COMPOSITION AND FATE OF TRICLOSAN IN THE SLUDGE FROM WASTEWATER TREATMENT IN GRAHAMSTOWN, SOUTH AFRICA AND TIARET, ALGERIA

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ABSTRACT

Physicochemical properties such as pH, specific surface area (SSA), cationic exchange capacity (CEC), loss on ignition (LOI), pathogens, plant nutrients (nitrates, ammonium and phosphates), and heavy metals (manganese, copper, lead and cadmium) were determined for sewage sludge from Grahamstown and Tiaret. The values obtained were log transformed thereafter a *t*-test at 5 % level of significance was used to test for the difference in each parameter for both sludges. The pH of sludge was determined in 1:3 water, 1:6 water, 1:3 0.01 M calcium chloride and 1:3 1 M potassium chloride. The pH for Grahamstown and Tiaret sludge were in the ranges of 6.66-7.11 and 7.88-8.18 respectively. The SSA values for Grahamstown and Tiaret were 218 ± 108 and 261 ± 99.9 m^2/g, and the CEC values were 119 ± 2.09 and 136 ± 6.03 mEq/100, respectively. The LOI values obtained were 1.33 ± 0.03 and 1.48 ± 0.11 % for Grahamstown and Tiaret, respectively. *E. coli* and heterotrophic bacteria were the pathogens determined, and were extracted from sludge using sterile saline and nutrient broth. The concentration of *E. coli* in Grahamstown and Tiaret sludge were 468 ± 7.63 and 7769 ± 1268 CFU/g d.w and for heterotrophic bacteria were $1.17\times10^9 \pm 7.42\times10^8$ and $1.43\times10^9 \pm 9.11\times10^8$ CFU/g d.w. For Grahamstown sludge, the concentration of nitrates, ammonium and phosphates were 55.61 ± 55.20 mg/g d.w, 6.60 ± 2.36 mg/g d.w and 1.40 ± 0.30 mg/g d.w, respectively. For Tiaret sludge, the concentration of nitrates, ammonium and phosphates were 2.56 ± 2.90 mg/g d.w, 0.64 ± 0.45 mg/g d.w and 0.24 ± 0.19 mg/g d.w, respectively. The concentration of Mn, Cu, Pb and Cd in Grahamstown sludge were 423 ± 101, 353 ± 92, 40.2 ± 20 and 0.0 mg/kg d.w respectively, and for Tiaret sludge, the corresponding concentrations were 358± 295, 549±50, 1427± 1352 and 1.54 ± 0.61 mg/kg d.w. Sewage sludge was found to contain Triclosan, and solubility studies of the compound were conducted using sodium deoxycholate and sodium lithocholate. The apparent solubilities and rate constants indicated in brackets of TCS at 37 ºC were 35.4 ± 1.21 mg/L (1.28 ± 0.36 Hr^-) and 14.4 ± 0.34 mg/L (0.99 ± 0.17 Hr^-) in sodium lithocholate and sodium deoxycholate, respectively. The apparent solubilities and rate constants indicated in brackets of TCS at 15 ºC were 32.3 ± 0.88 mg/L (2.16 ± 0.80 Hr^-) and 14.2 ± 0.39 mg/L (1.02 ± 0.17 Hr^-) in sodium lithocholate and sodium deoxycholate, respectively. Triclosan was extracted from sludge using 1 g/L sodium deoxycholate and the determined concentration were 142 ±

33.5 µg/g d.w for Grahamstown sludge and 0-12 µg/g d.w for Tiaret sludge. Finally plant growth studies were conducted on radish and garden cress plants using Grahamstown sludge at 0, 20, 40, 80 and 100 % treatments. Statistical analysis (*t*-test and Kruskal-Wallis) at 5 % level of significance was done to compare growth parameters between control and different sludge treatments. For radish plants, the values for plant height, root length, number of leaves, leaf length and dry mass were 28.4-80-7 mm, 4.3-44.7 mm, 3.3-17.0 mm, 2.3-4.0 leaves and 6.3-15.3 %, respectively. For garden cress, the values for plant height, root length, number of leaves, leaf length and dry mass were 13.7-25.0 mm, 7.7-20.3 mm, 5.7-8.3 leaves, 3.0-8.3 mm and 8.8-15.0 %, respectively. Twenty percent (20 %) sludge treatment gave the best results in radish and garden cress plants with respect to plant height, root length, number of leaves and dry mass. Triclosan concentration in radish and garden cress plants was below the detection limit of 32.4 µg/g d.w.

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ACRONYMS AND ABBREVIATIONS

AFNOR	Association Françoise de Normalization
$CaCl_2$	Calcium chloride
Cd	Cadmium
CEC	Cationic Exchange Capacity
COD	Chemical oxygen demand
Cu	Copper
dH_20	Distilled Water
d.w	Dry weight
DWAF	Department Of Water Affairs and Forestry
E. coli	Escherichia coli
EGME	Ethylene glycol monoethyl ether
ELISA	Enzyme Linked Immonosorbent Assay
GC	Gas Chromatography
H	Hour
KCl	Potassium chloride
LOI	Loss on Ignition
Min	Minutes

1	**Mn**	Manganese
2	**MS**	Mass spectrometry
3	**N**	Nitrogen
4	**NH_4Cl**	Ammonium chloride
5	**NH_4OH**	Ammonium Hydroxide
6	**Nm**	Nanometers
7	**P**	Phosphorus
8	**Pb**	Lead
9	**PEG**	Poly ethylene glycol
10	**SSA**	Specific surface area
11	**TCS**	Triclosan
12	**UV**	Ultra violet
13	**WHO**	World Health Organization
14	**Zn**	Zinc
15		

1 CHAPTER 1

LITERATURE REVIEW: SEWAGE SLUDGE MANAGEMENT PRACTICES IN SOUTH AFRICA AND ALGERIA

1.1 INTRODUCTION

In Africa, most countries are increasing in agricultural activity to improve economic development (DWAF, 1998; Snyman and Herselman, 2006). The most serious problem encountered by farmers is the degradation of agricultural soils through erosion and nutrient depletion of soils through incorrect agricultural practices (Snyman and Van der Waals, 2004). A study conducted by Kribaa et al., (2001) demonstrated that most Algerian soils are carbonate-rich soils, with low organic matter present in the soil. One of the most economically viable sources with organic material suitable for soil is sewage sludge (Snyman and Van der Waals, 2004). Studies conducted by Henning and Snyman, (1999); Snyman and De Jong, (1998) showed the beneficial use of sewage sludge under South African conditions in the short term (3 years). To ensure safe and sustainable use of sludge for soil amendment purposes, physicochemical analyses of sewage sludge analysis and effects of sludge on plants should be investigated.

The objective of this chapter is to highlight wastewater treatment process and sewage sludge management practices in South Africa and Algeria. This includes the benefits and challenges encountered when sewage sludge is used for beneficial use such as in agriculture, although more emphasis will be placed on the fate of Triclosan in wastewater treatment plants (WWTPs).

1.2 WASTEWATER TREATMENT IN SOUTH AFRICA AND ALGERIA

Wastewater treatment plants are biotechnology systems where domestic sewage and industrial effluents are treated and sludge solids produced as a by-product. The sludge composition is related to the type of treated wastewater, microbial composition of the active biofilms and hydraulic residence time.Sludge contains a mixture of solids (organic and mineral) and water; and potentially pathogenic microorganisms(Bitton, 1994; Okoh et al., 2007). According to national legislation and as function of the composition, the sewage sludge is considered a waste product in Algeria (Kehila, 2014)and South Africa(DWAF, 1998; Snyman and Herselman, 2006).Wastewater treatment involves physical, chemical or biological processes or combinations of these processes depending on the required outflow standards set by the legislation authorities in each country. An overview of the layout of a waste water treatment plant is shown in figure 1.1 below. The design of wastewater treatment plants is the same in both South Africa and Algeria as stated in the EPA guidelines (EPA, 1997) as this design has shown to be effective in the treatment of wastewater

(AFNOR, 1996; DWAF, 1996). The treatment processes differ and will be discussed in detail below.

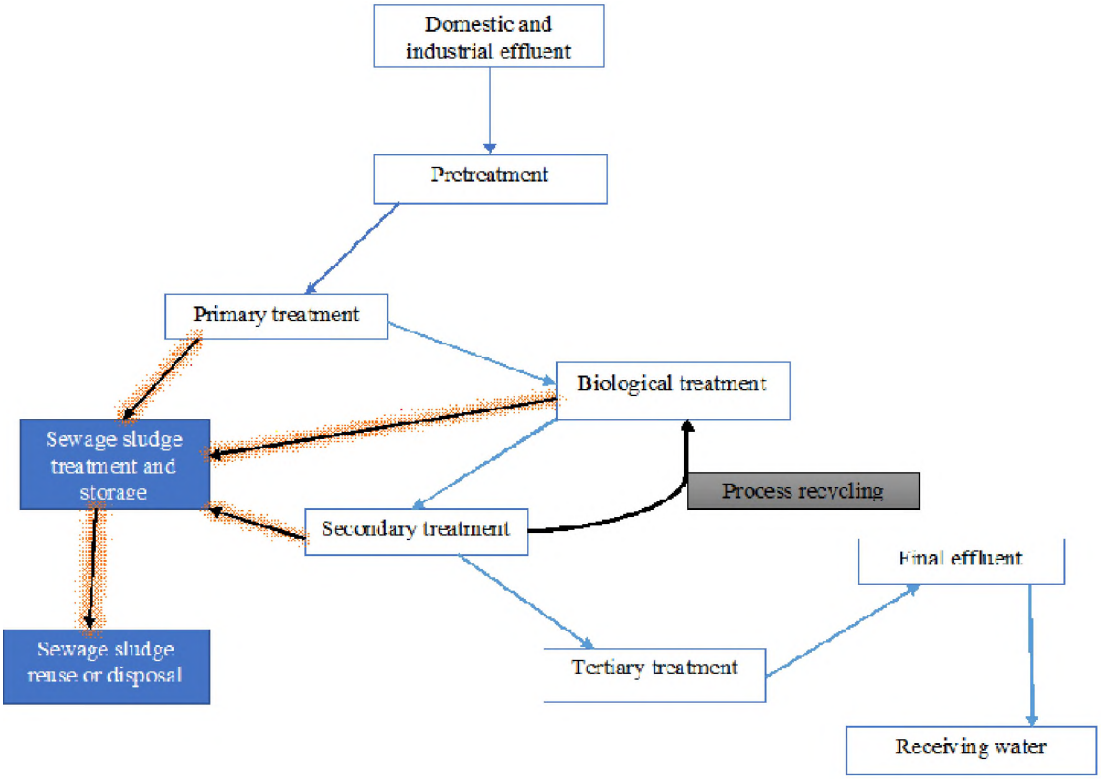


Figure 1.1:*Overview of wastewater treatment plants in South Africa and Algeria obtained from EPA guidelines redrawn using Windows Paint software* (EPA, 1997)

Wastewater treatment is divided into various stages, which are preliminary, primary, secondary and tertiary treatment. In preliminary treatment,material such as oils, fats, grease, grit, rags and large solids are removed(van Beelen, 2007). Subsequently, primary treatment follows whereby the suspended portion of wastewater settles at the bottom of the tank. Biological treatment of wastewater takes place in fixed media or suspended growth reactors using activated sludge, biofiltration, rotating biological contactors and constructed

wetlands(van Beelen, 2007)which are available in Belmont Valley WWTP and Tiaret WWTP. Nitrification or denitrification and biological phosphorus removal is incorporated at this stage (Biological treatment) and decreases nutrient concentrations in the outflow. Chemical treatment is used to improve the settling abilities of suspended solids prior to a solids removal stage or to adjust the properties or components of wastewater prior to biological treatment (e.g. pH adjustment, reduction of heavymetals or nutrient adjustment). It may also beused for precipitating phosphorus in conjunction with biological phosphorus treatment(AFNOR, 1996; DWAF, 1996; EPA, 1997).

Secondary treatment separates the sludge solids from the outflow of the biological stage with biological phosphorus treatment.Tertiary treatment refers to processes which are used to further reduce parameter values (turbidity and microorganisms) below the standards set out in national regulations. These are the guidelines for the utilization or disposal of waste (DWAF, 1998; Snyman and Herselman, 2006)and solid waste management standards(Kehila, 2014). The term is often used in reference to nutrient removal.Sludge treatment can be a significant part of a WWTP and involves the stabilization and or thickening and dewatering of sludge prior to reuse or disposal(DWAF, 1996; EPA, 1997).

1.2.1 PRIMARY TREATMENT

The purpose of primary treatment is to reduce the velocity of the incoming wastewater stream thereby allowing the largesolids to settle to the bottom of the tank(EPA, 1997). The

effluent from the preliminary treatment step is further treated in the primary treatment step where large solids and inorganic solids are removed by the sedimentation process(Maksimova et al., 2015). The water is left to stand in primary settlementtanks so that any large solids can sink and settle at the bottom of the tank (van Beelen, 2007). These solids are referred to as the sludge.Usually, 50-70 % of suspended solids are removed in primary settlement tanks. Furthermore, BOD is reduced by 20-50 % and the bacterial count by 25-75 %. The pH is usually unchanged by primary settlement(van Beelen, 2007; EPA, 1997).

1.2.2 BIOLOGICAL TREATMENT

The biology of wastewater treatment is based on the utilization of organic matter by microorganisms which include bacteria, viruses, algae and protozoa(Herselman et al., 2005;Bitton, 1994). Bacteria are the most common microorganisms used in wastewater treatment; thesemicroorganisms directly breakdown the polluting matter present in waste waters(van Beelen, 2007). Aerobic bacteria breakdown matter in the presence of oxygen; anaerobic bacteria breakdown matter in the absence of oxygen whilst facultative bacteria have the potential to function as both aerobic and anaerobic bacteria(van Beelen, 2007). Heterotrophic bacteria break down organic material like carbohydrates, fats and proteins. Thesebroken down products are characterized by the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of a wastewater(EPA, 1997). These compounds (carbohydrates, fats and proteins) are generally easily biodegradable and so the bacteria thrive and utilize them to increase cell growth (DWAF, 1996). Heterotrophic bacteria are responsible for the stabilization of concentrated organic sludges produced in wastewater

treatment(Bitton, 1994). Autotrophic bacteria derive their cell carbon from carbon dioxide and use a non-organic source of energy for cell growth, and these arenitrifying bacteria that oxidize ammoniato nitrite (NO_2^-) and nitrate (NO_3^-) under aerobic conditions(Bitton, 1994). Thesebacteria grow slower than heterotrophs and are sensitive to environmental changes such as toxic shock loads(EPA, 1997).

1.2.3 SECONDARY TREATMENT

The secondary treatment step is referred to as Biological Nutrient Removal (BNR). At this step, nutrient removal involves the reduction in phosphorus, nitrogen and chemical oxygen demand (COD) concentrations in wastewater. The removal of nitrogen and phosphorus prevents growth of algal and other photosynthetic aquatic organisms in the receiving waters.BNR has been found to be the most effective process in the decreasing nitrogen and phosphorus concentrations in wastewater. In Belmont Valley and Tiaret wastewater treatment plants (WWTP), biological nutrients are removed by activated sludge(AFNOR, 1996; DWAF, 1996; Kamizoulis et al., 2010), which is the most common method used internationally. Activated sludge treatment plants use a mass of microorganisms to aerobically treat wastewater. In this process, the organic contaminants in the wastewater provide the nutrients which promote microbial growth. Thereafter, wastewater is aerated in an aeration tank which converts the organic matter into microbial tissues and carbon dioxide, thus reducing COD of wastewater. The mixed liquor which consists of microorganisms and wastewater is then aerated for a short period of time and afterwards passed into a settling tank or secondary clarifiers where the biofilm or sludge settles to the bottom of the tank by

gravity. The sludge is pumped back into the aeration tank where it is mixed with the incoming wastewater, or the sludge is removed from the system in the process called wasting.In the Belmont Valley, the sludge is pumped back into the aeration tank at least 4-6 times(Mambo et al., 2014) whilst in Tiaret WWTP the sludge is pumped back into the aeration tank between 12-18 times(Barceló and Petrovic, 2011). As a result, the physicochemical properties of the sludge start to be differences in the nutrient and COD concentrations. The resultant effluent can be further treated or discharged.

Activated sludge consists of a variety of microorganisms, with aerobic bacteria being the predominant organisms; other microorganisms include protozoa and rotifers(Bitton, 1994).The presence of particular microorganisms indicate the conditions of the process, for example, the presence of nematodes and rotifers in the system is an indication that there has been longer aeration times(EPA, 1997).

1.2.4 TERTIARY TREATMENT

The final step of the wastewater treatment process is the tertiary treatment. This disinfection of wastewater as activated sludge fails to remove more than 90 % of microorganisms. The pathogenic microorganisms removed at this stage include faecal coliforms, streptococci, salmonella and enteric viruses(Bitton, 1994). In Belmont Valley, tertiary treatment involves treating the water with chlorine, ultra-violet light irradiation and using membrane technologies (0.1-1 μm membrane) to remove solids(Mambo et al., 2014). In Tiaret WWTP, tertiary treatment involves chemical disinfection using ozone and irradiation using ultra-

violet (UV) light(Barceló and Petrovic, 2011). The tertiary treatment is an additional treatment aftersecondary and this treatment can remove more than 99% of all the pollutants presentin wastewater, as a result producing an effluent of high quality(EPA, 1997).

1.3 SOURCES OF SLUDGE

Sewage sludge is an organic solid, semi-solid or liquid product of wastewater treatment process that contains human faecal waste as well as waste products and contaminants from domestic and industrial discharge(Herselman et al., 2005). The characteristics of sludge depend on waste stream of each treatment facility as well as treatment process (Marriot, 1998; Snyman and Van der Waals, 2004).

Growth of the human population has resulted in an increase in waste products including organic waste such as sewage sludge. Due to this increase, a challenge has been encountered by a majority of countries to dispose of the escalating amount of waste products (Herselman et al., 2005; Kehila, 2014). In South Africa, each person produces approximately 60 g of dry sludge a day(Lincoln, 2011). In Algeria, each person produces approximately 27 g of dry sludge(Kehila, 2014).Due to an increase in urbanization and industrialization in South Africa and Algeria, there is an increase in the amount of sludge produced(Herselman et al., 2005), thus as a consequence the current disposal routes (landfilling and incineration) are becoming increasingly unacceptable from an environmental point of view (NEMA, 2013; Sadek et al., 2013). Table 1.1 shows disposal practices of sludge in France, United Kingdom, USA, South Africa and Algeria.

Table 1.1: *Disposal practices of sewage sludge*

Country	Annual production (1000 dry tons)	Disposal method (%)			
		Agriculture	**Landfill**	**Incineration**	**Other**
France	700	50	50	0	0
United Kingdom	1075	51	16	5	28
USA	5357	36	38	16	10
South Africa	310	30	67	0	3
Algeria	10300	1	35	7	57

In WWTPs, sludge is derived from various processes and each type has its own characteristics which are mainly determined by moisture content, which is significant for stabilization and disposal route (Ross et al., 1992). The classification of different types of sewage sludge, is the same in South Africa and Algeria. Algeria has adopted guidelines set by World Health Organization (WHO)(WHO, 2010), and the same guidelines are the ones South Africa adopted as well. In South Africa, sewage sludge is classified as Type A, B, C and D, with decreasing order of potential to cause odor problems, fly breeding and transmission of pathogens to humans and environment. Type C and D sludges are parallel in hygienic quality only that Type D is produced for unrestricted use on land at an application rate of 8 tons/ha/year due to the low level of contaminants(DWAF, 1998; Snyman and Herselman, 2006).

Table 1.2: *Classification of sewage sludge to be disposed of on land(DWAF, 1998; Snyman and Herselman, 2006; WHO, 2010)*

Type of sewage sludge	Origin/Treatment	Characteristics of sludge
Type A Sludge	Raw sludge; Cold digested sludge; Septic tank sludge; Oxidation pond sludge	Unstable and can cause odour nuisances and fly breeding. Contains pathogenic organisms and variable metal and inorganic content
Type B sludge	Anaerobic digested sludge; Surplus activated sludge; Humus tank sludge	Fully or partially stabilized - should not cause significant odor nuisance or fly-breeding Contains pathogenic organisms and variable metal and inorganic content
Type C sludge	Pasteurized sludge; Heat-treated sludge; Lime-stabilized sludge; Composted sludge; irradiated sludge	Certified to comply with the following qualified requirement: Stabilized - Should not cause odour nuisances or fly-breeding; Contains no viable Ascaris ova per 10 g dry sludge; Maximum 0 Salmonella per 10 g dry sludge; Maximum 1000 Faecal coliform per 10 g dry sludge immediately after treatment; Variable metal and inorganic content
Type D sludge	Pasteurized sludge; Heat-treated sludge; lime-stabilized sludge; Composted sludge; irradiated sludge	Certified: Stabilized - Should not cause odor nuisances or fly-breeding; Contains no viable Ascaris ova per 10 g dry sludge; Max 0 Salmonella per 10 g dry sludge; Max 1000 Faecal coliform per 10 g dry sludge immediately after treatment; Maxi metal and inorganic content in dry sludge. User must be informed about the moisture and N, P and K content. User must be warned that not more than 8 t ha-1 year-1 may be applied to soil and that the pH of the soil should be preferably be higher than 6.5.

1.4 AGRICULTURAL UTILIZATION OF SLUDGE

According to table 1.1, beneficial use of sewage sludge for agricultural purposes is 30and 1 % in South Africa and Algeria, respectively(Benhamou and Fazouane, 2013; Herselman et al., 2005). This disposal route has started to be considered as it is an economic option for most WWTPs in these two countries(Herselman et al., 2005). However, the application of sewage sludge on agricultural soils in Algeria (Benhamou and Fazouane, 2013) and South Africa (Snyman and Van der Waals, 2004) is relatively not prominent because of lack of studies in the use of sewage sludge and high human health and environmental concerns. Sewage sludge could play a vital role in improving soil properties not only in South Africa and Algeria but furthermore globally as it the sludge could be of great economic and recycling value.

1.5 BENEFITS OF USING SEWAGE SLUDGE IN ENERGY PRODUCTION

In Algeria, due to the increase in municipal solid waste (Sadek et al., 2013), sewage sludge has been considered for beneficial use in methanisation so as to generate renewable energy(Benhamou and Fazouane, 2013). This increase in municipal solid waste, would possibly increase the generation of electricity to an estimated 5.85 terawatt-hours(TWh) in 2020 in Algeria (Kalloum et al., 2011). The major disadvantage of the use of sewage sludge in production of renewable energy are the end products formed such as pollutant gases and

ash with high metal composition which can be detrimental to public health and environment (Kalloum et al., 2011).

1.6 BENEFITS OF SEWAGE SLUDGE IN AGRICULTURE

The application of sewage sludge in agricultural soils is a common practice and has shown to improve soil properties as a result increasing plant productivity,and become a residual disposal route (Tamrabet et al., 2009; Wang et al., 2008). Sewage sludge is currently most frequently disposed offby use as fertilizer or for soil amendment purposes. Sewage sludge improves soil porosity, aggregate stability, bulk density, water retention and movement. In addition, organic matter and plant nutrient bioavailability increases (Fumagalli et al., 2013; Wang et al., 2008; Xu et al., 2013).South Africa has a variable climate, which ranges from subtropical to semi-arid or arid(South Africa Info, 2013), whilst Algeria has a semi-arid climate(Climate Zone, 2004). A semi-arid climate is known to favour rapid soil organic matter mineralization because of higher temperatures reached in summer(Kribaa et al., 2001). A study conducted by Maksimova et al., (2015) showed that the application of 25% of sludge on soil in growing lawn grass resulted in 2.8 times better growth than absence of sludge. Tamrabet et al., (2009) conducted a study on the growth of Durum Wheat at Agricultural Farm of the Field Crop Institute (Setif, Algeria) using sewage sludge obtained from Setif WWTP, and they observed that the sludge increased plant growthsignificantly when compared to plants grownusing mineralized fertilizer(Chata et al., 2002). A study conducted Tamrabet et al., (2009)showed that application of sewage sludge acts a seal as it maintains moisture content and the plants grown develop a deeper rooting system than untreated soils.

The physicochemical properties of sludge in soil amendment facilitates nutrient transport, increase water retention and improve soil texture (Ekama, 1993). Due to the incomplete removal of plant nutrients (nitrogen and phosphorus) in the wastewater treatment process, the presence of these nutrients in sludge could improve the soil's nutritional status after application. Nevertheless, long-term benefits of sludge use in soil agriculture as a source of plant nutrients is limited by the presence of organic pollutants, heavy metals and pathogens (Alvarenga et al., 2015; Benhamou and Fazouane, 2013; Wang et al., 2008). Therefore, considering the beneficial use of sewage sludge, benefits should be weighed up alongside limitations before and after treatment.

1.6.1 PLANT NUTRIENTS

The presence of high levels of organic matter and nutrients makes agricultural reuse of sludge be a great recyclable value (Snyman and Van der Waals, 2004). The total nitrogen (N) and total phosphorus (P) has been shown to be between 2.8-10.5% and 6.9-13.5% weight fraction of dry matter respectively (Alvarenga et al., 2015; Fumagalli et al., 2013). Approximately 40-60% of total nitrogen in sewage sludge exists in an inorganic form, that is nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) which is rapidly available for uptake by plants (Gilbert et al., 2011). As a consequence, nitrate-N is highly water-soluble and it could potentially lead to leaching to groundwater (Gilbert et al., 2011). N also exists as ammonium-N, which is vulnerable to be released to the atmosphere by volatilizing to form ammonia (NH_3) when applied to soil and thus nitrogen uptake by the soil should be quick to

prevent N losses (Gilbert et al., 2011). Organic N (mainly urea-nitrogen and amino-nitrogen) will become available over a period of time and it must be decomposed by soil microorganisms or mineralized to inorganic ammonium-N and nitrate-N before being available to the plants, therefore this makes sewage sludge release N over a prolonged period of time (Gilbert et al., 2011). On the other hand, the application of sewage sludge to meet N demands of plants results in more P than required, on the other hand the amount of P which ends up leaching to soil increases e.g. eutrophication(Gilbert et al., 2011).

Due to variability in sewage sludges, the nutrient content varies and therefore analysis needs to be done before applying the sludge onto the soil in order to calculate the appropriate rate of application(Gilbert et al., 2011). The use of sewage sludge for soil amendment purposes has risks, and these are (i) high ammonium-N which can contribute to ammonia emissions which reduce bioavailable N, (ii) high nitrate-N which can lead to leaching into groundwater, and(iii) potential contamination of soils due to the presence of pathogenic organisms(Alvarenga et al., 2015).

Considering the potential impacts of sewage sludge nutrients on the environment, it is necessary to deal with problems associated with N and P in agricultural soils Sludge type however plays a role to determine the extent to which nutrients will be present and available for beneficial crop growth (Snyman and Van der Waals, 2004). Legislation in South Africa(NEMA, 2013) and Algeria(Barceló and Petrovic, 2011)(WHO, 2010)are designed in such a way that inorganic-N or total Ncontent determines the maximum application rates.

Nonetheless, legislation does not take into consideration that some N exist in organic form, meaning that mineralization into organic form should first occur preceding plant uptake (Gilbert et al., 2011). Therefore, plant availability of N from sewage sludge is generally lower than commercial fertilizers(Gilbert et al., 2011; Korentajer, 1991). Table 1.3 shows the regulations set by WHO (WHO, 2010) and guidelines for the utilization and disposal on waste sludge for South Africa (DWAF, 1998; Snyman and Herselman, 2006).

Table 1.3: *Regulations of total nitrogen and phosphate content of dry sludge*

Nutrie	Range (%
Total N	3.2-4.5
Total P	1.5-1.7

1.6.2 HEAVY METALS

The determination of heavy metals present in sewage sludge is necessary prior to deciding the disposal route of the waste product (landfilling, agricultural purposes or production of recyclable energy). The use of sludge for soil amendment purposes also brings the likelihood of introducing toxic metals into the soil. The sludge contains potentially toxic elements which include Zinc (Zn), Copper (Cu), Nickel (Ni), Cadmium (Cd), Lead (Pb) and Mercury (Hg) (Alvarenga et al., 2015). The regulatory limits set by South Africa (NEMA, 2013) and Algeria (WHO, 2010)must be adhered to so as to prevent human health risksand environmental pollution.The guidelines for the utilization and disposal waste sludge

(DWAF, 1998; Snyman and Herselman, 2006) and National Environment Management Act(NEMA, 2013) have become the regulatory authorities in South Africa to regulate the standards of solid wastes to be disposed. In Algeria, Solid waste management organization (Kehila, 2014), Department of purification and the environment protection (DAPE) (DAPE, 2011) and EPA guidelines (Barceló and Petrovic, 2011)are responsible for the monitoring and management of solid waste.

The use of sewage sludge for soil amendment purposes has resulted in heavy metals being a concern in (i) human health when sludge is applied to agricultural soils, (ii) effects on surface and groundwater quality as some metals will leach down the soil profile over time (Gilbert et al., 2011; Lester et al., 1983). The transfer of these metals from the sludge treated soil have been found in the leaves and edible parts of the crops that have been grown using these soils, consequently this poses as a risk to the health of humans, animals and the plants(Xu et al., 2013).

Heavy metals are mainly found in sewage sludge because of increase in urbanization and industrialization, which therefore results in influents into the sewage system containing these heavy metals (Shamuyarira and Gumbo, 2014). Heavy metals are associated with the solid waste portion in the wastewater treatment process, and thus they are adsorbed onto sludge particles(Page et al., 1981; Tiruneh et al., 2014). In cases whereby domestic and industrial influents are treated in the same facility, domestic wastes have been observed to have lower heavy metal content than industrial wastes, thus heavy metals such as Pb, Cd, Hg and Ni

may be present in municipal influents because of high urbanization and entry of untreated industrial waste (McGrath et al., 2000; Singh et al., 2004). A study conducted by Shamuyarira and Gumbo, (2014) in South Africa, showed that the concentration of sewage Cd was between 0.82 and 3.10 milligrams per kilogram of dry weight (mg/kg dw), Cu was 263.68-626.00 mg/kg dw and Pb was 21.28-171.87 mg/kg dw. The results obtained from the study were all within limitsstated on the South African sludge guidelines (DWAF, 1998; Snyman and Herselman, 2006) and on the National Environment Management Act(NEMA, 2013). Table 1.4 shows the regulatory limits of sludge intended for agricultural use for Algeria and South Africa.

Table 1.4: *Regulatory limits of heavy metals in sewage sludge intended for agricultural application*

Heav meta	**EPA/WHO- Algeri guidelines**	**South African guidelines**	
	Limit value (mg/kg	Class A pollutant limit (mg/kg)	Class B Pollutant limit (mg/kg)
Cd	20-40	40	85
Cu	1000-1750	1500	4300
Pb	750-1200	300	840
Mn	Not specified	1000	Not specified

The huge risk of heavy metals in soils is their ability to leach from soils to groundwater. pH affects the leaching of heavy metals down the soil profile and consequently thelow pH increases water solubility of these metalsand vice versa (Alloway, 1995). This risk increases

with time because the metals are persistent in soils for long periods time, and metals do not undergo bio-chemical degradation, thereby increasing bioavailability of the metals and allowing them to leach to groundwater (Gadepalle et al., 2008). On a study conducted by Antoniadis and Alloway (2003)in soils amended with sludge, heavy metals showed to percolate down the soil profile up to a of 0.8 meters within the soil profile, indicating the potential of metals to leach into groundwater. Heavy metals may be leached through cracks (greater than 75 μm)within the soil profilevia a process called macropore transport.In this process, large pores open in the structure of the soil and allow fact percolation down the soil profile (McGrath and Lane, 1989; Williams et al., 1987).

1.6.2.1 Toxicity of heavy metals in humans and plants

Heavy metals such as Cd, Cu, Pb and Mn have a potential to accumulate in plants when sludge is used as a soil amendment, and may inhibit plant growth and can cause health problems in humans and animals that consume the plants(Tiruneh et al., 2014). A study conducted by Herselman and du Preez, (2000), found high heavy metal composition in spinach grown on soil amended sludge for 3 years. A study conducted bySnyman and Van der Waals, (2004), significant concentrations of Cd, Cu, Pb and Zn in maize, sunflower and soybean plants grown on soils amended with sludge. The concentrations Cd, Cu, Pb and Zn in sewage sludge were 1.8, 114.3, 66.0 and 679.0 mg/kg respectively. In sunflower, the heavy metal concentrations were Cu (18.0 mg/kg), Cd (0.23 mg/kg), Pb (10.0 mg/kg) and Zn (39.9 mg/kg) (Snyman and Van der Waals, 2004). This study confirmed that the use of sewage sludge with a high metal load will result in plant absorption of the metals, and thus

quantification of heavy metals in sludge and soils is important as it assists in the determination of application rates that may result in reduction of metal uptake by plants.

Cu concentration of more than 21 mg/kg of wet weight in plants, is an indication of toxicity (Gupta and Sinha, 2007). A study conducted by Gupta and Sinha, (2007) reported Cu concentrations ranging from 10 mg/kg in cucumber to 70 mg/kg in maize plants.Cu has a tendency to displace iron (Fe) (Mengel and Kirkby, 2001) causing chlorosis, Fe deficiency(Pais and Benton-Jones, 1997) and inhibits root growth (Mengel and Kirkby, 2001).Above toxic concentration (21 mg/kg of wet weight), Cu interferes with photosynthesis, resulting in leaf chlorosis and necrosis (Pais and Benton-Jones, 1997). In humans, Cu toxicity is possible as small quantities are likely to trigger an effectas stated in the South African Medicines Formulary (SAMF) (SAMF, 2010). A concentration of 0.1-0.2 mg/kg in the human body may result in gastrointestinal disturbances(SAMF, 2010).Cd is the most mobile heavy metal, which suppresses plant growth at a concentration of 3 mg/kg(Snyman and Van der Waals, 2004). In the human body, Cd accumulates in the kidney and to a lesser extent in the liver and spleen (Mengel and Kirkby, 2001). On consumption of plants containing Cd, the metal causes hypertension and is carcinogenic (Stewart-Pinkham, 1989).Pb mimics the behaviour of calcium (Ca) (Mengel and Kirkby, 2001). Due to the similarities of Pb and Ca(Mengel and Kirkby, 2001), Pbhas a tendency of accumulatingin the skeleton (Pais and Benton-Jones, 1997). In the human body Pb exposure leads to anaemia, gingival leadline (Langston, 1989),it is carcinogenic and impacts on brain development (Pais and Benton-Jones, 1997).

1.6.3 PATHOGENS

The presence of pathogens in sewage sludge potentially increases health risks to humans (Ross et al., 1992). There are five main types of pathogens in sludge and these are bacteria, viruses, fungi and yeasts, parasitic worms and protozoa (Snyman and Van der Waals, 2004). The presence of these pathogens in sludge consequently leads to contamination of surface water and groundwater by pathogens transported by runoff and filtration water (Korentajer, 1991; Snyman and Van der Waals, 2004). The pathogens present in sewage sludge originate from humans who suffer from acute or latent infections and reach the sewage plants through excretion of faeces and urine (Snyman and Van der Waals, 2004; Strauch, 1991). The varietyand concentrationsof pathogens are extended by other sources connected to the sewage system such as hospitals, abattoirs and livestock markets (Snyman and Van der Waals, 2004; Strauch, 1991). Exact species and quantities of pathogens present in sewage sludge from different WWTPs will be influenced by the health status of the humancommunity, and may vary substantially at different times(Okoh et al., 2007). Pathogens in sewage plants are associated with insoluble solids. Many of the pathogenic organisms become bound to solids after wastewater treatment and subsequently may be transferred to wastewater sludge (Bitton, 1994; Snyman and Van der Waals, 2004). During wastewater treatment process, these solids are concentrated into sewage sludge, as a result sewage sludge has higher quantities of pathogens than incoming wastewater (EPA, 1999). A study by Snyman and Van der Waals, (2004) observed the following pathogen concentrations in sewage sludge, 4*Ascaris* egg ova and faecal coliforms of 3800 CFU/g. Upon conducting growth of potatoes in the same study, 1800 CFU/g of *E. coli* were found in potato peels and there was no *Ascaris*or *Salmonella* present in potato tissue (Snyman and

Van der Waals, 2004). This study showed that the presence of pathogenic microorganisms may be found in plants grown in sludge amended soils, and thus it is important to determine pathogens present in sewage sludge prior to the use, as these microorganisms may influencehuman health.

Treatment processes such as lagoons, trickling filters and activated sludge in biological treatment may extensively reduce the number of pathogens in wastewater treatment, but the resulting sludge may still contain significant amounts of pathogens that may pose human and environmental health(EPA, 1999). Bacteria, viruses, protozoa and helminthes are the major human pathogenic organisms and may all be present in sludge and therefore may cause infections in humans or animals if they are exposed to high concentrations of these pathogens (EPA, 1999). Pathogenicity may differ in intensity from mild gastroenteritis to severe and sometimes fatal diarrhea, hepatitis, typhoid and dysentery(Okoh et al., 2007), therefore the beneficial reuse of sludge must be monitored to protect human health (EPA, 1999; Snyman and Van der Waals, 2004).

1.6.3.1 Bacteria

Bacteria pathogens of primary concern in sludge include *Salmonella, Shigella, Campylobacter, Escherichia coli (E.coli)* and heterotrophic bacteria (Snyman and Van der Waals, 2004). *E.coli* is predominantly abundant in human and animal faeces, and can have concentrations of up to 10^9 CFU/g(Okoh et al., 2007; Scotsman, 1998; Snyman and Van der Waals, 2004). A study conducted by Bubert et al., (1999) demonstrated that contamination of food material does not only occur during food processing, but also begins with the

production of raw materials in the environment. The survival times of pathogens in soil are affected by soil moisture, pH, temperature and organic matter(Snyman and Van der Waals, 2004).

E.coli is one of microorganisms found in the human gastrointestinal tract that often cause diseaseoutbreaks(Lee and Jones-Lee, 1993), and fruits and vegetables contain sufficient nutrients that promote bacterial growth of these pathogens. Therefore, if the barrier that was provided by the peel is broken, an opportunity to allow bacterial colonization is created (Janisewicz et al., 1999). *E.coli* was shown to cause hemorrhagic colitis and gastroenteritis for the first time in USA in 1982(Riley et al., 1983), and is known to be leading cause of childhood kidney failure (Janisewicz et al., 1999). These are heterotrophic microorganisms that breakdown organic material such as carbohydrates, fats and proteins and because of easy degradability of the these compounds, they form derivatives that increase bacterial growth (EPA, 1997).

