An-Najah National University

Faculty of Graduate Studies

Phytoremediation of organics and metals from Olive Mill Wastewater

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DEDICATION

This work is dedicated to My parents for their love, guidance, endless support and extraordinary encouragement.

To my wife, for her empowerment driving force, care, and

unconditional support.

To my sons: Abed el-Ruhman, A’mer and Soliman and my daughter: Wae’d with love.

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الاقرار

أنا الموقع أدناه مقدم الرسالة التي تحت عنوان:

Phytoremediation of organics and metals from olive Mill Wastewater

أقر بأن ما اشتملت عليه هذه الرسالة إنما هي من نتاج جهدي الخاص باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل ، أو أي جزء منها لم يقدم من قبل أية درجة عليمة أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced is my research own work, and has not been submitted elsewhere for any other degree or qualification.

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Phytoremediation of organics and metals from olive Mill Wastewater
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Abstract

The cultivation and processing of olives for olive oil production are among the most important industries in Palestine. The olive oil extraction process produces huge amounts of liquid waste called Olive Mill Wastewater (OMWW). Disposal of OMWW is a major environmental issue. In Palestine OMWW is being disposed of into the wadies. The present work aimed at studying the possibility of using the phytoremediation to remove polyphenol and heavy metals (Fe, Cu, Zn, and Ni) from OMWW.

Four groups of pots were cultivated by barley (*Hordeum vulgare L*) seeds in similar way like seeding in fields (approximately one seed per cm) and watered by OMWW (Zebar 100%), diluted OMWW (Zebar 50%), fresh water (FW) and prepared solution (SL) that has the same concentration of polyphenol and heavy metals (Fe, Cu, Zn, and Ni) in OMWW.

The concentration of polyphenol and heavy metals in OMWW and the soil before planting was measured. A week after germination, one pot from each group were taken, the soil were separated from the roots of barley and mixed to take a sample for analysis to measure the concentration of polyphenol and metals, these analyses were repeated for the rest of the pots

weekly. Flame atomic absorption was used for metals analysis. Total phenol content of each OMWW samples was analyzed according to Folin-Ciocalteau colorimetry method.

Eight days after planting, the seeds were germinated in three groups of pots (Zebar 100%, Zebar 50%, FW), while the fourth (SL) was not germinated at all. In the first group (Zebar 100%), the barely plants were shorter than those irrigated with diluted OMWW and fresh water, and the number of plants was less.

It is noticed that the concentration of polyphenol, Fe and Zn decreased during the experiment, while there were no significant reduction in concentrations of Cu and Ni.

The mass of polyphenol and Fe absorbed by barley plants were the most significant. Uptake ratio of polyphenol was 0.19 in the pots which were irrigated with Zebar 100% at the end of experiment. While it was 0.31 and 0.26 in the samples which were irrigated with Zebar 50% and fresh water respectively. It is noticed that the absorption ratio of Fe was the most significant among the other metals, it was 0.27, 0.42 and 0.49 in samples which were irrigated with Zebar 100%, Zebar 50% and fresh water respectively.

The uptake of polyphenol and metals (Fe and Zn) follow first order reaction with k value (day^{-1}) 0.006, 0.012 and 0.003 for polyphenol, Fe and

Zn respectively, and high R^2 value (nearly 0.98) in the pots which were irrigated with diluted OMWW (Zebar 50%).

CHAPTER 1

Introduction

1.1 General introduction

The cultivation and processing of olives for olive oil production are among the most important industries in Mediterranean countries. Olive production is the backbone of Palestinian agriculture. Olive farms covers almost half of the cultivated area in The West Bank, and oil production contributes by around 28.7% of the agriculture domestic income (El-Khatib, 2009). But on the other hand, the olive oil extraction process produces huge amounts of liquid waste called Olive Mill Wastewater (OMWW); known in Palestine as Zibar. OMWW is disposed off during the olive season extending for few months from October to December.

OMWW is a mixture of vegetation water and soft tissues of the olive fruit and the added water used in the various stages of the oil extraction process. Typical OMWW composition by weight is 83-94% water, 4-16% organic compounds and 0.4-2.5% inorganic compounds (mineral salts) (Davies et al., 2004).

The annual OMWW production of the Mediterranean olive growing countries is estimated to amounts ranging from 7 to over 30 million m3 (Niaounakis et al., 2004). Olive mills in the West Bank generate about 0.2 Million m3/year of OMWW (Subuh, 1999).

Disposal of olive wastes from olive mills is already a major environmental issue in several olive growing countries in the world. Wastewater from the different olive-mills located in and around the different villages in Palestine is being disposed of into the wadies. There, it is mixed with the untreated flowing municipal wastewater or with rainwater. The resulting high organic polluted wastewater affects the soil and water receiving bodies (Shaheen, 2007).

OMWW contain an enormous supply of organic matter very rich in phenolic compounds, which are toxic in addition very low quantity of heavy metals. These organic contaminants are harmful to nature, human being, and animals and therefore must be removed before disposal of OMWW to any water receiving body.

There are several treatment processes and technologies employed to reduce the negative environmental impact of OMWW. The efficiency, feasibility and sustainability of the treatment processes to remove organic matters must be taken into consideration when making a decision on the most suitable treatment of OMWW (Adham, 2012). All the traditional methods that were used until now to remove the organic contaminants from OMWW were inefficient.

In this research barley was used as the plant to adsorb and remove the organic matters and heavy metals from the OMWW. The aim was to study the efficiency and effectiveness of barley as the media for applying phytoremediation for OMWW.

1.2 Objective of Study

Reducing the negative environmental impact of OMWW is of great importance to protect the biophysical environment. The use of phytoremediation for removing heavy metals and polyphenol from OMWW was evaluated. Specifically, the following tasks were among the objectives and questions to be analyzed and answered:

- The use of barley as phytoremediation to remove organic compounds and heavy metals from OMWW.
- Prepare a solution that have same contents of OMWW (Zibar) and investigate if other materials in the Zibar will affect the phytoremediation.

CHAPTER 2

Literature Review

2.1 Olive oil production

Annually, approximately 1.8x106 tons of olive oil is produced worldwide, with a majority being produced in the Mediterranean basin (Paredson, 1999), (Tamburino, 1999). Olive oil production is the most important contribution to economic income in Palestine. According to the Palestinian international information, 2005 olive farms covers almost half of the cultivated area in The West Bank, and oil production contributes by around 28.7% of the agriculture domestic income, there are about 246 olive mills in the West Bank (El-Khatib, 2009).

The annual average production of olive fruits and olive oil reaches 120 and 24 thousand tons respectively. More than 200 olive mills are functioning in the West Bank generating about 200 thousand m3 per year of OMW (Subuh, 1999). According to the Palestinian Central Bureau of Statistics the total quantity of pressed olives in 2013 was 65,829.4 tons, the quantity of oil extracted was 17,143.9 tons in 2013, and there were 299 olive presses in Palestine (PCBS, 2013).

2.2 Pressing system

Three types of oil extraction processes applied in Palestine. These types are: the traditional oil extraction, two-phase system, and three-phase system.

2.2.1 Traditional method

It is based on pressing, generates one stream of olive oil and two streams of wastes, the Zibar and olive cake (Shaheen, 2004). Water and oil flow on the sides of the olive pulp piles thus separating from the solid part. Water and oil are later separated by centrifugation (MedPan, 2007).

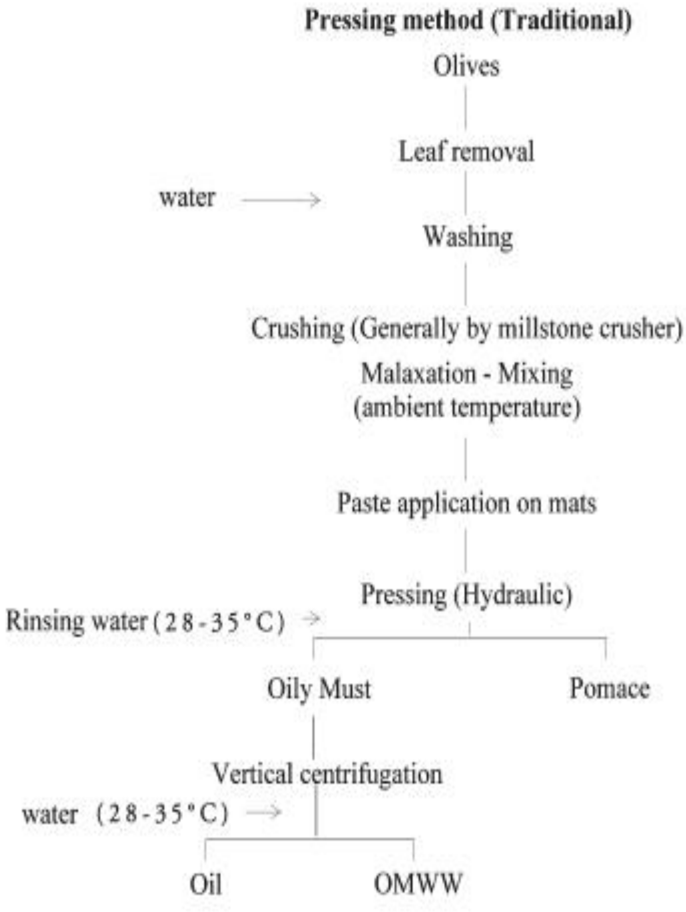

Figure (1): Extraction by the traditional pressing method (Petrakis, 2006).

