MITIGATION OF SOIL AND GROUND WATER POLLUTION CAUSED BY ON-LAND DISPOSAL OF OLIVE MILL WASTEWATER
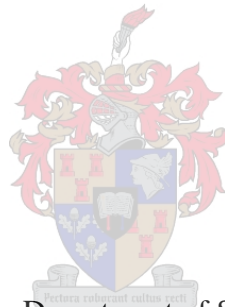
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Declaration

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ABSTRACT

Olive mill wastewater (OMW) is generated in large quantities, particularly in the regions with a Mediterranean climate where olive oil is produced on a commercial scale. Some producers collect the effluent and dispose of it as hazardous waste at significant expense, while others dispose of it directly on land, claiming the potential benefits to productivity from the plant nutrients present in the OMW. It was shown that the OMW also contains some phytotoxic phenols, which may have both immediate and cumulative negative effects on plant growth. The long-term effects on the soil and crop growth have been shown to be detrimental. Sandy soils are of particular concern due to the possibility of phenol penetration into deeper soil layers and potential ground water contamination.

The study explores *in-situ* (soil amendment with biochar prior to the OMW disposal) and *ex-situ* (OMW filtration through a biochar bed) options to mitigate the negative effects of the OMW on-land disposal.

A laboratory batch sorption experiment was set up using 0.2 g pinewood biochar to explore the possibilities of removing the phenols from 50 mL of the OMW or gallic acid (GA) solutions at different concentrations. The results showed that the sorption process was rapid and stabilized within one hour. The kinetic process followed a pseudo-second-order model and was described by the Freundlich multi-layer isotherm. The pinewood biochar had a sorption capacity of 30 mg·g^{-1} and 100 % removal was obtained with 300 g·l^{-1} of the OMW load. It was found that pinewood biochar could be used to remove the phenols contained in the effluent.

A column experiment was set up to determine the effectiveness of biochar and biochar-soil mixtures in removal of phenol and Chemical Oxygen Demand (COD) from the OMW compared to sand filtration. The breakthrough curves for phenol and COD were determined, while the pH and EC of the filtrates were monitored. Ten PVC columns of 30 cm height and 5 cm diameter were filled with five different materials: sand, sand + biochar, Hutton clay loam soil, Hutton clay loam soil + biochar and biochar alone. Two different treatments were given to the columns; five of the columns were prewashed with 2 liters of deionized water and the other five were not washed before the OMW filtration. The performance of the columns was determined in respect of the phenol and COD removal capacities, hydraulic conductivities and porosity changes. The results showed that washing enhanced the phenol sorption but not the COD sorption. The addition of the biochar at 2%wt load significantly improved the effectiveness of the filtration. The best performance was achieved in terms of COD removal in pure biochar columns, but in terms of the phenol, the best performance was on a pre-washed

Hutton clay loam soil with 2%wt biochar addition. Both the washing and biochar addition affected the porosity and reduced the hydraulic conductivity of the columns.

The greenhouse experiments were conducted to confirm the above statement using pot trials laid out in a 4 x 4 factorial Randomized Complete Design (CRD) to determine the effect of effluent and biochar on wheat and green beans on alkaline sand. Results showed that the increasing effluent rate up to 200 $m^3 \cdot ha^{-1}$ gave significantly negative results on wheat growth, even with fertilizer application. But the effect was different for beans where low effluent loads gave positive results though not significant while with fertilizer (N and P) 50 $m^3 \cdot ha^{-1}$ performed better. With the addition of biochar there was no significant effect on wheat, but it significantly affected beans at the application rate of 2.5 and 5%wt. The interaction of biochar and effluent showed that the best performance was at 5% biochar application and effluent loads of 50 and 100 $m^3 \cdot ha^{-1}$, but increased effluent rate decreased production even with a 5% wt biochar application rate. It was suggested that a leguminous crop should tolerate OMW application better compared to wheat even in the adverse conditions of the alkaline sand.

A second greenhouse experiment was conducted with another legume, an indigenous African crop, the bambara groundnut, on an acidic Hutton clay-loam soil (Oxisol) sourced locally. The experiment was laid out in a 2 x 6 CRD factorial design to determine the effect of the biochar and effluent combination on the yield and growth parameters of bambara as well as the effect on soil conditions and nutrient availability. The result showed that biochar addition improved seed germination, which was retarded by effluent loading. The effluent rate of 200 $m^3 \cdot ha^{-1}$ and biochar 2% gave the best yield performance. The biochar addition increased the pH and hence affected the release of P and N whereas Na and K availability were reduced.

We conclude that biochar may be used for both *ex-situ* filtration to treat the OMW, and as a soil amendment to allow safe on-land disposal of the OMW. The estimations of safe disposal loads and the required application rates of the biochar should be made individually for a specific soil type. Pinewood biochar was proven to be a cheaper source of activated carbon for the treatment of olive mill wastewater organic contaminants in South Africa.

DEDICATION

I would like to dedicate this dissertation to

- my late husband Christopher Okoye (ACA) who inspired me to continue my education.

  I will forever miss you, dear

  and

- to my son Dabeluchi, who is the reason I live.

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OLIVE MILL WASTEWATER: ON-LAND DISPOSAL AND MITIGATION OF SOIL AND GROUND WATER POLLUTION

1.1. Introduction

Soil is an effective filter for many contaminants, particularly organic. However, if the organic materials are not decomposed rapidly enough excessive accumulation may negatively affect soil properties and some of the contaminants may migrate to surface and ground waters. Indiscriminate on-land disposal of untreated effluents from olive oil mills is a common practice which leads to soil degradation and threatens ground waters, particularly on sandy soils (Jarboui et al., 2008). Research efforts are made globally to address this problem. This study intends to contribute by using locally available materials in combating the problems of this effluent disposal.

There is continuous increase in the quantity of olive (*Olea europaea*) oil produced, primarily in the regions with a Mediterranean type of climate, due to the high demand for olive oil. Olive oil is characterized by lesser fat content compared to many other vegetable oils and its wide use is part of the global striving for healthy living. It is believed and accepted that its consumption is beneficial to human health and reduces the risk of heart disease and could also prevent several types of cancer (Bendini et al., 2007). This reasoning has tremendously increased the rate of consumption of olive oil in the last few decades.

The olive oil production process generates large quantities of olive mill wastewater (OMW) which, due to its environmentally-hazardous composition, causes a problem for safe disposal. Over 98 % of olive oil production is done in the Mediterranean regions, with 2.5 million metric tons per year (McNamara et al., 2008). Justino et al. (2012) estimated the amount of olive effluent produced in the Mediterranean region to be between 7×10^6 and 3×10^7 m^3 annually. South Africa has joined the list of olive-oil-producing countries and the industry is growing continuously. According to Taylor and Atkinson (2013), the Western Cape, which is the most important and optimal commercial olive producing region of the country, has about 720 000 mature olive trees grown on 3 000 hectares of land. Olive farming in South Africa is one of the fastest growing sectors with, about 80% of the South African plantings consisting of olive oil cultivars for olive oil production (Taylor and Atkinson, 2013). This rate of production raises concern as enormous effluent will be generated.

Different types and quantities of wastes are generated from the different methods of extraction of the oil. According to Roig et al., (2006), there are two methods of extraction: the traditional method, which is almost obsolete and the centrifugation method (three phase and two phase olive oil extraction method). They explain that the traditional method generates a solid fraction called the olive husk and emulsion containing the olive oil and wastewater which is separated by decantation. Then the three phase methods also generates a solid fraction called olive husk or pomace, oil and more of olive mill wastewater (OMW) than the traditional method. These wastes are different from the two phase olive oil extraction method as it generates only two fractions; a solid fraction called different names (alperujo, olive wet husk or wet pomace) and a liquid one (olive oil) (Roig et al., 2006). Borja et al., (2006) classified the identifiable wastes from the two phase olive oil extraction as; 1) wash waters from the initial cleansing of fruit. 2), wash waters from the secondary centrifuge and 3) the aqueous solid residue from the primary centrifugation (TPOMW).

These different methods especially the three phase and the two phase oil extraction methods are different not only in the quantities and types of wastes generated but also in their characteristics. The two phase generates less volume of wastewater than the three phase as well as lesser contaminant values (Table 1.1). The three phase method is almost being replaced with the two phase method in most of the major olive oil producing countries including South Africa. The reason is basically because of the quality of the oil and reduced wastewater produced. Our study is focused on the liquid part of the waste called olive mill wastewater (OMW) from either of the two centrifugation methods (Table 1.1)

The untreated effluent is mostly spread on the land surface degrading the soil and threatening pollution of rivers and ground waters. Be that as it may, the two wastes (solid and liquid) from the two centrifugation methods affect the soil differently though similar. López-Piñeiro et al. (2008) noted depressed grain yield, N and P in the first year of using 40 Mg·ha⁻¹ of TPOMW on Typic Haploxeralf and Lithic Xerorthent. They concluded that TPOMW could be a potential valuable soil amendment because it maintained positive beneficial effect on soil properties after 2 years. In agreement, (López-Piñeiro et al., 2010) reported that olive grove soils amended with TPOMW could be an effective management practice for controlling ground water contamination by diuron. On the other hand, OMW was found to increase soil polyphenolic content with increased application rates (Magdich et al., 2012). While (Barbera et al., 2013) reviewed that OMW gives temporary positive effect on soil and its high salt content could result in disintegration of the soil structure. They also noted that it reduced soil hydraulic

conductivity but could provide micro soil nutrients as well as organic matter. Chaari et al., (2015) reported increased soil electrical conductivity. Though no pH changes were observed in their work but sodium adsorption ratio (SAR) and exchangeable sodium percentage (ESP) were effected and organic matter in the soil was increased.

Table 1.1. Average Volumes of the wastewaters generated in the different steps of the three- and two-phase olive oil extraction processes.

Effluent	Three phase process				Two phase process			
	Vol of wastewater (l/kg)	Solids %	Oil %	COD g/kg	Vol of wastewater (l/kg)	Solids %	Oil %	COD g/kg
Washing of olives	0.09	0.51	0.14	7.87	0.05	0.54	0.10	0.87
Horizontal centrifuge	0.90	6.24	0.96	73.82	0.00	0	0	0
Washing of olive oil (Vertical centrifuge)	0.20	0	0	0	0.15	1.43	0.57	1.17
General cleaning	0.05				0.05			
Final effluent	1.24	4.86	0.31	68.61	0.25	2.81	0.29	2.25

From Borja et al.,(2006).

In some instances, the wastewater is collected in dedicated concrete dams and disposed of as hazardous waste. The problem of oil mill effluent disposal is always a source of worry for researchers all over the vegetable oil producing areas. The severity of the problem resulted in some environmental agencies like United State Environmental protection Agency (USEPA)

regulating the quality and quantity of effluent disposed (Otles, 2012). In South Africa, the Department for Water Affairs and Forestry (DWAF) regulates the quality and quantities of wastewaters to be disposed or used in the country.

As good as these regulatory measures are, they are not a sustainable solution to the problems. An overview of properties of the olive oil mill effluents and the problems associated with their disposal on the soil are highlighted in this chapter as well as the technologies to combat the existing problems. Internationally, the treated effluent allowable limit for phenol and COD amount are 5 and 200 mg·L^{-1} (TERI and TVPL, 1974) while the South African water quality standard stated COD levels of 75 mg·L^{-1} for industrial use and no data available for the agricultural use. Meanwhile, the phenol limit for domestic use was stated as 1 mg·L^{-1} but there is no available data for irrigation (Department of Water Affairs and Forestry, 1996).

1.2. Effluent production volumes

South Africa is sharing in the 2% of the world production of olive oil with Argentina, the United States, Mexico, Australia, Asia (Afghanistan, Iran and China) (Zervakis and Balis, 1996; Hanifi and El Hadrami, 2009). The SA olive industry is only starting to grow. Its production has reached 10,000 tons in the first decade of the 21st century (Olives Go Wild, 2013). According to Besseling (2013), each ton requires about 10 kL of water for processing which results in about 100 million liters of OMW. The processing of 100 kg of olives generates roughly 100 L of OMW (Dhaouadi and Marrot, 2008). The country's olive oil production has reached 1 000 tons per annum (Louw, 2015) and it is estimated that 1 ton of olive oil generates 0.5 − 0.8 tons of OMW (Dhaouadi and Marrot, 2008) for a typical three phase extraction system. The diagram below illustrates the two different processes of extraction of olive oil and the quantity and type of products obtained (Figure 1.1). Total SA consumption of olive oil is about 3.5 million liters per annum while the local production is less than 20% and the rest is imported. The industry is growing by 20% per annum, doubling its size in four to five years. It has got over 300 growers and producers of varying sizes and intensity (Taylor and Atkinson, 2013).

The most widely used production method is centrifugation, which requires large quantities of water for extraction. It is important to note that the three phase method generates more wastewater than the two phase system (Figure 1.1). In South Africa, three phase system was conventionally used but majority of the farms are diverting to the two phase system of olive oil extraction. Part of the reasons being the large quantity of wastewater generated which no one

has clue on how to handle and the quality of the oil produced from the three phase (personal discussion with Gert van Dyk. Olive Shed manager at Tokara farm) The wastewater from olive oil production using three phase is equal to 1.1-1.5 times the weight of milled olives (Paraskeva et al., 2007). In the Mediterranean zone alone, approximately 15 million metric tons of olive mill wastewater is produced annually as at 2007 (Paraskeva et al., 2007). Justino et al. (2012) reported the Mediterranean zone is now producing more than 30 million m^3 of OMW annually.
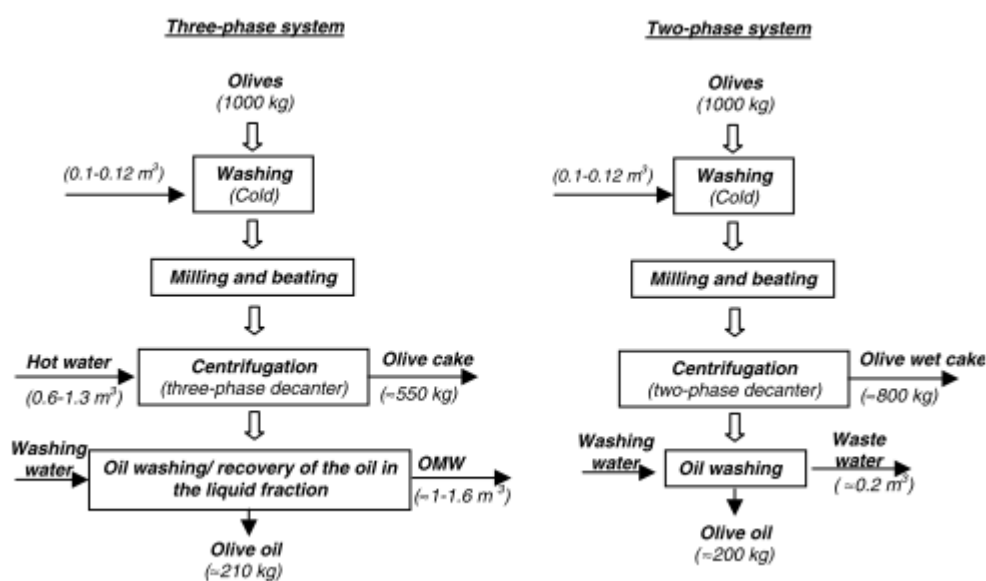
Figure 1.1 Three-phase and two-phase centrifugation systems showing quantity and type of wastes generated. From Roig et al., (2006)

1.3. Composition of the effluent

Olive mill wastewater has a complex composition and contains toxic compounds as well as plant nutrients. It contains contaminating organic and inorganic materials such as phenolic compounds, heavy metals and dyes (Al-asheh et al., 2003). Santi et al. (2008) reviewed the organic and inorganic constituents of OMW with COD (chemical oxygen demand) levels ranging from 70 000 to 150 000 mg·L^{-1}. According to Dhaouadi and Marrot (2008), OMW contains 83 – 92 wt % of water, organic matter 4 - 16 wt % and minerals 1 - 2 wt %, 4 - 5 % total solid and 2 - 4 % suspended solids. Specifically, OMW was further characterized by Sierra et al. (2001) to contain BOD (biological oxygen demand) of 100 000 mg·L^{-1}, COD of 200 000 mg·L^{-1}, polyphenols 3 000 – 24 000 mg·L^{-1}, oil and grease of 300 – 23 000 mg·L^{-1}, K content of 2100 mg·L^{-1}, P 300 – 1100 mg·L^{-1}, Ca 120 – 750 mg·L^{-1} , Mg 100 – 400 mg·L^{-1}, Na 80-

120 mg·L^{-1} and high EC of 8 – 22 dS·m^{-1}. The varied values of the different components of this effluent are due to the differences in fruit composition, as explained by Achak et al. (2009a), and determined by the cultivar, maturity stage, degree of damage, storage condition and processing method. The above stated values of the OMW parameters, especially the COD, is quite higher than the treated effluent disposal limits of South African (SA) standards according to Van Schoor. (2005). These are summarized in Table 1.2. This necessitates the need for treatment options.

Table 1.2. Summary of olive mill wastewater composition using three phase extraction system.

Properties	Value range	References	SA effluent legal requirement of effluent for irrigation	References
pH	3.6-5.4	(Borja et al., 2006)	6-9	(Van Schoor, 2005)
EC	5.5 – 12.0(dS·m^{-1})	(Roig et al., 2006b)	<2 (dS·m^{-1})	(Van Schoor, 2005)
O.M	30.57-57.40 (g·L^{-1})	(Mahmoud et al.,2012); (Vlyssides et al., 2004)	NA	
N	1.59-12.20 (g·L^{-1})	(Hanifi and El Hadrami, 2009)	<3, 5 – 10 (mg·L^{-1})	(Van Schoor, 2005)
Total P	31.00-63.87 (mg·L^{-1})	(Kapellakis et al., 2015) ;(Paredes et al., 1999b)	<10 (mg·L^{-1})	(Van Schoor, 2005)
Phenols	860-2 300 (mg·L^{-1})	(Dhaouadi and Marrot, 2008)	NA	
Oil & grease	8 370 (mg·L^{-1})	(Achak et al., 2009)	<2.5 (mg·L^{-1})	(Van Schoor, 2005)

BOD	25000-94000 (mg·L^{-1})	(Latif Ahmad et al., 2003); (Vlyssides et al., 1996)	NA	
Total Suspended Solids	52 (g·L^{-1})	(Achak et al., 2009b)	<25 (mg·L^{-1})	(Van Schoor, 2005)
COD	70 000-150 000 (mg·L^{-1})	(Santi et al., 2008)	<5000, 400 and 75 (mg·L^{-1})	(Van Schoor, 2005)
K	4300-4460(mg·L^{-1})	(Chaari et al., 2015);(Paredes et al., 1999b)	<5 (mg·L^{-1})	(Ryder, 1995)
Na	110-1400 (mg·L^{-1})	(Chaari et al., 2015);(Paredes et al., 1999b)	< 65 (mg·L^{-1})	(Ryder, 1995)
Mg	44-187 (mg·L^{-1})	(Mekki et al., 2009);(Vlyssides et al., 2004)	<25 (mg·L^{-1})	(Ryder, 1995)
Ca	137.5-300 (mg·L^{-1})	(Mahmoud et al., 2010);(Paredes et al., 1999b)	<60 (mg·L^{-1})	(Ryder, 1995)
SAR	4.02	Calculated using data from (Chaari et al., 2015)s	<5-9	(Van Schoor, 2005)

NA means values not available in the literature searched

The OMW is acidic (pH ranging from 3.5–6) and contains high amounts of nutrients, particularly K and organic matter, all of which can contribute to soil fertility. However, the presence of oil and grease as well as the high amounts of water-soluble phenols (Table 1.2) reduces its potential use in agriculture as a soil amendment. This is why its composition is both toxic and beneficial.

Hanifi and El Hadrami (2009) reported that the effluent has a high carbon to nitrogen content (C: N ratio). This property gives the nitrogen-fixing bacteria a conducive condition. It may be

a good idea to use this effluent on crops that can fix their own nitrogen to enhance production. Interestingly, the heavy metal content of the effluent is below the threshold of heavy metal toxicity, and OMW does not contain pathogenic microorganisms (Sierra et al., 2007). The effluent could be used as a source of plant nutrients but the toxic compounds should be removed prior to application on land. Consequently, the treatment of the effluent is necessary for optimal use.

1.4. Impacts of OMW disposal on-land disposal

1.4.1. Impact of OMW disposal on soil physical properties

Not much positive impacts has been recorded for OMW application on the soil physical properties apart from increased aggregation reported by Mahmoud et al. (2012) and an increase in soil porosity. The increase in soil porosity could be positive, depending on the condition of the soils of the area. Another factor is the quantity of OMW applied, doses more than 200 $m^3 \cdot ha^{-1}$ is considered detrimental especially to the soil structure (PROSODOL). High rates of application may negatively affect soil properties due to clogging and coating of the pores by oil and grease present in the effluent (Table 1.2). In agreement, Achak et al. (2009a) reported a decrease in the hydraulic loading rate with time which in their study is related to development of bio-films. This decrease in hydraulic loading rate increased after 10 days of filtering the OMW through a sand filter and it was attributed to clogging due to high total suspended solid (TSS) and the organic load of the effluent. Mahmoud et al. (2010) attributed the clogging to the coatings of the soil particles by the residues of oil and grease as well as other organic substances from the OMW. Mahmoud et al. (2012) reported that long term application of this OMW reduced diffusion into the soil aggregates, as well as decreased soil hydraulic conductivity. This was supported by the observation of Mahmoud et al. (2010) that the water penetration time increased from less than 1 second in the control site to 25.2 seconds and 36.1 seconds after 5 years and 15 years of application of the effluent. They also observed a decrease in the infiltration rate after 5 years of application due to reduction in drainage porosity. Achak et al. (2009) reported a reduced flow rate in the sand filter treated with OMW which was attributed to accumulation of organic pollutant on the surface of the filter. The association of the organic matter to the soil mineral components reduces the sorption capacity of the clay minerals. This condition causes slow diffusion of solutes into the aggregate, which then decreases the amount of adsorbed ions, thereby increasing the possibility of ground water

pollution by organic matter and phenolic compounds (Gámiz et al., 2012). Mahmoud et al., (2012) concluded that continuous application of OMW will alter the surface layer of the soil and make it fragmented by cracking, which may increase the risk for preferential solute transport. On the other hand, it has been reported by Mohawesh et al. (2014) that continuous application of OMW increased soil water retention capacity. The study went further to report that OMW treated soils which exhibited changes in pore size distribution, enhanced bromide diffusion because there was more water retention and increased organic matter. Positively, it could be used in compacted soils, as it has been recorded to increase total soil porosity by decreasing macropores and increasing micropores (Mahmoud et al., 2010).

Effluent Mobility

The surface layer alteration may lead to increased contaminant transport to the ground waters. Two processes often occur when this effluent is applied to soil for a long time: filtration and adsorption. Achak et al. (2009a) observed that the particulate organic matter in the effluent adsorbed on the surfaces of the soil, while the dissolved organic matter decomposed and the nitrogenous compounds in it were oxidized to nitrate form by the bacteria in the sand filter. The nitrate may also contribute to the ground water pollution. Nitrate is not the only component of the effluent that is mobile. The study of Mekki et al.(2007) showed that phenolic compounds of lower molecular mass moved deeper into the soil than those of high molecular mass where the polyphenols can be adsorbed by the soil and monomers can be mobilized and transported by infiltration of rain water to the ground water. The application of the effluent immediately increases the content of Fe^{2+} and Mn^{2+} in the soil which increases the oxidation of monomeric phenols. This process produces recalcitrant polyphenols which inhibit bacterial and fungal activities in the soil (Piotrowska et al., 2006). Other such wastewater mobility like the winery wastewaters were also studied by (Mulidzi et al., 2002). The study discovered that organic components of the effluent were detected at the depth of the water table (1 m depth) and the organic matter decomposition was slow.

1.4.2. Impact of OMW disposal on soil chemical properties and nutrient availability

OMW is characterized by high salinity and low pH with a reasonable quantity of soil nutrient. Its application on soil has given some contradictory results. It is expected that OMW application may have beneficial effect on the nutrient availability to plants (Chaari et al., 2014). Some researchers have reported no change in pH due to OMW application (Achak et al. 2009b;

Chartzoulakis et al. 2010; Chaari et al. 2015) while some reported increased soil pH. Chaari et al. (2014) noted pH increases on the surfaces of soil at the depth of 0-20 cm for 200 $m^3 \cdot ha^{-1}$ effluent application rates. Meanwhile, Mekki et al. (2007) did not observe any change in pH with depth, but Magdich et al, (2012) reported decreased soil pH which was attributed to the acidity of the OMW

Barbera et al. (2013) opined that OMW acidification impact is buffered by carbonates, hence application on soils rich in lime will reduce the acidification effect. Barbera et al. (2013) explained that high volumes of OMW application to soils can be detrimental due to the OMW acidity which may be aggravated by the acid produced by lipid hydrolysis especially when the soils have low base saturation and are not balanced by exchangeable cations. This implies that the acidic pH of the OMW will result in the long term loss of carbonates from the top soil (Mahmoud et al., 2010). Therefore use of OMW for soils rich in lime was recommended.

The EC content of the soils treated with effluent increases due to the high salt content of the effluent. However, the EC of the OMW is strongly related to the impact of olive oil extraction, while the increase in the EC of the soil is related to increase in quantity of OMW applied. Many studies have reported increased EC after the application of the OMW (Barbera et al., 2013; Di Serio et al., 2008; Mekki et al., 2009).

Increased quantity of organic matter, N, P, K, Mg, Ca and Na have been reported, but Chaari et al. (2015) reported high Ca/Mg ratio after application of OMW which was related to soil magnesium level decrease. They noted that the calcium and magnesium (Ca/Mg) ratio in the soil is an important factor that affects mineral nutrient availability. The other factor that was reported by them was the decrease in the calcium content due to the addition of Na from the OMW. The effluent induces the immobilization of certain nutrients and causes loss of some essential nutrients in the soil (Arienzo et al., 2009; Cabrera et al., 1997). Nitrification activities, lower nitrate and nitrite content were observed in the plots treated with OMW during the vegetative cycle of the harvest (Gamba and Piovanelli, 2005). Sierra et al. (2007) also noticed a decrease in nitrate formation with time and as the rate of OMW applied increased.

According to Sierra et al. (2007), who studied the agronomic use of OMW, there was an increase in pH, organic matter, N, P and K supply in soils treated with the OMW. Still, they recommended that quantities exceeding 180 $m^3 \cdot ha^{-1}$ should not be used as they observed immobilization of N, increased salinity and increased phenol concentration. Within the recommended limit, there was increased labile organic matter in the soil, which enhanced

degradation and release of nutrients as microbial activities also increased with OMW application. It has been widely reported that OMW increased soil fertility and plant growth due to its nutrient content (Komilis et al., 2005; Mekki et al., 2013) especially when they are applied in small quantities.

Phytotoxicity

Phytotoxicity of the olive mill wastewater has been often reported to be the reason for its most negative effects. It has been related to its phenol content, especially the monomeric phenols, reported by Mekki et al. (2013) as the cause of the phytotoxicity and antimicrobial effects. Barbera et al. (2013) also suggested that polyphenol is the main limiting factor for spreading OMW due to its phytotoxic and antimicrobial potentials. The lower molecular weight phenols are the more toxic ones. They inhibit bacterial activities in the soil and seem to be more persistent when the effluent is applied in large quantities (Barbera et al., 2013). Their detection also increases with depth (Mekki et al., 2007), indicating leaching and accumulation. Mekki et al. (2013) implied in their review that polyphenols inhibit certain microbial activities (particularly those of actinomycetes) which reduce the breakdown rate of organic matter and as a result slow down the nutrient release processes. This effect of the polyphenols, as reported by Sierra et al. (2007), manifests when the quantity of OMW applied is high and the labile organic carbon of the OMW cannot stimulate enough microorganism activity to decompose the phenols.

It is believed that the effluent is negatively affecting germination of plants and crop growth due to its polyphenolic content. This was confirmed by Mekki et al. (2013) when untreated OMW inhibited germination and plant growth compared to treated effluent samples. Barbera et al. (2013) also observed significant reduction in germination of wheat, maize and tomatoes after spreading OMW and related the effect to the polyphenolic content of the effluent. The effect was reported to be more pronounced when the seeds are planted immediately after the OMW application, probably because the phenols require some time to degrade.

Due to the polluting, phytotoxic and antimicrobial properties of OME, its on-land disposal is one of the main environmental concerns in all olive oil producing countries (Paredes et al., 1999; Piotrowska et al., 2006).

1.4.3. Effects of OMW application on plant growth

Most of the characteristics of OMW such as its high COD, BOD, phenol, oil and grease content have the potential to inhibit plant growth. Rusan et al. (2015) reported zero germination of

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barley when cultivated with untreated OMW and even with 50-75 % OMW dilution. Others reported inhibited growth when a large quantity of the effluent was applied (Casa et al., 2003; Komilis et al., 2005). Inhibition of seed germination and plant growth was studied by Mekki et al., (2013) and their study reported that seed germination was seriously inhibited by application of untreated effluent and even at 1/10 dilution of OME. They went further to explain that plant height as well as all the other plant parameters (number of spikes, chlorophyll, and yield) determined for *Vicia faba and Cicer arietinum* performed better with treated OMW than the control and untreated OMW. In contrast to the foregoing, other researchers reported increased growth after application of the OMW. Magdich et al. (2012) reported a 30% increase in the yield of olives after six years of application of OMW, while Barbera et al. (2013) reported that the effect of OMW application in literature varies. There is a confirmed record of modification of plant/soil relationship. The relationship seems to be species- or dose-dependent.

Table 1.3. Benefits and Risks of disposing OMW on soil

Benefits	References	Risks	References
Physical properties			
Increased soil aggregation		Clogging due to high rates	Mahmoud et al. (2012)
Increase soil porosity			
		Decreased hydraulic conductivity	Achak et al. (2009)
		Reduced water penetration time	
		Decreased infiltration rate	
Increase soil water retention capacity		Causes soil fragmentation	
		High mobility of organic components	
Soil Chemical properties			
Increased pH	Chartzoulakis et al. 2010; Chaari et al. 2015		
reduces acidity- with soils of high base saturation	Barbera et al. (2013)	Increased EC	
Increased N, P, K Mg, Ca, Na		High Ca/Mg ratio- which affects nutrient availability	
		Decrease in Ca content due to high Na	
		Immobilization of N	(Gamba and Piovanelli, 2005)
Increased soil fertility	Mekki et al., 2013)	Increased phenol concentration	

		Reduction in germination
		Slow degradation of organic materials- slow nutrient release
Effect on plant growth		
Increased yield	Magdich et al. (2012)	Inhibit plant growth

1.5. OMW treatment

Among the methods of solving the problems associated with effluent discharge, the most commonly used is direct disposal on agricultural soils (Paredes et al., 1999). Some farmers see the on-land spreading of the OMW as a simple and beneficial way of disposing of the effluent (Komilis et al., 2005) without considering the constraints of the high oil and grease content, salt and phenol contaminations. Several other methods have been employed in treating and making OMW a reusable waste, but each has its limitation(s). Some of the methods aim at destroying the toxic (phenols) compounds and some aim at removing the phytotoxins from the effluent. Methods include composting (García-Gómez et al., 2003), ponding, aerobic and anaerobic digestion (El Hajjouji et al., 2008; Hafidi et al., 2005), aerobic biodegradation (Hajjouji et al., 2007), membrane technologies (Pulido, 2015), and adsorbents used to remove phenols from the effluent (Achak et al., 2009a; 2014). Some other methods include oxidation, H_2O_2-AOP and electrochemical treatments (Belaid et al., 2013). Fenton treatments were used to destroy the toxic compounds in the solution (Khoufi et al., 2006). Different researchers have looked at this problem differently. Al-Malah et al. (2000) used a series of treatment steps composed of settling, centrifugation, and filtration to condition the OME, followed by post-treatment processes, including adsorption on activated clay. The study achieved maximum adsorption capacity with the different activated clays used. Some other researchers used other adsorbents like bentonites, zeolites, sands, fly ash, peat, activated charcoal and so on to remove the phenols from the effluent (Viraraghavan and de Maria Alfaro, 1998; Banat et al., 2000; Al-asheh et al., 2003; Yapar et al., 2005). Cici et al. (2001) used different physical and chemical methods in treating the OMW, among which are dissolved air floatation, acid cracking, which they used for emulsified oil and use of alum, ferric chloride and also an alum-clay process.

Some of the methods are seeking either to destroy or eliminate the toxic compounds from the effluent, but most of the efficient methods are too sophisticated for small-scale producers to apply. McNamara et al. (2008) suggested that the treatment of the OMW should be made simple for local farmers to use, since some of the producers are not at industrial level. From the above stated methods, adsorption has been reported to give the highest phenol removal percentage in OMW (Achak et al., 2009).

In view of this report, our study proposes to use a local and effective adsorbent "biochar" to treat the OMW. Several researchers have proposed the use of biochar as absorbent in restoring contaminated soils. Méndez et al. (2012) used sewage sludge biochar to reduce the leaching of mobile metals. Spokas et al. (2009) reported increased sorption of atrazine and acetochlor herbicide with biochar. Kasozi et al. (2010) observed more sorption of monomeric phenol (catechol) than humic acid onto biochar produced from pine, oak and grass. They reported pine biochar to have the least sorption capacity, but suggested soil amendment with biochar to sorb organic materials.

The abundance of pinewood in South Africa suggests the use of its biochar to sorb the contaminants in OMW The sorption capacity of biochar depends on the characteristics of the biochar, which is influenced by the process of pyrolysis (Uras et al., 2012). Biochars produced at temperatures of $300 - 450^{o}\,C$ have been reported to give the best properties for sorption.

The reaction of low temperature produced biochar and the effluent as regarding the availability of nutrients in the soil is not known. Cabrera et al. (2011) observed high sorption of fluometuron in the soils amended with biochar and lower leaching of the same herbicide in the same soil. Biochar has been recommended as an organic amendment because of its high stability against decay in soil environment and its apparent ability to influence nutrient availability as compared to other organic amendments. It has a highly condensed aromatic structure (Keiluweit et al., 2010) which makes it biologically inert. Therefore it has the ability of sequestering carbon in the soil and at the same time improving soil fertility.

Biochar sorption and nutrient availability are considered important in this study because the whole essence of the study is to mitigate the effect of the spreading and reuse of the OMW. Test crops have been chosen to elucidate the effect of the treatment on crop performance. Biochar is expected to neutralize the pH of the OMW and sorb the phenols and other organic contaminants from the effluent. The sorption capacity of biochar for organic contaminants has been studied by several researcher (Beesley et al., 2011; Ogbonnaya and Semple, 2013; Zhang

et al., 2013; Almaroai et al., 2014). The *in-situ* sorption may be better than use of bioremediation due to the reduced microbial activities during bioremediation (Zimmermann et al., 2010). It may be a better option than sorption on organic matter because of the higher reactive surface areas of the biochar. According to Ghezzehei et al. (2014), biochar sorbs nutrients, enhances biodegradation and then releases these nutrients gradually to plants (Laird et al., 2010).

1.6. Properties of Biochar

Biochar is a black carbon material realized from thermochemical decomposition of a biomass at elevated temperature in the absence of oxygen- a process called pyrolysis. McLaughlin et al. (2012) viewed biochar as the modern adaptation of 'Terra Preta' which is an example of anthropogenic char being credited with improving the soil fertility in the past. It is known as a carbon rich material capable of sequestering carbon to the soil for mitigating climate change and improving soil properties, enhancing soil fertility, microbial activities and hence increase crop productivity (Tang et al., 2013). The functionality of biochar is dependent on its property which is strongly affected by the biomass type and temperature of production. These factors (temperature and biomass type) result in the variations observed in biochar and its functionality. It is noteworthy to know that functional groups of biochar affects its properties. Li et al. (2013), further explained that biochars with high pH and EC could be produced to be applied on an acidic soils to ameliorate the acidity by controlling the high percentage of aromatic carbons using 2D correlation spectroscopy. The other inherent property of biochar which is high specific surface area, specific structure and porosity contributes to its contaminants adsorption capacity and increasing water and nutrient retention of the soil (Tang et al., 2013). The inclusion of this material into this study is therefore relevant as South Africa is not only experiencing poor soil conditions coupled with the contaminants effect from wastewaters but also the effect of climate change.

1.7. Gap in Knowledge

The overall question to be answered is what the effect of adding biochar to the soil contaminated with olive mill wastewater will be. How exactly the biochar is going to react with the effluent with respect to nutrients availability and toxic contaminants removal from the effluents is yet to be understood. It is also not clear how much of the pore spaces are coated with the organic matter from the effluent and what exactly is causing the coating. It is yet to be determined if the amendment with biochar will affect solute mobility in the soil and how it will

affect soil structure. The quantity of effluent that can be disposed of on the different soil types and the quantity of biochar that can be used on each soil type for sustainable disposal have also not been determined. Among recent studies there is no study on amendment of biochar on olive oil mill wastewater contaminated soil and whether the amendment will result in the decrease or increase in certain soil properties. Furthermore, it is unknown whether the biochar is going to enhance COD and phenol reduction and whether it will affect phenol mobility. This study is also going to explore biochar amendment on two different soil type, which will help in making wide range of recommendations to farmers.

1.8. Aims and organization of dissertation

Research Questions

What are the possible ways of solving the problems of olive oil effluent disposal on soil?