The beneficial reuse of sewage sludge in agriculture for growing plants and cropsis of great economic value, and thus if the sewage sludge is to be reused, pathogen load in the sludge should be examined to reduce human health risks. Guidelines for the utilization and disposal of wastewater sludge of South Africa (DWAF, 1998; Snyman and Herselman, 2006)and Algeria (WHO, 2010)standards for microbiology are shown in table 1.5. Different classifications exist, so as to identify where the sludge has to be used waste.

Table 1.5: *South African microbiological classification of faecal coliforms for sludge intended for beneficial use*

	Unrestricted use quality		**General use quality**		**Limited quality use**
	Class A		**Class B**		**Class C**
	Target value	Maximum permissible	Target value	Maximum permissible	
Faecal coliform (CFU/g d.w)	$< 1x10^3$	$1x10^4$	$< 1x10^6$	$< 1x10^7$	$> 1x10^7$

1.7 SOUTH AFRICAN WASTEWATER LEGISLATION

In South Africa, the use of water is governed by laws and the aim of the National Water Act 36 of 1998 is to recognize that water is a scarce resource and it is unevenly distributed which occurs in many different forms which are all part of a unitary, interdependent cycle.Recognizing that while water is a natural resource and belongs to everyone, the discriminatory laws and practices of the past have prevented equal access to water and the use of water resources.The protection of the quality of water is necessary to ensure the sustainability of the nation's water resource in the interest of all water users.

The National Water Act acknowledges the national government's overall responsibility for the authority over the nation's water resources and their use, including the equitable allocation of water for beneficial use, the redistribution of water and the international water matters. The act further recognizes that the aim of water resource management is to achieve a sustainable use of water for the benefit of all users, and recognizing the need for cohesive

management of all aspects of water resources and where fitting, the delegation of management function to a regional or catchment level so as to enable everyone to participate (DWAF 1, 2014).

The Water Services Act 108 of 1997 identifies the right of access to basic water supply and sanitation which is necessary to ensure sufficient water and an environment not harmful to health and well-being and that all government spheres must strive to provide water supply and sanitation services sufficient for sustainable economic activity, this act also recognizes that in striving to provide water supply services and sanitation services, all government sphere must observe and obey the principles of co-operative government, the act also recognizes that the delivery of water supply services and sanitation services is an activity distinct from the overall management of water service, it must be assumed in a manner consistent with the broader goal of water resource management (DWAF 2, 2014). These water acts govern or ensure the use of water in a sustainable manner in South Africa.

1.8 ALGERIAN WASTEWATER LEGISLATION

Algeria is presently looking at improving water availability to 600 m^3/inhabitants a year by adopting a new water resources policy and new alternatives that enable to ease the crisis. A total of 95 % of the population in rural and urban areas has access to safe and clean water(Sattar and Demmak, 2014), with an average of 170 liters of water/person/day. The total municipal water generated in 2013 was 3.1×10^9 m^3(Sattar and Demmak, 2014) and 6 ×

10^8 m^3 of treated wastewater was made available for agriculture compared to 9×10^7 m^3in 1999(Abbott, 2011). The use of wastewater for irrigation is governed by a legal frame work that sets health and environmental safety requirements(Monitoring & Evaluation for Water In North Africa) (Abbott, 2011). A policy developed by integrated water resources management (IWRM)is aimedat improving all forms of irrigation is currently taking place in Algeria byutilizing available water resources that will beused more effectively by adopting water-saving irrigation techniques such as trickle irrigation.

Treated wastewater represents a promising alternative that is not only constantly available but also increasingly available, with the development of cities, tourism and industry. In the agricultural sector, reuse of wastewater is a technique that adds to the value of the water resources while it protects the environment(Kamizoulis et al., 2010). Currently in Algeria, the reuse of treated wastewater is used in the irrigation of fodder crops, pasture and trees, but the legislation is developing guidelines to allow the reuse of water in irrigatingraw-eaten vegetable crops such as carrots, onions and tomatoes(AWC, 2011). The Algerian laws oblige also the cities of more than 100 000 inhabitants to treat their effluents, prior to any disposal or reuse, through a wastewater treatment station, and in less populated areas through wastewater stabilization ponds or sedimentation basins. Consequently, in the last few years, the Algerian authorities have initiated an ambitious program that enables mainly: (a) the rehabilitation of 56sewage treatment plants, (b) the construction of new 56 sewage treatment plants (activated sludge) for the cities of more than 100 000 inhabitants, and (c) for small populated areas, the construction of 67 lagoons(AWC, 2011). For the success of the program, there were efficient follow ups and periodic evaluation so that the wastewater

valorization becomes fruitful, and to safeguard the water resources and the environment from negative impacts of pollution (Tamrabet et al., 2009).

The use of pharmaceutical products containing antimicrobial agents has increased as these compounds have been detected at varying concentrations in wastewater effluent (Petrie et al., 2014). During wastewater treatment, many of the chemicals, including biocides, are removed, but some chemicals still reach surface waters. The efficiency with which WWTPs remove contaminants depends upon the particular wastewater treatment with some treatment plants reaching up to 98 % efficient removal from wastewater (Thompson et al. (2005). Wastewater and sewage sludge have been found to contain Triclosan which is antimicrobial found in most household products (Petrie et al., 2014). The high concentration of Triclosan in wastewater influent is due to increased use of soaps, in which more than 30 % of bar soaps contain Triclosan (Lozano et al., 2013). During wastewater treatment, Triclosan partitions to sewage biosolids such as sludge (Behera et al., 2010). Sewage sludge has been found to improve soil characteristics and has been used as a soil amendment in agricultural economies. The growing interest in the possible negative effects Triclosan can have on humans and the environment and the factors that lead to the Triclosan being found in receiving waters prompted the need to investigate and evaluate methods of detection of Triclosan in wastewater treatment systems.

1.9 THE FATE OF TRICLOSAN IN WASTEWATER TREATMENT PLANTS

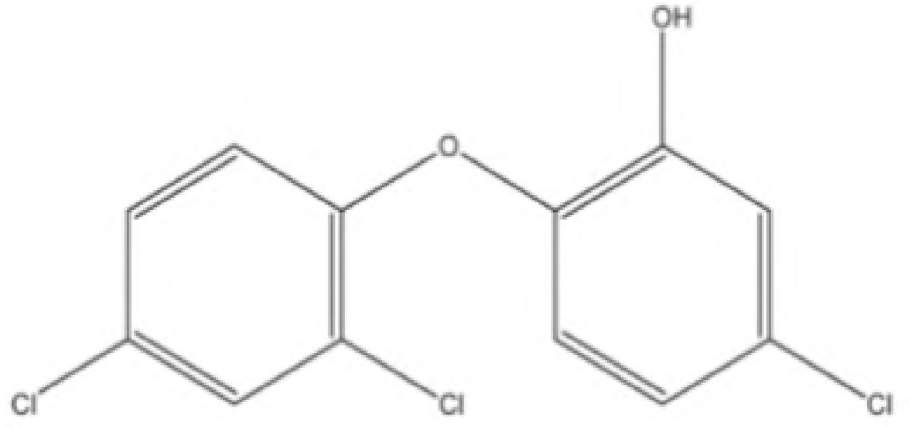


Figure 1.2: *Structure of Triclosan drawn using ACD/Chem sketch(Andrade et al., 2015).*

Triclosan (TCS) or 5-chloro-2-(2,4-dichlorophenoxy)phenol, is an active ingredient used in a variety of health care and consumer products(Chen et al., 2011). It is a chlorinated bisphenol, with a broad spectrum of antimicrobial, antifungal and antiviral action(Franz et al., 2008), which is used as an additive in many household products e.g. soaps, shampoos and toothpaste, and has been intensively used in improving environmental hygiene (Wu et al., 2007). TCS inhibits the enzyme enoyl reductase coenzyme A (enoyl-CoA), thus blocking fatty acid synthesis(Wu et al., 2009), which in bacteria produces lipid-containing components including cell membranes and in viruses, the mechanism of action against will likely differ from the mechanism of action against cellular microorganisms(Zheng et al., 2005). In bacteria, fatty acid biosynthesis is carried out by a group of individual enzymes collectively known as type II. In mammals it is carried out by a single multifunctional enzyme-acyl carrier protein (ACP) complex referred to as type I (McLeod et al., 2001;

Zheng et al., 2005).In mammals, enoyl-ACP reductase is required for the conversion of trans-2-butenoate to butanoate, and the inhibition of this enzyme will result in disruption of the fatty acid biosynthesis (Franz et al., 2008; McMurry et al., 1998). TCS has been found to be effective against various pathogenic viruses such as avian influenza A H5N1 virus, murine norovirus and feline calicivirus (Park et al., 2010) when used in concentrations between 0.05 and 0.5% (Dellano et al., 2009). TCS is also effective against various dermatophytes such as *Trichophyton mentagrophytes, Trichophyton rubrum* and *Epidermophyton floccosum*(Bondi et al., 2007).

Due to the widespread and extensive use of TCS, it has led to elevated concentrations of this compound in wastewater, wastewater treatment plants (WWTP) and in receiving waters (Chen et al., 2011). In wastewater TCSis removed from the liquid phase by concentrating the solids (Lozano et al., 2013). A study conducted by(Lozano et al., 2013) investigating TCS concentrations in wastewater in both the liquid phase and solid phase in a WWTP showed that the concentration of TCS in the influent was 8.05 ± 0.47µg/L which gradually decreased to 0.23 ± 0.13 µg/L in the effluent, and thus a removal of 97.1 ± 1.7% (Lozano et al., 2013). A study conducted by McAvoy et al., (2002) showed that the wastewater influent had a TCS concentration of 8.41 ± 0.17 µg/L and the concentration of TCS in the effluent decreased to 3.8 ± 1.16 µg/L.TCS has a K_{ow}value of 4.8 (Halden and Paull, 2005).Lozano et al., (2013)found that most TCS (around 80%) is attached to biosolids and in addition, the highest removal rates of TCS from the liquid phase isachieved in the primary treatmentwith removal of 75.4 ± 7.6%taking place mainly through sorption and settling or sedimentation of solids. In secondary treatment, the removal of TCS is reduced to 73.1 ± 4.8%. When comparing

mass removal, TCS found in sludge from primary treatment was 5.74 ± 0.65 kilograms per day (kg/day) (loading rate) and from secondary treatment was 2.31 ± 0.15 kg/day. Therefore TCS removed in the primary and secondary treatment waslargely present in the sewage sludge produced at the WWTP.

The type of wastewater and treatment process used at a given WWTP will control the sludge composition(González-Ubierna et al., 2012). The type of climate in every region controls the ambient temperatures and humidity, thus the speciation of organic pollutants such as TCS(Kolpin et al., 2002) will populate different parts of the biological WWTP (Gauthier et al., 2000).The current study, TCS was quantified in sewage sludge obtained from Grahamstown (South Africa) and Tiaret (Algeria) WWTPs. South Africa has a variable climate which ranges from subtropical to semi-arid (South Africa Info, 2013), whilst Algeria is mainly dryand hot with respect to climate(Climate Zone, 2004). Therefore composition of TCS will most likely differ between the two countries, as well as from previous studies which were conducted in mild climates(Smith, 2009; Thomas and Foster, 2005).

The removal of TCS from wastewater followed a seasonal trend, ranging from 42.6-97.1%, in a study conducted in 2014 at Lakefield, Canada (Hoque et al., 2014). The main challenge in the removal of TCS is that it is a relatively hydrophobic compound, and its removal is mainly through sorption (Petrie et al., 2014) and biodegradation by microorganisms (Hua et al., 2005). According to Thompson et al. (2005), 95-98% of TCS is removed by sorption in primary and biological treatment, which implies that it is mostly found in sludge. The

activated sludge plants use mechanisms to ensure that the dissolved oxygen is utilized to promote growth of microorganisms which remove the organic material, decreasing the load in the wastewater (van Beelen, 2007). As most wastewater treatment technologies are not particularly designed to remove micropollutants, therefore TCS can enter the environment, disperse and persist to a greater extent than expected (Ohe et al., 2011).

In the Mediterranean region, there are frequent water scarcity problems and as a result, there is low dilution capacity, which consequently poses environmental risks of TCS. A study conducted by Ricart et al., (2010), observed that between 0.5 and 500 μg/L of TCS had an effect on biofilm algae and bacteria. The lower no effect concentration (NEC), which is defined as the concentration at or below which microorganisms are not killed or whereby no effect of the compound is observed, is of 0.2 μg/L for TCS (Ricart et al., 2010). In as much as TCS affects both algal and bacterial communities in the biofilm, toxicity is higher in bacteria than algae. It is unknown if the algae has specific target sites for TCS, but studies on axenic algae hada EC_{50}value of between 0.7and 66 μg/L (Capdevielle et al., 2008). The nonexistence or absence of bacteria in biofilms on the study conducted by Ricart et al., (2010) suggested that the adverse effects were caused by TCS, and thus absence ofbacteria can be attributed to a direct mode of action of the bactericide (TCS).

According to Dinwiddie et al., (2014), TCS is a xenoestrogen, an oestrogen mimicking compound which interferes with the oestrogen binding receptors, this then affects mainly reproductive health, puberty and pregnancy. TCS has been linked to the decrease of

testosterone and thyroid hormone, and this is caused by the decreased levels of the thyroxine hormone in turn caused by an increase in glucoronidase activity, which can result in learning disabilities or lead to infertility(Gee et al., 2008). TCS has been detected in human breast milk, which raise health concerns with regards tobreast feeding mothers (Gee et al., 2008), thus the compound may influence child development is effectively absorbed by the body(Ayoola Saheed, 2012).

Traditionally, TCS has been used as surgical scrubs, hand and body washes and in dental care products. Its extensive use has led to concerns that it would exert a selective pressure for antibiotic-resistant strains of staphylococci and other bacteria arising in hospitals and domiciliary environments (Levy, 2002). At low concentration, TCS inhibits enoyl ACP reductase enzyme in *E.coli, P.aeruginosa* and *S.aureus.* Amutation to produce an altered enzyme occurs, or the overexpression of this gene can produce resistance to this agent, or resulting in the efflux of other antimicrobials out of the cell(Fan et al., 2002; Russell, 2003). Exposure to TCS of a TCS-sensitive mutant of *P.aeruginosa* switches on an efflux pump that renders the cell highly resistant to ciprofloxacin (Chuanchen et al., 2001), and furthermore, some mutants selected by TCS have shown to increase resistance to isoniazid (Bannerjee et al., 1994). Therefore, the use of TCS has contributed to antibiotic resistance due to its broad spectrum of antibacterial properties and multiple drug targets (Russell, 2003, 2004), and thus close monitoring on the compound should be done especially when biosolids are to be used for beneficial purposes.

1.9.1 SORPTION OF TRICLOSAN

Land application of sewage sludge is a common practice worldwide(Wu et al., 2009). This application alters the physicochemical properties of the soil by increasing the soil-water retention and organic matter content, asreported in both short and long term experiments. This change in soil properties can affect the interaction with many compounds(Wu et al., 2009). To demonstrate sorption of TCS, studies have been conducted using different types of soils. In a study by Wu et al., (2009), the solid/aqueous coefficient (K_d) of TCS, as calculated by equation (1.1) below(Petrie et al., 2014):

$$K_d = \frac{P}{A} \qquad (1.1)$$

Where P is the concentration of TCS in particulate (solid) phase (ng/kg), and $A$the concentration of TCS in the aqueous phase (ng/L).

$K_d$values reported for TCS range between 178 and 264 L/kg (Wu et al., 2009). A study conducted by Petrie et al., (2014), observed that TCS had an affinity for suspended solids with a relative distribution in the particulate phase of 29 ± 1 % and thus confirming that its removal in the wastewater treatment process is by sorption. TCS has a pK_a of 7.9(Halden and Paull, 2005), in consequence at pH 4 the compound exists in its unionized form and therefore sorption will increase at low pH. At pH above 8.5, TCS starts to exist in its ionic form and thus sorption of the compound is low at alkaline pH(Behera et al., 2010; Wu et al., 2009). In studies conducted by Behera et al., (2010) to demonstrate sorption of TCSusing activated carbon, kaolinite and montmorillonite further illustrated that TCS sorption is pH-dependent and at pH 3 the concentration of TCS in these media was 30 mg/g, 6.4 mg/g and 19.3 mg/g, respectively. To further elucidate the pH dependence of TCS, at pH 10 the concentration of

TCS in kaolinite and montmorillonite were found to be 1.5 mg/g and 3 mg/g respectively (Behera et al., 2010).

In conclusion, TCS can persist in soils from several days to months, and this persistence is dependent on the soil condition (aerobic or anaerobic) (Wu et al., 2009). There are various ways to removing TCS from the liquid phase besides sorption onto wastewater sludge and these include volatilization, photolysis depending on the pH and biodegradation (Thompson et al., 2005).The hydrophobic nature of TCS would suggest that it would be removed from the particulate phase and will be retained in the primary and secondary sludge, and if the sludge is intended for beneficial reuse, the analysis of sewage sludge is very critical (Thompson et al., 2005).

1.9.2 BIOACCUMULATION OF TRICLOSAN

Bioaccumulation refers to the buildup of toxic chemicals or compounds in various tissues of living organisms and this occurs when the rate of intake of a substance is greater than the rate of excretion or metabolic transformation of that substance(Coogan et al., 2007).The accumulation of anthropogenic chemical compounds in the aqueous environment and their potential deleterious effects on wildlife and humans isof increasing concern, with mainly agricultural and industrial persistent organic pollutants being the major cause(Fair et al., 2009).

Persistent chemicals are stable and only break down over long periods of time and tend to bioaccumulate in various organisms. These chemicals include polychlorinated biphenyls (PCBs), TCS, dichlorodiphenyltrichloroethane (DDT), dioxins and mercury (MDCH, 2011). In humans, TCS is absorbed through the skin, intestinal tract and mouth mucosa (Fair et al., 2009). In addition, it has a strong affinity for human liver, adipose and brain tissue. A study by Geens et al., (2012) showed concentrations of 1.48 ng/g, 3.78 ng/g and 0.91 ng/g in these organs respectively. TCS potential to bioaccumulatehas been observed in fish and marine mammals, with concentrationsranging between 0.75 and 10 ng/g (Fair et al., 2009; Geens et al., 2012). In a study conducted by Geens et al., (2012), upon intravaginal administration of ^{14}C-labelled TCS to Wistar rats, the tissue concentrations were highest in plasma, kidney and liver but extremely low in the brain, fat cells and skeletal muscle (Geens et al., 2012). Fair et al., (2009)conducted a study on dolphins, and found plasma concentrations between 0.025 and 0.27 ng/g of wet weight, with the effluent from the WWTPs ranging between 2800 and 3400 ng/L (Fair et al., 2009).

Therefore bioaccumulation is an important process to be studied as chemicals can persist in living organisms. The understanding of bioaccumulation is therefore important in protecting humans and other organisms from adverse effects of chemical exposure and it is important in regulation of chemicals (MDCH, 2011).

1.9.3 SURFACTANTS

Surfactants are a group of amphipathic chemicals and are designed to possess both disinfectant and solubilization properties, and the properties will depend on the compound in question (Ying, 2006). These compounds possess hydrophilic and hydrophobic characteristics, hence they are used in cleaning products, personal care products, polymers, pesticides, pharmaceuticals and various other products (Ying, 2006). The environmental contamination by surfactants is increasing due to the widespread use of detergents previously mentioned (Scott and Jones, 2000). There are two major surfactants which are currently in use and they are called linear alkylbenzene sulphonates (LAS) and alkyl phenol ethoxylates (APE); and these are partially degraded under aerobic digestion and as a result the surfactant metabolites are adsorbed onto sewage sludge which later on is applied to land (Scott and Jones, 2000).

When these surfactants are dissolved in water at low concentrations they exist as hemicelles. At higher concentrations they form micelles and the concentration at which this occurs is known as critical micelle concentration (CMC); with nonionic surfactants having a much lower CMC than both anionic and cationic surfactants (Ying, 2006). The formation of these micelles is what gives surfactants their detergency and solubilization properties (Ying, 2006). The formation of micelles depends on the polarity of the solvent and the characteristics of the surfactant e.g. chemical structure of the surfactant and the pH of the solvent. In a non-polar solution, the hydrophobic section of the surfactant turns towards the bulk of the solvent (figure 1.4) and the hydrophilic groups turn inside the micelles so as to

form an environment which can readily accommodate polar molecules such as water- in-oil emulsions, and in polar solvents, the reverse is true, this is to accommodate hydrophobic molecules such as oil in water emulsions (Cserháti et al., 2002).

1.9.3.1 Anionic surfactants

Anionic surfactants are not only responsible for changing the surface characteristic of solids by adsorption, but are capable of enhancing the solubility of hydrophobic compounds in water (Cserháti et al., 2002). These anionic surfactants are most commonly used and make up about 41% of all artificial surfactants used in industry (Liwarska-Bizukojc and Bizukojc, 2005), and as a result they have a high concentration in raw sludge and thus they strongly sorb onto sludge during treatment (Ying, 2006). Between 10 and 35% of LAS found in raw sewage has been found to adsorb onto particulate matter (Scott and Jones, 2000), and sediments that have been removed from the sludge settling tanks have been found to have a LAS concentration ranging between 5000 and 15000 mg/L with LAS having a half-life of less than 3 days (Ying, 2006). In a study conducted by Ying, (2006), on aerobic treated sludge, the concentration of LAS was between 100 and 500 mg/kg of dry weight (dw) of sludge while anaerobically treated sludge has concentrations ranging from 5000-15000 mg/kg dw (Ying, 2006). Nevertheless, LAS concentration in sludge depends on the individual WWTP because the input into sewage plant treatment method and efficiency are different (Ying, 2006).

1.9.3.2 Cationic surfactants

Cationic surfactants are quaternary ammonium compounds (QACs) which have a positive charge and have a strong affinity for the surface of particulates in sewage sludge which is predominantly negatively charged (Scott and Jones, 2000; Ying, 2006). In a study conducted by Scott and Jones, (2000), 95% of cationic surfactants were adsorbed onto activated sludge particulate matter (Scott and Jones, 2000). Cationic surfactants are biologically available in the environment with octadecyltrimethylammonium chloride with half-life of 2.5 hours in wastewater (Scott and Jones, 2000). Cationic surfactants with quaternary ammonium groups e.g. R_4N^+; where R is the alkyl chain and N is the quaternary nitrogen base, possess strong biocidal properties (Ying, 2006).

1.9.3.3 Nonionic surfactants

Nonionic surfactants include APE and fatty alcohol ethoxylates (AE) (Scott and Jones, 2000). Concern over the use of these nonionic surfactants has increased because of their relatively stable biodegradation products, nonylphenol (NP) and octylphenol (OP), which are toxic to both marine and freshwater species (Ying, 2006).

1.9.3.3.1 Fatty alcohol ethoxylates

This class of surfactants was developed as an alternative eco-friendly class to APE (Scott and Jones, 2000). These surfactants are easily biodegradable especially linear AE with greater than 80% primary degradation in 28 days and 40% for branched AE (Scott and Jones, 2000). The concentration of AE in sludge has been found to be less than 700 mg/kg and such concentrations suggest that AE are not entirely biodegradable under anaerobic conditions (Scott and Jones, 2000). Primary degradability of between 75 and 98% in aqueous environment is achieved in 10 days without significant accumulation of polyethylene glycol (PEG) and furthermore AE is readily biodegraded in a variety of soils and this suggests that they will not accumulate in aerobic sludge-amended soils (Ying, 2006).

1.9.3.3.2 Alkyl phenol ethoxylates

This class of nonionic surfactants undergoes almost complete primary degradation in aerobic digestion (Scott and Jones, 2000). Due to the amphiphilic nature of APE and their by-products, they have a higher affinity for particulate surfaces and therefore a significant proportion of APEs has been found in sludge (Scott and Jones, 2000). In aerobic digested sludge, the concentration of APE was found to be 0.3 mg/kg and in anaerobically digested sludge, the concentration was between 900 and 1100 mg/kg (Scott and Jones, 2000).

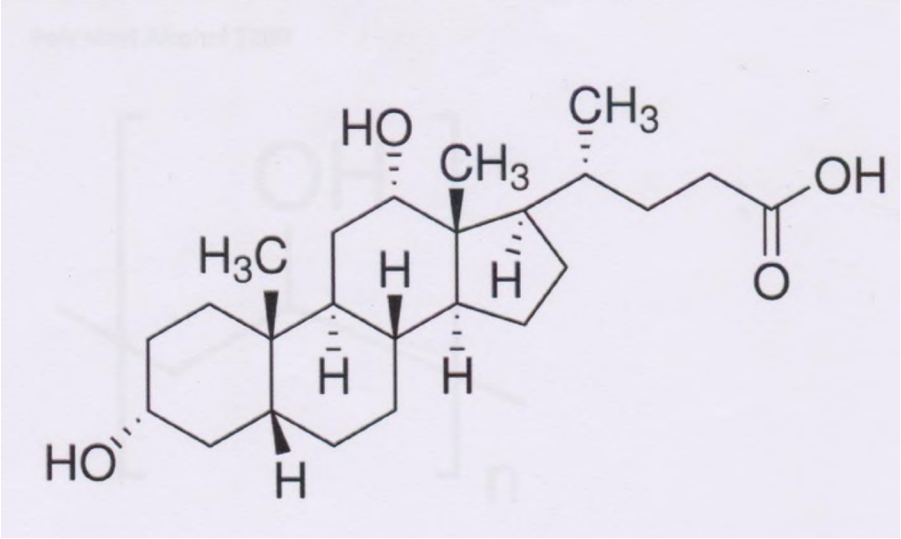
1.9.3.4 Bile acids as surfactants

Bile acids (BAs) are a group of water-soluble steroids formed during the catabolism of cholesterol, and synthesized in hepatocytes of the liver(Stamp and Jenkins, 2009). BAs are not only the water-soluble end products of cholesterol breakdown, but they are also amphipathic molecules with numerous physiological roles(Hofmann and Mysels, 1992).In bile, BAs solubilize cholesterol as mixed micelles, enhancing elimination ofsmall intestinal content and are found in faeces(Hofmann and Mysels, 1992). Moreover,BAs solubilize dietary lipids and their digestion products in mixed micelles, enhancing their absorption(Hofmann and Mysels, 1987). If the concentration of BAs anions is high, the BA molecules tend to self-associate to form micelles (Carey, 1985). Below CMC, added bile salt molecules dissolve in the form of monomers; and above CMC the molecules form micelles leaving the monomeric concentration constant (Hofmann and Mysels, 1992).

The hydrophobic regions of the BA molecules rest like a wedge between the heads of the alkyl chains of the lipid molecules and on the other hand the hydrophilic moieties of the BA faces the aqueous environment (Stamp and Jenkins, 2009). TCS has been found to be soluble in most organic solvents (Green Facts, 2010), but sparingly soluble in water (Aragón et al., 2008) and thus due to the presence of bile acids in sewage sludge, lithocholic acid and deoxycholic acid were used to investigate solubilization of TCS in the presence ofbile acids. As a result of low solubility of TCS (Lozano et al., 2013), BAs which are surface active agents (Hofmann and Mysels, 1987) were used to improve aqueous solubility of TCS in this study.In this thesis, deoxycholic acid and lithocholic acidwere used as surfactants to improve

1 the aqueoussolubility of TCS in this study, and the structures of these compounds is shown
2 below on figure 1.4 and 1.5, respectively.

3



4

5 ***Figure 1.3:*** *Structure of deoxycholic aciddrawn using ACD/Chem sketch(Hofmann and*
6 *Mysels, 1987).*

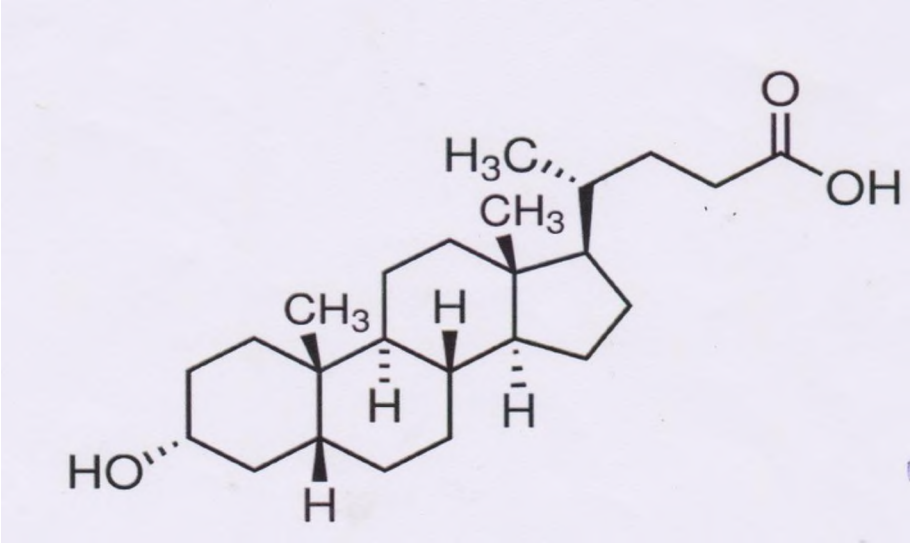


Figure 1.4: *Structure of lithocholic acid drawn using ACD/Chem sketch (Hofmann and Mysels, 1987).*

1.9.3.5 Environmental effects of surfactants.

Large amounts of surfactants and their by-products are released into the environment, and this may cause harmful effects, mainly due to their toxicity and enhanced solubility of toxic compounds e.g. pesticides, TCS(Tomczak-Wandzel et al., 2014). Domestic wastewaters reach wastewater treatment plants and the most essential biological treatment of wastewater is usually performed using the activated sludge process. The chemical pollutants that are found in the wastewater may have negative effects on microorganisms that are essential for the wastewater treatment process and surfactants are an example of these chemical pollutants (Fauser et al., 2003).

Sewage sludge used in agriculture for a soil amendment purposes, has shown to contain higher concentrations of artificial surfactants andthus there is major concern with the amount of surfactants that enter the environment through the WWTPs. Agricultural application of sludge poses a risk because of the presence of surfactants which have potential impact on the ecosystem due to their toxicity on organisms in the environment (Ying, 2006).

1.9.3.6 Effect of surfactants on Triclosan.

TCS mobility in the soil profile will be affected by the presence of artificial surfactants in the sludge particles (Tandlich and Balaz, 2011)and the ability of the native microflora to produce biosurfactants(Jain et al., 1991). This is because the surfactant molecules increase the aqueous solubility of hydrophobic molecules (Jain et al., 1991).Therefore, before sludge solids can be used as soil additives, the effects of TCS on the soil microflora will also have to be elucidated in detail.

2 CHAPTER 2

PHYSICHOCHEMICAL AND MICROBIOLOGICAL ANALYSIS OF SLUDGE

2.1 INTRODUCTION

The growth in human population has led to in an increase in the volume or weight of waste products such as sludge (Sadek et al., 2013). Currently regulatory authorities are striving to find ways to dispose of the escalating amount of waste products, this is more prominent around big towns and cities, where disposal of wastes is becoming an increasing problem (Benhamou and Fazouane, 2013; Kehila, 2014). In South Africa (Herselman et al., 2005)and Algeria(Benhamou and Fazouane, 2013), the current disposal practices are becoming an increasing problem as the sludge is not meeting the regulatory specifications, and thus inappropriate practices are causing implications in human and environmental health.

The presence of plant nutrients and organic matter in sewage sludge (Herselman et al., 2005), makes sludge solids a highly valuable fertilizer and therefore recovery and valorisation of the sludge from Belmont Valley and Tiaret wastewater treatment plants could be of great economic and recycling value. This is of particular interest in areas where agriculture constitutes a large part of economic activity such as the Eastern Cape Province of South Africa. In the Willaya of Tiaret in Algeria, increased agricultural activity could

diversify economic activity as the country is highly dominated by the petroleum industry and natural gas processing. Therefore, if sludge is to be used for beneficial purposes such as agriculture, physicochemical analyses need to be conducted to ensure the sewage sludge meets the criteria stated by the regulatory bodies in each country. The factors negatively influencing beneficial use of sludge in agriculture are presence of heavy metals, pathogenic organisms and plant nutrients, and these will be discussed in relation to the regulations below.

2.1.1 HEAVY METALS

Heavy metals are found in sewage sludge because they have been shown to be associated with the solid portion of wastewater (Page et al., 1981). Domestic waste have lower heavy metal contents than industrial wastes, therefore toxic metals such as lead (Pb), cadmium (Cd), mercury (Hg) and copper (Cu), end up being present in WWTP due to increased industrialization, and consequently these metals are found in sewage sludge after the wastewater treatment process (McGrath et al., 2000; Singh et al., 2004). On the basis of relative toxicity to plants and animals, heavy metals can be classified into two groups. The first group consisting of Cd, Pb and Hg are highly toxic to humans and animals but less toxic to plants. The second group consists of Zn, Ni and Cu and in excess are more damaging to plants than in humans (Gowrek and Ratenska, 2009).

The presence of Cd is sewage sludge is contributed by humans who have consumed meat of animals who ingested plants grown on soils contaminated with high levels of Cd and as a result humans suffer from adverse health effects (Chaney, 1988). Pb can enter the food chain from animals grazing on grass grown on sludge-soil mixtures contaminated with Pb (Chaney, 1988). Pb in sewage sludge be contributed by the use of Pb pipes in the sewage drainage system (Herselman et al., 2005), Pb-containing dust fall-out and roofing wearing off which reaches the drainage system with rain (Tiruneh et al., 2014). The presence of Cu in sewage sludge may originate from cosmetics and shampoos, paints and pigments (Tiruneh et al., 2014), the increase in the use of brass (alloy of Cu and Zn) products such as scrubbers for household cleaning and washing of pots which consequently enter the drainage system (Shamuyarira and Gumbo, 2014).

In agriculture, repeated application of sludge on soil may result in elevated metal concentrations that persist in the plough layer or top soil. A study conducted by Oliveira and Mattiazo, (2001)observed an increase in Cu, Cr, Ni and Zn concentrations in soils amended for two years with sewage sludge. Most natural soils act as a repository sink for metals without obvious effects on soil, but the accumulated heavy metals are depleted slowly by leaching or absorbed by plants (Kabata-Pendias and Pendias, 2001). The mobility of metals and other compounds is affected by the capacity of the amended soil to inhibit the passage of the contaminants to the groundwater and to subsurface run off, and thus the physicochemical properties of the soil determine this capacity of the soil to attenuate movement of contaminants (Wong et al., 2000). In South Africa, for sludge to be used for beneficial purposes the guidelines for the utilization and disposal of wastewater sludge (DWAF, 1998;

Snyman and Herselman, 2006) and National Environment Management Act(NEMA, 2013) set the standards, and in Algeria, Solid waste management agency (Kehila, 2014) and EPA guidelines of the Mediterranean (Barceló and Petrovic, 2011) set standards for sludge reuse. The limits of heavy metals stated in the South African (DWAF, 1998; Snyman and Herselman, 2006) and Algerian (WHO, 2010) guidelines are shown in table 2.1, and these will be referred to in this chapter.

Table 2.1:*Regulatory limits of heavy metals in sewage sludge intended for agricultural application set by WHO, EPA and guidelines for the utilization and disposal of wastewater sludge of South Africa*

Heavy metal	EPA guidelines	South African guidelines	
	Limit value (mg/kg)	Class A pollutant limit (mg/kg)	Class B pollutant limit (mg/kg)
Cadmium	20-40	40	85
Copper	1000-1750	1500	4300
Lead	750-1200	300	840
Manganese	1500	1000	2500

2.1.2 PLANT NUTRIENTS

Nitrogen (N) and phosphorus (P) in sludge solids contribute to 3-6 % and 2-12 % of dry matter, respectively (NCSU, 2013). This therefore makes sludge solids a potential high value

fertilizer. If sludge is applied to agricultural soils and the nutrients present are above a crop's nutrient requirement, this can be detrimental to plant growth (Tesfamariam et al., 2013). If in excess, they will leach through the soil profile and pollute groundwater as well as surface water due to run offs (Lotter and Pitman, 1997). N leaching is due to high concentration of nitrates such as NO_3^- and NO_3-N present in sewage sludge. P exists as PO_4-P, PO_4^{3-} and P_2O_5, and excess phosphorus washed from the soil may also increase the rate of eutrophication in nearby water bodies, and thus it is important to monitor the levels of N and P in sewage sludge (Sveda et al., 1992; Tesfamariam et al., 2013). The presence of N in sludge can cause soil and water pollution, therefore the application rate should be based on both N and heavy metal content which should be monitored closely (Herselman et al., 2005). Great caution should be taken in dedicated land disposal practices, where there are no restrictions on application rates of sludge, in these cases N leaching poses a serious pollution problem (Snyman and Van der Waals, 2004).

In South Africa, guidelines for the utilization and disposal of sludge (DWAF, 1998; Snyman and Herselman, 2006) set standards for nutrients that may be present in sludge used for beneficial purposes, and table 2.2 shows total N and total P limits in sewage sludge to be applied in agricultural soils. This table will be referred to in comparing values obtained from sludge analysis in this study, and determine if the regulations are met by sludge from Belmont Valley and Tiaret. In Algeria, no regulations have been determined at the moment, but the regulatory authorities use the WHO (WHO, 2010)guidelines which are similar to the South African guidelines.

Table 2.2: *Regulations of total nitrogen (nitrate-N, organic-N and inorganic-N) and phosphate content of dry sludge set by WHO and guidelines for the utilization and disposal of wastewater sludge of South Africa*

Nutrient	Range (%)
Total N	3.2-4.5
Total P	1.5-1.7

2.1.3 PATHOGENS

Sewage sludge obtained from Tiaret WWTP is expected to have a high concentration of pathogens, and the biological treatment is not highly effective as sludge is returned to the aeration tank between 12-18 times (Chapter 1, figure 1.1). This makes the sludge obtained from Tiaret WWTP rich in pathogens compared to sludge obtained from Belmont Valley WWTP. Lettuce grown in soils amended with sludge have shown to have *Escherichia coli (E. coli)* on the leaves (Janisewicz et al., 1999). A study conducted by Snyman and Van der Waals, (2004), showed the presence of *E. coli* on potato peels up to a concentration of 1800 CFU/g of potato peels. Therefore sludge should be monitored for the presence of pathogens, because of the serious health risks to the human population in close vicinity to land where is sludge is used for agricultural purposes; or consume raw foods grown on sludge amended soils. It should be noted that pathogens may be transmitted by air which can be inhaled by the

human population, and thus may poses health risks(Benhamou and Fazouane, 2013). In Algeria, there are no regulations that limit the number of microorganisms present in the sludge because of the lack of binding studies of epidemics in sewage sludge in the country (Benhamou and Fazouane, 2013), therefore WHO guidelines is what the regulatory authorities have adopted and these will be used in this chapter. The microbiological standards set in the guidelines of the utilization and disposal of wastewater sludge for South Africa(DWAF, 1998; Snyman and Herselman, 2006) are similar to WHO guidelines (WHO, 2010), and these guidelines will be used for comparison purposes. The table 2.3 shows microbiological classification of faecal coliforms for sludge intended for beneficial use, and this table will be referred to upon microbiological analysis in this chapter.

