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Ecological and Biochemical study of *Spinacia Oleracea* and *Ocimum Basilicum* Irrigated with Eastern Euphrates Drainage water

A thesis

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By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿أَوَلَمْ يَرَوْا أَنَّا نَسُوقُ الْمَاءَ إِلَى الْأَرْضِ الْجُرُزِ فَنُخْرِجُ

بِهِ زَرْعًا تَأْكُلُ مِنْهُ أَنْعَامُهُمْ وَأَنْفُسُهُمْ أَفَلَا يُبْصِرُونَ﴾

السجدة (27)

صدق الله العلي العظيم

Certification

I certify that the preparation of this thesis entitled (Ecological Impacts of Drainage Water on Some Terrestrial Plants) was made by (Imad Amer Subhi Abokalal) under my supervision at University of Babylon, College of Science, Department of Biology, as a partial fulfilment for the requirement of the degree of Master of Science in Biology, Ecology.

Supervisor

Prof. Dr. Batool Mohammed Al-Adily

In view of the available recommendations, I forward this thesis for debate by the examining committee.

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Date / /2022

Examination Committee

We, are the examining committee, certify that we have read this thesis entitled " **Ecological and Biochemical study of *Spinacia Oleracea* and *Ocimum Basilicum* Irrigated with Eastern Euphrates Drainage Water** " and examined the student " **Imad Amer Sobhi Habeeb** " in its content. In our opinion, it meets the standards of a thesis for the degree of Master of Science in Biology and accepted with "**Excellent**" degree.

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(Chairman)

Dedicated

I dedicate this work to.....

My Father & Mother

My brothers & sisters.....

My dear wife.....

My sons Saif & Taraf

Imad

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All Praise be to Allah Almighty for inspiring me with patience, perseverance and diligence to accomplish the present work. I must first of all bow to my Lord (Allah) to whom I thank very much, thanks are due to his Messenger Muhammad (peace and blessings of Allah be upon him and his family).

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SUMMARY

Summary

The current work was aimed at studying the possibility of using Eastern Euphrates Drainage water (Zaid Al-Shahed region) as suitable water resource in irrigation. Drainage water was collected in November 2021, then pH, Electric Conductivity, Total dissolved solids, total alkalinity, total hardness and chloride were studied, then it was frozen for later use in irrigation.

Results showed that drainage water had alkaline pH=8.08, total alkalinity was 280 mg/l with elevated concentration of chloride and Calcium hardness to 689.3 mg/l and 144.3 mg/l respectively, However, total hardness was less than its concentration in tap water that were 680 and 880 mg/l, respectively.

Electric Conductivity value in drainage water was elevated than in tap water but it was non- significant and their values were 1560 and 1465 μ mhos/cm, respectively.

Seeds of both *O. basilicum* and *S. oleracea* were planted in separate pots (each about 3 kg of soil) about ten pots for each species , then divided the pots of each plant species in two sub groups to irrigate, one with drainage water and latter with tap water. After one month, study the biochemical parameter of each plant group was included Chlorophyll a, Chlorophyll b, Carotene, Protein, Proline, Vitamin C, Malondialdehyde, Glutathione, Total Antioxidant Activity and Reactive oxygen species and recorded a significant variation among studied plant.

Soil characters were studied before and after irrigation with each type of water which included pH, Electric Conductivity, Total dissolved solids, Salinity, Ca content, Cl content, Cation exchange capacity and Organic matter.

Drainage water caused significant increase in Ca concentration in soil of both plant species but did not cause a significant difference in both pH and Cl. The organic matter was increase significantly in soil of both species that it

increased from 0.0 % to 0.03%, so Cation exchange capacity value was increased.

Drainage water causes decreased content of Chlorophyll a, Chlorophyll b and Carotenoid in both species, so the value in *O. basilicum* 3.43, 2.8 and 3.48 mg/g.f.w respectively, as well as in *S. oleracea* were 7.133, 9.31 and 2.27 mg/g.f.w respectively.

Protein content were decrease significantly in both species that irrigated with drainage water that is record 6.96 mg/ml and 4.62 µg/ml in *S. oleracea* and *O. basilicum* respectively .

S. oleracea showed a significant decrease in Vitamin C that is decrease from 31.82 to 28.84 µg/g.F.W in plant that irrigated with tap water and drainage water respectively, while *O. basilicum* showed a significant decrease in Total Antioxidant Activity that is concentration 17.64 and 35.48 mgAAE/g.F.W when irrigated with drainage water and tap water respectively.

The lower value of Malondialdehyde was in *O. basilicum* which irrigated with tap water 1.35 mol/l and the higher was 18.08 mmol/l in *S. oleracea* which irrigated with drainage water. Glutathione and Reactive oxygen species values in *O. basilicum* decrease significantly, while *S. oleracea* not showed significant change in their concentration.

Each of Cd, Fe, Cu, Pb and Zn were studied in drainage and tap water, in soil before and after irrigation and in both *S. oleracea* and *O. basilicum* in the end of experiment.

Elevated concentration of heavy metals in water receded in Cu 0.19 mg/l, while lowest one concentration recorded in pb 0.001 mg/l.

In soil, irrigation with drainage water was not caused significant different in each Cu, Fe, Zn and Pb, while Cd concentration increased in soil which planting in both species. *S. oleracea* was more accumulated to Cd, Cu, Fe and pb than *O. basilicum*.

CONCLUSION

AND

RECOMMENDATION

CHAPTER

ONE

INTRODUCTION

Introduction

The environment is formed from many biotic and abiotic factors that have an effect on all organisms on the earth (Gilpin., 1996). Agricultural drainage ditches are essential to sustaining food production in arid irrigation regions, with various sizes and drainage characteristics as important buffer ecotones in agricultural areas (Pan *et al.*, 2015).

Most plant problems are caused by environmental stress, either directly or indirectly. All environmental factors as climatic, biotic, physiographic and anthropic (socio economic) factors interact with one another to influence the crop growth and production. The major environmental factor (drought, high salinity, cold and heat) negatively influence the survival, biomass production and yields of major food crops up to 70% hence, threaten the food security worldwide (Singh *et al.*, 2016).

If one of the factors affecting the life of the plant approaches or exceeds the limits of environmental tolerance, which represents the limiting factor and that the different factors in terms of quality and quantity must be present at least within a certain limit in the environment in which the organism is located. Here it falls within the tolerance range of the organism in that ecosystem (Al-Saadi *et al.*, 2002).

Both physical and chemical elements that effect directly or indirectly on agriculture are very important for humans because they provide food, clothing and drugs. Scientific studies and research have proven that even a 1% increase in agricultural yield translates to a 0.6-1.2% decrease in the number of absolute poor households in the world (Thirtle., 2001). Increasing extreme water phenomena such as water pollution, water availability and temperature affecting productivity and having a large impact on agriculture (Noya *et al.*, 2018).

Plant exposed to various environmental pressure under natural and agricultural condition .The well –known of these factors are reduce water in plant tissues or increase water in soil, decrease water is one of important factors that effect on photosynthesis and decrease growth and productivity of plant that which causes crop loss worldwide and reduce more than 50% of average yield of most major crop plant (Wang *et al.*, 2003). Plant have many mechanisms to survive when water decrease such tolerant, escape, and avoidance dehydration of cell and tissue (Turner., 1986).

The total concentration of salts in drainage effluent is of major concern for irrigated agriculture. Salinity in the root zone increases the osmotic pressure in the soil solution. This causes plants to exert more energy to take up soil water to meet their evapotranspiration requirement. At a certain salt concentration, plant roots will not be able to generate enough force to extract water from the soil profile. Water stress will occur, resulting in yield reduction. The extent to which the plants are able to tolerate salinity in the soil moisture differs between crop species and varieties. The composition of the salts is also important for crop growth. Dominance of certain ions might cause an imbalance in ion uptake. This results in deficiencies of certain elements and depressed yields. The presence of high concentrations of sodium inhibits the uptake of calcium, causing nutritional disorders. Other ions can be toxic, causing characteristic injury symptoms as the ions accumulate in the plant. Toxic elements of major concern are chloride, sodium and boron (Tanji and Kielen., 2002). Iraq suffers from a lack of surface water and also suffers from a lack of rain water, especially in the current century (Hatfield and Brugger., 2015)

1.2 Aim of study

The current work was aimed to study the ability of use drainage water in the irrigation of terrestrial plant and study its effect on them by:

- 1- Study the main chemical characters of Eastern Euphrates Drainage Water in Al-Kifl region.
- 2- Determinate some heavy metals content (Cd, Fe, Zn, Pb, Cu) in water, soil and plant
- 3- Chose both *O. basilicum* and *S. oleracea* seeds to grow and irrigate with drainage water.
- 4- Study the effect of drainage water on the biochemical response of each plant.
- 5- Study the concentration of heavy metals onn both soil and plant when plant reach to picking stage.

CHAPTER
TWO
LITERATURES
REVIEW

1- Water scarcity problem

Iraq is one of the countries in which climate change and variability have had a severe impact (IPCC., 2014). As Iraq is part of a large area affected by climatic conditions and classified as having high degrees of aridity, high variability and very low annual precipitation, which is experiencing significant environmental changes due to climate change (Medany., 2008).

Most areas of Iraq are classified as dry to semi-arid, ranging from semi-arid in the southern regions to semi-humid in the northern regions due to the annual rainfall of less than 150 mm (Al-Ansari *et al.* , 2013). This makes Iraq highly affected by any slight change in the climate. The problem of water scarcity has emerged as an issue in large areas of the country, especially in the south, where this problem can increase due to climate change (IPCC, 2014). It can be said that climate change is one of the most important challenges that has an effect on the nature of the regions and has negative effects on water resources and also affects both environment and the economy, especially the agricultural sector. The climate has changed significantly in all regions of the world since the last century, where there has been a noticeable increase in surface air temperatures, since the fifties of the last century, when the temperature increased from 0.2 - 0.3°C (Zakaria *et al.*, 2013).

Many studies have shown that in the last three decades the temperature has increased by 0.7 °C and that this increase and climate variability may cause an increase in extreme weather events (Abbas *et al.*, 2017). Many negative issues have emerged due to climate change in Iraq, including desertification, temperature rise above 50, dust storms, prolonged drought conditions, storms and sudden heavy rains (Al-Ansari., 2014).

The effects of major changes in the climate are exacerbated by the inability to store water during rain Rains, abandonment of agricultural land

during drought, reduced flow rates in the Tigris and Euphrates rivers, shrinking marshes, soil erosion, and increased salinity of soil. Temperature is an important factor that contributes to plant development, and the increase in temperature associated with climate change has caused a decrease in agricultural productivity in large areas of Iraq (Hatfield and Brugger., 2015 and USIAD, 2017)

2.2: Drainage water

It is a water body used for reducing the increased water in the soil, so its type depended on many factors including: environmental factors, water resource and type of soil (Nlemadim *et al* ., 2019). Agricultural drainage ditches are essential to sustaining food production in arid irrigation regions, with various sizes and drainage characteristics as important buffer ecotones in agricultural areas (Pan *et al* , 2015).

Drainage channels are dug into the ground for the purpose of lowering groundwater levels by draining that water into marshes, depressions, major rivers, or seas away from the area they serve. The water is either directed to the drainage through surface water or through groundwater that goes towards the drainage dug in the area in the paths it creates through the pores between the soil atoms, and that water collects in the drainage and then heads towards the trough to which the drainage descends until it reaches its end and mixes with the water collected in the drainage system. The use of drainage water for the purpose of irrigation is very important if the properties of the water are in accordance with the accepted standards. The use of marginal quality water has the potential of causing serious problems of soil degradation and reduction in crop productivity because of irrigation water quality (Hoorn, 1970).

2.2.1 Types of Drainage water

Drainage are divided into three type (Ismail .,1988)

1- Open drain

It is one the oldest methods of drainage in the world, which is the transfer of excess water through a canal which is deep, open one of its advantages is that it can be used to get rid of the daily excess water that may come from heavy rain or emergency floods, in addition to its function in controlling the groundwater level. One of its shortcomings when slitting the drainage channels leads to a large loss of agricultural land, which may reach about 15% of the area of the region.

2-Covered drain

It is the drains located under the surface of the ground, meaning it is hidden from view. One of the advantages of this type of the drains are that there is no loss of agricultural land, as it is not possible to cultivate the land located directly above it drainage, thus saving 10-15% of agricultural land compared to open drains. Its disadvantages are the inability to get rid of superficial drainage water or excess water on the surface of the ground quickly and easily, and it is difficult to know whether the drains are working properly or not.

3-Vertical drain

It is intended to divert excess water vertically to the deep layers of the earth. The condition for the success of this type of drainage is the presence of deep layers with relatively high permeability that can accommodate large amounts of water. The water is either stored or transferred to distant infestations separated from the surface layer by another layer that is impermeable or has little permeability and one of its advantages is to get rid

of excess irrigation water in a short period before any damage to the plant occurs and to reduce the level of groundwater if it is high.

2-2-2- Drainage water characters

The drainage water characteristic are effected by surrounding soil thus it may be with high acidity when the soil with organic matter (Holden *et al.*, 2007), but in general drainage water tends to be neutral or alkaline due to action of carbonate specially calcium carbonate in the sediment (Miller *et al.*, 2001). The concentration of cations and anions are very effected with discharge geology and water sources (Gorham., 1956).

2-2-3 Importance of drainage

There are many important from use drainage in lands such prevent water logging, reduce soil salinity, increase the area which used in agriculture and finally make sustainability to both water resources and land (Ritzema, 2014).

Drainage water contains a varying amount of different kinds of anions and cations. The major cations are Ca^{+2} , Mg^{+2} , Na^{+} and K^{+} , which influence the suitability of water for human consumption, agricultural irrigation and other purposes. Some of these cations are beneficial to crops production at acceptable concentration, otherwise they cause toxicity to plants, affect properties of soil and management practices (Jawad., 2007). The concentrations of ions in irrigation water are particularly important because some crops are susceptible to these elements at high concentrations (Bohn *et al.*, 1985).

2.2.4 - limitations of drainage water uses

The limitations of drainage waters including the following (Skaggs *et al.*, 2012):-

- 1- Drainage water (in general) is saline which would effect on the yield of crops.
- 2-The percolation of drainage water on the soil can lead to ground water pollution.
- 3-Its water contaminated with toxic element due to effluents of human activities.
- 4-High concentration of Na in drainage water can lead to permeability problems.
- 5-The use of drainage water for long periods causes elevated concentration of salts in the Rhizospher.

2- 3 Heavy metals

Metals are designated as heavy metals when their molecular weight is over 20 g/mol and density higher than 5 g/mol (Stankovic *et al.*, 2014). More than 75% of known elements exhibit the properties of metals. We can divided metals in two groups due to their density, first are light where density is less than 5 gm/cm and second group that are higher than 5 g/cm .(Morkunas *et al.* , 2018). Heavy metals in nature cause high contamination because their height toxicity, persistence and easy movement through food chain (Duffus., 2003).

Contamination of soils with heavy metals is one of the problem related to metal toxicology because it resistance and exhibit high stability in soil and it no biodegradability such as Cd, Hg and Pb. Some metals are very important

because they are necessary for keeping homeostasis. Industrial activities causes disturbance in natural biogeochemical cycles that is accumulation of heavy metals and effect on environmental, nutritional and ecological system (Ali *et al.*,2013).

Two kinds of metals are found in soils, first essential metals, they are important for growth of plant such (Fe, Mn, Zn, Cu, Mo) and the second non-essential for growth plant such Cd, Cr, Pb, As, Co (Rascio and Navari., 2011)

2-3-1 Sources of heavy metals in the environment:

Heavy metals can inter the environment by both natural and anthropogenic resources (Dixit *et al.*, 2015) as illustrated in figure (2-1)

1- Natural resources

Heavy elements are released in the aquatic environment naturally through and from geological processes, like erosion and natural source (rocks and sediments), then they arrive to the aquatic environment. These released minerals are dissolved or suspended in rain water drifting on the surface of the earth or suspended in the air to be transmitted by wind from one place to another (Papagiannis *et al.*, 2004). Volcanic activities are also among the natural sources that contribute to the pollution of aquatic environment. It can reach through acid rain loaded with various pollutants, including heavy metals. This rain is able to dissolve the soil and release heavy elements into the water (Butu & Iguisi, 2013).

2-Anthropogenic resources

Industrial activity is a major source of pollution with heavy metals in the environment. There are many sources of this type of pollution, including the petroleum industries, oil refineries, iron and steel factories, Copper, aluminum, tanning factories, fertilizers, pesticides, gasoline and other various industries (figure 2-1) (Majed *et al.*, 2002 ; Butu and Iguisi.,2013). To that heavy metals can get into the water through pollution industrial or consumer waste.

