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Effect of Olive Mill Wastewater Pollution on Plant Litter Decomposition

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Dedication

To my dear parents who encourage and support me all the time

TO my soul my husband Shoa'a

To my lovely daughter “Reem" who lightens my future and draws the smile on my face

To my brothers, sisters, friends and all those who stood beside me while preparing this thesis

To all of these, I wish to accept my dedication

Bayan Abu Alwafa

Declaration

I certify that this thesis submitted for the degree of Master in environmental science is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not be submitted for a higher degree to any other university or institution.

Bayan Mohammad Abdullah Abu Alwafa

Signature: …………

Date: 5/ 5 /2019

Acknowledgment

As I take my last steps in this stage of education, all the thanks to GOD, who give me the power to finish this work.

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Abstract

Olive mill wastewater (OMW) become a major issue of environmental concern in the countries producing olive oil. Palestine is one of these countries. Applying of olive mill wastewater to soil could cause positive or negative effects. The present study aims to test the effect of olive mill wastewater on plant litter decomposition through testing the Tea Bag Initiative (TBI) under controlled lab conditions. The TBI is a simple, standardized method for measuring decomposition in soils.

In this study, a clay loam soil was collected from Al-Ubeidiya in October 2017, then the soil was incubated in pots each pot contains (500 g) of soil then tea bags (green and rooibos tea) was buried in soil and incubated under controlled lab conditions for nine months (270 day) at room temperature, the incubated soil containing the tea bags was irrigated with olive mill wastewater and the control irrigated with fresh water. A total of 60 tea bags used in the experiment (2 types of plant litter (green tea and rooibos tea) used * 2 types of irrigation used (fresh water as a control and OMW) * 3 replicates of each type of litter * for 5 periods of time).

The Cornell Soil Health Test (CSHT) protocols were used for analyzing physical and chemical soil properties, such as: pH, Ca, EC, K, Mg, Cl-, OM, Na, HCO3, TOC and TNb.

The results showed that the decomposition of green tea was faster than the decomposition of rooibos tea, and this is a consequences of the chemical composition of the two litter types. Also the decomposition of tea (green and rooibos) litters was higher when tea bags irrigated with fresh water more than that when tea bags irrigated with OMW. Since the decomposition rete constant (k) for the tea litters irrigated with fresh water was (0.01656), and for tea litters irrigated with OMW was (0.01375) and this indicates that the decomposition was faster when the soil was irrigated with fresh water. Also the stabilization factor (S) was (0.35153) for the tea litters irrigated with fresh water and it was higher when the soil was irrigated with OMW (0.39442), since the high S value indicates inhibition of decomposition, this result support that the OMW decreased the decomposition.

However, the results of soil properties showed that the pH, Ca, EC, K, Mg, Cl-, OM, Na, HCO3, TOC and TNb soil contents increased after applying OMW.

تأثير إضافة الزيبار للتربة على تحلل المادة العضوية في النبات

إعداد: بيان محمد عبدالله ابوالوفا

إشراف: د. جواد شقير

الملخص

أصبح الزيبار الناتج عن معاصر الزيتون قضية بيئية رئيسية في الدول المنتجة لزيت الزيتون وفلسطين هي واحدة من هذه البلدان. ويتسبب اضافة الزيبار إلى التربة بحدوث آثار إيجابية أو سلبية. تهدف هذه الدراسة إلى اختبار تأثير الزيبار على تحلل فضلات النباتات من خلال اختبار مبادرة أكياس الشاي في ظل ظروف المختبر الخاضعة للرقابة، وهذه الطريقة هي طريقة بسيطة وموحدة لقياس التحلل في التربة.

في هذه التجربة تم جمع تربة طينية من منطقة العبيدية في اكتوبر من عام 2017م، ثم حضنت التربة في أوعية كل وعاء يحوي (500غم) تراب وتم دفن أكياس الشاي (الأخضر والأحمر) في هذا التراب وحضنت لمدة تسعة أشهر (270 يوم) في ظروف المختبر الخاضعة للرقابة، في درجة حرارة الغرفة. سقي جزء من الأكياس بماء عذب وجزء أخر سقي بالزيبار. استخدم ما يقارب 60 كيس شاي في التجربة كالتالي: أكياس الشاي المستخدمة في التجربة نوعان (الشاي الأخضر والشاي الأحمر) * استخدمت طريقتين للري (المياه العذبة والزيبار) * كرر كل نوع من أكياس الشاي ثلاث مرات * تمت التجربة على خمس فترات زمنية (14 و 21 و 90 و 180 و 270 يومًا (أي تسعة أشهر)).

تم استخدام بروتوكولات اختبار صحة التربة بناءً على كتاب كورنيل لتحليل الخواص الفيزيائية والكيميائية للتربة، مثل درجة الحموضة، الكالسيوم، الموصلية الكهربائية، المادة العضوية، الصوديوم، المغنيسيوم، الكلور، مجموع الكربون الكلي، مجموع النيتروجين المرتبط و البايكربونات.

أظهرت النتائج أن تحلل الشاي الأخضر كان أسرع من تحلل شاي الأحمر وهذا نتيجة لاختلاف للتركيب الكيميائي لكل نوع من الشاي، كما كان التحلل في أكياس الشاي المسقية بالمياه العذبة أسرع من التحلل في أكياس الشاي المسقية بالزيبار، حيث كان معدل ثابت معدل التحلل في أكياس الشاي المسقية بمياه عذبة (0،01656) وفي أكياس الشاي المسقية بالزيبار (0،01375) مما يدل على أن التحلل كان أسرع عندما تم الري بالمياه العذبة، وكان معدل الثبات في أكياس الشاي المسقية بمياه عذبة (0،35153) وكانت أعلى في حالة أكياس الشاي المسقية بالزيبار (0،39442) وحيث أن الزيادة في هذا المؤشر تدل على نقصان معدل التحلل فان هذه النتيجة تدعم أن أضافة الزيبار تقلل من معدل التحلل.

وكما أظهرت نتائج خصائص التربة (درجة الحموضة ، الكالسيوم، الموصلية الكهربائية، المادة العضوية، الصوديوم، البوتاسيوم، المغنيسيوم، الكلور، مجموع الكربون الكلي، مجموع النيتروجين المرتبط و البايكربونات) زيادة بعد اضافة الزيبار اليها.

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List of Abbreviations

Abbreviations	**Full Name**
Al	Aluminum
Ba	Barium
C	Carbon
Co	Cobalt
Cr	Chromium
Cu	Copper
EC	Electrical conductivity
Gt	Gigantic (giga tone)
GHGs	Greenhouse
Gf	Green tea irrigated with fresh water
Rf	Rooibos tea irrigated with fresh water
Gz	Green tea irrigated with OMW
Rz	Rooibos tea irrigated with OMW
Li	Lithium
µg/g	Microgram/gram
Mo	Molybdenum
Sr	Strontium
SOC	Soil organic carbon
SOM	Soil organic matter
TBI	Tea bag index
%	Percentage

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Chapter One

Introduction

1.1. Background

Olive oil is a liquid fat obtained from olives, it has long been popular kitchen staple in the Mediterranean and Middle East countries. The cultivation of olive trees and olive production rely on many factors like soil fertility, climatic conditions and irrigation availability. According to the International Olive Oil Council, (2009) the olive oil production is considered as the most important agricultural industrial activities for the Mediterranean region in which over 95% of the world's olive oil is produced, about 3×10^6 ton/year. The average annual growth of olive oil production worldwide over the last 15 years is about 5 %. Spain, Italy and Greece are considered the major producers worldwide (Tsagaraki et al., 2007), then comes Turkey, Morocco, Tunisia and Syria. In Palestine, the olive farming and olive oil have economic and social importance (Figure 1.1). The olive production is used for local consumption in general, and small amount is being exported abroad. According to the Palestinian Central Bureau of Statistics (2013), about 52.5 % of the cultivated land, estimated at 881.9 km^2, is planted with olive trees; it accounts for 70 % of fruit production and contributes to 20% of agricultural income.

Figure 1.1: Traditional olive orchard in Palestine (left). Palestinian family during the olive harvesting season (right).

Typically, olive harvesting occurs during the autumn/winter season (Moraetis et al., 2011). The olive fruit is harvested, and then delivered to olive mills in order to extract the oil. The olive oil extraction is accomplished through a sequence of steps; firstly washing the olive fruit, then grind the olives together with their seeds and ending with milling and beating the olive pulp. In the milling step, water is added to further break down of the olives to create larger oil drops. Salt is also added for this purpose, which aids the osmotic break down of cells in the olives and to separate the oil and water from each other. The oil extraction technologies have advanced during the past decades, and it can be classified as described below.

First is the traditional or discontinuous press: is the oldest method and the most widespread one. During the milling stage a small quantity of water is added about 0.03-0.05 m^3 per ton of olive fruit (Tsagaraki et al., 2007). The byproducts of this method are high-quality olive oil, the solid waste called pomace, and the liquid wastewater called olive mill wastewater (OMW).

Secondly, the continuous press: is a new method of olive oil extraction, it uses an industrial decanter to separate oil from olive components by centrifugation. It operates by three-phase or two-phase decanter. The three-phase method produces better oil quality and requires small area for installation. But in the milling step, a large amount of water is required about 0.6-1.3 m^3 per ton of olive seed (Hanifi, 2009). Beside the olive oil, a large amount of OMW is produces in this technology and solid waste pomace. On the other side, the two-phase decanter is a new centrifugation system, which is similar to three-phase centrifugation system but adjusted to reduce the amount of OMW produced. Here there is only oil and humid solid waste. The advantage of this decanter over the three-phase one is in the limited amount of water added during the milling step which in return reduce the amount of OMW produced by up to 80 % (Figure 1.2).

Figure 1.2: Olive oil produced by three phase extraction technology in Palestine (left). Olive mill wastewater produced during the oil extraction process (right).

1.2 Olive mill wastewater (OMW)

OMW is one of the biggest challenges facing the olive oil industry. Generally, the extraction of olive oil produce about 1–1.2 m^3 of OMW per ton of olives (Hanifi, 2009), such a high amount of OMW is produced in a short period (~2 months) during the harvest season. And there is not yet a widespread solution to the release of OMW into the environment in an uncontrolled manner. This situation is highly undesirable due to possible contamination of freshwater resources and it trigger extensive damage to nearby ecosystems as well as potential adverse effects on soil quality and fertility. Since Soil is a major component of the terrestrial ecosystem and a major pool of carbon (C) on Earth (500 to 3000 Pg C) the effect of adding OMW at a long term will be critical.

OMW is a mixture of vegetation water, soft tissues of olive fruits and the water used in the milling process (Alburquerque et al., 2004). It is also a turbid liquid, black to dark brown in color and smells of oil (Sierra et al., 2001; Dermeche et al., 2013). OMW characteristics depend on many agents like the extraction technology used, the climatic conditions, the maturity of the olives, and the cultivation management (Sierra et al., 2001).

The OMW is typically acidic (pH 4–5) (Isidori et al., 2005), has high concentrations of cations and anions, nutrients, fats (oils and greases), high salinity (Roig et al., 2006), high biological oxygen demand (40–80 gl^{-1}) high chemical oxygen demand (50–150 gl^{-1}), and is high in polyphenolic compounds (Kistner et al., 2004), which is responsible for the typical black color of OMW (Piotrowska et al., 2006).

Many studies proposed the use of OMW for soil improvement, like Belaid et al. (2013), Charari et al (2015), Kallel et al (2009) and Mekki, et al (2006) they also reported that the treatment of OMW could generally reduce its negative effects, and make it serve as a fertilizer and safe water for use in agricultural irrigation. In addition, it could generally increase the overall soil respiration rate and number of heterotrophic microflora including fungi and coliforms (Mekki, et al., 2006). The idea of adding OMW as a fertilizer developed from the knowledge of its components. It contains considerable amounts of plant nutrients, like K (Roig et al., 2006), P, Mg (Mekki et al., 2006), and organic matter (OM) (Di Bene et al., 2013), which can be applied directly to the agricultural soils as an organic supplement (Chaari et al., 2014), and it could also modify the SOM properties.

However, the organic fraction of OMW is complex. It contains polyalcohols, carbohydrates, proteins, polyphenols, organic acids, glucosides, greases, and tannins (Mulinacci et al., 2001). By applying OMW to soil the probability of leaching of some of these components to the ground water is not excluded. Also some OMW compounds could affect soil quality and modify the absorptive capacity of soil to organic pollutants. For example, hydrophobic organic compounds present in the OMW like fatty acids can induce soil water repellency, this repellency of soil to water may accumulate with each new application of, this is why OMW can induce soil water repellency, which could later lead to surface runoff that may induce soil erosion. Also, the Polyphenolic compounds is highly recalcitrant and toxic to humans, animals and plants., when adding OMW directly to the soil these phenolic compounds could inhibit biodegradation of OMW-OM (Sierra et al., 2007) and render the soil highly phytotoxic for several weeks (Piotrowska et al., 2006).

several studies showed opposite results about toxicity and permanence of phenolic compounds in soil which can be attributed to the different amounts of toxic organic compounds present in OMW (Piotrowska et al., 2006), and different soil moisture and temperature through and after adding OMW which can affect the biological decomposition process of soil organic matter (SOM) constitutes (Steinmetz et al., 2015).