Table 2.3: *Microbiological classification of faecal coliforms for sludge intended for beneficial use set by WHO and guidelines for the utilization and disposal of wastewater sludge of South Africa*

	Unrestricted use quality		**General use quality**		**Limited quality use**
	Class A		**Class B**		**Class C**
	Target value	Maximum permissible Value	Target value	Maximum permissible value	
Faecal coliform (CFU/g d.w)	$< 1x10^3$	$1x10^4$	$< 1x10^6$	$< 1x10^7$	$> 1x10^7$

The aim of this chapter was to investigate the agricultural valorisation and sustainable use of sludge residues as soil amendment from WWTPs in Grahamstown Belmont and Tiaret.

Physical characterisation of sludge for soil amendments was done to provide information of sludge particle behaviour and aggregation. These were determined by measuring pH, determining specific surface area (SSA), cation exchange capacity (CEC) and loss of ignition of sewage sludge from Belmont Valley and Tiaret wastewater treatment plants. Chemical analysis of sludge was done to quantify concentration of heavy metals, namely Mn, Cu, Pb and Cd. Microbial characterisation of sludge samples was done by enumeration of *E. coli*and heterotrophic bacteria as indication of bacterial load in the sewage sludge. Plant nutritional value of sludge was done to quantify nitrates, ammonium and phosphates.

2.2 MATERIALS AND METHODS

2.2.1 MATERIALS

R-2A Agar (Lot: BCBG4776V), HiCrome™ m-Tec Agar (Lot: BCBG7584V), Glacial acetic acid (>99 %) (Lot: MFCD00036152), Eppendorf tubes (2 mL) (Product number: T2795), all glassware, Suprasil® 3500 µL quartz cuvettes (200-2500 nm spectral range) were purchased from Sigma Aldrich (Johannesburg, South Africa). Hydrochloric acid (HCl), 32 % (Product number: SAAR3063040LL), Sodium chloride (NaCl) (99.5 %) (Product number: 1.06404.0500), Calcium chloride ($CaCl_2$) (98 %) (Product number: 1.02378.0500), Ethylene Glycol Monoethyl Ether (EGME) (99.5 %) (Product number: 1.15118.2500), Manganese test kit (Product number: 1008160001), Manganese standard test solution (1000 mg, $MnCl_2$ in H_2O) (Product number: 1099880001), Copper test strips (Product number: 1100030001).

Potassium nitrate (>98 %) (Product number: 1156301), Potassium orthophosphate (>98.5 %) (Product number: 1058962) and Ammonium chloride (min 99 %) (Product number: 1034296) urine jars (40 mL), glass jars (100 mL) and polytope vials (5 mL) were purchased from Merck (Pty) Ltd (Johannesburg, South Africa). Potassium chloride (KCl) (99.5 %) (Product number: RPCK218), Nutrient broth (Lot: 52) was purchased from Biolab Chemicals (Pretoria, South Africa).

Masses were weighed using a Pioneer™ PA1214 analytical balance with 0.0001 g accuracy and Pioneer™ PA2102 analytical balance with 0.01 g accuracy purchased from Ohaus Corporation (Pine Brook, NJ USA). Mechanical shaking was done using Mechanical orbital shaker (Model number TS-520D) purchased from Already Enterprise Inc. (Taipei, Taiwan). Screw cap glass vials (5 mL) (Lot: 60352) were purchased from Supelco solutions (Pty) Ltd (Bellefonte, PA, USA). Loss of ignition was determined using Muffle furnace Gallenkamp App 9B 4152 SP (Leicestershire, United Kingdom). Dry weights were determined using UFE 700 Oven purchased from Memmert, (Schwabach, Germany). Incubation of plates was done using Labcon incubator (Model FSIM B) purchased from Labmark (Johannesburg, South Africa). Glassware was sterilized using Automatic Autoclave (Model RAU-530D) purchased from Rexall Industries. Co. Ltd, (Kaohsiung, Taiwan). pH was measured using Crison pH meter basic 20 purchased from Crison Instruments (Alella, Spain).

2.2.2 METHODS

2.1.1.1 Sampling of sewage sludge beds

The sampling of the sludge was done at the Belmont Valley Wastewater treatment plant in Grahamstown. The samples were collected from sludge drying beds at positions indicated in figure 2.1. A core sampler with one single compartment of 400 mm length and a diameter of 150 mm, was used for sampling the sludge beds. The sludge samples collected were transferred into sterile 100 mL glass jars. After sampling was done, the sludge samples were stored at 4 °C and analysed within a week after sampling.

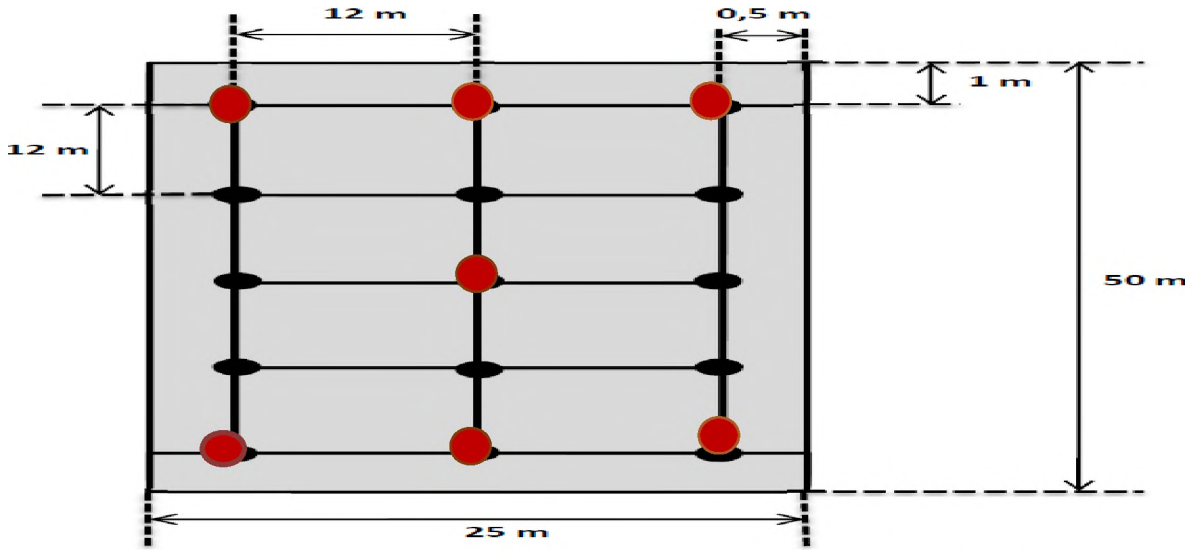


Figure 2.1: *Sludge sampling grid showing the sites (in red circles) at which the sludge samples were collected from Belmont Valley WWTP*

In Tiaret, sludge samples were collected at all 15 positions (black dots) indicated in figure 2.2. The sludge had been present in the sludge bed for more than six months prior to sampling. After sampling, sludge samples were stored in a plastic transparent bag. The samples were not analysed in Algeria due to challenges of procurement of consumables to conduct analysis. The samples were stored in room temperature, and sent to Grahamstown for analysis in November 2015, and upon arrival, the sludge samples were stored at 4 °C until analysis. The transport of the sludge samples to South Africa could have resulted in the decrease of the concentration of organic carbon and nitrogen and phosphorus. Therefore the measured values likely underestimate the real values.

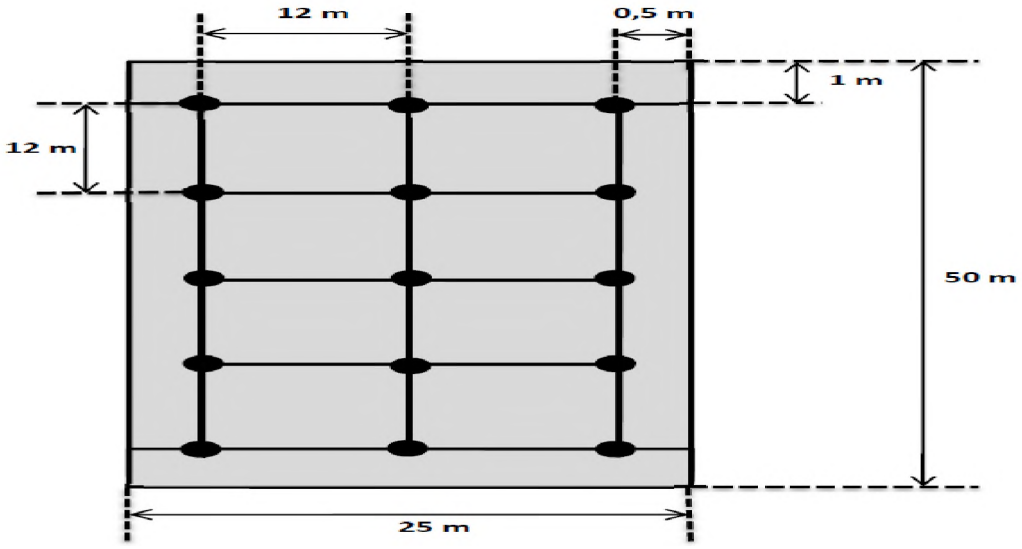


Figure 2.2: *Sludge sampling grid showing the sites (in red circles) at which the sludge samples were collected from Tiaret WWTP*

2.1.1.2 Bacterial quantification in sludge matrices

2.1.1.2.1 Sample preparation

To prepare sterile physiological saline solution, 9 g of NaCl was weighed using Pioneer™ PA2102 analytical balance into a 1000 mL Erlenmeyer flask, and thereafter, 1000 mL of distilled water was transferred into the Erlenmeyer flask using a graduated 1000 mL measuring cylinder. The Erlenmeyer flask was sealed with aluminum foil, thereafter placed in an Automatic Autoclave and steam sterilized at 121 ℃ for 15 minutes (min), and there after this solution was used to extract *E. coli* and heterotrophic bacteria from sewage sludge samples.

The extraction of bacteria from sewage sludge samples from Belmont Valley and Tiaret was performed using sterilized saline solution. Five grams(5 g) sewage sludge was weighed using Pioneer™ PA2102 analytical balance into sterile 250 mL Erlenmeyer flasks. Subsequently using a sterilized 50 mL graduated measuring cylinder, 50 mL of sterile physiological saline was added into each of the Erlenmeyer flasks and one Erlenmeyer flask was treated as a control which contained only sterile physiological saline. The Erlenmeyer flasks were placed in a Mechanical orbital shaker and shaken at 150 rpm for 20 min at 20 ℃.

Further extraction of bacteria was done using nutrient broth medium. Nutrient broth was prepared according to manufacturer's specifications and required masses were weighed using Pioneer™ PA2102 analytical balance and solution was prepared in a 1000 mL Erlenmeyer flask, and thereafter and sealed with aluminum foil, and subsequently sterilized using Automatic Autoclave at 121 ℃ for 15 min. Similar to extraction with sterile saline as

mentioned above, bacterial extraction was repeated with nutrient broth solution in the same manner. The only difference was that the samples were incubated in Labcon incubator at a temperature of 37 ± 0.2 ºC for 24 hours (h) instead of being shaken. After the incubation period, each sample was inoculated onto R-2A agar and HiCrome m-TEC agar.

2.1.1.2.2 Quantification of heterotrophic bacteria and Escheria coli

After shaking of the samples, three serial dilutions were performed (10^{-1} and 10^{-3}). For each dilution, 100 µl was pipetted and inoculated onto HiCrome m-Tec agar and R-2A agar under a laminar flow hood (Lab and Air laminar flow hood). The inoculated HiCrome m-TEC plates were incubated at 44.5 ± 0.2 ºC for 24 h and R-2A agar plates were incubated at 35 ºC for 72 h in a Labcon incubator. *E.coli* colonies appeared pink on HiCrome m-Tec agar and heterotrophic bacteria appeared white in R-2A agar plates. The number of colonies formed in HiCrome m-Tec agar were enumerated and recorded as colony forming units per gram of dry weight (CFU/g d.w). The following equations (2.1; 2.2 and 2.3) were used to calculate CFU/g d.w:

$$Multiplication\ factor = \frac{W_1}{W_2} \qquad (2.1)$$

$$\frac{CFU}{g\ of\ wet\ weig\ ht} = CFU \times dilution\ factor \qquad (2.2)$$

$$\frac{CFU}{g\ of\ dry\ \ weig\ ht} = \frac{CFU}{g\ of\ \ wet\ \ weig\ ht} \times Multiplication\ factor \quad (2.3)$$

Where W_1 is wet weight of the sludge (g); W_2 is the dry weight of sludge (g) and CFU is the colony forming units in the media plate.

2.1.1.3 Loss on ignition (LOI)

In calculating the dry weight (W_s) of sludge, equation (2.4) below was used. Porcelain high form crucibles, with a capacity of 15 mL, were acid washed (phosphate detergent, 10 % HCl and distilled water) and dried in an oven at 105 °C for 24 h and then placed in a desiccator containing silica gel for 24 h. The mass of the dried crucibles was determined (M_0) using a Pioneer™ PA1214 analytical balance. Two grams (2 g) of the sludge sample was weighed in the dried crucibles (M_1). The total mass of the crucible containing approximately 2 g of dried sludge was determined (M_2). The dry weight of sludge was calculated using the equation (3.4) below (Margesin and Schinner, 2005):

$$W_s = \frac{M_2 - M_0}{M_1 - M_0} \quad (2.4)$$

The crucibles containing the sludge samples were ignited at 550 °C in a muffle furnace for 4 h. The experiment was conducted in duplicates and a control which did not contain any sludge sample was included. LOI was calculated using the following equation (2.5) below:

$$\Delta m\ (g) = M_s - M_c \quad (2.5)$$

Percentage LOI can be calculated using the following equation (2.6) below:

$$LOI\ (\%) = \frac{\Delta m\ (g)}{M_s\ (g)} \times 100 \qquad (2.6)$$

Where $\Delta m\ (g)$ loss of mass is after ignition, M_s is sludge dry weight at 105 ºC and M_c is mass of sludge after ignition at 550ºC.

2.1.1.4 pH studies

2.1.1.4.1 Sample preparation

The pH of the sludge samples was measured using three media which were; 0.01 M $CaCl_2$, 1M KCl and distilled H_2O (dH_2O). Each sludge sample was weighed into a urine jar (40 mL) using Pioneer™ PA214 analytical balance and subsequently mixed with each solution in the following ratios of 1:3 [sludge: dH_2O], 1:6 [sludge: dH_2O], 1:3 [sludge: 0.01 M $CaCl_2$] and 1: 3 [sludge: 1 M KCl].

2.1.1.4.2 Measurement of pH

The samples were vigorously hand shaken at a temperature of 20 ± 2 ºC and after shaking, the suspension was allowed to stand for 5 min and subsequently the pH of each sample was measured using a Crison pH meter.

2.1.1.5 Cationic Exchange Capacity

2.1.1.5.1 Preparation of 1 M ammonium acetate (NH_4OAc) saturation solution

In a fume hood, 57 mL of glacial acetic acid was measured using a graduated 100 mL measuring cylinder and transferred into a 1000 mL volumetric flask containing 800 mL of distilled water (which was previously measured using a 1000 mL measuring cylinder and thereafter transferred into the 1000 mL volumetric flask). Thereafter, 68 mL of concentrated ammonium hydroxide (NH_4OH) was added to the volumetric flask and the contents were mixed in the flask by inverting the volumetric flask and thereafter allowed to cool. The pH of the solution was determined using a Crison pH meter adjusted to 7.0 usingNH_4OH and when the pH was 7.0, distilled water was added to make the solution up to 1000 mL.

2.1.1.5.2 Preparation of 1 M potassium chloride (KCl) solution

Using a Pioneer™ PA2102 analytical balance, 74.5 g of KCl was weighed and then transferred into a 250 mL beaker. Distilled water was added into the beaker, and the KCl powder was dissolved with the use of a stirring rod. After the powder was dissolved, the solution was transferred into a 1000 mL volumetric flask, water was used to make up to the mark.

2.1.1.5.3 Measurement of cationic exchange capacity

Ten grams(10 g) of sludge was weighed using Pioneer™ PA2102 analytical balance into a 500 mL Erlenmeyer flask and using a graduated 250 mL measuring cylinder, 250 mL of 1M ammonium acetate (NH_4OAc) of pH 7 was added into the flask and the flasks were sealed with aluminum foil and Parafilm™. The mixtures were shaken using a Mechanical orbital shaker at 100 rpm for 24 h after which the sludge samples were allowed to stand for 12 h. Thereafter, the suspension was filtered with light suction using a Buchner funnel lined with Whatman number 1 filter paper three times until the filtrate was clear (the sludge was not allowed to dry or crack). The sludge was leached with 1M NH_4OAc until no calcium (Ca^{2+}) and chloride (Cl^-) ions were detected. To ensure that all Ca^{2+} and Cl^- ions were removed, both calcium and chloride tests were done on the filtrate.

Calcium test: Into 10 ml of the leachate, a few drops of 1M NH_4Cl pH 7, 10 % ammonium oxalate, and dilute NH_4OH were added and the solution was heated to 90 ºC. The presence of calcium is indicated by white precipitation or turbidity of the resultant solution. The leachate was set aside, the sludge was then leached with 1M NH_4Cl pH 7 four times, and then leached with 0.25 M NH_4Cl once. The electrolytes were washed out with 99 % isopropanol, and the leachate then tested for calcium.

Chloride test: To the leachate a few drops of 0.10 M $AgNO_3$ were added. The presence of chloride ions is indicated by white precipitation.

Ammonium test: The adsorbed $NH_4^+$was extracted by leaching the sludge with 1 M KCl. The ammonium-saturated sludge was leached with 1 M KCl until 80 mL passed through the sludge sample. To determine the amount of leached ammonium ions, Ammonium (US EPA method 350.1) described in section 2.1.1.7.4 was used.CEC is calculated using the equation (2.7) below(Ross and Ketterings, 2011):

$$CEC\ \left(\frac{mEq}{100g}\right) = Conc(NH_4) + (0.25\ L \times 10\ g\ \ sludge) \times \left(1\frac{mEq\ N\ H_4}{18mg\ N\ H_4}\right) \times 100 \quad (2.7)$$

2.1.1.6 Specific Surface Area measurements

2.1.1.6.1 Drying of the sludge samples

The specific surface area of the sludge samples was determined using the modified ethylene glycol mono-ethyl ether / calcium chloride (EGME/$CaCl_2$) method (Tandlich and Balaz, 2011).Eight glass jars (100 mL) and 150 g of $CaCl_2$were ovendried using Memmert ovenat 105 °C for 24 h, then cooled in a desiccator for 2 h and the mass of each glass jar was recorded to four decimal places using a Pioneer™ PA214 analytical balance. The oven dried $CaCl_2$ was transferred into the separate desiccator, and the desiccator was covered with a lid. The glass jars were transferred into a desiccator with oven dried $CaCl_2$ for 48 h, and afterwards the mass of each glass jar was recorded. Using a Pioneer™ PA214 analytical balance,approximately 1.1g with accuracy of 0.0001 g of each sludge sample was weighed into each glass jar and the jars were placed into a desiccator for 24 h, with open lids, over $CaCl_2$ to dry the sludge samples. After 24 h, the individual glass jars were covered with lids

and weighed using an analytical balance to determine the dry weight of each sample. The mass of the glass jar and the sludgesample was recorded for each sample (Ws). As a control, an empty jar was treated the same way as the jars containing the sludge samples.

2.1.1.6.2 Preparation of $CaCl_2$/EGME solvate

One hundred and fifty grams(150 g) $CaCl_2$ was grounded, and sieved through a 400 µm sieve and baked at 160 °C for 24 h in an oven. One hundred and fifty grams(150 g) of hot $CaCl_2$ was removed from the oven (allowed to cool for 5 min) and then mixed into an aliquot volume of 150 mL of EGME to obtain the $CaCl_2$/EGME solvate(Tandlich, 2004). This solvate was used as it is sufficient to achieve constant pressure of EGME inside the desiccator (Tandlich, 2004).

2.1.1.6.3 Measurement of Specific Surface Area

The $CaCl_2$/EGME solvate was immediately placed into an empty desiccator (to create a stable EGME atmosphere over the sludge samples) and the sludge samples were allowed to equilibrate, with lids off, for 30 min. The desiccator was then evacuated at approx. 6.7 Pa using a vacuum pump for 45 min. After evacuation was completed, the desiccator was sealed and samples were allowed to equilibrate with the EGME atmosphere for 24 h. Samples were taken every 24 h and the desiccator re-pressurized. Samples were weighed using Pioneer™ PA214 analytical balance with the lids on. As the last step of the experimental procedure, the

desiccator content was again evacuated under conditions described above. The operations were repeated until a constant weight of the samples was obtained (0.0001g precision). The specific surface area was then calculated using equation 2.8 below (Segré, 2013):

$$SSA = \frac{Wa}{0.000286 \times Ws} \quad (2.8)$$

Where Wa is the mass (g) of ethylene glycol monoethyl ether retained in the sludge sample, Ws is the mass (g) of sludge sample and 0.000286 is the mass(g) of ethylene glycol monoethyl ether required to form a monolayer on a one square meter surface (m^2/g).

The figure (2.3) below shows the set-up for measuring SSA of sludge samples obtained from Belmont Valley and Tiaret. The diagram illustrates the layout of the glass jars in the desiccator and re-pressurizing the desiccator by the use of a vacuum pump.

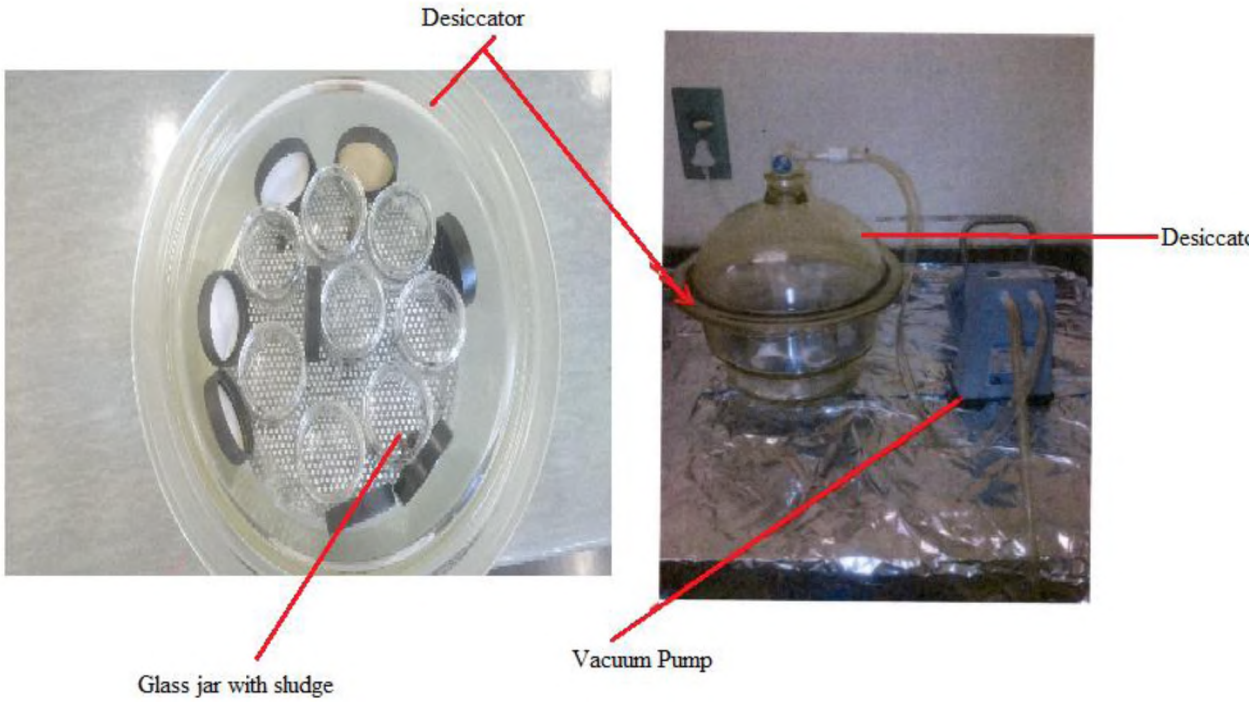


Figure 2.3:*Showing EGME set-up of determining SSA of sludge*

2.1.1.7 Quantification Nitrates, Ammonium and Phosphates in sewage sludge

2.1.1.7.1 Sample preparation

One gram (1 g) of sludge was weighed using Pioneer™ PA2102 analytical balance into 50 mL Erlenmeyer flasks, 20 mL of MilliQ water was added to the Erlenmeyer flask using a graduated 25 mL measuring cylinder. The Erlenmeyer flasks were placed in a Mechanical orbital shaker and shaken at 150 rpm for 1 h. After orbital shaking, the suspension was filtered and the filtrate was analyzed for nitrate-N (NO_3^-N), phosphates (PO_4^{3-}) and ammonium-N (NH_4-N) using the Nitrate, Phosphate and Ammonium test kits from Merck (Pty) Ltd, Johannesburg, South Africa.

2.1.1.7.2 Nitrate test (US EPA method 353.2)

The nitrateion was determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly coloured red azo dye (equation 2.9). The reaction mixture was transferred into Suprasil 3500 µL quartz cuvettes and the absorbancewas measured using UV/VIS spectrophotometerat 540 nm. The absorbance obtained was paralleled to the nitrate calibration curve shown in figure (3.5).

$$R - NH_2 + N0_2 \longrightarrow R - N_2 + R_{'} - NH_2 \longrightarrow R - N = N - R_{'} - NH_2(red) \quad (2.9)$$

For the quantitative analysis of nitrates, a calibration curve at 540 nm was constructed between 1 and 10 mg/L with three replicates each measured to construct the calibration curve.Potassiumnitratewas used in the construction of the calibration curve figure 2.5. The concentration of nitrate-N in potting soil and sewage sludge was determined using the equation (2.10) below:

$$nitrate - N\left(\frac{mg}{g} of\ dry\ weight\right) = \left(\frac{0.05L \times Conc.\left(\frac{mg}{L}\right)}{wet\ \ weig\ ht\ (g) \times dry\ \ weig\ ht}\right) \quad (2.10)$$

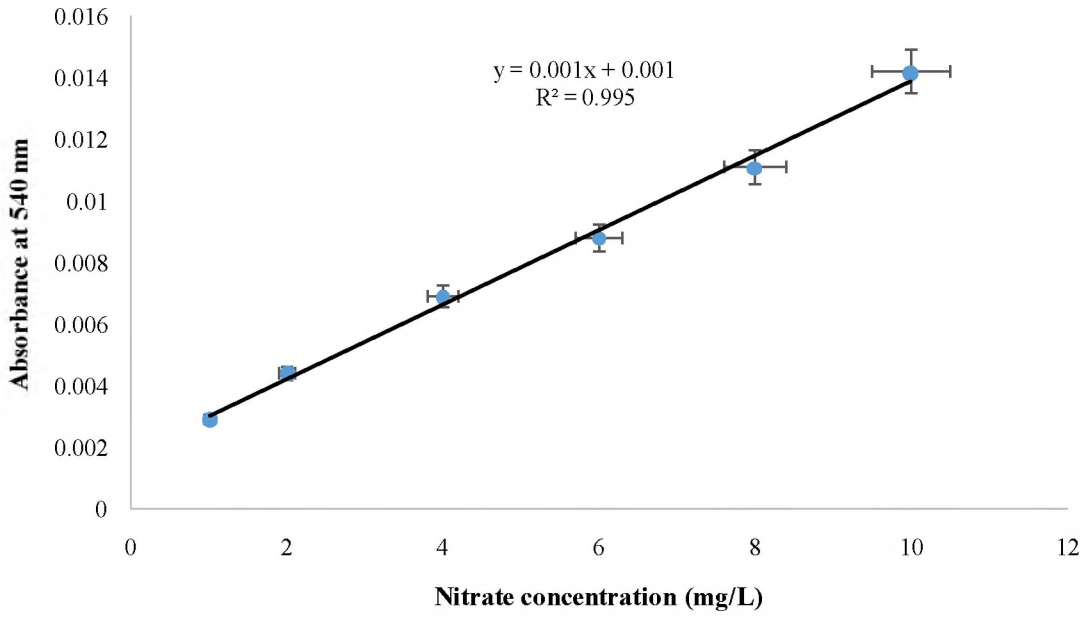


Figure 2.4: *Calibration curve for nitrates (n=3) at a range of 1-10 mg/L at 540 nm*

2.1.1.7.3 Phosphates (US EPA method 365.2)

In sulphuric solution, the orthophosphate ions react with the molybdate ions to form blue molybdophosphoricacid as shown in equation (2.11). Ascorbic acid reduced molybdophosphoricacid to intense dark blue phosphomolybdenum (PMB). The reaction mixture was transferred into Suprasil 3500 µL quartz cuvettes and absorbance was measured using UV/VIS spectrophotometer at 660 nm. The absorbance obtained was paralleled to the phosphate calibration curve shown in figure (2.6).

$$H_3PO_4 + 6Mo(VI) \longrightarrow 12MoPA(dark\ blue) + 9H^+ \qquad (2.11)$$

For the quantitative analysis of phosphate-P, a calibration curve at 660 nm was constructed between 1 and 10 mg/L with three replicates each measured to construct the calibration curve.Potassiumorthophosphatewas used in the construction of the calibration curve figure 2.6. The concentration of phosphate-P in potting soil and sewage sludge was determined using the equation (2.12) below:

$$phosphate - P\ \left(\frac{mg}{g} of\ dry\ weight\right) = \left(\frac{0.05L \times Conc.\left(\frac{mg}{L}\right)}{wet\ \ weig\ ht\ (g) \times dry\ \ weig\ ht}\right) \qquad (2.12)$$

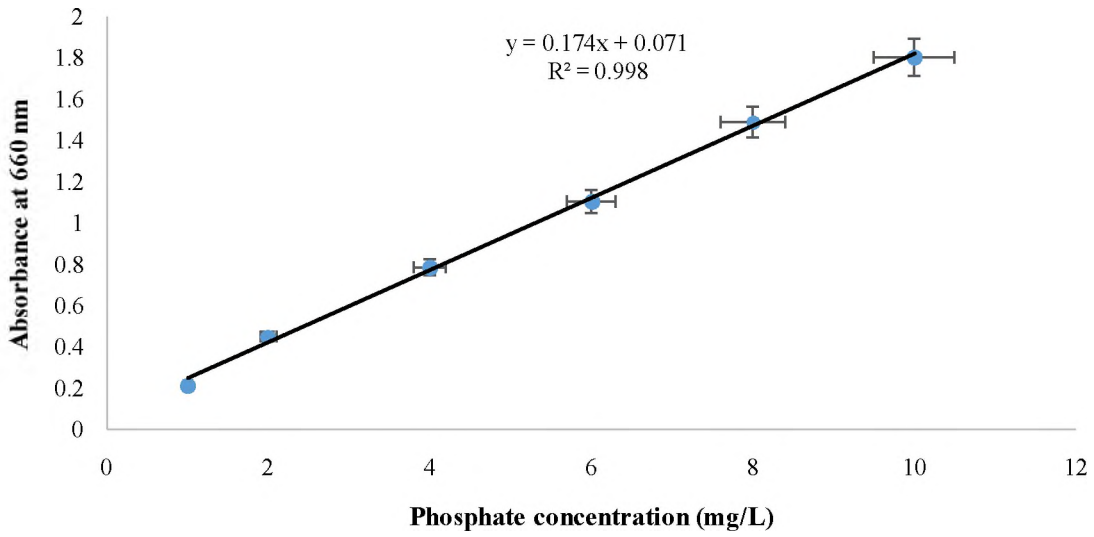


Figure 2.5: *Calibration curve for phosphates (n=3) at a range of 1-10 mg/L at 660 nm*

2.1.1.7.4 Ammonium (US EPA method 350.1)

Alkaline phenol and hypochlorite react with ammonium nitrogen is present almost entirely as ammonia (pH = 9.3)to form indophenol blue as shown in equation 2.13. The blue colour is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside.The blue reaction mixture was transferred into a Suprasil 3500 µL quartz cuvette and absorbance was measured at 660 nm using the UV/VIS spectrophotometer. The absorbance obtained was paralleled to the ammonium calibration curve shown in figure (2.7).

$$NH_3 + OCl^- + 2C_6H_5OH \longrightarrow OC_6H_8NC_6H_6O^- \ (blue) \quad (2.13)$$

For the quantitative analysis of ammonium-N, a calibration curve at 660 nm wavelength, was constructed between 1 and 10 mg/L with three replicates each measured to construct the calibration curve. Ammonium chloride was used in the construction of the calibration curve figure (2.7). The concentration of ammonium-N in potting soil and sewage sludge was determined using the equation (2.14) below:

$$ammonium - N\ \left(\frac{mg}{g}\ of\ dry\ weight\right) = \left(\frac{0.05L \times Conc.\left(\frac{mg}{L}\right)}{wet\ \ weig\ ht \times dry\ \ weig\ ht\ (g)}\right) \qquad (2.14)$$

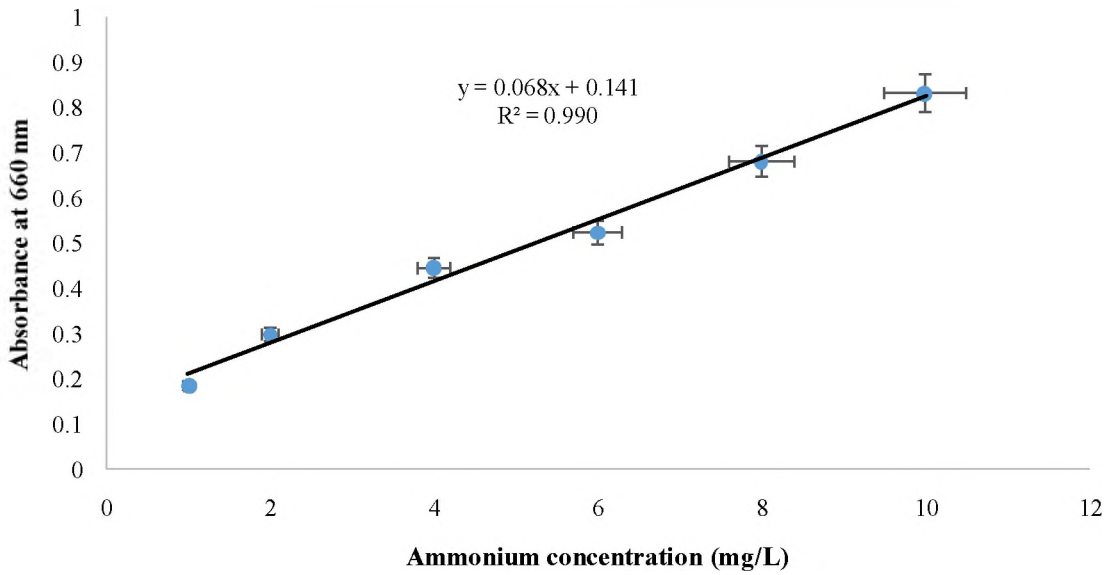


Figure 2.6: *Calibration curve for ammonium (n=3) at a range of 1-10 mg/L at 660 nm*

2.1.1.8 Quantification of heavy metals in sewage sludge and pit latrines

2.1.1.8.1 Sampling

In Belmont Valley WWTP, a core sampler with one single compartment of 400 mm length and a diameter of 150 mm (shown in figure 2.7) was used for sampling the sludge beds to investigate the distribution and quantification of heavy metals in sludge beds. Sludge samples were collected from seven different positions in the sludge beds as shown in figure 2.1, and thereafter the samples were transferred into 100 mL glass jars and stored at 4 °C until analysed for heavy metals.

Figure 2.8 below shows the core sampler that was used to obtain sludge samples from Belmont Valley sewage sludge beds.

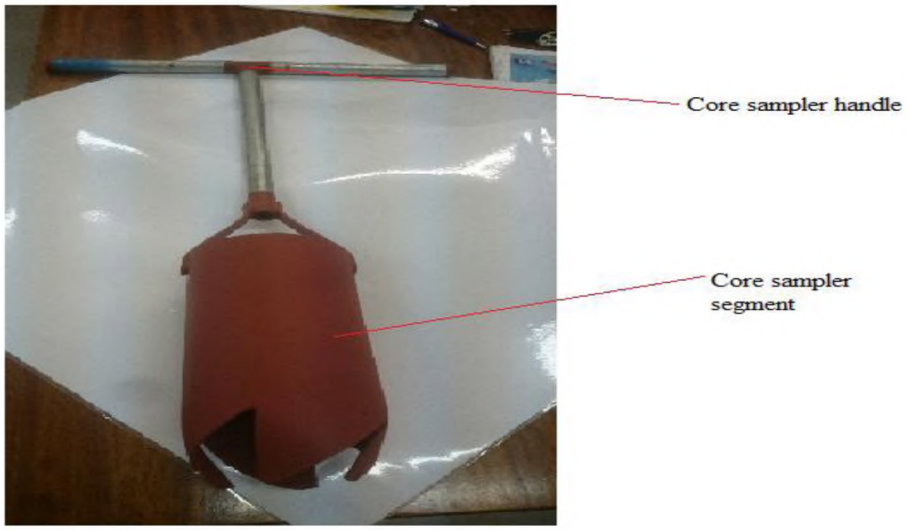


Figure 2.7: *Core sampler used to sample Grahamstown sludge beds*

In Tiaret WWTP, sludge samples were collected from 15 positions of the sludge bed as shown in figure 2.2. Ten sludge beds were sampled, and thus 150 sludge samples were analysed for metals. The sludge samples were placed in 5 mL Eppendorf tubes and sent to South Africa were they stored at 4 °C awaiting their analysis for heavy metals.

For sampling in pit latrines in Hlalani Township (Grahamstown), a core sampler was used for sampling at different layers of the pit latrine to investigate the heavy metal composition within the pit. The core sampler (figure 2.9) was segmented into seven segments and each segment was 250 mm in length and a diameter 90 mm. Each segment had an opening which allowed sampling to be possible at different layers. The core sampler was inserted into the pit latrine through turning it in a clockwise motion till all the seven segments were in the pit. Thereafter, in an anticlockwise motion, the core sampler was gradually withdrawn from the pit latrine with the opening in each segment closing and collecting faecal sludge in each layer. The samples were transferred into 40 mL urine jars, and then steam sterilized using an Automatic autoclave. The samples were stored at 4 ºC until heavy metal analysis.

The figure (2.8) below shows assembling of the core sampler that was used to obtain faecal sludge from pit latrines in Hlalani Township.