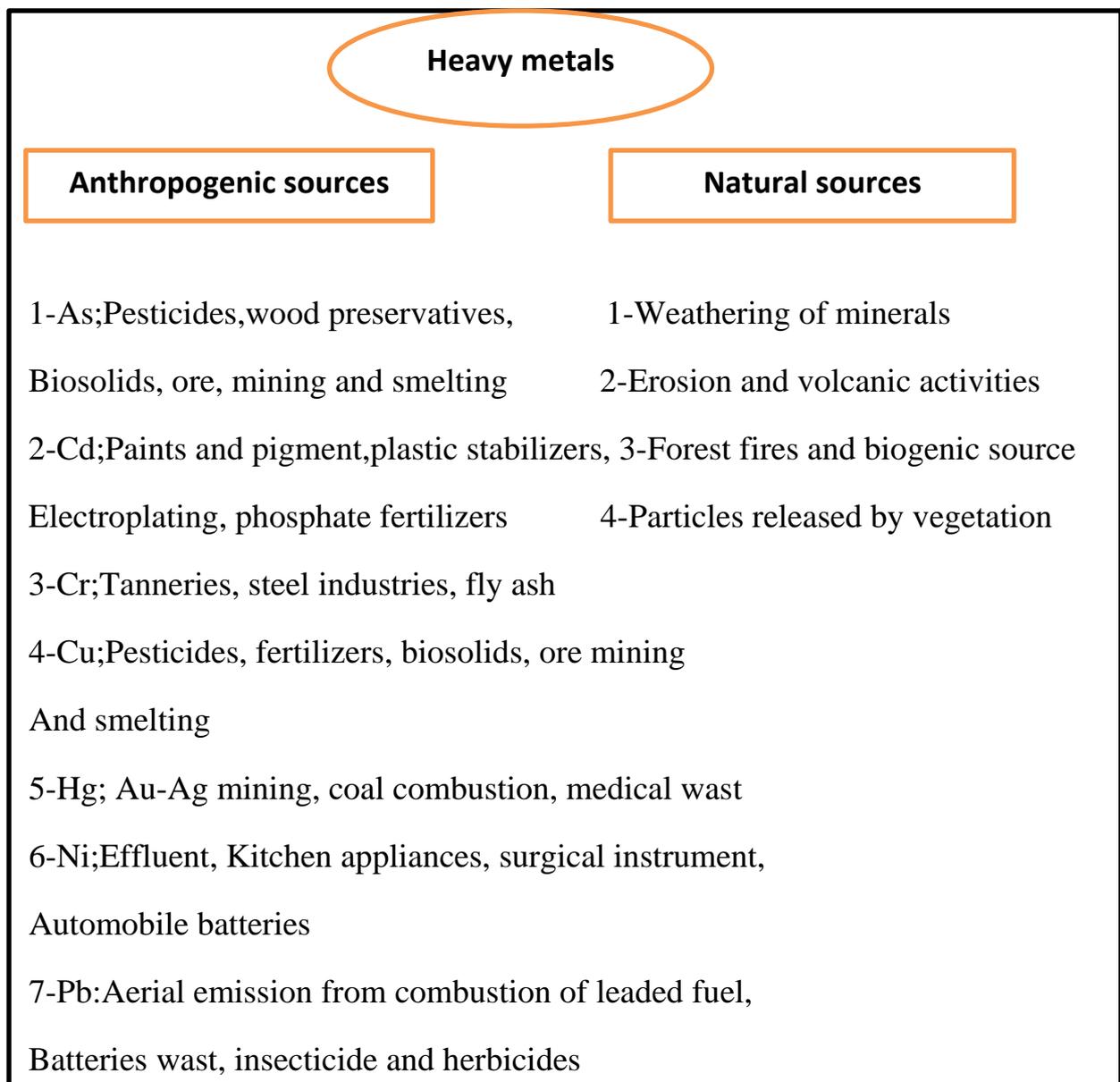


Figure (2-1) Heavy metals sources in the environment (Dixit *et al.*, 2015)

2-3-2 Effect of heavy metals on terrestrial plant.**A- Effect of essential heavy metal**

Copper is an essential metal for normal plant growth and development, although it is also potentially toxic. Copper (Cu) is considered as a micronutrient for plants (Thomas *et al.*, 1998) and plays important role in CO₂ assimilation and ATP synthesis (Jyotish *et al.*, 2015). Study conducted at Malanzkhand Copper Project (MCP) of Hindustan Copper Limited (HCL) at Malanzkhand, district Balaghat, M.P in which it was found that copper dust had adverse effect on various photosynthesis pigmentation secretions in many trees species leaves (Mohnish. and Kumar., 2015). Cu is also an essential component of various proteins like plastocyanin of photosynthetic system and cytochrome oxidase of respiratory electron transport chain (Jyotish *et al.*, 2015). But enhanced industrial and mining activities have contributed to the increasing occurrence of Cu in ecosystems. Cu is also added to soils from different human activities including mining and smelting of Cu containing ores. Mining activities generate a large amount of waste rocks and tailings, which get deposited at the surface. Excess of Cu in soil plays a cytotoxic role, induces stress and causes injury to plants. This leads to plant growth retardation and leaf chlorosis (Lewis *et al.*, 2001). Exposure of plants to excess Cu generates oxidative stress and ROS (Stadtman. and Oliver , 1991)

The function of zinc is to help a plant to produce chlorophyll. Leaves get discolor when the soil is deficient in zinc and plant growth is stunted (Kumar. N., 1984). Zinc deficiency causes leaf discoloration called chlorosis tissue of the veins to turn yellow. Chlorosis by zinc deficiency usually affects the base of the leaf near the stem. Chlorosis appears on the lower leaves first, and then gradually moves up to the plant. In severe cases, the upper leaves become chlorotic and the lower leaves turn brown or purple and die. When plants show symptoms this severe, it's best to pull them up and treat the soil

before replanting. Zinc (Zn) is an essential micronutrient that affects several metabolic processes of plants (Cakmak and Marshner ., 1993) and has a long biological half life. The phytotoxicity of Zn indicated by decrease in growth and development, metabolism and an induction of oxidative damage in various plant species such as *Phaseolus vulgaris* and *Brassica juncea* (Fernandes and Henriques.,1991). Zn have reported to cause alternation in catalytic efficiency of enzymes in *Phaseolus vulgaris* and pea plants (Van *et al.*, 1988). Concentrations of Zn found in contaminated soils frequently exceed to those required as nutrients and may cause phytotoxicity. Zn concentrations in the range of 150–300 mg/kg have been measured in polluted soils (Devries *et al.* ., 2002). High levels of Zn in soil inhibit many plant metabolic functions; result in retarded growth and cause senescence. Zinc toxicity in plants limited the growth of both root and shoot (Choi *et al.*, 1996).

Iron is mainly involved in the process of plant photosynthesis. The micronutrient's availability to plant roots depends on the pH level of the soil with iron more readily available in soil with a low pH. Iron and manganese both play an important role in plant growth and development, but often compete for absorption, as an abundance of one of these micronutrients makes the other less available to plant roots. Iron as an essential element for all plants has many important biological roles in the processes as diverse as photosynthesis, chloroplast development and chlorophyll biosynthesis. Although most mineral soils are rich in iron, the expression of iron toxicity symptoms in leaf tissues occurs only under flooded conditions, which involves the micro-bial reduction of insoluble Fe^{+3} insoluble Fe^{+2} (Becker and Asch., 2005)]. Iron toxicity in tobacco, canola, soybean and *Hydrilla verticillata* are accompanied with reduction of plant photosynthesis and yield and the increase in oxidative stress and ascorbate peroxidase activity (Sinha *et al.*, 1997).

B- Effect of Non-essential heavy metal on plant

Non-essential heavy metal like Cadmium decrease uptake of important nutrient, induce oxidative stress then alternate enzymes and antioxidant mechanisms as well as decrease chlorophyll synthesis (Padmaga *et al.*, 1990)

The toxicity of heavy metals causes abiotic stress on plant. Its effect on the growth of plants and inhibition life processes of plant that it causes decreases yields (Nagajyoti *et al.*, 2010).

The high concentration of heavy metals in plant causes accumulation in plants cell and causes several damage on plant and causes different alteration of biochemical, cellular and physiological level (Bhadur *et al.* ,2012). The plant has the ability to accumulate heavy metals from around environment that is very important in low concentration because some metals like Cu, Zn, Mn, and Fe are required for growth and development of plants because they are catalytic and structural compound of enzymes and proteins (Bhakuni *et al.*, 2012).

The impacts of heavy metals on plants including the reduction of plant growth and modification of root and shoot morphology and physiology (Chibuike and Obiora., 2014). Since they are structural and catalytic components of proteins and enzymes, high concentrations of them and others such as Al, Cd, Cr and Pb affect different physiological and metabolic processes at cellular and organelles levels (Kim *et al.*, 2014).

The impacts of heavy metals is strongly related to their doses, plant species and the plant developmental phase as well as environmental factors characteristic for a given climate zone (Woźniak *et al.*, 2017).

Plants on land tend to absorb lead from the soil and retain most of this in their roots. The uptake of lead by the plant may be reduced with the application of calcium and phosphorus to the soil. Lead (Pb) is one of the ubiquitously distributed most abundant toxic elements in the soil. It exerts

adverse effect on morphology, growth and photosynthetic processes of plants. Lead is known to inhibit seed germination of *Spartiana alterniflora*, *Pinus helipensis* (Morzck and Funicelli, 1982). Inhibition of germination may result from the interference of Lead with important enzymes (Mukherji and Maitra, 1976), observed 60 μM lead acetate inhibited protease and amylase by about 50% in rice endosperm. Lead also inhibited root and stem elongation and leaf expansion in *Allium* species barley and *Raphanus sativus*. The degree to which root elongation is inhibited depends upon the concentration of Lead and ionic composition and pH of the medium (Goldbold and Hutterman., 1986). A high lead level in soil induces abnormal morphology in many plant species. For example, lead causes irregular radial thickening in pea roots, cell walls of the endodermis and lignification of cortical parenchyma (Paivoke., 1983). High pb concentration also induces oxidative stress by increasing the production of ROS in plants (Reddy *et al.*, 2008).

2-4 Alternative water sources

Water demand increased in current century because of increase human population, large pressure of water supply and consequences to water shortage over the world because of fast growth of population, agriculture, and industry sectors. Besides, natural water sources such as lakes, river basins, and streams, alternative water source are also began eager by human to overcome the decrease of water in the future. Therefore, humans tend to be starting researching and developing on other water supplies. By exploring the new water resources and create new technologies to generate extra or replace current water supply to increase water source for current situation and future . (Boyle and Reynolds., 2010) .

Conventional sources of water are those that in principle are of high quality and low cost to exploit. Historically, surface water (river, lakes and dams) and shallow freshwater from the subsoil have been considered as the

conventional sources for water, making the ocean, brackish water from subsoil or estuaries, rainwater, water from irrigation drainage, storm water and very deep aquifers, as the non-conventional sources for water (Jiménez , 2001) .

Non- Conventional sources for water such springs , wells , rain water, derange , urban run-off , storm water and grey water (all wastewater generated in households or office buildings from streams without fecal contamination) are used when decreased water in site where the resource is scarce. It can facilitate the use alternative water by using many emerging technologies based on new development in physiochemical and biological processes. This will create jobs not only through technology development, because it enable new forms of small scale intensive uses of water such as cultivation of highly profitable crops in small plots and but also in the operation and maintenance of treatment plant to reclaims water and increasing worldwide use of municipal waste water and grey water along with the recycling of water within industries, as well the recycling of industrial waste water . Use of municipal waste water can represent up to 35% of the total water extracted for use in some countries (Jimenes and Asano , 2008) .

The use of green roof, rain water harvesting and other green infrastructure is gaining benefits in some urban environment. It has direct effect on decreasing water consumption in addition to decreasing risk of flood through increasing storage, decreasing emerge consumption through evaporative cooling and improving the urban environment. New technologies for water extraction and purification will can use new resources of water such as desalination , fog interception and rain water harvesting (Cashman., 2014) .Many studies explant ability of drainage water as alternative water resources like watering plants ,cooling of towers of energy ,etc (Gabr., 2019) .

2-5 The Previous Studies

Kazar, (1999) has done several tests for the irrigation project of Hilla - Kifil, where he took a group of samples from several sites of the main drainage water and conducted chemical analyses to determine the quality of the water for use in irrigating crops. He concluded from his study that the drainage water can be successfully used to irrigate different crops.

Al-Othman (2008) made a study on the possibility of using drainage water for irrigation in the city of Riyadh, Saudi Arabia, and he noticed in his research that the risks of salinity and sodium for drainage water in Riyadh, where it was from high to very high salinity and there are also risks for the proportion of sodium from slight to very high.

Al-Maliki, (2013) made a study on the possibility of using the Husseiniya drainage water in Kut, Iraq to irrigate agricultural crops. The results showed that there were no harmful effects of sodium indicators on crop production with the presence of the problem of salinity. So results showed that the drainage water of the Husseiniya sector can be used directly to irrigate wheat and barley without reducing the productivity of the crop, but not suitable for corn crop, unless mixed with fresh water to remove salinity.

Ali, (2014) conducted a study in the eastern Euphrates drainage system in the city of Babylon and included a study of the potential environmental pollutants of various decorations on the eastern Euphrates drainage system. The results showed that the electrical conductivity ranged from (4360-7400) micro Siemens/cm, and the salinity values during the study period ranged between (2.97-4.73) so the trocar water was prepared as saline, and the range of pH values were (7.5-8.72). The study showed that the average concentration of heavy metals concentration of iron, cadmium, lead and

copper were 113.89, 6.35, 1.5 and 0.8 μg / liter, respectively. The results confirmed the high concentrations of heavy metals in plant tissues, where the highest rate of iron was recorded at 1536.78 μg / g, while for copper it was the lowest concentration of elements in plant tissues was 46.65 μg / g, and this indicates the phenomenon of bioaccumulation in plants.

Habeeb (2015) studied the pollution in the eastern Euphrates drainage by determining some of the physical and chemical properties of water, as well as the concentrations of heavy metals in both water and drainage plants, and found that the drainage is not contaminated with any of the elements(Fe, Cd, Pb, Cu) according to the Iraqi determinants for the maintenance of a drain, the highest concentration of heavy metals recorded in iron (113.89) $\mu\text{g/L}$ and Copper recorded the lowest concentration as it was (0.8) $\mu\text{g/L}$ in drainage water. The concentrations of the studied elements tend to increase in aquatic plants in the drainage, where the highest content of lead, then cadmium, then copper and finally iron.

Hassan (2015) studied the eastern-Shamiya drainage system in Diwaniyah. She concluded that the drainage water can be used directly to irrigate rice, wheat and barley without any decrease in productivity. It is not possible to directly irrigate the corn crop due to salinity unless it is mixed with raw water to get rid of salinity.

Dhayef, (2015) made a study on the water of the Kifl drainage in Babylon to determine the concentrations of heavy elements and their effect on the Kifl drainage. Concentration of Zn, Pb, Cu, Cd and Cu were studied to show whether the study area is contaminated with heavy elements or not and shows the concentration of each element in soil and water, and the results showed that all samples were not contaminated with heavy metals, results explain that each samples of water and soils were not polluted with these elements.

Al-Tae, (2018), showed the effect of water of the main eastern drain on some physical and chemical properties of the water of the Euphrates River in the Samawah city during the seasons of the year for the period from Autumn/2007 to Summer/ 2008, where three stations were chosen for the study. Temperature, light transmittance, turbidity, electrical conductivity, salinity, pH, total available carbon dioxide, alkalinity, dissolved oxygen, oxygen saturation, total hardness, calcium and magnesium were measured. The study showed a clear effect of trough water on the physical and chemical properties of Euphrates River water in the study area. The results of the statistical analysis showed that there were significant differences in the studied characteristics among the study stations

CHAPTER THREE

MATERIALS

AND

METHODS

3. MATERIALS AND METHODS:**3-1 Laboratory Equipments and Instruments**

Laboratory Equipment and Instruments	Manufacturer/Origin
Bench centrifuge	Memmert(Germany)
Disposable and glassware	Cito(China)
Distillator	GFL(Germany)
Electric sensitive	Denver(USA)
Flame Atomic Absorption Spectrophotometer	Shemadzu
High speed cooling centrifuge Eppendorf centrifuge	Hitachi(Germany)
Jasco V-550 UV-vis spectrophotometer	
Micropipette	Capp(Denmmark)
Multi meter	PCSTESTR35, Oakton; U.S.A
Oven	Gallen Kamp
Refrigerator	Concord(Lebanon)
Sensitive Balance	Sartorius
Spectrophotometer SP-300	Optima
Ultra Centrifuge	Damon /IEC Division
UV-Vis spectrophotometer	Analytic Jena(Germany)
Water bath	Memmert(Germany)

3-2 Chemicals

Table (3-2): Chemical and Biological Materials

Chemical	Company/Origin
Ammonium Ferrous Sulphate [(NH ₄) ₂ So ₄ .FeSo ₄]	BDH\England
Ascorbic acid	BDA
Diphenyl amine	BDH\England
Erochrome blak T	Merck
Feron Indecator	BDH
Ferrous ammonium	BDH
Hydrofloric acid	Fluka
Metheline blue	Fluka
Nitric Acid (HNO ₃)	Central Drug House Ltd (CDH) \India
Normal saline	Karada Pharma\ Iraq
Perichloric acid(HClO ₄)	Himedia\U.S.A
Potassum dichromate	BHD\England
Sodium chloride	BHD\England
Sodium hydroxide	BDH
Sulphuric acid (H ₂ SO ₄)	Chem -Lab\Belgium
Sucarose	Fluka
Prolein	Fluka
Thiourea	Fluka
Toleoeen	Fluka
Nin Hydreine	Fluka
Phosphate buffer	Mercke

Bovine serum albumin	Mercke
Aceton	Mole
Murexied	Mercke
Potassium kromate	Mercke
Silver nitrate	Fluka
Ethelene diamene tetra acitiic acid	BDA
Xylenol orange	Mercke

3-3 Eastern Euphrates drainage water

The Eastern Euphrates Drain is located in Babylon city, 110 km south of the city of Baghdad. Its area between longitudes “13°44 - 26°44” and latitude “13°32-43°32”. It is 261 kilometres long and about 3.5 meters wide, and includes the area between the Hilla River to the east, Al-Kifl stream to the west, Saddat Al-Hindiya to the north, and the Hilla-Kifil road to the south. The water level in it is 19-30 m/min (Habeeb, 2015).