Management of SOM is significant from a large-scale perspective, and is important for improving soil water storage, biological activity, and nutrient availability in soil. It crucial for soil long-term performance and is a key attribute for soil quality and fertility, because it influences many of physical, biological and chemical properties in soil. It is also important for crop productivity since it's a source of major plant nutrients (Carter et al., 1997). Adding OMW as a source of OM to soil plays a great role on increase and enhance soil content of OM (Schaumann et al., 2010).

Chapter Two

Literature review

This chapter contains a brief description for the current situation, the problem statement and justification of the study. In addition, the research questions, the objectives for the study were included.

2.1 Decomposition of plant litter

Decomposition is the breakdown of organic material completed through the metabolic activity of saprotrophic organisms that may release decomposition products as gases, like CO_2, or as dissolved organic matter into soil solution (Laiho 2006). It is important for storage and recirculation of nutrients in the ecosystems. The main factors determining decomposition rates are climate, litter quality, soil properties, and microbial community structure (Trofymow et al., 2002).

Soil organic matter (SOM) is the organic matter component of soil, consisting of plant and animal residues at various stages of decomposition, cells and tissues of soil organisms, and substances synthesized by soil organisms. It consists of C, H, O, N, and P and S. its content can't be measured directly, but it can be determined by the balance between inputs from primary production, and losses through the decomposition of organic matter by time. But, there is a degree of variability in this balance and more researches is needed for more accurate estimation of present and future global carbon budgets and to understand the process of decomposition at multiple scales (Aerts 2006).

2.2 Decomposition stages

In terrestrial systems, decomposition of litter fall of plant is a primary pathway for nutrient return to the soil, since more than 70% of the aboveground litter fall in forests comes from leaf tissue, with the rest composed of small twigs, stems and reproductive structures (Robertson and Paul 1999).

Plant litter decomposition can be divided into at least two stages (e.g. Berg & Mc Claugherty, 2008), the early stage and the late stage. In the early stage of decomposition (0 to 40 %) of mass is lost, and is characterized by leaching of soluble compounds, also by decomposition of solubles and non-lignified cellulose (Couteaux et al., 1995). In this phase, a quick decrease in water-soluble substances concentrations (in a few months) before reaching relatively stable levels (Berg et al., 1987). Also, free unshielded holocellulose is degraded in this phase, whereas the recalcitrant lignin either does not decompose or only to a low extent. Thus, its concentration starts to increase due to the decomposition of other main compounds. Also the concentrations of some nutrients such as N, P and S, start to increase (Berg and B., 2000).

The other stage, is the late stage (40-100 %) of mass is lost, encompasses the degradation of lignified tissue. As decomposition proceeds the litter becomes enriched in lignin (Berg and Staaf, 1980). And the concentrations of N and P increased linearly with accumulated mass loss (Staaf and Berg, 1982).

The earlier studies reported that during litter decomposition as lignin concentrations increase the decomposition rates are suppressed (Fogel and Cromack, 1977; McClaugherty and Berg, 1987).

In general, in the early stage, the climate as well as concentrations of the major nutrients and water soluble had a clear influence on decomposition rate. In a later phase the decomposition of lignin dominate over the influence of nutrients and thus ruled the decomposition of litter (Berg and B., 2000).

2.3 Methods to estimate litter decomposition

Many experiments have been made focusing on litter decomposition as a fundamental soil process in order to understand factors control the terrestrial carbon transfer to the atmosphere. Decomposition studies across multiple sites using standardized methods already exist within observational networks or experimental studies such as GLIDE (Global Litter Invertebrate Decomposition Experiment– Wall et al., 2008), VULCAN (Vulnerability assessment of shrubland ecosystems in Europe under climatic changes - Emmett et al., 2004) and VAMOS (Variation of soil organic matter reservoir – Cotrufo et al., 2000), these studies of litter decomposition with standard techniques have all provided complementary information in different parameters used in the SOC simulation models and on the decomposition of litter, as well as information on carbon and nutrient cycling in forests, but have been limited to specific biomes or have used site specific litters (Djukic et al., 2018).

Comparisons of similar data across different experiments and sites posed a challenge due to the lack of common protocols and standard matrices, this added major uncertainty to syntheses across different experiments and sites.

2.3.1 Litter bag experiment

The approach is widely used to study decomposition at the soil surface. It has been a standard procedure used in soil ecology for the past 50 years (Kampichler & Bruckner, 2009). This methodology is a common method for estimating the decomposition rates, it can also be used to investigate the dynamics of decomposition and nutrient cycling. Here there are mesh bags filled with plant material that are buried, retrieved then weighed for measurement of the mass remaining over a certain period of time (Moore and Basiliko 2006). This method enables the researcher to study the decomposition dynamics under field conditions.

Mostly, one species of fresh litter is used, however, for a more realistic experiments a mixture of materials can be used, even small woody debris or reproductive structures (Karberg et al., 2008). Depending on research topic, the bags can be filled with different types of organic material, like leaves, straws or more complex mixes of organic materials (Karberg et al., 2008). The researcher can take control (restrict or permit) different types of soil fauna from entering the litterbag, by alternation of the mesh-size of the litterbags (Karberg et al., 2008). This to determine the effect of different soil fauna's contribution to the mass loss (Kampichler & Bruckner, 2009). The collected litterbags are then dried on oven, to compare pre- and post-decomposition mass. By using regression equations litter decomposition can be estimated (Karberg et al., 2008).

Nevertheless, litterbags have a few weaknesses concerning their use. That is certain macro-invertebrates are excluded from the litterbags, thereby lowering the rate of litter comminution and alter decomposition compared to real conditions, also the additional characteristics like the amount of litter used, litter bag construction and length of burial can vary between studies, further complicating cross-site comparisons (Karberg et al., 2008; Kampichler & Bruckner, 2009). In addition, filling Litter bags with local vegetation can inhibit comparison between study sites due to the ambiguity of environmental conditions versus litter characteristics (Keuskamp et al. 2013). So Keuskamp et al. (2013) have proposed the Tea Bag Index (TBI) method to solve these issues by using a widely available, standardized and pre-constructed tea bags

2.3.2 Tea bag index (TBI)

Few earlier studies used plant litter to test decomposition at global scale (Berg et al. 1993; Trofymow et al. 2002). In this regard, Keuskamp et al., in 2013, introduced a new methodology also uses a standardized plant litter to measure decomposition and stabilization in situ at a large scale and in a high resolution. The method also enables volunteers from all over the world to participate in the generation of a global database, by using the commercially available tea bags as highly standardized test kits containing tea as representative dead plant material. Tea bag index is characterized as a simple, standardized, time efficient and cheap, it's based on two types of tea with different decomposability: Rooibos tea (red or cortex) and Green tea (leaves). The rooibos tea is characterize with slow decomposition rate, while green tea is characterize as fast, Sarnell interpreted that Rooibos (cortex) tea, with a higher C/N-ratio of 40 is more recalcitrant and decomposes slower, on the other hand Green (leaves) tea have more water-soluble carbon and has a C/N-ratio of about 15 thus decomposes faster.

The bags themselves are non-degradable tetrahedron-shaped with mesh size of 0.25 mm, allowing microorganisms and smaller mesofauna to enter the bag, and preventing the macrofauna. The advantage of using commercially available tea, is that these tea bags constitute a pre made “litterbag” reducing any variation related to user differences in preparation.

Tea bags are buried at different locations and then retrieved (after 90 days), after that dried and weighted. Tea bag index (TBI) is derived based on the parameters (k and S). Where (k) is the decomposition rate and (S) is the litter stabilization factor. These parameters of TBI are estimators to characterize and compare carbon decomposition, where k is a measurement of short term effects and S long-term effects (Keuskamp et al., 2013). The difference in decay between two tea types allows the TBI to estimate the decomposition rate constant k in a single burial time interval (about 90 days), this period of time is the shortest compared to the two or more years recommended for previous litter bags (Moore and Basiliko 2006, Keuskamp et al. 2013). In addition, it good to know that, the litter stabilization factor S is influenced by temperature, while the decomposition rate k does not. Also TBI response to abiotic conditions e.g. temperature and soil moisture (Keuskamp et al., 2013).

On the other hand, the Rooibos bags sometimes destroyed and lost in the field because of animals, it may be due to its attractive odor. Over and above, results from another studies, reported that the analysis of TBI data was dependent on accurate assumptions, this means that the three months of incubation is sensible, whereas six months is too long, making the results less meaningful. So in shortage, even that the method of tea bag can give appropriate data on decomposition rates and stabilization factors, but it cannot substitute the precision and thoroughness of conventional litter bag methods (Keuskamp et al., 2013).

Numerous field experiments have been carried out in order to analyze the effect of olive mill wastewater (OMW) application to soil on various scales and parameters, like inorganic nutrients and salinization (e.g., Chaari et al. 2015); plant growth and yield quality (e.g., Ben Brahim et al. 2016), and SOM content and resulting soil properties like bulk density and porosity (e.g., Mohawesh et al. 2014). However, the influence of adding OMW to soil on plant litter decomposition has not been investigated yet systematically under controlled lab conditions. In this study, the TBI was used to investigate the influence of applying OMW to soil on plant litter decomposition systematically under controlled lab conditions.

2.4 Research questions:

- How tea bag initiative can contribute to the knowledge of unified litter decomposition method?
- How does OMW influence decomposition?

2.5 Research aims

Litter decomposition worldwide is a tool to test the effect of climatic and pollution in soil, therefor this research aims to test the effect of OMW on plant litter decomposition using the tea bag initiative proposed by International long term ecological research network.

To achieve this aim the following objectives has been assigned:

1. To test the effect of olive mill wastewater pollution on the litter decomposition through testing the TBI which might decrease the time of decomposition.
2. To test and monitor the litter decomposition under controlled lab conditions which can be used as a base for the Tea Bag Index (TBI).
3. To analyze soil properties and correlate it to the TBI.

Chapter Three

Methodology

This chapter includes a description of location, monitoring, sampling, analysis and all the related field and laboratory work during the period of the study. Furthermore, it includes the used mathematical equations and formulas.

3.1 Study site and site description

Soil samples were collected in October 2017 from Al-Ubeidiya (31°43′24″N 35°17′26″E), which is a Palestinian town in Bethlehem Governorate located 8.4km (horizontal distance) east the city of Bethlehem. It is located at an altitude of 532m above sea level. Al-Ubeidiya is bordered by the Dead Sea to the east, Sawahira al Sharqiya in Jerusalem Governorate to the north, Dar Salah village to the west, Tuqu' town and Dar Salah village to the south as indicated in (Figure3.1).

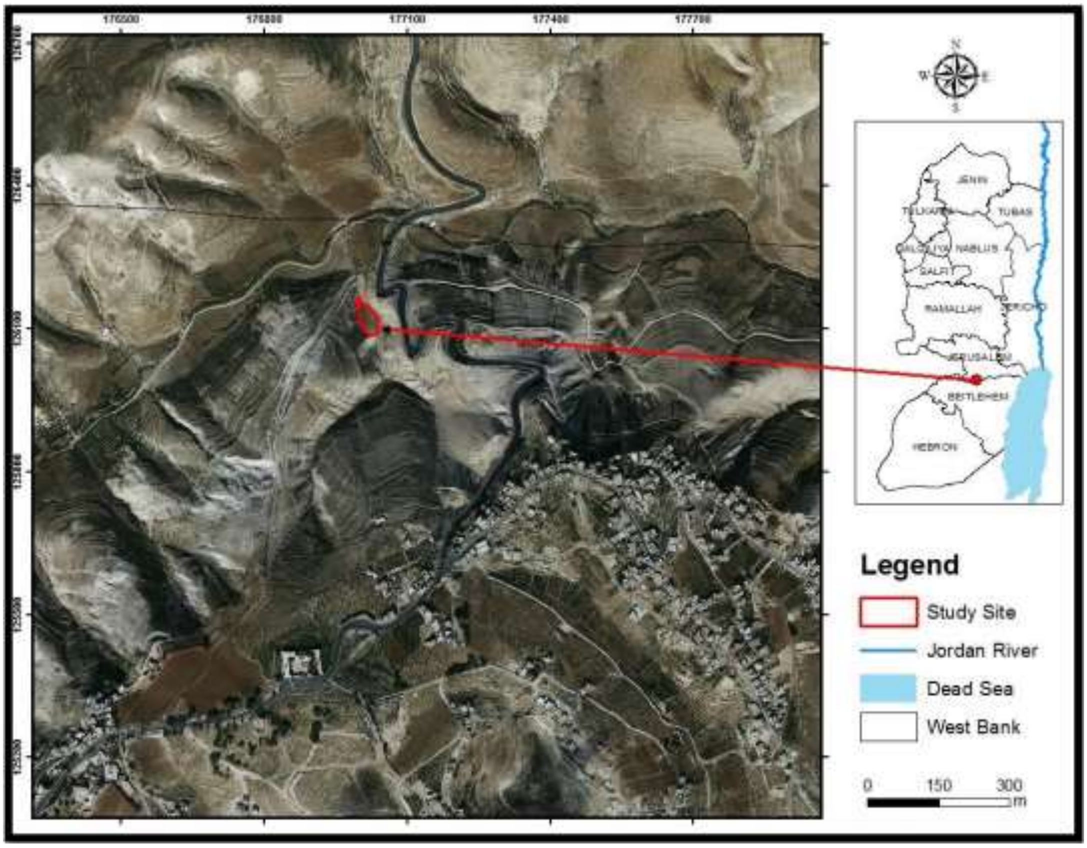

Figure 3.1: Description of the study site (left: The main road connecting the north and west of the West Bank, North: Wadi Nar stream is marked in white, south: residential communities in the town of Al-Ubeidiya, located in the Bethlehem district.

