors into two hydrogeological groups: the dynamic group (D and R) and the static group (A. S, T, I, C and L). For both groups, an independent correspondence analysis (CA) was performed, and a new set of upscaled factor weights was obtained for each season as mentioned in (Pacheco and Fernandes, 2013). CA deals with the subjectivity bias from using expert opinion for allocating factor weights.

This innovative approach of data treatment (static and dynamic factors) is different from the standard DRASTIC-based models, which integrates all factor weightings in one multivariate analysis. The multivariate eigenvector technique of CA has been selected because it has shown to be the most adequate method for transforming the interrelated DRASTIC factors into uncorrelated DRASTIC vectors as shown in Pacheco and Fernandes (2013). Details of the statistical tool of CA are described in Pacheco and Fernandes (2013), Pacheco (1998) and Pacheco et al. (2015).

Firstly, the seasonal factors (D), (R) plus a dummy factor (Q) with a constant value of (5) are resampled in ArcMap using a fishnet at regular intervals of 5 km, as data preparation step. The purpose of the sample is to extract factor rating values at each point. Next, another point resampling is performed for each of the static factors (A, S,

T, I, C, and L). Secondly, a CA is performed to calculate vector loadings for each group. In the case of the dynamic group, a dummy dataset Q with a mean of (5) in rating value is used to assist in the calculation of the vector loadings as CA can only be performed with more than two factors. CA is also performed for the static hydrogeological group using (A, S, T, I, C, A and L) factors. From both groups, factor loading values are derived from the CA, new vector loads are obtained and represent the uncorrelated value compared to other factors. Such values are called dynamic vector (D, R, Q) factor loadings and static vector (A, S, T, I, C, and L) factor loadings.
Finally, each vector load is rescaled to the DRASTICL values (1-5) using the harmonization Formula provided by Pacheco and Fernandes (2013), as seen in equation (6), these new set of weights are called seasonal vector DRASTICL factor weights.

$$v_j^* = \frac{W_{j.max} \text{ x } v_{j.max} - (W_{j.max} - W_{j.min}) \text{ x } (v_{j.max} - v_j)}{v_{j.max}}$$

$$1 \leq j \leq p \qquad (6)$$

where:
$w_{j.max}$= max factor weight in Pesticide DRASTICL
$w_{j.min}$= min factor weight in Pesticide DRASTICL
vj= Factor loadings

The CA was computed using R (R Core Team, 2019), an open-access software. New loading values for vector1 (V1) are calculated as mentioned earlier.

3.3.5.2 Step 4 Calculating new factor weights

Calculated loading values from the CA cannot be compared to Pesticide DRASTICL weighting values because loads are presented on a different scale. From equation (6) a new set of recoordinated weighting values from new vector-DRASTICL factor loadings were calculated. Each factor group was treated as a separate multivariate statistical data set. Recoordinated weighting values are known as vector-DRASTICL factor weights (Pacheco and Fernandes, 2013).

3.3.5.3 Step 5 Calculation of seasonal Pesticide DRASTICL vulnerability

As discussed above, to improve the outputs of the Pesticide DRASTICL method, factor ratings were reduced to a maximum value of 9 and an independent multivariate correspondence analysis (CA) was applied to both factor groups. The harmonization of scales method was applied to the resulting CA vector loading values recoordinating them from (1 to 5). As a result, seasonal Pesticide DRASTICL weighting values were used to calculate the GW vulnerability for each season.

The seasonality of the GW vulnerability assessment was quantified by assessing the aquifer's vulnerability using the corresponding seasonal factor weights at each season. This novel approach for estimating GW vulnerability identified areas where the vulnerability was larger at different times during the year. Therefore, the seasonal DRASTICL model accounts for spatial and temporal variations.

Eight raster layers from DRASTICL factors containing grid cells with the size of (50 x 50 m) were used to calculate the seasonal (I_D). Four new (I_D) were calculated for each grid cell of the study area. Once all map cells have a seasonal (I_D) value, the score was categorized in seven ranges, from the lowest to the highest, each range corresponds to unlikely, very low, low, medium, high, very high and extreme vulnerability. Results were mapped in the study area for each of the seasons.

Different values for vulnerability were expected for each season. The vulnerability values should be similar in places where the vulnerability had a small or no changes, this could represent that the aquifer hydrogeological properties are less sensitive to temporal variations in depth, recharge, and GW extraction effects. On the other hand, vulnerability values should be higher where the conditions of the aquifer are more sensitive to temporal variations in depth, recharge, and GW extraction.

3.4 Results

3.4.1 The seasonal vulnerability of the modified Pesticide DRASTICL

Results of this study are presented in the same sequence which corresponds to the methodology section. After rating categorization, a resample is made to obtain point values from each factor, resample was made using a fishnet. The fishnet is the base for a digital sampling of each of the factors taking part in the CA. Figure 3.7 shows a fishnet map.

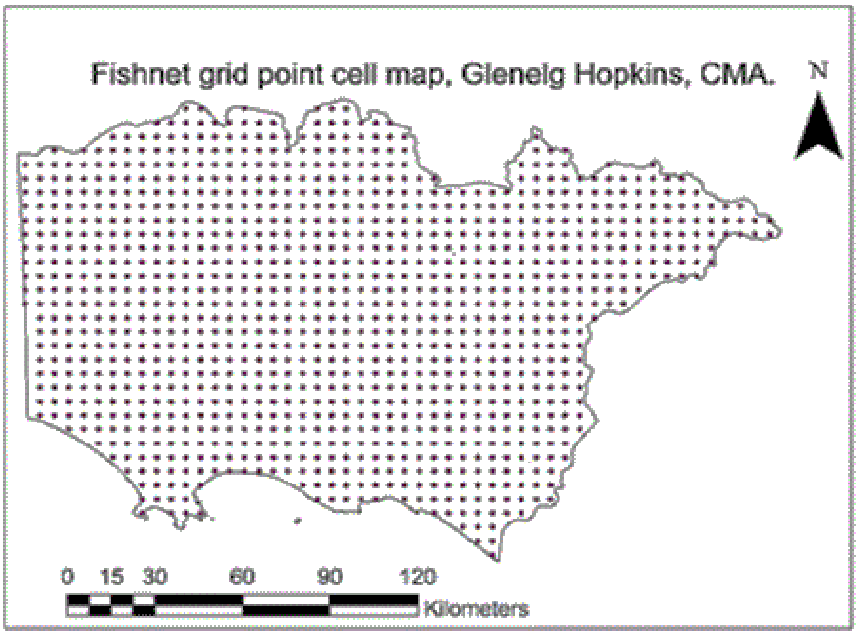

Figure 3.7 Fishnet map, a grid cell of 5x5 km

Vector loadings for the static group (L), (A), (S), (T), (I), and (C) are plotted in Figure 3.8 (a), where the biggest absolute value in loadings corresponds to (S). Figure 3.8 (b) presents the results for common vector loadings from the dynamic CA, vector 1 (D), vector 2 (R) and vector 3 (Dum) are mapped for spring, summer, autumn and winter. The application of CA to data for seasonal group resulted in the confirmation that the use of a dummy data set (Dum) does not impact the value of the cumulative percentage of the system variance. It can be seen that factor (R) has a larger load compared to (D). As seen in Figure 3.8, there are differences in the CA loading values for both groups; the bigger the absolute value, the higher the noncorrelation between factors. Such variables are called explicative factors, for the non-explicative factors, the values were lower or similar as seen in Pacheco and Fernandes (2013).

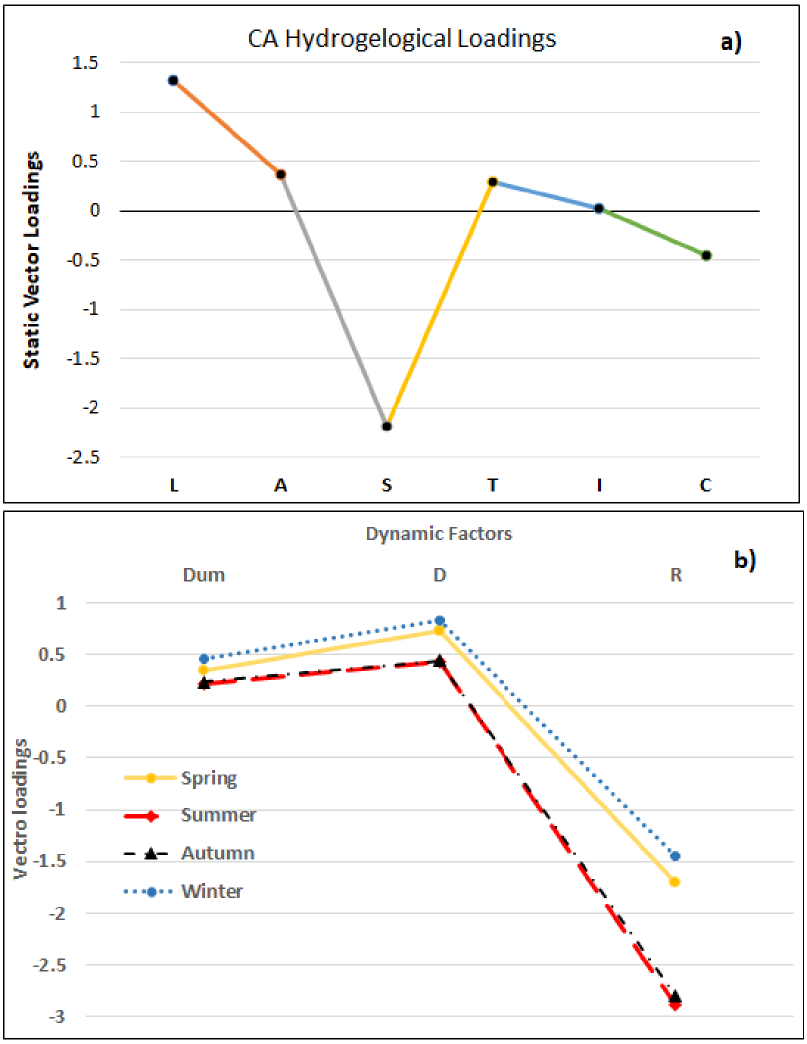

Figure 3.8 a) Distribution of static CA V1 loadings, b) Distribution of dynamic CA V1 loadings

In the case of the dynamic factor group, the (R) factor has a higher weight than factor (D). This could be explained because recharge is the driving hydrogeological process through which aquifer pollution occurs, the higher the recharge, the higher the chances for a pollutant to be transported to the water table (Robins, 1998). Because of differences in data management, data ownership, data availability and data quality regarding static and dynamic factors, the user can make informed decisions about excluding one or more of the less significant factors based on the results of CA. For this study, we did not perform a factor reduction, and we incorporated all factors in the GW vulnerability assessment of the study area.

The Figure 3.9 (a) shows the distribution of eigenvalues and the cumulative percentage of the system variance for common vectors V1, V2, and V3, two explicative factors for the dynamic group vector 1 (D) and vector 2 (R) can explain 100% of the system variance. Figure 3.8 (b) shows loading values for V1 and V2 plotted for each season, it can be seen a clear difference in the loads for (R).

Table 3.13 shows the cumulative percentage of the system variance and eigenvalues for each of the seasons. Table 3.14 shows the results for dynamic CA factor loading values at different percentages of system variance.

Table 3.13 Seasonal CA Eigenvalues and Cumulative % of system variance

Season	Eigenvalue			Cumulative % of system variance		
	D	R	dummy	V1	V2	V3
Spring	0.035428	0.006393	0.0	88.89	100	100
Summer	0.045634	0.00752	0.0	89.51	100	100
Autumn	0.056604	0.00689	0.0	89.39	100	100
Winter	0.038706	0.005426	0.0	88.49	100	100

Table 3.14 Seasonal factor loadings

Season	Factor loads		
	D	R	Dum
Spring	0.7324	-1.7068	0.345
Summer	0.4320	-2.8911	0.220
Autumn	0.4385	-2.8018	0.238
Winter	0.8322	-1.4440	0.463

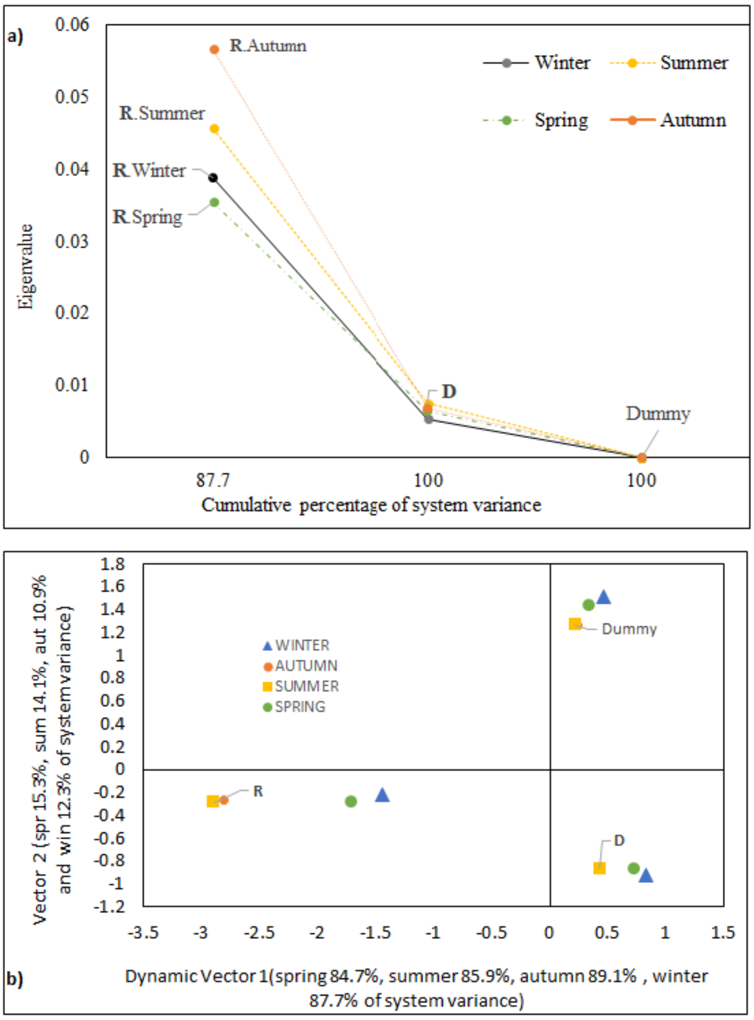

Figure 3.9 (a) Results of dynamic Correspondence Analysis eigenvalues. (b) Cartesian projection of vector loadings V1 and V2. A plot of (D), (R) and dummy loadings for spring, summer, autumn and winter

The Second correspondence analysis is performed for the static group, the variance in measurements are expected to be different from the variance in the dynamic group. That is, higher errors are expected to occur for the dynamic group related to their measurement methodology. Both measurements (D) and (R) have an inherent temporal variation and hold a bigger variance than the static factor group. For this reason, a CA is performed independently for each factor group. Table 3.15 shows the results of the CA static eigenvalues and cumulative percentage of variance. Figure 3.10 shows the results of CA for the static factors, common vectors V1, V2, V3 and V4 accounts for the cumulative percentage variance at about 98.6%.

Table 3.15 Static CA Eigenvalues and cumulative % variance

	Static Vectors					
Vector	V1	V2	V3	V4	V5	V6
Eigenvalue	0.0642	0.0418	0.030	0.007	0.0019	0.00
% Cum. var	44.2	73	93.7	98.6	100	100

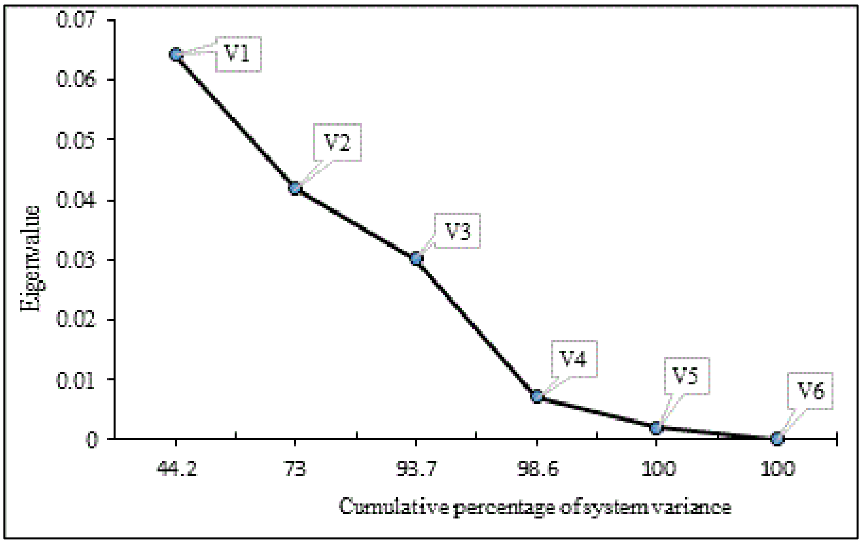

Figure 3.10 Results of static factors Correspondence Analysis. SCREE plot of common vectors

Figure 3.11 shows the distribution of static CA V1 and V2 loadings, it can bee seen a scatter plot for the static factors, vectors (S), (L) and (C) are the explicative factors of the static system. Vectors S, L and C are the ones with the highest loads and have highest distances from the centroid. Although results from static CA suggest that principal static vectors V1, V2 and V3 can explain up to 93.7% of system variance and that using only these three-factor should be enough to derive the vulnerability index, all static factor factors were considered for this study under the premise that each one of them are significant explicative factors that contribute with a different type of information in the seasonal DRASTICL vulnerability index.

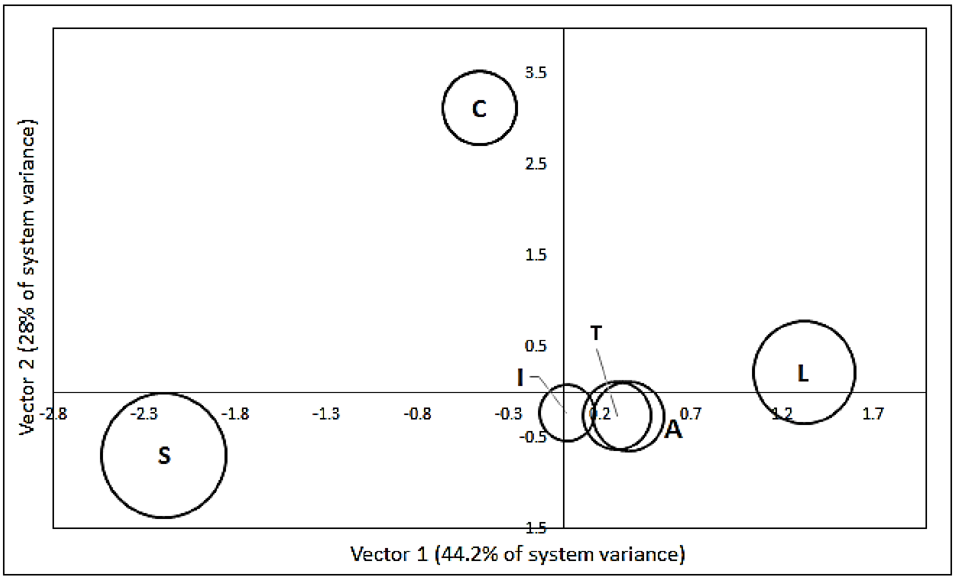

Figure 3.11 Results of static Correspondence Analysis. Cartesian projection of vector loadings V1, V2, V3, V4, V5 and V6

In Table 3.16 and Table 3.17 new seasonal vector DRASTCL weights are presented as a result of the harmonization of scales using the loading values for each of the factors, such weights will be used in the calculation for the seasonal Pesticide DRASTICL vulnerability. The new vulnerability will be referred to as seasonal vector DRASTICL Index and will be represented as I_{DSpr}, I_{DSum}, I_{Daut}, and I_{DWin}.

Table 3.16 Static Vector DRASTIC factor weights

Factors	L	A	S	T	I	C
v_j^*	3.42	1.66	5.00	1.54	1.04	1.83

Table 3.17 Dynamic Vector DRASTIC factor weights

v_j^*	D	R
Spring	2.72	5.00
Summer	1.60	5.00
Autumn	1.63	5.00
Winter	3.30	5.00

The seasonal vulnerability is presented in Figure 3.12. It can be seen that for each season there is a corresponding dimensionless scale, this should be approached as an independent representation of the vulnerability. Results show that the risk will be highly impacted by factors (R), (D), and (L), additionally, it can be seen that the model is capable of estimating seasonal changes of vulnerability, with summer presenting highest risk of contamination to GW.