3-4 Characteristics of studied water

Two types of water were used to irrigate plant. First one was collected from Eastern Euphrates drainage (near Zaid Al-shahed) as explained in figure (3-1) to explain its effects on both Basil (*O. basilicum*) and Spinach (*S. oleracea*), while the second was tap water used as control.

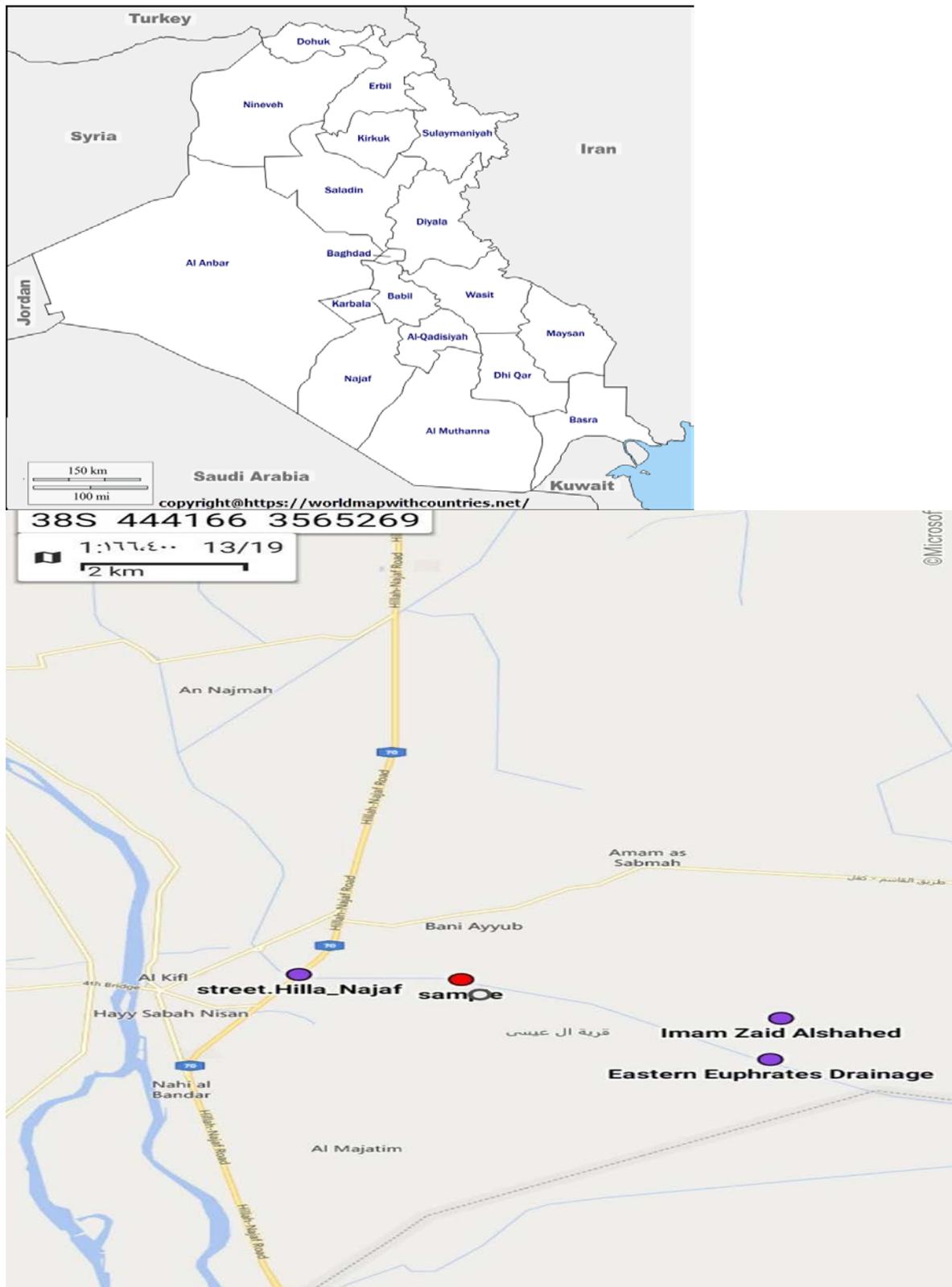


Figure 3-1 site of sample collection

3-4-1 pH

The pH was measured by pH-meter (multi-parameters), PCSTESTR35, Oakton; U.S.A in the field directly.

3-4-2 Electrical Conductivity (EC)

EC was measured in the field by multi-parameters, PCSTESTR35, Oakton ;U.S.A in the field directly and expressed using the units ($\mu\text{s}/\text{cm}$).

3-4-3 Total Dissolved Solid (TDS)

Total dissolved solid (TDS) was measured by multi-parameters, PCSTESTR35, Oakton ; U.S.A in the field directly and expressed using the units (mg/l).

3-4-4 Salinity

Salinity was measured by multi-parameters, PCSTESTR35, Oakton - U.S.A in the field directly and expressed with (‰).

3-4-5 Chlorides

The concentration of chloride in water were measured according to Argenometric methods by using potassium chromate as indicators and the following equation was to calculate as (mg/l). (APHA, 2013)

$$\text{mg } \backslash L(\text{Chlorid}) = \frac{a - b * N * 35450}{\text{mL}(V)} \times 1000$$

a : volume of AgNO_3 (ml) used with sample

b: volume of AgNO_3 (ml) used with blank

N: normality of AgNO_3

3-4-6 Total hardness

The total hardness determined by titration methods with Na₂- EDTA and used Eriochrom Blak T (EBT) as indicators (Lind,1979).

The concentration calculated as (mg/l) of Carbonate

$$mg \ L(Total \ hardness \ as \ CaCO_3) = \frac{a \times b}{mL(Sample)} \times 1000$$

a: EDTA volume (ml) used with sample

b: mg of CaCO₃ equivalent to 1 ml of EDTA

3-4-7 Alkalinity

To calculate Alkalinity, 10 ml of water were used then add some drops of methyl orange then titrated with 0.1N of HCl (APHA, 2013)

3-4-8 Calcium

To calculate calcium titration, EDTA-2Na were used and NaOH was added and used Mroxid was used as an indicator (Lind,1979), and calculate the concentration by following equation:-

$$mg / L(Ca) = \frac{a \times b}{V(ml)} \times 400.8$$

a: EDTA volume (mg) used with sample

b: mg of CaCO₃ equivalent to 1 ml of EDTA

3-5 Characters of soil

3-5-1 Soil texture

Soil Texture was estimated using Hydrometer (gm/l) capacitor (ASTM) (15H) and depending on the method described in Ryan (2000) using texture triangle .

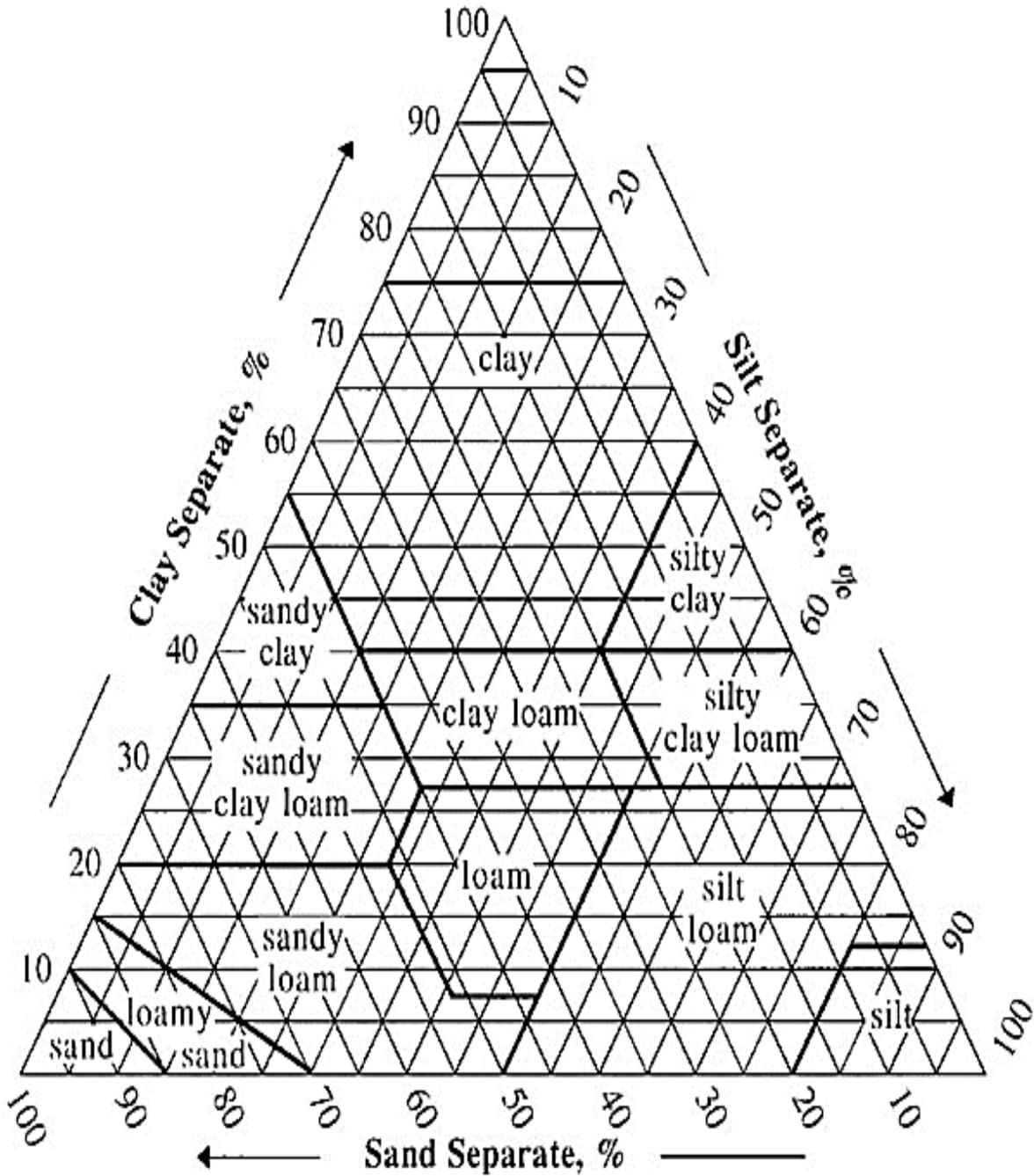


Figure (3-2) Soil texture triangle (Ryan, 2000)

3-5-2 pH

Soil extract (1:5) was used to determine soil pH by multi-parameters, PCSTESTR35, Oakton; U.S.A

3-5-3 EC

Soil extract (1:5) was used to determine soil EC by multi-parameters, PCSTESTR35, Oakton ; U.S.A

3-5-4 Total organic matter

Soil organic matter was estimated by wet oxidation method according to Walkley 1947 and described in (ECARDA) as follows:-

1-Weight 1gm air \ dry soils into a 500ml beaker .

2-Add 10ml 1.0 N-potassium dichromate solution, then add 20ml concentrated sulfuric acid by dispenser rotate the beaker for mix the suspension . leave it to settle down for 30 min .

3-Add 200 ml deionized water , then add (10ml) concentrated phosphoric acid ,leave the mixer to cool .

4-Add 10-15 drop diphenylamine sulphate as a indicator .

5-Titration with ferrous sulfate solution until the color change from blue – violet to bright green .

The percentage organic matter in soils

$$X = \frac{V_{\text{sample}}}{V_{\text{blank}}}$$

$$Y = 1 - X$$

$$\% \text{O.M (w/w)} = \frac{(Y * 0.67 * 10.5)}{W_t}$$

Where : W_t = Weight of (air-dry) soils (gm)

V_{blank} = Volume of (ferrous – ammonium sulfate solution) needful to titration the blank (ml).

V_{sample} = Volume of (ferrous – ammonium sulfate solution) needful to titration the sample (ml).

3-5-5 Calcium

The concentration of calcium was determined according to (ICARDA, 2001) by using meroxide as indicator and EDTA for titration.

3-5-6 Cation Exchange Capacity (CEC)

The exchange capacity of the positive ions of the soil under study was estimated by the methyl blue method (Savant, 1994) by placing 2 gm of soil sieved with a 2 mm sieve in a flask, adding 50 ml of 1% sodium carbonate solution, and shaking mechanically for a quarter of an hour, then crushed by adding 0.01 M of dye Methyl blue and stirring for one minute. Then a drop of the resulting solution was taken and placed on a Watman No.1 filter paper until a central halo is an indication of the end of the reaction. The value of the exchange capacity of the positive ions was calculated according to the following equation:

The exchange capacity of positive ions in soil mol/kg = ml of methyl blue dye *0.553

3-6- Agriculture of Plants

It was used two species of plants *Spinacia oleracea* and *Ocimum basilicum* to explain the effect of drainage water. 20 Plastic pots each contain about five kilograms of soil, and divided in tow groups according to plant species which planted its seed in pots. Then each group divided in two sub groups, one irrigated with drainage water while the other with tab water. This experiment was done in the green house in Biology Department/Collage of Sciences / Babylon University.

3-6-1 *S. oleracea*

Spinach (*Spinacia oleracea*) is a leafy green flowering plant native to central and western Asia (Subhash *et al.*, 2010). It is of the order Caryophyllales, family Amaranthaceae, subfamily Chenopodioideae. Its leaves are a common edible vegetable consumed either fresh, or after storage using preservation techniques by canning, freezing, or dehydration. It may be eaten cooked or raw, and the taste differs considerably; the high oxalate content may be reduced by steaming (Herbst, 2001)

Scientific Classification

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Order: Caryophyllales

Family: Amaranthaceae

Genus: *Spinacia*

Species: *S. oleracea*

Binomial name: *Spinacia oleracea*



Figure (3-3) *S. oleracea*

3-6-2 *O. basilicum*

Basil (*Ocimum basilicum*), also called great basil, is a culinary herb of the family Lamiaceae (mints). It is a tender plant, and is used in cuisines worldwide. In Western cuisine, the generic term "basil" refers to the variety also known as sweet basil or Genovese basil. Basil is native to tropical regions from Central Africa to Southeast Asia. In temperate climates basil is treated as an annual plant, however, basil can be grown as a short-lived perennial or biennial in warmer horticultural zones with tropical or Mediterranean climates (Simon and James, 2018).

Scientific Classification

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Asterids

Order: Lamiales

Family: Lamiaceae

Genus: *Ocimum*

Species: *O. basilicum*

Binomial name: *Ocimum basilicum*



Figure (3-4) *O. basilicum*

3-7 Plant biochemical responses

3-7-1: Photosynthetic pigments estimation.

Two hundred fifty mg of fresh leaves was homogenized with 85% acetone. The sample filtration by centrifuged and the absorbance taken at 663, 646, and 470 nm. The concentrations of the pigments were estimated and expressed as $\text{mg}\cdot\text{g}^{-1}$ fresh weight (Lichtenthaler, and Wellburn, 1983).

Chlorophyll a = $12.25 A_{663.2} - 2.79 A_{646.8}$ (mg per ml solution)

Chlorophyll b = $21.50 A_{646.8} - 5.10 A_{663.2}$ (mg per ml solution)

Total carotenoids: $C_{x+c} = (1000 A_{470} - 1.82 C_a - 85.02 C_b) / 198$ (mg per ml solution).

3-7-2: Estimation of protein

Five-hundred mg of sample was ground well with 15 ml of phosphate buffer (0.2M, pH7.2). The extract was centrifuged for 10 minutes at 3000rpm. The lower layer was discarded and the supernatant was taken. To the supernatant, an equal amount of cold 5% TCA was added. It was left for 30 minutes in an ice bath. The precipitated protein was taken and again centrifuged. The supernatant was discarded and the pellet was dissolved in 25 ml of 0.2N NaOH. From this, 1 ml was taken and mixed with 4 ml of alkaline copper reagent. It was shaken well and was allowed to stand for 10 minutes at room temperature. Then 0.1 ml of diluted Folin phenol reagent was added and mixed well. After 20 minutes, the optical density (O.D) was read at 650 nm using UV-VIS Spectrophotometer. Bovine Serum Albumin was used as the standard (Lowry *et al.*, 1951).