The study areas are located in long-term ecological research (LTER) semi-aired sites in Al-Ubeidiya. The site of the study is subject to graze and farm in previous years. The Herbaceous vegetation appears in the mid-winter after the rainfall begins and persists for 2–5 month. In this study, the soil is clay loam. Soil samples were taken in at a depth of (5-15) cm.

3.2 Experimental control conditions

Air temperature data was measured daily from the date of placement of the tea bags to the last date of retrieval. Also soil temperature was measured frequently using temperature probe at depths of 5 and 10 cm below the soil surface. The climatic conditions during the time period the tea bags remained in the lab (9 months) where variable. Mean air temperature ranged from 13℃ to 33 °C. Mean soil temperature for fresh water 19.5℃ and for OMW 21 °C.

3.3 Analysis of soil properties

The collected soil samples was placed in paper bags, dried at air at room temperature, crushed, homogenized, and passed through a 2 mm sieve as preparatory step for laboratory analysis. The Cornell Soil Health Test (CSHT) protocols were used for analyzing physical and chemical soil properties (Gugino et al. 2009). The analyzed chemicals and physical indicators are:

a. **Chemical indicators:** (Anions; (Cl^-, HCO3), Electrical Conductivity (EC), pH, Cations (Potassium (K^+), Sodium (Na^+), Magnesium (Mg^{+2}), Calcium (Ca^{+2})), Total organic carbon (TOC), Total Nitrogen Bound (TNb)**,** Organic matter (OM).
b. **Physical indicators:** (Soil texture (sand, silt, and clay), Soil moisture).

The pH and EC of the soil was measured by adding 5 g of air dry soil to 50 ml of deionized water, shaking for 1 minute and allowing the mixture to settle for 30 minute, stir suspension every 10 min during this period, after1 hour, stir the suspension the pH was measured using pH meter. The pH meter was calibrated following the manufacturer's instructions before it was inserted into the solution, also electrical conductivity is measured. Soil properties where analyzed by the Cornell Soil Health Test (CSHT) protocols.

3.4 OMW collection

OMW was collected without pre-treatment during the olive oil production season from September to November of 2017. OMW was collected directly from press and stored in 18L plastic containers in the dark at the laboratory. After that, the analysis was done in Al-Quds University at soil and hydrology research lab.

3.5 Olive mill wastewater characterization

The physicochemical characteristics of OMW were determined according to standard analytical methods. The measurements were conducted in triplicate. The pH, Electrical Conductivity (EC) were measured by using a WTW multi meter 3430.

The pH was measured in a suspension of 50ml of OMW at ambient temperature by pH-meter instruments previously calibrated with buffer solutions pH 4, 7 and 10 immediately after sampling, pH measurement is done directly in the raw effluent olive oil mills at room temperature according to multi meter (CCBA-SOP-005). Also the conductivity of the OMW was measured by a CCBA-SOP-006 multi meter.

Carbon content (CC), Total Nitrogen (N), Organic Matter (OM), Iron (Fe), Copper (Cu), chloride (Cl), Chromium (Cr), Antimony (Sb), Manganese (Mn), Total Organic Carbon (TOC), Arsenic (As), Lead (Pb), zinc (Zn), Tin (Sn), and Nitrate (NO_3^-) were measured also.

3.6 Litter decomposition (TBI) procedure

The tea bag method have been used to test the effect of olive mill waste water (OMW) on plant litter decomposition under lab conditions. The experiment was carried out with commercially available tetrahedron-shaped synthetic tea bags with sides of 5 cm containing 2 g of green tea or rooibos tea (Lipton, Unilever) (Figure 3.2). The two different types of tea used in the experiment manufactured by Lipton based on the request of the team of the TB initiative. The green tea consist of 89% green tea, and the rooibos tea consist of 93% rooibos tea, both were supplemented with natural flavoring. The standard protocol for TBI (Appendix 1) was used setting up the experiment, resulting in the following methodology.

Figure 3.2: The used tea bags of the type Green tea Sencha (EAN: 8722700055525) on the right and Rooibos tea (EAN: 8722700188438) on the left.

The initial weight of the content in the tea bags and all its components where determined on 3 bags of Green tea and 3 bags of Rooibos tea randomly selected from different boxes and weighed separately after drying in 70 C° for 48 hours. Results are shown in (Table 3.1).

Table 3.1: Mean initial weight of Green tea and Rooibos tea bag contents.

Content	Mean weight
Green tea	1.82 g
Rooibos tea	1.86 g

To determine decomposition of tea litters (green and rooibos tea) over time, the tea was incubated in pots, each pot contains 500g soil, under lab conditions and the soil containing tea bags was irrigated daily with 150 ml of OMW or 150 ml fresh water, this was done at a temperature ranged from 13 °C to 33 °C, which are well within its expected range. (Table 3.2) shows a total of 60 tea bags used in the experiment (2 types of plant litter (green tea and rooibos tea) used * 2 types of irrigation used (fresh water and OMW) * 3 replicates of each type of litter * for 5 periods of time). Two types of irrigation system was applied: fresh water as a control and OMW as a source of pollution. All the tea bags were incubated for 14, 21, 90, 180 and 270 days (Table 3.2).

Table 3.2: Tea bags sampling in tow irrigation systems.

type of irrigation	litter type	time periods				
		14	21	90	180	270
fresh water	G	3	3	3	3	3
	R	3	3	3	3	3
OMW	G	3	3	3	3	3
	R	3	3	3	3	3
Total		12	12	12	12	12

For each date of measurements, three tea bags of Green tea, and three bag of Rooibos tea where used per replicate. The tea bags were retrieved after five different time periods; 14, 21, 90, 180 and 270 days (n=5). The tea bags from each replicate were harvested at the same time. The three tea bags of each brand were placed at different pots (Figure 3.3, Figure3.4 and Table 3.2).

Figure 3.3: The incubated rooibos and green teas were irrigated with fresh water and OMW under lab conditions.

Figure 3.4: Three tea bags of Green tea, and three bag of Rooibos tea where used per replicate under lab conditions.

Each tea bag was identified on its label with a black permanent marker and gently placed in the soil at about 7 cm depth in separate pipe using a shovel. The label where still above the ground after the placement.

After the specified time, the tea bags from each replicate where retrieved and then dried in oven for 48 hours at 70 °C. Then the tea bags where opened and its contents where weighted separately.

3.7 Calculations of TBI parameters k and S

Litter bag studies measures decomposition by weight loss of plant material in time. Keuskamp et al. (2013) described in detail the concept of the TBI and the underlying assumptions behind the derivation of the two parameters, here only a brief detailing is given.

The tea bag index consists of two factors; the decomposition rate constant k and the stabilization factor S. k is calculated for both the labile and recalcitrant compounds using the equation (1)* (Keuskamp et al., 2013);

$$W(t) = ae^{-k_1 t} + (1-a)e^{-k_2 t} \quad (1)$$

Where W (t) is the weight of the substrate after incubation time t,

α is the labile fraction.

1-α is the recalcitrant fraction.

k_1 and k_2 are the decomposition rates for the labile and recalcitrant fractions, respectively.

In the first phase, the labile fraction (a) breaks down quickly and the weight loss of the litter is determined by k_1. The recalcitrant fraction (1-a) decomposes slower, and k_2 is very low (about zero), and assumed negligible in short-time incubations. This reduces equation 1a to exponential decay function, as presented in equation (2)* (Keuskamp et al., 2013);

$$W(t) = ae^{-k_1 t} + (1 - a) \qquad (2)$$

TBI uses two litter types with different decomposition rates. The decomposition rate of rooibos tea is lower than that of green tea. This means that the decomposition of labile material still continues in rooibos tea after all the labile material in green tea has been finished. In other words, the decomposable fractions (labile) are Green tea α_g, and the recalcitrant is Rooibos tea α_r. To solve the equation above, α_r can be estimated from α_g assuming that the relation between the decomposable fraction α and hydrolysable fraction H, the chemically expected labile fraction (i.e. chemically labile), only depends on environmental conditions. During decomposition, some of the labile fractions stabilize and become recalcitrant and the stabilization factor S for the labile compounds can be calculated through the equation (3)*(Keuskamp et al., 2013);

$$S = 1 - \frac{a_g}{H_g} \qquad (3)$$

Where H_g is the hydrolysable factor of Green tea.

The decomposable factor of Rooibos tea α_r is calculated by using the hydrolysable fraction of Rooibos tea H_r and the stabilization factor S, giving the equation (4)* (Keuskamp et al., 2013);

$$a_r = H_r(1 - S) \quad (4)$$

When knowing W (t) and α_r, these figures can be used in equation (2) to retrieve k for Rooibos tea.

Chapter Four

Results and Discussion

The result and discussion sections consist of chemical and physical indicators and the results of the tea bag initiative. This section represents the analysis of soil samples and their interpretation.

4.1 Physical and chemical properties of soil

All the results of physical and chemical analysis were repeated three times, the reported results are averaged, and the results of soil characteristic that located in Al-Ubeidiya are shown in (Table 4.1).

Table 4.1: The analyzed physical and chemical properties of control soil.

parameter	Amount	Unit
Cl	1.73±0.35	meq/l
HCO3	0.42±0.03	meq/l
Clay	25.5±9.2	%
Mg	0.39±0.03	meq/l
Ca	0.37±0.57	meq/l
K	0.13±0.07	meq/l
Na	0.24±0.04	meq/l
OM	6.08	%

soil moisture	1.24±0.38	%
EC	283±55.5	µS/cm
pH	7.61±0.24	-
TOC	44	mg/l
TNb	4.1	mg/l
Co	0.01	µg/g
Sr	0.33±0.06	µg/g
Mo	0.01	µg/g
Cu	0.011±0.003	µg/g
Ba	0.06±0.02	µg/g
Cr	0.01	µg/g
Al	0.21±0.34	µg/g
Li	0.01	µg/g

* (pH), (Ca) Calcium, (EC) Electrical Conductivity, (K^+) Potassium, (Mg^{+2}) Magnesium, (Cl^-) Chloride, lithium (Li), (Al) Aluminum, (Mo) Molybdenum, (Sr) strontium, (Co) cobalt, (Cu) copper, (Ba) Barium, (OM) Organic Mater, (Cr) Chromium, (Na^+) Sodium, (HCO_3) Bicarbonate, (TOC) Total Organic Carbon and (TNb) Total Nitrogen bound.

4.2 Physical and chemical properties of OMW

The analysis of physical and chemical characteristics of OMW were repeated three times, the reported results are averaged, and the results of OMW characteristics are shown in (Table 4.2).

According to (Table 4.2), the analyzed OMW was rather acidic and particularly rich in Iron (Fe), Copper (Cu) and chloride (Cl). This is most probably because of differences in fruit composition, maturity stage and harvesting method. Thus, it is difficult to define specific doses of OMW that must be applied to specific area, also prior work to

Characterize the composition OMW is necessary before the application.

Table 4.2: The analyzed physical and chemical properties of the OMW.

Parameter of OMW	amount	unit
EC	10.8	µS/cm
Ph	4.6	-
OM	7.06	%
Carbon content	33.9	%
Nitrogen content	0.9	%
TOC	40.3	g/L
Cl	1028	mg/L
NO3-	1.3	mg/L
Fe	82.7	mg/L
Zn	2.2	mg/L
Mn	1	mg/L
Cu	166	µg/L
As	4.4	µg/L
Sb	12.8	µg/L
Pb	47.7	µg/L
Sn	5.2	µg/L
Cr	56.9	µg/L

*(Mn) Manganese, (Cu) copper, (CC) Carbon content and (TN) Total Nitrogen, (As) Arsenic, Lead (Pb), (Sn) Tin, (NO_3^-) Nitrate, zinc (Zn), Iron (Fe), Antimony (Sb), (OM) Organic matter.