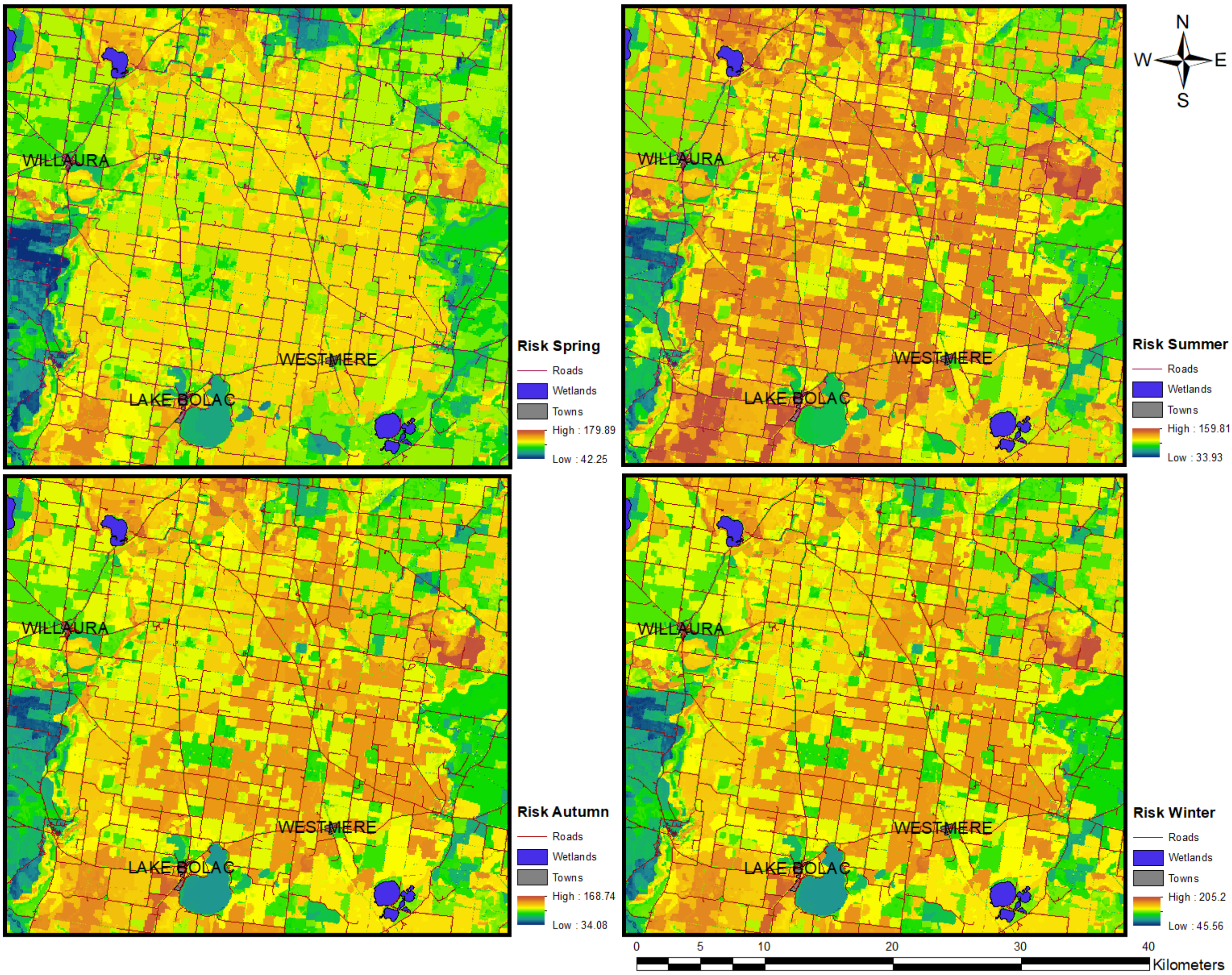

Figure 3.12 Seasonal vulnerability for the central region of the GH CMA, Vic, Au.

3.5 Discussion

Aforementioned results are different from former research as seen in the latest literature review of the models in Hamza et al. (2015) and Gogu and Dassargues (2000), the inclusion of a seasonal approach produces four different scales. Therefore, in the resulting seasonal GW vulnerability as an independent estimation, the reader should avoid the temptation to integrate the four different scales into a unified scale. Each seasonal vulnerability estimation is the representation of different conditions in the aquifer that reflects physical changes in the environment and should be interpreted accordingly. We suggest that the categorization of the vulnerability (very high, high, medium, low and very low) as mentioned in most of the previous research may lead to loss of valuable information for the reader, in this study a scale represents better the changes in the seasonal interpretation of results.

The scale for spring ranges from 42.25 – 179.89, the scale for summer ranges from 33.93 – 159.81, the scale for autumn ranges from 34.08 – 168.74 and the scale of winter ranges from 45.56 – 205.20. The lower vulnerability value between scales occurs in summer, which, for the GH CMA represents the season where less water is recharged into the aquifer because of less precipitation and high-temperature averages which produces the highest evapotranspiration rates having a direct impact on the groundwater net recharge. Conversely, the highest vulnerability value between scales occurs in winter where the environmental conditions are the opposite of summer. This means, that the vulnerability scales behaviour is consistent with the behaviour of the transient model in terms of groundwater net recharge from DELWP. (R) as been acknowledged as the key to groundwater pollution and groundwater vulnerability (Robins, 1998).

Only two studies have reported seasonal estimations of the vulnerability, Sangam et al. (2016) who estimated GV for pre-monsoon, monsoon and post-monsoon seasons, and Sekhar and Kumar (1999) who estimated the vulnerability for pre-monsoon and post-monsoon seasons. However important, those studies failed to comprehensibly assess factor's weights for each season considering all factors weights to be static over time. Results from this study suggest that the weights could vary between seasons. The novelty of the proposed seasonal Pesticide DRASTICL model focuses on the separation of the factors (static and dynamic) and in the comprehensible calculation of factor's weighting.

The seasonal CA suggest that the highest weight corresponds to the dynamic factor (R) followed by the static factor (L). It should be noticed that (D) showed a significant variation of weighting values between seasons being highest in winter and spring. That is, in the season with the highest precipitation (D) is weighted heavily than in summer with the lowest weighting value.

A fundamental step in standard DRASTIC-based models is the calibration process, authors have reported different methods (Rupert, 2001), however, in this study there are no seasonal data for the calibration step. We suggest that calibration of the proposed seasonal Pesticide DRASTICL model should be carried out with seasonal nitrogen and/or phosphorus concentration data.

3.6 Conclusions

In current DRASTICL-based models, the vulnerability index is represented as a map with a fixed vulnerability scale assuming the vulnerability remains the same for the catchment or study area throughout the year. These models ignore temporal changes in vulnerability. This study has attempted to address this knowledge gap by differentiating the influencing factors into two hydrogeological groups (static and dynamic) to investigate seasonal GW vulnerability. In this novel approach, a Pesticide DRASTICL model was combined with a double Correspondence Analysis.

The results indicated that the vulnerability index significantly varies within the seasons and is highly dependent on the two dynamic factors: recharge and water depth. We found that GW recharge and soil media are the explicative factors in the seasonal quantification of GW vulnerability. The quantification of temporal and spatial variations of the GW vulnerability will provide additional information to farmers and water administrators to better manage GW pollution prevention programs. It will also assist with the efficient use of fertilisers and pesticides in agricultural areas. Further research needs to be performed in the calibration step for the model to improve the estimation of the seasonal vulnerability of the aquifer.

Chapter 4

Are triazines and nutrients from farms making their way into groundwater and wetlands at the Glenelg Hopkins, Victoria, Australia?

4.1 Abstract

Groundwater sampling and groundwater testing for monitoring pesticides and nutrients are complex tasks. In the Glenelg Hopkins Catchment in Victoria, Australia, intensive farming of oil and other crops are causing significant use of pesticides and nutrients. It is imperative to quantify the extension and concentration of pollution in aquifers and groundwater dependent ecosystems in the vicinity of intensive agriculture farmlands. This Chapter studies pesticide occurrences in groundwater and wetlands tested for triazines (atrazine, hexazinone, metribuzin, prometrym, simazine, desisopropyl-atrazine and desethyl-atrazine), nitrogen and phosphorus. In selecting groundwater monitoring bores prone to agricultural pollution, we used a seasonal Pesticide DRASTICL model as per (Cervantes-Servin et al., in prep). In groundwater, atrazine, metribuzin, simazine, and desisopropyl atrazine tested positive at high levels in one out of 23 monitoring bores (19.43 µg/L total pesticide concentration). Nitrogen (below 51.6 mg/L) and phosphorus (below 0.30 mg/L) tested positive in most of the groundwater bores. Three out of the ten wetlands tested positive for atrazine occurrence (below 0.064 µg/L), and four of them showed simazine occurrence (below 1.2 µg/L). DRASTIC vulnerability estimation value showed a low correlation with nitrogen (NOx) concentrations at 0.25 R^2. The results showed a low occurrence of triazines

contamination in groundwater, however, occurrences of nitrogen were higher. This study shows that traces of pesticides can be found in wetlands and groundwater and that nutrients contamination is affecting the aquifer at the study site. Further research needs to be done with a larger monitoring sample size that includes wetlands in agricultural areas.

4.2 Introduction

Water availability is a major limiting factor that controls natural and agricultural ecosystems (Nemani et al., 2003). Water quality in surface and groundwater (GW) systems is also of great concern to ensure optimal use of water suitable for this purpose (Khatri and Tyagi, 2015). GW quality and quantity are critical for human development and lotic and lentic GW-dependent ecosystems (Eamus et al., 2006). In intensive agricultural areas, surface water and GW have an inherent vulnerability to pollution by agricultural practices. In Victoria, Australia, farmers apply triazine herbicides to triazine tolerant canola (TTC) cropping and the government uses triazines for weed control in landscaping and road maintenance (Regional Roads Victoria, 2019). Pesticides pose a direct threat to the aquatic life such as algae, fish, amphibians, invertebrates, plants and various other microorganisms (Schäfer et al., 2011). Similarly, pesticides can have serious adverse effects on humans (Sengupta and Banerjee, 2014; Cohen, 2007; Swan, 2006), as atrazine showed to have a detrimental effect on sperm quality in small mammals (Dehkhargani et al., 2011), and is toxic for embryos (Bigsby et al., 1999). Atrazine and atrazine mixtures are safe to use in Australia under the APVMA (2008) and were declared to be safe in Canada by the PMRA (2017), atrazine and atrazine mixtures have been banned in Europe by the EC (2004). There is a need to better understand the mobility and fate of pesticides from the application area into other components of the environment and their impact on receiving waters (Kearney and Helling, 1982).

The Glenelg Hopkins Catchment (GHC) encompasses over 26,000 km^2 of area, of which 80% area has been cleared for agricultural activities (Agriculture Victoria, 2018b). Agricultural activity in this catchment is one of the most productive in Australia (GH CMA, 2018). Additional to its economic importance, there are some ecologically important wetlands in this region such as the Victorian Western Districts

Lakes (DNRE, 2002b) mentioned in DNRE (2002a). Seasonal herbaceous and permanent wetlands provide a natural habitat for threatened species such as "*Dwarf Galaxias*, *Yarra Pygmy Perch*, *Swamp Antechinus*, *Swamp Skink*, *Growling Grass Frog*, *Ancient Greenling*, *Australasian Bittern*, *Ground Parrot*, *Swamp Greenhood* and *Maroon Leek-orchid*" at Long Swamp wetland as per GH CMA (2014). Some of these wetlands are totally or partially GW dependent and therefore fluctuation in GW availability and quality has direct effects on these wetlands (GH CMA, 2006). However, to our knowledge, there is no published research documenting the impact of agriculture on GW and wetlands in this catchment. Although some studies attempted to correlate nutrients in GW and their effect on wetlands the results were inconclusive mainly because of the small sample size of the studies as per Raisin et al. (1999). The Victorian Strategic Direction Statement does not mention agricultural pollution in GW as a potential threat to wetlands.

Using triazine-based products in Victorian agriculture has escalated over the last 20 years associated with the increase of canola production as can be seen in the VLUIS. In the GH CMA farmers use primarily triazines to control weed in canola, lentils, and maize helping farmers to increase grain production. The seeding time for crops and pasture ensues in early May with an initial application of herbicides and fungicides, followed by fertilizer application in June and second spraying of herbicides and fungicides in mid-July or mid-August. Harvesting time for canola is in mid-November to early-December for cereals and winter pastures are harvested in September and November. The common pesticide products for canola are altiplano, triflur, atradex, ultra, avadex, burst, and butisan. Use of two or more products in a single spray mix is common depending on the agricultural practice. The active chemical ingredients of these herbicides are shown in Table 4.1.

Table 4.1 Herbicide and their chemical active ingredients (Conboy et al., 2018)

Altiplano	Triflur	Atradex/ Atrazine	Ultra	Avadex	Burst	Butisan
Clomazone, Napropamide	Trifluralin, Liquid hydrocarbon	Atrazine	Potassium salt of glyphosate	Triallate, Liquid Hydrocarbon	Propyzamide	Metazachlor

The branch of Regional Roads Victoria (RRV) in the GH CMA suggested a list of herbicides for road furniture for the year 2019 (Regional Roads Victoria, 2019). In road maintenance, herbicides play a key role in road conservation improving safety and visibility for drivers. The product list includes the use of Brush-Off (metsulfuron-methyl), Cavalier (oxyfluorfen), Glyphosate (isopropylamine salt), Lontrel Clopyralid (trisopropanolamine salt), and Sulfometuron 750 (sulfometuron-methyl). Road maintenance work includes rotation of herbicides to prevent weed resistance (Regional Roads Victoria, personal communication, 2019). However, some of the herbicides used in agriculture and road conservation showed to be persistent in the environment and could be toxic for the aquatic ecosystems. Wetlands of international importance such as the ones declared in the Ramsar convention could be at risk from agricultural chemicals. Furthermore, excessive amounts of nutrients may cause noxious and toxic algal blooms in aquatic ecosystems (Rabalais, 2002). Atrazine has adverse hermaphroditic effects in amphibians at 0.1 ppb (Hayes et al., 2003; Hayes et al., 2002) and is highly toxic for frogs (Orton and Tyler, 2015). Oxyfluorfen can be highly toxic for *Scenedesmus sp.* algae (Rojíčková and Maršálek, 1999), it poses a low risk for GW, as the risk for leaching into GW exists (Alister et al., 2009; Yen et al., 2003; US EPA, 2001). Clopyralid has shown to be more mobile than other products in GW and more stable in the environment (Munira et al., 2018). Conversely, sulfometuron-methyl is unlikely to occur in GW (Steinheimer et al., 2000). In Victoria, the presence of atrazine in GW of unconfined aquifers has been reported at agricultural areas (Chapman and Stranger, 1994, 1993) and throughout different agricultural sites of Australia (Bauld, 1994).

Research for pesticides and nutrients pollution in Victoria are limited as reported in Mossop et al. (2013), Gagliardi (2012), Wightwick and Allinson (2007), Rose and Kibria (2006), (SKM, 2002), Ivkovic et al. (2001), Watkins et al. (1999a), Watkins et al. (1999b), Wenig (1997), Moore et al. (1996), SKM (1995), Bauld (1994), McKenzie-Smith et al. (1994) and Leitch and Fagg (1985). All pesticide studies in GW were conducted before 2007, and there has not been much attention given to current status after increasing use of pesticide over last 20 years that might lead to increased vulnerability of GW and wetland contamination, the increasing use of pesticides can be observed in the changes of the land use in the VLUIS as seen in Agriculture Victoria (2018b). The latest pesticide review in Victoria mentions that atrazine, simazine, chlorpyriphos and DDT were the most commonly detected pesticides (Wightwick and Allinson, 2007). In New Zealand, a recent national pesticide survey showed that 17% of the monitoring bores tested positive for pesticides with triazines being the most detected over a sample of 165 bores (Humphries and Close, 2015). Nitrate pollution in GW is shown to be directly related to agricultural activities as per Benson et al. (2006). The Victorian government offers data for some pollutants such as nitrogen and phosphorus in the national GW information system within the GW explorer which includes the previous pollutants. (http://www.bom.gov.au/water/).

Nutrients data present spatial and temporal discontinuity and in most of the cases, no data has been registered for pesticides. Pesticide surveys in GW face significant hindrances due to costs such as laboratory analysis, technical labour, the extent of the study area, and fieldwork travelling expenses. Additionally, in most cases, GW pesticide surveys are deployed with no guidance in selection/identification of GW monitoring bores. The Victorian government has mentioned the use of a hydrogeological assessment before the sampling campaign (EPA, 2006), but such method does not include comprehensible guides for estimating the vulnerability of the aquifer system (EPA, 2000a).

This Chapter attempts to address all the limitations and knowledge gaps mentioned above to identify and select unconfined aquifers that may have a high risk of pollution from agricultural activities. Pesticide DRASTICL is a GW vulnerability assessment (GWVA) model that can predict the aquifer's vulnerability to pollutants and has proven to be a valid tool in GW pollution prevention plans (Bartzas et al., 2015; Rupert, 2001; Aller et al., 1987). DRASTICL is the acronym of eight factors included in the vulnerability model (depth to water table (D), net recharge (R), aquifer media (A), soil (S), slope topography (T), impact to vadose zone (I), aquifer conductivity (C), and land use (L) (Aller et al., 1985).

This study then focuses on assessing the effects of agriculture on the GW quality in shallow aquifers and on wetlands at the study area by sampling and testing of collected water samples from high, medium and low-risk zones. It includes laboratory testing for triazine herbicides (atrazine, hexazinone, metribuzin, prometryn, simazine, desisopropyl atrazine and desetilpropyl atrazine), nitrogen (NO^{-2} + NO^{-3} as NOx), phosphorus as dissolved reactive phosphorus (DRP), pH, temperature and electric conductivity (EC). Overall, this study shows the implementation and evaluation of a seasonal Pesticide DRASTICL model for identifying GW bores at agricultural areas with a high risk of contamination.

4.3 Methodology

This section presents an overview of the study area and the methodology used for the collection and analysis of the GW and wetlands samples. The methodology includes three steps as follow: 1) seasonal GW risk assessment, 2) GW sample collection, and 3) water quality analysis. The first step of the methodology entails the use of a seasonal pesticide DRASTICL model to estimate areas prone to agricultural pollution. The second step includes sample size and site selection for GW bores and wetlands. Furthermore, it includes the methodology followed to extract water from bores as well as to collect water samples from the wetlands. Finally, the third step is the laboratory analysis applied to GW and wetlands samples.

4.3.1 Study area

The GH CMA (see Figure 4.1) is in the south-west of the Victorian State. The GH region geographical boundaries are defined to the north with the Wimmera CMA, to the east with the Corangamite CMA, to the west with South Australia Estate and to the south with the Tasman Sea (GH CMA, 2013). To see a full description of the study area and the hydrogeological conformation of the aquifer please refer to Cervantes-Servin et al. (in prep). Water table depth varies with the seasons in GH Victoria, Australia, as it is closer to the surface during the winter and is deeper in the summer season. On average, GW depth can be localised from 0.0m, up to 6m below the surface. Diverse landscaping can be seen within the 26,000 square kilometres of land. The main dominant features are the iconic Grampians National Park, the Flat Volcanic Plains, Central Highlands and Dundas tablelands (GH CMA, 2018, 2013). This region combines agriculture, water reservoirs, nature conservancy, lakes, wetlands, energy production and urban areas. The main crops in agriculture are canola, wheat, barley, oat, and winter pastures (Conboy et al., 2018). The Glenelg catchment is within the most productive agricultural areas in Australia, it is the fourth CMA in Australia and the topmost in Victoria for agricultural productivity. The total revenue it had in 2017-2018 from agricultural production was reported at about AU$2,184 million (GH CMA, 2018).

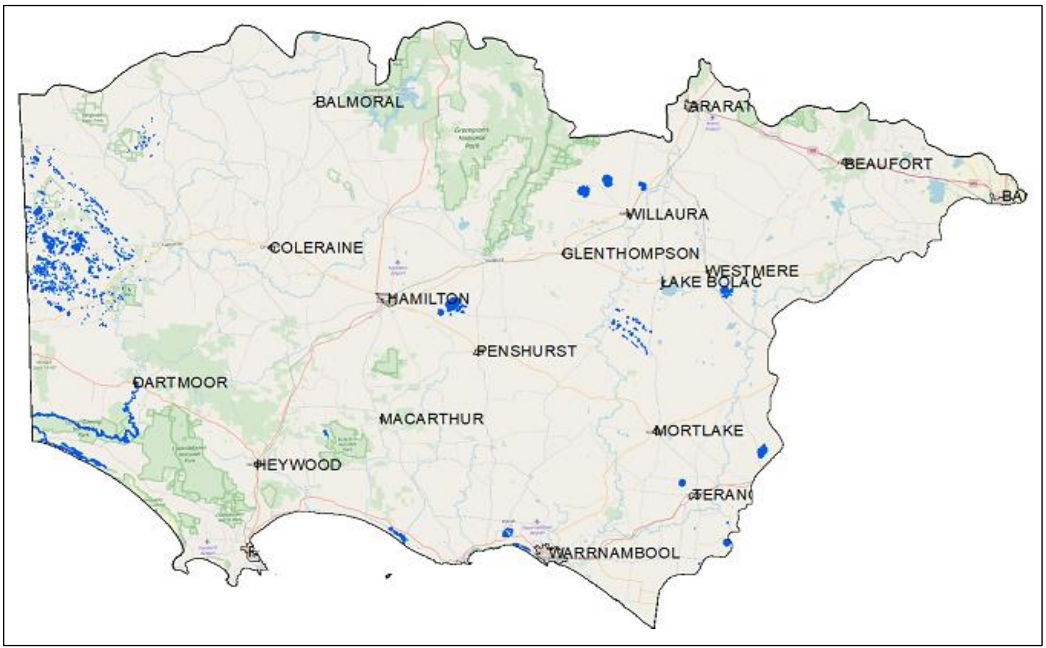

Figure 4.1 The GH CMA, Vic. Au.

4.3.2 Seasonal groundwater vulnerability assessment (Pesticide DRASTICL)

The purpose of using a Seasonal Pesticide DRASTICL model was to estimate areas that may be vulnerable to agricultural pollution. This model uses seven hydrogeological factors as mentioned in the Introduction section. Factors (D), (R), (A), (S), (T), (I) and (L) are independently weighted and rated, with weights (w) having a range from (1 to 5) and ratings (r) a range from (1 to 9). After estimating the GWV map as per (Cervantes-Servin et al., in prep), it was used in conjunction with the actively monitoring bores map to select the size and location of bores to be sampled. At the same time, GWV map was used to identify wetlands close to the high vulnerability areas within the catchment.

