**An-Najah National University**

**Faculty of Graduate Studies**

# The Use of Plant Growth-Promoting Rhizobacteria (PGPR) to Improve Plant Growth in Heavy Metal Contaminated Soil for Phytoremediation

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**This thesis was defended successfully on 16/03/2016 and approved by:**

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# Dedication

I dedicate this dissertation to the soul of my beloved late father; Fawzi, may GOD let him rest in mercy and peace

To my mother Khawla

To my darling wife; May

To my cherished daughter; Khawla

To my precious son; Fawzi

To my well-regarded sisters and brothers, and

To my late cousin Mohammad Salahat

To those who treasured Palestine as a home land.

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Thank you GOD for the opportunity to learn…

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# الإقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان

**The Use of Plant Growth-Promoting Rhizobacteria (PGPR) to Improve Plant Growth in Heavy Metal Contaminated Soil for Phytoremediation**

أقر بأن ما شملت عليه الرسالة هو نتاج جهدي الخاص, باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو لقب علمي أو بحثي لدى أي مؤسسة علمية أو بحثية

## Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degrees or qualifications.

**Student's Name:** **اسم الطالب: حافظ فوزي دراغمة**

**Signature** **التوقيع:**

**Date** **التاريخ :16/03/2016**

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# List of Acronyms and Abbreviations

| | |
|---|---|
| **ACC** | 1-Amino Cyclopropane-1-Carbozylate |
| **BSN** | Bunch Stem Necrosis |
| **CEC** | Cation Exchange Capacity |
| **DDH2O** | De-ionized and Distilled water |
| **EC** | Electrical Conductivity |
| **FW** | Fresh Water |
| **IAA** | Indole-3-Acetic Acid |
| **OD** | Optical Density |
| **PGPR** | Plant Growth Promoting Rhizobacteria |
| **PPM** | Part Per Million |
| **ROS** | Reactive Oxygen Species |
| **SAM** | S-Adensoyl-Methionine |
| **SAR** | Sodium Adsorption Ratio |
| **SW** | Saline Water |
| **TDS** | Total Dissolved Salts |

# The Use of Plant Growth-Promoting Rhizobacteria (PGPR) to Improve Plant Growth in Heavy Metal Contaminated Soil for Phytoremediation

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## Abstract

Plant growth-promoting rhizobacteria (PGPR) are free-living, soil-borne bacteria that colonize the rhizosphere and, when applied to crops, enhance the growth of plants. Bioremediation is the submission of biological progression for cleanup of pollutants from the environment. The main aim of this study was to use the *Barely* (*Hordeum vulgare L.*), and *clover (Trifolium)* metal tolerant plants with bacteria to extract metals from soil focusing on *Iron (Fe)* and *Magnesium (Mg)*.

Trials were conducted by incorporating them with plant growth promoting rhizobacteria (PGPR): *Pseudomonas putida* (UW3 and UW4).

This study was conducted in two places with two experiments; the first experiment was carried out in greenhouse conditions, while the second one was conducted at the lab of An-Najah National University, Collage of Science, Nablus, Palestine, during the year 2015. The mother plant material was collected from the Palestinian Ministry of Agriculture. Iron and magnesium were taken as reference values to study the change in their concentrations after planting in vivo medium. Three types of water were used, while control seed pots used in experiments: one pot used with fresh

water, another irrigated with saline with metals while the last one with saline water without metals. *Pseudomonas putida* strains (UW3 and UW4) possessed the direct growth promoting characteristics and the ability of strain to the uptake and accumulation of the metals.

Results determined that the average barley shoot length denoted to 38cm and the average shoot weight was 10g, while the clover shoots also increased to an average length of 28cm after 30 days, and an average weight of 8g. Barley plant had the average root value biomass after 30 days (17.7cm) while the average root weight was observed to have 8g. The root growth plant height of the clover after 30 days recorded an average of 15cm length and an average weight of 6g after 30 days. PGPR inoculation also increased the average root wet weight more than root dry weight (137-141%). Accumulations and uptakes of Fe and Mg in barley which were conducted in vitro, for the treated barley seeds with bacteria C (UW3+UW4) irrigated with saline water with metals (SW) were 0.575 and 0.542 gram/liter respectively, while that of Fe and Mg in clover for the treated clover seeds with bacteria C irrigated with SW were 0.69 and 0.48 gram/liter respectively. The growth attributes were increased due to PGPR inoculation due to uptake and accumulation of heavy metals. The overall growth performance of inoculated seedlings was higher in compare to un-inoculated control. Conclusions indicated that all bacterial strains increased the average shoot and root growth of barley and clover in comparison with the untreated control, thus, this study suggests that UW3 and UW4 strains in combination have a great potential to increase

photosynthesis, transpiration, leaves chlorophyll content, and could be used as crop-enhancer and bio-fertilizer for vigor seedling and production of both plantlets. This study recommended that PGPRs are the potential tools for sustainable agriculture and trend for the future. For this reason, there is an urgent need for researchers to clear definition of what bacterial traits are useful and necessary for different environmental conditions and plants, so that optimal bacterial strains can either be selected and/or improved. Furthermore, the reason to use Fe and Mg in this study is its vital importance to the plants as well as animals. Throughout the use of barley and clover, it is likely to produce feed nutrients containing enhanced excellent nutrients to the animals, consequently, instead of industrialized fodder.

# Chapter One

# Introduction

## 1.1 Introduction

The unremitting global industrialization, agricultural practices and numerous anthropogenic actions have caused widespread environmental tribulations attributable to the discharge of contaminants, such as heavy metals, organic pollutants, etc. Physical, chemical and biological methods have been used for the purging of pollutants from the environment. Heavy metals are the principal inorganic pollutants accumulate in environment due to their non-biodegradable nature and afterward taint the food succession (Rajkumar et al., 2010). Bioremediation is the submission of biological progression for cleanup of pollutants from the environment. It is a cost effective and convenient solution for remediation of heavy metal contaminated soil compared to physico-chemical remediation technologies which are furthermore precious and detrimental for soil characteristics (Quartacci et al., 2006).

Phytoremediation is a technique of bioremediation procedure by the help of hyperaccumulator plants. The attainment of phytoremediation is reliant on the likely of plants to yield eminent biomass[1] and endure the metal stress. The efficiency of phytoremediation can be enhanced by increasing the heavy metal recruitment or solubility in the soil and rising plant biomass by endorsing plant growth (Zhuang et al., 2007). This can be achieved by

[1] Biomass is biological material derived from living, or recently living organisms. In the context of biomass for energy this is often used to mean plant based material, but biomass can equally apply to both animal and vegetable derived material.

developing the relationship of hyperaccumulator plants with heavy metal resistant bacteria (Figure 1.1). The plant growth promoting rhizobacteria (PGPR) merit extraordinary consideration because it can unswervingly improve the phytoremediation process by varying the metal bioavailability during altering pH, release of chelators and production of phytohormones, along with the rhizosphere microorganisms involved in plant interactions with metal contaminated soil environment, etc. (Ma et al., 2011).

This depicts the use of PGPR to hasten plant biomass production and control plant metal amassing or stabilization with better performance abilities such as adaptive strategies, metal mobilization and immobilization mechanisms.

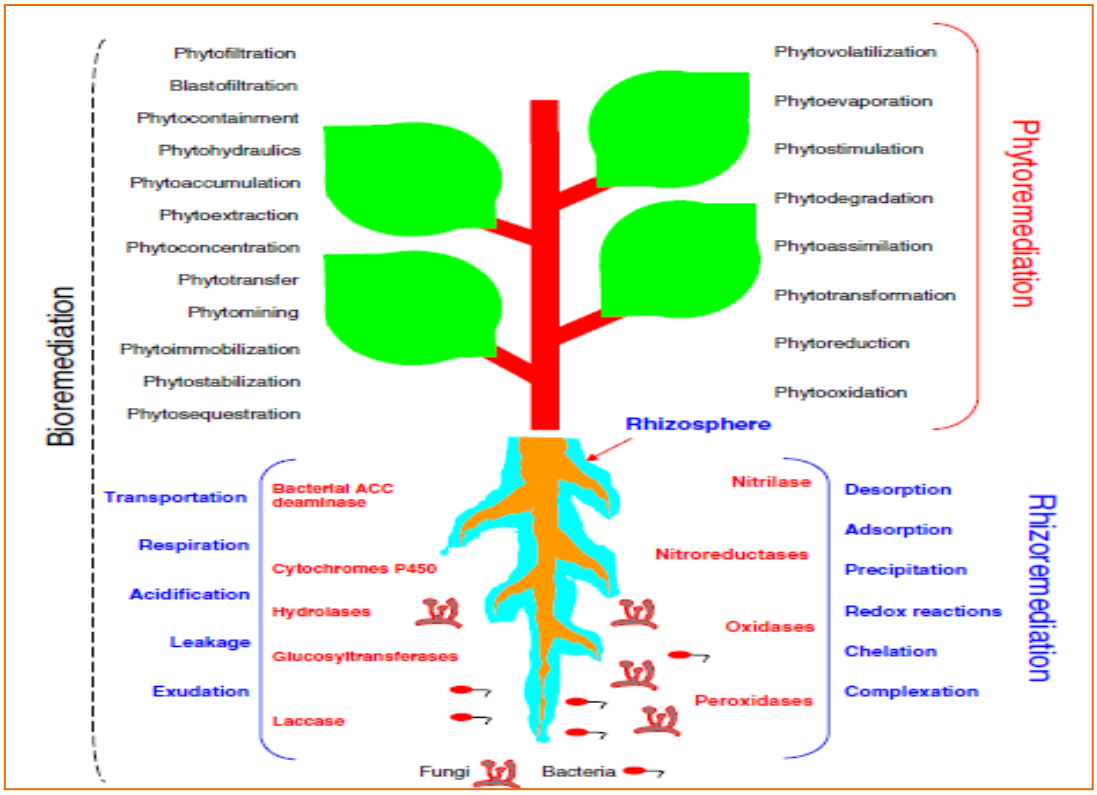



**Fig 1.1:** Importance of soil-microbe interactions in bioremediation for the cleanup of metals and organics (Ma et al., 2011).

## 1.2 Historical Background

The conception of using plants to crackdown-tainted milieu is not new-fangled. Three hundred (300) years ago, plants were projected for use in the treatment of wastewater (Hartman, 1975). Latter end of the 19th century, *Thlaspi caerulescens* and *Viola calaminaria* were the foremost plant species documented to accumulate high levels of metals in leaves. In 1935, Byers reported that plants of the genus *Astragalus* were capable of accumulating up to 0.6% selenium in dry shoot biomass. One decade later, Minguzzi and Vergnano (1948) identified plants able to accumulate up to 1% Ni in shoots. More recently, Rascio (1977) reported tolerance and high Zn accumulation in shoots of *Thlaspi caerulescens*. Despite subsequent reports claiming identification of Co, Cu, and Mn hyperaccumulators; (a hyperaccumulator is a plant capable of growing in soils with very high concentrations of metals, absorbing these metals through their roots, and concentrating extremely high levels of metals in their tissues (Rascio., 2011), the existence of plants hyperaccumulating metals other than Cd, Ni, Se, and Zn has been questioned and requires additional confirmation (Salt et al., 1995). The idea of using plants to extract metals from contaminated soil was reintroduced and developed by Utsunamyia (1980) and Chaney (1983), and the first field trial on Zn and Cd phytoextraction was conducted in 1991 (Baker et al.). In the last decade, extensive research has been conducted to investigate the biology of metal phytoextraction. Despite significant success, our understanding of the plant mechanisms that allow metal extraction is still emerging. In addition, relevant applied aspects,

such as the effect of agronomic practices on metal removal by plants are largely unknown. It is conceivable that maturation of phytoextraction into a commercial technology will ultimately depend on the elucidation of plant mechanisms and application of adequate agronomic practices. Natural occurrence of plant species capable of accumulating extraordinarily high metal levels makes the investigation of this process particularly interesting.

### 1.3 Prompting Plant Growth

Plant coupled with bacteria play an indispensable role in host adaptation to a varying environment. The mechanism of plant growth stimulation incorporates synthesis of ACC deaminase, siderophores and phytohormones production, nutrients uptake, biocontrol agents (Figure 1.2). Phytohormones are responsible for plant growth as well as metal uptake (Zaidi et al., 2006).

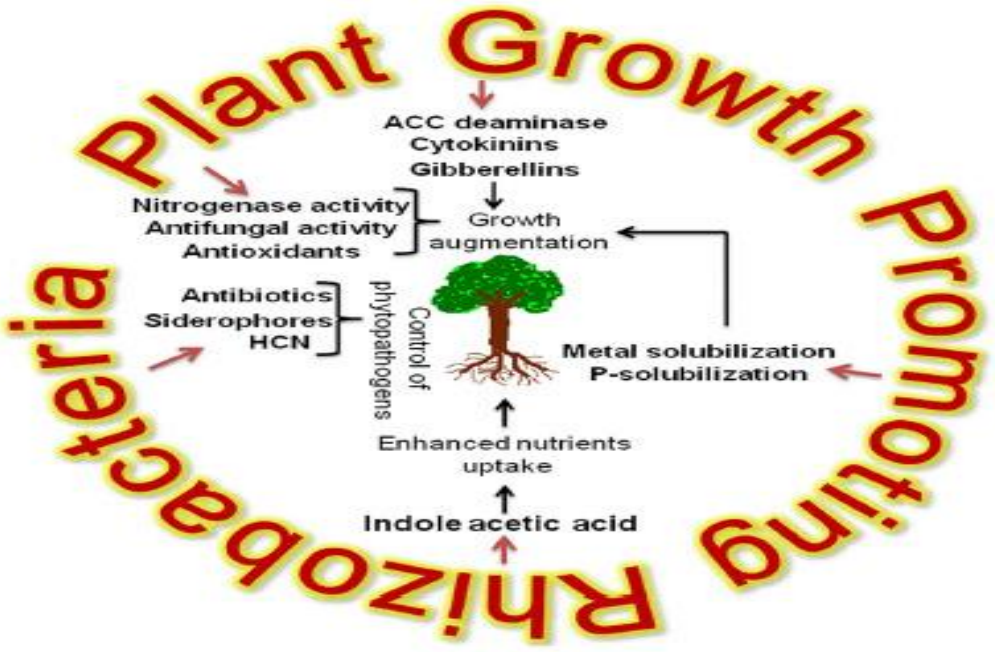



**Fig 1.2:** Mechanism of plant growth promotion by rhizobacteria (Ahmed et al., 2013)

## 1.4 Iron

Iron is an imperative plant nutritional element. Green plants have the need to absorb Fe as (Fe2+ (ferrous)) during growth because it is not relocated from the old to the young foliage. Concentrations of Fe in ionic forms are very little, and so plants perk up iron availability through specific uptake mechanisms for example, plants extend more root hairs and they exude more protons (H+ ions), phenolic compounds and organic acids, some of which have chelating properties and plants exude increasing amounts of iron chelating substances known as ‘phytosiderophores’, which form chelates with Fe3+ in the rhizosphere and on that way facilitate the iron uptake (Bergmann, 1992). Factors that power the iron uptake include interactions of Fe with other elements, soil and environmental factors.

Deficit of Fe typically broaden in vines, particularly on soils that are rich in lime, with the pH value exceeding 6.5 (Bergmann, 1992; Fregoni, 1997). Iron chlorosis is considered one of the diseases that result from iron deficiency that lessens the quantity of fruit harvest production (Tagliavini and Rombolà, 2001).

## 1.5 Magnesium

Mg is a central atom of the chlorophyll molecule. The observable marker of Mg deficiency is chlorosis of older basal leaves, which results from the Mg transference from the older to young leaves, growing organs and chiefly to the seed and fruit, where it is frequently needed. Lack of Mg is common in sandy soils in areas with high rainfall, feebly drained sites, or alkaline soils because Mg is leached from the soil (Marschner, 1995). Mg deficiency is

noticed by the low concentration in the soil and high concentration of other ions such as H+ (low pH), K+ (heavy application of fertilizers), NH4+ (ammonium sulphate as a fertilizer), Ca2+, Mn2+, and Al3+ ions (in acid soils with pH ≤ 5 (Bergmann, 1992). The widespread symptoms of Mg deficiency in vines incorporate 'bunch stem necrosis' (BSN) or 'stalk necrosis' or 'stem dieback'. BSN is one of the main dangerous physiological diseases that affect grapevine and causes harms to clusters in the ripening phase failure in the amount and quality of the crop (Capps and Wolf, 2000). BSN is caused by an imbalance between $K^+$ ions and $Ca^{+2}$ and Mg ions (Haefs et al., 2002).

## 1.6 Objectives

The main aim of this study is to use the *Barely* (*Hordeum vulgare L.*), and *clover (Trifolium)* metal tolerant plants with bacteria to extract metals from soil focusing on *Iron* (Fe) and *Magnesium* (Mg) and used for phytoremediation. In order to increase their productivity and tolerance to Iron (Fe) and magnesium (Mg) metals conditions, trials were conducted by incorporating them with plant growth promoting rhizobacteria (PGPR): UW3 and UW4 (*Pseudomonas putida*). These strains will be used in coating seeds separately. The effect of plant growth promoting rhizobacteria (PGPR) on plants in terms of biomass production and photosynthetic activity under metals stress will be examined. Also metal accumulations in plants will be measured and compared with fresh water irrigated control plants.

## 1.7 Justification

In our country, there are many sources of soil contamination with heavy metals, especially the use of fertilizers and pesticides, which led to the loss of soil fertility in addition to the adverse effects on the environment and humans. In addition, in some territories, namely in Jericho, the underwater supply encloses high levels of salinity and metals that cannot be used in irrigation and much costly to be treated. Large amounts of generated brine water (10-12 million $m^{3)}$ are produced yearly from five stations of reverse osmosis plants in Jericho districts. Brackish water was disposed in unfriendly environmental ways by dribbling it in soils and/or rivulets which created further environmental tribulations (Palestinian Water Authority, 2013). Accordingly, there is a growing need for more efficient and cost effective methods to remove heavy metals from soil. Recent researches prove the efficiency of phytoremediation technique in treating the soil polluted with heavy metals, which justifies the conduction of this research.

Consequently, in this research, phytoremediation techniques will be implemented as method in treatment of the soil polluted with heavy metals through using forms of plants that are germinated with PGPR and then they will be cultivated in soil irrigated with water that contains heavy metals and determine the high ability for plants to up take heavy metals from the soil.

## 1.8 Literature Review

The plant classes for the study were selected based on biomass and phytoremediation efficiency for different contaminants as per the literature review. *Barely* (*Hordeum vulgare* L.), and *clover (Trifolium repens)* were

selected since they were proven effective in remediating for contaminated soil of metals and organic contaminants.

Locally, Hamed (2014) carried out a study and tested PGPR which was implemented to investigate the efficiency of phytoremediation techniques for treatment of generated brackish water. Two strains of PGPR *Pseudomonas Putida* (UW3 and UW4) had been selected with the two plants; barley (*Hordeum valgare L.*) and malt (*Panicum maximum Jacq*.). Trials included the treatment of these plants with and without PGPR in order to study its effect on plant responses toward brackish water irrigation. The results showed that PGPR had significant effects on plant growth, photosynthetic activity, membrane stability and root and shoot lengths that increased under salt stress compared to control trials treated without PGPRs and irrigated with fresh water and brackish water. Furthermore, greenhouse study showed that plants treated with PGPRs and irrigated with brackish water increased significantly in biomass percentage for trials with fresh water. In addition, it was noted that PGPR's treated plants had (150%) and (283%) increase in their roots and shoots lengths respectively. Also, several chlorophyll fluorescence parameters showed that treated plants with PGPR resulted in improvement in their photosynthesis under brackish water.

Another local study was conducted by Alhousani (2012) in Hebron, Palestine, who applied the green technology “phytoremediation” approach in Wadi Alsamin in Hebron-Palestine to evaluate the plant efficiency in remediation of polluted soil. An open field controlled experiment was

conducted to assess the efficiency of two plant species namely: corn (*Zea mays*) and tobacco (*Nicotianatabacum*) plants for bioaccumulation of heavy metals under natural growth without chemical assistance. The concentrations of three heavy metals (Cr, Mn, Zn) were determined in all plant parts (root, stem, leaf and fruit) for both plants by using Inductively Coupled Plasma–Atomic Emission Spectrometry (ICP-AES). The accumulation of heavy metals in leaves was higher than in the other parts for both plants. Results showed that the bioaccumulation factor ($f$) of corn plant for Cr as a pollutant metal 0.05 was higher than in tobacco 0.02 while bioaccumulation factor ($f$) for Mn in tobacco 0.13 was higher than in corn 0.09 where bioaccumulation factor ($f$) for Zn in both plant was 0.3.

Many researchers have dealt with the methods to be used in cleanup the soil from heavy metals. Most researches focused on mechanically or physio-chemically based remediation. However, more recent studies have been focusing on a biology-based emerging technology and phytoremediation. Below are some of these researches:

Madrid and Kirkham (2002) studied the heavy metals uptake by barely and sunflower grown in abandoned animal lagoon soil. The results showed that barely was the better choice in phytoremediation. Plants grew for 60 days in pots (16 cm diameter; 18.5 cm tall) with soil. Control pots had no plants. The plants, especially sunflower, germinated poorly in the lagoon soil. Of 240 barley seeds planted, 45 germinated (19%); of 360 sunflower seeds planted, 7 germinated (2%). High penetration resistance of the lagoon soil appeared to be the cause of the poor growth.