4.3 Characterization of physical and chemical properties of soil irrigated with OMW

The result of applying OMW to soil regarding the physical and chemical soil properties. Applying OMW to soil shows significant differences in most of soil properties. The results shows an increase in the soil moisture, pH, Electrical Conductivity, Potassium, calcium, Magnesium, Chloride, Sodium, Bicarbonate, Total Organic Carbon and Total Nitrogen bound. The characteristics of soil irrigated with OMW are shown in (Table 4.3).

Table 4.3: The analyzed physical and chemical properties of the soil after applying OMW.

Parameter	Rz9	Gz9	Unit
EC	2158	1971	μS/cm
OM	40.6	73.1	%
Ph	9.49	9.41	
Cl	249	253	meq/l
Mg	59	59	meq/l
Ca	10.66	12	meq/l
soil moisture	2.67	2.79	%
TOC	913	723	mg/l
TNb	48.97	39.45	mg/l
Na	159	159	meq/l
K	123070	119017	meq/l
HCO3	20.01	20.01	meq/l
Clay	10.80	17.90	%

* (pH), (Ca) Calcium, (EC) Electrical Conductivity, (K+) Potassium, (Mg+2) Magnesium, (Cl-) Chloride, (Na+) Sodium, (HCO3) Bicarbonate, (TOC) Total Organic Carbon and (TNb) Total Nitrogen bound.

4.3.1 Chemical properties of soil irrigated with OMW

Taking into consideration the deviation of the soil pH, electrical conductivity, Cl, Na, Mg, TOC, TNB, Ca and K also increase with OMW rates (Table 4.3). All this parameters increased after applying OMW compared to the control soil.

4.3.1.1 pH

Generally, soil pH indicates how acidic or alkaline soil conditions are. pH of the soil solution affects both solubility and ionization of elements. It also affects the solubility of salts, that is as the alkalinity increases the solubility of salts deceases. Also low pH indicates an acidic soil, and this can have a major impact on the decomposition of organic matter.

According to (Table 4.2) the pH value of OMW was (4.6). The acidic pH is a fundamental characteristic of OMW with values between (4.5 and 5.32) due to organic acids present in OMW.

However, the results show that there is an increase in the average pH after applying OMW to soil from (7.61±0.24) to (9.41) in Gz and (9.49) in Rz. And this in agreement with Mkhabela and Warman (2005) who reported that the increase of pH could be due to the mineralization of carbon which product OH ions by ligand exchange, such as K^+, Ca^{2+} and Mg^{2+}, or to the Na brought by this waste which generates $NaCO_3$ of more alkaline hydrolysis than the $CaCO_3$.

4.3.1.2 Electrical conductivity (EC)

The conductivity measurement is a good assessment of the degree of mineralization of OMW, where each ion is characterized by its concentration and specific conductivity, the electrical conductivity is strongly related to the concentration of dissolved substances and to their nature.

The EC of OMW was (10770 $\mu S \cdot cm^{-1}$), this result is comparable to those found in the literature such as Bouknana et al. (2014). And for control soil it was (283±55.55 µS/cm), this in agreement with Piotrowska et al. (2006). However, after applying OMW the EC was significantly affected (Figure 4.1), it increased to (1971 µS/cm) in Gz and increased to a higher extent in Rz to (2158 µS/cm). The result were consistent with related studies, which showed an increase in EC during the irrigation times (Chartzoulakis et al., 2010; Moraetis et al., 2011). Generally, the EC is proportional to the concentration of dissolved ions, especially to sodium and chloride. Thus the increase in soil EC maybe due to ionic species (Na^+ and Cl^-) present in the applied OMW. In this regard, high ion loads in the soil after applying OMW confirmed the significance of mineral input from OMW.

Even though the EC values remained below the salinity threshold (4000 µS/cm), but, if a long-term application of OMW takes place on soils and at a high rates, the increase in soil salinity can be of a major concern.

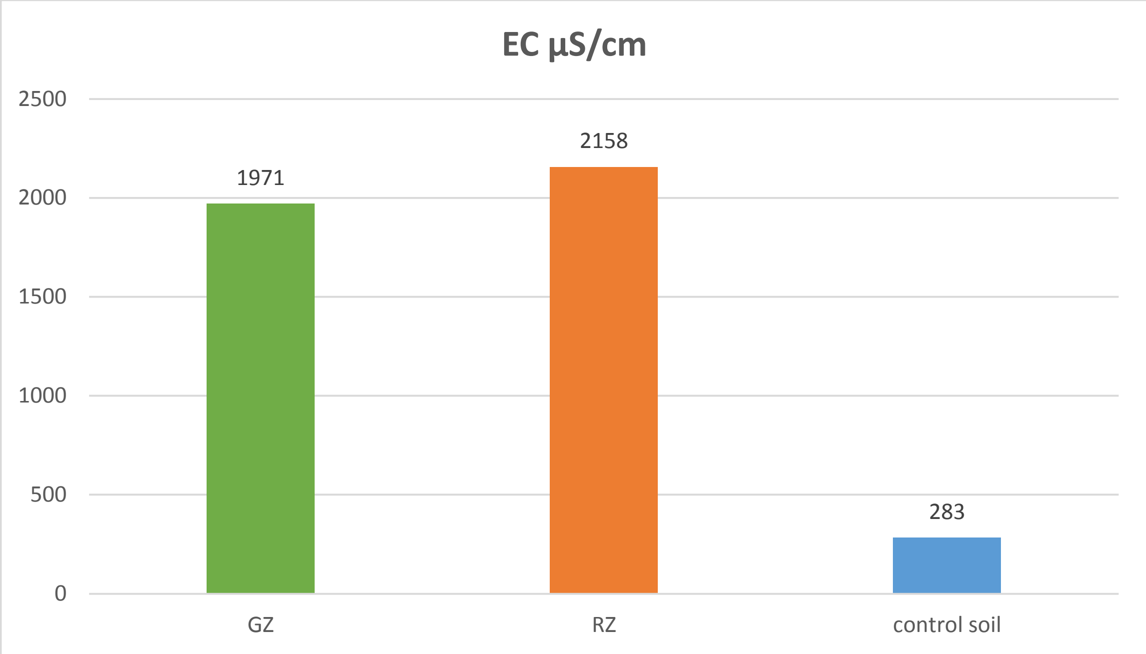

Figure 4.1: The electrical conductivity (EC) in (µS/cm) for the control soil, soil irrigated with OMW incubated with green tea and soil irrigated with OMW incubated with rooibos tea.

4.3.1.3 Sodium and Chloride

Soil content of Na^+ and Cl^- increased significantly after applying OMW to soil. Cl^- increased from (1.73±0.35) in control soil to (253) in Gz and to (249) in Rz soil (Figure 4.2). Na^+ increased from (0.24 ±0.04 meq/l) in control soil to (159 meq/l) in Gz and to (159 meq/l) in Rz (Figure 4.3).

The increase of nutrient contents Mg and K at all OMW treated pots (Table 4.3), may have a beneficial effect on the soil fertility. But, on the other hand, the main ionic species like sodium and chloride coming from the OMW could increase the salinity in the soil. The high amounts of exchangeable monovalent cations like Na^+ and K^+ might decrease the aggregate stability of soil and thus enhance soil erosion potential when replacing Ca^{+2} in humus–clay bonds. In this regard, Zenjari and Nejmeddine (2001) reported that the long-term applications of OMW may replace soil Ca by the cations of Na, K and Mg and could lead to the formation of saline soil and the degradation of the soil structure.

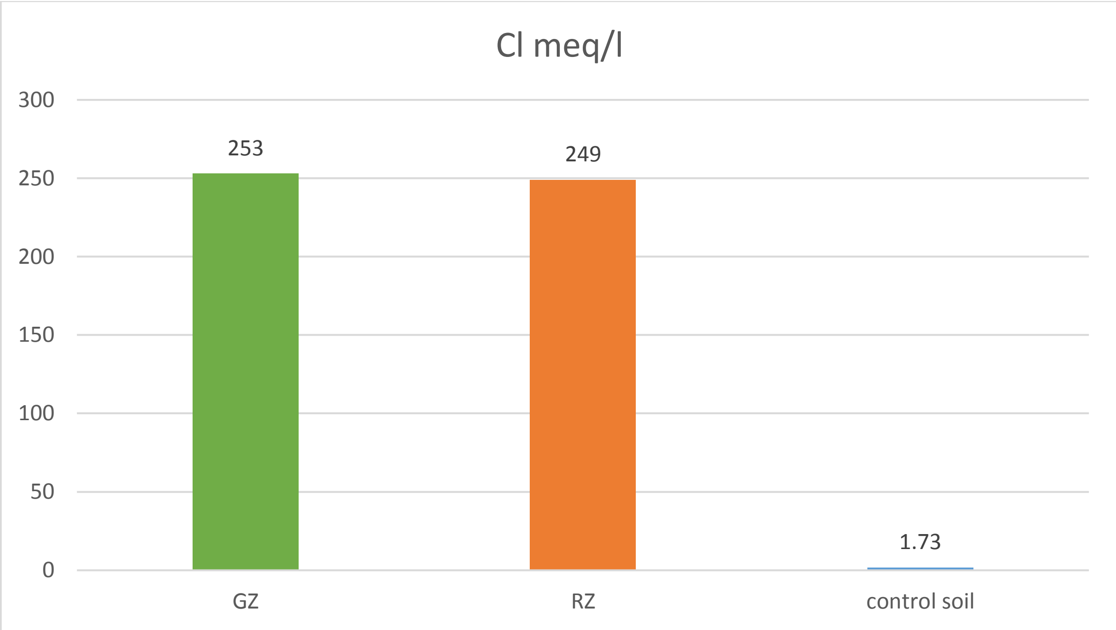

Figure 4.2: The concentrations of chloride (Cl^-) in (meq/l) for the control soil, soil irrigated with OMW incubated with green tea and soil irrigated with OMW incubated with rooibos tea.

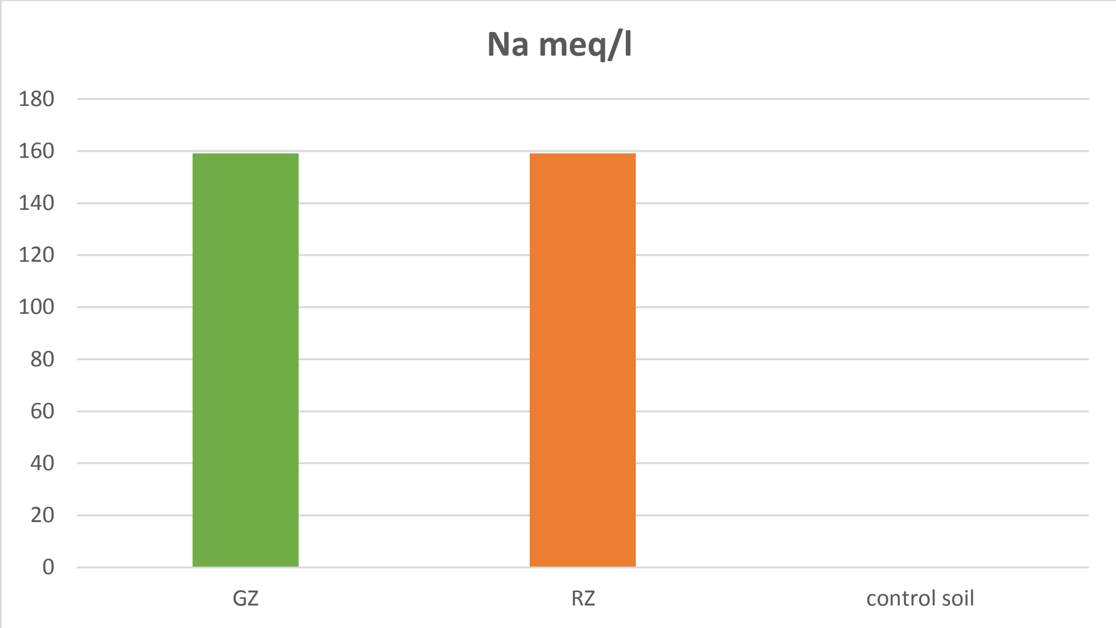

Figure 4.3: The concentrations of sodium (Na^+) in (meq/l) for the control soil, soil irrigated with OMW incubated with green tea and soil irrigated with OMW incubated with rooibos tea.

4.3.1.4 Potassium (K)

Soil content of potassium increased significantly after applying OMW to soil. It increased from (0.13±0.07 meq/l) in control soil to (119017 meq/l) in Gz and to (123070 meq/l) in Rz as shown in (Figure 4.4). This is in line with Di Bene et al. (2013) who reported that the extractable K was significantly affected by the OMW treatment at spreading sites and just after OMW disposal, also Di Bene et al. (2013) reported a surplus of approximately 80 mg kg^{-1} of exchangeable K after Six months of application of 8 L OMW m^{-2} in spring. It seems that The K^+ content is proportional to the applied quantity of OMW.