2.2.2 centrifugation method

This separation method is based on the principle that any combination of immiscible liquids with differing densities tends to split up spontaneously into its individual constituents. The reason is that the natural force of gravity affects liquids differently, depending on the density (Petrakis, 2006). The continuous centrifugation involves the steps of: leaf removal and washing, crushing of the olives, malaxing the olive paste, and centrifuging with or without water addition according to the “three-phase” or “two-phase” mode, respectively.

1. Two-phase system

In this case, no water is added, while oil and solid wastes of high moisture (approximately 65 %) are produced, due to a more effective centrifugal system (MedPan, 2007). In addition, energy consumption is reduced and water utilization in the olive mill decreases considerably (Niaounakis et al., 2004).

According to Ranalli, 1996 the tow-phase centrifugal extractor renders better qualitative characteristics in the oil. Also, it has been reported that the best method of oil extraction from olive crops is the two-phase separation, as it is more cost efficient and utilizes fewer amounts of water and electrical energy, while the produced oil has, in general, better qualitative characteristics and higher oxidant stability. Finally, the yield of the two-phase oil-mills is high, as they convert the 83.3% of olive paste and the 3.6% of the high moisture solid waste into oil (MedPan, 2007).

2. Three-phase system

This method takes advantage of the difference in the specific gravity between water and oil. By centrifuging the olive paste and the addition of hot water, three final exit products are received: olive oil, liquid wastes (from the olive juice and the added water) and solid wastes (the core and pulp of the olive crop) (MedPan, 2007).

Disadvantages of this process include increased amounts of wastewater that is produced due to increased water utilization (1.25 to 1.75 times more water than press extraction), loss of valuable components (e.g. natural tioxidants) in the water phase, and problems of disposal of the Oil Mill Waste Water (Petrakis, 2006).

Table (1)summarizes the main advantages and disadvantages between the two basic centrifugal systems used in olive oil extraction

Table (1) : Advantages and disadvantages of the two basic olive oil extraction methods.

Method	Advantages	Disadvantages
Three-phase system	Relatively dry waste, convenient control of oil extraction, continuous flow.	Large quantities of water and energy (for warming the water), phenols are lost through wastes, large amount of liquid wastes, requires two vertical centrifuges.
Two-phase system	Small amount of liquid wastes, more polyphenols in oil, less required water, continuous flow, only one vertical centrifuge.	Dry waste of high moisture, difficulties in assessment of extraction effectiveness.

Source: (MedPan, 2007).

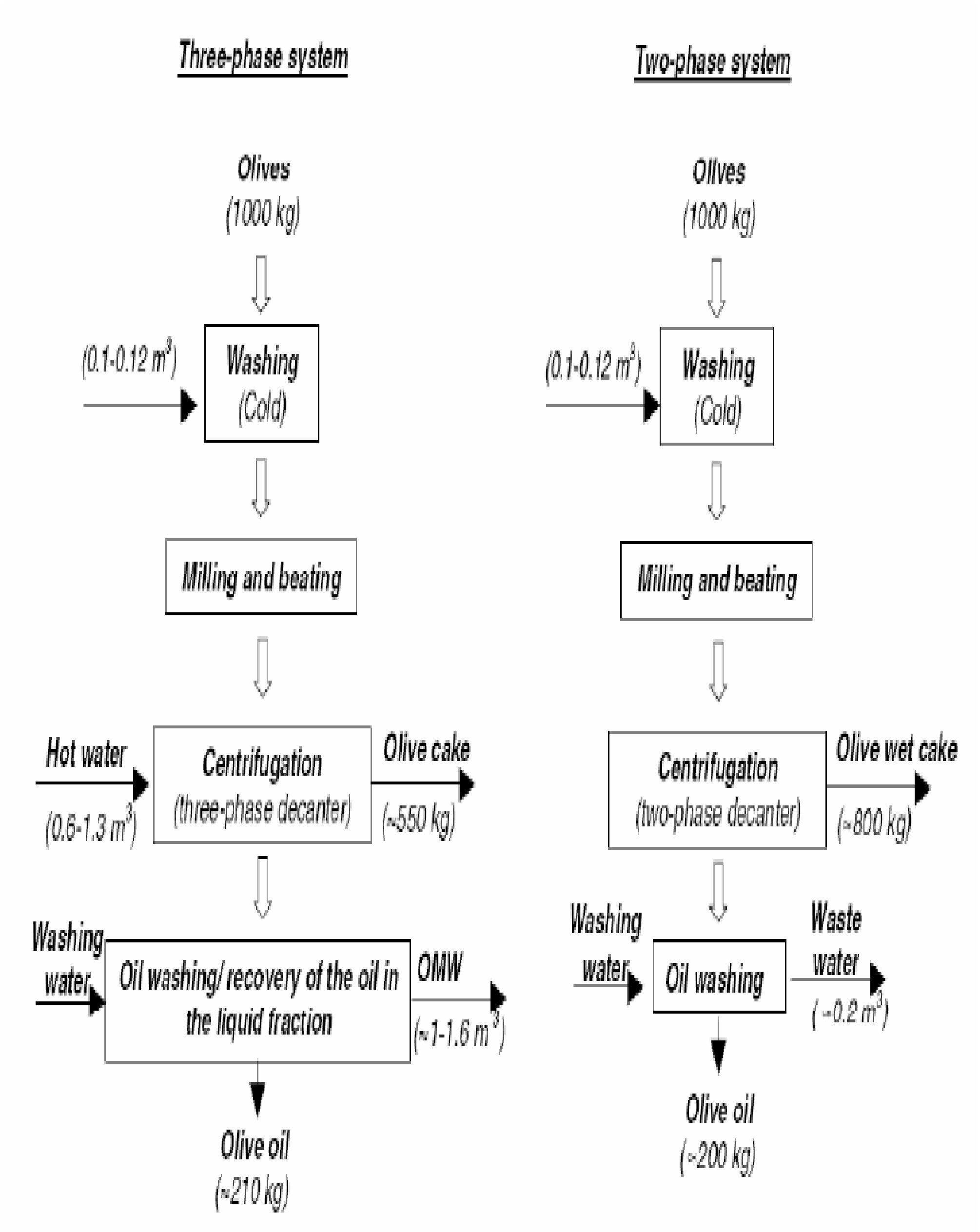

Figure (2): Flow diagram of the three and two-phase centrifugation systems (MedPan, 2007).

2.3 OMWW characteristics

OMWW is a mixture of vegetation water and soft tissues of the olive fruit and the added water used in the various stages of the oil extraction process. Typical OMWW composition by weight is 83-94% water, 4-16% organic compounds and 0.4-2.5% inorganic compounds (mineral salts) (Davies et al., 2004). The organic load in OMW is considered one of the highest of all concentrated effluents, being 100-150 times higher than the organic load of domestic wastewater. In general, OMW produced in discontinuous mills contains higher organic load than those generated in continuous mills (El-Khatib, 2009).

The characteristics of OMWW in terms of its quantity and quality are highly dependent on the extraction process (Shaheen, 2007). The characteristics of OMWW is rather variable depending on crop, variety of fruit and in particular on the technological system used for oil extraction (press, centrifugation or filtration) (Lopez and Ramos-Cormenzana, 1996).

The olive mill liquid wastes have the following characteristics : Dark brown to black color, strong odor of olive oil, high organic load (COD up to 220 g/L), pH 3-6, high electrical conductance, high polyphenol concentration (0.5 - 24 g/L), large concentration of suspending particles (MedPan, 2007). Table (2) show the general characteristics of OMWW which has a wide range in terms of the concentration of components.

Table (2): General characteristics of OMWW

Parameter	Unit	Value	Parameter	Unit	Value
COD (total)	mg/l	40000 - 220000	Potassium	mg/l	2800 – 11600
COD (soluble)	mg/l	32000 – 176000	Polyphenols	mg/l	500 – 80000
BOD5	mg/l	23000 – 100000	Carbohydrates	mg/l	3000 – 24000
pH	mg/l	3.0 – 5.9	Oil Content	mg/l	1000 – 23000
Alkalinity as CaCO3 (total)	mg/l	1170	Total Solids	mg/l	30600 – 58200
Organic Nitrogen	mg/l	154 – 1106	Total Volatile Solids **	mg/l	21300 – 45900
Phosphorous	mg/l	100 – 900	Total Suspended Solids **	mg/l	1400 – 3600
Sodium	mg/l	100 – 500	Total Bacteria *	106 col/ml	5
Magnesium	mg/l	200 – 900	Total yeasts and fungi *	106 col/ml	5
Calcium	mg/l	100 - 700			

Source: (Naser et al., 2007; * Gonzalez-lopez et.al., 1994; **Esra et al., 2001).

All the above parameters must be taken into consideration in the design of a well integrated treatment process of OMWW.

according to Aladham, Ruba the characteristic of OMWW in the West Bank as in the table below.