3.7.3: Determination Total Antioxidant Activity

The total antioxidant activity was determined by phosphomolybdenum method. It is based on the reduction of MO (VI) to MO (V) by the sample and subsequent formation of a green Phosphate/ MO(V) complex at acidic pH. The absorbance is measured at 695nm using an UV/Vis spectrophotometrically. The Antioxidant capacity was expressed as Ascorbic acid equivalent (AAE) by using the standard Ascorbic acid.

Standard solution was prepared as: 50mg of Ascorbic acid was dissolved in 50ml standard flask using distilled water (conc., 1mg/ml).

Extract solution was prepared by dissolved 50mg of methanolic dried extract in 50ml standard flask using distilled water (conc., 1mg/ml), then the following steps dependent to determine total antioxidant activity concentration: (50-250 µg) of the buffer was prepared and extracted, 0.3 ml of each sample was taken in succession and 3.0 ml of phosphomolybdenum reagent was added to all tubes and 0.3 ml of water and 3.0 ml of reagent alone was used empty and all tubes were incubated at 97 °C for 90 min, then cooled and the absorbance measured at 695 nm using a UV/Vis spectrophotometer against vacuum. The antioxidant capacity was expressed as equivalent to ascorbic acid using standard ascorbic acid.

3-7-4: Estimation of proline

Proline was estimated according to the method of (Bates *et al.*, 1973). Briefly, a sample of 0.5 g fresh leaf tissue was homogenized in 10 mL of 3% sulfosalicylic acid and the homogenate was centrifuged at 13000 rpm for 10 min at 4 °C. Then 2 ml of the supernatant was mixed with 2 mL of acid ninhydrin and 2 mL of glacial acetic acid. This mixture was incubated at 100 °C for 1 h and then cooled at room temperature. Finally, 4 mL of toluene was added. Proline was extracted from the toluene layer and its absorbance was noted at 520 nm using toluene as a blank or reference. The proline concentration was determined from a standard curve.

3-7-5: Estimation of Malondialdehyde (MDA):

Malondialdehyde was estimated by Thiobarbituric acid (TBA) assay method of (Buege & Aust, 1978) on spectrophotometer .

Stock TCA – TBA – HCl Reagent:

It was prepared by dissolving 15% W/V trichloroacetic acid and 0.375% W/V thiobarbituric acid and 0.25N HCl to make 100 ml (2.1 ml of concentrated HCl in 100 ml). This solution was mildly heated to assist in the dissolution of TBA. Dissolved 15 gm TCA and 0.375 mg thiobarbituric acid in 0.25 N HCl and volume was made up to 100 ml with 0.25 N HCl.

Calculation:

$$\text{Malondialdehyde}(\mu\text{mol/l}) = \frac{\text{Absorbance of sample}}{E_o \times L} \times D$$

Where:

E_o = Extinction coefficient $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$

L= light path cm.

D = dilution factor = 6.7×10^6

3-7-6: Reactive Oxygen Species determination

Reactive oxygen species (ROS) were evaluated according to the FOX2 method (Erel, 2005). The FOX2 test system is based on the oxidation of ferrous ion–odanisidine complex to ferric ion by the various types of oxidants contained in the plasma samples. The Fe(III) ion produced is bound by xylenol orange, forming a complex with an absorption peak at 560 nm. The FOX2 assay works with two reagents that are stable for at least 6 months at 4°C: reagent 1 contained 150 μM xylenol orange, 140 mM NaCl and 1.35 M glycerol in 25 mM H_2SO_4 ; reagent 2 contained 5 mM ferrous ammonium

sulfate and 10 mM *o*-dianisidine dihydrochloride in 25 mM H₂SO₄. For each sample, the procedure was as follows: 140 µl of the sample were added to 900 µl of reagent 1 and 44 µl of reagent 2. The samples were vortexed and incubated at room temperature for 30 min. Following incubation, absorbance was measured at 560 nm using a Jasco V-550 UV-vis spectrophotometer.

3-7-7 Estimation of Vitamin C

3 g of fresh sample was ground with 25 ml 4% oxalic acid and filtered. 2 ml aliquots of the above extract were pipetted into each of the different test tubes and the volume was made up to 3 ml with distilled water. 1 ml of 2% DNPH (2, 4-dinitro phenyl hydrazine) reagent and 1 or 2 drops of 10% thiourea were added to each test tube. The contents of the test tubes were mixed thoroughly and incubated at 37°C for 3 hours. After incubation, 7 ml of 80% sulphuric acid was added to each test tube to dissolve the orange red oxazole crystals and the absorbance was measured at 540 nm against a reagent blank. The ascorbic acid content present in the sample was calculated by referring to a standard graph of ascorbic acid (Sadasivam and Manickam, 1992).

3-7-8: Determination of reduced glutathione (GSH)

Reduced glutathione in the plant tissue was determined according to the method of (Moron *et al*, 1979) which included

100 µl of plant extract was mixed with 100 µl of 25 % TCA, kept on ice for few minutes. These were then subjected to centrifugation at 3000 g for few minutes to settle the precipitate. 300 µl of the supernatant was mixed with 700 µl of 0.2 M sodium phosphate buffer (pH 8) and 2 ml of 0.6 mM DTNB (prepared in 0.2 M buffer, pH 8). The yellow color obtained was measured after 10 min at 412 nm against a blank which contained 0.1 ml of 5% TCA in place of the supernatant.

3-8 Heavy Metals concentration:

3-8-1 Heavy Metals in water

Concentrated hydrochloric acid (5 ml) was added to 1 L of the filtrate and concentrated by oven (800C) until evaporate to 100 ml and then measured by Flame Atomic absorption - spectrophotometer model (7000) Aa type Shimadzu, Japan was used to determine the concentrations (mg/l) of the elements (APHA, 2013).

3-8-2 Heavy Metals in soil

Soils samples for heavy metals determination were digested according to the procedure described by Sharidah (1999). One gram of dried soil samples was digested with (10ml) di-acid mixture (9ml HNO₃, 4ml HClO₄). The mixture was boiling gently in sandy bath on hot plate until fumes are released, after cooling and filtering through Whatman No.42 filter paper and <0.45µm Millipore filter paper and transferred quantitatively to 25 volumetric flask by adding distilled water. The concentration of (Cu,Fe,Cd,Pb) were determined by Flame Atomic absorption - spectrophotometer model (7000) Aa type Shimadzu, Japan was used to determine the concentrations (mg/l) of the elements (APHA, 1985) (Figure 2-2).

$$M. Con = \frac{A * B}{W}$$

Where :

M.C= Metal Concentration in mg/kg

A= Element concentration on sample (reading devise) in mg/l

B= The final volume of the sample in ml =25

W= Weight of the sample in gram =1 gm

3-8-3 Heavy Metals in plant

Digest plant samples (root and shoot) using Haynes (1988) method , (0.2 gm) dry plant samples were weighted put in a flask of digestion add 3ml sulfuric acid quit samples for 1 day after that 1ml from mixture (sulfuric acid + perchloric acid) the flasks are placed on the digestive system hot plate and the fumes are then detected and the color of the samples is gradually changed until the colorless solution is obtained cool the samples and complements the size of each sample to 50 ml with add D.D.W.

All samples are preserved in polyethylene bottle until measuring by Atomic Absorption Spectrophotometer type 6300 (Shimadzu, Japan) .All results were expressed as $\mu\text{g/L}$ (APHA, 2013). Dissolved heavy metal concentration was calculated according to the following equation UNESCO (1992) which was clarified by Al-Taei (1999).

3-9 Statistical analysis

Data was analyzed using SPSS(version 28, SPSS Inc. Chicago, Illinois, USA). Descriptive statistics (mean, standard deviation), Student's t-test was used.

CHAPTER

FOUR RESULTS

AND

DISCUSSION

4- Results and Discussion

4-1 Characteristics of water:

Water is the most important factors among the other environment factors in the distribution, survival and growth of plants, having an effect on all their biotic processes (Marshall, 1988).

The results of the current study for drainage water explained that each of total alkalinity and chloride concentration were less in their concentration in tap water with a significant decrease ($P \leq 0.05 = 0.00$), so the total hardness recorded a significant variation but it was higher in tap water compared with the drainage water as explained in table (4-1), with pH 7.99 which was less pH in tap water but without significant variation. Calcium concentration in drainage water was significant increase (144.3) mg/l while record 96.3 in tap water.

So both TDS and EC were less in their levels in tap water, but drainage water not record a significant increase in them, as explained in table (4-1). All drainage systems tend to be elevated concentration of TDS and may reach several thousand of mg/l depending on soil characteristics, ground water and environmental factors (Skaggs *et al.*, 2012), the decrease of some studied parameter of water may be due to decrease pollution in the drainage water (Habeeb, 2015), and may it has a feeding from Euphrates River because according to our observation it was with moderate flow rate (drainage usually with very slow flow rate), lead to a decrease in the level of ground water in Iraq regions (Sulaiman, 2013)

Total alkalinity is an important parameter to evaluate ability of all uses of water like plant irrigation (APHA, 2013). It is effected by CO_2 concentration, microorganisms, algae and aquatic plants in water (Al-Saadi *et al.*, 1989), as well as pH value as when pH less than 9 bicarbonate level increases which

raises alkalinity (Wetzel., 2001). Result of water analysis showed concentration of total alkalinity was 280 mg/l which according to drains limits is low, which may due to slight alkalinity pH =8.08.

Table (4-1) Some studied parameters of drainage water and tab water

Parameter	Units	Tab water	Drainage water	P value
pH		7.99	8.08	0.934
Total Alkalinity	mg/l	240	280	0.00
Chlorides	mg/l	639.4	689.3	0.00
Total hardness	mg/l	880	680	0.00
Calcium hardness	mg/l	96.19	144.3	0.002
EC	ms/cm	1465	1560	0.080
TDS	mg/l	102	107	0.341

4-2: Characteristics of Soil

Soil is an essential component in in the earth ecosystem and it characters with several physical and chemical feathers that changeable these soil has a significant effect on terrestrial plants (Reddy *et al.*, 2008).

Both *O. basilicum* and *S. oleracea* are well growing in a sandy soil (Parwada *et al.*, 2020). The soil used in this experiment was sandy with 85.59 %, 8.55% and 4.97% of sand, silt and clay, respectively (figure 4-1). Sandy soils less charge of ions so they are less capable of absorbing ions (Sonmez, 2008). Soil texture influences the EC, anion and cataion concentrations (Ozcan *et al.*, 2006).

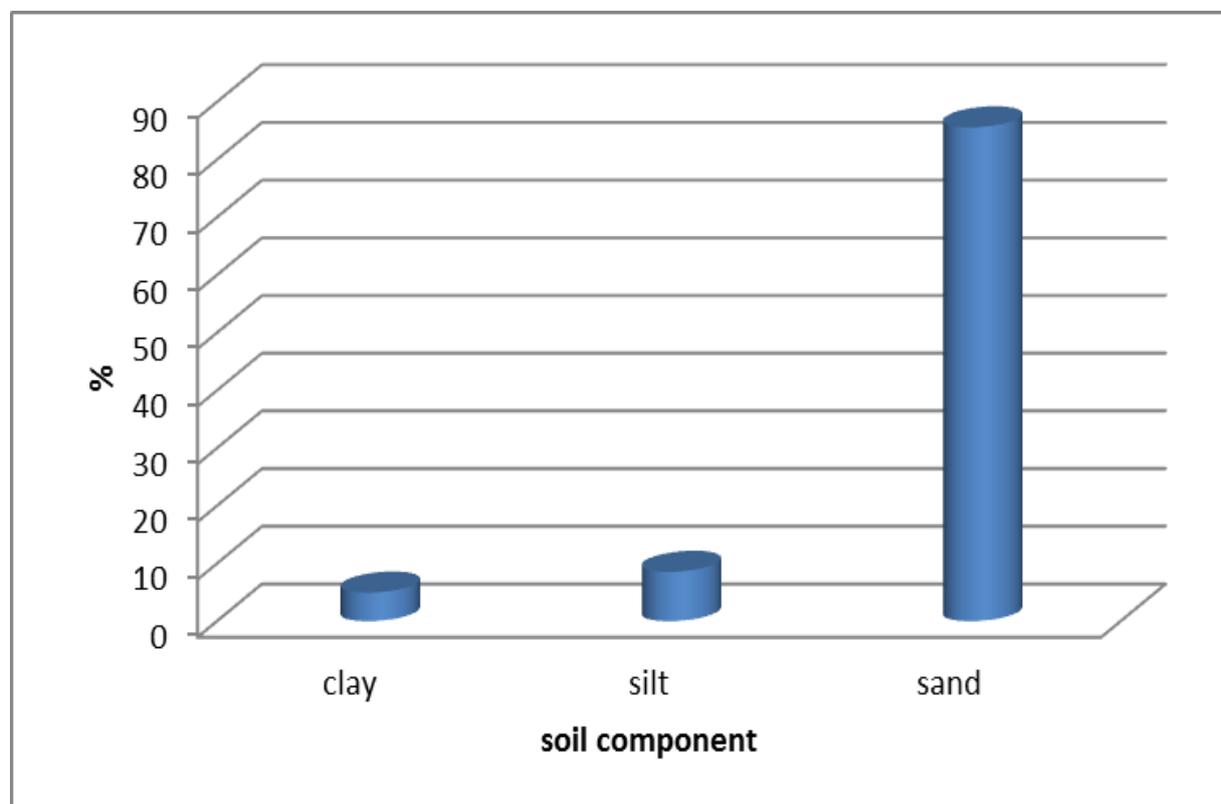


Figure (4-1) Used Soil Component

Main chemical characteristics of used soil in this work are illustrated in table (4-2). pH is an active factor that controls the solubility of ions in soil (Conklin, 2005). Results showed a significant variations among pH values of soil before planting and after irrigation that it was 7.4 then became 7.6 ($p \leq 0.05=0.008$). The increase in the pH due to the nature of used water which as explained in table (4-1), was alkaline (as total alkalinity content). Most terrestrial plants prefer to live in neutral to slightly alkaline soil (Agren *et al.*, 2012). Thus, it is very important to evaluate effect of irrigated water on pH soil.

Table (4-2a): Characters of soil before planting and after irrigated with tap water in studied species

Plant species	Parameter	Before planting (mean± SD)	After irrigated with Tap water	P value
<i>O. basilicum</i>	Ca (mg/l)	5.00±2.00	15±1.00	0.000
	pH	7.40±0.10	7.6±0.00	0.092
	EC (mmoh/cm)	0.46±0.01	0.83±0.01	0.007
	CEC (meq/100gr)	1.61±0.001	0.54±0.001	0.001
	CL (mg/l)	11±1.00	14±1.00	0.041
	TDS (mg/l)	241±1.732	279±3.00	0.261
	Organic matter	0	0.067	0.00
<i>S. oleracea</i>	Ca (mg/l)	5.00±2.00	20±1.00	0.000
	pH	7.40±0.10	7.6±0.00	0.421
	EC (mmoh/cm)	0.46±0.01	0.36±0.01	0.017
	CEC (meq/100gr)	1.61±0.001	1.07±0.01	0.021
	CL (mg/l)	11±1.00	13±1.00	0.057
	TDS (mg/l)	241±1.73	260±5.00	0.021
	Organic matter	0	0.066	0.00

Table (4-2 b): Characters of soil before planting and after irrigated with drainage water in studied species

Plant species	Parameter	Before planting (mean± SD)	After irrigated with Drainage water	P value
<i>O. basilicum</i>	Ca (mg/l)	5.00±2.00	35±1.00	0.00
	pH	7.40±0.10	7.6±0.00	0.092
	EC (mmoh/cm)	0.46±0.01	0.44±0.01	0.081
	CEC (meq/100gr)	1.61±0.001	1.07±0.01	0.031
	CL (mg/l)	11±1.00	12±1.00	0.620
	TDS (mg/l)	241±1.732	288±56.012	0.041
	Organic matter	0	0.03	0.00
<i>S. oleracea</i>	Ca (mg/l)	5.00±2.00	25±1.00	0.00
	pH	7.40±0.10	7.6±0.00	0.421
	EC (mmoh/cm)	0.46±0.01	0.44±0.01	0.084
	CEC (meq/100gr)	1.61±0.001	1.61±0.001	1.00
	CL (mg/l)	11±1.00	10±1.00	0.062
	TDS (mg/l)	241±1.732	310±2.00	0.022
	Organic matter	0	0.03	0.000

Soil electric conductivity (EC) refers to the amount of dissolved salts in soil (Shakir, 2011). It refers to the ability of solution to transfer the electricity supply. The drainage water caused a significant decrease in EC of soil for both *O. basilicum* and *S. oleracea* (EC=0.44 mmohs/cm). The decrease in EC may be due to plant are used the ions in drainage water to grow that because used soil was not rich enough with nutrients.

Cation exchange capacity (CEC) refers to all cations concentration that are adsorbed by soil particles at specific PH value (Lorenz and Kahr., 1996).