The increase of available K could be ascribed to the acidic nature of OMW and to its capacity to increase salinity (Saviozzi et al., 1991; Lo´pez etal., 1996). This result supports the proposal of OMW for application as an alternative K fertilizer

(Montemurro et al., 2004). The increase in soil K can upgrade soil fertility and minimize the use of chemical fertilizers.

Furthermore, the probability of K^+ leaching to deeper soil layers or groundwater is considered a negligible risk regarding its low toxicity and high sorptivity to soil particles (Arienzo et al., 2009).

However, it should be mentioned that the high amounts of K^+ could decrease the aggregate stability of soil and enhance soil erosion potential.

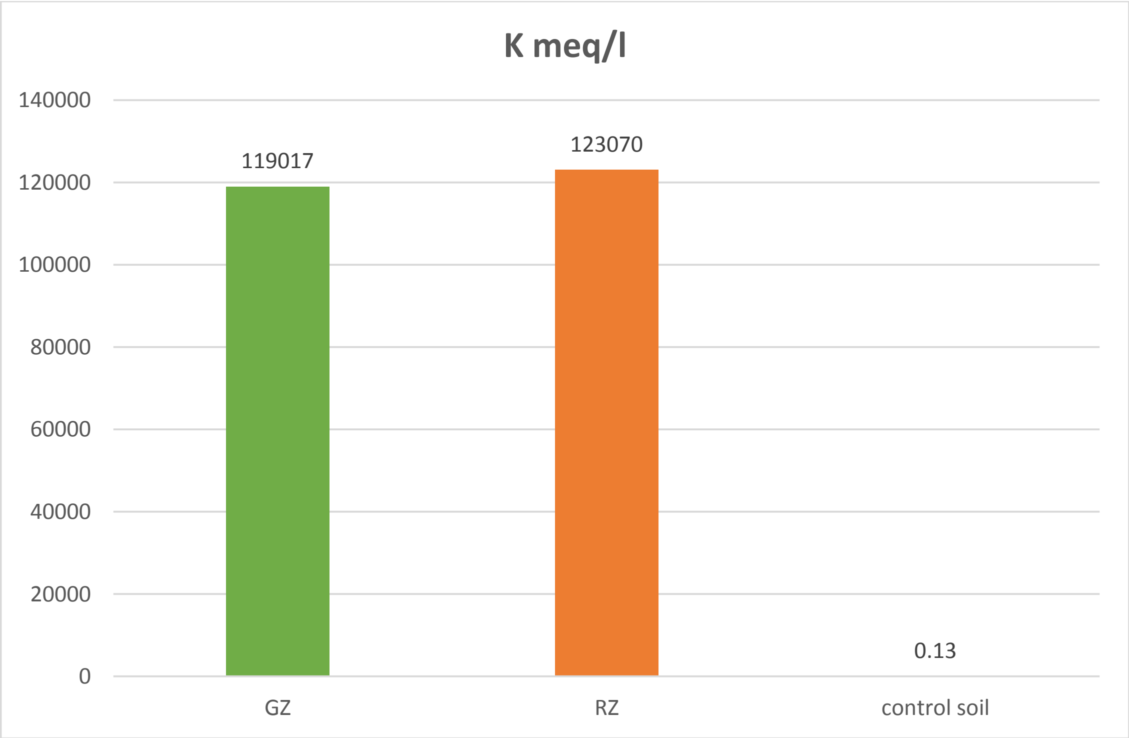

Figure 4.4: Concentration of potassium (K^+) in meq/l for the control soil, soil irrigated with OMW incubated with green tea and soil irrigated with OMW incubated with rooibos tea.

4.3.1.5 Magnesium and calcium

Mg and Ca serve as secondary macronutrients. Applying OMW to soil increased soluble Mg^{2+}, Ca^{2+} contents with respect to control soil. Mg increased from (0.39±0.03) in the control soil to (59) in both Gz and Rz (Figure 4.5), also Ca increased from (0.37±0.57) to (12) in Gz and to (10.6) in Rz (Figure 4.6).

The higher Mg^{2+} and Ca^{2+} contents in the soil after applying OMW indicated the impact of OMW application on soil properties. However, Paredes et al. (1999) reported that the Mg from OMW can be less available to plants compared to control soil bound Mg.

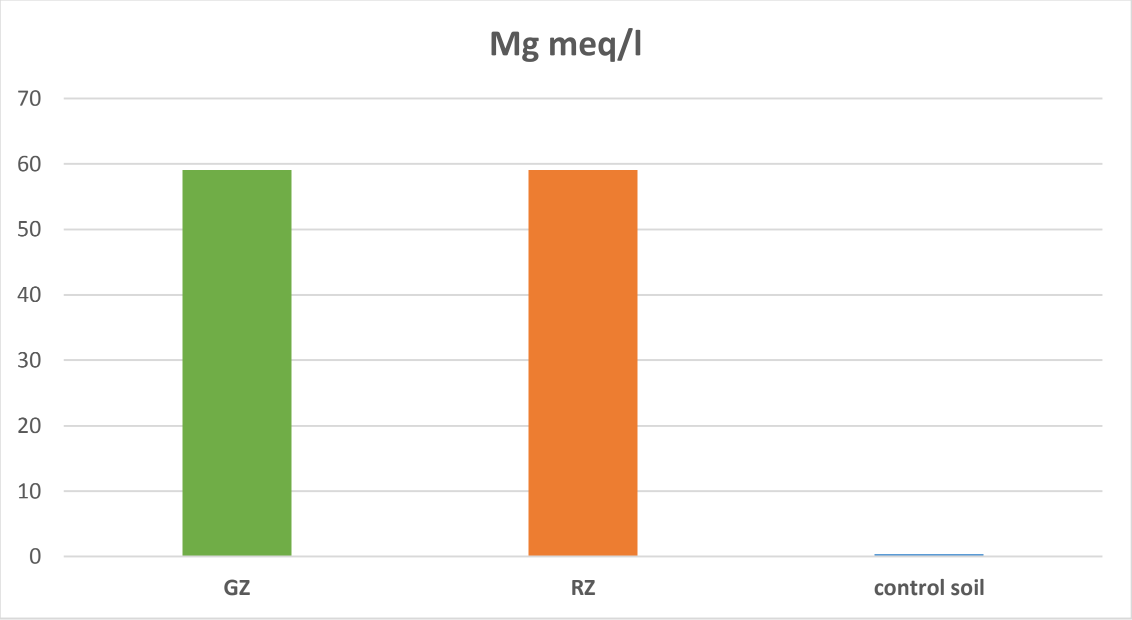

Figure 4.5: Concentration of magnesium (Mg^{+2}) in meq/l for the control soil, soil irrigated with OMW incubated with green tea and soil irrigated with OMW incubated with rooibos tea.

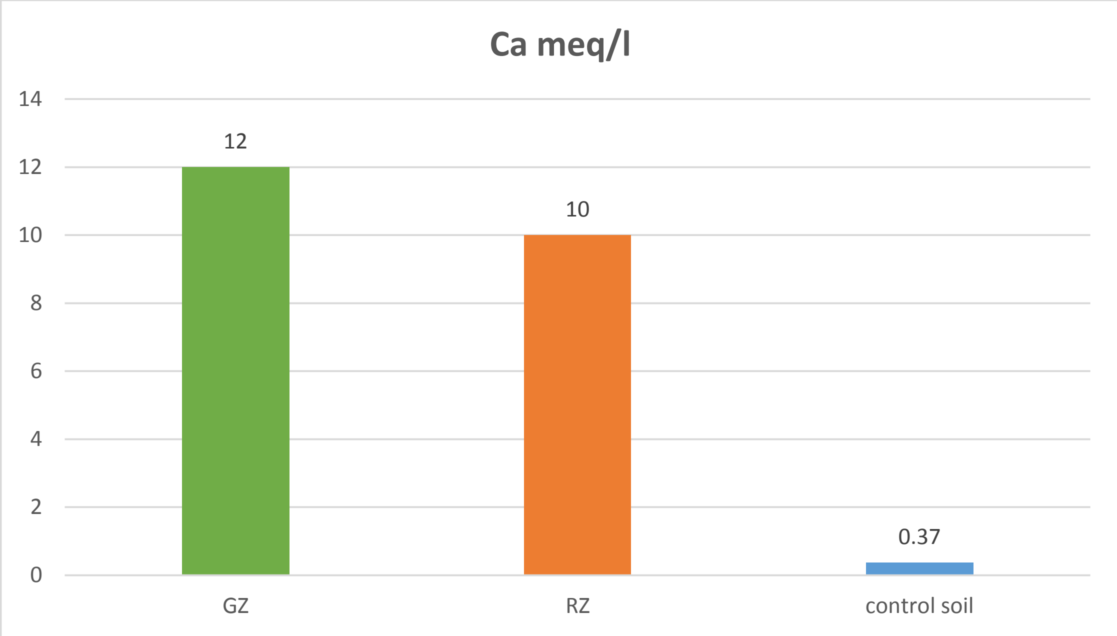

Figure 4.6: Concentration of calcium (Ca^{+2}) in meq/l for the control soil, soil irrigated with OMW incubated with green tea and soil irrigated with OMW incubated with rooibos tea.

4.3.1.6 Bicarbonate (HCO_3^-)

Bicarbonate in the control soil was (0.42 meq/l) and it showed a high increase after applying OMW to (20meq/l) in both Gz and Rz.

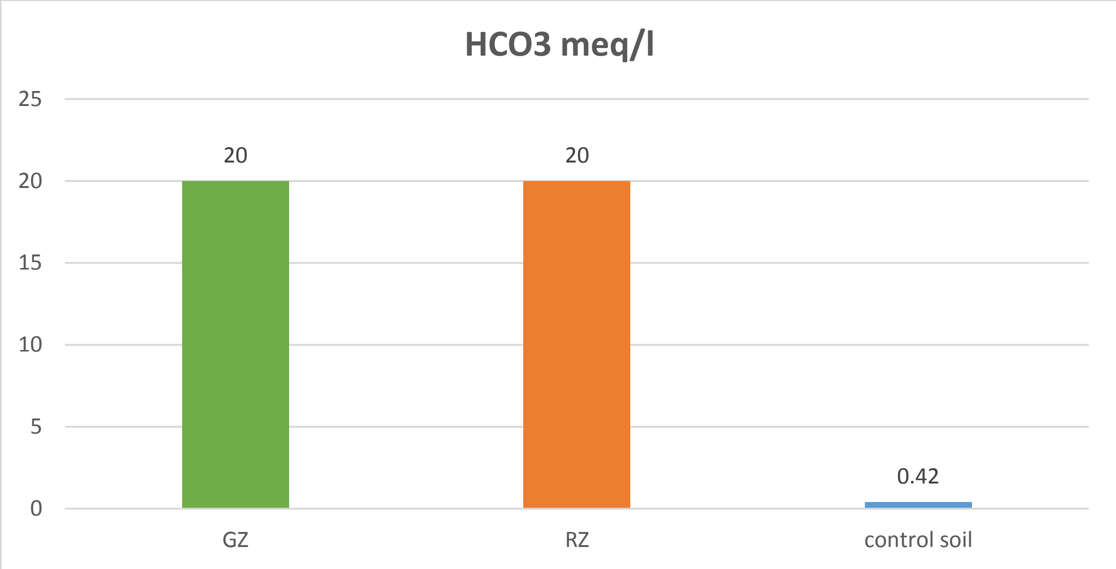

Figure 4.7: Concentration of Bicarbonate (HCO3-) in meq/l for the control soil, soil irrigated with OMW incubated with green tea and soil irrigated with OMW incubated with rooibos tea.

4.3.1.7 Total nitrogen bound and total organic carbon (TOC and TNb)

An increase in total Nitrogen bound (TNb) following irrigation with OMW was observed. The TNb increased from (4.1 mg/l) in the control soil to (39.45 mg/l) in Gz and to (48.97 mg/l) in Rz (Figure 4.9). The total organic carbon (TOC) also increased from (44.3) in control soil to (723) in Gz and to (913.4) in Rz (Figure 4.8).

OMW significantly increased the soil N. Several studies like Mekki et al. (2006) and Brunetti et al. (2007) showed an increase in the total Nitrogen bound (TNb) following the irrigation with OMW and this could have a beneficial effect on soil fertility.

However, Dakhli et al. (2013) found that after two years of application of OMW, the total N diminishes considerably with time, as nitrate formation increases due to the mineralization of organic nitrogen brought by OMW.