4.3.3 Sample size and collection

4.3.4 Selection of groundwater monitoring bores

The GH CMA contains more than 351 actively GW monitoring bores according to the "GW monitoring of the State Observation Bore Network (SOBN)" (http://data.water.vic.gov.au/) as seen in Figure 2. The State monitoring wells were plotted on the CMA region map, followed by clipping the monitoring wells corresponding upon the GH region map, this was performed in ArcMap10.4.

This new map contains all the monitoring maps that are reported as active as seen in Figure 4.4 (left). However, not all monitoring bores are accessible, whether some of them are privately owned, do not physically exist, or the bore diameter is very small (less than 6 cm), therefore data filtering is required to select only those wells that can be used to sample aquifers.

Data filtering consisted of deleting all inaccessible for sampling bores that were mentioned before. Data filtering was performed in Microsoft Excel-365 and the resulting file was saved as comma-separated values (CSV). The CSV file was plotted again in ArcMap. Figure 4.4 (left) shows the bores that can be included in the selection of bores.

The available bores were plotted in the study area and above the GW vulnerability assessment map. Using this tool, bores that were in the very high GW vulnerable zone were identified. The selection of bores was made by identifying the low, medium and high vulnerability values. The selection of the bores included in this study is showed in Figure 4.4 (right).

4.3.5 Wetlands and groundwater sample collection

Wetlands water quality samples

Water samples were collected manually from wetlands, during 2017-2018. In all cases, wetland's water samplings were conducted close to the road entrance of the wetland. The purpose was to obtain a water sample that could be collected without complex equipment, but at the same time could offer information regarding the state of the water quality. The point of sampling for each of the wetlands is shown in Table 2. Water samples were taken from the shore of the wetland and within the first 4.0m from the waterline. A closed 500ml dark glass bottle was immersed into the water at a depth of 0.5m. At this depth, the lid of the bottle was opened to allow water to fill the bottle. Samples were treated as mentioned in “A guide to the sampling and analysis of waters, wastewaters, soils and wastes” (EPA, 2000b) and “Sampling and analysis of waters, wastewaters, soils and wastes” (EPA, 2009).

Groundwater quality sampling

GW quality samples were also collected and preserved in 500ml dark glass bottles. For each bore, three bottles of 500ml were filled with water. The bottles were then preserved in a cooler with ice, during transportation. After transportation, the samples were stored at 4°C in a dark room before laboratory analysis.

The methodology used for the GW bores sampling was the low flow GW sampling; this included the use of a pneumatic low flow pump (Sample Pro – Micropurge Portable Pump), together with a water quality multiparameter (YSI Pro) and a 2.0L probe. The pump was actioned by a pneumatic compressor and an air controller. The aforementioned equipment is known as the GW sampling kit. The stabilizing parameters for the GW flow were pH, temperature and conductivity. Samples were extracted from the bores following the Victorian GW sampling guidelines (EPA, 2000a).

4.3.6 Sample analysis

Surface water and GW samples were sent to accredited laboratories National Measurement Institute (NMI) (first GW sampling campaign) and to the Department of Industry, Innovation and Science at The Australian Government and the Australian Laboratory Services (ALS) (second GW sampling campaign) both located in Melbourne, Victoria. Samples were tested for nitrogen as NOx (NO_2^- and NO_3^-) and phosphorus as dissolved reactive phosphorus (DRP) by the discrete analyser method. Likewise, samples were tested for the triazine herbicides suite. The triazine herbicide suit comprises of atrazine, hexazinone, metribuzin, prometrym, simazine, desisopropyl atrazine, and desetilpropyl atrazine. The triazine herbicides detection were performed by liquid chromatography. The limit for reporting of NOx is 0.01mg/L, the limit for reporting of DRP is 0.01mg/L and the limit for reporting of the triazine suit is 0.01µg/L.

4.4 Results

4.4.1 Sample size

As a result of implementing a GWVA, 47 bores were and 10 wetlands were identified across the GH CMA. The total monitoring bores and sites are presented in Table 2. Out of the 47 selected monitoring bores, 24 could not be accessed for various reasons, which included bores inside private land, bores with an internal diameter less than 60mm, and physically unavailable bores. As a result, only 23 bores were available for testing. Additionally, 10 wetlands were included in this survey, a summary of the field survey is presented in Table 4.2. Results are presented in two groups: the first one is for wetlands and the second one is for GW monitoring bores.

Table 4.2 Sample size, the GH CMA

Sample size and total monitoring bores
47 Total sites visited
24 Bores were inaccessible or with no water
17 Bores visited in the first field survey
6 Bores were sampled in a second field survey
10 Wetlands

4.4.2 Groundwater vulnerability assessment

The Pesticide DRASTICL that was used corresponds with the autumn season. Figure 4.2 shows, the areas with high vulnerability (red and orange) and areas with low vulnerability (blue and deep blue), whilst, medium vulnerability is represented in light green. Values of the GWV estimation ranged from 34.08 (low) to 168.74 (High) these estimations are dimensionless. It can be appreciated that the Grampians National Park and the east area of the region (between Casterton, Coleraine and Merino) have the lowest vulnerability values and that the most vulnerable areas are localized on the ocean side and the area between the Grampians, Dunkeld, Ararat, and Skipton). It is paramount to consider that this area is as well the most important area for cropping in the region.

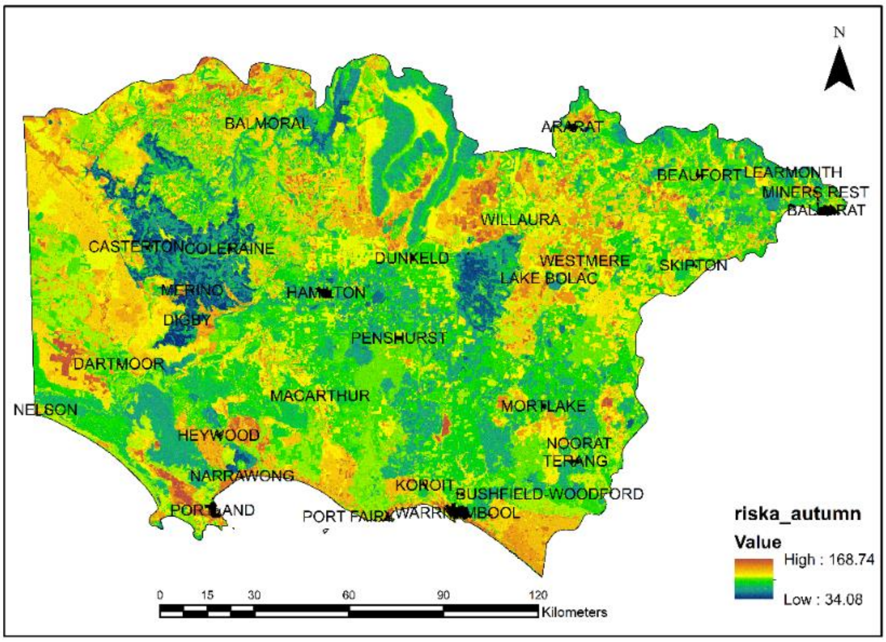

Figure 4.2 GW risk assessment

4.4.3 Groundwater monitoring bores and wetlands selection

As a result of data filtering of monitoring bores from SOBN, 351 active monitoring bores were found to be suitable for testing. These bores are plotted in Figure 4.3. By overlaying of the Pesticide DRASTIC with the SOBN monitoring bores, we could detect areas and corresponding monitoring bores that were prone to agricultural pollution. Figure 4.4 (left) shows the overlay of autumn GW vulnerability and monitoring bores. Figure 4.4 (right) shows a map of the GH CMA that contains both GW vulnerability for autumn and sites selection including wetlands. Wetlands selection was conducted using the GW vulnerability map together with the selection of the GW bores. As a result, 23 bores and 10 wetlands were selected across the GH CMA.

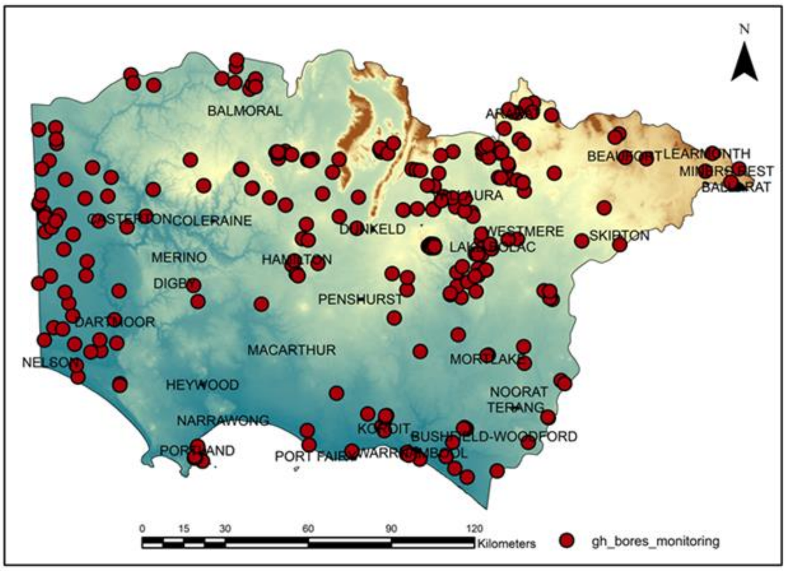

Figure 4.3 GW actively monitoring bores at GH CMA

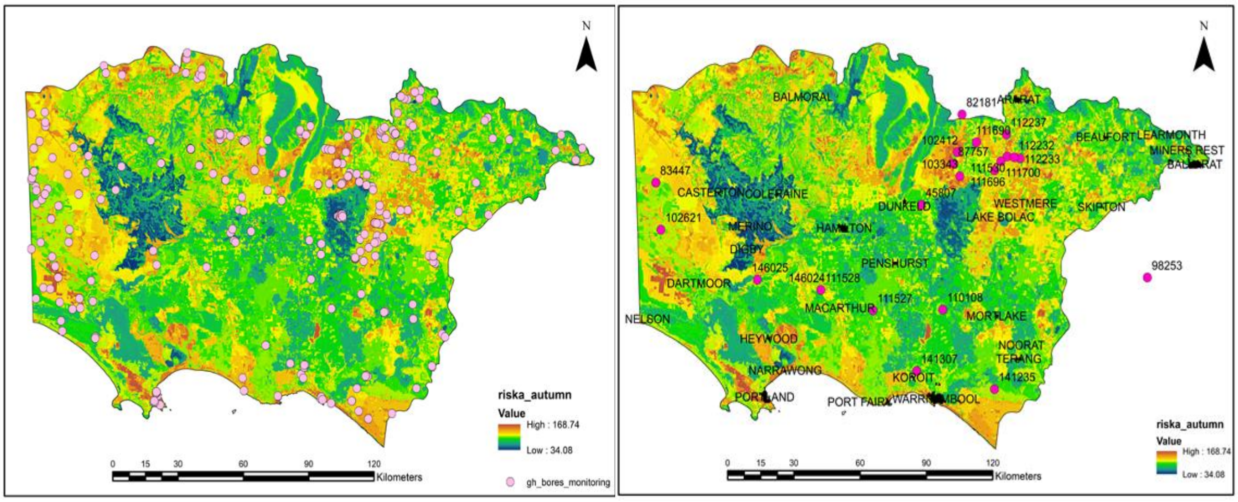

Figure 4.4 Monitoring bores overlay on the vulnerability Map 49(left). Selected GW bores and wetlands (right)

4.4.4 Groundwater nutrients results

Results for NOx and DRP are shown in Table 4.3. The results show the largest concentration for NOx in GW was at about 51.6 mg/L in the bore 112233 which is significantly above the Australian threshold values for nitrogen as NOx in freshwater at 0.04 mg/L. The maximum values for DRP were reported at about 0.30mg/L in the same bore. The value for DRP in GW for bore 112233 was above the Australian trigger values at 0.015mg/L. The trigger values were chosen as for the slightly disturbed ecosystems in low land river ecosystems as presented in Table 3 of the

ANZECC/ARMCANZ (2000) guidelines. Bore 98253 was located out of the study area and is localized in a confined aquifer. For this reason, it is not considered in the linear correlation. 65% of the bores showed the occurrence of NOx above threshold values of the Australian guidelines. 43.47% of the bores showed DRP pollution concentration above the threshold values.

Table 4.3 Results for NOx and DRP in GW bores

N	Bore ID	NOx	DRP	N	Bore ID	NOx	DRP
1	11170	6.54	0.02	12	111528	< 0.01	0.08
2	45807	0.52	0.04	13	111530	0.08	0.06
3	82181	< 0.01	< 0.01	14	111690	0.02	0.01
4	83447	0.12	< 0.01	15	111696	0.03	0.09
5	87757	0.03	< 0.01	16	112232	2.17	0.11
6	98253	0.25	0.01	17	112233A	51.6	0.30
7	102412	< 0.01	< 0.01	18	112237	0.06	0.03
8	102621	1.16	< 0.01	19	141235	0.01	< 0.01
9	103343	4.20	< 0.01	20	141307	4.37	0.07
10	110108	2.49	0.08	21	146024	< 0.01	< 0.01
11	111527	1.00	< 0.01	22	146025	< 0.01	< 0.01
				23	112233B	2.53	< 0.01

4.4.5 Groundwater triazines results

Results for herbicides in GW are reported in Table 4.4 It can be seen that from the 23 GW monitoring bores, only the bore 112233 showed the occurrence of pesticides. For this reason, the bore 112233 was sampled and tested again in a second field survey. The second sampling for the bore is treated as a separate sample. However, no pesticides were detected in the second sampling of the bore 112233. The limit of reporting for triazines was at about 0.1µg/L. The total triazines concentration found in this bore was 19.52µg/L in August 2017. However, in a second visit in April 2018 there were no traces of triazines as it can be seen in the following Table. In a subsequent laboratory test, evidence of exceeding amount of coal from the burning of agricultural paddocks at 800m from the GW monitoring bore was found. These burnings are used to clear agricultural lands from the remains of the last cropping plants. Coal in this shape acts as active coal and adsorbs pollution from nutrients and herbicides.

Table 4.4Triazines concentrations [µg/L]

N	Bore ID	Atrazine	Hexazinone	Metribuzin	Prometryn	Simazine	Desisopropyl-atrazine	Desethyl-atrazine
17	112233A	0.017	< 0.01	6.3	< 0.01	0.12	13	< 0.1
23	112233B	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

4.4.6 Wetlands nutrients results

The results for nutrients in wetlands are shown in Table 4.5. It can be seen that the concentrations for nutrients are in most cases below the threshold values compared to the wetlands water quality guidelines (ANZECC/ARMCANZ, 2000). The Victorian guidelines threshold values for inland lakes indicate values for NOx at 0.04 mg/L and values for DRP at 0.015mg/L (EPA, 2010). With just a few wetlands with values slightly above 0.015mg/L for DRP (Bull Rush, Lake Bookar, and Linlithgow), phosphorus concentrations were within the threshold values. The limit of detection for nutrients was at about 0.01mg/L. The nutrients concentration in wetlands should be treated carefully as all results represent a single sampling point within the littoral zone.

Table 4.5 Wetlands nutrients results [mg/L]

Wetland	NOx (mg/L)	DRP (mg/L)
Bull rush	< 0.01	0.16
Buninjon Lake	< 0.01	0.02
Cobden Terang Volcanic	< 0.01	< 0.01
Dergholm Park	< 0.01	< 0.01
Lake Bookar	0.04	0.47
Lake Kennedy	< 0.01	0.09
Linlithgow	< 0.01	0.22
Maroona Wetland	0.05	< 0.01
Rookies Hill	0.05	0.02
Yatmarone Wetland	0.02	< 0.01

4.4.7 Wetlands triazines results

Traces of atrazine and simazine were found on five wetlands. Atrazine was detected in Bull Rush, Lake Kennedy, and Dergholm Park at 0.049, 0.064 and 0.036µg/L respectively. Simazine was detected in Bull Rush, Lake Kennedy, Buninjon Lake and Lake Bookar at about 0.16, 1.2, 0.064 and 0.097µg/L respectively. Table 4.6 shows the occurrence of atrazine and simazine in some of the wetlands at the GH CMA. The highest concentration for atrazine was found in Lake Kennedy (0.064µg/L) and the maximum concentration for simazine was found in Lake Kennedy as well at 1.2µg/L. The threshold values for atrazine and simazine in freshwater ecosystems are 13.0µg/L and 3.2µg/L (ANZECC/ARMCANZ, 2000). All the recoded results were well below these thresholds. However, these results are to be treated carefully as these concentrations were taken in a single point sample within the littoral zone. Bore 112233 showed extremely high concentrations of total triazine herbicides. 30% of the wetlands showed atrazine pollution and 40% of the wetlands showed the occurrence of simazine.

Table 4.6 Triazine detection in wetlands at the GH CMA, Victoria, Aus. [µg/L]

Wetland	Atrazine	Hexazinone	Metribuzin	Prometryn	Simazine	Desisopropyl-atrazine	Desethyl-atrazine
Bull Rush	0.049	<0.01	<0.01	<0.01	0.16	<0.1	<0.1
Lake Kennedy	0.064	<0.01	<0.01	<0.01	1.2	<0.1	<0.1
Buninjon Lake	<0.01	<0.01	<0.01	<0.01	0.064	<0.1	<0.1
Cobden Terang	<0.01	<0.01	<0.01	<0.01	<0.01	<0.1	<0.1
Dergholm Park	0.036	<0.01	<0.01	<0.01	<0.01	<0.1	<0.1
Lake Bookar	<0.01	<0.01	<0.01	<0.01	0.097	<0.1	<0.1
Linlithgow	<0.01	<0.01	<0.01	<0.01	<0.01	<0.1	<0.1
Yatmarone	<0.01	<0.01	<0.01	<0.01	<0.01	<0.1	<0.1
Rookie Hills	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Maroona	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

4.4.8 Performance of the model

Generally, after the model for GWV (DRASTICL-based) are built, the next step should be model calibration. However, in this there was no seasonal data available for calibration, the development of the model is the core in most of the available research in this field. Therefore, it was not possible to perform a calibration of the model with nitrogen concentration values. It is fundamental to mention that the model predicts the risk or the vulnerability to be polluted from agricultural chemicals and does not predict pollution by itself. To make the most efficient use of the model, the user needs access to good quality nitrogen concentrations measurement data in GW on a seasonal basis and then to calibrate the model using this data. Ongoing data collection will lead to improved model performance. The most commonly used method to measure the model's performance is to derive a linear correlation between the scale of the vulnerability and observed nitrogen concentrations. Figure 4.5 shows that within all the possible values of vulnerability at different sites the correlation with the actual nitrogen concentrations in GW is promising, at 0.55 R^2. In this correlation, all the seasons are included. The effect on the seasonality of pollutants in GW is expected to have a direct impact on the correlation value and therefore observed data for each season should be used.

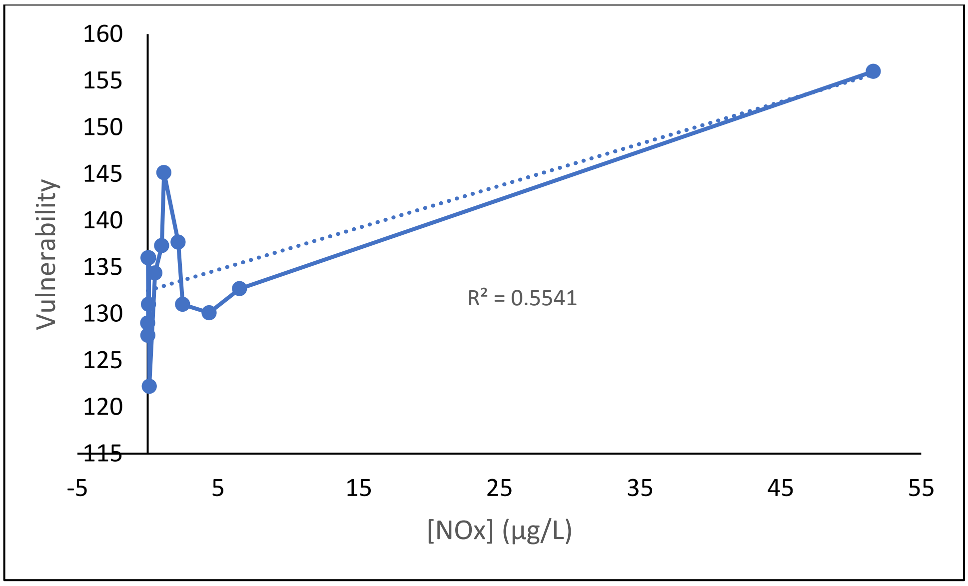

Figure 4.5 Linear correlation of the model (Nitrogen concentrations in X-axis and Vulnerability values in Y-axis)

4.5 Discussion and conclusions

Results found in this research suggest that GW is being affected by agricultural activity in the GH CMA region. 15 out of 23 (65%) bores showed the occurrence of NOx above threshold values of the Australian guidelines. For DRP ten out of 23 (43.47%), bores showed pollution concentration above the threshold values and Bore 112233 showed extremely high concentrations of total triazine herbicides. Atrazine, metribuzine, simazine and desethyl-atrazine showed occurrence in this bore. Bore 112233 represents the 4.3 percentile of the sampling size. However, its high concentrations of total triazines make it of significant importance as the dimension of the GW pollution is still unknown.

Analysis of the results for wetland aquatic ecosystems showed that nutrients do not represent an environmental concern and values for nitrogen as NOx and phosphorus as DRP were within the environmental guidelines for Victorian Lakes trigger values. However, wetlands could be affected by herbicides. Three out of ten (30%) wetlands showed atrazine pollution and four out of ten (40%) wetlands showed the occurrence of simazine. The sampling methodology performed in wetlands sampling could not be representative of the entire ecosystem and results should be used as a reference for future research. Sources of pesticides in wetlands need to be investigated further.

