Phytoextraction of Zinc from Hydroponic Solutions

By

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A Thesis
in
The Department
of
Building, Civil and Environmental Engineering

Presented in Partial Fulfillment of the Requirements

for the Degree of Master of Applied Science (Civil Engineering) at

Concordia University
Montreal, Quebec, Canada

May, 2010

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ISBN: 978-0-494-71089-0
Our file Notre référence
ISBN: 978-0-494-71089-0

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ABSTRACT

Phytoextraction of Zinc from Hydroponic Solutions

Eyad Abbadi

Heavy metals can have adverse health effects on both humans and biota if they exist in high concentrations in the soil or in water bodies. Rhizofiltration is a specific type of Phytoremediation which involves plants to extract contaminants from large wetland areas that have a low level of contamination. The objective of this research is to elucidate the interaction of the plant species Solanum lycopersicum (tomato plant) with the surrounding zinc contaminated solution, after being subjected to environments of darkness, illumination, and alternate current gradient. To perform this objective, tomato plants were grown in the hydroponic solutions containing 0, 100, 200 and 500 mg/L of zinc under illuminated conditions. Subsequently, these plants were grown in solutions of 100mg/L and were subjected to varied environmental conditions stated earlier. At the end of each experiment, which lasted seven days, concentration of zinc in plant roots, shoots and fruits were analyzed separately. The effects of plants and alternate current on solution pH and electrical conductivity (EC) were also investigated concurrently. It was found that the accumulated amount of zinc in plant roots is proportional to its concentration in hydroponic solution. However, the augmentation of zinc concentration in plant roots attains a plateau with increasing concentration of zinc in the hydroponic solution. As in roots, accumulation of zinc in plant shoots generally reached a saturation level of zinc when zinc concentration increased. However, in the fruit, zinc concentration appears to be not dependent on solution concentration of zinc. It was found that plants subjected to a light environment accumulated more zinc in their roots and shoots than plants subjected to a dark environment. Further, it was also noted that for the specific environment which consisted of an applied alternate current gradient of 1Volt/cm at 16HZ , plant capability to extract zinc in both shoot and root was observed to be reduced. A brief series of tests related to the effect of nutrients on the uptake of zinc indicated that they modify the plant uptake of zinc.

ACKNOWLEDGEMENT

I would like to thank my advisors Professor Ramamurthy, Amruthur S. and Professor Anna Smoczynska (Warmia-Mazury University) for suggesting the problem and for the advice provided during the completion of the project.

Also, I would like to take the opportunity to acknowledge Professor Maria Electrowicz for funding this work in the first four years, and also for providing much attentions and contributions to the scientific work. My thanks extend to the BCEE department members for their support during my stay at Concordia.

Lastly, I sincerely acknowledge the love and support of my family and friends both in Jordan and in Canada who are always provided a helping hand during the past few years.

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LIST OF ABBREVIATIONS

AC:	Alternate current
C_o:	Concentration of zinc in the plant xylem
C_{soln}:	Concentration of zinc in solution.
D:	Diffusion Coefficient
D:	Conditions of darkness
DC:	Direct current
DGT:	Diffusive gradient in thin films tool
DM:	Dry mass
DOC:	Dissolved organic carbon
E:	Voltage gradient
EC:	Electrical conductivity
E-L:	Conditions of illumination while applying alternate current
ET:	Evapotraspiration
F:	Faraday constant
L:	Conditions of illumination
LMWOCs:	Low molecular weight organic carbons
TF:	Translocation factor
THSU:	Total hydroponic solution uptake
R:	Gas constant
T:	Solution temperature
U:	Ionic mobility
V:	Ion velocity
Z:	Ion charge

CHAPTER ONE
INTRODUCTION

1.1 GENERAL

Heavy metal contamination of soils and waters is a challenging problem in the current era. Soil contamination receives much attention since it is the constituent of most plant rhizospheres. Consequently, it is the media through which heavy metals can be transferred to human through the food chain. Likewise, these metals can be leached to groundwater or it can be transferred to water bodies through runoff and soil erosion, contaminating sea resources and drinking water supplies. The following section describes the routes through which metals may accumulate in soil and water systems. In addition, toxicity effects of the metal, zinc, and the regulations specifying its limits will be discussed subsequently.

1.2 SOIL POLLUTION WITH HEAVY METALS; TOXICITY AND REGULATIONS

Heavy metal contamination of soil and surface water can be attributed to variable sources. As illustrated in Fig. 1.1, factories, mines and transportation vehicles can emit heavy metals to air, which eventually will be deposited or precipitated with the falling rain (Li et al. 2001). In addition, run off from roads and roofs will carry several types of heavy metals that may terminate in soil and water bodies. For example, it may be sent to a municipal treatment plant and adsorbed to the sludge that may eventually be applied to the agricultural soil as a fertilizer. Meanwhile, contaminated wastewater effluent can be discharged into water systems. Alternatively, runoff water can be used to recharge ground water and hence it will accumulate heavy metals on the soil by which it may pass (Boller 1997). Likewise, this runoff water can be sent to the water bodies contaminating its resources. Presence of heavy metals on roads and roofs is attributed to corrosion and to vehicles of which fuel, tires, oil lubricants may contain several metals including zinc, lead, and cadmium (Li et al. 2001). Among these metals, zinc will be discussed in more detail subsequently.

Zinc is used in storage batteries, in alloys, and for cathodic protection against corrosion of steel. Besides being used as a plant nutrient, zinc is required by the human body for optimum function of three hundred enzymes. If contacted with water, it librates highly flammable gases. It can be absorbed by ingestion or by inhalation. If absorbed, it can affect the pancreas, but it does not cause kidney and liver damage. Gastrointestinal absorption of zinc is inhibited by an organic phosphate compound present in grain and vegetables. Guideline of European commission (EC) suggest that its concentration should be not more than 100 μg/L at supply, and 5000 μg/L after 12 hours contact with consumer's pipe work. The Environmental quality objective for potable fresh water is 3000 μg/L total zinc. While it is 8-125 μg/L for protection of sensitive aquatic life and it is 25 to 500 μg/L for protection of other aquatic life. If it present at concentration of 0.1 - 0.2 μg/g in soil, it will retard the growth of most soil microorganisms. In addition, if it present at a concentration above 10 mg/L, it will affect negatively the nitrification in treatment plants (Richardson and Gangolli 1994). According to Quebec regulations, zinc concentration in soil should not exceed 1500 μg/g if the land is intended to be used for industrial purposes; meanwhile, for commercial purposes, its concentration should not exceed 500 µg/g. It is established that the concentration of zinc in the basin of Saint Laurent river to be 110 µg/g which represents its original concentration in soil with no anthropogenic contamination (Gouvernement du Québec 2002). However, these regulations that are related to soil are based on total zinc concentration and not on the bioavailable zinc. Therefore, it will not be considered in this study.

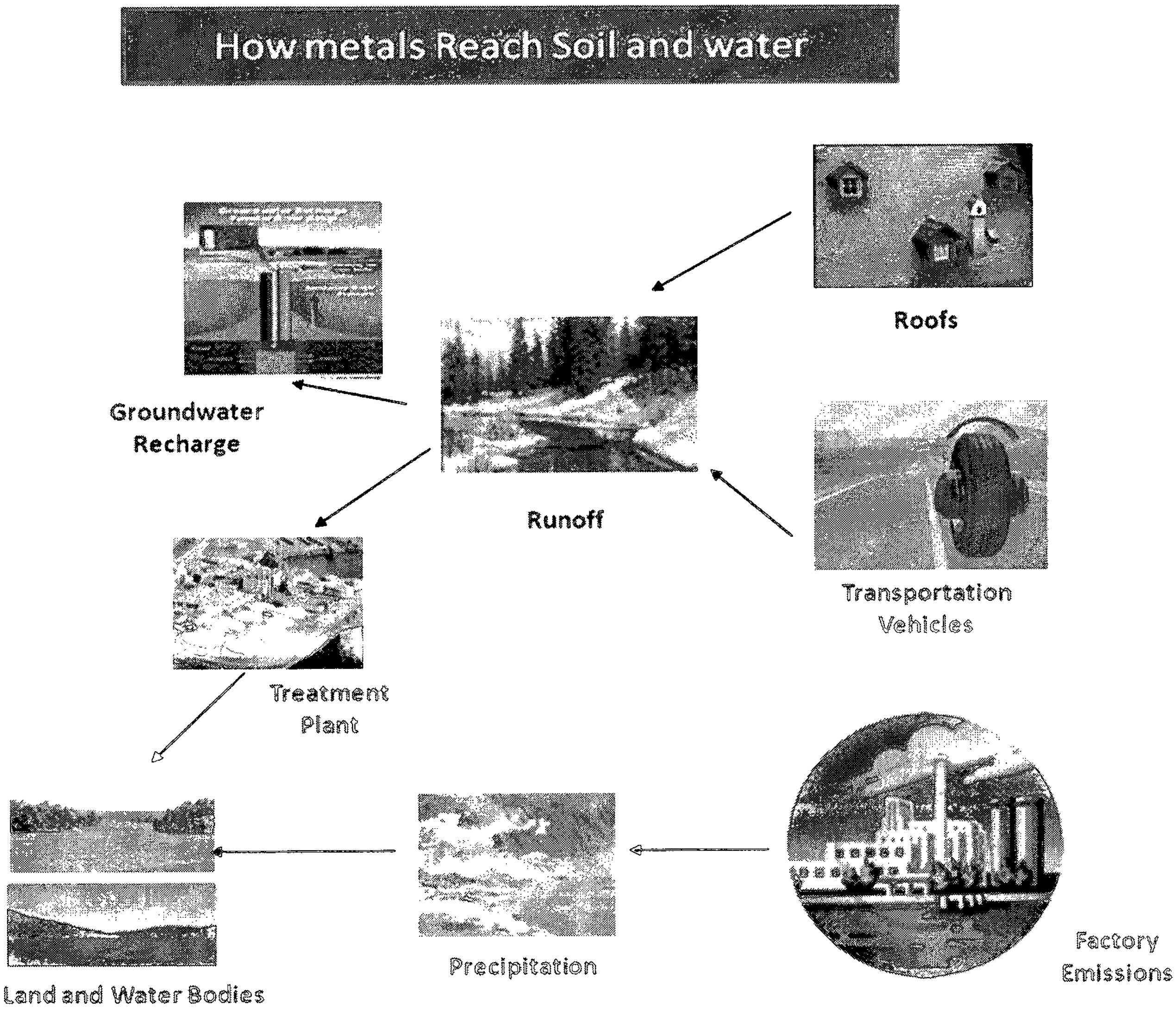


Fig. (1.1): Schematic representation of possible routes through which metals may contaminate soil and water system