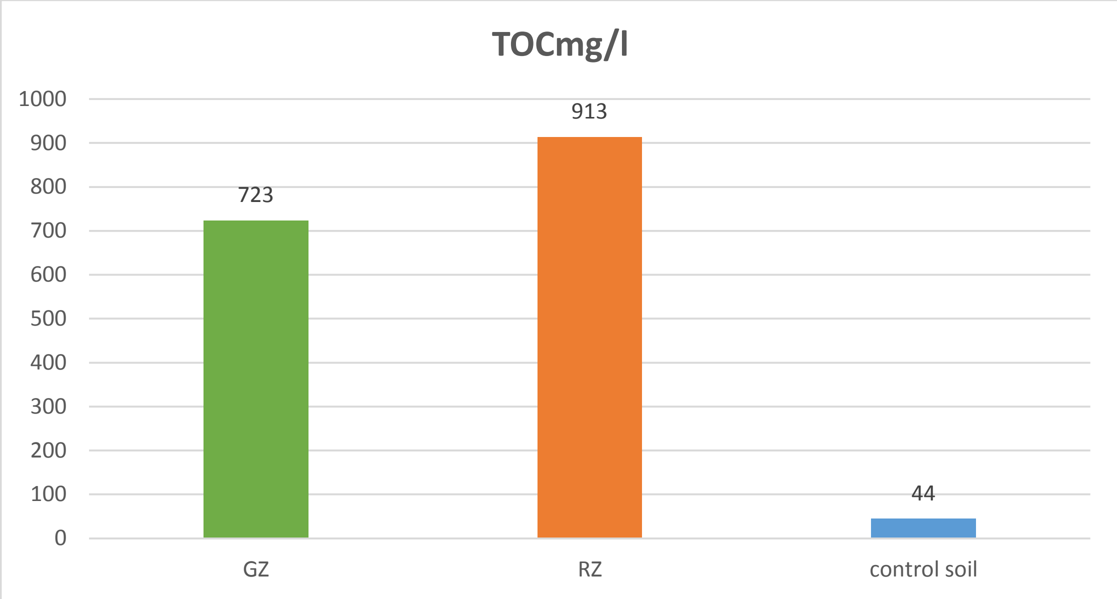

Figure 4.8: The total organic carbon in mg/l for the control soil, soil irrigated with OMW incubated with green tea and soil irrigated with OMW incubated with rooibos tea.

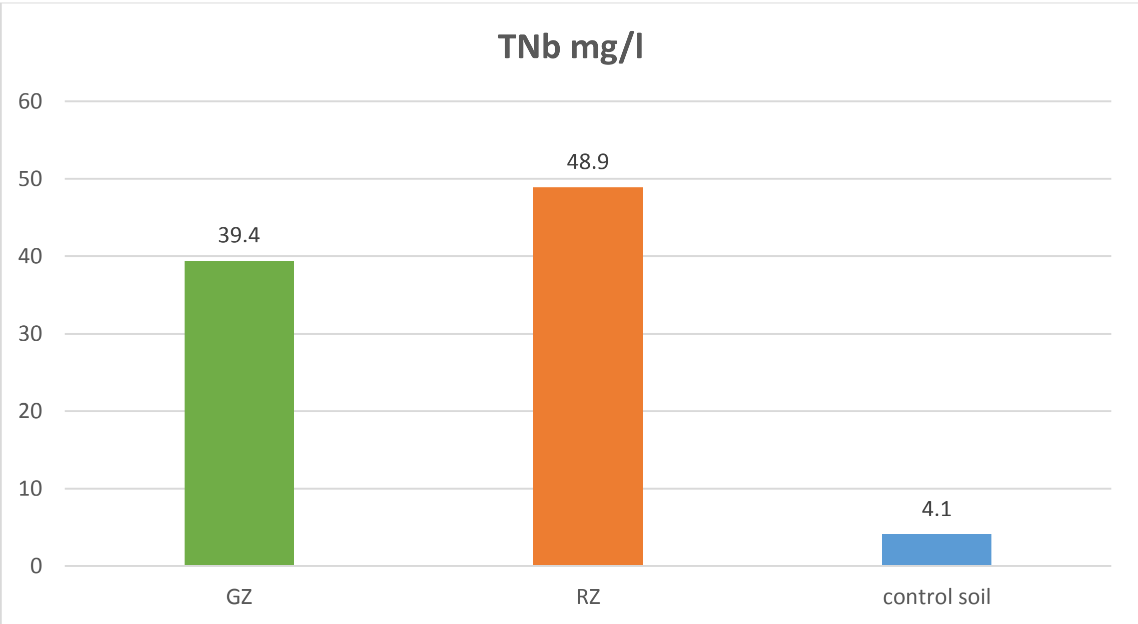

Figure 4.9: The total nitrogen bond in mg/l for the control soil, soil irrigated with OMW incubated with green tea and soil irrigated with OMW incubated with rooibos tea.

4.3.1.8 Organic Matter

Generally, OMW is known to increase soil organic matter and the concentrations of essential inorganic elements for plant growth resulting in enhanced soil fertility (Paredes et al., 1999).

This in agreement with the results obtained in this study, which show that the application of OMW significantly increased the OM in the soil with respect to the control soil, during the nine months of the experiment (Table 4.3). It increased from (6.08%) in the control soil to (40.6%) in Rz and to a higher extent in Gz to (73.1%) (Figure 4.10).

These results are consistent with Madejón et al. (2003), López-Piñeiro et al. (2002) and Montemurro et al. (2004) whom indicated that olive mill waste application raise soil organic C, and have a beneficial effect on soil fertility (Mechri et al., 2008).

However, the organic fraction of OMW is often hydrophobic (Mahmoud et al., 2010) consequently it is likely remains in the top soil. Under convenient environmental conditions, easily degradable carbon compounds are mineralized by microorganisms directly after application. Consequently the soil fertility and soil stability increases due to this mineralization (Magdich et al., 2013, Mekki et al., 2006a).This is important in semi-arid conditions where agricultural soils poor in OM, and are being subjected to acute processes of degradation. However, it should be mentioned that application of insufficiently stable OM may cause several negative effects on soil properties like increase in mineralization rate of native soil organic carbon, generation of anaerobic conditions and release of phytotoxic substances that may adversely affect plant growth.

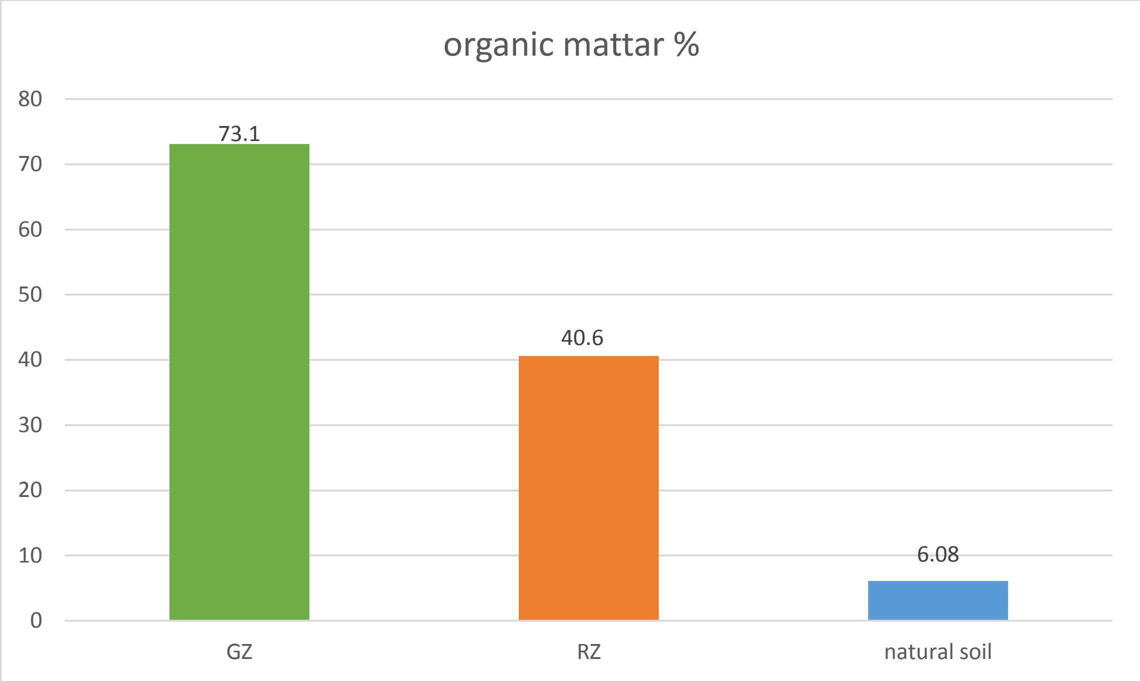

Figure 4.10: Organic matter for the conrol soil, soil irrigated with OMW incubated with green tea and soil irrigated with OMW incubated with rooibos tea.

4.3.2 Physical properties of soil irrigated with OMW

4.3.2.1 Soil moisture

The soil water content affects the decomposition rate of organic matter both indirectly and directly. Indirectly, a wet soil results in a slower break down because water fills the air spaces in the soil, and thus prevent the microbes of oxygen. However, all living organisms also need water too. Thus, soil that is too dry directly decreases organic matter decomposition, as the microorganisms found in soil cannot survive without water.

In this study, soil moisture before applying OMW was (1.24±0.38 %), and after the OMW was applied this percent increased to (2.79 %) in Gz and to (2.67 %) (Figure 4.11). Carer et al. (2010) reported that the moisture capacity of soil related soil particles had a significant effect on decomposition because of possible interactions between matrix potential and microbial activity.

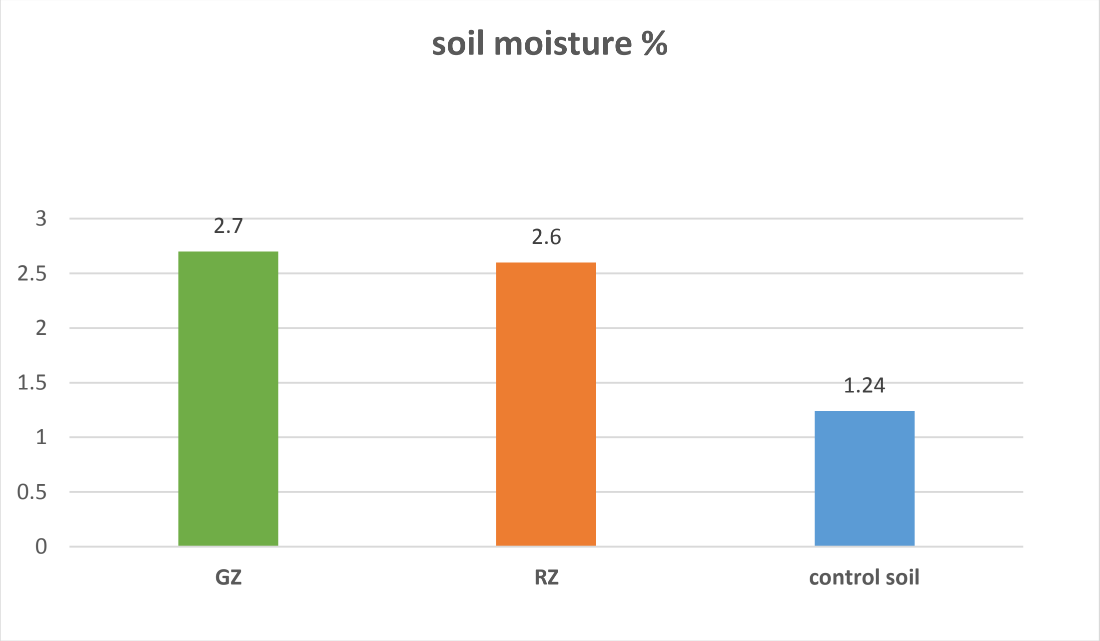

Figure 4.11: Soil moisture for the control soil, soil irrigated with OMW incubated with green tea and soil irrigated with OMW incubated with rooibos tea.

4.3.2.2 Soil type

The type of soil is one of the most important factors that have effects on decomposition of any organic material in soil after the structure of any decomposing organic matter. The Clay substratum is capable to hold most of the organic load and nutrients. Schimel et al. (1994) found that the clay has big impact on moisture-respiration relationships, and thus positively correlates with SOC. The higher the clay content, the lower the decomposition rates. This could, with caution, be translated into that higher clay content leads to higher stabilization and SOC levels. In the present study the soil texture characteristic is a clay loam (clay 25.5±9.2%, silt 46.4±6.8%, sand 28±8.9%). The clay content of soil after applying OMW is (17.9) in Gz and (10.8) in Rz, which is within the original soil value (25.5 ±9.2).

4.4 Tea bag index (TBI)

This method was derived from the standard TBI protocol. However, we designed the experiment so that we could have sub-replicates with teabags within each of the experimental plots.

A total of 60 tea bags used in the experiment (2 types of plant litter (green tea and rooibos tea) used * 2 types of irrigation used (fresh water and OMW) * 3 replicates of each type of litter * for 5 periods of time). Two types of irrigation system was applied: fresh water as a control and OMW as a source of pollution. All the tea bags were incubated for 14, 21, 90, 180 and 270 days. The tea bag initiative results are shown in (Table 4.4).

