LONG-TERM BIOSOLIDS USE IN AGRICULTURAL LANDS: EFFECTS ON SELECTED SOIL CHEMICAL PROPERTIES, ACCUMULATION OF TRICLOSAN AND SULFAMETHOXAZOLE IN THE SOIL AND MAIZE PLANT

By

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DECLARATION

I undersigned, declaring that this thesis, which I hereby submit for the degree of **Masters of Science (MSc) in Agriculture (with specialization in Soil Science)** at the University of Pretoria, is my work, except where acknowledged in the text, and has not previously been submitted by me for a degree at this or any other tertiary institution.

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ABSTRACT

Biosolids are the transformed products of sewage sludge produced through various sludge stabilization processes including drying, dosing and digestion. Biosolids are commonly applied to agricultural lands to improve soil organic matter, supply plant nutrients, and thus rehabilitate degraded lands. Hence, improving crop production and playing a role in poverty alleviation. However, there is little or no information on the long-term effects of biosolid use in agricultural lands on soil chemical parameters and accumulation of emerging contaminants in sub-tropical Africa in general and in South Africa in particular. The aim of this study was, therefore, to investigate the long-term (> 15 years) effects of biosolid use in agricultural lands on a) soil organic matter, nitrogen, salinity, and soil pH, b) the accumulation of sulfamethoxazole and triclosan in the top 30 cm soil layer, and c) uptake of sulfamethoxazole and triclosan by maize crop. It was hypothesized that long-term (> 15 year) biosolids use in agricultural lands will, a) improve soil organic matter and nitrogen content, b) not compromise soil productivity through salt accumulation as long as soil pH is maintained through liming, c) not lead to significant accumulation of sulfamethoxazole and triclosan in the top 30 cm soil layer to compromise soil quality, and d) not lead to significant uptake of sulfamethoxazole and triclosan by plants to cause food safety related health concerns.

This study was conducted on a controlled long-term field trial (> 15 years) consisting of rainfed-irrigated and rainfed maize plots arranged in a completely randomized block design. Both rainfed and rainfed-irrigated plots were treated annually with three biosolid application rates (4, 8, and 16 t ha^{-1}), one commercial inorganic fertilizer (according to soil analysis and crop nutrient requirements) and a zero control (without biosolid and commercial inorganic fertilizer). At crop maturity, maize plants and soil samples were collected and prepared for selected soil chemical parameters (soil organic carbon, pH, electrical conductivity and total nitrogen) and emerging contaminants (triclosan and sulfamethoxazole) analyses. Soil samples were collected only from the top 30 cm soil layer (the plough layer (20 cm) plus 10 cm below the plough layer considering possible illuviation) due to high analytical costs. Soil organic matter was determined by oxidation method following Walkley-Black's procedure. Soil pH and electrical conductivity were measured in soil-water suspension of 1:2.5. Total soil nitrogen was analysed by dry combustion method using Carlo Erba NA1500 C/N analyser. Extraction of triclosan and sulfamethoxazole from soil and maize samples was performed using

Ultrasound Assisted Extraction (UAE) method. Prior to extraction, samples were spiked with a known concentration of a surrogate standard (triclosan, TCS $^{13}C_{12}$) to assess the method performance. The mean recovered concentrations of TCS $^{13}C_{12}$ ranged from 75 % (in leaves) to 105 % (in soil). Clean-up was performed using Supelclean™ ENVI™-18 cartridges. Identification and quantification of the two targeted compounds, triclosan and sulfamethoxazole, was achieved with the aid of Shimadzu Liquid Chromatograph Mass Spectrometer (LC-MS/MS).

Long-term biosolid applications to agricultural land significantly ($p \leq 0.0001$) improved soil organic matter and total nitrogen in the uppermost 30 cm soil layer under both rainfed-irrigated and rainfed maize cropping systems. This was, however, associated with a significant ($p \leq 0.0001$) increase in soil acidity (declining soil pH) and salinity (EC), which may limit soil productivity subsequently lowering crop production. The level of increment in soil salinity was, however, too low to cause concerns in soil productivity. Therefore, hypotheses a and b were accepted. Long-term biosolid applications to agricultural lands at 16 t ha^{-1} yr^{-1} and lower rates did not result in traceable concentration levels of triclosan and sulfamethoxazole in the top 30 cm soil layer under both rainfed and rainfed-irrigated maize cropping systems (i.e. below the detection limits, which were 1.963 ng/g for triclosan and 0.64 ng/g for sulfamethoxazole). Similarly, sulfamethoxazole concentration in maize crop planted to long-term biosolid amended soils was below the detection limits (i.e. 14.30 ng/g). However, triclosan was detected in different above ground biomass parts of maize planted to soils that received 8 and 16 t ha^{-1} biosolids annually under both rainfed-irrigated and rainfed cropping systems. Maize stems recorded the highest triclosan concentration (666 - 892 ng/g dry weight), whereas maize grains recorded the least concentration (35.5 - 42.8 ng/g dry weight). The estimated daily triclosan intakes (EDIs) from the current study ranged between 0.21 µg Kg^{-1} day^{-1} for adults (individuals older than 18 years) and 1.25 µg Kg^{-1} day^{-1} for toddlers (children younger than 5 years), which was well below the Acceptable Daily Intake (ADI) of triclosan (83 µg/Kg/day).

It can be concluded that, long-term biosolids use in agricultural lands could serve as a climate change action strategy through carbon sequestration, as a poverty alleviation by improving soil fertility, as a land rehabilitation strategy by increasing soil organic matter, and for cleaner water and environment by reducing nitrate and phosphate contamination of surface and ground water.

Therefore, biosolids of similar quality with the current study could be applied at rates of 16 t ha^{-1} yr^{-1} and lower in agricultural lands under similar agro-ecological zones without contaminating the soil with triclosan and sulfamethoxazole and causing concerns in human health risks via dietary intake. It is recommended that biosolid application practices be synchronised with proper lime application strategies to remediate soil acidification associated with biosolids use in agricultural lands. To clearly understand the fate of triclosan and sulfamethoxazole in the soil-plant system, future studies should focus on monitoring the dynamics of triclosan, sulfamethoxazole as well as their transformation products in the whole soil profile and plant biomass.

Keywords: Biosolids, Triclosan, Sulfamethoxazole, Rainfed-Irrigated, Rainfed.

CHAPTER 1

INTRODUCTION

1.1 Background information

Food insecurity is one of the fundamental challenges of the century in sub-Saharan African countries. Sub-Saharan African countries remain at the top of the world for having people living in hunger and starvation (Clover, 2003). Close to 33 % of people living in Africa are malnourished (Kidane *et al.*, 2006). The major causes of food insecurity are the degradation of agricultural lands, rapid rise in human population and the prevailing climate variability (Hendrix and Glaser, 2007; Vlek *et al.*, 2010). Land degradation is mainly caused by continuous cultivation with low remediation, the use of low organic matter soil amendments as well as land-use change practices (Mwangi, 1997; Sheffield *et al.*, 2014). There is a need to restore the degraded lands in sub-Sahara African countries including South Africa, in order to secure food security.

The use of biowaste products in agricultural lands could help to address food insecurity challenges faced by the sub-Saharan African countries. Biowaste fertilizers can improve soil organic matter, supply crop nutrients and thus rehabilitate degraded agricultural lands (Eden *et al.*, 2017). This will lead to increase crop production in agricultural lands. The use of biowastes in agricultural lands could also play a significant role in (i) a circular economy by recovering valuable non-renewable crop nutrient such as phosphorous which at the moment its level is rapidly declining on our planet (Chojnacka *et al.*, 2020) and (ii) farms by lowering crop production costs through the reduction of the use of commercial inorganic fertilizers which are relatively expensive for most smallholder farmers (Lazarova *et al.*, 2012).

The negative perception of the society about biowastes due to the lack of knowledge has played a huge role in the use of biowastes as fertilizers in agricultural lands. Many people perceived biowastes as being harmful to the environment and human health (Collivignarelli *et al.*, 2019). This is highly expected because of their origin (i.e. dumbing sites, animal facilities and wastewater treatment plants). However, efforts have been made in this regard and guidelines

have been developed in many countries to classify biowastes as suitable and non-suitable for use in agricultural lands in order to limit the risks of using biowaste in agricultural lands. In South Africa, for example, the South African sludge guideline is used to classify the sludge suitability for use in agricultural lands based on their microbiological, pollutant, stability classes (Snyman and Herselman, 2006).

Biosolids are one of the most important biowaste products that can be used in agricultural lands to improve soil organic matter, supply crop nutrients and thus rehabilitate degraded agricultural lands (Bravo-Martín-Consuegra *et al.*, 2016; Antonelli *et al.*, 2018; Hamdi *et al.*, 2019). The rehabilitation of degraded agricultural lands could play significant role in improving land productivity and subsequently playing crucial role in poverty alleviation (Mohamed *et al.*, 2018). Biosolids applied in agricultural lands improved the grain yield of maize crop (Tesfamariam *et al.*, 2009), cowpea (Tomocski *et al.*, 2016), barley (Antolın *et al.*, 2005), carrots (Neilsen *et al.*, 1998) and chard (Neilsen *et al.*, 1998). Similarly, the use of biosolids in agricultural lands improved soil water-holding capacity (Nicholson *et al.*, 2018), infiltration rate (Nicholson *et al.*, 2018), aggregate stability (Nicholson *et al.*, 2018), soil microbial biomass (Banerjee *et al.*, 1997), total nitrogen (Antonelli *et al.*, 2018) and available phosphorus (Cortellini *et al.*, 1996). It is also apparent that the recycling of biosolids in agricultural lands is aligned to the United Nation Sustainable Development Goals (UNSDGs) especially to SDG 12 “Responsible consumption and production, target 12.5, promote the substantial reduction of waste generation through prevention, reduction, recycling and reuse” (UN, 2015). Other SDGs aligned to the use biosolids in agricultural lands include SDG 1 (poverty alleviation), SDG 2 (zero hunger), SDG 6 (clean water and sanitation), SDG 13 (climate action) and SDG 15 (life on land).

1.2 Problem statement

In spite of the above merits on biosolids, there are human and environmental health concerns associated with the use of biosolids in agricultural lands. These concerns include: pathogens, microplastics, trace metals and emerging contaminants added with biosolids in agricultural lands. The detection of emerging contaminants in biosolids amended soils (Gottschall *et al.*, 2012) and crops planted in biosolid amended soils such as, radish (Fu *et al.*, 2016), wheat (Jachero *et al.*, 2016; Li and Ma, 2016), spinach (Navarroa *et al.*, 2017), tomato (Navarroa *et*

al., 2017), carrots (Fu *et al.*, 2016; Li *et al.*, 2020b) and celery (Li *et al.*, 2020b) have been reported. Human health risk assessment from most previous studies, however, suggest that emerging contaminants pose negligible or minimal toxicity on human health via dietary intake of contaminated plants grown on biosolids amended soils (Prosser and Sibley, 2015). This is mainly due to the low concentration of emerging contaminants in plants grown on biosolids fertilized soils (Prosser and Sibley, 2015). Triclosan and sulfamethoxazole are two of the commonly detected emerging contaminants of concern in biosolids and biosolids amended agricultural soils as a consequence of the failure of wastewater treatment plants to completely degrade them from wastewater (Gogoi *et al.*, 2018). Triclosan and sulfamethoxazole accumulation in plants grown from soils amended with biosolids have been reported (Holling *et al.*, 2012; Pannu *et al.*, 2012; Prosser *et al.*, 2014a; Prosser *et al.*, 2014b; Jachero *et al.*, 2016). However, there is still no published information on the levels of triclosan and sulfamethoxazole uptake by crops and their accumulation in the soil profile from a long-term biosolid amended agricultural soil, which is crucial information required to clearly understand the impacts of long-term biosolids use in agricultural lands with regards to sulfamethoxazole and triclosan groundwater contamination and trophic level transfer.

The present sludge guideline for South Africa does not include emerging contaminants among the factors that determine the use of biosolids on agricultural lands even though it provides restrictions based on heavy metal and pathogen contents. This is mainly due to the lack of conclusive scientific findings globally and locally.

1.3 Justification of the study

There is quite an extensive literature related to the effect of biosolids application on soil chemical parameters including pH, organic matter, electrical conductivity and total nitrogen under real field conditions. The information comprises of studies conducted all over the globe including in Europe (Bravo-Martín-Consuegra *et al.*, 2016; Calleja-Cervantes *et al.*, 2017; Protano *et al.*, 2020), Asia (Keramati *et al.*, 2010; Dede *et al.*, 2017), Africa (Zoghlami *et al.*, 2016; Hamdi *et al.*, 2019) and Indian and Pacific Ocean (Wijesekara *et al.*, 2017) under different soils including sandy (Ozores-Hampton *et al.*, 2011; Bravo-Martín-Consuegra *et al.*, 2016; Zoghlami *et al.*, 2016; Hamdi *et al.*, 2019; Amorim Júnior *et al.*, 2021), silt (Dede *et al.*, 2017), clay loam (Roig *et al.*, 2012a; Tziachris *et al.*, 2017; Skowrońska *et al.*, 2020; Ippolito

et al., 2021), silt clay loam (Calleja-Cervantes *et al.*, 2017), loam (Schroder *et al.*, 2008) and sandy loam (Keramati *et al.*, 2010; Hamdi *et al.*, 2019). Information is also available for studies conducted under a wide range of climatic conditions such as the continental (Bravo-Martín-Consuegra *et al.*, 2016; Calleja-Cervantes *et al.*, 2017; Tziachris *et al.*, 2017), tropical (Amorim Júnior *et al.*, 2021), temperate (Protano *et al.*, 2020) and arid (Zoghlami *et al.*, 2016; Hamdi *et al.*, 2019) conditions. These studies used biosolids and sludges of various wastewater treatment origins including anaerobic digestion (Roig *et al.*, 2012a; Ippolito *et al.*, 2021), aerobic digestion (Dede *et al.*, 2017; Skowrońska *et al.*, 2020) and activated sludge (Zoghlami *et al.*, 2016; Hamdi *et al.*, 2019).

Despite this, there is little or no information on the long-term effects of biosolid use in agricultural lands on soil organic matter, pH, electrical conductivity, total nitrogen and soil chemical parameters in general in the sub-Saharan Africa in general and in Southern Africa in particular. The information is crucial to update the current South African sludge guideline and adopting the best sustainable method of managing and using biosolids in agricultural lands. However, in order to achieve this goal, a wide range of locally based studies under different cropping systems, soils and climate should be conducted. It is with hope that the current study will help inform policy makers, researchers, farmers and the general public about the effects of long-term (> 15 years) consecutive biosolid applications in agricultural lands on selected soil chemical parameters, in particular soil organic matter, pH, electrical conductivity and total nitrogen under rainfed and rainfed-irrigated maize cropping systems.

The accumulation of triclosan in biosolids amended soils depends on various factors including, but not limited to, its concentration in the biosolids used (Langdon *et al.*, 2012), biosolids application rate (Lozano *et al.*, 2010; Langdon *et al.*, 2012; Jachero *et al.*, 2016) and the frequency of biosolid applications (once-off or repeated application) (Lozano *et al.*, 2010; Chen *et al.*, 2014a; Chen *et al.*, 2014b). Chen *et al.* (2014a) reported that multiple biosolid applications and the use of high biosolid application rates can lead to high accumulation of triclosan in the soil. The general idea is that, once triclosan is introduced to the soil, it can interact with the soil and living organisms, translocate to deeper soil layers, and can be taken up by plant roots. These pathways influence triclosan dissipation and its availability in the soil. However, it is important to highlight that there are factors controlling or affecting these routes,

hence triclosan availability in the soil following biosolid application (s). These factors include among others, the type of biosolids used (liquid or dewatered) (Lapen *et al.*, 2008; Al-Rajab *et al.*, 2009), biosolids application rate (Fu *et al.*, 2016; Jachero *et al.*, 2016), biosolid placement method (surface or subsurface placement) (Topp *et al.*, 2008; Al-Rajab *et al.*, 2015), presence of vegetation in the soil (Davis *et al.*, 2015), climatic factors (Butler *et al.*, 2012; Gottschall *et al.*, 2012; Dodgen *et al.*, 2015), soil parameters (Butler *et al.*, 2012; Langdon *et al.*, 2012; Chen *et al.*, 2014b; Jachero *et al.*, 2016; Chen *et al.*, 2020), and triclosan chemical parameters (mobility, solubility, partitioning ability) (Edwards *et al.*, 2009; Wu *et al.*, 2013; Al-Rajab *et al.*, 2015).

Previous studies have investigated the dissipation of triclosan in biosolids amended soils (Lozano *et al.*, 2010; Xia *et al.*, 2010; Butler *et al.*, 2012; Langdon *et al.*, 2012; Lozano *et al.*, 2012; Chen *et al.*, 2014a; Chen *et al.*, 2014b; Bourdat-Deschamps *et al.*, 2017; Chen *et al.*, 2020). Some of these studies were conducted in soils that received a single low biosolid application rate (Butler *et al.*, 2012), some in soils that received single high biosolid application rate (Langdon *et al.*, 2012; Lozano *et al.*, 2012), whereas some in soils that received few consecutive years of both low and high biosolid application, up to four years (Lozano *et al.*, 2010; Chen *et al.*, 2014b). There are very few exceptional long-term studies such as that by Xia *et al.* (2010). However, Xia *et al.* (2010) study was conducted in mine rehabilitated lands (calcareous spoils) hence does not represent typical agricultural soils physically, chemically and biologically.

Triclosan mobility within the soil profile and its uptake by crops is a serious concern for ground water contamination and trophic level transfer, respectively. To date, studies have only determined the levels of triclosan uptake by crops (Gottschall *et al.*, 2012; Holling *et al.*, 2012; Sabourin *et al.*, 2012; Prosser *et al.*, 2014b) and its accumulation in the soil profile (Lapen *et al.*, 2008; Edwards *et al.*, 2009; Gottschall *et al.*, 2012) following both single low and high biosolid application rate to agricultural soil. Most of these studies (Lapen *et al.*, 2008; Edwards *et al.*, 2009; Gottschall *et al.*, 2012; Sabourin *et al.*, 2012; Prosser *et al.*, 2014b), however, were conducted in Canada under silt clay loam, silt loam and loam soils receiving high rainfall and variable seasonal temperatures. There is also a variation on how previous studies have treated their soils with biosolids. Some used liquid biosolids pre-spiked with triclosan (Lapen *et al.*,

2008), others used dewatered biosolids in soils that had never received biosolids (Gottschall *et al.*, 2012; Sabourin *et al.*, 2012), whereas some used dewatered biosolids but in soils that had been amended with triclosan pre-spiked liquid biosolids (Edwards *et al.*, 2009). Despite this, there is still no published information, to our knowledge, on the level of triclosan uptake by crops and its accumulation in the soil profile from a long-term biosolid amended agricultural soil, which received biosolids on yearly bases according to crop nutrient requirements. Such information is crucial to understand the impacts of long-term biosolids use in agricultural lands with regards to triclosan groundwater contamination and trophic level transfer. The information is also crucial to amend the current South African sludge guideline which currently does not have restrictions and regulation related to risks from emerging contaminants added with biosolids in agricultural lands. The current study will inform the levels of triclosan uptake in crops and accumulation in the soil from a long-term (> 15 years) biosolid amended agricultural soil under rainfed and rainfed-irrigated maize cropping system.