1.3 REVIEW OF TECHNOLOGIES USED FOR SOIL REMEDIATION OF HEAVY METALS

Several methods can be used to decontaminate metals from soil and water system. In water, metals can be removed by applying a specific chemical that can decrease their solubility. Likewise, metals can be separated based on their size or on their charge. The former can be accomplished by reverse osmosis process, where the solution passes through a membrane acquiring pore size smaller than that of the metal. Likewise, resins can be used to adsorb metals in a process known as ion exchange. Not only physicochemical methods can be used to remove metals from water, but biological methods can also be employed for this purpose. For instance, metals can be sorbed to microorganisms in a process known as biosorption. Finally, plants have also been exploited to accumulate metals from wetlands and shallow water bodies in a process referred to as rhizofiltration. This process will be explained more in the next chapter.

On the other hand, a limited number of technologies are available for heavy metal remediation from soil. These technologies aim to both immobilize metals and reduce their toxicity or to separate them from the soil matrix. However, implementing these techniques should not transfer metals to humans through another media like groundwater. Heavy metal immobilization can be achieved by site containment, vitrification, solidification, chemical stabilization, and by phytostabilization.

Containment is applied to hinder the transport of heavy metals from contaminated area to surrounding clean soil or groundwater, or to prevent groundwater flow from passing through the contaminated area. Soil containment involves capping, and constructing vertical or horizontal barriers. Examples of vertical barriers are slurry walls, grout curtains, sheet pile walls, and geomembrane curtains. The most economically feasible barrier is the slurry wall. Geomembranes could be attached to these walls if the contaminant is not compatible with the slurry material. On the other hand, grout curtains are economically feasible when used to close the voids among the underlined rocks. These vertical walls should be attached to an impermeable barrier; otherwise Groundwater should be pumped to prevent its contact with the contaminated area (smith et al. 1995).

Vitrification is another immobilization process in which soil constituents are melt using electrical heating. This process may need a supply of silicates to form a glassy material that decreases the solubility of the incorporated heavy metals. This method can be applied in situ or ex situ for sand or clay soils (Smith et al. 1995).

Similar to vetrification, Solidification and stabilization can be applied either in-situ or ex-situ. Solidification aims to decrease soil permeability and to increase its bearing strength (LaGrega et al. 2001). Macro encapsulation and micro encapsulation are among the varieties of this method (Smith et al. 1995). Likewise, stabilization aims to decrease heavy metal mobility and toxicity through a chemical reaction; for instance Cr^{+6} can be reduced to insoluble Cr^{+3} hydroxides through this process (Suthersan 1997). In addition to the chemical means occurring in stabilization, heavy metal detoxification can be accomplished through bioremediation (The Hazardous Waste Consultant 1996). An emerging technology that makes use of the plants to decrease the mobility of heavy metals is referred to as phytostabilization. This technology is described in the next chapter.

In order to remove the contaminants permanently from the soil, separation techniques should be implemented. Among these techniques is the pyrometallurgical separation. This method can be economically feasible, if the value of the extracted metal can offset the cost of the process (Smith et al. 1995). Removal of heavy metals from soil to an aqueous solution is referred to as soil washing, if done *ex-situ*, or soil flushing, if done *in-situ*. Soil washing or flushing works more efficiently with high permeable soils (The Hazardous Waste Consultant 1996). In addition, microbiological processes could be exploited to separate heavy metal from soil as in bioleaching method making use of sulphide oxidation by bacteria to enhance heavy metal mobility (Karavaiko et al. 1988). Consequently, separation of metals from low permeable soils seems to be more challenging than its separation from sandy soils as fewer solutions are available to tackle such a task in clayey soils. This demands to gain more insight into other alternatives to solve this problem.

Electrokinetics and phytoextraction are emerging processes that can deal with heavy metal contamination of soil, especially for the low permeable soils. Electrokinetics implies applying a direct voltage gradient in soil, so that ions can migrate to the electrode carrying the opposite charge. There, they can be extracted from the soil. Moreover, this gradient will cause water to flow towards the negatively charged electrode mobilizing neutral metal species with it. Meanwhile, phytoextraction refers to accumulating metals from soil into the harvestable part of the plants. However, this process is considered to be very slow. A previous study showed that it can be accelerated by applying an alternating current in soil (Bi et al. 2007). However, the main factors that contributed to this enhancement still need more understanding. From another perspective, plant phytoextraction of metals has not been studied previously while being subjected to darkness and illumination environment. Such a dark environment can be dominating in winter in the northern regions which make it necessary to understand how it might affect plant behaviour.

This study aims to shed more light on the effect of a dark environment as also the effects of the presence and absence electrical stimulation in an illuminated environment. To this end, the ***Solanum lycopersicum*** (tomato) plant was selected as the test plant species which could extract the zinc from aqueous solution. It is hypothesized that the same effect occurring in these solutions would be present in the wastewater effluent as well as in the soil. A detailed discussion of phytoextraction as well as electroremediation and the potential benefit of their hybrid use will be described in Chapter two. Even though electroremediation utilizes direct current to achieve its objectives, applying an alternate current is thought to have the same effect, except that the flow of water and ions will not be directed toward a specific direction. Moreover, it should be emphasized that water movement occurs only when the electrokinetic gradient is applied in clayey soil. However, when applied in water, as in this study, only migration of charged colloids and ions can occur.

1.4. OBJECTIVES AND HYPOTHESIS

1.4.1 Objectives

The objectives of this study are listed below

1) Investigate the effect of light and dark environments on the ***Solanum lycopersicum*** (tomato) plant's capability to extract zinc from the hydroponic solution.
2) Understand the effects of an alternate current on the accumulation of zinc by plants when subjected to an illuminated environment.
3) Briefly determine the effects of nutrients (N, P, K) on the accumulation of zinc in the plant under different environmental conditions (darkness, light, with and without current).

1.4.2 Hypothesis

It is expected that the application of alternate current will stimulate electrochemical reactions which will change zinc speciation augmenting its bioavailability. Since metals are transported into the root apoplast (Fig. 2-1) as a result of diffusion from a region of higher to a region of lower concentration, application of an alternate current is expected to alter this diffusion factor and subsequently to modify the absorbed mass of metal into this fragment. Likewise, the alternate current is anticipated to affect the active uptake of zinc, since it has been established in literature that a specific voltage gradient might stimulate plant metabolic activities (Stenz et al 1998). Hence, it is likely that both root symplast and apoplast will experience a modified zinc concentration, which might be reflected in the shoot and fruit fragments.

Subjecting plants to dark environment might hinder active uptake of zinc in plant roots. In other words, only passive uptake will exist in the dark. Hence, it is possible that plant illumination would enhance the root uptake of zinc. Taking into account that evapotranspiration is usually less in the dark (SAPS 2010), subjecting plants to light might increase zinc uptake in plant shoot and fruit sections.

Moreover, it is expected that plant uptake of zinc has a threshold value, after which no increase in accumulated zinc in plant dry mass (DM) would be observed. This can be justified by the fact that plants receive limited amount of solar energy thereby limiting their active uptake of metals.

Chapter two will discuss a few more details of the concepts related to the study. Moreover, case studies that lead to the idea of this study will be reviewed in chapter three. Subsequently, chapter four will describe the detailed experimental methodology used to accomplish the planned objectives. Following this, the generated results will be discussed in chapter five; and finally, the derived conclusions and recommendations will be summarized in chapter six.

CHAPTER TWO
THEORETICAL BACKGROUND

2.1 PHYTOREMEDIATION

2.1.1 General

Phytoremediation is defined as "the engineered use of plants *in-situ* and *ex-situ* for environmental remediation" (Suthersan 2002). It can also be defined as "the destruction, inactivation or immobilization of pollutants in a harmless form" through the aid of photosynthetic plants. The latter include higher plants and algae. Detoxification of the soil or aquatic medium can be done through the plant or through bacteria and fungi which are stimulated by the presence of that plant (Horne 2000).

Phytoremediation has several advantages over other conventional methods for both soil and water systems, among these are:

- The cost of growing the plant and maintenance of the system is low (Suthersan 2002, Nyer et al. 2001).
- It improves the aesthetics of the contaminated site (Suthersan 2002, Nyer et al. 2001)
- For soil, it does not deteriorate its fertility, structure, texture, and biota, and it leaves it in usable condition (Suthersan 2002).
- The volume of harvested plants that need to be disposed is much less than the original volume of the contaminated medium (Brooks 1998)

On the other hand, phytoremediation has some drawbacks, and these are stated below:

- The harvested crops are considered as waste that needs treatment and disposal (Suthersan 2002).
- It takes a long time to achieve its goal, especially when used for phytoextraction (Suthersan 2002, Nyer et al. 2001).
- The right plant needs to be selected so that it can grow in the specific climate of concern (Brooks 1998).
- Plant activity decreases in cold temperatures (Suthersan 2002).
- It could not be used in sites contaminated with phytotoxic concentrations of heavy metals (Suthersan 2002, Nyer et al. 2001).
- It could not be applied to remove contaminants at depths lower than root zone (Suthersan 2002, Nyer et al. 2001).
- There is a risk that the contaminants can get transferred into the wild life food chain through the above ground portion of plants (Nyer et al. 2001).