Also, the average relative remaining mass of the tea bags were calculated for each collection-time. The averages were then transformed through Excel as shown in (Table 4.5, Figure 4.12). The litter types differed significantly from each other. Rooibos tea was the most nutrient rich, for both TNB (913 mg/l) and TOC (48.9 mg/l), while values of green tea were lower with TOC (723mg/l) and TNB (39.4 mg/l). Also the EC, K^+, and to a low extent the pH of rooibos tea was higher than green tea. On the other hand the Cl^- and Ca^{+2} values of green tea was higher than that in rooibos tea. However, for Mg^{+2}, Na^+ and $HCO3^-$ the results were equally for both litter types, as presented in (Table 4.3).

Table 4.4: The accumulated results of the lab incubation experiment during the time periods (14, 21, 90, 180 and 270 days) over five intervals (n=5) from fall to summer seasons of 2017.

Sample ID	Initial weight green tea (w1) tea only	Initial weight red tea (w1) tea only	Final weight green tea (w2) tea only	Final weight red tea (w2) tea only	Fraction decomposed green tea (ag)	Predicted labile fraction red tea (ar)	Fraction remaining red tea (Wt)	Incubation time red and green tea (t)	S	K
14 days/ Fall season										
G1f-R1f	**1.6484**	**1.8299**	**1.2811**	**1.6501**	**0.223**	**0.146**	**0.902**	**14**	**0.735**	**0.079762**
G2f-R2f	**1.7248**	**1.8847**	**1.0459**	**1.6966**	**0.394**	**0.258**	**0.900**	**14**	**0.533**	**0.03493**
G3f-R3f	**1.586**	**1.8693**	**1.0584**	**1.6701**	**0.333**	**0.218**	**0.893**	**14**	**0.605**	**0.047905**
G4z-R4z	**1.6751**	**1.8948**	**1.2052**	**1.763**	**0.281**	**0.184**	**0.930**	**14**	**0.667**	**0.033942**
G5z-R5z	**1.7578**	**1.7764**	**1.3269**	**1.6648**	**0.245**	**0.161**	**0.937**	**14**	**0.709**	**0.035415**
G6z-R6z	**1.7014**	**1.8438**	**1.4784**	**1.7914**	**0.131**	**0.086**	**0.972**	**14**	**0.844**	**0.028685**
21 days/ Fall season										
G7f-R7f	**1.819**	**1.886**	**0.8626**	**1.6567**	**0.526**	**0.345**	**0.878**	**21**	**0.376**	**0.020713**
G8f-R8f	**1.7629**	**1.853**	**0.7894**	**1.6281**	**0.552**	**0.362**	**0.879**	**21**	**0.344**	**0.019446**
G9f-R9f	**1.7629**	**1.9117**	**0.9694**	**1.8073**	**0.450**	**0.295**	**0.945**	**21**	**0.465**	**0.009745**
G10z-R10z	**1.7606**	**1.8832**	**1.0943**	**1.6749**	**0.378**	**0.248**	**0.889**	**21**	**0.551**	**0.028108**
G11z-R11z	**1.8142**	**1.8619**	**1.2095**	**1.7194**	**0.333**	**0.219**	**0.923**	**21**	**0.604**	**0.020532**
G12z-R12z	**1.6741**	**1.8997**	**1.1327**	**1.6856**	**0.323**	**0.212**	**0.887**	**21**	**0.616**	**0.036114**
90 days/ Winter and Spring										
G13f-R13f	**1.713**	**1.774**	**0.634**	**1.563**	**0.630**	**0.413**	**0.881**	**90**	**0.252**	**0.003761**
G14f-R14f	**1.721**	**1.792**	**0.619**	**1.535**	**0.640**	**0.420**	**0.856**	**90**	**0.240**	**0.004653**
G15f-R15f	**1.797**	**1.880**	**0.677**	**1.564**	**0.623**	**0.409**	**0.832**	**90**	**0.260**	**0.005907**

G16z-R16z	**1.745**	**1.844**	**0.668**	**1.707**	**0.617**	**0.405**	**0.926**	**90**	**0.267**	**0.002239**
G17z-R17z	**1.698**	**1.888**	**0.707**	**1.857**	**0.584**	**0.383**	**0.983**	**90**	**0.307**	**0.000492**
G18z-R18z	**1.779**	**1.901**	**0.775**	**1.861**	**0.565**	**0.370**	**0.979**	**90**	**0.329**	**0.000644**
180 days/ Winter, Spring and Summer										
G19f-R19f	**1.706**	**1.844**	**0.495**	**1.6987**	**0.710**	**0.465**	**0.921**	**180**	**0.157**	**0.001028**
G20f-R20f	**1.739**	**1.821**	**0.531**	**1.3148**	**0.695**	**0.455**	**0.722**	**180**	**0.175**	**0.005243**
G21f-R21f	**1.701**	**1.798**	**0.485**	**1.4536**	**0.715**	**0.469**	**0.808**	**180**	**0.151**	**0.00292**
G22z-R22z	**1.704**	**1.789**	**0.213**	**1.587**	**0.875**	**0.574**	**0.887**	**180**	**-0.040**	**0.001219**
G23z-R23z	**1.872**	**1.798**	**0.441**	**1.7281**	**0.765**	**0.501**	**0.961**	**180**	**0.092**	**0.000449**
G24z-R24z	**1.681**	**1.738**	**0.622**	**1.5663**	**0.630**	**0.413**	**0.901**	**180**	**0.252**	**0.001521**
270 days/Fall, Winter, Spring and Summer										
G25f-R25f	**1.807**	**1.892**	**0.902**	**1.3968**	**0.501**	**0.328**	**0.738**	**270**	**0.405**	**0.005909**
G26f-R26f	**1.674**	**1.809**	**0.827**	**1.3986**	**0.506**	**0.332**	**0.773**	**270**	**0.399**	**0.004262**
G27f-R27f	**1.819**	**1.790**	**0.558**	**1.425**	**0.693**	**0.455**	**0.796**	**270**	**0.176**	**0.00220**
G28z-R28z	**1.743**	**1.871**	**0.927**	**1.176**	**0.468**	**0.307**	**0.629**	**270**	**0.444**	**0.00580**
G29z-R29z	**1.758**	**1.858**	**0.456**	**1.0639**	**0.740**	**0.485**	**0.573**	**270**	**0.121**	**0.007856**
G30z-R30z	**1.775**	**1.808**	**0.511**	**1.313**	**0.712**	**0.467**	**0.726**	**270**	**0.154**	**0.003269**

*calculations are based on the link: http://www.teatime4science.org/researchers/

*Where:

ag= 1-final weight green/initial weight green

ar =Hr*(1-S)

Wt =final weight red/initial weight red

S =1-(ag/Hg)

k =LN(ar/(Wt-(1-ar)))/t

*(G) Green Tea Bag, (R) Rooibos Tea Bag, (f) Fresh water, (z) OMW, (k) Decomposition Rate Constant, (S) Stabilization Factor.

*For example G10f-R10f: means sample of Green tea bag number 10 irrigated with fresh water and sample of Rooibos tea bag number 10 irrigated with fresh water.

4.4.1 Relative mass remaining

The overall litter mass loss was highly different between litter types (Table 4.5, Figure 4.12). The overall mass loss of green tea was higher than that in rooibos tea.

During the first two periods of the experiment (14 and 21 day) the tea bags experienced a rapid weight decline. After the 21 day period rooibos tea lost weight gradually slower. Rooibos tea seems to have hit a stabilized post-leaching phase after 21days with a relative mass remaining of 0.9 (in both Rf and Rz), and green tea after 90 day with relative mass remaining of ~0.39 in Gf and ~ 0.41 in OMW (Gz).

On the one hand, the mass loss of rooibos Rf tea was higher than that of Rz from the beginning of the experiment period and till the 220 day period. And the decomposition of Rz during the (21-220 day) period was very slow. But after the 220 day it starts to decompose faster than Rf and still till the end of the experiment with relative remaining mass (0.642).

On the other hand, during the 120 day of the experiment period, Gf experienced a weight loss more than that in Gz. past the 120 day and till the end of the experiment period, Gz starts to lose weight more than Gf.

Table 4.5: Relative mass remaining of rooibos and green tea as measured in laboratory incubations on temperate soil at 19.5°C for soil irrigated with fresh water and 21°C for soil irrigated with OMW. The tea bags were incubated in soil and retrieved after 14, 21, 90, 180 and 270 days of incubation (n = 5).

Time /day	Relative mass remaining (g/g)			
	Gf	**Gz**	**Rf**	**Rz**
14	**0.684**	**0.781**	**0.898**	**0.946**
21	**0.491**	**0.655**	**0.901**	**0.900**
90	**0.369**	**0.411**	**0.856**	**0.963**
180	**0.294**	**0.243**	**0.817**	**0.916**
270	**0.433**	**0.360**	**0.769**	**0.642**

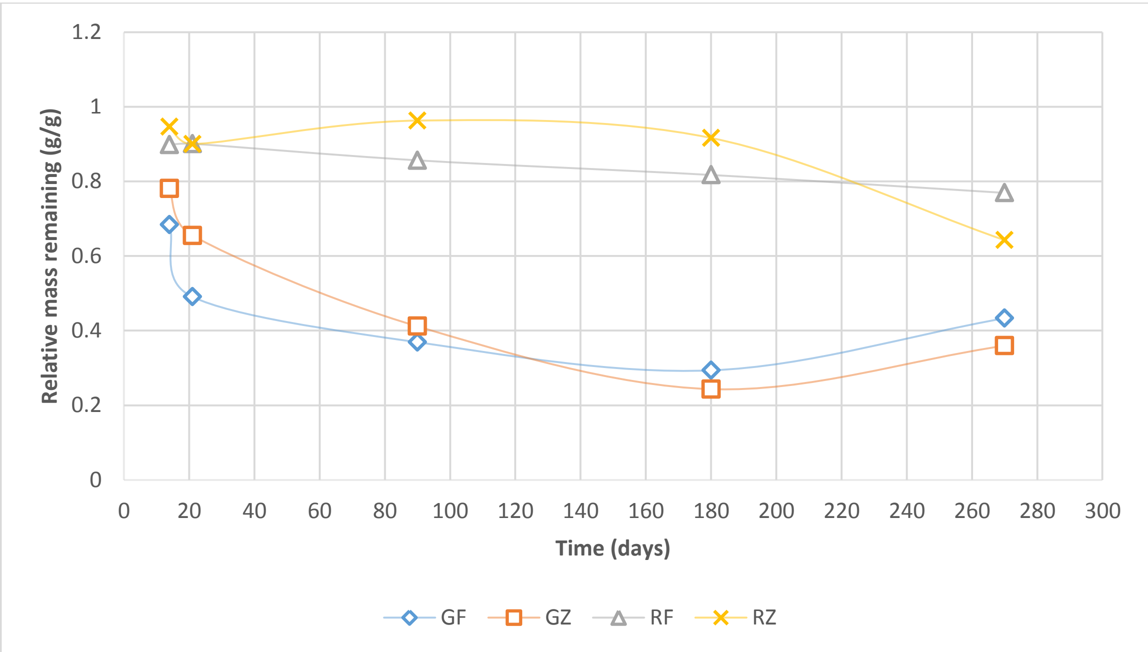

Figure 4.12: Relative mass remaining of rooibos and green tea as measured in laboratory incubations on temperate soil at 19.5°C for soil irrigated with fresh water and 21°C for soil irrigated with OMW. The tea bags were incubated in soil on top and retrieved after 14, 21, 90, 180 and 270 days of incubation (n = 5). Each data point is the mean of three (n = 3) replicates.

4.4.2 TBI parameters k and S

TBI values for decomposition rate (k_{TBI}) and stabilization factor (S) were conforming to general litter mass loss patterns (Table 4.4). The k_{TBI} of tea litters varied considerably between irrigation types. Generally, It ranged from (0.07976) to (0.00045). The maximum k_{TBI} for tea litters irrigated with fresh water was observed on (G10f-R10f) sample after two weeks (0.07976), however, for tea litters irrigated with OMW the maximum k_{TBI} was observed on (G11z-R11z) sample after 21 days (0.03611).

While the average k_{TBI} was (0.01375) in tea litters irrigated with OMW irrigation type, it was higher in tea litters irrigation with fresh water to (0.01656) (Table 4.5, Figure 4.13).

Table 4.6: Average decomposition rate constant (k) and average stabilization factor (S) after the 270 day of the experiment.