Ekwumemgbo et al., 2013, conducted a study and confirmed that *B. pinnatum* as one of the plants that could be employed in phytoremediation of soil polluted by heavy metals. The plants were left in ambient conditions and watered periodically. After the first 2 weeks, the plant and soil samples were collected and analysed for total concentration of Cd, Cr, Cu, Ni, Pb V and Zn. Subsequently, the plant and soil samples were collected monthly and analysed for the total concentrations of these heavy metals, using Atomic Absorption Spectrophotometry.

In (2013), Munees Ahmed and others studied the mechanism and application of plant growth promoting rhizobacteria. The results of their study showed increased health and productivity of different plant species by the application of plant growth promoting rhizobacteria under both normal and stressed conditions.

In addition, Chirakkara and Reddy (2013) carried out a study on twelve plants. They selected for the study; Helianthus annuus (sunflower), Brassica Juncia (mustard), Brassica Rapa (Field mustard), Tagetes patula (marigold), Avena sativa (Oat plant), Lolium perenne (perennial rye grass), Festuca arundinacea (tall fescue), Medicago sativa (alfalfa), Trifolium repens (white clover), Vigna Radiata (green gram), Allium fistulosum (green onions) and Solanum nigrum (black nightshade). This study concluded that the mixed contamination soil had significant effect on growth characteristics of almost all the plants studied. All plants showed delayed germination and survival rates in contaminated soil compared to the control. No seeds germinated in the contaminated soil for white clover.

All plants except oat plant had considerable reduction of biomass in contaminated soil. Based on the % germination, % survival and growth characteristic, out of the 12 plant species studied, oat plant, rye grass and tall fescue performed best mixed contaminated soil.

# Chapter Two

# Background

## 2.1 Metals' Classification and Sources

Nutritional elements that are absorbed by the soil and air adjoining the plants, as well as water, are indispensable for plant growth. A well-equiponderant nutrient outfit is vital for the whole harvest in order to avoid needless growth or mineral deficiency since mineral elements impinge on plant physiology and plant maturity.

There are about 16 nutritional elements required to grow crops (Table 1). While three essential nutrients; carbon (C), hydrogen (H) and oxygen (O2) are riveted from atmospheric carbon dioxide and water, the remainder 13 nutrients are taken up from the soil and are frequently classified as primary nutrients, secondary nutrients and micronutrients.

**Table 2.1: Nutritional elements required to grow crops.**

| **Basic elements in organic matter** | | **Mineral elements** |
|---|---|---|
| **(Organic nutrients)** | **Macro-elements** | **Micro-elements** |
| Carbon (C) | Calcium (Ca) | Boron (B) |
| Hydrogen (H) | **Magnesium (Mg) a** | Chlorine (Cl) |
| Oxygen (O) | Nitrogen (N) | Copper (Cu) |
| | Phosphorus (P) | **Iron (Fe)** |
| | Potassium (K) | Manganese (Mn) |
| | Sulphur (S) | Molybdenum (Mo) |
| | | Zinc (Zn) |
| | | (Aluminium (Al)) |
| | | (Cobalt (Co)) |
| | | (Sodium (Na)) |
| | | (Nickel (Ni)) |
| | | (Silicon (Si)) |
| | | (Vanadium (V)) |

The ***primary nutrients*;** Nitrogen (N), Phosphorus (P) and Potassium (K) are taken from intermingled fertilizers. They are consumed in superior quantities by yields matched up to secondary nutrients and micronutrients.

The **s*econdary nutrients*;** Calcium (Ca), Magnesium (Mg) and Sulfur (S) are generally consumed in smaller amounts than the primary ones. The foremost source for affording the soil with calcium and magnesium is dolomitic lime (aglime. Sulfur is being found in fertilizers; such as potassium and magnesium sulfate, gypsum (calcium sulfate) and elemental sulfur).

***Micronutrients*;** Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Boron (B) and Molybdenum (Mo) are needed in smaller amounts compared to secondary nutrients. They are available in manganese, zinc and copper sulfates, oxides, oxy-sulfates and chelates, and boric acid and ammonium molybdate.

Soils diverge in terms of the quality and quantity of the mineral nutrients (macro and microelements). They also differ in the extent of the uptake ease of use by the roots. The degree of uptake depends on factors such as: soil composition, rooting depth, and organic matter content and is customized by soil moisture and pH. These factors influence the chemical and biochemical processes that take place in the soil and manage the mobility and plant-availability of nutrients. Plant-available nutrients are taken up as ions, dissolved in the soil solution and their uptake depends on the water flow in the course of the soil-root-shoot pathway.

Soil nutrients metals sources are either natural resources or anthropogenic. Natural sources originate from close relative material because of chemical and biological disintegration of rocks, stones and organic remnants, air and water.

Anthropogenic sources include involvements of micro-elements throughout the use of fertilizers, pesticides, organic manures, industrial and public wastes, irrigation using waste waters and wet or dry consigns from industry sources like iron and steel industry, metal smelters and metal refineries.

**Heavy Metals:** Heavy metallic elements have a high density compared to water. Taking into consideration that heaviness and toxicity are inter-related, heavy metals enclose metalloids, such as arsenic, that are toxic at low level of exposure. Lately, the environmental pollution caused by these metals has been inducing ecological and global public health concern. Inhabitants are prone to these metals at the present time more than before because of their use in several industrial, agricultural, domestic and technological submissions.

**Importance of Metals:** It is widely recognized that micronutrients such as Iron (Fe), Manganese (Mn), Copper (Cu), magnesium (Mg) and Zinc (Zn) are essential metals for plant growth. However, plants may accumulate heavy metals that are found in soils, such as Cadmium (Cd), Nickel (Ni), Chromium (Cr) and Lead (Pb) which are not important for plant growth and cause serious problems to the environment. The concentration of heavy metals in soil solution plays a vital role in determining the density of ions in plants.

Some human activities such as mining and smelting of metals, electroplating, gas exhausting, energy and fuel production, using fertilizer, controlling sewage and overusing pesticide, municipal waste generation, etc. have led to metal pollution and have consequently become a hazardous source of threats to the environment. High degrees of concentration of heavy metals in water and soil form an enormous source of danger to environment and human health.

Excessive accumulation of heavy metals such as Cadmium (Cd), Nickel (Ni), Chromium (Cr) and Lead (Pb) is toxic to most plants. When heavy levels of metals ions are absorbed by roots, they are translocated to shoot, and this will result in impaired metabolism and reduced growth. Thus, it is important to check and experience methods to control the levels of metal concentration in soil.

The clean-up of metal-contaminated soils by traditional physicochemical methods is both expensive and also affects the normal properties of the soil. On the other hand, phytoremediation focuses on the use of green plants to reduce the levels of metals in the soil. This modern technique has many advantages. Firstly, it is less expensive compared to the traditional techniques, and moreover, it is more effective and environmentally sustainable. The success of this technique relies heavily on factors such as the identification of suitable plant species that tolerates and accumulates heavy metals and produces large amounts of biomass using suitable agricultural techniques.

In addition to cleaning the soil up from high concentrated metals, the biomass produces though this technique is harvested and used for many purposes such as feeding animals. Nevertheless, this technique is not widely used up to now because the growth of the plants that are used in the application of this technique is reduced and gagged by the high levels of metals.

In this study, phytoremediation technique will be implemented for treatment-contaminated water with Iron (Fe) and magnesium (Mg) metals using barely plant (*Hordeum vulgare L.*) and clover *(Trifolium)* plant. These plants germinated with plant growth promoting rhizobacteria (PGPR).

**Heavy Metals Sources:** Environmental sources of heavy metals are gelogenic, industrial, agricultural, pharmaceutical, domestic effluents and/or atmospheric sources. While heavy metals are naturally found throughout the earth's coating, environmental infectivity and individual exposure result from anthropogenic actions such as mining and smelting operations, use of domestic and agricultural use of metals and metal-containing compounds. Industrial sources include metal processing in refineries, coal burning in power plants, petroleum combustion, nuclear power stations and high tension lines, plastics, textiles, microelectronics, wood preservation and paper processing plants.

The environmental contaminators include metal corrosion, atmospheric deposition, soil erosion of metal ions and leaching of heavy metals,

sediment re-suspension and metal evaporation from water resources to soil and ground water.

Other reasons of environmental contamination with heavy metals refer to natural phenomena such as weathering and volcanic eruptions.

**Impacts of Heavy Metals:** Metals such as Cobalt (Co), Copper (Cu), Chromium (Cr), Iron (Fe), Magnesium (Mg), Manganese (Mn), Molybdenum (Mo), Nickel (Ni), Selenium (Se) and Zinc (Zn) are vital nutrients for biochemical and physiological functions. Lack of these nutrients causes several diseases and syndromes. Their bioavailability is influenced by either physical factors such as temperature, adsorption and sequestration, chemical factors such as complexation kinetics, lipid solubility and octanol/water partition coefficients or biological factors such as species characteristics, and biochemical/physiological adaptation.

The heavy metals are significant as well as essential constituents of several enzymes that play important roles in various oxidation-reduction reactions, e.g., copper functions as an essential co-factor for several oxidative stress-related enzymes such as catalase. In addition, it is incorporated into a number of metalloenzymes implicated in hemoglobin production, carbohydrate metabolism, catecholamine biosynthesis and hair keratin. Despite the consequences of its role as a nutrient, copper can be toxic because the transitions between Cu (II) and Cu (I) can cause the production of superoxide and hydroxyl radicals. Besides, excessive exposure to copper causes cellular damage leading to Wilson disease in humans.

The other metals that are essential elements for biological functioning produce cellular and tissue damage leading to a variety of human diseases such as Aluminum (Al), Arsenic (As), Cadmium (Cd), Gallium (Ga), Lead (Pb), Lithium (Li), Mercury (Hg), Nickel (Ni).

Heavy metals in biological systems have an effect on cellular organelles and components such as cell membrane, mitochondrial, lysosome, endoplasmic reticulum, nuclei, and some enzymes that take part in metabolism, detoxification and damage repair.

Metal ions interact with cell components like DNA and nuclear proteins, causing DNA damage and conformational changes that may lead to cell cycle modulation and carcinogenesis.

Several studies from our laboratory have demonstrated that reactive oxygen species (ROS) production and oxidative stress play a key role in the toxicity and carcinogenicity of metals such as arsenic, cadmium, chromium, lead, and mercury. Because of their high degree of toxicity, these five elements rank among the priority metals that are of great public health significance. They are all systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure. According to the United States Environmental Protection Agency (U.S. EPA), and the International Agency for Research on Cancer (IARC), these metals are also classified as either “known” or “probable” human carcinogens based on epidemiological and experimental studies showing an association between exposure and cancer incidence in humans and animals.

**Metals' Tolerance Level in Plant and its Mechanisms:** Metal toxicity is in charge for many visual symptoms in plants. Root growth is often abridged, and foliage may change color. Competition with nutrient ions for

uptake by roots can cause deficiency symptoms (e.g. aluminium causes calcium or magnesium deficiency). Similarly, binding of toxic metals with proteins and other compounds because they resemble essential metals, can also cause toxicity. The plasma membrane of root cells is often damaged by exposure to toxic metals, resulting in leakage of cellular solutes.

Despite the fact that most plants are badly affected by high concentrations of toxic metals, others are able to tolerate toxic environments. Some plants emerge to put up with metals either by excluding them from the shoot or by accumulating metals in older leaves and then dropping them. Others are hyper-accumulators and enclose very high concentrations of metals up to four orders of magnitude higher than those found in most plants.

**Table 2.2: How plants cope with heavy metals.**

| | |
|---|---|
| Avoidance | plants restrict the uptake of metals within root tissue by:<br>1. Plants can prevent metal uptake by exploring less contaminated soil.<br>2. Mycorrhizal fungi, where they can extend their hyphae outside the plants rooting zone up to several tens of meters and transfer the necessary elements to the plant.<br>3. Plants can also restrict contaminant uptake in root tissues by immobilizing metals e.g. through root exudates in the rhizosphere.<br>4. A role of root exudates is to chelate metals and stop their entry inside the cell. The cell wall has also been found to be involved in restricting metal uptake into the cells cytoplasm. |
| Tolerance | Controlling different plant physiological processes, including ROS and MG detoxification. Heavy metals uptake, translocation, chelation, and detoxification. |
| Accumulation | Metal accumulators/hyperaccumulators are plants that increase internal sequestration, translocation and accumulation of metals in their harvestable biomass to levels that far exceed those found in the soil. |

**Physiological Mechanisms of Metal Resistance:** Defiant plants are proficient to grow on metal tainted soil due to avoidance and/or tolerance strategies. Plant resistance to high levels of heavy metals in soils can be caused by either reduced uptake or once taken up; metals have to be altered into a physiologically tolerable form.

**Plant Uptake and Transport of Metals:** The majority of metal accumulating types were revealed in regions having a high metal attentiveness, and majority of such areas exist in steamy regions. These natural plant hyperaccumulators of metal represent diverse sorts, although the majority exist in the family Brassicaceae, e.g., Indian mustard (*Brassica juncea*) rapidly concentrates Cd (II), Ni (II), Pb (II), and Sr (II) into root tissues at levels 500 times bigger than the liquid medium in which they are growing (Salt et al., 1995; Salt and Kramer, 1999). Uptake of metals into root cells, which is the point of entry into living tissue, is a key pace in the phytoextraction course. Nonetheless, for phytoextraction to be successful, the absorbed metals ought to be transported from root to shoot. The mechanisms by which metals are absorbed into the plant root are complex. This route engages move of metals from the soil solution to the root–surface, and subsequently penetration through the root membranes to root cells. Metal ions cannot move unreservedly across the cellular membrane because of their charge. As a result, ion transport into cells must be mediated by membrane proteins that have a transport function, and these are generically referred to as transporters. These transporters acquire an extracellular domain to which the ions attach just before the transport, and

a transmembrane binding structure that connects extracellular and intracellular media. This is an oversimplification, and the uptake process is actually rendered even more complex by the nature of the rhizosphere (Laurie and Manthey, 1994).

Hyperaccumulator plants take metals up from soil in direct proportion to their bioavailability (Wenzel et al., 2003). Bioavailability is regulated by electrochemical potential gradient that exists for each metal ion across the plasma membrane of root cells (Welch, 1995). On the other hand, the precise nature of the membrane transporters that control the incursion athwart the plasma membrane into the cytoplasm is not known yet. **Restriction of Metal Uptake:** Plasma membrane is the primary constitution of living cells prone to heavy metals. The membrane serves as a fence for the movement of heavy metals into cytoplasm. The restriction of metals at the plasma membrane inhibits the uptake and accumulation of metals by thwarting their entry into the cytoplasm. This could be done by changing the ion binding capacity of the cell wall and/or decreasing the uptake of metal ions through modified ion channels, and/or by removing metals from cells with vigorous efflux pumps and/or with root exudates. The cell wall and membrane interface could be a site of metal tolerance since a noteworthy amount of metals has been reported to be accumulated there. Divalent and trivalent metal cations can bind plant cell walls because of the presence of functional groups such as COOH, OH and SH. Pectins are polymers that include carboxyl groups that enable the binding of divalent and trivalent heavy metals ions. In enriched heavy metal

environments, some plants will raise the capacity of their cell wall to bind metals by increasing polysaccharides, such as pectin. Resistant plants can also restrict the entry of metals by immobilizing them in the rhizosphere with root exudates outside the plasma membrane, for example, Ni exclusion in plants involving Ni-chelating exudates which include histidine and citrate. In non-hyperaccumulator plants, these Ni chelators accumulate in their root exudates which, in turn, decrease Ni uptake. The copper exclusion could be due to its chelation with citrate and malate exudates in the rhizosphere of wheat roots.

**How Does PGPR Combat Heavy Metal Stress:** Contrasting a lot of noxious waste, which experience biodegradation to produce less toxic, less mobile, and less bioavailable products, removing heavy metals from an infected environment is much thornier. Heavy metals cannot be despoiled biologically and are ultimately indestructible, though the speciation and bioavailability of metals may change as environmental factors change. Some metals (e.g., zinc, copper, nickel, and chromium) are essential and beneficial micronutrients for plants, animals, and microorganisms (Olson et al., 2001), whereas others (e.g., cadmium, mercury, and lead) have no known biological or physiological function (Gadd, 1992). Nevertheless, high concentrations of these heavy metals greatly affect microbial communities, and possibly reduce their total microbial biomass (Giller et al., 1998), their activity (Romkens et al., 2002), or change microbial community structure (Gray and Smith, 2005). Thus, at higher concentrations, either heavy-metal ions entirely restrain a microbial

population by inhibiting its various metabolic activities or these organisms may develop resistance or tolerance to such elevated levels of heavy metals. This ability to live and grow under in the presence of high metal concentration exists in many rhizospheric microorganisms. Ledin, 2000, explained the difference between microbial tolerance and resistance; he defined tolerance as the ability to cope with metal toxicity by means of intrinsic properties of the microorganisms, whereas resistance is the ability of microbes to detoxify heavy metals by being activated in direct response to the high heavy-metal concentrations. Toxic heavy-metal pollutants should be either completely removed from the contaminated soil or transformed or immobilized in ways that submit them safe. For survival under metal-stressed environment, PGPR have developed a range of mechanisms by which they can immobilize, mobilize, or transform heavy metals, thereby, rendering them inactive (Nies, 1999). These mechanisms include:

1) Exclusion metal ions that are kept away from target sites.
2) Extrusion-metals that are pushed out of the cell through chromosomal/plasmid mediated events.
3) Accommodation: metals that form complexes with metal-binding proteins, e.g., mettalothioneins, low molecular weight proteins (Kao et al., 2006; Umrania, 2006), and other cell components.
4) Biotransformation, in which the toxic metal is reduced to less toxic forms, and

5) Methylation and demethylation. One or more of the above-mentioned mechanisms allow the microbes to function metabolically in metal-contaminated sites/soils. Interest in exploiting these bacterial properties to remediate heavy-metal contaminated sites is growing, and early results from their application are promising (Lloyd and Lovley, 2001; Hallberg and Johnson, 2005).

## 2.2 Plant Nutrients and Their Functions

Vegetation requires adequate quantities of macro- and micro-elements for their physiological and biochemical function. Every element has its physiological tasks. Distant from N and P, other micro and macro elements can substitute one another to definite ranks according to the element and its function. However, N and P play an inimitable character that cannot be played by other elements in the plant metabolism.

Table 2.3 below recapitulates the major physiological functions and deficiency symptoms of the basic elements.

**Table 2.3: Major physiological functions and deficiency symptoms of the basic elements.**

| Mineral nutrient | Major functions | Deficiency symptoms |
|---|---|---|
| *N* | *Structural component of amino and nucleic acids, proteins, nucleotides, chlorophyll and metabolic enzymes* | *Chlorosis of basal leaves; yield reduction* |
| *P* | *Used in high-energy bonds (ATP); structural component of nucleic acids, phospholipids, phosphoproteins* | *Chlorosis of leaves (reddening on leaves of red cultivars); reduced berry set and yield* |
| *K* | *Involved in enzyme activation (carbohydrate metabolism and transport); act in osmosis and ionic balance; control the acidity and pH of grape juice* | *Chlorosis of basal leaves; yield reduction* |
| *Mg* | *Cofactor and activator of many enzymes involved in protein synthesis, RNA formation, synthesis of chlorophyll and other leaf pigments, phosphorylation processes and others* | *Chlorosis of basal leaves* |
| *Fe* | *Enzyme activator (part of prosthetic groups, involved in redox reactions, bridging element between enzyme and substrate)* | *Chlorosis of apical leaves first* |
| *Zn* | *Functional, structural, and regulatory cofactor of enzymes (synthesis of* | *Stunted shoot growth, „little leaves", chlorosis of apical* |

| | | |
|---|---|---|
| | *RNA, indoleacetic acid, and others)* | *leaves* |
| *N* | *Structural component of amino and nucleic acids, proteins, nucleotides, chlorophyll and metabolic enzymes* | *Chlorosis of basal leaves; yield reduction* |
| *P* | *Used in high-energy bonds (ATP); structural component of nucleic acids, phospholipids, phosphoproteins* | *Chlorosis of leaves (reddening on leaves of red cultivars); reduced berry set and yield* |

## 2.3 Brackish Water

Brine water defined as a solution contains significant concentrations of dissolved salts ions. Typically it contains high levels of free ions such as $Na^{+}$, $Cl^{-}$ as major ions, and minor ions than normal levels for $Ca^{+2}$, $Mg^{+2}$ ,$K^{+1}$ ,$SO^{-2}$ and $CO_3^{-2}$. These concentrations are usually expressed as total dissolved salts per liter in units of parts per thousand (per mile, %) or parts per million (ppm) (Al Agha et al, 2005; Arnot et al, 2011).

TDS (total dissolved salts) parameter for generated brine water produced form reverse osmosis plants in Jericho districts range from 5000-10000 mg/LTDS. Where NaCl ionic compound considers as main compound exists in large quantity within this range (Marie and Vengosh, 2011).