2.1.2 Applications of phytoremediation

Generally the phytoremediation of metals involves the following four processes:

a) Phytoextraction/phytovolatalization: phytoextraction entails the uptake of metals in plant roots followed by their translocation into the harvestable part of the plant (Kumar et al. 1995). On the other hand, phtytovolatalization entails phytoextraction accompanied by volatilization of accumulated metals into the ambient air. However, phytovolatalization can be applied to a few metals including mercury, selenium, and arsenic (Brooks 1998). If a chelating agent is applied to metal-contaminated soil, then the contaminant should be monitored to make sure that it does not leach to ground water (Vangronsveld et al. 1998).

b) Phytostabilization: The use of plants to immobilize metals and to decrease its bioavailability in the rhizosphere. Immobilization can be applied by adsorption on or by absorption in roots without significant translocation into the above ground portion of the plant so that metals do not transfer into the food chain. Likewise, metals can be stabilized by being precipitated and hence immobilized due to root activity in changing its surrounding environment. Moreover, protecting soil from being eroded is one of the main goals of phytostabilization. Alternatively, this method can be applied to organic toxicants, as well as to ground water (Nyer et al. 2001). To enhance the efficiency of phytostabilization, application of amendments, such as phosphate, can be coupled with plantation (Ruby et al. 1994)

c) Rhizofiltration: the use of plants to absorb, adsorb, or precipitate heavy metals from an aqueous solution. However, it is not preferable to translocate metals into the upper portion of the plant in order to decrease the amount of disposed plants (Dushenkov et al. 1995, Nyer et al. 2001).

d) Hydraulic containment: The use of plants to control the flow of pore water and hence to prevent leaching of a contaminant to ground water (Nyer et al. 2001)

2.1.3 Plant interaction with metal in contaminated sites

For a plant to be efficient, phytoextractors it should have sufficient accumulation of metals, tolerance to site condition, and fast growth rate with large biomass, in addition to being easily harvested, treated and disposed (Vangronsveld et al. 1998). Generally, plant type, physiology and genetic characteristics are important factors to be considered while choosing it for phytoremediation; however, it is not only important to know about plant characteristics before remediating a site, but it is also important to know about chemistry and fertility of the contaminated soil and water for more comprehensive evaluation (Kumar et al. 1995).

Plants are classified into three kinds according to heavy metal accumulation patterns. First, the excluders are the ones which prevent ion uptake and translocation, or in other words, plants which accumulate metals in concentrations less than their surrounding medium. The second type are the indicators, which accumulate metals in proportional concentrations to that in the soil; therefore, these are suitable for biomonitoring. The third kind are the hyper accumulators, which accumulate metals in concentrations more than that available in the soil, and these are often called hyperaccumulators (Bargagli 1998).

Explanations of heavy metal hyper accumulation by plants include: inadvertent uptake, metal tolerance or sequestration, drought resistance, pathogen defense, and interference with other plants. The latter term refers to the competition with other plants that are less tolerant to metal toxicity; for instance, one of the competition mechanisms a plant may use is by dropping their leaves which have a high concentration of metals inflicting toxicity effects on the competitors (Brooks 1998).

Mechanisms of heavy metal accumulation include extra and intra cellular chelation, precipitation, compartmentalization, and translocation into the plant vascular system (Kumar et al. 1995). In a case study, physical and chemical processes like ion exchange and chelation by root surface was faster than other processes that need energy from plants like intracellular uptake or precipitation of metal by exudating phosphorous ions into soil solution (Dushenkov et al. 1995). In another case study, it was shown that soil fertility can affect the effectiveness of the plant in accumulating a certain metal (Kumar et al. 1995).

To understand phytoremediation more thoroughly, it is necessary to understand root structure through which ions will pass. The next section supplies a short summary about this structure.

2.1.4 Plant root fragments and their significance for ion uptake

For an ion to pass to the vascular system of the plant, which is the media through which ions are translocated to shoots. For this, it has to pass either through plant cells or in the gaps separating them (Fig. 2-1). The former pathway has been referred to as the symplast, while the latter is called the apoplast.

Moreover, as illustrated in Fig. 2-2, plant roots can be subdivided into several sections. The outer layer of the root is referred to as the epidermis. This layer is followed by the cortex, the endodermis, and the stele subsequently. In the outer layer of the cortex, exodermises cells can develop in some plants. These cells may exudate suberin chemical which can fill the gap that exists in their vicinity. It has been indicated that if this suberin is composed of aliphatic compounds, then it may hinder the passage of ions through the apoplast; meanwhile, if it is composed of aromatic compounds, then it might be permeable to these ions (Schreiber et al. 2005, Hose et al. 2001).

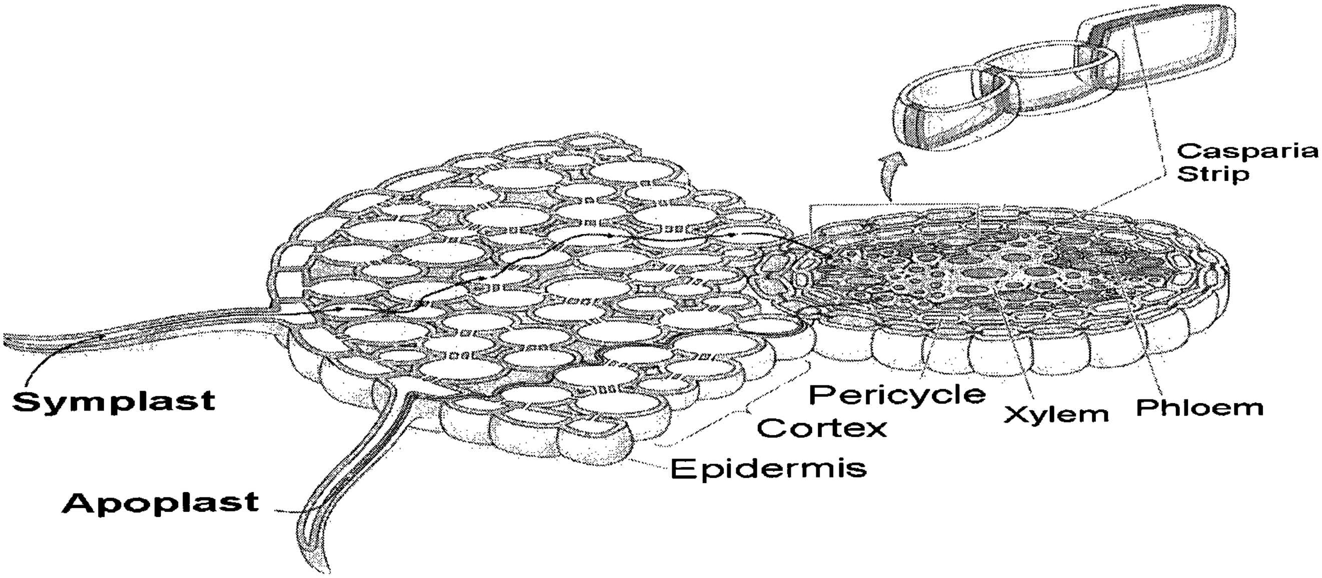


Fig. (2-1): A schematic representation of symplastic and apoplastic pathways in the root section (adapted from Taiz and Zeiger, 2002)

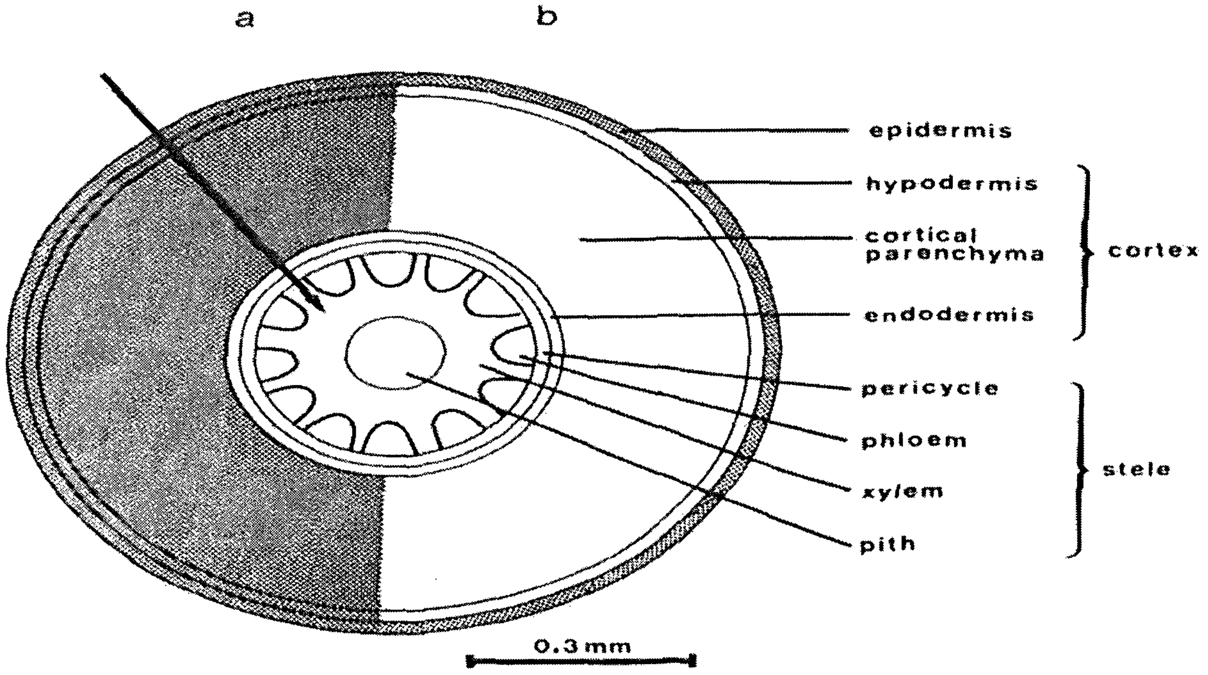


Fig. (2-2): Root section of corn plant, where hypodermis is considered as an exodermis if it has a casparian band of which is constituted of suberin. The arrow indicated the direction of water flow inside the root (Peterson 1988)

At the end of the ion pathway in the cortex, an apoplastically impermeable layer exists called the endodermis. This layer separates the cortex from the root stele. For an ion to pass to the stele, it has to pass by the symplastic route since the apoplastic one is interrupted with suberin exudates. The outer layer of the stele is the pericycle, which is responsible for the growth of secondary roots. This layer surrounds the vascular tissue of the plant which is composed of xylem and phloem. The xylem is responsible for translocating ions and water from roots to plant shoots; while the phloem transfers the plant metabolic products from shoot back to roots. These two tissues are separated by the cambium which is composed of meristemic cells.