Figure 2.8: *Showing core sampler used to sample pit latrines*

2.1.1.8.2 Dry weights of Algerian samples for heavy metal analysis

In calculating the dry weight (W_s) of sludge, equation (2.15) below was used. Polytope vials with a capacity of 10 mL, were acid washed and dried in a Memmert oven at 60 °C for 48 hand then placed in a desiccator containing silica gel for 24 h. The mass of the dried polytope vials was determined (M_0). Between 0.15 and 0.20 g of the sludge samples was weighed using a Pioneer™ PA214 analytical balance into the dried polytope vials (M_1). The total mass of the polytope vial containing between 0.15 and 0.20 g of dried sludge was determined (M_2). The dry weight of sludge was calculated using the equation (3.15) below (Margesin and Schinner, 2005):

$$W_s = \frac{M_2 - M_0}{M_1 - M_0} \quad (2.15)$$

2.1.1.8.3 Sample preparation

Heavy metals were extracted from sludge using 1 M HCl (Tuin and Tels, 1990). Five grams(5 g) of sewage sludge samples were weighed using Pioneer™ PA2102 analytical balance and then transferred into separate 250 mL Erlenmeyer flasks. Using a 50 mL graduated measuring cylinder, 50 mL of 1 M HCl was transferred into each Erlenmeyer flask. The flasks were sealed with Parafilm™ and aluminum foil. Seven Erlenmeyer flasks each containing sludge and 1 M HCl, were placed in the Mechanical orbital shaker and shaken at 150 rpm at 20 °C for 24 h. The samples were left to stand for 15 min, after which the supernatant was pipetted into 5 mL glass vials.

For sludge samples obtained from Tiaret WWTP, the mass of each 2 mL eppendorf tube containing sewage sludge was weighed using Pioneer™ PA214 analytical balance. The samples were then transferred into distinct 5 mL polytope vials and the mass of the empty eppendorf tube was determined. Into each polytope vial containing sludge, 5 mL of 1 M HCl was pipetted. The polytope vials of each sample were placed in a Mechanical orbital shaker and shaken for 24 h at 150 rpm. After 24 h, the sludge samples were left to stand for 15 min and afterwards the supernatant of each sludge sample was transferred into 25 mL volumetric flasks and subsequently 1 M HCl was added to make up to the final volume of each flask.

The flasks were inverted five times to ensure homogeneity of the solution, and thereafter the samples were transferred into 5 mL glass vials.

2.1.1.8.4 Quantification of heavy metals in sewage and faecal sludge.

The heavy metal composition of samples obtained in Belmont Valley WWTP was determined using inductively coupled plasma/optical emission spectrometry (ICP/OES) at Bemlab (Pty) Ltd, Cape Town, South Africa. Method 3132 was used for determination of Mn and Cu, and method 3225 was used to determine concentration of Pb and Cd. Metal concentrations were converted to mg/kg d.w. The concentration of each heavy metal was calculated using the equation (2.15) below:

$$Heavy\ metal\ (mg/kg\ \ d.w) = \left(\frac{Conc\ (mg\ /L) \times 0.05\ L}{weig\ ht\ wet\ \ sludge\ \ \ (g) \times dry\ \ weig\ ht}\right) \times 1000 \quad (2.15)$$

For samples obtained from Tiaret WWTP, Mn and Cu were analysed using manganese and copper test kits. The determination of copper was done semi quantitatively due to the lack of commercially available quantitative test kits at the time of the analyses. When the supplier Merck South Africa was contacted about the lead time on the quantitative copper kits, the company stated it would be six months. The analyses were urgent and needed to be done before then. Pb and Cd analysis was done byBemlab (Pty) Ltd, Cape Town, South Africa using ICP/OES method 3225, and the limits of detection (LOD) for the methods were 0.001

mg/L. These concentrations (mg/L) were converted to mg/kgd.w using the equation (2.16) below:

$$Heavy\ metal\ (mg/kg) = \left(\frac{Conc.(mg/L) \times 0.025\ L}{weig\ ht\ of\ sludge\ \times dry\ \ weig\ ht\ (g)}\right) \times 1000 \qquad (2.16)$$

Faecal sludge samples obtained from pit latrines were in semi-solid state, and were transferred into 5 mL glass vials and sent for analysis at Bemlab (Pty) Ltd, Cape Town, South Africa. The samples were analysed for Mn, Cu, Pb and Cd using ICP/OES, and method 3132 was used for determination of Mn and Cu, and method 3225 was used to determine concentration of Pb and Cd. The LODs for both methods was 0.001 mg/L.

2.1.1.8.5 Manganese (Merck method DIN 38406-2)

In alkaline solution, manganese (II) ions reacted with an oxime to form a red-brown complex (equation 2.17). The reaction mixture was transferred into a Suprasil 3500 µL quartz cuvette and absorbance was measured at 395 nm.

$$Mn^{2+} + R - N(OH) - R_{'} + NaOH \longrightarrow R - N - (O^{-}Mn^{+}) - R_{'}(red) + H_2O + Na^{+} \qquad (2.17)$$

For the quantitative analysis of manganese, a calibration curve at 395 nm wavelength, was constructed at the following concentrations: 0.01 mg/L, 0.5 mg/L, 1 mg/L, 2 mg/L, 3 mg/L and 5 mg/L. The manganese standard test solution (1000 mg, $MnCl_2$ in H_2O) was used in the

construction of the calibration curve figure (2.10). The result obtained was in mg/L and subsequently converted into mg/g for each sample using the equation (2.16).

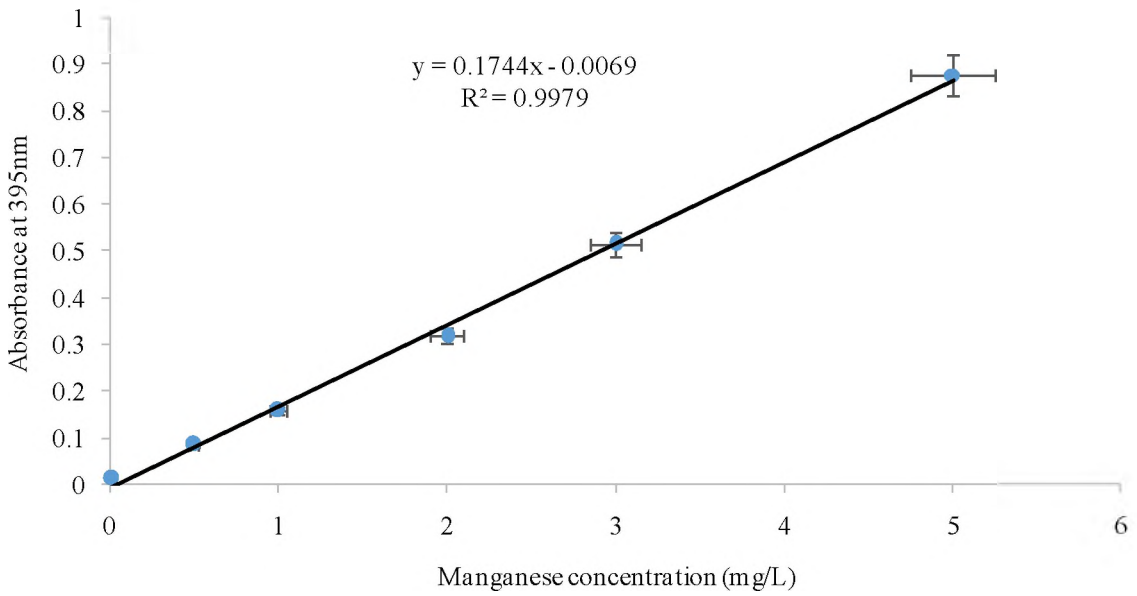


Figure 2.9: *Calibration curve for Mn (n=3) in distilled water at a range of 0.01-5 mg/L at 395 nm*

2.1.1.8.6 Copper (Merck method1.10003.0001)

In this method, copper (II) ions are reduced to copper (I) ions by a reducing-agent mixture, thereafter copper (I) ions react with 2, 2'-biquinoline (cuproin) to form a violet complex (equation 2.18). Copper concentration was done by colorimetric comparison of the reaction zone of the test strip with the fields of a color scale on the test kit. The LOD was 9 mg/L and the concentration of Cu (mg/L) was recorded. This result was converted into mg/g of dry weight for each sample.

$$Cu(II) + Reducing\ agent \rightarrow Cu(I) + cuprion \rightarrow Cu(I)(violet) \quad (2.18)$$

2.2.3 DATA ANALYSIS

Data analysis was done using Microcal™ Origin 6.0 software package (Microcal Software, Inc. Northampton, MA, USA).Each value for LOI, SSA, CEC, nutrients and heavy metal was converted to the logarithmic value. A t-test statistical analysis was done at significance level of 5 %so as to test the differences between the sludge composition in South Africa and Algeria.

2.3 RESULTS AND DISCUSSION

The sewage sludge samples from Belmont Valley and Tiaret were compared and data analyses were done using *t-test*(5 % level of significance) after log-transformation to ensure normal distribution. Table 2.4 below shows the summary of results obtained for pH, LOI, SSA, CEC, plant nutrient composition, microbiological analysis and heavy metal composition of sewage sludge obtained from Belmont Valley and Tiaret. The values of each parameter analysed were obtained from the methods described in section 2.2, and will be elucidated in detail in this section.

Table 2.4: *Comparison of physicochemical properties microbiological composition of sludge obtained from Grahamstown and Tiaret.*

Parameter		South Africa (N=7)	Algeria (N=4)	Statistical analysis (p = 0.05)	
				t-critical	*p-value*
pH	**1:3 [sludge:dH$_2$O]**	6.66 ± 0.40	8.18 ± 0.20	5.04	0.00149
	1:6 [sludge:dH$_2$O]	7.11 ± 0.20	8.08 ± 0.64	1.52	0.173
	1:3 [sludge:CaCl$_2$]	6.73 ± 0.20	7.88 ± 0.35	6.3	0.0004
	1:3 [sludge:KCl]	6.69 ± 0.24	7.78 ± 0.23	5.68	0.0007
LOI	**(%)**	1.33 ± 0.03	1.48 ± 0.11	2.382	0.076
SSA	**(m^2/g)**	218 ± 108	261 ± 99.9	0.674	0.517
CEC	**(mEq/100g)**	119 ± 2.09	136 ± 6.03	7.259	0
PO_4^{3-}-P	**(mg/g d.w)**	1.40 ± 0.30	0.24 ± 0.19	5.551	0.0004
NO_3^-- N	**(mg/g d.w)**	57.61 ± 55.20	2.56 ± 2.90	5.17	0.0006
NH_4^+-N	**(mg/g d.w)**	6.60 ± 2.36	0.64 ± 0.45	-6.637	0.0001
***E.coli* (Saline)**	**(CFU/g d.w)**	468 ± 73.6	7769 ± 1268	1.977	0.187
***E.coli* (N. broth)**	**(CFU/g d.w)**	>1.17E+09	>1.43E+09		
***Heterotrophic bacteria* (Saline)**	**(CFU/g d.w)**	1.17E+09 ± 7.42E+08	1.43E+09 ± 9.11E+08	0.681	0.62
***Heterotrophic bacteria* (N.broth)**	**(CFU/g d.w)**	>1.17E+09	>1.43E+09		

2.3.1 pH

The pH of the sludge is the measure of the activity of hydrogen ions, which can give an indication of whether the sludge is acidic or alkaline (Segré, 2013). Measurement of pH of sewage sludge in 0.01 M calcium chloride ($CaCl_2$) is an interpretation for the degree of saturation of sludge particles by cations other than hydrogen (Segré, 2013). The difference between pH in water and in $CaCl_2$ can range from 0-1.1, depending on the salt content of the soil. Soils with low amount of alkaline cations such as calcium, will observe larger deviations from their pH in water when exposed to 0.01 $CaCl_2$ solution. According to Ahern et al., (1995), measuring pH using 0.01 M $CaCl_2$ is more accurate than pH measurement in water. In most cases, pH values obtained when 0.01 M $CaCl_2$ is usually lower than that of water (Ahern et al., 1995). The use of 0.01 M $CaCl_2$ and 0.01 M KCl brings results closer to the true sewage sludge pH as these salts solubilize more hydrogen ions that bind to sludge or other organic particles, thus influencing pH (Ahern et al., 1995). The use of 0.01 M KCl solution is also used to obtain the exchangeable acidity of the soil. Soil acidity comes from hydrogen ions that are released when high levels of aluminium in the soil react with water molecules and thus the KCl use in determination of soil pH indicates the true soil pH by displacing hydrogen ions that might influence the pH of the soil (Ahern et al., 1995; Segré, 2013). In sewage sludge, the presence of heavy metals (Tiruneh et al., (2014) and other organic compounds(Butler et al., 2012) might influence the pH, and thus causing differences between soil and sludge pH values.

The pH values obtained in this study were ranging between 6.66-7.11 and 7.78-8.11 for Belmont Valley and Tiaret sewage sludge, respectively. Statistical analysis indicated significant difference (p =0.00149) in pH measured in 1:3 (sludge:dH_2O) between sludge samples obtained from Belmont Valley and Tiaret. Statistical analysis indicated significant difference (p = 0.173) in pH measured in 1:6 (sludge:dH_2O) between sludge samples obtained from Belmont Valley and Tiaret. Statistical analysis indicated significant difference (p =0.0004) in pH measured in 1:3 (sludge:0.01M $CaCl_2$) between sludge samples obtained from Belmont Valley and Tiaret.Statistical analysis indicated significant difference (p =0.0007) in pH measured in 1:3 (sludge:0.01 M KCl) between sludge samples obtained from Belmont Valley and Tiaret.The pH values obtained in literature were between 6.5 and 7.5 (Fytili and Zabaniotou, 2008) and an average of 6.73 ± 0.81 (Wang et al., 2008), and these were comparable to the values obtained in this study. More precisely, the pH values obtained from table 2.4 were 6.66-8.18 (1:3dH_2O); 7.11-8.08 (1:6dH_2O); 6.73-7.88 (1:3$CaCl_2$) and 6.69-8.18 (1:3KCl). In general, Belmont Valley samples were slightly acidic (except 1:6dH_2O), whereas Tiaret samples were slightly alkaline. The differences in pH might have been the result of longer resident times in stabilizing chambers of the WWTP were lime is used, and thus the pH of the sewage sludge might have been shifted towards the alkaline range (Herselman et al., 2005).

It should be noted that pH is an important parameter to be determined in sewage sludge as it may improve amended soil properties (Wang et al., 2008) and affect cationic exchange capacity (CEC) and heavy metal bioavailability (Alloway, 1995; Fytili and Zabaniotou, 2008).Triclosan (TCS), a compound mainly found in vast personal care products has been

detected in sewage sludge due its widespread use(Butler et al., 2012; Verlicchi and Zambello, 2015). TCS has a pK_a of 7.9 (Halden and Paull, 2005), thus below this pH it will exist in its unionized form. In addition, TCS has a log K_w (partition coefficient) of 4.76 (Lozano et al., 2013), therefore pH will govern its sorption onto sludge particles (Wu et al., 2009) and be retained.

2.3.2 LOSS ON IGNITION (LOI)

Benefits of land application of sewage sludge amongst others include improved soil organic matter (SOM) content. The average LOI (%) of sewage sludge samples from Tiaret and Belmont Valley were 1.48 ± 0.11 % and 1.33 ± 0.03 % respectively as shown in table 2.4. Upon conducting a t-test statistical analysis to compare LOI values between Belmont Valley and Tiaret sludge samples, there was no significant difference (*p-value* = 0.076) in LOI. These values were comparable to studies conducted by Sanchez-Monedero et al., (1998),Snyman and Van der Waals, (2004)that obtained a values of 1.81 ± 0.22 % and 1.0-2.7 % respectively. Soil organic matter (SOM) constituents have shown to possess a high affinity for heavy metals (Nogueira et al., 2010) and similarly sewage sludge is known to contain a considerable percentage of organic compounds (Kabata-Pendias and Pendias, 2001), thus some heavy metals such as Zn, Cd and Pb can be bound to OM and retained in the sludge(Xu et al., 2013). OM has been found to be associated to increase sorption capacity of sludge for organic compounds (Gorga et al., 2014; Wu et al., 2009). Thus the presence of OM is expected to increase the sorption of TCS onto sewage sludge particles and as a result it may be bound onto sludge and be retained. The binding of organic and inorganic

compounds onto sewage sludge will be affected by the total concentration of SOM which also includes soil organic carbon which has to be above 0.2 % to affect sorption (Huang et al., 2003; McGroddy et al., 1998). The value of SOM found using LOI in this current study was greater than 0.2 %, and upon conducting a t-test statistical analysis at 0.2 % between sludge samples from both sites, there was no significant difference (*p-value* = 0.07583) in the LOI and thus SOM in sewage sludge will result in TCS and heavy metals being retained by sludge particles (Lozano et al., 2013; Xu et al., 2013).

2.3.3 SPECIFIC SURFACE AREA (SSA)

The specific surface area (SSA) of a material is the ratio of the surface per unit mass (m^2/g) (Segré, 2013). This property is very important for sewage sludge as it may vary depending on mineralogy, particle size distribution and organic matter (OM) (Segré, 2013). Fine grained soils are more likely to have a higher SSA than coarse grained materials for example, kaolinite (10-20 m^2/g) and vermiculite (40-80 m^2/g) (Mitchell, 1993). The SSA of sewage sludge is an indicator of the space available cations and organic compound adsorption and (Van de Graaff and Patterson, 2001). The average SSA for sewage sludge obtained from Belmont Valley and Tiaret were 218.16 ± 108.09 and 261.04 ± 99.90 m^2/g, respectively as shown on table 2.4. A statistical t-test indicated no significant different (p =0.517) in SSA of Belmont Valley and Tiaret sludge. A study conducted by Tiruneh et al., (2014)on sewage sludge obtained from Swaziland, they obtained SSA values ranging between 192 and 284 m^2/g. The values obtained in this study were comparable to the values in literature for

sewage sludge. The SSA values of sewage sludge obtained from Tiaret and Belmont Valley were both high because, after wastewater treatment sludge was stored in stockpiles and as a result the sludge particles might have aggregated, resulting in large particle size. Therefore it should be noted that the high SSA values of sewage sludge may imply that the surfaces are likely to be charged and thus have a higher cationic exchange capacity (CEC) (Segré, 2013). The higher values of CEC may potentially indicate high levels of heavy metals present in the sewage sludge (Segré, 2013). In addition, SOM is also negatively charged (Mitchell, 1993), and therefore may increase the CEC of sewage sludge. In conclusion, SSA and CEC should be determined as it as important parameter that may affect other variables if the sewage sludge is to be considered for reuse.

2.3.4 CATIONIC EXCHANGE CAPACITY (CEC)

Cationic exchange capacity (CEC) is the measurement of the quantity of negatively charged sites on soil surfaces that can retain positively charged ions (cations) such as Ca^{2+}, Mg^{2+} and K^{+} by electrostatic forces (Ross and Ketterings, 2011). Cations which are electrostatically retained are easily exchangeable with cations present in the sludge solution, therefore sewage sludge with higher CEC has a greater capacity to maintain adequate quantities of cations as compared to soils with lower CEC (Ross and Ketterings, 2011). A directly proportional relationship exists between CEC and SSA(Churchman and Burke, 1991), as SSA is an indicator of space available for cations to adsorb, and furthermore the ability to attract cations is not only dependent only on SSA of the material but also its charge (Segré, 2013).

The CEC values of Belmont Valley and Tiaret are 119.41 ± 2.09 and 136.03 ± 6.03 mEq/100g, respectively. On t-test statistical comparison of the two sites, the CEC values were significantly different (*p-value* = 0.0000), and thus a one-sided t-test on at 5 % significance level was done and it was observed that (*p-value* = 0.01069) Tiaret sludge samples had a higher CEC than Belmont Valley sludge samples. On literature, the values obtained for sewage sludge were 61 ± 7.4 mEq/100g (Hyland et al., 2012) 144-259 mEq/100g (Tiruneh et al., 2014) and thus the CEC obtained from the study were comparable to the latter study. The method used above has an advantage that it will give the value close to the pH of the sludge, as the buffers used have a neutral pH which is close to the sewage sludge pH, and thus there will be no overestimates of CEC values. Sludge pH values ranging from slightly acidic to slightly alkaline, may indicate that the sewage sludge has the potential to retain metals, and thus if sewage sludge is to be used for soil amendment purposes, metals may be transferred into the amended soil. The presence of cations or heavy metals in the sewage sludge may imply that TCS can be retained within the sludge when it exists in its ionic form ($pH>pK_a$) as it may electrostatically bind to these metals.

2.3.5 NUTRIENTS

The average concentrations of NO_3^- were 57.61 ± 55.20 mg/g d.w (Belmont Valley) and 2.56 ± 2.90 mg/g d.w (Tiaret) (*p-value* = 0.0006); PO_4^{3-} were 1.40 ± 0.30 mg/g d.w (Belmont Valley) and 0.24 ± 0.19 mg/g d.w (Tiaret) (*p-value* = 0.0004) and NH_4^+ were 6.60 ± 2.36 mg/g d.w (Belmont Valley) and 0.64 ± 0.45 mg/g d.w (Tiaret) (*p-value* = 0.0001). Generally

higher nutritional values for sludge samples were obtained from Belmont Valley than Tiaret as shown in table 2.4. The lower values of Tiaret sludge samples might have been influenced by the storage conditions between sampling and analysis, and may not be a true indication of the nutrients present. The total inorganic P concentration constituted 0.14 % and 0.02 % of dry sludge for South Africa and Algerian sludge, respectively. Total inorganic N was 6.42 % and 0.32 % of dry sludge for South Africa and Algerian sludge, respectively. On comparing values with the guidelines for utilization and disposal of waste sludge as shown in table 2.2 (DWAF, 1998; Snyman and Herselman, 2006), the total inorganic N was higher for Belmont Valley sludge and for Tiaret, the sludge met the regulatory values. The total inorganic P was lower than the regulation values for both sites. On conducting a statistical t-test there was significant difference in nitrate, phosphate and ammonium concentrations from both sites. A study by Xu et al., (2013) obtained total nitrogen of 45.23 mg/g and total phosphorus of 16.52 mg/g; and Tiruneh et al., (2014) obtained total nitrogen concentration ranging between 21.2 and 36 mg/g and total phosphorus of 8.7 and 10 mg/g. The results in literature were higher for phosphorus than the values obtained our study and total nitrogen concentration was higher in our study than the values in literature. The reasons that might have caused the differences between values obtained from our study and literature was that no quantification of organic P and N in our study was done. The presence of nitrogen and phosphorus in sewage sludge shows that these nutrients can be detected in wastewater treated in the WWTP, and would add nutritional value in agricultural sludge amended soils (Ekama, 1993). The presence of N and P in sewage sludge provide significant source of inorganic fertilizer replacement value as it contains these major plant nutrients (Hall, 1985; Tamrabet et al., 2009). The high concentrations of total N and total P present in sewage sludge,

introduces the risk of leaching out nitrates to groundwater, whereas phosphates are usually not leachable from soils (Epstein et al., 1976; Herselman et al., 2005). In addition, the high concentration of N in amended soils, leads to ground and surface water contamination (Smith, 1996). The fate of nitrates following sludge application as soil amendment has been reported, not only absorbed by plants, but moreover they may leach onto groundwater leading to human and environmental health issues (Chang et al., 1988; Lotter and Pitman, 1997). Nitrate-N is toxic to humans and animals, as when it enters the human body it is reduced to NO_2^-, which when absorbed converts oxyhaemoglobin (oxygen-carrier in blood) to methaemoglobin causing methaemoglobinemia in adults and blue-baby syndrome in children (Snyman and Van der Waals, 2004; Sveda et al., 1992).

2.3.6 MICROBIOLOGICAL ANALYSIS

*E.coli*and heterotrophic bacteria are the dominant species of bacteria found in wastewater treatment plants (WWTPs), with protozoans and fungi also present (Bitton, 1994). Around 95 % of the total bacteria population plays a role in purification of wastewater (Fang et al., 2006). Due to the present of different microorganisms (MOs) in sewage sludge, it must be known that these species are harmful to humans, animals and plants (Herselman et al., 2005), therefore in this study, *E.coli* and heterotrophic bacteria were species of interest. The quantification of these bacteria is important if sewage sludge is to be recycled for land refilling and composting (Kehila, 2014). The concentration of *E.coli* in Belmont Valley and Tiaret sewage sludge using saline was 468.84 ± 73.6 CFU/g d.w and 7769 ± 1268 CFU/g d.w respectively as shown in table (2.4). On the other hand when *E.coli* was extracted with

nutrient broth, the concentration was greater than $1.17x10^9$ and $1.43x10^9$ CFU/g d.w for Belmont Valley and Tiaret sludge samples. Leachability index is defined as the ratio of CFU/g d.w of bacteria extracted using sterile saline to CFU/g d.w of bacteria extracted using nutrient broth, and is expressed using by equation (2.19) below:

$$Leachability\ index = \frac{\frac{CFU}{g} dw\ (saline\ )}{\frac{CFU}{g} dw\ (nutrient\ \ brot\ h)} \qquad (2.19)$$

Using the equation above, the leachability index values obtained were 0.0000004 for Belmont Valley sludge and 0.000005 for Tiaret sludge. The presence of high *E.coli* concentrations through by physiological saline implies that these MOs are loosely bound to sewage sludge particles and might leach down the soil profile if the sludge is used for agricultural purposes, and consequently enter groundwater as a result causing human and environmental contamination (Mezrioui and Baleux, 1994; Scotsman, 1998). According to the Guidelines for the utilization and disposal of wastewater sludge of South Africa, Belmont Valley sludge met the unrestricted use quality specifications and was classified under Class A and thus the sludge would be considered for reuse (*E.coli* below 1000CFU/g d.w)(DWAF, 1998; Snyman and Herselman, 2006). For Tiaret sludge, both concentrations of *E.coli* and heterotrophic bacteria were high and the fact that there are no regulations that limit the number of MOs in sewage sludge because of the lack of binding studies of epidemics in sewage sludge, the health risk is still possible even if the MOs are not absorbed by the plants, they can be transmitted by air or by binding to injuries of plants such as vegetables which cause health risks to humans who are in close proximity to places where sludge is reused (Benhamou and Fazouane, 2013). Therefore, the leachability index may be used to determine

type of irrigation that the sludge may be used for due to the risks previously stated. The unrestricted irrigation will be based on the saline extracted bacteria, not the nutrient broth.

E.coli, faecal coliforms and *Salmonella*spp. have been reported to thrive in soils for prolonged periods of time (Strauch, 1991).Snyman and Van der Waals, (2004) found *E.coli* (1800 CFU/g) on potato peels in soils treated with sewage sludge after 16 weeks post application. Therefore the presence of MOs indicates the potential hazard to public health, and thus disposal of sludge must be controlled based on the Guidelines for the utilization and disposal of wastewater sludge of South Africa (DWAF, 1998; Snyman and Herselman, 2006) and solid waste management guidelines of Algeria (Kehila, 2014). The amount of heterotrophic bacteria present in Belmont Valley sludge and Tiaret sludge samples was very high, and furthermore in both countries there are no regulations that state permissible levels of heterotrophic bacteria. These bacteria breakdown organic material such as carbohydrates, fats and proteins, and due to easy biodegradability of these organic compounds, bacterial growth is rapid (EPA, 1997). Heterotrophic bacteria affect denitrification processes in soils (Brookes et al., 1986), and in anaerobic conditions they utilize oxygen in NO_3^- by forming nitrogen gas (Brookes et al., 1984). There are no guidelines in South Africa and Algeria that regulate the limits for heterotrophic bacteria in sludge for reuse. Regulatory authorities in Europe (EU Directive, 1991) and USA (USEPA, 1986) have not stated the amount of heterotrophic bacteria to be present in sewage sludge if it is to be reused. Thus no regulatory values were present to compare heterotrophic bacteria present in sewage sludge for reuse. Determination of heterotrophic bacteria is important as these bacteria may have an influence on soil fertility once the sludge is disposed on soil. The heterotroph composition may

indicate the ability of the sludge amended soils to remove organic matter, which is essential for soil properties. Therefore is it important to determine the regulatory values of heterotrophic bacteria in sewage sludge, as these bacteria will influence the organic matter present in amended soils and consequently affect soil fertility. Nonetheless, it is important for microbiological analysis to be done because in both South Africa and Algeria sewage sludge is used in agriculture (Morrison et al., 2004; Snyman and Van der Waals, 2004; Tamrabet et al., 2009), land refilling (Kehila, 2014) and production of renewable energy (Sadek et al., 2013). Thus the proliferation of waste without appropriate treatment is harmful to human and environmental health, and more over leads to the loss of recyclables and energy (Sadek et al., 2013).

2.3.7 HEAVY METALS

After sampling of sewage sludge beds in Belmont Valley and Tiaret, the heavy metal composition was determined. Faecal sludge samples obtained from pit latrines in Hlalani township will be discussed in this section with respect to heavy metal load. The metals analysed were Mn, Cu, Pb and Cd, and these will be discussed in detail in this section, and the sludge samples will be statistically analysed using a t-test at 5 % level of significance.

Table 2.5: *Concentrations of heavy metals in pit latrines in Hlalani Township*

	Average amount of heavy metal			
	Mn (mg/L)	**Cu** (mg/L)	**Pb** (mg/L)	**Cd** (mg/L)
***SA guidelines**	0.5	2	0.01	0.0003
P2	0.34 ± 0.36	0.38 ± 0.30	0.01 ± 0.03	0.00 ± 0.00
P3	0.75 ± 1.68	0.22 ± 0.38	0.01 ± 0.02	0.00 ± 0.00
P4	0.41 ± 0.87	1.70 ± 3.23	0.00 ± 0.0	0.00 ± 0.00
P5	0.36 ± 0.51	0.52 ± 0.52	0.00 ± 0.0	0.00 ± 0.00
P6	0.00 ± 0.0	0.00 ± 0.00	0.00 ± 0.0	0.00 ± 0.00
P7	0.00 ± 0.0	0.21 ± 0.18	0.00 ± 0.0	0.00 ± 0.00

Notes: * Guidelines for the Utilization and disposal of Wastewater (DWAF, 1998; Snyman and Herselman, 2006). #highlighted figures exceed SA guidelines.

Table 2.6: *Comparison of heavy metals in sewage sludge from Grahamstown and Tiaret*

Metal	***SA Guidelines** (mg/kg d.w)	****AFNOR/WHO Algeria guidelines** (mg/kg)	**Average experimental values**	
			South Africa (mg/kg d.w)	**Algeria** (mg/kg)
Mn	1000	***NS	423 ± 101	358± 295
Cu	1500	1750	353 ± 92	549± 50
Pb	300	1200	40.2 ± 20	1427± 1352
Cd	40	40	0.00 ± 0.00	1.54 ± 0.61

Notes: * Guidelines for the Utilization and disposal of Wastewater (DWAF, 1998; Snyman and Herselman, 2006). **EPA(Barceló and Petrovic, 2011) and AFNOR (AFNOR, 1996) and WHO (WHO, 2010) guidelines for metal limit for soil receiving high sludge loads. ***NS values not specified.

Table 2.7: *Concentrations of heavy metals in seven sampling position from Belmont Valley*

Metal	Sample Number							***SA guidelines**
	1	**2**	**3**	**4**	**5**	**6**	**7**	Maximum Permissible Level
Mn	269 ± 41	417 ± 18	558 ± 32	524 ± 50	409 ± 33	453 ± 49	331 ± 69	1000
Cu	236 ± 16	348 ± 10	473 ± 77	454.± 72	321 ±62	382 ± 92	252 ± 58	1500
Pb	14 ± 0.7	40 ± 2.5	65 ± 6.6	67 ± 2.9	33 ± 6.2	38 ± 4.2	22 ± 2.4	300
Cd	0	0	0	0	0	0	0	40

Notes: * Guidelines for the Utilization and disposal of Wastewater (DWAF, 1998; Snyman and Herselman, 2006). **EPA (Barceló and Petrovic, 2011).

1 ***Table 2.8:*** *Concentration of Manganese and Copper in sewage sludge obtained from Tiaret*

	Metal	
	Mn (mg/kg)	**Cu (mg/kg)**
***EPA guidelines**	**NS	500
Sample		
L141	277	628
L551	727	532
L843	406	584
L812	1006	597
L213	49.8	467
L441	151	553
L623	231	537
L822	445	575
L553	637	468
L112	528	528
L742	558	550
L531	154	595
L842	138	609
L823	521	566
L442	523	572
L121	239	543
L133	389	532
L214	112	537
L223	69.1	564
L222	115	480
L452	471	605
L424	543	539
L252	63.3	585
L154	64.1	592
L241	331	516
L752	395	578
L821	125	468
L742	310	551
L841	64.1	479
L554	243	556
L144	192	580
L534	432	522
L444	452	545
L341	909	496

	Metal	
	Mn (mg/kg)	Cu (mg/kg)
***EPA guidelines**	**NS	500
Sample		
L313	1083	558
L851	306	519
L253	486	486
L113	649	475
L621	655	505
L731	1445	554
L641	1105	567
L523	856	609
L814	914	606
L332	302	567
L331	709	606

1

2 Notes: *L denotes sampling bed and numbers denotes unique coding of each sample.

3

	Metal	
	Mn (mg/kg)	Cu (mg/kg)
*EPA guidelines	**NS	500
Sample		
L231	292	597
L844	1127	517
L414	524	525
L724	177	627
L711	997	526
L423	428	542
L234	681	585
L232	157	576
L744	451	526
L431	272	496
L412	246	544
L254	307	519
L652	825	555
L132	735	628
L234	1087	585
L143	559	483
L422	764	531
L751	765	574
L114	412	536
L541	89.1	596
L614	126	524
L451	301	543
L311	259	518
L653	195	535
L634	337	582
L511	109	631
L533	959	576
L732	319	575
L514	206	547
L151	556	621
L714	358	570
L521	240	552
L723	233	520
L721	107	556
L323	248	559

1

	Metal	
	Mn (mg/kg)	**Cu (mg/kg)**
***EPA guidelines**	**NS	500
Sample		
L513	216	570
L543	102	551
L552	318	559
L421	321	646
L523	130	609
L713	173	576
L722	234	525
L524	102	495
L834	354	352
L231	281	597
Average	**419.90**	**549.78**
S.D	**304.65**	**50.80**

Notes: *L denotes sampling bed and numbers denotes unique coding of each sample. * Guidelines for the Utilization and disposal of Wastewater (WHO, 2010).

Table 2.9: *Concentration of Cadmium (Cd) and Lead (Pb) in sewage sludge obtained from Tiaret.*

	Metal	
	Cd (mg/kg)	Pb (mg/kg)
***EPA guidelin**	5.00	1200
Sample		
L141	1.40	4134
L551	1.63	554
L843	0.16	1275
L812	1.99	327
L213	1.17	33.33
L441	2.15	57.14
L623	2.84	1751
L822	1.60	3392
L553	0.39	1766
L112	1.32	3444

L624	1.46	558
L742	1.53	3912
L531	1.82	45.45

1

	Metal	
	Cd (mg/kg)	Pb (mg/kg)
***EPA guidelin**	5.00	1200
Sample		
L842	2.03	300
L823	1.73	3395
L442	1.59	327
L121	1.21	4087
L133	1.18	3712
L214	1.49	940
L223	1.25	156
L452	2.35	166
L424	1.05	446
L252	1.63	17.50
L154	0.16	2725
L241	1.00	3360
L752	3.69	1875
L821	1.95	190
L742	1.38	3477
L841	0.67	395
L554	1.70	2506
L144	1.13	3596
L534	2.03	451
L444	1.97	128
L341	1.38	1162
L313	1.40	2027
L851	1.01	414
L253	0.81	179.17
L113	1.59	264
L621	1.40	1732
L731	1.08	2510
L641	1.42	1691
L523	1.69	332
L814	1.35	1725
L332	1.58	3735
L331	1.68	3505
L231	1.66	387
L844	1.15	3143
L414	1.46	247
L724	1.92	304

1

	Cd (mg/kg)	Pb (mg/kg)
***EPA guidelin**	5.00	1200
Sample		
L711	1.17	1884
L234	1.46	363
L232	1.44	280
L744	1.46	4110
L412	1.51	135
L254	1.01	300
L652	0.46	666
L132	3.84	1604
L143	1.21	2894
L422	1.18	371
L114	1.49	3522
L541	1.66	217
L614	1.89	265
L451	0.76	840
L311	1.58	3270
L653	1.64	2129
L634	1.78	3154
L511	1.40	3.13
L533	1.76	1643
L732	1.44	3163
L514	1.98	880
L151	1.90	1304
L521	2.30	590
L723	1.45	2707
L721	1.39	702
L323	1.55	2887
L513	2.06	273
L543	2.45	46.8
L552	2.95	85.5
L421	1.97	443.18
L523	0.68	268.
L713	1.44	113
L722	1.75	1981
L524	1.24	608
L834	0.78	362
Average	**1.54**	**1425.58**
***S.D**	**0.61**	**1352.05**

1

Notes: *L denotes sampling bed and numbers denotes unique coding of each sample.* Guidelines for the Utilization and disposal of Wastewater(WHO, 2010). #highlighted figures exceed WHO guidelines.

In this study, acid extraction was used for heavy metal extraction. Dilute 1 M HCl was used to extract the metals from sewage sludge as reported extraction efficiencies for Cu (80-90 %), Cd (90-93 %), Pb (98-100 %) and Mn (80-88 %) were high in the extraction from soil according to Tuin and Tels, (1990). Several studies have been reported on extraction of heavy metals from soils (Rauret, 1998; Sánchez-Martín et al., 2007; Tuin and Tels, 1990). Metal extraction from soils can be done several ways, which include: (a) acid extraction using 2 M Nitric acid (HNO_3), 0.1-1 M HCl. These acids dissolve trace elements associated to different fractions such as exchangeable carbonates, metal oxides and OM; (b) use of chelating agents such as 0.01 M $CaCl_2$, 0.015 M ammonium fluoride (NH_4F), 0.01-0.05 ethylenediaminetetraacetic acid (EDTA). These agents dissolve the exchangeable element fraction and the element fraction forming OM complexes and the element fraction fixed on soil hydroxides; (c) extraction using buffered solutions such as 1 M ammonium acetate or acetic acid buffer at pH 7; and (d) extraction using unbuffered salt solution such as 1 M ammonium nitrate (NH_4NO_3) and 0.1 M barium chloride ($BaCl_2$) (Rauret, 1998). Both buffered and unbuffered solutions on extraction dissolve mainly the cation exchangeable fraction (Rauret, 1998).