**Table 2.4: Classification of water salinity based on dissolved salts.**

| **Water salinity based on dissolved salts** | | | |
|---|---|---|---|
| **Fresh water** | **Brackish water** | **Saline water** | **Brine** |
| < 0.05% | 0.05–3% | 3–5% | > 5% |
| < 0.5 ‰ | 0.5 – 30 ‰ | 30 – 50 ‰ | > 50 ‰ |

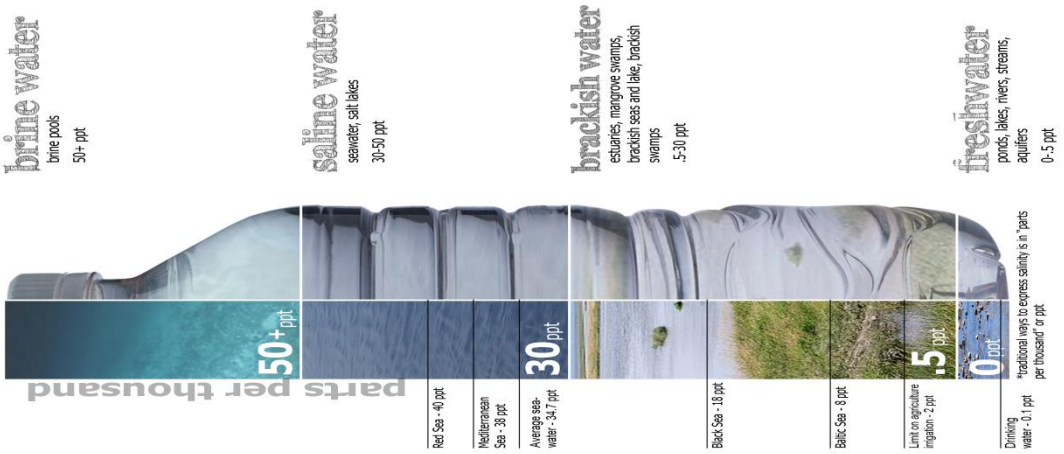



**Figure 2.1:** Graphic breakdown of water salinity, defining freshwater, brackish water, saltwater, and brine water.

### 2.3.1 Brackish Water Impacts:

Brine affects soil structure where ions in brine water effect soil texture and increase salinity of soil, especially $Na^+$ and $Cl^-$ according to amount of ions impact soil (Bohn et al, 1985).

Sodium is a particular concern for soil quality where negatively charged particles form soil structure is typically matched with divalent cations that they are calcium and magnesium. This composition connects clay particles into large flocs. These flocs do not pack tightly to allow for air, water and roots to pass through it easily. Additions of sodium ions as monovalent cations result in exchange between monovalent and divalent cations at negative charges in soil particles. These exchange results in variation in soil structure cause disruption on flocculation of soil, where flocs scatter, and soil particles pack more tightly (Bohn et al., 1985; Cramer, 2002).

Impact of brine water is the most severe environmental stress on plants. The common ions stress and inhibit plant growth are sodium and chloride.

When these ions enter the soil and surround the rhizosphere, it causes differences between water potential in roots above water potential in soils. This alteration lessens the movement of water from soil into rhizosphere, limiting water and nutrient uptake (Aard, 2007; Ashraf, 2004; Das and Parida, 2005).

$Na^+$ is the principal cause of confusion from enzyme activation to protein synthesis. It is considered more toxic than $Cl^-$ ion. Once high concentration of $Na^+$ enters rhizosphere, it rapidly translocates to shoots via the xylem, and then it accumulates in leaves and results in necrosis and short of lifetimes of individual leaves.

Furthermore, sodium possesses copious physiological effects. It causes deficiencies of other nutrients by interfering with ion transporters $K^+$ that is essential to activate more than 50 enzymes and for synthesis of protein which plays a role in cellular functions. This interference, which happens due to Na+, is similar to ionic radius to $K^+$. This similarity allows for competition between these two ions while competition results in superfluity of sodium in tissue compared to potassium, and enters in coordination with t-RNA, resulting inhibited protein synthesis and leading disruption to these cellular functions (Blaha et al., 2000; Blumwald and Aharon, 2000; Carden et al., 2003).

The same competition is found with displacement with calcium ion by sodium ion where it reduces calcium concentration within plant while his competition impairs gas exchange rate for photosynthesis. Even deficiencies of magnesium due to sodium entrance inhibit photosynthetic

rates in plants, further chlorophyll synthesis and functions (Parida and Das, 2005).

Chloride ion in plants requires to some limited levels as vital ions inside plants. It is involved in photosynthetic mechanisms, in adjusting osmotic potential, and maintains electrical charge through membrane (Naidoo and Somaru, 2008).

Excess levels than required for plants process cause toxicity and inhibition of photosynthetic process while its accumulation causes toxicity to leaves (James et al., 2006; Naidoo and Somaru, 2008).

## 2.4 Plant Growth Promoting Rhizobacteria (PGPR)

The useful free-living soil bacteria that exist in association with the roots of many different plants are generally referred to as plant growth-promoting rhizobacteria (PGPR) (Kloepper and Schroth, 1978). Depending on their relationship with the host plants, PGPR can be divided into two major groups:

1. Symbiotic rhizobacteria, which may invade the interior of cells and survive inside the cell (also called intracellular PGPR, e.g., nodule bacteria), and, Free-living rhizobacteria that exist outside plant cells (called extracellular PGPR, e.g., Bacillus, Pseudomonas, Burkholderia, and Azotobacter) (Khan, 2005; Babalola and Akindolire, 2011). The major factor that affects the high concentration of bacteria found in the rhizosphere is the presence of high nutrient levels (especially small molecules such as amino acids, sugars, and organic acids) that are exuded from the roots of most

plants (Bayliss et al., 1997; Penrose and Glick, 2001). PGPR can positively influence plant growth and development in three different ways.

2. Synthesize and provide growth-promoting compounds to the plants (Glick, 1995) (Table 2.5).
3. Facilitate the uptake of certain environmental nutrients such as nitrogen, phosphorus, sulfur, magnesium, and calcium (Bashan and Levanony, 1990; Belimov and Dietz, 2000; Cakmakci et al., 2006), and Reduce or prevent some harmful effects caused by phytopathogenic organisms or other diseases (Khan et al., 2002; Lugtenberg and Kamilova, 2009).

Overall, rhizobacteria improves plant growth by synthesizing phytohormone precursors (Ahmad et al., 2008), vitamins, enzymes, siderophores and antibiotics (Burd et al., 2000; Noordman et al., 2006). PGPR also increases plant growth by synthesizing specific enzymes, which induce biochemical changes in plants. For example, ethylene plays a critical role in diverse plant developmental processes, such as leaf senescence and abscission, epinasty, and fruit ripening (Vogel et al., 1998). Ethylene also regulates node factor signaling, nodule formation, and has primary functions in plant defense systems. Moreover, as a result of the plant infection by rhizobacteria, ethylene production is increased (Boller 1991), which, at higher concentrations, will hold back plant growth and development (Morgan and Drew, 1997; Grichko and Glick, 2001). However, bacterial 1-aminocyclopropane -1- carboxylate (ACC), a

deaminase synthesized by PGPR (Babalola et al., 2003; Madhaiyan et al., 2006; Rajkumar et al., 2006), assuages stress induced by such ethylene-mediated impact. In addition, rhizobacterial strains can solubilize inorganic P (Glick et al., 1998; Yu et al., 2012), or mineralize organic P, and thus enhance plant stress tolerance to deficiency, salinity, and metal toxicity (Ponmurugan, 2006; Khan et al., 2007). Pishchik et al, mathematically simulated the succession of events that began with phytohormone (IAA and ethylene) synthesis and ended with higher uptake of ions by roots, under conditions of cadmium stress. Probably, synthesis of phytohormones might be enthused by exposure to heavy metals. On the other hand, these processes may be stalled by high heavy-metal concentrations (DellAmico et al., 2005), because many rhizobacteria cannot survive when such concentrations are high. Many different microbial communities are able to survive high heavy-metal concentrations when living in association with rhizospheric soils and the rhizoplane.

**Table 2.5: Plant Growth Promoting Rhizobacteria Strains**

| PGPR | Plant growth promoting traits |
|---|---|
| *Pseudomonas putida* | IAA, siderophores, HCN, ammonia, exo-polysaccharides, phosphate solubilization |
| *Pseudomonas aeruginosa* | IAA, siderophores, HCN, ammonia, exo-polysaccharides, phosphate solubilization |
| *Klebsiella* sp. | IAA, siderophores, HCN, ammonia, exo-polysaccharides, phosphate solubilization |
| Enterobacter asburiae | IAA, siderophores, HCN, ammonia, exo-polysaccharides, phosphate solubilization |
| *Pseudomonas* sp. A3R3 | IAA, siderophores |
| *Psychrobacter* sp. SRS8 | Heavy metal mobilization |

| | |
|---|---|
| *Bradyrhizobium* sp. | IAA, siderophores, HCN, ammonia, exo-polysaccharides |
| *Pseudomonas aeruginosa* 4EA | Siderophores |
| *Bradyrhizobium* sp. 750, *Pseudomonas*sp., *Ochrobactrum cytisi* | Heavy metal mobilization |
| *Bacillus* species PSB10 | IAA, siderophores, HCN, ammonia |
| *Paenibacillus polymyxa* | IAA, siderophores |
| *Rhizobium phaseoli* | IAA |
| *Stenotrophomonas maltophilia* | Nitrogenase activity, phosphate solubilization, IAA, ACC Deaminase |
| *Rahnella aquatilis* | Phosphate solubilization, IAA, ACC deaminase |
| *Pseudomonas aeruginosa*,*Pseudomonas fluorescens*, *Ralstonia metallidurans* | Siderophores |
| *Proteus vulgaris* | Siderophores |
| *Pseudomonas* sp. | Phosphate solubilization, IAA, siderophore, HCN, biocontrol potentials |
| *Azospirillum amazonense* | IAA, nitrogenase activity |
| *Mesorhizobium* sp. | IAA, siderophores, HCN, ammonia |
| *Pseudomonas* sp. | ACC deaminase, IAA, siderophore |
| *Serratia marcescens* | IAA, siderophore, HCN |
| *Pseudomonas fluorescens* | ACC deaminase, phosphate solubilization |
| *Acinetobacter* sp. | ACC deaminase, IAA, antifungal activity, $N_2$- |

| | |
|---|---|
| , *Pseudomonas* sp. | fixation, phosphate solubilization |
| *Enterobacter* sp. | ACC deaminase, IAA, siderophore, phosphate solubilization |
| *Burkholderia* | ACC deaminase, IAA, siderophore, heavy metal solubilization, phosphate solubilization |
| *Pseudomonas jessenii* | ACC deaminase, IAA, siderophore, heavy metal solubilization, phosphate solubilization |
| *Azotobacter* sp., *Mesorhizobium* sp.,*Pseudomonas* sp., *Bacillus* sp. | IAA, siderophore, antifungal activity, ammonia production, HCN |
| *Bradyrhizobium* sp. | IAA, siderophores, HCN, ammonia |
| *Rhizobium* sp. | IAA, siderophores, HCN, ammonia |
| *Mesorhizobium ciceri, Azotobacter chroococcum* | IAA, siderophores |
| *Pseudomonas, Bacillus* | Phosphate solubilization, IAA and siderophores |
| *Klebsiella oxytoca* | IAA, phosphate solubilization, nitrogenase activity |
| *Bacillus* spp., *Pseudomonas* spp.,*Azotobacter* spp., *Rhizobium* spp. | IAA, ammonia production |
| *Pseudomonas fluorescens* | Induced systemic resistance, antifungal activity |
| *Pseudomonas chlororaphis* | Antifungal activity |
| *Baciilus subtilis* | Antifungal activity |
| *Gluconacetobacter diazotrophicus* | Zinc solubilization |
| *Brevibacillus* spp. | Zn resistance, IAA |
| *Bacillus subtilis* | IAA, phosphate solubilization |
| *Pseudomonas* sp., *Bacillus* sp. | IAA, siderophore, phosphate solubilization |
| *Pseudomonas putida* | Antifungal activity, siderophore, HCN, phosphate solubilization |

### 2.4.1 Effect of PGPR on Plant Growth:

The physiological effect of microbial indole acetic acid (IAA) on plant growth eventually depends on the quantity of hormone obtainable to the plant, which is supported by the interaction between the plant and the bacterium (Patten and Glick, 1996). Common forms of potential involvement between the plant and the bacterium are imperative in order to make use of a positive effect. The forms are:

a)Convey of IAA genes straight into the host genome as is the case in *Agrobacterium* species,

b) Infection of internal regions of the plant and secretion of IAA into the surrounding tissue and,

c) Colonization of the outside plane and secretion of IAA as an exogenous source to plants. The effect is primarily thought to be valuable when the bacteria are colonizing the peripheral facade of the plant (Del Gallo and Fendrik, 1994).

Untimely work demonstrated that PGPR such as *Azotobacter paspali* secreted IAA into culture media and drastically enlarged the dry weight[2] of leaves and roots of numerous plant species following root treatment (Barea and Brown, 1974). *Azospirillum brasilense*, which had the ability to fabricate plant growth-promoting substances such as indole acetic acid (IAA), indole lactic acid, gibberellin and cytokinin when applied to pearl millet (*Pennisetum americanum* L.), increased the number of lateral roots which were densely covered by root hairs (Tien et al., 1979).

[2] *The dry matter (or otherwise known as dry weight) is a measurement of the mass of something when completely dried and all water has been removed.*

### 2.4.2 Mechanisms of Plant Growth Promotion by PGPB:

Plant growth promotion bacteria **(**PGPB) interceded plant growth promotion takes place by the modification of the completely microbial community in rhizosphere position throughout the fabrication of different substances. In the main, PGPB promote plant growth frankly by either help resource acquisition (essential minerals) or modulating plant hormone levels, or indirectly by lessening the inhibitory possessions of various pathogens on plant growth and development in the forms of biocontrol agents (Glick, 2012; Ahemad and Kibret, 2014).

## 2.5 Remediation Techniques

Soil remediation, also known as soil washing, is a term that refers to various processes designed to remove contaminants such as hydrocarbons (petroleum and fuel residues), heavy metals, pesticides, cyanides, volatiles, creosote, and semi-volatiles from soil. Soil remediation is needed to clean and maintain high quality standards of soil, water and air that can consequently benefit commercial cultivation, and wild flora and fauna. Remediation of heavy metal infectivity in soils is further complicated. Heavy metals cannot be destroyed biologically, but are transformed from one oxidation state or organic complex to another. So far, methods used for their remediation such as physical and chemical methods are not suitable for practical applications, because of their high cost, low efficiency, large devastation of soil construction and fecundity and high confidence on the contaminants of concern, soil properties and site conditions. Thus, natural and environmental friendly technology, cost-effective, aesthetically

pleasant, soil organism friendly, diversity enhancer, energy derivation from sunlight, and more importantly, it is able to retain the fertility status of the soil even after the removal of heavy metals this new technology called phytoremediation.

**Phytoremediation:** Phytoremediation is an in situ biomediation process that uses green plants and the microorganisms that are associated with them to extract, sequester, or detoxify pollutants. Plants have the capacity to take up, accumulate, degrade, or eliminate metals, pesticides, solvents, crude oil, and many industrial contaminants. Phytoremediation is a clean, cost-effective, environment-friendly technology, especially for treating large and diffused areas that are contaminated. Depending on the method used and nature of the contaminant involved, phytoremediation areas where metals and other inorganic compounds exist may employ one of several techniques:

1) phytoextraction,
2) phytostabilization,
3) phytostimulation,
4) phytovolatilization/rhizovolatilization,
5) phytodegradation, and/or
6) rhizofiltration.

The mechanism carried out in this study is phyto-extraction mechanism in which plants take up salts ions during irrigation with brine water, and accumulate it in above ground portions of plant. After biomass reached its crop coefficient (Kc), it can be harvested to clean soil. Plants that can

potentially accumulate large quantities of metals by natural methods have been identified, and are being studied for their use to remediate heavy metal contaminants. These plants are called hyperaccumulators. Unluckily, at high enough metal levels, even hyperaccumulating plants are slow growing and manage only a small size. As a result, high metal levels restrain plant growth, even in plants that are capable of hyperaccumulating them. Depending upon the amount of metal at a meticulous site and the type of soil, even hyperaccumulating plants may require 15–20 years to remediate an encrusted site. This time frame is usually too slow for practical application.

**Phytoextraction:** is the uptake/absorption and translocation of contaminants by the plant roots into the above ground portions of the plants (shoots) that can be harvested and burned gaining energy and recycling the metal from the ash (Erakhrumen and Agbontalor, 2007; Erdei et al., 2005; Ibeanusi, 2004).

**Phytostabilisation:** also known as phytoimmobilization, is the use of certain plant species to immobilize the contaminants in the soil and groundwater through absorption and accumulation in plant tissues, adsorption onto roots, or precipitation within the root zone preventing their migration in soil, as well as their movement by erosion and deflation ash (Erakhrumen and Agbontalor, 2007; Erdei et al., 2005; Ibeanusi, 2004).

**Rhizofiltration:** is the adsorption or precipitation onto plant roots or absorption into and sequesterization in the roots of contaminants that are in

solution surrounding the root zone by constructed wetland for cleaning up communal wastewater ash (Erakhrumen and Agbontalor, 2007; Erdei et al., 2005; Ibeanusi, 2004).

**Phytovolatilization:** is the uptake and transpiration of a contaminant by a plant, with release of the contaminant or a modified form of the contaminant to the atmosphere from the plant. Phytovolatilization occurs as growing trees and other plants take up water along with the contaminants. Some of these contaminants can pass through the plants to the leaves and volatilize into the atmosphere at comparatively low concentrations ash (Erakhrumen and Agbontalor, 2007; Erdei et al., 2005; Ibeanusi, 2004).

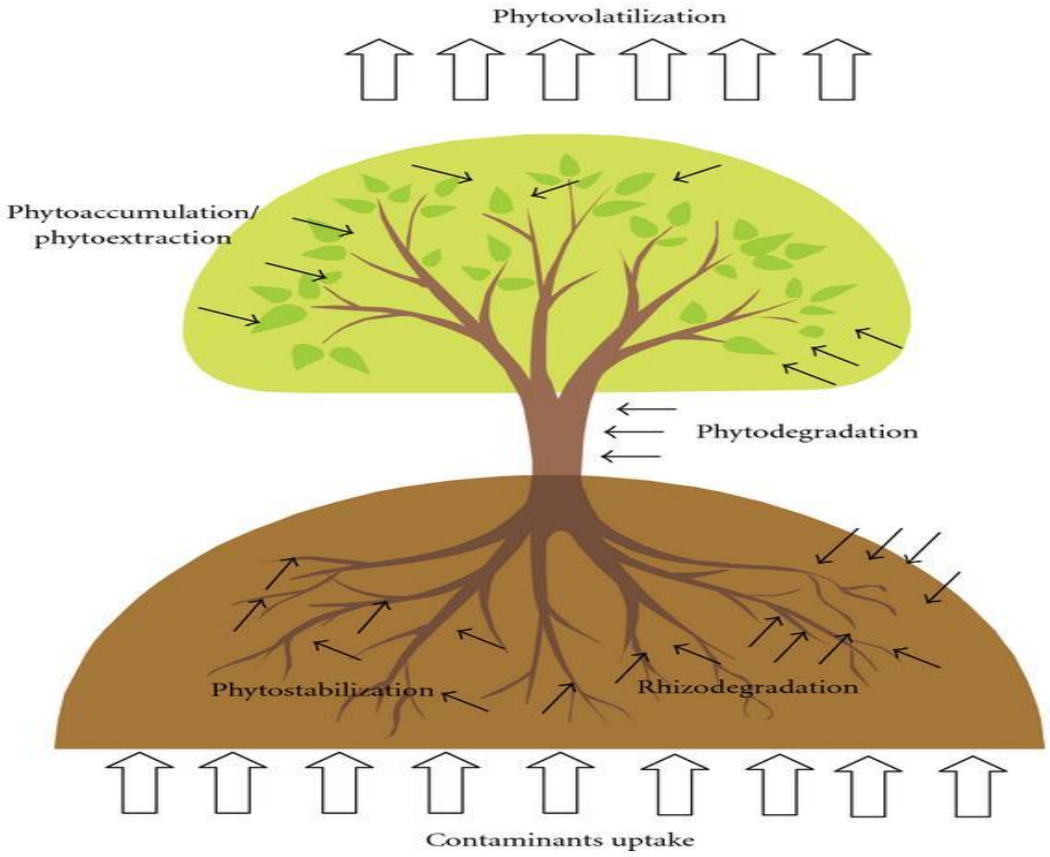



**Figure 2.2:** The mechanisms of heavy metals uptake by plant through phytoremediation technology.

## 2.6 Factors Affecting the Uptake Mechanisms

There are several factors that can affect the uptake mechanism of heavy metals, as shown in Figure 2.3. By having knowledge about these factors, the uptake performance by plant can be greatly improved.