Results of studied CEC showed decreased significant in soil that planting with *O. basilicum* to be 1.07 meq/100gm, this decrease may due to nature of *O. basilicum* which need high concentration of cation (Balakrishnan *et al*, 2018). In contrast, soil of *S. oleracea* does not show any difference in CEC .The significant decrease of CEC in soil of the two studied species may be due to the increase in the concentration of organic matter which is very effecting on solubility of ctaion then make them can be absorbed by plant (Astera, 2010). The used soil was without organic matter that due to used soil was vary sandy and was washed before using it for planting so the elevated temperature in month before planting may oxidize organic matter. Root of plant adding some organic matter to soil (Cochrane and Aylmore., 1994). That explained the significant increasing in organic matter content after planting and irrigated with drainage water (0.03% , $P \leq 0.05 = 0.00$). The same increase in both soils that planting with *O.basilicum* and *S.oleracea* and irrigation with drainage water is related to the same value of pH (7.6) which is an effecting factor on organic matter content (Benavides., 2014), or may due to same concentration of organic matter in drainage water. The significant increase of organic matter in soil of two species that are irrigated with tap water (0.063%) may be related to polluted tab water with organic matter and many studies indicate same case (Al-Yasari., 2012)

Total dissolved solids (TDS) in water describe the content of all solids that dissolved in water and pass through a filter paper (0.45 μ) and it used as parameter to determine soil salinity in some cases (WHO, 1996). The results in table (4-2a) showed non-significant variation in TDS among used soil and *O. basilicum* soil that was irrigated with tap water and drainage water. The high value in soil of *S. oleracea* irrigated with drainage water was (310 mg/l) as in table(4-2b), which may be due to nature of Spanish which needs certain salts in high amount but not all dissolved solids (Fekry and Nawar., 2017) .

Chloride ions are very important in any soil to determine soil if saline or not (Moore, et al, 2006). Drainage water does not have any significance on soil Cl⁻ content but it recorded significant increase in tap water in two species as in table (4-2b) , so plants are used all Cl in their physiological process to control osmosis (Colmenero *et al.*, 2019).

Calcium is one of the important element in plant growth (Adily, 2014). The high concentration of Ca was (35 Mg/l) in soil of *O. basilicum* irrigated with drainage water then in soil of *S. oleracea* (25mg/l) irrigated with same water. The significant increase ($P \leq 0.05-0.00$) in soils irrigated with drainage water due to elevated Ca concentration in this water (144.28mg/L) as shown in table (4-1b).

4.3: Biochemical responses of studied plants after irrigated with drainage water

4-3-1 Chlorophyll a and b

Higher plant is identified with their content of both chlorophyll a and chlorophyll b. Chlorophyll is used to as a bio indicator to explain the effect of environmental factor (Sitko, 2017). Plant growth and yield are directly related to photosynthetic performance. Chlorophyll is the main photosynthetic pigment and plays an

important role in plant photosynthesis (Tang *et al.*, 2020). Salt stress decreases chlorophyll synthesis and inhibits photosynthesis (Barhoumi *et al.*, 2007).

This study showed non-significant decrease in Chlorophyll a in both *O.basilicum* and *S.oleracea* when irrigated with drainage water, as explain in figure (4-3a and 4-3b). Higher value (7.5) mg/g.f.w recorded in *S.oleracea* irrigated with tap water. Drainage water caused a significant decrease in Chlorophyll b content in *S. oleracea* when used drainage water but its record significant decrease Chl b in *O.basilicum* (Pvalue \leq 0.05 =0.025), as shown in figure (4-2a and 4-2b). In contrast chlorophyll b in *S. oleracea* showed non-significant decreased by drainage water (9.31) mg/g.f.w and (9.87) mg/g.f.w in tap water.

The reason of variations in chlorophyll concentrations between *O.basilicum* and *S. oleracea* plants is due to the nature of each plant, as it varies in the amount of chlorophyll according to several factors including growth condition and type of plant (Zeren *et al.*, 2017). The decrease in chlorophyll in plants irrigated with drainage water is attributed to the high chlorides in it Table (4-1), as chlorides inhibit calcium absorption, which in turn collects in the cell wall (Wisniewki *et al* 1995) and regulates the entry of essential nutrients responsible for the growth and effectiveness of organelles, including plastids, and thus affects the Chlorophyll content. Since the calcium concentration in drainage water is higher that record (144.28) mg/l compared to tab water (96.19) mg/l and because of *O. basilicum* needs low concentrations of calcium in growth (Ramchunder *et al.*, 2009), the change in chlorophyll become significant for *O. basilicum* irrigated with tap water and drainage water.

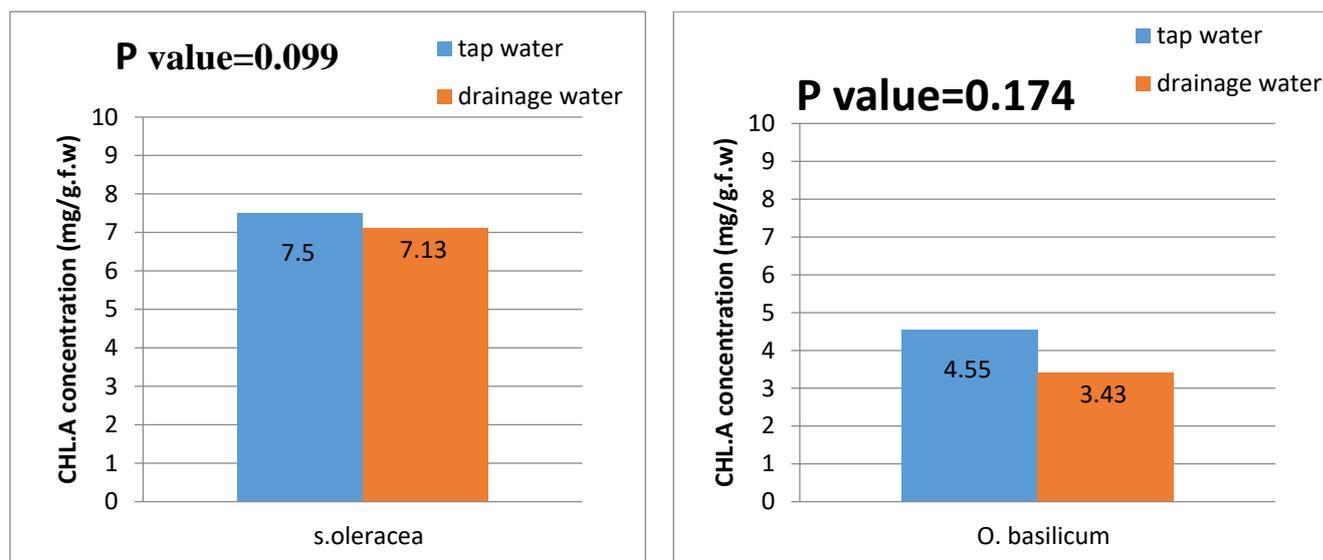


Figure (4-2) : CHL.a concentration (mg/g.f.w) in a- *S. oleracea* and b- *O. basilicum*

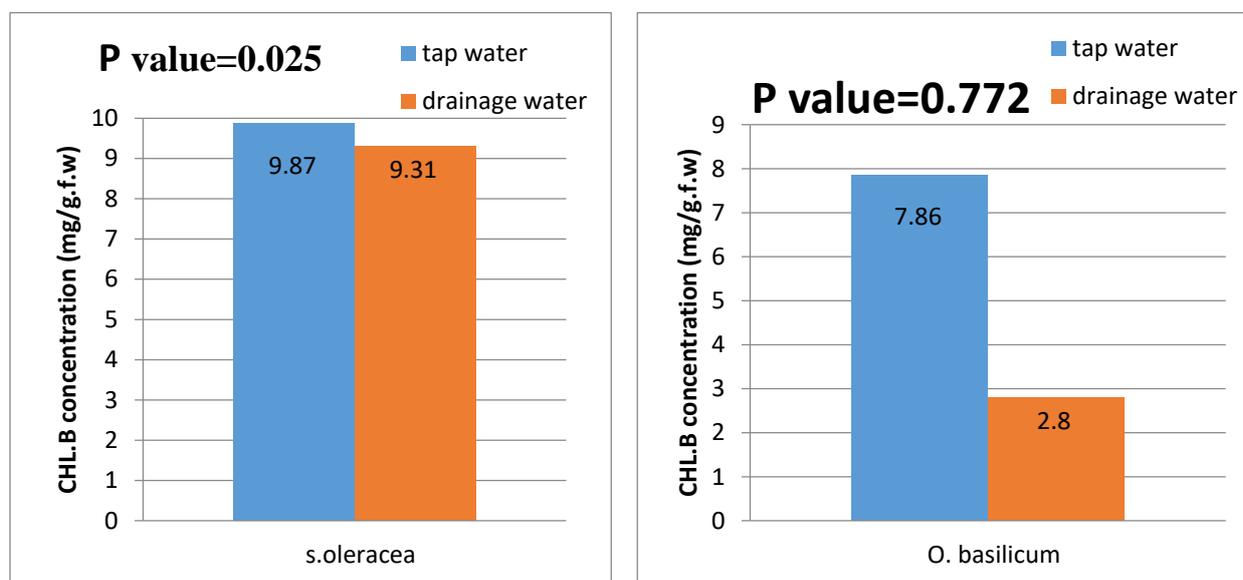


Figure (4-3) : CHL.b concentration (mg/g.f.w) in a- *S. oleracea*
b- *O. basilicum*

4.3.2: Carotenoids

Carotenoids are pigments with several functions in plants, besides their direct role in photosynthesis, they are involved in the oxidative stress defence mechanisms (Gill and Tuteja., 2010).

Less reading of carotenoids recorded in *S. oleracea* is (2.27) mg/g.f.w when irrigated with drainage water, while highest value recorded (4.80) mg/g.f.w in same plant but used tap water, which shows significant decrease (Pvalue \leq 0.05). The value of carotenoids concentration record in *O .basilicum* (4.37) mg/g.f.w when irrigated with tap water and this recorded decrease when used drainage water to (3.48) mg/g.f.w, that is showed non-significant decrease as shown in the figure (4-4a) and (4-4b). Plant need carotenoid as a antioxidant substance, the decrease in carotenoid content due to stress of chloride , calcium and total alkalinity in drainage water. The significant decrease in *S. oleracea* may be due to the physiological pattern of this species more than other antioxidant (Mcelroy& Kopsell, 2009).

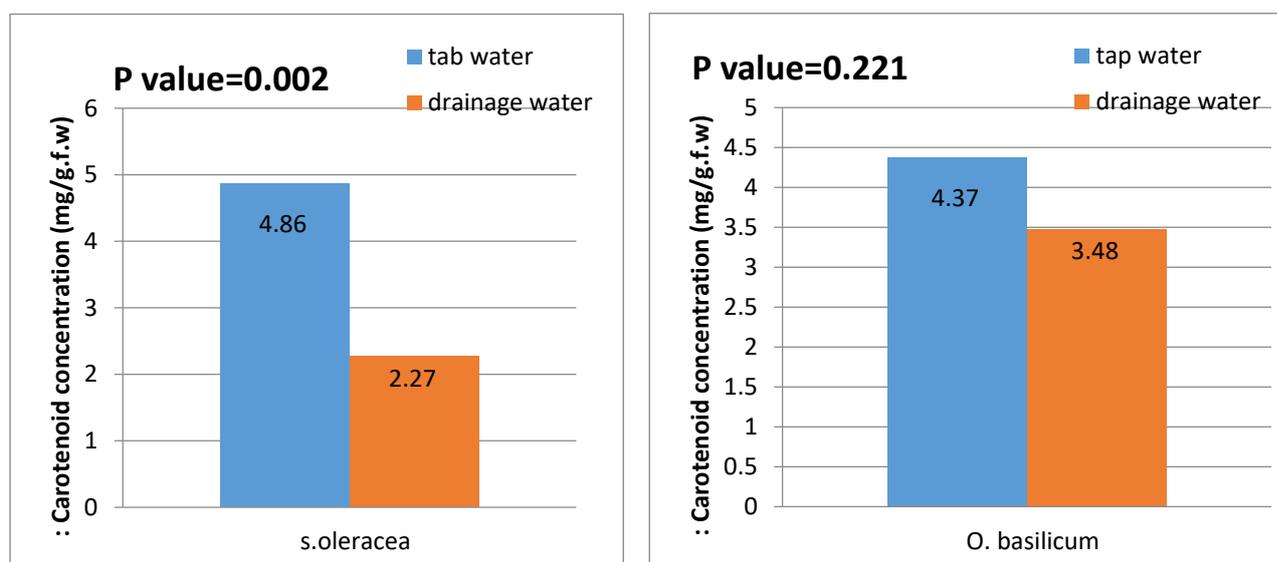


Figure (4-4) : Carotenoid concentration (mg/g.f.w) a- *S. oleracea*

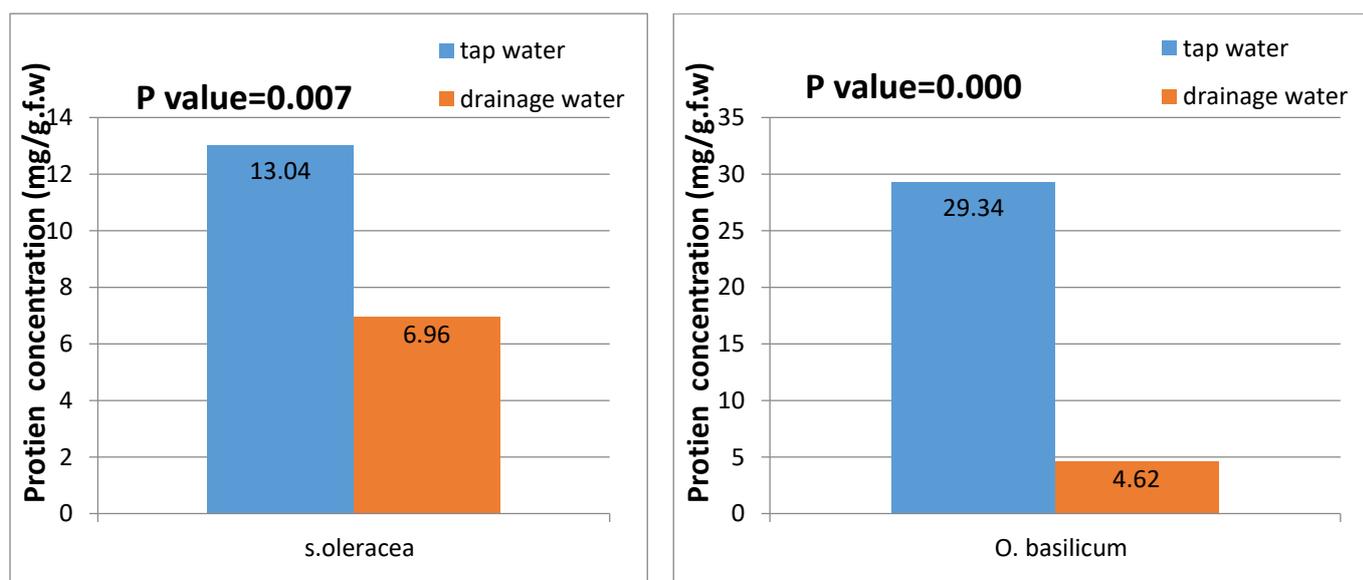
b- *O. basilicum*

4.3.3: Total Proteins

Proteins are considered to be the building blocks of life and, in addition to their nutritional role, have other functions in living organisms. All vital activities of plants are carried out through the interference and influence of proteins, and any interaction with external and internal factors depends on whether the plant has a mechanism for the production of supporting proteins or not (Musidlak ., 2017). This feature varies with different plants and their varieties in different regions. Therefore, these plants, climatic or environmental differences that affect protein synthesis clearly indicate that the amount of some proteins, they can be an indicator of resistance or sensitivity to an external or internal factor and how resistant a plant is to environmental stresses (Hasanuzzaman., 2013).

In this study, the value protein concentration in *O. basilicum* was noted when using tap water record (29.43) mg/g.f.w and the value of protein in *S. oleracea* when used same water (13.04) mg/g.f.w . The results showed significant decrease in both species when using drainage water, that is record (4.62) mg/g.f.w in *O. basilicum* and record (6.96) mg/g.f.w in *S. oleracea* ($P \leq 0.05 = 0.00$ and $P \leq 0.05 = 0.007$, respectively).

Many studies explain that protein content in plant is very affected with external environmental stress specially irrigated water type. The results of the current study agree with other studies which investigated the effect of drainage water on protein content on other terrestrial plants (Adily *et al.*, 2021).



**Figure (4-5) Protein concentration (mg/g.f.w) in a- *S. oleracea*
b- *O. basilicum***

4.3.4: Proline

A huge studies and data indicate a positive relationship between proline accumulation and plant stress. Proline, an amino acid, plays a very beneficial role in plants exposed to various stress conditions. In addition to being an excellent osmolality (Meena., 2019).

In this studied noted non-significant increase in concentration of proline when used drainage water in both *O. basilicum* and *S. oleracea* that is shown in figure (4-6a and 4-6b), results showed non-significant increase in concentration of proline when drainage water is used when compared with irrigation with tap water. The high concentration was (4.14) $\mu\text{mole/g .F.W}$ *S. oleracea* and the lower (1.34) $\mu\text{mole/g .F.W}$ in *O. basilicum* that irrigated with tap water.