The advantage of using 1 M HCl is that it dissolves greater that 20% of solid material (Rauret, 1998; Tuin and Tels, 1990), therefore increasing the likelihood of all metals being extracted. The extraction efficiencies of Pb and Cd are high because of the tendency of these

metals to form stable chloride complexes in soils, which in consequence increase their extraction from sludge (Tuin and Tels, 1990). On the other hand Cu is difficult to extract from soils because of its small ionic radius 0.72 Å, and as a result it tends to bind onto interlayer sites, and in some instances substitute iron (Fe) or magnesium (Mg) which are similar in size, with an ionic radii of 0.74 Å and 0.66 Å, respectively (Tuin and Tels, 1990). For these reasons, the extraction of metals are in the following order Cd>Pb>Cu(Tuin and Tels, 1990).

The sludge samples from Tiaret had a higher heavy metal content than sludge samples from Belmont Valley. On statistical analysis, there was significant difference in Cu, Pb and Cd levels from both sites, and no significant difference was observed in Mn levels from both sites as shown in table 2.6. Furthermore, on sampling different positions in Belmont Valley, each heavy metal was compared to the mean of all sampled positions to determine if sampling position played a role in heavy metal composition. A statistical analysis t-test, showed no significant difference (*p-value* = 0.9153) on where the sample was collected, as the concentrations were almost the similar.

On analysis of the results, samples from both sites were compared to the values on guidelines for the utilization and disposal of wastewater sludge and national environmental act for South Africa (DWAF, 1998; Snyman and Herselman, 2006; NEMA, 2013), and for Algeria Solid waste management guidelines and Guidelines for Wastewater Treatment and

Reuse in the Mediterranean Region(AFNOR, 1996; Barceló and Petrovic, 2011; Kehila, 2014).

2.1.1.8.7 Copper

The concentration of Cu (mean ± standard deviation) is shown in table 2.6 for comparison of Belmont Valley and Tiaret sludge samples. Table 2.7 shows concentration of Cu in Belmont Valley sludge and table 2.8 shows concentration of Cu from Tiaret sludge samples. The average concentration of Cu from Belmont Valley was 352.84 ± 91.81mg/kg d.w and was 548.56 ± 49.96 mg/kg. Literature reports Cu concentrations in sewage sludge of 246-626 mg/kg (Shamuyarira and Gumbo, 2014) and 245-411 mg/kg (Morrison et al., 2004), which are comparable to the values obtained in our study. Statistical analysis, indicated a significant difference between sludge obtained from Belmont Valley and Tiaret, with Tiaret samples containing higher Cu concentrations. Furthermore, on assessment of the influence of sampling position in heavy metal concentration, from the study it was shown that sampling position does not influence the heavy metal, therefore on statistical analysis there was no significant difference in the amount of Cu in all sampled seven positions. The concentrations of Cu from both sites were below the values stated in the guidelines the utilization and disposal of wastewater sludge and national environmental act for South Africa(DWAF, 1998; Snyman and Herselman, 2006; NEMA, 2013), and for Algeria Solid waste management guidelines and guidelines for Wastewater Treatment and Reuse in the Mediterranean Region(AFNOR, 1996; Barceló and Petrovic, 2011; Kehila, 2014). Sludge samples obtained from Belmont Valley, was classified under pollutant class A (Cu level

below 1750 mg/kg) based on the guidelines the utilization and disposal of wastewater sludge for South Africa (DWAF, 1998; Snyman and Herselman, 2006). On the National environmental act for South Africa(NEMA, 2013), the sludge was classified under Type 3 waste (concentration between 16 and 19500 mg/kg), and thus it implies that the sludge could be considered for beneficial use in South Africa. For sludge samples obtained from Tiaret, the Cu concentration was below (threshold of 1500 mg/kg) the stated values on the AFNOR guidelines (AFNOR, 1996) and EPA guidelines in the Mediterranean Region(Barceló and Petrovic, 2011). According to solid waste management policies in Algeria (Kehila, 2014), the sludge was considered low risk to environment contamination, and thus it would be considered for beneficial reuse and disposal in land filling application(WHO, 2010).

In faecal sludge, Cu concentrations were in the range 0.02-7.54 mg/L (table 2.5) and most of the values obtained were in accordance with the DWAF guidelines and only one pit latrine had a value (7.54) which was higher than the permissible values stated in Guidelines the utilization and disposal of wastewater sludge for South Africa (DWAF, 1998; Snyman and Herselman, 2006). Most of the faecal sludge samples were classified under pollutant Class A (Cu below 0.13 mg/L), and thus they could be used of unrestricted application in agricultural soils.

The high values of Cu in sewage sludge would be probably as a result of corrosion of water supply pipes which are predominantly made of copper, as this was further substantiated when in Australia where 46% of Cu in sewage sludge was from water supply connected to

household pipes (AWA, 2008). Other sources of Cu might have been due to surface runoff into storm drainage pipes then eventually reach the sewage plant (Herselman et al., 2005) and one of the most explanatory reasons was the extensive use of brass (copper and zinc) products such as scrubbers for household cleaning and washing pots which consequently the water is disposed into a drainage (reaching the WWTP) or in the pit latrine(Herselman et al., 2005). Cu is highly adsorbed onto soil particles(Alloway, 1995), and thus sewage sludge has a potential to increase levels of Cu in amended soils if applied in agriculture and thus may accumulate in the top horizons of the soil. The Cu mobility is governed by pH, therefore the more acidic the soil is, the greater the mobility of Cu (Kabata-Pendias and Pendias, 2001). OM appears to be a dominant factor controlling Cu mobility due to electrostatic bonding, and thus the increase in OM content, the greater chance of increasing Cu content and mobility(Alloway, 1995). Cu greater than 21 mg/kg increases the likelihood of toxicity of plants (Gupta and Sinha, 2007). In a study conducted by Gupta and Sinha, (2007), they reported Cu levels of 10 mg/kg and 70 mg/kg in cucumber and maize crops, respectively. Cu has a tendency to replace Fe in physiological centres (iron-sulphur centres in hemoglobin)(Mengel and Kirkby, 2001)which leads to chlorosis and Fe deficiency in plants (Pais and Benton-Jones, 1997). Moreover Cu is responsible for the inhibition of root growth (Mengel and Kirkby, 2001). In humans, Cu toxicity is rare and is usually associated with gram quantities (greater than 0.1 mg/kg)(Chaney, 1988; Tiruneh et al., 2014). Approximately between 0.1 and 0.2 mg/kg of Cu body weight can result in gastrointestinal disturbances (Tiruneh et al., 2014).

2.1.1.8.8 Cadmium

The average of Cd (mean ± standard deviation) is shown in table 2.9 and distinct concentrations of Cd in sludge samples from Tiaret and Belmont Valley are shown in table 2.6. In sewage sludge obtained from Belmont Valley, there was no Cd detected, whereas Tiaret sludge samples had a concentration of 1.54 ± 0.61 mg/kg. The individual concentrations of Cd from sampling positions are shown in table 2.7. In literature, the Cd sludge concentrations were 1.9 mg/kg d.w (Morrison et al., 2004), 3.10 mg/kg d.w (Shamuyarira and Gumbo, 2014) and 0.01-14.15 mg/kg (Maas et al., 2010), which are comparable to the values found in our study. Statistical analysis indicated a significant difference in Cd concentration between Tiaret and Belmont Valley sewage sludge. On investigating the role of the sampling position in sludge beds, no significant difference in Cd concentration amongst the samples obtained from Belmont Valley sludge beds was observed. On comparing the Cd concentration for Belmont Valley sludge to guidelines for the utilization and disposal of wastewater sludge and National Environmental Act for South Africa(DWAF, 1998; Snyman and Herselman, 2006; NEMA, 2013), the sludge met the given standards and Belmont Valley sludge was classified under pollutant A (less than 40 mg/kg of Cd). The sludge could therefore be considered for beneficial use based on Cd level (DWAF, 1998; Snyman and Herselman, 2006). On comparison to National Environment Management Act of South Africa, the sludge was considered as Type 4 waste (Cd less than 40 mg/kg), making the sludge safe to dispose of in the environment with no human and environmental risks arising. (NEMA, 2013). For Tiaret sludge samples, the average concentration was below AFNOR guidelines (AFNOR, 1996) and EPA guidelines in the

Mediterranean Region(Barceló and Petrovic, 2011). The Tiaret sludge samples were below 15 mg/kg of Cd which was stated in both AFNOR guidelines and EPA guidelines in the Mediterranean Region. Based WHO guidelines, the sludge could safely be reused for beneficial use such as land filling (WHO, 2010), production of biogas and in agriculture (Sadek et al., 2013).

There was no Cd detected in faecal sludge samples obtained from Hlalani Township as shown in table 2.5, and thus all the faecal sludge samples met the standards and was classified as Class A pollutant (Cd below 0.031 mg/L) (DWAF, 1998; Snyman and Herselman, 2006). Therefore the faecal sludge may be safe to the environment and thus may be considered for beneficial reuse.

Cd has no essential biological function, but it is highly toxic to plants and animals (Snyman and Van der Waals, 2004). Sources of Cd include vehicle tyres which wash off into storm waters, domestic products and industrial effluent (Alloway, 1995), and these might have been the cause of Cd levels detected in sludge obtained from Tiaret. In plants, Cd concentration greater than 3 mg/kg suppresses growth (Snyman and Van der Waals, 2004), and in addition, interferes with photosynthesis and causes leaf chlorosis and necrosis (Pais and Benton-Jones, 1997). In humans, Cd accumulates in the kidney, and to some extent the liver and spleen (Mengel and Kirkby, 2001), and can cause hypertension, carcinogenesis and nausea (Stewart-Pinkham, 1989).

2.1.1.8.9 Lead

The average concentration of Pb (mean ± standard deviation) is shown in table 2.6, and table 2.7 and table 2.9 show Pb concentration in Belmont Valley and Tiaret, respectively. The average concentration of Pb was 40.2 ± 20.1 mg/kg d.w and 1425 ± 1352 mg/kg from Belmont Valley and Tiaret, respectively. Literature reports Pb concentrations in sewage sludge of 69-365 mg/kg d.w (Morrison et al., 2004) and 21.3-171.85 mg/kg d.w (Shamuyarira and Gumbo, 2014) and which are comparable to the values obtained in our study for Belmont Valley sludge samples not Tiaret sludge samples. Statistical analysis to compare Pb levels between the two sites indicated that Pb concentrations were significantly different (p = 0.01546). The sludge met standards set in the guidelines for the utilization and disposal of wastewater sludge and National Environmental Management Act for South Africa(DWAF, 1998; Snyman and Herselman, 2006; NEMA, 2013). Using the same guidelines, the sludge from Belmont Valley met the requirements (Pb less than 300 mg/kg), and was classified under pollutant A, implying that, based on the Pb levels, the sludge would be considered for unrestricted use in agricultural soils (DWAF, 1998; Snyman and Herselman, 2006). Upon evaluation using the National Environment Management Act, Belmont Valley sludge was classified as Type 3 waste (20-1900 mg/kg), and the sludge would need to be disposed with caution (NEMA, 2013). Sludge obtained from Algeria did not meet the specifications stated on AFNOR guidelines (AFNOR, 1996) and EPA guidelines in the Mediterranean Region(Barceló and Petrovic, 2011) as the Pb concentration was above 1200 mg/kg. as a consequence, the sludge will not be considered for recycling,

land filling and in agriculture as it may result in toxicity human and environmental species (Kehila, 2014).

For faecal sludge obtained from pit latrines in Hlalani Township, the average concentration of Pb was 0.00 ± 0.01 mg/L as shown in table 2.5, and all faecal sludge samples met the guidelines for the utilization and disposal of wastewater sludge of South Africa (DWAF, 1998; Snyman and Herselman, 2006), and thus the faecal sludge may be considered for agricultural applications after the other risks such as the microbial concentrations of the sludge have been evaluated.

Pb binds strongly to soils, and forms insoluble precipitates with phosphates (Laperche, 2000) and thus root uptake is low. In soils, Pb is the least mobile element, and therefore plant uptake is small and has low concentrations in plants or crops (Laperche, 2000). In humans, Pb mimics the behaviour of Ca and as a result may inhibits enzyme systems (Mengel and Kirkby, 2001) and mainly accumulate in the skeleton (Pais and Benton-Jones, 1997). Prolonged exposure to Pb leads to anaemia, gingival leadline (Langston, 1989) and is both carcinogenic and teratogenic , moreover decreases brain development (Pais and Benton-Jones, 1997).

2.1.1.8.10 Manganese

The average concentration on Mn (mean ± standard deviation) is shown in table 2.6 for Belmont Valley and Tiaret sewage sludge. Individual site Mn composition are shown in table 2.7 and 2.8 for Belmont Valley and Tiaret, respectively. The average concentration of Mn was 423.35 ± 101.32mg/kg d.w and 358.05 ± 294.88mg/kg for Belmont Valley and Tiaret, respectively. In literature, Mn concentrations in sewage sludge have been reported between 15-60 mg/kg d.w (Obrador et al., 1997) and 83-565 mg/kg (Tiruneh et al., 2014)which were comparable to the values obtained in our study. Statistical analysis, showed no significant difference (p = 0.06142) in Mn concentration present in sewage sludge from both sites (Belmont Valley and Tiaret). The Mn concentration in Belmont Valley was equated to the specifications in the guidelines for the utilization and disposal of wastewater sludge (DWAF, 1998; Snyman and Herselman, 2006)and National environmental management Act (NEMA, 2013), and it met the specified standards. According to guidelines for the utilization and disposal of wastewater sludge, Belmont Valley was classified under pollutant class A (Mn concentration below 1000 mg/kg), meaning that the sewage sludge possibly could be considered for agricultural application (DWAF, 1998; Snyman and Herselman, 2006). Contrasting the Mn concentration in Belmont Valley sewage sludge to National Environmental Management Act, the sewage sludge was classified as Type 4 waste, implying that it has a very low risk on causing human and environmental health implications and therefore be disposed of without any challenges and moreover could be considered for reuse based on the Mn concentration (NEMA, 2013). For sewage sludge obtained from Tiaret, the Mn concentration was below the specified standards on AFNOR guidelines (AFNOR, 1996) and EPA guidelines in the Mediterranean Region(Barceló and Petrovic, 2011), and thus the sludge would be considered for production

of recyclable energy (Sadek et al., 2013) and use as agricultural fertilizer, (WHO, 2010, Benhamou and Fazouane, 2013). Relating the Mn values of sewage sludge to solid waste management policies (Kehila, 2014), the sludge was considered low risk and thus may be specifically used for land filling and in agricultural soils (Tamrabet et al., 2009).

In faecal sludge obtained from pit latrines, the average concentration of Mn in was 0.36 ± 0.84 mg/L as shown in table 2.5. Most of the faecal sludge samples met the guidelines for the utilization and disposal of wastewater sludge of South Africa (DWAF, 1998; Snyman and Herselman, 2006), and thus the faecal sludge may be considered for beneficial reuse. The high levels of Mn in both sewage and faecal sludge might have been due to industrial manufacture of manganese alloy products which becomes a component of industrial effluent and in addition incorrect disposal of dry cell batteries (either into the pit latrine or some cases flushed down the toilet), which as a consequence may enter sewage systems (Herselman et al., 2005).

Mn makes up a significant portion of heavy metals in sewage sludge because of high water solubility and thus is highly plant available of metals present in sewage sludge (Chaney et al., 2001). Mn plays a role as a macronutrient for microorganisms and higher plants (Alloway, 1995). Upon release of Mn in sludge amended soils at pH values close to neutral, the metal precipitates due to low solubility at this pH (Snyman and Van der Waals, 2004) resulting in low absorption by the roots and entering the crops or plants. The concentration at which Mn is toxic to plants is between 400 and 1000 mg/kg, and at these levels Mn causes

stunted growth in plant and crops (Smith, 1996). Mn is an important metal which plays a role in retention of other metals in sewage sludge, though this is dependent on conversion to manganese oxide states, which can be terminated or reversed by flooding of the sludge-amended soils (Herselman et al., 2005).

2.4 CONCLUSION

It can be seen that regulations play a pivotal role in the amount of heavy metals, plant nutrients and pathogens that may be present in sewage sludge especially if it is to be reused for beneficial purposes such as agricultural application in soils. The total metal concentrations present in sewage sludge samples taken from Belmont Valley and Tiaret WWTP showed a range of variations in accordance with the characteristics of the wastewater generated from the respective towns and the level of industrial establishments present in the cities. Tiaret WWTP sludge samples showed generally higher heavy metal and pathogen levels compared with the sludge samples taken from Belmont Valley WWTP. This is because Tiaret is an industrial area and dominated by petroleum production, with several of the effluents generated from the industries having minimal treatments, such as equalization basins before being discharged to the municipal sewage system, whilst Belmont Valley serves as smaller population and little or no industrial activity in the town.

In terms of the regulatory limits of total metal concentrations in the guidelines for the utilization and disposal of wastewater sludge in South Africa,National Environment Management Act, Solid waste management of Algeria, WHO and EPA guidelines most of the sludge samples largely show compliance for agricultural application with respect to microbiological analysis and heavy metal composition. The experimental results indicated that most of the heavy metals and pathogens are present in moderate concentrations well below the regulatory limits (except Tiaret sewage sludge), thus the sludge generated from the

wastewater treatment plants may be considered further for agricultural application. Nonetheless, N levels from Belmont Valley sludge did not meet the values stated in the guidelines, thus beneficial use of the sludge would not be a major challenge as nitrates can leach out and cause environmental pollution, and consequently affect human health. In the event sludge is used for beneficial purposes, close monitoring needs to be done especially on determining N levels present on soils before application, plant N uptake and the application rate of sludge.

In conclusion it was demonstrated that sludge from WWTPs has great recyclable value considering the nutrients and soil conditioning values of the sludge, and therefore could provide agriculture with an economical and environmentally viable option. This can be of great benefit to Belmont Valley and Tiaret WWTPs where sewage sludge is stored in large stockpiles and thus suggesting a possible future option for valorisation of sludge by use in agricultural application and this will be demonstrated in Chapter 5 whereby plants will be grown using sludge amended soils.

3 CHAPTER 3

TRICLOSAN SOLUBILITY STUDIES

3.1 INTRODUCTION

TCS is an antimicrobial agent that has been incorporated into many skin care formulations, toothpastes, shampoos and soaps (Chen et al., 2011; Farré et al., 2008). TCS is known to inhibit the enzyme enoyl reductase, which therefore results in blocking the fatty acid synthesis (Wu et al., 2009), which in bacteria is accountable for the production of vast lipid-containing components including cell membranes (Zheng et al., 2005). It is a white to off-white crystalline powder with a faint aromatic smell. TCS has a meltingpoint of between 55 and 57 ºC (MSDS, 2015), a pK_a of 7.9(Halden and Paull, 2005), and water solubility of less than 10^{-6} g/mL(Aragón et al., 2008). The use of surfactants increases aqueous solubility of hydrophobic compounds (Ying, 2006) and in this chapter, bile acids which have surface active agent properties (Carey, 1985), were used to improve aqueous solubility of TCS and determine the extent of solubilization of TCS.

Bile acids are a group of water-soluble steroids formed during the catabolism of cholesterol, and synthesized in the hepatocytes of the liver (Stamp and Jenkins, 2009). In bile, bile acids solubilize cholesterol as mixed micelles, enhancing its elimination; in small intestinal

content and have been found to be present in human faeces (Hofmann and Mysels, 1992). If the concentration of BAs anions is high, the BA molecules tend to self-associate to form micelles (Carey, 1985). Micelles have a tendency to aggregate and the midpoint concentration range where micellar aggregation occurs is called critical micellar concentration (CMC) (Stamp and Jenkins, 2009). Bile acids are excreted in salt form, with lithocholate having the highest concentration of between 0.38 and 2.03 μmol/g of dry faeces and deoxycholate subsequently having a concentration ranging from 0.18-2.78 μmol/g of dry faeces. It should be noted that bile acids are resistant to biodegradation in the environment(Hofmann and Mysels, 1992), and thereforethey could increase the aqueous solubilities of the environmental pollutants(Peysson and Vulliet, 2013). The presence of bile acids in human faeces, implies that they have the potential to solubilize TCS molecules that are found in the environment (Hofmann and Mysels, 1992). No such studies have been done to date and this is the knowledge gap this chapter is aimed at addressing. The aim of this chapter was to study the extent of solubilization of TCS in the presence of bile acids (sodium deoxycholate and sodium lithocholate) at 15 and 37 ºC. Chromosorb G and Silica gel powder was used in the assessment of aqueous solubility of TCS, as these compounds were coated with TCS. The studies on aqueous solubility of TCS will be elucidated in detail in this chapter.

3.2 MATERALS AND METHODS

3.2.1 MATERIALS

Methanol (>98%) (Cat. number: 34860-2.5L-R), Acetone (>97.5%) (Cat. number: 42631-2.5L-R), Chromosorb G acid washed (80-100 mesh) (Cat. number: C-7264), Lithocholic acid (>95%) (Cat. number: L6250-25G), Deoxycholic acid (sodium salt) (>97%) (Catalogue number: 264101-25GM), Irgasan (Triclosan) (>98%) (Catalogue number: PHR1338-1G)and Suprasil® 3500 µL quartz cuvettes (200-2500 nm spectral range) were purchased from Sigma Aldrich(Johannesburg, South Africa). Sodium hydroxide pellets (>98%) was purchased from Minema Spellbound laboratory solutions(Port Elizabeth, South Africa). Silica gel 60 powder (0.040-0.063 mm) (Product number: 1.09385.1000) and all glassware used in this chapter was purchased from Merck (Pty) Ltd (Johannesburg, South Africa). All masses were measured using a Pioneer™ PA214 analytical balance purchased from Ohaus Corporation(Pine Brook, NJ USA). Mechanical orbital shaker Model number TS-520D was purchased from Already Enterprise Inc. (Taipei, Taiwan). Crison pH meter Model: Basic 20 was purchased from Crison instruments (Alella, Spain). Magnetic stirrer Model STR-N11 was purchased from FMH Instruments(Johannesburg, South Africa). Absorbance was measured using Shimadzu UV-1240 spectrophotometer (Shimadzu, Johannesburg, South Africa). Rotavapour Model number R-215 was purchased from Büchi labortechnik Inc. (Flawil, Switzerland). MilliQ water used in this chapter was prepared by reverse osmosis, using a Milli-RO® 15 water purification system purchased from Millipore®(Bedford, MA,

USA). Mechanical orbital shaker water bath Type N085-57L25 was purchased from Labdesign Engineering (Pty), Ltd, Maraisburg(Johannesburg, South Africa).

3.2.2 METHODS

3.2.2.1 Determination of maximum absorption wavelength of triclosan using UV/VIS Spectrophotometer

The maximum wavelength (λ_{max}) of TCS was determined using Shimadzu UV-1240 spectrophotometer. Nine concentrations ranging between 1 to 50 mg/L of TCS were prepared inmethanol.The absorbance of TCS was measured at UV spectrum between 200–800 nmfor each solution, and each solution was measured in triplicates. Subsequently a calibration curve of absorbance at 281 nm against concentration was plotted. The calibration curve for the TCS quantification is shown in figure 3.1.

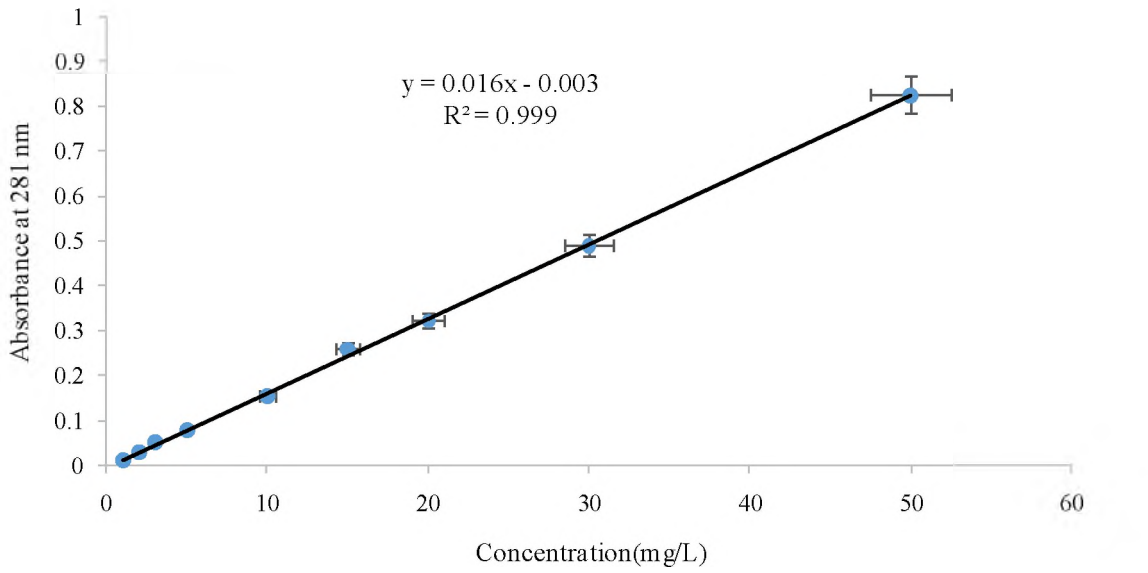


Figure 3.1: *TCS calibration curve (n=3) with a range of 1-50 mg/L in methanol at 281 nm wavelength.*

3.2.2.2 Quantification of triclosan using UV/VIS spectrophotometry

3.2.2.2.1 Preparation of 0.5 g/L of triclosan solution in acetone.

To prepare the 0.5 g/L of TCS solution, 0.25 g of Irgasan was weighed using aPioneer™ PA214 analytical balance and thereafter transferred into a 250 mL volumetric flask. Fifty milliliters (50 mL) of acetone was graduallytransferred to the volumetric flask to dissolve the TCS powder. To ensure all the TCS powder dissolves, thecontents of the volumetric flask were hand-shaken until the TCS particles were completely dissolved.After TCS powder had dissolved, acetone was added up to 250 mL mark. To ensure all the TCS powder dissolves, thecontents of the volumetric flask were hand-shaken until the TCS particles were

completely dissolved. The volumetric flask was sealed with a glass stopper to avoid sorption of TCS onto the seal(Fauser et al., 2003). The flask was wrapped with aluminum foil, so as to prevent photodegradation of TCS (Son et al., 2007).

3.2.2.2.2 Evaporation of acetone from triclosan solution with chromosorb G and silica gel powder

Chromosorb Gwas a standard medium for the solubility measurements, but the product was commercially discontinued and thus a comparison for future studies was done with Silica gel. One gram(1g) of Chromosorb G and 1 g of Silica gel powder were weighed using Pioneer™ PA214 analytical balance, and then transferred into two separate 500mL round bottom flasks. Using a graduated measuring cylinder, 100mL of 0.5g/L of TCS (prepared in 3.3.2.1.1) was transferred into each of the 500mL round bottom flasks containing Chromosorb G and Silica gel powder. The solvent (acetone) was evaporated from each flask using a Rotavap, so as to coat the each of the Chromosorb G and Silica gel adsorbents with TCS. The parameters of the Rotavap were: rotation speed of 85rpm; set temperature of 40°C; and vapour temperature of 21°C. Whenacetone had evaporated in each round bottom flask, the flasks were detached from the Rotavap, then sealed with a glass stopper and wrapped with aluminum foil. The round bottom flasks was stored in the fridge at a temperature of 4 °C for until use.

3.2.2.2.3 Preparation of 1g/L of deoxycholic acid (sodium deoxycholate)

One gram(1 g) of sodium deoxycholate saltwas weighed using Pioneer™ PA214 analytical balance. The weighed powder was transferred into a 1000 mL volumetric flask and subsequently MilliQ water was added gradually to make up to the volume (1000 mL). The volumetric flask was inverted three times to ensure all the sodium deoxycholate powder dissolves. The resultant concentration of the final solution was 1 g/L. A Crison pH meter was used to measure the pH of the solution and the reading obtained was recorded. The solution was stored in the fridge at a temperature of 4°C for 24h.

3.2.2.2.4 Preparation of 1 g/L of lithocholic acid (sodium lithocholate)

One gram(1 g) of lithocholic acidand 0.15 g sodium hydroxide pellets were weighed using Pioneer™ PA214 analytical balance with 0.0001 accuracy. The sodium hydroxide pellets were transferred into a 150 mL beaker and dissolved in 50 mL of MilliQ water using a glass rod to stir the contents in the beaker. The sodium hydroxide solution in the beaker was transferred to a 1000 mL volumetric flask. The weighed out lithocholic acid powder was transferred into a 250 mL beaker, and dissolved in MilliQ water. Thereafter the dissolved solution was transferred into a 1000 mL volumetric flask. MilliQ water was added up to make up to the required volume.When the total volume required was reached, a glass stopper was used to seal the flask. The resultant solution was not clear, thus the contents were mixed using a Magnetic stirrer at a temperature of 35 °C for five minutes so as to have a

homogeneous clear solution. The final concentration of the sodium lithocholate solution was 1 g/L. A Crison pH meter was used to measure the pH of the solution and the reading obtained was recorded.

3.2.2.3 Solubility studies of triclosan from chromosorb G and silica gel powder in the presence of bile acids

3.2.2.3.1 Solubility studies of triclosan from chromosorb G and silica gel powder in the presence of sodium deoxycholate

Assessment of TCS solubilization was performed by in assessing the solubilization of TCS in the presence of sodium deoxycholate. Into six of the 250 mL amber coloured bottles, 200 mg of the adsorbent (Chromosorb G powder or Silica gel powder) coated with TCS (from 4.2.2.3.2) was weighed using Pioneer™ PA214 analytical balance. The weighed Chromosorb G coated with TCS was transferred into three of the amber coloured bottles, and the other three amber coloured bottles each contained 200 mg of Silica gel coated with TCS. Using a graduated 250 mL measuring cylinder, 250 mL of 1 g/L of sodium deoxycholate solution was transferred into each amber coloured bottle. The control (seventh flask) only contained 250 mL of 1g/L sodium deoxycholate solution.

The samples were placed in a Mechanical orbital shaker and incubated for 72 h, at 200 rpm and at between 12 and 15 °C. Upon sampling, the sampling position was 30 mm from the

bottom of the amber coloured bottle. The samples were collected at: 0 h, 1 h, 2 h, 4 h, 8 h, 20 h, 24 h, 36 h, 48 h, 60 h and 72 h intervals.

The experiment was repeated with all conditions similar to the above, however at 37 ± 0.5 ºC at 150 rpm. The solubility of TCS in BAs was determined in the previously stated conditions.

3.2.2.3.2 Solubility studies of triclosan from chromosorb G and silica gel powder in the presence of sodium lithocholate.

Assessment of TCS solubilization was performed by in assessing the solubilization of TCS in the presence of sodium lithocholate. The method to assess solubilization was similar to the above method in section 3.2.2.3.1, and the difference was the use of 1 g/L of sodium lithocholate solution.

3.2.2.3.3 Analysis of samples using UV/VIS spectrophotometry

The amount of TCS released was determined using UV/VIS spectrophotometer by measuring absorbance at 281 nm over a 72 h period. The blank was the respective bile acid solution for the given solubilization study, i.e. 1 g/L ofsodium deoxycholate was the blank in solubilization by sodium deoxycholate and; 1g/L sodium lithocholate was the blank in solubilization by sodiumlithocholate. Each sample collected was transferred into a Suprasil® 3500 µL quartz cuvette, and the absorbance of each sample measured using UV/VIS

spectrophotometer. The obtained absorbance was concentration determined from the calibration curve.

3.2.2.4 Extraction of TCS from Chromosorb G

Two hundred milligrams (200 mg) of chromosorb G coated with 0.5 g/L TCS solution was weighed using a Pioneer™ PA214 analytical balance. The weighed powder was transferred into a 10 mL polytope vial, and extracted with 5 mL of methanol three times. Thereafter, the extracts were combined and a subsample of was used to fill up a Suprasil 3500 µL quartz cuvette and absorbance was measured at 281 nm using the UV/VIS spectrophotometer. The absorbance obtained was paralleled to the TCS calibration curve shown in figure (3.1). The extraction efficiency or entrapment efficiency was used to demonstrate through the extractions that even at the end of the TCS solubilization experiment there was enough TCS left on the Chromosorb G or Silica gel powder to provide excess amount of TCS, and the TCS amount in the system did not influence your measurement results. The extraction efficiency or entrapment efficiency of TCS from chromosorb was then determined using the equation below:

$$Ext.efficiency(\%) = \frac{Amount\ \ of\ TCS\ extracted\ \ \ from\ \ adsobent}{Theoretical\ \ \ amount\ \ \ of\ TCS} \times 100 \qquad (3.1)$$

3.2.2.5 Extraction of TCS from Silica gel powder

The extraction efficiency or entrapment efficiencyof TCS from silica gel powder was determined similarly to the method above. The difference was that Silica gel powder was used instead of Chromosorb G. The extraction efficiency of TCS was determined using equation 3.1.

3.2.2.6 Data analysis

Data analysis was done using non-linear regression by Microcal™ Origin 6.0 software package (Microcal Software, Inc. Northampton, MA, USA). The mathematical model of first-order dissolution kinetics was followed as shown in equation (3.2).

$$C = C_{max} \times (1 - e^{-bt}) \qquad (3.2)$$

In equation (3.2), C is the dissolved TCS concentrations in the bile acid solution (mg/L), while, t is the time of the experiment (hours). The adjustable parameters that were derived using the non-linear regression were C_{max} which is the TCS solubility in BA solution in question (mg/L) and b is the first-order dissolution rate constant ($hours^{-1}$). The calculated aqueous solubility of TCS in water is 4.621 mg/L (based on the CAS number and the WSwin software package).

3.3 RESULTS AND DISCUSSION

3.3.1 UV/VIS SPECTROSCOPY

The use UV/VIS spectroscopy was used to determine the maximum wavelength of TCS. The analytical method was used because of the chemical nature of TCS, it is expected to absorb electromagnetic radiation in the ultraviolet region due to its aromatic ring structure. The maximum absorption wavelength of TCS was 281 nm, and this wavelength was used for TCS analysis. According to a study conducted by Lavecchia and Zuorro, (2014), the maximum absorption wavelength of TCS in aqueous ethanol was obtained at 282 nm. On another study conducted by Son et al., (2007) using ethanol as a solvent, they obtained a maximum absorption wavelength of 280 nm using HPLC with a UV/VIS detection, and sharp symmetrical peaks were observed at this wavelength. Therefore from these studies, it was shown that TCS absorbs between 280 and 282 nm, and the maximum wavelength of 281 nm which was obtained in this study was comparable to the values in literature. Neither lithocholic nor deoxycholic acid interfered with the TCS determination using UV/VIS spectrophotometer, and the pH of the solutions were 8.4 and 8.6, respectively. A calibration curve of TCS at 281 nm was plotted and used in assessing solubility of TCS in the presence of sodium deoxycholate and sodium lithocholate.

3.3.2 DISSOLUTION CURVES OF TCS

TCS belongs to a class of compounds known as hydroxyphenyl ethers. It is a lipophilic compound that is poorly soluble in water, but soluble in numerous organic solvents such as acetone, hexane, methanol and propylene glycol(Duan et al., 2005; Green Facts, 2010). The calculated aqueous solubility of TCS at pH 6, in pure water at room temperature has been determined at4.621 mg/L.

The addition of small amounts of deoxycholic acid and lithocholic acid to aqueous media showed enhanced solubility of TCS. This enhancement of aqueous solubility of TCS was due to bile acids' tendency to aggregate and the midpoint concentration range where micelles formed and entrapped the compound (Stamp and Jenkins, 2009). Numerous studies have been done to improve aqueous solubility of TCS including use of glycospheres (Ding, 2001), amino alcohols and amino acids (Duan et al., 2005). From our study, lithocholic acid enhanced TCS solubility more than the deoxycholic acid due to the lower CMC values for lithocholate. In literature, the CMC value of lithocholic acid is 0.009-0.030 g/L and for deoxycholic acid is 0.083-0.249 g/L (Hjelm et al., 1995; Stamp and Jenkins, 2009). Therefore, the low CMC values of lithocholic acid explain the ease of the compound to form micelles, and thus the higher aqueous solubility of TCS observed with lithocholic acid than deoxycholic acid. Bile acids are naturally occurring surfactants and their influence on the solubility of TCS has not been studied yet. As they are naturally occurring they might play a significant role in the solubilization of TCS in the environment, which in turn might have

significant environmental health and toxicity implications. The presence of bile acids in the environment may result in environmental pollution of water sources, as TCS concentrations might be increased by these bile acids. The persistence of TCS residuals or by-products that are not degraded during wastewater treatment process can enter the aquatic environment in wastewater effluents and sludges (Capdevielle et al., 2008), and moreover some of the TCS can persistent in WWTP thus potentially reaching fluvial ecosystems (Ricart et al., 2010). If the WWTP effluent containing TCS is discharged to the environment for example rivers, the presence of bile acids in cattle excreta next to the rivers may increase in concentration of TCS (due to solubilization) in these sources, and as a result aquatic organisms, environmental flora might be affected (Chalew and Halden, 2009; Tatarazako et al., 2004).

In our study, the apparent solubilities and rate constants indicated in brackets of TCS at 37 °C were 35.4 ± 1.21 mg/L (1.28 ± 0.36 Hr^{-}) and 14.4 ± 0.34 mg/L (0.99 ± 0.17 Hr^{-}) in sodium lithocholate and sodium deoxycholate, respectively. The apparent solubilities and rate constants indicated in brackets of TCS at 15 °C were 32.3 ± 0.88 mg/L (2.16 ± 0.80 Hr^{-}) and 14.2 ± 0.39 mg/L (1.02 ± 0.17 Hr^{-}) in sodium lithocholate and sodium deoxycholate, respectively. Upon conducting a t-test statistical analysis, it was shown that there was significant difference (p = 0.01265) between the TCS released by sodium deoxycholate and sodium lithocholate. On further conducting another t-test statistical analysis, there was no significant difference in C_{max} for each bile acid when temperature was changed from 15 °C to 37 °C.

The pH of the solubility studies was done at 8.4 for sodium lithocholate and 8.6 for sodium deoxycholate. The pH of South African drinking, surface and ground water ranges between 8.2-8.6 (DWAF, 2008), and thus this study was done to mimic the environmental conditions present in South Africa. Since the pH of South African water is above the pK_a of TCS (Halden and Paull, 2005), it implies that TCS will be ionized in these waters. Increase in ionisation of TCS implies that the water solubility of the compound is increased, and thus bioaccumulation in organisms might occur.

The increased solubility will likely be the result of the formation of the sodium salt of TCS on the phenolic group in its structure. The reported aqueous solubility of the TCS at pH = 6.0 is an underestimation of the actual TCS solubility in South African waters. In literature, Duan et al., (2005) used cyclodextrine derivatives to improve aqueous solubility of TCS and they obtained a value of 54.1 mg/mL and the results that were measured in this chapter were lower than the literature values, but nonetheless bile acids significantly increased the aqueous solubility of TCS. The extraction efficiency of TCS from Chromosorb G was 92.4 ± 0.001 % and for Silica gel powder was 78.6 ± 0.003 %. This implied that more than 94 % of the 0.5 g/L of TCS solution was coated onto the Chromosorb G powder. The high percentage of coating of TCS onto Chromosorb G adsorbent meant that amount of TCS was sufficient to saturate the bile acid solutions with TCS.