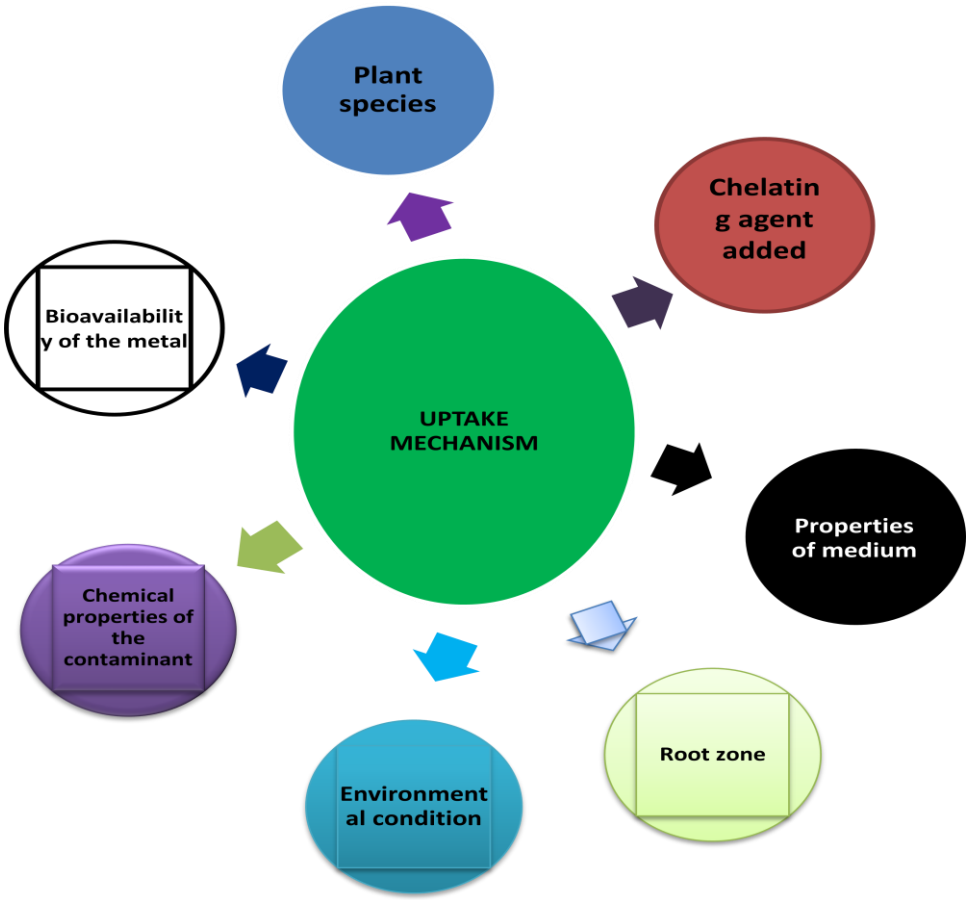



**Figure 2.3:** Factors affecting the uptake mechanisms of heavy metals.

**The Plant Species:** Plants species or varieties are screened, and those with superior remediation properties are selected (Prasad and Oliveira Freitas, 2003). The uptake of a compound is affected by plant species characteristics (Burkan and Schnoor, 1996). The success of phytoremediation technique depends upon the identification of suitable plant species that hyperaccumulate heavy metals and produce large amounts

of biomass using established crop production and management practices (Rodriguez et al., 2005).

**Properties of Medium:** Agronomical practices are developed to enhance remediation (pH adjustment, addition of chelators, fertilizers) (Prasad and Oliveira Freitas, 2003). For example, the amount of lead absorbed by plants is affected by the pH, organic matter, and the phosphorus content of the soil. To reduce lead uptake by plants, the pH of the soil is adjusted with lime to a level of 6.5 to 7.0 (Traunfeld and Clement, 2001).

**The Root Zone:** It is of special interest in phytoremediation. It can absorb contaminants and store or metabolize it inside the plant tissue. Degradation of contaminants in the soil by plant enzymes exuded from the roots is another phytoremediation mechanism. A morphological adaptation to drought stress is an increase in root diameter and reduced root elongation as a response to less permeability of the dried soil (Merkl et al., 2005).

**Vegetative Uptake:** It is affected by the environmental conditions (Burken and Schnoor, 1996). The temperature affects growth substances and consequently the root length. Root structure under field conditions differs from that under greenhouse condition (Merkl et al., 2005). The success of phytoremediation, more specifically phytoextraction, depends on a contaminant-specific hyperaccumulator (Tu et al., 2004). Understanding mass balance analyses and the metabolic fate of pollutants in plants are the keys to proving the applicability of phytoremediation (Mwegoha, 2008).

Metal uptake by plants depends on the bioavailability of the metal in the water phase, which in turn, depends on the retention time of the metal, as well as the interaction with other elements and substances in the water. Furthermore, when metals have been bound to the soil, the pH, redox potential, and organic matter content will all affect the tendency of the metal to exist in ionic and plant-available form. Plants will affect the soil through their ability to lower the pH and oxygenate the sediment, which affects the availability of the metals (Fritioff and Greger, 2003), increasing the bioavailability of heavy metals by the addition of biodegradable physicochemical factors, such as chelating agents and micronutrients (Van Ginneken et al., 2007).

**Addition of Chelating Agent:** The increase of the uptake of heavy metals by the energy crops can be influenced by increasing the bioavailability of heavy metals through addition of biodegradable physicochemical factors such as chelating agents, and micronutrients, and also by stimulating the heavy-metal-uptake capacity of the microbial community in and around the plant. This faster uptake of heavy metals will result in shorter and, therefore, less expensive remediation periods. However, with the use of synthetic chelating agents, the risk of increased leaching must be taken into account (Van Ginneken et al., 2007). The use of chelating agents in heavy-metal-contaminated soils could promote leaching of the contaminants into the soil. Since the bioavailability of heavy metals in soils decreases above pH 5.5–6, the use of a chelating agent is warranted, and may be required, in alkaline soils. It was found that exposing plants to EDTA for a longer

period (2 weeks) could improve metal translocation in plant tissue as well as the overall phytoextraction performance. The application of a synthetic chelating agent (EDTA) at 5 mmol/kg yielded positive results (Roy et al., 2005). Plant roots exude organic acids such as citrate and oxalate, which affect the bioavailability of metals. In chelate-assisted phytoremediation, synthetic chelating agents such as NTA and EDTA are added to enhance the phytoextraction of soil-polluting heavy metals. The presence of a ligand affects the biouptake of heavy metals through the formation of metal-ligand complexes and changes the potential to leach metals below the root zone (Seuntjens et al., 2004).

## 2.7 Synergistic Interaction of PGPR and Plants in Heavy Metal Remediation

While numerous plant microbe interactions have been explored, the studies performed to date have largely emphasized plant pathogen interactions. A decade ago, researches on the ecology of microbes in the rhizosphere focused on the microbiological detoxification and decontamination of soil as affected by heavy metals. The fact that PGPR promotes plant growth is well documented (Reed and Glick, 2004; Babalola et al., 2007; Babalola, 2010), and more recently, PGPR has been effectively used to lessen plant stress in metal contaminated soils. The microorganisms that are associated with roots institute a synergistic relationship with plant roots that enhance nutrient absorption and improve plant performance, as well as the quality of soils (Tinker, 1984; Yang et al., 2009). Bacteria interact with and affect plant growth in a variety of ways. A number of bacteria are

phytopathogenic and actively inhibit plant growth, others (e.g., PGPR) facilitate plant growth through several mechanisms, while many soil bacteria do not appear to affect the plant growth at all, although a change in soil conditions could reverse this (Glick 1995). Some microbial communities have the ability to sequester heavy metals, and therefore may be useful for bioremediating contaminated areas (Hallberg and Johnson, 2005; Umrania, 2006). When microbes are used to bioremediate a contaminated site, plant-associated bacteria can be potentially used to improve phytoextraction activities by changing the solubility, availability, and transport of heavy metals, and nutrients as well, by reducing soil pH and releasing chelators (Ma et al., 2011). Among the metabolites produced by PGPR, siderophores play a momentous role in metal mobilization and accumulation (Rajkumar et al., 2010). Lately, Cr and Pb were found to be unconfined into the soil solution after soil was immunized with Pseudomonas aeruginosa (Braud et al., 2009). Pseudomonas aeruginosa can pragmatically only serve as a model system, because it is a well-known pathogen, and regulators would not allow premeditated release of it to the environment. Although field success has not been yet achieved by doing so, the concept of inoculating seeds/rhizospheric soils with selected metal-mobilizing bacteria to improve phytoextraction in metal-contaminated soils has advantage.

## 2.8 1-Amino Cyclopropane-1-Carbozylate (ACC) Deaminase and Stress Reduction from Ethylene

Ethylene is formed under standard plant-growth circumstances and legalizes plant growth, although it is toxic to plants at higher concentrations (Bestwick and Ferro, 1998). In a step-wise metabolic reaction, methionine is first transformed into S-adenosyl-L-methionine by SAM synthetase (Giovanelli et al., 1980) and SAM is then hydrolyzed to ACC and 5-methyl thioadenosine (Kende, 1989) by ACC synthetase. Eventually, ACC is metabolized to ethylene, $CO_2$, and cyanide by ACC oxidase as shown in Figure 2.4 (John, 1991). Quite a few chemicals have been used to manage ethylene-mediated stress in plants. Unluckily, use of such matter is environmentally unfriendly. Applying cyclopropenes can wedge the action of ethylene and it can potentially be used to expand the ledge life of flowers, and sealed plants. Extra compounds are known to inhibit ethylene biosynthesis, although they are potentially harmful to the environment, e.g., silver thiosulfate (Bestwick and Ferro, 1998). In the main, it is imperative that ethylene levels in plants kept at minimum levels possible. This can be accomplished by diminishing the ethylene precursor ACC, which is subject to degradation by an ACC enzyme isolated from a pseudomonas sp. strain ACP and from yeast hansenula saturnus (Honma and Shimomura, 1978; Minami et al., 1998). An ACC deaminase has also been detected in the fungus Penicillium citrinum , and bacterial strains originating from the soil that have ACC deaminase activity have been reported (Glick, 1995; Jia et al., 2000; Belimov et al., 2001; Babalola et al., 2003). This enzyme, ACC

deaminase, degrades ACC to ammonia and α-ketobutyrate, and can be employed to protect plants from ethylene-generated stress (Glick et al., 1998). Many plant kinds necessitate ethylene for seed germination, and ethylene production increases during seed germination and seedling growth (Abeles et al., 1992). However, elevated ethylene levels may inhibit root elongation and depress growth (Morgan and Drew, 1997).

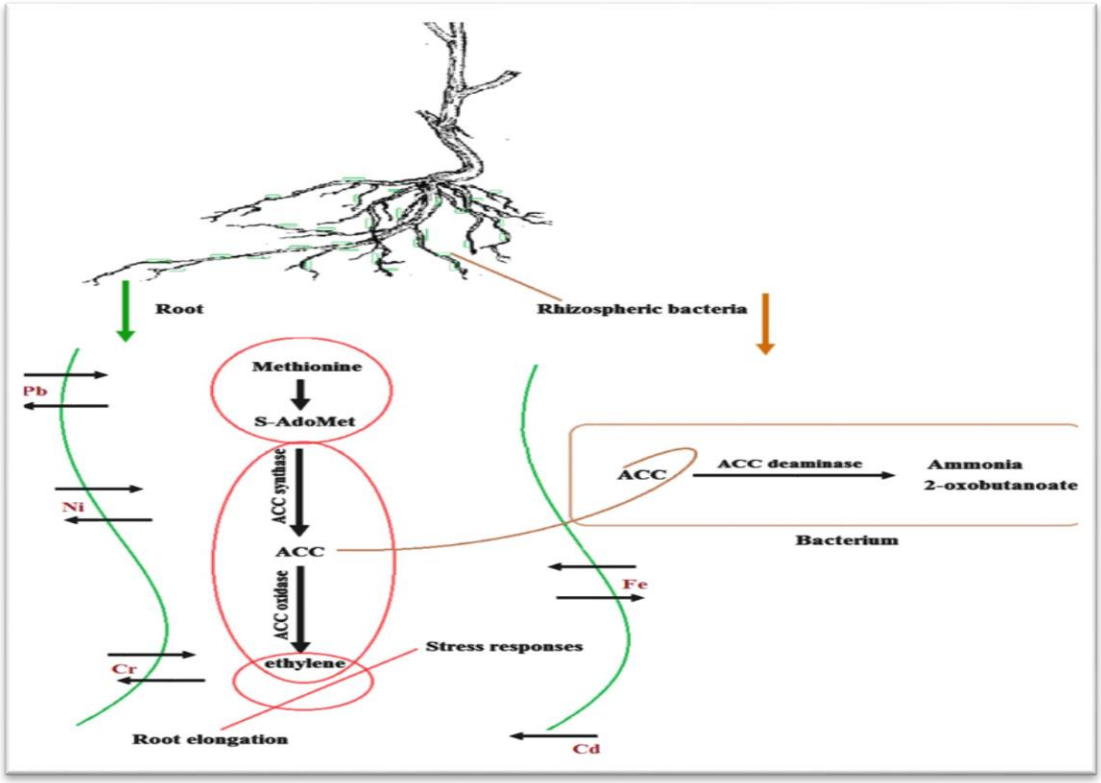



**Figure 2.4:** Diagrammatic model showing the process for reducing ethylene levels in roots by using bacterial 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase.

In higher plants, S-Adenosyl-L-methionine (S-AdoMet) is manufactured from methionine and ACC is synthesized and converted into ethylene by ACC oxidase. Further ethylene production in plants is managed by regulating the expression of ACC synthase and ACC oxidase genes (Kim et al., 2001). In Fig.2.4, a model of the process by which ethylene levels are

reduced in roots is presented, along with the role played by the ACC enzyme (Glick et al., 2007). PGPR synthesize the indole-3-acetic acid (IAA) utilizing tryptophan excreted by roots in the rhizospheric region. The synthesized IAA molecules are then secreted and transported into plant cells. These auxins have dual roles. One is to partake in plant cell growth while the other is to endorse ACC synthase activity to increase the ethylene titer. Stress induces an increase in ACC levels and, therefore, emulates the action of IAA molecules. Increased ACC molecules then diffuse from plants and are imported into PGPR cells where they are subjected to the action of ACC deaminase. Because of this, microbes and plants are more tolerant to stress-induced growth inhibition that is mediated by ethylene. When tested, strains of ACC deaminase-containing plant growth-promoting bacteria were found to condense the sum of ACC that was detectable by HPLC, and the ethylene levels in canola seedlings were also lowered (Penrose and Glick, 2001). Thus, PGPR can potentially be used to offset ethylene-mediated stress, although field trials are needed to elucidate the mechanism by which this occurs.

## 2.9 The Kinetics of Metal Extraction by Plant

It was not possible for metal ions to budge unreservedly athwart the cellular membranes, which are lipophilic configurations. Consequently, ion transport must be mediated into cells by membrane proteins with transport functions, basically known as transporters. Transmembrane transporters acquire an extracellular requisite sphere to which the ions join just before the transport, and a transmembrane structure, which unite extracellular and

intracellular media. The binding domain is approachable only to definite ions and is responsible for transporter specificity. The transmembrane structure eases the relocation of bound ions from extracellular gap through the hydrophobic environment of the membrane into the cell. Transporters that are typified by certain kinetic parameters, such as transport capacity (Vmax) and affinity for ion (Km). Vmax measures the maximum rate of ion transport across the cellular membranes. Plant biologists get imminence to specificity and selectivity of the transport system by studying kinetic parameters; Km and Vmax. A significant note is that of the entire amount of ions coupled with the root; only a part is absorbed into cells. A noteworthy ion portion is physically adsorbed at the extracellular negatively charged sites (COO-) of the root cell walls. The cell wall-bound division cannot be translocated to the shoots, hence, cannot be removed by harvesting shoot biomass (phytoextraction). As a result, it is potential for a plant displaying momentous metal accumulation into the root to express a inadequate capacity for phytoextraction. For instance, numerous plants accumulate Pb in roots, but Pb translocation to shoot is very low. Blaylock and Huang (1999) concluded that the limiting step for Pb phytoextraction is the long-distance translocation from roots to shoots. Strapping to the cell wall is not the only plant mechanism responsible for metal control into roots and ensuing reticence of ion translocation to the shoot. Metals can also be sequestered in cellular structures (e.g., vacuole), becoming engaged for translocation to the shoot (Lasat, 2002). Besides, some plants, coined excluders, seize particular mechanisms to curb metal uptake into roots.

Conversely, the concept of metal barring is not well understood (Peterson, 1983). Uptake of metals into root cells, the point of entry into living tissues, is a step of foremost magnitude for the process of phytoextraction. On the other hand, for phytoextraction to take place, metals must also be transported from the root to the shoot. Two processes primarily control movement of metal-containing sap from the root to the shoot, termed translocation; root pressure and leaf transpiration. Subsequent translocation to leaves, metals can be reabsorbed from the liquid into leaf cells.

# Chapter Three

## Material and Methods

### 3.1 Introduction

This study was conducted in two places with two experiments; the first experiment was carried out in a greenhouse model, while the second one was conducted at the laboratories of An-Najah National University, Collage of Science, Nablus, Palestine, during the year 2015. The mother plant material was collected from the Palestinian Ministry of Agriculture. To achieve research objectives, it was realized that the needed tests could be conducted in two stages: preparation of required conditions for test percolation, and sample collection and analysis.

### 3.2 Preparation of Test Requirements

In this phase, bacteria culturing and growth promoting were made, then the selected seeds are sterilized. After that, seed coating with bacteria was carried out, then, soil was prepared and sterilized. Known concentrations of metals were added to water which was used to irrigate the selected seeds. Finally, the coated seeds were planted.

#### 3.2.1 Selecting and Culturing (PGPR):

Two bacterial strains: *Pseudomonas putida* (UW3 and UW4) were used. The two strains were grown in Tryptic Soy Broth (TSB) media in the laboratories of An-Najah National University. 100 mg/l of Ampicillin antibiotic (AMP) were added to the media of UW3. For preparing the solid

media, 7.5g of agar were added. The selected bacterial strains were cultured on solid and liquid media at $30^0$C for overnight. Some of the samples of the cultivated bacterial strains were stored at $^-80^0$C to be used in further studies. For liquid cultures preparations, bacterial inoculums had been transferred to 50 ml falcon tubes containing proper TSB media and incubated at 30ºC with shaking at 200 r.p.m in rotatory shaker (orbital shaking incubator, labtech, LSI-3016 A) for 26 hours.

Cultures for each strain were transferred into two 50 ml falcon tubes separately, centrifuged for 20 minutes at 2000 r.p.m using (Universal 320 R). The pellets were suspended by (10 ml) and the Optical Density (OD) was measured for each strain at wavelength 600 nm by using an UV-spectrophotometer (Spectro UV-Vis Dual Beam -8 Auto cell, UVS- 2700) to have 1.5 (OD) for UW3 and 2.0 (OD) for UW4.

### 3.2.2 Seed Sterilization and Testing:

In this research, seeds suitable to be used as livestock foods were selected. The selected seeds were sterilized by soaking them in bleach sodium hypochlorite, and then they were washed three times with distilled–deionized water ($ddH_2O$) before coating the seeds with the mixture. The selected seeds were tested according to germination test procedure to ensure that all seeds are in good condition.

### 3.2.3 Seed Treatment with (PGPR):

For coating of bacterial cells to the seeds surfaces, methylcellulose white gel polymer was prepared by using 7g of methylcellulose powder that were

dissolved in 500 ml of distilled–deionized water ($ddH_2O$); stirred for one hour until most of clumps were dissolved, before they were autoclaved for 20 minutes at 110 $^0$C and 100 psi using autoclave (EQUS steam sterilization autoclave). The resulted polymer was white gel and it became clear gel upon cooling. The next step was adding 2.5 volumes of methylcellulose polymers to one volume of bacterial suspension to make the mixture to be added to the seeds. The mixture then was added to the seeds by (2.5:1) volume for clover seeds and up to (7:1) volume for barely seeds. After treating the seeds with PGPR, they were dried for 5 minutes and then transferred into sealed autoclaved plastic bags. Finally, the seeds were put in a refrigerator for a weak at 4 $^0$C for the seeds to be ready to use.

### 3.3 Soil Preparation

Soil samples were collected and filled in bags and autocleaved to ensure removal of any bacteria and fungi suspensions. Then, allowed to dry to remove moisture, and sieved using 10 mm particle size. Randomly chosen samples were tested for Electrical conductivity (EC), soil PH, Nitrogen content concentrations. Finally, 100-200 $cm^3$ of sieved soil were filled in plastic pots which have holes at the bottoms to enable water drainage (Figure 3.1).


**Figure 3.1:** Miniature greenhouse model built in backyard. Plastic pots (13*14*15cm: length*width*height) were filled with sieved soil and placed in rows to ease irrigation. Temperature was measured twice daily without human interference to the temperature or light intensity during the period of experiments.

## 3.4 Preparation of Water Samples

Two types of water were used; the first was taken from Arabic Project in Jericho, because they have salt water samples from the groundwater and working to have water desalination, while the second was prepared in lab using distilled water. The first sample was analyzed in order to determine the metals concentration inside it. Iron and magnesium were taken as reference values to study the change in their concentrations after planting in vivo medium. The second type was prepared by adding 1 g of Iron, 3 g of NaCl and 1g of $KNO_3$, $MgCl_2$, and $CaCO_3$ separately to 1 liter of warm distilled water. The solution was stirred and used to irrigate plants in vitro medium.