- How does the on-land disposal affect the germination and growth of field crops in various soil conditions?
- Can the negative effects of effluent disposal be mitigated by application of biochar as a soil amendment?
- What is the possibility of sorbing the contaminants with biochar ex-situ prior to effluent disposal?

Hypothesis

The hypothesis of this study is that biochar will remove the phenol and COD from OMW to acceptable level which will be below the guideline application levels stated for both the South African and international effluent disposal limits. The biochar amendment can mitigate the pollution from olive oil mill wastewater on-land disposal by adsorbing the organic contaminants. The OMW filtration through biochar prior to disposal may be a viable effluent treatment option.

Main research objectives

The main objective is to explore *in-situ* and *ex-situ* options for pollution prevention by way of laboratory and greenhouse experiment.

Specific Objectives

- Determine the effectiveness of adsorption of organic contaminants using biochar;

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- Determine the contaminant breakthrough curves and sorption capacity of total phenol and COD removal in columns packed with biochar, sand, Hutton clay loam soil and their mixtures with biochar;

- Determine the effect of olive mill wastewater disposal on porosity and hydraulic properties of soil columns;

- Assess the crop growth parameters on soils amended with biochar *in-situ* in the case of continued effluent disposal

Chapter 2
SORPTION, KINETICS AND MECHANISM OF PHENOL ADSORPTION FROM OLIVE MILL WASTEWATER ONTO PINEWOOD BIOCHAR

2.1. Introduction

Olive mill wastewater (OMW) on-land disposal is a common practice in olive-oil-producing regions (Barbera et al., 2013; McNamara et al., 2008). The phenolic compounds present in OMW are considered the major contributors to the toxicity and antimicrobial activity of this wastewater (Ena et al., 2012; Achak et al., 2014). They are the major cause of the low germination of crops in the OMW polluted soils (Casa et al., 2003). Phenolic compounds limit the microbial degradation of OMW and microbial activity in the soil. Hameed and Rahman (2008) considered the polyphenols as priority pollutants due to their harmful effects on organisms even at low concentrations and many of them have been classified as hazardous pollutants (Hachicha et al., 2009) because of their potential harm to human health. The United States Environmental Protection Agency (EPA) called to lower polyphenol content in wastewater to less than 1 mg·L^{-1} (Banat et al., 2000).

Olives are a source of at least 30 phenolic compounds, including hydroxytyrosol, oleuropein, tyrosol, caffeic acid, gallic acids, vanillic acid, etc. (Tuck and Hayball, 2002). The total phenolic content of OMW is quite high and may range from 500 to 23 000 mg·L^{-1} (McNamara, 2008; Belaid et al., 2013) in all cases exceeding the EPA guidelines. About 1 % of the polyphenols in the olive fruits goes into the oil, and the majority of the olive phenols go into the wastewater and the sludge (Hachicha et al., 2009) which are later often disposed of on land. However, OMW is known to contain a considerable amount of major plant nutrients for soil amendment (Mekki et al., 2013) and should not be discarded, but rather used in agricultural production provided the phenolic content is reduced to acceptable levels.

The high organic load and the polyphenol content of OMW give rise to limited treatment options. Of the several treatment methods used so far, adsorption has proven to be the best. Adsorption is an equilibrium separation process and an effective method for water decontamination application. Achak et al. (2009b) reported that adsorption is superior to other techniques for water reuse in terms of initial cost, flexibility and simplicity of design and ease of operation.

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Phenolic compounds have been adsorbed on different matrices: peat, fly ash and bentonite (Viraraghavan and de Maria Alfaro, 1998; Srivastava et al., 2006), activated carbon (Hameed and Rahman, 2008; Gundogdu et al., 2012; Hegazy et al., 2013) and many other adsorbents. However, the kinetic experiment reported by Viraraghavan and de Maria Alfaro (1998) showed that it took longer equilibrium time (15 h) for polyphenols to be adsorbed on some materials other than activated carbon.

The sorbent properties of biochars could largely be affected by the treatment it received. Pyrolysis temperature for instance determines the porosity of charred biomass (Uras-Postma et al., 2014). Accordingly, the sorption ability of a biochar is dependent on its surface area, surface chemistry (Kasozi et al., 2010) and the molecular structure which are all affected by both precursor and pyrolysis conditions. The sorption of polyphenol from OMW is also affected by the properties of the OMW itself. Some studies have focused on the sorption of individual phenols (Achak et al., 2014). Kasozi et al.(2010) investigated the sorption of an individual phenol (catechol) and humic acid (HA) onto biochar and discovered that it took the catechol 14 days to reach equilibrium while HA reached equilibrium in 1 day. Lower concentration of catechol had direct affinity for micropores unlike HA, which were excluded from the micropores. Gallic acid (GA) is one of the commonly found monomeric phenols in OMW and its mechanism of sorption compared to the OMW on pinewood biochar needs to be investigated because of the polycomponent nature of OMW. Moreover, we have used GA as the standard for phenol determination in this experiment. The sorption of a compound is dependent on the properties of the compound and structures and to an equal extent, on the surface chemistry of the biosorbent (Dávila-Jiménez et al., 2003). Tseng et al. (2003) stated that pinewood adsorption of phenol from wastewater did not necessarily occur within the micropores of the activated pinewood while it fitted nicely to the Freundlich isotherm compared to the dyes that fitted to Langmuir. That result could be due to the nature of the phenols they used, which are, 3-chlorophenol and o-cresol). Notwithstanding, pinewood biochar is cheap and an abundantly found waste in South Africa and has been proven by Núñez-Delgado et al. (2015) to give better sorption than oak wood ash in removing Cr(VI). In this regard, the objective of this research is to study the kinetics and mechanisms of interaction between the OMW and the pinewood biochar and compare the sorption capacity result to the sorption capacity of gallic acid as well as the effect of biochar doses and biochar properties to sorption.

2.2. Materials and Methods

2.2.1. Chemicals

Sodium carbonate anhydrous (min. 99.5 % assay), gallic acid (anhydrous) for synthesis, Folin-Ciocalteu phenol reagent, and methanol (assay min. 99%) were obtained from Merck (Pty) Ltd., South Africa.

2.2.2. Olive mill wastewater and its characterization

The OMW used in this experiment was collected from an olive farm situated 5.8 km north of the Stellenbosch University. The farm uses a two- phase system of production, which has the advantage of reduced effluent rate production (Borja et al., 2006). This system of extraction is known to have two major outputs (oil and wet solid) as well as little wastewater (Figure 1.1). The system contains a separator in the design of the plant used to extract the water that could be suspended in the oil after it comes from a two phase decanter. The two phase decanter theoretically should not leave any water in the oil but due to the nature of the design and the original function of the horizontal centrifuge, it does however leave marginal amounts of wastewater in the oil after centrifugation. This water is removed with the use of the separator to improve the quality of the oil produced. The wastewater on this farm was stored in a concrete dam for further disposal as hazardous waste. The wastewater sample was collected from the dam and was kept refrigerated at 4°C until the time of use. A representative sample from shaken 20 L containers was filtered through a 0.45 µm nylon filter for characterization.

Major elements were analyzed on a Thermo ICap 6200 ICP-AES. The instrument was calibrated using NIST (National Institute of Standards and Technology, Gaithersburg MD, USA) traceable standards to quantify selected elements. A NIST-traceable quality control standard of a separate supplier than the main calibration standards was analyzed to verify the accuracy of the calibration before sample analysis. The main elements present in the effluent are given in Table 2.1 along with other characteristics determined as follows: The total phenol content was determined using the Folin-Ciocalteu method described by Ainsworth and Gillespie (2007), as modified by Alhakmani et al. (2013), with undiluted 0.5 mL of the effluent. Gallic acid was used as the standard for plotting the calibration curve. The absorbance was read off with a Pharmacia Ultrospec III UV/Visible spectrophotometer at a wavelength of 765 nm. The procedure was described in detail in section 2.2.6. The pH of the effluent was read off with a EUTHECH 700 pH meter with 30 mL of the sample. Chemical oxygen demand (COD), total

organic carbon (TOC), total nitrogen (TN) and fats oil and grease (FOG) were determined as described in the Standard Methods (2012).

The effluent properties determined in this study (Table 2.1) is similar to the three phase wastewater characteristics and it showed that the organic contaminant (COD, TSS, FOG and phenols) values of the effluents were very high. Comparing these values to the South African allowable limits of effluent disposal, there is strong need to treat the effluent before disposal. The pH, EC, N and P content of the OMW were also higher than the South African legal standards of effluent disposal (Table 1.2).

Table 2.1.Characteristics of the Olive mill wastewater used in the study

Parameters	Values	Parameters	Values
COD mg·L^{-1}	13700	Ca mg·L^{-1}	13.2
Total Phenol mg·L^{-1}	971	Mg mg·L^{-1}	14.9
Kjeldahl N mg·L^{-1}	82	Na mg·L^{-1}	19.93
TOC mg·L^{-1}	2000	K mg·L^{-1}	339.7
pH	4.6	P mg·L^{-1}	24
C:N	24.4	SAR	0.62
FOG mg·L^{-1}	3440	EC dS·m^{-1}	2.41
Suspended solid mg·L^{-1}	7819	Total N mg·L^{-1}	35

COD Chemical oxygen demand, TOC Total organic carbon, FOG Fat oil and grease

2.2.3. Biochar and its properties

The pinewood biochar used in this study was produced by slow pyrolysis at 450° C and previously characterized by Sika and Hardie (2014). It was crushed and passed through a 2 mm sieve before analysis. The pH of the biochar was determined as described in Cheng and Lehmann (2009) by using a 1:20 ratio of biochar and water. Proximate analysis was carried out using thermal gravimetric analysis (TGA) to determine the fixed carbon, moisture, volatiles and ash content. The Brunauer-Emmett-Teller (BET) specific surface area was determined using nitrogen gas on a Micrometrics ASAP 2010 (Micrometrics, Norcross, GA, USA) system. Elemental analysis was done using the dry combustion (Elemental Analyzer 3000 series, EuroVector, Milan, Italy) to determine the total carbon (C), nitrogen (N) and hydrogen (H) content of the biochar and oxygen was obtained by subtraction. The functional groups of the biochar were determined using Fourier Transform Infrared (FTIR) spectroscopy. The FTIR analysis was carried out with 64 scans on potassium bromide (KBr) pellet using 1% biochar

sample with 50 mg of KBr. The KBr and biochar were properly mixed and pressed into stable pellets using a hydraulic press and pelleted under pressure. The infrared (IR) of the pellet was analyzed using Thermoelectrron Nexus 6700 FTIR spectroscopy. OMNIC® software package version 7.2 was used for analysis of the data. The properties of the biochar are given in table 2.2

Table 2.2: Pinewood biochar properties

pH (H$_2$O)	% C	% N	% O	% H	O/C	H/C	C:N	Fixed C (%)	Volatile (%)	Ash (%)	BET m^2/g
9.30	82.71	0.53	11.11	2.95	0.13	0.04	156.1	74.45	19.90	3.04	346.42

2.2.4. Kinetic Experiment

The equilibrium time of OMW adsorption onto pinewood biochar was determined by pipetting 50 mL of undiluted OMW with GA equivalent phenol concentration of 93.98 mg·L^{-1} using ball pipette into a 100 mL bottle and adding 0.2 g of pinewood biochar. The batches of 100 mL bottles containing the same sorbent and sorbate concentrations were loaded on an industrial horizontal shaker and shaken at 200 rpm and removed at different time intervals, filtered and analyzed for total phenol concentrations using Folin-Ciocalteu reagent. The experiment was done in duplicate and the average values for the two replicates were used in all the calculations. The equilibrium time and GA sorption onto pinewood biochar was determined in a similar way using a 100 mg·L^{-1} GA solution.

2.2.5. Batch sorption

Two adsorption isotherm experiments were conducted with GA and OMW to determine the phenol adsorption capacity of pinewood biochar. Batch experiments were carried out to determine the adsorption isotherm of total phenol onto pinewood biochar using an industrial shaker (IKA-WERKE, Type IKA®KS 260 basic from GMBH and Co Kg, No. D-78219 Staufen, made in Germany) at 200 rpm at room temperature of $\pm 22^o$ C in 100 mL plastic bottles. A known mass of 0.2 g of biochar was added into 100 mL bottle containing 50 mL of OMW sample. The same mass of biochar was added into a 200 mL bottle containing 100 mL of GA sample with GA equivalent concentrations of 10.03, 41.35, 60.15, 81.45 and 93.98 mg·L^{-1} of total phenol in OMW and 0, 10, 20, 40, 60, 80 and 100 mg·L^{-1} of GA. The bottles were shaken for 1 h (OMW) and 3 h (GA) to reach equilibrium as determined in the kinetic experiment above.

The different concentrations of the OMW were obtained by diluting the effluent with different quantities of distilled water and measuring the total phenol content. These experimentally determined values of the different concentrations were recorded as the initial concentration values before shaking. The mixture was filtered using a Buchner funnel and 0.45 μm nylon membrane filters sucked under pressure after shaking. The filtrate GA equivalent total phenol concentration values were recorded as the equilibrium concentration. This experiment was carried out in triplicate.

Another set of solutions was prepared to determine the total phenol removal efficiency with different doses of biochar 0, 0.5, 1, 2.5, 5, 7.5, and 10 g with 50 ml of OMW diluted to initial concentration of 31.33 mg·L^{-1}. The same range of doses was prepared for 50 ml gallic acid of 500 mg·L^{-1} concentration in 200 ml bottle. The mixture was centrifuged after shaking for 20 mins at 2500 rpm and filtered through a Buchner funnel with a 0.45 μm nylon filter. The filtrate was analyzed for total phenol content using the Folin-Ciocalteu reagent. The quantity sorbed was calculated using equation 2.1.

$$q_e = \frac{(C_o - C_e)V}{m}$$

$$(2.1)$$

Where q_e is the quantity of phenol adsorbed, C_o and C_e (mg·L^{-1}) is the initial and equilibrium concentration, V (L) is the volume of phenol solution and m (g) is the mass of adsorbent. The percent removal (%R) was calculated as:

$$\%R = \frac{C_o - C_e}{C_o} \times 100$$

$$(2.2)$$

2.2.6.   Phenol sorption studies with pinewood biochar

Total phenol determination

The residual or dissolved total phenolic content was measured using the Folin-Ciocalteu method described in Ainsworth and Gillespie (2007) as modified by Alhakmani et al. (2013). We used gallic acid as our reference standard for plotting the calibration curve. We pipetted 0.5 mL of the filtrate and mixed with 2 mL of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water). The mixture was neutralized with 4 mL of sodium carbonate solution 7.5%, (w/v). The solution mixture was incubated at room temperature for 30 minutes and shaken at 10-minute intervals for color development. The absorbance of the total phenol concentration was measured using Pharmacia Ultrospec III UV/Visible spectrophotometer and read at a wavelength of 765 nm. The total phenol concentration was calculated from the linear equation

of the standard curve prepared with gallic acid. The concentration of the total phenols was expressed as mg·L^{-1} of gallic acid equivalent. All the experiments were done in triplicates and the average values were reported. The quantity absorbed or rather the uptake was calculated from the difference between the initial and final concentration (Equation 2.1).

2.3. Results and discussion

2.3.1. Adsorption Kinetics

The adsorption of phenol from OMW on biochar is a complex sorption reaction considering the complex nature of OMW and the polydisperse state of the biochar. It is important to understand its sorption mechanism in order to design an efficient treatment plant. The mechanism of this sorption was studied using the adsorption kinetics which according to El-Naas et al. (2010) describe the reaction pathway and the time needed to reach equilibrium, which is dependent on both physical and chemical characteristics of the adsorbent. Considering that OMW is a polycomponent material, the gallic acid solution sorption on the same biochar was studied for comparison to eliminate the effect of competing reactions and processes.

Figure 2.1 shows the effect of phenol sorption from OMW and GA solution on pinewood biochar over time. The sorption rate was quite rapid. A total of 73% of the phenol concentration was removed in the first 20 minutes and increased to 88% in the next 20 minutes before decreasing to 84% at one hour. The OMW total phenol sorption levelled at one hour, while GA sorption increased up to the three hour point, and then levelled. This trend of slightly decreased removal after peak removal indicated that there were large surface sites available for sorption on the sorbent. Achak et al.(2009a) observed this rapid sorption at the initial time with the banana peel used to sorb phenol from OMW and they explained that the rapid accumulation of the sorbate on the surface of the sorbent resulted in the formation of repulsive forces between the sorbed phenolic compounds and those still present in the sorbate. Hence, the little decrease. Mukherjee et al. (2007) opined that the rapid accumulation was due to the affinity of the organic compound to activated carbon instead. Vadivelan and Kumar (2005), who observed exactly the same trend as is the case in this study, explained that the decrease in sorbed quantity and percentage removal quantity and at which peak sorption point is passed and the processes levelled out is due to saturation of the vacant sites on the sorbent. The adsorption equilibrium of the phenol was obtained in one hour for the OMW and three hours for GA with an adsorption

of 19.27 and 25.26 mg·g^{-1} for OMW and GA, respectively. It can be seen that 84% of the phenol in OMW was removed in the first hour (See appendix 1, Table 1). The reaction showed no significant improvement after the equilibrium time and hence the equilibrium time was considered to be one hour.

For the gallic acid sorption, it was seen that more GA was sorbed by the same biochar than the phenol from OMW, indicating that other compounds present in OMW (fats, oils, etc.) may have also occupied the vacant sites and not only the phenol was sorbed. It is interesting to note that it took a longer time for the GA to reach equilibrium than the OMW. This was also observed by Kasozi et al. (2010), who suggested that the GA has smaller molecules than some of the OMW components, and hence did occupy the micropores of the char which may have been rapidly blocked by larger molecules from the OMW. Thus some OMW components may have blocked the micropores, making their interior surface unavailable and restricting most sorbate to reactions in the macropores. This could possibly explain why OMW sorption on biochar reached equilibrium faster than sorption of the GA.
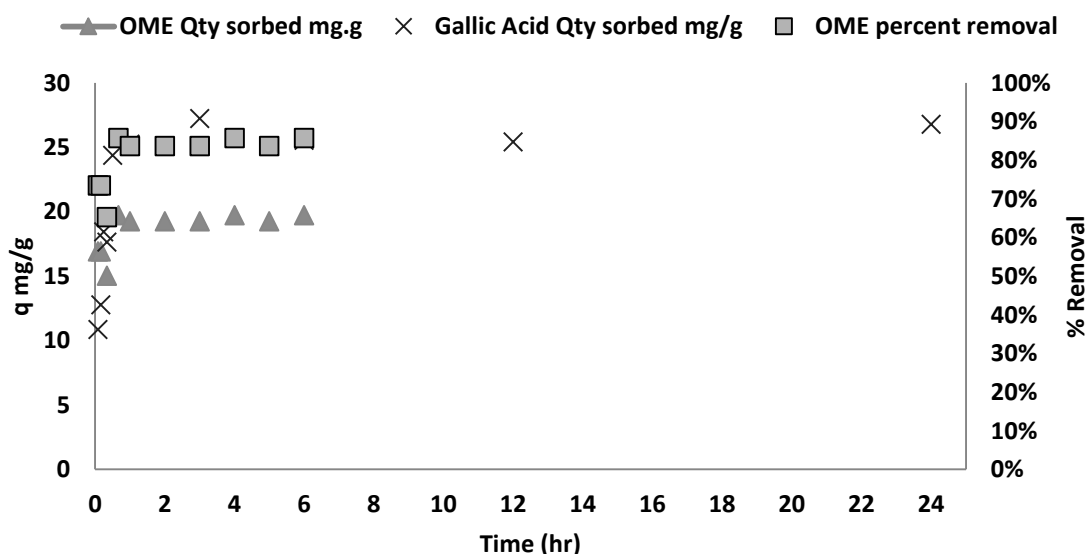
Figure 2.1. Effect of contact time on sorption of GA and total phenol from OMW on pinewood biochar

The mechanism of the sorption behavior involved in this process was investigated with the following kinetic models to better understand total phenol sorption into pinewood biochar.

2.3.1.1.   Pseudo-first-order equation

The pseudo-first order equation was fitted using (Lagergren, 1898)

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$$\frac{dq_t}{dt} = k_t \ (q_e - q_t) \tag{2.3}$$

where q_t and q_e are the amount sorbed at time t and at equilibrium and K_t, the rate constant of the pseudo-first-order sorption process. The first order dependence of concentration was evaluated with the integrated form of Equation (2.3) using the initial conditions as t = 0, q_t = 0:

$$Log \ (q_e - q_t) = Log q_e - \frac{k_1 t}{2.303} \tag{2.4}$$

The plot of $Log \ (q_e - q_t)$ versus t gives a straight line for pseudo-first-order kinetics. The slope and intercept of this plot were used to compute the sorption rate, k_1 as in Table 2.3. The positive slope of OMW sorption (1.094) indicated that it did not fit the pseudo-first-order reaction (Sparks, 2003). Conversely, the GA sorption had a negative slope of -2.8241 (See appendix 1 Figure 1 and 2) but with lower R^2 value than the pseudo-second-order model. The assumption is that the rate-limiting step may be chemical sorption or chemisorption involving valence forces through sharing or exchange of electron between sorbate and sorbent (Ho and McKay, 1999). Hence, we can state that this is not a diffusion controlled process.

2.3.1.2. Pseudo-second-order kinetic model

The data was further fitted into the pseudo-second-order kinetic model. The pseudo-second-order as explained by Hameed and Rahman (2008) is based on the sorption capacity of the solid phase and it predicts the behavior over the whole range of adsorption mechanism, being the rate controlling step and it is in agreement with the chemisorption mechanism. The equation is expressed as:

$$\frac{dq_t}{d_t} = k_2 (q_e - q_t)^2 \tag{2.5}$$

where the k_2 is the equilibrium rate constant of pseudo-second-order adsorption ($mg \cdot g \cdot h^{-1}$). Integrating Equation 2.5 for boundary conditions t = 0 to t = t and q_t = 0 to qt = t gives:

$$\frac{1}{q_e - q_t} = \frac{1}{q_e} \ k_2 t \tag{2.6}$$

which is the integrated law of the pseudo-second-order reaction. Equation 2.6 can be rearranged to obtain the linear form of:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \qquad\qquad (2.7)$$

The constant rate of the pseudo-second-order kinetics was calculated from the slope and intercept of plotting the t/q versus t (Figure 2.2). The high R^2 values (Table 2.3) of these correlations for both OMW and GA confirm that the sorption processes followed the pseudo-second-order mechanism, indicating that surface reaction was dominant. This result agrees with the result of El-Naas et al. (2010), who further explained that the phenol adsorption on the surface exchange sites continues until the surface functional sites are fully occupied, and thereafter the phenol molecules diffuse into the sorbent network for further interaction. Achak et al. (2009a) reported that the higher R^2 value of the pseudo-second-order plot is an indication of a chemical reaction which may be partly due to the hydrogen-binding between the hydroxyl groups of phenolic compound and the active functional group in the sorbent.
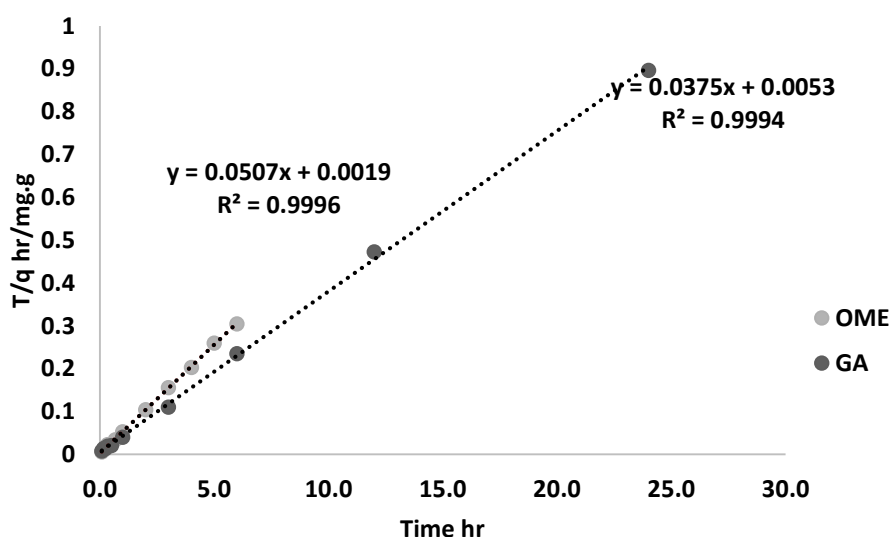
Figure 2.2. Pseudo-second-order kinetics for the adsorption of GA and total phenol from OMW onto pinewood biochar

Table 2.3. Model parameters for the different suggested sorption mechanisms

	OME	Gallic acid (GA)
Pseudo-first-order model		
R^2	0.8929	0.8578
Q_e cal (mg·g^{-1})	1.75	34.36
Q_e exp (mg·g^{-1})	19.27	25.26
K_1 (1/h)	2.52	-0.001
Pseudo-second-order model		
R^2	0.9996	0.9994
Qe cal (mg·g^{-1})	19.72	26.66
Q_e exp (mg·g^{-1})	19.27	25.26
K_2 (mg·g·h^{-1})	1.35	0.27
Elovich model		
R^2	0.5725	0.714
β	0.8257	2.6449
α	3.29	1.11
Q_e exp (mg·g^{-1})	19.27	25.26
Intra-particle diffusion		
R^2	0.4487	0.4378
K_i (mg·g^{-1}·h$^{0.5}$)	1.5035	2.5788
Q_e exp (mg·g^{-1})	19.27	25.2
Intercept	16.898	17.303

2.3.1.3.   Elovich kinetic model

To elucidate the further interaction in the sorbent network the Elovich model equation was also used to calculate the rate constant using the plot of qt against ln (t). This model does not predict any definite mechanism, but it is useful in describing adsorption on highly heterogeneous adsorbents (Bulut et al., 2008). This model is also useful for describing activated chemical adsorption where the rate decreases with time (Wu et al., 2009). The equation is given thus:

$$\frac{dq_t}{dt} = a \, exp \, (-bq_t) \tag{2.8}$$

where a is the initial adsorption rate (mg·g·h^{-1}). Wu et al., (2009) noted that the initial rate may be determined as the dqt/dt approaches a when qt approaches $0;$ b is related to the extent of surface coverage and activation energy for chemisorption (g·mg^{-1}) (El-Naas et al., 2010). Applying the boundary conditions qt = 0 at t = 0 and qt = qt at t = t. The linear form of the equation becomes:

$$q_t = \beta \ln(\alpha\beta) + \beta \ln t \tag{2.9}$$

The constants were obtained from the slope and intercept of the linear plot of qt versus ln(t) (See Appendix 1 Figure 3). The linear relationship established with the graph gave an R^2 value of 0.5725 and 0.714 for OMW and GA respectively (Table 2.3). These values are much lower than those for the pseudo-second-order and the pseudo-first-order models and do not describe the observed sorption process.

2.3.1.4. Intra-particle diffusion

It is important to understand the sorption processes involved in the phenol removal from the OMW. There could be a possibility of intra-particle diffusion (film or pore diffusion) or mass transfer and this was investigated using the intra-particle diffusion model equation:

$$q = k_i t^{0.5} + c \tag{2.10}$$

where q is the amount of phenol adsorbed (mg·g^{-1}) at time t, ki is the intra-particle diffusion constant (mg·g^{-1}) h$^{0.5}$ and c is intercept (Hameed and Rahman, 2008).
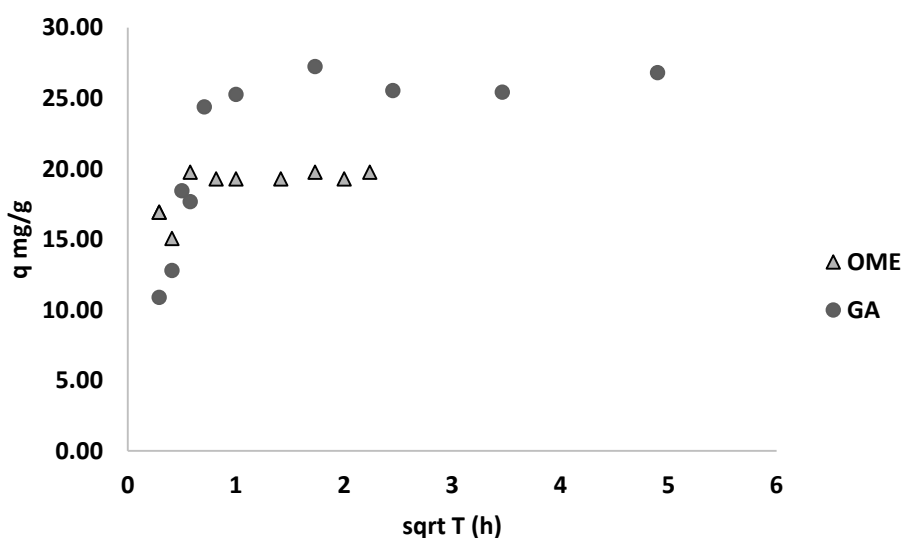
Fig. 2.3. Intra-particle diffusion of the OMW and GA sorbed onto pinewood biochar

The plot of q (mg·g⁻¹) versus $t^{0.5}$ ($h^{0.5}$) showed more than one linearity indicating that the sorption process proceeds by surface sorption and is followed by a chemical reaction stage (Fig. 2.3). The points were not continuously linear, strengthening the idea of multi-linearity. This type of condition was illustrated by the experiment of Ofomaja (2007) which showed three phase linearity of the plot. The slope of the first portion indicates the type of diffusion controlling the system. If the slope of the first portion is not zero, then film (boundary layer) diffusion is controlling the adsorption rate at the beginning as explained by Hameed and Rahman, (2008). The larger the intercept, the greater the contribution of surface sorption in the rate limiting step. The GA had a greater intercept value (Table 2.3) indicating the larger surface sorption of phenol from GA solution than from the OMW. Meanwhile these R^2 values were not higher than those of the pseudo-second-order model R^2 value (Table 2.3). It only indicated that more than one mechanism affected the adsorption. This therefore suggests that a pseudo-second-order model is the dominant adsorption mechanism in this system, which means that the overall rate of the adsorption was controlled by the chemical reaction between phenol and biochar.

2.3.2. Effect of pinewood biochar (adsorbent) dosage

Adsorbent dosage plays a significant role in adsorption system efficiency because it determines the adsorption capacity of an adsorbent for a given initial concentration of the adsorbate. It can determine the cost effectiveness of using a particular adsorbent against the other. Tan et al., (2015) reported that it is important to apply an optimum dose of biochar to contaminant removal for its cost-effective use. Increasing doses of adsorbent increased also the contaminant uptake due to the increased number of vacant sites available for sorption. This may not hold for different biochars with different adsorption properties. Figure 2.4 shows that the increased adsorbent dose increased the adsorption rate of phenol from both OMW and gallic acid. The different adsorbent doses used in this work ranged from 20-400 $g \cdot l^{-1}$ and 20-200 $g \cdot l^{-1}$ for the OMW and gallic acid respectively. The gallic acid experiment showed a maximum of 96 % removal at 200 $g \cdot l^{-1}$ dose while the OMW expressed maximum removal of 100% with 300 $g \cdot l^{-1}$ dose (Fig. 2.4). The total phenol removal from the gallic acid solution was achieved with a lesser dose of pinewood biochar compared to OMW with the same GA equivalent phenol concentration. This result is in line with the result of El-Naas et al. (2010) who showed that the effect of the dose is stronger for the synthetic contaminant than for the refinery water. It is possible as mentioned above that the OMW contains other non-phenolic substances that occupied the sites of the adsorbent and hence reduced the effectiveness of the phenol sorption on the biochar.
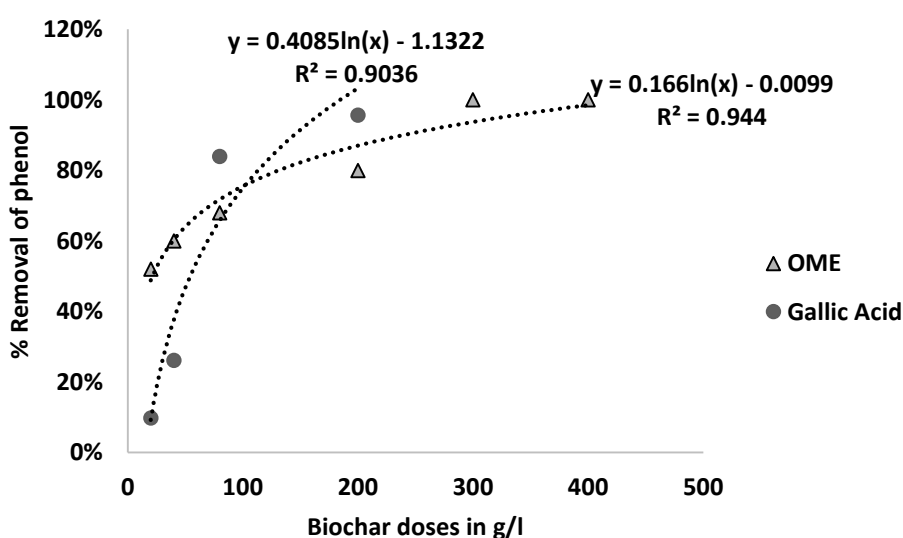
Figure 2.4. Effect of biochar doses to total phenol removal from OMW and Gallic acid synthetic solution

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2.3.3. Adsorption isotherms

In this type of work where we are trying new adsorbent and new adsorbate (OMW and pinewood biochar), it is very important to know the appropriate adsorption correlation to be able to predict adsorption behavior (Foo and Hameed, 2010). A batch adsorption experiment was explored in describing the interaction of solutes with adsorbent and it is critical in optimizing the use of adsorbent. Two isotherm models (Langmuir and Freundlich) were used to understand the sorption in these systems. The Langmuir model which was originally developed to describe gas-solid-phase adsorption onto activated carbon but has been used to quantify the performance of different sorbents, was formulated on the assumption of monolayer adsorption. This model is valid for monolayer adsorption on a surface containing a finite number of identical sites. It is assumed that the substances can only get sorbed once on the surfaces of the sorbent.

The linear form of the Langmuir is represented thus:

$$\frac{C_e}{q_e} = \frac{1}{Q_o b} + \frac{C_e}{Q_o} \tag{2.11}$$

Where Ce (mg·L^{-1}) is the equilibrium concentration of the phenols in solution, qe (mg·g^{-1}) is the amount of phenol adsorbed per unit mass of adsorbent, Q_o and b are Langmuir constants related to adsorption capacity and rate of adsorption or affinity of binding sites, respectively. The plot of Ce/qe versus Ce was plotted using the experimental data and the R^2 values were 0.8749 and 0.8127 for OMW and gallic acid solution respectively. The adsorption capacity of the gallic acid (56.180 mg·g^{-1}) is 46 % higher than that for the OMW, on pinewood biochar whereas the OMW adsorption rate was higher than that of the gallic acid adsorption rate (Table 2.3). The fast rate and lower sorption capacity is an indication of other contaminants competing for the same sites which reduced the sorption of phenols from the OMW. However, this result is different from that of Wang et al. (2013) who found that co-existence of humic acid and metal cations enhanced the sorption of polychlorinated biphenyls (PCB) on biochar. Our study, agrees with the findings of Utrera-Hidalgo et al. (1992) that gallic acid adsorbed on different activated carbons, adsorbed flat (completely) because it had no string competition from the solvent. The adsorption capacities for the different activated carbons used by Utrera-Hidalgo et al., (1992) ranged from 189-245 mg·g^{-1} which compared to our result of 56.108 mg·g^{-1} sorption into biochar, which shows a comparable, though inferior biochar performance. This result means that pinewood biochar could be used in place of the high-cost activated carbon

for sorbing phenols (gallic acid) and mainly economic considerations should determine the choice of adsorbent.

The adsorption of the phenolic compounds on the pinewood biochar can be classified using the equilibrium parameter R_L. This is an essential characteristic of the Langmuir isotherm for determining the favorability of the adsorption process. It is defined as:

$$R_L = \frac{1}{1 + bC_o} \tag{2.12}$$

where b is the Langmuir constant, C_o is the initial phenolic compounds concentration (mg·L^{-1}) and R_L values indicate the type of isotherm to be irreversible ($R_L = 0$), favorable ($0 < R_L < 1$), linear ($R_L = 1$) or unfavorable ($R_L > 1$) (Achak et al., 2009a). Our R_L value was calculated as 0.0063 and 0.0130 for OMW and GA, respectively, indicating that the Langmuir adsorption isotherm was favorable for describing the adsorption of phenol onto the pinewood biochar used in this study.