Table (3): General characteristics of OMWW in the Northern West Bank

Parameter	Unit	Jenin	Nablus	Tulkarem	Safit	Qalqilya	Average
BOD_5	mg/l	8830	8755	13698	12580	13010	11375
COD	mg/l	136750	130625	145000	136750	138500	137525
Total Phenol	mg/l	5276	4032.4	6232.7	3179.1	4239.7	4592.0
TS	mg/l	73970	46250	87800	62450	66920	67478
TSS	mg/l	58070	38150	68600	45680	49570	52014
TDS	mg/l	15900	8100	19200	16770	17350	15464
pH		4.8	4.9	4.9	4.6	4.9	4.8

Source: Aladham, Ruba , 2012

2.3.1 Organic compounds

The olive fruit is very rich in phenolic compounds, but only 2% of the total phenolic content of the olive fruit passes in the oil phase, while the remaining amount is lost in the OMWW (approx. 53%) and in the pomace (approx. 45%) (Deep et al, 2012).

OMWW contains an enormous supply of organic matter very rich in phenolic compounds, which are toxic. Phenolic compounds are divided into low-molecular weight (caffeic acid tyrosol, hydroxytyrosol, p-cumaric acid, ferulic acid,syringic acid, protocatechuic acid etc.) and high molecular weight compounds (tannins, anthocianins, etc.) (Davies et al., 2004).

Large fraction of polyphenols is lost in OMWW, in the range from 0.5 to 2.4 g/l (Sorlini et al., 1986). The olive oil extraction process leads to partition of the olive fruit phenolic content into two main groups; the group of polyphenols that posses beneficial antioxidant effect and which ends up in the olive oil, and the other group of polyphenols that has higher affinity to the aqueous phase and attributed to antimicrobial and phytotoxic effect of OMWW (Davies et al., 2004). The chemical structures of the seven pure compounds are reported in Figure (3) (Deep et al. 2012).

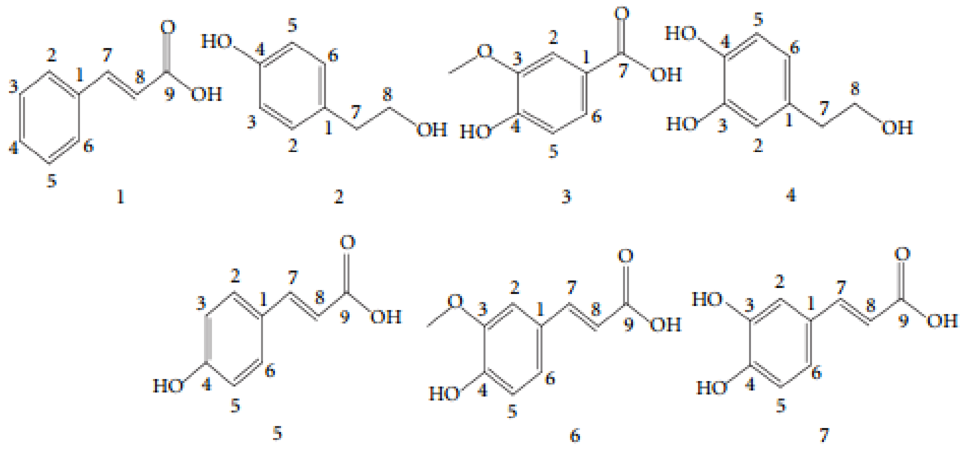

Figure (3): Structures of the phenolic compounds (Deep, et. al. 2012).

2.3.2 Inorganic compounds

OMWW contains very low quantity of heavy metals and regular supply of $50m^3$/ha/annum provides 30 to 100 times less of heavy metals than the limits allowed by the EU standards for the environment (Naija et al, 2014).

The amount of heavy metals naturally present in the OMWW, as researched by Fatih Vuran and Mustafa Demir: Zn was the predominant metal (3.907 ppm) followed in decreasing order by Cu (1.376 ppm), Mn

(1.359 ppm), Ni (0.545 ppm), Pb (0.180 ppm), Co (0.075 ppm), and Cd (0.036 ppm).

The below table show the mineral composition of OMWW according to Naija et al., 2014 study.

Table (4): mineral composition of OMW by extraction process.

Characteristic of the OMWW	Classic oil factory (kg/m^3)	Continued oil factory (kg/m^3)
P	1.1	0.3
K	7.2	2.7
Ca	0.7	0.2
Na	0.9	0.3
Fe	0.07	0.02
CO_3	3.7	1.0
SO_3	0.4	0.15
Cl_2	0.3	0.1
SiO_2	0.05	0.02

Source: (Naija et al., 2014)

2.4 Environmental impact of OMWW

The environmental impact of olive mill wastewater (OMWW) has been addressed by many studies. The effect on the environment is negative, leading to a saturation of the soil, causing pollution of superficial groundwater and of the water table itself. This unfavorable effect of OMWW on the environment is exacerbated by its acidity and high phenol content (El-Hajjouji et al., 2007). The effects can be summarized as follows:

2.4.1 Antimicrobial effect

One of the most important characteristics of the olive mill liquid waste is its antimicrobial activity. This activity has been described and attributed mainly to its phenolic content (Medpan, 2007).

2.4.2 Genotoxicity

phenols exert other toxic and genotoxic effects on animal and human cells For example, the exposure of Syrian hamster embryo cells to phenol and catechol induced cell transformation, gene mutations, unscheduled DNA synthesis, chromosomal aberrations and sister chromatid exchange (El-Hajjouji et al, 2007).

2.4.3 Phytotoxicity

It has been reported that the OMWWs inhibit seed vegetation and plant growth (Della Greca et al., 2001). This phytotoxicity has been attributed to their phenolic content as well as to some organic acids, such as acetic, produced during storage (Medpan, 2007).

2.4.4 Effect on soil

Doula et al, (2009) concluded that disposal of untreated OMWW at evaporation lagoons without using protective materials (e.g. impermeable membranes) has significant effect on soil chemical properties.

On the other hands Mohawesh et al., (2014) concluded that although OMW application affected relative total porosity, the pore space available, for nutrient exchange processes, for instance, was less. Even though OMW could cause soil and water pollution, its use in agriculture is promoted because of the high content of plant nutrients, such as N, P, and K and OM.

2.5 Treatment of OMWW

The treatment of OMWW is extremely difficult due to its large volume and the high concentration of organic matter. The major factor of the environmental problems imposed by the OMWW is the high concentration of polyphenols. These compounds are difficult to decompose and present phytotoxicity, toxicity against aquatic organisms, or suppression of soil microorganisms (Deeb et al., 2012).

The great variety of pollution components found in OMWW requires

different technologies to eliminate them (shaheen, 2007). There are several treatment methods and processes to reduce the impact of OMWW.

Many researchers have studied and discussed methods of Olive mill wastewater treatment.

According to shaheen, 2007 the modification of oil-extraction process using the ecological 2-phase decanters in combination with forced evaporation are concluded as the most appropriate management and treatment option for OMWW in Palestine.

Jodeh, 2014 concluded that the P.I.A (polyItaconic acid) polymer in basic medium was effective for phenol adsorption from OMWW more than in neutral medium which lead to a higher percent of phenol removal ~ 50% occurs when the amount of dosage is 0.1g with phenol concentration of 50 mg/L.

Khatib, 2009, showed that 84% of COD removal was achieved using UASB (upper flow anaerobic sludge blanket reactor) technology under the specified parameters. This COD removal makes the OMWW within the OMWW disposal standards.

In practice, the most common elimination method is through evaporation in storage ponds in the open because of the low investment required and the favorable climatic conditions in Mediterranean countries. The evaporation of OMWW produces sludge. Most of the studies about revalorization of OMWW sludge focus on composting (MedPan, 2007).

2.6 Bioremediation

Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site. According to the EPA, bioremediation is a “treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non toxic substances”.

For bioremediation to be effective, the right temperature, nutrients, and food also must be present. Proper conditions allow the right microbes to grow and multiply—and eat more contaminants. If conditions are not right, microbes grow too slowly or die, and contaminants are not cleaned up(EPA, 2012).

2.7 Phytoremediation

Phytoremediation (from Ancient Greek φυτο (phyto), meaning "plant", and Latin remedium, meaning "restoring balance") describes the treatment of environmental problems (bioremediation) through the use of plants that mitigate the environmental problem without the need to excavate the contaminant material and dispose it of elsewhere.

Phytoremediation consists of mitigating pollutant concentrations in contaminated soils, water, or air, with plants able to contain, degrade, or eliminate metals, pesticides, solvents, explosives, crude oil and its derivatives, and various other contaminants from the media that contain them.

Phytoremediation is a developing technology which uses plants for the remediation of soil contamination. Research in the field of phytoremediation is aiming at developing innovative, economical and environmentally compatible approaches to remove heavy metals from the environment (Mathew, 2001).

Phytoremediation applications can be classified based on the contaminant fate: degradation, extraction, containment, or a combination of these. Phytoremediation applications can also be classified based on the mechanisms involved. Such mechanisms include extraction of contaminants from soil or groundwater; concentration of contaminants in plant tissue; degradation of contaminants by various biotic or abiotic processes; volatilization or transpiration of volatile contaminants from plants to the air; immobilization of contaminants in the root zone; hydraulic control of contaminated groundwater (plume control); and control of runoff, erosion, and infiltration by vegetative covers (EPA, 2000).

Table (5) explain the different types of phytoremediation, and table (6) show the advantages and disadvantages of different forms of phytoremediation.