Each plastic pot was put in wide container to collect the leached water to be used for re-irrigating the plants in order to prevent the loss in metal concentration not resulting from plant absorption.

### 3.5 Parameters

Soil and plant parameters were assessed to evaluate the use of plant growth-promoting rhizobacteria (PGPR) to improve plant growth in heavy metal contaminated soil for phytoremediation. Soil parameters include heavy metals content, pH and EC. Plant parameters include plant height and weight and heavy metals.

#### 3.5.1 Soil pH

Soil pH was measured using electronic pH meter (827. pH Lab, Metrohm). Table 3.1 shows the mean pH for each level. Soil pH was measured using 1:5 w.v-1 soil extracts. These extracts were then measured to obtain the pH of the samples to be 8.72.

#### 3.5.2 Soil Electrical Conductivity (EC)

EC was measured using the conductivity meter (4010 Jenway). Table 3.1 shows the mean of EC. Soil salinities were measured using 1:5 w.v-1 soil extracts. The EC of soil in assessed plots measured 100.1 µS. Soil electrical conductivity is usually influenced by a combination of physio-chemical factors, including soluble salts, clay content, minerals, organic matter, bulk density, water content and soil temperature (Corwin and Lesch, 2005). The EC variation affects mainly the anions types, whereas cation types are not noticeably affected with relatively low cation exchange capacity. In

addition, there is a clear correlation between pH and EC values. EC increases with pH decrease pH decrease (Ouhdai and Goodarzi, 2007).

**Table 3.1: Soil sample inside a black plastic sac carried out in the Poison Control & Chemical/Biological Center of An-Najah National University.**

| Test | Units | Before | After | Ref. |
|---|---|---|---|---|
| EC | µS | 100.1 | 101.2 | Instruction Manual Method of Conductivity meter |
| N | ppm | 490 | 580 ppm | Instruction Manual Method of Vapo dest 20/Gerhardt |
| pH | --- | 8.72 | 5.58 | Instruction Manual Method pH meter |

EC values separating variations in soil texture, EC has been shown to relate closely to other soil properties used to determine a field's productivity (Figure 3.2).

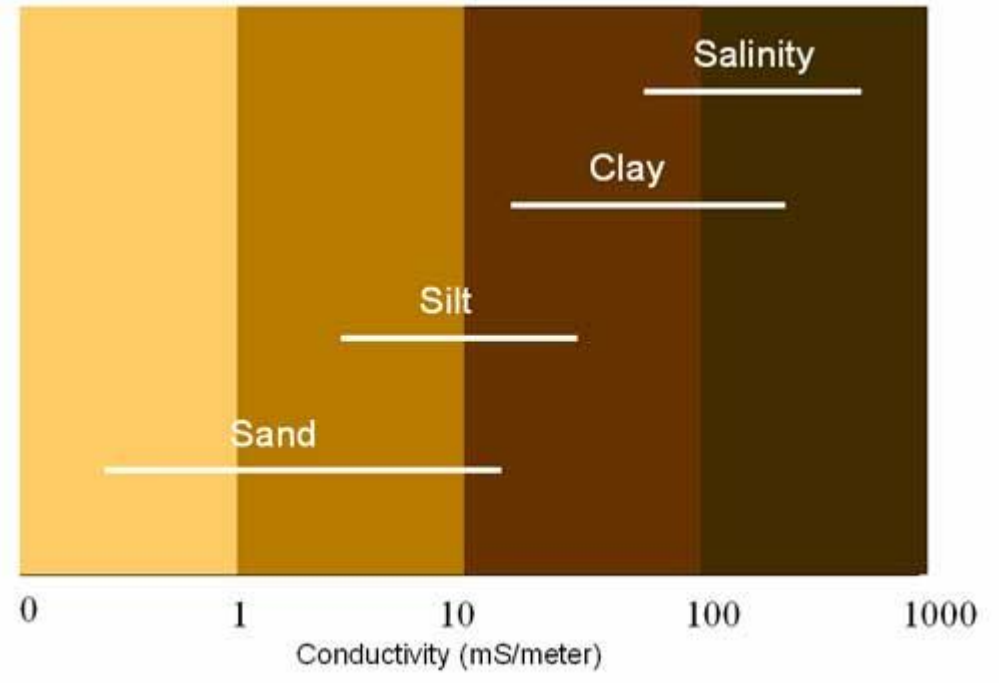



**Figure 3.2:** Scale of soil EC.

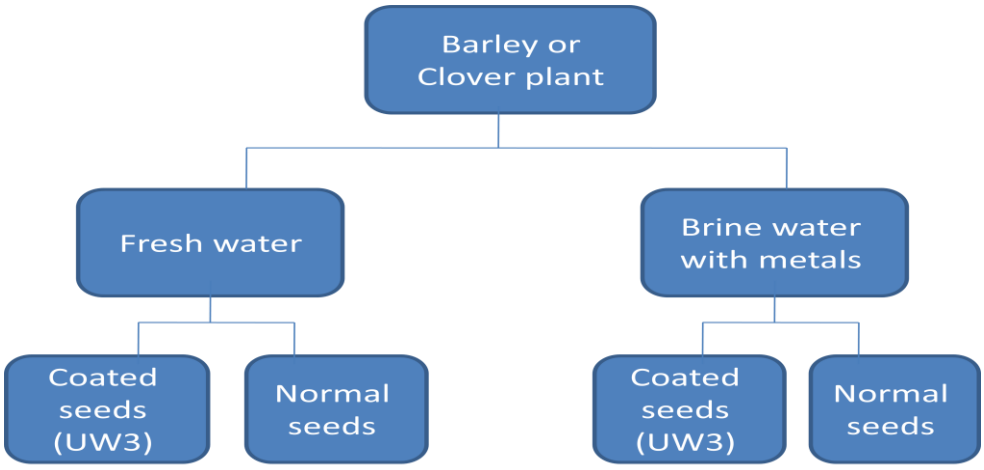
### 3.6 Greenhouse Plant Germination and Growth Assays

Two salt tolerant plant species were used in this research which are (Barley and Clover plants) obtained from the National Agriculture Resource Center (NARC) - Jenin district. Each plastic pot includes about 20 seeds of barely and clover, distributed at the surface of the pot, then thin layer about 5cm of sandy soil covered the seeds. All 24 pots were planted in March, 2015, placed in rows to ease irrigation to imitate Jericho climate. Small green house model was constructed at the green house model fixed with soil and stones to model in vivo planting, and the same number of pots were planted in lab to model in vitro planting. All pots were irrigated by fresh water twice daily for five days until seeds' germination. After that, each pot was irrigated according to the assumed trails. Temperature was measured twice daily and was found to range from 18-25$^0$C. Sun light and electrical lighting were used as a source of light for in vivo and vitro planting respectively. After 30 days, all plants were taken from pots and subjected to the required assumed test.

Control seeds pots used in experiments: One pot used with fresh water (FW), another irrigated with saline with metals (SW) and the last one with saline water without metals (S).

#### 3.6.1 In vivo:

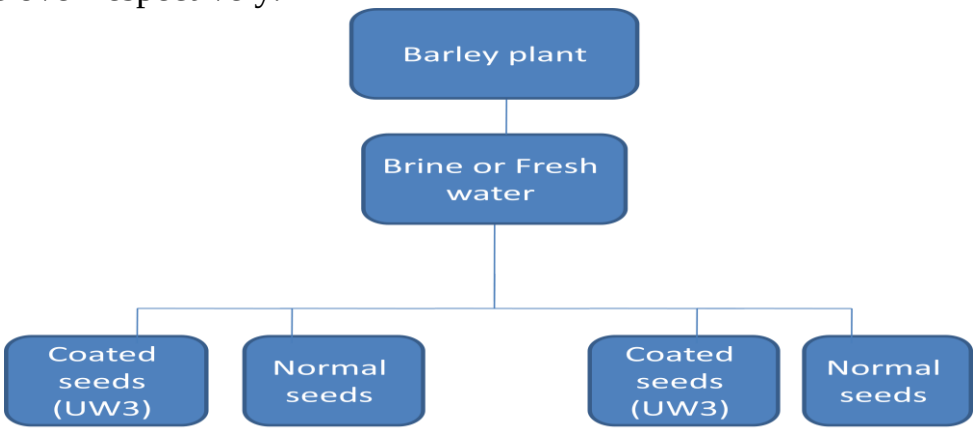
Each trail has been examined for three replicates. The following scheme illustrates barely and clover respectively.



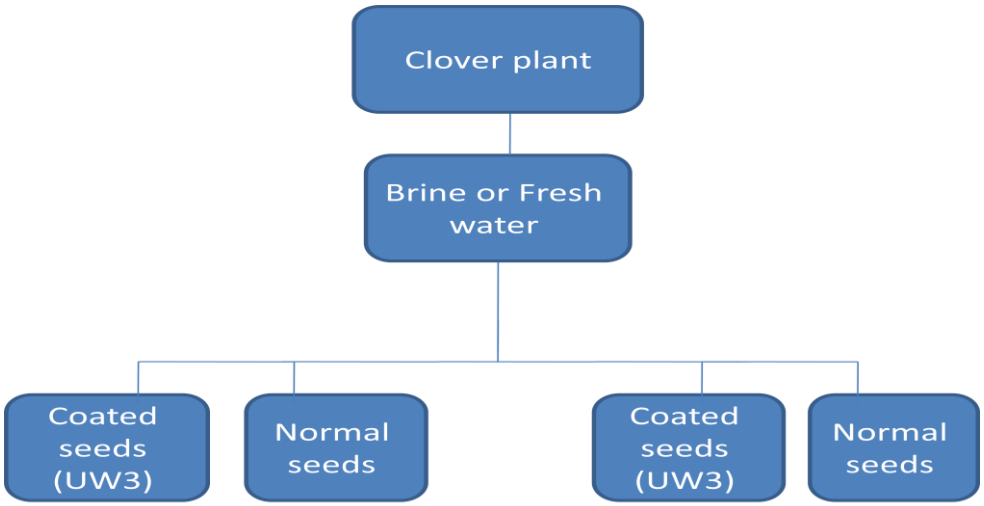

**Scheme 3.1:** Treated seeds germinated with UW3 in this experiment. One pot was irrigated with fresh water; the second one was irrigated with brine water with metals. Each pot contained an average of twenty seeds for barley and/or clover plants.

### 3.6.2 In vitro:

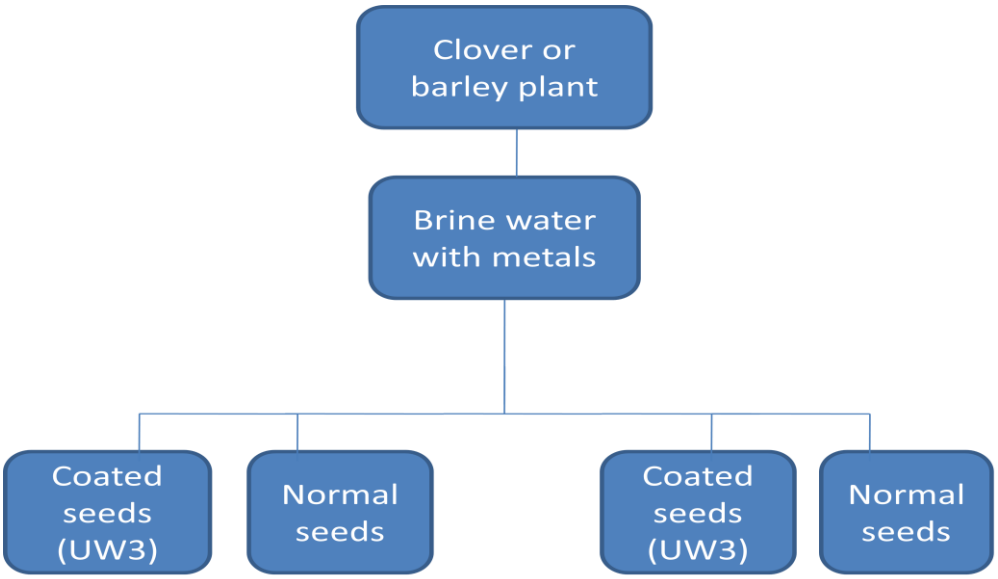
In this type of planting, the seeds were coated by two types of bacteria; UW3 and UW4 which have two strains. The seeds were irrigated by three different types of water; fresh water, saline water and saline water with known concentration of metals. The following scheme illustrates barely and clover respectively.




**Scheme 3.2:** Treated seeds germinated with UW3 in this experiment. One pot was irrigated with fresh water while the second was irrigated with brine water without metals. Each pot contained an average of twenty seeds for barley.




**Scheme 3.3:** Treated seeds germinated with UW3 in this experiment. One pot was irrigated with brine water without metals while the second one was irrigated with fresh water. Each pot contained an average of twenty seeds for clover.




**Scheme 3.4:** Treated seeds germinated with UW3 in this experiment. One pot was irrigated with brine, water while the second one was irrigated with brine water with metals for clover and/or barley.

## 3.7 Sample Collection and Analysis

Statistical tests were done using SPSS software-15.0 while Microsoft Office Excel 2007 was used to obtain figures. Analysis was carried out in two phases; the first was for soil and the second was for the plants.

### 3.7.1 Soil Analysis:

Sample was taken from each pot in a weekly basis for five weeks to measure the reduction in metals' concentration in soil. This reduction is a result for plant absorption. The reduction also gives indicator about kinetics of ions in roots.

These samples were put in an oven $70^0$C degree for two hours to get dried. Then, 1g of each sample was put in dry flask, 10 ml of $HNO_3$ and 3 ml of HCL were added to flask. After that, the flask was heated till all vapor disappeared and solution became colorless. After that, the solution was filtered in 100 ml flask. Finally, the 100 ml flask containing the filtration residue was filled with distilled water and put in ISE 3000 Series Atomic Absorption Spectrophotometry (AAS).

### 3.7.2 Plant Analysis:

In this phase, lengths of plants were measured after 14 and 30 days of planting, respectively. Then, the plants were extracted from pots to measure the roots' lengths. After that, the roots and shoots of plants were cut and each of them was weighted separately. One week later, the roots and shoots were re-weighted to get the dry weight. The dry weight was compared with the wet weight[3] to assess the photothensis and to compare the plant response to high concentrations of saline and metals.

[3] *Wet weight of a tissue or biological sample is obtained after blotting the sample to remove an arbitrary amount of water adhering externally to the sample.*

# Chapter Four

## Results and Discussion

### 4.1 Introduction

Phytoremediation experiments were conducted in combination with plant growth promoting rhizobacteria (PGPR) to test the performance of PGPR on two plant species; "Barley and Clover" to assess uptake of Fe and Mg, and to increase the ability of tolerance under fresh and brine water and to improve plant growth in heavy metal contaminated soil for phytoremediation.

### 4.2 Accumulation of Fe and Mg in Barley, 1st Experiment.

In table 4.1.below, barley measurements with fresh water and bacteria showed that the average accumulation and uptake of Fe and Mg (in the four replicates which were proceeded at the green house model) in wet weight of the shoot was 9.475g, the root weighted 5.77g, while the average dry weight of the shoot counted 4.45g, and the root weighted 2.12g. The shoot metering after 14 days has denoted to 9cm, post 30 days grown up to 28cm, while the root measured a length of 20.25cm post 30 days (Table 4.1).

**Table 4.1: Average measurements of accumulation and uptake of Fe and Mg in barley with fresh water and bacteria, first experiment.**

| **Barley with FW with Bacteria (average)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Weight** | | | | | | **Length** | | |
| **Replicate** | **Wet weight (g)** | | | **Dry weight (g)** | | | **Shoot (cm) 14 days** | **Shoot 30 days (cm)** | **Root 30 days (cm)** |
| | **shoot** | **root** | **total** | **shoot** | **root** | **total** | | | |
| 1 | 9.9 | 4.8 | 14.7 | 4.3 | 1.9 | 6.2 | 9 | 29 | 19 |
| 2 | 9.3 | 6.2 | 15.5 | 4.5 | 2.1 | 6.6 | 8 | 27 | 20 |
| 3 | 8.8 | 6.7 | 15.5 | 4.4 | 2.2 | 6.6 | 9 | 26 | 21 |
| 4 | 9.9 | 5.4 | 15.3 | 4.6 | 2.3 | 6.9 | 10 | 30 | 21 |
| **Average** | **9.47** | **5.77** | **15.25** | **4.45** | **2.12** | **6.57** | **9** | **28** | **20.25** |

In Table 4.2, barley measurements with fresh water and without bacteria showed that the average accumulation and uptake of Fe and Mg (in the four replicates which were proceeded at the green house model) in wet weight of the shoot was 5.8g, the root weighted 2.82g, while the average dry weight of the shoot added up 1.33g, and the root weighted 0.75g. The shoot height after 14 days denoted to 4cm, expanded to 15.25cm length post 30 days, while the root after 30 days measured a loftiness of 11.51cm (Table 4.2).

**Table 4.2: Average measurements of accumulation and uptake of Fe and Mg in barley with fresh water and without bacteria, first experiment.**

| **Barley with FW without Bacteria** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Weight** | | | | | | **Length** | | |
| **Replicate** | **Wet weight (g)** | | | **Dry weight (g)** | | | **Shoot 14 days (cm)** | **Shoot 30 days (cm)** | **Root 30 days (cm)** |
| | **shoot** | **root** | **total** | **shoot** | **root** | **total** | | | |
| 1 | 4.8 | 2.4 | 7.2 | 1.1 | 0.6 | 1.7 | 4 | 14 | 11 |
| 2 | 5.9 | 2.3 | 8.2 | 1.4 | 0.7 | 2.1 | 5 | 16 | 10 |
| 3 | 6.5 | 3.5 | 10 | 1.5 | 0.8 | 2.3 | 4 | 16 | 13 |
| 4 | 6 | 3.1 | 9.1 | 1.2 | 0.7 | 1.9 | 3 | 15 | 12 |
| **Average** | **5.80** | **2.82** | **8.62** | **1.33** | **0.75** | **2** | **4** | **15.25** | **11.51** |

Table 4.3 below shows that barley measurements with saline water and bacteria demonstrated that the average accumulation and uptake of Fe and Mg (in the four replicates which were proceeded at the green house model) in wet weight of the shoot was 10.12g, the root weighted 6.27g, while the average dry weight of the shoot counted 4.83g, and the root has weighted 2.25g. The shoot height after 14 days indicated 10.5cm, and developed post 30 days to 30.75cm, while the root measured a rise up to 22.5cm following 30 days (Table 4.3).

**Table 4.3: Average measurements of accumulation and uptake of Fe and Mg in barley with saline water and bacteria, first experiment.**

| Barley with SW with Bacteria (average) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Weight | | | | | | Length | | |
| Replicate | Wet weight (g) | | | Dry weight (g) | | | Shoot 14 days (cm) | Shoot 30 days (cm) | Root 30 days (cm) |
| | shoot | root | total | shoot | root | total | | | |
| 1 | 10.4 | 6.3 | 16.7 | 4.6 | 2.1 | 6.7 | 12 | 33 | 24 |
| 2 | 10.2 | 6.1 | 16.3 | 4.7 | 2.2 | 6.9 | 11 | 30 | 22 |
| 3 | 10 | 6.2 | 16.2 | 5 | 2.4 | 7.4 | 9 | 29 | 21 |
| 4 | 9.8 | 6.5 | 16.3 | 4.9 | 2.3 | 7.2 | 10 | 31 | 23 |
| **Average** | **10.12** | **6.27** | **16.37** | **4.83** | **2.25** | **7.05** | **10.5** | **30.75** | **22.5** |

In Table 4.4 below, barley measurements with fresh water and without bacteria showed that the average accumulation and uptake of Fe and Mg (in the four replicates which were proceeded at the green house model) in wet weight of the shoot was 2.77g, the root weighted 1.55g, while the average dry weight of the shoot counted 0.87g, and the root weighted 0.27g. After 14 days, the shoot height indicated a 4.25cm, post 30 days grown up to 13cm, while the root after 30 days measured a height of 9.75cm (Table 4.4).

**Table 4.4: Average measurements of accumulation and uptake of Fe and Mg in barley with saline water and without bacteria, first experiment.**

| Barley with SW without Bacteria (average) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Weight | | | | | | Length | | |
| Replicate | Wet weight (g) | | | Dry weight (g) | | | Shoot 14 days (cm) | Shoot 30 days (cm) | Root 30 days (cm) |
| | shoot | root | total | shoot | root | total | | | |
| 1 | 2.5 | 1.4 | 3.9 | 0.7 | 0.4 | 1.1 | 4 | 12 | 9 |
| 2 | 3.1 | 1.6 | 4.7 | 0.9 | 0.3 | 1.2 | 5 | 14 | 10 |
| 3 | 2.6 | 1.5 | 4.1 | 1 | 0.2 | 1.2 | 5 | 15 | 12 |
| 4 | 2.9 | 1.7 | 4.6 | 0.9 | 0.2 | 1.1 | 3 | 11 | 8 |
| **Average** | **2.77** | **1.55** | **4.32** | **0.87** | **0.27** | **1.15** | **4.25** | **13** | **9.75** |

Barley measurements with fresh water and with bacteria showed that the average accumulation and uptake of Fe and Mg (in the four replicates which were proceeded at the green house model) in wet weight of the shoot and root of the barley plant, showed that germination increased with bacteria after two weeks (15.25g) (Table 4.2). Accumulation of Fe and Mg in dry weight for roots and shoots of barley seeds in fresh water with bacteria treated with UW3 gave higher weights (5.57g) compared to wet and dry weight without bacteria (total average=8.62g, 2g) respectively.