The use of a Seasonal Pesticide DRASTICL model proved to be a significant tool in GW pollution estimation of pesticides and nutrients. The changes of pesticides occurrences in GW in Bore 112233 showed that GW pollution is more complex than expected and that contamination is capable to reflect changes on land by farmers, this indicates that there is the need for further investigation to better comprehend GW pollution behaviour in agricultural areas.

Suggestions for future work include: (1) Use a pesticide DRASTICL model for each season; (2) deploy a GW field survey that includes triazines and nutrients; (3) implement the results for nitrogen in the model calibration for each seasonal GWVE.

Acknowledgements

This research was sponsored by Melbourne Water, Melbourne School of Engineering at The University of Melbourne, RMIT School of Science at The Royal Melbourne Institute of Technology and “El Consejo Nacional de Ciencia y Tecnologia CONACYT-Mexico” under the international postgraduate scholarship program.

Chapter 5

Expanding the horizon of green algal ecotoxicology to the groundwater environment

5.1 Abstract

Given the significance of groundwater (GW) in meeting various demands including domestic, industrial and agricultural water demand as well as feeding various GW dependent ecosystems, the need to monitor and assess GW quality at agricultural areas will play a key role in future human development. Algal ecotoxicity tests have been used thrivingly in marine, riverine, estuarine and lentic ecosystem waters however, GW toxicity in aquifers from agricultural areas has not been tested through algal bioassay techniques. In this study, we present a GW toxicity test using green algae (*Scenedesmus sp.*). Testing for contamination in GW is associated with complex laboratory techniques and use of sophisticated and expensive equipment and tests only certain selected chemicals. An ecotoxicity test can provide a cheap and faster alternative that is both capable to estimate toxicity (with confidence levels as the traditional laboratory tests) and are less technology-dependent. Results showed growth inhibition in control samples spiked at low atrazine concentrations at 0.1 mg/L. The green algae showed not to be tolerant to high salinity levels above (10,790 - 22,170µs/cm). Results suggest that the most significant effect on algae growth was due to low nutrient concentrations. Application of this toxicity tests to GW in agricultural regions could expand the area of ecotoxicology to GW ecotoxicology, defined as the area of knowledge that studies the toxic response of physical-chemical factors occurring in GW on living organisms.

5.2 Introduction

Nutrients and pesticides are impacting GW and freshwater ecosystems in Victoria, Australia (Cervantes-Servin et al., in prep). Algal toxicity tests are laboratory standard procedures that use algae as a test organism to conduct ecotoxicological screens of water to see if this organism is affected by contaminants such as nutrients, salinity, and herbicides. In such bioassays, different test organisms are more sensitive to some substances than others. Toxicity tests protocols are validated tools used and recognized globally (Gustavson et al., 2000) while producing quantitative estimations of toxicity with confidence levels comparable to chemical-physical standard methods (Coombe et al., 1999). Algal toxicity tests are sensitive to toxicants in the wide range (Geis et al., 2000) and can be applied in diverse environmental studies under different situations such as environmental management of chemical discharges in wastewaters and leachates (Nyholm and Källqvist, 1989). To our knowledge, algal toxicity tests have not been applied in the GW environment in agricultural areas when testing for pesticides and agrochemicals contamination. Despite the importance of GW to human development, laboratory tests applied to quantify contamination from agriculture are conditioned to the use of traditional methods, which are expensive and time-consuming resulting in scarce monitoring of GW for agricultural chemicals.

Previous research using green algal toxicity shows that this method can be applied to quantitatively measure the toxicity of pollutants in GW from fixed point sources of contamination, where previously GW has been tested with traditional methods such as high-performance liquid chromatography (HPLC), gas-liquid chromatography (GC) and mass spectrometry (MS) or similar (Naddy et al., 2011; Neuwoehner et al., 2009; Baun et al., 2000; Gustavson et al., 2000). The use of latter technology represents a burden in developing and undeveloped countries, such methods that target preselected compounds, are commonly slow, costly and not able to detect biologic responses (Gustavson et al., 2000). Standard guidelines for toxicity tests have not been developed to test for contaminants in GW (Avramov et al., 2013). There is a need for a method that is statically robust but uses less complex technology. The limitations of the standard analytical methods for scanning contaminants is that frequently they do not assess many other toxic chemicals in water samples (Gustavson et al., 2000; Thomas et al., 1999). Herbicides and nutrients were detected in the Glenelg Hopkins CMA GW in Victoria, Australia (Cervantes-Servin et al., in prep), however, in such study, not all herbicides were chemically tested. In this case, a toxicity test could assess the overall impact of all the herbicides.

GW is different from surface water regarding its water quality. Aquifers are GW bodies that share similar chemical and physical properties (pH, salinity, and temperature). In contrast with surface water, GW shows different values of the aforementioned parameters. Additionally, there is a difference from surface waters because of inherent aquifer conformation material and hydrogeological processes such as GW recharge, evapotranspiration, precipitation, GW gain or GW loss within the catchment. On the contrary, unless there is a large impact on the freshwater ecosystem, water quality parameters will remain similar for a river reach or a wetland system but will show seasonal changes.

Green algae toxicity tests can detect herbicides and herbicides mixture toxicity (Zhao et al., 2018) in a broad range of salinities, nitrogen and phosphorus concentrations. The confounding factors in freshwater such as organism toxicant exposure regime variability and environmental conditions (Castro et al., 2003; Moreira-Santos et al., 2002; Ringwood and Keppler, 2002; Chappie and Burton Jr, 2000; Culp et al., 2000; Sibley et al., 1999; Chappie and Burton Jr, 1997) could interfere between exposure and toxicity effects linkage (Chappie and Burton Jr, 2000; Culp et al., 2000). Confounding factors on freshwater systems are less significant in GW because in general GW shows long term chemical and physical stability. Thus, the application of a laboratory green algal bioassay could be a suitable methodology when assessing atrazine toxicity in GW.

We have chosen green algal species for ecotoxicity test, as they are easily available and easy to maintain under laboratory reproducible conditions (Nalewajko and Olaveson, 2018). Therefore, green algal tests are amongst the most used commonly used and recommended for freshwater toxicity testing, and its use has been endorsed by governments and agencies throughout published guidelines (Environment Canada, 2007; Weyers et al., 2000; Lewis et al., 1994a; Lewis, 1990). For example, these tests have been used to detect contamination from copper sulphate and chlormequat chloride pesticides (Radix et al., 2000). Additionally, it has shown to be versatile organisms used on *in situ* essays (Moreira-Santos et al., 2004; Cherry et al., 2001; Culp et al., 2000; Clements and Kiffney, 1994).

Physical, chemical and biological processes are interrelated in the freshwater aquatic environment and such processes are complex to replicate in the laboratory. The same chemical and physical processes take place in GW, however, they show less intensity, fewer changes and have lower reaction time making GW different from freshwater in terms of chemical, physical and biological composition and production. GW ecotoxicology is defined as the area of knowledge that studies the toxic response of physical-chemical factors occurring in GW on living organisms.

In a study of seven algal species including *Scenedesmus sp*. (*S. obliquus* and *S. quadricauda*), all of the species showed inhibitory effects to toxicity from personal care products (PCP's), disinfectants and antidepressant drugs (triclosan and fluoxetine) (Bi et al., 2018). In addition to pesticides, algal *Scenedesmus sp.* could be applied to detect a broader range of pollutants in GW studies. The few studies in GW ecotoxicology are divided into two groups: 1) stygofauna studies (Di LorenzoDi Marzio et al., 2019), and 2) hyporheic and freshwater organisms studies. For the first group, the underlying assumption is that environmental risk assessments should include the endemic or original fauna and that those organisms could better respond to a sudden change in the GW quality conditions (Reboleira et al., 2013). The chemicals tested using stygofauna are heavy metals (Cd^{2+}, CU^{2+}, Zn^{2+}, Pb^{2+}, Cr^{6+}, $NaASO_2$), pesticides (S-metholachlor, desethylatrazine, permethrin, fluoroxypyr, chlorpyrlid and MCPA, Imazamox), fungicide (Thiram) and KCL. For the second group *C. dubia*, *P. promelas*, *Ceriodaphnia dubia*, submitochondrial particle and Microtox ® bioassays have been used to test for pesticides (organochlorine, organophosphorus and carbamate insecticides) (Gustavson et al., 2000). *Desmodesmus subcapitatus* has been applied in GW to assess quinoline and hydroxylated derivatives (Neuwoehner et al., 2009).

Within the bioassay organisms, stygofauna appears to be less sensitive to pollutants than freshwater organisms (Reboleira et al., 2013; Gustavson et al., 2000). Green alga bioassay methods are more adaptable than stygofauna as the handling and culturing has been studied and applied with good results, while on stygofauna these burdens still represent a complex task, in both cases, there is limited research applied to GW ecotoxicity for GW samples from agricultural areas. GW ecotoxicity studies need the integration of hydrogeology that can describe the chemicals and physical condition of the aquifer before the bioassay test can be applied as it can add pivotal information that could explain better toxicity results.

This study aims to design an herbicide toxicity test for GW using green algal *Scenedesmus sp*., with the purpose to estimate the sensitivity of the species to a broad range of salinities, nutrients, and atrazine to determine whether such chemicals could affect growth rates. This will determine whether the salinity, nutrients or atrazine of a GW sample may affect the outcome of the toxicity test. In this study, we explore the development of a green algal *Scenedesmus sp*. toxicity test for GW in agricultural areas when testing for the toxicity of atrazine herbicides.

5.3 Methodology

5.3.1 Study site

This study was conducted using the samples collected from the Glenelg Hopkins Catchment Management Authority (CMA) region. This region is located in the south-west part of Victoria in Australia. This region is important because of its economic significance in agriculture for Victoria. This region has national (The Grampians National Park) and internationally important natural assets where some Ramsar wetlands sustain international species (GH CMA, 2013). Figure 5.1 shows the location of the GH CMA. For this study, four different field campaigns were deployed in this region. The black/hollow circles in Figure 5.1 indicate the bores and the sites that were used to sample the unconfined aquifers in this region.

5.3.2 Groundwater sampling, handling and storing.

Groundwater sampling was performed accordingly to the (Commonwealth, 2013; EPA, 2000a). For Test One 21 sites were sampled, for Test Two 8 sites were sampled, for Test Three 12 sites were sampled and Test Four includes a replica of four sites from Test Three. Samples were transported in an isolated container at 4°C and stored in dark glass bottles supplied by an accredited laboratory, samples were collected in clean 0.5L bottles for a total volume of 2L. For each groundwater field survey samples were filtered in the laboratory with a glass filter at 4.5µm.

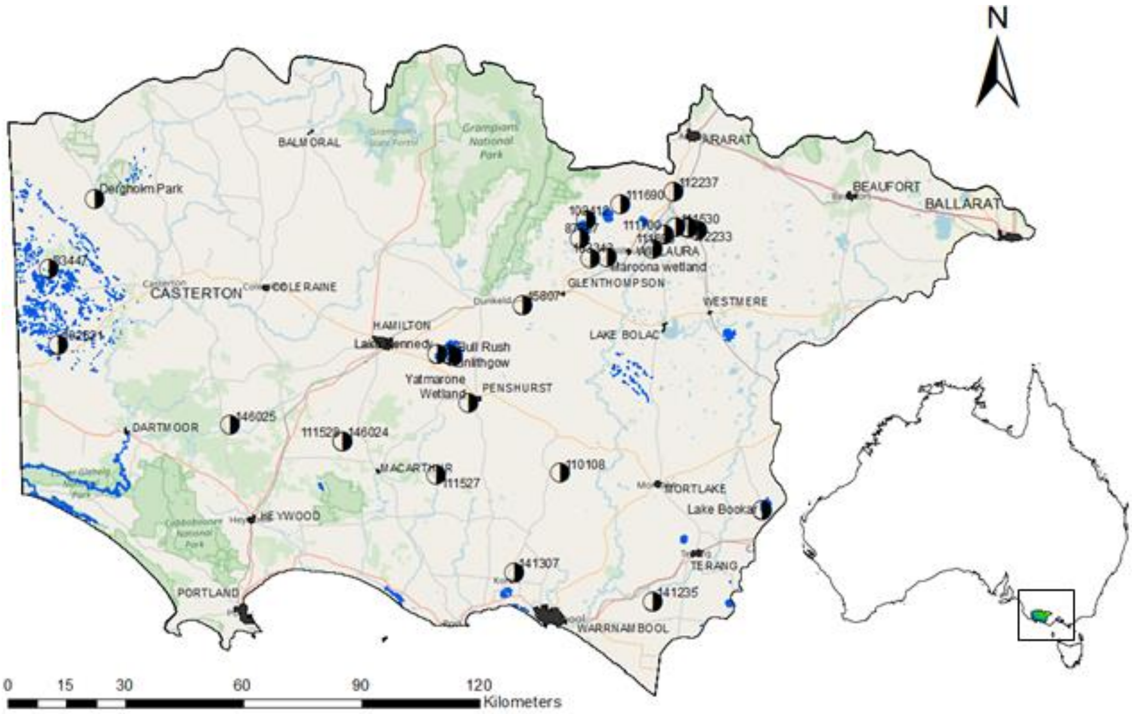

Figure 5.1 Location of the GH CMA region in Australia

5.3.3 The test organisms and culture conditions

The species used in this study was the *Scenedesmus sp.* kindly provided by the RMIT University, School of Science laboratories. Slants were used to prepare the inoculum and each 120h *Scenedesmus sp.*, a study was tested as per Lewis et al. (1994b). After cleaning glassware was sterilized before its use at 100°C for 12h. The media was prepared with Milli-Q® water. The inoculum was concentrated to an average of 7.566 x 10^6 cells mL^{-1} and used in the first hour before initiation of the test. The inoculum was aggregated to the test solution at 0.30mL inoculum to 30mL of the solution to target 75 x 10^4 cells/mL. The culture medium was prepared as per OECD (1984).

5.3.3.1 Conductivity, pH, nutrients and atrazine determination.

pH and electrical conductivity as (microsiemens/cm) were measured on-site with a YSI-Pro Sounder probe in a continuous slow flow regime. For the triazine family screening, samples were sent to an accredited laboratory as well as for nutrient determinations of nitrite plus nitrate as N (NOx) and dissolved reactive phosphorus as (DRP). Both determinations were made by discrete Analyser EPA-136, EPA-126.

Groundwater samples were sent to accredited laboratories to test for triazines. Herbicides included in this study were atrazine, hexazinone, metribuzin, prometryn, simazine, desisopropyl-atrazine, and desethyl-atrazine. Ecotoxicological testing

The proposed method estimates the sensitivity of the test species *Scenedesmus sp*. to a broad range of salinities to determine whether it could affect growth rates. This allows us to determine whether the salinity of a groundwater sample may affect the outcome of a toxicity test or to determine if the lack of nutrients or other toxicants are causing inhibitory effects on the algal growth.

This method includes an experimental design of two tests. Test one estimates algal growth inhibition effects of low nutrients concentration, this test is identified as nutrient uncalibrated. The second test includes a nutrient calibration step the concentration calibration levels is the one reported in the constitution of the growth media (MLA) for nitrogen and phosphorus respectively. Each test was replicated with different groundwater samples from different sites. For both tests, NOx and DRP are quantified before the starting of the test.

The modification of the standard algal growth inhibition test 201 OECD (2011) was based on the toxicity identification evaluation (TIE) methodology (Coombe et al., 1999). Incorporating the TIE approach in this study produced a test that is sensitive to triazine pollutants with no interference from salinity or nutrients limitations, both are standard tests and are not the focus of this research.

Test flasks were 50ml clear crystal bottles containing 30ml of the test solution. Six replicates were tested for each of the controls in all of the tests and three replicates were tested for each of the sites. Measurements were taken every 24 hours for each test where the biological response was growth (fluorescence) in a fluorometer.

5.3.4 Experimental design

The experimental design used in this study was developed to test if atrazine was causing toxicity in GW samples and to see the impacts of nutrients on *Scenedesmus sp.* In all samples MLA controls, salinity and atrazine were replicated with 6 samples, and sites samples were tested by triplicate. Table 5.1 shows the design of the proposed groundwater toxicity test.

Table 5.1 Test design

Test	Samples	Replicas	Nutrients calibrations (NOx and DRP)	Nutrients determination before and after
MLA	-----	6	-------	Yes
Salinity	-----	6	-------	Yes
Atrazine	-----	6	-------	Yes
1	24	3	No	Yes
2	8	3	No	Yes
3	12	3	Yes	Yes
4	4	3	Yes	Yes

5.3.5 Nutrient uncalibrated toxicity test

This test includes Test One and Test Two, both 120h tests were performed as a standard test for the green algal growth inhibition test 201 (OECD, 2011), no manipulation was performed for these tests (See Table 5.1).

Test One included an MLA control with six replicates, high salinity control (21,720µs/cm) with six replicates, atrazine control (0.1mg/L) with six replicates, and the rest of the sites by triplicate. 24 samples from the sites across the Glenelg Hopkins Region were included in this test.

Test Two included an MLA control with six replicates, high salinity control (22,300µs/cm) with six replicates, atrazine1 control (0.1mg/L), atrazine 2 control (0.5mg/L) and atrazine 3 control (1.0mg/L) each one with six replicates and eight sites with three replicates.

5.3.6 Nutrient calibrated toxicity test

Test Three and Four were performed including a nutrient calibration step. Previous modifications of the test are presented in (Norberg-King et al., 1991). This test manipulated the nutrient content as it is known that nitrogen and phosphorus levels in the water are significant factors influencing algal growth kinetics (Xin et al., 2010).

Microorganisms growth is also limited by the salinity content in the water sample (Dewhurst et al., 2005), normally this consideration has not been taken into account in recent studies of groundwater ecotoxicity where it is common that test is performed in only one type of aquifer with low salinity concentrations, with salinity EC values no more than 1,000µs/cm (Guimarães et al., 2019; Alvarenga et al., 2016; Naddy et al., 2011). This test is designed to withdraw the effect of low nutrient levels.

The calibration step was performed using the stoichiometric relationship of $NaNO_3$ and NK_2HPO_4 as the molecular weight percentage of $NO^{3—}$ and $PO^{3—}$. Nitrogen and phosphorus are measured at the beginning and at the end of the test for all of the treatments, we run the test for 5 days. After measuring the NOx and DRP of the samples, both NOx and DRP were calibrated in the laboratory targeting the MLA media nutrients concentration values (124.02mg/L and 18.98mg/L respectively). In the treatment for nitrogen and nitrogen + phosphorus, all samples were calibrated in the laboratory to target the MLA nutrient concentration. The phosphorus treatment and the nitrogen + phosphorus treatment were calibrated in the laboratory individually to target the MLA nutrients concentration.

Test three included an MLA control with six replicates, high salinity control (20,030µs/cm) with six replicates, atrazine control (0.1mg/L) with six replicates and 12 sites with three replicates per treatment.

Test Four included an MLA control with six replicates, high salinity1 control (22,230µs/cm) with six replicates, high salinity2 control (10,790µs/cm) with six replicates, atrazine control (0.1mg/L) with six replicates and four sites with three replicates per treatment.

5.4 Results

The test was performed in two major groups, nutrients uncalibrated test and nutrients calibrated test, results are presented in the same sequence as described in the methodology section as follows.

5.4.1 Test One and Two - Chemical laboratory analysis

The results for the nutrients uncalibrated test are showed in Table 5.2 and Table 5.3. Results show that the values for nutrients as NOx and phosphorus as Dissolved Reactive Phosphorus (DRP) are lower compared to the media inoculation values (MLA). Only six sites showed values for NOx above 1.0mg/L and none of them showed values above 0.5mg/L for DRP. Salinity values ranged from 240 to 19330µs/cm, and only three sites showed salinity values above 10,000 µs/cm. pH values were in all cases close to the value of seven. Bore 112233 showed values of NO_X at about 51.6mg/L and 0.3mg/L for DRP.

Table 5.2 Uncalibrated Test 1. Salinity and nutrient values

Site	pH	Salinity (µs/cm)	NOx (mg/L)	DRP (mg/L)
MLA.CTRL	7.13	535	124.02	18.97
Atzn.CTRL	7.41	129.1	124.02	18.97
Sal.CTRL	7.00	21720	124.02	18.97
b82181	7.29	240	0.01	0.01
b111696	7.53	9410	0.03	0.09
b111530	7.86	6520	0.08	0.06
b112233	7.6	874	51.6	0.30
b83447	8	1160	0.12	0.01
b102621	7.72	1738	1.16	0.01
b146025	7.44	707	0.01	0.01
b146024	7.43	1502	0.01	0.01
b110108	7.9	2800	2.49	0.08
b141235	7.65	743	0.01	0.01
b141307	7.35	824	4.37	0.07
b111527	7.55	174.2	1.00	0.01
b45807	7.49	7470	6.54	0.04
b102412	7.51	9600	0.01	0.01
b103343	7.53	13000	4.2	0.01
b98253	7.52	5380	0.25	0.01
Bull.Rush	8.81	1436	0.01	0.16
L. Kennedy	8.03	19330	0.01	0.09
Derg.Park	7.88	462	0.01	0.01
Linlithgow	7.87	1349	0.01	0.22
Yatmarone	7.66	540	0.02	0.01
L. Bookar	8.08	10380	0.04	0.47
C. Terang	7.76	2530	0.01	0.01
L. Bunninjon	7.41	8250	0.01	0.02

For the second test, salinity results showed values above 10,000 in four out of eight samples as seen in Table 5.3, with a maximum of 17690 µs/cm. Only three sites showed concentrations of NOx above 1.0mg/L and for DRP none of them showed values above 0.5mg/L. Results from Test 1 and Test 2 shows that values for nutrients are lower than the values from the MLA, all sites showed NOx concentrations below 7.0mg/L and DRP below 0.15mg/L. The maximum value for salinity was at 17520µs/cm and the lowest was at 7890µs/cm (see Table 5.3).