The availability of sulfamethoxazole in the soil following biosolid application (s) is controlled by various factors. Some of these factors include, sulfamethoxazole concentration in biosolids (Gonod *et al.*, 2022), the type of biosolid used (e.g. liquid or dewatered) (Wu *et al.*, 2015), climatic factors (Wu *et al.*, 2012; Srinivasan and Sarmah, 2014; Zhang *et al.*, 2016b), soil parameters (Liu *et al.*, 2010; Srinivasan and Sarmah, 2014; Zhang *et al.*, 2016b), sulfamethoxazole chemical parameters (Dodgen *et al.*, 2015), and plant processes (e.g. uptake and transpiration rate) (Dodgen *et al.*, 2015). These factors also affect the plant uptake and mobility of sulfamethoxazole within the soil profile. There are major environmental and human health concerns related to sulfamethoxazole availability in agricultural soils. Some of which include groundwater contamination through leaching and the transfer of sulfamethoxazole to various trophic levels including humans through crops uptake. To date, studies have only reported the level of sulfamethoxazole uptake by crops and its accumulation in the soil profile following a single low biosolid application rate to the soil under both greenhouse (Holling *et al.*, 2012) and real field conditions (Lapen *et al.*, 2008; Edwards *et al.*, 2009). Most of these studies (Lapen *et al.*, 2008; Edwards *et al.*, 2009) were conducted in Canada under silt clay loam soils receiving high rainfalls and variable seasonal temperatures. Both liquid (Lapen *et al.*, 2008) and dewatered (Edwards *et al.*, 2009) biosolids were used in the previous studies. Despite this, there is still no published information on the level of sulfamethoxazole uptake by crops and its accumulation in the soil profile from a long-term biosolid amended agricultural

soil, which is crucial information required to clearly understand the impacts of long-term biosolids use in agricultural lands with regards to sulfamethoxazole groundwater contamination and trophic level transfer. The information is also crucial and needed towards amending the existing South African sludge guideline. The current study will inform the level of sulfamethoxazole uptake in crops and accumulation in the soil from a long-term (> 15 years) biosolid amended agricultural soil under rainfed and rainfed-irrigated maize cropping system.

1.4 Objectives

1.4.1 Main objective

The overall objective of this study was to investigate the effects of long-term biosolids use in agricultural lands on selected soil chemical properties, the fate of triclosan and sulfamethoxazole in the soil, uptake by maize crop, and potential human health risks via dietary intake under rainfed and rainfed-irrigated systems.

1.4.2 Specific objectives

1. To investigate the effects of long-term biosolids land applications on soil organic matter, electrical conductivity, pH and total nitrogen,

2. To quantify the concentration of triclosan and sulfamethoxazole in soils amended with biosolids for more than 15 years under rainfed and rainfed-irrigated conditions,

3. To quantify the uptake of triclosan and sulfamethoxazole by maize planted in soils amended with biosolids for more than 15 years under rainfed and rainfed-irrigated conditions and

4. To determine human health risks of triclosan and sulfamethoxazole from maize grain consumption planted to biosolids amended soils.

1.5 Hypotheses

It was hypothesized that the long-term (> 15 year) use of biosolids in agricultural lands will:

1. Improve soil organic matter and nitrogen content,

2. Not compromise soil productivity through salt accumulation as long as soil pH is maintained through liming,

3. Not lead to significant accumulation of sulfamethoxazole and triclosan in the top 30 cm soil layer to compromise soil quality, and

4. Not lead to significant uptake of sulfamethoxazole and triclosan by plants to cause food safety related health concerns.

1.6 Thesis subdivisions

The thesis consists of seven chapters. The content of each chapter is summarized below.

Chapter 1 is an introduction. It provides background information, problem statement, justification, objectives and hypothesis.

Chapter 2 is literature review. This chapter provides a synthesized literature review on the role of biosolids use in agricultural lands in a circular economy, the benefits and limitation. It also provides a review of literature on the presence and fate of triclosan and sulfamethoxazole in biosolids amended soils.

Chapter 3 is Materials and Methods section. This chapter provides the description of the study area, sludge used at the study area, experimental set-up and the weather data of the study area. Chapter 3 also explains the sampling procedures, laboratory analysis as well as statistical method followed to analyze results.

Chapter 4 is the Results section. This chapter presents and interprets the obtained results on the effects of long-term biosolid use on: a) selected soil chemical properties, b) the accumulation of triclosan and sulfamethoxazole in the soil, and c) plant uptake of trclosan and sulfamethoxazole.

Chapter 5 is the Discussion section. Under this chapter, the effects of long-term biosolid use on: a) selected soil chemical properties, b) the accumulation of triclosan and sulfamethoxazole in the soil, and c) plant uptake of triclosan and sulfamethoxazole are discussed using the results presented in Chapter 4.

Chapter 6 is the Human health risk assessment section. This chapter quantifies human health risks associated with triclosan and sulfamethoxazole consumption via dietary food intake of maize grain planted to biosolid amended soils.

Chapter 7 is the Conclusion and Recommendation session. This chapter summarizes the core findings of this study and outlines the recommendations for future studies.

CHAPTER 2

LITERATURE REVIEW

2.1 The use of biosolids in agricultural lands in a circular economy

The world is faced with many challenges such as the scarcity of food, natural resource depletion, climate change and rapid population growth. To sustain economic growth under these conditions, it is essential to adopt sustainable ways of managing and consuming available resources and conserve them for future generations. The United Nations sustainable developmental goal (SDG) 12 "responsible consumption and production pattern", under target 12.2 encourage countries to achieve sustainable management and efficient use of natural resources by the year 2030 (UN, 2015). Similarly target 12.5 of SDG 12 promotes "substantial reduction of waste generation through prevention, reduction, recycling and reuse" (UN, 2015). This includes the use of biosolids in agricultural lands. The use of biosolids in agricultural lands play a role in a circular economy by improving soil productivity, increasing agricultural production and attract tourists in cities through the creation of greener cities (Brown *et al.*, 2020). Biosolids use in agricultural lands can also minimize crop production costs by reducing the use of commercial inorganic fertilizers which are relatively expensive for most smallholder farmers (Lazarova *et al.*, 2012).

2.1.1 Background information and historical perspective

The negative perception of the society about biosolids due to the lack of knowledge has played a huge role in the use of biosolids as fertilizers in agricultural lands. Besides, the absence of reliable treatment technologies that can produce safe and good quality sludge also contributed to this (Apedaile, 2001). Hence, many people perceived biosolids as being harmful to the environment and human health (Collivignarelli *et al.*, 2019). Recently, the perception of many people seem to have changed as new treatment technologies emerged and the knowledge of the society about the role of biosolids in the economy improved (Collivignarelli *et al.*, 2019).

2.1.2 Biosolids use in agricultural lands from the UNSDG perspective

The use of biosolids in agricultural lands could help to address several UNSDGs mandated to be achieved by 2030. The use of biosolids in agricultural lands directly or indirectly contributes to the following UNSDGs: "zero Hunger (SDG 2), climate action (SDG 13), life on land (SDG 15), and responsible consumption and production pattern (SDG 12)".

"Zero hunger (SDG 2)"

Soil organic matter enhances soil properties and thus leading to increased crop production (Barnard and du Preez, 2004). This is because organic matter improves both the nutrients and water retention in the soil by enhancing sorption of nutrient ions and water molecules and thus creating favorable conditions for root uptake. Most South African soils lack organic matter (Mills and Fey, 2004), which significantly affected the productivity of the land and thus negatively affecting crop production.

Several strategies or practices have been tested and proved to improve and maintain soil organic matter in agricultural lands. Such strategies or practices include among others, conservation agriculture, agroforestry, cover cropping, rotational grazing and the addition organic matter rich soil amendments (Lal, 2016). Biosolids are also good sources of organic matter as they consist of about 50 to 70 % organic matter (Lu *et al.*, 2012). Besides, biosolids improve soil fertility and crop yield (Qasim *et al.*, 2001; Nicholson *et al.*, 2018) because they are good sources of macronutrients (nitrogen, phosphorous, potassium, calcium, magnesium and sulfur) (Cogger *et al.*, 2006) and micronutrients (iron, boron, manganese, cobalt, cadmium, zinc, copper, lead, nickel, copper and molybdenum) (Lu *et al.*, 2012).

"Life on land (SDG 15)"

According to Töpfer (2016) land degradation costs about 300 billion United States dollars per annum globally in farming areas. About 22 % of the total cost is accounted for in the sub-Saharan African region (Töpfer, 2016). Land degradation is mainly caused by deforestation, land use change, overgrazing, climatic factors, continuous cultivation with low remediation

and the use of low organic matter soil amendments (Sheffield *et al.*, 2014). Despite farming being amongst one of the major sources of income for most people living in rural areas of the sub-Saharan African countries, the size of the cultivated land is shrinking due to degradation. Therefore, there is an urgent need to restore the degraded lands of the sub-Sahara African countries including South Africa to secure food security.

Land degradation can be minimized and rehabilitated through various practices and techniques such as conservation tillage (e.g. minimum tillage or no tillage) (Li *et al.*, 2019a; Li *et al.*, 2020c), residue retention to the soil (Li *et al.*, 2019a; Li *et al.*, 2020c), intercropping (Chen *et al.*, 2019a; Gitari *et al.*, 2019), controlled grazing (e.g. rotational grazing) (Byrnes *et al.*, 2018), vegetation regeneration (dos Santos Falcao *et al.*, 2020), cover cropping (Dozier *et al.*, 2017) and the use of organic matter input soil amendments (Wijesekara *et al.*, 2016; Li *et al.*, 2018). The use of biosolids in agricultural lands can help restore degraded lands (de Andres *et al.*, 2007) because it is a good source of organic matter (50 to 70 % by mass) and crop nutrients (Lu *et al.*, 2012), improve crop production (Tesfamariam *et al.*, 2013) and thus secure food production.

"Climate action (SDG 13)"

Climate change continues to be a major global challenge as more and more greenhouse gases such as carbon dioxide, methane, nitrous oxides and chlorofluorocarbons are being released to the atmosphere by various anthropogenic activities (Mirolyubova *et al.*, 2017). Between the years 1750 and 2020, the global atmospheric carbon dioxide levels increased exponentially from 280 to 409.8 ppm (Lindsey, 2020). Climate change impacts include severe drought, floods, soil erosion, melting of glaciers and rising sea levels. Reports show that Africa would be the worst to face severe drought as a consequence of climate change (Wallace, 2000). One of the serious concerns with low rainfall and extreme high atmospheric temperatures in agricultural lands is the resulting reduction in crop production (Mafakheri *et al.*, 2010; Iqbal *et al.*, 2020). There is an undoubtedly urgent need to reduce the emission of greenhouse gases including carbon dioxide to limit the impacts of climate change on food security.

There are a number of practices that can be used to limit the release of greenhouse gases including carbon dioxide from agricultural land. Such practices include but not limited to, conservation tillage (Lal and Kimble, 1997; Dong *et al.*, 2021), growing nitrogen fixing crops (Bayer *et al.*, 2016), cover cropping (Bayer *et al.*, 2016), grazing management (e.g. rotational grazing) (Carvalho *et al.*, 2014) and the use of organic materials in agricultural lands to enhance carbon sequestration (Sarfraz *et al.*, 2019; Wijesekara *et al.*, 2021). The use of biosolids in agricultural lands is viewed as one of the climate change action strategy by enhancing carbon sequestration in the soil and thus reducing its emission to the atmosphere (Wijesekara *et al.*, 2016). For instance, biosolids application to clay loam and sandy loam soils at a single 70 Mg ha^{-1} increased total organic carbon of the two studied soils by more than 45 % in the top 15 cm soil layer (Wijesekara *et al.*, 2017).

"Responsible consumption and production pattern (SDG 12)"

There is a serious concern with regard to the depletion of non-renewable natural resources in the world. Such resources include freshwater (Wyman, 2013), natural gas (Bentley, 2002), oil (Bentley, 2002), coal (Hook and Tang, 2013) and phosphorous (Cordell and White, 2011). Phosphorous as one of the most important macronutrients responsible for plant growth is expected to reach its peak depletion soon (Cordell and White, 2011). Shortages of phosphorous would results in low crop production in farming areas and thus lead to food scarcity problems (Cordell *et al.*, 2009; Chowdhury *et al.*, 2017). The United Nations general assembly has, therefore, sets a goal (SDG 12) as a mandate to all countries to "achieve sustainable management and efficient use of natural resources by the year 2030" (UN, 2015). One of the agreement was that, there must be reduction in waste generation and the increase in recycling and reuse of generated waste materials (UN, 2015). The recycling of biosolids in agricultural lands could serve as both waste management practice and a sustainable nutrient management practice by returning essential nutrients including phosphorous to the soil. Biosolids contain between 1.5 and 3.0 % phosphorous (Cogger *et al.*, 2006) and have been used as a source of phosphorous in agricultural lands to improve crop production (Tesfamariam *et al.*, 2009; Tesfamariam *et al.*, 2013).

2.1.3 Current trend and perspectives

Biosolids use in agricultural lands seems to be the most common sludge management or disposal option in most European countries (Table 2.1). Spain, United Kingdom and Ireland use more than 70 % of the total sludge produced in agricultural lands (Table 2.1). The mean and median biosolid use in agricultural lands within the European countries is 36 and 27, respectively. While the maximum and minimum usage of biosolids in agricultural lands is 80 % (Spain) and 2 % (Austria), respectively. In South Africa, less than 30 % of the sludge produced annually is used in agricultural lands (Lotter and Pitman, 1997). Considering the level of land degradation and the associated low productivity as well as large proportion of low income small holder farmers in South Africa, there is great potential to expand the use of biosolids in agricultural lands. Some of the main reasons for the low usage of biosolids in agricultural lands include: the lack of knowledge or awareness and access to biosolids in rural areas because most treatment plants are based in cities or urban areas where farm activities are minimal.

Table 2. 1: Total sludge produced in selected European countries and the % of sludge used in agricultural lands (Eurostat, 2021)

Country	**Annual total sludge produced (thousand tons (year))**	**% of sludge applied to agricultural lands**
Austria	234481 (2018)	2.05
Belgium	176.3 (2010)	9.81
Denmark	141 (2010)	52.48
France	1174 (2017)	25.47
Germany	1794443 (2016)	23.60
Greece	119768 (2016)	17.97
Ireland	58773 (2017)	79.10
Italy	11027 (2010)	28.62
Luxembourg	9156 (2015)	34.36
Poland	58307 (2018)	20.29
Portugal	11917 (2016)	11.65
Spain	11744 (2016)	80.18
Sweden	2109 (2018)	39.02
United Kingdom	11367 (2012)	74.29

2.2 Limitations of using biosolids in agricultural lands

Biosolids improve soil physical (Nicholson *et al.*, 2018), chemical (Tesfamariam *et al.*, 2013) and biological (Banerjee *et al.*, 1997) properties hence enhancing soil productivity (Tesfamariam *et al.*, 2013) and crop yield (Tesfamariam *et al.*, 2013) contributing to poverty alleviation. However, there are concerns from the public in general and the scientific community in particular about the health and environmental safety associated with heavy metals, pathogens, microplastics and emerging contaminants added with sludge into agricultural lands (Collivignarelli *et al.*, 2019). These concerns are briefly discussed in the following sub-sections.

2.2.1 Heavy metals

Despite the fact that trace metals are needed for plant development and growth at low amounts, excess accumulation of heavy metals in the soil can result in plant toxicity and subsequently lower crop production (Fliebach *et al.*, 1994). Biosolids may contains heavy metals which could be accumulated in the soil and transferred to plants through the roots when biosolids are applied as fertilizers to the soil (Ogbazghi *et al.*, 2015). Heavy metals of concern in biosolids include arsenic, zinc, cadmium, lead, mercury, selenium, nickel and molybdenum (EPA, 1994; Snyman and Herselman, 2006). To date, several countries including South Africa and United states have included restrictions in their guidelines with regard to the levels of heavy metals in biosolids considered to be feasible for use in agricultural lands (EPA, 1994; Snyman and Herselman, 2006).

The amount of heavy metals that accumulates in the soil from sludge application depends on a number of factors including the sludge application rate (Logan *et al.*, 1997; Eid *et al.*, 2017), sludge type (Gove *et al.*, 2001) and sludge origin (Silveira *et al.*, 2003). Application of liquid biosolids has been reported to cause greater accumulation of heavy metals as opposed to the application of dry biosolids (Gove *et al.*, 2001). This is because dewatering processes reduces the content of heavy metals in sludge (Gove *et al.*, 2001). The amounts of heavy metals in municipal sludge are lower than that from industrial origin (Silveira *et al.*, 2003). According to Evanylo *et al.* (2006), the higher the application rate of biosolids, the greater the accumulation

of heavy metals. This is due to an increase in the amount of heavy metals added to the soil for every increase in biosolid applications rate (Evanylo *et al.*, 2006).

2.2.2 Pathogens

Biosolids may contain human pathogens which could be transferred to agricultural lands when biosolids are applied as fertilizers to the soil (Al-Gheethi *et al.*, 2018; Collivignarelli *et al.*, 2019). The amount of pathogens transferred to agricultural lands will, however, depend on the wastewater treatment method used to produce biosolids (Wang *et al.*, 2008). Some wastewater treatment methods such as pasteurization, thermal hydrolysis, aerobic digestion and anaerobic digestion are more effective in reducing pathogens from biosolids as compared with some treatment methods such as lime stabilization (Wang *et al.*, 2008). Telles Benatti *et al.* (2002) investigated the removal of *P.aeruginosa* pathogenic bacteria by anaerobic digestion treatment and reported more than 94 % removal of the bacteria. Pathogens which have been added with sludge into agricultural lands may bring onsite human health risks to people living around the farm and offsite health risks through transportation by runoff water to aquatic environments (Clarke *et al.*, 2017). Several countries including South Africa have included restriction in their guidelines with regard to the level of micro-organisms in biosolids considered to be feasible for use in agricultural lands (Snyman and Herselman, 2006).