Based on the CAS number of TCS (4.621 mg/L) as previously mentioned, both sodium lithocholate and sodium deoxycholate improved the aqueous solubility, by up to 3.1 fold for

sodium deoxycholate and 7.7 fold for sodium lithocholate. Due to extensive use of personal care products (Ricart et al., 2010), the compound has been detected in domestic effluent that reach WWTP (Chen et al., 2011). During the wastewater treatment process, TCS partitions onto the sludge or sewage biosolids (Bahman and Droste, 2014; Wu et al., 2009).

After the study confirmed that the use of bile acids increase the aqueous solubility of TCS, this therefore meant that their presence in sewage sludge may solubilize TCS (Mulligan, 2005). As a result, this may determine whether the compound is retained in the sewage sludge during wastewater treatment process or is solubilized by these surfactants and therefore end up in receiving waters where it may give rise to human and environmental health implications (DeLorenzo et al., 2008; Durán-Álvarez et al., 2015). It should be noted that the presence of deoxycholic acid and lithocholic acid in wastewater treatment plants are potential indicators of wastewater contamination and furthermore these bile acids are not biodegradable (Scott and Jones, 2000) and they may be present in sludge biosolids. Use in agriculture or the use suggested for the Belmont and Tiaret sludge in chapter 2 might of sewage sludge that contains TCS and surfactants (Mulligan, 2005; Thompson et al., 2005), may enhance TCS solubility. This could result in the compound being absorbed by plants grown in amended soils. Entering the food chain may result in human and environmental complications such as effects on reproductive health in humans (Dinwiddie et al., 2014) and affecting microbial communities in the environment (Tatarazako et al., 2004) and this will be demonstrated by plant growth studies in Chapter 5.

In as much as lithocholic acid and deoxycholic acid increase the aqueous solubility of TCS (as shown in our study), further investigation on various microorganisms such as bacteria which might produce biosurfactants (Noha et al., 2004) must be done so as to fully gain an understanding of all forms of surfactants that are present in sludge biosolids. Because of the increase in aqueous solubility of TCS by lithocholic acid and deoxycholic acid, these compounds were in the extraction of TCS from sewage sludge as this will be investigated in the next chapter.

Figures 3.2-3.9 show dissolution curves of TCS obtained from solubility studies. The curves indicate studies using Chromosorb G and Silica gel powder at 12-15 and 37 ± 0.5 ºC. These curves have been discussed above and will be further elucidated in detail below.

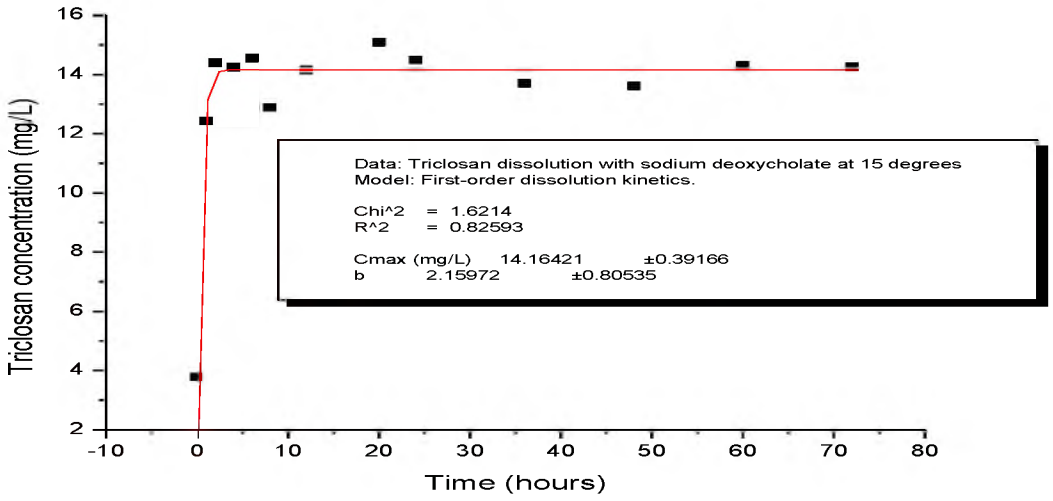


Figure 3.2: *Dissolution of TCS from chromosorb G in sodium deoxycholate at 12-15 ºC*

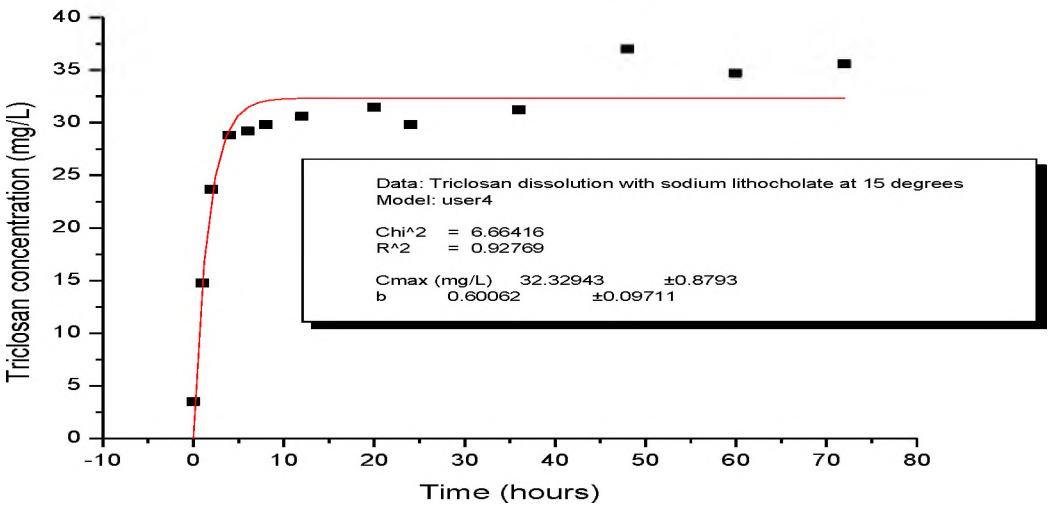


Figure 3.3: *Dissolution of TCS from chromosorb G in sodium lithocholate at 12-15 ºC*

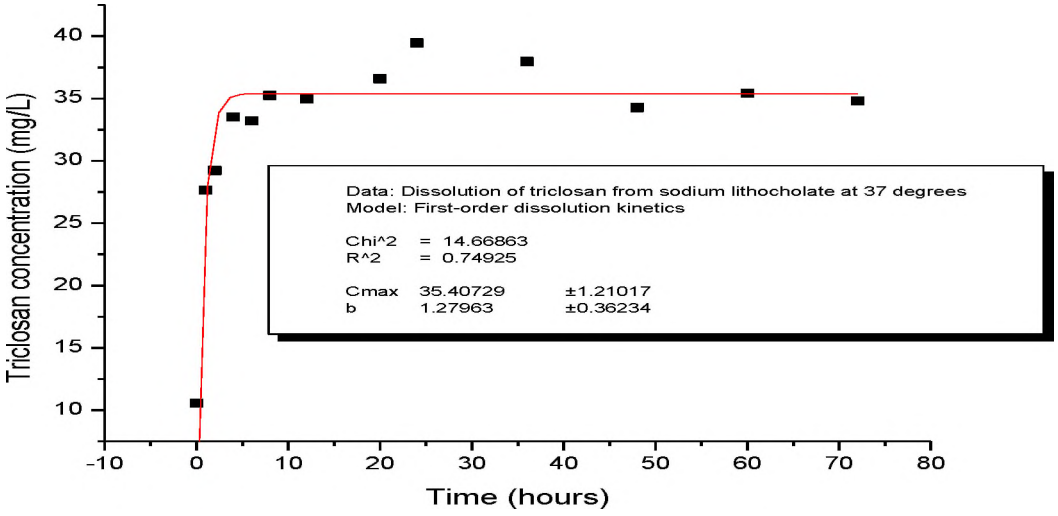
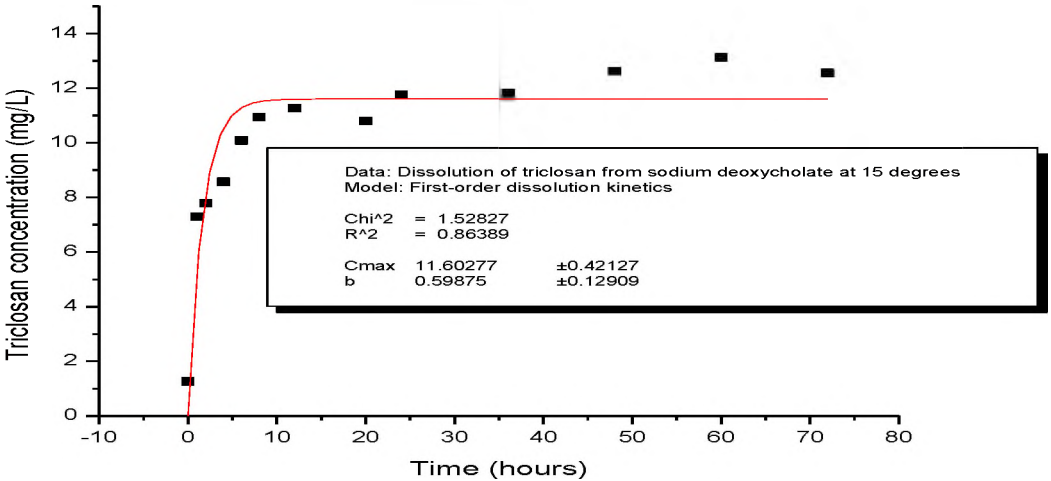


Figure 3.4: *Dissolution of TCS from chromosorb G in sodium lithocholate at 37 ºC*



4

5 ***Figure 3.6**: Dissolution of TCS from silica gel in sodium deoxycholate at 12-15 ℃*

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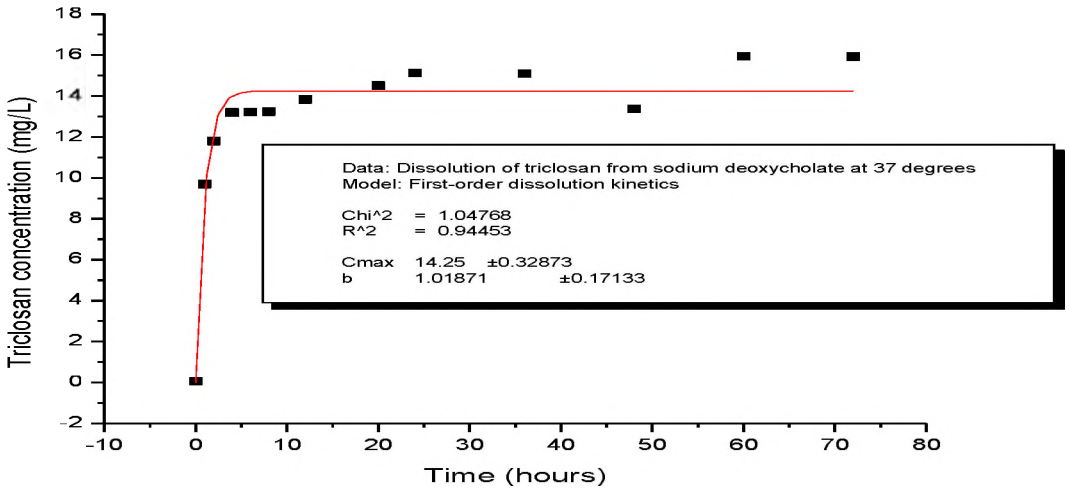

Figure 3.5: *Dissolution of TCS from chromosorb G in sodium deoxycholate at 37 ℃*

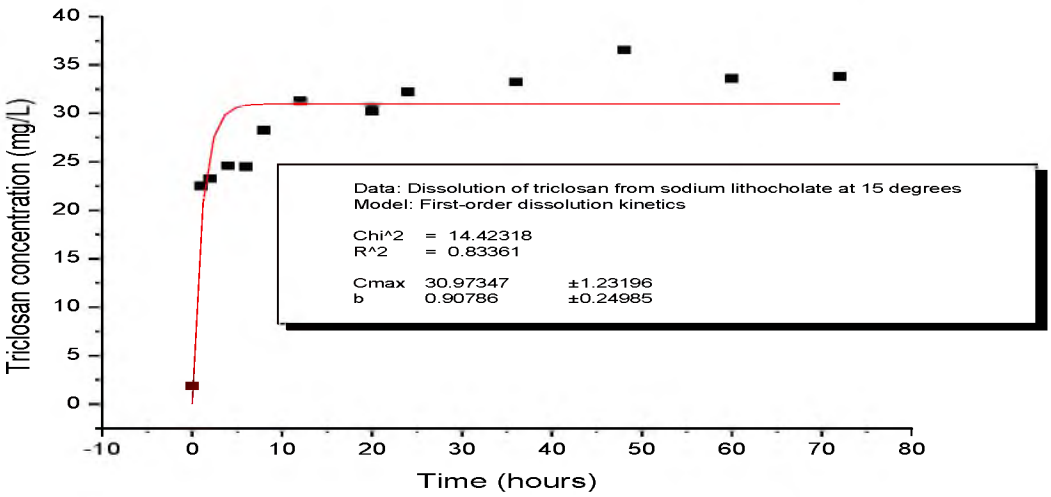

Figure 3.7: *Dissolution of TCS from silica gel in sodium lithocholate at 12-15 ℃*

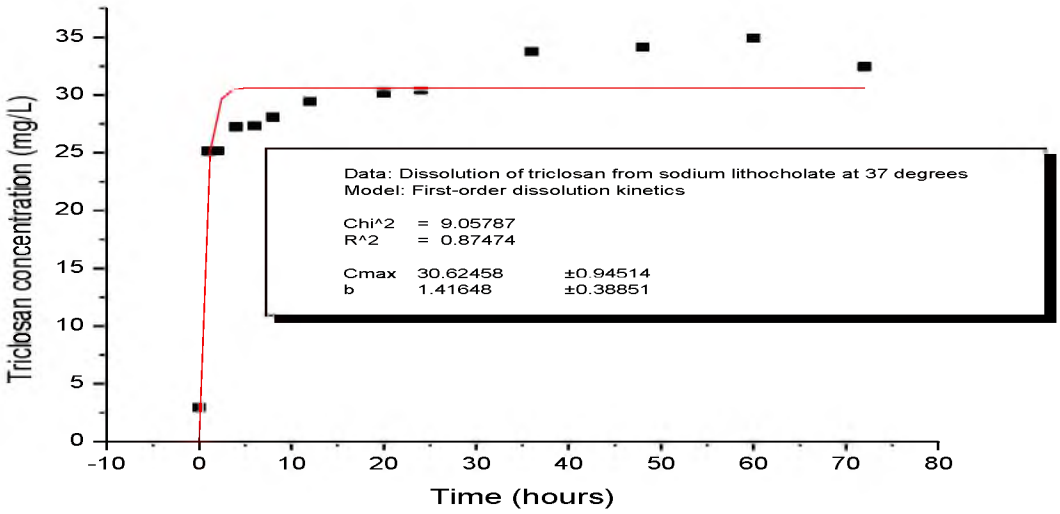

Figure 3.8: *Dissolution of TCS from silica gel in sodium lithocholate at 37 ℃*

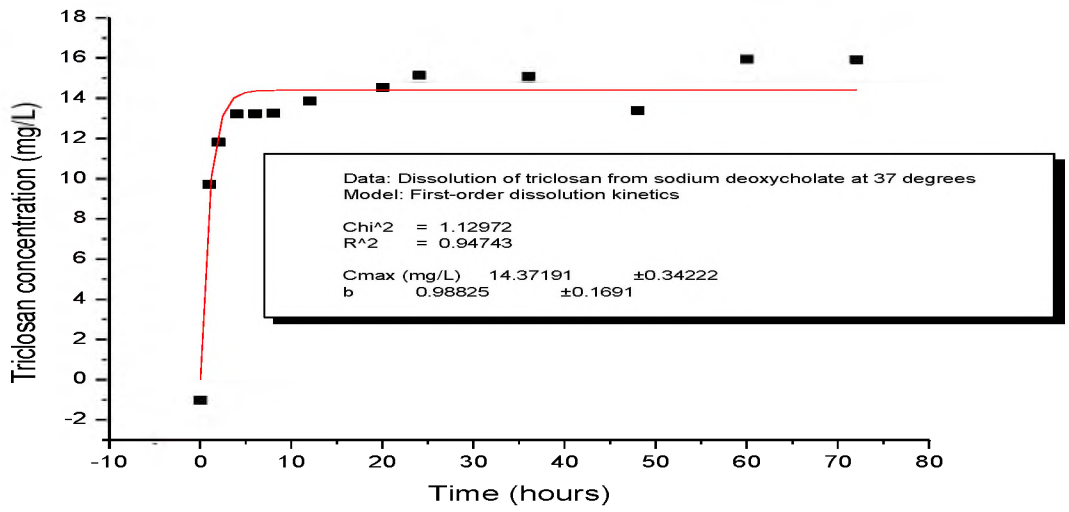

Figure 3.9: *Dissolution of TCS from silica gel in sodium deoxycholate at 37 ℃*

Table 3.1: *Data analysis for lithocholate solubility studies*

	Adsorbent used	C_{max} (mg/L)	T_{max} (hours)	Rate constant (hr^{-})	R^2
15 ℃	Si-gel	31.0 ± 1.23	13.5	0.91 ±0.25	0.8336
	ChG	32.3 ± 0.88	13	0.60 ± 0.09	0.9277
37 ℃	Si-gel	30.6 ± 0.95	13	1.42 ± 0.39	0.8747
	ChG	35.4 ± 1.21	13	1.28 ± 0.36	0.7493

Table 3.2: *Data analysis for deoxycholate solubility studies*

	Adsorbent Used	C_{max} (mg/L)	T_{max} (hours)	Rate constant (hr^-)	R^2
15 ºC	Si-gel	11.6 ± 0.42	17	0.60 ± 0.13	0.8639
	ChG	14.16 ± 0.39	17	2.16 ± 0.80	0.8259
37 ºC	Si-gel	14.37 ± 0.34	17.5	0.99 ± 0.17	0.9474
	ChG	14.25 ± 0.33	18	1.02 ± 0.17	0.9445

From table 3.1, it can be seen that sodium lithocholate significantly increased the aqueous solubility of TCS more than sodium deoxycholate. The time it took reach C_{max} for sodium lithocholate system was between 10-14 hours. The rate constants of dissolution for sodium lithocholate system ranged from 0.60-1.42 Hr^-, with the highest C_{max} (35.4 ± 1.21 mg/L) having a rate constant of 1.28 ± 0.36 Hr^-. The high rate constant indicates that Cmax was reached at a rapid and fast rate. The rate constants obtained in this study were not comparable to the values in literature, where Duan et al., 2005 obtained 2.586 ± 0.26 Hr^-. All the experiments in the sodium lithocholate system showed a good regression analysis (R^2) values which ranged between 0.7493 and 0.9277, which thus showed that the first-order dissolution model describes the experimental data well.

For sodium deoxycholate system, it took between 10-18 hours to reach C_{max} as shown in table 3.2. The C_{max} observed with sodium deoxycholate system was significantly (p =0.1293) lower than the C_{max} observed for sodium lithocholate for reasons mentioned above. The R^2 values for the sodium deoxycholate system had a more positive correlation than those obtained for the sodium lithocholate system which implied better relationship between TCS

concentration and time variable in which the experiment occurred. From the solubility studies conducted, due to lithocholic acid and deoxycholic acid ability to increase the aqueous solubility of TCS, these compounds were used to extract TCS from sewage sludge as this will be shown in the next chapter.

3.4 CONCLUSIONS

Our results show that bile acids significantly increase the solubility of TCS. The apparent solubilities and rate constants indicated in brackets of TCS at 37 ºC were 35.4 ± 1.21 mg/L (1.28 ± 0.36 Hr^{-}) and 14.4 ± 0.34 mg/L (0.99 ± 0.17 Hr^{-}) in sodium lithocholate and sodium deoxycholate, respectively. The apparent solubilities and rate constants indicated in brackets of TCS at 15 ºC were 32.3 ± 0.88 mg/L (2.16 ± 0.80 Hr^{-}) and 14.2 ± 0.39 mg/L (1.02 ± 0.17 Hr^{-}) in sodium lithocholate and sodium deoxycholate, respectively. This in turn suggests that bile acids may be considered in the extraction of TCS from sewage sludge, as this will be investigated in the next chapter. As a consequence, bile acids found in sewage sludge may potentially increase aqueous solubility of TCS, and as a result the compound may not be fully removed from wastewater. Further studies need to be conducted on other surfactants that may be present in sewage sludge, so as to understand extent of solubilization from different surfactants.

4 Chapter 4

QUANTIFICATION OF TRICLOSAN FROM SEWAGE SLUDGE MATRICES

4.1 INTRODUCTION

Due to widespread and extensive use of personal-care products containing Triclosan (TCS), the consumption has risen up to approximately 350 tonnes per annum worldwide(Singer et al., 2002). Due to the broad spectrum antimicrobial activity of TCS,it is used in personal-care products such as toothpastes, shampoos and soaps (Chen et al., 2011; Farré et al., 2008). TCS inhibits bacterial growth by blocking fatty acid biosynthesis (Lim et al., 2012). TCS has been used in the cosmetic industry as a stabilizing agent in many detergents and cosmetics (Chen et al., 2011). As a result of the extensive use of TCS containing products in domestic households, it is washed down the drain after use and thus it has led to elevated concentrations in wastewater, wastewater treatment plants (WWTPs) and receiving waters (Aragón et al., 2008; Chalew and Halden, 2009; Chen et al., 2011).

Several studies have shown that TCS is of one of the most frequently detected organic wastewater contaminants 8.05 ± 0.47 µg/L (Davis et al., 2012), 8.41 ± 0.17 µg/L (Durán-Álvarez et al., 2015) 3.8 ± 1.16 µg/L (Davis et al., 2012a; Durán-Álvarez et al., 2015; Lozano et al., 2013). Attributable to the difficulty in removing TCS in WWTP, it ends up reaching wider water and soil environment through effluent discharge or sewage sludge use

in agriculture (Butler et al., 2012; Thompson et al., 2005). Sewage sludge is used as a soil fertility aid in agricultural soils because it contains nitrogen (N) and phosphorus (P) which are plant nutrients (Yilmaz and Temizgül, 2014), but may contain micropollutants such as TCS (Butler et al., 2012). The presence of TCS in sewage sludge presents a mechanism for the introduction of substantial amounts of the compound into the environment (Heidler and Halden, 2007), and as a consequence it is not only present in the environment initially but remains in the environment after the wastewater treatment process.

Wastewater treatment contamination by TCS is a serious health threat. The wastewater treatment process cannot render TCS harmless, and thus regulations identify compound as either harmful or harmless as it is free to reenter the environment and ultimately cause human and environmental health issues(Wallace et al., 2010). Once in the environment, TCS can have detrimental effects on aquatic ecosystems as it is highly toxic to different types of algae and bacteria (Ricart et al., 2010) and thus as a may result in an imbalance in the ecosystem (Chalew and Halden, 2009; Tatarazako et al., 2004). In algal species, TCS induces mitochondrial depolarization and impairment of energy metabolism in animals cells as well as inhibition of sulfotransferases important in phase II detoxification mechanisms(Coogan et al., 2007). The TCS mode of action in bacteria involves the blockage of fatty acidbiosynthesis. The trans-2-enoyl-ACP reductase in *E. coli*, known as FabI, regulates fatty acid synthesis and isinhibited by TCS (Sivaraman et al., 2004). This has led to concern that chronic exposure of natural algal and bacterial total abundance are reduced when exposed to TCS, and thus causing an imbalance in the ecosystem as symbiotic relationships in environmental species are affected (Coogan et al., 2007).

As a human health concern, TCS has shown to bioaccumulate in human bodies and in turn disrupts the endocrine system, thus threatening thyroid function and hasbeen shown to affect puberty, reproductive health and pregnancy (Dinwiddie et al., 2014). In addition, TCS may give rise to bacterial resistance to antibiotic medications and antibacterial cleansers. At low concentration, TCS inhibits enoyl ACP reductase enzyme in *E. coli, P. aeruginosa* and *S. aureus*. A mutation to produce an altered enzyme occurs or overexpression of this gene can produce resistance to this agent or causing efflux of other antimicrobials out of the cell (Fan et al., 2002; Russell, 2003). Exposure to TCS of a TCS-sensitive mutant of *P. aeruginosa* switches on an efflux pump that renders the cell highly resistant to ciprofloxacin (Chuanchen et al., 2001), and furthermore, some mutants selected by TCS have shown to increase resistance to isoniazid (Bannerjee et al., 1994). Such resistance may cause multiple threats, and consequently affecting the treatment of conditions such as Tuberculosis which is very prevalent in South Africa (WHO, 2013)because of isoniazid resistance. Treatment of respiratory tract and urogenital infections becomes a challenge as ciprofloxacin is the first-line treatment of treatment of such conditions (SAMF, 2010).

Guidelines that have set standards for reuse of sewage sludge and wastewater were developed a long time ago, and they did not state the levels of TCS as it was not a concern then. As it has been shown above, now it is an issue as it is extensively used (Singer et al., 2002) and it possess environmental and human health concerns. Several studies have been reported on the fate of TCS in WWTPs (Butler et al., 2012; Lozano et al., 2013; Samaras et al., 2013; Thompson et al., 2005). A study conducted by Ricart et al., (2010), observed that between 0.5 and 500 µg/L of TCS had an effect on biofilm algae and bacteria. The lower no

effect concentration (NEC), which is defined as the concentration at or below which microorganisms are not killed or whereby no effect of the compound is observed, is of 0.2 µg/L for TCS (Ricart et al., 2010). In as much as TCS affects both algal and bacterial communities in the biofilm, toxicity is higher in bacteria than algae. It is unknown if the algae has a specific target sites for TCS, but studies on axenic algae had a EC_{50} value of between 0.7 and 66 µg/L (Capdevielle et al., 2008). Therefore at above 0.2 µg/L of TCS, bacterial and algal communities in the environment are affected, and consequently could cause an imbalance in the ecosystem. It is important to determine the concentration of TCS of sources that may introduce the compound to the environment, so as it will be of great value to prevent toxicity towards environmental species. Few studies have been conducted in the quantification of TCS from sewage sludge. On a study conducted by Smith, (2009), he obtained a concentration of TCS to be 551 ± 61 µg/g d.w, whereas Butler et al., (2012) obtained between 11.22 and 28.22 µg/g d.w and Thompson et al., (2005) observed a concentration of 128-156 µg/g d.w.A gap exists in the quantification of TCS in sewage sludge and moreover no guidelines have been developed in South Africa and Algeria for TCS if sewage sludge is to be used for soil amendment purposes in agriculture. Therefore, the aim of the study was to analyze the sewage sludge from Belmont Valley (South Africa) and Tiaret (Algeria); and determine the concentration of TCS present in the sludge.

4.2 MATERIALS AND METHODS

4.2.1 MATERIALS

Acetone (>97.5%) (Catalogue number: 34860-2.5L-R), Deoxycholic acid (sodium salt) (>97%) (Catalogue number: 264101-25GM), Irgasan (Triclosan) (>98%) (Catalogue number: PHR1338-1G) and all glassware used were purchased from Sigma Aldrich (Johannesburg, South Africa). Whatman filter paper 1 was purchased from MiNEMAa Spellbound laboratory solutions (Port Elizabeth, South Africa). Sodium sulfate anhydrous (>99%) (Batch number: MKOM603561), 32% hydrochloric acid (HCl), n-Hexane (>98%) and TR 300 thermoreactor were purchased from Merck (Pty) Ltd (Johannesburg, South Africa). All masses were measured using a Pioneer™ PA214 analytical balance purchased from Ohaus Corporation, Pine Brook, NJ USA. Mechanical orbital shaker Model number TS-520D was purchased from Already Enterprise Inc.(Taipei, Taiwan). Crison pH meter Model: Magnetic stirrer Model STR-N11 was purchased from FMH Instruments (Johannesburg, South Africa). Rotavapour Model number R-215 was purchased from Büchi labortechnik Inc. (Flawil, Switzerland). Abraxis Triclosan assay kit (PN 530114) was purchased from Abraxis LLC (Warminster, PA, USA). MilliQ water used in this chapter was prepared by reverse osmosis, using a Milli-RO® 15 water purification system Millipore®(Bedford, MA, USA). An Agilentgas chromatography (GC) system with MassHunter software Model 7820A and Mass spectrometry (MS) detection Model 5977E

MSD, Automatic sampler injector Model G4513A ICES-001 were purchased from Chemetrix(Johannesburg, South Africa).

4.2.2 METHODS

4.2.2.1 Detection with GC/MS parameters

The samples were analysed using an Agilent7820A GC interfaced to 5976 MSD mass spectrometric detector, equipped with system with an AgilentG4513A ICES-001 automatic liquid sampler injector. The instrument was equipped with a HP-5MS GC column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) for chromatographic separation with helium (purity>99.999 %) as the carrier gas at a constant flow rate of 1.5mL/min. Injector temperature was 300 ºC. The GC oven temperature was programmed from 100 ºC (held for 3 minutes), then raised to 200 ºC at 20 ºC/min and then to 280 ºC at a rate of 5 ºC/min. One microliter (1 μL) sample was injected at splitless mode and the total analysis run time for GC run was 24 minutes (min). The MS parameters were: full scan, solvent delay of 6 min and scan range from 10-550 mass-to-charge ratio (m/z). All the analysis in this chapter were conducted under the above parameters.

4.2.2.2 Quantification of triclosan in sludge using Gas chromatography and Immunological assay kits

4.2.2.2.1 Preparation of 1 g/L sodium deoxycholate

The preparation of 1 g/L solution of sodium deoxycholate was conducted in the same manner described in Chapter 3, section 3.3.2.2.3.

4.2.2.2.2 Preparation of 15 g/L of Triclosan solution

One hundred and fifty milligrams (150 mg) of TCSwas weighed using Pioneer™ PA214 analytical balance. The weighed powder was transferredinto a 10 mL volumetric flask and afterwards, 5 mL n-hexanewas added into the volumetric flask. The flask was hand shaken vigorously to ensure TCS powder dissolves, and after the powder dissolved, n-hexane was added to make up to the volume and a glass stopper was used to seal the flask. The flask was wrapped using aluminum foil and then stored in the fridge at a temperature of 4°C until use.

4.2.2.2.3 Extraction of Triclosan from sewage sludge

Five grams(5 g) of sewage sludge from Belmont Valley and Tiaret was separately weighed using Pioneer™ PA2102 balance and the weighed sludge was transferred intosix separate 250 mL Erlenmeyer flasks. Using a 100 mL graduated cylinder, 100 mL of 1 g/L sodium deoxycholate was transferred into each 250 mL Erlenmeyer flask, and thereafter the each Erlenmeyer flask was covered with aluminum foil and sealed with Parafilm™. The Erlenmeyer flasks were placed on a Mechanical orbital shaker and shaken for 48 h at 150 rpm and temperature of 20 °C. The pH of sodium deoxycholate solution was 8.6, and it would mimic the pH of the water in the Eastern Cape of South Africa. Based on the previous

chapter on solubility studies, the sodium deoxycholate was used to extract TCS from sewage sludge as it showed to increase aqueous solubility of TCS by a 3.7 fold.

After 48 h, the Mechanical orbital shaker was stopped, and the samples were left to set for 30 minutes. After 30 min, the supernatant of each sample was decanted into separate 250 mL separation funnels. Into each funnel, 20 mL of a mixture of n-hexane/acetone (v/v) (9:1) was transferred using a graduated 50 mL measuring cylinder and thereafter 0.7 mL of 5 M HCl was pipetted into each separating funnel with sodium deoxycholate extract. The separating funnel was shaken relieving pressure at 10 second intervals for 2 min.

After shaking the separating funnel, a resultant of two phases appeared which consisted of the aqueous layer and the lipophilic (oily) layer as shown in figure 4.1. Since the pH of each sample was reduced to be below 5, TCS was expected to partition into the lipophilic layer because it was highly unionized at pH 5 and thus the unionized form of the compound will partition towards the oily phase (Hyland et al., 2012). The aqueous phase was extracted three times, and the oily phase (extracts) were collected into separate 100 mL Erlenmeyer flasks. One thousand five hundred milligrams (1.5 g) of sodium sulphate (anhydrous) was weighed using a Pioneer™ PA2102 balance, and thereafter transferred into a funnel lined with Whatman filter paper 1. The oily phase was passed through a funnel lined with Whatman filter paper 1 containing 1.5 g sodium sulphate (anhydrous). The filtrate was collected in a 250 mL round bottom flask. Sodium sulphate in the funnel was rinsed with 5 mL of acetone into the round bottom flask containing the filtrate. Using a Rotavap, the solvent in the round

bottom flask was evaporated up until approximately 10 mL of the extract was left in the round bottom flask. By means of a Pasteur pipette, the remaining 10 mL extract was used to rinse the walls of the round bottom flask, and afterwards the extract was transferred into a 10 mL volumetric flask and covered with aluminum foil. The samples were then screened for TCS using immunological method described in section 4.2.2.2.4, and using GC/MS using the methods and parameters mentioned in section 4.2.2.1. For each sludge sample, the sample was analysed in triplicates. The use of tributylphosphate (TPB) as an internal standard was approached, but the method failed and thus TCS calibration curve was used as an external standard for quantification purposes.

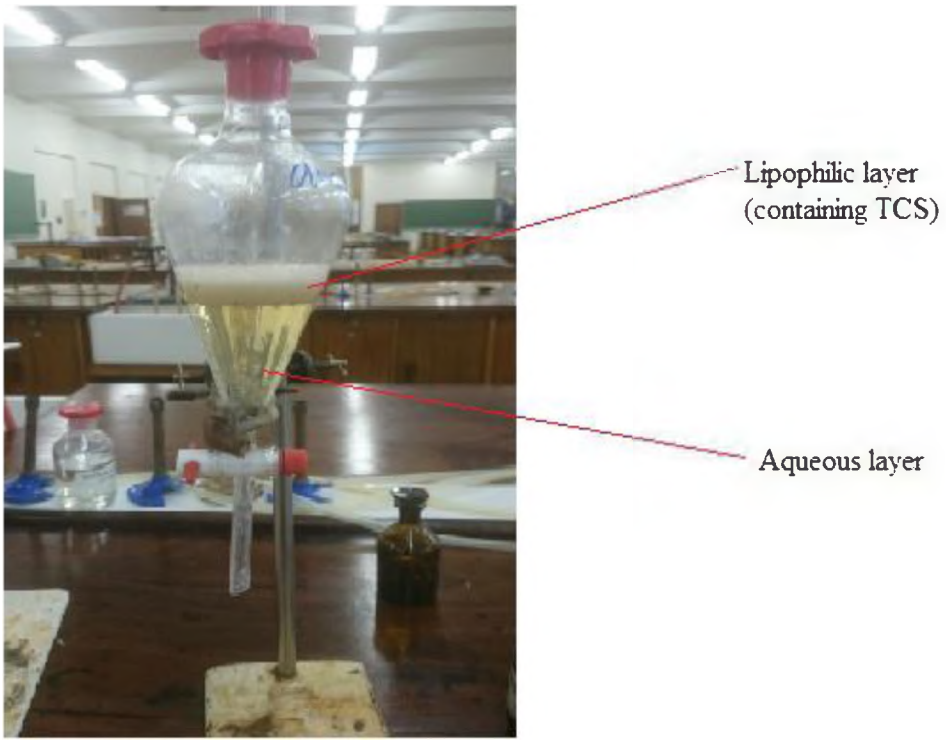

Figure 4.1: *Extraction of TCS from sodium deoxycholate sludge extract*

4.2.2.2.4 Analysis of sludge samples using Triclosan plate assay

Using a pipette, 50 µL of each sludge extract (duplicates) was pipetted into microtiter plate coated with Goat-Anti Rabbit Antibody. Afterwards, 50 µL of TCS antibody solution was in turn added to each well with the sample. The wells with the samples were covered with Parafilm™ and then the contents of the wells were mixed by moving the strip holder in a gentle horizontal and circular motion on a benchtop for 30 seconds. The well plate with the contents was incubated using Labcon low temperature incubator LTIE 10 at 20 ± 0.5 ºC for 30 min. After incubation, 50 µL of TCS enzyme conjugate solution was pipetted successively into 96 well plate containing the samples and afterwards covered withParafilm™. The contents of the well were mixed by moving strip holder in a gentle horizontal and circular motion on the benchtop for 30 seconds. The samples in the well were incubated using Labcon low temperature incubator for further 30 min at 20 ± 0.5 ºC. After the 30 min had lapsed, Parafilm™ was removed and the contents were shaken into a waste container. The strips were washed with diluted Wash buffer by the addition of 250 µL of wash buffer to each well (wash step was repeated three times). The remaining buffer in the wells was removed by tapping the plate on a dry stack of paper towels. By the use of a pipette, 100 µL of Colour solution was pipetted into each washed well and the contents were covered with Parafilm™. Similarly to the above, the contents of the wells were mixed by moving the well in a gentle horizontal and circular motion for 30 seconds. Thereafter the samples were incubated at 20 ºC in an oven for 20 min. After 20 min had lapsed, 50 µL of stopping solution was successively added into each well. The absorbance of each sample well was measured using a Power Wave at 450 nm wavelength and the absorbance was

recorded. This method is applicable to Abraxis Triclosan Assay kit, 96T PN530114. The concentration of TCS (ng/g d.w) in each sewage sludge sample was obtained using the following equation (4.1) below:

$$C(ng\ TCS\ per\ 1\ g\ dry\ weight) = \frac{10\ mL \times Conc.\ from\ \ calib\ .curve}{wet\ \ mass\ \ of\ \ sludge\ \ \times dry\ \ weig\ ht} (4.1)$$

A calibration between 0.05 and 2.5 parts per billion (ppb) of TCS was constructed using the standards that came with the Triclosan plate assay kit. The concentrations of the calibration curve were 0.05, 0.1, 0.25, 0.5, 1.0 and 2.5 ppb. The TCS standard solutions were analysed in the same manner as the samples above, and a calibration curve of absorbance (B/B_0) against concentration was constructed (figure 4.2), where B_0 was the absorbance of 0 ppb TCS solution.