Table (5): the different types of phytoremediation

Types of phytoremediation	Descriptions
Phtoextraction	The use of pollutant accumulating plants to remove metals of organics from soil by concentrating them in harvestable parts.
Phytotransformation (phytodegradation)	The partial or total degradation of complex organic molecules or their incorporation into plant tissues.
Phytostimulation	The release of plants exudates / enzymes into the root zone (rhizosphere) stimulates the microbial and fungal degradation of organic pollutants.
Phytostabilisation	The use of plants to reduce the mobility and bioavailability of pollutants in the environment, preventing thus their migration to groundwater or their entry into the food chain.
Phytovolatilisation	The use of plants to volatilize pollutants or metabolites.
Rhizo-filtration	The use of plant roots to absorb or adsorb pollutants, mainly metals, but also organic pollutants, from water and aqueous waste streams.
Pump and Tree	The use of trees to evaporate water and to extract pollutants from the soil.
Hydraulic control	The control of the water table and the soil field capacity by plant canopies.

Source**:** (Schwitzguebel, 2004)

Various soil and plant factors such as soil’s physical and chemical properties, plant and microbial exudates, metal bioavailability, plant’s ability to uptake, accumulate, translocate, sequester and detoxify metal amounts for phytoremediation efficiency (Hooda, 2006).

Table (6): the advantages and disadvantages of different forms of phytoremediation

Methods	Advantages	Disadvantages
Phytoextraction	1. Cost of phytoextraction is fairly inexpensive.	1. Metal hyperaccumulators are generally slow- growing with a small biomass and shallow root systems.
	2. The contaminant is permanently removed from the soil (Henry, 2000).	2. Plant biomass must be harvested and removed, followed by metal reclamation or proper disposal of the biomass (Prasad, 2004).
Rhizofiltration	1. The ability to use both terrestrial and aquatic plants for either in situ or ex situ applications.	1. The constant need to adjust pH.
	2. The contaminants do not have to be translocated to the shoots (Henry, 2000).	2. Plants may first need to be grown in a greenhouse or nursery (Henry, 2000).
Phytostabilization	1. The disposal of azardous biomass is not required.	1. Contaminant remaining in soil.
	2. The presence of plants also reduces soil erosion and decreases the amount of water available in the system (Henry, 2000).	2. Application of extensive fertilization or soil amendments, mandatory monitoring is required (Henry, 2000).
Phytovolatilization	1. Contaminants could be transformed to less-toxic forms, such as elemental mercury and dimethyl selenite gas.	1. The contaminants or a hazardous metabolite might accumulate in vegetation such as fruit or lumber.
	2. Contaminants or metabolites released to the atmosphere might be subject to more effective or rapid natural degradation processes such as photodegradation (Prasad, 2004).	2. Low levels of metabolites have been found in plant tissue (Prasad, 2004).

Source: (singh, 2012)

2.8 Phytoremediation of metals contaminants

phytoremediation of heavy metals from the soil and water were discussed by many researchers. Some of those researchers:

Singh et al., 2012 showed that aquatic plants such as pistia, duckweed, water hyacinth and hydrilla can have remediatry effects on lead removal from wastewater.

Preeti et al., 2011 concluded that there is a direct relationship between the concentration of heavy metals and morphological and biochemical responses of plants and chemical characteristics of soil.

Greger and Landberg, 1999 calculated that the removal rate of Cd from soil was 216.7 g/ha per year (Pivetz, 2001).

Poniedziałek et al, 2010 concluded that in metal reduction efficiency, maize and red beet may be indicated as potential phytoremediants of Cd, cabbage and field pumpkin of Pb, and cabbage of Zn.

Based on the above, it seems that the phytoremediation of heavy metals is a beneficial method to reduce the concentration of these metals.

2.9 Phytoremediation of organic contaminants

In general there are two approaches for the phytoremediation of organic-polluted soils based on the difference in remediative mechanism. First, organic pollutants can be taken up directly by plants, resulting in the sequestration or degradation of pollutants inside of plants, which is called phytoextraction. Second, organic pollutants can be degraded by plant-secreted enzymes or plant-modified microbial community in rhizosphere, which is called plant-assisted rhizoremediation (Chen et al., 2013).

CHAPTER 3

Materials and Methods

3.1 Samples collection

3.1.1 OMWW sample

Approximately15 litter OMWW were taken from Zeta olive-mill facility on January, 2015. Sample was taken from OMWW after shaken to ensure homogeneity and analyzed to determine the content of phenol and heavy metals (Ni, Zn, Cu, and Fe).

Flame atomic absorption (FLAA) was used for metals analysis. are reported from (FLAA) using units of mg/l.

polyphenol content of OMWW sample was analyzed in the Poison Control and Chemical /Biological Center in An-Najah National University according to Folin-Ciocalteau colorimetry method (Singleton and Rossi, 1965). Values are reported from UV-spectrophotometer (Shimadzu, model UV1601 PC) in Gallic acid equivalents (GAE) using units of mg/l.

The sample was also analyzed for OMWW content of chemical oxygen demand (COD), 5-day biological oxygen demand (BOD5), total solids (TS), total suspended solids (TSS), total dissolved solids (TDS), and (pH).

- **chemical oxygen demand (COD)**

COD was analyzed according to the "Standard Methods for Examination of Water and Wastewater", 5220 C, Closed Reflux Method (Clescer et al., 1999).

- **5-day biological oxygen demand (BOD5)**

BOD5 was analyzed according to the "Standard Methods for Examination of Water and Wastewater", 5210 B, 5-day BOD Test (Clescer et al., 1999).

- **total solids (TS)**

(TS) was analyzed according to the "Standard Methods for Examination of Water and Wastewater", 2540 B, Total Solids (Clescer et al., 1999).

- **total suspended solids (TSS)**

(TSS) was analyzed according to the "Standard Methods for Examination of Water and Wastewater", 2540 D, Total Suspended Solids (Clescer et al., 1999).

- **total dissolved solids (TDS)**

(TDS) was analyzed according to the "Standard Methods for Examination of Water and Wastewater", 2540 C, Total Dissolved Solids (Clescer et al., 1999).

- **pH measurement**

pH was measured by calibrated membrane pH meter (Hanna HI98100).

3.1.2 Soil sample preparation

The soil aggregation from the top 0-20 cm layer and mixed to ensure homogeneity. 60 plastic pots with a diameter of 12 cm and a height of 10 cm were filled with 0.9 kg soil, soil samples were taken from the pots and mixed together and prepared for analysis. This sample dubbed code (bp) before planting.

3.2 planting

The soil in the pots was soaked in water before planting the seeds. After 3 days, barley seeds were cultivated in those pots and seeded in similar way like seeding in fields (approximately one seed per cm). The pots were distributed into 4 groups. The first group was watered by OMWW (Zibar) after knowing the exact concentration of organics and metals in both the soil and OMWW. The second group was watered by diluted sample of OMWW with 50% concentration in order to know if the acidity is very high as to avoid Inhibition of seed germination. To see if other contents in OMWW will affect phytoremediation, the third group was watered with the prepared solution that is to have just organics and metals which appeared in analysis of OMWW. The fourth group was watered with fresh water as a control group.

Three days after cultivation the first group was irrigated with 100 ml of OMWW (zebar 100%). After a week, it was irrigated with 300 ml of OMWW every 3 days. In the same time the other groups were treated in the same way and quantities with diluted OMWW (zebar 50%), prepared solution and fresh water (fw) respectively.

3.3 Experimental design

The pots were aligned in 4 groups as shown in figure (4). The control pots which were irrigated with fresh water designated by F.W and, Z 100% , Z 50%, and SL represents the pots which were irrigated with OMWW (zebar 100%), with diluted OMWW (zebar 50%), and with the prepared solution, respectively.

3.4 Soil analysis

A week after the plant samples grow up, one pot from each group were taken. The soil were collected separately from the pots and separated from the roots of barley and mixed to take a sample for analysis to see how much the barley plant extracted organics and metals from the soil. These analyses were to be repeated in the rest of the pots during about 7 days period.