2.2.3 Microplastics

Microplastics are the division products of larger plastic materials and are generally classified by a diameter size less than 5 mm (Crossman *et al.*, 2020). The use of biosolids in agricultural lands is one of the most common practices that result in the accumulation of microplastics in agricultural environments (Mohajerani and Karabatak, 2020; Rolsky *et al.*, 2020). It is estimated that more than 90 % of microplastics that reach wastewater treatment plants are removed from wastewater and end up in the sludge (Li *et al.*, 2020a). Eliminating microplastics from sludge is not easy (Talvitie *et al.*, 2017). This is because during sludge treatment processes, microplastics may be further divided into smaller particles (Nano plastics) which are even more difficult to remove (Mahon *et al.*, 2017). However, some sludge treatment process such as anaerobic digestion (Mahon *et al.*, 2017) have shown the effectiveness in reducing microplastics from sludge compared to lime stabilization processes (Mahon *et al.*, 2017). The presence of microplastics in agricultural lands can affect soil properties (de Souza

Machado *et al.*, 2019) and plant performance (de Souza Machado *et al.*, 2019). There are currently no restrictions included in sludge guidelines from various countries including South Africa with regard to the level of microplastics in biosolids considered to be feasible for use in agricultural lands.

2.2.4 Emerging contaminants

The term "emerging contaminants" refers to groups of unregulated/regulated compounds originating from various sources such as hospitals, industries, livestock vaccination facilities, household and agricultural lands (Gogoi *et al.*, 2018). Most common emerging contaminants groups include pesticides, hormones, industrial chemicals, personal care product compounds, and pharmaceutical compounds (Stefanakis and Becker, 2016). Biosolids may contain emerging contaminants which could be transferred to agricultural lands when they are applied as fertilizers to the soil (Mohapatra *et al.*, 2016). However, it is important to highlight that the amount of emerging contaminants transferred to the soil from biosolids application depend on their concentration in biosolids (Langdon *et al.*, 2012), biosolids application rate (Lozano *et al.*, 2010; Langdon *et al.*, 2012; Jachero *et al.*, 2016) and wastewater treatment method (s) used to produce biosolids (Ratola *et al.*, 2012). Commonly used wastewater treatment methods including aerobic and anaerobic digestion do not completely remove emerging contaminants from sludge (Ratola *et al.*, 2012). However, further treatment techniques such as composting and thermal drying showed the ability to additionally remove contaminants from sludge (Roig *et al.*, 2012b). Despite these efforts, emerging contaminants continue to accumulate in the final sludge or biosolids due to sorption on organic matter (Ratola *et al.*, 2012). There are no restrictions included in sludge guidelines from various countries including South Africa with regard to the level of emerging contaminants in biosolids considered to be feasible for use in agricultural lands. Many countries including South Africa have only regulated the levels of heavy metal and pathogens in sludge used for agricultural production (Snyman and Herselman, 2006). The presence of emerging contaminants in the soil can alter soil processes (Chen *et al.*, 2019b) and affect plant performance (An *et al.*, 2009). The current study focus is on two of the most commonly persistent emerging contaminants, triclosan and sulfamethoxazole, and this literature review will provide a brief summary on them.

2.3 Triclosan and sulfamethoxazole

2.3.1 Triclosan and sulfamethoxazole in biosolids

Personal care product compounds such as hand soaps, deodorants, shampoos and toothpaste contain the antimicrobial substance triclosan, which can form part of the wastewater stream (Gottschall *et al.*, 2012). Triclosan is characterized by a high octanol-water partitioning coefficient (Log K_{ow} = 4.8), which enables it to strongly sorb onto sludge and persist during wastewater treatment (Chen *et al.*, 2014a). Land application of biosolid leads to the addition of triclosan to agricultural soils (Lozano *et al.*, 2010; Butler *et al.*, 2012). Sulfamethoxazole is often prescribed by health professionals as an antibiotic to treat urinary tract bacterial infection in humans (Wang and Wang, 2018). However, human bodies do not completely metabolize antibiotics (Daughton and Ternes, 1999). As a result, antibiotics such as sulfamethoxazole may form part of the municipal wastewater stream after being excreted by human bodies (Daughton and Ternes, 1999). In addition, direct flushing of antibiotics in toilets from household level can also contribute to wastewater contamination (Daughton and Ternes, 1999). Similar to triclosan, sulfamethoxazole is removed from wastewater during wastewater treatment and form part of the sludge (Gottschall *et al.*, 2012). Consequently, sulfamethoxazole end up in the soil when biosolids are used as fertilizers (Holling *et al.*, 2012).

2.3.2 Triclosan and sulfamethoxazole in agricultural lands

There are several sources of triclosan and sulfamethoxazole in agricultural lands. Some of which includes the use of animal manure (Mullen *et al.*, 2019), wastewater (Sallach *et al.*, 2015; Zhang *et al.*, 2016b) and biosolids (Lozano *et al.*, 2010; Butler *et al.*, 2012). For the purpose of this study, focus is mainly on the presence of triclosan and sulfamethoxazole in agricultural lands from biosolids application. The presence of triclosan and sulfamethoxazole in agricultural lands can have negative impacts on: (i) the soil, (ii) plants, (iii) humans and (iv) aquatic environment.

I. Soil

The presence of triclosan and sulfamethoxazole in the soil can affect the soil microbial population (Waller and Kookana, 2009; Buttler *et al.*, 2011) and activity in general. Triclosan is capable of reducing soil respiration (Waller and Kookana, 2009), changing nitrogen cycle (Chevillot *et al.*, 2018; Chen *et al.*, 2019b) and inhibiting enzyme activities in the soil (Liu *et*

al., 2009). Soil triclosan level of 10 mg Kg^{-1} was reported to inhibit soil respiration (Waller and Kookana, 2009; Buttler *et al.*, 2011). Sulfamethoxazole can alter the function of microbial community (Liu *et al.*, 2012) and their composition (Archudia *et al.*, 2021) in the soil.

II. Plants

Triclosan and sulfamethoxazole can affect plant development and performance. Triclosan can affect seed germination (Stevens *et al.,* 2009) and reduce plant growth (Stevens *et al.,* 2009; Prosser *et al.*, 2014a). Wheat roots elongation was reduced when wheat plants were subjected to triclosan levels between 50 and 250 mg L^{-1} (An *et al.*, 2009). Sulfamethoxazole was reported to cause phytotoxic effect on plants (Zhu *et al.*, 2020) by damaging plant roots and reducing photosynthetic efficiency (Lv *et al.*, 2021).

III. Human health

Most previous studies have shown that consumption triclosan from contaminated plants grown from biosolids amended soils pose negligible risks to human health (Prosser and Sibley, 2015). This is because the concentrations of triclosan in plants grown from biosolids amended soils are generally low (Prosser *et al.*, 2014b) due to enhanced triclosan sorption in the soil caused by organic matter from biosolids (Fu *et al.*, 2016). Nonetheless, there is little if any study related to the impact of biosolids-derived sulfamethoxazole on human health via dietary intake.

IV. Aquatic ecosystem

Biosolids application to agricultural lands can have offsite negative impacts from triclosan and sulfamethoxazole contamination in aquatic environments through transportation by runoff water (Rabiet *et al.,* 2006; Matamoros *et al.,* 2012). Triclosan and sulfamethoxazole presence in aquatic environment can affect the growth, reproduction and functioning of organisms. Veldhoen *et al.* (2006) observed the reduction in the overall body weight and rate of thyroid hormone production in a tadpole after being exposed to triclosan. Delayed hatchment and reduced growth in Zebrafish was also reported after exposing Zebrafish to environmentally relevant concentrations of triclosan and sulfamethoxazole (Oliveira *et al.*, 2009; Liu *et al.*, 2020a).

2.5 Fate of sulfamethoxazole and triclosan in biosolids amended soils

Emerging contaminants including triclosan and sulfamethoxazole undergo several pathways after being introduced to the soil. According to Pullagurala *et al.* (2018), emerging contaminants can undergo microbial degradation and transformations, sorbed by the soil colloids, volatilize to the atmosphere, leached to groundwater, and taken up by plant root and translocate to aerial plant parts (Figure 2.1).

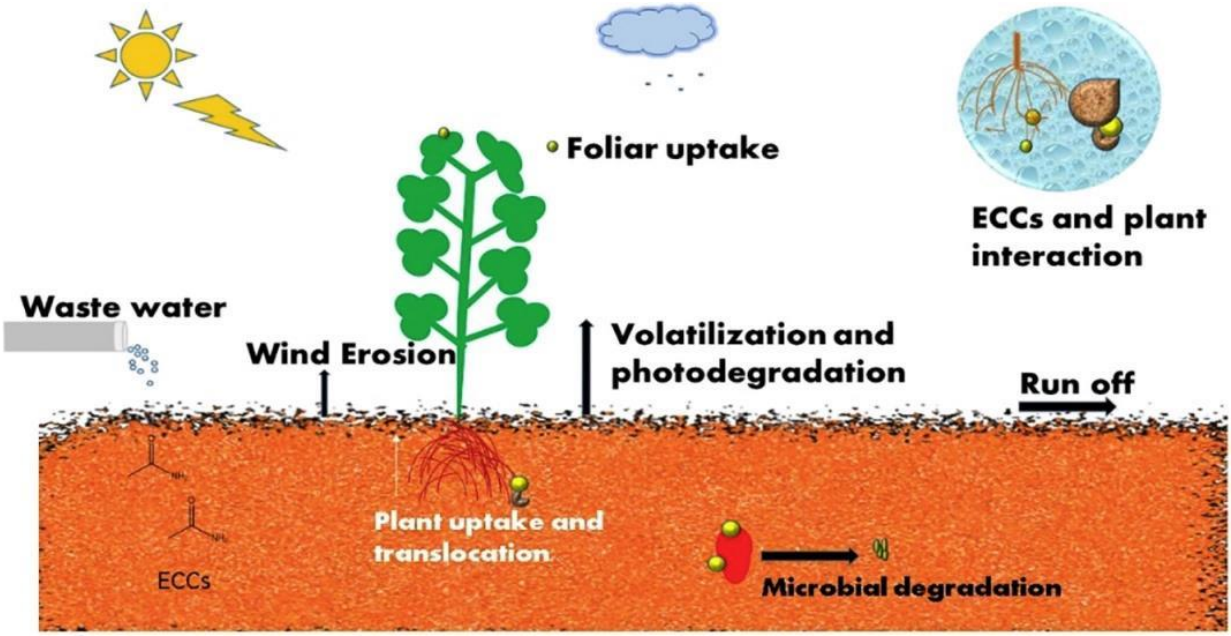

Figure 2. 1: Fate and transport of emerging contaminants in the soil (Pullagurala et al., 2018)

The fate of triclosan and sulfamethoxazole in biosolid amended soils is discussed in the following sub sections:

2.5.1 Microbial degradation and transformation

Triclosan and sulfamethoxazole can undergo microbial degradation in the soil to form methyl triclosan and N-acetyl sulfamethoxazole, respectively (Wu *et al.*, 2009; Butler *et al.*, 2012; Macherius *et al.*, 2014; Andriamalala *et al.*, 2018; Chen *et al.*, 2020). Butler *et al.* (2012) and Lozano *et al.* (2012) observed the appearance of methyl triclosan (transformation product of

triclosan) in the soil as triclosan (parent compound) concentration reduces, indicating methyl triclosan is a product of triclosan. It is worth mentioning that, the transformations of triclosan and sulfamethoxazole in the soil is a microbial driven process and it will, therefore, depend on the capability of the type of microorganisms that are present in the soil (Chen *et al.*, 2020).

2.5.2 Sorption in the soil

Chemical sorption is the ability of a compound to be held tightly on the soil colloidal surfaces. Sorption is determined by the compound octanol-water partitioning coefficient (Log K_{ow}) (Hu *et al.*, 2019). The higher the Log K_{ow}, the higher the sorption and vice versa (Hu *et al.*, 2019). Sulfamethoxazole is characterized by low Log K_{ow} (i.e. 0.89), whereas triclosan is characterized high Log K_{ow} (i.e. 4.8) (Holling *et al.*, 2012). Hence, triclosan can be strongly held on soil colloidal surfaces as opposed to sulfamethoxazole. However, it is worth mentioning that there are factors influencing triclosan and sulfamethoxazole sorption in the soil such as soil pH, organic matter and clay content (Wu *et al.*, 2009; Cantarero *et al.*, 2017; Hu *et al.*, 2019). Increase in soil organic matter enhances triclosan sorption in the soil (Fu *et al.*, 2016).

2.5.3 Volatilization

Volatilization is one of the most important features of the compound that determines its dissipation from the environment including in the soil. Chemical volatilization mainly depends on compound vapour pressure. Compounds with low vapour pressure do not easily volatilize from the environment as opposed to compounds with high vapour pressure. Triclosan and sulfamethoxazole have low vapour pressures of 7×10^{-4} Pa at 25 ^{O} C (Reiss *et al.*, 2009) and 6.9 mm Hg at 25 ^{O}C (Andriamalala *et al.*, 2018), respectively. Hence, triclosan and sulfamethoxazole volatilization is unlikely from the environment.

2.5.4 Leaching

One of the concerns with triclosan and sulfamethoxazole availability in biosolids amended soil is the contamination of ground water through leaching. Previous studies have reported triclosan and sulfamethoxazole mobility towards ground water and their presence in ground water from

biosolids amended soils (Edwards *et al.*, 2009). Triclosan and sulfamethoxazole leaching from the soil is influenced by a number of factors including triclosan and sulfamethoxazole solubility and sorption, soil organic matter, texture and climatic conditions (i.e. rainfall) (Butler *et al.*, 2012). Triclosan has a low water solubility (10 mg/L at 25 ^{O}C) and strong sorption (Log K_{ow} = 4.8) as opposed to sulfamethoxazole which has high solubility in water (610 mg/L at 25 ^{O}C) and weak sorption (Log K_{ow} = 0.89) (Holling *et al.*, 2012). Hence, sulfamethoxazole is more susceptible to leaching as opposed to triclosan. Soil organic matter enhances triclosan and sulfamethoxazole sorption in the soil (Xu *et al.*, 2009; Hu *et al.*, 2019) and thus reducing their leaching potential. Climatic factors such as rainfall can accelerate the extent of leaching of contaminants including triclosan and sulfamethoxazole from the soil (Edwards *et al.*, 2009; Gottschall *et al.*, 2012). Gottschall *et al.* (2012) investigated the leaching potential of emerging contaminants including triclosan from soils amended with a single 22 Mg ha^{-1} of municipal sludge. None of the studied compounds were present in the ground water two months post biosolid applications to the soil (Gottschall *et al.*, 2012). However, two days following a single heavy rainfall event led to the presence of emerging contaminants including triclosan in tile drainage and ground water (Gottschall *et al.*, 2012).

2.5.5 Plant uptake and translocation

One of the concerns with triclosan and sulfamethoxazole availability in agricultural lands is their transfer to plants through root uptake and their translocation to various organs including the edible parts. Previous studies have shown that different crops including cabbage (Holling *et al.*, 2012), wheat (Jachero *et al.*, 2016), lettuce (Pannu *et al.*, 2012), radish (Pannu *et al.*, 2012; Prosser *et al.*, 2014b), carrot (Prosser *et al.*, 2014b) and soybean (Prosser *et al.*, 2014b) can take up triclosan from biosolids amended soils and translocate it to various aerial organs. Sulfamethoxazole can also be taken up by plants from biosolids amended soils (Holling *et al.*, 2012). Despite this, there is still no published information, to our knowledge, on the level of triclosan and sulfamethoxazole uptake by crops and its accumulation in the soil profile from a long-term biosolid amended agricultural soil, which received biosolids on yearly bases according to crop nutrient requirements. Emerging contaminants including triclosan and sulfamethoxazole are taken up by plants through the partitioning on soil particles to the high lipid outer portion of the root tissue (epidermis or cortex) and subsequently accumulate in the roots (Trapp and Legind, 2011; Pannu *et al.*, 2012). Once absorbed by plant roots, emerging contaminants are transported by bulk water flow driven by transpiration to various plant organs

including the edible plant parts (Cantarero *et al.*, 2017; Nie *et al.*, 2020). Factors affecting plant uptake and translocation of triclosan and sulfamethoxazole are discussed in the following sub sections.

Plant characteristics

Plant characteristics differ from one plant species to another and they influence the plant uptake and translocation of contaminants in plants (Wu *et al.*, 2013). Wu *et al.* (2013) investigated the uptake of 20 personal care products and pharmaceutical compounds by pepper, cucumber, spinach and lettuce from the soil. Lettuce absorbed 13, spinach 12, cucumber 17 and peppers 15 of the studied personal care products and pharmaceutical compounds (Wu *et al.*, 2013). Figure 2.2 highlights the potentials of different crops to take up emerging contaminants (Figure 2.2). Most root (e.g. carrot and radish) and leafy (e.g. spinach and cabbage) vegetables have high potential to uptake emerging contaminants as compared to cereal crops (e.g. wheat, rice and corn) and fruit vegetable (e.g. pepper and tomatoes) (Figure 2.2). Plant roots are one of the main important features of the plant that determines its ability to take up contaminants from the soil (Pullagurala *et al.*, 2018). Plants with dicotyledonous root system (i.e. lettuce and radish) were found to have accumulated high concentrations of triclosan from the soil as opposed to plants with monocotyledonous root system (*bahiagrass*) (Shahmohamadloo *et al.*, 2017). Shahmohamadloo *et al.* (2017) associated these high triclosan accumulations in dicotyledonous plants with high lipid content found in both lettuce and radish roots.