Heights of the shoots and the roots of the barley were also noted to have a lot of accumulation and absorption of Fe and Mg carried out with bacteria, and this signifies a rising growth with bacteria within time intervals from 14 days to 30 days. In the fresh water, the length of the root with bacteria after 30 days =20.25cm, while that without bacteria =11.5cm, while the root in the saline water with bacteria after 30 days =30.5cm, and that without bacteria after 30 days =13cm.

### 4.3 Accumulation of Fe and Mg in Clover, 1st Experiment.

In Table 4.5 below, clover measurements with fresh water and bacteria showed that the average accumulation of Fe and Mg (in the four replicates which were proceeded at the green house model) in wet weight of the shoot was 5.82g, the root weighted 2.35g, while the average dry weight of the shoot counted 3.75g, and the root weighted 1.42g. The shoot metering after 14 days has implied to 7.75cm, post 30 days grown up to 17.75cm, while the root measured a length of 15.25cm post 30 days (Table 4.5).

**Table 4.5: Average measurements of accumulation and uptake of Fe and Mg in clover with fresh water and bacteria, first experiment.**

| Clover with Fresh water with Bacteria (average) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Weight | | | | | | Length | | |
| Replicate | Wet weight (g) | | | Dry weight (g) | | | Shoot 14 days (cm) | Shoot 30 days (cm) | Root 30 days (cm) |
| | shoot | root | total | shoot | root | total | | | |
| 1 | 6 | 2.5 | 8.5 | 3.46 | 1.5 | 4.96 | 8 | 18 | 14 |
| 2 | 6.2 | 2.7 | 8.9 | 4.33 | 1.7 | 5.03 | 10 | 20 | 16 |
| 3 | 5.8 | 2.1 | 7.9 | 3.78 | 1.3 | 5.08 | 7 | 17 | 17 |
| 4 | 5.2 | 2.1 | 7.3 | 3.43 | 1.2 | 4.63 | 6 | 16 | 14 |
| **Average** | **5.82** | **2.35** | **8.15** | **3.75** | **1.42** | **4.92** | **7.75** | **17.75** | **15.25** |

In Table 4.6 below, clover measurements with fresh water and without bacteria showed that the average accumulation of Fe and Mg (in the four replicates which were proceeded at the green house model) in wet weight of the shoot was 2.53g, the root weighted 0.64g, while the average dry weight of the shoot added up 0.45g, and the root weighted 0.17g. The shoot height after 14 days designated to 3cm, expanded to 9cm post 30 days, while the root after 30 days measured a loftiness of 6.62cm (Table 4.6).

**Table 4.6: Average measurements of accumulation and uptake of Fe and Mg in clover with fresh water and without bacteria, first experiment.**

| Clover with Fresh water without Bacteria (average) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Weight | | | | | | Length | | |
| Replicate | Wet weight (g) | | | Dry weight (g) | | | Shoot 14 days (cm) | Shoot 30 days (cm) | Root 30 days (cm) |
| | shoot | root | total | shoot | root | total | | | |
| 1 | 2.8 | 0.5 | 3.3 | 0.39 | 0.2 | 0.59 | 3 | 9 | 6 |
| 2 | 1.9 | 0.4 | 2.3 | 0.4 | 0.18 | 0.58 | 2 | 8 | 5 |
| 3 | 2.7 | 1 | 3.7 | 0.6 | 0.16 | 0.76 | 4 | 11 | 8 |
| 4 | 2.6 | 0.5 | 3.1 | 0.43 | 0.14 | 0.57 | 3 | 8 | 6 |
| **Average** | **2.53** | **0.64** | **3.13** | **0.45** | **0.17** | **0.62** | **3** | **9** | **6.25** |

Clover measurements with fresh water and with bacteria showed that the average accumulation and uptake of Fe and Mg (in the four replicates which were proceeded at the green house model) in wet weight of the shoot and root of the clover plant, showed that germination increased with bacteria (8.15g), while accumulation of Fe and Mg in dry weight for roots and shoots of clover seeds in fresh water with bacteria treated with UW3 gave higher weights (total=4.92g), compared to wet without bacteria (3.1g) and dry weight (0.62g) respectively.

Heights of the shoots and roots of the clover were also noted to have a great deal of accumulation and absorption of Fe and Mg carried out with bacteria, and this signifies a rising growth with bacteria within time intervals from 14 days to 30 days. In the fresh water with bacteria, the length of the shoot post 14 days grew up to 7.75cm continued to grow up to 17.75cm compared to 3cm and grew up to 9cm without bacteria respectively for the same intervals. The root with bacteria after 30 days equals 15.25cm, while that without bacteria =6.25cm.

Moreover, measurements of accumulation and uptake of Fe and Mg absorbed by clover plant with saline water and with and without bacteria showed that the average accumulation of Fe and Mg (in the replicate which was proceeded at the green house model in the $4^{th}$ week), clover plant dried up and died.

Grossly, it is noted that the accumulation and uptake of Fe and Mg in barley and clover with bacteria was more than that without bacteria, as well as, it was noted that germination increased in wet weights than in the dry

ones about three folds and sometimes up to four folds, which, in turn, increased the process by which green plants and some other organisms use sunlight to synthesize foods from carbon dioxide and water (photosynthesis), and this eventually increased phytoremediation of the biomass product.

It is worth mentionining that Bacteria A (UW3) strains were chosen only for this experiment. Four replicates were conducted at the green house model and eventually the average was calculated.

Shan (2009) study showed some plant species such as barley plant with PGPR showed high performance of photosynthesis activity in saline soil. Also, Mcneill (2011) study illustrated photosynthesis activities for different plants species such as barley, Oats, and Tall Wheatgrass treated with PGPR and grown in saline soil field and high performance of their photosynthesis activity.

## 4.4 Uptake of Fe and Mg Absorbed by Barley and Clover Plants in ppm[4] in 5 Weeks, 1st Experiment.

In Table 4.7 below, results of the trial revealed that the amount of Fe absorbed and accumulated by barley plant during five weeks irrigated with saline water treated with UW3 bacteria had increased to the highest peak of 878ppm (0.878mg/g) compared to the trial without bacteria.

Calculated measurements of accumulation and uptake of Fe absorbed by barley plant with fresh water and bacteria showed that the average

[4] *PPM is a term used in chemistry to denote a very, very low concentration of a solution. One gram in 1000 ml is 1000 ppm and one thousandth of a gram (0.001g) in 1000 ml is one ppm.*
*1 ppm = 0.001 mg/g; 1 mg/g = 1000 ppm, 1000 ppm = 1 g/L*

accumulation of Fe (in the replicate which was proceeded at the green house model) in the 1st week was 710ppm, increased in the 2nd week to 720ppm, and continued to rise up to 810ppm in the 5th week.

Also, measurements of accumulation and uptake of Fe absorbed by barley plant with fresh water and without bacteria showed that the average accumulation of Fe (in the replicate which was proceeded at the green house model) in the 1st week was 425ppm, improved in the 2nd week to 455ppm, and continued to rise up to 517ppm in the 5th week.

Moreover, measurements of accumulation and uptake of Fe absorbed by barley plant with saline water and with bacteria showed that the average accumulation of Fe (in the replicate which was proceeded at the green house model) in the 1st week was 740ppm, augmented in the 2nd week to 790ppm, and sustained to rise up to 878ppm in the 5th week.

Furthermore, measurements of accumulation and uptake of Fe absorbed by barley plant with saline water and without bacteria showed that the average accumulation of Fe (in the replicate which was proceeded at the green house model) in the 1st week was 360ppm, increased in the 2nd week to 380ppm, and continued to grow up to 430ppm in the 5th week of the trial.

Through this experiment which was carried out at the green house model, it was noted that the average Fe accumulated and uptaken inside barley plant within five (5) weeks and barley plant with saline water and bacteria had the highest absorption compared to barley without bacteria.

**Table 4.7: Measurements of accumulation and uptake of Fe absorbed by barley plant in ppm in 5 weeks, first experiment.**

| **Barley with FW with Bacteria** | | | | | |
|---|---|---|---|---|---|
| Replicate | Weeks | | | | |
| | 1st | 2nd | 3rd | 4th | 5th |
| Average | 710 | 720 | 730 | 740 | 810 |
| **Barley with FW without Bacteria** | | | | | |
| Replicate | weeks | | | | |
| | 1st | 2nd | 3rd | 4th | 5th |
| Average | 425 | 455 | 467 | 467 | 517 |
| **Barley with SW with bacteria** | | | | | |
| Replicate | weeks | | | | |
| | 1st | 2nd | 3rd | 4th | 5th |
| Average | 740 | 790 | 850 | 875 | 878 |
| **Barley with SW without Bacteria** | | | | | |
| Replicate | weeks | | | | |
| | 1st | 2nd | 3rd | 4th | 5th |
| Average | 360 | 380 | 400 | 410 | 430 |

In Table 4.8 below, calculated measurements of accumulation and uptake of Mg absorbed by barley plant with fresh water and bacteria showed that the average accumulation of Mg (in the replicate which was proceeded at the green house model) in the 1st week was 510ppm, increased in the 2nd week to 525ppm, and continued to rise up to 620ppm in the 5th week.

Also, measurements of accumulation and uptake of Mg absorbed by barley plant with fresh water and without bacteria showed that the average accumulation of Mg (in the replicate which was proceeded at the green house model) in the 1st week was 195ppm, improved in the 2nd week to 210ppm, and continued to rise up to 270ppm in the 5th week.

Moreover, measurements of accumulation and uptake of Mg absorbed by barley plant with saline water and with bacteria showed that the average accumulation of Mg (in the replicate which was proceeded at the green

house model) in the 1st week was 620ppm, augmented in the 2nd week to 640ppm, and sustained to rise up to 675ppm in the 5th week.

Furthermore, measurements of accumulation and uptake of Mg absorbed by barley plant with saline water and without bacteria showed that the average accumulation of Mg (in the replicate which was proceeded at the green house model) in the 1st week was 180ppm, increased in the 2nd week to 195ppm, and continued to grow up to 255ppm in the 5th week of the trial.

**Table 4.8: Measurements of accumulation and uptake of Mg absorbed by barley plant in ppm in 5 weeks, first experiment.**

| **Barley with FW with Bacteria** | | | | | |
|---|---|---|---|---|---|
| Replicate | Weeks | | | | |
| | 1st | 2nd | 3rd | 4th | 5th |
| Average | 510 | 525 | 545 | 585 | 620 |
| **Barley with FW without Bacteria** | | | | | |
| Replicate | weeks | | | | |
| | 1st | 2nd | 3rd | 4th | 5th |
| Average | 195 | 210 | 228 | 245 | 270 |
| **Barley with SW with bacteria** | | | | | |
| Replicate | weeks | | | | |
| | 1st | 2nd | 3rd | 4th | 5th |
| Average | 620 | 640 | 650 | 655 | 675 |
| **Barley with SW without Bacteria** | | | | | |
| Replicate | weeks | | | | |
| | 1st | 2nd | 3rd | 4th | 5th |
| Average | 180 | 195 | 215 | 230 | 255 |

It was noted that the average uptake of Mg by processed barley by bacteria, absorbed and accumulated a large amount of Mg.

Shan (2009) study showed some plant species such as barley plant with PGPR showed high performance of photosynthesis activity in saline soil. Also, Mcneill (2011) illustrated photosynthesis activities for different plants species such as barley, Oats, and Tall Wheatgrass treated with

PGPR and grown in saline soil field and high performance of their photosynthesis activity.

Also, the test can be applied in future researches to study if performance of PGPR can be differentiating with different time intervals, which will indicate more biomass production.

In Table 4.9 below, calculated measurements of accumulation and uptake of Fe absorbed by clover plant with fresh water and bacteria showed that the average accumulation of Fe (in the replicate which was proceeded at the green house model) in the 1st week was 650ppm, increased in the 2nd week to 720ppm, and continued to rise up to 890ppm in the 5th week.

Also, measurements of accumulation and uptake of Fe absorbed by clover plant with fresh water and without bacteria showed that the average accumulation of Fe (in the replicate which was proceeded at the green house model) in the 1st week was 300ppm, descended in the 2nd week to 310ppm, and continued to rise up to 390ppm in the 5th week.

Moreover, measurements of accumulation and uptake of Fe absorbed by clover plant with saline water and with bacteria showed that the average accumulation of Fe (in the replicate which was proceeded at the green house model) in the 1st week was 670ppm, decreased in the 2nd week to 650ppm, and decreased to 644ppm in the 3rd week, while in the 4th week, clover plant dried up and died.

Furthermore, measurements of accumulation and uptake of Mg absorbed by clover plant with saline water and without bacteria showed that the average accumulation of Fe (in the replicate which was proceeded at the

green house model) in the 1st week was 500ppm, increased in the 2nd week to 510ppm, and continued to grow up to 640ppm in the 3rd week of the trial, clover plant dried up and died (Figure 4.1, below with arrows).

**Table 4.9: Measurements of accumulation and uptake of Fe absorbed by clover plant in ppm in 5 weeks, first experiment.**

| **clover with FW with Bacteria** | | | | | |
|---|---|---|---|---|---|
| Replicate | Weeks | | | | |
| | 1st | 2nd | 3rd | 4th | 5th |
| Average | 650 | 720 | 720 | 740 | 890 |
| **clover with FW without Bacteria** | | | | | |
| Replicate | Weeks | | | | |
| | 1st | 2nd | 3rd | 4th | 5th |
| Average | 300 | 310 | 325 | 348 | 390 |
| **clover with SW with Bacteria** | | | | | |
| Replicate | Weeks | | | | |
| | 1st | 2nd | 3rd | 4th | 5th |
| Average | 670 | 650 | 660 | 0 | 0 |
| **clover with SW without Bacteria** | | | | | |
| Replicate | Weeks | | | | |
| | 1st | 2nd | 3rd | 4th | 5th |
| Average | 270 | 280 | 310 | 0 | 0 |

Followed pictures represent photos for some trials in comparing between them in visual differences;

**Figure 4.1:** Photo represents trial of accumulation and uptake of Fe absorbed by clover plant in the 3rd week where clover plant dried up and died (Red arrows).

The average amount of Fe accumulated and absorbed inside clover plant with the addition of saline water and elements, germination took place in the first two weeks, and then all clover plants dried up and died. This may be attributed to the genesis of toxic substances resulting from the elements or because the iron levels were high as clover does not tolerate this amount of iron.

In Table 4.10 below, calculated measurements of accumulation and uptake of Mg absorbed by clover plant with fresh water and bacteria showed that the average accumulation of Mg (in the replicate which was proceeded at the green house model) in the 1$^{st}$ week was 395ppm, increased in the 2$^{nd}$ week to 398ppm, and continued to rise up to 418ppm in the 5$^{th}$ week.

Also, measurements of accumulation and uptake of Mg absorbed by clover plant with fresh water and without bacteria showed that the average accumulation of Mg (in the replicate which was proceeded at the green house model) in the 1$^{st}$ week was 220ppm, descended in the 2$^{nd}$ week to 222ppm, and continued to rise up to 240ppm in the 5$^{th}$ week.

Moreover, measurements of accumulation and uptake of Mg absorbed by clover plant with saline water and with bacteria showed that the average accumulation of Mg (in the replicate which was proceeded at the green house model) in the 1$^{st}$ week was 420ppm, lessened in the 2$^{nd}$ week to 402ppm, and fell down to 320ppm in the 3$^{rd}$ week, while in the 4$^{th}$ week, clover plant dried up and died.

Furthermore, measurements of accumulation and uptake of Mg absorbed by clover plant with saline water and without bacteria showed that the

average accumulation of Mg (in the replicate which was proceeded at the green house model) in the 1st week was 120ppm, decreased in the 2nd week to 112ppm, and fell down into 95ppm in the 3rd week of the trial, while in the 4th week, clover plant dried up and died.

**Table 4.10: Measurements of accumulation and uptake of Mg absorbed by clover plant in ppm in 5 weeks, first experiment.**

| **clover + FW + bacteria** | | | | | |
|---|---|---|---|---|---|
| Replicate | weeks | | | | |
| | 1st | 2nd | 3rd | 4th | 5th |
| Average | 395 | 398 | 403 | 410 | 418 |
| **clover + FW - bacteria** | | | | | |
| Replicate | weeks | | | | |
| | 1st | 2nd | 3rd | 4th | 5th |
| Average | 220 | 222 | 232 | 235 | 240 |
| **clover + SW + bacteria** | | | | | |
| Replicate | weeks | | | | |
| | 1st | 2nd | 3rd | 4th | 5th |
| Average | 420 | 402 | 320 | 0 | 0 |
| **clover + SW - bacteria** | | | | | |
| Replicate | weeks | | | | |
| | 1st | 2nd | 3rd | 4th | 5th |
| Average | 120 | 112 | 95 | 0 | 0 |

It has been well documented that all the plants which have been germinated, survived by the end of the experimental period, however, some plants showed phytotoxicity yellowish-like-color and reduced growth, and eventually dried up. Here, survival is expressed as the presence of green/live plant in the pot at the end of the test period. Out of the five week intervals, clover survived only for three weeks.

The average amount of Mg accumulated and absorbed inside clover plant with the addition of saline water and elements, germination took place in the first two weeks, and then all clover plants dried up and died. This may

be attributed to the genesis of toxic substances resulting from the elements or because the iron levels were high as clover does not tolerate this amount of iron.

## 4.5 Lengths of Shoots and Roots for Barley and Clover Plants, Second Experiment.

Table 4.11 shows lengths of roots and shoots for barley plant; PGPR contributed to increase lengths for barley plants shoots and roots more than controls. Length measurements were more for trials treated with PGPR and irrigated with saline water SW compared to trials irrigated with fresh water. Lengths of shoot of barley seeds after 14 days with treated barley seeds with bacteria C (UW3+UW4) irrigated with SW were the tallest (24cm) among the treated barley seeds, while that after 30 days elongated to 38cm. In addition, lengths of roots after 30 days reached up to 27cm with treated barley seeds with bacteria C irrigated with SW.

The root length of 30-day-old barley plants was notably increased to 27cm compared to the control barley irrigated with SW (11).

**Table 4.11: Lengths of barley plant for shoot (cm) after 14 days and 30 days, and root length (cm) after 30 days, carried out in vitro, second experiment.**

| No: | Treatment | Length of Shoot after 14 days | Length of Shoot after 30 days | % Length of Shoot after | Length of root after 30 days | % Length of root after 30 days |
|---|---|---|---|---|---|---|
| 1 | Control barley irrigated with FW | 8 | 18 | 100 | 12 | 100 |
| 2 | Control barley irrigated with SW | 7 | 16 | 89 | 11 | 92 |
| 3 | Control barley irrigated with S | 7 | 14 | 78 | 10 | 83 |
| 4 | Treated barley seeds with bacteria A irrigated with FW | 16 | 36 | 200 | 16 | 133 |
| 5 | Treated barley seeds with bacteria A irrigated with SW | 18 | 27 | 150 | 19 | 158 |
| 6 | Treated barley seeds with bacteria A irrigated with S | 17 | 32 | 178 | 18 | 150 |
| 7 | Treated barley seeds with bacteria B irrigated with FW | 17 | 35 | 194 | 18 | 150 |
| 8 | Treated barley seeds with bacteria B irrigated with SW | 14 | 28 | 156 | 17 | 142 |
| 9 | Treated barley seeds with bacteria B irrigated with S | 12 | 27 | 150 | 19 | 158 |
| 10 | Treated barley seeds with bacteria C irrigated with FW | 20 | 31 | 172 | 22 | 183 |
| 11 | Treated barley seeds with bacteria C irrigated with SW | 24 | 38 | 211 | 27 | 225 |
| 12 | Treated barley seeds with bacteria C irrigated with S | 22 | 35 | 194 | 23 | 192 |
| | | | | 156% | | 147% |

***Bacteria A: UW3, Bacteria B: UW4: Bacteria C: UW3 + UW4, FW: Fresh Water, SW: Saline with Metals, S: Saline Water without Metals.***

Table 4.12 below shows lengths of roots and shoots for clover plant; PGPR contributed to increase lengths for clover plant shoots more than

controls. It is noted that trials of treated lengths of roots were generally high compared to controls. The lengths of shoots after two weeks showed that treated clover seeds with bacteria C irrigated with SW have had the tallest lengths of shoots (12cm), while those after 30 days also expanded to have the tallest shoots (24cm). The lengths of roots after 30 days showed that treated clover seeds with bacteria C irrigated with SW have had the tallest lengths of root (12cm).

All bacterial strains increased the shoot and root growth of barley and clover in comparison with the untreated control (Tables 4.11 and 4.12).

Length measurements were more for trials treated with PGPR and irrigated with saline water compared to trials irrigated with fresh water. This leads to PGPR to promote vigorous growth for both plants under salt stress. For trials treated with UW3, UW4 and UW3+UW4 compared to trials treated separately with strains, differences in lengths were significant. This indicates performance of trials with both strains show high effective to tolerate salinity and same performance of photosynthetic, as trials treated separately.