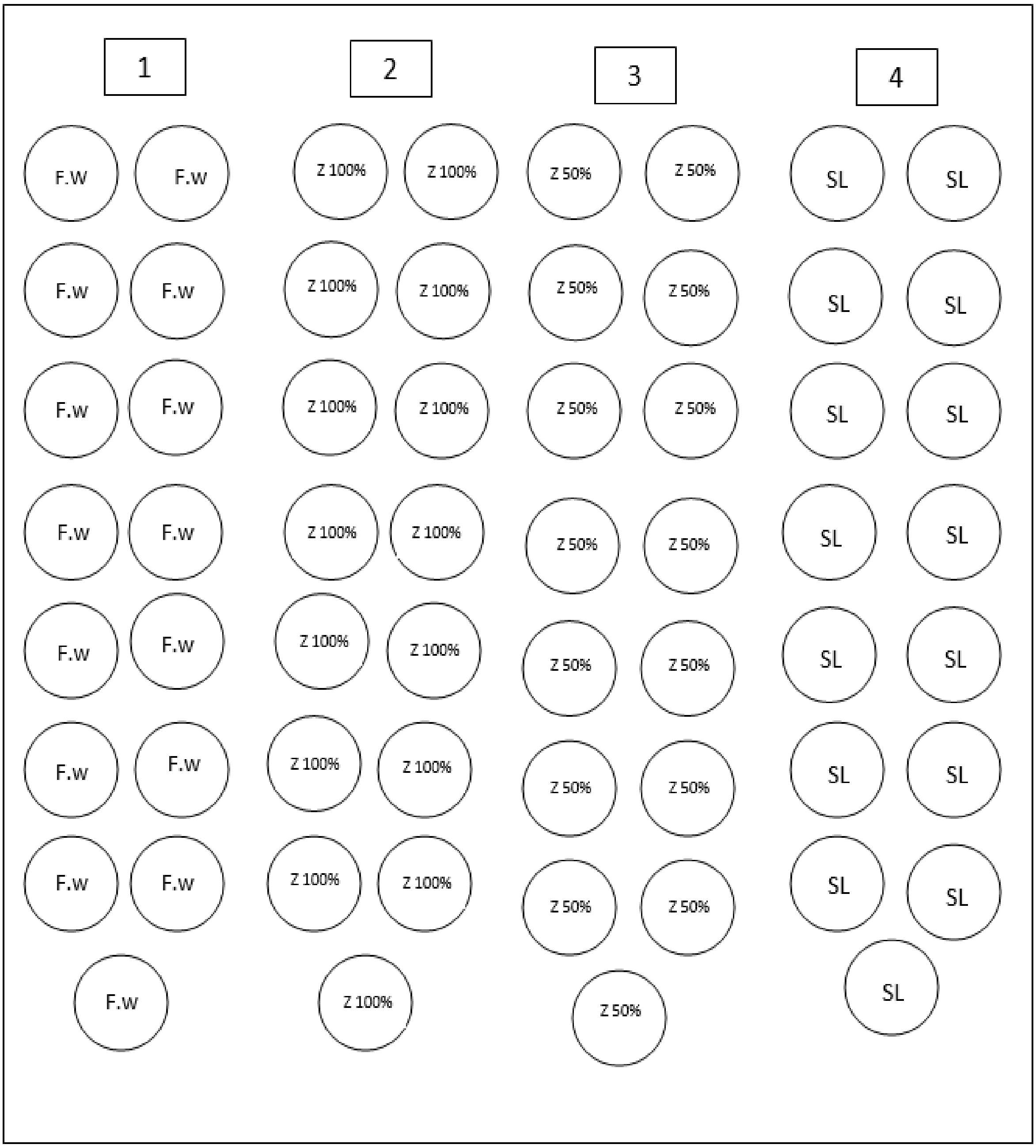

Figure (4) : Experimental set up

3.5 Analytical analysis

Various analytical analyses were done on each soil samples from all groups and the sample which was taken before planting.

3.5.1 Heavy metals concentration in the soil samples

For determining the heavy metals concentration in the soil samples, the samples were put in the oven at a temperature of 70 ° C for two hours to dry. For the digestion of samples, a representative 1 g (dry weight) sample was mixed with 10 ml of nitric acid (HNO_3) and 3 ml of hydrochloric acid (HCl) in dry flask. The mixture in the flask was heated on a hotplate located in a fume hood for 15 minutes . Absence of brown fumes from the solution indicates the completion of digestion. The digested sample was then filtered through filter paper and collected in a 100 ml volumetric flask and made up to 100 ml with distilled water. filtered sample were diluted to 10% and prepared to analysis by flame atomic absorption.

For all calibrations, standards of each metal were prepared. The concentration of calibration standards is shown in table (7).

Table (7): The concentration of calibration standards for metals

Metal	concentration of calibration standards mg/l		
Fe	10	30	50
Cu	2.5	10	20
Zn	2.5	10	20
Ni	5	10	20

3.5.2 Phenol concentration in the soil samples

determination of polyphenol concentration in the soil samples was done in the Poison Control and Chemical /Biological Center in An-Najah National University. The concentration of total phenols was determined colorimetrically using the Folin-Ciocalteau reagent. The absorbance was measured at 725 nm (in the range 0.01-1.00 mg ml-1) against a blank, using a UV-VIS spectrophotometer (GBS model 916). Results were expressed in mg/ kg (Gutiérrez et al., 1977).

3.6 Up take by plant

Mass up taken by the plant was calculated by the following equation.

$$A = Cb + Cz - Cn$$

A: Mass of polyphenol and the heavy metals up taken by plant (mg).

Cb: Mass of polyphenol and the heavy metals in the soil sample before planting (mg).

Cz: Mass of polyphenol and the heavy metals in OMWW added (mg).

Cn: Mass of polyphenol and the heavy metals in the soil sample after planting (mg).

The absorption indicates to the phytoremediation of polyphenol and the heavy metals.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 General characteristic of OMWW

Table (8) shows the general characteristics of olive mill wastewater (OMWW). According to results OMWW is acidic and contain high concentration of total polyphenols, total suspended solid, and total dissolved solid. Also BOD_5 and COD are high when compared with values reported by other researchers.

Table (8): General characteristics of olive mill wastewater from the sample.

Parameter	Unit	Value
BOD_5	mg/l	33532
COD	mg/l	246652
TS	mg/l	75328
TSS	mg/l	62117
TDS	mg/l	13211
Total polyphenols	mg/l	21000
pH		5.1

BOD_5 / COD ratio is low (about 0.14). This ratio has been commonly used as an indicator for biodegradation capacity (Abdalla and Hammam, 2014).

There are periodic and spatial variations in the investigated parameters in Palestine. Table (9) shows the OMWW characteristics in this study and the characteristics which were reported by other researchers.

Table (9): Characteristics of OMWW in Palestine presented by different researchers:

Parameter	Unit	This study	Aladham (2012)	Khtib et al., (2009)	Basheer et al., (2004)
BOD_5	mg/l	33532	11375	45624.67	27500
COD	mg/l	246652	137525	98999.67	163500
TS	mg/l	75328	67478		
TSS	mg/l	62117	52014	16963.67	86840
TDS	mg/l	13211	15464	35212.67	
Total polyphenols	mg/l	21000	4592	3149.33	6800
pH		5.1	4.9	4.99	5.0

According to table (10) the characteristics of OMWW far exceed the standards for OMWW disposal. It is indication to the toxicity and hazards of OMW to the environment

Table (10): Comparison between OMWW in this study and the Maximum Allowable Limit-Jordanian standards (Discharge to sanitary systems).

Parameter	Unit	This study	Maximum Allowable Limit-Jordanian standards (Discharge to sanitary systems)*
BOD_5	mg/l	33532	2100 **
COD	mg/l	246652	1100
TS	mg/l	75328	
TSS	mg/l	62117	800
TDS	mg/l	13211	
Total polyphenols	mg/l	21000	10
pH		5.1	5.5-9.5

*source: Khatib et al., 2009

** This value equals 2000 mg/l according to Palestinian law (16) in 2013

4.2 Heavy metals concentration in the OMWW

The heavy metal concentrations in the OMWW were measured by analysis in an flame atomic absorption. Table (11) shows the concentration of heavy metals (Fe, Cu, Zn, and Ni) in OMWW sample.

Table (11): the concentration of heavy metals in OMWW sample.

Parameter	Unit	Value
Fe	mg/l	0.1487
Cu	mg/l	0.0012
Zn	mg/l	0.0055
Ni	mg/l	0.0068

Concentration of heavy metals which is presented in table (11) are very low when compared with the concentration values of OMWW reported in literature. Table (12) shows comparison between the concentration of heavy metals in this study and the concentrations which were reported by other researchers.

Table (12): comparison between the concentration of heavy metals by different authors:

Parameter	Unit	This study	Other authors
Fe	mg/l	0.1487	20-70*
Cu	mg/l	0.0012	1.376**
Zn	mg/l	0.0055	3.907**
Ni	mg/l	0.0068	0.545**

Source: * Naija et al., 2014. ** Fatih Vuran and Mustafa Demir

4.3 Polyphenol and heavy metals concentration in the soil before planting.

During soil preparation, soil samples were taken from the pots and mixed together and prepared for analysis. The results of this analysis are shows in table (13).

Table (13): concentration of polyphenol and heavy metals in the soil sample before planting.

Parameter	Unit	Value
Polyphenol	mg/kg	3100
Fe	mg/kg	3243.98
Cu	mg/kg	1.99
Zn	mg/kg	9.06
Ni	mg/kg	8.51

4.4 Seed germination

Eight days after planting, the seeds were germinated in the pots which were irrigated with OMWW (Zebar 100%), diluted OMWW (Zebar 50%), and fresh water (FW). While the pots which were irrigated with prepared solution were not germinated at all.

The barley plants which were irrigated with OMWW was shorter than those irrigated with diluted OMWW and fresh water. The number of plants in the pots which were irrigated with OMWW (Zebar 100%) was 10, while in the pots which were irrigated with diluted OMWW (Zebar 50%) and fresh water (FW) was 15 and 18 respectively.

4.5 Samples taken from the pots

A week after plant germination, the process of taking samples from the pots for analysis has been started. It should be noticed that this process was restricted to those that were irrigated with OMWW (Zebar 100%), diluted OMWW (Zebar 50%), and fresh water (FW).