These data were also fitted into the Freundlich adsorption isotherm model. This model can be expressed as

$$q_e = K_d C_e^{\frac{1}{n}} \tag{2.13}$$

Where q_e is the quantity adsorbed, C_e is the equilibrium concentration, K_d is the distribution coefficient, and n is a correction factor. To linearize Eqn. (2.13), we have

$$\log q_e = \frac{1}{n}\log C + \log K_d \tag{2.14}$$

where the slope is the value of 1/n and the intercept is equal to log k_d. The plot of log qe versus log Ce is shown in Figure 2.6 with R^2 values of 0.9776 and 0.9762 for OMW and gallic acid solution. These R^2 values were higher than that of the Langmuir isotherm. The value of n > 1 indicates that the adsorption model is favorable (Hameed and Rahman, 2008). In our case phenol adsorption onto the pinewood biochar was favorable with n value of 1.64 and 1.48 for OMW and GA respectively. The values of 1/n > 1 are indicative of cooperative adsorption, which was not observed here.
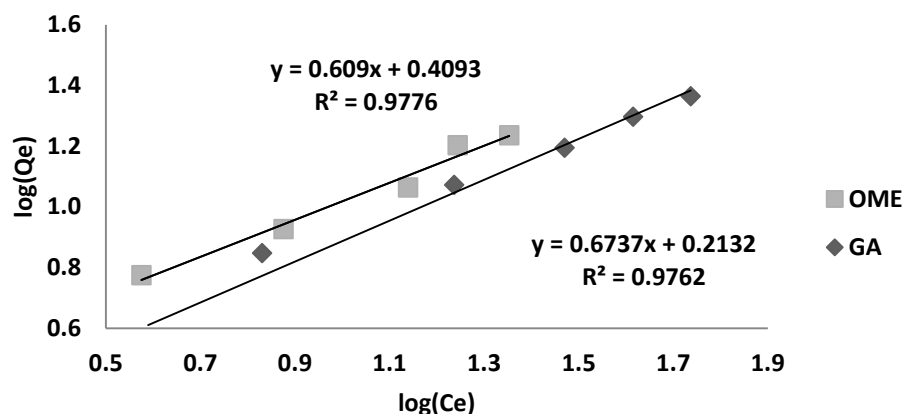
Figure 2.6. Freundlich Isotherm for phenol adsorption onto pinewood biochar

The Freundlich model may be applied to multilayer adsorption and has affinities over the heterogeneous surface (Foo and Hameed, 2010). The slope is the measure of adsorption intensity or surface heterogeneity, becoming more heterogeneous as its value gets closer to zero whereas the value below unity is an indication of chemisorption process (Foo and Hameed, 2010). The phenol sorption from the OMW showed more heterogeneity than sorption from the gallic acid solution (Table 2.4).

Table 2.4. Isotherm constants for adsorption of phenol and OMW on pinewood biochar

	OME	Gallic acid	GA sorption on activated carbon (literature)
Langmuir Isotherm			
R^2	0.8749	0.8127	
Q_0 cal (mg/g)	30.211	56.180	189 (Utrera-Hidalgo et al., 1992)
k (L/mg)	1.690	0.760	
Freundlich Isotherm			
R^2	0.9776	0.9762	
k_d (mg/g (l/mg)$^{1/n}$)	3.091	1.630	
n	1.640	1.480	
1/n	0.609	0.673	

2.3.4. The effect of the biochar properties on the adsorption of gallic acid and phenols from the OMW

We observed greater sorption of gallic acid from solution than phenols from the OMW on the same pinewood biochar (Table 2.4), most likely due to the inherent characteristics of both the sorbate and the sorbent. It is obvious that the OMW contains other contaminants which probably occupied the surfaces of the adsorbent first before the phenols. At the same time, the surface chemistry of the sorbent also influences adsorption. The oxygen containing functional groups may determine the mechanism and extent of phenolic compound adsorption.

The pinewood biochar sorbent used in this study was found to have polar functional groups like hydroxyls, carboxylic acids etc., as shown in (Figure 2.7), which enhanced the sorption of polar substances. According to the opinion of Chun et al. (2004), the higher the proportion of polar functional groups, the more hydrophilic the char. Hence the water molecules in the OMW could have formed clusters to prevent the non-polar substances from adsorbing on the surfaces. Their experiment explored the sorption of nitrobenzene and benzene from water so they can have that conclusion. But we rather say that it was the oil and fat (Table 2.1) component of the OMW that occupied the surfaces of the char instead. The gallic acid solution is more polar and would sorb more on the sorbent than the apolar molecules present in the OMW.

Oxidized biochar has a higher capacity for removing phenols than non-oxidized charred materials. The high carbon value (Table 2.2) and decreased O and H indicated the biochar is strongly carbonized (Chun et al., 2004). The calculation of the (O+N)/C ratio giving a high value of 0.14, is also an indication of presence of polar functional groups. The O/C ratio which is indicating the carbonized state of the biochar and the degree of hydrophilicity, shows that the value of 0.13 is hydrophilic. Chen et al. (2008) reported that H/C ratios >1 are an indication that biochar contains a good quantity of original organic residue. The H/C ratio of 0.04 confirms the sufficiently carbonized state of this pinewood biochar and relatively small contribution of oxidation to ionic interactions.
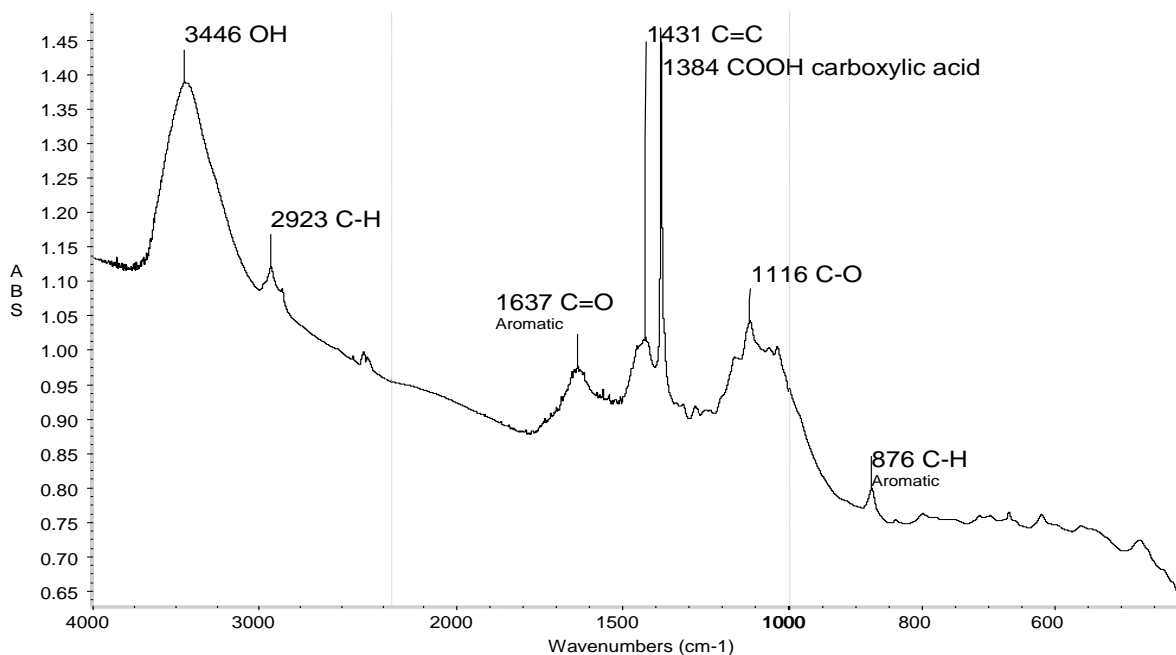
Figure 2.7. FTIR spectral characteristic showing the main functional groups of the pinewood biochar

2.4. Conclusion

The sorption of GA and phenols from OMW was quite rapid. The system stabilized within one to three hours. It implies that a filtration system using pinewood biochar may be designed with rather short retention times.

In both cases of GA and OMW the sorption process was mainly chemisorption, kinetically following the pseudo-second-order model (R^2 = 0.9994 and R^2 = 0.9996 respectively). The sorption process is described by the Freundlich multi-layer model for both GA and the phenols sorbed from the OMW, supporting the surface sorption and chemical sorption process.
Sorption capacity of phenols from the OMW on biochar is significantly (46%) lower compared to sorption of GA from solution. This may be attributed to direct clogging of biochar micro pores by fats, oils and greases as well as competing reactions (sorption of non-phenolic compounds) present in the OMW.

Although sorption of GA on pinewood biochar was significantly lower compared to sorption of GA on AC reported in literature, pinewood biochar may still be a viable solution for some filtration systems, and economic considerations along with material availability should determine the choice of a suitable sorbent. Conclusively, pinewood biochar can be used to

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remove the phenols contained in the OMW though more biochar than the quantity needed for GA will be required since the OMW has other interfering contaminants compared to single phenol GA.

Pinewood biochar was shown to fully sorb phenols from OMW at the rates of 300 $g \cdot L^{-1}$ and 200 $g \cdot L^{-1}$ from GA. Such rates applied to the soils allowed partial mitigation of the negative effects of OMW on-land disposal on plant growth. The polar functional groups of the pinewood biochar enhanced its sorption abilities.

Chapter 3

COLUMN DYNAMICS AND BREAKTHROUGH CURVES DETERMINATION FOR PHENOL AND COD REMOVAL FROM OLIVE MILL WASTEWATER USING SAND AND OXISOL TO PROTECT GROUNDWATER POLLUTION

3.1. Introduction

Phenolic inhibition of biological activities of microorganisms is responsible for the stabilization of organic load in industrial and domestic effluents (Moustafa, 2004). In addition, the fat, oils and greases (FOG) content of OMW reduces its treatment efficiency with bio-digesters by causing fouling of the machines (Pulido, 2015). Moreover, the phenols present in the olive mill wastewaters are very mobile, as reported by Mekki et al. (2007). The mobility of these contaminants threatens ground waters as well as causes soil degradation at on-land disposal sites (Kapellakis et al., 2015; Mekki et al., 2013).

On the other hand, COD which is a measure of the oxidizability of a substance, expressed as the equivalent amount of oxygen in water, largely measures organic matter content in water. This test predicts the oxygen requirement of the effluent and is commonly used for assessing treatment plant performance (Mekki et al., 2013). COD and phenol contents of more than 250 and 5 mg·L^{-1} in water respectively indicate poor water quality (TERI and TVPL, 1974). Where the South African legal requirement for winery wastewater allows COD level 0f 400 mg·L^{-1} but up to 5000 mg·L^{-1} for irrigation quantity of not more than 50 m^3·day^{-1} (Van Schoor, 2005).

Chhonkar et al.(2000) found that, despite the increase in organic matter and plant nutrient content of the soils treated with the effluent, the application of large amounts of these effluents with high COD and BOD may have adverse effects on soil health by increasing pCO$_2$ and temperature. Mahmoud et al. (2010) also reported that OMW may be characterized by high salinity apart from high organic matter content. They have showed that suspended solids in this wastewater may block soil pores and decrease saturated hydraulic conductivity because of accumulation of grease and oil in upper soil horizons. Therefore, direct on-land disposal of such effluents is not encouraged, and hence there is a need for prior removal of both phenols and the COD.

Column experiments used in treating wastewater in soil infiltration treatment (SIT) proved to be a good alternative for wastewater treatment with the considerations of efficiency and cost. Such filtration systems are widely used for wastewater treatment and reuse in the United States, France, Israel and other countries (Zhang et al., 2007). However, on-land disposal demands a large land area. Therefore, column filtration of the OMW and other wastewater to reduce and possibly reuse the effluent (Chowdhury et al., 2012; Welz et al., 2012; Achak et al., 2014) may be a better alternative. Sand washing has been proposed as possible remediation technologies for soils contaminated by petroleum and other hydrophobic organic chemicals. It has often resulted in up to 60 % reduction of contaminants to be treated with further clean up methods (Bhandari et al., 2000). Introduction of biochar into this process, knowing the potential of biochar in sorption, is believed to improve the whole process.

Here we explore the breakthrough curve to show the behavior of water contaminants to be removed from the OMW effluent in a fixed bed filtration system. The area under the breakthrough curve obtained by integrating the adsorbed concentration (C_{ad} mg·l^{-1}) versus the throughput volume (L) plot can be used to find the column capacity (Oztürk and Kavak, 2005). Other models could also be used to understand the column capacity. The capacity of the filter is affected by the processes developed during the movement of particles through a column. The deposition of the materials will affect the pores and the filtration capacity of the filter. We therefore aim to achieve the following objectives:

1. Determine the effect of pre-washing and biochar addition to the column capacities for the sorption of phenol and reduction of COD levels;

2. Understand the breakthrough curves (BTC) of phenol and COD in the different columns with different treatments;

3. Determine the effect of prewashing and biochar addition on the pH and the EC of the filtrates.

3.2. Materials and methods

3.2.1. Materials used

Volumetric flasks of 1000 mL was purchased from Sigma, PVC pipe and tubing purchased from Agrimark stores in Stellenbosch, South Africa. Other apparatus used, included burette stand and 300 mL collecting flasks.

The olive mill wastewater and biochar used in this study have been described in Chapter 2 while the sand and Hutton clay loam are described in Chapters 5 and 6. Some key properties of the sand, Hutton clay loam soil, and biochar and soil-biochar mixtures are given in Table 3.1.

Table 3.1. Characteristics of the sand, Hutton clay loam, biochar and soil-biochar mixtures used

Sample	pH (H_2O)	EC (mS/m)	% C	% N
Sand (S)	9.15	12.10	0.04	0.006
Sand Biochar (SB)	9.13	15.3	0.06	0.007
Hutton Clay loam (H)	4.20	14.0	0.74	0.05
Hutton Clay loam Biochar (HB)	4.30	14.5	0.78	0.04
Biochar (B)	9.38	0.75	82.71	0.53

3.2.2. Effluent

The effluent used for this study was collected from olive producer located in the vicinity of Stellenbosch University, but represented a new batch of effluent accumulated towards the end of the 2014-2015 processing season. The company uses a two-phase method of extraction of olive oil. The raw effluent was collected fresh from the separator point of processing. This is the point after the pulp has been removed and water and oil that is remaining is passed through the separator to separate the oil from the water (OMW) to enhance the quality of the oil produced. After this point, the wastewater is piped to the factory's dam, where the sample was collected. The wastewater was stored in a refrigerator at 4° C until use. The sample for ICP-AES analysis was filtered with a 0.45 micron filter using a Buchner funnel. The effluent characteristics are tabulated below (Table 3.2).

Table 3.2 Characteristics of the olive mill wastewater used in this study

Parameter Analyzed	Values
Total organic carbon (mg·l^{-1})	2450
Chemical Oxygen Demand (mg·l^{-1})	62591
Total Nitrogen (mg·l^{-1})	58
Suspended Solids (mg·l^{-1})	7819
Fat Oil and greases (mg·l^{-1})	3440
Total phenol (mg·l^{-1})	1388
pH	4.67
EC (mS·m^{-1})	1

3.2.3. Packed bed column system

Ten PVC columns of 30 cm length and 5 cm diameter were used to determine the breakthrough curves and filter performance. The column design included an outlet pipe of 290 mm length and 5 mm diameter as shown in Figure 3.1. The experiments were carried out at room temperature (day time 22± 1° C).

Figure 3.1. Column experimental setup showing the inverted volumetric flask maintained at constant pressure head and the effluent pipe inserted into the collecting flask

The columns were packed with different types of soils and soil mixtures (sand, Hutton clay loam, sand mixed with 2% pinewood biochar, Hutton clay loam mixed with 2% biochar, and biochar alone) (Table 3.3). The column was first layered with 100 g of the pure sand at the bottom to serve as filter and gauze to prevent particle discharge into the collected filtrate.

Table 3.3 Different treatments' designations and column bulk densities, mass of materials in the column and the quantities of de-ionized water used in pre-washing of the columns

Treatment	Designations	Bulk densities (g·cm⁻³)	Mass of soil in column (g)	Pre-wetting DI (mL)
Sand	S	1.56	779.48	
Sand + biochar	SB	1.52	760.50	
Sand washed	SW	1.58	790.30	-
Sand + biochar washed	SBW	1.52	709.93	250
Hutton clay loam	H	1.52	718.62	
Hutton clay loam washed	HW	1.52	715.78	200
Hutton clay loam + biochar	HB	1.50	751.93	
Hutton clay loam + biochar washed	HBW	1.52	757.65	190
Biochar	B	0.56	280.11	
Biochar washed	BW	0.60	302.23	390

The treatments were marked as shown in Table 3.3. The columns were filled with air-dry soil sieved through 2 mm sieve to approximately 24 cm column height and were packed with a systematic rhythm with resulting weights and bulk density as reported in Table 3.3.

The columns were classed into two sets. The first set of columns was pre-washed with deionized water. The washing of the columns was to simulate the effect of OMW disposal onto wet polluted soil for we anticipate that disposing OMW after rains could affect the downward movement of the contaminants and their transport to the ground waters. This initial flushing was also to remove the small charcoal particles from the filters and reduce the filtrate turbidity before applying the effluent.

The pre-wetting of the columns was done column by column. Different amounts of de-ionized water (DI) were used to pre-wet each column as shown in Table 3.3. Pre-wetting was done by

adding 10 mL of DI slowly on top of the column at short intervals repeatedly. Care was taken to make sure that no air was entrapped in the column while it got saturated to avoid preferential flow during the experiment.

At saturation, the columns were made up to 2000 mL with deionized water which was passed through five of the washed columns (SW, SBW, HW, HBW, BW) before OMW of 1000 mL was passed through each of the columns. The saturated hydraulic conductivity calculated from these pre-washing stages were recorded in Table 4.1 as K_{sat} before.

The second set of columns were subjected to OMW filtration from air-dry state. The air-dry columns were brought to saturation with 195, 145, 245,145 and 245 mL of the OMW for H, SB, B, S and HB respectively. After saturation, the OMW was added up to the total volume of 1000 mL to each column and the filtration process was monitored.

A constant head of 5 cm above the surface of the soil was maintained throughout the period of the OMW and DI steady-state flow filtration in all the columns as shown in Figure 3.1. The volume of the eluent and time of collection was recorded and used to determine the flow rates for each column. The individual column flow rates were determined by dividing volume eluted with time taken, and the values are given in Table 3.4.

The values of COD, pH, EC and total phenol concentration calculated as GA equivalent of the eluent were determined and plotted as breakthrough curves to characterize the filtration process and filter performance of the filters.

3.2.4. Analytical methods

The chemical parameters COD, pH, EC of the eluent were determined according to standard methods (Standard Methods, 2012). Total phenol determination was done by the Folin Ciocalteu method as described by Ainsworth and Gillespie (2007) and modified by (Alhakmani et al. (2013) and the absorbance was read at 765 with a UV vis spectrophotometer and expressed in $mg \cdot L^{-1}$ of gallic acid equivalent. The detailed procedure of phenol determination is in Chapter 2.

3.3. Results and discussion

3.3.1. Breakthrough curves of the different columns for total phenol and COD removal

A breakthrough curve is a plot of concentration measured at a fixed point in the column usually at or near the outlet versus time or cumulative volume. Our study measure the concentration at the outlet versus the cumulative volume of applied OMW. The different columns used in this experiment showed different breakthrough curves, owing to their different adsorption capacities and inherent properties. However, the breakthrough curves seem more similar within column groups (sand, clay loam and biochar). Hence, the comparison within the groups. The breakthrough curves (BTC) were determined as defined by Hodaifa et al. (2014) by plotting the normalized concentration (effluent concentration divided by the inlet concentration, C_f/C_o) vs cumulative volume.

The two important parameters to determine here in order to understand the operations and dynamic response of an adsorption column are the volume for the breakthrough appearance and the shape of the breakthrough curves (BTC). The 'S' shape of the curve is normal and indicates that as the fluid travels along the column, adsorption takes place and the column gets exhausted at a point where the effluent concentration levels up at the upper part of the 'S' shape. This is different from the equilibrium model described by Neuman and Walker (1992), where the fluid in the interstitial void spaces of the column is assumed to be in equilibrium with the fluid in the soil particles throughout the column at all times. This assumption means that at any point in the column the concentration of the fluid is the same and hence no adsorption of solute occurred. In the case of differences in the effluent concentration, it signifies that adsorption and not equilibrium has occurred as observed in this study. The breakthrough point as defined by Hodaifa et al. (2014) is that point on the S-shaped curve at which the solute concentration reaches its maximum allowed (usually 5% with respect to its value in the inlet concentration). This point has been adopted in this study because it agrees with the maximum allowed concentration of the phenol and COD in treated effluent (TERI and TVPL, 1974; Dwaf, 1996)

Table 3.4. Thomas model parameters for phenol and COD removal by the different columns using linear regression analysis

Column	Phenol				COD		
	Flow rate, ml·min^{-1}	K_{Th} mL·mg·min^{-1}	q_o mg·g^{-1}	R^2	K_{Th} mL·mg·min^{-1}	q_o mg·g^{-1}	R^2
SB	0.18	2.33×10^{-7}	506	0.75	2.14×10^{-8}	6497	0.92
SBW	1.81	7.43×10^{-6}	607	0.81	9.48×10^{-7}	4024	0.86
S	1.00	1.72×10^{-6}	33	0.76	1.64×10^{-7}	11261	0.82
SW	3.15	4.77×10^{-6}	397	0.51	8.44×10^{-7}	3222	0.30
HB	0.03	6.48×10^{-8}	2704	0.78	1.03×10^{-8}	7542	0.92
HBW	0.07	4.03×10^{-8}	8145	0.75	1.56×10^{-8}	14716	0.98
H	0.03	8.21×10^{-8}	1719	0.90	2.67×10^{-10}	19797	0.63
HW	0.10	1.72×10^{-7}	2063	0.95	8.93×10^{-9}	18565	0.96
B	0.25	6.84×10^{-7}	4454	1.00	2.98×10^{-8}	71053	0.76
BW	0.15	1.11×10^{-6}	5868	0.52	3.79×10^{-8}	32346	0.84

3.3.1.1. *Phenol breakthrough curves*

The breakthrough curves shown below showed that the treatments given to the filters affected the shapes of the curves and their phenol adsorption patterns. The washed filters with or without biochars retained the phenols more than the unwashed filters, while the biochar filters retained the phenols more than the same filters without biochar. Their phenol breakthrough points (BTP) were in the order 143, 193, 298, 328, 546 and 1113 mL for the SBW, SW, H, HW, B and BW respectively. However, HBW and HB (Figure 3.2 C) never reached their breakthrough points (BTP), which means they could still clean more effluent while S (Figure 3.2 B) and SB (Figure 3.2 A) never had BTP meaning they were inefficient as their first release was more than 5 % of the initial concentration and this value is more than the maximum allowed value for phenol in treated water. The sand filters all got exhausted after about 600 mL of effluent was collected except for the S (Figure 3.2 B) filter that got exhausted after collection of only 200 mL of the effluent. This means that very little phenol was retained by the sandy soil and that biochar addition did help to alleviate the problem in this soil a little. The inefficiency of the sandy soil in retaining the phenol is in agreement with the study of Mulidzi et al., (2002),

that water soluble organic compounds from winery wastewater disposed on sandy soil were found leaching to 1 meter depth of the soil profile. However, in contrary to the finding of Achak et al. (2009b) who reported 92 % removal of phenol from olive effluent with sand filter. Their result could be right because they applied the wetting and drying method to the sand filter used which could have enhanced its performance.

Whereas the addition of biochar had no much effect to the sandy soil, it had a definite effect in the Hutton soil. All the Hutton columns showed small upward trends at some point before being exhausted. H and HW (Fig 3.2 D) got exhausted after a longer time (about 30% of the applied phenol was still going through), when 723 and 878 mL volume of effluent respectively was collected while the HB, HBW, B and BW (Figures 3.2 C and E) never got exhausted. The addition of biochar to the Hutton columns reduced the exhaustion stage of HB and HBW to 4% and 3% respectively. That is, about all the phenols remained behind in the column. This is a very effective combination.

Biochar alone was not as effective as the combination of biochar and Hutton soil. Biochar washed stayed at about zero to a very high cumulative application before it went up and then stabilized at about 0.10 (10%). This is still very effective. B also remained at zero and then shot up to over 0.30 (30%) where it did not get level off. It is interesting to note that washed columns retained the phenols more than even the biochar columns despite the increased surface area of the biochar columns. Interestingly, HBW and BW cleaned the OMW phenol content to meet the South African water quality standard for domestic use which is 1 mg·L^{-1} according to (Department of Water Affairs and Forestry, 1996).
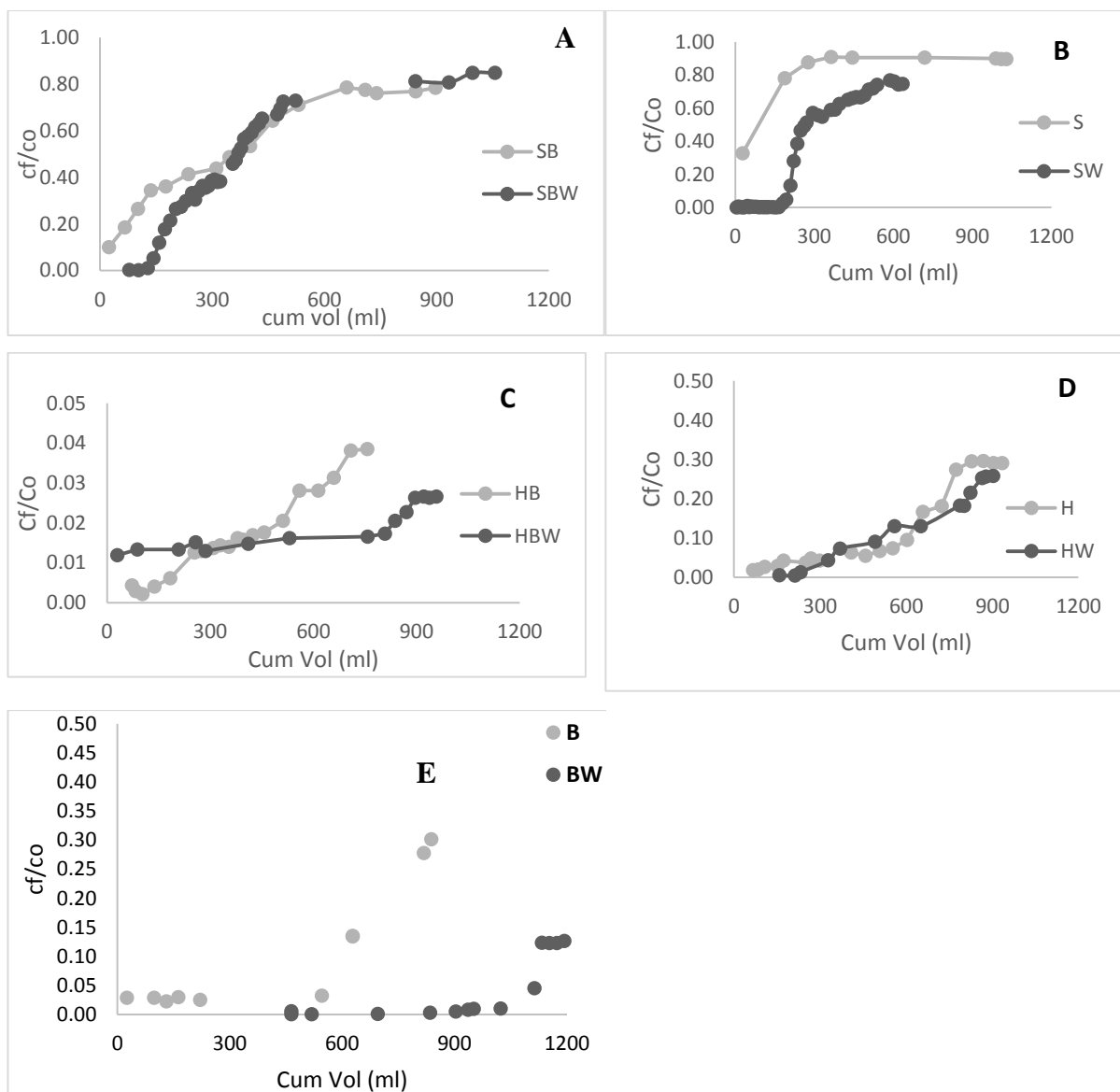
Figure 3.2. Phenol breakthrough curves for washed and unwashed columns. A) Sand biochar washed and sand biochar unwashed; B) Sand washed and Sand Column; C) Hutton clay loam + biochar washed and unwashed; D) Hutton clay loam washed and unwashed and E) biochar washed and unwashed.

3.3.1.2.   COD Breakthrough curves

For the COD breakthrough curves, some of the treatments (SB, S and H) showed non-ideal S shaped curves indicating multiple equilibrium conditions (Figure 3.3 ABD). This type of condition was experienced by Hodaifa et al. (2014) and was explained that it indicates the occurrence of another type of adsorption that may be chemical or ion exchange (slower than physical adsorption). They went further to explain that it could also mean that the adsorbent (filter) saturated very quickly and the effect of inlet concentration became less significant. The COD adsorption capacities of these filters (Table 3.4) followed this order: SW< SBW< SB<

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HB< S< HBW< HW<H< BW< B indicating that B and BW gave the best COD adsorption. Figure 3.3 also showed the COD breakthrough point (BTP) followed this order: 106, 150, 232, 380, 519, and 521 mL for H, SW, HW, HBW, BW and B columns respectively. The S, SB, SBW, HB, did not have BTP for COD which reflected also in their low adsorption capacities (Table 3.4). The sand retention of the COD causing compounds was much better than phenol retention. Contrary to the phenol sorption result, sand columns (S, SB) removed the COD better than the sand washed columns (SBW, SW) which had more COD in the eluted samples and showed breakthrough (especially the SW), meaning that washing did not enhance COD adsorption as in the phenol sorption. Biochar addition without washing was a little better as it got exhausted at 0.02 (20%). COD removal was enhanced by the biochar addition instead.

The Hutton columns did not perform better than S and SB columns as with the phenol. HBW (Figure 3.3 C) never got exhausted, but HB got exhausted at 457 mL of effluent collected. HW served a little longer than its unwashed counterpart and got exhausted after collecting 878 mL of effluent. The washing of the Hutton clay loam + biochar filter HBW elongated its capacity to lower COD better than the HB. It was the same scenario with the B and BW (Fig 3.3 E) where B got exhausted after only 546 mL of effluent and BW took more samples. It seems to record more COD but it could be attributed to the black color of the biochar. It had more adsorption capacity than the rest of the filters. Most of the filters (H, HBW, B and BW) were able to clean the OMW COD content to meet the South African water quality standard for industrial use which is 75 mg·L^{-1} as recorded in the report of the (Department of Water Affairs and Forestry 1996).
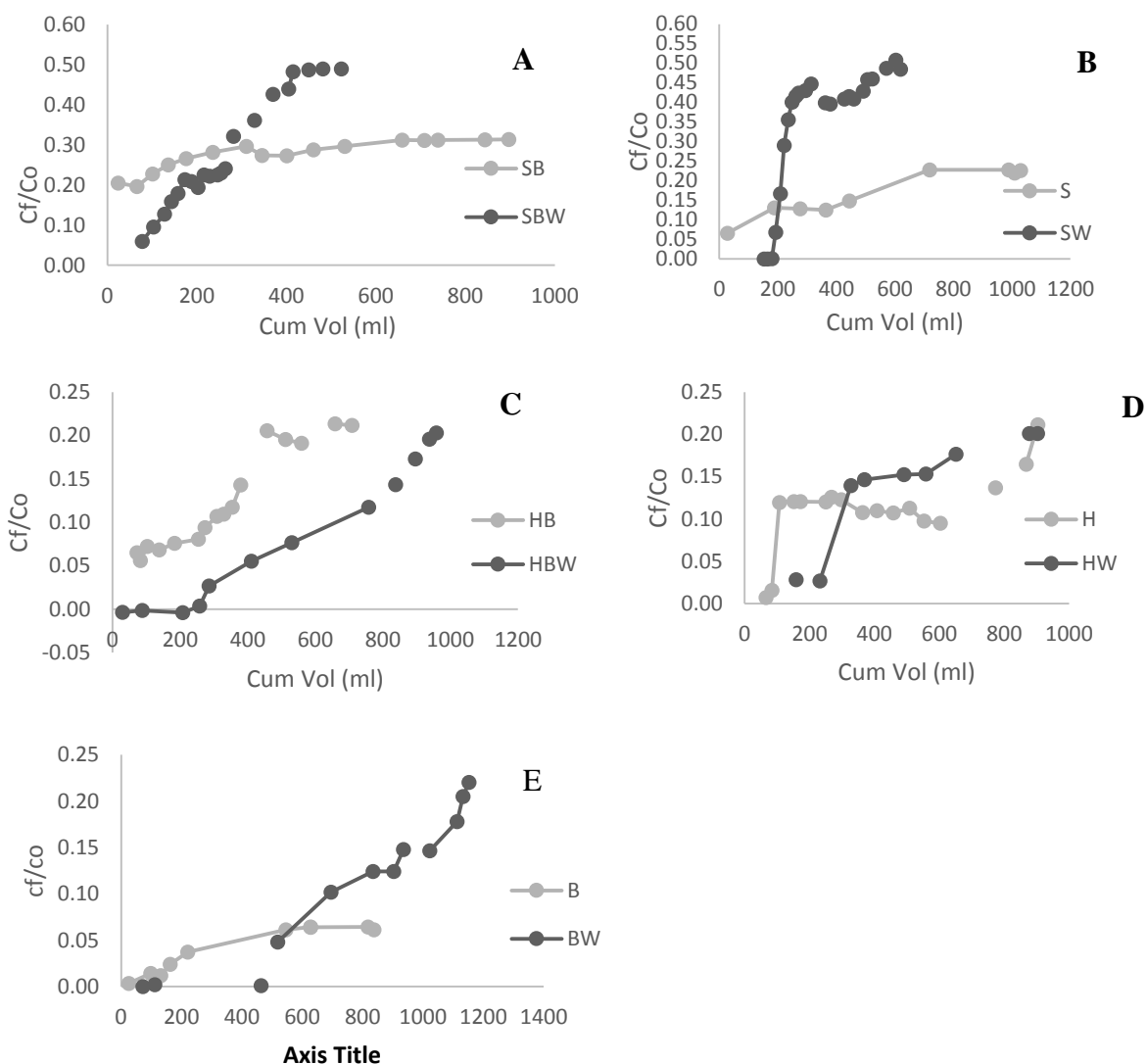
Figure 3.3 COD breakthrough curves for the washed and unwashed columns, A) Sand biochar washed and sand biochar unwashed; B) Sand washed and Sand Column; C) Hutton clay loam + biochar washed and unwashed; D) Hutton clay loam washed and unwashed and E) biochar washed and unwashed column.

3.3.2. Column dynamics study

Having looked at the BTC's of these filters, their adsorption capacities also need to be understood. These was explored using the Thomas model for the columns starting with the normalized concentration levels of 5% BTP. For the columns that did not reach the BTP, the entire values recorded in the eluent concentration was used. The 5 % level BTP was chosen because of the 5 and 250 mg·L^{-1} wastewater treatment quality standard for phenol and COD respectively (TERI and TVPL, 1974). The South African untreated winery wastewater legal

requirement for irrigation requires COD of <400 mg·L^{-1}. The international standard was used instead for our calculations because it enforces lower COD levels.

3.3.2.1. *Application of the Thomas model for phenol and COD removal*

This model is applied based on the assumption that the adsorption experiment discussed in Chapter 2 followed both the Langmuir and the Freundlich isotherm and obeyed pseudo-second-order kinetics as was also seen in the research of Chowdhury et al. (2012) for cation sorption through the columns. The Thomas model is traditionally used to understand the maximum adsorption capacities of adsorbent in column experiments with a continuous flow. This model has the following forms as stated by (Zou et al., 2009):

$$\frac{C_f}{C_o} = \frac{1}{1 + exp\left[\frac{K_{Th}}{Q}\left(q_o M - C_o\, V_{eff}\right)\right]} \qquad\qquad 3.1$$

where K_{Th} is the Thomas rate constant (L/ (min·mg^{-1}) and Q is the volumetric flow rate (mL·min^{-1}). The equation can be linearized to:

$$ln\left[\left(\frac{C_o}{C_f}\right) - 1\right] = \left(\frac{K_{Th}q_o\, M}{Q}\right)\left(\frac{K_{Th}C_o}{Q}V_{eff}\right) \qquad\qquad 3.2$$

where K_{Th} has been explained above, C_f and C_o are the concentration of the outward solution and its initial concentration respectively (mg·L^{-1}), q_o (mg·g^{-1}) is the maximum adsorption capacity and *M* is the total amount of adsorbent in the column, Q is the volumetric flow rate (mL·min^{-1}), V_{eff} is the total volume of the effluent collected. The K_{Th} values were calculated from the slope and the intercepts of the linear plots of $ln\left[\left(\frac{C_o}{C_f}\right) - 1\right]$ plotted against V_{eff} (the graphs not shown). The parameters determined are listed in Table 3.4.

From the regression coefficient (R^2) values and the other parameters determined, it could be observed that the different treatments affected the sorption of phenol and COD differently. Washing of the column with 2 liters of deionized water before passing the OMW through the column increased the flow rates (mL·min^{-1}) of the S (1.00-3.15), SB (0.18-1.18), HB (0.03-0.07) and H (0.03-0.10) but not for the B column, where the washing decreased its flow rate from 0.25 to 0.15 (Table 3.4). This finding is in line with reduced porosity of the same column as observed in Chapter 4 (Table 4.1). The result of washing increasing the flow rate was expected due to the removal of organic debris in the column but the reduction of the flow rate

in biochar columns could be due to biochar expansion after imbibing water. Interestingly, the increased flow rate did not increase phenol mobility due to adsorption onto biochar sites and it is encouraging as it will prevent underground water pollution. On the other hand, the non-washed columns experienced higher water retention and slow flow rates which could cause clogging. It was also pointed out by Achak et al. (2009b), that clogging of the upper layers of the sand filter increased the average retention time in the filter and reduced the effective area available for the infiltration to a point where ponding occurred.