**Table 4.12: Lengths of clover plant for shoot (cm) after 14 days and 30 days, and root length (cm) after 30 days, carried out in vitro, second experiment.**

| No: | Treatment | Length of Shoot after 14 days | Length of Shoot after30 days | % Length of Shoot after | Length of root after 30 days | % Length of root after 30 days |
|---|---|---|---|---|---|---|
| **1** | Control clover irrigated with FW | 4 | 8 | 100 | 3 | 100 |
| **2** | Control clover irrigated with SW | 3 | 7 | 88 | 2 | 67 |
| **3** | Control clover irrigated with S | 3 | 8 | 100 | 2 | 67 |
| **4** | Treated clover seeds with bacteria A irrigated with FW | 6 | 14 | 175 | 6 | 200 |
| **5** | Treated clover seeds with bacteria A irrigated with SW | 8 | 18 | 225 | 4 | 133 |
| **6** | Treated clover seeds with bacteria A irrigated with S | 7 | 17 | 213 | 4 | 133 |
| **7** | Treated clover seeds with bacteria B irrigated with FW | 6 | 14 | 175 | 7 | 233 |
| **8** | Treated clover seeds with bacteria B irrigated with SW | 9 | 16 | 200 | 6 | 200 |
| **9** | Treated clover seeds with bacteria B irrigated with S | 5 | 14 | 175 | 5 | 167 |
| **10** | Treated clover seeds with bacteria C irrigated with FW | 10 | 20 | 250 | 8 | 267 |
| **11** | Treated clover seeds with bacteria C irrigated with SW | 12 | 24 | 300 | 12 | 400 |
| **12** | Treated clover seeds with bacteria C irrigated with S | 11 | 19 | 238 | 9 | 300 |
| | | | | 186% | | 189% |

***Bacteria A: UW3, Bacteria B: UW4: Bacteria C: UW3 + UW4, FW: Fresh Water, SW: Saline with Metals, S: Saline Water without Metals.***

**Figure 4.2:** Root formation carried out in vitro, second experiment.

**Figure 4.3:** Shoot formation carried out in vitro, second experiment.

Tables 4.11, 4.12 showed measurements of barley and clover plant lengths for shoots (cm) after 14 days and 30 days, and root lengths (cm) after 30 days, carried out in vitro. Shoots and roots of barley plants treated with PGPR were taller, thicker and green darker color compared to untreated ones, besides their roots were longer compared to untreated plants. Thus, PGPR affected photosynthetic activity even under irrigation with salt solution. Bacteria have increased the length of shoots and roots to resist the stress from elements and salts. The reason for decrease in photosynthesis in trials without PGPR can be related to accumulation of high concentration of salts in tissue that is responsible for photosynthesis process. It could be as a result of swelling of thylakoids, and distortion of chloroplast membrane; which lead to disrupt all process in plant (Mcneill, 2011).

Differences between plants species which one responded more to bacteria strain were clear. Barley and clover plants consider tolerant species to salty conditions, but response of barley plant to these microbes was more than clover's. This could be attributed to large surface area for barley seeds compared to clover seeds, more bacteria strains have been adhesive to surface of barley seeds. Another reason may be related to some specie–specific differences in physiology and anatomy as well as specific differences in conditions required for optimal growth for clover plant differs from barley plant. These indicate that clover plant may need different PGPR strains other than those UW3, UW4 for their optimal growth condition.

In Table 4.13, trials of barley seeds treated with UW3 irrigated with 6000 ppm of saline water (SW) gave total biomass values as (15.2g for wet weight, 6.26g for dry weight) compared to control ones (7.3g, 2.34g respectively).

Trials of barley seeds treated with UW4 irrigated with 6000ppm of saline water (SW) gave total biomass values as (17.31g for wet weight, 6.89g for dry weight) compared to control ones (7.3g, 2.34g respectively).

Trials of barley seeds treated with UW3+UW4 irrigated with 6000ppm of saline water (SW) gave total biomass values as (24.87g for wet weight, 14.75g for dry weight) compared to control ones (7.3g, 2.34g respectively).

**Table 4.13: Wet and dry biomass for shoots and roots (gm) of barley plant trials, carried out in vitro, second experiment.**

| No: | Treatment | Wet weight | | | | Dry weight | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | shoot | root | total | % | shoot | root | total | % |
| 1 | Control barely irrigated with FW | 7.69 | 1.63 | 9.32 | 78 | 2.65 | 0.65 | 3.3 | 100 |
| 2 | Control barely irrigated with SW | 5.66 | 1.66 | 7.32 | 60 | 1.94 | 0.44 | 2.38 | 72 |
| 3 | Control barely irrigated with S | 4.66 | 0.9 | 5.56 | 131 | 1.81 | 0.43 | 2.24 | 68 |
| 4 | Treated barely seeds with bacteria A irrigated with FW | 9.56 | 2.66 | 12.22 | 163 | 4.15 | 1.17 | 5.32 | 161 |
| 5 | Treated barely seeds with bacteria A irrigated with SW | 12 | 3.2 | 15.2 | 156 | 4.9 | 1.36 | 6.26 | 190 |
| 6 | Treated barely seeds with bacteria A irrigated with S | 11 | 3.5 | 14.5 | 143 | 3.95 | 1.4 | 5.35 | 162 |
| 7 | Treated barely seeds with bacteria B | 9.96 | 3.4 | 13.36 | 186 | 3.68 | 2.2 | 5.88 | 178 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | irrigated with FW | | | | | | | | |
| 8 | Treated barely seeds with bacteria B irrigated with SW | 12.85 | 4.46 | 17.31 | 164 | 4.65 | 2.24 | 6.89 | 208 |
| 9 | Treated barely seeds with bacteria B irrigated with S | 11.67 | 3.66 | 15.33 | 219 | 3.94 | 2.83 | 6.77 | 205 |
| 10 | Treated barely seeds with bacteria C irrigated with FW | 14 | 6.4 | 20.4 | 267 | 6.8 | 3.85 | 10.65 | 322 |
| 11 | Treated barely seeds with bacteria C irrigated with SW | 16.7 | 8.17 | 24.87 | 240 | 8.82 | 5.93 | 14.75 | 447 |
| 12 | Treated barely seeds with bacteria C irrigated with S | 14.72 | 7.63 | 22.35 | 78 | 7.15 | 4.93 | 12.08 | 366 |

***Bacteria A: UW3, Bacteria B: UW4: Bacteria C: UW3 + UW4, FW: Fresh Water, SW: Saline with Metals, S: Saline Water without Metals.***

In Table 4.14, trials of clover seeds treated with UW3 irrigated with 6000ppm of saline water (SW) gave total biomass values as (5.8g for wet weight, 2.28g for dry weight) compared to control ones (2.1g, 0.93g respectively).

Trials of clover seeds treated with UW4 irrigated with 6000ppm of saline water (SW) gave total biomass values as (6.78g for wet weight, 3.88g for dry weight) compared to control ones (2.1g, 0.93g respectively).

Trials of clover seeds treated with UW3+UW4 irrigated with 6000ppm of saline water (SW) gave total biomass values as (5.7g for wet weight, 1.08g for dry weight) compared to control ones (2.1g, 0.93g respectively).

Results revealed that shoots and roots of the treated clover seeds with bacteria C (UW3+UW4) irrigated with SW had the heaviest weights in

grams (12.6g) in the wet weight while in the dry one had also the over most weight (7.08g).

A noteworthy effect was observed each week during the trial. A considerable interaction of treatment was observed for root wet weight and shoot wet weight in the trial. It was noted that wet weights of the shoots and roots have had high values than dry ones.

Potential for phytoremediation depends upon the interactions among soil, heavy metals, bacteria, and plants. These complex interactions are affected by a variety of factors, such as characteristics and activity of plant and rhizobacteria, climatic conditions, soil properties, etc.

**Table 4.14: Wet and dry biomass for shoots and roots (g) of clover plant trials, carried out in vitro, second experiment.**

| No: | Treatment | Wet weight | | | | Dry weight | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | shoot | root | total | % | shoot | root | total | % |
| 1 | Control clover irrigated with FW | 2.1 | 0.87 | 2.97 | 100 | 0.94 | 0.08 | 1.02 | 100 |
| 2 | Control clover irrigated with SW | 1.9 | 0.2 | 2.1 | 70 | 0.9 | 0.03 | 0.93 | 91 |
| 3 | Control clover irrigated with S | 1.8 | 0.8 | 2.6 | 87 | 0.82 | 0.07 | 0.89 | 87 |
| 4 | Treated clover seeds with bacteria A irrigated with FW | 3.4 | 1.4 | 4.8 | 162 | 1.36 | 0.4 | 1.4 | 137 |
| 5 | Treated clover seeds with bacteria A irrigated with SW | 3.9 | 1.9 | 5.8 | 195 | 1.38 | 0.9 | 2.28 | 223 |
| 6 | Treated clover seeds with bacteria A irrigated with S | 3.7 | 1.7 | 4.8 | 162 | 1.46 | 0.78 | 2.24 | 219 |
| 7 | Treated clover seeds with bacteria B irrigated with FW | 4.1 | 1.6 | 5.7 | 194 | 2.25 | 0.66 | 2.91 | 285 |
| 8 | Treated clover seeds with bacteria B irrigated with | 4.8 | 1.98 | 6.78 | 228 | 2.9 | 0.98 | 3.88 | 380 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | SW | | | | | | | | |
| 9 | Treated clover seeds with bacteria B irrigated with S | 5 | 2.3 | 7.3 | 246 | 2.42 | 1.11 | 3.53 | 346 |
| 10 | Treated clover seeds with bacteria C irrigated with FW | 7.1 | 2.9 | 10 | 337 | 3.65 | 1.26 | 4.91 | 481 |
| 11 | Treated clover seeds with bacteria C irrigated with SW | 8.9 | 3.7 | 12.6 | 424 | 4.72 | 2.36 | 7.08 | 694 |
| 12 | Treated clover seeds with bacteria C irrigated with S | 8.3 | 3.2 | 11.5 | 387 | 3.71 | 1.98 | 5.69 | 558 |

***Bacteria A: UW3, Bacteria B: UW4: Bacteria C: UW3 + UW4, FW: Fresh Water, SW: Saline with Metals, S: Saline Water without Metals***

## 4.6 Uptake of Fe and Mg in Barley and Clover Conducted in Vitro, the 2nd Experiment.

Table 4.15 and Figure 4.4 below shows accumulation and uptake of Fe in barley conducted in vitro in the second experiment. In the 1st week, control barley irrigated with FW recorded 220ppm, while in the 2nd week counted 230ppm and continued to raise up to 270ppm in the 5th week.

Control barley irrigated with SW showed 222ppm in the 1st week, moved up to 228ppm in the 2nd week, while in the 5th week elevated to 278ppm.

Treated barley seeds with bacteria A (UW3) irrigated with SW showed 370ppm in the 1st week and raised up to 378ppm in the 2nd week, while in the last week elevated to 440ppm.

Treated barley seeds with bacteria B (UW4) irrigated with SW showed a number of 360ppm in the 1st week while in the 2nd week heaved to 375ppm and hoisted to 420ppm in the 5th week.

Treated barley seeds with bacteria C (UW3+UW4) irrigated with SW showed 490ppm in the 1st week and continued to reach up to 508ppm in the 2nd week, while in the 5th week increased to 575ppm.

**Table 4.15: Accumulation and uptake of Fe in barley conducted in vitro, the second experiment.**

| No: | Treatment | Weeks | | | | |
|---|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd | 4th | 5th |
| 1 | Control barley irrigated with FW | 220 | 230 | 245 | 260 | 270 |
| 2 | Control barley irrigated with SW | 222 | 228 | 246 | 262 | 278 |
| 3 | Treated barley seeds with bacteria A irrigated with SW | 370 | 378 | 388 | 400 | 440 |
| 4 | Treated barley seeds with bacteria B irrigated with SW | 360 | 375 | 382 | 390 | 420 |
| 5 | Treated barley seeds with bacteria C irrigated with SW | 490 | 508 | 535 | 550 | 575 |

***Bacteria A: UW3, Bacteria B: UW4: Bacteria C: UW3 + UW4, FW: Fresh Water, SW: Saline with Metals, S: Saline Water without Metals.***

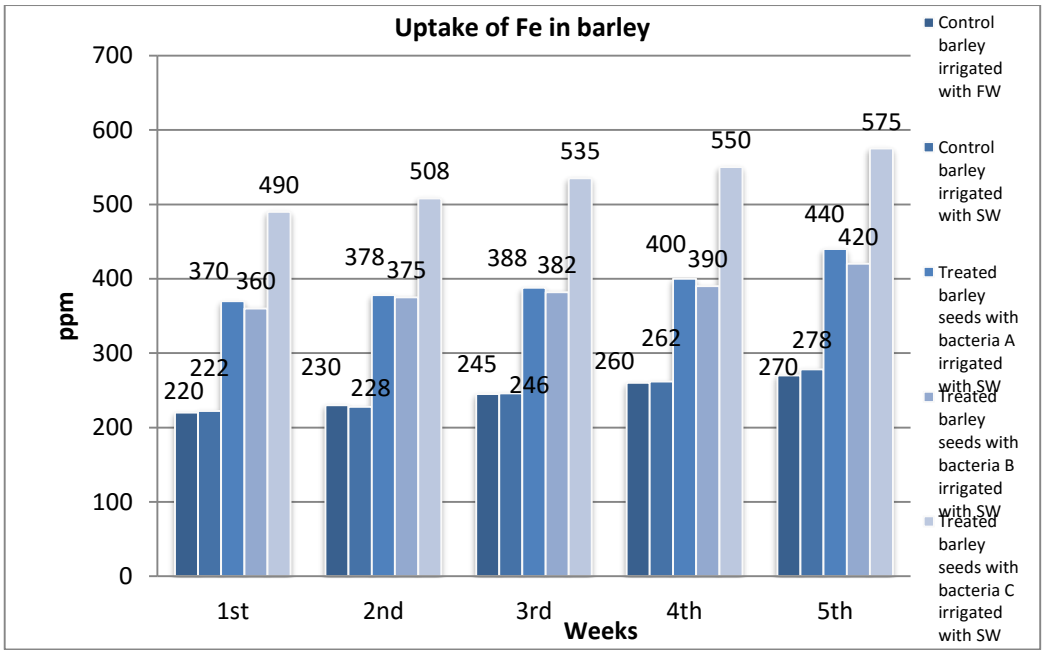



**Figure 4.4:** Accumulation and uptake of Fe in barley conducted in vitro, the second experiment.

Table 4.16 and Figure 4.5 below show accumulation and uptake of Fe in clover conducted in vitro in the second experiment. In the 1st week, control clover irrigated with FW recorded 230ppm, while in the 2nd week counted 238ppm and continued to raise up to 258ppm in the 5th week.

Control clover irrigated with SW showed 225ppm in the 1st week, moved up to 228ppm in the 2nd week, while in the 5th week elevated to 245ppm.

Treated clover seeds with bacteria A (UW3) irrigated with SW showed 345ppm in the 1st week and raised up to 354ppm in the 2nd week, while in the last week elevated to 404ppm.

Treated clover seeds with bacteria B (UW3) irrigated with SW showed a number of 355ppm in the 1st week while in the 2nd week heaved to 365ppm and hoisted to 409ppm in the 5th week.

Treated clover seeds with bacteria C (UW3+UW4) irrigated with SW showed 510ppm in the 1st week and continued to reach up to 545ppm in the 2nd week, while in the 5th week increased to 690ppm.

Table 4.16: Accumulation and uptake of Fe in clover conducted in vitro, the second experiment.

| **No:** | **Treatment** | **Weeks** | | | | |
|---|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd | 4th | 5th |
| 1 | Control clover irrigated with FW | 230 | 238 | 245 | 250 | 258 |
| 2 | Control clover irrigated with SW | 225 | 228 | 235 | 240 | 245 |
| 3 | Treated clover seeds with bacteria A irrigated with SW | 345 | 354 | 368 | 384 | 404 |
| 4 | Treated clover seeds with bacteria B irrigated with SW | 355 | 365 | 378 | 398 | 409 |
| 5 | Treated clover seeds with bacteria C irrigated with SW | 510 | 545 | 580 | 648 | 690 |

***Bacteria A: UW3, Bacteria B: UW4: Bacteria C: UW3 + UW4, FW: Fresh Water, SW: Saline with Metals, S: Saline Water without Metals.***

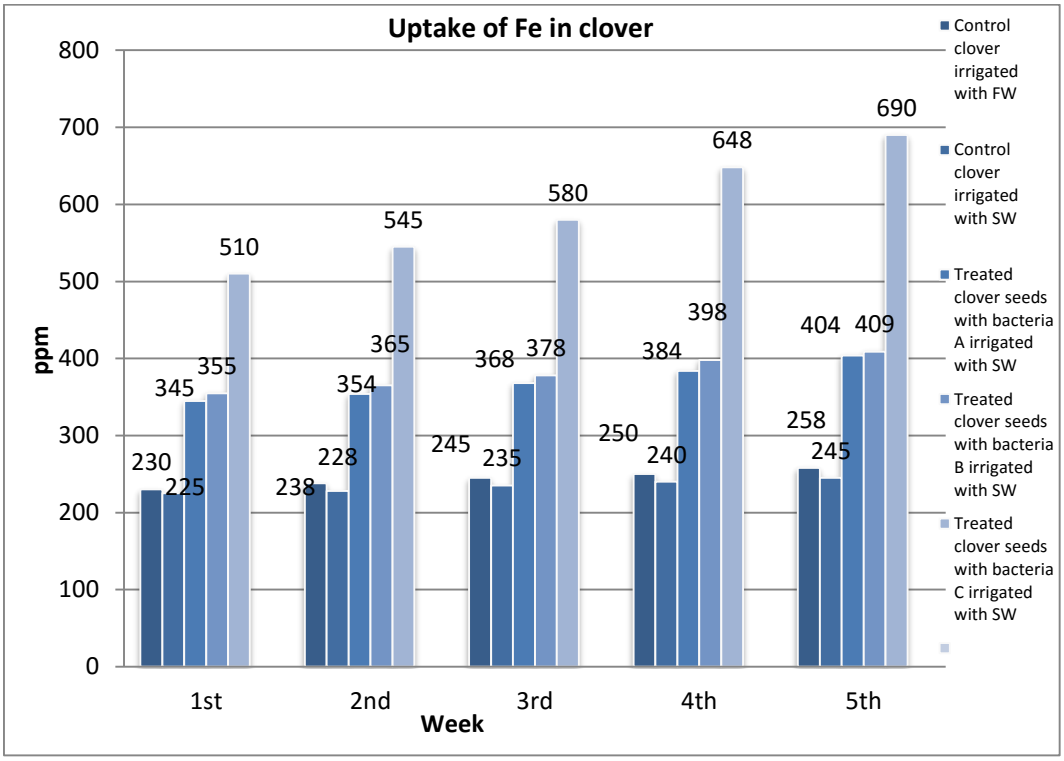



**Figure 4.5:** Accumulation and uptake of Fe in clover conducted in vitro, the second experiment.

Table 4.17 and Figure 4.6 below show accumulation and uptake of Mg in clover conducted in vitro in the second experiment. In the 1st week, control clover irrigated with FW recorded 180ppm, while in the 2nd week counted 194ppm and continued to raise up to 226ppm in the 5th week.

Control clover irrigated with SW showed 188ppm in the 1st week, moved up to 204ppm in the 2nd week, while in the 5th week elevated to 235ppm.

Treated clover seeds with bacteria A (UW3) irrigated with SW showed 240ppm in the 1st week and raised up to 258ppm in the 2nd week, while in the last week elevated to 295ppm.

Treated clover seeds with bacteria B (UW3) irrigated with SW showed a number of 257ppm in the 1st week while in the 2nd week heaved to 264ppm and hoisted to 316ppm in the 5th week.

Treated clover seeds with bacteria C (UW3+UW4) irrigated with SW showed 410ppm in the 1st week and continued to reach up to 445ppm in the 2nd week, while in the 5th week increased to 480ppm.