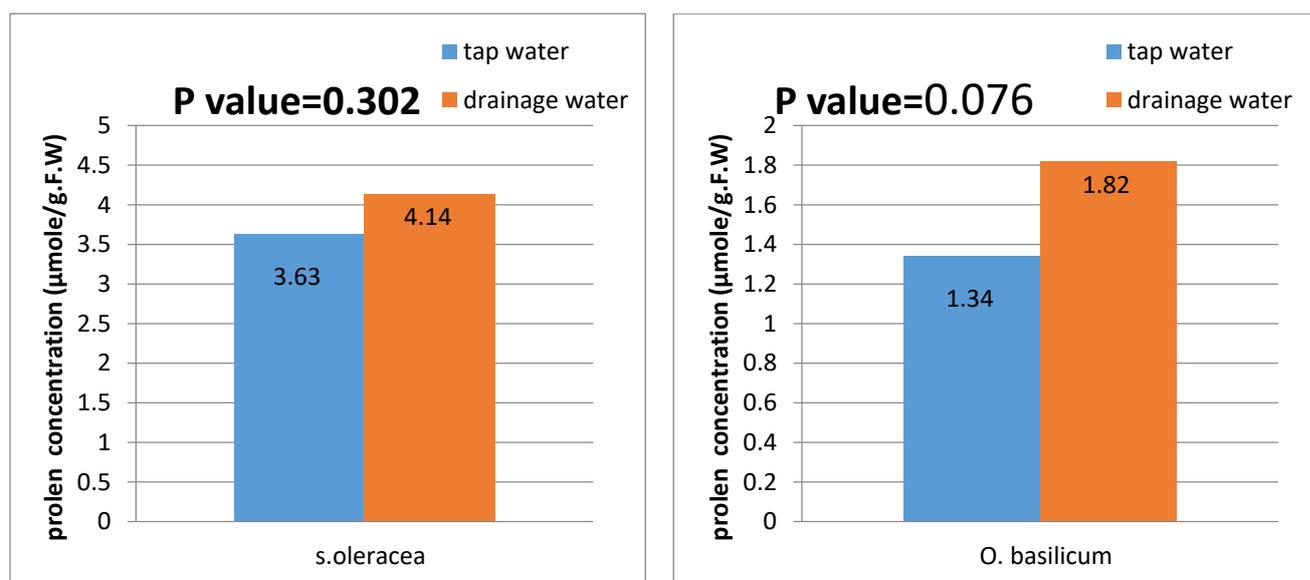


Figure (4-6) : prolen concentration (µmole/g.F.W) in a- *S. oleracea*

b- *O. basilicum*

4.3.5 : Vitamin C

Vitamin C (ascorbic acid) is a key component and an essential requirement of tolerance. Ascorbic acid (vitamin C) is an abundant component of plants. It reaches a concentration of over 20 mm in chloroplasts and occurs in all cell compartments, including the cell wall. It has proposed functions in photosynthesis as an enzyme cofactor (including synthesis of ethylene, gibberellins and anthocyanins) and in control of cell growth, Vitamin C (ascorbic acid) is very popular for its antioxidant properties (Arrigoni and De Tullio., 2002).

The results in figure (4-7a and 4-7b) showed values of vitamin C concentration. That is record (29.51) µg/g.F.w in *O. basilicum* when used tap water and its decrease non-significantly to (29.14) µg/g.F.w when irrigated with drainage water.

In *S. oleracea* the results record (31.82) when used tap water but it decreased significantly when irrigated with drainage water.

Decreasing vitamin C concentration in both species due to the role of this vitamin in cell plant which used in antioxidant system then its concentration will decrease (Paciolla *et al.*, 2019).

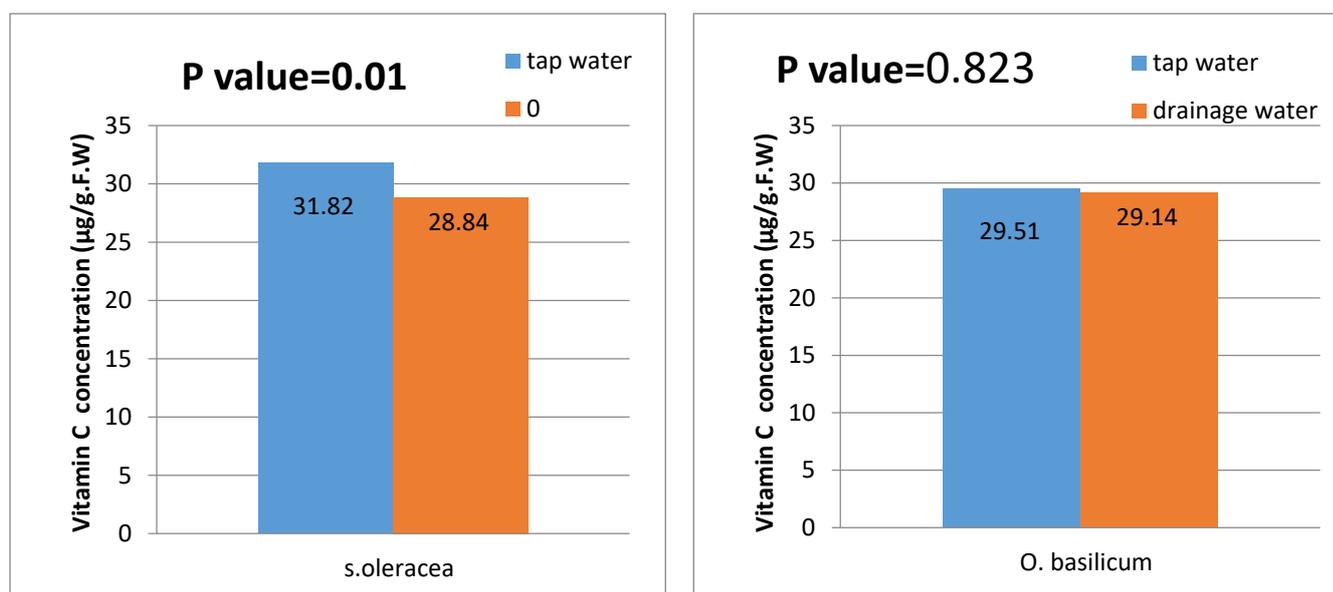


Figure (4-7) : Vitamin C concentration (µg/g.F.W) in a- *S. oleracea*
b- *O. basilicum*

4.3.6 : Total Antioxidant Activity (TAO)

The study TAO concentration showed significant decrease in both *O. basilicum* and *S. oleracea* when using drainage water as explained in figure (4-8a and 4-8b), that is record (35.48) mgAAE/g.F.w in *O. basilicum* when using tap water and became (17.64) mgAAE/g.F.w when irrigated with drainage water ($P < 0.05 = 0.002$), while in *S. oleracea* the TAO was (49.96) in plant that irrigated with tap water, but it decrease significantly when used drainage water to (44.66) mgAAE/g.F.w ($P < 0.005 = 0.004$).

Both studied species of plant showed decrease in TAO when irrigated with drainage water as a way to tolerant elevate chloride and total alkalinity, because increase chloride induces using of antioxidant substance (Kumar *et al.*, 2021).

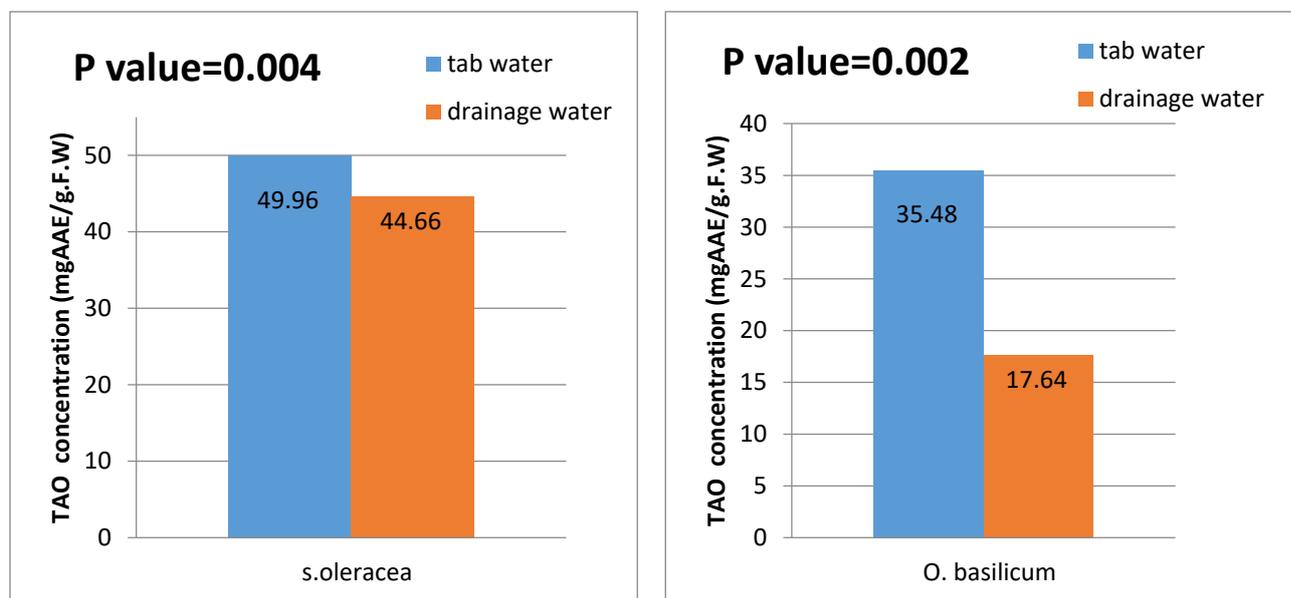


Figure (4-8) : TAO concentration (mgAAE/g.F.W) in a- *S. oleracea*
b- *O. basilicum*

4-3-7: Malondialdehyde (MDA)

MDA is one of the non-enzymatic antioxidants LPO and is one of the most effective indicators of the oxidative degradation of organisms prone to stress. Malondialdehyde (MDA) is a cytotoxic product and is an important indicator of the production of free radicals that cause lipid peroxidation and hence the free expression (Lu *et al.*, 2010).

The values of MDA is (1.35) mmol/l in *O. basilicum* and (8.81) mmol/l in *S. oleracea* when irrigated with tap water, as shown in figure (4-9a and 4-9b). Drainage water caused significant increase in MDA in both studied species and content became (14.59) mmol/l in *O. basilicum* and record (18.08) mmol/l in *S. oleracea* ($P \leq 0.05 = 0.005$ and $P \leq 0.05 = 0.004$ respectively).

Statistical analysis explain that MDA has a positive correlation with Cu and Fe concentration in plants, and this results is agree with many studies which explain the effect of heavy metal and drainage water on MDA of plant but it is not in general because studies explain non-significant effect of heavy metals on MDA concentration like Raphanus and Solanum (Al-jaryan., 2019)

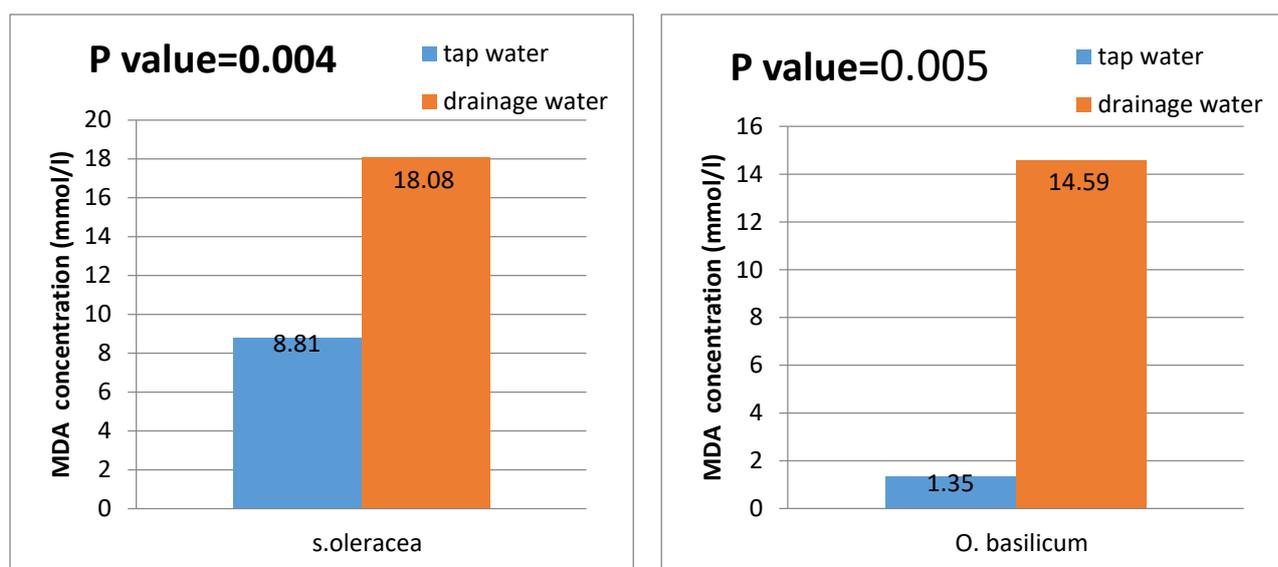


Figure (4-9) : MDA concentration (mmol/l) in a- *S. oleracea*

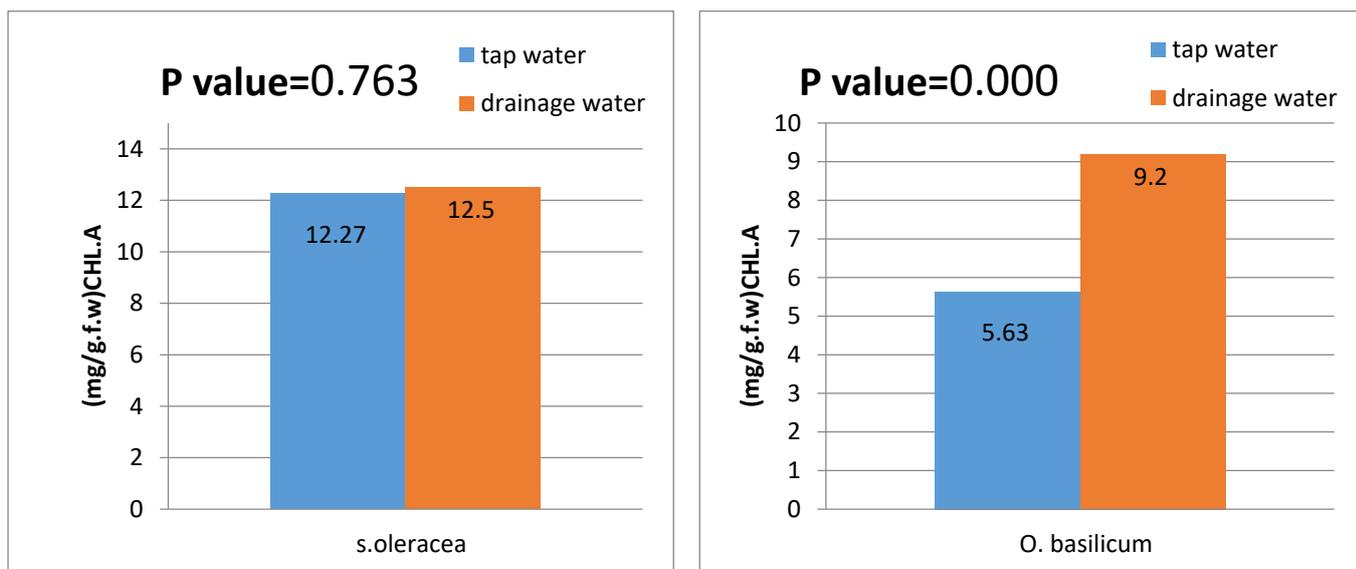
b- *O. basilicum*

4-3-8 Reactive oxygen spices (ROS)

Increase in the level of reactive oxygen species (ROS) is a common response to stress factors, including exposure to metals ROS which can be formed during normal aerobic metabolic processes like photosynthesis and respiration. Thus, the majority of ROS are produced in the mitochondria, chloroplast, peroxisomes, plasma membrane and apoplast (Flora *et al.*, 2008).

The results in figure (4-10a) and (4-10b) showed significant increase in ROS concentration in *O. basilicum* when used drainage water that is (5.63) mg/g.f.w when using tap water and increased to (9.2) mg/g.f.w when using

drainage water ($P \leq 0.05$). In *S. oleracea* the value ROS concentration did not show any significant change according to type of irrigated water and the concentration where (12.27) mg/g.f.w and (12.5) mg/g.f.w in plant that irrigated with tap water and drainage water respectively. The increase in ROS content due to increase of calcium and chloride in drainage water which both are stimulate ROS formation (Pradibha *et al.*, 2022) .