The adsorption capacity (q_o) values agreed with the BTC that washing increased phenol sorption in all the columns. However, COD sorption (q_o) was not improved with washing except for the HB column (Table 3.4). All the columns positively correlated for phenol and COD removal meaning they could all remove phenol and COD at different degrees and that they fitted the Thomas model. The phenol sorption was highest with HBW (8144.53 $mg \cdot g^{-1}$) though the B column had the highest ($R^2 = 1$) while the least sorption occurred in S (33.39 $mg \cdot g^{-1}$). The addition of biochar enhanced phenol sorption and COD removal. Actually, the high COD q_o values in the biochar columns could be attributed to the colors detected as a result of the biochar used. The biochar enhancing phenol removal could be due to the increase in adsorbent sites. The low COD removal especially in the washed columns could be attributed to the anaerobic condition of the filter since the flow was continuous. The continuous flow is expected to reduce the oxygen content of the filter that perhaps reduced oxidation or degradation of the organic contents of OMW. The better performance of the biochar filters could be attributed to their aeration capacities and hence the B had more capacity than the BW. The BW column had more reduced pore sizes than the B (Table 4.1). It was observed that biochar addition increased the aeration of the columns. The more aerated the filter is, the better COD removal. This is because the elimination of COD is realized through biological degradation under oxygenated conditioning of the porosity of the columns (Achak et al., 2009b). The results of the COD analysis show that the biochar columns followed by the Hutton clay loam columns sorbed the organic load content of the OMW reasonably well.
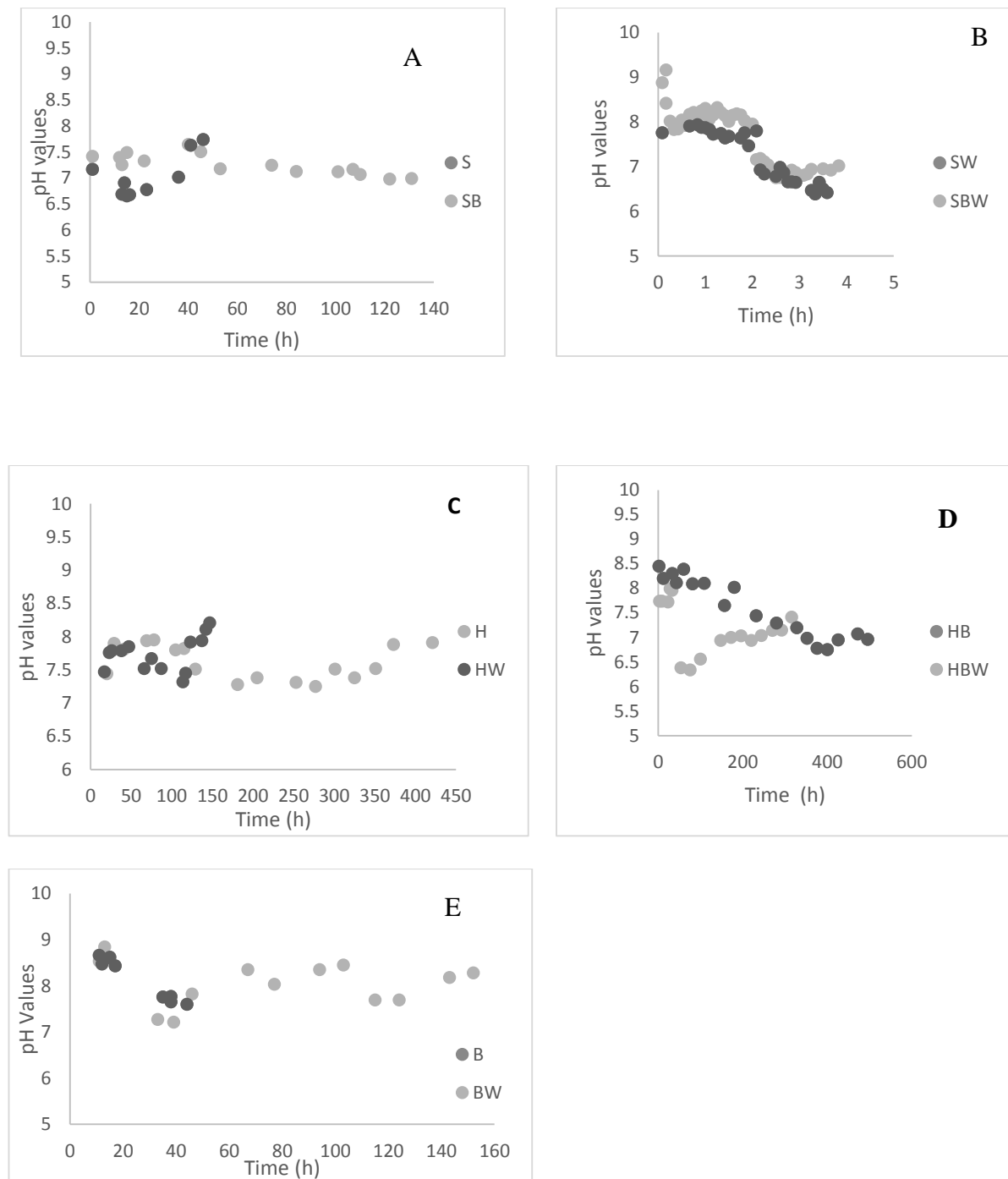
Figure 3.4. Effect of washing and biochar treatment on pH of the eluted effluents from the columns. A) Sand (S) and Sand Biochar (SB). B) Sand biochar washed SBW and Sand washed (SW) Column; C) Hutton clay loam (H) and Hutton clay loam washed (HW); D) Hutton clay loam + biochar washed (HBW) and Hutton clay loam + biochar unwashed (HB) and E) biochar washed and unwashed column (B and BW).

3.3.3. Treatment effect on pH and EC

The effect of the different treatments on pH was considered over time and the filters showed different results to the pH of the eluted effluent. The washed sand column (SW) and sand columns with biochar washed (SBW) and unwashed (SB) showed decrease in pH from 7.4-6.4,

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9.4-6.9 and 7.4-7.1 respectively (Figures 3.4 A and B). The pH of the sand column also decreased initially from 7.1 to 6.66 and then increased to 7.8 (Fig 3.4 A).

The Hutton clay loam columns; HBW, HW and H showed a decrease in pH from 7.99-6.33 and then increased to 7.41; 7.79 - 7.41 and increased to 8.21; 7.94 – 7.20 and increased to 7.90 respectively, with continuous flow of the effluent (Fig 3.4 C and D). Also, the HB column showed a continuous decrease in pH from 8.45 to 6.96 after 400 hours but did not show any further increase.

According to the equilibrium study discussed in section 2.3.1, the mechanism of sorption was shown to be dependent on the physical and chemical characteristics of the adsorbent such as porosity and pH. It was therefore observed that there could be a possibility of a neutralization reaction occurring between the alkaline sand and the acidic effluent used. This could explain why the pH value of the sand column at 1h was 7.1. The system however experienced decrease in pH, which could be attributed to the production of CO_2 from the reaction of calcium carbonate contained in the soil and the hydrogen ion from the acidic effluent. The decreasing effect of pH observed could also be due to the washing off of the carbonates contained in the soil. Whereas the increase of pH in the columns could be attributed to the complexation reactions resulting from the presence of the weak acids from the polyphenols.

The Hutton clay loam columns showed a different trend of a depression after about 12 days before the pH started increasing. This is likely to be due to reduction of Fe (III) oxide and its possible effect on carbonate occurring in the columns. Reduction of Fe (III) by microorganism via organic matter oxidation can potentially increase the alkalinity of the system through production of HCO^- and OH^- especially in increased water saturation with respect to carbonate phases (Donahoe et al., 1998). The time was long enough to allow for some microbial degradations to occur. The reduction in pH could also be attributed to adsorption whereas the increase could be as a result of ion exchange. Adsorption and ion exchange are the principal physical-chemical surface reactions that occur on sand during the transit of wastewater through the medium (Achak et al., 2009b). The B column reduced the pH more than the BW due to its cation content, so it is believed that washing reduced this pH during the process (Figure 3.5 E).

The Hutton clay loam columns and the BW column recorded lower ECs in the eluent than the sand column and the B column (Fig. 3.5).
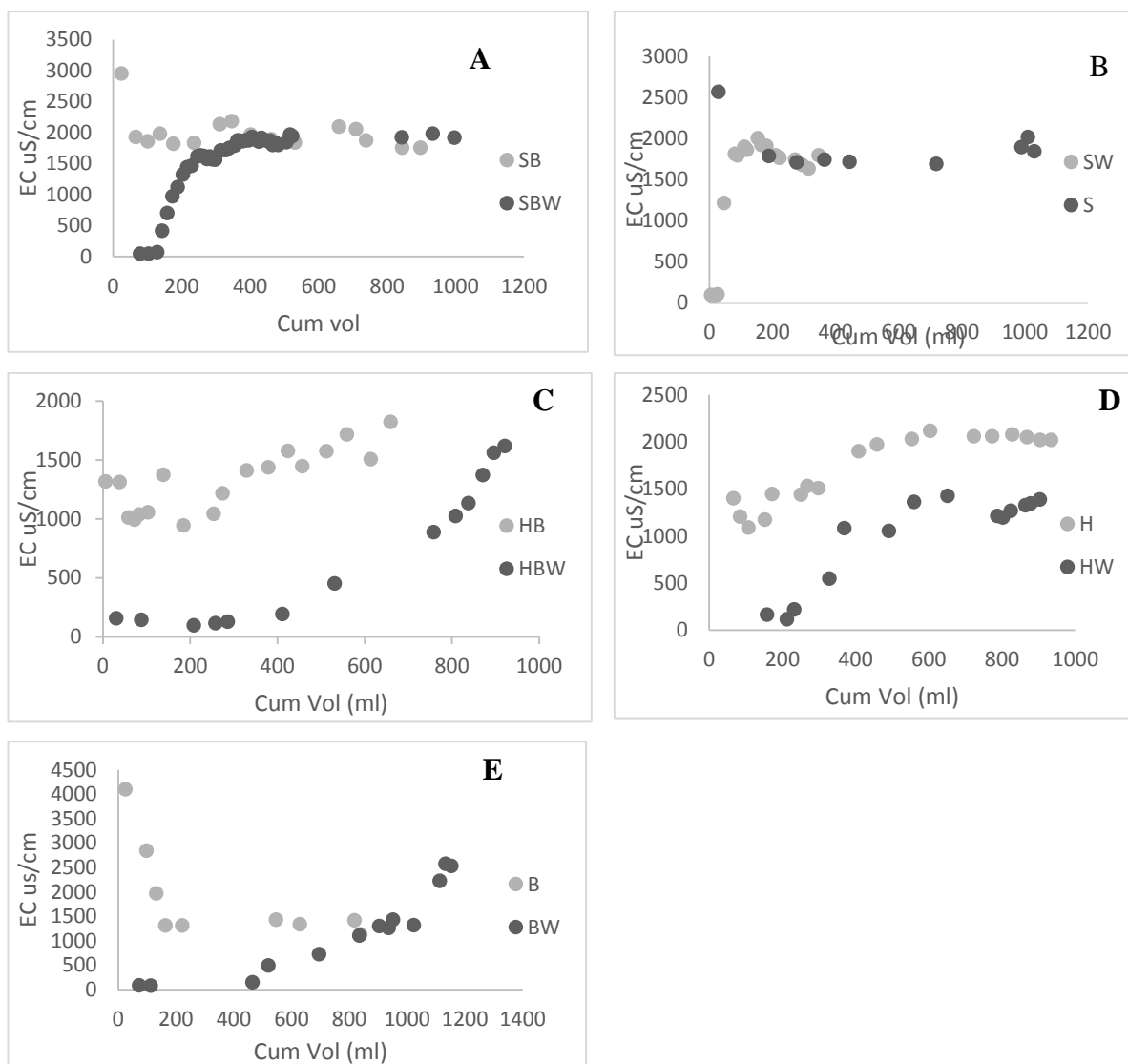
Figure 3.5. Pre-washing and biochar addition effects on EC. A) Sand biochar washed and sand biochar unwashed; B) Sand washed and Sand Column; C) Hutton clay loam + biochar washed and unwashed; D) Hutton clay loam washed and unwashed and E) biochar washed and unwashed.

The pre-washing of the columns with water resulted in the removal of soluble salts (ash) associated with biochar (Fig 3.5). The subsequent filtration of the effluent through pre-washed columns initially pushed out the fresh water used for washing the columns. Due to diffusion of salts from the OMW into residual water stored in the columns the EC of the filtrate increased gradually until reaching the levels associated with the OMW.

The columns containing biochar (Fig 3.5) that were not pre-washed showed immediate release of the large volumes of salt stored in the ash fraction of the biochar. Subsequent filtration of the OMW showed stable EC values associated with the OMW.

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It is quite clear that any exchange reactions between salts in the OMW and the biochar cannot remove the salts from the OMW and additional dilution of the filtrate by fresh water may be necessary prior to on-land disposal depending on the EC of the filtrate. Hence, monitoring of the filtrate EC is essential to prevent soil salinization.

3.4. Conclusions

Biochar addition to both sand and Hutton clay loamy soil significantly improved phenol removal during the filtration process mainly due to a significant reduction in flow rate (10 times for Hutton clay loam and 5 times for sand) allowing for longer retention time within the column. The increased retention time increased sorption of phenols in the mixtures. The sorption capacity for phenol in the sand-biochar mix increased almost 20 times, while in the clay-loam-biochar mix it almost doubled.

It was observed that under saturated flow conditions, pre-washing improved phenol sorption in all cases. It implies that on-land disposal may be mitigated best in pre-wetted soil conditions – shortly after the rains.

In the case of biochar, COD in the filtrate increased due to the migration of fine biochar particles. The capacity of biochar to remove COD significantly was observed in pure biochar columns, which showed the best overall performance. Most of the columns (H, HBW, B and BW) removed COD even below the South African wastewater treatment standard of 75 mg·L^{-1}.

The Hutton clay loam and biochar mix showed the best performance for phenol removal, possibly due to longer retention time, while COD removal was best achieved with pure biochar. These columns did not reach their breakthrough points indicating very high removal potential.

The study also found that continuous disposal of the effluent increased the COD accumulation in all the columns. It is therefore advised to dispose of effluent intermittently to allow time for degradation of the organic load.

Pre-washing was an important treatment as it both reduced the pH and the EC. The Hutton clay loam soils and the biochar filters reduced the pH and EC via adsorption and ion exchange to neutrality and averaged 7 and 1.5ds.cm^{-1} for pH and EC respectively. These treatments were

therefore good as they could reduce the phenol and COD level below the South African water quality standard and internationally acceptable limits of wastewater disposal.

There is need to study the degradation process of the disposed OMW with or without biochar in relation to nutrient release.

Chapter 4

SATURATED HYDRAULIC CONDUCTIVITY AND TOTAL POROSITY CHANGES IN ALKALINE SAND, OXISOL AND PINEWOOD BIOCHAR TREATED WITH OLIVE MILL WASTEWATER IN A SATURATED COLUMN EXPERIMENT

4.1. Introduction

Rühle et al. (2013) reported that column experiments are often used to study transport properties of sediments to predict the fate of contaminants in the subsurface. They emphasized that water flow in column experiments is rarely monitored over the entire length of an experiment, despite the known impact of biogeochemical processes on pore structures. Water flow through soil column affects the hydraulic properties of the soil, especially the flow of oily waters like the olive oil effluent. Olive mill wastewater (OMW) is known particularly to contain substances that have the tendency to reduce water flow in the soil via pore blockage. Mahmoud et al. (2010) reported that OMW is characterized by high salinity, high organic matter content, suspended solids and mineral elements and that irrigation with this wastewater has been shown to decrease saturated hydraulic conductivity because of accumulation of grease and oil in upper soil horizons. Zenjari and Nejmeddine (2001) experienced the filling-in property of the olive effluent in their experiment, which they suspected could seal the large pores of the soil. Meanwhile, it has been reported that OMW was shown to reduce large pores and increase small pores (Cox et al., 1997). Mohawesh et al. (2014) also noted that OMW movement through the soil columns was considerably slower in the soils irrigated for 5 years (T5) and 15 years (T15) treatment than the control (irrigated with fresh water) due to changes that occurred in the pore size distribution.

Several studies have been done to study the hydraulic soil properties especially clay and silt loam in the field and in soil columns (Dikinya et al., 2008) but only a few investigations have been done with olive oil effluent and pinewood biochar. Few studies, if any, have been done to see the effect of applying biochar in relation to porosity in a soil column. The use of biochar is in line with the principle that sorption is one of the main processes reducing the mobility of chemicals in soil. Cox et al. (1997) noted that the addition of exogenous organic matter to soil has been suggested as a possible method to reduce pesticide leaching. There is not much work on OMW effect on soil porosity and hydraulic conductivity changes as a results of using pinewood biochar in a soil column. Our study therefore set the following objectives:

1. Determine the effect of washing and biochar addition on the hydraulic conductivity of different soil columns treated with olive mill wastewater in a saturated condition;

2. Determine the effect of washing and biochar addition on porosity changes in the alkaline sand, clay loam soil and pinewood biochar treated with olive mill wastewater in a saturated column.

4.2. Materials and methods

4.2.1. Materials used

The alkaline soils, pinewood biochar and Hutton clay loam soils used in this experiment are described in Chapters 5 and 6. The other materials used were described in Chapter 3. The soils and the biochar were sieved to pass through a 2 mm sieve. The packing of the column and density of each columns were explained in the preceding chapter.

Five basic different mixtures were used for this experiment, as follows: B (biochar), S (alkaline sand), SB (alkaline sand + 2 % biochar mixed), H (Hutton clay loam), BH (Hutton clay loam mixed 2 % biochar). These materials were given two treatments each; (a) washing with two liters of deionized water before passing one liter of OMW through the column and (b) passing one liter of OMW through the column without washing. In total, we had 10 columns consisting of 5 mixtures replicated twice, one replication of five mixtures washed with water (2 L) and the other five not washed. The columns washed with water were denoted with WO, for example; BWO, BHWO, HWO, SWO and SBWO and samples not washed with water are denoted as BO, BHO, HO, SO, and SBO. Original samples before any treatment was applied were denoted as B, BH, H, S and SB.

The saturated hydraulic conductivities of the columns were determined by using the constant head method of klute and Dickson (1986) with the pressure head maintained at 5 cm. The 5 cm pressure head was achieved by inverting a 1000 mL volumetric flask filled with liquid over the column at 5 cm height above the soil surface of the column. The solution was allowed to pass through the column and a known volume was collected at the recorded time. Assuming Darcian flow, saturated hydraulic conductivity was calculated using equation 4.1:

$$K_{sat} = \frac{VL}{tA\Delta H} \hspace{4cm} 4.1$$

where A is the cross sectional area of the flow through the soil column (L^2); ΔH is the hydraulic head; L is the length of the column and V is the volume of water (L^3) flowing across the column in time t.

The porosities of the five basic materials used for this experiment (B, BH, S, SB, and H) were determined with the CT (computer tomography) scanner before the experiment started just after packing the columns. After the experiment was done, the same samples were scanned again using the same scanner to determine the changes in porosities as stated in Table 4.1.

4.2.2. CT analysis

The holistic change in porosity of the ten columns used for this experiment was assessed using a computer tomography (CT) scanner. This was done to get a non-destructive analysis of the columns as well as to observe the oil coatings on the surfaces of the soil.

The instrument used was a microfocus X-ray CT scanner model General Electric Phoenix V|Tome|X L240 with an NF180 tube. The machine uses x-ray projection images at a rate of up to 3000 angles around the object to reconstruct a complete 3D image that can be used to accurately measure, analyze and view the external and internal structure of an object as well as to provide more advanced analyses of materials in a non-destructive way (Ikram et al., 2015). The machine was set to operate at 100 KV, with a current of 100 µA for x-ray generation with acquisition time of 500 ms per projection three thousand images recorded during one full scan were reconstructed to a volume data set making about 60 minutes at a scan resolution of approximately 100 µm.

Porosity analysis was carried out on a subsection designated as ROI (Region of Interest). Three regions of interest were selected per soil column and an analysis was performed on each ROI to determine the porosity changes. A measurement of 4 cm length and 4 cm width of the 3D column image was done and at approximately 4 cm, 11 cm and 16 cm from the top of the column denoting the top, middle and bottom parts respectively. A cylinder ROI selection tool was used to create the ROIs. A 2D slice was cut from the column at 3, 9 and 12 cm depth starting the measurement from the top of the column to visualize the porosity changes on the different columns due to the treatments compared to original state. The total porosity calculations were done at the same regions within a 4 cm^2 square area of the 3D column image

measuring from top at 4 (top), 11 (medium) and 16 cm (bottom) length of the columns. The volume of pore spaces of these cut sections was determined by manually thresholding the images using VGStudioMax 2.2.6-Defect Detection Module 64 bit software. The calculation of the percent total porosity was done using the bulk volume of the selected 4 cm square and the pore volume of the same area.

4.3. Results

4.3.1. Olive mill treatment effect on total porosity and hydraulic conductivities of the columns

The results of the porosity differences and saturated hydraulic conductivities of the different columns used in this experiment were shown in Table 4.1. It can be seen that the total porosity values in this study were very low compared to the expected total porosities when calculated with bulk densities. According to Jassogne et al.(2007), this occurs because measurements made from CT images are resolution dependent, the values were much smaller than the values calculated from bulk density calculations. They noted that bulk density calculations are also sensitive to resolutions defined by the diameter of the cores and its relationship with the scale of the structure. A measurement of 4 cm long of 2D section (4 cm^2) of the 3D column image was done at approximately 4 cm, 11 cm and 16 cm from the top of the column denoting the top, middle and bottom parts respectively to calculate the total porosities. To visualize the porosity changes of the column, a cross sectional slice cut was made at 3, 9 and 12 cm depth starting the measurement from the top to fall within the sections above (4, 11 and 16 cm).

Table 4.1. Effect of treatment on total porosities determined from the top, middle and bottom parts of the columns and hydraulic conductivities measured before and after the experiment

Column Treatments	Total Porosity (%)			K_{sat} mm·h^{-1}
	Top (4 cm)	Middle (11 cm)	Bottom (16 cm)	
B	3	6	5	3.902
BO	9	3	5	1.68
BWO	7	5	3	0.9
HB	9	7	7	1.169
HBO	7	6	6	0.24
HBWO	7	6	5	0.48
H	2	2	2	0.321
HO	3	3	4	0.24
HWO	3	3	3	0.66
S	3	3	3	
SO	2	3	3	4.68
SWO	4	5	3	17.22
SB	9	11	11	56.46
SBO	8	10	10	1.44
SBWO	10	9	9	13.32

4.3.2. Effect of the different treatments on the porosity changes and hydraulic conductivities of the biochar columns

The porosities of the biochar (B) columns before the treatment applications were lower at the top of the column and increased with depth but the reverse was the case after washing (BWO) and passing oil through them without washing (BO). The passing of the oil treatments increased porosity of the biochar columns especially at the top (Figure 4.1). The effect observed from the washed columns was a little better than the columns passed with oil without washing. The porosity of the washed biochar columns (BWO) had a 28.5% and 57% decrease with depth while the unwashed biochar column decreased by 66% and 44% from the top to the middle and bottom of the column respectively (Table 4.1). This result shows that the washing and oil

passing, both affected the top soil's porosity more that than the middle and the bottom depths. The washed column treatment (BWO) seems to have restricted flow of the oil at the depth of 12 cm more than the unwashed (BO) column (Figure 4.1) and this could explain the reason the (B) column had higher hydraulic conductivity than the unwashed column (Table 4.1). Notwithstanding, the two treatments (washing and biochar addition) decreased the hydraulic conductivity of the biochar (B) columns.

	BO	BWO	B
3 cm			
9 cm			
12 cm			
Entire Column Length			
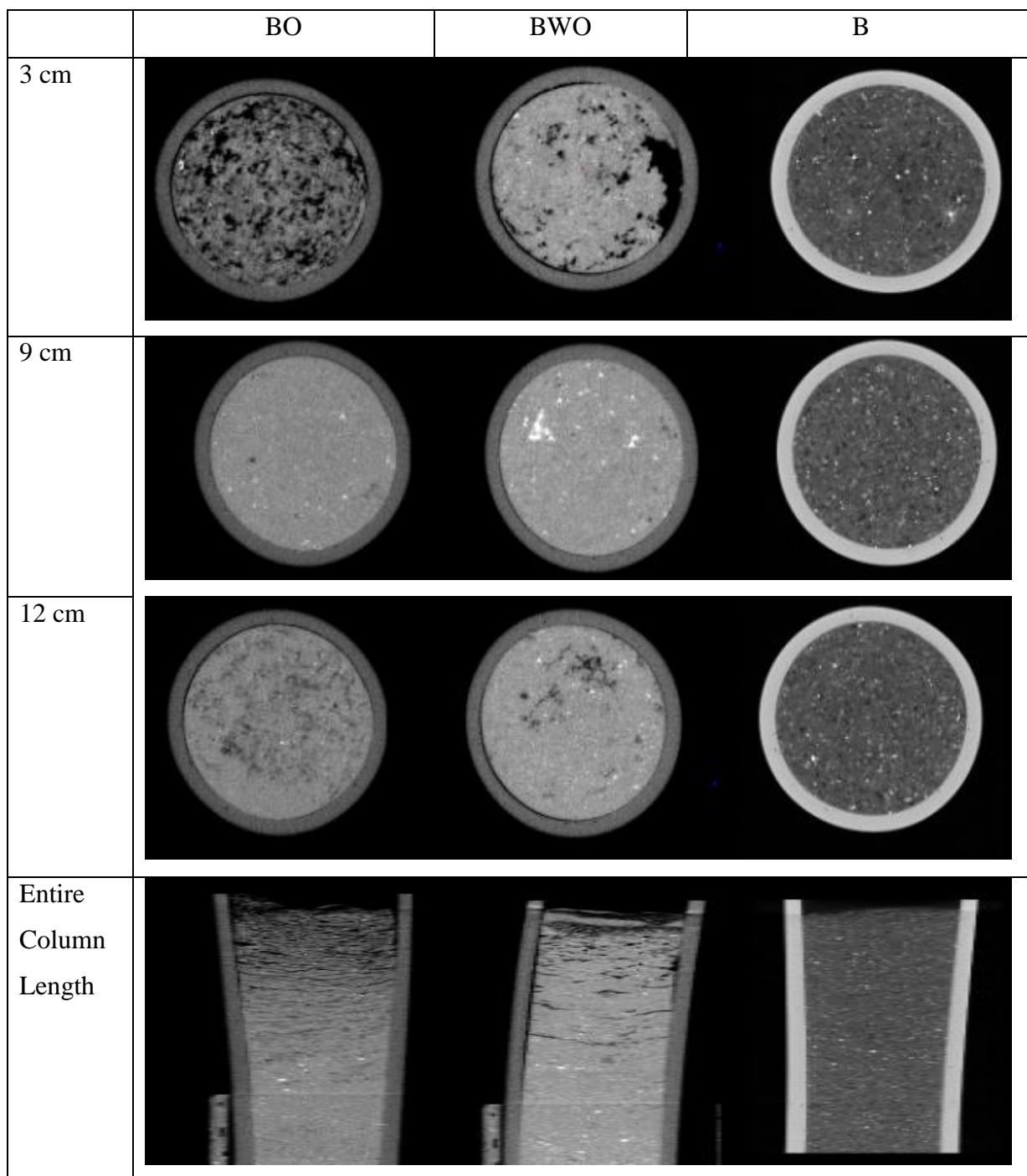
Figure 4.1. Effect of porosity changes on the biochar column at different depths

4.3.3. Effect of the treatments on the porosity changes and hydraulic conductivities of the Hutton clay loam +Biochar (HB) columns

The Hutton clay loam columns mixed with 2% biochar (HB) showed a similar trend with the biochar column by recording the highest porosity at the top of the column. But in this case the two treatments (HBO) and HBWO) decreased porosity compared to the control with all the columns recording decreasing porosity with depth (Table 4.1). The HBWO recorded 14% and 28 % decrease in porosity from the top to 11 cm and 16 cm depths respectively while the HBO showed only a 14% decrease from the top to the next depth and then remained constant (Table 4.1). Figure 4.2 illustrated reduced porosity with depth on the washed column (HBWO) compared to the HBO column, meaning that the HBO column had more pores than BHWO. However, the washed column had higher hydraulic conductivity than the column treated with oil without washing. Meanwhile, they both decreased compared to HB. The two treatments decreased the hydraulic conductivities of the HB column (Table 4.1).
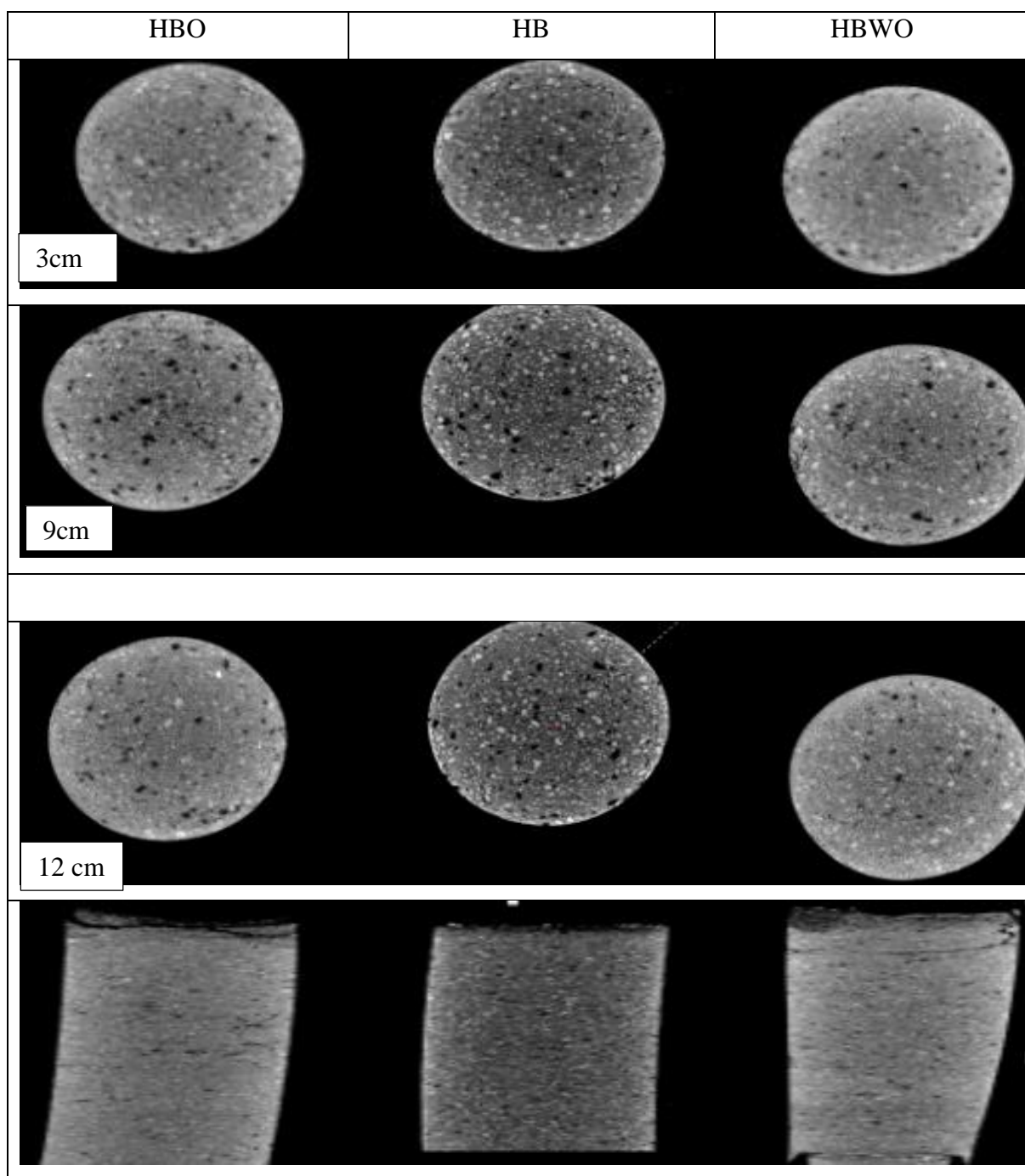
Figure 4.2. Porosity changes at the different depths of the Hutton clay loam mixed with biochar treated by washing and unwashed before passing olive effluent through them.

4.3.4. Effect of the treatments on the porosity changes and hydraulic conductivities of the Hutton clay loam (H) columns

The Hutton clay loam columns (H) without biochar had uniform porosity before the application of any of the treatments (washing and OMW). The results in Table 4.1 showed that the Hutton clay loam column had uniform porosity except for the unwashed column (HO) which had more porosity at the 16 cm depth. It is interesting to note that passing of the oil increased the porosity throughout the entire column unlike the washing with water before passing the oil. Figure 4.3 clearly shows the restricted oil and suspended solid movement in the HWO column to the first few centimeters in the top part of the column. The rest of the column was not shown because there was no much differences.

This result is contrary to the previous results in B and HB columns where the treatments reduced hydraulic conductivity, the washing of the column before applying the effluent increased the hydraulic conductivity from 0.321 to 0.66 mm·hr^{-1} while passing of oil decreased it to 0.24 mm·hr^{-1} (Table 4.1). With the increased porosity observed in the Hutton clay loam with biochar rather than in the H columns, it is noteworthy to observe that the biochar application seemed to reduce porosity increase down the column, unlike in the HO column (Figure 4.3).

H	HWO	HO
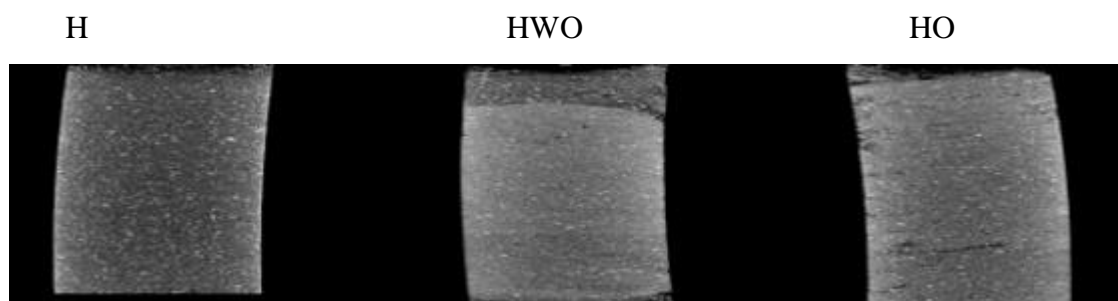
Figure 4.3. Effect of the washing and oil leaching through the Hutton clay loam (H) on porosity changes

4.3.5. Effect of the treatments on the porosity changes and hydraulic conductivities of the sand (S) columns

The sand columns (S) showed a little different result from the biochar mixed with Hutton clay loam (HB) and the Hutton clay loam only (H). The sand columns also had a uniform porosity with depth before the treatment application (Fig. 4.4), but the porosities of the washed column (SWO) at both 3 cm and 9 cm depth were increased compared to the unwashed column (SO)

(Figure 4.4). The hydraulic conductivity was also reflected in an increased manner with washing (Table 4.1)
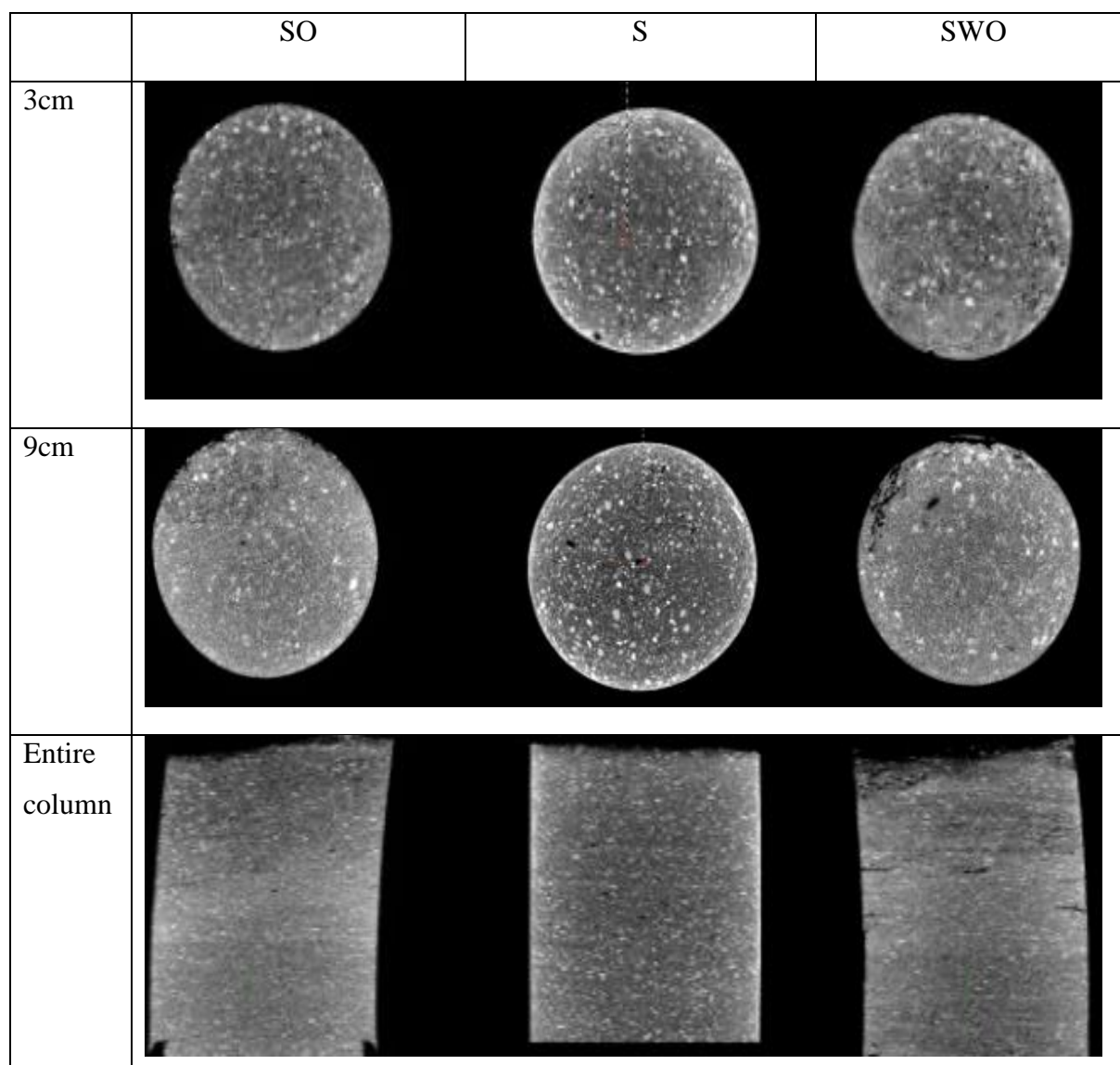
Figure 4.4. Porosity changes in sand treated with oil and deionized water at different depths

4.3.6. Effect of the treatments on the porosity changes and hydraulic conductivities of the sand + biochar (SB) columns

The addition of biochar to the sand column (SB) caused quite large increased porosity, which is similar to the result of biochar addition to the Hutton clay loam (HB) soil. The porosity also increased with depth before the treatments were administered (Table 4.1). The washed column showed increased porosity at the top compared to the other SB columns but down the depth

showed lower porosity compared to control deeper down (Table 4.1). Also, the washing greatly improved the hydraulic conductivity that was greatly reduced by the passing of oil first from 56.46 to 1.44 mm·hr^{-1} (Table 4.1).