Sample ID	Gf-Rf	Gz-Rz
K	0.01656	0.01375
S	0.35153	0.39442

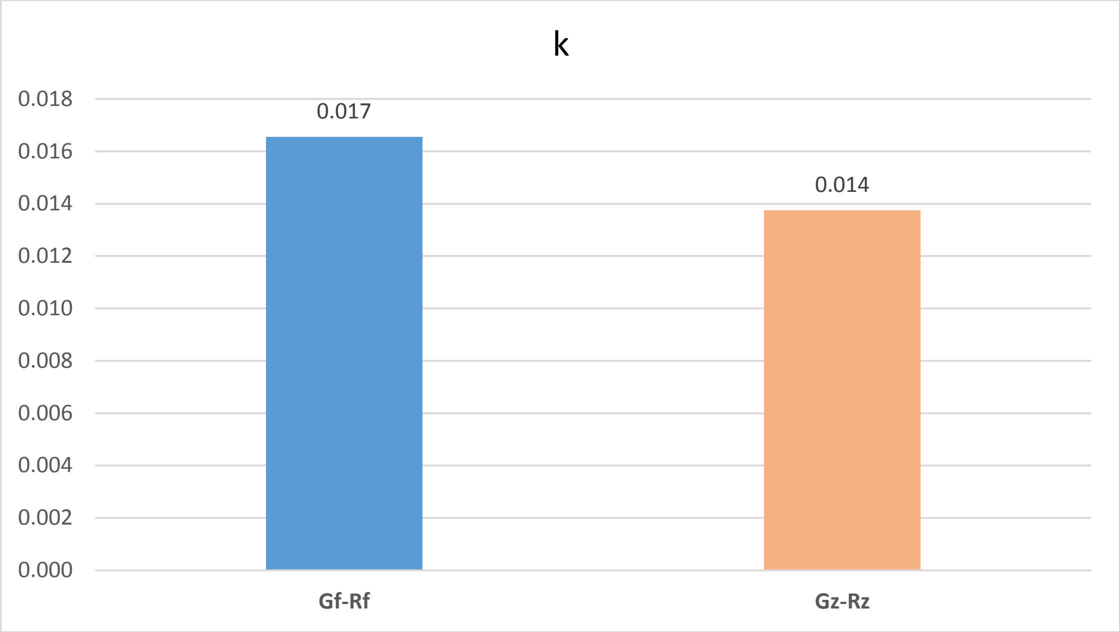

Figure 4.13: Tea Bag Index decomposition rates k in response to type of irrigation (fresh water, OMW).

The stabilization factor (S) ranged from (-0.039) to (0.844) in general. While the average S for tea litters in soil irrigated with fresh water was (0.735), it was higher in tea litters irrigated with OMW (0.39442) (Table 4.5, Figure 4.14).

High value of S indicate inhibition of decomposition and vice versa. the maximum stabilization factor S for fresh water was observed on G10f-R10f sample after two weeks (0.735), and for OMW, the maximum S value was observed on G5z-R5z sample after two weeks (0.844), both values was in the fall season (i.e. lower temperature decreases the decomposition).

The minimum S value for tea litters irrigated with fresh water was (0.151) in G20f-R20f sample during summer after 180 day. The minimum S value for tea litters irrigated with OMW was (-0.03958) in G21z-R21z sample also during summer after 180 day of the experiment period.

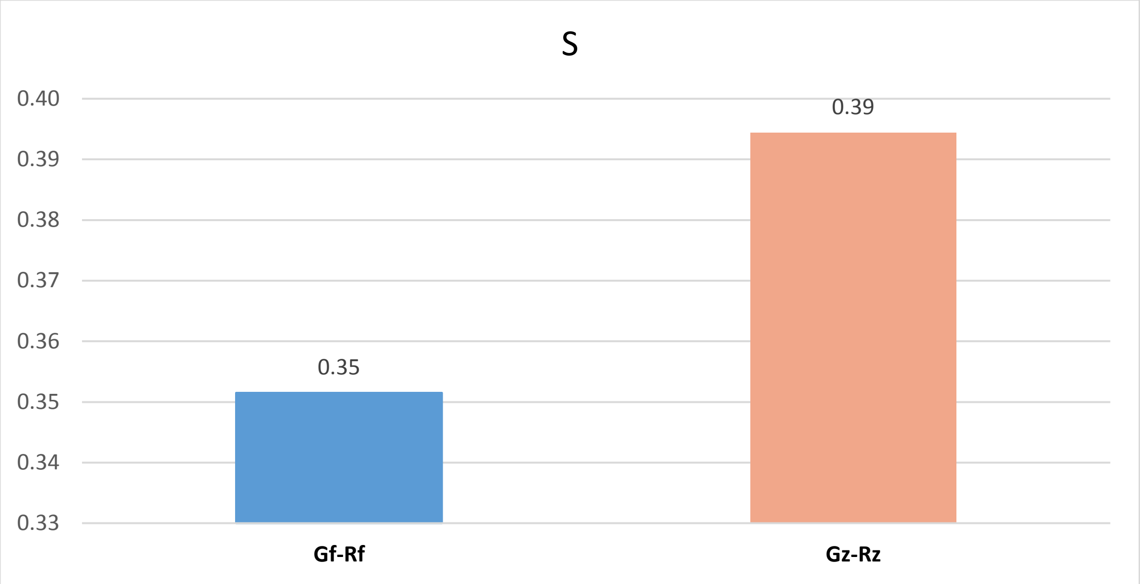

Figure 4.14: Tea Bag Index stabilization factor S in response to type of irrigation (fresh water, OMW).

4.5 Methodology discussion

The decomposition over the 270 day period (Figure 4.12) were consequences of the chemical composition of the litter types. the decomposition of green tea was faster than that of rooibos tea for the two types of irrigation (fresh water and OMW), however, for a large proportion of the incubation time, green tea had already reached a limit value, while the labile fraction of rooibos tea was still actively decomposing, this is on line with (Keuskamp et al,. 2013). This may because green tea contained high fraction of the easily decomposable water soluble compounds (Appendex2). For this reason, it decomposed quickly at first, however, the decomposition slowed down after the majority of the original water soluble compounds fraction was decomposed. Also green tea has a C/N-ratio of (~12), while the Rooibos has a C/N-ratio of (~42), so rooibos tea is more recalcitrant and decomposes slower, in this regard the microbial decomposition is easier when the C/N ratio decline and the material is more labile, thus the decomposition of green tea was faster than that of rooibos tea. On the other hand rooibos tea had a high amount of the more resistant N fraction (Figure 4.9), thus it decomposed slower over the entire 270 days period.

The observed increase in mass after 180 days of green tea decomposition (Gf and Gz) (Figure 4.12) was likely due to colonization by fungi, which was also observed in similar studies like (Didion et al., 2016; Li et al ., 2003).

In this regard, De Santo et al. (2009) suggested that the increase in ash content could be responsible for the mass increase. However, ash content was not analyzed, thus, this hypothesis could not be examined.

In general, during the 120 day for green tea and 220 day for rooibos tea, the weight loss of the tea bags irrigated with fresh water (i.e. Gf and Rf) was higher than that of tea bags irrigated with OMW (i.e. Gz and Rz). However, after these two periods and till the end of the experiment the weight loss of (Gz, Rz) was higher than that in (Gf, Rf).

When irrigation with OMW the decomposition decreased during the first part of the experiment, however it increased in the long term of the experiment. This may be related to the fats present in OMW, which can reduce oxygen availability for some time, which may decrease microbial activity, and hence decrease the decomposition rate constant k and the stabilization factor S increased. At the same time, OMW may have increased the amount of nutrients available for microbial communities, this may result in the breakdown of more recalcitrant material and thus increase the decomposition in the later stages for both tea types.

The average k_{TBI} for tea litters in soil irrigated with OMW was (0.01375), and was higher foe tea litters in soil irrigated with fresh water (0.01656).

Also, the maximum k_{TBI} for fresh water was observed after two weeks to be (0.07976) and it was higher than that of tea litters irrigated with OMW (0.03611) which was after 21 days. And since k indicate how fast the decomposition process is, this support the hypothesis that OMW delayed the decomposition of tea litter.

The stabilization factor (S) accounts for the transformation of some components of the tea bags from fast decomposing into slow decomposing molecules, and these components are said to be stabilized. In other words high value of S indicate inhibition of decomposition, and in this study the average S for tea litters irrigated with fresh water was lower than that of tea litters irrigated with OMW. And this in line with the previous hypothesis.

Chapter Five

Conclusion and Recommendations

5.1 Conclusion

This study provided attempt to further develop and apply a recent method, and to investigate its potential for improving our understanding of the decay process of plant litter. The results presented here give the evidence for the suitability of the tea bag approach using rooibos and green tea litters as standard litter to study the effect of applying OMW on litter decomposition.

After the application of OMW to the soil for 9 months, a positive and negative effects were observed on soil. Results indicated that the controlled application of OMW could increase soil fertility, since it contains high amounts of organic matter and macronutrients especially K; the total K fertilization with OMW resulted in more potassium than control. This also make OMW considered as a low cost fertilizer. Electrical conductivity in the soil remained below the salinization threshold therefore no impact was observed on the soil quality. However, long-term OMW application could affect the soil salinity.

On the other hand, OMW decreased the decomposition of tea in the first stages and increased it in the later stages, the decomposition rate constant (k) and stabilization factor (S) parameters also indicated that, since the k decreased when the soil irrigated with OMW than in the soil irrigated with fresh water, while S increased in the soil irrigated with OMW than the soil irrigated with fresh water.

Although the experiment presented here, is limited by the laboratory, controlled conditions adopted (i.e. same temperature and precipitation and absence of soil fauna), they may be appropriate for estimating the temporary response of soil to an applied perturbation. Furthermore, these investigations may be useful guidelines for further studies to extrapolate data to control status.

5.2 Recommendations

In order to understand the long-term effects of OMW on soil properties and decomposition of plant litter longer experiments under different soil conditions are still needed. It should also be evaluated if the results derived from the TBI method are comparable with other methods used to estimate S and k in long-term field experiments.

The overall results from this study showed that the TBI is a useful approach to investigate the effect of OMW on SOC dynamics. However, further research is needed where each parameter is investigated in more detail, and where all potential factors influencing S and k are included. It should also be evaluated if the results derived from the TBI approach are comparable with other approaches used to estimate stabilization and decomposition rates in long-term field experiments.

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5.4 Appendixes

Appendix I – TBI protocol

As described in Keuskamp et al. (2013).

1. Use one bag of Lipton Green tea (EAN: 87 22700 05552 5) and one Lipton Rooibos tea (EAN: 87 22700 18843 8) per replicate. To obtain better estimates of TBI, bury more replicates per site.

2. Measure the initial weight of the tea bag and subtract the weight of an empty bag (see also Table 1) to determine the initial weight of the tea.

3. Mark the tea bags on the white side of the label with a permanent black marker.

4. Bury the tea bags in 8-cm deep, separate holes while keeping the labels visible above the soil and mark the burial site with a stick.

5. Note the date of burial, geographical position, ecotype and experimental conditions of the site.

6. Recover the tea bags after c. 90 days.

7. Remove adhered soil particles and dry in a stove for 48 h at 70°C (not warmer!).

8. Remove what is left of the label but leave the string, weigh the bags and subtract the weight of an empty bag without the label to determine the weight after incubation. To get a more precise estimation, open the bag and weigh its content; combust the content at 550°C and subtract what is left from the content weight.

9. Calculate stabilization factor S and decomposition rate k using eqn 1b.

10. More (facultative) instructions and tips on how to incorporate the TBI in scientific experiments can be found on our website: http://www.decolab.org/tbi.

Appendix II

Table 1. Results from ANOVAS of quality parameters and weights of four batches of green tea and rooibos tea with different production numbers ($N = 3$). Asterisks denote significance levels (*P<0·05, **P<0·01, ***P<0·001)

	Green tea			Rooibos tea		
	Mean ± SD	F(3,8)	P	Mean± SD	F(3,8)	P
Nonpolar extractable fraction ($g\ g^{-1}$)	0·066 ± 0·003	5·062	0·030*	0·049 ± 0·013	12·950	0·002 **
Water soluble fraction ($g\ g^{-1}$)	0·493 ± 0·021	0·975	0·451	0·215 ± 0·009	0·418	0·745
Acid soluble fraction ($g\ g^{-1}$)	0·283 ± 0·017	0·625	0·618	0·289 ± 0·040	2·149	0·172
Acid insoluble fraction ($g\ g^{-1}$)	0·156 ± 0·009	0·356	0·787	0·444 ± 0·040	1·166	0·381
Mineral fraction ($g\ g^{-1}$)	0·002 ± 0·0009	7·084	0·012*	0·004 ± 0·0006	3·158	0·086
Hydrolysable fraction (H) ($g\ g^{-1}$)	0·842 ± 0·023	0·295	0·828	0·552 ± 0·050	1·189	0·374
Total carbon (%)	49·055 ± 0·109	0·243	0·864	50·511 ± 0·286	2·769	0·111
Total nitrogen (%)	4·019 ± 0·049	0·151	0·926	1·185 ± 0·048	0·727	0·564
C : N ratio	12·229 ± 0·129	0·145	0·930	42·870 ± 1·841	0·774	0·541
Total tea bag weight (g)	2·019 ± 0·026	1·260	0·351	2·152 ± 0·013	0·848	0·506
Empty bag weight (g)	0·246 ± 0·001	2·058	0·184	0·245 ± 0·001	0·487	0·701