In addition to plant root structure, it is essential to be acquainted with the microenvironment of the plant root zone to gain more comprehensive image about phytoremediation mechanisms in soil and water. This zone is called the rhoizosphere if the plant is present in the soil. A similar root effect is expected to occur in water except that ions and micro biota are more free to move. A brief description of some of the processes that occur in this zone will be discussed in the following section.

2.2 RHIZOSPHERE

Rhizosphere zone represents the soil section that surrounds the root by 2 mm distance away from its plane (Zoysa et al. 1997). Wang et al (2002) states that this zone has a different characteristics from the bulk soil since it is affected by plant root exudates which change its microenvironment. These exudates are composed mainly of dissolved organic carbon (DOC) which is dominated by low molecular weight organic carbons (LMWOCs). LMWOCs are comprised of organic acids, amides, amino acids and sacharides (Wenzel et al. 2001). Typical concentrations of organic acids in vegetated soil solution are less than 50 ηM. They are comprised of mono carboxylic acids in higher concentration than di or tri carboxylic ones. Examples of mono carboxylic acids are formic, propeion, butyric, lactic, and valeric acids. Examples of di and tri carboxylic acids are oxalic, malic, malonic, succinic and citric acids (Sandnes et al. 2005). Generally, the composition and concentration of exudated organic acids depend on plant species, method of sampling, and plant age (Sandnes et al. 2005). Therefore, due to the latter factor, root enhanced changes vary with time (Tao et al. 2003), but its effect is most pronounced in the first day (Wenzel et al. 2001). Besides the previous factors, nutrient deficiency can enhance root exudation (Fitz et al. 2003b). DOC are not only introduced in rhizosphere by plant roots, but they can also be supplied by ectomicorhizzal fungi (Sandnes et al. 2005). In addition to organics, roots can exudate other inorganic ions like bicarbonates (Tao et al. 2003) and phosphates (Dushenkov et al. 1995). Root effects on soil are not only demonstrated by increasing its DOC content, but can also be manifested by changing its pH (Fitz et al. 2003b , Tao et al. 2003) and by decreasing its redox potential (Fitz et al. 2003b).

Being acquainted with the interactions occurring in the rhizosphere that may affect the extractability of these metals, it is necessary for a more thorough understanding of the factors that may control phytoremediation efficiency. This extractability phenomenon of heavy metals is termed bioavailability which will be discussed in section 2.3.

2.3 BIOAVAILABILITY

Bioavailability represents the extent to which a chemical can be absorbed, or cross a living cell membrane (Batley et al. 2004; Thompson and Nathanail 2003). However, absolute bioavailability can hardly be measured; instead, relative bioavailability, or in other words, bioaccessibility can be used to substitute the latter term. Bioaccessibility stands for the fraction of a chemical that is attainable for intake by a living organism (Thompson and Nathanail 2003). In the following discussion, bioaccessibility will be referred to as bioavailability.

For an ion to be bioavailable for plant roots, it has to be released into the soluble and the exchangeable pools (Tao et al. 2003). Several parameters can affect heavy metal bioavailability; and these include redox potential (Fitz et al. 2003b, Wang et al. 2002, Zhang et al. 2004), DOC (Fitz et al. 2003b), pH (Fitz et al. 2003b, Zhang et al. 2004), soil texture (Fitz et al. 2003b, Zhang et al. 2004), microbial effects , stage of root growth and development, aging of the contaminant in soil (Zhang and Davison 2000), organic complex lability (Downard et al. 2003), presence or absence of another competing ion (Fitz et al. 2003b), and presence of the different solid phase pools like the organic matter (Fitz et al. 2003b, Zhang et al. 2004), and the oxides (Fitz et al. 2003b). On the other hand, bioavailability might not be a direct function of the total heavy metal concentration nor of its labile fraction, where labile metals corresponds to the fraction of metal that can be displaced by a neutral salt solution or by any other weak extractant (Fitz et al. 2003b).

Among the previously mentioned factors, soil acidity affects ion speciation and distribution among the variable soil fractions. For instance, under acidic conditions, ions can be released from carbonate fraction into the soluble one rendering it more bioavailbale (Tao et al. 2003). Moreover, microbial activity thrives in the rhizosphere, and this may affect the ion mobility or its bioavailability (Zoysa et al. 1997, Tao et al. 2003). Likewise, Bacteria and fungi may stimulate plant roots to increase their exudation (Sandnes et al. 2005) which can affect the ion's bioavailability too.

Altering the oxidation potential can change the ion valence, which may lead to the dissolution or formation of new soil components, and hence it may affect the distribution of ions among the variable soil pools. In other words, heavy metal lability can be affected by the oxidation state of the soil (Fitz et al. 2003b). For instance, oxidation of the soil microenvironment can release the ions attached to the organic matter into solution; alternatively, its reduction can release the ones which are attached to ferrous and manganese oxides. Hence, the factors that can affect this potential should be taken into account.

Several processes contribute to changing the redox potential of soil; for example, air drying can raise it, which may render the measured bioavailability non representative of the real case (Wang et al. 2002). In addition, redox potential can be affected by the application of electrokinetic gradient, which develops a reduced environment at the cathode and an oxidized one at the anode (Acar and Alshawabkeh 1993). Furthermore, plant roots can reduce the oxidation potential of soil by exudating biodegradable organic matter (Fitz et al. 2003b). Meanwhile, the value of soil potential depends on its acidity; hence, to compare two soil redox environments, the effective potential which is calculated at neutral pH can be used (Fitz et al. 2003b). If it is not possible to supply a reliable measurement of this potential, then it can be indicated by the presence or absence of specific ions; for instance, in a study, redox potential has been verified to be maintained, by ensuring that the nitrate, ferrous and manganese ions' concentrations have not been affected; indicating an aerobic condition due to the absence of denitrification process (Ernsberger et al. 2005).

Complex lability can be described as the degree of attachment between a metal and an organic ligand. The more the lability of the heavy metal from its organic complexes the more is its measured bioavailable fraction (Downard et al. 2003). Therefore, if a heavy metal is not specifically adsorbed to the organic compound, it will be more labile (Tusseau_Vuillemin et al. 2003). For instance, heavy metals adsorbed to fulvic acid are labile or partially labile (Downard et al. 2003); another example of labile complexes is the Cu-citrate compound. When ions are specifically adsorbed to organic ligands, as it may occur with EDTA, it will not be labile; however its hydrated radius may not be affected (Tusseau_Vuillemin et al. 2003). On the other hand, formation of this latter complex might enhance the absorption of a metal by some plants (Cunningham and David 1996) indicating that the measured bioavailability can be deviated from the actual one. Alternatively, this measured value can indicate the toxicity level of a heavy metal to plants.

Aging of a contaminant is the time during which it resides in the soil. This effect can lead to the formation of inert organic complexes, which renders the ions less labile (Zhang and Davison 2000). In addition, the more the aging of the metal contaminant, the more it will be partitioned into the less available soil fractions, even though it is not expected to be partitioned into the residual one (Tao et al. 2003). For instance, in a study, aging of spiked heavy metal on clays allowed it to transfer slowly into the organic and oxide fractions within 50 days (Daramarwan and Wada 2002).

Existence or absence of some specific ions can have an effect on the targeted ion bioavailability to plants. For instance, fertilizers can interfere with metal absorption by plant roots (Fitz et al. 2003b). On the other hand, the absence of some nutrients may affect root exudation (Tusseau_Vuillemin et al. 2003). Hence, it may be preferable to apply low quantity of fertilizers to ensure that plant growth is not affected by nutrient deficiency nor that ion uptake and release in soil will be disturbed by this factor (Song et al. 2004).

For better measurement of heavy metal bioavailability, freezing of a sample is not recommended to preserve it before the measurement since it can make the metals more soluble; in other words, it can transfer metals from different phases to the soluble one (Fitz et al. 2003a). In addition, freezing can lead to microbial cell ruptures and precipitation of some constituents in water (Batley et al. 2004). Hence, freezing will change the measured properties of the specimen.

Bioavailability of heavy metals can be measured by diffusive gradient in thin films tool (DGT). However, if the soil moisture content is less than 80% of soil field capacity, it may be more reliable to extract metals by common low molecular weight organic acids (LMWOCs). This latter method produced results that correlated with metal extraction by plants (Wang et al. 2003).

Heavy metal bioavailability is critical for better management of phytoremediation efficiency. Application of other processes while phytoremediation is taking place can modify the rhizosphere environment, which might affect the heavy metal bioavailability to plants. Electrokinetics is one of these techniques. This technique will be described in section 2.4.

2.4 ELECTROKINETICS

Application of an electrokinetic gradient within a soil or a liquid sample will result in the development of an electromagnetic field as well as in stimulating variable electrochemical reactions. This electromagnetic field can develop an alternate or a direct electrical current. This study is concerned with the effect of the alternate current on metal behaviour in liquid solutions. However, the implications of applying direct current in soil have been more studied since it is dominantly applied in remediation applications. These implications will be discussed briefly to better understand the effect of electrical current on liquid samples. Subsequently, a brief review of the electrochemical reactions that result from voltage application will be given. Finally, a brief comparison between the effects of applying a direct and an alternate current will be presented.