4.4. Discussion

Effect of washed and unwashed treatments on the column total porosity and hydraulic conductivities

It was observed from this experiment that biochar and biochar mixed columns gave the highest porosity changes compared to the columns without biochar. This could easily be attributed to the porous nature of biochar that it has transferred to the systems herein. Tang et al. (2013) defined biochar as carbon-rich and porous with high specific surface area and this specific structure has been proved to have the capability of increasing water and nutrient retention of soil. That specific property of biochar nature can restrict oil movement down the column via its adsorption capacity as we have observed. Tang et al. (2013) reviewed several research works that have proven biochar adsorption capacities. For example, Obst et al., (2011) found that phenanthrene accumulated in the interconnected pore-system along primary ''cracks'' in the biochar particles, hence confirming an adsorption mechanism.

It was interesting to note that despite the increased porosity due to biochar addition, there was a decreased in porosity with depth in all the columns except in sand. The decrease in porosity with depth of the biochar columns (BO, BWO and B) as a result of passing of the oil and especially washing could be attributed to the movement of the biochar particles under saturated conditions. Zhang et al. (2010) stated that the percentage of biochar particles eluted in saturated experiments was greater in unsaturated experiments by 38-302% under otherwise same experimental conditions. This could be regarded as particle removal and rapid self-filtration (migration and re-arrangement of particles and removal of particles within the porous medium) of soil particles (Dikinya et al., 2008). This implies that the washing technique prevents ground water pollution in the case of contaminant mobility.

The decrease in porosity with depth was noticed in biochar columns (B) as well as Hutton clay loam with biochar (HB), and sand biochar washed column (SBWO). The sand biochar and clay loam biochar (SBO and HBO) recorded decreased porosity compared to the control. Cox et al. (1997) also reported a decrease in porosity of clay soil irrigated with OMW for three years

which they attributed to the solubilization-precipitation of the original soil CaCO$_3$, promoted by presence of fulvic and humic acid content of the OMW. They assumed that the association of the OMW organic matter and CaCO$_3$ will reduce Cu (II) and Zn (II) retention by OMW amended soils and contribute to porosity reduction. This phenomenon could be accepted for the clay loam and sand biochar amended soil columns of our study and could be supported with the increased salt content as reflected in their ECs (Figure 3.6). The following column (BO, BWO, HO, HWO, SWO and SBWO) recorded increased porosity compared to the control.

It was not only the biochar addition that increased the porosities of the columns; the passing of the OMW without washing in the Hutton clay loam (HO) also exhibited increased porosities. This is in agreement with the studies of Mahmoud et al. (2012) who reported that olive mill wastewater application increased porosity of the soil after long term application. Long term application causes changes in pore size distribution by decreasing macro pores and increasing micro pores (Cox et al., 1997). It can be seen that OMW increased the porosity of the column which is in line with the findings of the studies of Mahmoud et al. (2012) that OMW application caused formation of macro aggregates from micro aggregates, which resulted in larger pore formation which enhanced downward movement. They attributed the larger pores experienced in the soils treated with OMW for 15 years to the frequent plowing of the soil. This column research contradicts their argument as there was no plowing involved in the columns and yet the pores were more in the OMW treated columns compared to the control. The reason is more likely to be due to increased carbon content which acted as glue to cement the micro-aggregates together to form macro aggregates and form larger pore spaces between micro aggregates as reported by Wei et al. (2006). Their work expected a larger impact on a sandy soil as they worked with a silt loam soil, but our result which was on both Hutton clay loam and sandy soils showed not much difference in the porosity changes of the two soils after passing OMW through the saturated soil columns (Table 4.1).

Wei et al. (2006) reported decreased hydraulic conductivity on the sites with increased higher porosity and this is in line with our findings. They attributed their greater porosity to enhanced root growth but we attribute it rather to the accumulation of more organic compound. They also argued it could be as a result of more concentrated deposits near the column inlet due to

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deposit compaction. The results of these columns (S, SB and BW) top soil porosities with lower hydraulic conductivity agree with this argument.

Our findings of hydraulic conductivities ranged from biochar to sand particles in this order (0.24, 0.24, 0.48, 0.66, 0.9, 1.44, 1.68, 4.68, 13.32 and 17.22) representing (BHO, HO, BHWO, HWO, BWO, SBO, BO, SO, SBWO and SWO) indicating that the samples with smaller sized particles had the lower hydraulic conductivities because they retained more water. Studies by Mays and Hunt (2005) also reported that the release and increase of particles in the pore stream caused more reductions in hydraulic conductivity when fine grained material was present in soil columns. The work of Mohawesh et al. (2014) reported that OMW significantly increased the water retention capacity of the soil due to the increased organic matter content despite the reduction of the macro pore percentage. As a result, the aggregates were coated with complex organic molecules that hinder inter and intra aggregates solute exchange and thereby reduced hydraulic conductivity. Bisdom et al. (1993) stated that the oil and grease fraction of the OMW forms coatings on the pore walls of the soils and this may reduce saturated hydraulic conductivity.

4.5. Conclusion

It can therefore be concluded that application of the OMW without washing significantly affected the macro-porosity and reduced hydraulic conductivity of the system. We also found that biochar application and column pre-washing seemed to restrict the movement of the suspended particles of OMW down the column. That is due to the removal of hydrophilic compounds from pore walls by pre-washing and the addition of hydrophobic surfaces from biochar. Both treatments lead to an overall increase in lipophilic surfaces, thus increasing sorption and retarding movement of the organic compounds, particularly fats and greases. The pre-washing effect was not observed in the sand columns due to lack of hydrophobic (organic) compounds in the native sand sample.

Generally, pre-washing increased the hydraulic conductivity and also increased the porosity.

We can therefore recommend that for better OMW on-land disposal management soil amendment with biochar and the OMW disposal after rains would be preferred.

Considering the porosity changes and hydraulic conductivity, we would recommend the disposal to be effected on heavier, rather than sandy soils. The results achieved in Chapter 3 supports this argument.

Chapter 5
MITIGATION OF OLIVE MILL WASTEWATER EFFECTS ON GROWTH OF WHEAT AND GREEN BEANS USING BIOCHAR ON ALKALINE SAND IN GREENHOUSE POT TRIALS

5.1. Introduction

Olive mill wastewater is a major by-product of olive oil production and it is a controversial substance (Mekki et al., 2009; Mekki et al., 2013), showing both positive and negative effects on soils and crops. It is the most polluting agro-alimentary by-product constituting a real and major environmental threat (Casa et al., 2003). This production is major in the Mediterranean regions of the world, where alkaline sands are common in coastal areas (Angiolini et al., 2013; Fenu et al., 2013). Alkaline sands are a significant impediment to crop production on their own, and application of acidic effluent may be regarded as beneficial.

The effluent is characterized by acidity (pH 4-6), high Chemical Oxygen Demand (COD) ranging from 50 000 to 150 000 mg·L^{-1}, high polyphenol content of 500 to 23000 mg·L^{-1} and high salt content (Belaid et al., 2013; McNamara, 2008). Application of the effluent in agriculture has been reported to inhibit seed germination of barley (Rusan et al., 2015), reduce plant growth parameters of tulip plants (Lykas et al., 2014) and generally inhibit plant growth (Ouzounidou et al., 2008).

However, Mekki et al. (2013) reviewed the ferti-irrigation properties and abilities of the OMW and pointed out that olive mill wastewater, unlike many other industrial effluents, contains large amounts of plant nutrients. Approximately 65 % of the olive mill waste is made up of organic matter, as noted by Barbera et al. (2013). The organic matter content of the OMW as reported by Roig et al. (2006) ranges from 46.5 g·L^{-1} to 62.1 g·L^{-1}. OMW improves the soil organic matter (Mekki et al., 2009), as well as the nitrogen and phosphorus content of the soil (Umeugochukwu and Ezeaku, 2013). It may be regarded as a low-cost source of nutrients and water for agricultural purposes especially in the Mediterranean region (Angelakis et al., 1999).

The OMW has a low nitrogen content which Di Serio et al. (2008) indicated that may increase the soil C:N ratio to as much as 50. Jiménez Aguilar (2010) showed that ammonium and nitrate ions were complexed with diluted olive mill wastewater. It was therefore recommended that

an application rate of 180 $m^3 \cdot ha \cdot yr^{-1}$ should be used to avoid the temporary immobilization of nitrate at higher rates. This action is recommended because high rates increase soil salinity and phenolic compounds in the soil, which negatively impact plant production (Sierra et al., 2007).

Apart from the on-land disposal of olive oil effluent, researchers have tried different methods of effluent treatment: activated clay (Al-Malah et al., 2000; Aziz et al., 2012), bio-augmentation (Mekki et al. 2013), and sand filtration (Achak et al., 2009a). Paraskeva and Diamadopoulos (2006) reported that OMW adsorbed onto activated carbon, gave 71 % removal of COD and 81 % removal of phenol. Of all their reviewed physicochemical methods, apart from the combined methods, adsorption onto activated carbon, though most expensive, had the highest percent contaminant removal. It is, therefore, feasible to test the use of biochar in the treatment of OMW to reduce cost.

Barrow (2012) listed nutrient affinities of biochar, which included retention of plant nutrients, notably retention of N in permeable soils under rainy conditions as one of the possible benefits of biochar. It is therefore assumed that biochar could possibly correct the low nitrogen problem by retaining N and making it available for plant to use later. Schulz and Glaser (2012) reported that though compost gave the best result for plant growth and plant nutrients, but compost + biochar performed also better than mineral fertilizer. To reduce the high C:N ratio resulting from the high doses of OMW application, Barbera et al.(2013) suggested the possibility of adding N to influence the kinetics of OMW degradation. Nitrogen can be added via nitrogen fixing leguminous crops, hence the use of beans as a test crop in this study to see the combined effect of N fixation, biochar and the effluent.

A study by Sekar (2012) combined biochar and digested effluent and found that it reduced the pH lower than that of the biochar alone but the change was not significant. So it is expected that the acidic nature of the OMW will influence the pH of the soil combined with biochar. According to these authors, the biochar tends to have a negatively charged surface area in alkaline conditions, suggesting that the cations from the effluent have bound to the surfaces of the biochar. This phenomenon was expected to improve production of crops under alkaline conditions.

Meanwhile the concentration of the biochar to be used to sorb a certain quantity of contaminants needs to be studied. Chen and Chen (2009) showed that an increased

concentration of solutes affected the sorption capacity of biochar. This finding indicates that it is necessary to know the corresponding rate of biochar that can sorb a certain solute concentration. In this present study, we shall also consider the reaction of biochar with the effluent in terms of nutrient release to the crops. We therefore in this study are associating the different effluent and biochar mixture properties to the response of the plant growth parameters.

Considering the findings from literature given above, the following objectives were formulated:

1. Determine separately the effect of effluent or biochar application at different rates (with or without mineral fertilizer) on growth of wheat and green beans in a pot filled with alkaline sand;

2. Determine the combined effects of the OMW and biochar application on growth parameters of wheat and green beans in a randomized pot trial on alkaline sand.

5.2. Materials and methods

5.2.1. Sand Properties

Alkaline sand was purchased from the GH Supplies (Pty) Ltd Stellenbosch, South Africa. The sand was air-dried and sieved through a 2 mm sieve. The characteristics of the sand are given in Table 5.1. The pH was determined using the 1:2.5 ratio in water and KCl and read using a EUTHECH pH 700 meter (Thomas, 1996). Electrical resistance was determined in saturated soil-water paste and then the EC calculated from it as:

$$EC\ (mS/m) = \frac{25000}{Resistance\ (ohms)} \qquad 5.1$$

The 1% citric acid was used to extract the amount of available phosphorus (P), extractable calcium, magnesium, potassium and sodium by Inducted Coupled Plasma spectroscopy (ICP). All the methods were determined as described in the handbook of standard soil testing methods for advisory purposes (Non-Affiliated Soil Analysis Work Committee and Soil Science Society of South Africa, 1990). Soil organic carbon was determined by Walkley-Black method (Nelson and Sommers (1996) and nitrogen by Kjeldahl method (Bremner, 1996). The soil texture was determined using the pipette method as adapted from the Gee and Or, (2002).

Table 5.1 Properties of biochar, alkaline sand, and sand-biochar-effluent mixture

Effluent (m³/ha)	Biochar rates (%)	pH (H₂0)	pH (KCl)	Ca (cmol⁺/kg)	Ca (mg/kg)	Mg (cmol⁺/kg)	Na (cmol⁺/kg)	K (cmol⁺/kg)	P (mg/kg)	(%) C	(%)N	EC (mS/m)	Ca: P	C:N
0	0	9.15	9.1	70.6	14120	2.63	0.71	0.03	24	0.04	0.006	12.10	588.33	6.67
0	0.5	9.0	9.1	83.70	16740	2.60	0.70	0.05	24	0.04	0.006	12.30	697.50	6.67
0	2.5	9.1	9.0	81.25	16250	2.47	0.67	0.10	24	0.06	0.007	15.50	677.50	8.57
0	5	9.1	9.0	66.40	13280	2.20	0.59	0.24	24	0.12	0.004	23.80	553.33	30.00
50	0	8.9	9.0	66.10	13220	2.55	0.70	0.06	38	0.04	0.007	27.20	347.89	5.71
50	0.5	9.2	9.2	65.05	13010	2.56	0.70	0.08	25	0.05	0.005	16.00	520.40	10.00
50	2.5	9.2	9.0	73.00	14600	2.31	0.60	0.13	25	0.04	0.006	21.00	584.00	6.67
50	5	9.3	9.0	62.75	12550	2.17	0.60	0.25	25	0.12	0.005	25.80	502.00	24.00
100	0	9.4	9.2	71.70	14340	2.42	0.68	0.09	26	0.04	0.006	24.00	551.54	6.67
100	0.5	9.3	9.2	85.50	17100	2.39	0.67	0.11	25	0.04	0.006	24.80	684.00	6.67
100	2.5	9.3	9.1	71.10	14220	2.33	0.65	0.16	27	0.08	0.007	25.00	526.67	11.43
100	5	9.4	9.1	62.35	12470	2.11	0.61	0.28	26	0.14	0.007	32.00	479.62	20.00
200	0	9.5	9.3	86.45	17290	2.34	0.71	0.13	27	0.04	0.008	29.40	640.37	5.00
200	0.5	9.5	9.2	74.35	14870	2.37	0.70	0.15	28	0.05	0.010	33.30	531.07	5.00
200	2.5	9.7	9.2	65.40	13080	2.28	0.66	0.20	26	0.1	0.009	31.60	503.08	11.11
200	5	9.7	9.1	66.20	13240	2.15	0.66	0.33	30	0.13	0.005	39.70	441.33	26.00

Table 5.1 cont'd. Properties of biochar, alkaline sand, and sand-biochar-effluent mixture

	pH (H₂0)	pH (KCl)	Ca (cmol⁺/kg)	Ca mg/kg	Mg (cmol⁺/kg)	Na (cmol⁺/kg)	K (cmol⁺/kg)	P (mg/kg)	(%) C	(%) N	EC (mS/m)	Ca: P	C:N
Biochar only	9.3	8.5	4.75	950	1.35	1.05	3.74	90	82.71	0.530	0.007	10.56	156.05
Sand only	9.1	9.1	70.60	14120	2.63	0.71	0.03	24	0.04	0.006	12.10	588.33s	6.67

5.2.2. Biochar properties

The biochar used in this experiment, produced from pinewood sawmill waste by a small-scale commercial producer from the Eastern Cape, South Africa has been characterized by Sika and Hardie (2014). The sawmill waste was slow-pyrolyzed at approximately 450° C, and then crushed and sieved through 2 mm sieve. Biochar chemical properties are given in Table 5.1. The proximate analysis of the biochar analyzed showed the following results: % Volatiles (19.90), % fixed carbon (74.45), % ash (3.04), % C (82.71), and % N (0.53), C: N (156:1), O/C (0.13), H/C (0.04), % H (2.95).

5.2.3. Olive oil mill wastewater sampling and characterization

The OMW was collected from the storage dam of a nearby olive industry near the University of Stellenbosch, RSA. The method of production of the effluent was a two-phase system which has the advantage of reduced water consumption of the mills (Borja et al., 2006). Effluent characterization was done with samples digested with 1 ml HNO_3 and analyzed using Induced Coupled Plasma-Acid digestion (ICP-AES). The macro and micro elements of the effluent are stated in Table 5.2. The total phenol content was determined using the Folin-Ciocalteu method described below, using 0.5 mL of the effluent. The pH of the effluent was read off with the EUTHECH 700 pH meter with 30 mL of the sample. Chemical Oxygen Demand (COD), Total organic carbon (TOC) and Total Nitrogen (TN) were determined as described in the (Standard Methods (2012).

Table 5.2 Olive mill wastewater characteristics

Parameters	Values
pH	5.14
Total phenol mg·L^{-1}	971
EC uS/cm	1005
Ca mg·L^{-1}	47.17
K mg·L^{-1}	408.60
Mg mg·L^{-1}	11.21

Table 5.2 cont'd. Olive mill wastewater characteristics

Parameters	Values
Na mg·L^{-1}	165.70
P mg·L^{-1}	20
N mg·L^{-1}	82
TOC mg·L^{-1}	2000
COD mg·L^{-1}	13700
C:N ratio	24

5.2.4. Soil-biochar-effluent mixture characteristics

Soil with a mass of 3.5 kg was mixed with 0, 0.5, 2.5 and 5% (w/w) biochar and 0, 50, 100, and 200 m^3·ha^{-1} of OMW respectively (See appendix 2), using a wheelbarrow and mixing each treatment at a time to ensure uniform mixture. The mixing rates were calculated by assuming 15 cm soil depth and calculated soil bulk density of 1.45 g·cm^{-3}. A total of 16 pooled soil samples of each soil-biochar-effluent mixtures was collected and analyzed after mixing the samples before planting. The properties determined on the different treatment mixtures were detailed in Table 5.1. The analysis of these mixtures was conducted in the same manner as for the sand properties stated above.

5.2.5. Experimental setup and design

A greenhouse trial was conducted at the tunnel section of the Welgevallen experimental farm of the Stellenbosch University in South Africa (lat. 33º 56' 34.65S, long. 18º 51'55.84 E). Wheat and green bean pot trials were laid out in a 4 x 4 factorial experiment in a complete randomized (CRD) design with three replications without fertilizer to elucidate the effect of biochar and effluent amendment. The biochar levels were designated as B0, B0.5, B2.5, and B5 reflecting approximately 0, 10, 50 and 100 tons·ha^{-1} which is equivalent to 0, 0.5, 2.5, and 5% w/w biochar sand mixture while the effluent levels were 0, 50, 100 and 200 m^3·ha^{-1} reflected as, E0, E50, E100, and E200.

In addition, sand mixtures with the four levels of effluent and four levels of biochar respectively were treated with one dose of fertilizer optimized for the first N application according to the split application system used for wheat and bean production in the Swartland area of the Western Cape, South Africa.

These sets of treatments were designated as FE0, FE50, FE100, FE200 and B0F, B0.5F, B2.5F, B5F and they were done to see the a) combined effect of biochar and fertilizer as well as the b) combined effect of effluent and fertilizer. The rates of fertilizers used were 28 kg·N·ha^{-1} and 10 kg·P·ha^{-1} for the short plant period of 70-73 days of beans and wheat respectively.

The soil mixtures of 3.5 kg were deposited into four-liter containers. The bags were perforated below to allow for sufficient drainage. There was a total of 16 unfertilized treatments and 8 fertilized treatments using winter wheat (*Triticum aestivum L.*) and green beans (*Phaseolus vulgaris*) as test crops.

The treatment combinations without fertilizer were as follows: B0E0, B0E50, B0E100, B0E200, B0.5E0, B0.5E50, B0.5E100, B0.5E200, B2.5E0, B2.5E50, B2.5E100, B2.5E200, B5E0, B5E50, B5E100, and B5E200 for the two crops, while the treatments with fertilizers were E0F1, E50F1, E100F1, E200F1, B0F1, B0.5F1, B2.5F1, and B5F1. Two different crops were used for specific reasons. Green beans was used to check the response of a legume to the treatments and compare it to the cereal (wheat) since we knew that the nitrogen component of the system was low. The highest rate of 200 m^3·ha^{-1} effluent rate was chosen because of the water holding capacity of the sand which was low and considering the limit of 200 m^3·ha^{-1} known to be detrimental as mentioned by Mekki et al. (2013).

After mixing the sand with effluent and biochar, the mixtures were left for seven days before planting to allow the effluent and biochar to fully interact and for the biochar to have sorbed the contaminants in the effluent. Mekki et al., (2007) reported that there was no germination for crops that were planted immediately after amending the soil with OMW.

All the pots were irrigated when the soil water depletion level reached 50 % of the plant available water. Field capacity was determined as described in the physical methods of soil analysis (Romano and Santini, 2002). Four bean seeds and 10 wheat seeds respectively were planted per pot and thinned down to 1 bean and 4 wheat plant respectively 40 days after

planting because emergence was delayed by 2 weeks and the growth rate was slow. Micro nutrients, namely 0.2 g of Omnia nutrition (Hidrospoor) containing 86.9 Fe, 18 Mn, 10.7 Zn, $1.2 \text{g} \cdot \text{g}^{-1}$ Cu, 22 B and $1.9 \text{ g} \cdot \text{kg}^{-1}$Mo were dissolved in 10 L of water and sprayed 60 days after planting when chlorosis was noticed. Micro nutrient deficiencies were to be expected on a soil with such very high pH. There was no further fertilizer application to the pots until they were harvested. The bean seeds were soaked overnight before planting to enhance germination. The water added was the same for all the pots irrespective of the differences in the treatment water holding capacities.

5.2.6. Plant growth parameters: Plant height, number of leaves, above and below ground biomass

The following measurements were recorded for the crops tested: plant height and number of leaves were determined on a weekly basis. The height of the tallest group of plants in the pot was recorded. Plant biomass [above ground biomass (AGB) and below ground biomass (BGB)] was harvested destructively after 70 days for beans and 73 days for wheat and was dried at 80° C for 48 hours and then weighed. Total dry biomass (TDB) was the sum of above ground biomass (AGB) and below ground biomass (BGB).

5.2.7. Statistical analysis

The wheat and bean growth parameters were all normally distributed within the treatments. The plant height and the number of leaves data that were collected over time were analyzed using the mixed model repeated measures ANOVA. The following main effects were measured: biochar main effect, effluent main effect, time main effect and all the second and third order interactions effects. The biomass and the nutrient data were analyzed using the general linear model using two-way factorial-ANOVA to test biochar main effect, effluent main effect and biochar and effluent interactions effect. The Fisher Least Significant Difference (LSD) post hoc testing was also done to determine the significant differences between the treatments with ($p < 0.05$). The data were statistically analyzed using STATISTICA 12.6 software.

5.3. Results

5.3.1. Effluent effects on plant growth parameters of wheat and beans

Effluent negatively affected the wheat plant growth parameters. The control had the highest plant height with means of 14.58 > 13.07> 11.48 and >10.93 cm recorded for E0 control > E50 > E100> E200 for the wheat (p= 0.000020) (Fig. 5.1). The number of leaves for wheat also decreased with increasing effluent rate. The effects were not the same in the bean trial (Figure 5.1). The 50 and 200 $m^3 \cdot ha^{-1}$ gave the highest plant height of beans while 200 $m^3 \cdot ha^{-1}$ was higher than the control with 30% increase for the number of leaves (Figure 5.1).
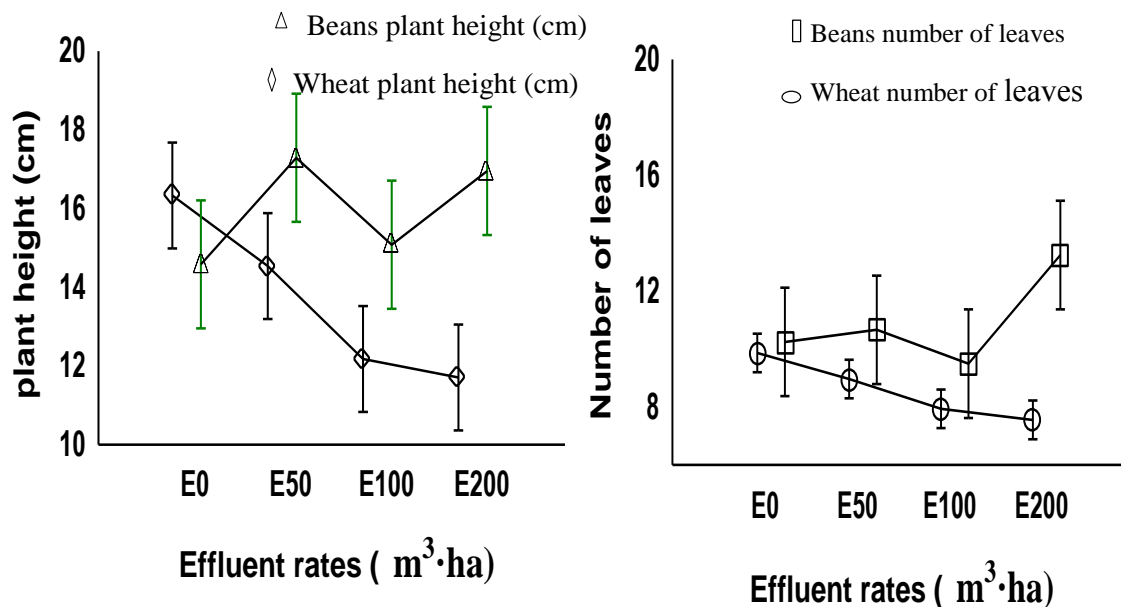
Figure 5.1. Effluent effect on the plant height and number of leaves of wheat and beans. Error bars shows the standard deviation

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A decreasing growth effect with increased OMW application was observed in the wheat biomass production, where the high rate of effluent (E100 and E200) decreased the above ground, below ground and total dry biomass of wheat (Figure 5.2). Meanwhile, the highest mean AGB and TDB of the wheat was recorded with the 50 $m^3 \cdot ha^{-1}$ effluent treatment and it differed significantly at 5% from the control treatment (p=0.02). The increased effluent rate of 100 and 200 $m^3 \cdot ha^{-1}$ AGB significantly decreased from 50 $m^3 \cdot ha^{-1}$ with 16% and 25% decrease respectively (Figure 5.2a). Effluent application had no significant effect on the beans' biomass parameters (Figure 5.2b) however it did not decrease growth with increased rate of 200 $m^3 \cdot ha^{-1}$ as opposed to wheat (Figure 5.2b). The highest rate of 200 $m^3 \cdot ha^{-1}$ gave the same result in beans as 50 $m^3 \cdot ha^{-1}$ for the AGB. Although BGB and TDB decreased by 15.7% and 8% respectively compared to the 50 $m^3 \cdot ha^{-1}$, these reductions were not significant.
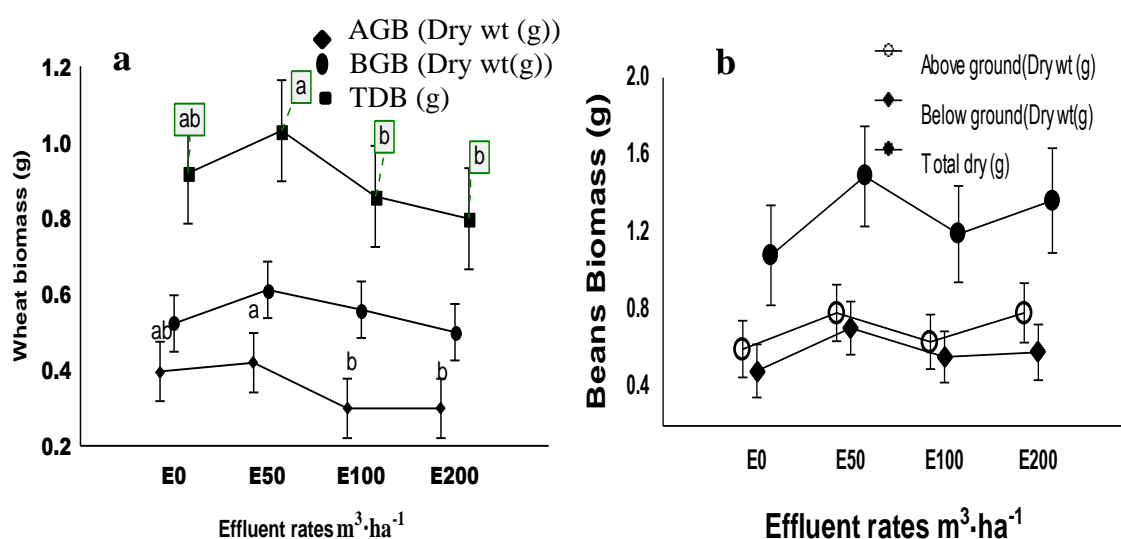
Figure 5.2 Effluent effect on the plant biomass of wheat (a) and beans (b). Error bars shows the standard deviation

Fertilizer addition to the effluent-treated pots did not have any significant effect on the production decline pattern in wheat with increasing effluent application rates. The control pot with fertilizer gave a significant poorer result than without fertilizer for plant height. Then there was a linear increase up to E100 and drops at E200 with fertilizer. The wheat plant height showed that improvement only occurred at the 100 $m^3 \cdot ha^{-1}$ namely a 38% increase compared to the same rate of non-fertilized wheat but an 11% decrease compared to the control (Figure 5.3). The plant height of beans decreased with fertilizer application for all the effluent rates

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except the 200 m$^3 \cdot$ha^{-1} application which showed the same plant height response as the non-fertilized treatment at the same rate (Fig 5.3). Only the 50 m$^3 \cdot$ha^{-1} rate of the beans plant height performed better without fertilizer; the rest decreased with increasing rates of application of effluent. The number of leaves of wheat followed the same pattern as the plant height while increasing effluent application decreased the number of leaves.in beans (data not shown).
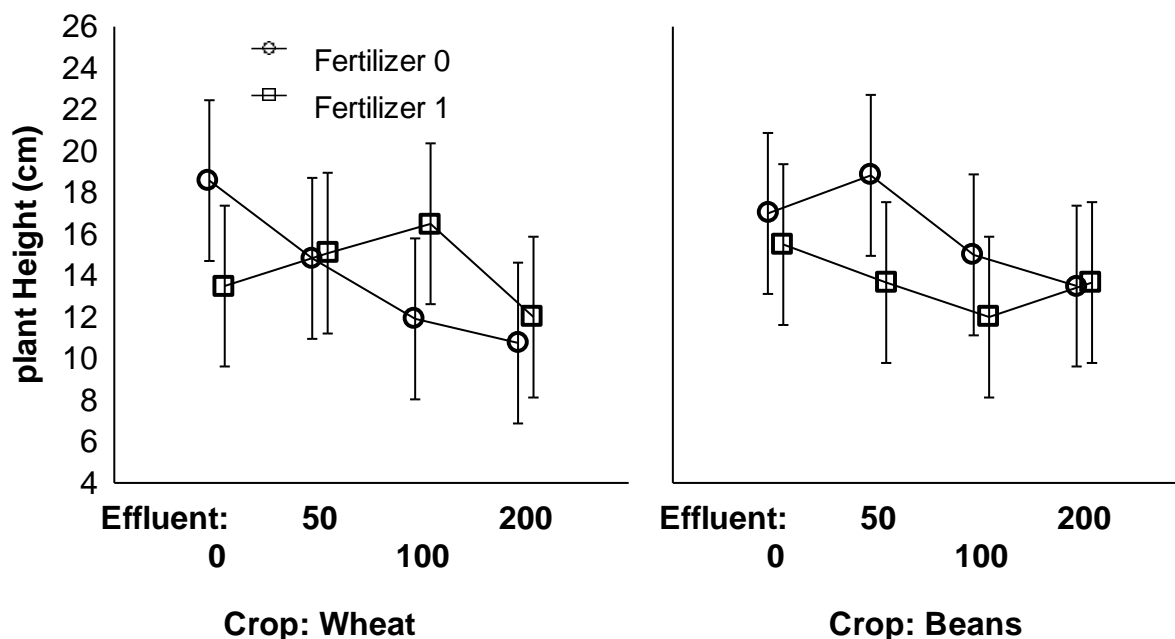
Figure 5.3. Effluent and fertilizer effect on the plant height of beans and wheat

Fertilizer did not affect the AGB and TDB of the wheat and beans; however there was little non-significant improvement for the BGB at the 50 m$^3 \cdot$ha^{-1} rate for wheat compared to the control with fertilizer There was a decreasing effect in production with fertilizer as the effluent rate increased for beans (Figure 5.4). From this result it was clear that the N and P nutrition were not the restricting factors in this case, but the effluent itself had a significant inhibiting effect on plant growth of wheat. Unlike wheat, the green beans were not significantly affected by the OMW application to the soil.