Table 5.3 Uncalibrated Test 2. Salinity and nutrient values

Site	pH	Salinity (µs/cm)	NOx (mg/L)	DRP (mg/L)
MLA.CTRL	7.07	451	124.02	18.98
Sal.CTRL	7.59	22300	124.02	18.98
Atrazine1	7.16	362	124.02	18.98
Atrazine2	6.9	355	124.02	18.98
Atrazine3	7.20	487	124.02	18.98
b111696	7.33	8070	0.03	0.09
b111700	7.27	11270	6.54	0.02
b112233	7.36	9570	2.53	0.00
b112232	7.59	10340	2.17	0.11
b112237	7.7	7820	0.06	0.03
b87757	7.41	17520	0.03	0.00
Maroona	7.41	17290	0.05	0.02
Rookies	7.44	7890	0.05	0.02

The results for the laboratory analysis are presented in Table 5.4, only bore b112233 showed herbicide occurrence in groundwater. Atrazine, metribuzine, simazine and desisopropyl atrazine were the herbicides detected where desisopropyl atrazine was the largest followed by metribuzine (see Table 1.3). For Test Two and there was no detection for the triazine herbicides.

Table 5.4 Triazine pesticides modified from Cervantes-Servin et al. (in prep)

Bore ID	Atrazine	Hexazinone	Metribuzin	Prometryn	Simazine	Desisopropyl-atrazine	Desethyl-atrazine
b112233A	0.017	< 0.01	6.3	< 0.01	0.12	13	< 0.1

5.4.2 Test One and Two - Uncalibrated algal growth results

The results for the 120 hours algal growth toxicity for Test One are shown in Figure 5.2. The green bar on the left corresponds to the MLA control sample, atrazine control value was 0.1mg/L and salinity control at 21720µs/cm. The line represents MLA control value, both atrazine and salinity controls showed growth inhibition. 12 sites out of 24 showed growth inhibition, in general, all samples contained low nutrient concentrations. For the sites that showed growth inhibition, the origin of the toxicity couldn’t be determined in standard laboratory analysis as the triazine family (atrazine, desisopropyl-atrazine, desethyl-atrazine, hexazinone, metribuzin, prometryn and simazine) was not detected in samples except for the bore b112233 that showed atrazine concentrations at 0.17µg/L, metribuzine at 6.30.17µg/L, simazine at 0.120.17µg/L and Desisopropyl-atrazine at 13.0µg/L. The growth estimation for this bore is slightly above the MLA control, the concentration of the nutrients for this bore was 51.6mg/L for NOx and 0.30mg/L for DRP, this bore showed the highest nutrients concentration from all the samples. All pH values were within the range of 6.9 to 8.0, there were no effects from this parameter on the algal inhibition test. Figure 5.3 shows the algal growth results for Test Two nutrients uncalibrated. It can be seen that the effects of low nutrients affected all the samples and inhibition occurred in all of the samples.

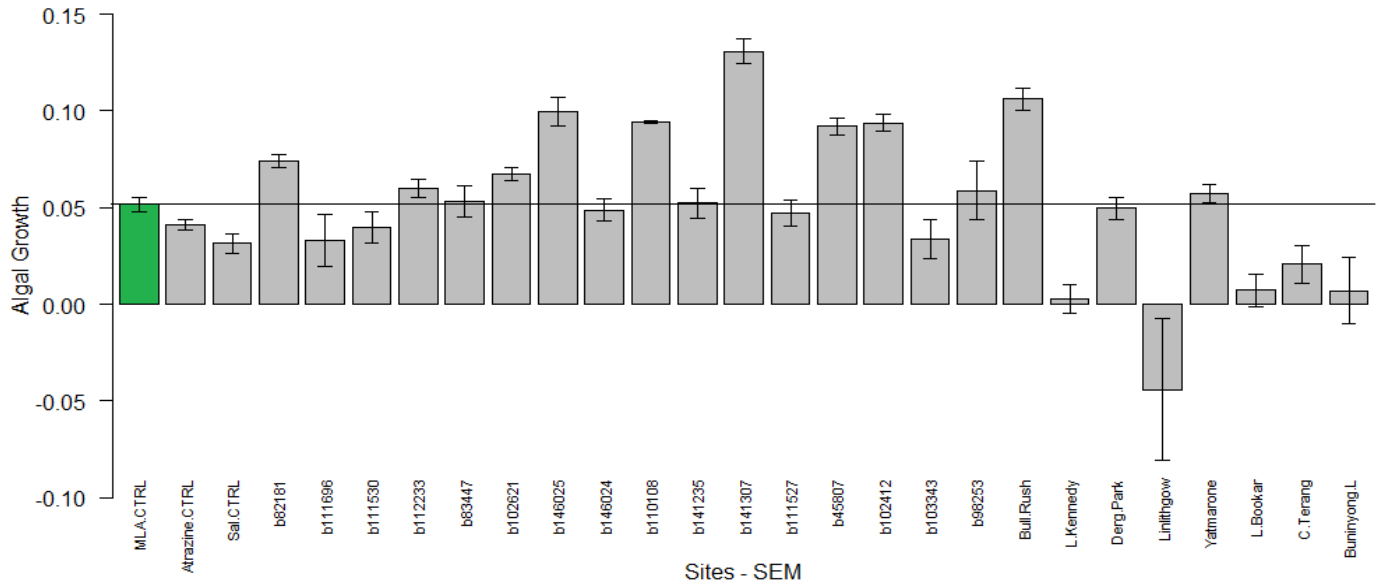

Figure 5.2 Test 1. Algal growth

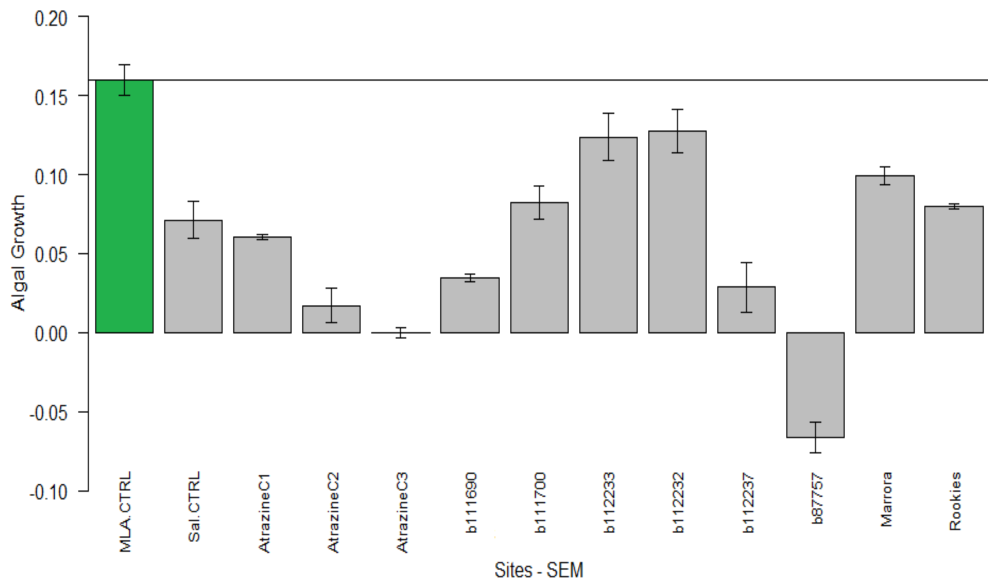

Figure 5.3 Test 2. Algal growth

5.4.3 Test Three and Four - Chemical laboratory analysis

The results of nutrients and salinity for the nutrient calibrated test are presented in Table 5.5. For test Three, it can be seen that according to the calibration method there is a calibration for NOx and another calibration for DRP for each of the treatments in the test (Plain water, nitrogen calibrated, phosphorus calibrated and nitrogen + phosphorus calibrated). Plain water does not include any addition of nutrients, nitrogen calibration treatment includes only the addition of nitrogen, phosphorus calibration only includes the addition of phosphorus and the compound treatment of nitrogen + phosphorus includes both nitrogen and phosphorus. In all cases, calibration concentrations were targeted to the MLA media concentration values NOx (123.86mg/L) and DRP (16.9mg/L). As determined in the preliminary laboratory tests all pH values were within the range of 6.9 to 7.9, it was observed that there were no effects from this parameter on the algal growth inhibition test.

Results show that except for salinity control, all the samples were calibrated to the MLA concentrations. For the sites EV1, EV2, SF1 and SF2 calibration step were not included to contrast both categories (calibrated vs uncalibrated) within the same algal growth inhibition test. Bores b87757 and Maroona site presented low values of NOx in the nitrogen calibrated treatment, this is assigned to a human error on the preparation of the test. Site Rookies presented low NOx concentration in the treatment for nitrogen + phosphorus. Nutrients uncalibrated category presented low concentration for both NOx and DRP. Table 5.6 shows the nutrients concentrations for Test Four (corroboration test). This table shows that all the sites have been calibrated for NOx and DRP.

Table 5.5 Calibrated Test 3. Salinity and nutrient values

		Calibration NOx(mg/L)				Calibration DRP(mg/L)			
Site	(µs/cm)	Plain W	N	P	N+P	Plain W	N	P	N+P
MLA.CTRL	391	123.86	123.86	123.86	123.86	16.90	16.90	16.90	16.90
Atzne.CTRL	400	127.79	127.79	127.79	127.79	17.35	17.35	17.35	17.35
Sal.CTRL	22,300	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
b111696	8,840	0.03	91.46	0.02	119.00	0.21	0.23	13.30	15.60
b111700	12,600	2.58	48.74	2.58	62.00	0.06	0.08	17.10	16.55
b112233	10,820	0.5	78.20	0.62	98.33	0.17	0.2	15.05	16.55
b112232	11,560	2.58	82.62	3.08	40.39	0.22	0.31	15.50	16.95
b112237	9,140	0.04	98.33	0.03	74.76	0.07	0.09	17.45	17.00
b87757	20,150	0.04	0.50	0.04	0.62	0.17	0.19	16.95	16.35
Maroona	18,410	0.05	1.11	0.06	1.11	0.06	0.03	16.25	15.75
R. Hills	8,300	0.05	39.90	0.06	3.07	0.08	0.05	17.00	17.50
EV1	11440	0.05	0.05	0.05	0.05	1.87	1.87	1.87	1.87
EV2	6330	0.04	0.04	0.04	0.04	0.60	0.60	0.60	0.60
SF1	9900	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01
SF2	3900	0.04	0.04	0.04	0.04	0.09	0.09	0.09	0.09

Table 5.6 Calibrated Test 4. Salinity and nutrient values

Site	(µs/cm)	Calibration NOx (mg/L)				Calibration DRP (mg/L)			
		Plain W	N	P	N+P	Plain W	N	P	N+P
MLA. CTRL	3,910	123.8	123.8	123.8	123.8	16.75	16.75	16.7	16.7
Atzne. CTRL	4,000	129.3	129.3	129.3	129.3	19.8	19.80	19.8	19.8
Sal. CTRL1	10,270	125.4	125.4	125.4	125.4	16.1	16.10	16.1	16.1
Sal. CTRL2	2,020	123.9	123.9	123.9	123.9	17.1	17.10	17.1	17.1
EV1	11,440	1.11	96.8	0.07	83.1	0.2	0.90	18.3	18.0
EV2	6,330	0.62	12.2	0	106.18	0.1	0.15	18.6	18.1
SF1	9,900	0	68.4	0	85.56	0.15	0.30	14.4	13.0
SF2	3,900	0	119.4	0	158.72	0.3	0.16	14.2	16.6

5.4.4 Test Three and 4 - Calibrated algal growth results

Test Three incorporates nutrients calibration and a set for uncalibrated sites (EV1, EV2, SF1 and SF2). Figure 5.5 shows that for these two groups, nutrients uncalibrated shows a strong inhibition rate compared to the calibrated category. These inhibition effects can be correlated to the low concentrations of the nutrients as seen in Table 5.5.

For the category of nutrients calibrated Test, four sites over eight showed effects of algal inhibition. At site b111700, salinity was 12600µs/cm with nutrient concentrations in the range of the calibration values. This site indicates that salinity above

10,000µs/cm starts to show inhibitory effects on the green alga growth. Site b112232 showed inhibition, values for salinity were at 11,560µs/cm with nutrient concentrations values within the range of calibration values. Site b887757 showed inhibition only in the treatment of nitrogen + phosphorus at a salinity of 20,150µs/cm nutrients concentrations for NOx were out of the calibration values below 0.7mg/L and for DRP within the calibration range. For the site R. Hills, inhibition effects can be observed at salinity 8300µs/cm, NOx showed to be out of the calibration range and phosphorus within the calibration values as seen in Table 1.4. In the sites where the nitrogen was out of calibration values for the treatments of nitrogen + phosphorus, the results suggest that nitrogen plays a key role in the algal growth inhibition.

Test Four was performed as a corroboration test. Figure 5.5 shows that only one sample (site SF2) showed inhibition effects even at values lower than 10,000µs/cm with nutrients concentrations within the range of calibration values. This test shows that the calibration step is a complex task and should be executed at the best laboratory practices. The origin of the toxicity was not determined for this site; however, this toxicity is not related to the lack of nutrients or high salinity.

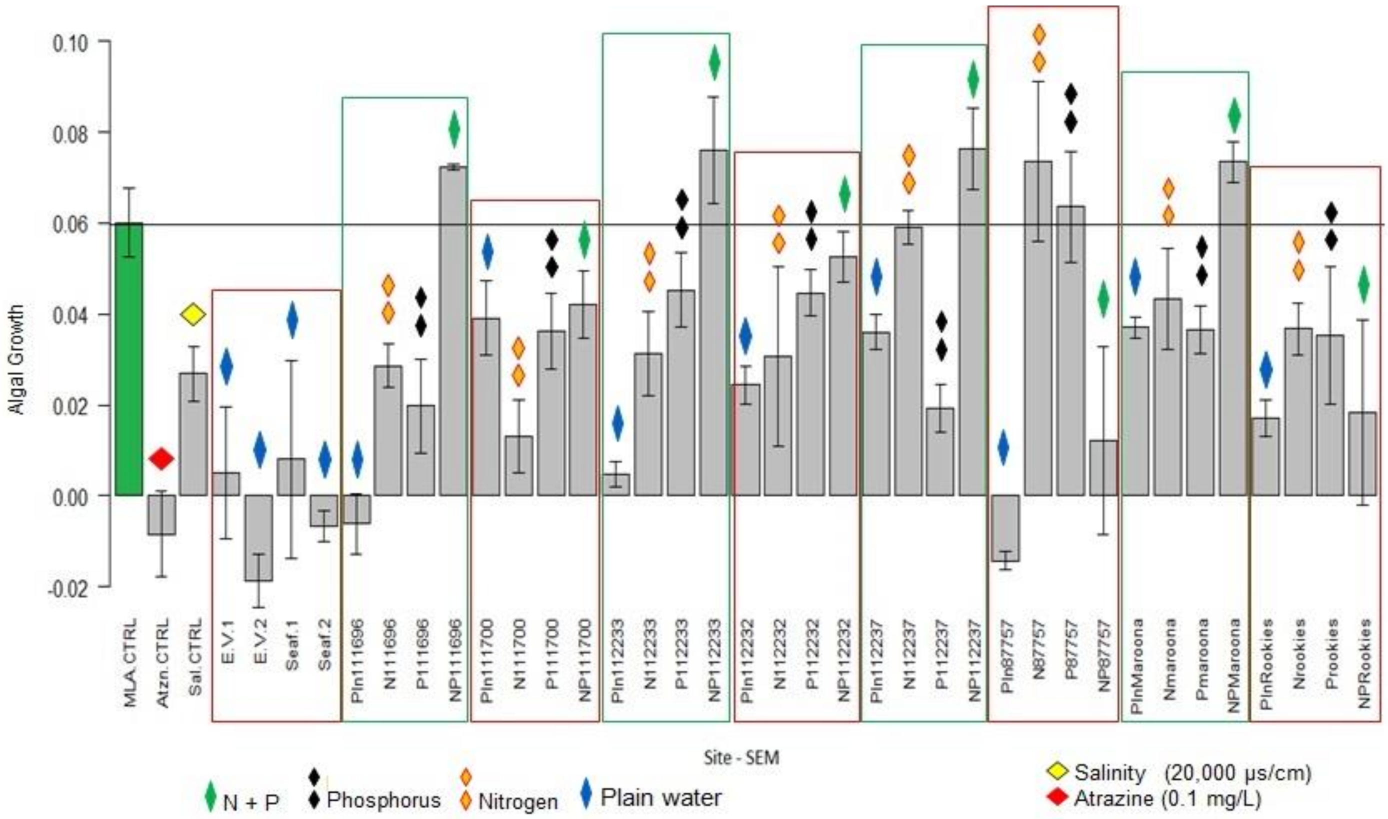

Figure 5.4 Test 3. Algal growth

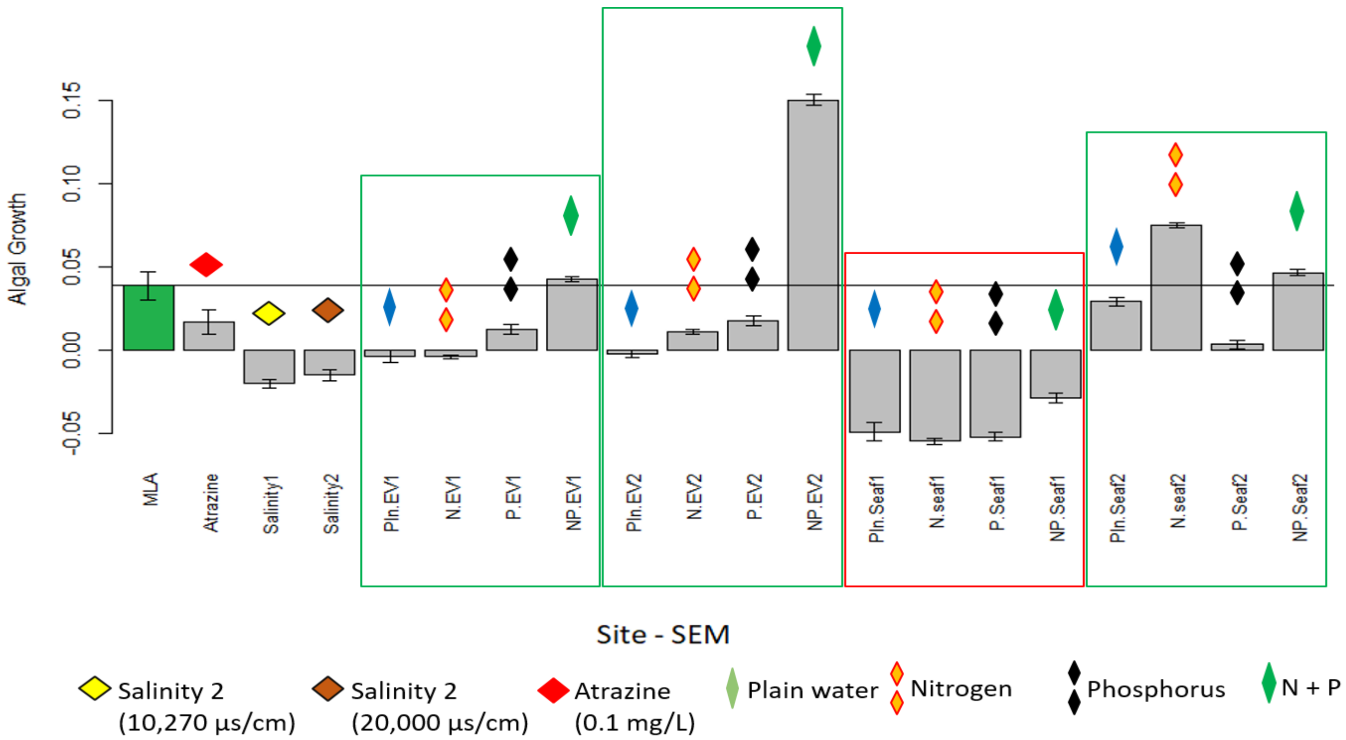

Figure 5.5 Test 4. Algal growth (Corroboration Test)

5.5 Discussion

In this study, four toxicity tests were performed for samples collected from different sites in the agricultural area of the GH CMA region. Those samples were collected at different times during the year 2018. Test one and test two correspond to the application of a standard algal growth inhibition toxicity test where results showed inhibition in all the samples with nutrients values close to zero. In Test Three, both categories (nutrient calibrated and uncalibrated) were included in the same test, it was possible to observe the direct effect of nutrients in the inhibition of the algal growth (see Figure 1.4). In Test Four, results showed that even though the calibration step was performed, the accuracy for the calibration when adding the calculated amount of nutrients not always derives the best results, the accuracy of the calibration should be taken in consideration in future studies to guarantee that the calibration is accurate to the target concentrations.

The initial concentration of nutrients (NOx and DRP) will have a direct effect on the algal growth and showed to be a significant factor with direct impact on algal growth kinetics as also seen in Xin et al. (2010).

This study corroborated the study reported in (Dewhurst et al., 2005) that indicates that high salinity measured as Electric Conductivity (EC) of the samples has a direct effect on the test sensitivity and that this effect could be masking other pollutants toxicity to the green algal. However, the test was able to detect toxicity due to other toxicants under salinity levels bellow 10,000µs/cm. These findings suggest that it is possible to conduct toxicity tests using *Scenedesmus sp.* if the conductivity of the water is suitable for this species.