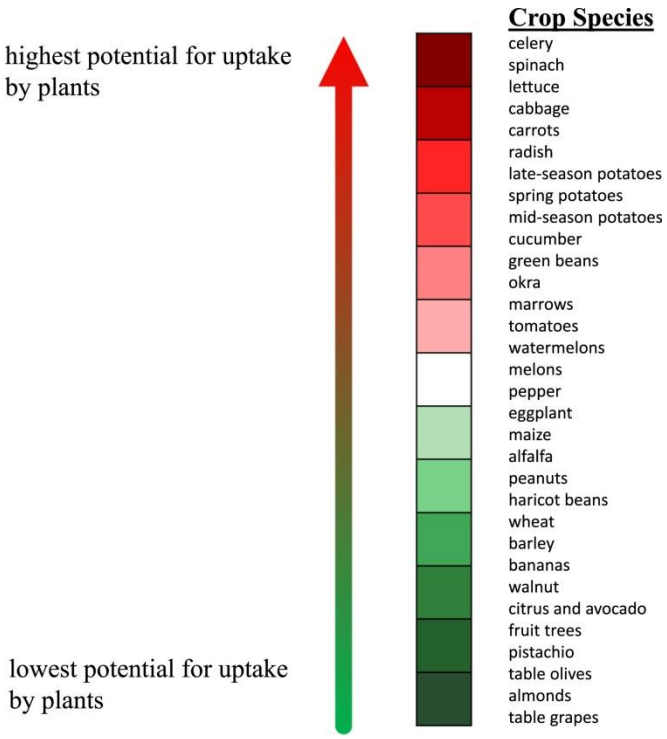

Figure 2. 2: Potential levels of different crops to take up emerging contaminants (Christou *et al.*, 2019)

Contaminant properties

Chemical properties of a contaminant can determine the contaminant uptake by plants from the soil (Jachero *et al.*, 2016; Chuang *et al.*, 2019). One of the chemical properties of a contaminant that determines its ability to be taken up by plants is the charge form that it exists in the soil (Jachero *et al.*, 2016). Studies have reported that positively charged contaminants are easily taken up by plant roots than the negatively charged contaminants (Jachero *et al.*, 2016). This is because positively charged compounds pass through the root membranes much faster than the negatively charged molecular compounds which are mostly repelled by plant roots surfaces (Trapp, 2000). Molecular size of a compound also influences the plant uptake of a compound from the soil and their translocation within the plant (Chuang *et al.*, 2019). Compounds with small molecular size are easily absorbed and distributed within the plant as opposed to compounds with large molecular size large-sized compounds (Chuang *et al.*, 2019).

Soil properties

Soil properties affect the plant uptake of emerging contaminants including triclosan and sulfamethoxazole from the soil (Butler *et al.*, 2012; Biel-Maeso *et al.*, 2019). Soil pH affects the plant uptake of triclosan and sulfamethoxazole from the soil by controlling the charge form that triclosan (Butler *et al.*, 2012) and sulfamethoxazole (Gottschall *et al.*, 2012) exist in the soil. Gottschall *et al.* (2012) reported that soil pH levels closer or higher than triclosan acid dissociation constant (pK_a = 7.9) lead to more triclosan being present in the soil in their anionic (negatively charged) forms. Similarly, Biel-Maeso *et al.* (2019) reported that soil pH levels of 7.2 or higher result in the formation of more anionic forms of sulfamethoxazole in the soil. Anionic (negatively charged) compounds are highly repelled from soil colloidal surface and, therefore, easily leached from the soil (Butler *et al.*, 2012). Soil organic matter is another factor that affects the plant uptake of emerging contaminants including triclosan and sulfamethoxazole from the soil by influencing their sorption on the colloids (Jachero *et al.*, 2016; Cantarero *et al.*, 2017; Biel-Maeso *et al.*, 2019). High soil organic matter enhances strong sulfamethoxazole and triclosan sorption and thus lowering their bioavailability in the soil for plant uptake (Fu *et al.*, 2016; Jachero *et al.*, 2016; Cantarero *et al.*, 2017; Biel-Maeso *et al.*, 2019).

Soil texture also affects the plant uptake of emerging contaminants including triclosan and sulfamethoxazole from the soil by influencing their sorption (Zhang *et al.*, 2016b). Generally, the plant uptake of emerging contaminants from coarse-textured soil is much easier and faster as opposed to the uptake from fine-texture soils due to poor sorption and low tension under coarse-textured soils (Biel-Maeso *et al.*, 2019). For example, Zhang *et al.* (2016b) observed an increase in sulfamethoxazole concentration in plants as sand fraction was increased. Soil moisture is one of the most important parameters of the soil that influences the plant uptake of emerging contaminants from the soil by enhancing their solubility (Zhang *et al.*, 2016b; Li *et al.*, 2019b) and their translocation within plants through mass water flow driven by transpiration (Kinney and Heuvel, 2020).

CHAPTER 3

MATERIALS AND METHODS

3.1 Study area

This study was conducted on a long-term field trial (> 15 years) at the East Rand Water Care Works (ERWAT), Ekurhuleni, South Africa. The study area is located at an altitude of 1577 m and receives a summer rainfall of about 700 mm annually. The soil at ERWAT is classified as Hutton form (clay loam) with 36 - 46 % clay content (Tesfamariam *et al.*, 2009). Figure 3.1 shows the location of the study area.

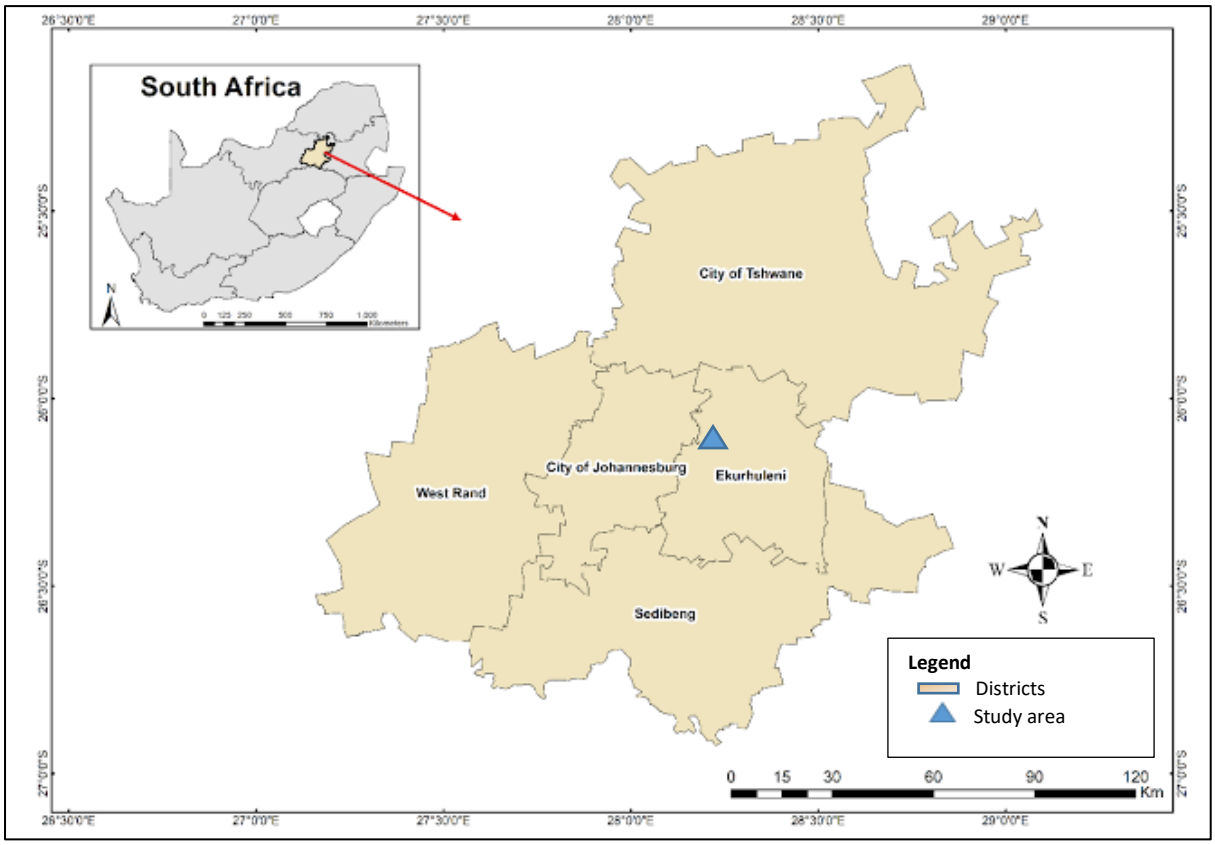

Figure 3. 1: Map of South Africa (top right) and Gauteng Province (middle) showing different districts of the province and the location of the study area

3.2 Sludge Information

The biosolid used for the current study was anaerobically digested and paddy dried municipal biosolids from Vlakplaat wastewater treatment plant, one of the ERWAT plants. In most of the cases, the biosolids were applied shortly after collection. Nutrient and moisture content of the sludge was determined the day before the treatment application. Selected chemical properties of the biosolids used in the study are presented in Table 3. 1.

Table 3. 1: Selected chemical properties of biosolids (mean value) used at ERWAT for over 15 years (Tesfamariam *et al.*, 2013)

Element	Unit	(Average ± SD)
Nitrogen (N)	%	2.51 ± 0.45
NH4_N	mg/Kg	3751.5 ± 1785.24
NO3_N	mg/Kg	87.88 ± 72.15
Total carbon	%	20,22 ± 2.09
EC	mS/m	1586.63 ± 667.44
Potassium	mg/Kg	1446.10 ± 942.17
Calcium	mg/Kg	21584.88 ± 7182.66
Magnesium	mg/Kg	2788.38 ± 1719.49
pH (H_20)		6.05 ± 0.95
Cadmium	mg/Kg	8.21 ± 8.06
Mercury	mg/Kg	0.80 ± 0.70
Chromium	mg/Kg	239.21 ± 197.39
Arsenic	mg/Kg	6.17 ± 5.49
Lead	mg/Kg	55.42 ± 35.65
Zinc	mg/Kg	2478.76 ± 2206.60
Nickel	mg/Kg	83.01 ± 63.89
Copper	mg/Kg	349.33 ± 270.43

SD is standard deviation

3.3 Field experimental design and treatments

A long-term field trial consisting of two cropping systems (rainfed maize and rainfed-irrigated maize) was carried out at ERWAT. Each cropping system consisted of three biosolid application rates (4, 8, and 16 Mg $ha^{-} yr^{-}1$), a commercial inorganic fertiliser, and a zero control. The treatments were laid out on the field in a randomised block design. Each experimental plot had an area of 25 m^2 and was replicated four times. The biosolids and inorganic fertilizer was

incorporated into the top 30 cm soil layer immediately after applications using rotovator. Maize (PAN 6966) was planted each year (between November and December) immediately after biosolids and inorganic fertilizer incorporation into the soil. Harvesting of a maize crop was performed at physiological maturity between April and June each year. Unlike biosolids, inorganic fertilizers were split applied at different plant growing stages. Table 3.2 presents the types, composition and the amount of inorganic fertilizers applied at different maize growing stages. Moisture content under rainfed-irrigated plots was maintained at field capacity. Weeding was performed manually as needed using hand hoe.

Table 3. 2: Types, composition and the amount of inorganic fertilizers applied during different maize growing period at ERWAT (study area)

Time of fertilizer application during maize growing period	Fertilizer type and their composition	Application rate (Kg ha^{-1})					
		Rainfed			Irrigated		
		N	**P**	**K**	**N**	**P**	**K**
At planting	NPK 2:3:2 (22 %)	13	20	13	6	9	6
	LAN (28 % N)	17					
	KCl (50 % K)			7			14
Three weeks post planting	Limestone ammonium nitrate (LAN)				90		
	Super phosphate (18.5 % P)					31	
Five weeks after emergence	LAN				64		
	Super phosphate						
	KCl						40
Seven weeks after emergence	LAN	66			66		
	Super phosphate						
	KCl			40			40
Cumulative annual application		**96**	**20**	**60**	**226**	**40**	**100**

3.4 Weather data

Weather data (rainfall and temperatures) was collected from an automatic weather station (AWS) situated closer to the experimental site, about 100 m away. The AWS was equipped

with an electronic rain gauge Model TR-525M-R2 (Texas Electronic Inc., Dallas, Texas, USA) and temperature sensors (Thermistor Humitter 50Y) for measuring rainfall and temperatures, respectively. Table 3.3 present monthly average temperatures and cumulative average monthly rainfall at ERWAT.

Table 3.3: Mean monthly temperatures (^{O}C) (Tesfamariam et al., 2013) and cumulative average monthly rainfall (mm) (Tesfamariam et al., 2009) at ERWAT, Johannesburg, South Africa

	(Average ± SD)	
Months	**Temperature (^{O}C)**	**Rainfall (mm)**
January	20,75 ± 1,26	138,25 ± 61,59
February	21,00 ± 0,91	84,25 ± 46,59
March	18,75 ± 1,32	84,50 ± 53,74
April	16,38 ± 0,85	30,25 ± 21,87
May	12,75 ± 1,50	2,50 ± 2,12
June	10,88 ± 1,11	
July	10,88 ± 1,03	
August	12,63 ± 0,95	
September	17,25 ± 1,55	18,00 ± 1,00
October	19,00 ± 2,12	55,00 ± 49,62
November	20,63 ±1,31	100,75 ± 31,96
December	20,75 ± 1,85	100,00 ± 27,13

SD is standard deviation.

3.5 Sampling and sample preparation

3.5.1 Sampling

Maize plant samples were harvested 5 cm above the soil surface manually using sickle at physiological maturity in June, 2019. The maize plant samples were harvested randomly from the inner rows to avoid border effects. A total of six plant samples were collected from each

plot and immediately stored in an electric cooler box (CAMP master) during transportation to the laboratory. Soil samples were collected after maize harvest from the top 30 cm soil layer and immediately stored in an electric cooler box (CAMP master) (those for emerging contaminants analysis only) during transportation to the laboratory. Prior to soil sampling, all obstacles including stones and plant materials were gently removed from the soil surface. A total of six soil subsamples was taken with a manual hand auger from each plot and mixed in a pre-cleaned bucket to obtain a 100g composite sample. The Auger was washed with water and rinsed with ethanol each time before sampling the next plot to minimize contamination between treatments or plots.

3.5.2 Sample preparation

The procedures followed during plant and soil sample preparation for soil physicochemical properties and emerging contaminant (triclosan and sulfamethoxazole) determnation are presented below.

Preparation of soil samples for physicochemical analysis

In the laboratory, composite soil samples were spread on clean steal drying trays. Composite soil samples (100 g) collected from the field were then allowed to air-dry to constant weight, sieved with a pre-cleaned 2 mm steal sieve and stored at -4 ^{O}C until analysis.

Preparation of plant and soil samples for triclosan and sulfamethoxazole analysis

Maize samples were each partitioned into stems, leaves and grains. The partitioned maize samples were washed with deionized water and chopped (leaves and stems only) into smaller pieces. Partitioned and washed maize parts were then freeze-dried, ground with an IKA® MF 10 basic mill, sieved with a 2 mm pre-cleaned steal sieve and stored at -20 ^{O}C before extraction and analysis. Similar to maize samples, soil samples were freeze-dried, sieved with a 2 mm pre-cleaned steal sieve and stored at -20 ^{O}C before extraction and analysis.

3.6 Analytical method

3.6.1 Soil organic carbon, nitrogen, electrical conductivity and pH

Soil organic matter (SOM) was measured according to the Walkley-Black's procedure (Walkley and Black, 1934). Soil pH (H_2O) and electrical conductivity (EC) were determined in solution of a 1:2.5 soil: water ratio using Consort pH (C830 model) and EC (C861 model) meters, respectively (McLean, 1983). Total nitrogen was analysed by dry combustion method using Carlo Erba NA1500 C/N analyser (Carlo Erba Strumentazione, Italy) (Verardo *et al.*, 1990).

3.6.2 Triclosan and sulfamethoxazole

Chemicals and reagents used, extraction and clean up procedures followed, and method validation measures for the determination of triclosan and sulfamethoxazole from the soil and plant samples are presented below.

Chemicals and reagents used

The following standards were purchased from Sigma-Aldrich (Aston Manor, South Africa): analytical standards of triclosan (TCS) and sulfamethoxazole (SMX), a surrogate standard of triclosan (TCS $^{13}C_{12}$), and an internal standard of sulfamethoxazole (SMX $^{13}C_6$). HPLC grade methanol, acetone, ammonium acetate and Supelclean^{TM} ENVI^{TM}-18 cartridges (6 mL) were also purchased from Sigma-Aldrich (Aston Manor, South Africa). All working standards were prepared in methanol (HPLC grade) and stored at 4 ^{O}C in glass amber bottles. LC MS grade water (treated by SIEMENS Labostar water purifying equipment) was used for the preparation of the mobile phase (ammonium acetate) and the rinsing of the laboratory glassware. Physicochemical properties of the two target compounds, triclosan and sulfamethoxazole are presented in Table 3.4.

Table 3. 4: Physicochemical properties of pharmaceutical and personal care product compounds (Holling et al., 2012)

Compound CAS-Number	CAS number	pK_a	Log K_{ow}	water solubility (25 ^{O}C)	Molecular weight (g/mol)
Triclosan	3380-34-5	7.9	4.8	4.6 mg L^{-1}	289.54
Sulfamethoxazole	723-46-6	1.7, 5.7	0.89	610 mg L^{-1}	253.28

Extraction and clean-up procedure

Extraction of the soil and plant samples was performed following previous published methods (Hu *et al.*, 2010; Pannu *et al.*, 2012; Fu *et al.*, 2016) with some modifications. In short, 1 g of each sample (soil or plant) was placed in a 50 mL centrifuge tube, spiked with 1 mL of a known concentration of TCS$^{13}C_{12}$ surrogate standard and allowed to equilibrate for at least one hour. A 10 mL of methanol: acetone (50:50 by volume) ratio solvent was added to an impregnated sample and agitated using IKA® VORTEX 3 for 2 minutes. The sample mixture was then extracted using ultrasound-assisted extraction (UAE) for a period of 15 minutes using Fisher Scientific FS110H sonicator. The sample mixture was then centrifuged for a period of 30 minutes at a speed of 1500 rotation per minute (rpm). The same extraction procedure was repeated twice from the same sample that was extracted using the same volume of solvent, sonication time and centrifuge speed and time. The extracts from the same samples were each removed, combined and dried gently under nitrogen gas (N_2). Prior to sample clean-up, the Supelclean™ ENVI™-18 cartridges were preconditioned with methanol and LC MS grade water. After conditioning, the extracts mixture was carefully loaded into a Supelclean™ ENVI™-18 cartridge. About 10 mL of a solvent was used to elute the analytes. The extracts were then dried with N_2 gas, reconstituted with 950 µL methanol and transferred to LC MS vials. Finally, a 50 µL of an internal standard (SMX $^{13}C_6$) was added to the extracts, vortexed and taken to the instrument (LC-MS/MS) for analysis.