**Table 4.17: Accumulation and uptake of Mg in clover conducted in vitro, the second experiment.**

| No: | Treatment | Weeks | | | | |
|---|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd | 4th | 5th |
| 1 | Control clover irrigated with FW | 180 | 194 | 204 | 217 | 226 |
| 2 | Control clover irrigated with SW | 188 | 204 | 220 | 228 | 235 |
| 3 | Treated clover seeds with bacteria A irrigated with SW | 240 | 258 | 267 | 284 | 295 |
| 4 | Treated clover seeds with bacteria B irrigated with SW | 257 | 264 | 276 | 299 | 316 |
| 5 | Treated clover seeds with bacteria C irrigated with SW | 410 | 445 | 456 | 468 | 480 |

***Bacteria A: UW3, Bacteria B: UW4: Bacteria C: UW3 + UW4, FW: Fresh Water, SW: Saline with Metals, S: Saline Water without Metals.***

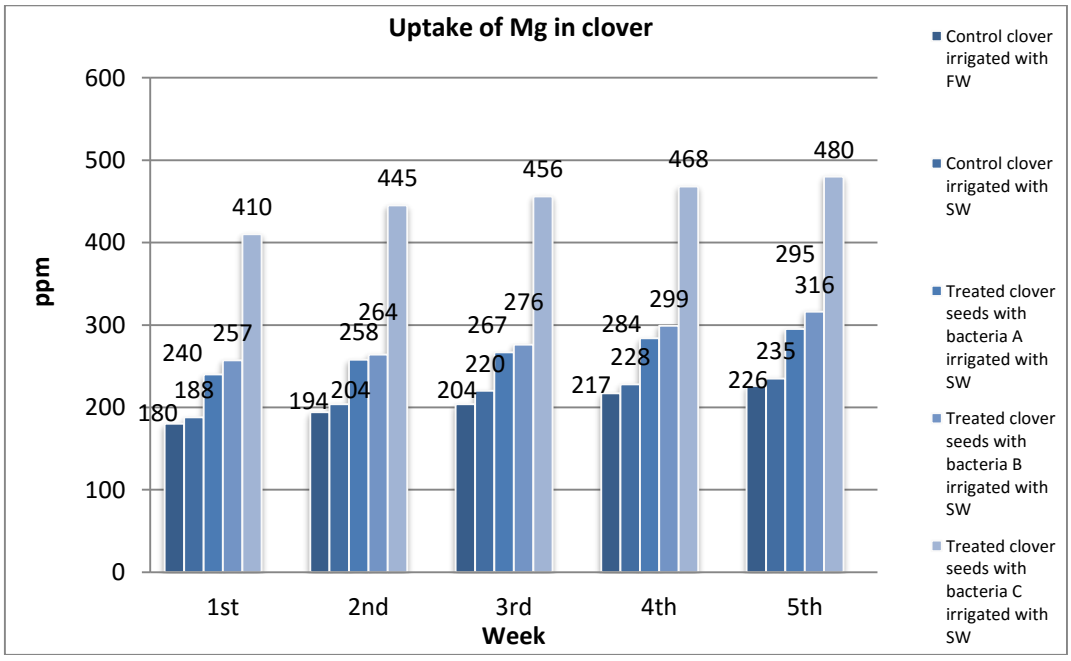



**Figure 4.6:** Accumulation and uptake of Mg in clover conducted in vitro, the second experiment.

Table 4.18 and Figure 4.6 below shows accumulation and uptake of Mg in barley conducted in vitro in the second experiment. In the 1st week, control barley irrigated with FW recorded 195ppm, while in the 2nd week counted 208ppm and continued to raise up to 245ppm in the 5th week.

Control barley irrigated with SW showed 205ppm in the 1st week, moved up to 214ppm in the 2nd week, while in the 5th week elevated to 248ppm.

Treated barley seeds with bacteria A (UW3) irrigated with SW showed 355ppm in the 1st week and raised up to 368ppm in the 2nd week, while in the last week elevated to 397ppm.

Treated barley seeds with bacteria B (UW3) irrigated with SW showed a number of 370ppm in the 1st week while in the 2nd week heaved to 388ppm and hoisted to 417ppm in the 5th week.

Treated barley seeds with bacteria C (UW3+UW4) irrigated with SW showed 488ppm in the 1st week and continued to reach up to 495ppm in the 2nd week, while in the 5th week increased to 522ppm.

**Table 4.18: Accumulation and uptake of Mg in barley conducted in vitro, the second experiment.**

| No: | Treatment | Weeks | | | | |
|---|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd | 4th | 5th |
| 1 | Control barley irrigated with FW | 195 | 208 | 218 | 232 | 245 |
| 2 | Control barley irrigated with SW | 205 | 214 | 218 | 240 | 248 |
| 3 | Treated barley seeds with bacteria A irrigated with SW | 355 | 368 | 374 | 389 | 397 |
| 4 | Treated barley seeds with bacteria B irrigated with SW | 370 | 388 | 395 | 404 | 417 |
| 5 | Treated barley seeds with bacteria C irrigated with SW | 488 | 495 | 508 | 528 | 542 |

***Bacteria A: UW3, Bacteria B: UW4: Bacteria C: UW3 + UW4, FW: Fresh Water, SW: Saline with Metals, S: Saline Water without Metals.***

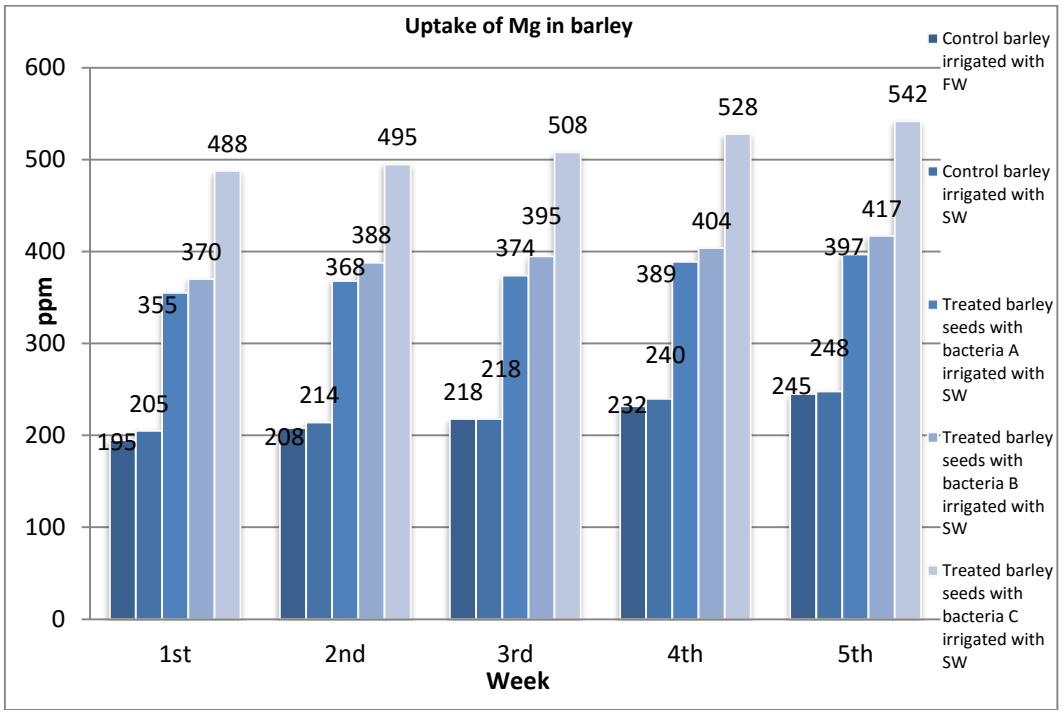



**Figure 4.7:** Accumulation and uptake of Mg in barley conducted in vitro, the second experiment.

## 4.7 Discussion

Bacteria are involved in various biotic activities of the soil ecosystem to make it dynamic for nutrient turn over and sustainable for crop production. They stimulate plant growth through mobilizing nutrients in soils, producing numerous plant growth regulators, protecting plants from phytopathogens by controlling or inhibiting them, improving soil structure and bioremediating the polluted soils by sequestering toxic heavy metal species. Indeed, the bacteria lodging around/in the plant roots (rhizobacteria) are more versatile in transforming, mobilizing and solubilizing the nutrients.

Plant growth promoting rhizobacteria, which possess the enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, facilitate plant

growth and development by decreasing ethylene levels, inducing salt tolerance and reducing drought stress in plants. Several forms of stress are relieved by ACC deaminase producers, such as effects of phytopathogenic microorganisms (viruses, bacteria, and fungi etc.), and resistance to stress from polyaromatic hydrocarbons, heavy metals, radiation, wounding, insect predation, high salt concentration, draft, extremes of temperature, high light intensity and flooding.

In the first experiment, the average amount of $Mg^{+}$ accumulated and absorbed inside clover plant with the addition of saline water and elements, germination took place in the first two weeks, and then all clover plants dried up and died. This could be attributed to the genesis of toxic substances resulting from the elements or because the iron levels were high as clover does not tolerate this amount of iron.

Accumulations and uptakes of Fe and Mg in barley which were conducted in vitro, for the treated barley seeds with bacteria C (UW3+UW4) irrigated with saline water with metals (SW) were 0.575 and 0.542 gram/liter respectively, while that of Fe and Mg in clover for the treated barley seeds with bacteria C irrigated with SW were 0.69 and 0.48 gram/liter respectively.

### 4.7.1 Root Growth:

Plant root activity: Plants take up nutrients in the form of ions ($NO_3^{-}$, $NH_4^{+}$, $Ca^{2+}$, $H_2PO_4^{-}$, etc.), and often, they take up more cations than anions. However, plants must maintain a neutral charge in their roots. In order to compensate for the extra positive charge, they will release $H^{+}$ ions from the

root. Some plants will also exude organic acids into the soil to acidify the zone around their roots to help solubilize metal nutrients that are insoluble at neutral pH, such as iron (Fe).

PGPR inoculation stimulated the root formation (Fig 4.2). Inoculation greatly increased the production of primary, secondary and tertiary roots. The initiation of more root hairs might be due to the results of bacterial interactions with the root surface of the host plant. This interaction resulted in more root hair formation. PGPR inoculation also increased the root wet weight more than root dry weight (137-141%), there were significant differences among plant growth rhizobacteria (PGPR) on rooting percentage in barley and clover plants ($P < 0.01$). Barley plant had the average root value biomass after 30 days (17.7cm) while the root weight was observed to have 8g. The root growth plant height of the clover after 30 days recorded 15cm length and a weight of 6g after 30 days.

### 4.7.2 Shoot Growth:

The effect of PGPR inoculation resulted in more shoot growth compared to un-inoculated control plants. PGPR inoculation stimulated the shoot elongation (Fig 4.3).

A comparable study by Hamed (2014), signified to have consistent results using two salt tolerant plants; barley and malt plants germinated with PGPR (UW3+UW4) that showed a very clear and significant improvements of high salt uptake and thus high phytoremediation activities of these plants once they were treated with PGPRs.

Similar results were found in different cereal crops and tomato seedlings where PGPR inoculation enhanced the appearance of root hairs (Okon, 1985; Hadas and Okon, 1987). In our study, the PGPR inoculation in barley and clover has been shown to result in enhanced roots and shoots of both species, and promoted their development and branching which caused alteration in arrangement of root and shoot length and weight. Similarly, inoculation also increased the root and shoot growth of tomato seedlings (Hadas and Okon, 1987). Azospirillum (Sp7) has the potential to synthesize plant hormone which can replace indole acetic acid (IAA) to stimulate root growth in vegetable soybean (Molla et al., 2001). Also, this is consistent with that of Dobbelarere et al. (1999) who suggested that secretions of plant growth promoting substances such as auxins, gibberellins and cytokinins by the bacteria seem to be responsible for these effects. Sarig et al. (1988) further suggested that growth promoting effects of PGPR inoculation are mainly derived from morphological and physiological changes in inoculated sorghum roots and enhancement in water and plant nutrient uptake.

From these results, it can be said that barley plant had the highest value of root and shoot biomass. Also, barley plant had a better survival status than clover, while found to be more effective to improve rooting than control. This is consistent with the study conducted by Madrid and Kirkham who studied the heavy metals uptake by barely and sunflower grown in abandoned animal lagoon soil. The results showed that barely was the better choice in phytoremediation. Shoot and root lengths and weights were

greater and showed effective performance when treated with UW3 and UW4 bacteria in combination compared to other treatments.

This increased growth of inoculated plants might be due to the higher Fe and Mg accumulation by bacterial fixation and better root growth, which promoted the greater uptake of water and nutrients.

In our study, N has increased from 490ppm into 580ppm (Table 3.3). The higher N incorporation has apparently increased the formation of protein and enzyme for better physiological activities. The higher N also contributed to the formation of chlorophyll, which consequently, increased the photosynthetic activity. PGPR inoculation increased the physiological properties of the host plants namely, photosynthetic rate (Mia et al., 2005).

## 4.8 Conclusions

- In conclusion, our study showed that treated barley and clover seeds with bacteria C (UW3+UW4) irrigated with SW were clearly more consistent in improving different root and shoot parameters, as well as accumulations and uptakes of Fe and Mg in barley and clover plants for the treated barley seeds with bacteria C (UW3+UW4) irrigated with saline water with metals (SW). The results indicated that PGPR inoculation significantly increased the root properties (length and weight), shoot growth, (length and weight), plantlets. A substantial increase in chlorophyll content was also observed in the plantlets inoculated with PGPR. Combinations of beneficial bacterial strains that interact synergistically are currently being devised and

numerous recent studies show a promising trend in the field of inoculation technology

- In addition, the application of the heavy metal resistant and plant-beneficial bacteria can be considered as bioremediating tools with great economical and ecological relevance.
- All bacterial strains increased the shoot and root growth of barley and clover in comparison with the untreated control, thus, the present study suggests that UW3 and UW4 strains alone or in combination have a great potential to increase photosynthesis, transpiration, water use efficiency, leaves chlorophyll content and grain yield. PGPR strains can indirect enhance stress tolerance as a consequence of increasing activity of some antioxidant enzymes during periods with intense photosynthesis. The PGPR strains improved the nutritive value of the barley and clover by enhancing the soluble protein and reducing carbohydrates content.
- The most critical factor in determining how efficient phytoremediation of metal contaminated soil will be is the rate of uptake of the metal by plants. In turn, this depends on the rate of bioavailability. Using beneficial bacteria, which can alter metal bioavailability of plants, improves the performance of phytoremediation of the metal contaminated sites.
- In our country, large amount of generated brine water (about 400 $m^3$) are produced daily from five stations of reverse osmosis plants in Jericho districts. Brine water is being disposed in unfriendly

environmental ways by spilling them out in soils and/or streams which created further to environmental problems. This problem can be solved by the use of bacteria.

- Finally, applying PGPR-associated phytoremediation under field conditions is important, because, to date, only locally contaminated sites have been treated with this technique, by using microbes cultured in the laboratory.

## 4.9 Recommendations

- PGPRs are the potential tools for sustainable agriculture and trend for the future. For this reason, there is an urgent need for research to clear definition of what bacterial traits are useful and necessary for different environmental conditions and plants, so that optimal bacterial strains can either be selected and/or improved.
- PGPB exhibiting multiple plant health and development enhancing traits coupled with the excellent potential to lower down the heavy metal stress in soils, may eventually find wide-ranging applications in the development of bioremediation strategies for heavy metal decontamination.
- The reason to use Fe and Mg in this study is its vital importance to the plants as well as animals. Throughout the use of barley and clover, it is possible to produce feed nutrients containing enhanced excellent nutrients to the animals, consequently, instead of industrialized fodder.

- Our study suggests that the two PGPR strains may be used as crop-enhancer and biofertilizer for vegetable production in sustainable and ecological agricultural systems. However further studies are necessary in order to evaluate the impact of beneficial bacteria introduction into soil ecosystems with other strains and plants.
- There is a need to optimize the agronomic practices to maximize the cleanup potential of remediative plants. Since in many instances metal absorption in roots is limited by low solubility in soil solution, it is important to further investigate the use of chemical amendments to induce metal bioavailability. Significant results have been obtained in this area. However, there is a need to find cheaper, environmentally benign chemical compounds with metal chelating properties.
- More information is also needed to optimize the time of harvest. Plants should be harvested when the rate of metal accumulation in plants declines. This will minimize the duration of each growth cycle and allow more crops to be harvested in a growing season.
- Research is also needed to identify phytoremediating other species rather than barley and clover capable of being rotated to sustain the rate of metal extraction and raise the nutrient values e.g. to solve iron deficiency in children.
- Our results suggested that simultaneous screening of rhizobacteria for growth and yield promotion under pot and field experiment is a good tool to select effective PGPR for biofertilizer development

biotechnology. PGPR are highly beneficial for plant growth and can serve as potential substitute for pesticides and chemical fertilizers. Even under unfavorable and stress conditions, PGPR can enhance seed germination and can exert a beneficial effect on plant growth.

- The isolation and development of bacteria from the Palestinian environment that has the ability to accommodate with miscellaneous environmental circumstances, and not only dependent on bacteria from outside that may affect the biodiversity and have negative effects on the environment.

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جامعة النجاح الوطنية
كلية الدراسات العليا

# "إستخدام بكتيريا الجذور في تحسين تنقية التربة من العناصر الثقيلة"

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# "إســـتـخـدام بكـتـيـريا الـجـذور فـي تـحـسـيـن تـنـقـيـة الـتـربـة مـن الـعـنـاصـر الـثـقـيـلـة"

**إعداد**

**حـافـظ فـوزي دراغـمـة**

**إشراف**

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## الملخص

من المعروف أن البكتيريا هي كائنات حية دقيقة تستطيع أن تنتج مواد عديدة تنشط في تنظيف البيئة من الملوثات, كما أن لديها القدرة على تعزيز نمو النبات إذا أُضيفت للتربة, لذلك, فإن هذه الميكروبات تلعب دورا هاما في معالجة وخصوبة التربة الزراعية. يمكن تعزيز كفاءة علاج النبات عن طريق زيادة إستخدام المعادن الثقيلة أو ذوبانها في التربة وارتفاع الكتلة الحيوية للنبات.

والهدف من إجراء هذه الدراسة هو إثبات ما إذا كان النبات الذي إستخدمناه (الشعير والبرسيم) مع البكتيريا والماء المالح والذي يحتوي على العناصر (الحديد والمغنيسيوم) أكثر فعالية ونمواً وعمليات حيوية من النبات الذي لا يحتوي بكتيريا.

تكمن أهمية هذه التجارب بإضافة بكتيريا محفزات النمو (PGPR) إلى التربة أو تُلوث بها البذور قبل الزراعة بواسطة نوعين من البكتيريا (UW3,UW4). أُجريت هذه الدراسة بتجربتين في مكانين مختلفين؛ التجربة الأولى في منزل الباحث؛ حيث تم عمل بيت بلاستيكي يماثل الظروف الطبيعية, بينما تم إجراء التجربة الثانية في مختبر جامعة النجاح الوطنية, كلية العلوم, نابلس, فلسطين. تم إحضار عينات النباتات من وزارة الزراعة الفلسطينية, وقد إستخدمنا ثلاثة أنواع من الماء؛ ماء عذب, ماء مالح بدون عناصر وماء مالح مع عناصر.

أظهرت النتائج أن أطوال السيقان للشعير والبرسيم بعد ثلاثين يوماً هي 38 سم, 28 سم, والأوزان 10 غم, 8 غم على التوالي, بينما أظهرت النتائج أن أطوال الجذور للشعير والبرسيم بعد ثلاثين يوما هي 17 سم, 15 سم والأوزان هي 8 غم, 6 غم على التوالي. إن

تلقيح ال (PGPR) للوزن الرطب للجذور بزيادة مقدارها (137–141%) عن الوزن الجاف للجذر قد يعزى إلى تطور نمو المجموع الجذري للنباتات المعاملة ونشاط هذه النباتات في تحلل المواد العضوية في التربة ومن ثم زيادة جاهزية العناصر المغذية في منطقة الرايزوسفير وتحفيز قدرة النبات في زيادة امتصاصه للعناصر الغذائية. بينت النتائج أن تراكم وإمتصاص الحديد والمغنيسيوم في الشعير المعالج بالبكتيريا (UW3,UW4) معا بالماء المالح مع العناصر قد وصل إلى 0.575 و 0.542 غم/لتر على التوالي, بينما أوضحت الدراسة الحالية أن تراكم وإمتصاص الحديد والمغنيسيوم بعد ثلاثين يوما في البرسيم هو 0.69 و 0.48 غم/لتر على التوالي. وقد تُعزى هذه الزيادة في النمو إلى قدرة هذه البكتيريا (UW3,UW4) على إفراز بعض منظمات النمو والتي تؤثر ايجابياً في نمو النبات بزيادة نمو ونشاط المجموع الجذري وقدرته على إمتصاص المغذيات ونشاط هذه البكتيريا في تثبيت النتروجين الجوي، كل هذا له دور في زيادة الحاصل مقارنة إلى النباتات غير الملقحة بالبكتيريا.

مما سبق, وُجد من خلال هذه الدراسة نجاح تأثير اللقاح البكتيري (UW3,UW4) في زيادة أوزان وأطوال الجذور والسيقان لنباتي الشعير والبرسيم, والقدرة على زيادة التمثيل الضوئي، والنتح، والأوراق ومحتوى الكلوروفيل. واستمرت الزيادة في أعدادها مع تقدم عمر النبات وخلال مراحل نموه إلى نهاية الشهر, وهذا يدل على حيوية ونشاط اللقاح البكتيري خلال مدة التجربة في تفاعلها حيويا مع نمو وفعالية هذين النباتين مما تنعكس إيجابياً على خصوبة التربة ونمو النبات.

لذلك, توصي هذه الدراسة باستعمال اللقاحات الحيوية البكتيرية لنباتي الشعير والبرسيم لما لها من تأثير إيجابي في تحسين الحالة التغذوية للنبات مما ينعكس على معايير نمو النبات المختلفة في المستقبل.

وعلاوة على ذلك، فإن سبب إستخدام الحديد والمغنيسيوم في هذه الدراسة يعود لأهميتها الحيوية للنباتات وكذلك الحيوانات, فمن خلال إستخدام الشعير والبرسيم مع البكتيريا، فإنه من المرجح إنتاج مواد مغذية تحتوي على عناصر غذائية محسنة للحيوانات، بديلاً من العلف الصناعي.