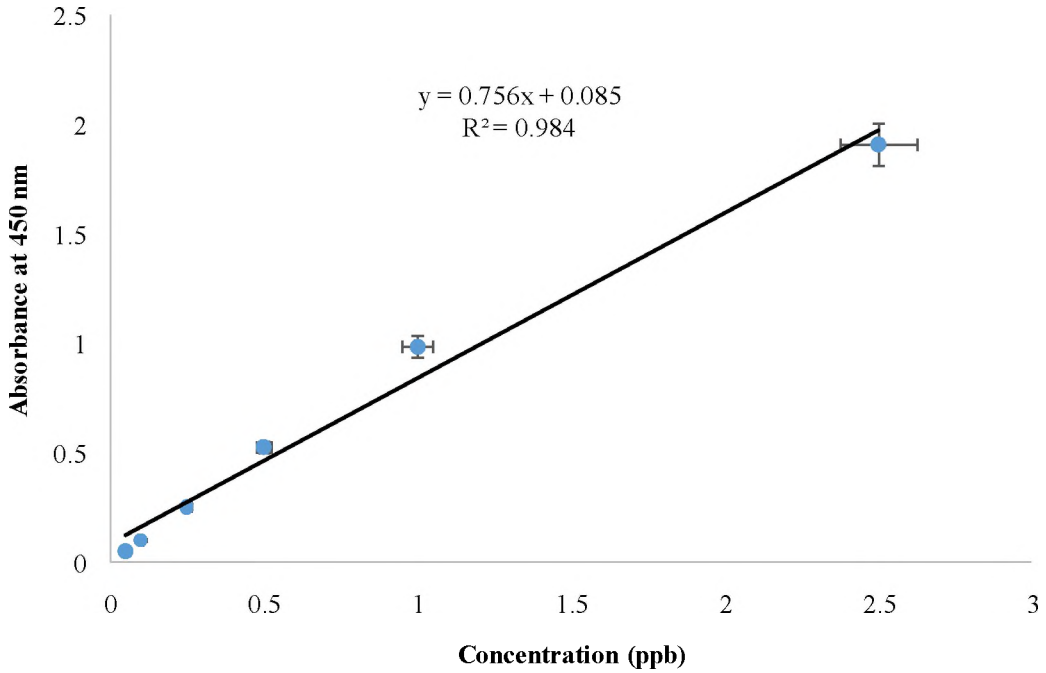

Figure 4.2: *Calibration curve for TCS (n=3) at a range of 0.05-2.5 ppb at 450 nm signal*

4.2.2.2.5 Calibration curve of TCS using GC/MS

To construct a calibration curve, parameters described in section 4.2.2.1 were used. A calibration between 10 and 100 mg/L of in n-hexane was constructed. The concentrations of the calibration curve were 10, 20, 50 and 100 mg/L, and three solutions of each calibration curve concentration were prepared in n-hexane. The samples were analysed using methods specified in Guidance for the Validation of Analytical Methodology and Calibration of Equipment used for Testing of Illicit Drugs in Seized Materials and Biological Specimens(UNODC, 2009). A calibration curve of peak area against concentration was constructed (figure 4.2). The retention time of TCS obtained was 12.76 ± 0.013 min as shown in figure 4.5. A power calibration curve was constructed using Microsoft excel software package, and regression analysis value (R^2) of 0.9873 was obtained. After the calibration curve was constructed, interday variability and intraday variability was calculated and expressed as a percentage (%). For interday variability, it was determined for 50 mg/L TCS solution which was analysed within three days of preparation, on the other hand, intraday variability was determined for 50 mg/L which was prepared and analysed during an 8 h period. The percentage of TCS detected was 99 % (figure 4.4), and this was obtained from 50 mg/L solution. The limit of detection (LOD) was determined to be 11 mg/L. The accuracy of the method used to construct calibration curve was 122 ± 11.6 %. The precision of the method was determined on seven 50 mg/L solution of TCS, and the precision of the method was 111 ± 8.90 %. To ensure the method was reproducible, the calibration solution were repeatedly analysed over a 30 min intervals for three times. The concentration of TCS

in sludge samples was determined by paralleling the results onto the calibration curve, and thereafter calculated using equation (4.2) below:

$$TCS\ conc.\ in\ sludge\ \left(\frac{\mu g}{g}dw\right) = \frac{10\ mL \times sample\quad conc\ .(from\ \ cal\ .curve\ )}{extraction\quad efficiency\quad \times 5\ g \times dry\ \ weig\ ht} \qquad (4.2)$$

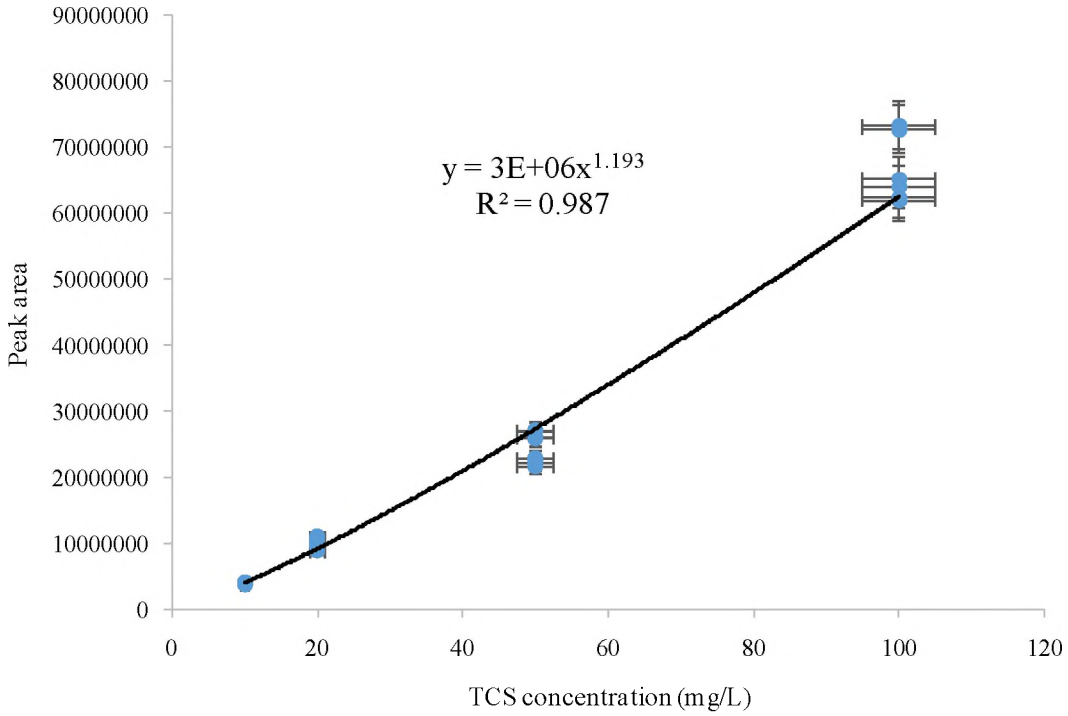

Figure 4.3: *Calibration of TCS (n=3) at a range of 10-100 mg/L from GC/MS*

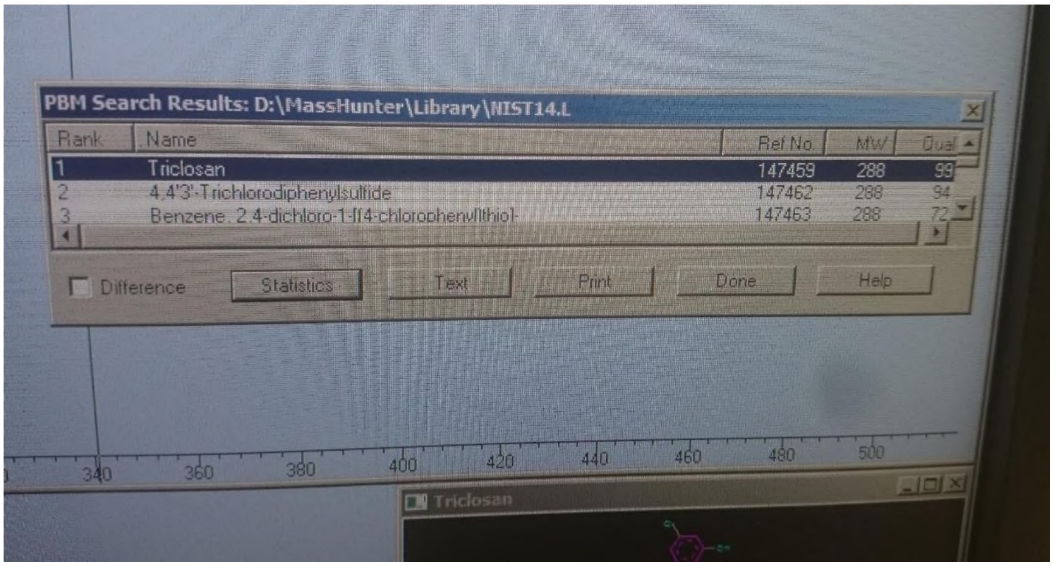

Figure 4.4: *Percentage of TCS from 50 mg/L solution from GC/MS analysis*

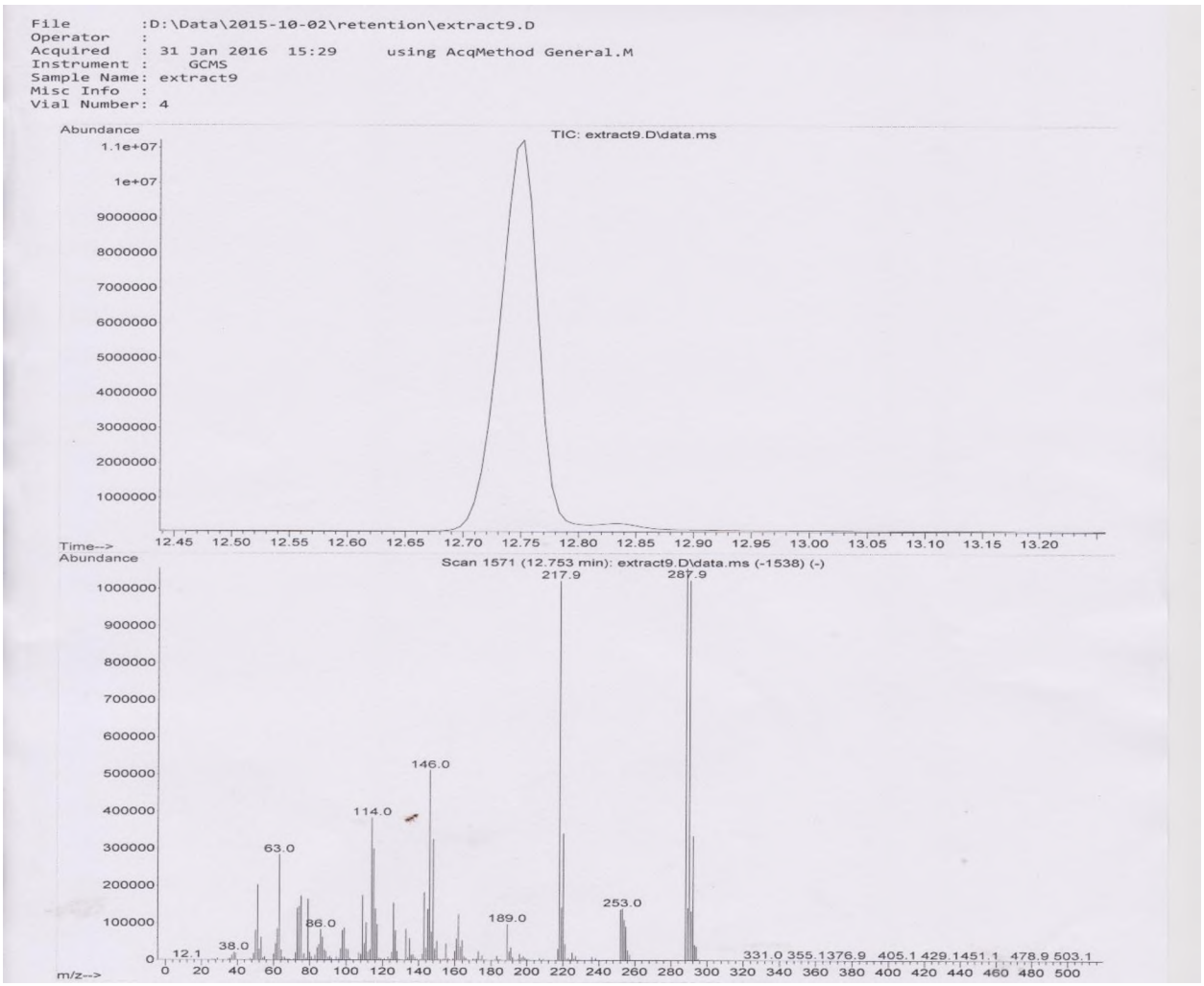

Figure 4.5: *Chromatogram showing retention time of TCS from GC/MS analysis*

The retention time of TCS obtained from the method used as described in section 4.2.2.1, was 12.76 ± 0.013 min. A power function calibration curve was obtained, with a regression analysis value (R^2) of 0.9873. From this calibration curve, intraday and interday variabilities were calculated for the 50 mg/L TCS solution and the values obtained were 9.61 % and 10.44 %, respectively. The higher interday variability might have been a result of degradation of TCS (Chen et al., 2011) within the three days in which the 50 mg/L solution

was analysed, thus consequently affecting the TCS concentration in solution. Accuracy and precision of the method were determined to be 122 ± 11.6 % and 111 ± 8.90 %, respectively.

4.2.2.2.4 Extraction efficiencies

4.2.2.2.4.1 Preparation of synthetic faeces

To determine extraction efficiencies of TCS, extraction efficiencies based on TCS simulated in synthetic faeces which simulate human faeces and mimic the true water retention properties of human faeces, chemical composition and consistency of human faeces (Wignarajah et al., 2006). The only difference was that the *E.coli* bacteria were replaced with coarse sand. Table 4.1 shows the masses of each component were weighed using Pioneer™ PA2102 balance, and mixed in a 250 mL beaker.

Table 4.1: *Showing components used in the preparation of synthetic faeces.*

Component	% weig	Working formula (
Loom sand	30	4.50
Cellulose acetate phthalate	15	2.25
Polyethylene glycol (PEG) 60(	20	3.00
Psyllium	5	0.75
Peanut oil	20	3.00
Miso	5	0.75
Calcium carbonate	5	0.75
Dried vegetables	50 mg	0.05 mg

4.2.2.2.4.2 Determination of dry weight of synthetic faeces

The dry mass of synthetic faeces was determined using the method described in Chapter 2 (section 2.1.1.3). The dry mass of synthetic faeces was calculated using the equation (4.3) below (Margesin and Schinner, 2005):

$$W_s = \frac{M_2 - M_0}{M_1 - M_0} \quad (4.3)$$

4.2.2.2.4.3 Quantification of Nitrates, Ammonium and Phosphates in synthetic faeces

The nitrate, ammonium and phosphates were determined using the methods described in chapter 2 sections 2.1.1.7.2, 2.1.1.7.3 and 2.1.1.7.4, respectively. The concentrations of the inorganic compounds are shown in table 4.2.

4.2.2.2.4.4 Measurement of chemical oxygen demand

Chemical oxygen demand (COD) was measured using the closed-reflux colorimetric method (APHA, 1998) in the concentration ranges from 100 to 2000 and from 500 to 10000 mg/L. The KHP was used as the standard to prepare solutions and the COD values were converted into KHP concentrations (mg KHP eq/L) where eq/L refers to equivalent per liter based on equation 4.4.Digestions were performed according to the manufacturer‘s instructions using the TR 300 thermoreactor. After completion of the digestions, the respective solutions were

cooled and the spectrophotometric measurements were performed using a UV/VIS spectrophotometer.

$$KC_8H_5O_4 + 7.5\ O_2 \longrightarrow KOH + 8CO_2 + 2H_2O \qquad (4.4)$$

For the quantitative analysis of KPH, a calibration curve at 600 nm wavelength, was constructed at the following between 100 and 2000 mg/L with three replicates each measured to construct the calibration curve.This was then plotted as the dependence of the absorbance at 600 nm on the COD concentration in mg KHP eq/L. The calibration curve for the COD measurements is shown in figure 4.6.The concentration of KHP in synthetic faeces was determined using the equation (4.5) below:

$$KHP\left(\frac{mg}{g}of\ dry\ weight\right) = \left(\frac{0.1L \times Conc.\left(\frac{mg}{L}\right)}{wet\ weig\ ht \times dry\ weig\ ht\ (g)}\right) \qquad (4.5)$$

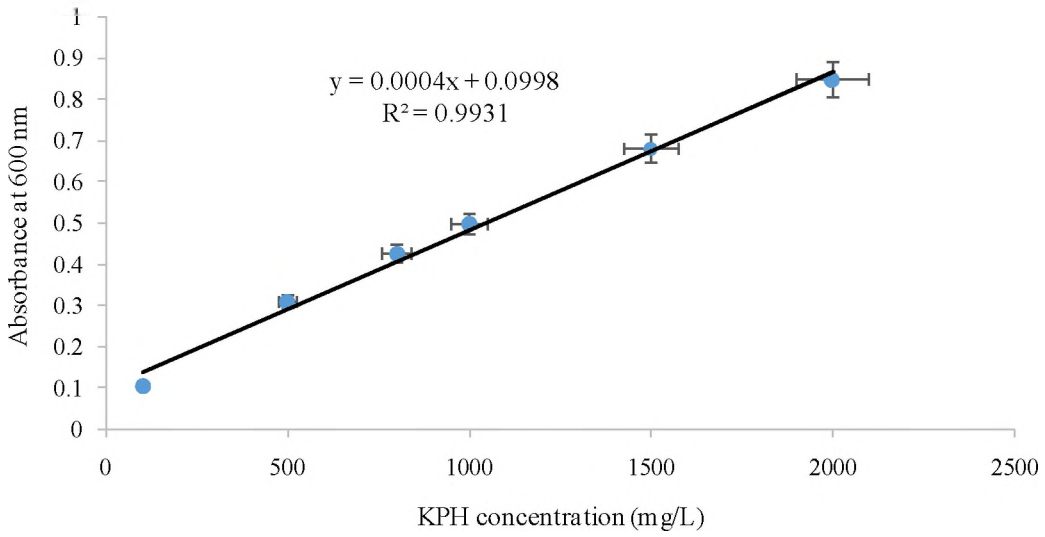

Figure 4.6: *Calibration curve for COD (n=3) at a range of 100-2000 mg/L KPH as a standard solution*

Table 4.2: *Concentration of nitrates, phosphates and ammonium in synthetic faeces*

Compound	Concentration
Nitrate (mg/g d.w)	1.61
Ammonium (mg/g dw)	2.00
Phosphate (mg/g d.w)	3.70
COD (mg KHP/g d.w)	105.4

The chemical composition of synthetic faeces resembled sewage sludge obtained from Belmont Valley and Tiaret (as discussed in Chapter 2) and as well as values obtained in literature which were discussed in Chapter 2. The comparison between synthetic faeces and sewage sludge will be elucidated in detailon the results section.Becauseof similarities in

chemical composition, synthetic faeces were used to determine extraction efficiencies in the next section.

4.2.2.2.4.5 Determination of extraction efficiencies of synthetic faeces

Five grams (5 g) of synthetic faeces were weighed using Pioneer™ PA2102 and thereafter transferred into separate three 250 mL Erlenmeyer flasks. Using a graduated 100 mL measuring cylinder, 100 mL of 1 g/L of sodium deoxycholate was added into each Erlenmeyer flask and subsequently, 20 µL of 15 g/L of TCS solution was pipetted into each Erlenmeyer flask. The three flasks were placed on a Mechanical orbital shaker, and the samples were shook at 150 rpm for 48 h. After 48 h, TCS was extracted from the samples using the method in 4.2.2.4.1, and the amount of TCS in each sample was determined using GC/MS using conditions and parameters mentioned in section 4.2.2.1. The extraction efficiency of TCS from synthetic faeces was calculated using the equation (4.4) below. The extraction efficiency was repeated 9 times and 30 % of the extraction efficiency samples produced outlier results (Dickson test at 5 % level of significance, *p-value* = 0.0001). The most probable reasons for this observation was the retention of deoxycholate in the injector of the GC/MS system. This might have caused problems with extraction efficiency reproducibility. Determination with calibration curves nor the samples results were compromised with this observation as the liner of the GC/MS system was changed once the extraction efficiency experiments were completed.

$$Ext.efficiency(\%) = \frac{Amount\ \ of\ TCS\ extracted\ \ from\ synthetic\ faeces\ \ (\frac{\mu g}{g} dw)}{Theoretical\ \ amount\ \ of\ TCS\ in\ synthetic\ faeces\ \ (\frac{\mu g}{g} dw)} \times 100 \quad (4.4)$$

4.3 RESULTS AND DISCUSSION

4.3.1 QUANTIFICATION OF TRICLOSAN FROM SEWAGE SLUDGE

On quantitative analysis using GC/MS, the parameters were retention time and peak area which were then used to calculate the slope and accuracy of each concentration. A calibration curve was constructed between 10 and 100 mg/L, and from the analysis the retention time of TCS obtained was 12.76 ± 0.013 minutes. A power function calibration curve was obtained, with a regression analysis value (R^2) of 0.9873. From this calibration curve, accuracy, precision, intraday and interday variabilities were calculated for the 50 mg/L TCS solution. The accuracy, precision, intraday and interday variabilities values were 122 ± 11.6 %, 111 ± 8.90 %, 9.61 % and 10.44 % respectively. The higher interday variability might have been a result of degradation of TCS by esterification with deoxycholic acid (Chen et al., 2011) within the three days in which the 50 mg/L solution was analysed, thus consequently affecting the TCS concentration in solution.

TCS was extracted from sewage sludge using sodium deoxycholate. For the experiments conducted in the previous chapter, it was shown that sodium deoxycholate increases the aqueous solubility of TCS by 3.1 fold, thus it was the surfactant of choice for the extraction process. Nonetheless, sodium lithocholate showed to increase the aqueous solubility of TCS by 7.7 fold, but it was not used due to failure in procurement and being delivered in time as results needed to be presented for this thesis. Before the quantification of TCS using GC/MS,

the presence of TCS using Abraxis triclosan assay kits was used to determine the presence of the compound in sewage sludge. TCS was found to be present in sewage sludge, and thus quantitative analysis was thereafter done using GC/MS to determine the exact concentrations of the compound. On conducting analysis to determine TCS concentration in sewage sludge, immunological and GC/MS methods were used. Quantitative results from immunological analysis vary widely from GC/MS methods, therefore the results from immunological elisa are orientational and the GC/MS is the golden standard and these results are the guiding ones(Zajicek et al., 2000). Therefore in this chapter, immunological elisa was used for qualitative analysis by to screening for the presence of TCS in sewage sludge, and GC/MS was used for quantitative analysis to determine the concentration of TCS in sewage sludge from South Africa and Algeria.

The concentration of TCS in sewage sludge obtained from Belmont Valley (South Africa) was 142 ± 33.5 μg/g d.w. The concentration of TCS in sewage sludge obtained from Tiaret (Algeria) was between 0 and 12 μg/g d.w, and this value was low because of stratification of the sample. Upon conducting a t-test statistical analysis, there was significant difference (p = 0.0165) in TCS concentration between Belmont Valley and Tiaret sludge. These values obtained might have been not been a true indication of the exact quantities of the compound in sewage sludge because sodium deoxycholate showed to increase the aqueous solubility of TCS by less than half of sodium lithocholate. Therefore, if sodium lithocholate was used as an extraction medium, the TCS levels would have been expected to be higher than the value observed. From the sampling period to the quantification of TCS, there was a 40 day difference. TCS is known to be biodegradable in the presence of wastewater microorganisms

such as heterotrophic bacteria (Lee et al., 2012), and thus due to long storage periods before analysis, the TCS might have degraded and subsequently the low concentrations of the compound observed. In literature, very few studies have been conducted to determine the concentration of TCS in sewage sludge. On a study conducted by Smith, (2009), a TCS concentration in sewage sludge was 551 ± 61 µg/g d.w, whereas Butler et al., (2012) obtained between 11.22 and 28.22 µg/g d.w. On comparison of the results obtained in this study and in literature, the values results were comparable to the figures in literature. The differences might have been caused by population differences amongst the WWTPs, and thus the greater the population that uses personal care products containing TCS, the higher the concentration in the sewage sludge. In Belmont Valley, the WWTP serves the small fraction of the Grahamstown population and thus, the concentration of TCS in sludge is expected to be lower than sewage sludge obtained from WWTP that service larger populations (Butler et al., 2012).

To determine the effectiveness of the extraction using sodium deoxycholate, synthetic faeces were used to simulate sewage sludge particles. The formulated faeces are designed in a way to represent water-holding capacity, chemical composition and consistency of human faeces (Wignarajah et al., 2006), and thus were used to measure extraction efficiency as they mimic sewage sludge to some extent. From table 4.2, it can be shown that the chemical composition of synthetic faeces was similar to sewage sludge characterized in Chapter 2 (table 2.4). The parameters measured were nitrates, ammonium, phosphates and COD. The data obtained was log-transformed before conducting statistical analysis. On conducting statistical t-test, there was no significant difference between Grahamstown sludge and synthetic faeces with respect

to nitrates (p = 0.3793) and ammonium (p = 0.1185). Statistical analysis indicated significant difference in phosphates concentration (p = 0.00038) between Grahamstown sludge and synthetic faeces. On conducting a statistical t-test on comparing the dry weight between Belmont Valley sewage sludge and synthetic faeces, there was significant difference (p = 0.01723) in the values obtained. A statistical analysis indicated that there was no significance difference (p = 0.78782) in nitrate concentration in sludge obtained from Tiaret and synthetic faeces. On conducting another statistical analysis, it was observed that there was no significant difference (p = 0.07388) in ammonium concentration in sludge obtained from Tiaret and synthetic faeces. And lastly, on comparing the phosphate concentration between synthetic faeces and Tiaret sludge, statistical analysis indicated there was significant difference (p = 0.00052). Therefore, because of the chemical composition which was similar between the sludge and synthetic faeces, the extraction efficiency of TCS was determined in this media. The extraction efficiency of TCS from synthetic faeces was 84.3 ± 12.5 %. The result meant that the method used to extract TCS was fairly efficient as most of the spiked TCS solution was extracted from the synthetic faeces. From the spiked TCS 15 g/L solution, the theoretical concentration of TCS to be obtained was to be 74.2 µg/g d.w, and the results obtained show an average concentration of 62.5 ± 9.93 µg/g d.w. This method shows that more than 84 % of TCS was extracted using sodium deoxycholate, and that the extraction method was very efficient.

4.4 CONCLUSION

In a nutshell, TCS was found to be present in sludge from both countries but the concentrations significantly differed as South African sludge had a higher TCS concentration. It is of great importance to monitor the presence of micropollutants (such as TCS) in sludge if it is to be used for beneficial reuse such as in agriculture. The concentrations of TCS present in sludge may result in the compound accumulating in plants grown in amended soils. It is therefore important to understand the chemical characteristics of compound and other compounds present in sewage sludge that might affect the bioavailability of TCS. The next chapter on plant growth studies will highlight fate of TCS in plants grown in sludge amended soils.

There are no regulations in South Africa and Algeria that state the permissible levels of TCS, it is important to develop guidelines that will prevent accumulation of TCS in plants grown in sludge amended soils. Accumulation in plants consumed by humans, will give rise to health implications such as affecting reproductive health, puberty and breast cancer, therefore it will be important to regulate the TCS levels in sources that might introduce the compound to humans. In addition, the guidelines must ensure that the algal and bacterial communities are not affected by TCS concentrations as toxicity may result in an imbalance in the ecosystem.

5 CHAPTER 5

PLANT GROWTH STUDIES

5.1 INTRODUCTION

Interest in environmental issues are constantly increasing and at the same time, environmental issues have gradually been broadened with concepts, such as sustainable development, which denotes not only ecological, but also economic and social responsibilities(Fytili and Zabaniotou, 2008). The handling of sewage sludge is one of the most significant challenges in wastewater management(Alvarenga et al., 2015; Fytili and Zabaniotou, 2008).Nonetheless, the reuse of sewage sludge for agricultural purposes or as a soil fertilizer faces both social and technical obstacles(Caldeira et al., 2014; Chata et al., 2002; Fytili and Zabaniotou, 2008). Technical hindrances arise because sludge is continuously produced throughout the whole year whilst its application is only needed once or twice, and as a result the sludge has to be stored(European Commision, 2002; Herselman et al., 2005; Herselman and du Preez, 2000).

The generation of sewage sludge is increasing globally due to the increase in urbanization and industrialization (Tiruneh et al., 2014). The current disposal methods of sewage sludge include land filling, incineration, stockpiling and in some countries reuse in agriculture

(Alvarenga et al., 2015; European Commision, 2002; DWAF, 1998; Snyman and Herselman, 2006). Therefore the only viable option in some places is the utilization in agriculture as a soil fertility aid and for amendment purposes (Özyazıcı, 2013). Sewage sludge has been found to contain nitrogen (N), resulting especially from nitrification–denitrification phases in wastewater treatment process(Tchobanoglous et al., 1991). From Chapter 2 on the characterisation of sewage sludge, it was noted that sewage sludge had a high concentration of inorganic N and inorganic phosphorus (P). The concentration of nitrates, ammonium and phosphates were 57.61 ± 55.20 mg/g, 6.60 ± 2.36 mg/g and 1.40 ± 0.30 mg/g, respectively, thus the sludge was useful as a soil fertility aid, as these elements are vital for plant growth (NCSU, 2013). Nonetheless, sewage sludge may contain elements or compounds which give rise to human and environmental health issues, such as the presence of heavy metals, pathogens (Tiruneh et al., 2014) and organic micropollutants such as TCS (Butler et al., 2012). The concentration of TCS in Belmont Valley sewage sludge used in this chapter was determined to be 142 ± 33.5 μg/g d.w from the previous chapter, and thus the presence of the compound gives rise to human and environmental health concerns (Butler et al., 2012) and will be discussed in the next section. Nonetheless, the presence of heavy metals should not be ignored, and thus it is important to determine optimum sewage concentration in the growth of plants so as to prevent phytotoxicity (Tiruneh et al., 2014).

Therefore the recovery and valorisation of the sludge from wastewater treatment plants can be of great economic and recycling value. This is of particular interest in an area where agriculture constitutes a large part of the economic activity such as the Eastern Cape Province of South Africa such as the Makana Municipality in the Eastern Cape Province of

South Africa. This together with the structure and chemical identity of the organic components of sludge biosolids will have a strong influence on the bioavailability of nutrients from sewage sludge.

5.1.1 TRICLOSAN

Triclosan (TCS) is a widely used antimicrobial agent in pharmaceuticals and personal care products (Lei et al., 2013), has attracted worldwide attention due to its frequent detection in domestic effluent into sewage plants (Davis et al., 2012b). During wastewater treatment process, the TCS partitions onto biosolids (Bahman and Droste, 2014; Pannu et al., 2012) resulting in parts per billion concentrations in sludge (Butler et al., 2012; Davis et al., 2012b). The beneficial reuse of sewage sludge for agricultural purposes introduces TCS into the amended soil and consequently the compound may accumulate in plants grown in these soils (Kumar et al., 2009).

Chemical toxicity and accumulation is dependent upon the type of chemical and plant species. A study conducted by Kumar et al., (2009), showed that if the uptake of any particular chemical is to be studied in plants, the compound should have molecular weight (M_r)< 450, log K_{ow} < 3, number of hydrogen donors< 3and number of hydrogen bond acceptors < 6. TCS has a pK_a of 7.9, log K_{ow} of 4.76 and M_r of 288 g/mol (Halden and Paull, 2005). TCS meets all the requirements except for log K_{ow}, nonetheless the risk of potential

TCS accumulation in plants grown in sludge amended soils is high (Pannu et al., 2012; USEPA, 2009).

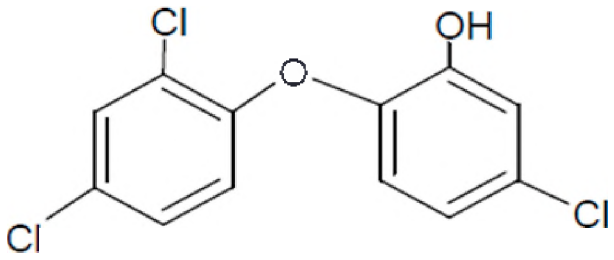

Figure 5.1: *Structure of TCS drawn using ACD/Chem sketch (Andrade et al., 2015).*

Only a few studies have been done on the accumulation of TCS in plants grown on sludge amended soils. A study conducted by Xia et al., (2010), they showed the presence of 0.0065 mg/kg of TCS in corn, Pannu et al., (2012) observed TCS a concentration of 0.09 ± 0.05 mg/kg in radish plants. Furthermore, other studies have shown that toxicity and plant accumulation of TCS varies with plant species and characteristics of soil (Duarte-Davidson and Jones, 1996; Suter, 2007). On a study conducted by Wu et al., (2010), soybeans were grown for 110 days on sludge amended soil at pH of 5.1 and organic content of 16 g/kg, and the TCS was found the whole plant was 0.012 ± 0.002 mg/kg, thus this study showed the potential for TCS to accumulate in plants when sludge solids are used in agriculture.

In this chapter, the aim of the study was the valorisation of sewage sludge as a fertility aid through plant growth studies and the quantification of TCS in plants grown in sludge amended soils, with radish and garden cress being used as the plants. In this study, TCS

accumulation was assessed for the whole plant (grown at different sludge concentrations), by extraction with bile acids based on the increase in aqueous solubility of TCS which was shown in the previous chapter. Radish (*Raphanus sativus)* is a tuber forming plans widely used as a vegetable and is an essential source of vitamin A and C (Sun et al., 2015); proteins and carbohydrates(Jilani et al., 2010). Garden cress (*Lepidium sativum*) is a vegetable mainly used in salads. High nutrient value of the plant is in its seeds, with leaves and roots also showing the presence of vitamin A and D (Ali, 2013; Januskaitiene, 2008). The hypothesis was that TCS can bioaccumulate in plants and the objective was to evaluate the extent of plant uptake of TCS in sludge amended soils by growing radish and garden cress.

5.2 METHOD AND MATERIALS

5.2.1 MATERIALS

Potting soil, planting pots, garden cress seeds and radish seeds were purchased from Buco (Pty) Ltd(Grahamstown, South Africa). Sludge was obtained from the sludge beds in Belmont Valley, Grahamstown, South Africa. All masses were measured using a Pioneer™PA2102 balance (with 0.01 accuracy) and a Pioneer™PA214 balance (with 0.0001 accuracy) purchased from Ohaus Corporation, Pine Brook, NJ USA. All glassware was purchased from Sigma Aldrich (Johannesburg, South Africa).Potassium nitrate (>98 %) (Product number: 1156301), Potassium orthophosphate (>98.5 %) (Product number: 1058962) and Ammonium chloride (min 99 %) (Product number: 1034296) were purchased from Merck (Pty) Ltd(Johannesburg, South Africa). A Power Wave EN STD 61010-1 was

purchased from Bio-Tek Instruments, Inc., Winooski, VT, USA. An Abraxis Triclosan Assay kit PN 530114 was purchased from Abraxis LLC(Warminster, PA, USA). Dry weights of the plants were determined using UFE 700 Oven purchased from Memmert, Schwabach, Germany. Orbital shaking was done using Lasec mechanical shaker Model number TS-520D purchased from Already Enterprise Inc.(Taipei, Taiwan). Labcon low temperature incubator Model LTIE 10 was purchased from Labmark (Johannesburg, South Africa).

5.2.2 METHODS

5.1.1.1 Loss on ignition (LOI)

The LOI and dry mass of sewage sludge and potting soil were determined using the method described in Chapter 2 (section 2.1.1.3). The dry weight of sludge and potting soilwere calculated using the equation (6.1) below (Margesin and Schinner, 2005):

$$W_s = \frac{M_2 - M_0}{M_1 - M_0} \quad (6.1)$$

LOI was calculated using the following equation (6.2) below:

$$\Delta m\,(g) = M_s - M_c \quad (6.2)$$

Percentage LOI can be calculated using the following equation (6.3) below:

$$LOI\ (\%) = \frac{\Delta m\ (g)}{M_s\ (g)} \times 100 \qquad (6.3)$$

Where $\Delta m\ (g)$ loss of mass is after ignition, M_s is potting soil dry weight at 105 °C and M_c is mass of sludge after ignition at 550 °C.

5.1.1.2 pH measurements

5.2.2.1.1 Sample preparation

The pH of the potting soil and sludge samples was measured in 0.01 M $CaCl_2$. Each sample was weighed into a urine jar (40 mL) using a Pioneer™ PA214 analytical balance and subsequently mixed with 0.01 M $CaCl_2$ solution in the following ratio of 1:3 [sludge: 0.01 M $CaCl_2$].

5.2.2.1.2 Measurement of pH

The samples were shaken vigorously at 20 °C, and after shaking the suspension was allowed to stand for 5 minutes and subsequently the pH of each sample was measured using Crison pH meter.

5.1.1.3 Quantification of plant nutrients in potting soil and sludge

5.2.2.1.3 Sample preparation

One gram (1 g) of potting soil and sewage sludge were separately weighed using the Pioneer™ PA2102 balance into separate 50 mL Erlenmeyer flasks, subsequently 20 mL of MilliQ water was added to the Erlenmeyer flasks using a graduated 25 mL measuring cylinder. The Erlenmeyer flasks were placed in Mechanical orbital shaker and shaken at 150 rotations per minute (rpm) for 1 h. After orbital shaking, the suspension was filtered and the filtrate was analyzed for nitrate-N (NO_3^--N), phosphates (PO_4^{3-}-P) and ammonium-N (NH_4^+-N) using the Nitrate, Phosphate and Ammonium test kits.