Here we assume that the negative effects are mainly associated with the presence of phenolic compounds, which may be sorbed on biochar and, thus, reduce the negative effects of OMW on on-land disposal.
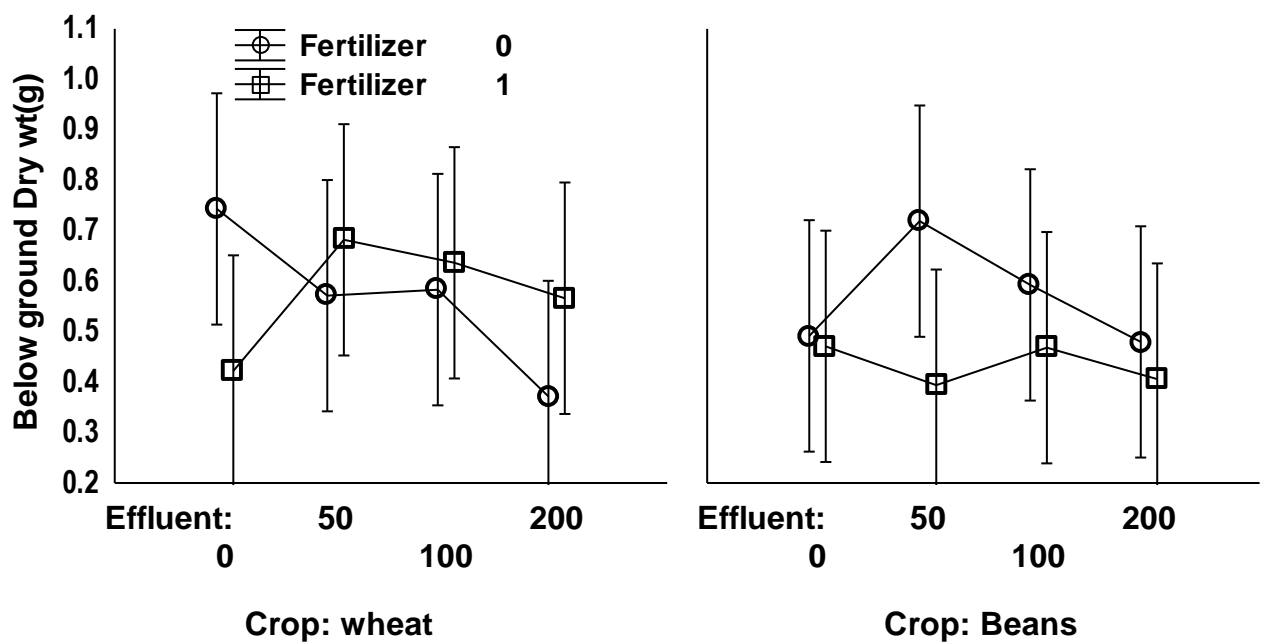
Figure 5.4. Effluent and fertilizer effect on the biomass of beans and wheat

5.3.2. Effects of biochar on the plant growth parameters

Biochar addition without fertilizer had a non-significant effect on wheat plant height and number of leaves (Fig. 5.5). However biochar without fertilizer had a significant positive effect on beans' plant height and number of leaves (Figure 5.5). The 2.5 and 5% biochar rates (the highest rates) gave an increased effect to beans' number of leaves and plant height. The increasing rates of biochar consistently decreased the AGB production in wheat with the control recording the highest mean of 0.5 g without fertilizer. The control and the 0.5% biochar rates in wheat trials did not differ from each other but differed from the 2.5 and 5% levels for the AGB (Figure 5.6). The BGB and TDB also decreased though not significantly with increasing rates of biochar application. For bean biomass results, the increasing rates of biochar application significantly increased the AGB, BGB, and TDB. The total dry biomass of beans had a 30% increase compared to the control when 5% biochar (w/w) was added. Here we say that the biochar application to alkaline soil had little effect on wheat growth with a tendency towards reduction of the AGB, but improved the biomass production of green beans. The lack of biomass production improvement in wheat may be explained by immobilization of N in the presence of biochar. This can be tested by the addition of N fertilizer to the system
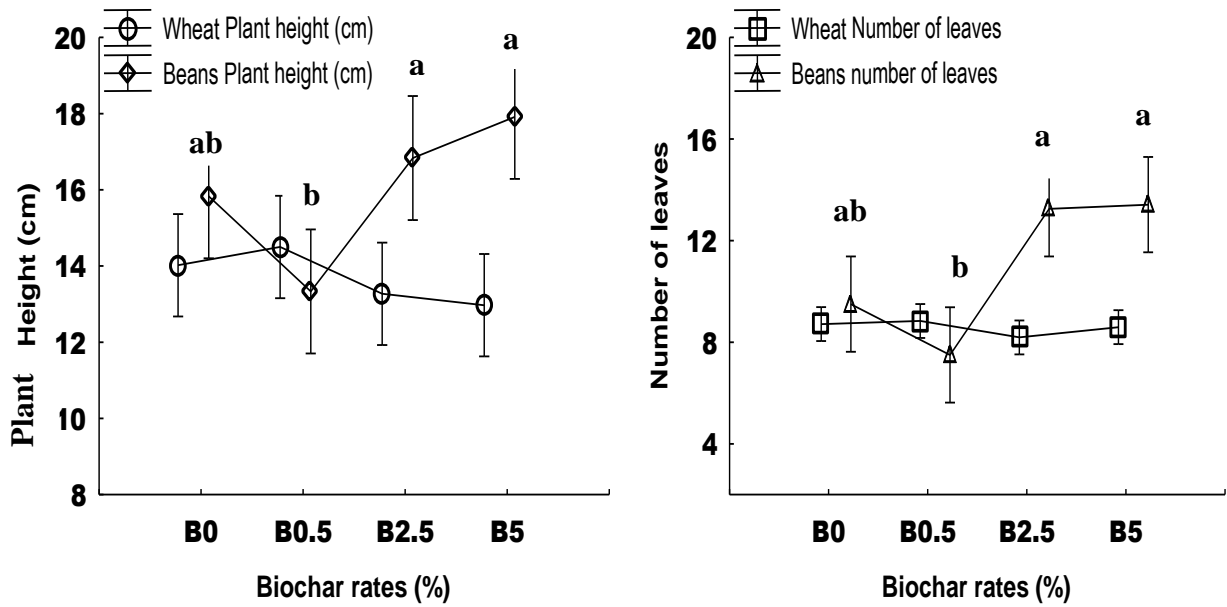
Figure 5.5. Effect of biochar on the plant height and number of leaves of beans and wheat without fertilizer
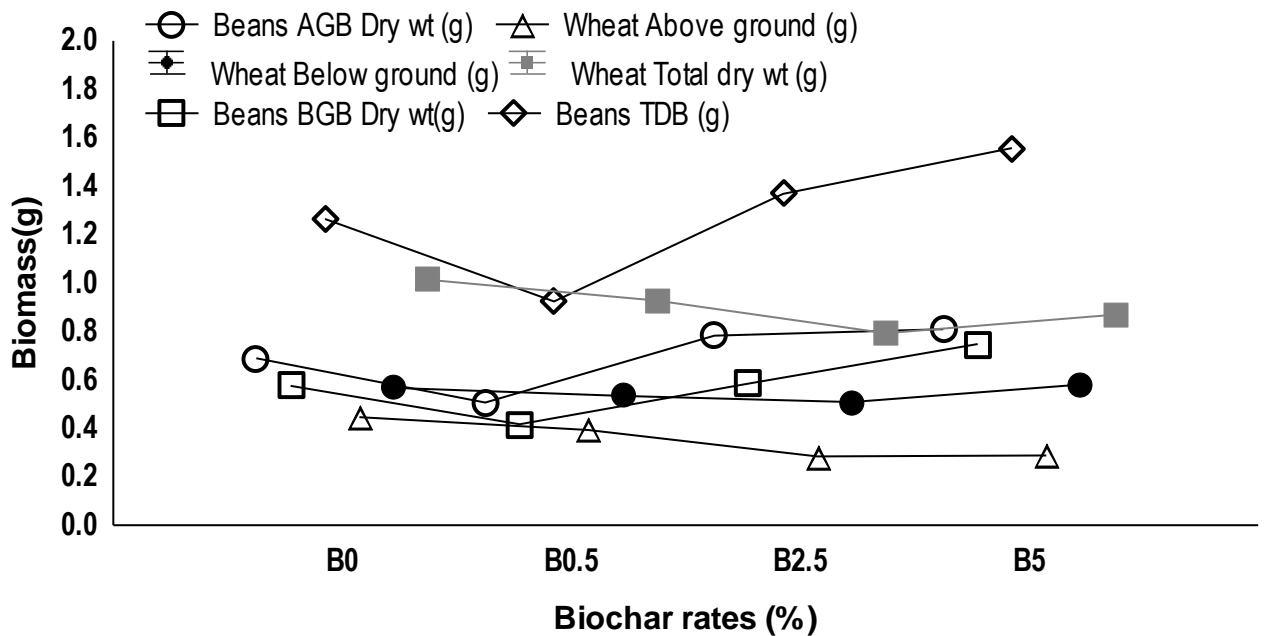
Figure 5.5 Biochar effect on the wheat and beans biomass production

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Addition of fertilizer significantly improved the effect of biochar application on the wheat and bean plant height. There was a positive increase of 11% at 0.5% biochar rate for wheat. The control with fertilizer significantly performed poorer than without fertilizer. The beans plant height had 15.6% increase at the 0.5% (10 tons·ha^{-1}) biochar rate compared to the control (Figure 5.7). The positive effect decreased with increased biochar rate above (10 tons·ha^{-1}). The number of wheat leaves did not show any significant response to the biochar and fertilizer application, although the 0.5% biochar rate showed the highest mean of 11.66 with fertilizer application (data not shown). Green beans number of leaves had better performance with fertilizer at 0 and 0.5% biochar application rate but decreased when the biochar rate increases to 2.5 and 5%. In other words, fertilizer application at high biochar rates decreased the number of leaves of beans (data not shown) Fertilizer application improved the biochar effect for AGB and TDB of beans which performed best at 0.5% biochar application rate with 11% increase compared to the control. Fertilizer application improved the performance of beans but biomass production decreased at 5% (100 tons·ha^{-1}) rate of biochar application (data not shown).
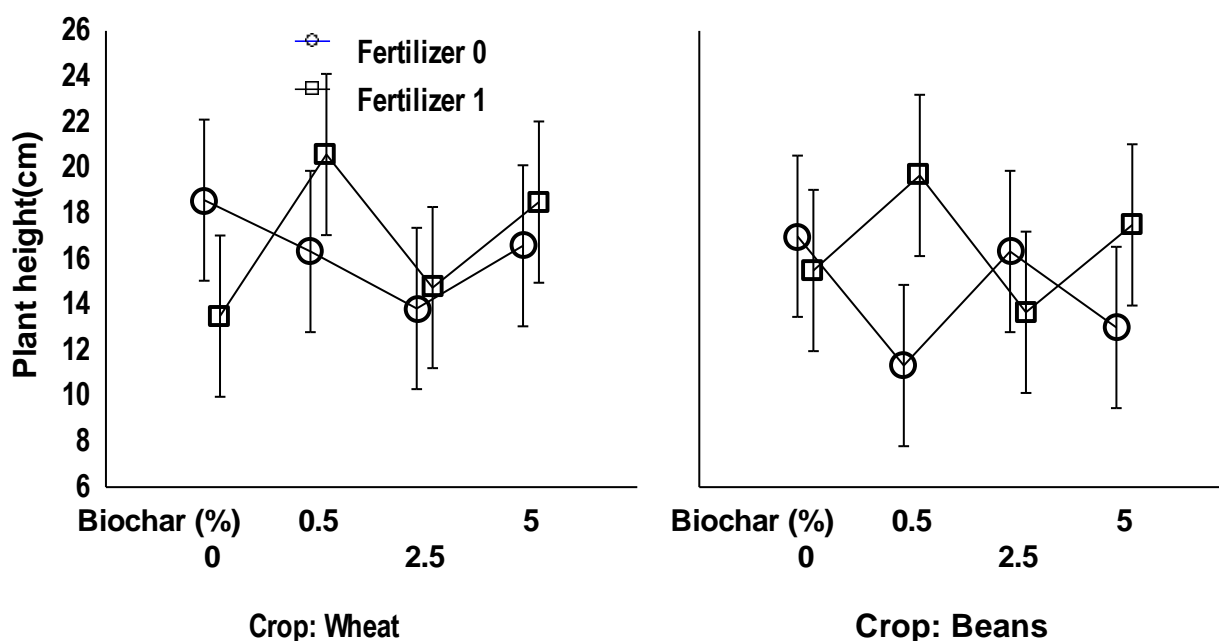
Figure 5.7.   **Fertilizer and biochar effect on the plant height of beans and wheat**

5.3.3. Combined biochar and effluent effects on the growth parameters of wheat and beans

There was no significant effect of the biochar and effluent interaction on the wheat plant height and number of leaves. The treatments applied did not improve the growth of wheat (plant height, the number of leaves and AGB) (data not shown). The beans showed a slight positive growth reaction. The biochar rate of 5% with the highest mean of 18.7 cm had a curvilinear effect on the plant height of beans with the peak at 50 and 100 $m^3 \cdot ha^{-1}$ rate but decreased on increased rates of effluent (Figure 5.8). The number of leaves significantly differed from the control with a 68% increase at the 2.5 and 5% biochar rates and effluent rate of 200 $m^3 \cdot ha^{-1}$ (Appendix 3,Table 5.4 and Fig. 5.9).
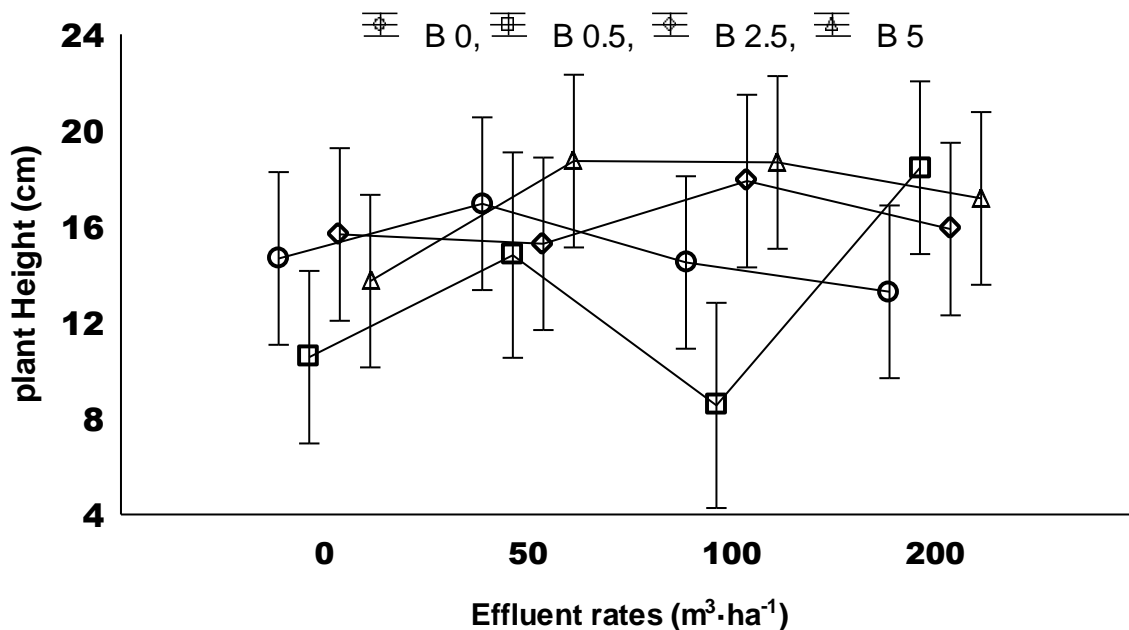
Figure 5.8.Combined effluent and biochar effect on the plant height of beans. Error bars shows the standard deviation.
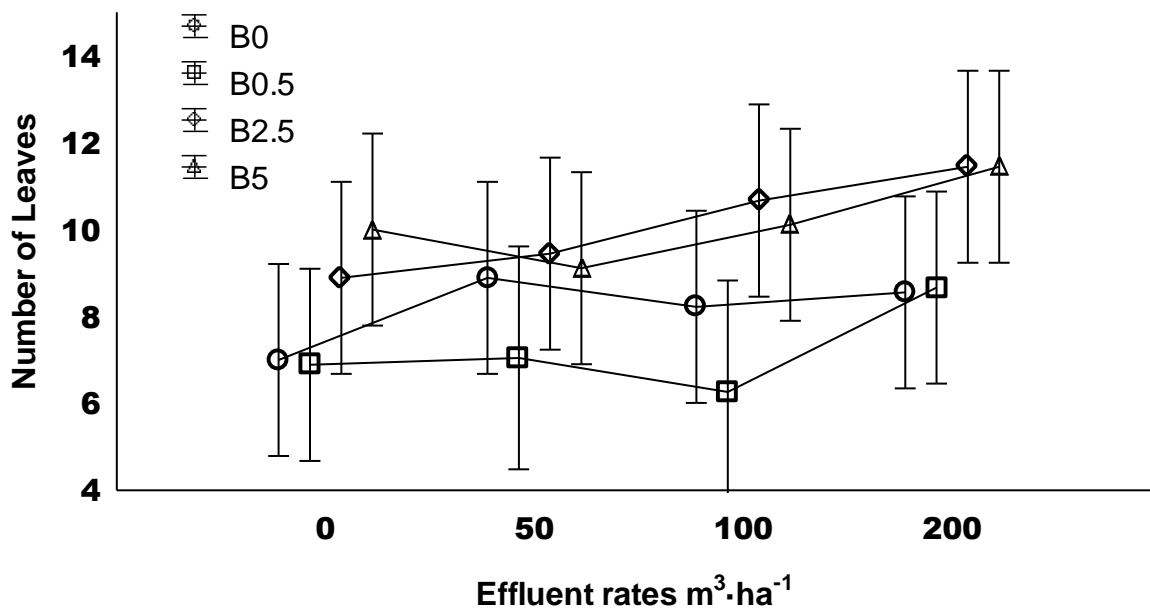
Figure 5.9. Biochar and effluent effect on the number of leaves of beans Error bars shows the standard deviation (**see data in Appendix 3 Table 5.4**)
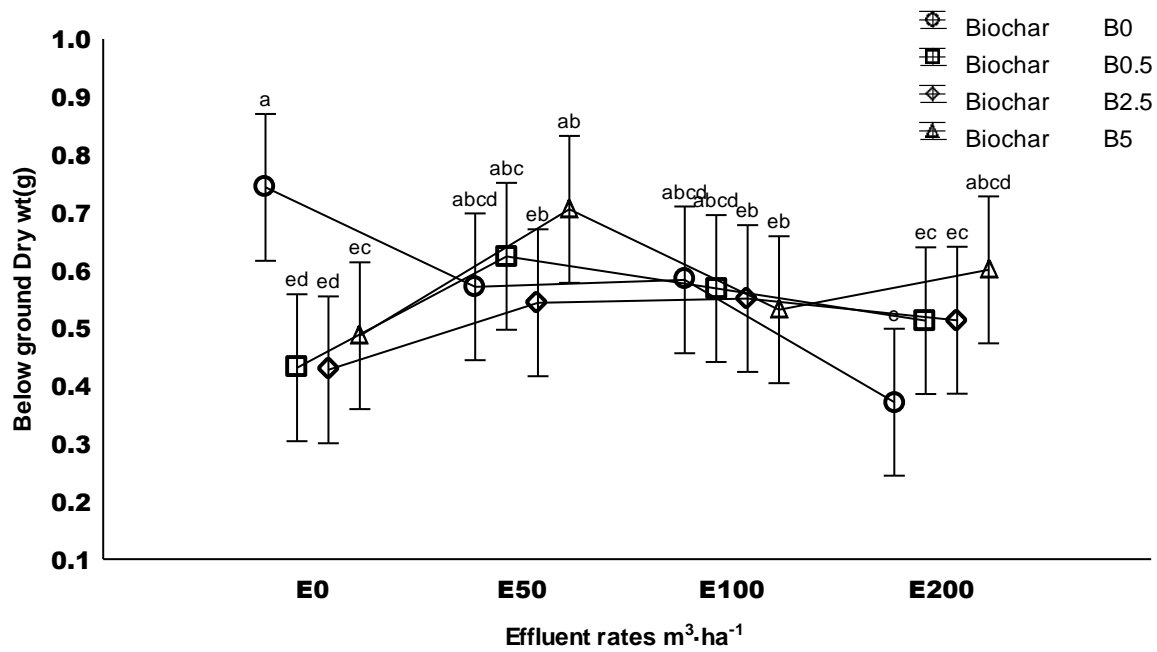
Figure 5.10. Combined effect of effluent and biochar on BGB of wheat. Error bars shows the standard deviation (see data in Appendix 3 Table 5.4)
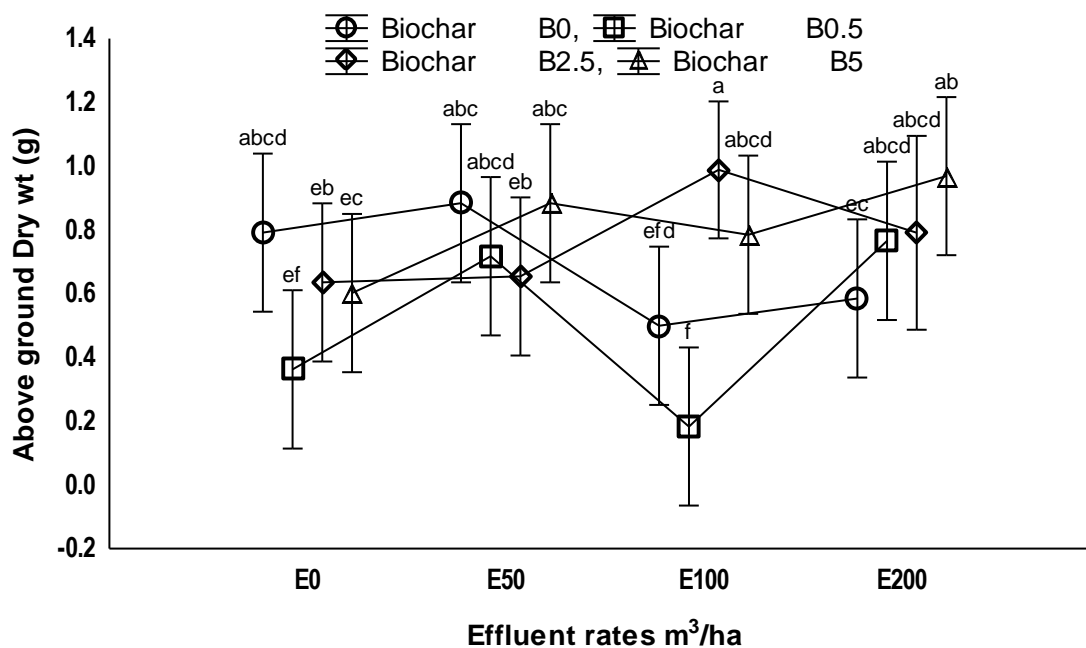
Figure 5.11.   Combined effect of biochar and effluent on the AGB of beans. Error bars shows the standard deviation

The wheat below ground biomass (BGB) showed significant interaction between the biochar and effluent combination (p=0.02) with the control giving the best mean biomass yield of 0.743 g (Figure 5.10 and Table 5.4). Biochar rates of 0.5% and 5% combined with the effluent rate of 50 m^3·ha^{-1} partially counteracted the negative effluent effect and it decreased (not significantly) by 16% and 5% respectively from the control (Figure 5.10). The wheat TDB was significantly affected by the biochar and effluent interactions (p=0.02). Though the combination did not improve the production of wheat, the negative effects of the effluent application were reduced (data not shown).

There was significant interaction between biochar and effluent treatments applied for the AGB of beans (p=0.01). A biochar rate of 2.5 % improved effluent 100 m^3·ha^{-1} performance and had the highest mean yield of 0.99 g (Figure 5.11). The effect on the BGB and TDB was not significant.

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5.3.4. Soil-biochar-effluent mixture characteristics

The parameters determined on the soil-biochar-effluent mixtures showed significantly increased pH, EC, and K. The increase was significant with increased effluent addition as shown for EC (mS·m^{-1}), pH (Fig 5.12) and K (mg·kg^{-1}) (Fig 5.13). Meanwhile, biochar production negatively correlated with Mg (Cmol//kg) and Na (Cmol//kg) and positively correlated with K (mg·kg^{-1}) and C (%) at the following R values and p-values (Table 5.6). Increased biochar addition decreased total N content as was observed in this study.
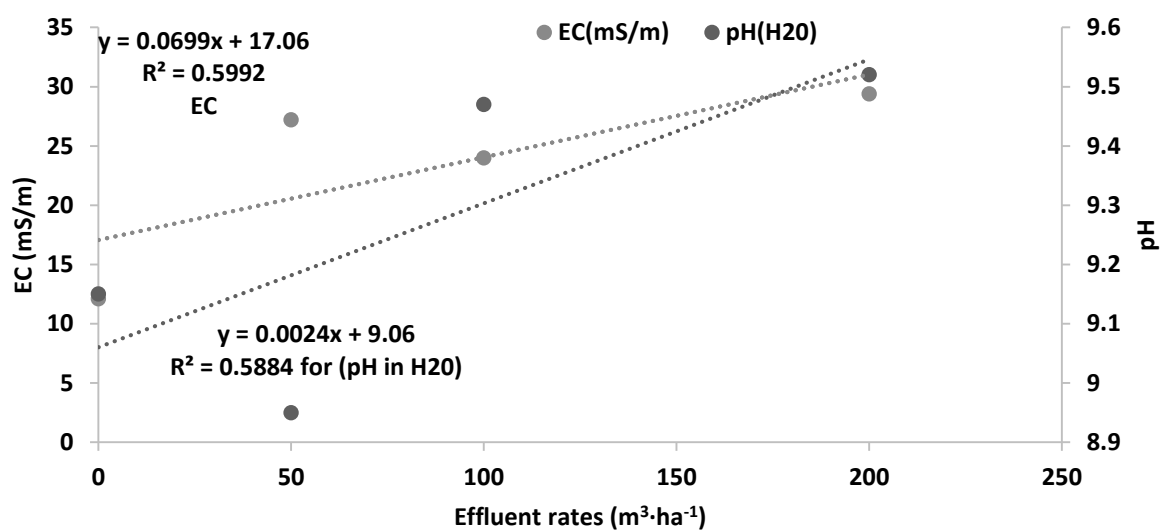
Figure 5.12   **increased pH and EC as effluent rates increased**
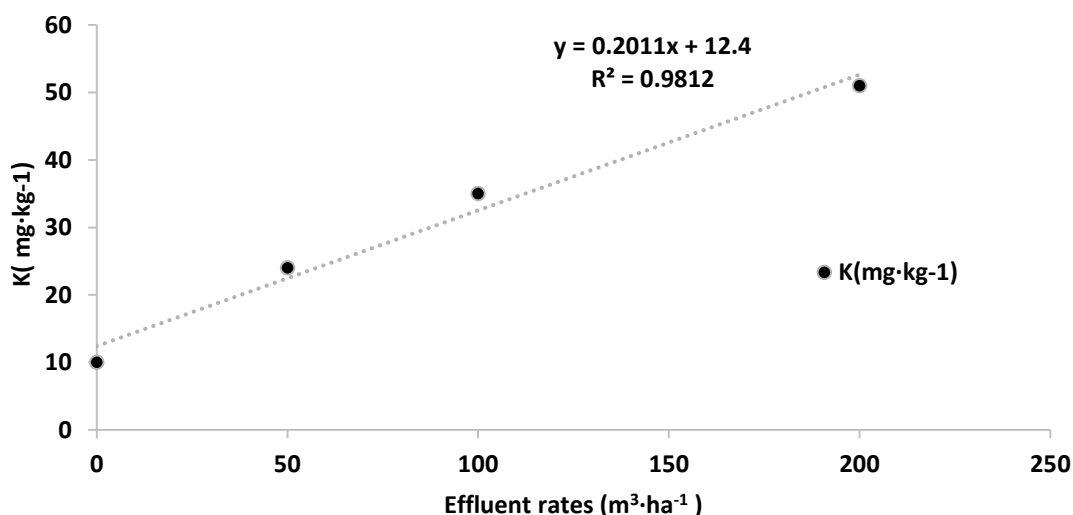
Figure 5.13   **Increased K content as effluent rate increases**

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5.4. Discussion

5.4.1. Effluent effect on the plant growth parameters of wheat and beans

Effluent negatively affected the plant growth parameters observed which was attributed to the phenol toxicity of the effluent to the plant (Hachicha et al., 2009). This confirmed the result of Mekki et al. (2013) who opined the tolerable limit of the effluent to be 50 $m^3 \cdot ha^{-1}$ as increased rates negatively affected wheat growth parameters. A small positive effect of the effluent application was observed in the growth of green beans. The 50 $m^3 \cdot ha^{-1}$ of effluent rate without fertilizer resulted in a little better performance of beans, in the plant height and AGB production. Fertilizer application worsened the beans performance though had a little improvement on wheat. The negative effect was attributed to high phenolic content of the effluent. Hence, treating effluent polluted soil with biochar to neutralize the phenolic effect prior to effluent disposal may be a viable option. The poor performance of the beans with increased fertilizer and effluent application could also be attributed to increased EC (Figure 5.12) considering lower tolerance of green beans to soil salinity compared to wheat.

5.4.2. Effect of biochar doses on phenol adsorption from the effluent

The increasing adsorbent concentration at a fixed phenol concentration of the effluent provided more available adsorption sites for phenol and hence the removal was enhanced. At 7.5 g of biochar dose, there was 100% removal of the phenols in the effluent (Figure 2.4). This is in line with the result of El-Naas et al. (2010) who observed the same results in their aqueous water and refinery wastewater adsorption experiment. The rate of 300 g of biochar per liter of effluent (Fig. 2.4) may be seen as reasonable to completely neutralize the phenol effects on plant growth. With this result, and introducing a safety margin, high rates of biochar (2.5% and 5%) were applied to eliminate all the effects of the phenols in effluent and phenol limitations on plant growth (Table 5.5).

Table 5.5. Biochar and effluent quantity applied per pot in relation to the rates used in the study

Effluent rates ($m^3 \cdot ha^{-1}$)	Effluent ($ml \cdot pot^{-1}$)	Biochar rates %	Biochar Qty/pot (g)	Biochar $g \cdot pot^{-1}$
50	100	0.5	17.5	30
100	200	2.5	87.5	60
200	400	5	175	120

5.4.3.   Biochar effect on plant growth parameters

The differences in the effect of biochar on plant growth parameters indicated that the biochar itself had little effect on wheat in alkaline soil. This is opposite of what was observed in an acidic environment (Sika and Hardie, 2014). However, in calcareous sand used in this study, the increased rates of biochar improved the plant growth parameters of beans. This led us to conclude that the nutrient (particularly N) availability and not only toxicity of phenol played a role in further combination of biochar and effluent treatment. The sorption experiment indicated that 300 g of biochar could completely neutralize the phenol in one liter of the effluent (Fig 2.4). The 2.5% and 5% rates had enough biochar to neutralize all the phenols at the 100 $m^3 \cdot ha^{-1}$ and 200 $m^3 \cdot ha^{-1}$ effluent rates (Table 5.5). The wheat performance remained poor, though they improved slightly compared to effluent treatment without biochar priming. At these two biochar rates there was an increasing positive effect for the beans.

Fertilizer was applied here with the intent to correct the negative effects from biochar application. The result showed that with fertilizer application there was an improved biochar effect for both wheat and beans at 0.5% biochar rate but this effect decreased with increased rates of biochar application. This result was in accordance with the other researchers who recommended that a 0.5% (10 $t \cdot ha^{-1}$) rate of biochar application is the best for plant growth (Sika and Hardie, 2014). The appropriate rate for low temperature biochar application on coarse textured soils may be up to 1 % (Butnan et al., 2015) roughly equivalent to 20 $t \cdot ha^{-1}$. High biochar rates to especially coarse textured soils have been reported to have a negative effect on plant growth. Biochar rates higher than 60 $t \cdot ha^{-1}$ applied to ryegrass perennial crop on a sandy loam resulted in declined production (Baronti et al., 2010).

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5.4.4. Soil–biochar-effluent mixture effect on nutrient availability

It is important to understand the biochar effects on nutrient availability in these mixtures. Biochar is generally known for its liming capacity but some biochars used in a calcareous soil could lower the soil's pH depending on their surface properties (Marks et al., 2014) and initial soil pH. The lowering of the pH with biochar application could be the reason for the little improvement in wheat when biochar was added. The biochar-mediated pH changes may be related to its surface properties such as functional group composition, which may include carboxyls (strong acids), phenols and carbonyls (weaker acids) (Joseph et al., 2009). The pinewood biochar used in this work contained reasonable peaks of acidic functional groups on its surfaces like the carboxylic, the aromatic and aliphatic carbons (Figure 2.7) and hence negatively correlated with the pH of the mixture (soil-biochar-effluent) though at low significance level (Table 5.6). It reduced the effect on the pH that increased due to the effluent addition. The increase in pH due to effluent rate increase is in line with the work of Sekar et al. (2012), who reported that a digested effluent increased the pH of the soil. Also Carter et al. (2013) noted a pH increase in the fertilized + liquid compost + biochar + soil mixture. In their study, the effluent was left for some weeks to ferment before application and decomposition took place. Di Serio et al. (2008) also reported a small increase in pH from 20 to 40 cm after applying 160 $m^3 \cdot ha^{-1}$ of effluent on a sandy loam soil with pH of 6.4.

The elemental concentrations (Table 5.1) showed the extent of effluent and biochar correlation to nutrient availability in the soil-effluent-biochar mixtures which is to elucidate the effect on pH. Effluent had a positive correlation with %N though only at 10% (p=0.06) significance level and biochar had a negative correlation with %N (Table 5.6). The biochar negative correlation indicates that biochar has bound almost all the available nitrogen in the biochar mixture. This is contrary to Ducey et al. (2013), who said that in alkaline soils, the addition of biochars of lower pH potentially reduced ammonia volatilization and therefore increase plant available N. Other researchers have reported that biochar has the ability to retain nitrogen and release it gradually back to the soil (Steiner et al., 2008). Considering the high C: N ratio (24) of the effluent compared to the optimal level and that of the biochar which was very high (156), the effect was a reduction of N availability.

Table 5.6. **The correlation of the treatments with the soil parameters of the soil-biochar-effluent mixture and their significant levels**

Pair of Variables	Correlation R	p <.05000 p-value
Effluent & pH(kCl)	0.660401	0.005361
Effluent & K(mg/kg)	0.497564	0.049864
Effluent & P(mg/kg)	0.790787	0.000265
Effluent & EC(mS/m)	0.848875	0.000032
Effluent & C (%)	0.183854	0.495488
Effluent & C:N	-0.123185	0.649464
Effluent & Na(mg/kg)	0.073030	0.788098
Effluent & Mg (cmol/kg)	-0.460818	0.072429
Effluent & Ca (cmol/kg)	0.012127	0.964447
Effluent & N (%)	0.479010	0.060486
biochar & pH(kCl)	-0.469900	0.066270
biochar & Ca (cmol/kg)	-0.460818	0.072429
biochar & Mg (cmol/kg)	-0.800368	0.000196
biochar & Na(mg/kg)	-0.858099	0.000021
biochar & K(mg/kg)	0.825228	0.000083
biochar & P(mg/kg)	-0.117382	0.665055
biochar & C (%)	0.874891	0.000009
biochar & EC(mS/m)	0.278916	0.295511
biochar & N (%)	-0.317266	0.231164
biochar & C:N	0.856135	0.000023

Potassium was positively correlated with both biochar and effluent application. The result showed that more K was released from the biochar than from the effluent. This is understandable since K is released in plant-available form during low temperature pyrolysis (Zeelie et al., 2012) and the biochar used in this work was a slow pyrolyzed material at the temperature of 450°C.

Wastewaters are known to have a high carbonate concentration and this concentration has been known to disturb the phosphorus recovery (Song et al., 2002). The P precipitation is affected by the calcium content and pH values as in generally known. The higher the calcium content of the soil, the more the P is unavailable through the secondary precipitation of Ca-P minerals and /or a strong sorption reaction of P with $CaCO_3$ Chaari et al.(2015). In our study, the available P values were positively affected by the effluent application rates and were affected negatively by the biochar rates though not significantly. This can be explained with the formation of calcium phosphate that resulted in super saturation phenomenon as explained below. The increase in carbonate addition at pH of 8 and 9 increases the concentration of $CaCO_3$ and $Ca(HCO_3)_2$ ion pairs and increases free calcium ions resulting in super saturation and increase in P (Table 5.1) precipitation (Song et al., 2002). It was assumed that increased effluent addition would have increased carbonate formation in the soil as explained above and hence the super saturation which led to more phosphorus availability. The slight negative correlation of the biochar can be understood as a result of increased concentration of divalent cations in the soil solution as suggested by Chintala et al. (2014) thereby increasing precipitation of P as Ca or Mg phosphates.

5.4.5. Effects of effluent-biochar-soil interactions on soil nutrient status

Biochar has been reported to increase crop yields when combined with other additives like mineral fertilizer (Zhang et al., 2010) and with chicken manure (Utomo et al., 2012). However, pinewood biochar added to a silt loam soil reduced or negated the yield improvements achieved with both bio-solids fertilizer and mineral fertilizer. The authors explained that biochar could have retained the nutrients making them inaccessible to plants (Knowles et al., 2011). In contrast, Sekar et al. (2012) observed a non-significant results of biochar application and a "nearly significant" increase of biochar + effluent addition on crop yield on a neutral soil with pH 7.2. Their result signified that the effluent had a positive impact on nutrient release to the crop in combination with biochar.

A biochar application on calcareous soil has been reported to have a negative effect on nutrient availability and plant growth; for instance, it lowered maize silage production (Lentz and Ippolito, 2012). Many researchers related the negative results of plant growth from biochar application to biochar negative correlation to NO_3^- (Ventura et al., 2012; Ducey et al., 2013). In view of that, Marks et al. (2014) suggested potential fertilization of slow pyrolysis wood char under such conditions. They stated that a number of studies of biochar effects in calcareous soils induced changes in N and P availability and plant growth. They noted an exception, which was the study of Zhang et al. (2012a), who reported the increased maize production in a biochar + mineral fertilizer treatment on a calcareous soil of the Chinese Great Central plain. They attributed the good result to the increased N-use efficiency caused by the increased organic carbon content from the biochar addition. Our experiment showed improved wheat and beans biomass production with biochar + fertilizer treatment in alkaline sand compared pairwise to unfertilized treatments (Fig 5.7). This could be attributed to the increased nutrient supply from the fertilizer

In this work, pH negatively correlated significantly with plant height and number of leaves for beans with R Values of (-0.312871, and -0.362458) respectively, while the respective values of wheat were (-0.52971, -0.453418). It can be concluded that an increased effluent rate increased the EC and hence salinity aggravated the negative effects of high pH on plant growth. Although phosphorus present in both effluent and biochar is an important nutrient in plant growth, low nitrogen content and binding to abundant Ca limited its performance (Havill et al., 1974). More so, the cation exchange sites of the soil boosted by the biochar might have contributed to immobilizing nitrogen. This confirms the suggestion from Marks et al. (2014) that it is nutrient immobilization and not volatilization that causes the reduced N availability in biochar treated soils. It agrees with the work of Ventura et al., (2012) who speculated that strong alkalinity around the biochar particles might have caused N changes. This experiment recorded the best result of the BGB and TDB of wheat in the control (Figure 5.2). The addition of the effluent did not enhance the AGB which indicated that the acidity of the effluent was not high enough to neutralize the alkalinity of the soil solution.

Contrary to the results of Sekar (2012) who stated that effluent application positively affected the soil treated with biochar. Our study showed a curvilinear effect with the best result at the $50 \text{ m}^3 \cdot \text{ha}^{-1}$ rate of effluent application + 5% biochar rate for beans plant height and a significant decrease with increased rate for both the TDB and AGB. This can be explained with the positive correlation of effluent and pH while biochar negatively correlated with P and N

availability. The added biochar decreased the wheat biomass production and it agreed with the result of Farrell et al. (2014) who found that biochar seemed to have suppressed wheat yield without fertilizer addition and concluded that biochar has an impact on both soil N and P availability.