Instrumental analysis using LC-MS/MS

Target compounds identification and quantification was done with the aid of a Liquid Chromatograph Mass Spectrometer (LC-MS/MS model 8030). An LC-MS/MS instrument equipped with electrospray ionization source and a C18 column (3 µm particle size, 2.1 × 150 mm) was used to perform chromatographic separation of triclosan and sulfamethoxazole. Ammonium acetate (20 mM in LC MS grade water) and HPLC grade methanol (100 %) were used as mobile phase A and B, respectively. Mobile phase A and B were delivered at flow rates of 0.2500 mL min^{-1} and 0.1500 mL min^{-1}, respectively. The injection volume of the aliquot was 10 µL (injected twice per sample). The run-time of the test samples and standards were set to be 4 minutes. Multiple reactions monitoring (MRM) mode was used on mass spectrometer, sulfamethoxazole in positive (+) ionization mode and triclosan in negative (-) ionization mode. The specifics of the MRM transition for the studied compounds together with the precursor and product ions and their retention time are shown in Table 3.5.

Table 3. 5: MRMs for target compounds and standards as well as their average retention times

Compounds	**Precursor ion** m/z	**Product ion** m/z	**Retention time** (minutes)
Sulfamethoxazole	254.1	155.95	0.9
Sulfamethoxazole $^{13}C_6$	260.1	162	0.9
Triclosan	289.1	34.9	1.2
Triclosan $^{13}C_{12}$	301.1	34.85	1.2

Quality assurance/ quality control measures

Soil, grains, stems and leaves were divided into four batches. Each batch consisted of its own blanks (procedural and methods blanks). Analytes of triclosan and sulfamethoxazole were identified based on the retention time and MRM transition. To determine the concentration of the analytes from the samples, the internal standard dilution method was used using a relative response factor (RRF) derived from the calibration curve of each compound. To assess the accuracy of the extraction procedure, samples were first spiked with a surrogate standard (TCS

$^{13}C_{12}$) prior to extraction. Method recoveries, detection limit (LOD) and quantification limit (LOQ) for different sample matrixes are presented in Table 3.6. The values of the LOD's and LOQ's were calculated as 3 times and 10 times the value of the blank standard deviation, respectively (Wang *et al.*, 2015). In case analyte concentration in blank was zero, LOD and LOQ were calculated as 3 times and 10 times the lowest concentration of analyte on the calibration curve, respectively (Wang *et al.*, 2015). Linearity of the calibration curves for the two target compounds triclosan and sulfamethoxazole were above 0.99.

Table 3. 6: Method recoveries for surrogate standard (TCS $^{13}C_{12}$), LOD's and LOQ in each sample matrix

Sample matrix	Mean Recoveries (%)	LOD (ng/g)	LOQ (ng/g)
Soil	105	TCS: 1.9625	TCS: 5.9494
		SMX: 0.64	SMX: 1.9438
Stems	79	TCS: 5.595[a]	TCS: 18.65[a]
		SMX: 14.295[a]	SMX: 47.65[a]
Leaves	75	TCS: 7.3683	TCS: 22.3283
		SMX: 14.295[a]	SMX: 47.65[a]
Grains	85	TCS: 5.595[a]	TCS: 18.65[a]
		SMX: 14.295[a]	SMX: 47.65[a]

[a] LOD's and LOQ's calculated from calibration curve of analyte.

3.7 Statistical analyses

SAS software version 9.0 was used to carry out statistical analyses. Two-way Analysis of Variance (ANOVA) test of significance was used to assess statistical differences between treatment means. When significant differences between treatment means were detected by ANOVA, separation of treatments means was performed using Student t-test at $p \leq 0.05$.

CHAPTER 4

RESULTS

This chapter presents the long-term effects of biosolid application on selected soil chemical properties (soil organic carbon, soil pH, soil electrical conductivity, and total soil nitrogen), on the accumulation of selected emerging contaminants (Triclosan and Sulfamethoxazole) in the uppermost 30 cm soil layer and on plant aboveground biomass tissue.

4.1 Effects of long-term biosolids application on selected soil chemical properties

4.1.1 Soil organic carbon

There was no cropping system (rainfed vs rainfed-irrigated) x treatment interaction effects on soil organic matter (Table 4.1). However, there was a statistically significant ($p \leq 0.0001$) biosolids treatment effect on soil organic carbon (Table 4.1).

Table 4. 1: Degrees of freedom (df), sum squares, mean squares, F value, and F probability for analysis of variance for soil organic carbon (t-Test) at ERWAT experimental site, Johannesburg (Ekurhuleni), South Africa

Source of variation	df	Sum squares	Mean square	F value	Pr > F
Cropping system (CS)	1	0.1381	0.1381	11.67	0.0018
Biosolids (B)	4	1.6544	0.4136	34.97	<.0001
CS x B	4	0.0985	0.0246	2.08	0.1081

Generally, long-term biosolids application enhanced soil organic carbon sequestration both under rainfed and rainfed-irrigated maize cropping systems (Figure 4.1). The general trend shows that soil organic carbon increased as the biosolids application rate doubled for both rainfed and rainfed-irrigated maize cropping systems. This shows that soil organic carbon

increment under both cropping systems was primarily caused by the addition of biosolids. The difference was, however, statistically significant ($p \leq 0.05$) only under rainfed-irrigated maize cropping systems (Figure 4.1). Biosolids application rates of 16 t ha^{-1} incurred the highest soil organic carbon content both for rainfed (1.64 %) and rainfed-irrigated maize (1.90 %) systems. Regardless of the cropping system, inorganic fertiliser (< 1.4 %) and the zero control (< 1.3 %) treatments had the lowest soil organic carbon content. Generally, soil organic carbon under inorganic fertilizer treatment was slightly higher than the control but almost similar to the 4 t ha^{-1} (< 1.50 %) biosolids treatment.

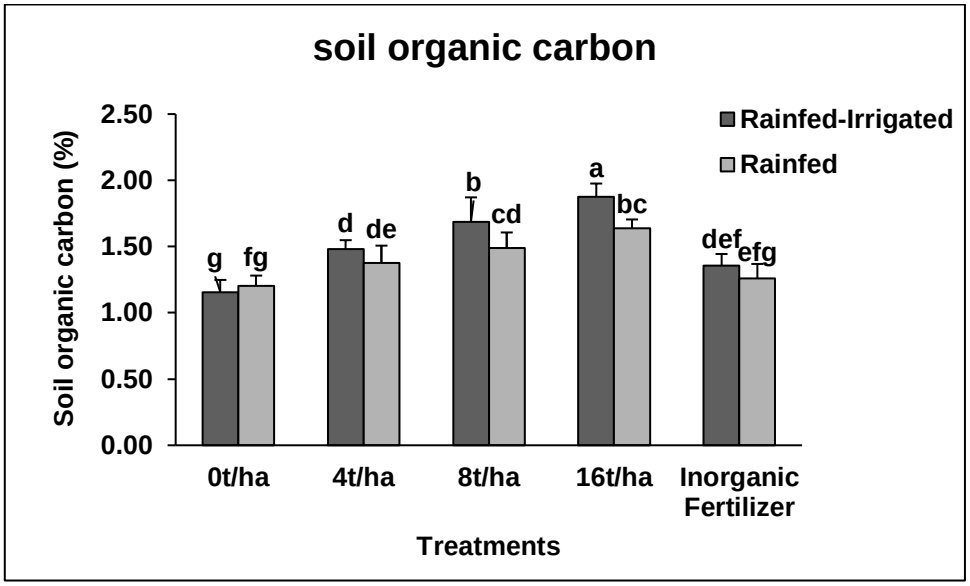

Figure 4. 1: Effect of cropping system and biosolids application rate on % soil organic carbon. Bars sharing the same letter are not statistically significant at ($p \leq 0.05$) and vice versa

4.1.2 Soil pH

Long-term biosolids application reduced soil pH under both rainfed and rainfed-irrigated maize cropping systems (Table 4.2). According to the ANOVA, there was no cropping system x biosolid interaction effect on soil pH (Table 4.2). There was, however, a statistically highly significant ($p \leq 0.0001$) cropping system effect as well as treatment (biosolids application rate) effect on soil pH. Generally, rainfed-irrigated maize cropping systems had higher soil pH than rainfed maize cropping systems (Figure 4.2). When comparing soil pH between rainfed-irrigated and rainfed maize systems, there was significant ($p \leq 0.05$) soil pH differences at all

biosolids application rates, and rainfed-irrigated systems had higher soil pH than rainfed systems (Figure 4.2).

Table 4. 2: Degrees of freedom (df), sum squares, mean squares, F value, and F probability for analysis of variance for soil pH (t-Test) at ERWAT experimental site, Johannesburg (Ekurhuleni), South Africa

Source of variation	df	Sum square	Mean square	F value	Pr > F
Cropping system (CS)	1	2.65225	2.65225	41.87	<.0001
Biosolids (B)	4	11.2299	2.80749	44.32	<.0001
CS x B	4	0.6462	0.16155	2.55	0.0596

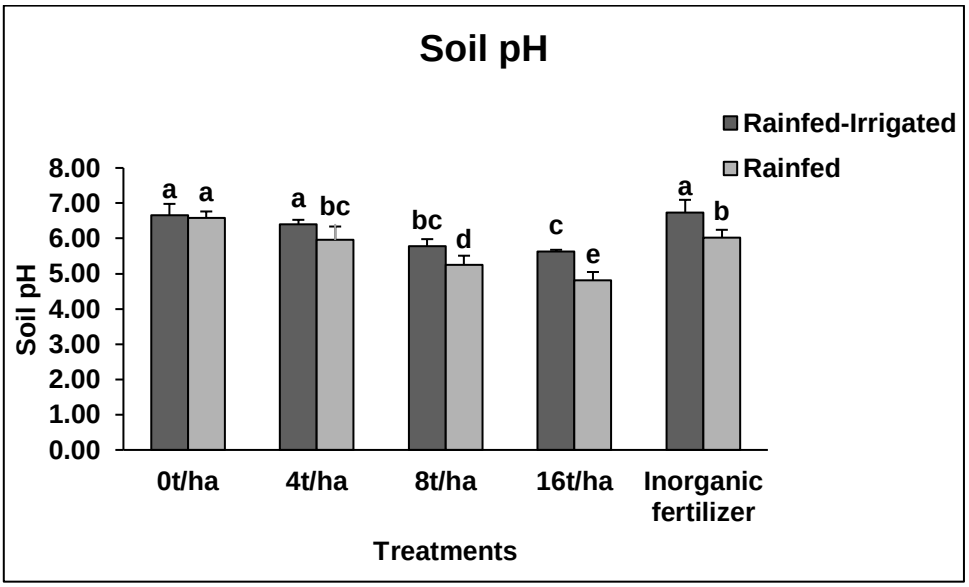

Figure 4. 2: Effect of cropping system and biosolids application rate on soil pH. Bars sharing the same letter are not statistically significant at ($p \leq 0.05$) and vice versa

However, no significant differences in soil pH were observed between the two control treatments (Figure 4.2). The results also showed that there was a statistically significant ($p \leq$

0.0001) biosolids treatment effect on soil pH (Table 4.2). The general trend shows that soil pH decreased as the biosolids application rate increased (Figure 4.2). The differece was, however, significant ($p \leq 0.05$) only under rainfed maize cropping system. Inorganic fertilizer treatment experienced the highest soil pH but was statistically similar to that observed under the zero control and 4 t ha^{-1} rainfed-irrigated maize cropping system treatments (Figure 4.2). Biosolids application rates of 16 t ha^{-1} had the least soil pH (Figure 4.2).

4.1.3 Soil electrical conductivity

Generally, long-term biosolids application enhanced soil electrical conductivity both under rainfed and rainfed-irrigated maize cropping systems. According to the ANOVA, there was a cropping system x biosolid treatment interaction effect ($p \leq 0.05$) on soil electrical conductivity (Table 4.3). Cropping system did not affect soil electrical conductivity significantly ($p \leq 0.05$). There was, however, a statistically highly significant ($p \leq 0.0001$) biosolids treatment effect on soil electrical conductivity (Table 4.3). Hence, the interaction effect is mainly attributed to the inconsistent EC values reported at various biosolid application rates.

Table 4. 3: Degrees of freedom (df), sum squares, mean squares, F value, and F statistics for analysis of variance of soil electrical conductivity from long-term biosolids amended soils at ERWAT experimental site, Johannesburg (Ekurhuleni), South Africa

Source of variation	**df**	**Sum square**	**Mean square**	**F value**	**Pr > F**
Cropping system (CS)	1	7884.864	7884.864	1.19	0.2849
Biosolids (B)	4	303028.47	75757.117	11.39	<.0001
CS x B	4	105005.71	26251.427	3.95	0.0109

The general trend indicates that an increase in biosolids application rate increases soil electrical conductivity for both rainfed and rainfed-irrigated cropping systems (Figure 4.3). This indicates that biosolids application is the main cause of the increase in soil electrical conductivity. Regardless of the cropping system, the electrical conductivity of the control,

inorganic fertiliser, and the 4 t ha^{-1} treatments had the least soil electrical conductivity and were statistically similar to each other (Figure 4.3).

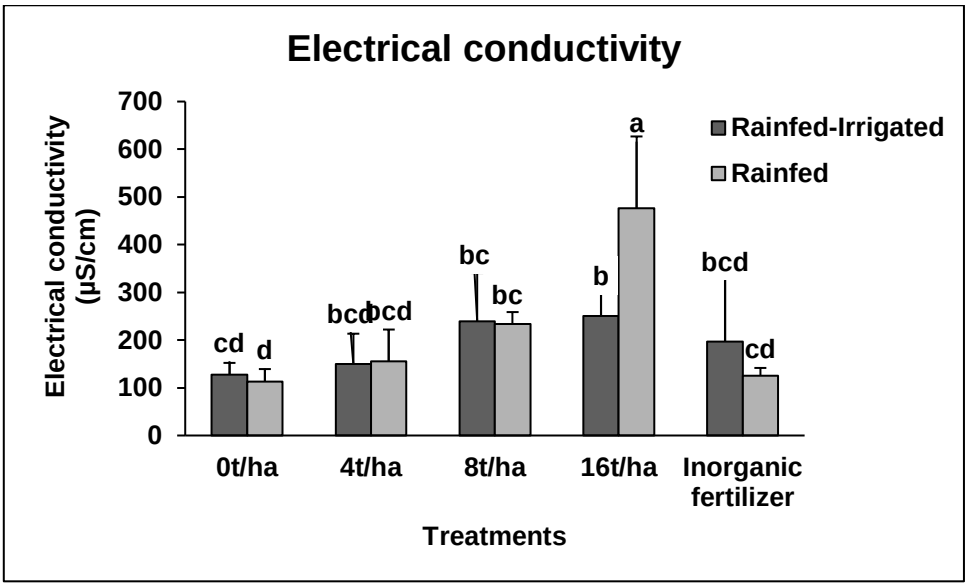

Figure 4. 3: Effect of biosolids application rate and cropping system on soil electrical conductivity. Bars sharing the same letter are not statistically significant at ($p \leq 0.05$) and vice versa

4.1.4 Soil nitrogen

Generally, long-term biosolids application improved total soil nitrogen for both rainfed and rainfed-irrigated maize farming systems. Statistical analyses using ANOVA indicated that there was no cropping system x treatment interaction effect (Table 4.4). However, there was a statistically significant ($p \leq 0.0001$) biosolids treatment effect on the total soil nitrogen (Table 4.4).

Table 4. 4: Degrees of freedom (df), sum squares, mean squares, F value, and F probability for analysis of variance for total soil nitrogen at ERWAT experimental site, Johannesburg (Ekurhuleni), South Africa

Source of variation	df	Sum square	Mean square	F value	Pr > F
Cropping system (CS)	1	0.00324	0.00324	13.99	0.0008
Biosolids (B)	4	0.0300784	0.0075196	32.47	<.0001
CS x B	4	0.0007048	0.0001762	0.76	0.5591

Total soil nitrogen increased as the biosolids application rate doubled for both rainfed and rainfed-irrigated maize cropping systems (Figure 4.4).

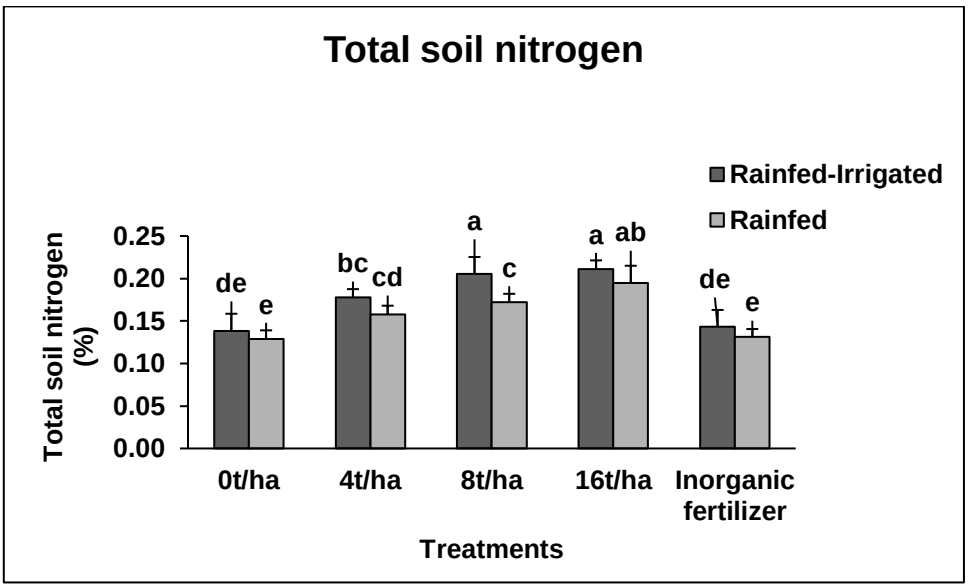

Figure 4. 4: Effect of biosolids application and cropping system on soil total nitrogen (%). Bars sharing the same letter are not statistically significant at ($p \leq 0.05$) and vice versa

However, the significant ($p \leq 0.05$) soil nitrogen increase related to biosolids application was observed only between 4 t ha^{-1} and 8 t ha^{-1} for the rainfed-irrigated maize and between 8 t ha^{-1} and 16 t ha^{-1} for the rainfed maize cropping systems. The nitrogen contents of the zero control

and inorganic fertiliser treatments, had the lowest nitrogen contents, which were also similar to each other.