One pot has been taken from each group, then the soil separated from the barley roots. The soil of each group was separately mixed to guarantee the homogeneity. On sample from each group has been taken for analysis. These analyses were to be repeated for the rest of the pots weekly. It has also been observed that after 7 weeks from the first sample the plants were no longer growing, and had reached a stagnant point. So taking samples was stopped at this point. Sampling date is shown in table (14).

Table (14): The date of sampling

sample number	Sampling date
1	3/16/2015
2	3/23/2015
3	3/30/2015
4	4/6/2015
5	4/13/2015
6	4/20/2015
7	4/27/2015

4.6 Soil samples analysis

The heavy metal concentrations in the soil samples were measured using the conventional digestion procedure followed by analysis in an flame atomic absorption, while polyphenol concentration was done in the Poison Control and Chemical /Biological Center in An-Najah National University according to Folin-Ciocalteau colorimetry method.

4.6.1 Concentration of polyphenol and heavy metals

Soil pH and other factors such as the presence of competing ligands, the ionic strength of the soil solution and the simultaneous presence of competing metals are known to significantly affect the sorption processes of particular elements through a soil profile (Harter and Naidu, 2001).

The concentration of polyphenol and heavy metals (Fe, Cu, Zn, and Ni) in the soil samples which were irrigated with Zebar 100%, Zebar50%, and FW are presented in table (15), table (16), and table (17) respectively.

In general, it can be observed that the concentration of polyphenol and heavy metals (Fe and Zn) decreased during the experiment, while there was no significant reduction in concentration of Cu and Ni. This is due to high concentrations in each of the polyphenol and Fe. Each concentration of Ni and Cu was very low, it is possible that these metals were absorbed in a small concentration but have not been detected by Flame atomic absorption.

Table (15): Concentration of polyphenol and heavy metals in samples which were irrigated with OMWW (Zebar 100%).

Sample number	polyphenol mg/kg	Fe mg/kg	Cu mg/kg	Zn mg/kg	Ni mg/kg
1	23500	3117.26	2.1	9.14	9
2	23100	2889.32	2	8.92	9.11
3	23200	2775	1.99	8.65	8.99
4	22000	2715.7	2.05	8.63	8.97
5	21800	2617	2.03	8.59	9.1
6	22000	2434.45	2.11	8.41	9
7	21500	2354.3	1.98	8.05	8.89

According to table (16) it is noticed that the uptake rate in samples which were irrigated with diluted OMWW (Zebar 50%) was higher than the uptake rate in samples irrigated with OMWW (Zebar 100%). It is possible that the high concentration of organic compounds and heavy metals prevent the germination and plant growth. It is also possible that the compounds found in OMWW trying to catch transitional compounds to form complex compounds.

Table (16): Concentration of polyphenol and heavy metals in samples which were irrigated with diluted OMWW (Zebar 50%).

Sample number	polyphenol mg/kg	Fe mg/kg	Cu mg/kg	Zn mg/kg	Ni mg/kg
1	12700	3138.62	1.99	9.07	8.78
2	12300	2946.86	2.03	8.81	8.7
3	11500	2793.5	2	8.55	8.88
4	10600	2542.01	1.97	8.37	8.53
5	10300	2283.62	2.02	8.22	8.01
6	10100	2027.36	2.07	8.02	8.79
7	9600	1893.52	1.97	7.77	8.8

Table (17): Concentration of polyphenol and heavy metals in samples which were irrigated with fresh water (FW).

Sample number	polyphenol mg/kg	Fe mg/kg	Cu mg/kg	Zn mg/kg	Ni mg/kg
1	2800	3028	1.95	8.5	8.33
2	2700	2832.3	1.98	8.18	8.48
3	2650	2588.52	1.97	7.91	8.37
4	2600	2272.35	1.98	7.71	8.5
5	2500	1927.6	1.97	7.59	7.98
6	2400	1878.54	1.97	7.35	8.26
7	2300	1665.89	1.95	7.11	8.02

4.7 Kinetics of polyphenol and heavy metals depletion

The figures below show the ln[con.] versus time (day) for polyphenol, Fe and Zn. From these figure, it is concluded that the uptake of polyphenol and metals (Fe and Zn) follow first order reaction.

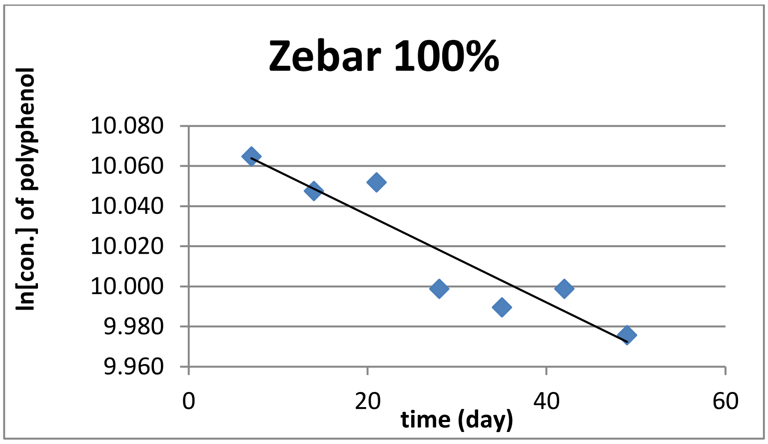

Figure (5): ln [con.] 0f polyphenol vs time in pots which were irrigated with OMWW (Zebar 100%).

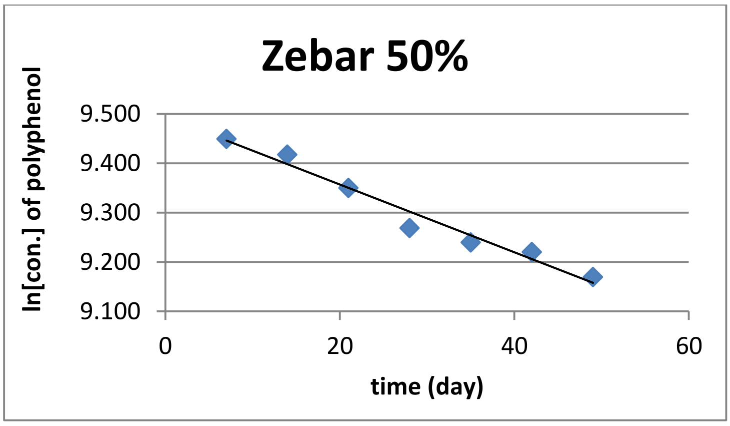

Figure (6): ln [con.] of polyphenol vs time in pots which were irrigated with diluted OMWW (Zebar 50%).

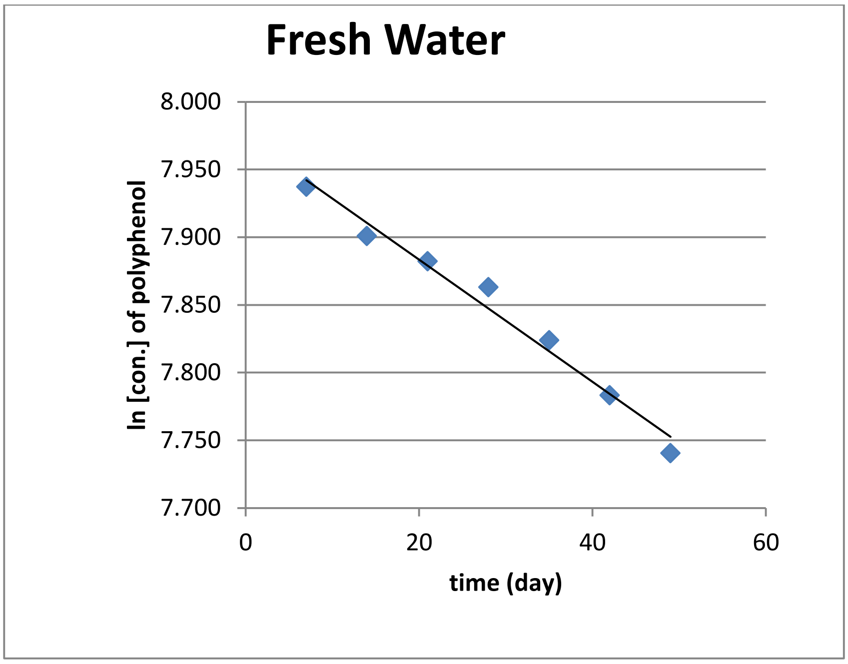

Figure (7): ln [con.] 0f polyphenol vs time in pots which were irrigated with fresh water (FW).