The effect of the applied treatments on beans was different from that of the wheat treatments. The performance was better than that of wheat due to symbiotic N fixation by the beans. Here K significantly correlated positively with plant height and number of leaves, while the effect of soil N supplied with the effluent was not significant. The Ca, Mg and Na in these soils had strong negative correlations with the plant height and number of leaves. Crop yield responses to biochar depend on the chemical and physical properties of the biochar, climatic conditions, soil conditions and crop type (Van Zwieten et al., 2010; Haefele et al., 2011). Zhang et al. (2012b) recorded increased maize yield compared to the control when they applied 20 and 40 t·ha^{-1} of biochar in a poor calcareous soil. The study attributed the yield increases to the biochar effect in improving the soil and making the nutrients available as earlier stated by Zhang et al. (2010). They noted that the yield increase did not correspond to the biochar amendment rates and they suspected that N availability could be decreased under high biochar application rates (Lehmann et al., 2003) due to a high C:N ratio. The condition is the same as in our study where biochar addition also negatively correlated with N but had a positive significant correlation with C and C: N ratio. Their study revealed that the N use efficiency was significantly increased under biochar amendment, which was first and also noted by Pan et al. (2009) namely that soil organic carbon accumulation due to biochar amendment could enhance N use efficiency and increase crop yield. The biochars with higher N, referred to as N-species, resulted in significant plant response according to Marks et al. (2014). This indicates that N has a significant role to play in plant growth. This is shown by the beans performing better than the wheat. It showed that the N could have been possibly supplied by the fixation process of the bean crop as a nitrogen fixing legume.

The bean AGB showing the best result at B2.5E100 and showing significant interaction between the effluent and biochar, indicated there was a positive effect of applying the treatment to the crop. Increased biochar rates significantly favored the BGB and TDB. The effluent and biochar applied had a positive correlation with all the green bean plant parameters determined. This can be explained by the negative correlation with pH, Ca, Na, and Mg and positive

correlation with K, P, C and N. The N addition from the bean's fixing ability reduced the C: N ratio and made other nutrients available.

5.5. Conclusion

The olive mill wastewater had a strong negative effect on growth parameters of wheat on alkaline sand. The deterioration of wheat growth parameters with increasing effluent application rates was observed with and without mineral fertilizer addition. This effect was less pronounced in green beans. The negative effect was primarily attributed to high concentration of phenols on the OMW and increased pH due to effluent addition which contributed to immobilization of other nutrients.

Pinewood biochar application to calcareous alkaline sand resulted in negative effects on wheat growth, which was partially mitigated by addition of mineral fertilizer. Biochar improved bean performance but biochar + fertilizer addition had a more or less negative effect on the performance of bean. The high pH of the system could also be the reason the wheat performance was very poor as wheat has very low tolerance for high pH.

It can be concluded that it was not only the phenol contamination that contributed to the low yield obtained but also the availability of nutrients. The nutrient supply was optimized at the effluent application rate of 50 $m^3 \cdot ha^{-1}$ and the negative effects from phenol neutralized by biochar application at the rate of 0.5% w/w (10 $t \cdot ha^{-1}$). The increased P availability and N added by N fixation in case of beans explains why beans performed better than wheat in this environment. Therefore, on-land disposal of OMW may be possible at higher than currently recommended rates in combination with biochar soil priming and careful crop choice. It could therefore be beneficial to try OMW + biochar combination on an acidic soil with a leguminous plant.

Chapter 6
EFFECT OF PINEWOOD BIOCHAR ON IMPROVING BAMBARA GROUNDNUT PRODUCTION ON OXISOL POLLUTED WITH OLIVE MILL WASTEWATER IN POT TRIALS

6.1. Introduction

The bambara groundnut (*Vigna subterranea*) crop is a very important legume of West African origin. Its production is neglected even in its place of origin despite its usefulness to humans as food and the soil as a nitrogen fixer. It is a good alternative food to beans and recently, research into commercialization of this crop has been started in South Africa (Chibarabada et al., 2015). Bambara is known for its high protein content. Yet bambara is neglected due to several reasons, including low yield (Akpalu et al., 2013), high cost of the product and pest and disease attack (Hillocks et al., 2012). The high cost of the product is probably due to low yield which could easily be attributed to low nutrient status of highly weathered tropical soils. This low nutrient status is also a problem in the Mediterranean regions where, in addition to low nutrient status, there is also low water availability for agriculture.

Oxisol (Hutton) that is used in this study is normally a tropical soil but was also dug from a non-agricultural land near the University of Stellenbosch. This soil has a problem of phosphorus deficiency due to strong fixation into unavailable forms and as explained in the previous chapter and would therefore be suitable for this study. It is also a soil type that requires a lot of organic matter addition for better performance.

The quantity of OMW generated in the Mediterranean region and the problem with its composition have been discussed in the previous chapters of this work. There are reports that its toxic compound contents counteract the beneficial effect of nutrient supplements (Piotrowska et al., 2006; Ouzounidou et al., 2008). Despite its high nutrient content, it is unfortunate that the effluent has a high C: N ratio which according to Hanifi and El Hadrami (2009) leads to N immobilization in the OMW treated soils. The low N content could be improved by the use of an N-fixing crop. Since the lack of N is the main limiting factor in crop productivity, it was decided to use bambara groundnut as a test crop. It has been shown that OMW also reduced the germinability of crops due to its phytotoxic properties at high rates (Di

Serio et al., 2008; Nwoko et al., 2010; Mekki et al., 2013) and inhibition of soil microbial dehydrogenase activity (Nwaogu et al., 2012). In spite of these problems, OMW is still used as a soil amendment for enhancing crop production (Nwoko et al., 2010), limiting ammonia loss (Siva et al., 1999) and irrigation to offset water scarcity (Chaari et al., 2014) especially in the Mediterranean region. The main concern regarding this effluent is that microbial activities may be reduced due to the toxicity of some effluent components, and thus possibly reduce the nutrient release from the effluent degradation.

The toxic component phenol of the OMW may possibly be adsorbed by biochar which has been found to be one of the effective means of treating wastewater (Southavong et al., 2015). Ogbonnaya and Semple (2013) concluded in their review that biochar influences the bioavailability of hydrocarbon organic contaminants through sorption whilst the mineral contained in the biochar serves as a nutrient source to the soil microorganisms. Several other studies have shown that biochar can be used to sorb organic contaminants in the soil (Chen and Chen, 2009; Vithanage et al., 2014; Kupryianchyk et al., 2015). The effect of biochar on nutrient availability is uncertain as it was reported to either increase or decrease yield depending on crop and soil type (Jeffery et al., 2011). Biochar addition has been reported to increase the N fixation by both free living and symbiotic diazotrophs (Jeffery et al., 2011). The biochar and its low nutrient status makes it a non-fertilizer on its own. Hence soil needs the addition of a fertilizer to realize its potential. Steiner et al. (2007) strongly emphasized that charcoal with chicken manure amendment would mimic the favorable properties of Terra Preta best. We proposed to use OMW instead of chicken manure in combination with pinewood biochar to enhance nutrient availability from poor subsoil (B horizon) of an Oxisol, simulating highly weathered and poor nutrient acidic soils as well as manage the disposal of OMW. The aims of this study were:

1. To investigate the effects of pinewood biochar and OMW on bambara plant growth parameters and yield'
2. To evaluate the changes of soil condition and nutrient availability in controlled pot experiments.

6.2. Materials and methods

6.2.1. Study site description

The greenhouse experiment was conducted at the Welgevallen experimental farm of the Faculty of Agrisciences, Stellenbosch University, South Africa (lat.33º 56' 34.65S, long. 18º 51'55.84E). Bambara pot trials were arranged in a tunnel in a complete randomized design to determine the effect of biochar in mitigating OMW toxicity and enhancing the nutrient status of the OMW amended soils.

6.2.2. Soil and biochar material and their characteristics

Soil classified as an Oxisol in US Soil Taxonomy (Soil Survey Staff, USDA 2006) was collected from a non-agricultural land at Helshoogte pass (Lat. 33° 55′ 17.55″ S, Long.18° 55′ 3.01″ E), Stellenbosch, South Africa. The top 20 cm of the soil was removed and composite samples were collected from the acidic nutrient-poor subsurface B horizon (20 - 60 cm depths) and used to fill the pots. The soil was air dried and sieved through a 2 mm sieve prior to the experiments. The particle size analysis was conducted using pipette method (Gee and Or, 2002). The soil texture was classified as clay loam (37.75% sand fraction, 37% clay, and 25.25% silt). The pH of the soil was determined using the 1 : 2.5 ratio in distilled water and a EUTHECH pH 700 meter (Thomas, 1996). The soil bulk density was determined as 1.01 g·cm^{-3} using the core method (Blake and Harte, 1986). The available P, of the soil was determined with 1% citric acid extract using Inducted Coupled Plasma spectroscopy (ICP) as shown in the (Non-Affiliated Soil Analysis Work Committee and Soil Science Society of South Africa, 1990). While the cations; Ca, Mg, Na, K, and ECEC were determined with ammonium acetate extract. Soil organic carbon was determined by the Walkley-Black method of Nelson and Sommers, (1996) and Nitrogen by the Kjeldahl method (Bremner, 1996) (Table 6.1).

The biochar used for this work was produced from pinewood sawmill waste by a small-scale commercial producer (S & P Carbon (Pty) Ltd, Kareedouw, Eastern Cape, South Africa). The sawmill waste was slow pyrolyzed at approximately 450° C, and then crushed to 5 cm particle size and sieved through 2 mm sieve before use. The pH of the biochar was measured as described by Cheng and Lehmann (2009) with 1:20 solution ratio owing to the low bulk density of the biochar (approximately 0.4 g·cm^{-3}). They stated that this ratio is approximately volumetrically equal to a 1:2.5 soil to solution ratio. The plant available P and K (Table 6.1)

were determined in the biochar using the ammonium bicarbonate (AB) diethylenetriamine pentacetic acid (DTPA) method (Soltanpour and Schwab, 1977) as reported by Sika (2012). This method was selected due to the alkaline nature of the biochar and because it can be used to determine plant available P and other elements simultaneously in the extracting solution by inductively coupled plasma mass spectroscopy (ICP-MS) using the Thermo ICap 6300 radial instrument. The C and N of the biochar were determined using the EuroVector Elemental Analyzer 3000 by a dry combustion method after ball milling the samples series (Nelson and Sommers, 1996) (Table 6.1).

Table 6.1 Selected chemical properties of the biochar and pooled soil-biochar-effluent mixture determined before planting

Treatment	pH KCl	pH H20	cmol (+)·kg⁻¹ Acidity	Ca	Mg	Na	K	ECEC	Total % C	NH₄ % N	% Acid Saturation
B0E0(Soil only)	4.2	4.7	2.10	0.18	0.32	0.14	0.21	2.96	0.74	0.050	71
B0E50	4.1	4.8	2.26	0.28	0.34	0.25	0.30	3.43	0.78	0.022	66
B0E100	4.2	4.9	1.80	0.28	0.42	0.31	0.33	5.66	0.75	0.017	32
B0E200	4.2	4.8	2.04	0.48	0.35	0.33	0.38	3.59	0.78	0.017	57
B0E400	4.2	4.9	1.96	0.26	0.35	0.46	0.52	3.55	0.83	0.047	55
B0E800	4.4	6.2	1.60	0.44	0.41	0.65	0.80	3.90	0.91	0.050	41
B2E0	4.3	6.2	1.94	0.30	0.37	0.16	0.29	3.07	0.78	0.040	63
B2E50	4.2	6.4	1.87	0.32	0.36	0.25	0.36	3.17	0.78	0.048	59
B2E100	4.2	6.3	1.89	0.33	0.36	0.24	0.36	3.20	0.83	0.046	59
B2E200	4.3	6.4	1.87	0.39	0.40	0.33	0.45	3.45	0.72	0.049	54
B2E400	4.3	6.3	1.71	0.51	0.42	0.48	0.57	3.70	0.91	0.050	46
B2E800	4.3	6.4	1.86	0.37	0.37	0.62	0.67	3.88	1.37	0.048	48
BC only		9.3	0	4.75	1.35	0.02	4.41	10.90	82.71	0.530	

The treatments represent interaction between biochar and effluent applied at the different rates; 0 and 2 % for biochar and 0, 50, 100, 200, 400, and 800 $m^3 \cdot ha^{-1}$ for effluent.

6.2.3.   Analysis of soil-biochar-effluent mixtures

The sieved soil and biochar were properly mixed by hand, pot by pot, to ensure proper mixing. The biochar application rates of 0 and 2% denoted by B0 and B2, approximately equivalent to 0 and 40 tons·ha^{-1}, respectively, were mixed with 3 kg of the sieved soil. The biochar rate calculation was done assuming 15 cm depth. After the soil-biochar mixing, raw unfiltered liquid effluent of 0, 50, 100, 200, 400, and 800 m^3·ha^{-1} equivalent application rates, denoted as E0, E50, E100, E200, E400, and E800, were added to the soil-biochar mixture. The soil-biochar-effluent mixture was now thoroughly mixed by hand to get a homogenous mixture that was analyzed as stated below.

The pH of the soil-biochar-effluent mixture was determined using the 1: 2.5 ratio in distilled water and read using a EUTHECH pH 700 meter (Thomas, 1996). The available P, Ca, Mg, Na, K and ECEC of the mixtures were determined also with 1% citric acid extract using inducted coupled plasma spectroscopy (ICP) as stated in section above. Soil organic carbon was determined by the Walkley-Black method of Nelson and Sommers (1996) and nitrogen by the Kjeldahl method (Bremner, 1996). These initial determinations of soil and soil-biochar-effluent mixtures were done using pooled samples (mixing the 3 replications together and collecting a representative sample) from every set of treatments and were not duplicated (Table 6.1). After harvest, the same methods were used to characterize the treatments as presented in Table 6.2 (Mean ± S.D, n=3).

6.2.4. Olive oil mill effluent sampling and characterization

The olive mill wastewater was collected from a nearby farm situated 5.8 km from the Stellenbosch University. The effluent was collected from the storage dam and it had a density of 1.04 g·cm^{-3}. The method of production of the effluent was a two-phase system which has the advantage of reduced water consumption (Borja et al., 2006). Effluent characterization was done with 1 mL of the representative samples digested with 7 mL HNO$_3$ and analyzed using Induced Coupled Plasma-Acid digestion (ICP-AES) to determine the acid extractable major and minor cations. Acid extraction was used because of the abundant organic content of the effluent. For the purpose of this study, only a few of the major cations (Ca, Mg, Na and K) were tabulated (Table 6.2). These elements were analyzed on a Thermo ICap 6200 ICP-AES. The instrument was calibrated using NIST (National Institute of Standards and Technology, Gaithersburg MD, USA) traceable standards to quantify selected elements. A NIST-traceable quality control standard of a separate supplier than the main calibration standards was analyzed to verify the accuracy of the calibration before sample analysis. The pH of the effluent was read off with the EUTHECH 700 pH meter with 30 mL of the sample. Total phenol was determined using 0.5 mL of undiluted raw sample using the Folin Ciocalteu method as explained in the section below. COD, TOC, TN, suspended solids, fats, oil and grease (FOG) were determined as described in the Standard Methods (2012) (Table 6.3).

Table 6.2 Olive mill wastewater characteristics determined before use

Parameters	Values
COD mg·L^{-1}	13700
Total Phenol mg·L^{-1}	971
TN mg·L^{-1}	82
TOC mg·L^{-1}	2000
pH	4.60
C:N	24.39
Phosphorus (P) mg·L^{-1}	28.50
FOG mg·L^{-1}	3440
Suspended solid mg·L^{-1}	7819
Calcium (Ca) mg·L^{-1}	47.21
Potassium (K) mg·L^{-1}	339.7
Magnesium (Mg) mg·L^{-1}	14.90
Sodium (Na) mg·L^{-1}	165.70

6.2.5.   Adsorption isotherm

The isotherm explored in this experiment was the Langmuir isotherm due to its importance in determining the quantity adsorbed and maximum quantity of sorbate sorbed by the sorbent. Langmuir isotherm relates to the coverage or adsorption of molecules on a solid surface to gas or a concentration of a medium of interest on the solid surface at a fixed temperature. The Langmuir equation is stated as

$$q = \frac{KC}{1 + Kc} \, qmax \qquad\qquad 6.2$$

where q is the quantity adsorbed, K is the constant, $q\ max$ is the maximum quantity of sorbate adsorbed on sorbent and C is the concentration.

The inverse of this equation on both sides will give the lineweaver-Burk equation

$$\frac{1}{q} = \frac{1}{qmax} + \frac{1}{qmax.KC} \qquad\qquad 6.3$$

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The plot of 1/q versus 1/c gives a linear graph of slope 1/Kqmax and intercept of 1/q max (Fig 6.1). The q max was calculated to be 23.53 mg·g^{-1} and Kd equated to 3.09 using the intercept (0.0425) and slope (0.4901) of the graph below (Fig 6.1). The sorption was carried out as stated in section 2.2.5 and 2.2.6.
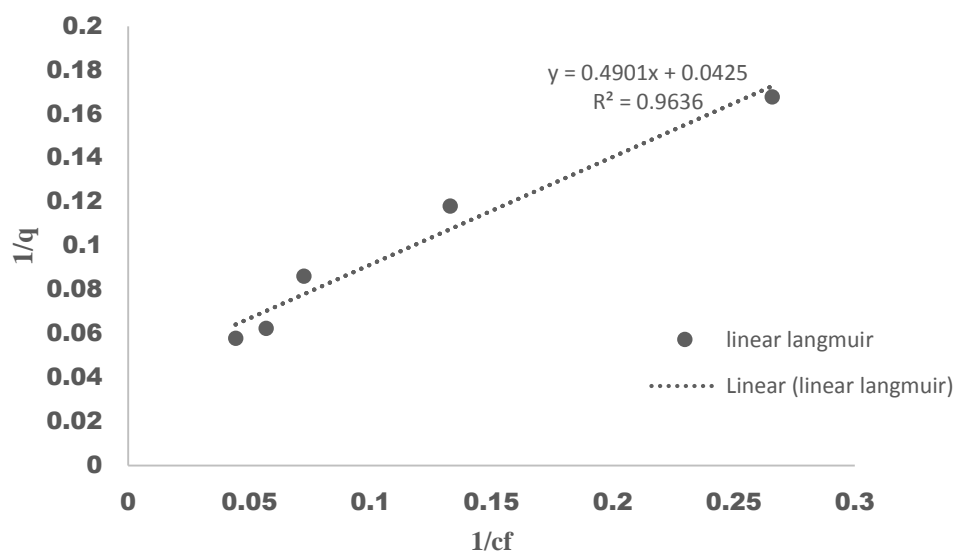
Figure 6.1. Linearized Langmuir adsorption isotherm for phenol used for q max determination

6.2.6.   Experimental set up and design

Bambara pot trials were laid out in a 2 x 6 factorial in a complete randomized design. Different effluent rates of 0, 50, 100, 200, 400, 800 $m^3 \cdot ha^{-1}$ (E0, E50, E100, E200, E400, E800) were combined with biochar rates of 0 and 2% (B0 and B2) (w/w) giving a total of 12 treatments.The treatments were replicated three times and were designated as, B0E0, B0E50, B0E100, B0E200, B0E400, B0E800, B2E0, B2E50, B2E100, B2E200, B2E400 and B2E800. Five-liter plastic pots with 20 cm diameter and height of 17 cm were filled with 3 kg soil which was mixed with 2% w/w (60 g) of biochar representing the 40 $t \cdot ha^{-1}$ (2% BC). The 2% rates were used based on the adsorption experiment (Figure 6.1) which showed that using the q max calculation of the Langmuir isotherm, less than 60 g of biochar is needed to sorb the phenol contained in 1 L of the effluent. The previous chapter also revealed that biochar rates of 2.5% and effluent combination of 100 $m^3 \cdot ha^{-1}$ gave the best above-ground performance for beans. In addition, the biochar rates ranging from 0.5-2.5% w/w was recommended by Olivier (2011), who reported that the biochar rates of 0.5-2.5 % w/w significantly improved biomass production, though with fertilizer. The dry soil was mixed with biochar and the effluents were applied on top to simulate on-land disposal of the effluents. The seeds were not soaked before planting in order to model the actual effect of the treatments on seed germination/emergence. The application of OMW and biochar did not follow sowing as recommended by Barbera et al. (2013) but preceded it. The soil-biochar-effluent mixtures were left for seven days before planting to allow for oxidation of the biochar and adsorption of the organic components (Steiner et al., 2007). The pots were watered to 75% capacity as recommended for bambara by Chibarabada et al. (2015). On the first day of planting, the same quantity of water was given to all the pots at 75% field capacity. Two seeds were sown per pot and thinned down to one plant 18 days after planting. The pots were fertilized hydroponically after one month of planting with 200 mL of the nutrient solution, supplying nutrient as given in Table 6.3 and applied daily. The fertilizer was composed using the electrical conductivity of 2.1 considering the subsoil's EC of 44 $\mu S \cdot cm^{-1}$. The bambara pot trials lasted for five months from October 2014 to March 2015 and upon harvest, the 0.5 cm layer from every pot was collected for soil analysis. The plant biomass and yield were sampled destructively and dried at 80° C for 48 hours and the weights were recorded.

Table 6.3. Composition of the nutrient solution used for fertigation

Element	g 1000 L^{-1}	EC = 2.1
KNO$_3$	690.84	
K$_2$SO$_4$	120.06	
K H$_2$PO$_4$	34.00	
NH$_4$ H$_2$PO$_4$	92.00	
Ca(NO$_3$)$_2$. 2H$_2$0	689.00	
MgSO$_4$. 7H$_2$O	419.43	
Hidrospoor / Omnispoor	20	

6.2.7. Plant growth parameters determined

Germination data was recorded from the time of planting to two weeks after planting and the date of emergence noted. The plant height and the number of spikes were recorded on a weekly basis and the height of the tallest plants in the pot was recorded. Above ground biomass (AGB) was measured by cutting the plant above the soil surface and below ground biomass (BGB) was determined destructively at the end of five months and the materials were oven dried at 80° C for 48 hours and then the weight was recorded. The seeds harvested after uprooting the BGB were washed and dried for 48 hours at 80° C and the weight recorded as the yield.

6.2.8. Statistical analysis

The bambara parameters determined were all normally distributed within the treatments. The plant height and the number of spikes data were collected over time and were analyzed using the mixed model repeated measures ANOVA. The effects measured in the mixed model repeated ANOVA were the biochar main effect, effluent main effect, time main effect and all the second order and 3rd order interaction effects. Pots were treated as a random effect nested in biochar and effluent treatment. The biomass and the nutrients data were analyzed using the general linear model using two-way factorial-ANOVA to test two effects. The effects tested were biochar main effect, effluent main effect and biochar and effluent interaction effect. The Fisher Least Significant Difference (LSD) post hoc testing was also done to determine the significant differences between the treatments. The data were statistically analyzed using STATISTICA 12.6 software.

6.3. **Results**

6.3.1. Bambara germination

The germination was delayed with effluent rate of 200 and 400 $m^3 \cdot ha^{-1}$ before it came down at 800 $m^3 \cdot ha^{-1}$, but was improved with biochar application. This trend showed no significant interaction effect at (p=0.21). The application of biochar reduced the number of days for germination to occur. The control (B0E0) germinated within 11.56±2.36 mean number of days and with biochar (B2E0) it was slightly reduced to 10.78±1.90 days. The high rates of 200 $m^3 \cdot ha^{-1}$ and 400 $m^3 \cdot ha^{-1}$ of effluent delayed germination to 12.00±2.37 and 12.50±2.74 mean number of days respectively. The biochar and effluent interaction was not significant for the germination but biochar addition had an effect. The reduction of up to 40% was observed in the 200 $m^3 \cdot ha^{-1}$ (10.00±1.00) mean number of days with biochar from the same rate without biochar while the same rate (200 $m^3 \cdot ha^{-1}$) had approximately 40% delay compared to the control. It was interesting to note that 800 $m^3 \cdot ha^{-1}$ effluent treatment did not delay germination further, but rather reduced it to 10.50±2.74 mean number of days. It showed earlier germination without biochar (9.67±0.58) than with biochar (11.33±3.21) (Figure 6.2).
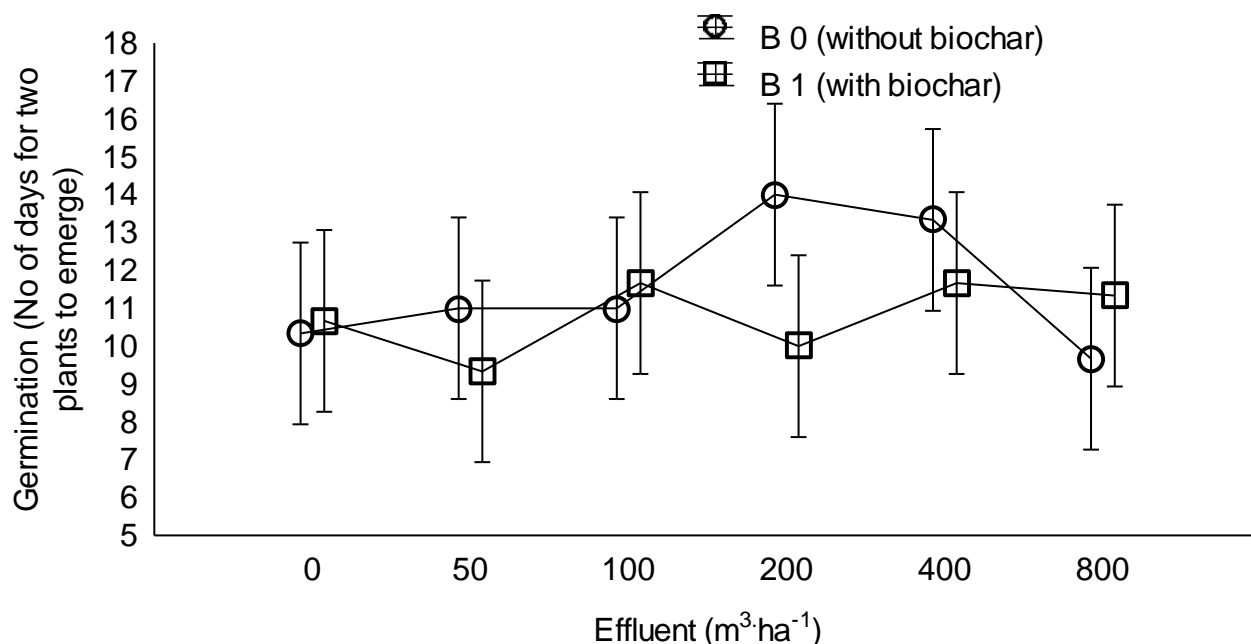
Figure 6.2.   Effect of biochar and effluent combinations on bambara ground nut germination

6.3.2. Bambara plant height and number of spikes

Biochar and effluent interaction effect was not significant on the plant height and number of spikes observed. Notwithstanding, biochar addition showed an increased plant height trend from control to 400 m^3·ha^{-1} with 21% at 200 m^3·ha^{-1} which came down to 5.3 % at 400 m^3·ha^{-1} with 2.8% decrease at 800 m^3·ha^{-1} compared to their non-biochar rates (Figure 6.3). With biochar addition, the highest mean of 23.51±4.65 was observed with the 200 m^3·ha^{-1} effluent rate followed by 21.49±4.90 and 21.17±4.80 mean at 800 m^3·ha^{-1} without and with biochar respectively. Biochar and time treatment interaction was significant for plant height while effluent and time interaction significantly affected the number of spikes. There was a significant increase in plant height when biochar was added, especially in the first four weeks before the addition of the fertilizer through irrigation. This effect was evidenced by the non-significance of all the effluent effect in the second order and third order interaction as stated in the table below (Table 6.4).

The number of spikes had a different trend than the plant height. The biochar addition improved all performance with highest influence at the 400 m^3·ha^{-1} with 43% increase from the non-biochar rate. The best result was obtained from the 800 m^3·ha^{-1} without biochar with mean of 26.78±17.73, data not shown.
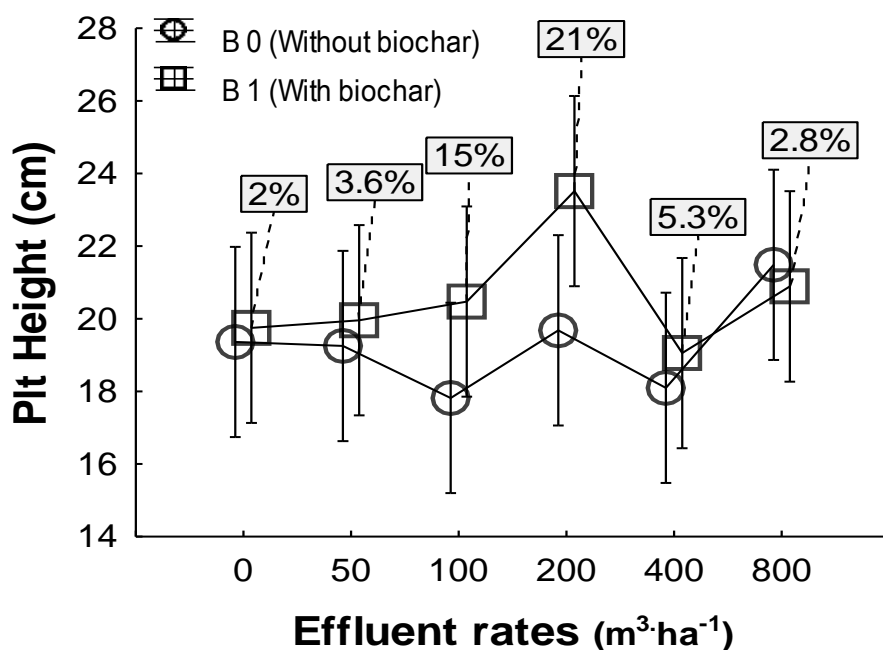
Figure 6.3. Effect of different rates of effluent and biochar combination on plant height with the different labels showing percent increase and decrease compared to the control and same effluent rates with or without biochar

Table 6.4. Fixed Effect Test for plant height of biochar and effluent treatment collected every week until point of harvest.

Treatment Effects tested	Num. DF	Den DF	F	P <0.05
Biochar (B)	1	24	3.2642	0.083363
Effluent (E)	5	24	1.7506	0.161470
Time (T)	11	263	128.3681	0.000000
B *E	5	24	0.8148	0.550910
B* Time (weeks)	11	263	4.0669	0.000016
E*Time(weeks)	55	263	0.9269	0.622605
B*E*T (weeks)	55	263	0.7672	0.880791

Fixed effects: "B" + "E" + "Time (wks)" + "B" "E " + "B * "Time (wks)" + "E "*"Time (wks)" + "B "*"E "*"Time (wks)".
 Random: "Pot nested in (B and E).

6.3.3.   Bambara plant biomass and yield

The above ground biomass (AGB) did not show any significant effects of either biochar or effluent treatments. The 800 $m^3 \cdot ha^{-1}$ effluent rate with 2% biochar gave the highest mean of 10.81±5.41. In contrast to the AGB response, the effluent and biochar mixture significantly affected the BGB (B*E p=0.06). The p-value was not significant at 5% but significant at 10 %. The best performance was obtained at the 400 $m^3 \cdot ha^{-1}$ effluent with biochar at a 14% increase from the control and a mean of 2.26 g ±0.38 (Figure 6.4). The biochar treatments for 0, 50, 100 and 200 $m^3 \cdot ha^{-1}$ were not significantly different and perform tended to lower than the 0, 50 and 800 $m^3 \cdot ha^{-1}$ effluent rate treatment (Figure 6.4). The interaction of the biochar and effluent here implies different effects from effluent and biochar. Effluent gave a decreasing effect as the rates increased while biochar rather maintained a steady effect until 200 $m^3 \cdot ha^{-1}$ rate.

The yield showed a significant interaction effect with the biochar and effluent treatments applied at 5% (p<0.05). The effluent rate of 50 $m^3 \cdot ha^{-1}$ without biochar gave the best performance with mean of 14.60±1.90 but performance decreased significantly at 200 and 400 $m^3 \cdot ha^{-1}$ without biochar (Fig 6.5). Combining biochar with effluent, the soil could tolerate more effluent and this combination gave a significantly higher yields at 200 $m^3 \cdot ha^{-1}$ with 42% increase compared to the control. The continued effluent and biochar mixtures higher than 200 $m^3 \cdot ha^{-1}$ of effluent decreased yields at 800 $m^3 \cdot ha^{-1}$ compared to control. However, there was a good result at 800 $m^3 \cdot ha^{-1}$ (14.15 g ±32) without biochar. In fact, adding biochar at 800 $m^3 \cdot ha^{-1}$ reduced yield by 60 %. (Figure 6.5).

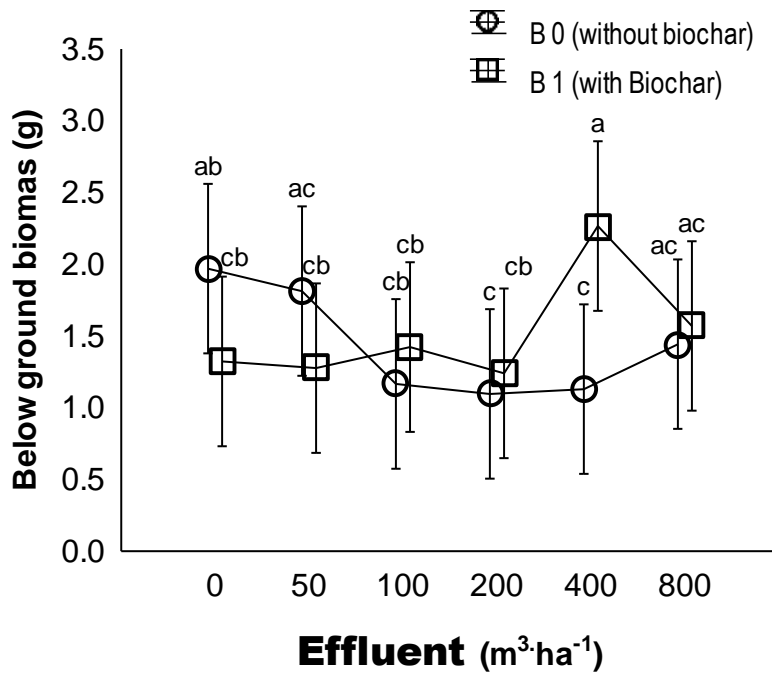**Figure 6.4.** **Effect of effluent and biochar on below ground biomass production of bambara. The different letters above represent the differences at p<0.05 and same letters lack significant difference.**
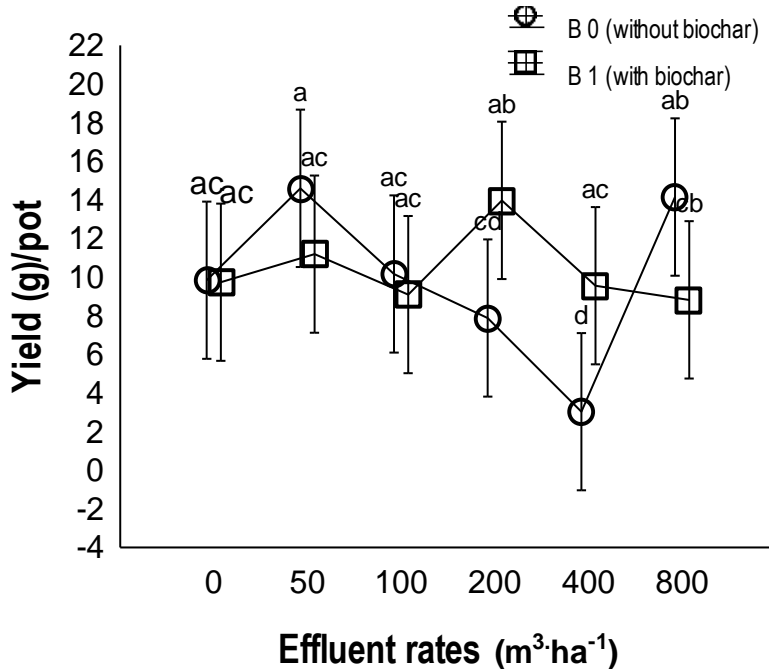

Figure 6.5. Effect of different rates of biochar and effluent combinations on the yield of bambara groundnut with the letters on top representing the treatment differences at p<0.05. Same letters lack significant difference

6.3.4.   Nutrient concentrations in relation to biochar and effluent applied

Biochar and effluent application to the soil affected nutrient status as well as its availability. The pH was increased by both biochar and effluent addition, but the biochar was not increasing the pH as continuous as effluent alone was increasing it. Effluent continuously increased the soil pH as the rates increased. However, the 800 $m^{3.}ha^{-1}$ of effluent rate application without biochar decreased the acidity more than with biochar (Table 6.1) before planting. After harvest (Table 6.5) biochar addition increased pH better than the effluent concentration till 200 $m^{3.}ha^{-1}$. Though, the biochar and effluent interaction was not significant at 0.5% level of probability. Biochar added at 0, 200 and 400 $m^{3.}ha^{-1}$ did not differ significantly but significantly differed from 800 $m^{3.}ha^{-1}$ with and without biochar which significantly increased pH. Percent acid saturation calculated before treatment was very high especially in samples with pH >6 (Table 6.1) and this is a potential hazard to plant growth. This condition was reduced after harvest though, acid saturation was still significant to be a problem but much lower than before planting (Table 6.5).