**Figure (4-10): ROS concentration ($\mu\text{g}/\text{gm}$) in samples a- *S. oleracea*
b- *O. basilicum***

4-3-9 Glutathione (GSH)

GSH is a low molecular weight thiol tripeptide that acts as an antioxidant defines through the Ascorbate /GSH cycle and plays an important role in plant defences against biological and abiotic stresses. It also participates in the detoxification of heavy metals (Foyer and Rennenberg, 2000), it plays an important role in controlling heavy metal concentrations in cells (Larson, 1988). It is mostly found in its reduced form GSH in plant tissues and is found in all cellular parts such as cytoplasm, vacuoles, endoplasmic reticulum as well as mitochondria and chloroplasts (Jimenez *et al.*, 1998).

Results that showed the value of GSH concentration in both *O. basilicum* and *S. oleracea* are explained in figure (4-11a) and (4-11b). Drainage water causes significant increase in GSH content in *O. basilicum* that is increase from 2.62 $\mu\text{mol/l}$ in tap water 6.18 $\mu\text{mol/l}$ when using drainage water ($P \leq 0.05$). While concentration of GSH in *S. oleracea* type of water does not cause any significant effect. The higher value record (4.59) $\mu\text{mol/l}$ when using drainage water.

The increase in GSH content due to increased calcium and chloride in drainage water which both stimulate GSH formation (Mori and Schroeder., 2004), and high elevation of GSH in *O. basilicum* may be due to the nature of this species.

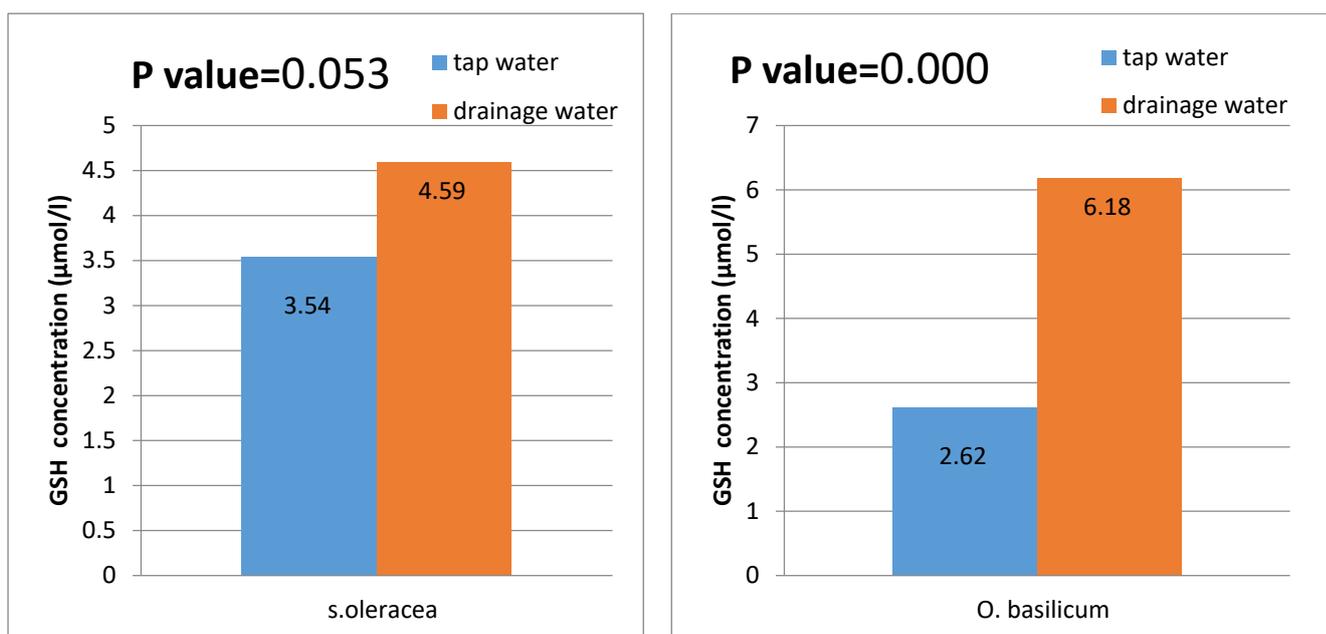


Figure (4-11) : GSH concentration ($\mu\text{mol/l}$) in a- *S. oleracea*

b- *O. basilicum*

4-4: Heavy metals concentration

4-4-1 Heavy metals concentration in water

The concentration of heavy metals in drainage water is explained in figure (4-12) . All studied heavy metals were elevated significantly in drainage water compared with their concentrations in tab water, except Cd which record non-significant increase . Heavy metals inter the drainage systems from different ways like agricultural activities and ground waters (Abd-Elaty *et al.*, 2022).

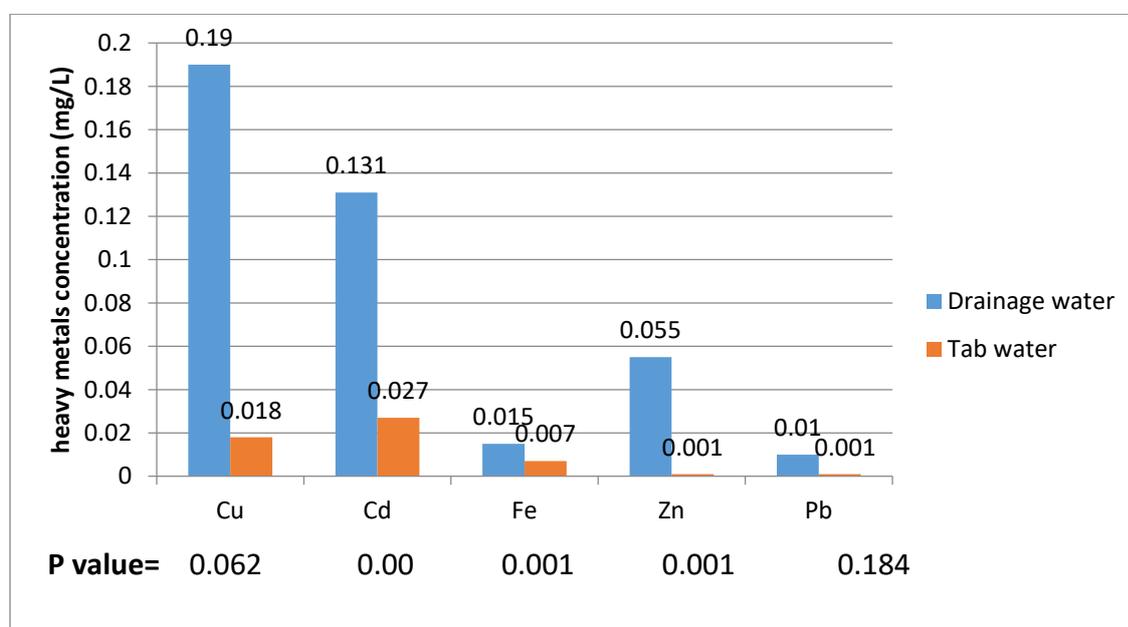


Figure (4-12) heavy metals concentrations (mg/l) in water samples

The concentrations of both Cu and Cd are more than Iraqi standards of drinking water but all studied element were within standards of FAO for irrigation, as explained in table (4-3).

The results of this study agree with other studies on drainage like results of Habeeb (2015) who found In her study on same drainage that concentrations of Fe, Pb , Cd and Cu were 113.98, 1.5, 6.35, 0.8 $\mu\text{g/g}$ respectively.

Table (4-3) Heavy metal concentrations in studied water and local and some global limited (mg/l)

Parameter	Indian standard (Awashthi,2000)	FAO1985	Iraq Standard (Ministry of Environme nt,2001)	Limited concentration in drawing(Rowe and Abdel-Magid ,1995)	Current study	
					Tab water	Drainage water
Cu	0.05	0.20	0.05	0.2	0.02	0.12
Cd	0.01	0.01	0.005	0.01	0.03	0.13
Fe	-	-	0.3	5.0	0.01	0.02
Zn	5.0	2.0	0.5	2.0	0.001	0.06
Pb	0.10	5.0	0.05	5.0	0.001	0.01

4-6-2 Heavy metals concentration in Soil

There are many sources of soil pollution with heavy metals especially human activities (Raymond *et al.*, 2011). Table (4-4) explain concentrations of studied heavy metals in soil before and after planting with *O. basilicum* and *S. oleracea*. Copper (Cu) concentration for used soil was 0.63 mg/l and it decreased at the end of experiment. This decrease is related with used this element by plants as a important essential element (Bjuhr., 2007). Cd elevated in all soils after irrigation due to its elevated concentrations in both tap and drainage water compared with other elements. So its solubility is very effected with soil content of clay which was form the lowest component among soil texture (Chrostowski *et al.*, 1991).

Fe is an essential element in plant growth, thus the concentrations decrease after the end of the experiment table (4-5) and the lowest value was 0.047 mg/l in soil where *S. oleracea* grow and irrigated with drainage water because Fe in

drainage water was less than in tap water and due to *S. olereacea* is well known is rich in Fe by up take from soil (Snehalatha and Vandana., 1992). The non-significant increase in pb concentration due to its concentration in used water. The elevated value were 0.027 and 0.025 mg/l in soils of *S.olereacea* and *O. basilicum* which irrigated with tap water which may be related with soil characters after irrigated with tap water. Lead was within soil limits which allow concentration to be less than 300 mg/l (Shiowatana *et al.*, 2001). All studied heavy metals were less than global limits, as explained in table (4-4).

Table (4-4) Heavy metals in soil (mg/Kg) before planting and after irrigated with tap water in studied species

Parameter	Indian standard (Awashthi,2000)	USA (Burt et al, 2005)	European Union Standard (EU 2002)	H.M in soil Basra (Khwedim et al, 2009)	Current study				
					Soil Before planting	Tap water		Drainage water	
						<i>O.basilicum</i>	<i>S.olereacea</i>	<i>O.basilicum</i>	<i>S.olereacea</i>
Cu	135-270	17.3	140	16.9	0.63±0.150	0.57±0.142	0.47±0.123	0.43±0.076	0.51±0.158 a
Cd	3-6	0.16	3.0	5.5	0.001±0.00	0.06±0.015	0.05±0.007	0.001±0.00	0.001±0.008
Fe	-	n.a		0.07	0.06±0.001	0.05±0.003	0.05±0.003 b	0.05±0.003	0.05±0.004 b
Zn	300-600	n.a	300	na	0.163±.005	0.22±0.028	0.22±0.025	0.17±0.002	0.18±0.002
Pb	250-500	10.1	300	39.4	0.024±0.003	0.03±0.003	0.03±0.002	0.02±0.002	0.02±0.001

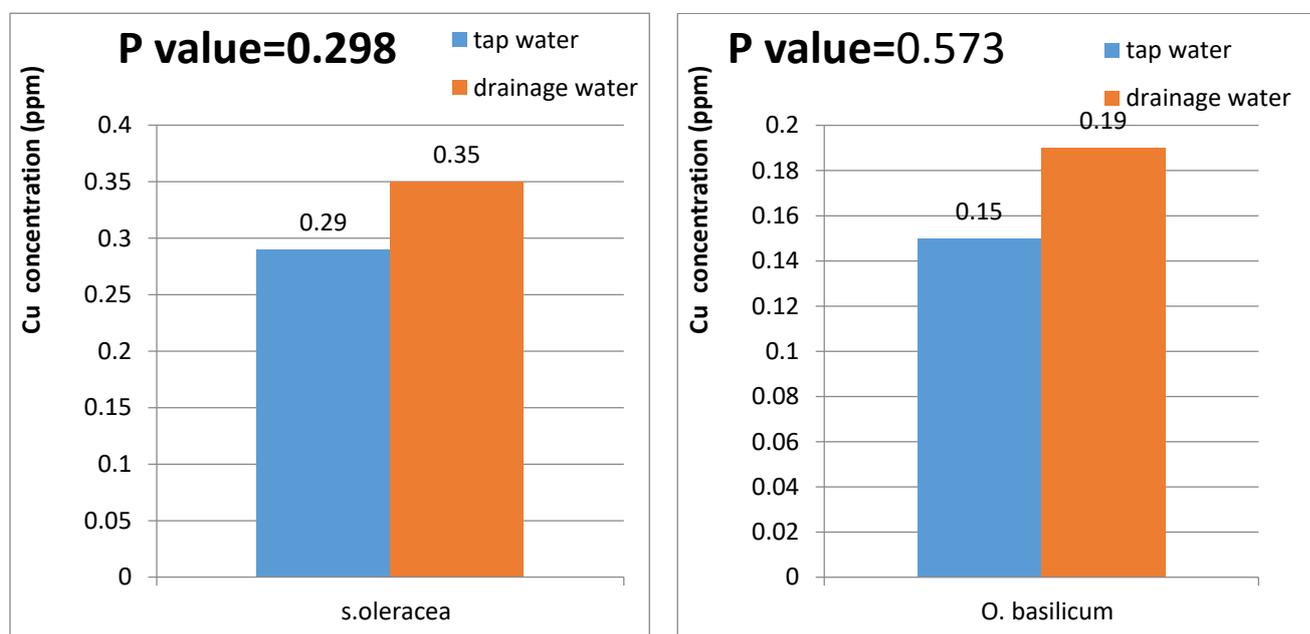
4-6-3 Heavy metals concentration in plants

Table (4-5) summarized the concentration of studied heavy metals in both *S. oleracea* and *O. basilicum* after irrigation with tap and drainage water.

Table (4-5) Heavy metal concentration in *S. oleracea* and *O. basilicum* before planting and after irrigated with tap water in studied species

Parameter	Indian standard (Awashthi,2000)	WH0/FAO (1985)	European Union Standard (EU 2002)	Conten of H.M in Tomatoes in Mansoor-Bagdad(Ahmed et al, 2020)	Current study			
					Tab water		Drainage water	
					<i>O.basilicum</i>	<i>S.oleracea</i>	<i>Basilicum</i>	<i>S.oleracea</i>
Cu	30.0	40.0	-	42.7	0.19±0.091	0.29±0.07 4	0.19±0.05 3	0.35±0.03 4
Cd	1.5	0.2	0.2	0.38	0.001±0.00	0.01±0.00 2	0.03±0.00 3	0.03±0.01 1
Fe	na	na	na	105.6	0.007±0.00 1	0.01±0.00 2	0.01±0.00 1	0.02±0.00 1
Zn	50.0	60.0	-	n.a	0.135±0.00 2	0.21±0.00 5	0.111±0.0 03	0.19±0.01 0
Pb	2.5	5.0	0.30	61.24	0.003±0.00 2	0.004±0.0 03	0.003±0.0 04	0.01±0.00 2

Elevated concentration was 0.354 mg/l of Cu in *S.oleracea* which was irrigated with drainage water. Statistically analysis showed non-significant variations in Cu concentration plant, and *S.oleracea* accumulated more Cu than *O. basilicum* as explained in figure (4-13a) and (4-13b) due to this species of plant is very prefer Cu ions (Chetan and Patel., 2015).



**Figure (4-13) : Cu concentration (mg/l) in a- *S. oleracea*
b- *O. basilicum***

The concentration of Cadmium is increased in *S. oleracea* and *O. basilicum* when irrigated with drainage water that it record 0.011mg/l and 0.007 mg/l in *S. oleracea* and *O. basilicum* respectively in tap water and then elevated to 0.034 mg/l and 0.0087 in *S. oleracea* and *O. basilicum* respectively, the elevated Cd in drainage water caused elevated concentration of this element in both plant, but *O. basilicum* showed a significant increase in Cd after irrigated with drainage water (figure 4-14a) and (4-14b) All Cd values were less than standers of WHO/FAO and India standers.