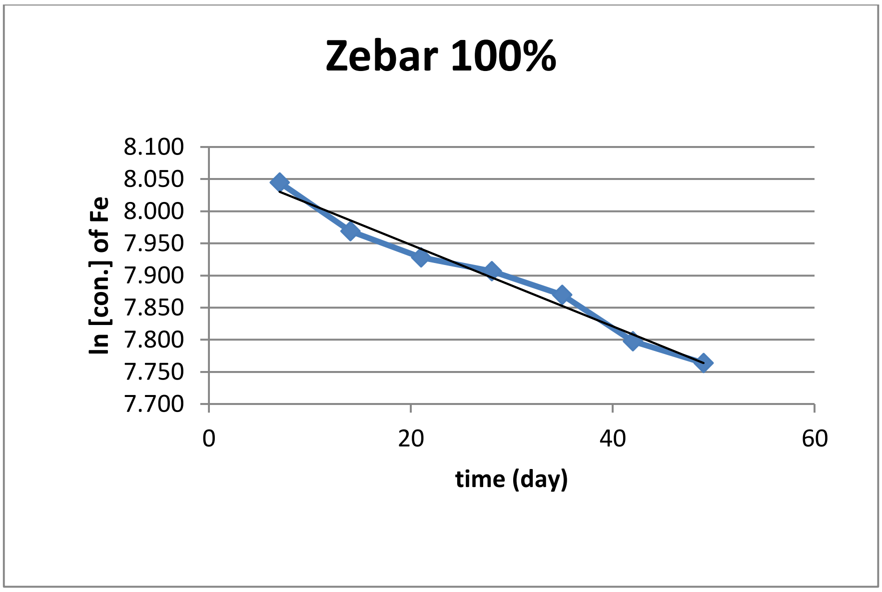

Figure (8): ln [con.] of Fe vs time in pots which were irrigated with OMWW (Zebar 100%).

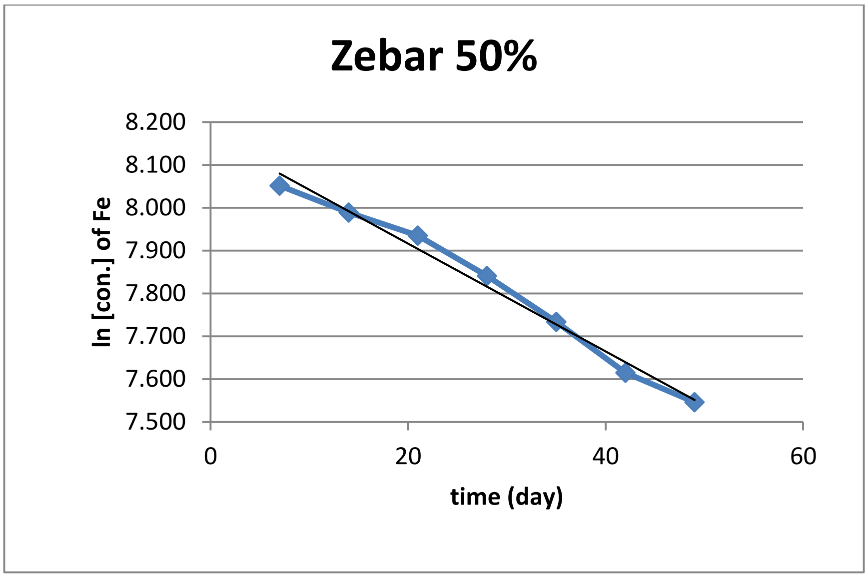

Figure (9): ln [con.] of Fe vs time in pots which were irrigated with diluted OMWW (Zebar 50%).

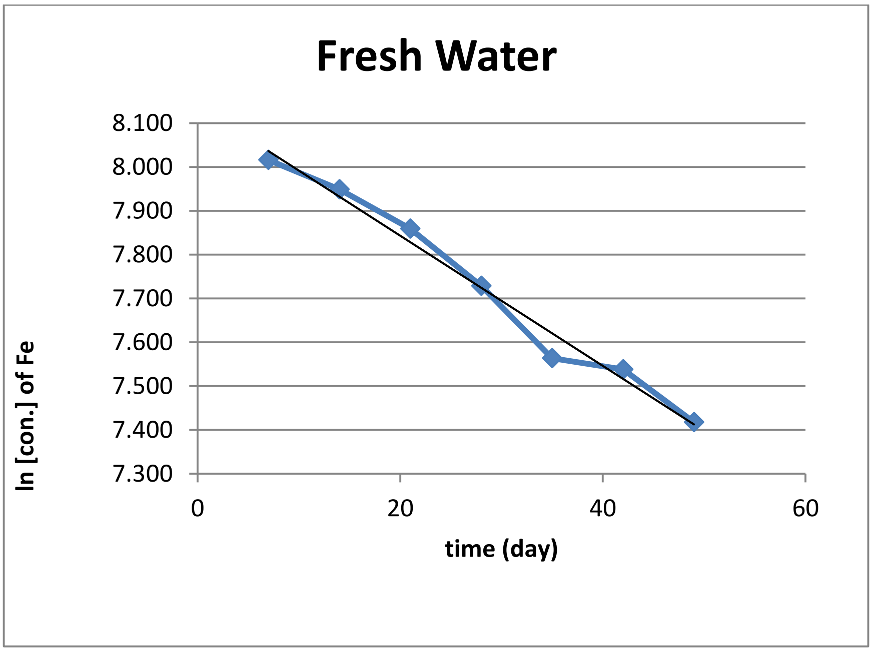

Figure (10): ln [con.] of Fe vs time in pots which were irrigated with fresh water (FW).

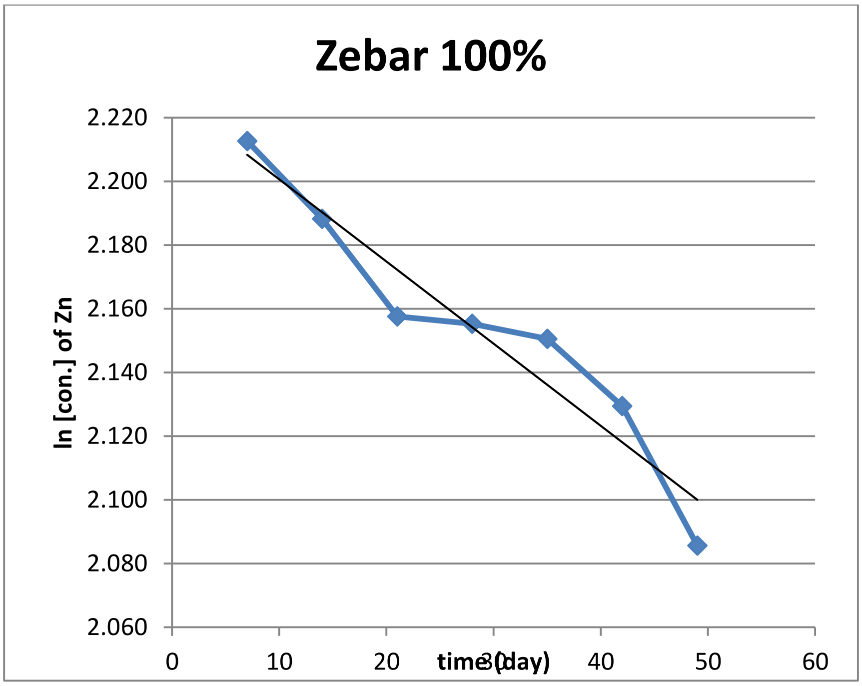

Figure (11): ln [con.] 0f Zn vs time in pots which were irrigated with OMWW (Zebar 100%).

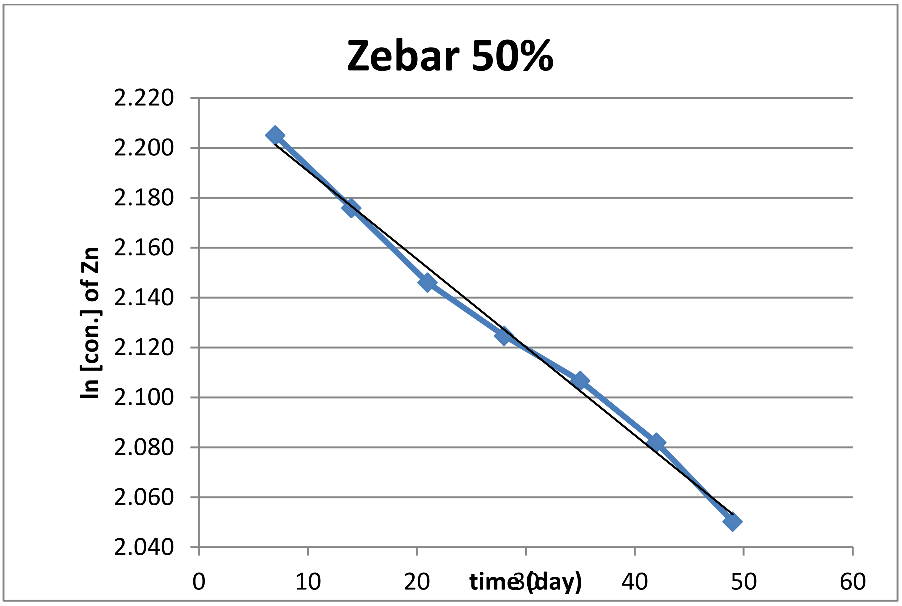

Figure (12): ln [con.] of Zn vs time in pots which were irrigated with diluted OMWW (Zebar 50%).