When a voltage gradient is applied in a soil sample, an electromagnetic field develops between the electrodes. This field may cause three phenomena. First, charged colloids may be displaced towards the electrode of their opposite charge in a process named electrophoresis. In general, this process is only significant when applied to slurries or when charged micelles are formed through the application of surfactants (Acar and Alshawabkeh 1993). Bacteria can move through this process in a wave pattern. However, their movement can be slow which may necessitate application of a surfactant

(Wick et al. 2004). Likewise, through electromigration each existing ion transfers towards the electrode that carries its opposite electrical charge (Cox 1996, Virkutyte et al. 2002). Finally, solvents can move through the soil pores towards the electrode which acquires a similar charge to the one developed on the soil mineral itself. This flow results from frictional forces with the moving charged layer attached to these soil minerals during an application of a voltage gradient. This latter phenomenon is called electroosmosis (Chilingar et al. 1997, Virkutyte et al. 2002). Since the current research focuses on the application of an electrical current in solutions containing dissolved metals, only electromigration will be discussed in this section.

During electromigration, several factors can influence the efficiency of the applied current in transporting the existing metals in the soil. For instance, this efficiency correlates with the relative concentration of the target ion to that of the other ions. Moreover, ions should be desorbed before being transported (Acar and Alshawabkeh 1993), which emphasize the importance of the used type of the enhancement solution. In addition to the previous factors, soil mineralogy and structure, conductivity of soil pore water, and the mobility of the ion itself, all play a role in its migration. Furthermore, moisture content affects the pore water conductivity and the path length of the migrating ion which renders it as an important controlling parameter (Virkutyte et al. 2002). Hence, it can be concluded that, in liquids, the factors that control electro migration efficiency are constituted of the target metal concentration and its mobility, in addition to the concentration of other ions as well as the electrical conductivity of the solution.

The ion's electromigration velocity correlates with its mobility as well as with the applied voltage gradient. Ion mobility depends on its charge and diffusion coefficient, in addition to solution temperature. An equation that is used to calculate ion mobility can be represented as follows (Acar et al. 1993)

$$U = \frac{D\,Z\,F}{RT} \qquad (2.1)$$

where, U = ionic mobility (m^2/Vs), D= diffusion coefficient (m^2/s), Z= ion charge
F= faraday constant (96485.3399 C/mol), R= gas constant (8.314472 J/ K mol) and
T= solution temperature (K)

Consequently, to predict ion velocity, the following equation can be used (page and page 2002):

$$V = UE \qquad (2.2)$$

Where, E= voltage gradient (V/m) and V= ion velocity (m/s)

Electrochemical reactions that occur while applying an electrical current should be considered to better understand its effect on solution chemistry. While applying an electrokinetic gradient, oxidation reaction occurs at the anode and reduction reaction occurs near the cathode. At anode, polarized solutions are decomposed; for instance, water is hydrolyzed into hydrogen ions and oxygen gas (Acar and Alshawabkeh 1993), and EDTA is transformed, after sequential removal of acetate group, into carbon dioxide, formaldehyde, and ethylene diamine (Allen and Chen 1993). Likewise, at the cathode, water is hydrolyzed into hydroxide ions and hydrogen gas. These produced hydroxide ions will move by diffusion, and by electro migration toward the anode, while the hydrogen ions will be transferred toward the cathode. However, hydrogen ions exhibit more ionic mobility than hydroxides and move in the same direction as electro-osmosis when present in soil; this develops an advancement of an acid front toward the cathode. By the time this front passes by the cathode, the secondary reactions that produce hydrogen gas form hydrogen ion reduction will dominate over the water hydrolysis process (Acar et al. 1993). Likewise, another secondary reaction that may occur, nearby the cathode too, is the reduction of the oxidation state of the existing metals (Yeung et al. 1997, Acar and Alshawabkeh 1993).

Even though direct current is dominantly used in soil electroremediation, it suffers from several limitations. First, precipitation of metal can occur near the cathode (Chilingar 1997) rendering it unaffected by the applied electromagnetic field. Generally, low solubility of the target metal, whether thermodynamically or kinetically, is detrimental to its migration (Acar and Alshawabkeh 1993, Virkutyte et al. 2002, Cox 1996). On the other extreme, near the anode, the soil can be subjected to a very low acidic condition which may dissolute metal precipitates excessively (Virkutyte et al. 2002) so that it can be transferred to other areas. Furthermore, during the voltage application, the existing electrodes will be covered by hydrogen and oxygen gas bubbles in addition to the precipitated ions (Virkutyte et al. 2002). These insulations will generally decrease the efficiency of the applied electromagnetic field. Moreover, if an enhancement solution is applied to desorb metals from soil, then its reaction by products should be monitored to verify that it will not pose any risk on the humans (Virkutyte et al. 2002). On the other hand, applying an alternate current is not expected to change the solution pH. However, since electrochemical reactions occur continuously in this process, applying an alternate current is expected to produce gas bubbles and to change the speciation of ions in the solution.

Studies that compared the effects of alternate current with that of direct current have been investigated when they are applied during bioremediation process. It has been established that alternate current provides more uniform injection and distribution of ions along the soil profile (Rabbi et al. 2000). Likewise, it maintains the soil pH and moisture content while accelerating the biodegradation of organic contaminants. However, an alternate current consumes more electrical energy than the direct one (Luo et al. 2005).

Understanding the limits and the opportunities of both phytoremediation and electrokinetics facilitate the analysis of the promises that may result from the hybrid combination of both of these processes. Section 2.6 will discuss this issue in more detail.

2.5 PHYTOELECTROREMEDIATION

Phytoelectroremediation refers to a process that makes use of plants while applying an electrokinetic gradient to diminish the bioavailable concentration of a metal contaminant in soil. During the application of this process, phytoremediation and electroremediation may show various synergetic mechanisms since each will modify the rhizosphere environment for the other. In addition, both may affect another unknown factor which may play a role in the remediation process.

Plants exhibit its effect on the fate of the contaminants in the rhizosphere through its root exudates. For instance, these exudates can form a chelating complex with metals enhancing their mobility in the rhizosphere. On the other hand, some root exudates may render some pollutants insoluble. Likewise, direct electrical current (DC) may dissolute clay minerals transforming it to amorphous ones by extracting its interlayer water out (Chilingar et al. 1997), which will affect heavy metal distribution among soil phases and hence will affect its uptake by plants. Brayman and Miller (1986) described root behavior by the following statement: "When exposed to an electric field, the root acts as an open structure with dielectric characteristics similar to a suspension of isolated cells". In their study, they showed that root growth can be enhanced by the application of a voltage gradient up to a threshold value using an alternate current within a specific frequency range.

Microbiological factors could be affected by any of these remediation technologies which may alter the expected results. For instance, plant roots provide nutrients to bacteria through its root exudates rendering the microorganisms in rhizosphere more versatile and intense than the ones in the bulk soil. Microorganisms can degrade the organic matter in the rhizosphere, and that can enhance the micorhizal formation. This can supply more nutrients to plants and enhance their growth. Likewise, applying an electrokinetic gradient can affect the distribution of microorganisms in the rhizosphere. For instance, Elektorowicz et al. (1999) showed that a direct current can mobilize biosurfactant forming bacteria in low permeable soils. However, in another study related to the activity of the microbes to degrade PAH (Hernesmaa et al. 2005), the current did not have a synergetic effect on microbiological processes.

Applying fertilizers can interfere with the above remediation methods. During phytoremediation of mined lands, fertilizers are necessary for the survival of plants even though it may increase the plant tissue mass without increasing its heavy metal phytoextraction (Xia 2004). In some cases, fertilizers may affect the phytoavailability or the mobility of a certain metal. For instance, application of a relatively large amount of phosphates or sulfates may precipitate these metals (Acar and Alshawabkeh 1993, Kumar et al. 1995) rendering them to be less subjected to decontamination by phytoextraction or by electro-migration. To better understand the gap in the knowledge of the processes that may occur while using the hybrid combination of these phytoremediation and electroremediation, a few case studies will be discussed in the next chapter.

CHAPTER THREE
REVIEW OF SELECTED STUDIES

Previous studies have concluded that combining bioremediation with electroremediation may remove contaminants more efficiently than using each of these technologies separately if other factors did not interfere with any of these processes. For instance, Luo et al. (2005) indicated that a small polarity reversing during electro-bioremediation can induce higher and more uniform phenol removal but with higher energy consumption. However, Kim et al. (2005) indicated that an increase of soil temperature to 45° C due to electrical current may inhibit bacterial activity. Another synergetic effect between bioremediation and electroremediation has been investigated by Mani et al. (2000). In their experiment, sulphur oxidizing bacteria was used to enhance the efficiency of electroremediation process through solubilising Cu^{2+} ions. Likewise, electrokinetic gradient improved the soil microenvironment for these bacteria by eliminating the inhibitory factors for their growth.

Other studies investigated the potential benefit of using electro-kinetics in enhancing plant growth conditions. One of these studies has been conducted by Lamont-Black et al. (2003) who utilized a direct current (DC) in managing grass growth. This management has been facilitated by reversing the electrode potential making it easier to control rhizosphere pH, water content, and oxygenation of plant roots. It has been found that electrokinetic flow may enhance root growth and reduce the stress on plant that is attributed to low lighting. The latter has been inferred by noting the increase of plant chlorophyll content after being subjected to a voltage gradient of (0.24 V/cm).