5.2.2.1.4 Nitrate test (US EPA method 353.2)

To determine the nitrate ions present in potting soil, the nitrate test conducted in Chapter 2 (section 2.1.1.7.2) was used to quantify nitrates present in potting soil. Thereafter, concentration of nitrate-N in potting soil and sewage sludge was determined using the equation (6.5) below:

$$nitrate - N\left(\frac{mg}{g} of\ dry\ weight\right) = \left(\frac{0.05L \times Conc.\left(\frac{mg}{L}\right)}{wet\ weig\ ht\ (g) \times dry\ weig\ ht}\right) \quad (6.5)$$

5.2.2.1.5 Phosphates (US EPA method 365.2)

To determine the phosphate ion concentration present in potting soil, the phosphate (US EPA method 365.2) conducted in Chapter 2 (section 2.1.1.7.3)was used. Thereafter, concentration of phosphate-P in potting soil and sewage sludge was determined using the equation (6.7) below:

$$phosphate - P\ \left(\frac{mg}{g} of\ dry\ weight\right) = \left(\frac{0.05L \times Conc.\left(\frac{mg}{L}\right)}{wet\ \ weig\ ht\ (g) \times dry\ \ weig\ ht}\right) \qquad (6.7)$$

5.2.2.1.6 Ammonium (US EPA method 350.1)

To determine the ammonium ion concentration present in potting soil, the ammonium (US EPA method 350.1) conducted in Chapter 2 (section 2.1.1.7.4)was used. The concentration of ammonium-N in potting soil and sewage sludge was determined using the equation (6.9) below:

$$ammonium - N\ \left(\frac{mg}{g} of\ dry\ weight\right) = \left(\frac{0.05L \times Conc.\left(\frac{mg}{L}\right)}{wet\ \ weig\ ht\ (g) \times dry\ \ weig\ ht}\right) \qquad (6.9)$$

5.1.1.4 Plant growth studies using radish and garden cress seeds.

Based on the characteristics of the sewage sludge and potting soil shown in table (6.1), five treatments were used in the growth of radish and garden cress seeds. The plant growth media concentrations were 0% (control), 20%, 40%, 80% and 100% weight fraction (w/w) sewage sludge, and potting soil was used as the diluent. Sludge and soil masses were weighed using a Pioneer™ PA2102 balance and each weighed mass was transferred into plant pot (Buco

(Pty) Ltd, Grahamstown, South Africa) and each plant pot had a total mass of 50 g. A total of three plant pots per treatment were prepared for each of the seeds. Seeds of both garden cress and radish were transferred into separate petri dishes, and soaked in water for 48 hours at 22 ± 0.5 ºC to allow the seeds to germinate. Once germinated, five seedlings of radish were planted at 3 mm depth in each treatment plant pot. Five garden cress seedlings were planted out in the same manner. A total of 3 flower pots per treatment were prepared and five germinated seedlings of garden cress were planted in each pot per concentration resulting in 15 seedlings per concentration (n=15). For radish, five seedlings were planted per flower pot per concentration resulting in 15 seedlings per concentration (n=15). The seedlings were planted at a depth of 3 mm in each pot. The plants were irrigated with 10 mL distilled water from Monday to Thursday, and 15 mL of water on Friday (plants were not watered on Saturday and Sunday). The plants were grown for 21 days in a controlled environment under the following conditions: light for 12 hours and darkness for 12 hours a day and at a temperature of between 20-21 ºC being maintained in the room. From preliminary studies that were conducted on radish and garden cress plants, it was shown that there was no difference in plants grown for 21 days and 25 days. A light source consisted of 15 watts fluorescent bulbs (Eveready Cool day light) and two fluorescent strip lights which were Osram L36watts/33-640 cool white and Osram L36watts/77 Fluora. The radiance of light ranged between 70-90 μmol/cm^2/sec.

Figure 5.2 below shows garden cress plants grown using 20 % (w/w) of sewage sludge. The plants shown in the picturewere obtained before sampling after 14 days. At this sludge

concentration best plant growth in terms of number of leaves and plant height were observed to be best at these conditions.

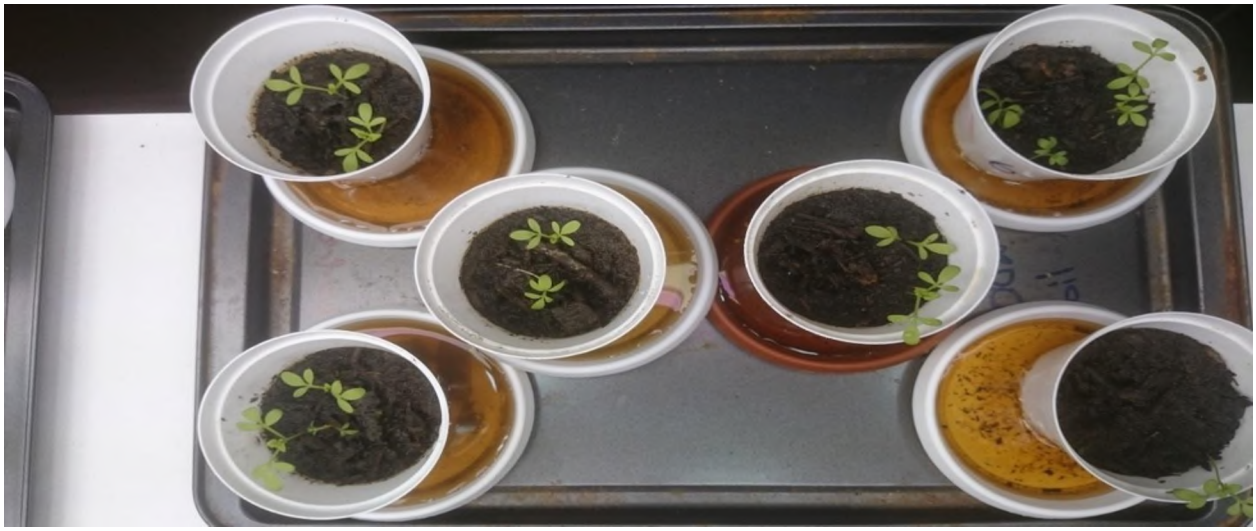

Figure 5.2: *Garden cress plants grown at 20 % sludge treatment*

Figure 6.8 shows radish plants grown at 20 % (w/w) sludge in sewage sludge. The plants shown in the picturewere obtained at day 14 before sampling, and at this sludge concentration the highest plant height and number of leaves were observed.

Figure 5.3: *Radish grown at 20 % sludge treatment*

5.2.2.1.7 Plant analysis

For the assessment of growth of the plants, sampling was done on day 7, 14 and 21 and three plants of radish and 3 plants of garden cress were collected. The number of leaves was determined by numerical counting; leaf length, plant height and root length were measured using a ruler; dry mass of each plant was determined on day 7 and day 21 by weighing fresh weight and dry weight of each plant using a Pioneer™ PA214 balance. To determine the fertilizer value of the sewage sludge, the sludge treatments were compared with the control which only composed of potting soil only.

5.2.2.1.7.1 Dry mass of plants

In calculating the dry mass (D_s) of radish and garden cress, equation (6.10) below was used. The mass of the dried crucibles (M_0) was determined. The fresh mass of each of the radish and garden cress was determined (M_1) using a Pioneer™ PA214 balance. The plants were then placed on top of aluminum foil and thereafter dried at 60 °C in an oven for 72 h. The total mass of each dried plant was determined (M_2) using Pioneer™ PA214 balance. The dry mass of radish and garden cress was calculated using the equation (6.10) below:

$$D_s = \frac{M_2}{M_1} \qquad (6.10)$$

5.2.3 DETERMINATION OF TRICLOSAN IN RADISH AND GARDEN CRESS

5.1.1.5 Extraction of triclosan from radish and garden cress

After the growth studies were complete, three plants each of radish and garden cress from each treatment were wrapped in aluminum foil and stored in the fridge at 4 ± 0.5 ºC until analysis. On analysis, the total weight of the three plants was determined using Pioneer™ PA214 analytical balance and the mass was recorded. After the weight of the three plants from each treatment was determined, the plants were evenly cut in squares in the dimensions of 5 mm x 5 mm using a scissors and placed in a 250 mL Erlenmeyer flask. Into each Erlenmeyer flask, 50 mL of 1 g/L of deoxycholic acid was transferred. The Erlenmeyer flasks with plants from the different treatments were then placed onto a Mechanical orbital shaker and shaken at 150 rpm at 20 ºC for 48 h.

After 48 h, the orbital shaker was stopped and the samples were left to stand for 15 minutes, thereafter the supernatant was transferred into 50 mL amber coloured jars. Spectrophotometric blanks (deoxycholate and lithocholate) and were run as plain extracts to compensate for any particle interference with the colour readings for TCS. Each sample in the well plate was screened for the presence of TCS using the Abraxis Triclosan Assay kit, as shown in the next section.

5.1.1.6 Screening of triclosan using Triclosan plate assay (Abraxis Method 96T PN530114)

To screen for the presence of TCS in radish and garden cress plants grown in sewage sludge, the methods used in Chapter 4 (section 4.2.2.2.4) was used. A calibration construction was similar to method described in Chapter 4 section (4.2.2.2.5).The limit of detection (LOD) was determined to be 32.4 μg/g. The concentration of TCS (ng/g d.w) in each plant was obtained using the following equation (5.11):

$$C(ng\ TCS\ per\ g\ dry\ weight) = \frac{50\ mL \times Conc.\ from\ \ calib.\ curve}{fres\ h\ \ weig\ ht\ of\ \ plant\ \ (g) \times \%\ dry\ \ weig\ ht} \quad (5.11)$$

5.2.4 DATA ANALYSIS

Data analysis was done using Microcal™ Origin 6.0 software package (Microcal Software, Inc. Northampton, MA, USA). Each parameter for radish and garden cress was statistically analysed. All data in this chapter was log transformed to ensure normal distribution and thereafter a t-test statistical analysis was done at significance level of 0.05 and values from each parameter were considered as independent populations in comparison of the means (average values).Paleontological Statistics software for education (PAST) version 2.17c (Hammer, et. Al, 2013) was used to conduct ANOVA and Kruskal-Wallis statistical analysis at the5 % level of significance.

5.3 RESULTS AND DISCUSSION

5.3.1 CHARACTERISATION OF SLUDGE AND POTTING SOIL

Upon conducting the plant growth studies and investigating bioaccumulation of TCS in radish and garden cress plants grown at different sludge treatments, physicochemical analysis of both sewage sludge and garden cress was conducted. Table 5.1 shows the physicochemical characteristics of sewage sludge obtained from Belmont Valley in Grahamstown and potting soil which was used as a diluent. In literature, the textural class of Eastern Cape soils is mainly medium expansive clay(Diop et al., 2011). The soil characteristics of the Eastern Cape in South Africa are listed in table 5.2, and it can be seen that the pH is about 7.8 (Diop et al., 2011) whereas the sludge pH was 6.73 ± 0.20. Introducing sewage sludge for soil amendment purposes will influence pH, and in this case use of sewage sludge will slightly reduce pH of the soil. pH of the sludge and soil will govern nature of TCS, and thus if the pH is higher than pK_a of TCS (7.9), the compound will ionize and leach out as it is ionized. If the pH of the soil or sludge is lower than pK_a, TCS will be bound to sludge particles(Hyland et al., 2012)and consequently be absorbed by the plants grown on amended soils (Pannu et al., 2012). The use of sewage sludge might increase physicochemical properties of soil, such as organic matter, water holding capacity and plant nutrients which might be of great agriculturalbenefit to farmers (Diop et al., 2011).

.***Table 5.1:*** *Characteristics of sewage sludge and potting soil used in this study.*

Parameter		Sludge (N=7)	Potting soil (N=3)
pH	**1:3 [sludge:$CaCl_2$]**	6.73 ± 0.20	7.08 ± 0.22
Dry weight	**(%)**	0.22 ± 0.04	0.38 ± 0.06
LOI	**(%)**	1.33 ± 0.03	0.08 ± 0.04
PO_4^{3-}- P	**(mg/g d.w)**	1.40 ± 0.30	1.33 ± 0.01
NO_3^--N	**(mg/g d.w)**	57.61 ± 55.20	1.55 ± 0.00
NH_4^+-N	**(mg/g d.w)**	6.60 ± 2.36	1.51 ± 0.00

Table 5.2: *Classification of soils in the Eastern Cape of South Africa*(Diop et al., 2011)

Parameters		
Clay	**%**	56.3
Silt	**%**	36.2
Sand	**%**	7.6
pH		7.8
Organic matter	**%**	2.77
Textural class		Medium expansive clay

5.3.2 ANALYSIS OF PHYSICAL PARAMETERS OF RADISH AND GARDEN CRESS

5.1.1.7 Root length

Plant roots in plants play a vital in nutrient and water uptake in plants. The presence of high concentrations of salts, heavy metals or insects may damage roots, consequently having an

impact on nature of roots and nutrient uptake (Januskaitiene, 2008). Absorption of nutrients by roots is influenced by the diffusion gradient between the soil and the plant (Boxal et al., 2006). In our study, we observed that radish grown in 20 % sludge had the longest tubers (roots) which increased from 13.7 ± 3.8 mm (day 7) to 44.7 ± 4.2 mm (day 21). On conducting a t-test statistical analysis, there was no significant difference (p = 0.28212) in radish root length between control and 20 % treatment. On conducting ANOVA and Kruskal-Wallis statistical analysis, on day 7 (p =0.04864) and 21 (p =0.04834) there was significant difference in root length of the radish plants grown in different treatments as shown in table 5.3.There was no significant difference (p =0.1427) at day 14 in root length for radish plants grown in different sludge treatments, and the highest root length was observed with 20 % sludge treatment. The lowest root length for radish was observed between 40 and 100 % treatments, which were less than the control. For garden cress, the highest root length obtained was at 20 % treatment, with 20.3 ± 4.5 mm (day 7) and 18.5 ± 2.1 mm (day 21), whereas the shortest root length was observed at 80 % treatment with 10.3 ± 1.5 mm after 21 days. Upon conducting a t-test statistical analysis, there was no significant difference (p = 0.06491) between the control and 20 % treatment garden cress plants. On conducting ANOVA and Kruskal-Wallis statistical analysis, on day 7 (p =0.04552) there was significant difference in root length of the garden cress grown in different treatments as shown in table 5.3. Further ANOVA and Kruskal-Wallis statistical analysis, showed no statistical differences in root length at day 14 (p =0.2539) and 21 (p =0.09816) in garden cress plants. A study conducted by Pannu et al., (2012), the longest root length for radish obtained was 84.4 cm and Jilani et al., (2010) obtained 11 cm, and therefore the values obtained in our study for radish root length were not comparable to the values in literature. A

study conducted by Buss and Masek, (2014)the highest root length obtained was 42 mm for garden cress and Aminidehaghi et al., (2006)obtained 32.04 mm. The values in literature were not comparable to the results obtained in our study because the studies in literature conducted plant growth studies over a period of 60 days. The reasons that might have influenced root length of both radish and garden cress was the having five plants in one plant pot might have resulted in competition for nutrients (Baloch, 2014)and thus low development of roots make the plants low on nutritional value for human consumption. Presence of heavy metals in sewage sludge (Tiruneh et al., 2014) could have caused phytotoxicity in radish and garden cress plants therefore limiting plant development.

Table 5.3: *Kruskal-Wallis statistical analysis for root length*

		H_c	*p-value*
Radish	Day 7	9.37	0.04864
Root length	Day 14	6.83	0.1427
	Day 21	9.47	0.04834
Garden cress	Day 7	9.71	0.04552
Root length	Day 14	5.34	0.2539
	Day 21	7.83	0.09816

Table 5.4: *Average root length (mm) of radish and garden cress plants at different sludge treatments*

		Sludge Treatment				
		0%	**20%**	**40%**	**80%**	**100%**
Radish	Day 7	10.6 ± 3.5	13.6 ± 3.7	6.0 ± 2	5.6 ± 3.7	4.3 ± 0.6
Root length (mm)	Day 14	21.6 ± 3.1	37.3 ± 2.3	23.3 ± 8.0	24.0 ± 7.5	24.0 ± 7.5
	Day 21	37.0 ± 9.8	44.7 ± 4.2	17.0 ± 9.5	21.3 ± 4.2	23.0 ± 3.0
Garden cress	Day 7	10.0 ± 2.7	20.3 ± 4.5	14.3 ± 1.5	11.33 ± 5.03	7.7 ± 0.6
Root length (mm)	Day 14	17.7 ± 8.0	17.3 ± 6.0	16.7 ± 8.3	8.0 ± 2.0	13.3 ± 8.7
	Day 21	19.0 ± 1.0	18.3 ± 2.1	15.0 ± 4.6	10.3 ± 1.5	11.0 ± 2.0

5.1.1.8 Number of leaves and leaf length

The presence of plant leaves plays a vital role in the life cycle of plants especially during the vegetative stage. Damage to leaves would have an impact on photosynthesis and food storage in the plant (Mondal et al., 2013). For radish, the highest number of leaves was observed at 20 % sludge treatment with 2.7 ± 0.6 leaves (day 7) and 4.0 ± 0.0 leaves (day 21), with 100 % sludge treatment recording the least number of leaves of 3.3 ± 0.6. From our study, it was shown that increasing the concentration of sewage sludge treatment decreased the number of leaves, and thus on day 21 the treatments with the least number of leaves were 40 % (3.3 ± 0.6 leaves), 80 % (3.7 ± 0.6 leaves) and 100 % (3.3 ± 0.6 leaves). Before conducting a t-test, the data was log-transformed. On conducting a t-test statistical analysis, there was significant difference (p = 0.01161) between the control and 20 % sludge treatment radish plants, but there was significant difference amongst control compared to 40 % and 100 % sludge treatments. On conducting ANOVA and Kruskal-Wallis statistical

analysis at, on day 7 (p =0.3034), day 14 (p =0.2247) and day 21 (p =0.2311) there was no significant difference in number of leaves in radish grown in different treatments as shown in table 5.5. For garden cress plants, the highest number of leaves observed was with 20 % treatment whereby there was 5.0 ± 1.0 leaves (day 7) and 8.7 ± 0.6 (day 21), whilst the least number of leaves was observed with 80 % treatment where the maximum value obtained was 6.7 ± 1.2 leaves (day 21). Before conducting a t-test, the data was log-transformed. On conducting t-test statistical analysis, there was no significant difference (p = 0.03739) in the number of leaves between the control and each treatment. On conducting ANOVA and Kruskal-Wallis statistical analysis, on day 7 (p =0.7381) and day 21 (p =0.1452) there was no significant difference in number of leaves in garden cress grown in different treatments as shown, but there was significant difference in the number of leaves on day 14 (p =0.03579) in table 5.5. On comparison of each treatment to the control, both radish and garden cress showed no significant difference in the number of leaves with increasing sludge concentration. A studies conducted by Semhi et al., (2014)and Kumari and Patel, (2013), observed similarities to this study, with no increase in the number of leaves with increase in sludge concentration.

Table 5.5: *Kruskal-Wallis statistical analysis for number of leaves*

		Hc	***p-value***
Radish	Day 7	4.85	0.3034
Number of leaves	Day 14	5.68	0.2247
	Day 21	5.61	0.2311
Garden cress	Day 7	1.98	0.7381
Number of leaves	Day 14	10.29	0.03579
	Day 21	6.82	0.1452

Table 5.6: *Kruskal-Wallis statistical analysis for leaf length*

		Hc	*p-value*
Radish	Day 7	11.01	0.0256
Leaf length	Day 14	4.49	0.3281
	Day 21	8.82	0.06045
Garden cress	Day 7	5.82	0.2128
Leaf length	Day 14	2.39	0.6653
	Day 21	6.86	0.1437

Table 5.7: *Average number of leaves in radish and garden cress plants at different sludge treatments*

		Sludge Treatment				
		0 %	**20 %**	**40 %**	**80 %**	**100 %**
Radish	Day 7	2.3 ± 0.6	2.7 ± 0.6	2.3 ± 0.6	2.7 ± 0.6	3.3 ± 0.6
Number of leaves	Day 14	3.7 ± 0.6	3.3 ± 1.2	2.3 ± 0.6	2.7 ± 0.6	3.3 ± 0.6
	Day 21	4.0 ± 0.0	4.0 ± 0.0	3.3 ± 0.6	3.7 ± 0.6	3.3 ± 0.6
Garden cress	Day 7	5.7 ± 0.6	5.0 ± 1.0	5.7 ± 0.6	5.3 ± 1.2	6.0 ± 1.0
Number of leaves	Day 14	8.0 ± 1.0	8.7 ± 0.6	6.7 ± 0.6	6.3 ± 0.6	8.3 ± 0.6
	Day 21	7.3 ± 0.6	8.7 ± 0.6	7.3 ± 0.6	6.7 ± 1.2	7.3 ± 0.6

Table 5.8: *Average leaf length (mm) of radish and garden cress plants at different sludge treatments*

		Sludge Treatment				
		0 %	**20 %**	**40 %**	**80 %**	**100 %**
Radish	Day 7	7.7 ± 1.5	14.5 ± 1.5	7.3 ± 2.5	6.7 ± 1.5	3.3 ± 0.6
Leaf length (mm)	Day 14	11.7 ± 3.5	16.0 ± 3.6	13.0 ± 2.7	12.7 ± 2.1	11.0 ± 1.0
	Day 21	10.0 ± 2.0	17.0 ± 1.0	13.7 ± 2.1	11.3 ± 2.1	12.0 ± 3.0
Garden cress	Day 7	3.7 ± 1.5	6.3 ± 1.5	3.0 ± 1.7	4.7 ± 2.1	5.0 ± 1.0
Leaf length (mm)	Day 14	6.3 ± 1.5	5.7 ± 2.5	6.0 ± 1.0	4.3 ± 1.5	5.3 ± 2.3
	Day 21	6.0 ± 1.0	8.3 ± 0.6	7.3 ± 2.1	6.3 ± 1.5	5.0 ± 1.0

5.1.1.9 Plant height

Plant height is an indicator of vegetative growth. The results shown in table 5.10, show that 20 % of sludge concentration resulted in the highest plant height for radish, with 69.00 ± 4.00 mm (day 7) and 79.0 ± 4.2 mm (day 21), whilst the lowest plant height was observed at 100 % treatment recording 16.3 ± 1.5 mm (day 7) and 51.6 ± 3.5 mm (day 21). Before conducting a t-test, the data was log-transformed. Upon conducting a t-test statistical analysis, there was significant difference (p = 0.01135) between the control and radish grown at 20 % treatment. On conducting ANOVA and Kruskal-Wallis statistical analysis, on day 14 (p =0.1051) and day 21 (p =0.09367) there was no significant difference in plant height in radish plants grown in different treatments, but there was significant difference in plant height of radish plants at day 7 (p =0.01019) as shown in table 5.9. This implied that use of sewage sludge in day 14 and 21 did no influence plant height of radish whereas on day 7, the use of sewage sludge influenced plant height of radish plants as 20 % sludge treatment

showed to have the highest plant height (69.0 ± 4.0mm). For garden cress, the highest plant height was observed at 20 % with 15.0 ± 2.7 mm (day 7) and 25.3 ± 4.4 mm (day 21), whereas the lowest plant height was at 80 % treatment with plant height of 17.0 ± 4.0 mm (day 7) and 18.7 ± 7.4 mm (day 21). Before conducting a t-test, the data was log-transformed. On conducting a t-test statistical analysis, there was no significant difference (p = 0.00431) between the control and each treatment. On conducting ANOVA and Kruskal-Wallis statistical analysis, on day 7, (p =0.3083), 14 (p =0.5735) and 21 (p =0.445) there was no significant difference in plant height in garden cress plants grown in different treatments as shown in table 5.9. This implied that the use of sewage sludge did not significantly influence plant height of garden cress plants.

Studies conducted in literature indicate that radish grows up to 83 cm (Pervez, 2004) and 20 cm (Semhi et al., 2014). From our study, the observed radish plant height for radish was not comparable to the ones in literature because of the duration of the study which might have played a significant role as the plants might have not been allowed to grow for a long period of time. Reports in literature have observed garden cress may grow up to 118 cm in height (Diwakar et al., 2008; Kumari and Patel, 2013) and the results from this study were not comparable to the these values. N which is essential for plant growth and development (Baloch, 2014), and since five plants were planted in each flower pot this might have resulted in competition for N, and thus contributing to low radish and garden cress development. N is essential for cell division in plant development (Baloch, 2014)and insufficient levels of this element may significantly influence the growth of plants. Futhermore, plant development was not comparable to other studies because of the presence

of heavy metals that were present in the sewage sludge (Tiruneh et al., 2014) and thus resulting in phytotoxicity in radish and garden cress plants therefore limiting plant development.

Table 5.9: *Kruskal-Wallis statistical analysis for plant height*

		H_c	*p-value*
Radish	Day 7	13.23	0.01019
Plant height	Day 14	7.65	0.1051
	Day 21	7.94	0.09367
Garden cress	Day 7	4.8	0.3083
Plant height	Day 14	2.91	0.5735
	Day 21	3.72	0.445

Table 5.10: *Average plant height (mm) of radish and garden cress plants at different sludge treatments*

		Sludge Treatment				
		0 %	**20 %**	**40 %**	**80 %**	**100 %**
Radish	Day 7	56.3 ± 7.5	69.0 ± 4.0	46.3 ± 3.5	28.4 ± 7.5	16.3 ± 1.5
Plant height (mm)	Day 14	61.0 ± 14.0	80.7 ± 9.5	63.0 ± 18.0	53.0 ± 9.9	53.0 ± 9.9
	Day 21	58.0 ± 7.2	79.0 ± 4.2	51.0 ± 14.5	53.1 ± 11.1	51.6 ± 3.5
Garden cress	Day 7	17.6 ± 3.8	15.0 ± 2.7	21.3 ± 5.1	17.0 ± 4.0	20.3 ± 2.5
Plant height (mm)	Day 14	16.7 ± 2.1	20.3 ± 5.7	13.7 ± 6.4	15.7 ± 4.2	15.0 ± 3.0
	Day 21	22.0 ± 2.0	25.0 ± 4.4	21.0 ± 3.6	18.7 ± 7.4	19.7 ± 1.5

5.1.1.10 Dry mass

Dry mass of the plant indicates the amount of lipids, carbohydrates and proteins after the removal of moisture in the plant (Kumari and Patel, 2013). In our study, the highest dry mass observed was 15.1 ± 7.3 % for radish grown at 20 % sludge treatment. Upon conducting a statistical t-test analysis, there was no significant difference (p = 0.1023) between the control and radish plants grown in different sludge concentrations as shown in table 5.12. Before conducting a t-test, the data was log-transformed. On conducting ANOVA and Kruskal-Wallis statistical analysis, on day 7, (p = 0.0221) and 21 (p = 0.03373), there was significant difference in dry mass of radish plants grown in different treatments as shown in table 5.11. This therefore implied that the use of sewage sludge significantly influenced dry mass of radish plants. For garden cress, the highest dry mass was observed at 20 % with 12.5 ± 3.6 % (day 7) and 15.0 ± 6.3 % (day 21), whereas the least dry mass was at 40 % treatment with of 10.9 ± 6.9 % (day 7) and 11.7 ± 5.3 % (day 21).Upon conducting a statistical t-test analysis, there was no significant difference between the control and garden cress plants grown in different sludge concentrations. On conducting ANOVA and Kruskal-Wallis statistical analysis, on day 7, (p =0.03069) and 21 (p =0.6216). On day 7, there was significant difference in dry mass of garden cress plants grown in different treatments the use of sewage sludge did not affect the dry mass of garden cress plants whereas, in day 21 there was no significant difference in dry mass of garden cress plants grown in different treatments as shown in table 5.11. This implied that the use of sewage sludge at different concentrations significantly influenced dry mass of garden cress plants. A study conducted by Pannu et al., (2012) obtained a dry mass of between 8-13.7 % and Verma et al., (2007) obtained a dry

mass of 24.8 % of radish plants. A study conducted by Buss and Masek, (2014) obtained a dry mass of 16.5 % of garden cress, whereas Aminidehaghi et al., (2006) obtained a dry mass of 18.6 %. The values obtained in our study were lower than the values in literature, and this was because of the presence of heavy metals that were present in the sewage sludge (Tiruneh et al., 2014) and thus resulting in phytotoxicity in radish and garden cress plants therefore limiting plant development. The ANOVA and Kruskal-Wallis statistical analysis showed that the use of sewage sludge influences dry mass of radish and garden cress, thus high dry mass values will indicate high nutritional value of in the plants.

Table 5.11: *Kruskal-Wallis statistical analysis for dry mass*

		H_c	*p-value*
Radish	Day 7	11.43	0.0221
Dry mass	Day 14	10.43	0.03373
Garden cress	Day 7	8.89	0.03069
Dry mass	Day 14	2.63	0.6216

Table 5.12: *Average dry mass (%) of radish and garden cress plants at different sludge treatments*

		Sludge Treatment				
		0 %	**20 %**	**40 %**	**80 %**	**100 %**
Radish	Day 7	6.3 ± 4.1	11.0 ± 2.7	7.1 ± 4.1	10.0 ± 5.7	12.3 ± 6.4
Dry mass (%)	Day 21	10.5 ± 1.1	15.3 ± 7.3	11.3 ± 2.3	13.2 ± 2.1	13.7 ± 3.7
Garden cress	Day 7	9.1 ± 6.8	12.5 ± 3.6	10.9 ± 6.9	11.8 ± 6.8	8.8 ± 4.1
Dry mass (%)	Day 21	13.3 ± 2.7	15.0 ± 6.3	11.7 ± 5.3	13.5 ± 7.8	14.4 ± 4.7

Dry mass therefore is an indication of plant nutritional value. Radish is a high source of ascorbic acid, folic acid and dietary fiber (Jilani et al., 2010). Therefore, dietary intake of the plants may reduce incidences of low collagen and gum development (SAMF, 2010). Garden cress is a high source of vitamin A and C (Jilani et al., 2010), and thus intake of radish assists in keeping good vision and collagen fiber formation (SAMF, 2010).In agricultural economies where subsistence farming is practiced, growth of plants such as radish and garden cress may be of great benefit especially in South Africa were malnutrition in children under the age of 12 has been a problem in the health system (WHO, 2013). Malnutrition is one of the major causes of death and disability and the major cause of malnutrition are directly related to inadequate dietary intake (WHO, 2013), and thus if radish and garden cress plants are grown using sewage in areas prone to malnutrition, the incidence of malnutrition might be reduced.

In conclusion, after conducting Kruskal-Wallis statistical analysis, it was shown that generally there was no significant difference in the number of leaves, length of leaves, plant height and dry mass of the plants when sewage sludge was used as a fertility aid. This therefore might be of great advantage as WWTPs in South Africa encounter challenges in disposal of sewage sludge. The option of disposal of sewage sludge for beneficial use in agriculture might be viable. Nonetheless, it should be noted as discussed in chapter 2 that sewage sludge contains heavy metals such as manganese (Mn), copper (Cu), lead (Pb) and cadmium (Cd); nitrates and pathogens such as Escherichia coli *(E. coli)* and heterotrophic bacteria. Thus therefore, in as much as disposal of sewage sludge for agricultural purposes might be a viable option, environmental concerns might arise as metals and nitrates might

leach onto groundwater, and consequently affect human health. Even though leaching onto groundwater might occur, phytoaccumulation of heavy metals in plants might occur with high concentrations of sewage sludge being applied. The presence of pathogens such *E. coli* in sewage sludge must not be neglected, as shown in chapter 2 must not be neglected as the leachability index of *E. coli* was 0.0000004, showing that the pathogens might leach onto groundwater or become absorbed by plants, thus giving rise to human health risks.

From this study, best results with respect to the number of leaves, length of leaves, plant height and dry mass were observed with 20 % sludge treatment. It is important to monitor the sludge composition especially if it is to be used for agricultural purposes. This is because as mentioned in the previous paragraph, sludge can contain heavy metals, pathogens and nitrates which might give rise to environmental concerns, therefore to determine application rates in agricultural soils, characterisation of sludge and soil composition will be important so as to reduce entry of heavy metals, pathogens and nitrates into the environment and food chain (Herselman et al., 2005; Herselman and du Preez, 2000).

5.3.3 SCREENING OF TRICLOSAN IN RADISH AND GARDEN CRESS PLANTS

The concentration of TCS in radish and garden cress plants was assessed by screening for the presence of the compound using the Abraxis Triclosan Assay kit. The limit of detection (LOD) of the TCS assay kit was 32.4 µg/g d.w, and from our study, preliminary results from immunological kit indicated that the plants you analysed not accumulate the TCS. The A

study conducted by Pannu et al., (2012), it showed low accumulation of TCS in radish plants of up to 0.004 ± 0.002 mg/kg, and these results were not comparable to the results obtained in our study whereby TCS concentration was below 32.4 µg/g d.w for radish and garden cress plants grown at 0, 20 40, 80 and 100 % sludge treatments. The concentration of TCS in sludge used in this chapter was 142 ± 33.5 µg/g d.w as it was calculated in Chapter 4. The major reason that might have influenced the uptake of TCS by radish and garden cress was the low concentration of TCS in the sludge, hence extent of absorption was minimal and the compound could not accumulate in radish and garden cress plants. There was qualitative agreement between the immunological and GC/MS and therefore the lack of detection of TCS in radish and garden cress plant tissue by immunological methods indicated that there was no TCS in the plants.

In some studies, TCS accumulation has been quantified by calculating a parameter known as bioaccumulation factor (BAF) which is expressed as a ratio of TCS concentration in plants to TCS concentration in soil (Pannu et al., 2012); and TCS accumulation is expected to occur when the concentration of TCS in soil is higher than 20 mg/kg. Accumulation is further affected by lipid characteristics of the plant, which differs amongst plants and moreover Suter(2007) suggested that water and lipid content in plant tissues affects contaminant uptake by plants, thus in this study, accumulation might have occurred but it was below the limit of detection (LOD) due to lipid and water content influencing uptake. Water content in plants may be affected by transpiration, and thus humidity and temperature will play a vital role in plant water content (Suter, 2007). Due to the molecular weight, log K_{ow} and pK_a(Aragón et al., 2008), TCS was expected to accumulate in different parts of the plants due to its

similarity to other antimicrobials such as trimethoprim and diazimon which both accumulated in carrot plants (Boxal et al., 2006; Simonich and Hites, 1995).

US EPA(USEPA, 2009)introduced the use of empirical formulas to determine bioaccumulation of hydrophobic compounds including pesticides, dioxins (Pannu et al., 2012) and Travis and Arms, (1988) derived the following equation for compounds with log K_{ow} of between 1.75 and 6.15, and TCS falls within the range. The only disadvantage of the formula is that accumulation is less likely to follow the model described in equation (6.12) if the chemical, soil and plant species are different from than those used to derive the equation.

$$logUp = -0.578logK_{ow} + 1.588 \quad (6.12)$$

Where Up is the uptake coefficient (similar to BAF) and numerical values are regression parameters.

The calculated uptake coefficient (Up) for TCS was 0.065 for all the sludge treatments. Our observation from this study showed that TCS might have accumulated in radish and garden cress, but the concentration was below the LOD. In literature, some studies have shown that TCS is expected to accumulate in plants grown in contaminated soils based on the model shown in equation 6.12 (Boxal et al., 2006; Pannu et al., 2012).Our observation in this study was in accordance with Suter(2007)who suggested that chemical accumulation is less likely to follow the model prediction if the chemical, soil, and plant species are different than those used to derive the model (equation 6.12). A conclusive prediction using the experimentalmodels can only be made if the models are validated to a wide range of

chemicals, soils, and plants. Thus, the models such empirical equations (such as equation 6.12) will tend to overestimate TCS phytoaccumulation and should be used with caution.

Chemicals enter plants by: (a) partitioning for contaminated soil to roots, and then transported through the xylem to upper parts of the plants, (b) directly by gas-phase and particle phase deposition on leaves enter leaf pores (stomata) and transported via phloem; and (c) partitioning from soil particles to root epidermis or cortex and accumulate in the root (Leewen and van Vermeire, 2007). This therefore implies that chemical uptake by plants depend on the chemical properties of the compound (water solubility, lipophilicity, vapour pressure), environmental conditions (temperature and soil organic content) and plant characteristics (surface area of leaves and root mass) (Pannu et al., 2012; Trapp and Legind, 2011). Furthermore, it should be noted that Henry's constant (H) at 25 ºC is low at 10^{-9}, as a result vapour movement in this study was low implying minimal movement of TCS from sludge amended soil to plants tissue additionally confirming the low concentrations of TCS in radish and garden cress (Trapp and Legind, 2011). In addition, lipophilic compounds with log K_{ow} greater than 4 (such as TCS), have demonstrated to display limited movement across endodermis membrane from amended soils (Leewen and van Vermeire, 2007; Waria et al., 2011), and therefore this justifies the low concentration in radish and garden cress plants from this study, and further studies are to be done on each plant segment to assess the distribution of TCS in the whole plant. On a study conducted by Trapp and Legind, (2011), they demonstrated that movement of non-ionized compounds through xylem is mainly by water flow, but the low water solubility of TCS in water makes the compound less mobile and thus might have resulted in the low or no TCS present in plants. In conclusion, TCS is

known to be biodegradable under aerobic conditions, but persists under anaerobic conditions (Chen et al., 2011; Wang et al., 2014; Wu et al., 2009). Thus as a consequence, the presence of aerobic bacteria (such as heterotrophs) in sludge amended soils may significantly reduce the concentration of TCS and resulting in low bioavailability for accumulation (Chen et al., 2011). This hence might have been one of the factors that led to concentration of TCS being below LOD due to biodegradation of the compound.

In conclusion, the absence of TCS in plants implied that when radish and garden cress are consumed, none of the TCS will be in the human body to trigger any physiological response. The absence of TCS in plants is of great advantage as it will reduce human health related issues that may affect reproductive health, puberty and pregnancy (Ayoola Saheed, 2012). Moreover, TCS being associated with oestrogen mimicry (Dinwiddie et al., 2014), thus it may increases the rates of breast cancer tumours in females and in males it may cause development of mammary glands(Gee et al., 2008). Therefore, in the nutritional value contributed to by radish and garden cress and discussed earlier on will be of significant value as no health risks will be associated with the consumption of these plants. In a nutshell, the use of sewage sludge for agricultural purposes will be of great value if the sludge has low concentration of TCS, which will not be absorbed by plants. It should be noted that besides accumulation of TCS in plants, sludge should be used with great caution as it might contain heavy metals, nitrates and pathogens that might accumulate in plants and cause phytotoxicity in plants, and thus defeating the beneficial purpose of the sludge. To reduce plant accumulation of heavy metals, nitrates and pathogens, sludge and soil characterisation is

important so as to determine application rates that will not cause plant phytotoxicity, environmental risks and food chain contamination.

5.4 CONCLUSION

The application of sewage sludge in the growth of radish and garden cress plants did not show any form of toxicity in the plants with respect to TCS concentration in the amended soil. Phytotoxicity of sewage sludge was observed at and above 40 % (w/w) sludge concentration, with plant height and root length being incomparable to the control and 20 % treatment plants. The limit of detection (LOD) of the Abraxis triclosan assay kit was 32.4 µg/g d.w, and thus plant uptake of TCS by radish and garden cress was very minimum with most of the plant TCS concentrations being below LOD, thus making these plants suitable for both human and animal consumption with little or no TCS entering the food chain affecting human and animal health. Accounting for TCS degradation, plants grown in sludge amended soils at agronomic application rates for long periods of time will not experience any toxicity, but may accumulate TCS in different plant tissues. BAF and logUp must be studied or determined for plants grown in amended soils so as to prevent transfer of these micropollutants into the food chain. Further research needs to be done on these studies to assess accumulation over longer periods of time so as to have an understanding of the risk posed by biosolids and understand the valorization of the sewage sludge if it is to be considered for agricultural reuse.

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