5.6 Conclusions

Results suggest that in the absence of nutrients, salinity could be the growth inhibitor factor above 15,000µs/cm. Within the nutrients uncalibrated Test One and Test Two, Test One showed that the lesser the nutrients content in the sample higher was the effects of toxicity (less nutrients the higher the inhibitory effect). The nutrients calibration step is fundamental to eliminate the inhibition effect due to the lack of nutrients in the test. Comparing the nutrients uncalibrated test to the nutrients calibrated results showed that after calibration the algal growth values increased and that in the cases where the test showed inhibition, further laboratory analysis is required, this will allow the researchers to focalize resources on the samples that are effectively contaminated from agricultural pollutants. Test Four was deployed to corroborate the toxicity test proposed in this study, results indicate that in the bore where the inhibition was higher there was an unidentified pollutant in the sample. This test represents an alternative to chemical laboratory analysis that can be applied in groundwater pollution prevention strategies and groundwater monitoring programs.

Chapter 6

Discussion and conclusion

6.1 Introduction

This thesis was motivated by the need for a groundwater vulnerability assessment model that can account for climatic variations and offers a time-variant estimation of the vulnerability. DRASTIC-based groundwater vulnerability estimation that are well recognized and used worldwide for groundwater vulnerability assessment (Machiwal et al., 2018), but they only provide annual estimates of vulnerability, without due regard to the seasonal changes and its impact on the GW quality. The lack of this capability to incorporate time variance has been identified as the major limitation in existing models. Another major limitation identified in the literature is the time consuming and costly analysis of the collected groundwater samples that has led to almost non-existent data on the groundwater quality, especially agricultural contamination. Therefore, this thesis attempted to develop a fast, inexpensive and accessible laboratory test based on algal toxicity to the water as a preliminary screen before more expensive analytical pesticide tests are conducted.

This thesis presented the research outcomes of both the development of a time-variant groundwater vulnerability estimation model (DRASTIC-based) and exploring the performance of an algal ecotoxicity test using *Scenedesmus sp*. to detect atrazine and other herbicide contamination in groundwater. This thesis demonstrated the application of both the aforementioned methodologies in the Glenelg Hopkins Catchment Management Authority region.

6.2 Summary

This Chapter summarises the major results from the three thesis components in line with the three research questions outlined in Chapter 1: 1) the development of a time-variant DRASTICL model for groundwater vulnerability estimation, 2) A field survey and quantification of triazines and nutrients pollution in the groundwater and wetlands in the Glenelg Hopkins CMA region, and 3) the development of a green algal (*Scenedesmus Sp.*) toxicity test as a pre-screen for all herbicides that would affect this specie. This test Conclusions from each research question are mentioned followed by its contribution to the scientific knowledge and further research needed. The findings for each stage were presented as three peer-reviewed journal papers (see Chapters Three, Four, and Five). Each Chapter addresses the research objectives as seen in the Thesis Introduction (Chapter One).

In Chapter Three, a time-variant model was developed by separating the variables comprising the DRASTICL model (Groundwater depth, groundwater net recharge, aquifer media, soil type, slope topography, impact of vadose zone, hydraulic conductivity and land use). Analysis of factor's conformation and variable outcomes (units of measurement or description of the aquifer media) provided a broader scope to estimate vulnerability in aquifers. Identification of variables that are descriptive of the aquifer's physical condition and variables that provide an actual measure of the aquifer response to climatic changes were central to differentiate hydrogeological groups. Hydrogeological factor groups were identified as dynamic (variables that change seasonally due to the influence of climatic conditions) and static (fixed variables).

Standard DRASTIC-based models are based under the assumption that all factors participate equally within a resulting vulnerability index, therefore, such models consider factors to be as equally constituted and assign an importance value over the others regarding on the degree of participation for pollution transport to the aquifer (Aller et al., 1987; Aller et al., 1985). However, factors are not equally constituted, a group of factors is a measurement unit of a response from the aquifer's conditions to climatic changes. For example, groundwater depth reflects the extent of changes of the aquifer after a precipitation event, whilst groundwater recharge is the measure of the quantity of water that the aquifer has gained either from precipitation, water intrusion from other aquifer or water gain from an adjacent surface water system as rivers or lakes. Groundwater depth is measured as the water table elevation from the Australian Head Datum (AHD) its units are in meters, as for the groundwater recharge factor it is measured as the amount of water gained by the aquifer, its units are measured in (mm) of water. Therefore, these 2 are dynamic variables and this variability needs to be accounted for while determining the GW vulnerability to pollution.

Rest of the variables are considered as static variables, as they do not change with time. Aquifer media corresponds to the aquifer characteristic as described in Section 3.1. This is a qualitative factor that represents the type of aquifer. The selected study site GH CMA has 13 different types of aquifers including aquitards and the base rock (See Chapter 3, Section One). This study also includes a diverse compilation of different soils types (See Chapter Three). The Slope topography is another variable that does not experience significant changes through time in a short scale, so this factor was considered a descriptive variable as well. The factor Impact to the vadose zone is another descriptive factor that depicts the potential for a pollutant to reach the water table level once it has crossed the soil horizon (See Chapter Three). Finally, within the static group of factors, the hydraulic conductivity factor is a description of the ease for the aquifer to drive water to the downgradient of the aquifer, however, this factor has been reported to be the most complex factor to extract a precise estimation. Its units are in m/day. However, this a measurement of the aquifer characteristic, it is mostly only a good approximation. In this thesis, it is reported as a range of values over different aquifer types.

The hydrogeological factors were separated into dynamic factors and static factors. The dynamic group was observed to be consisting of variables that were the time-variant measurement of the aquifers' conditions (D and R). The static group was observed to be the rest of the factors A, S, T, I, C and L. To extract the more realistic factor weights (called vector weights) the model used a double Correspondence Analysis (CA) statistical method for each of the hydrogeological group. For calculating the CA a set of 1,300 data points were extracted for each of the seven factors (DRASTICL). The time-variant model was applied to the study area and the results were used in Chapter Four to identify the groundwater bores more prone to pollution from agricultural activities.

Chapter 4 addressed Research Question Two, which focuses on quantifying the concentrations of triazines, nitrogen and phosphorus in groundwater to assess the potential of agricultural chemicals to contaminate GW. For this purpose, three groundwater field surveys were carried out during the year 2018. The triazine herbicides tested in this thesis included atrazine, hexazinone, metribuzine, prometryn, simazine, desisopropyl-atrazine and desethyl-atrazine. The nutrients concentrations were estimated as NOx for nitrogen and Dissolved Reactive Phosphorus (DRP) for phosphorus. The selection of the sites was performed using the system “Visualizing Victoria’s groundwater” available at https://www.vvg.org.au/ which offers the location, description, tag (serial number for the bore), groundwater levels, chemical analysis records and the active monitoring bores, such bores were of interest for this thesis. The selection of the final sample was made by using both the Victorian groundwater system and the time-variant Pesticide DRASTICL model. This thesis included samples from wetlands as well that were localized within high vulnerability areas identified by the model.

This Chapter also studied the relationship between the groundwater vulnerability scores from the time-variant DRASTICL model and the concentrations of herbicides and nutrients detected in the groundwater sampled from the field surveys. The results from this research question indicate that the contamination of aquifers is happening at the agricultural area between Ararat, The Grampians National park, Hamilton, Mortlake, and Ballarat, that is, that agricultural activities could be impacting aquifers at low rates.

The third results Chapter (Chapter Five) was dedicated to the development of an ecotoxicity test that could be applied as pre-screen to reduce the number of samples requiring the expensive analytical tests to detect atrazine (which is part of the triazine herbicide family) in groundwater samples. Only the samples showing toxicity need a full analytical scan. The use of organisms to estimate the toxicity of freshwater has been addressed by several authors, but has not been tested in GW (Guimarães et al., 2019; Zhao et al., 2018; Dewhurst et al., 2005) (See Chapter Five). In this thesis, the test was designed to account for the differences between freshwater and groundwater physico-chemical conditions. This Chapter is intended to test a proposed green algal toxicity test using *Scenedesmus sp*. This test was based on the toxicity identification evaluation (TIE) method, which is used universally to identify the pollutant in the water sample that is causing toxicity. To test this method, toxicity tests were carried out on controls, collected wetlands and groundwater samples and as well groundwater with nutrients to avoid toxicity effects due to lack of nutrients as it was observed in other studies that the alga is sensitive to the initial concentration values (Xin et al., 2010). Results suggested that algal test can be successfully applied to groundwater samples to estimate toxicity as an indicator of potential atrazine and triazine herbicide pollution.

6.3 Research contributions

This thesis has developed a time-variant groundwater vulnerability assessment model that has been used to identify the potentially contaminated groundwater bores in the agricultural areas within GH CMA region. Also, it offers an accessible, quick and cost-effective method to test for groundwater toxicity from atrazine and other contaminants, that can be applied as a pre-screen to test the water quality condition of aquifers. Both of these innovative steps coupled together provide an accessible, robust, and flexible method for use in groundwater pollution prevention strategies. Major scientific contributions from this thesis are as follows:

❖ Construction of a time-variant Pesticide DRASTICL model for assessing groundwater vulnerability in the Australian unconfined aquifers.
  - Development of the range of rating values for the Victorian groundwater depths (D).
  - Development of the rating values for the aquifer media (A) that is present in the Victorian groundwater framework and for the soil types for the Australian top-layer soil system.
  - Development of the rating values for the Impact to the vadose zone (I) factor that includes different aquifer conformation materials from the Victorian groundwater framework.
  - Development of the range rating values for the hydraulic conductivity (C) for the aquifer in the Victorian groundwater framework.

- Development for the rating values for the land use (L) factor that incorporates different land uses in the Victorian Land use Information System.
- Development of a statistical method by using a Correspondence Analysis to estimate time-variant DRASTICL weighting factors.
- This thesis redefined the existing estimation of GWV by including separation of two hydrogeological factor groups (the Dynamic and the Static) and by incorporating a double correspondence analysis for each of the seasons. For instance, the results from this study show that the time-variant model was able to estimate the GWV for each season at the GH CMA, the model was capable to detect changes within the seasons being the biggest resulting scale for the rainy (winter) season and the lower for the dry (summer) season
- The study estimated the GWV in an economically important are for Victoria and unlike existing research, the extension of the study is (140 x 140 km) overcomes previously published research as this area is the biggest area studied that has been reported in the literature.

The results from the field survey show that the model can accurately predict the vulnerability of groundwater. The model can also account for the seasonal variability of the GW vulnerability for the pollution and thus provides a useful guide to select the best timing for fertiliser and pesticide application in conjunction with weather forecasts.

❖ With respect to the development of ecotoxicity test for groundwater presented in this thesis, significant original contributions have been made, including:
  - Development of a green algal groundwater toxicity test using the *Scenedesmus sp.*
  - Development of a modification of a TIE to overcome the nutrients deficiency growth effects in the groundwater samples.
  - This thesis expanded the application of green algal species to the groundwater environment by using a modified method “Freshwater growth inhibition test- 201” (OECD, 2011) which is an international guideline for the testing of chemical and water quality around the world.

Advances in published research

This thesis has advanced the contributions of previous research in both areas the GWVA based on the DRASTICL models and in the GW ecotoxicology. in GWVA methods researchers have tried to incorporate seasonal changes in the estimation of GWV (Saha and Alam, 2014), such models around the world have not changed their approach since 1985 when Aller et al. (1985) presented their method and the subsequent research around the world as shown by (Hamza et al., 2015). The major advance from published research in DRASTIC-based models has been to address the subjectivity problem of the model by incorporating various techniques to quantify the new factor weights (the relative importance of a factor over the others). The need for incorporating seasonal changes in the estimation of GWV has been emphasized in the latest literature review as seen in (Machiwal et al., 2018), however, to the author's knowledge there are no publications proposing a time-variant approach in estimating the GWV.

In the are of GW ecotoxicology, this thesis has advanced the contribution of previous research that has applied ecotoxicological tests to quantify pollution in groundwater where most of the published work has been focused in using stygofauna species as reported in Di LorenzoCastaño-Sánchez et al. (2019) where currently such methods have developed standard guidelines as mentioned in "Guidelines on laboratory practices with subterranean fauna". However, green algal studies are on its infant stage (Guimarães et al., 2019). Unfortunately, there is not enough work published for groundwater quality assessments using green algal toxicity tests. There are some examples of the use of green algal toxicity tests applied to detect fixed point industrial pollution (quinoline) in groundwater (Neuwoehner et al., 2009), organic chemical pollution from a landfill (Baun et al., 2000) and pesticides pollution from a pesticide pollution facility (organochlorine, organophosphorus and carbamate) using bioassay technique (Gustavson et al., 2000), however, to the knowledge of the author, there is no research published using green alga growth inhibition methods applied to assess groundwater quality in agricultural areas with extensive use of agrochemicals.

6.4 Limitations and assumptions

Though this research has made significant contributions to the existing knowledge, there are some limitations of the research as established in each Chapter under the discussion sections. The following points offer a general review of the limitations which should be considered while using the results presented in this thesis:

- ❖ Geographic limitations: This study was limited by the regional boundaries of the Glenelg Hopkins CMA, the application of the model may not be suitable for other regions and continents. The model has been specifically designed to include most of the range ratings for the factors that best represent the Victorian Aquifer at the CMA region. The use of the model in even within Australia might require site-specific information, though the model is flexible to incorporate the local information to be applicable for any CMA in the Australian continent.

- ❖ Model limitations: The model is restricted to the Victorian Groundwater Framework. It could not be applied to other places within Australia that may have different aquifer configuration. However, the procedure will be similar, results may vary if applied using the same values for factor ranges and factor weightings.

- ❖ Data limitation regarding the factors of the model: This model depends heavily on the first explicative factors (D) and (R) and (S) as seen in (Chapter Three). In cases where the estimation of (R) factor does not originate from a transient groundwater recharge model, it is advisable to obtain the recharge values from the deep drainage derived from the Australian Landscape Water Balance (AWRA model) at http://www.bom.gov.au/water/landscape

- ❖ Green algal toxicity limitations: The green algal test is limited by the salinity, as seen in the discussion section of Chapter Five for values above 10,000µs/cm. The inhibition could be masked by the high salinity content in the groundwater samples. Another limitation of the test is that it could not differentiate toxicity from high levels of nutrients (NOx and DRP).

6.5 Conclusions

DRASTIC-based models have been criticised in the literature because of its inability to account for climatic variations and thus reflect seasonal changes in the estimation of the vulnerability. Some researchers tried to force the model by creating independent models for different seasons, e.g. one DRASTIC model for the monsoon and one DRASTIC model for the post-monsoon season while creating different ranges for both seasons. However, such studies failed to incorporate the time series data to create independent variables for groundwater depth and groundwater recharge that could reflect temporal changes and that could account for climatic changes with spatial variations within a catchment.

Therefore, this thesis has developed a time-variant DRASTICL model as described in Chapter Three, Section 3, which can account for the seasonal variability of the GW vulnerability for the pollution and thus provides a useful guide to select the best timing for fertilisers and pesticide’s application in conjunction with the weather forecast.

The fieldwork performed to test the aquifers for triazines and nutrients showed that groundwater pollution is a threat in the Victorian agricultural area and that herbicides and nutrients could be detected in ground and surface waters in the agricultural area of the Glenelg Hopkins CMA region, and that groundwater pollution showed to be time variable within 12 month time period.

Regarding the groundwater toxicity test, this research for the first time created a definition and a framework for groundwater ecotoxicology (See Chapter Four). While doing this, the differences from freshwater and groundwater were established creating a common language where researches can communicate their findings. Additionally, a novel green algal toxicity test for groundwater was proposed and tested. As a result of the original contributions of this part of the research, the tests for groundwater toxicity could be improved. While it was a common practice to ignore the salinity sensitivity of the green algal and the nutrients calibration step in most of the previous studies

Finally, this thesis provides significant insights for improved groundwater monitoring and groundwater pollution prevention programs by offering an alternative to test aquifers for herbicides pollution with an efficient and accessible coupled methodology.

6.6 Future research

Studies in time-variant groundwater vulnerability estimation that accounts for climate variability are scarce, the same as for studies in groundwater ecotoxicology. As mentioned in Section 6.4, there are some limitations in this study and there is a huge opportunity to address some of these limitations through further research:

- Model application to other agricultural regions within Australia and/or elsewhere.

  - Understand the effect of extreme values of groundwater depth and groundwater recharge on the temporal estimation of the groundwater vulnerability assessment.

  - Understand the limitations for regions with data scarcity of depths and net recharge and for other factors that may not be available for use.

- Construction of a methodology for model calibration

  - Model calibration remains a challenge in the absence of long-term data for a reasonable sample size. Future work should attempt to develop new techniques that could calibrate the novel time-variant approach of DRASTICL-based models presented in this thesis.

  - Apply and compare the best three calibration methods against the original time-variant mode, including the error estimation for each of the calibration methodologies comparing the calibrated model against data for NOx and DRP from the BoM (Groundwater section).

- Salinity and atrazine mixed effects on algal growth inhibition

  - Test the groundwater green algal toxicity for mixed effects of salinity, nutrients and triazines toxicity, this will estimate the salinity limits for the test and could propose a new method to integrate to the test to make the toxicity test more robust and flexible.

- Triazines individual and mixed effects on algal growth inhibition

- Test the individual and mixed effects of the triazines on the algal inhibition to identify the highest toxicity of the triazine family members.

- ❖ Green algal test standardization and guidelines for groundwater ecotoxicology

  - Create a framework and guidelines for groundwater ecotoxicity tests for green algal organisms to standardize the test to make the test more reliable and robust and accessible as a regulatory tool in groundwater pollution prevention programs and strategies.

References

AG. (1999). Environment protection and biodiversity conservation act 1999. Canberra: Australian Government

Agriculture Victoria. (2018a). A land resource assessment of the Glenelg-Hopkins region.

Agriculture Victoria. (2018b). Victorian land use information system (VLUIS). Retrieved from http://vro.agriculture.vic.gov.au/dpi/vro/vrosite.nsf/pages/vluis

Ahmed, I., Nazzal, Y., Zaidi, F. K., Al-Arifi, N. S. N., Ghrefat, H., & Naeem, M. (2015). Hydrogeological vulnerability and pollution risk mapping of the Saq and overlying aquifers using the DRASTIC model and GIS techniques, NW Saudi Arabia. *Environmental Earth Sciences, 74*(2), 1303-1318. doi:10.1007/s12665-015-4120-5.

Al-Abadi, A. M., Al-Shamma'a, A. M., & Aljabbari, M. H. (2017). A GIS-based DRASTIC model for assessing intrinsic groundwater vulnerability in northeastern Missan governorate, southern Iraq. *Applied Water Science, 7*(1), 89-101. doi:10.1007/s13201-014-0221-7.

Alam, F., Umar, R., Ahmed, S., & Dar, F. A. (2014). A new model (DRASTIC-LU) for evaluating groundwater vulnerability in parts of central Ganga Plain, India. *Arabian Journal of Geosciences, 7*(3), 927-937. doi:10.1007/s12517-012-0796-y.

Alister, C. A., Gomez, P. A., Rojas, S., & Kogan, M. (2009). Pendimethalin and oxyfluorfen degradation under two irrigation conditions over four years application. *Journal of Environmental Science and Health, Part B, 44*(4), 337-343. doi:10.1080/03601230902800986.

Aller, L., Bennett, T., Lehr, J. H., Petty, R. J., & Hackett, G. (1987). DRASTIC: A standardized system for evaluating groundwater pollution potential using hydrogeological settings. US Environmental Protection Agency. Oklahoma.

Aller, L., Lehr, J. H., Petty, R., & Bennett, T. (1985). DRASTIC: A standardized system to evaluate groundwater pollution potential using hydrogeologic setting. *J Geol Soc India, 29*(1), 23-37.

Allouche, N., Maanan, M., Gontara, M., Rollo, N., Jmal, I., & Bouri, S. (2017). A global risk approach to assessing groundwater vulnerability. *Environmental Modelling & Software, 88*, 168-182.

Alvarenga, P., Mourinha, C., Farto, M., Palma, P., Sengo, J., Morais, M.-C., & Cunha Queda, C. (2016). Ecotoxicological assessment of the potential impact on soil porewater, surface and groundwater from the use of organic wastes as soil amendments. *Ecotoxicology and environmental safety, 126*, 102-110.

Anane, M., Abidi, B., Lachaal, F., Limam, A., & Jellali, S. (2013). GIS-based DRASTIC, Pesticide DRASTIC and the susceptibility index (SI): Comparative study for evaluation of pollution potential in the Nabeul-Hammamet shallow aquifer, Tunisia. *Hydrogeology Journal, 21*(3), 715-731. doi:10.1007/s10040-013-0952-9.

Angeliki, A., Philip, J. B., Hans-Jorgen, A., & Poul, l. B. (2015). Modeling factors impacting pesticide concentrations in groundwater. *National Groundwater Asociation, 53*(Denmark), 722-736.

Australian and New Zealand guidelines for fresh and marine water quality, (2000).

APVMA. (2008). Atrazine final review report and regulatory decision. Australian Pesticides and Veterinary Medicines Asociation , Symonston, ACT. Australia.

Aschonitis, V. G., Castaldelli, G., Colombani, N., & Mastrocicco, M. (2016). A combined methodology to assess the intrinsic vulnerability of aquifers to pollution from agrochemicals. *Arabian Journal of Geosciences, 9*(7), 503. doi:10.1007/s12517-016-2527-2.

Askarimarnani, S., & Willgoose, G. (2014). Using the DRASTIC model for determination of groundwater vulnerability in shallow aquifer in broke, NSW, Australia.

ASRIS. (1968). Digital atlas of Australian soils. Retrieved from http://www.asris.csiro.au/themes/Atlas.html

Avramov, M., Schmidt, S. I., & Griebler, C. (2013). A new bioassay for the ecotoxicological testing of VOCs on groundwater invertebrates and the effects of toluene on *Niphargus Inopinatus*. *Aquatic Toxicology, 130-131*, 1-8. doi:10.1016/j.aquatox.2012.12.023.