The direct effect of alternate current (AC) on plant growth has been studied by Stenz et al. (1998). In their study, an alternate current was applied to an illuminated garden Cress plant (Lipidium Sativum L.) roots while being present in aqueous solution at a voltage gradient of 0.1V/cm, and frequencies of 0.1, 1, 10, 100, 1000, and 10000 Hz. In this experiment, the frequency of (10 Hz) was the one which enhanced plant growth the better. Later, using the same latter frequency, they applied weak voltage gradients of 0.0001, 0.001, 0.01, 0.1, 1 V/cm for one day. It was found that maximum growth rate occurs between 0.001 and 0.1 V/cm. The authors also found that, under an illuminated condition, the maximum growth rate occurred at a voltage range between 10^{-3} and 10^{-1} V/cm and at a field frequency of 10 Hz. These results have been attributed to the probable stimulation of H-ATPase in the plasma membrane of the root cells. The authors concluded that a weak voltage gradient of 1V/cm or less is expected to enhance plant growth. However in another experiment done by Brayman et al. (1986), an application of an AC gradient of 3.5 V/cm at a frequency of 60 Hz decreased the root growth rate of Cucumis Sativus at the segment located 4 to 8 mm from root tips. The affected segment of this root was increased to a distance of 2 to 8 mm from root tips, when a voltage gradient of 4.5 V/cm was applied. Furthermore, applying a gradient of 2.25V/cm on Cucurbita Maxima decreased the growth rate of root fragment located at 4 to10 mm from root tips. A gradient of 3.5 V/cm increased the retarded section to 2 to10 mm from these tips.

Takamura (2006) investigated the effect of imposing AC on the growth rate of two plants, *Vigna Mungo*(L.) , a dicotyledon, and *Zea Mays*(L.), a monocotyledon, utilizing various frequencies and voltage gradients. The author found that an electro-kinetic gradient can enhance root growth only if the plants are subjected to sufficient daylight. Moreover, he found that the availability of nutrients is necessary while applying an alternate current to better enhance root growth rate. After subjecting the plants to 75% daylight, and after applying the square wave mode, it was revealed that the optimum voltage gradient and frequency in stimulating plant growth was about 0.18V/cm and 30Hz for Vigna Mungo as well as 0.29V/cm and 20Hz for Zea Mays. Moreover, Takamura found that square wave mode is more efficient in stimulating root growth than both the sinusoidal wave and the DC mode.

Goldsworthy (2006) suggested a hypothesis that may explain the existence of optimal voltage and frequency values at which an AC may stimulate plant growth. This hypothesis was able to explain many of the results generated in previous research. Goldsworthy indicated that Ca^{+2} ions decrease the permeability of plasma membrane, while K^{+}, which are abundant in the intracellular plant compartments, increase it. Increasing the permeability will allow more Ca^{+2} ions to penetrate into cytoplasm. These penetrating Ca^{+2} ions will interfere with gene transcription enhancing plant growth. Hence, to increase plant growth, Ca^{+2} ions that link plasma membrane ligands should be replaced by K^{+}. In order to replace Ca^{+2}, sufficient voltage gradient should be applied so that it can mobilize them as specifically as possible without mobilizing the rest ions which have lower mobility. Mobilizing Ca^{+2} ions will allow the less mobilized K^{+} to replace it on the cell membrane. If the applied voltage gradient is more than the optimal range, it may induce two possible implications. First, this high gradient may dissociate all the ions from plasma membrane, and return them back in the same initial fraction from which it was dissociated. In other words, since the ions which are associated with plasma-membrane have not been altered, its permeability will not be enhanced. Moreover, even if Ca^{+2} is replaced by K^+, increasing plasma membrane permeability excessively will lead to augmenting the Ca^{+2} concentration in cytoplasm to a degree that is perceived by plants as an indication of stress, which may eventually cause plants to close plasmodesmata and hence preventing the action potential from being propagated to other cells. Goldsworthy (2006) indicated that low frequency is necessary to allow sufficient time for Ca^{+2} to be replaced by K^+. Likewise, square wave mode is anticipated to release Ca^{+2} from plasma membrane for sufficient time that allows them to be replaced by K^+ while they are away from the membrane. Moreover, if a frequency equal to the cyclotron resonance of K^+ is applied, which is 16Hz, it will increase K^+ activity thus enhancing the plasma membrane permeability and eventually stimulating root growth. Meanwhile, at a frequency of 32 Hz, the cyclotron resonance of Ca^{2+}, is expected to decrease plasma membrane permeability.

Electrokinetics is not only utilized in enhancing plant growth, but it has also been explored as a potential enhancing agent of phytoremediation. O'Connor et al. (2003) studied decontamination of two soils, containing copper (Cu^{+2}) and cadmium (Cd^{+2}) using DC current and the plant Perennial ryegrass (Lollium Perenne cv Elka). The former metal was not accompanied by other metals while the latter was mixed with Arsenic. In their experiment, DC has been applied with a voltage gradient of about 1.67 V/cm under an illuminated condition while the temperature was varied between 18-25°C. The authors established a correlation between Cu^{2+} concentration in soil and its phytoextracted amount. Even though plants were able to extract Cd^{2+}, the phytoextracted amount was not correlated with its concentration in soil. O'Connor et al. (2003) showed that phytoremediation can proceed during the application of a voltage gradient. It has been concluded that electrical current shows no adverse direct effect on plant. However, it is its indirect effect on pH and on heavy metal concentration that affects plant growth.

Likewise, this technology has been considered in two patents. The first has been authored by Raskin et al. (1998) who suggested the use of direct current to increase metal mobility thereby increasing its availability to the plant Brassica. The authors proposed applying phosphorous fertilizers through plant leaves to avoid metal precipitation in the vicinity of the roots. However, Hodko et al. (1998) criticized this approach since soil pH can be rendered too low to support plant growth. Alternatively, they suggested changing the electrode's polarity each hour during the direct current implementation. In a test conducted on mustard plant grown over lead contaminated soil, it has been demonstrated that applying a direct current of 0.5 A for a period of 12 days while reversing the electrode polarity prevented high acidity build up near the anode. Moreover, in this experiment, it has been shown that plant phytoextraction of lead has been enhanced. Meanwhile, reversing the electrode polarity was as efficient as adding chemicals in ameliorating soil pH condition. Furthermore, in another test, it has been shown that applying a direct electrokinetic gradient of 150 mA for forty days on soil planted with Brassica Juncea (L.) Czern plant (Florida broadleaf Indian mustard) at initial soil pH values of 4, 6 and 7.5, while being illuminated for a period of 16 hours per day enhanced the phytoextraction of lead from this soil . In this experiment, light was supplied through combined florescent/ incandescent (320 watt/180 watt) or through 1000 W halide lamp. The authors implied that the electrokinetic effect supplied more ions to plant roots increasing their availability. Another aspect of this patent is the proper arrangement of the electrodes for the purpose of transferring the contaminants from deep soil horizons into the root zone. Likewise, it has been suggested to use electrokinetic phenomena in mobilizing metals from soil that have phytotoxic concentration of metals by growing plants on a newly laid layer of soil on top of contaminated soil.

To differentiate between the effect of AC and DC on phyto-extraction of metal contaminants, Bi et al. (2007) applied these currents for 60 days on contaminated soil planted with potato in an illuminated experiment, while comparing the results with the ones generated with no current application. The current in this experiment was constant at an intensity of 500 mA, while AC frequency was 50 Hz. The results showed that the electrokinetic gradient increased water as well as the chlorophyll content of plant leaves. Moreover, the alternate current increased plant biomass from 1 Kg to 1.8 Kg, whereas DC decreased it to 0.75Kg. Likewise, AC enhanced plant extraction of the metals (Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}). On the other hand, DC enhanced only a little the plant extraction of Zn^{2+} and Cu^{2+} metals. It decreased its extraction of Pb^{2+} and Cd^{2+} ones. The authors stated that the negative effects of the direct current on plant uptake of Cd^{2+} and Pb^{2+} could be attributed to the effect of the low soil pH near the anode, which was 3.8, on plant growth.

Understanding the factors that affect the bioavailability of metals to plants is also important to gain more insight into this hybrid technology. A study conducted by Siegel et al. (2003) demonstrated the multitude of variables that may affect root exudates' concentration and hence ion bioavailability to plants. These variables include moisture content, nutrient concentration, oxygen supply, temperature, microbial degradation and root density. A simple mass balance has been used to predict the distribution of Cs^+ among three pools in the rhizosphere which are the soluble, the bound, and the phytoextracted fractions. In this study, a simple empirical adsorption model was used to aid in the estimation of the adsorbed phase.

To better understand the effect of the environmental factors on heavy metal accumulation and fractionation in plant root fragments, like an electrokinetic gradient, it is essential to comprehend the mechanisms through which these metals are absorbed. These mechanisms are composed of passive and active transport. A study conducted to distinguish between these two fractions has been conducted by Fritioff and Greger (2007). Based on their review, the authors hypothesized that apoplast accumulate ions passively, while symplast may accumulate it via active and passive modes. The authors tested the presence of active uptake by comparing Cd^{2+} accumulation in live plant roots with that in metabolically inhibited roots as well as in dead roots. It has been indicated that the active uptake can be obtained by subtracting the uptake in living plants from that in dead ones. These dead plants were obtained by drying live roots at 40°C overnight. It was found that Cd^{2+} uptake in Elodea Canadensis plant was mostly passive, since the accumulated metal in live plants was neither higher than that in metabolically inhibited plants nor in dead ones. Radiography was used to check the distribution of Cd^{2+} in plant sections. The similarity of Cd^{2+} distribution in both dead and living plants was explained as an indication of passive traslocation occurring through the apoplast rather than an active one through the symplast. Likewise, the even distribution of Cd^{2+} in the plant sections suggests its translocation through the apoplast rather than the symplast.

In another study, Mitani and Ma (2005) conducted an experiment on rice cucumber and tomato plants and found that their uptake of Si^{4+} in cortical cells utilized both passive and active transport. Meanwhile, it is suggested that silicon is transferred to the xylem by active transport in rice, but by passive transport in both cucumber and tomato. The authors maintained that apoplastic uptake of Si is done through passive transport while symplastic uptake is achieved by both active and passive transport in all of the tested plants. In their experiment, passive uptake has been perceived by the investigated similarity between Si^{4+} in bulk solution and in plant root fragments. Likewise, active transport is inferred by the presence of higher concentration of Si^{4+} in root's symplast than in bulk solution. However, active transport was diminished by either subjecting the roots to low temperature of 4°C, or by mixing it with a metabolic inhibitor. Under these conditions, Si^{4+} concentration in symplast was similar to that in apoplast as well as in bulk solution leading to the conclusion that part of the accumulated metal in the symplast has to be transported passively.