The K increased with effluent application but increased more with biochar application (Table 6.1) before planting. After planting, the interaction between biochar and effluent showed that higher rates of effluent, 400 $m^{3.}ha^{-1}$ differed significantly from the control with and without biochar. 800 $m^{3.}ha^{-1}$ differed significantly from the 50 $m^{3.}ha^{-1}$ with or without biochar (Table 6.5). Effluent application increased Na more than biochar at a lower rate of 0 and 50 $m^{3.}ha^{-1}$. The addition of biochar reduced Na content especially at 0 and 50 $m^{3.}ha^{-1}$ rates of effluent applied and its effect significantly differed from the higher rates of effluent without and with biochar at higher rates (Table 6.5). Biochar and effluent interaction revealed a trend that from 200 $m^{3.}ha^{-1}$ effluent rate, biochar could no longer reduce the Na and hence Na content was more. The Ca content showed no significant difference among all the treatments while the Mg effect leveled up after 100 $m^{3.}ha^{-1}$ of effluent and biochar combined. The Mg biochar and effluent interaction was not significant at 5% but significant at 10 % and showed no effect after 100 $m^{3.}ha^{-1}$. The CEC showed an increasing trend with the biochar and effluent mixture as the effluent rates increased (Tables 6.1 and 6.5). The initial soil characterization showed increased N and P as effluent rates increased with biochar (Fig.6.6). After planting, the soil mixture indicated increasing N reduction with biochar at 0 to 100 $m^{3.}ha^{-1}$ while biochar reduced P from 200 $m^{3.}ha^{-1}$ rate of application (Figure 6.7).

Table 6.5. Two-way ANOVA of biochar and effluent effect (mean ± SD, n = 3) on selected soil nutrients determined after harvest

Treatments	pH (H$_2$0)	Acidity	Ca	Mg	Na	K	ECEC	%C	% Acid Saturation
				Cmol (+)·kg^{-1}					
B0E0	5.57±0.35^{ad}	1.30±0.16^d	3.29±1.38^{NS}	1.51±0.59^a	0.26±0.09^{ab}	0.75±0.44^{ab}	9.20±2.98^{ab}	1.14±0.13^{ab}	40
B0E50	5.40±0.36^d	0.99±0.23^{ac}	4.19±0.46^{NS}	2.67±1.02^b	0.55±0.21^{bc}	1.34±3.28^{abc}	14.33±1.29^a	1.21±0.19^{ab}	24
B0E100	5.60±0.30^{abd}	0.99±0.19^{ac}	4.24±0.95^{NS}	1.97±0.30^{ab}	0.40±0.11^{abc}	1.59±0.33^{ac}	14.33±1.87^a	1.21±0.16^{ab}	23
B0E200	5.60±0.00^{abd}	1.12±0.02^{cd}	3.26±0.15^{NS}	1.45±0.33^a	0.27±0.14^{abc}	0.81±0.59^{ab}	9.62±2.86^{ab}	1.27±0.07^b	35
B0E400	5.86±0.05^{abc}	0.93±0.12^{abc}	3.39±1.39^{NS}	1.67±0.71^a	0.37±0.25^{abc}	0.89±0.49^{ab}	10.33±4.42^{ab}	1.22±0.09^{ab}	27
B0E800	6.00±0.40^c	0.87±0.00^{ab}	3.81±1.13^{NS}	1.76±0.24^{ab}	0.46±0.25^{abc}	1.27±0.55^{abc}	11.89±2.09^{ab}	1.33±0.18^b	23
B2E0	5.76±0.11^{abcd}	0.96±0.11^{abc}	3.13±0.23^{NS}	1.12±0.09^a	0.18±0.03^a	0.55±-.02^b	7.80±0.28^b	1.04±0.07^a	31
B2E50	5.83±0.15^{abc}	0.86±0.01^{ab}	3.26±0.45^{NS}	1.16±0.04^a	0.17±0.04^a	0.57±0.02^b	7.55±0.78^b	1.02±0.01^a	26
B2E100	5.80±0.20^{abc}	0.84±0.06^{ab}	3.79±1.31^{NS}	1.97±0.81^{ab}	0.35±0.23^{abc}	1.55±1.00^{ac}	13.45±6.33^a	1.01±0.01^a	22
B2E200	5.63±0.15^{abcd}	0.90±0.04^{ab}	3.46±0.25^{NS}	1.96±0.47^{ab}	0.46±o,25^{abc}	1.32±0.71^{abc}	12.52±3.60^{ab}	1.12±0.14^{ab}	26
B2E400	5.73±0.05^{abcd}	0.76±0.03^b	3.46±0.70^{NS}	1.76±0.58^{ab}	0.60±0.25^{abc}	1.60±0.2^c	14.36±4.34^a	1.05±0.09^a	22
B2E800	5.96±0.15^{bc}	0.81^{ab}	3.28±0.71^{NS}	1.75±0.41^{ab}	0.60±0.30^c	1.60±0.2^{ac}	12.92±2.54^{ab}	1.27±0.17^b	27

The rows with different superscripts are significantly different (p=0.05). The treatments represent interaction between biochar and effluent applied at the different rates; 0 and 2 % for biochar and 0, 50, 100, 200, 400, and 800 m^3·ha^{-1} for effluent. NS means not significant. The superscripts denoted as A-D rank from 1-4, lowest to highest.
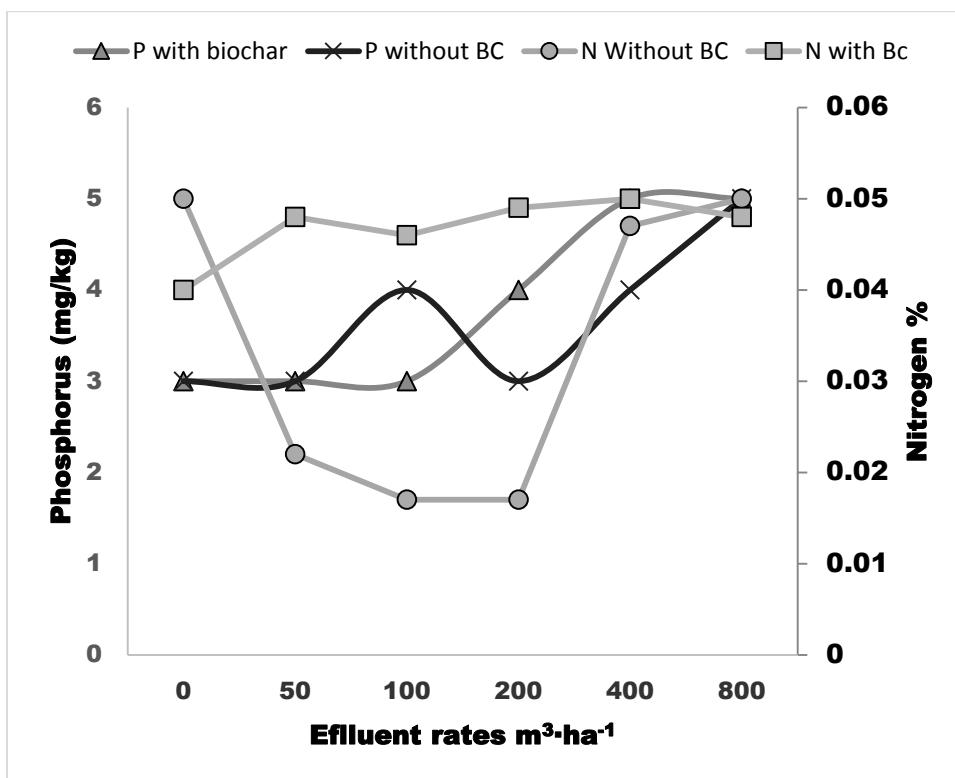
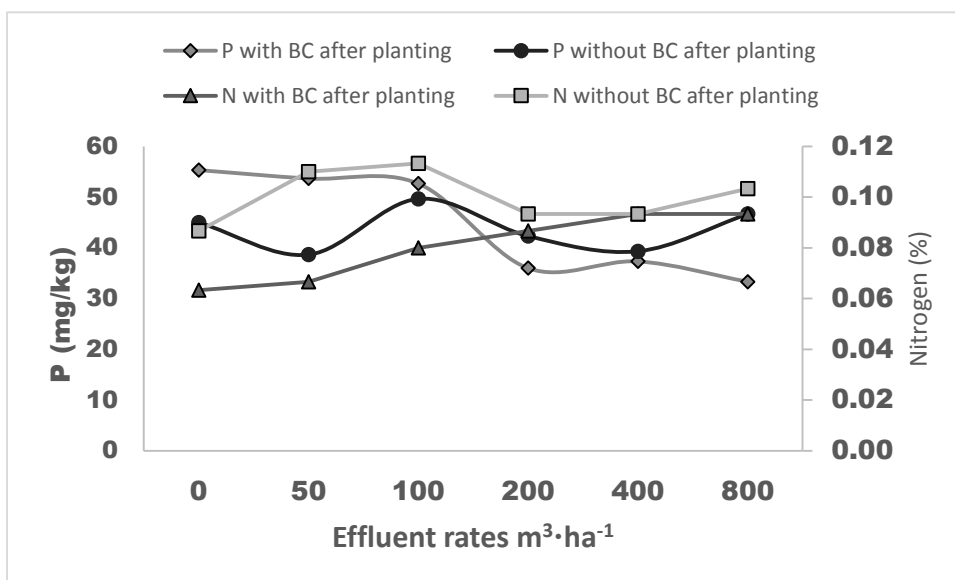**Figure 6.6.** **Soil Nitrogen (N) and Phosphorus (P) content before planting**

Figure 6.7. Contribution to soil N and P after harvest

6.4. **Discussion**

6.4.1. **Germination and adsorption**

The adsorption isotherm conducted with pinewood biochar showed that only 41.26 g of biochar was needed to sorb the phenols contained in one liter of the effluent used. This calculation was obtained using the q max value (23.53 mg·g^{-1}) and the phenol content of the effluent. In view of this 41.26 g·L^{-1}, we decided to use biochar at a rate of 20 g·kg^{-1} of soil. This quantity was expected to give a good result up till 400 m^3·ha^{-1} effluent rate assuming the only cause of germination and plant growth delay was phenol toxicity of the OMW (Lykas et al., 2014). At 400 m^3·ha^{-1} we expected to obtain approximately 65.6 mg N from the effluent.

It was observed that effluent delay to germination up till 400 m^3·ha^{-1} rate was reduced with biochar application and this improvement to germination was not effective above 400 m^3·ha^{-1}. It could be assumed that the phenol content has been adsorbed by the biochar. Rondon et al. (2007) explained that reasons for improvement of biological nitrogen fixation after the addition of biochar are most likely a combination of factors related to nutrient release and stimulation of plant microbes. The high P and N value of the medium at the effluent rate of 800 m^3·ha^{-1} explained the reason for earlier germination at that rate (Figure 6.6). This shows that effluent delay to germination is not only based on phenol toxicity as indicated in Mekki et al. (2013) but also nutrient availability which biochar provided with lower rates of effluent. Baronti et al. (2010) also observed accelerated germination in pots where biochar was added.

6.4.2. **Biochar and effluent effect to bambara production**

Most of the studies done with effluent (mostly bio-digester effluent) have reported improved response to the effluent levels on the aerial part of the vegetables studied, mostly water spinach (Sophea and Preston, 2001; Bunyeth and Preston, 2004). Their results showed a positive response to the yield of water spinach up to 140 kg N ha^{-1}. One of the very few studies with effluent and biochar on legumes (Southavong et al. 2015) also reported a yield increase of yardlong beans at 100 kg N ha^{-1} combined with 4 kg.m^2 of biochar. This was attributed to the ability of biochar to retain nutrients and increase CEC of the soil which in turn improved crop yield by making other nutrients available especially with improved pH. Our study also showed improvement of the effluent performance with biochar added for the plant height and number of spikes up 400 m^3·ha^{-1} of OMW. This supports the fact that phenol toxicity contributes to decrease in crop performance. The weekly data collected for the plant height also supported

the fact that biochar improved the performance of the mixture significantly at p<0.05 (Table 6.3). This implies that with biochar addition to the OMW amended soil, most of the other nutrients were released and made available for plants. Nitrogen was increased as shown in Figure 6.6 and hence reduced the C: N ratio which improved nutrient availability to plants.

Significant results obtained for the below ground biomass and yield with combined effluent and biochar are similar to the work of Rodriguez and Salazer. (2009) who reported an increase in maize growth when biochar and bio-digester effluent were applied to a poor subsoil and recorded better performance than fertile soil. The BGB and yield corresponds to the same trend as the P and N in Figure 6.6 where BGB with biochar was improving as nitrogen with biochar improved. The good yield result obtained with combined effluent and biochar at 200 $m^3.ha^{-1}$ rate (Figure 6.5) indicated that biochar reduced the quantity of the effluent to be used by making available quite a lot of nutrient that even when 800 $m^3 \cdot ha^{-1}$ of OMW had more nutrients (Tables 6.1 and 6.5). This reduction in the quantity of effluent to be used is good, as more effluent contained more Na and K as well (Tables 6.1 and 6.5) which can cause soil sodicity. The 50 $m^3 \cdot ha^{-1}$ gave the best result, it had more Na content than the 200 $m^3.ha^{-1}$ effluent rate (Table 6. 5) with biochar after harvest which is not a good soil improvement and it showed a decreasing yield at the 400 $m^3.ha^{-1}$. It was found here that biochar did not only release the nutrient in the 200 $m^3 \cdot ha^{-1}$ but also reduced the Na content and therefore improved soil health. Perhaps, using higher more than 200 $m^3.ha^{-1}$ effluent rate with 2% biochar is not advisable as it contained more Na and K (Table 6. 1). This experiment is an improvement to the work of Mekki et al. (2013) who recommended 50 $m^3.ha^{-1}$ of effluent augmented with *P. chrysosporium* as the best performance rate. It can be seen that with 2% biochar, higher effluent rates of up to 200 $m^3 \cdot ha^{-1}$ could be used.

6.4.3.   Biochar and effluent effect on nutrient availability

The amendment of biochar improved the nutrient content of the soil system after planting. There was an increase in the pH, C, P, K, ECEC and reduction of Na as a result of biochar addition. It is expected that the biochar would increase the ECEC of the soil by the formation of organo-mineral complexes due to the oxidation at the edges of the char (Steiner et al., 2007). They went further to explain that the slow reduction of acidity might be explained by the steady formation of organic material with functional groups such as carboxyl and phenolic groups during decomposition. There was an increase in ECEC which is in agreement with Cheng et al, (2006) that four months is enough for a significant increase in ECEC due to oxidation.

The effluent continued to increase pH (Table 6.5) and it is in line with the review reported by Barbera et al. (2013) that a small increase in pH after applying 160 $m^3 \cdot ha^{-1}$ of OMW resulted from production of ammonia during breakdown of OMW. This could explain the large increase in N (Figure 6.6) as effluent and the bambara N fixing ability could have contributed positively. Perhaps, the knowledge of biochar reducing N availability as reported by Sika and Hardie (2014), and also observed in the previous chapter, was slightly remedied with the effluent and bambara combination under acidic condition. The increase in P and other nutrients could be understood from this phenomenon. Lehmann et al., (2003) and Rondon et al., (2007) found that biochar addition did not increase the extractable P in their experiment. But in our study we noted an increase in extractable P after harvesting (Figure 6.7), which could be from the effluent which increased P, K, and other cations as effluents were added. The availability of N and lower pH will influence the increased P content. The organic P in soil prevents the leaching of K and N (Brady and Weil, 2001).

The lower acidity showed that most of the exchange sites were no longer blocked by the Al or Fe and this increased Ca and Mg availability (Steiner et al., 2007). Biochar addition reduced Na due to the active SOM sites which will help to maintain the SOM and not lose them due to no cations in the soil. This will in turn improve crop growth.

6.5. Conclusion

Biochar addition partially mitigated the soil pollution by the OMW in the acidic condition under the bambara crop. The best results were achieved with 200 $m^3 \cdot ha^{-1}$ application of OMW preceded by 2% biochar addition to the soil. The biochar not only removed the phenols from the soil solution, but made other nutrients available for, crop growth and sustainable crop conditions for early germination. The combination of this effluent with biochar greatly affected the availability of nutrients like N, P, K by lowering acidity and increasing pH and ECEC. High effluent rate application alone was detrimental to crop growth and high biochar alone was also affecting crop growth negatively. This study has found that 2% biochar could increase the use of effluent up to 200 $m^3 \cdot ha^{-1}$. It could mean that more biochar could take a higher rates of effluent but the commercial viability of such high application rates should be considered. The 800 $m^3 \cdot ha^{-1}$ gave the slightly better yield performance of 14.1 g/pot against the 13.9 g of the 200 $m^3 \cdot ha^{-1}$ plus 2% biochar. But considering the detrimental effect of the high effluent rate in parameters determined like the germination and plant height, then 200 $m^3 \cdot ha^{-1}$ with biochar is

recommended for sustainable use. The study also erases the notion that it was only phenol toxicity that delays germination.

Chapter 7
Summary, Conclusion and Recommendation

Wastewater management is an important issue around the world and especially when treating water from vegetable oil industries, because of the fouling effect from the oil while using treatment plants. Olive mill wastewater treatment has attracted a lot of research in the mediterranean world but this is the first research that solved this problem in South Africa. This study explored the possibilities of treating the olive mill wastewater with pinewood biochar and generally concluded that pinewood biochar can be used to mitigate the degradation effects of olive mill wastewater on-land disposal *in-situ* and *ex-situ*. The pinewood biochar filtration was found to be a simple and effective mechanism for mitigating the degradation effect of the olive mill wastewater by reducing the cost of plant installation and by application of biochar as a soil amendment. The use of biochar in treating OMW was first ever explored in this research with positive results obtained.

The previous studies showed that the phytotoxicity of the effluent is the main reason why the effluent negatively affects plant germination and growth. The high COD, phenol and the fats, oils and greases (FOG) components of the effluent significantly contributed to soil degradation in general. While phenol was revealed to inhibit germination and plant growth, the FOG and OM measured as COD clogged fine soil pores.

The pH of the effluent affected the availability of nutrients in soil. The methods of using activated carbon to sorb the toxic components of the effluent were effective but costly and hence the use of pinewood biochar as a cheaper and readily-available (in South Africa) alternative.

The first objective was to determine the effectiveness of biochar in sorbing the phenol and COD contaminants from olive mill wastewater. The sorption of total phenols from olive mill wastewater with pinewood biochar was found to be rapid and it equilibrated fast within a few (1-3) hours. The research further compared the sorption with a single synthetic phenol (gallic acid) to elucidate the interference from the other particles like the suspended solids from the effluent. It was found that the pinewood biochar was effective in cleaning the effluent within a short retention time. Nevertheless, the sorption of the phenol from the effluent was 46 % lower than the sorption from a single compound solution (GA solution). The reason for the lower sorption in the OMW was understood to be due to the presence of the fats, oils and greases in OMW which probably blocked the biochar pores. The two sorption processes (GA and OMW)

kinetically followed chemisorption of the pseudo-second-order model ($R^2 = 0.9994$ and $R^2 = 0.9996$ respectively). The sorption process followed the Freundlich multi-layer model for both GA and OMW. The pinewood biochar was shown to fully sorb phenols from the OMW at the rates of 300 g·L^{-1},

The effluent was further filtered with soil columns to determine the phenol and COD breakthrough curves and column dynamics with sand and Hutton clay loam under saturated flow condition. The pre-washing and biochar additions to the columns were also monitored regarding their effect to column capacities and pH and ECs.

The sand filters used, did not perform well, even with the addition of biochar and the washing techniques applied. The Hutton clay loam filters and the biochar alone were found to remove the contaminants much below the allowable limits of disposal both internationally and in South Africa. They did not even reach their breakthrough points. The prewashing of the filters was found to enhance the removal of total phenol. Notwithstanding, it was found that prewashing the filters increased the flow rate, except for pure biochar beds, where washing decreased the flow rate. This result may be due to the expansion of the chars. The addition of the biochar to the sand and clay loam filters was more effective in the COD removal than in the phenol removal although the biochar-enhanced soil filters removed more phenols than the filters, which were not amended with char. These deductions indicated that biochar significantly improved the COD and phenol removal by soil filters, but as to COD, aeration of the filters was necessary to enhance degradation. The pH and the EC of the filtered effluent were further examined, and the result showed that the Hutton clay loam soils and the biochar filters reduced the pH and EC over time via adsorption and ion exchange to neutrality and an average of 1.5 ds.cm^{-1} for pH and EC respectively.

The study examined the effect of the OMW disposal on the structure of the soil. The effect of the washing of the columns and addition of biochar on hydraulic conductivity and porosity was studied. This study found that both biochar addition and washing only slightly affected the macro-porosity, but significantly reduced the hydraulic conductivity. Filter prewashing restricted the oil movement down the column. It was therefore recommended that in the case of continuous disposal, it will be good practice to add biochar and dispose of the OMW on land shortly after the rains. Moreover the best soil type to restrict downward movement of the

contaminants in the disposal environment would be Hutton clay loam soil amended with biochar.

It was therefore necessary to test these observations in greenhouse trials to confirm the effect on the different plants. This led to the investigation of in-situ adsorption of the contaminants in the top soil (alkaline sand, regarded as a largely adverse soil environment) using biochar and mineral fertilizer to improve crop (wheat and green beans) production in the case of continuous effluent disposal.

The study revealed that olive mill wastewater had a strong negative effect on the growth parameters of wheat on alkaline sand. The deterioration of wheat growth parameters was observed with and without mineral fertilizer addition. This effect was less pronounced in green beans. The negative effect was primarily attributed to a high concentration of phenols in the OMW. But the application of biochar to an alkaline soil has to be amended with additional fertilizer especially for wheat. In this study, the effluent did not have enough nitrogen to buffer the effect of biochar retention of N in the case of the wheat but there was a little improvement when beans were able to fix nitrogen. It can be concluded that it was not only the phenol contamination that contributed to the low yield obtained, but also the availability of nutrients. The nutrient supply was optimized at the effluent application rate of 50 m^3 and neutralized by biochar application at the rate of 0.5 % (w/w) (10 t/ha). The increased P availability and N added by N fixation explains why the beans performed better than the wheat in this environment. The little improvement experienced with the wheat trial when biochar was added also confirmed that the high pH of the system also contributed to the poor performance. Therefore, on-land disposal of OMW may be possible at higher than currently recommended rates in combination with biochar incorporation and careful crop choice.

The study further examined the effect of the biochar and OMW combined on growth parameters and yield of the bambara nut on an acidic Hutton clay loam. The study showed that the best results were achieved with 200 $m^{3 \cdot}ha^{-1}$ application of OMW preceded by 2%wt biochar addition to the soil. In the case of this soil, more effluent was added to give a better result. The biochar not only removed the phenols from soil solution, but made other nutrients available for crop growth and sustainable crop conditions for early germination. The combination of this effluent with biochar greatly affected the availability of nutrients like N, P, and K by lowering acidity and increasing pH and ECEC. The result showed that a high effluent application rate alone was detrimental to crop growth, and high biochar alone also affected crop growth negatively. This study has found that 2% biochar could allow an increase in the rates of the on-

land effluent disposal to 200 m^3·ha^{-1}. It could mean that more biochar could bear higher effluent disposal rates, but commercial viability of such high application rates should be considered.

This study has shown that the negative effects of the OMW on-land disposal may be partially mitigated by prior amendment of the soil with pinewood or similar biochar. Such an *in situ* treatment may be an inexpensive solution for prevention of soil and ground water pollution in olive-oil-producing regions.

An *ex-situ* filtration of the effluent through biochar or biochar clay-loam mixtures may be an alternative solution to the problem. Good results have been achieved for developing an industrial-scale filtration system as a practical solution for OMW disposal in future

Recommendations

1. Further research into the effects of OMW on water quality with emphasis on EC and SAR is strongly recommended.

2. Experimentation on the effects of the biochar purified OMW on-land application in crop is also good to research on

3. Further pot and field experiments with biochar- filtration of OMW on different soils for crop production is necessary

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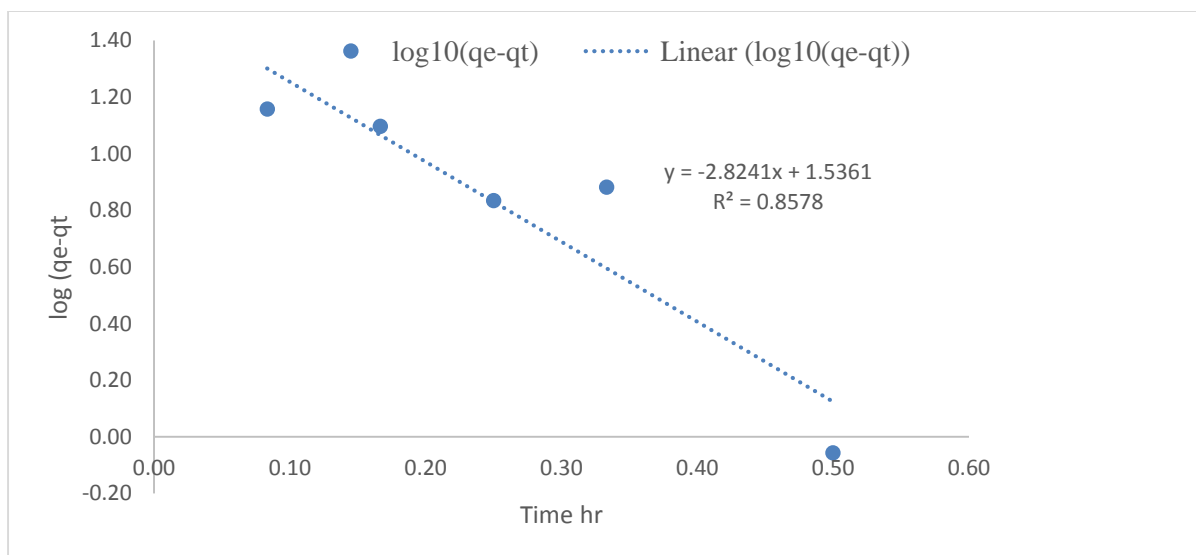
Appendix 1.

Time	phenol Abs	Cf.mg/l	initial conc	Average Cf	Co-Cf*V	q mg/kg	q=mg/g	Average q	Time min/60	% Remov
5min	0.007	26.32	92.11	24.44	3.29	16448.6	16.45	16.92	0.1	73%
5min	0.006	22.56	92.11		3.48	17388.4	17.39			
10min	0.007	26.32	92.11	24.44	3.29	16448.6	16.45	16.92	0.2	73%
10min	0.006	22.56	92.11		3.48	17388.4	17.39			
20min	0.011	41.35	92.11	31.95	2.54	12689.2	12.69	15.04	0.3	65%
20min	0.006	22.56	92.11		3.48	17388.4	17.39			
40min	0.003	11.28	92.11	13.16	4.04	20208.0	20.21	19.74	0.7	86%
40min	0.004	15.04	92.11		3.85	19268.1	19.27			
60min	0.004	15.04	92.11	15.04	3.85	19268.1	19.27	19.27	1	84%
60min	0.004	15.04	92.11		3.85	19268.1	19.27			
120min	0.003	11.28	92.11	15.04	4.04	20208.0	20.21	19.27	2	84%
120min	0.005	18.80	92.11		3.67	18328.3	18.33			
180min	0.005	18.80	92.11	15.04	3.67	18328.3	18.33	19.27	3	84%
180min	0.003	11.28	92.11		4.04	20208.0	20.21			
240min	0.004	15.04	92.11	13.16	3.85	19268.1	19.27	19.74	4	86%
240min	0.003	11.28	92.11		4.04	20208.0	20.21			
300min	0.004	15.04	92.11	15.04	3.85	19268.1	19.27	19.27	5	84%
300min	0.004	15.04	92.11		3.85	19268.1	19.27			
360min	0.003	11.28	92.11	13.16	4.04	20208.0	20.21	19.74	6	86%
360min	0.004	15.04	92.11		3.85	19268.1	19.27			

Co and Cf means Initial and final concentrations. V= volume of effluent used. Q=quantity adsorbed
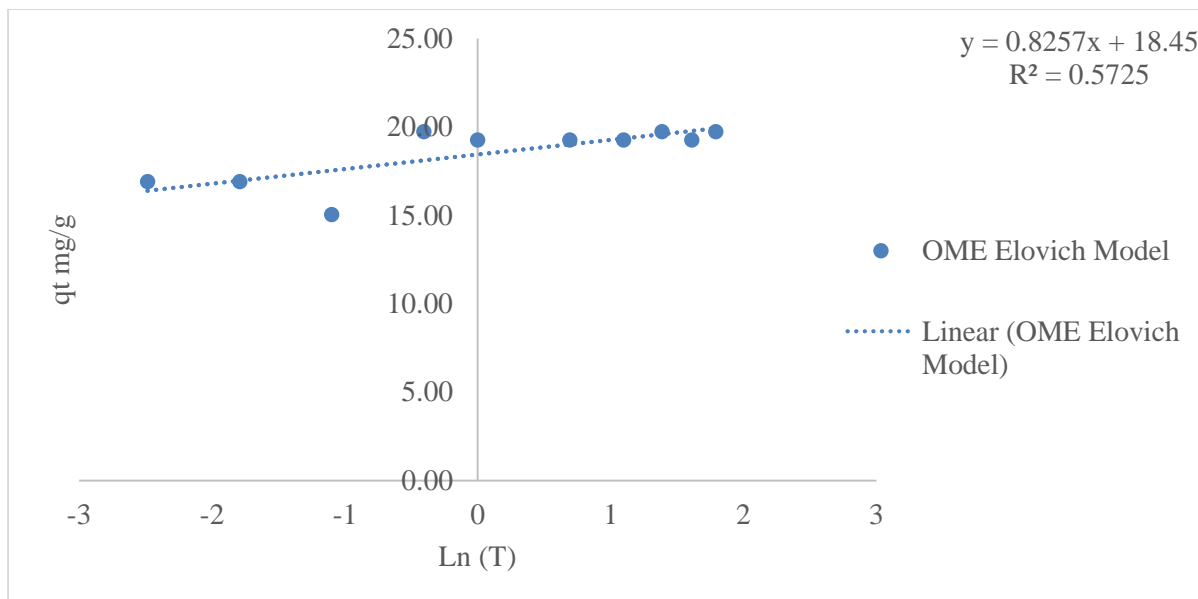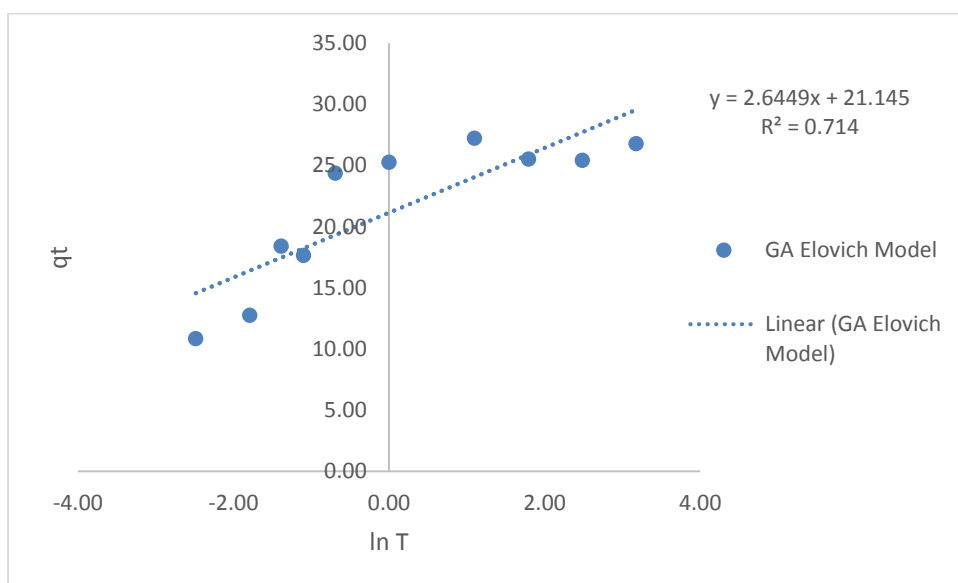And % removal =percentage removal.
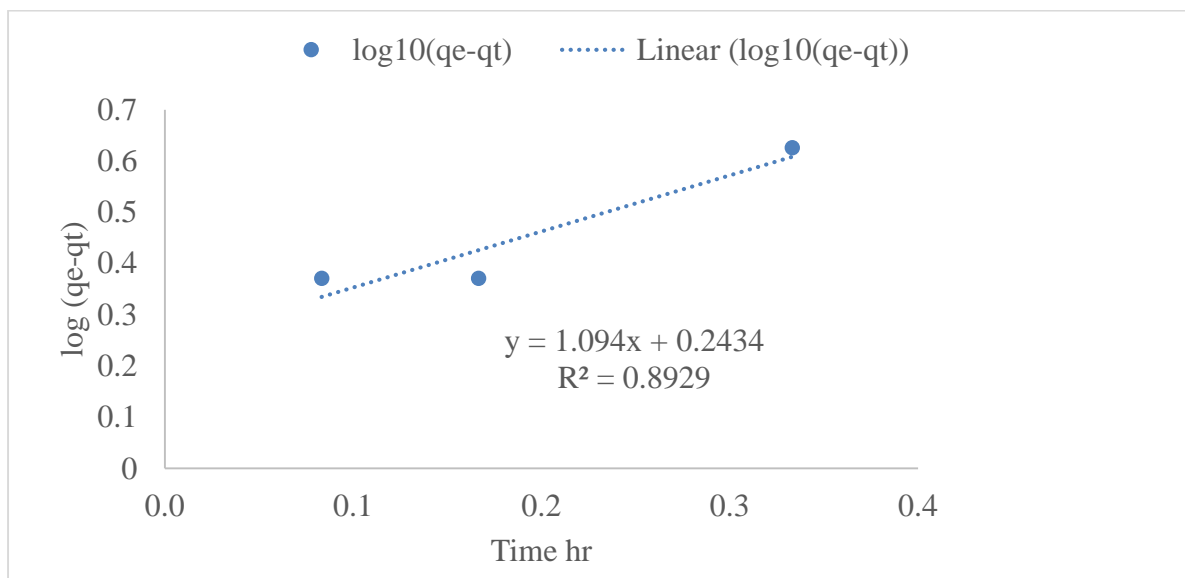
Figure. 1. Graph of the Pseudo first order reaction of OMW showing the slope and intercept

Figure.0.1 Graph of the Pseudo first order reaction of GA showing the slope and the intercept

$y = 0.8257x + 18.45$
$R^2 = 0.5725$

Figure 0.2 Graph of the OMW Elovich model showing the intercept and slope

$y = 2.6449x + 21.145$
$R^2 = 0.714$

Figure 0.3 Graph of GA Elovich Model showing slope and intercept

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Appendix 2

Calculation showing the quantity of biochar used in the study

Given:

Bulk density of 1.45 g/cm^3

15 cm depth of soil was assumed =0.15m

 1 ha = 10000 m^2

Volume of the soil = 0.15 m X 10000 m^2 = 1500 m^3

Mass of soil = Vol of soil X Bulk density = 217500 kg/ha

For the 10 tons/ha (0.5%) application rate = (quantity of soil in the pot (kg) multiplied by 10000)/ mass of the soil in one ha (m^2)

10 tons = 10000 kg

$$\frac{Mass\ of\ Soil\ in\ the\ pot\ (3.5\ kg)\ X\ 10000\ kg}{Mass\ of\ soil\ assuming\ 15\ cm\ depht\ 217500\ m2} = mass\ of\ biochar\ used\ (g)$$

Calculating the quantity of effluent used in the study

50 m3/ha =50, 000, 000 ml of effluent

Mass of soil in one ha was calculated to be = 217500 kg

Mass of soil in the bag = 3.5 kg

$$\frac{50\ 000\ 000\ ml}{2175000\ kg} = 22.988\ ml/kg$$

So for 3.5 kg/pot = 22.988 ml/kg X 3.5 kg =80.5 ml/pot

 Same calculation was done for the effluent rates of 100 m^3, 200 m^3 etc.

Appendix 3

Table 5.4 Combined effect of effluent and biochar on the beans number of leaves and the wheat BGB

Treatments	Beans Number of leaves Mean ± SD	Wheat BGB Mean ± SD
B0E0	**7.00 ±3.24**	**0.74±0.24^a**
B0E50	8.89 ±3.18	0.57±0.02^{abcd}
B0E100	8.22 ±0.83	0.58±0.02^{abcd}
B0E200	8.56 ±2.55	0.37±0.04^e
B0.5E0	6.89 ±1.54	0.43±0.03^{ed}
B0.5E50	6.78 ±1.79	0.62±0.12^{abc}
B0.5E100	6.11 ±1.69	**0.57±0.12^{abcd}**
B0.5E200	8.67 ±2.18	0.51±0.14^{cde}
B2.5E0	8.89 ±2.26	0.43±0.11^{ed}
B2.5E50	9.44 ±3.17	0.54±0.05^{bcde}
B2.5E100	10.67 ±3.81	0.55±0.07^{bcde}
B2.5E200	**11.44 ±5.15**	0.51±0.17^{cde}
B5E0	10.00 ±3.97	0.49±0.06^{cde}
B5E50	9.11 ±3.02	**0.71±0.07^{ab}**
B5E100	10.11 ±2.42	0.53±0.09^{bcde}
B5E200	**11.44 ±4.61**	0.60±0.02^{abcd}

The cells with different superscripts are significantly different at (p=0.5). The treatment represent interaction between biochar and effluent applied at different rates: Biochar rates of, 0, 0.5, 2.5 and 5% and effluent rates of 0, 50, 100 and 200 $m^3 \cdot ha^{-1}$ m NS means not significant. The superscripts denoted as A-E ranks from 5-1, highest to lowest.