4.2 Accumulations of selected emerging contaminants in the uppermost 30 cm soil layer and plant aboveground biomass

4.2.1 Triclosan accumulation in the uppermost 30 cm soil layer

Triclosan concentration in the uppermost 30 cm soil layer under both rainfed-irrigated and rainfed maize cropping systems was below the limit of detections (LOD) for all biosolids treatment levels (Table 4.5). Hence, long-term land application of biosolids did not result in traceable accumulation of triclosan in the top 30 cm soil layer of both rainfed-irrigated and rainfed maize cropping systems.

Table 4. 5: Concentrations of triclosan and sulfamethoxazole (ng g^{-1} dry w.t) in the top 30 cm soil layer of rainfed-irrigated maize and rainfed maize

	Triclosan		Sulfamethoxazole	
Treatments	Rainfed-Irrigated maize	Rainfed maize	Rainfed-Irrigated maize	Rainfed maize
0t/ha	< LOD	< LOD	< LOD	< LOD
4t/ha	< LOD	< LOD	< LOD	< LOD
8t/ha	< LOD	< LOD	< LOD	< LOD
16t/ha	< LOD	< LOD	< LOD	< LOD
Inorganic fertilizer	< LOD	< LOD	< LOD	< LOD

Limit of detection (ng g^{-1}): Triclosan in soils, 1.963; Sulfamethoxazole in soils, 0.64.

4.2.2 Sulfamethoxazole accumulation in the uppermost 30 cm soil layer

Long-term land application of biosolids did not result in the accumulation of both sulfamethoxazole in the uppermost 30 cm soil layer of both rainfed-irrigated and rainfed maize cropping systems (Table 4.5). Similar to triclosan, sulfamethoxazole concentration in the uppermost 30 cm soil layer was below the limit of detections (LOD) for all biosolids treatment levels (Table 4.5).

4.2.3 Triclosan uptake by maize

Triclosan was detected in different aboveground biomass parts (i.e. stems, leaves and grains) of maize planted to land that received 8 t ha^{-1} and higher biosolid application rates (both under rainfed-irrigated and rainfed systems) as well as irrigated inorganic fertilizer (Table 4.6).

Table 4. 6: Mean triclosan (ng g^{-1} dry w.t) concentration in maize stems, leaves and grains planted to biosolid amended land under rainfed and rainfed-irrigated cropping system

	Rainfed maize			Irrigated maize		
Treatments	Stems	Leaves	Grains	Stems	Leaves	Grains
0 t/ha	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
4 t/ha	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
8 t/ha	393 ± 37	525 ± 50	32.5 ± 7	898 ± 41	862 ± 97	39.6 ± 7
16 t/ha	666 ± 446	450 ± 94	35.5 ± 4	892 ± 67	635 ± 34	42.8 ± 7
Inorganic fertilizer	N.D.	N.D.	N.D.	420 ± 283	402 ± 310	N.D.

N.D.: not detected.

Concentration of triclosan in maize stems

There was no significant ($p \leq 0.05$) biosolid x cropping system interaction effect on maize stems triclosan concentration (Table 4.7). Similarly, biosolids application rates did not have significant ($p \leq 0.05$) effect on maize stems triclosan concentration (Table 4.7). The concentration of triclosan in maize stems was, however, significantly ($p \leq 0.05$) affected by the cropping system (Table 4.7).

Table 4. 7: Degrees of freedom (df), sum squares, mean squares, F value, and F probability for analysis of variance for triclosan concentration in maize stems

Source of variation	df	Sum square	Mean square	F value	Pr > F
Cropping system (CS)	2	344367.035	172183.518	3.01	0.0798
Biosolids (B)	1	533613.704	533613.704	9.32	0.0081
CS x B	1	77922.28	77922.28	1.36	0.2617

Triclosan concentration in maize stems planted to the zero control, 4 t ha^{-1} biosolid treatment as well as the rainfed inorganic fertiliser treatments were below the detection limit (Figure 4.5). Generally, the concentration of triclosan in maize stems was higher under rainfed-irrigated than under rainfed systems for similar sludge application rate treatments. The difference was, however, statistically significant ($p \leq 0.05$) only for the 8 t ha^{-1} treatment. It was also apparent that there was no statistically significant ($p \leq 0.05$) triclosan concentration difference between the 8 t ha^{-1} and 16 t ha^{-1} treatments under similar cropping systems. Nonetheless, triclosan concentration increased as the sludge application rate doubled from 8 to 16 t ha^{-1} under rainfed-irrigated system, though not statistically significant. The concentration of triclosan in maize stems under the rainfed-irrigated inorganic fertilizer treatment was significantly ($p \leq 0.05$) lower than that of the 8 and 16 t ha^{-1} irrigated treatments.

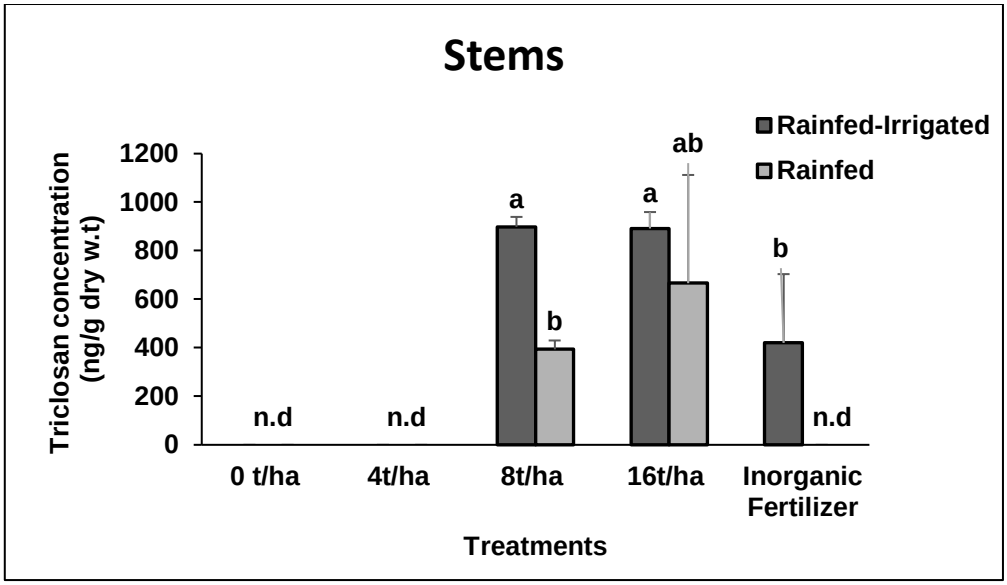

Figure 4. 5: Effect of cropping system and biosolids application rate on the accumulation of triclosan in maize stems. Bars sharing the same letter are not statistically significant at ($p \leq 0.05$) and vice versa. (n.d means not detected, w.t means weight)

Concentration of triclosan in maize leaves

There was a significant ($p \leq 0.05$) biosolids x cropping system interaction effect on triclosan concentration of maize leaves (Table 4.8).

Table 4. 8: Degrees of freedom (df), sum squares, mean squares, F value, and F probability for analysis of variance for triclosan concentration in maize leaves

Source of variation	df	Sum square	Mean square	F value	Pr > F
Cropping system (CS)	2	241518.47	120759.2349	26.04	<.0001
Biosolids (BS)	1	277511.76	277511.7604	59.85	<.0001
CS x B	1	21387.571	21387.5708	4.61	0.0485

The concentration of triclosan in rainfed maize leaves followed similar trends to that of the rainfed-irrigated system indicating the difference was magnitudnal. Biosolid application rates and cropping system highly significantly ($p \leq 0.0001$) affected triclosan concentration of maize leaves (Table 4.8). Similar to that of maize stems, triclosan was not detected in maize leaves planted to the zero control, 4 t ha^{-1} biosolids treatment, and rainfed inorganic fertiliser treatment (Figure 4.6). Generally, the concentration of triclosan in maize leaves was significantly ($p \leq 0.05$) higher under rainfed-irrigated than rainfed systems for similar sludge application rate treatments. It was also apparent that there was no statistically significant ($p \leq 0.05$) triclosan concentration difference between the 8 t ha^{-1} and 16 t ha^{-1} treatments under rainfed cropping systems. Nonetheless, triclosan concentration decreased as the sludge application rate doubled from 8 to 16 t ha^{-1} under both rainfed-irrigated and rainfed systems. The concentration of triclosan in maize leaves under the rainfed-irrigated inorganic fertilizer treatment was lower than that of the 8 and 16 t ha^{-1} rainfed-irrigated sludge treatments. The differences was, however, statistically significant ($p \leq 0.05$) relative to the 8 t ha^{-1} treatment.

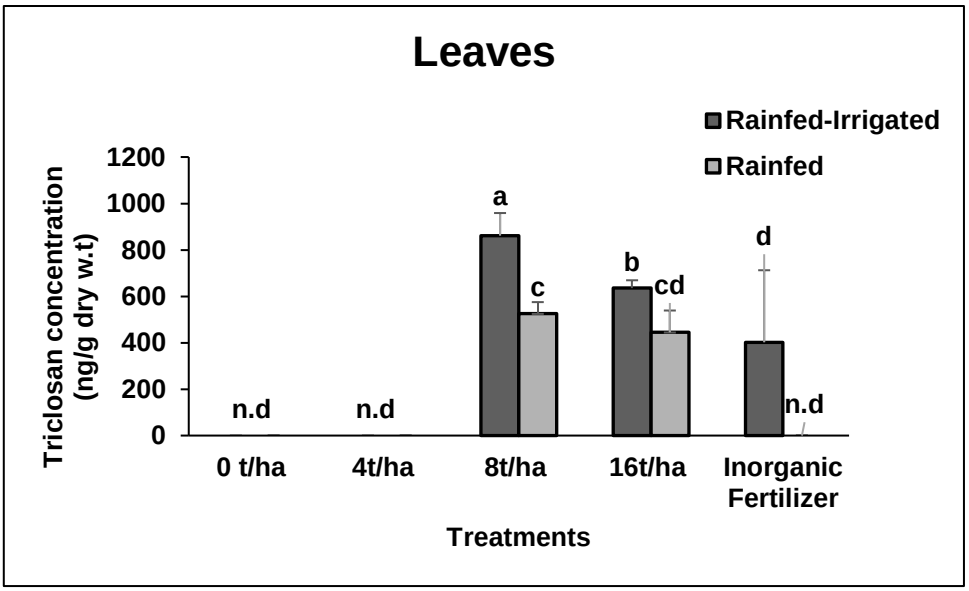

Figure 4. 6: Effect of cropping system and biosolids application rate on the accumulation of triclosan in maize leaves. Bars sharing the same letter are not statistically significant at ($p \leq 0.05$) and vice versa. (n.d means not detected, w.t means weight)

Concentration of triclosan in maize grains

There was no significant ($p \leq 0.05$) biosolids x cropping system interaction effect on maize grains triclosan concentration (Table 4.9). Similarly, cropping system did not have significant ($p \leq 0.05$) effect on maize grains triclosan concentration (Table 4.9). Nonetheless, the concentration of triclosan in maize grain was significantly ($p \leq 0.05$) affected by biosolid application rate.

Table 4. 9: Degrees of freedom (df), sum squares, mean squares, F value, and F probability for analysis of variance for triclosan concentration in maize grains

Source of variation	**df**	**Sum square**	**Mean square**	**F value**	**Pr > F**
Cropping system (CS)	1	38.04289	38.0428	0.91	0.3601
Biosolids (B)	1	204.6571	204.6571	4.87	0.0475
CS x B	1	0.02353	0.0235	0	0.9815

Similar to maize stem and leaves, triclosan was not detected in maize grains planted to the zero control, 4 t ha^{-1} biosolid application rate, and inorganic fertilizer treatments (Figure 4.7). Maize grains triclosan concentration did not vary significantly ($p \leq 0.05$) between the 8 and 16 t ha^{-1} biosolids application rates of similar cropping systems. Generally, triclosan concentration of maize grains planted to similar biosolids application rates was relatively higher under rainfed-irrigated than rainfed systems but was not significantly ($p \leq 0.05$) different. Maize grains triclosan concentration was lowest under rainfed 8 t ha^{-1} biosolids treatment and was significantly ($p \leq 0.05$) lower than that planted to the rainfed-irrigated 16 t ha^{-1} biosolids application rate.

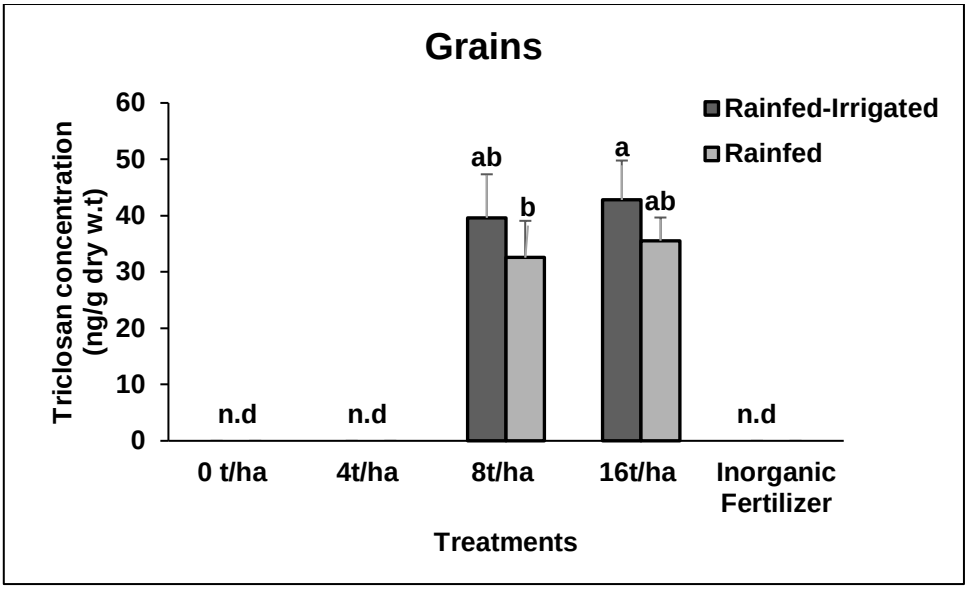

Figure 4. 7: Effect of cropping system and biosolids application rate on the accumulation of triclosan in maize grains. Bars sharing the same letter are not statistically significant at ($p \leq 0.05$) and vice versa. (n.d means not detected, w.t means weight)

4.2.4 Sulfamethoxazole uptake by maize

Irrespective of the cropping system (rainfed or rainfed-irrigated maize), sulfamethoxazole concentration in the stems, leaves and grains of maize planted to biosolids amended land was below the detection limit (Table 4.10).

Table 4. 10: Mean sulfamethoxazole (ng g^{-1} dry w.t) concentration in maize stems, leaves and grains planted to biosolid amended land under rainfed and rainfed-irrigated cropping system

	Rainfed maize			Rainfed-Irrigated maize		
Treatments	Stems	Leaves	Grains	Stems	Leaves	Grains
0 t/ha	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
4 t/ha	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
8 t/ha	< LOD	N.D.	N.D.	< LOD	N.D.	N.D.
16 t/ha	< LOD	N.D.	N.D.	< LOD	N.D.	N.D.
Inorganic fertilizer	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

N.D.: not detected.

Limit of detection (ng g^{-1}): Sulfamethoxazole in the stems, 14.295.

CHAPTER 5

DISCUSSION

This chapter discusses the following topics based on the reported results in chapter 4:

- Effects of long-term biosolid applications on selected soil chemical properties.
- The accumulations of selected emerging contaminants in the uppermost 30 cm soil layer and plant aboveground biomass.

5.1 Effects of long-term biosolid applications on selected soil chemical properties

5.1.1 Soil organic carbon

An increase in soil organic carbon associated with increase in biosolid applications rate is a commonly reported trend in agricultural lands (Poffenbarger *et al.*, 2017; Dong *et al.*, 2020). This trend is the result of direct addition of organic carbon from biosolids (Wijesekara *et al.*, 2017) and the increase in the leaf and root biomass, which contributes to the soil organic carbon through litter decomposition (Tanaka *et al.*, 2013; Gotoh *et al.*, 2018). Similarly, in the current study, soil organic carbon increased as biosolid applications rate was doubled under both rainfed and rainfed-irrigated maize cropping systems, which is in line with previous findings (Poffenbarger *et al.*, 2017; Dong *et al.*, 2020). The reported increase in soil organic carbon with doubling of biosolid application rate under the current study is attributed to the addition of organic carbon from biosolids (Wijesekara *et al.*, 2017) and due to the increase in biomass (Tesfamariam *et al.*, 2009), which might have contributed to the soil organic matter through decomposition.

Climatic conditions affect soil organic carbon accumulation (Dolan *et al.*, 2006; Zhang *et al.*, 2016a). Generally, carbon sequestration is reported to be higher in areas receiving higher rainfall than those receiving lower rainfall because plant biomass production is affected by the availability of water (Alvarez, 2005; Sinoga *et al.*, 2012). According to Tesfamariam *et al.* (2009), maize above-ground biomass increased with doubling of the biosolid application rate and it was significantly higher under rainfed-irrigated than rainfed systems. This should have contributed to the reported higher soil carbon sequestration under the current rainfed-irrigated

system relative to the rainfed system, which is in accordance with previous discoveries (Alvarez, 2005; Sinoga *et al.*, 2012).

It is evident from the current and other previous studies that, the use of biosolids in agricultural lands is an effective mechanism of sequestering carbon in the soil and could serve as one of the greenhouse gas emission reduction mitigation strategies (Wijesekara *et al.*, 2017). The benefit of using biosolids in agricultural lands is double fold. Besides sequestering carbon and reducing greenhouse gas emission, biosolid application in agricultural lands improves the physical and chemical properties of soils (Bravo-Martín-Consuegra *et al.*, 2016; Zoghlami *et al.*, 2016; Dede *et al.*, 2017), hence enhancing the retention of both nutrients and water in the soil (Speir *et al.*, 2004; Tsadilas *et al.*, 2005). Therefore, biosolids use in agricultural lands contributes to the practice of climate change action strategy through organic carbon sequestration besides improving the physical and chemical properties of agricultural lands thus enhancing crop production.