To gain a more comprehensive image about the processes that occur in the vicinity of plant roots while applying a voltage gradient, charge distribution around these roots should be taken into consideration. A study that employed this characteristic to better elucidate the factors affecting root absorption of ions has been conducted by Kinraide (2003). In this study, the author investigated the electrostatic effect of the cations: Ca^{2+}, Mg^{2+}, Sr^{2+}, and H^+ on plant root uptake of SeO_4^{2-}anion. During his experiment, the author inferred that ion toxicity caused decreasing root growth. The electrostatic effect of the root surface charge on the ions in solution has been calculated using prepared software. The results of this experiment showed that adding a cation like Ca^{2+} can increase the toxicity effect of the anion SeO_4^{2-} as demonstrated by the negative correlation between root growth and the cation concentration. However, due to the fact that H^+ itself is a toxicant, the relation between decreasing the solution pH and the toxicity effect of SeO_4^{2-} on root length was less clear. The author suggested that either the co-transportation of the anion with H^+ across the cell membrane or the active uptake of SeO_4^{2-} in roots may play a role in increasing the absorbed mass of SeO_4^{2-} in plant as when compared to the case where only passive uptake is the dominant process.

A parameter that can indicate electrical potential distribution as well as the surface charge of these roots is the zeta potential. This parameter represents the electrical potential of the root at the boundary between the stern layer and the diffuse double layer. A study that incorporated this parameter to better understand plant processes has been concluded by Ahn et al. (2001). In this study, the effect of Al^{3+} ions on root growth as well as on the zeta potential and on H_ATPase enzyme activity has been investigated. During their experiment, the roots were subjected to variable concentrations of Al^{3+} up to 100μM for a period of 24 hours. The authors found that increasing Al^{3+} concentration would decrease root growth, specially at the fragment located at 2 to 4 mm from the root tip. Likewise, it was found that this latter fragment accumulated the maximum amount of Al^{3+} ions. In another set of experiments, plasma membrane vesicles of roots subjected to the above mentioned Al^{3+} solutions not only showed a decrease in the negativity of zeta potential, but also it manifested a reduction in H_ATPase activity. This trend was more obvious at the root fragment located at distance of 0-5 mm from the root tip. The authors concluded that Al^{3+} would cause irreversible changes to plasma membrane of roots due to its strong binding which decrease the membrane's capacity to adsorb more ions. However, in other tests, the authors observed that Ca^{2+} has less effect on zeta potential when compared to Al^{3+} ion. The former ion decreased zeta potential only by 10%.

It can be noticed that a few aspects such as the effect of dark environment and the effect of AC with illumination on uptake of metals under hydroponic conditions has not been studied well in the past. These aspects will be investigated in the present study. The proposed methodology for the present study will be discussed in Chapter Four.

CHAPTER FOUR
EXPERIMENTAL METHODOLOGY

4.1 SUMMARY

For phytoremediation using the hydroponic system, the tomato plant (*Solanum lycopersicum),* was selected for tests in the greenhouse. This plant has been used in the past for such studies (Bao and Sun 2008). The tomato variety used in this study is called *Tiny Tim*. Several sets of experiments were conducted by changing the plant environment to gain more insight into phytoremediation mechanisms. In the first set of experiments (Fig. 4-1a), the effect of nutrients (N, P and K) on phytoextraction of zinc by roots and shoots was studied. Following this, zinc (zinc sulphate) was added in the absence of other nutrients to avoid any interference effect. During the tests, plants were subjected to well illuminated and dark environments separately. Some of the illuminated plants were also subjected to an alternate current to explore if this could modify the phytoremediation process.

In a separate series of tests, in the absence of plants (Fig. 4-1b), the effects of alternate current on the hydroponic solution was studied. The change in the system parameters such as temperature, electrical conductivity (EC) and pH were noted in these test series for one voltage gradient (1V/cm).

The hydroponic conditions developed were adequate to support healthy plant growth. Moreover, concentration of zinc in plants was high enough to be measured by the available atomic absorption analyser. The next section will describe the experimental set up used to conduct this study.

4.2 EXPERIMENTAL SETUP

4.2.1 Plant preparation

Tomato plants (*Solanum lycopersicum)* have a well established root system as well as a high zinc hyperaccumulation capacity. The plant seeds were placed in nursery trays filled with peat moss. Each tray was irrigated with water and placed in a relatively dark laboratory for a period that varies between one week and two weeks till the seeds germinated. Subsequently, these trays were transferred into the greenhouse, where they were irrigated daily till the plant length reached about three inches. Later, these plants were transferred into bigger peat moss pots inside the greenhouse and irrigated daily. A drain hole at the bottom of the pot allowed water to drain off after the plant was watered. After four weeks, five plants were separated from the peat moss by gently flushing their roots with a gush of tap water. Immediately, these plants were transferred into water bottles (Fig. 4-1a) to form the hydroponic system.

4.2.2 Elements of hydroponics

This study utilized hydroponic system rather than soil to investigate the genuine plant interaction with changing its environment while eliminating confounding soil factors. Hydroponics implies "growing plants in a medium other than soil using mixtures of the essential plant nutrient elements dissolved in water" (Harris 1992). A schematic representation of a hydroponic arrangement used in this study is shown in Fig. 4-1a.

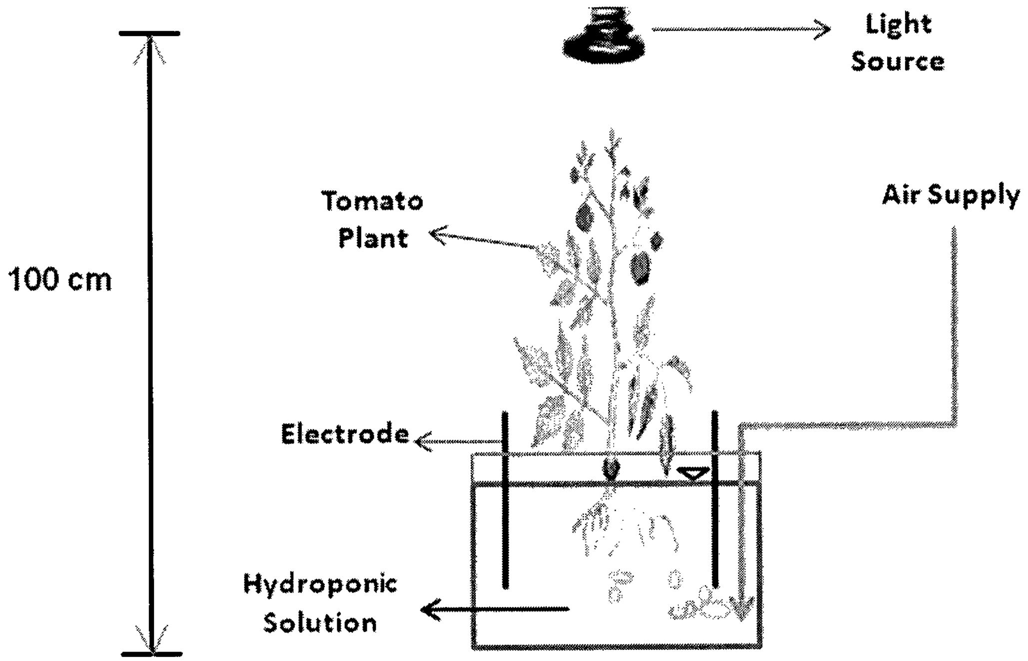


Fig. (4-1a): Hydroponic system (Adapted from "Figure 1-1" , by G.N. Agrios, 1997, *Plant Pathology*, p.5. copyright 1997, 1988, 1978, 1969 by Academic Press, and " Tomato Disease Identification Key By Affected Pant Part: Stem and Whole Plant Symptoms " by Cornell University Department of Plant Pathology, n.d., *Vegetable M. D. Online*).
Typical depth of water in the jar = 15 cms. Distance between electrodes= 10 cms.

Oxygen was supplied to the roots of the plants by pumping air into the water tanks. The duration of each experiment was seven days.

For test with nutrients, macronutrients were supplied to the system by adding 50 mg of $(NH_4)_2SO_4$ and 15mg of KH_2PO_4 per one litre. These quantities have been added so that the molar ratio of nitrogen (N) to potassium (K_2O) to phosphorous (P_2O_5) would be approximately 20:10:10. These fertilizers were added just in the beginning of the experiment.

During the experiments, plants were grown in tap water containing 0, 100, 200, and 500 mg/L of zinc in the form of $ZnSO_4.7H_2O$. One of the transparent reactors (plastic test bottles) that acquired 100mg/L of total zinc was placed in a dark box. Plastic is transparent and electrically insulated material. Transparency is important to facilitate monitoring plant roots. Another bottle containing the same zinc concentration was subjected to an alternate current for which the voltage gradient was 1V/cm and the frequency was 16HZ. This tank was placed under a yellow growing light (Fig. 4-1a). The aeration tubes and thermometer electrodes were placed inside each reactor. The reactors were 15 cm wide with an approximate initial solution depth of about 15cm. One plant was placed in each reactor and was supported by a fixed styrofoam layer on the plastic cover to reduce leaks. Styrofoam is a flexible material and it will not cause any injury to plant parts. Moreover, it has been illustrated that Styrofoam can keep the plants in a vertical direction. Carbon electrodes were placed on the edge of the plastic covers while keeping the distance separating them constant in each reactor (bottle) to maintain the same voltage gradient during the experiments which was 1V/cm. Meanwhile, the electrical current in the hydroponic solution was 39mamp in the absence of nutrients.