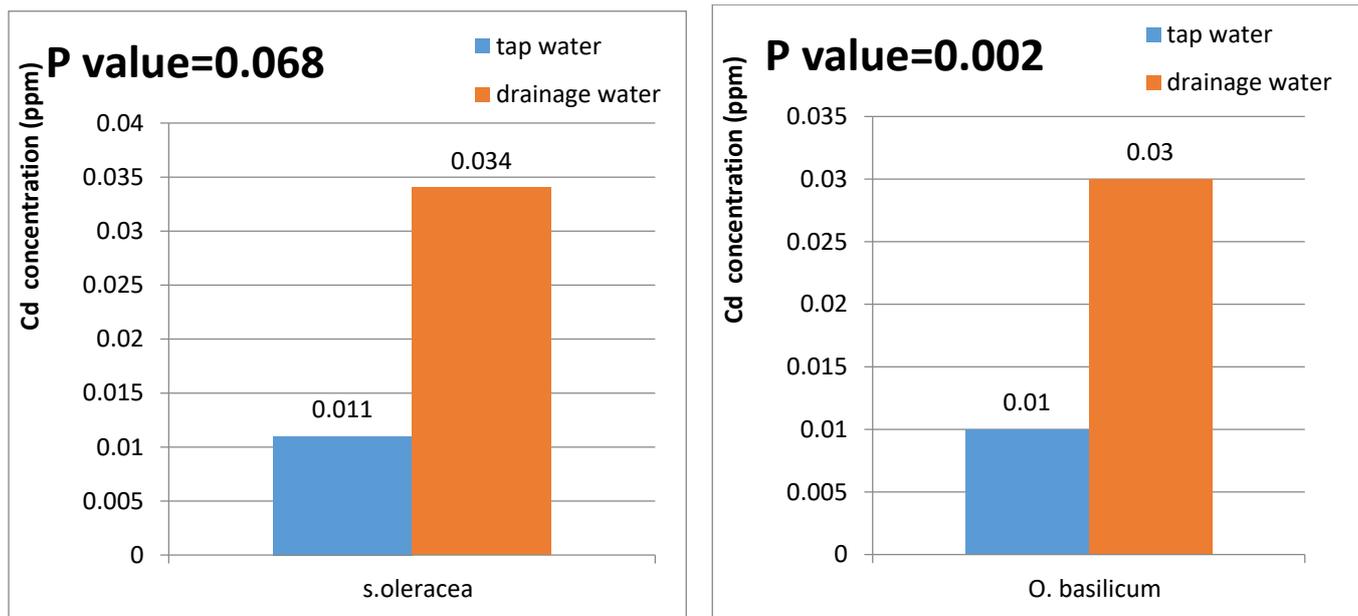
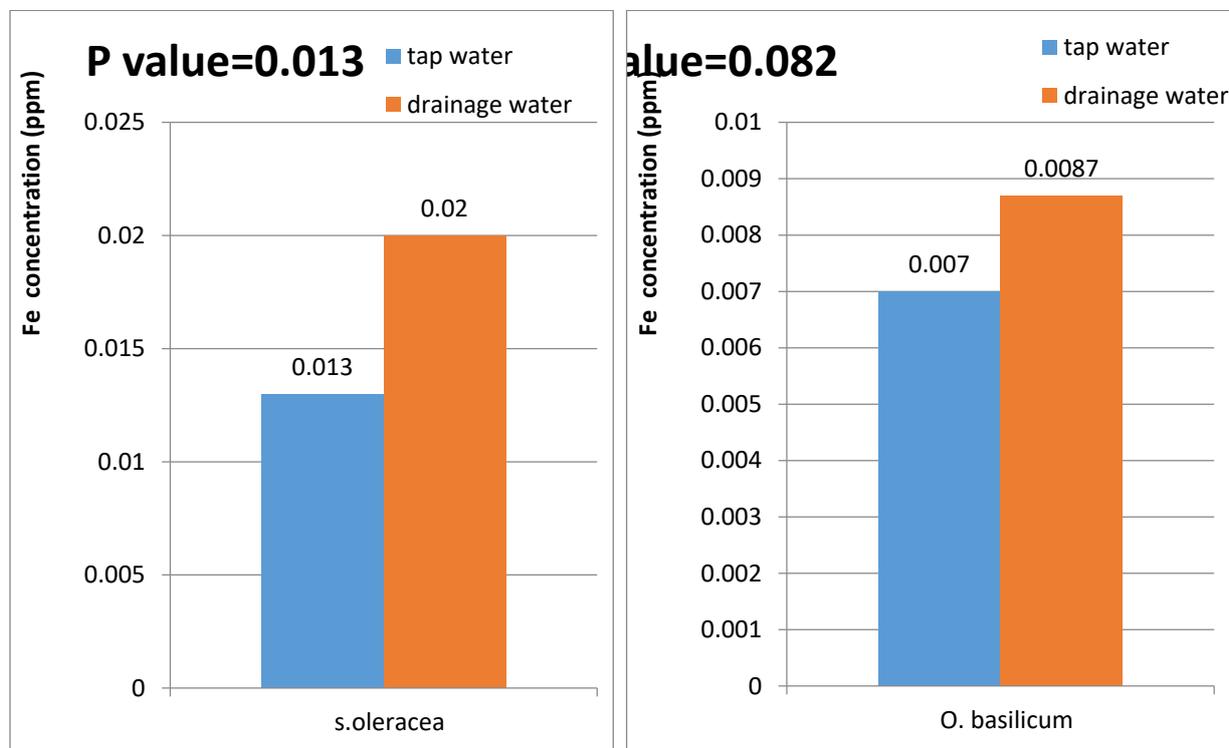


Figure (4-14): Cd concentration (mg/l) in a- *S. oleracea* b- *O. basilicum*

Fe was more accumulated in *S. oleracea* and drainage water cause a significant increasing in this plant, as explained in figure (4-15a) and (4-15b).



**Figure (4-15) : Fe concentrations (mg/l) in in a- *S. oleracea*
b- *O. basilicum***

A significant variation was recorded among concentrations of Zn in all plant groups as explained in figure (4-16a) and (4-16b). The elevated value was 0.214 mg/l in *S. oleracea* where used two water for irrigation.

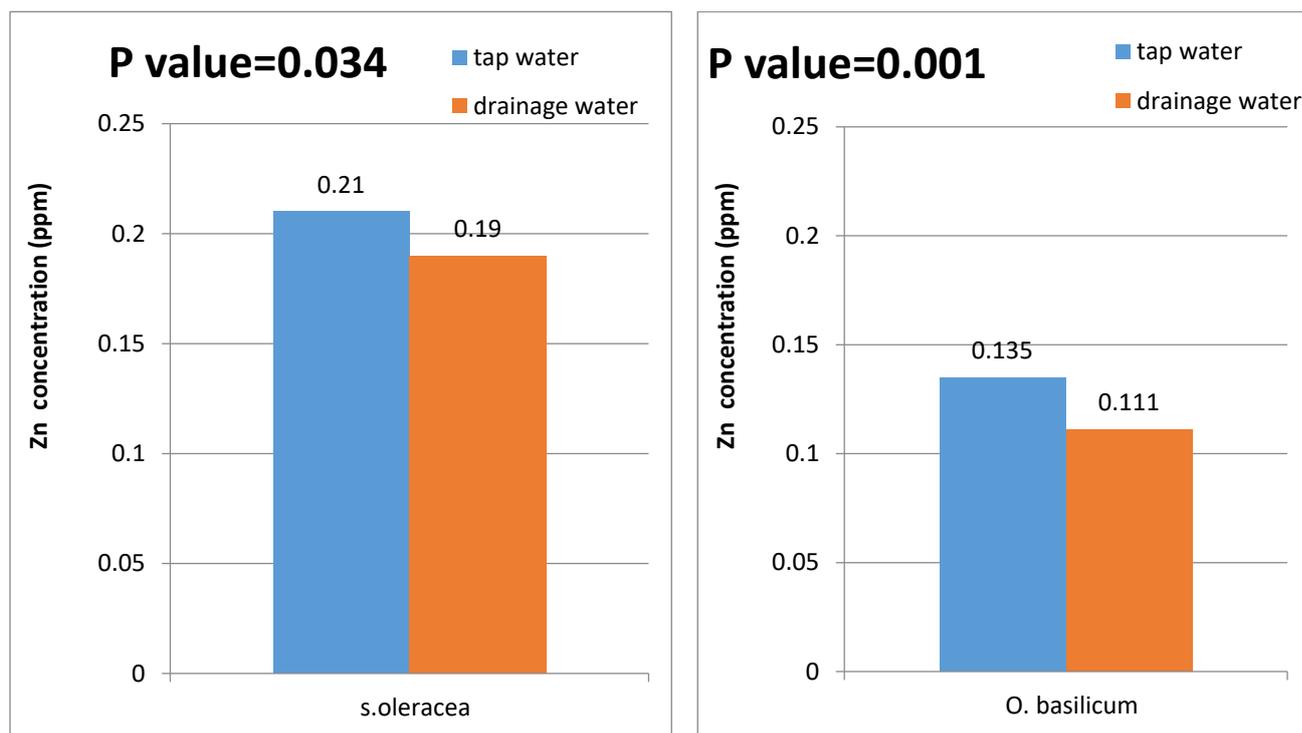
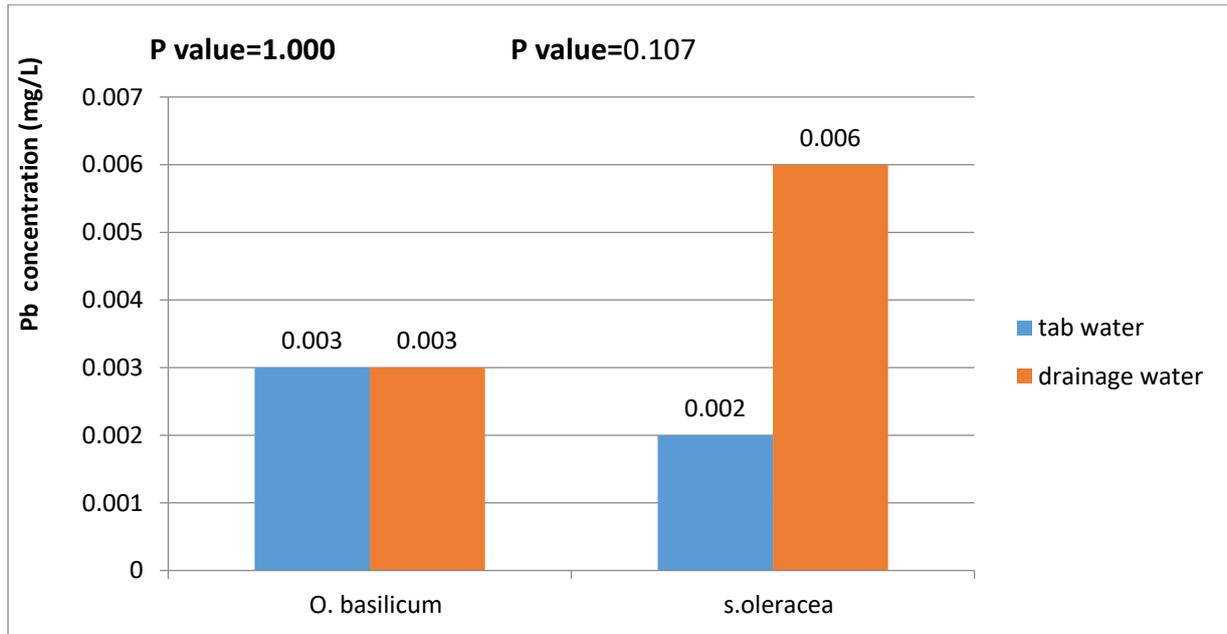


Figure (4-16) : Zn concentrations (mg/l) in a- *S. oleracea*

b- *O. basilicum*

The lowest concentration among studied heavy metals was lead (figure 4-17). Lead not recorded any significant variations among Pb concentrations. All studied heavy metal concentrations in plants were within the standard limits of them except Cd. This results are agree with study of Habeeb (2015) who explained that heavy metals concentration were within Iraq standard of drainage water.



**Figure (4-17) : Pb concentration (mg/l) in *S. oleracea*
b- *O. basilicum***

CONCLUSION

AND

RECOMMENDATION

Conclusions and Recommendations

Conclusions

- 1- Both *O. basilicum* and *S. oleracea* are showed good growth after irrigation with studied drainage water.
- 2-The heavy metals (Cd, Fe, Cu, Zn, Pb) in Eastern Euphrates drainage water (in studied area) were within limitations of Iraqi drainage water.
- 3- The main characteristics of used soil were not changed after irrigation with drainage water.
- 4- Drainage water showed non-significant variations in almost biochemical responses in both studied species.
- 5- *S. oleracea* showed more accumulation to almost studied heavy metals.

Recommendations

- 1- Study the possibility of using other drainage system in irrigation.
- 2- Study the effect of long term use of drainage water on the genetic content of economic plants.
- 3- Using a suitable method to treat tab water before using it.

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الخلاصة

هدف العمل الحالي الى دراسة امكانية استخدام مياه مزل الفرات الشرقي (منطقة زيد الشهيد) كمصدر مياه مستدام لأغراض السقي.

تم جمع عينات المياه من البزل خلال شهر تشرين الثاني ٢٠٢١ وتم قياس كل من الكلوريدات وقيمة الاس الهيدروجيني والعسرة الكلية والمواد الصلبة الذائبة والقاعدية والتوصيلية الكهربائية ثم جمدت كميات كبيرة من مياه البزل لغرض استخدامها في الري فيما بعد.

اظهرت نتائج دراسة خواص المياه ان مياه البزل قاعدية ($pH = 8.08$) وذات محتوى من القاعدية الكلية 280 ملغم/لتر، وتراكيز الكلوريدات ارتفعت الى 689.3 ملغم/لتر، وتركيز الكالسيوم ارتفع الى 44.3 ملغم/لتر غير ان تراكيز العسرة الكلية كانت اقل من تلك المسجلة لماء الحنفية المستخدم اذ كانت 680 و 880 ملغم/لتر على التوالي وان قيمة التوصيلية الكهربائية كانت اعلى في ماء البزل ولكن ليس بفرق معنوي ($P < 0.05 = 0.8$) اذ كانت 1560 و 1465 مايكرو موز/سم في ماء البزل والحنفية على التوالي.

تمت زراعة بذور *O. basilicum* و *S. oleracea* كلا على حدا في اصص بلاستيكية سعة 3 كلغم تربة وبواقع عشرة اصص لكل نوع نباتي ثم تم تقسيم اصص كل نبات الى مجموعتين حيث يتم ري احدهما بماء البزل والاخرى بماء الحنفية وبعد مرور شهر تم قياس كل المتغيرات الكيموحياتية لكل نبات وتشمل Chlorophyll a, Chlorophyll b) Carotenoid, Protein, Proline, Vitamin C, MDA, (GSH, TAO, ROS

كما درست صفات التربة بعد السقي بكل نوع ماء والتي شملت قيمة الاس الهيدروجيني والملوحة وقدرة التبادل الكاتيوني والتوصيلية الكهربائية والمواد العضوية والمواد الصلبة الذائبة والكلوريدات.

ان استخدام مياه البزل سبب زيادة معنوية بتركيز الكالسيوم في ترب النباتين الا انه لم يؤدي الى تغيير معنوي قيمة الاس الهيدروجيني والكلوريدات وتم تسجيل زيادة معنوية في تركيز المادة العضوية اذ ازدادت من 0.0 % الى 0.03 % وكذلك ازدادت قيم (CEC) في تربة *O. basilicum* و *S. oleracea* بعد السقي بمياه البزل اذ بلغت (1.07) و (1.06 mg/100gm) على التوالي.

بينت النتائج ان لمياه البزل تاثير على بعض الصفات الكيموحياتية لكلا النوعين اذ وجد ان تراكيز الكلوروفيل a و b والكاروتينات قد انخفضت في النباتات المسقية بمياه البزل حيث كانت التراكيز بالنسبة لنبات *O. basilicum* (3.43)، (2.8) و (3.48) mg/g.f.w على التوالي و كذلك بالنسبة لنبات *S. oleracea* حيث بلغت (7.133)، (9.31) و (2.27) mg/g.f.w على التوالي.

سجل البروتين انخفاض معنوي لكلا النوعين النباتيين المسقيين بمياه البزل اذ كانت (6.96) و (4.62)
($\mu\text{g/ml}$ ل *S. oleracea* و *O. basilicum* على التوالي.
اظهر *S. oleracea* انخفاض معنوي في قيمة فيتامين C اذ انخفض التركيز من (31.82) الى (28.84)
(mg/g.F.W في ماء الحنفية والبزل على التوالي فيما اظهر *O. basilicum* انخفاض معنوي في تركيز
TAO اذ كان (17.64) (mgAAE/g.F.W في النباتات المسقية بماء البزل مقارنة (35.48)
(mgAAE/g.F.W المسقية بمياه الحنفية.
ان اقل قيمة سجلت لل MDA كانت في *O. basilicum* المسقي بمياه الحنفية وكانت (mmol/11.35) فيما
كانت اعلى قيمة (18.08) (mmol/l في *S. oleracea* المسقي بمياه البزل واخيرا انخفضت قيمة
ال ROS و GSH في نبات *O. basilicum* معنويا ($P<0.05=0.00$) في النباتات المسقية بمياه البزل
فيما لم يظهر *S. oleracea* اي تغير معنوي في قيمهما .
تمت دراسة كل من الكاديوم والحديد والنحاس والرصاص والزنك في مياه البزل ومياه الصنبور وايضا في
التربة قبل وبعد الري وفي النباتات في كل من *S. oleracea* و *O. basilicum* في نهاية التجربة.
كان اعلى تركيز المعادن الثقيلة في الماء في بالنحاس (0.19) ، وسجل أقل تركيز بالرصاص (0.001).
في التربة لم يتسبب الري بمياه البزل في اختلاف معنوي في كل من (النحاس ، الحديد ، الزنك ، الرصاص)
، بينما زاد تركيز الكاديوم في التربة التي تزرع في كلا النوعين.
في النبات ، تراكمت (Cd ، Cu ، Fe ، pb) في *S. oleracea* أكثر من *O. basilicum*.



وزارة التعليم العالي والبحث العلمي

جامعة بابل/كلية العلوم

قسم علوم الحياة

دراسة بيئية وكيموحيوية لنباتي السبانغ *Spinacia Oleracea* والريحان
Ocimum Basilicum المرورية بمياه مبزل الفرات الشرقي

رسالة مقدمة إلى

مجلس كلية العلوم / جامعة بابل وهي جزء من متطلبات نيل درجة

ماجستير/ علوم الحياة

من قبل

عماد عامر صبحي حبيب البوكلل

(بكالوريوس علوم، علوم بيئية، 2011-2012)

بإشراف

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