5.1.2 Soil pH

A decline in soil pH is a well known phenomenon experienced in biosolid amended soils (Dede *et al.*, 2017; Protano *et al.*, 2020; Skowrońska *et al.*, 2020). This phenomenon is caused by organic acids released during organic matter decomposition (Angin *et al.*, 2012) and the proton ions (H^+) released during the nitrification of ammonium ion (NH_4^+) from biosolids (Moreno *et al.*, 1997). During the nitrification of one NH_4^+, two proton ions ($2H^+$) are released into the soil which reacts with carbon dioxide, thereby forming carbonic acids which increases soil acidity (Bolan *et al.*, 1991). In the current study, the application of biosolids decreased soil pH. This decline in soil pH was further aggravated as the biosolid application rate was doubled under both maize cropping systems, which is in line with previous findings (Dede *et al.*, 2017; Protano *et al.*, 2020; Skowrońska *et al.*, 2020). This is most probably attributed to the release of organic acids during the decomposition (Angin *et al.*, 2012), as stated earlier, and the release of proton ion ($2H^+$) during the nitrification of ammonium ion (NH_4^+) (Bolan *et al.*, 1991).

Irrigation water can alter and affect soil pH (Tarchouna *et al.*, 2010). Generally, soil pH is reported to be higher under rainfed-irrigated than rainfed soils because irrigation water contains soluble salts which are directly added to the soil during irrigation (Tarchouna *et al.*, 2010; Bedbabis *et al.*, 2014). Previous studies have shown that supplemental irrigation under water

limiting conditions enhances biomass production (Tesfamariam *et al.*, 2009), hence the deposition of plant litter and root residues to the soil. Plant residue contains cations which enhance the alkalinity of the soil when residues are returned to the soil (Xu *et al.*, 2006) and nitrogen which undergoes ammonification during mineralization, hence forming NH_4^+ which contribute to the raise in soil pH (Tang and Yu, 1999; Xu *et al.*, 2006). In the current study, cropping system had a significant ($p < 0.0001$) effect on soil pH, hence soil pH was significantly ($p < 0.0001$) higher under rainfed-irrigated than under rainfed cropping system, which is in agreement with previous findings (Tarchouna *et al.*, 2010; Bedbabis *et al.*, 2014). It was also apparent that, cropping system was not a limiting factor for biomass production under rainfed-irrigated cropping system, hence biomass produced under the current cropping system was significantly higher than that of rainfed cropping system as stated in the previous section (Tesfamariam *et al.*, 2009). The reported significant higher soil pH under rainfed-irrigated cropping system relative to rainfed cropping system under the current study is attributed to the direct addition of soluble salts (cations) from irrigation water and plant residue deposits (Xu *et al.*, 2006; Tarchouna *et al.*, 2010; Bedbabis *et al.*, 2014) as well as the contribution of NH_4^+ produced from the ammonification of plant residue nitrogen (Tang and Yu, 1999; Xu *et al.*, 2006).

We can conclude from the current study that, long-term biosolid applications to agricultural lands could lead to soil acidification problems. Soil acidity enhances the uptake of heavy metals by crops (Qin *et al.*, 2020), plant nutrient uptake imbalances (Qin *et al.*, 2020), and could negatively affect crop yield. Therefore, synchronizing sludge application practices with proper lime application strategies is crucial for sustainable agricultural use of biosolids, in order not to compromise both crop production and environmental health (Neina, 2019).

5.1.3 Soil electrical conductivity

A positive response of soil electrical conductivity to an increase in biosolid applications rate is a commonly reported phenomenon observed in agricultural lands (Zoghlami *et al.*, 2016; Cucina *et al.*, 2019; Hamdi *et al.*, 2019). This relationship is the result of a direct addition of soluble salts from biosolids (Miranda *et al.*, 2018; Cucina *et al.*, 2019). In the current study, soil electrical conductivity increased as the biosolid application rate was doubled under both rainfed-irrigated and rainfed maize cropping systems, which is in line with previous findings (Zoghlami *et al.*, 2016; Cucina *et al.*, 2019; Hamdi *et al.*, 2019). The increase in soil electrical

conductivity with the doubling of biosolid applications rate under the current study is attributed to the addition of soluble salts from biosolids, whose average electrical conductivity was 1600 mS m^{-1} (Table 3.1). The dominant cation contributing largely to the salinity of the biosolid used in the current study is Ca (2.16 % by mass) followed by Mg (0.28 % by mass).

Soil salinity negatively affects crop growth and final harvested yield (Becker *et al.*, 2017; Tian *et al.*, 2020). This is because increase in soil salinity decreases osmotic potential (i.e. osmotic potential becomes more negative), thereby reducing soil water potential and subsequently reducing the plant available water resulting in crop stress, negatively affecting crop growth hence the final crop yield (Sheldon *et al.*, 2017). Soil salinity is also capable of negatively affecting the soil microbial population (Jun-yu *et al.*, 2017). The results from the current and other previous studies indicate that, long-term biosolid applications to agricultural lands could lead to soil salinity problems (Mohamed *et al.*, 2018). Therefore, soil profile leaching strategies must be synchronized with biosolid applications to limit excessive build-up of soluble salts in the soil profile, which could lower soil productivity (Jun-yu *et al.*, 2017) and reduce crop yield (Becker *et al.*, 2017; Tian *et al.*, 2020).

5.1.4 Total soil nitrogen

Generally, biosolid applications to agricultural lands enhances the nitrogen content of soils (Zoghlami *et al.*, 2016; Miranda *et al.*, 2018). This phenomenon is a result of direct addition of nitrogen from biosolids (Kabirinejad and Hoodaji, 2012; Cucina *et al.*, 2019) and nitrogen deposited from plant litter grown in the biosolid amended land (Xu *et al.*, 2006). In the current study, total nitrogen increased as biosolid applications rate was doubled under both rainfed-irrigated and rainfed maize cropping systems, which is in accordance with previous discoveries (Zoghlami *et al.*, 2016; Miranda *et al.*, 2018). The reported increase in total soil nitrogen with doubling biosolid applications rate under both rainfed-irrigated and rainfed system is attributed to the direct addition of nitrogen from the biosolids (Kabirinejad and Hoodaji, 2012; Cucina *et al.*, 2019) and most probably due to nitrogen deposited from plant residue (Xu *et al.*, 2006), mainly the roots because the aboveground biomass was removed from the field.

Plant residues return to the soil is one of the best sustainable nutrient management practices, which demonstrated to be effective in enhancing the nitrogen content of the soil (Xu *et al.*,

2006). According to Tesfamariam *et al.* (2009), maize grain nitrogen content significantly increased with biosolid applications rate and was higher under rainfed-irrigated than rainfed cropping system. This should have contributed to the reported significantly ($p \leq 0.05$) higher total soil nitrogen content under rainfed-irrigated relative to the rainfed system, possibly due to more residue returned to the soil under rainfed-irrigated compared to the rainfed system (Xu *et al.*, 2006).

Nitrogen is an important macronutrients capable of increasing crop yield (Wang *et al.*, 2019; Ata-Ul-Karim *et al.*, 2020). This is because nitrogen increases the photosynthetic rate by increasing leaf area index (Njuguna *et al.*, 2016) and also improving soil fertility (Su-mei *et al.*, 2020). It is evident from the current and other previous studies that, biosolids use in agricultural lands serves as an effective sustainable nutrient management practice which improves the fertility status of the soil. Besides improving the nitrogen status of the soil through the direct addition of nitrogen (Kabirinejad and Hoodaji, 2012; Cucina *et al.*, 2019), biosolids also improve soil nitrogen through enhanced plant biomass production, which contributes to soil nitrogen via litter decomposition. Therefore, biosolids could be used in agricultural lands as one of the sustainable nutrient management practice to improve nutrient status of the soil not only through the direct addition of nitrogen to the soil but also as a tool to improve biomass production which adds nitrogen to the soil through plant litter and root deposits.

5.2 Accumulations of selected emerging contaminants in the uppermost 30 cm soil layer and plant aboveground biomass

5.2.1 Triclosan accumulation in the uppermost 30 cm soil layer

Personal care product compounds such as hand soaps, deodorants, shampoos and toothpastes contain the antimicrobial substance, triclosan, which can form part of the wastewater stream (Gottschall *et al.*, 2012). Triclosan is characterized by a high Log K_{ow} = 4.8, which enables it to strongly sorb onto sludge and persist during wastewater treatment processes (Chen *et al.*, 2014a). According to Heidler and Halden (2009), about 50 % or more triclosan that enter the wastewater treatment plants, can partition or sorb to biosolids. As a consequent of the low triclosan elimination of conventional wastewater treatment processes (Chen *et al.*, 2014a), land application of biosolids leads to triclosan accumulation in agricultural lands (Lozano *et al.*, 2010; Butler *et al.*, 2012).

In the current study, triclosan level in the uppermost 30 cm soil layer was below the limit of detection for both rainfed-irrigated and rainfed maize cropping systems following 15 years of biosolid application at various rates. Similar findings had been reported by Cha and Cupples (2009) following municipal biosolid applications of 7.29 t ha^{-1} yr^{-1} for over 4 years. According to Cha and Cupples (2009), triclosan was not detected in 6 of the 10 agricultural sites. In another study conducted by Aryal and Reinhold (2011), the level of triclosan in a soil amended with biosolids at a rate of 7.3 t ha^{-1} was also below the detection limit. The findings from our study and that of Cha and Cupples (2009) as well as Aryal and Reinhold (2011), are in contrast with the findings of other studies such as that of Gottschall et al. (2012) and Mcherius et al. (2014). Gottschall *et al.* (2012) reported triclosan concentrations of 98 ng g^{-1} (dry mass basis) in the top 30 cm layer of a sandy loam soil amended with municipal sludge (containing 10 900 ng g^{-1} triclosan) at a single application rate of 22 t ha^{-1} six months after biosolid applications. Similarly, Macherius *et al.* (2014), reported a median triclosan concentrations of 1.5 ng g^{-1} (dry mass basis) in the top 10 cm soil layers four years after a single 22 t ha^{-1} biosolid application in the same area.

Generally, the concentration of triclosan in the soils from the studies by Gottschall *et al.* (2012) and Macherius *et al.* (2014) were very low, 98 ng g^{-1} and 1.5 ng g^{-1} (median), respectively, relative to the initial concentration in the biosolids (10 900 ng/g). This is most probably due to a dilution effect as it gets mixed with large volume of soil. It could also have been due to the degradation of the compound. Various authors reported varying triclosan half-life ($t_{0.05}$): 107.4 days (Lozano *et al.*, 2010), 104 days (Lozano *et al.*, 2012), 106 days (Chen *et al.*, 2014b) and 43 - 84 days (Chen *et al.*, 2014a) in biosolid amended soils. This variation is possibly due to variations in their study's environmental conditions (e.g. temperature) (Butler *et al.*, 2012) and soil properties (Chen *et al.*, 2020), because these factors influence triclosan dissipation in the soil. One of the possible reasons for the detection of triclosan in the soils reported by Gottschall *et al.* (2012) and Macherius *et al.* (2014) could possibly be due to the combination of high biosolid application rate and high triclosan concentration of the biosolids applied (Langdon *et al.*, 2012; Jachero *et al.*, 2016). The persistence of triclosan in soils amended with high biosolid application rates have also been reported by the following authors: soils amended with 20 t ha^{-1} yr^{-1} (dry mass basis) (Rivier *et al.*, 2019), 33 t ha^{-1} yr^{-1} (Xia *et al.*, 2010), 50 t ha^{-1} yr^{-1} (dry

mass basis) (Butler *et al.*, 2012), 60 t ha^{-1} yr^{-1} (dry mass basis) (Chen *et al.*, 2014b; Rivier *et al.*, 2019), 67.2 t ha^{-1} yr^{-1} (Xia *et al.*, 2010) and 72.3 t ha^{-1} yr^{-1} (wet mass basis) (Lozano *et al.*, 2012) of biosolids. The persistence of triclosan in soils amended with biosolids is influenced by several factors. These factors include compound properties, soil properties, phytoaccumulation by plants and soil biota activities (Butler *et al.*, 2012).

Compound properties

Chemical volatilization is one of the well-known dissipation pathways of chemicals from the soil. However, triclosan has a very low vapor pressure (7 x 10^{-4} Pa at 25 ^{O} C) (Reiss *et al.*, 2009), hence the volatilization of triclosan is very unlikely.

Soil properties

Organic matter of the soil affects the persistence of triclosan in the soil, by influencing its sorption (Jachero *et al.*, 2016; Cantarero *et al.*, 2017; Biel-Maeso *et al.*, 2019). High soil organic matter encourages strong triclosan sorption, hence reducing its bioavailability and increases its persistence in the soil (Jachero *et al.*, 2016; Cantarero *et al.*, 2017; Biel-Maeso *et al.*, 2019). In the current study, biosolids application improved soil organic matter under both rainfed-irrigated and rainfed cropping systems. Nonetheless, the concentration of triclosan in the soil remained below detection limit regardless of the availability of water and biosolid application rate.

Soil pH influences the charge form that triclosan exist in the soil (Butler *et al.*, 2012). Soil pH levels closer or higher than triclosan acid dissociation constant (pK_a = 7.9), lead to more presence of anionic triclosan forms rather than the neutral triclosan forms in the soil (Gottschall *et al.*, 2012). Anionic triclosan forms are highly repelled from soil colloidal surface and are less absorbed by plant roots (Butler *et al.*, 2012), hence they are not persistent in the soil. In the current study, soil pH levels ranged between 5 and 6.74, which are below triclosan pK_a value (7.9), hence low fraction of triclosan should have been in their anionic forms (Butler *et al.*, 2012). Soil pH levels of less than 7 were reported to account to just less than 10 % formation of anionic triclosan forms in clay and sandy clay loam soils (Butler *et al.*, 2012). Therefore, the

impact of soil pH on triclosan dynamics within the soil profile for the current study is expected to be low, possibly due to less formation of anionic triclosan forms (Butler *et al.*, 2012).

Soil temperatures can influence the biodegradation of triclosan in the soil (Butler *et al.*, 2012). According to Al-Rajab *et al.* (2009), an increase in the soil temperature enhances the degradation of triclosan in the soil. In their study, Al-Rajab et al. (2009) reported a rapid degradation of triclosan (95 % of the initial concentration) under a controlled laboratory incubation study at 30 ^{O}C soil temperature compared with open field study, having a soil temperature range of 10 to 20 ^{O}C, where only 40 % of initial triclosan concentration was degraded. The increase in triclosan degradation as a function of the increase in soil temperature is attributed to enhanced microbial activities (Srinivasan and Sarmah, 2014). In the current study, soil temperatures ranged between 10.5 ^{O}C and 25 ^{O}C (rainfed-irrigated) and 15.6 ^{O}C and 25 ^{O}C (rainfed) in the top 15 cm soil depth. Soil temperature ranges under the current study (Table 3.3) are similar to that of Al-Rajab *et al.* (2009) field study, where 40 % of the initial triclosan concentration was degraded.

Phytoaccumulation by plants

The presence of vegetation or plants in the soil play a crucial role in the dissipation of triclosan from the soil (Davis *et al.*, 2015). Many previous studies have reported that, plants can reduces soil triclosan concentration through phytoaccumulation mechanism (Aryal and Reinhold, 2011). In the current study, triclosan accumulation in stems, leaves and grains of maize planted to biosolid amended soils under both rainfed-irrigated and rainfed cropping systems was evident. This indicates that, phytoaccumulation of triclosan was also involved in the dissipation of triclosan from the soil.

Soil biota activities

Biological transformation has been pointed as one of the major dissipation pathway of triclosan from biosolids amended soils (Chen *et al.*, 2020). Soil microorganisms transform triclosan in biosolid amended soils to methyl triclosan and other non-extractable residues (Lozano *et al.*, 2012; Chen *et al.*, 2020). According to Butler *et al.* (2012), large portion of the initial triclosan

that accumulate in soils from biosolid applications is recovered as methyl triclosan, indicating that, transformation is the major dissipation pathway of the triclosan from the soil. In the current study, however, formation of methyl triclosan from the parent triclosan was not measured in biosolids amended soil, as it was not part of the project scope. Hence, a conclusive statement cannot be drawn regarding the transformation of triclosan.

Triclosan availability in the soil can affect soil ecological processes (Buttler *et al.*, 2011; Chen *et al.*, 2019b). Soil triclosan level of 10 mg Kg^{-1} was reported to inhibit soil respiration (Waller and Kookana, 2009; Buttler *et al.*, 2011). Triclosan is also capable of interfering with nitrogen cycle, by inhibiting the denitrification process in the soil (Chen *et al.*, 2019b). This is because triclosan is capable of reducing both the activities (Zaayman *et al.*, 2017) and the abundance of microorganisms responsible for both respiration and denitrification processes in the soil (Park *et al.*, 2013; Chen *et al.*, 2019b). It is evident from the current study that, biosolids application to agricultural lands at 16 t ha^{-1} yr^{-1} and lower did not lead to traceable concentration quantities of triclosan in the soil. However, previous findings have shown that, biosolids application to agricultural lands at 20 t ha^{-1} yr^{-1} (dry mass basis) (Rivier *et al.*, 2019), 33 t ha^{-1} yr^{-1} (Xia *et al.*, 2010), 50 t ha^{-1} yr^{-1} (dry mass basis) (Butler *et al.*, 2012), 60 t ha^{-1} yr^{-1} (dry mass basis) (Chen *et al.*, 2014b; Rivier *et al.*, 2019), 67.2 t ha^{-1} yr^{-1} (Xia *et al.*, 2010) and 72.3 t ha^{-1} yr^{-1} (wet mass basis) (Lozano *et al.*, 2012) resulted in traceable concentrations in the soil. However, this will depend on climatic factors and the quality of biosolids in terms of triclosan concentration levels. Therefore, biosolids of similar quality, as the one used in our study, in terms of their triclosan concentration, could be applied at rates lower than 16 t ha^{-1} yr^{-1} in agricultural lands under similar agro-ecological zones as a strategy to improve soil fertility and support plant growth (Tesfamariam *et al.*, 2013) without compromising the soil ecological processes due to triclosan accumulation. However, the findings from the current study should be interpreted with caution, taking into account the fact that, only parent compounds were measured and none of the transformation products or metabolites was measured in the soil. Previous studies have shown that, triclosan can undergo transformation to form methyl triclosan and other non-extractable residues in the soil (Butler *et al.*, 2012). Therefore, future studies should monitor the distribution of triclosan and its transformation products in the whole soil profile to have a clear understanding of the behaviour of triclosan in soils amended with biosolids.