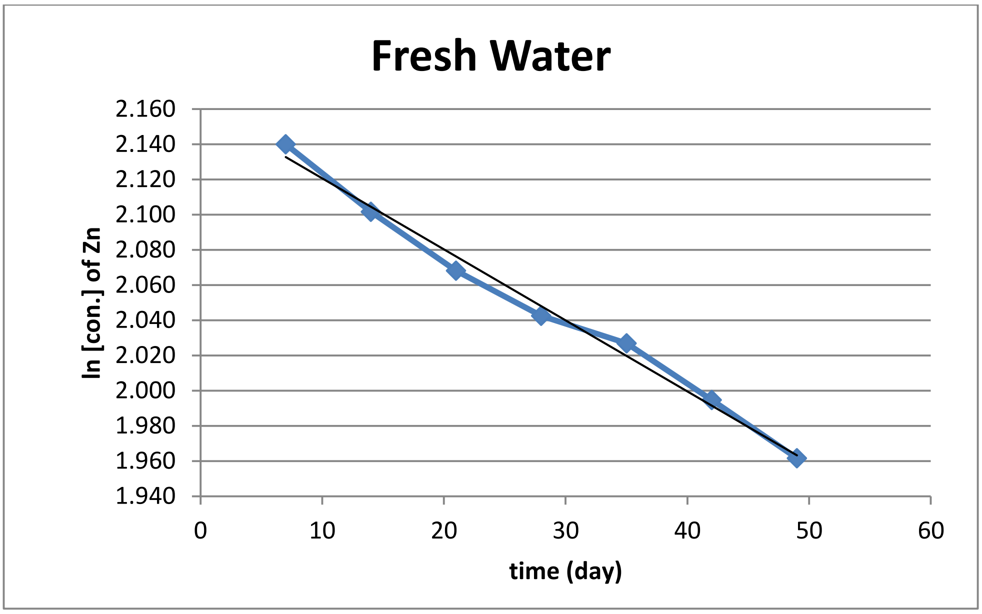

Figure (13): ln [con.] of polyphenol vs time in pots which were irrigated with fresh water (FW).

Table (18) shows the first order reaction constant (k) in (day^{-1}) and the R^2 for the pots which were irrigated with OMWW (Zebar 100%), diluted OMWW (Zebar 50%) and fresh water.

Table (18): First order reaction constant k (1/day) and R^2

parameters	Zebar 100%		Zebar 50%		Fresh Water	
	K. constant	R^2	K. constant	R^2	K. constant	R^2
polyphenol	0.002	0.863	0.006	0.979	0.004	0.921
Fe	0.006	0.969	0.012	0.985	0.014	0.994
Zn	0.002	0.979	0.003	0.982	0.004	0.990

According to table (18) the first order reaction constant (k) for the pots which were irrigated with diluted OMWW (Zebar 50%) was higher than those irrigated with OMWW (Zebar 100%). The obtained fitting R^2 values are high, especially for the pots which were irrigated with diluted OMWW (nearly 0.98), and so confirming Linearity.

4.8 The mass of polyphenol and heavy metals in soil after irrigation

The mass of polyphenol and metals in the soil after being irrigated with OMWW, diluted OMWW, and fresh water can be measured if the mass of polyphenol and metals in the soil sample before planting were collected with the mass in water added, as shown in the table (19).

Table (19): The mass of polyphenol and heavy metals in soil after irrigation

	polyphenol (mg)	Fe (mg)	Cu (mg)	Zn (mg)	Ni (mg)
Zebar 100%	23790	2919.73	1.7922	8.1595	7.6658
Zebar 50%	13290	2919.66	1.7916	8.15675	7.6624
FW	2790	2919.58	1.791	8.154	7.659

4.9 Uptake ratio of polyphenol

Uptake ratio of polyphenol was calculated and presented in table (20). It was 0.19 in the samples which were irrigated with Zebar 100% at the end of experiment. While it was 0.31 and 0.26 in the samples irrigated with Zebar 50% and fresh water respectively.

According to Kapellakis et al. (2012), the removal efficiency average is 74%- 87% in constructed wetlands which were operated with diluted OMWW (1:10) and planted with *Phragmites australis* plant.

Table (20):Uptake ratio of polyphenol during taking of samples.

samples number	Uptake ratio of polyphenol		
	Zebar 100%	**Zebar 50%**	**Fresh water**
1	0.11	0.14	0.10
2	0.13	0.17	0.13
3	0.12	0.22	0.15
4	0.17	0.28	0.16
5	0.18	0.26	0.19
6	0.17	0.32	0.23
7	0.19	0.31	0.26

4.10 Uptake ratio of metals

Tables (21)and (22) show the uptake ratio of metals (Fe, and Zn) during the taking of samples. It be noticed that the uptake ratio of Fe was the most significant, it was 0.27, 0.42 and 0.49 in samples irrigated with Zebar 100%, Zebar 50% and fresh water respectively.

Table (21):Uptake ratio of Fe during taking of samples.

samples number	uptake ratio of Fe		
	Zebar 100%	Zebar 50%	Fresh water
1	0.04	0.03	0.07
2	0.11	0.09	0.13
3	0.14	0.14	0.20
4	0.16	0.22	0.30
5	0.19	0.30	0.41
6	0.25	0.38	0.42
7	0.27	0.42	0.49

Table (20): Uptake ratio of Zn during taking of samples.

samples number	Uptake ratio of Zn		
	Zebar 100%	Zebar 50%	Fresh water
1	-0.01	0.00	0.06
2	0.02	0.05	0.10
3	0.05	0.06	0.13
4	0.05	0.08	0.15
5	0.05	0.08	0.16
6	0.07	0.10	0.19
7	0.11	0.14	0.22

Conclusions

- Olive mill wastewater constitutes a serious environmental problem because it contains high concentration of polyphenol.

- Barley plant uptake polyphenol and Fe from diluted OMWW (Zebar 50%) more than undiluted OMWW (Zebar 100%).

- 0.31 of polyphenol and 0.42 of Fe was absorbed by barley plant from the pots which irrigated with diluted OMWW.

- The uptake of polyphenol and metals (Fe and Zn) was calculated as first order kinetics reaction with k value (day^{-1}) 0.006, 0.012 and 0.003 for polyphenol, Fe and Zn respectively, and high R^2 value (nearly 0.98) in the pots which were irrigated with diluted OMWW (Zebar 50%).

Recommendations

- Quantitative survey of environmental aspects related to olive oil industry in Palestine must be included in the annual olive mills survey carried out by Palestinian Central Bureau of Statistics (PCBS) and Palestinian Ministry of Agriculture (MoA).

- Seasonal OMWW quality monitoring program must be implemented as part the olive mill waste management policy within the national olive oil sector strategy.

- Study the fate of polyphenol and heavy metals by photosynthesis analysis for the barley plants.

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كلية الدراسات العليا

استخلاص المواد العضوية والمعادن من مخلفات الزيتون "الزيبار" باستخدام النبات

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قدمت هذه الأطروحة استكمالا لمتطلبات الماجستير في العلوم البيئية بكلية الدراسات العليا في جامعة النجاح الوطنية، نابلس– فلسطين .

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الملخص

تعتبر زراعة الزيتون وإنتاج الزيت من أهم الصناعات في فلسطين. إلا أن عمليات استخراج الزيت يصاحبها إنتاج كميات كبيرة من المخلفات السائلة التي تعرف بالزيبار. ويعتبر التخلص من الزيبار من أهم القضايا البيئية العالمية، حيث يتم التخلص من الزيبار في فلسطين بشكل عام من خلال طرحه في الوديان.

يهدف هذا البحث إلى دراسة إمكانية استخلاص المواد العضوية "البولي فينول" والعناصر الثقيلة مثل الحديد Fe والنحاس Cu والخارصين Zn والنيكل Ni من الزيبار باستخدام النبات الشعير *"Hordeum vulgare L"*.

بعد قياس تركيز كل من البولي فينول و العناصر "Fe, Cu, Zn, Ni" في عينة من الزيبار والتربة قبل الزراعة، تم زراعة أربعة مجموعات من الأواني ببذور الشعير *"Hordeum vulgare L"* وتم ري المجموعة الأولى منها بالزيبار والثانية بزيبار مخفف بنسبة 50% والثالثة بالماء والرابعة بمحلول محضر يحتوي على نفس تركيز البولي فينول والعناصر المراد دراستها في الزيبار.

بعد أسبوع من الإنبات تم أخذ عينة واحدة "إناء" من كل مجموعة حيث تم فصل الجذور عن التربة وأخذ عينة تربة لفحصها ومعرفة تركيز البولي فينول والعناصر "Fe, Cu, Zn, Ni" في كل منها. وتكرر أخذ عينات من الأواني الباقية أسبوعياً.

بعد ثمانية أيام من الزراعة بدأ ظهور النباتات في الأواني التي تم ريها بالزيبار والزيبار المخفف والماء، في حين أن الأواني التي تم ريها بالمحلول لم تنبت فيها النباتات نهائيا.

تحليل وفحص عينات التربة لكل من المجموعات الثلاثة التي ظهرت فيها النباتات أشار إلى انخفاض في تركيز كل من البولي فينول والحديد والخارصين، في حين لم يلاحظ انخفاض في تركيز كل من النحاس والنيكل. وقد كانت نسبة الامتصاص للبولي فينول في نهاية التجربة في العينات التي تم ريها بالزيبار 0.19 بينما كانت 0.31 و0.26 في العينات التي تم ريها بالزيبار المخفف والماء على التوالي. ولوحظ أيضاً أن نسبة الامتصاص للحديد كانت الأكثر من بين العناصر الأخرى حيث بلغت 0.27، 0.42، 0.49 في العينات التي رويت بالزيبار والزيبار المخفف والماء على التوالي.