Bartzas, G., Tinivella, F., Medini, L., Zaharaki, D., & Komnitsas, K. (2015). Assessment of groundwater contamination risk in an agricultural area in north Italy. *Information Processing in Agriculture, 2*(2), 109-129. doi:10.1016/j.inpa.2015.06.004.

Bauld, J. (1994). Groundwater quality in irrigation areas of Australia: Interactions of agriculture and hydrogeology. *Water Down Under 94: Groundwater Papers; Preprints of Papers*, 423.

Bauld, J., Ivkovic, K. M., Please, P. M., & Watkins, K. L. (1998). Groundwater quality benchmarks for areas of irrigated agriculture. In W. TR & L. CR (Eds.), *Proceedings of the International Groundwater Conference 1998, Groundwater Sustainable Solutions, University of Melbourne* (pp. 395-399). Melbourne, Australia: International Association of Hydrogeologists.

Baun, A., Jensen, S. D., Bjerg, P. L., Christensen, T. H., & Nyholm, N. (2000). Toxicity of organic chemical pollution in groundwater downgradient of a landfill (Grindsted, Denmark). *Environmental Science 'I&' Technology, 34*(9), 1647-1652.

Baxter, N. M., & Robinson, N. J. (2001). A land resource assessment of the Glenelg Hopkins region. *Agriculture Victoria, Department of Natural Resources and Environment: Bendigo, Vic*.

Bear, J., Beljin, M. S., & Ross, R. R. (1992). Fundamentals of ground-water modeling*: Superfund Technology Support Center for Ground Water, Robert S. Kerr Environmental Research Laboratory.*

Benson, V. S., Vanleeuwen, J. A., Sanchez, J., Dohoo, I. R., & Somers, G. H. (2006). Spatial analysis of land use impact on ground water nitrate concentrations. *Journal of Environment Quality, 35*(2), 421-421.

Bi, R., Zeng, X., Mu, L., Hou, L., Liu, W., Li, P., . . . Xie, L. (2018). Sensitivities of seven algal species to triclosan, fluoxetine and their mixtures. *Scientific Reports*, *8*(1), 15361-15361.

Bigsby, R., Chapin, R. E., Daston, G. P., Davis, B. J., Gorski, J., Gray, L. E., . . . Vom Saal, F. S. (1999). Evaluating the effects of endocrine disruptors on endocrine function during development. *Environmental Health Perspectives*, *107*(Suppl 4), 613-613. doi:10.2307/3434553.

Bondarenko, S., Gan, J., Haver, D. L., & Kabashima, J. N. (2004). Persistence of selected organophosphate and carbamate insecticides in waters from a coastal watershed. *Environmental Toxicology and Chemistry*, *23*(11), 2649-2654.

Böttger, R., Schaller, J., & Mohr, S. (2012). Closer to reality — The influence of toxicity test modifications on the sensitivity of Gammarus roeseli to the insecticide imidacloprid. *Ecotoxicology and Environmental Safety*, *81*, 49-54.

Castro, B. B., Guilhermino, L., & Ribeiro, R. (2003). In situ bioassay chambers and procedures for assessment of sediment toxicity with *Chironomus Riparius*. *Environmental Pollution*, *125*(3), 325-335.

Centner, T. J. (1998). Unwanted agricultural pesticides: State disposal programs. *Journal of Environmental Quality*, *27*(4), 736.

Cervantes-Servin, A. I., Arora, M., Peterson, T. J., & Pettigrove, V. (in prep). Seasonal assessment of groundwater vulnerability to agricultural pollutants using DRASTICL.

Chapman, R. N., & Stranger, J. W. (1993). Horticultural pesticide residues in water. A survey of pesticide residues conducted in the Mitchell Valley, Victoria, 1992-1993. *Research Report Series-Victorian Department of Agriculture (Australia)*.

Chapman, R. N., & Stranger, J. W. (1994). Horticultural pesticide residues in water: A survey of pesticide residues conducted in Gippsland, Victoria. Victorian Department of Agriculture.Australia. (1039-3846)

Chappie, D. J., & Burton Jr, G. A. (1997). Optimization of in situ bioassays with *Hyalella azteca* and *Chironomus Tentans*. *Environmental Toxicology and Chemistry: An International Journal*, *16*(3), 559-564.

Chappie, D. J., & Burton Jr, G. A. (2000). Applications of aquatic and sediment toxicity testing in situ. *Soil 'I&' Sediment Contamination*, *9*(3), 219-245.

Cherry, D. S., Currie, R. J., Soucek, D. J., Latimer, H. A., & Trent, G. C. (2001). An integrative assessment of a watershed impacted by abandoned mined land discharges. *Environmental Pollution*, *111*(3), 377-388.

Chui, T. F. M., Low, S. Y., & Liong, S.-Y. (2011). An ecohydrological model for studying groundwater–vegetation interactions in wetlands. *Journal of Hydrology*, *409*(1–2), 291-304.

Clark, J. R., Lewis, M. A., & Pait, A. S. (1993). Pesticide inputs and risks in coastal wetlands. *Environmental Toxicology and Chemistry*, *12*(12), 2225-2233.

Cleland, J. (2013). World population growth; Past, present and future. *Environmental and Resource Economics*, *55*(4), 543-554. doi:10.1007/s10640-013-9675-6.

Clements, W. H., & Kiffney, P. M. (1994). Integrated laboratory and field approach for assessing impacts of heavy metals at the Arkansas River, Colorado. *Environmental Toxicology and Chemistry*, *13*(3), 397-404.

Cohen, M. (2007). Environmental toxins and health-The health impact of pesticides. *Australian Family Physician*, *36*(12), 1002-1004.

Commonwealth. (2013). Guidelines for groundwater quality protection in Australia. Australia

Conboy, C., Jhonson, K., Mclean, B., Maconachie, A., & Connor, J. (2018). Gorst Rural - 2018 Trial results.Lake Bolac, Victoria, Australia.

Coombe, V. T., Moore, K. W., & Hutchings, M. J. (1999). TIE and TRE: an abbreviated guide to dealing with toxicity. *Water Science and Technology, 39*(10-11), 91-97. doi:10.2166/wst.1999.0635.

Costelloe, J. F., Peterson, T. J., Halbert, K., Western, A. W., & McDonnell, J. J. (2015). Groundwater surface mapping informs sources of catchment baseflow. *Hydrol Earth Syst Sc, 19*(4), 1599-1613. doi:10.5194/hess-19-1599-2015.

Council, N. R., Studies, D. E. L., Commission on Geosciences, E. R., & Vulnerability, C. (1993). Ground Water Vulnerability Assessment: Predicting Relative Contamination Potential Under Conditions of Uncertainty.*: National Academies Press.*

Croskrey, A., & Groves, C. (2008). Groundwater sensitivity mapping in Kentucky using GIS and digitally vectorized geologic quadrangles. *Environmental Geology, 54*(5), 913-920. doi:10.1007/s00254-007-0899-z.

Culp, J. M., Lowell, R. B., & Cash, K. J. (2000). Integrating mesocosm experiments with field and laboratory studies to generate weight-of-evidence risk assessments for large rivers. *Environmental toxicology and chemistry, 19*(4), 1167-1173.

Dahlhaus, P., Heislers, D., & Dyson, P. (2002). Groundwater flow systems of the Glenelg Hopkins catchment management authority region.

Dehkhargani, F. S., Malekinejad, H., Shahrooz, R., & Sarkhanloo, R. A. (2011). Detrimental effect of atrazine on testicular tissue and sperm quality: implication for oxidative stress and hormonal alterations. *Iranian Journal of toxicology, 5*(12), 426-435.

Dewhurst, Dewhurst, R. E., Callaghan, A., Connon, R., Crane, M., Mather, J. D., & Wood, R. (2005). Toxicity testing of groundwater quality. *Water Environ J, 19*(1), 17-24.

Dhananjayan, V., & Muralidharan, S. (2010). Organochlorine pesticide residues in inland wetland fishes of Karnataka, India and their implications on human dietary intake. *Bulletin of environmental contamination and toxicology, 85*(6), 619-623.

Di Lorenzo, T., Castaño-Sánchez, A., Di Marzio, W. D., García-Doncel, P., Nozal Martínez, L., Galassi, D. M. P., & Iepure, S. (2019). The role of freshwater copepods in the environmental risk assessment of caffeine and propranolol mixtures in the surface water bodies of Spain. *Chemosphere, 220*, 227-236. doi:10.1016/j.chemosphere.2018.12.117.

Di Lorenzo, T., Cifoni, M., Fiasca, B., Di Cioccio, A., & Galassi, D. M. P. (2018). Ecological risk assessment of pesticide mixtures in the alluvial aquifers of central Italy: Toward more realistic scenarios for risk mitigation. *Science of The Total Environment, 644*, 161-172. doi:10.1016/j.scitotenv.2018.06.345.

Di Lorenzo, T., Di Marzio, W. D., Fiasca, B., Galassi, D. M. P., Korbel, K., Iepure, S., . . . Hose, G. C. (2019). Recommendations for ecotoxicity testing with stygobiotic species in the framework of groundwater environmental risk assessment. *Science of the Total Environment, 681*, 292-304.

Di Lorenzo, T., Di Marzio, W. D., Sáenz, M. E., Baratti, M., Dedonno, A. A., Iannucci, A., . . . Galassi, D. M. P. (2014). Sensitivity of hypogean and epigean freshwater copepods to agricultural pollutants. *Environmental Science and Pollution Research, 21*(6), 4643-4655. doi:10.1007/s11356-013-2390-6.

Di Marzio, W. D., Castaldo, D., Di Lorenzo, T., Di Cioccio, A., Sáenz, M. E., & Galassi, D. M. P. (2013). Developmental endpoints of chronic exposure to suspected endocrine-disrupting chemicals on benthic and hyporheic freshwater copepods. *Ecotoxicology and Environmental Safety, 96*, 86-92. doi:10.1016/j.ecoenv.2013.06.029.

Di Marzio, W. D., Castaldo, D., Pantani, C., Di Cioccio, A., Di Lorenzo, T., Sáenz, M. E., & Galassi, D. M. P. (2009). Relative sensitivity of hyporheic copepods to chemicals. *Bulletin of Environmental Contamination and Toxicology, 82*(4), 488-491. doi:10.1007/s00128-008-9603-6.

Di Marzio, W. D., Cifoni, M., Sáenz, M. E., Galassi, D. M. P., & Di Lorenzo, T. (2018). The ecotoxicity of binary mixtures of imazamox and ionized ammonia on freshwater copepods: Implications for environmental risk assessment in groundwater bodies. *Ecotoxicology and Environmental Safety, 149*, 72-79. doi:10.1016/j.ecoenv.2017.11.031.

Díaz, F. J., Anthony, T. O., & Dahlgren, R. A. (2012). Agricultural pollutant removal by constructed wetlands: Implications for water management and design. *Agr Water Manage, 104*, 171-183.

DNRE. (2002a). Management of Vicotria's Ramsar wetlands: Strategic directions statement. Victoria, Australia: Department of Natural Resources and Environment

DNRE. (2002b). Western district lakes Ramsar site: Strategic management plan. Victoria, Australia: Department of Natural Resources and Environment

Donald, D. B., Syrgiannis, J., Hunter, F., & Weiss, G. (1999). Agricultural pesticides threaten the ecological integrity of Northern Prairie wetlands. *Science of The Total Environment, 231*(2–3), 173-181.

DPIE. (2018). Groundwater quality. Department of Planning, Industry and Environment. New South Wales. Retrieved from https://www.industry.nsw.gov.au/water/science/groundwater/quality

Eamus, D., Froend, R., Loomes, R., Hose, G., & Murray, B. (2006). A functional methodology for determining the groundwater regime needed to maintain the health of groundwater-dependent vegetation. *Australian Journal of Botany, 54*(2), 97-114. doi:10.1071/BT05031.

EC. (2004). Decision 2004/248/CE of 10 March 2004 concerning the non-inclusion of atrazine in Annex I to council directive 91/414/EEC and the withdrawal of authorizations fro plant protection prodycts containing this active substance *Official Journal of the European Communities*.

Ekström, M., Abbs, D., Bhend, J., Chiew, F., Kirono, D., Lucas, C., . . . Webb, L. (2015). Central slopes cluster report, climate change in Australia projections for Australia's natural resource management regions. CSIRO and Bureau of Meteorology. Australia.

El Amri, A., Makram, A., Manel, S., & Dridi, L. (2016). Mapping of vulnerability to pollution of Oued Laya phreatic aquifer.

Environment Canada. (2007). Biological test method: Growth inhibition test using a freshwater alga.

EPA. (2000a). Groundwater sampling guidelines. (669). 40 City Road, Southbank Victoria 3006, Australia: Environment Protection Authority

EPA. (2000b). A guide to the sampling and analysis of waters, wastewaters, soils and wastes.Victoria, Australia.

EPA. (2006). Hydrogeological assessment (groundwater quality) guidelines.Victoria.

EPA. (2009). Sampling and analysis of waters, wastewaters, soils and wastes.Victoria, Australia.

EPA. (2010). Guidelines; Water quality/water ecosystem monitoring. Environment Protecttion Authority Victoria Victoria, Australia.

Falconer, K. E. (1998). Managing diffuse environmental contamination from agricultural pesticides: An economic perspective on issues and policy options, with particular reference to Europe. *Agriculture, Ecosystems & Environment, 69*(1), 37-54.

Flury, M. (1996). Experimental evidence of transport of pesticides through field soils—A review. *Journal of Environmental Quality*, *25*(1).

Foster, S. M. (1988). *Groundwater pollution risk assessment: A methodology using available data.*

Foster, S. S. D., & Hirata, R. (1988). Groundwater pollution risk assessment a methodology using available data. *Pan Ame. Cent. for Sanit. Engin. and Envir. Scien.(cepis). Lima*.

Fritch, T. G., McKnight, C. L., Yelderman, J. C., & Arnold, J. G. (2000). An aquifer vulnerability assessment of the Paluxy aquifer, central Texas, USA, using GIS and a modified DRASTIC approach. *Environ Manage*, *25*(3), 337-345. doi:10.1007/s002679910026.

Gagliardi, B. (2012). Groundwater quality in the Woori Yallock catchment and its potential impacts on *Euclyptus Camphora* in the Yellingbo Nature conservation reserve, Victoria. Center for Aquatic Pollution and Management Technical Report

Gagliardi, B., & Pettigrove, V. (2013). Removal of intensive agriculture from the landscape improves aquatic ecosystem health. *Agriculture*, *ecosystems & environment, 176*, 1-8.

Geis, S. W., Fleming, K. L., Korthals, E. T., Searle, G., Reynolds, L., & Karner, D. A. (2000). Modifications to the algal growth inhibition test for use as a regulatory assay. *Environmental Toxicology and Chemistry*, *19*(1), 36-41. doi:10.1002/etc.5620190105.

Gevao, B., & Jones, K. C. (2002). Pesticides and persistent organic pollutants. *Agriculture, hydrology and water quality*, 83-106.

GH CMA. (2006). Glenelg Hopkins regional wetlands status report. Glenelg Hopkins CMA, Hamilton

GH CMA. (2008). Climate change in the Glenelg Hopkins region. Department of Sustainability and Environment.Melbourne.

GH CMA. (2013). Glenelg Hopkins regional catchment strategy 2013-2019.Victoria, Australia.

GH CMA. (2014). Glenelg Hopkins CMA 2015-18 Projects. Glenelg Hopkins Catchment Management Authority.Victoria, Australia.

GH CMA. (2018). Glenelg Hopkins CMA: Annual report 2017-2018.Victoria, Australia. (20)

Gilliom, R. J. (2007). Pesticides in U.S. streams and groundwater. *Environ Sci Technol, 41*(10), 3408-3414.

Gogu, R. C., & Dassargues, A. (2000). Current trends and future challenges in groundwater vulnerability assessment using overlay and index methods. *Environmental Geology, 39*(6), 549-559. doi:10.1007/s002540050466.

Gonçalves, C. M., Esteves da Silva, J. C. G., & Alpendurada, M. F. (2007). Evaluation of the pesticide contamination of groundwater sampled over two years from a vulnerable zone in Portugal. *Journal of Agricultural and Food Chemistry, 55*(15), 6227-6235.

Guimarães, L., Guilhermino, L., Afonso, M. J., Marques, J. M., & Chaminé, H. I. (2019). Assessment of urban groundwater: towards integrated hydrogeological and effects-based monitoring. *Sustainable Water Resources Management, 5*(1), 217-233. doi:10.1007/s40899-019-00301-w.

Gustavson, K. E., Sonsthagen, S. A., Crunkilton, R. A., & Harkin, J. M. (2000). Groundwater toxicity assessment using bioassay, chemical, and toxicity identification evaluation analyses. *Environmental Toxicology, 15*(5), 421-430. doi:10.1002/1522-7278(2000)15:5<421::Aid-tox10>3.0.Co;2-z.

Hallberg, G. R. (1989). Pesticides pollution of groundwater in the humid United States. *Agriculture, Ecosystems & Environment, 26*(3–4), 299-367.

Hamza, S. M., Ahsan, A., Imteaz, M. A., Rahman, A., Mohammad, T. A., & Ghazali, A. H. (2015). Accomplishment and subjectivity of GIS-based DRASTIC groundwater vulnerability assessment method: A review. *Environmental Earth Sciences, 73*(7), 3063-3076. doi:10.1007/s12665-014-3601-2.

Hancock, P. J., Hunt, R. J., & Boulton, A. J. (2009). Preface: hydrogeoecology, the interdisciplinary study of groundwater dependent ecosystems. *Hydrogeology Journal, 17*(1), 1-3. doi:10.1007/s10040-008-0409-8.

Hayes, T., Haston, K., Tsui, M., Hoang, A., Haeffele, C., & Vonk, A. (2003). Atrazine-induced hermaphroditism at 0.1 ppb in American leopard frogs (*Rana pipiens*): laboratory and field evidence. *Environmental health perspectives, 111*(4), 568-568.

Hayes, T. B., Collins, A., Lee, M., Mendoza, M., Noriega, N., Stuart, A. A., & Vonk, A. (2002). Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. *Proceedings of the National Academy of Sciences, 99*(8), 5476-5480.

Hildebrandt, A., Guillamón, M., Lacorte, S., Tauler, R., & Barceló, D. (2008). Impact of pesticides used in agriculture and vineyards to surface and groundwater quality (North Spain). *Water Research, 42*(13), 3315-3326.

Himel, C. M., Loats, H., & Bailey, G. W. (1990). Pesticides sources to the soil and principles of spray physics. (Vol. 2). *Wisconsin.*

Humphries, B., & Close, M. (2015). National survey of pesticides in groundwater 2014. *ESR Report CSC15003*.

Ivkovic, K. M., Watkins, K. L., Cresswell, R. G., & Bauld, J. (2001). A groundwater quality assessment of the Upper Shepparton formation aquifers: Cobram Region, Victoria.

Kearney, P. C., & Helling, C. S. (1982). Problems caused by pesticides with particular reference to the impact on the agricultural environment*: na.*

Khatri, N., & Tyagi, S. (2015). Influences of natural and anthropogenic factors on surface and groundwater quality in rural and urban areas. *Frontiers in Life Science, 8*(1), 23-39. doi:10.1080/21553769.2014.933716.

Kim, G.-B., Ahn, J.-S., & Marui, A. (2009). Analytic hierarchy models for regional groundwater monitoring well allocation in Southeast Asian countries and South Korea. *Environmental Earth Sciences, 59*(2), 325. doi:10.1007/s12665-009-0029-1.

Köck-Schulmeyer, M., Ginebreda, A., Postigo, C., Garrido, T., Fraile, J., López de Alda, M., & Barceló, D. (2014). Four-year advanced monitoring program of polar pesticides in groundwater of Catalonia (NE-Spain). *Science of The Total Environment, 470–471*, 1087-1098.

Kumar, P. (2015). Index-based groundwater vulnerability mapping models using hydrogeological settings: A critical evaluation. *Environmental impact assessment review*, *51*, 38-49. doi:10.1016/j.eiar.2015.02.001.

Lapworth, D. J., Gooddy, D. C., Stuart, M. E., Chilton, P. J., Cachandt, G., Knapp, M., & Bishop, S. (2006). Pesticides in groundwater: some observations on temporal and spatial trends. *Water Environ J, 20*(2), 55-64.

Larsen, L., Jørgensen, C., & Aamand, J. (2001). Potential mineralization of four herbicides in a ground water–Fed wetland area. *Journal of environmental quality*, *30*(1), 24-30.

Leitch, C., & Fagg, P. (1985). Clopyralid herbicide residues in streamwater after aerial spraying of a *Pinus radiata* plantation. *New Zealand Journal of Forestry Science*, *15*(2), 195-206.

Lewis, M. A. (1990). Are laboratory-derived toxicity data for freshwater algae worth the effort? *Environmental Toxicology and Chemistry, 9*(10), 1279-1284.

Lewis, M. A. (1995). Use of freshwater plants for phytotoxicity testing: a review. *Environmental Pollution, 87*(3), 319-336.

Lewis, P. A., Klemm, D. J., Lazorchak, J. M., Norberg-King, T. J., Peltier, W. H., & Heber, M. A. (1994a). Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. In: US Environmental Protection Agency, Environmental Monitoring Systems Laboratory.

Lewis, P. A., Klemm, D. J., Lazorchak, J. M., Norberg-King, T. J., Peltier, W. H., & Heber, M. A. (1994b). Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms*: US Environmental Protection Agency, Environmental Monitoring Systems Laboratory*.