Photodegradation of triclosan is also possible via the following pathways (Sanchez-Prado et al., 2006):

Reductive dechlorination leading to dichlorohydroxydiphenyl ether

Photoinduced hydrolysis leading to 2,4 dichlorophenol

Reductive photocyclization leading to 2,8- dichlorodibenzo-p-dioxin

5.2.2 Sulfamethoxazole accumulation in the uppermost 30 cm soil layer

Sulfamethoxazole is often prescribed by health professionals as an antibiotic to treat urinary tract bacterial infections in humans (Wang and Wang, 2018). However, human bodies do not completely metabolize antibiotics (Daughton and Ternes, 1999). As a result of incomplete metabolism, antibiotics such as sulfamethoxazole may form part of the municipal wastewater stream after being excreted by human bodies (Daughton and Ternes, 1999). In addition, direct flushing of antibiotics in toilets from household level can also contribute to wastewater contamination (Daughton and Ternes, 1999). Similar to triclosan, sulfamethoxazole is removed from wastewater during wastewater treatment and form part of the sludge (Gottschall *et al.*, 2012). As a result, land application of biosolids leads to the accumulation of sulfamethoxazole in agricultural soils (Holling *et al.*, 2012). In the current study, the quantity of sulfamethoxazole in the uppermost 30 cm soil layer was below the limit of detection for both rainfed and rainfed-irrigated maize cropping systems following 15 years of biosolid application at various rates. The findings from our study differ from that of Holling *et al.* (2012), who detected sulfamethoxazole concentrations of 67.4 ng g^{-1} in a soil that received 8 t ha^{-1} of sludge, 71 days following its application. One of the possible reasons for this difference is that, Holling *et al.* (2012) study was conducted under controlled laboratory conditions for a short period of time (71 days). While our study was conducted in a long-term field trail, which has been running for the past 15 years, where biosolids were applied on a yearly basis at the planting time. In addition, the soil samples from our study were collected 150 days after the last sludge application, following the maize crop harvest. The early sampling of soils by Holling *et al.* (2012) might have limited further sulfamethoxazole biodegradation and leaching to deeper soil layers or to the ground water because sulfamethoxazole has weak sorption and therefore high mobility. Sulfamethoxazole is characterized by high solubility in water (610 mg L^{-1} at 25 ^{O}C) and low Log K_{ow} (0.89) (Lucida et al., 2000; Halden and Paull, 2005; Raquel et al., 2010; Zhao

et al., 2013). This means that, sulfamethoxazole has a weak sorption (as indicated by low Log K_{ow}, i.e. 0.89) and high mobility (as a result of high solubility, i.e. 610 mg L^{-1}) in the soil (Kodesova *et al.*, 2019).

The presence of sulfamethoxazole in agricultural soil can affect soil properties (Demoling *et al.*, 2009; Wang *et al.*, 2016; Cheng *et al.*, 2020). Sulfamethoxazole concentration levels of 20 mg Kg^{-1} soil was reported to have inhibited the bacterial growth (Demoling *et al.*, 2009). In the current study, biosolids applications rates of up to 16 t ha^{-1} yr^{-1} did not lead to traceable accumulation of sulfamethoxazole in the soil (Sulfamethoxazole concentration was below LOD (0.64 ng/g)). Therefore, biosolids of similar quality, as the one used in our study, in terms of sulfamethoxazole concentrations could be applied to agricultural lands under similar agro-ecological zones as the current study sites to enhance agricultural productivity without compromising soil ecological processes due to sulfamethoxazole accumulation. Future studies should monitor the variation in the concentration of sulfamethoxazole across various sludge source streams and the distribution of sulfamethoxazole and its transformation products in the whole soil profile to have a clear understanding of the fate of sulfamethoxazole in biosolids amended soils.

5.2.3 Triclosan uptake by maize

Triclosan uptake by plants is very common in biosolids amended soils (Pannu *et al.*, 2012; Prosser *et al.*, 2014b; Jachero *et al.*, 2016). This is because biosolid contains triclosan and is added to the soil when biosolids are used as fertilizers in agricultural lands (Lozano *et al.*, 2010). As a result of triclosan contamination to the soil via biosolid applications, triclosan is transferred to plants through the process called phytoaccumulation (Prosser *et al.*, 2014b). In the current study, triclosan was detected in different parts of the maize planted to biosolid amended soils at an annual rate of 8 t ha^{-1} and higher. Previous studies by (Pannu *et al.*, 2012; Prosser *et al.*, 2014b; Jachero *et al.*, 2016) also reported similar findings. The major pathway of triclosan uptake in plants from the soil is through the process which involves the partitioning of triclosan from soil particles to the high lipid outer portion of the root tissue (epidermis or cortex) and the subsequent accumulation in the roots (Trapp and Legind, 2011; Pannu *et al.*, 2012). This conclusion was reached following the realisation that volatilization and triclosan gas-phase deposition through the plant leave stomata opening (stoma) is very unlikely due to

low vapour pressure of triclosan (7 x 10^{-4} Pa at 25 ^{O}C) (Pannu *et al.*, 2012). Also, due to low water solubility of triclosan (10 mg/L at 25 ^{O}C), the uptake through partitioning from soil solution to the root mechanism is also limited. Once triclosan has reached the xylem tissue, it is transported by bulk water flow driven by transpiration to the aboveground portion of the plant (Kinney and Heuvel, 2020). Previous studies have shown that, translocation of triclosan from roots (xylem) to the aboveground biomass (stems, leaves and fruits) of the plant is possible but is a limited process (Aryal and Reinhold, 2011; Pannu *et al.*, 2012). This is because there is less formation of water-soluble forms of triclosan (anions) in the xylem tissue caused by the pH of the xylem solution (i.e. approximately 5) being lower than triclosan pK_a (7.9) (Trapp and Legind, 2011). According to Holling *et al.* (2012), when solution media pH (e.g. soil) is less than the triclosan pK_a (i.e. 7.9), triclosan exists mostly in its neutral (protonated) form which could be easily trapped in the roots and thus have limited translocation to other parts of the plant.

Concentration of triclosan in maize stems

Soil water facilitates the movement of triclosan from the soil to the plant, by enhancing both its solubility and bioavailability (Zhang *et al.*, 2016b; Li *et al.*, 2019b). In this study, triclosan concentrations of maize stems were significantly ($p \leq 0.05$) influenced by cropping system (due to water availability variations), hence triclosan concentration of maize stems were higher under rainfed-irrigated than under rainfed cropping systems, thus supporting the claims made by Zhang *et al.* (2016b). It was also apparent that, triclosan was detected in maize stems planted to rainfed-irrigated inorganic fertilizer treatments as opposed to maize stems planted to rainfed inorganic fertilizer treatments. The variations in the detection of triclosan in maize stems planted to rainfed-irrigated and rainfed inorganic fertilizer treatments can be explained by the differences in the amount of water received between the two cropping systems. Rainfed-irrigated inorganic fertilizer treatments received additional amount of water from drip irrigation which should have enhanced triclosan solubility and its bioavailability (Zhang *et al.*, 2016b; Li *et al.*, 2019b) hence enhancing the translocation by bulk water flow (Kinney and Heuvel, 2020).

Concentration of triclosan in maize leaves

Generally, triclosan mobility and its bioavailability decreases with an increase in organic matter of the soil (Fu *et al.*, 2016; Cantarero *et al.*, 2017). This is because increase in soil organic matter enhances triclosan sorption and thus reduce its mobility and bioavailability in the soil (Jachero *et al.*, 2016; Cantarero *et al.*, 2017; Biel-Maeso *et al.*, 2019). In the current study, doubling biosolid application rates from 8 t ha^{-1} yr^{-1} to 16 t ha^{-1} yr^{-1} resulted in a significant ($p \leq 0.05$) (irrigated) decline in triclosan concentration of maize leaves following 15 consecutive years of biosolid applications. It was also apparent that doubling biosolid application rates resulted in a significant increase in soil organic matter (Figure 4.1), hence supporting the claims by Cantarero *et al.* (2017). Therefore, the decrease in triclosan concentration of maize leaves with doubling biosolid applications rates from 8 t ha^{-1} yr^{-1} to 16 t ha^{-1} yr^{-1} under the current study is most probably attributed to the increase in soil organic matter, which probably enhanced sorption and reduced the bioavailability of triclosan for maize plant uptake (Jachero *et al.*, 2016; Cantarero *et al.*, 2017; Biel-Maeso *et al.*, 2019). However, this was not a similar trend in the case of maize stems, where doubling biosolid application rates from 8 t ha^{-1} yr^{-1} to 16 t ha^{-1} yr^{-1} resulted in an increase in triclosan concentrations. There is no clear explanation for the observed variations in the latter case as biosolid applications rate doubled.

As highlighted earlier in the case of maize stems, the significantly ($p < 0.05$) higher triclosan concentration of maize leaves under rainfed-irrigated systems as opposed to rainfed is attributed to enhanced triclosan solubility and bioavailability in the soil caused by the additional water received under the rainfed-irrigated system (Zhang et al., 2016b; Li et al., 2019). This might have led to enhanced bulk water flow translocating triclosan to the leaves (Kinney and Heuvel, 2020).

Concentration of triclosan in maize grains

Similar to what was observed on maize stem and leaves, triclosan concentration of maize grain was relatively higher under rainfed-irrigated systems than the rainfed systems, but was not statistically significant ($p \leq 0.05$), which is in agreement with the claims made by Zhang *et al.* (2016b).

It is evident from the current study that, long-term biosolid applications to agricultural lands at previous South African biosolid application upper limit (8 t ha^{-1}) and higher, lead to the addition of triclosan in maize plant parts as a result of triclosan-soil-root partitioning, followed by the bulk water flow distribution of triclosan through the xylem to aerial plant parts including the edible parts (grains). The presence of triclosan in the soil and the subsequent uptake by plants can have phytotoxicity on plants (An *et al.*, 2009). Wheat roots elongation was reduced when wheat plants were exposed to triclosan concentrations between 50 and 250 mg L^{-1} (An *et al.*, 2009). Triclosan concentration in the soil from the current study was below LOD (i.e. less than 1.96 ng g^{-1}) and was also below the concentration of triclosan in the study of An *et al.* (2009). Therefore, based on our results, we can conclude that, applying biosolids of similar quality to agricultural lands under similar agro-ecological zones at rates of 16 t ha^{-1} yr^{-1} and lower may not cause phytotoxicity effect on plants. However, future studies should also monitor the formation and the distribution of the transformation products of triclosan in the whole plant biomass.

5.2.4 Sulfamethoxazole uptake by maize

Sulfamethoxazole can have phytotoxic effect on plants (Zhu *et al.*, 2020) by damaging plant roots and reducing photosynthetic efficiency (Lv *et al.*, 2021). Meanwhile, studies have shown that, organic matter from biosolids enhances sulfamethoxazole sorption in the soil and reduce its bioavailability for plant uptake (Hu *et al.*, 2019). Most previous research have focused on sulfamethoxazole uptake from soils irrigated with wastewater (Sallach *et al.*, 2015; Zhang *et al.*, 2016b), contaminated water (Kodesova *et al.*, 2019), manure amended soils (Mullen *et al.*, 2019), hydroponics (Herklotz *et al.*, 2010) and sulfamethoxazole spiked (fortified) soils (Cheng *et al.*, 2020). Few studies reported on the fate of sulfamethoxazole from biosolid amended soils such as that of Holling et al. (2012). Holling *et al.* (2012) grown cabbage plants in biosolid amended soil (sulfamethoxazole concentration of the soil at the end of the experiment 67.4 ng/g), and detected sulfamethoxazole only in roots of one cabbage plant. Despite the fact that, Holling *et al.* (2012) detected sulfamethoxazole in cabbage plant grown on biosolids amended soils, they reported low sulfamethoxazole phytoaccumulation (as indicated by its presence in only one plant) and translocation (as indicated by the detection in roots only) in plants. Kodesova *et al.* (2019) observed similar low uptake and limited translocation of sulfamethoxazole in plants and associated this behaviour of sulfamethoxazole with its rapid

degradation in the soil as a result of high mobility (as indicated by high water solubility, i.e., 610 mg/L at 25 ^{O}C) in the soil. Such high water solubility may result in high leaching (Pan and Chu, 2017), making sulfamethoxazole not to be available in the soil for plant uptake. In the current study, sulfamethoxazole concentration was below the detection limit (i.e. 0.64 ng/g) in maize planted to biosolids amended soils under both rainfed and rainfed-irrigated maize cropping system. This could be most probably attributed to the rapid degradation of sulfamethoxazole in the soil (Kodesova *et al.*, 2019) and possibly due to leaching (Pan and Chu, 2017) hence reducing its bioavailability in the soil for plant uptake. Also, based on pH values of our soils (between 4.8 and 6.74), which are mostly higher than sulfamethoxazole pK_as (1.7 and 5.7), except for the 16 t ha^{-1} y^{-1} biosolid treatments, the sulfamethoxazole should have been in its ionic form in most of the treatments (Holling *et al.*, 2012). Ionic charged compounds cross the root cell membrane at slow speed as opposed to the neutral (protonated) charged compounds (Kinney and Heuvel, 2020).

Therefore, based on the current study, it can be concluded that, applying biosolids with similar sulfamethoxazole concentrations to agricultural lands under similar agro-ecological zones at rates of 16 t ha^{-1} yr^{-1} and lower may not result in accumulation within the uppermost 30 cm soil layer, cause phytotoxicity and transfer to the edible portion of maize grain. However, future studies should monitor the distribution of sulfamethoxazole and its transformation products in the whole plant biomass in order to have a clear understanding of the uptake, behaviour and the distribution of sulfamethoxazole in plants.

CHAPTER 6

HUMAN HEALTH RISK ASSESSMENT

To assess the potential human health risks which may be encountered via maize dietary intake of plants grown in biosolids amended soils, the human health risk assessment test was performed.

Maize grains triclosan concentrations were used to evaluate the potential human health risk of ingesting biosolids-derived triclosan via dietary intake. Triclosan concentrations of maize stems and leaves were excluded for health risk assessment as they are not representing the edible portion of a maize plant by humans. The maximum average triclosan concentration of maize grains was used as it represents the worst-case scenario.

To evaluate the possibility of appreciable human health risk that could result via dietary intake, the value of the Estimated Daily Intake (EDI) of triclosan was calculated. Using the calculated EDI and the reported Acceptable Daily Intake (ADI) of triclosan, i.e., 83µg kg^{-1} day^{-1} (Prosser and Sibley, 2015), the human health hazard quotient ratio (HQ) was calculated. EDI (Eq. 1) and HQ (Eq. 2) were calculated after (Prosser and Sibley, 2015; Liu *et al.*, 2020b; Nie *et al.*, 2020) as follows:

$$EDI_{(\mu g/g/day)} = \frac{C_{(\mu g/g)} \times GI_{(g/day)}}{BW_{(Kg)}} \quad \text{(Eq. 1)}$$

$$HQ = \frac{EDI}{ADI} \quad \text{(Eq.2)}$$

Where C_{grains} represented the maximum average concentration of triclosan in maize grains (Table 4.6). GI which is 350g day^{-1} represented the average maximum maize intake per person/day in the Sub- Sahara African region (Kornher, 2018). BW represented the average adult (individuals older than 18 years) or toddler (children's aged between 1 and 4 years) body

weights in South Africa which is estimated to be 70 Kg and 12 Kg, respectively (Abafe and Martincigh, 2016). Contaminant concentration in plant tissue is considered a potential hazard to human health via dietary intake when the calculated HQ ratio is > 0.1.

Using the recorded maximum average grain triclosan concentration of 42.8 ng g^{-1} (0.0428 µg/g) (Table 4.6), health risk assessment indicates that for adults, the triclosan EDI was calculated as 0.21µg/kg/day, whereas for toddlers it was calculated as 1.25µg/kg/day which are far below the tissue biosolids-derived acceptable daily intake (ADI) of triclosan (83µg/kg/day) reported by Prosser and Sibley (2015). Other previous studies have also reported triclosan EDI values in edible plant tissues below the ADI (83µg/kg/day) (Liu *et al.*, 2020b; Nie *et al.*, 2020). These EDI values represent the raw grains (Uncooked) and are likely to be lower in the cooked or boiled grains due to high temperature exposure. Further assessment indicated that the HQs for adults and toddlers were 0.003 and 0.01, respectively which were also well below the 0.1 (potential HQ value).

CHAPTER 7

CONCLUSIONS AND RECOMMENDATION

7.1 Conclusions

Long-term (> 15 years) biosolid applications to agricultural land enhanced total soil nitrogen and soil organic matter in the uppermost 30 cm soil layer under both rainfed-irrigated and rainfed maize cropping systems. This demonstrates that biosolids use in agricultural lands serves as both a climate change action strategy and a sustainable nutrient management practice by enhancing soil carbon sequestration and soil fertility, respectively. Hence, improving crop production and playing role in poverty alleviation. This is, however, associated with an increase in soil acidity (declining soil pH) and soil salinity (EC), which may limit soil productivity and subsequently lower crop production. Therefore, it is of most importance to lime biosolid amended soils to alleviate problems associated with soil acidification. The observed increment in soil salinity due to biosolid application is within acceptable limits to be a cause for concern.

Long-term biosolids applications to agricultural lands at 16 t ha^{-1} yr^{-1} and lower rates did not results in traceable concentration levels of triclosan and sulfamethoxazole in the top 30 cm soil layer under both rainfed and rainfed-irrigated maize cropping systems. Similarly, sulfamethoxazole concentration in maize crop planted to long-term biosolids amended soils was below the detection limits. However, triclosan was detected in different above ground biomass parts of maize planted to soils that received 8 and 16 t ha^{-1} biosolids annually under both rainfed-irrigated and rainfed cropping systems. We can conclude that, biosolids of similar quality in terms of their triclosan and sulfamethoxazole concentration could be applied at rates lower than 16 t ha^{-1} yr^{-1} in agricultural lands under similar agro-ecological zones as a strategy to improve soil fertility and support plant growth without contaminating the soil with triclosan and sulfamethoxazole at traceable levels and without causing human health risks via dietary intake. However, it is worth mentioning that, only parent compounds were measured and none of their transformation products or metabolites were measured.

7.2 Recommendations

- To ensure sustainable agricultural production, proper lime applications and soil profile leaching strategies must be synchronized with biosolid applications to raise soil pH and lower the build-up of soluble salts in the top soil layer.
- Future studies should monitor the variation in the concentration of triclosan and sulfamethoxazole across various sludge source streams and the distribution of triclosan, sulfamethoxazole and their transformation products in the whole soil profile to have a clear understanding of their fate in biosolids amended soils.
- The distribution of sulfamethoxazole, triclosan and their transformation products in the whole plant biomass should also be monitored to have a clear understanding of their fate in the plants.

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