Liu, S., Ryu, D., Webb, J., Lintern, A., Guo, D., Waters, D., & Western, A. (2018). Characterisation of spatial variability in water quality in the Great Barrier Reef catchments using multivariate statistical analysis. *Marine Pollution Bulletin, 137*, 137-151. doi:10.1016/j.marpolbul.2018.10.019.

Maazouzi, C., Coureau, C., Piscart, C., Saplairoles, M., Baran, N., & Marmonier, P. (2016). Individual and joint toxicity of the herbicide S-metolachlor and a metabolite, deethylatrazine on aquatic crustaceans: Difference between ecological groups. *Chemosphere, 165*, 118-125. doi:10.1016/j.chemosphere.2016.09.030.

Machiwal, D., Jha, M. K., Singh, V. P., & Mohan, C. (2018). Assessment and mapping of groundwater vulnerability to pollution: Current status and challenges. *Earth-Science Reviews, 185*, 901-927. doi:doi.org/10.1016/j.earscirev.2018.08.009.

McKenzie-Smith, F., Tiller, D., & Allen, D. (1994). Organochlorine pesticide residues in water and sediments from the Ovens and King rivers, north-east Victoria, Australia. *Archives of environmental contamination and toxicology, 26*(4), 483-490.

McManus, S.-L., Richards, K. G., Grant, J., Mannix, A., & Coxon, C. E. (2014). Pesticide occurrence in groundwater and the physical characteristics in association with these detections in Ireland. *Environmental Monitoring and Assessment, 186*(11), 7819-7836.

Milhome, M. A. L. (2015). Influence The USE of pesticides in the quality of surface and groundwater located in irrigated areas of Jaguaribe, Ceara, Brazil. *International Journal of Environmental Research, 9*(1), 255.

Moore, S., Lewin, K., Talman, S., & O'Connor, N. (1996). Effects of past and present biocide usage on aquatic fauna in the Ovens catchment. *Water Ecoscience. Melbourne, Water Ecoscience, 12*, 44-46.

Moreira-Santos, M., Moreno-Garrido, I., Gonçalves, F., Soares, A. M. V. M., & Ribeiro, R. (2002). An in situ bioassay for estuarine environments using the microalga *Phaeodactylum Tricornutum*. *Environmental toxicology and chemistry, 21*(3), 567-574.

Moreira-Santos, M., Soares, A. M. V. M., & Ribeiro, R. (2004). A phytoplankton growth assay for routine in situ environmental assessments. *Environmental toxicology and chemistry, 23*(6), 1549-1560.

Morris, B. L., Lawrence, A. R. L., Chilton, P. J. C., Adams, B., Calow, R. C., & Klinck, B. A. (2003). Groundwater and its susceptibility to degradation: a global assessment of the problem and options for management (Vol. 3)*: United Nations Environment Programme.*

Mossop, D., Kellar, C., Jeppe, K., Myers, J., Rose, G., Weatherman, K., . . . Leahy, P. (2013). Impacts of intensive agriculture and plantation forestry on water quality in the Latrobe catchment, Victoria*: EPA Victoria.*

Munira, S., Farenhorst, A., Sapkota, K., Nilsson, D., & Sheedy, C. (2018). Auxin herbicides and pesticide mixtures in groundwater of a canadian prairie province. *J Environ Qual, 47*(6), 1462-1467. doi:10.2134/jeq2018.05.0202.

Naddy, R. B., Tapp, K., Rehner, A. B., Pillard, D. A., & Schrage, L. (2011). Identifying the cause of toxicity in an algal whole effluent toxicity study - An unanticipated toxicant. *Chemosphere, 85*(6), 1026-1032.

Nalewajko, C., & Olaveson, M. M. (2018). Ecophysiological considerations in microalgal toxicity tests. In *Microscale Testing in Aquatic Toxicology* (pp. 289-309): CRC Press.

National Research Council US. (1993). Ground water vulnerability assessment: Predicting relative contamination potential under conditions of uncertainty. *Washington, D.C.: The National Academies Press.*

Nemani, R. R., Keeling, C. D., Hashimoto, H., Jolly, W. M., Piper, S. C., Tucker, C. J., . . . Running, S. W. (2003). Climate-driven increases in global terrestrial net primary production from 1982 to 1999. *Science, 300*(5625), 1560-1563.

Neuwoehner, J., Reineke, A.-K., Hollender, J., & Eisentraeger, A. (2009). Ecotoxicity of quinoline and hydroxylated derivatives and their occurrence in groundwater of a tar-contaminated field site. *Ecotoxicology and Environmental Safety, 72*(3), 819-827.

Norberg-King, T. J., Mount, D., Durhan, E., Ankley, G. T., & Burkhard, L. (1991). Methods for aquatic toxicity identification evaluations. Phase 1. Toxicity characterization procedures. Second edition.United States.

NRC. (1993). Ground water vulnerability assessment: Predicting relative contamination potential under conditions of uncertainty. *Washington, DC: The National Academies Press.*

Nyholm, N., & Källqvist, T. (1989). Methods for growth inhibition toxicity tests with freshwater algae. *Environmental Toxicology and Chemistry, 8*(8), 689-703.

OECD. (1984). Guideline for testing of chemicals 201 - Algal growth inhibition.Paris, France.

OECD. (2011). Test No. 201: Freshwater alga and cyanobacteria, growth inhibition test. In *OECD Guidelines for the Testing of Chemicals, Section 2* (pp. 25-25): OECD Publishing, Paris.

OECD. (2019). Test No. 203: Fish, acute toxicity test.

Orton, F., & Tyler, C. R. (2015). Do hormone-modulating chemicals impact on reproduction and development of wild amphibians? *Biological Reviews, 90*(4), 1100-1117.
Pacheco, F. A., & Fernandes, L. F. S. (2013). The multivariate statistical structure of DRASTIC model. *Journal of hydrology, 476*, 442-459. doi:10.1016/j.jhydrol.2012.11.020.
Pacheco, F. A. L. (1998). Application of correspondence analysis in the assessment of groundwater chemistry. *Mathematical Geology, 30*(2), 129-161. doi:10.1023/a:1021718929576.
Pacheco, F. A. L., Pires, L. M. G. R., Santos, R. M. B., & Sanches Fernandes, L. F. (2015). Factor weighting in DRASTIC modeling. *Science of The Total Environment, 505*, 474-486. doi:10.1016/j.scitotenv.2014.09.092.
Patrikaki, O., Kazakis, N., & Voudouris, K. (2012). Vulnerability map: a useful tool for groundwater protection: an example from Mouriki Basin, North Greece. *Fresenius Environ Bull, 21*(8c), 2516-2521.
Peel, M. C., Finlayson, B. L., & McMahon, T. A. (2007). Updated world map of the Köppen-Geiger climate classification. *Hydrol. Earth Syst. Sci., 11*(5), 1633-1644. doi:10.5194/hess-11-1633-2007.
Pérez-Lucas, G., Vela, N., El Aatik, A., & Navarro, S. (2018). Environmental risk of groundwater pollution by pesticide leaching through the soil profile. In *Pesticides, anthropogenic activities and the health of our environment*: IntechOpen.
Peterson, T. J., Cheng, X., Western, A. W., Siriwardena, L., & Wealands, S. R. (2011). *Novel indicator geostatistics for water table mapping that incorporate elevation, land use, stream network and physical constraints to provide probabilistic estimation of heads and fluxes.* Paper presented at the Proceeding of the 19th International Congress on Modelling and Simulation, Perth, Australia.
Peterson, T. J., & Western, A. W. (2014). Nonlinear time-series modeling of unconfined groundwater head. *Water Resour Res, 50*(10), 8330-8355. doi:10.1002/2013WR014800.
Peterson, T. J., & Western, A. W. (2018). Statistical Interpolation of groundwater hydrographs. *Water Resour Res, 54*(7), 4663-4680. doi:10.1029/2017wr021838.
Peterson, T. J., & Western, A. W. (in-prep). State-wide geostatistical estimation of the water table level from 1985-2014.
Special review decision: Atrazine, (2017), Re-evaluation Note.
R Core Team. (2019). R: A language and environment for statistical computing. Viena, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org/
Rabalais, N. N. (2002). Nitrogen in Aquatic Ecosystems. *AMBIO: A Journal of the Human Environment, 31*(2), 102-112.
Radix, P., Léonard, M., Papantoniou, C., Roman, G., Saouter, E., Gallotti-Schmitt, S., . . . Vasseur, P. (2000). Comparison of four chronic toxicity tests using algae, bacteria, and invertebrates assessed with sixteen chemicals. *Ecotoxicology and Environmental Safety, 47*(2), 186-194.
Raisin, G., Bartley, J., & Croome, R. (1999). Groundwater influence on the water balance and nutrient budget of a small natural wetland in Northeastern Victoria, Australia. *Ecological Engineering, 12*(1-2), 133-147.

RDA. (2010). The Great South Coast regional strategic plan. Regional Development Australia and Regional Development Victoria.Victoria, Australia.

Reboleira, A. S. P. S., Abrantes, N., Oromí, P., & Gonçalves, F. (2013). Acute toxicity of copper sulfate and potassium dichromate on *Stygobiont Proasellus*: General aspects of groundwater ecotoxicology and future Perspectives. *Water, Air, & Soil Pollution, 224*(5), 1550. doi:10.1007/s11270-013-1550-0.

Regional Roads Victoria (2019, May 30). [Road herbicides furniture list 2019].

Rhymes, J., Jones, L., Lapworth, D. J., White, D., Fenner, N., McDonald, J. E., & Perkins, T. L. (2015). Using chemical, microbial and fluorescence techniques to understand contaminant sources and pathways to wetlands in a conservation site. *Science of The Total Environment, 511*, 703-710.

Ribeiro, L., Pindo, J. C., & Dominguez-Granda, L. (2017). Assessment of groundwater vulnerability in the Daule aquifer, Ecuador, using the susceptibility index method. *Science of The Total Environment, 574*, 1674-1683. doi:10.1016/j.scitotenv.2016.09.004.

Ringwood, A. H., & Keppler, C. J. (2002). Comparative in situ and laboratory sediment bioassays with juvenile *Mercenaria mercenaria*. *Environmental Toxicology and Chemistry, 21*(8), 1651-1657.

Robins, N. S. (1998). Recharge: The key to groundwater pollution and aquifer vulnerability. *Geological Society, London, Special Publications, 130*(1), 1-5. doi:10.1144/gsl.sp.1998.130.01.01.

Rojíčková, R., & Maršálek, B. (1999). Selection and sensitivity comparisons of algal species for toxicity testing. *Chemosphere, 38*(14), 3329-3338.

Rose, G., AnhDuyen, B., Pei, Z., Mahendrra, R., Michael, L., Mayumi, A., & Graeme, A. (2010). Agrochemicals in the corner inlet catchment 2009-10 pilot project report.Victoria, Australia.

Rose, G., & Kibria, G. (2006). Pesticide Monitoring in Goulburn-Murray water's irrigation supply channels covering the six irrigation areas (2004-2005 irrigation season study report). In: Department of Primary Industries, Victoria, Australia.

Rupert, M. G. (2001). Calibration of the DRASTIC ground water vulnerability mapping method. *Ground Water*, *39*(4), 625-630. doi:10.1111/j.1745-6584.2001.tb02350.x.

Saaty, T. L. (1990). How to make a decision: The analytic hierarchy process. *European Journal of Operational Research, 48*(1), 9-26. doi:10.1016/0377-2217(90)90057-i.

Saha, D., & Alam, F. (2014). Groundwater vulnerability assessment using DRASTIC and Pesticide DRASTIC models in intense agriculture area of the Gangetic plains, India. *Environmental monitoring and assessment, 186*(12), 8741-8763.

Sampat, P. (2000). Deep trouble: The hidden threat of groundwater pollution. *Worldwatch Paper*, 46.

Sánchez-Bayo, F., Baskaran, S., & Kennedy, I. R. (2002). Ecological relative risk (EcoRR): Another approach for risk assessment of pesticides in agriculture. *Agriculture, Ecosystems & Environment, 91*(1–3), 37-57.

Sangam, S., Dickson, J., & Vishnu, P. P. (2016). Assessment of groundwater vulnerability and risk to pollution in Kathmandu Valley, Nepal. *Science of the Total Environment, 556*, 23-35.

Sangam, S., Ranjana, K., & Vishnu, P. P. (2017). Evaluation of index-overlay methods for groundwater vulnerability and risk assessment in Kathmandu Valley, Nepal.

Science of the Total Environment, 575, 779-790. doi:10.1016/j.scitotenv.2016.09.141.
Schäfer, R. B., van den Brink, P. J., & Liess, M. (2011). Impacts of pesticides on freshwater ecosystems. *Ecological impacts of toxic chemicals*, 111-137.
Schwartz, F. W., & Zhang, H. (2003). Fundamentals of ground water. *New York: Wiley*.
Sekhar, M. C., & Kumar, K. K. (1999). The use of DRASTIC modelling indices for the assessment of groundwater pollution potential. *IAHS PUBLICATION*, 111-116.
Sener, E., & Davraz, A. (2013). Assessment of groundwater vulnerability based on a modified DRASTIC model, GIS and an analytic hierarchy process (AHP) method: The case of Egirdir Lake basin (Isparta, Turkey). *Hydrogeology Journal*, *21*(3), 701-714. doi:10.1007/s10040-012-0947-y.
Sengupta, P., & Banerjee, R. (2014). Environmental toxins: Alarming impacts of pesticides on male fertility. *Human & Experimental Toxicology, 33*(10), 1017-1017.
SGV. (2011). Western region sustainable water strategy. Victorian Government. Department of Sustainability and Environment.Melbourne, Victoria.
Sibley, P. K., Benoit, D. A., Balcer, M. D., Phipps, G. L., West, C. W., Hoke, R. A., & Ankley, G. T. (1999). In situ bioassay chamber for assessment of sediment toxicity and bioaccumulation using benthic invertebrates. *Environmental toxicology and chemistry, 18*(10), 2325-2336.
SKM. (1995). Summary of 1994/95 Pesticide sampling program, groundwater report No. 37.
SKM. (2002). Goulburn-Murray water sediment toxicants - Biocide and metal levels in drain sediments of the Shepparton irrigation region. Goulburn-Murray Water.Victoria, Australia.
SKM. (2009). Victorian aquifer framework - Summary report. Department of Sustainability and Environment.Victoria, Australia.
SKM. (2010). Glenelg Hopkins CMA groundwater model - Final model development report. Department of Sustainability and Environment.Victoria, Australia.
SKM. (2012). Australian groundwater modelling guidelines. National Water Commission.Canberra, Australia.
Steinheimer, T. R., Ross, L. J., & Spittler, T. D. (2000). Agrochemical movement: Perspective and scale-of-study overview. In *Agrochemical Fate and Movement* (Vol. 751, pp. 2-18): American Chemical Society.
Stigter, T. Y., Ribeiro, L., & Dill, A. M. M. C. (2006). Evaluation of an intrinsic and a specific vulnerability assessment method in comparison with groundwater salinisation and nitrate contamination levels in two agricultural regions in the south of Portugal. *Hydrogeology Journal, 14*(1), 79-99. doi:10.1007/s10040-004-0396-3.
Swan, S. H. (2006). Semen quality in fertile US men in relation to geographical area and pesticide exposure. *International Journal of Andrology, 29*(1), 62-68. doi:10.1111/j.1365-2605.2005.00620.x.
Teixeira, J., Chaminé, H. I., Espinha-Marques, J., Carvalho, J. M., Pereira, A. J. S. C., Carvalho, M. R., . . . Rocha, F. (2015). A comprehensive analysis of groundwater resources using GIS and multicriteria tools (Caldas da Cavaca, Central Portugal): environmental issues. *Environmental Earth Sciences*, *73*(6), 2699-2715. doi:10.1007/s12665-014-3602-1.

Thirumalaivasan, D., Karmegam, M., & Venugopal, K. (2003). AHP-DRASTIC: Software for specific aquifer vulnerability assessment using DRASTIC model and GIS. *Environmental Modelling & Software, 18*(7), 645-656.

Thomas, K. V., Thain, J. E., & Waldock, M. J. (1999). Identification of toxic substances in United Kingdom estuaries. *Environmental Toxicology and Chemistry, 18*(3), 401-411.

Timbal, B., & Drosdowsky, W. (2013). The relationship between the decline of Southeastern Australian rainfall and the strengthening of the subtropical ridge. *International Journal of Climatology, 33*(4), 1021-1034. doi:10.1002/joc.3492.

Timbal, B., Ekström, M., Fiddes, S., Grose, M., Kirono, D. G., Lim, E.-P., . . . Wilson, L. (2016). Climate change science and Victoria*: Bureau of Meteorology.*

Tocker, J., Berrisford, T., & Gilmour, D. (2010). South West farm monitor project–summary of results 2008–09. *Department of Primary Industries: Melbourne*.

Tomlin, C. (2003). The pesticide manual: a world compendium (C. D. S. Tomlin Ed. 13th ed. ed.)*: Alton : British Crop Protection Council, 2003.*

UN. (2019). World population prospects 2019: Highlights. United Nations, Department of Economic and Social Affairs, Population Division

Uricchio, V. F., Giordano, R., & Lopez, N. (2004). A fuzzy knowledge-based decision support system for groundwater pollution risk evaluation. *Journal of environmental management, 73*(3), 189-197. doi:10.1016/j.jenvman.2004.06.011.

US EPA. (1993). Review of methods assessing aquifer sensitivity and ground water vulnerability to pesticide contamination. United States Environmental Protection Agency.Washington, DC.

US EPA. (2001). Oxyfluorfen: toxicology chapter for RED. Pesticides and toxic substances. US Environmental Protection Agency Office of Prevention. Unied States Environmental Protection Agency.Washington, D.C.

Memorandum. Atrazine: finalization of interim reregistration eligibility decision and completion of tolerance reassessment and reregistration eligibility process, (2006a).

Triazine cumulative risk assessment, (2006b).

US EPA. (2016). Ecological effects test guidelines OCSPP 850.1000 background and special considerations-Tests with aquatic and sediment-dwelling fauna and aquatic microcosms. United States Environemental Protection Agency(EPA 712-C-16-014)

van der Werf, H. M. G. (1996). Assessing the impact of pesticides on the environment. *Agriculture, Ecosystems & Environment, 60*(2–3), 81-96.

VP. (1998). Black Range state park management plan. (0731131223). Melbourne, Australia: Parks Victoria

VSG. (1994). Catchment and land protection act. Victoria, Australia: Victorian State Government. Environment, land, water and planning

Wang, Y., Merkel, B. J., Li, Y., Ye, H., Fu, S., & Ihm, D. (2007). Vulnerability of groundwater in Quaternary aquifers to organic contaminants: A case study in Wuhan City, China. *Environmental Geology, 53*(3), 479-484. doi:10.1007/s00254-007-0669-y.

Watkins, K. L., Bauld, J., & Ivkovic, K. M. (1999a). A groundwater quality assessment of the Goulburn Catchment Victoria: Kyabram-Tongala*: Bureau of Rural Sciences.*

Watkins, K. L., Bauld, L., & Ivkovic, K. (1999b). A groundwater quality assessment of the Goulburn Catchment Victoria: Nagambie-Mangalore*: Bureau of Rural Sciences.*

Wenig, D. (1997). Pesticide contamination of shallow aquifers at Ardmona, Girgarre and Kyvalley, Shepparton irrigation region, Victoria*: University of Melbourne, School of Earth Sciences.*

Wenig, D., & Lawrence, C. (1998). Pesticide contamination of shallow aquifers at Ardmona, Girgarre and Kyvalley, Shepparton irrigation region, Victoria. In W. T.R. & L. C.R. (Eds.), *International Groundwater Conference 1998, Proceedings: Groundwater Sustainable Solutions, University of Melbourne* (pp. 586-590). Melbourne, Australia: International Association of Hydrogeologists.

Weyers, A., Sokull-Klüttgen, B., Baraibar-Fentanes, J., & Vollmer, G. (2000). Acute toxicity data: A comprehensive comparison of results of fish, daphnia, and algae tests with new substances notified in the European Union. *Environmental toxicology and chemistry, 19*(7), 1931-1933.

Wightwick, A., & Allinson, G. (2007). Pesticide residues in Victorian waterways: A review. *Australasian Journal of Ecotoxicology, 13*(3), 91.

Wu, C., Luo, Y., Gui, T., & Huang, Y. (2014). Concentrations and potential health hazards of organochlorine pesticides in shallow groundwater of Taihu Lake region, China. *Science of The Total Environment, 470–471*, 1047-1055.

Wu, H., Chen, J., & Qian, H. (2016). A modified DRASTIC model for assessing contamination risk of groundwater in the northern suburb of Yinchuan, China. *Environmental Earth Sciences*(6), 1. doi:10.1007/s12665-015-5094-z.

Xin, L., Hong-ying, H., Ke, G., & Ying-xue, S. (2010). Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga Scenedesmus sp. *Bioresource Technology, 101*(14), 5494-5500.

Yadav, I., Devi, N., Syed, J., Cheng, Z., Li, J., Zhang, G., & Jones, K. (2015). Current status of persistent organic pesticides residues in air, water, and soil, and their possible effect on neighboring countries: A comprehensive review of India. *Science of the total environment, 511*, 123-137.

Yen, J.-H., Sheu, W.-S., & Wang, Y.-S. (2003). Dissipation of the herbicide oxyfluorfen in subtropical soils and its potential to contaminate groundwater. *Ecotoxicology and environmental safety, 54*(2), 151-156.

Zhao, F., Li, Y., Huang, L., Gu, Y., Zhang, H., Zeng, D., & Tan, H. (2018). Individual and combined toxicity of atrazine, butachlor, halosulfuron-methyl and mesotrione on the microalga Selenastrum capricornutum. *Ecotoxicology and Environmental Safety, 148*, 969-975.