An estimate of the water used towards the plant evapotranspiration and the increase of the plant biomass was obtained by noting the difference in hydroponic solution weight before and after the experiment. This is the total hydroponic solution uptake (THSU).

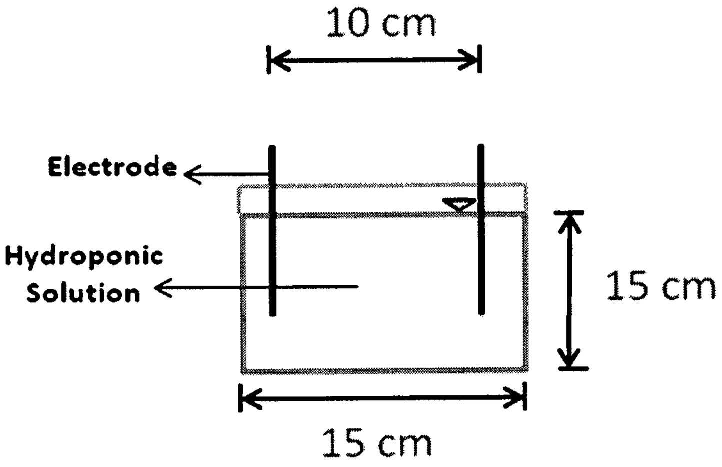


Fig. (4-1b) Hydroponic system without plants

4.3 METHOD OF ANALYSIS

4.3.1 Analysis for zinc concentration in the plant

After seven growing days, plants were washed, divided into root, shoot and fruit and then air dried for one day. Subsequently, shoots and roots were cut into small segments and transferred with fruits into an oven that maintained the temperature at 105^0C. The fruits were dried for two days, while the rest of the plant parts were dried just for one day. Afterwards, plant parts were minced using a grinder and placed separately in labelled and sealed clean glass containers. Subsequently, 0.500 grams of each sample was placed in an aluminum dish which was heated in the furnace at 550°C for about four hours. The samples were cooled and later placed in a plastic tube. 15 ml of 1M HCL was added to these tubes to digest the samples. After placing the tubes on the shaker for half an hour, they were kept in the refrigerator at 4^0C. Finally, all samples were analyzed using the atomic absorption analyzer. Atomic absorption analyzer was calibrated for zinc concentrations of 1, 3, and 6mg/L. This requires dilution of most analyzed samples so that their concentrations would stay between 1 and 6mg/L. The next chapter will discuss the results achieved.

CHAPETER FIVE
RESULTS AND DISCUSSION

5.1 GENERAL

The effects on metal uptake, acidity, and electrical conductivity of hydroponic solutions caused by changing the environmental conditions are discussed in this chapter. The environmental changes include subjecting the plant to darkness and illumination with and without AC current.

It was noticed that plants remain green, when grown in tap water and in solutions containing 100mg/L of Zinc nutrient. However, even a concentration of 200mg/L caused mild toxicity, since some plant leaves acquired yellow color. A concentration of 500mg/L was more toxic and caused more leaves to turn yellow. This trend explains the reason for using the concentration of 100 mg/L in most tests. This is especially true for plants grown in the dark and the others which were subjected to electrical gradient. This will eliminate toxicity effect from interfering with the results.

Some plants were subjected to different nutrients while some others were subjected to solutions of different zinc concentrations. The plants grown in zinc solutions were subdivided into two groups, group1 and group2. Group 1 results relate to preliminary experiments where mainly the effect of light on biomass zinc accumulation was investigated. In group 2 studies, the effects of light, darkness and AC voltage under conditions of illumination were studied. Zinc concentration in the shoots and roots were determined for the various conditions of the tests. Generally, the final pH and electrical conductivity (EC) data was determined and then reported for the tests conducted. Finally, evapotranspiration and translocation efficiencies will be discussed briefly within the last section. During the experiments the temperature of the hydroponic solution varied between 19 to 25 °C. Illumination period was 12 hours per day using lamps of 240 Watt.

5.2 EFFECTS OF VARIOUS CONDITIONS ON THE HYDROPONIC SOLUTION

5.2.1 Effect of AC on hydroponic solutions without plants

A separate set of experiments were conducted to investigate the validity of the results generated while applying an alternate current. Solution temperature, electrical conductivity and acidity were of concern. To understand these factors, tap water was mixed with zinc sulfate in four reactors so that two of them had a concentration of 100mg/L and the other two contained 200mg/L of zinc. The results of these experiments are shown in table 5-1. In this table, numbers following E denote zinc concentration. The results indicate that the effect of alternate current on these factors were not significant.

Table 5-1: Temperature, EC, pH and Zn concentration of solutions before and after application of AC voltage gradient
Conditions: E = Sample subject to AC voltage gradient for two days

Initial Zn concentration (mg/L)	EC(µS/cm)	pH	Temperature (C)	Soluble Zn concentration (mg/L)
100	549	7.24	21.7	75
E-100	576	7.24	22.2	75
200	831	7.13	21.6	190
E-200	821	7.05	22.2	190
Tap Water	288	7.66	21.7	-

5.2.2 The effect of test conditions on solution pH and electrical conductivity

For individual tests, Fig. 5-1 represents the variation of the final pH values of the hydroponic solutions for plants of group 2 under conditions of darkness (D), illumination (L) and AC current (L-E), for various total initial zinc concentrations. Fig. 5-2 shows the average experimental results for the final pH values for the same experiments. However, the pH variation when zinc is mixed with other nutrients (K, P and N) in the hydroponic system is shown in Fig. 5-3. In these Figures, control 1 represents tests with tap water, control 2 represents tap water with 100mg/L zinc concentration, and control 3 refers to tap water with 200mg/L of zinc.

Fig. 5-2a for control 1 denoting the system without nutrients, indicated a pH value of 7.66. When plants are grown in the hydroponic system with no zinc (Fig. 5-2b&c), the pH values varied between 8.08 and 8.29. This type of increase in pH caused by plant exudates have been recorded by Tao et al 2003 in their studies related to Cu extraction by maize. However, for the hydroponic system with nutrient and no zinc (Fig. 5-3 b) the pH value was 7.7 . For similar reasons, this value of pH in Fig. 5-3b was also higher than 7.46 (Fig. 5-3a) attributed to control 1 (case with nutrient and without zinc). Comparing the average pH values in Fig. 5-2 b and c indicates that, on the average, hydroponic solution pH was slightly more basic when the plants were illuminated. This indicates, for the system with no zinc, that there was more root exudates caused possibly by higher activity.

A solution containing a 100mg/L of zinc (Fig. 5-2 e, f) appears to stimulate the plant to change its root environment to become slightly more acidic than control 2 (Fig. 5-2 d). Possibly plants generate more hydrogen ions that might result from the active uptake of zinc. Even for a zinc concentration of 200 mg/L (Fig. 5-2 i, j), pH of the solution was less than that for control 3 (Fig. 5-2 h), indicating that plant active uptake might still exist under this concentration, although at this level of zinc, some leaves have turned yellow.

The application of AC under illuminated conditions (Fig. 5-2 g) appears to generate higher pH than in the other systems (Fig. 5-2 e, and f) subjected to the same initial total zinc concentration. It will be noted later that AC environment reduced zinc uptake (plant activity), this might indicate also the reduction in the increase of plant exudates.

When zinc is added to solution, the average pH (Figs 5-2 e, f, i and j) was slightly lower under illuminated conditions compared to dark conditions. This can be traced to the higher plant activity under illuminated conditions. Possibly, plants generate more hydrogen ion that might result from the active uptake of zinc. In Figs 5-2b, c, f, e, i and j, the pH of illuminated condition show more variability (standard deviation) than their counterparts in the dark environment. This further confirms that higher variability can be traced to more uptake in the active mode.

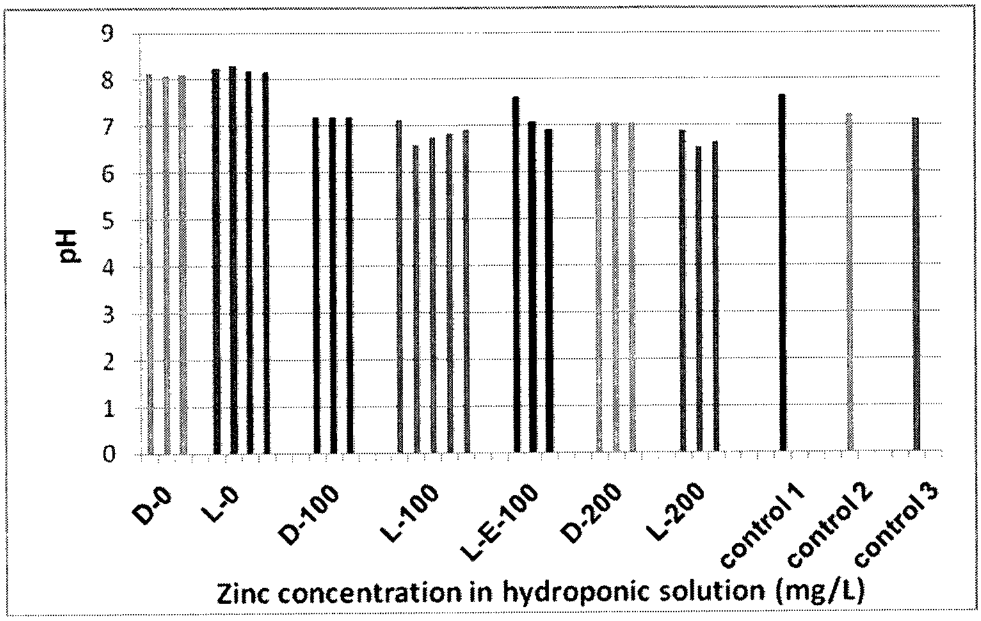


Fig. (5-1) Individual test pH values without nutrients;
D –dark , L- illumination and E- electricity, numbers represent initial zinc concentrations (mg/L), (group2).

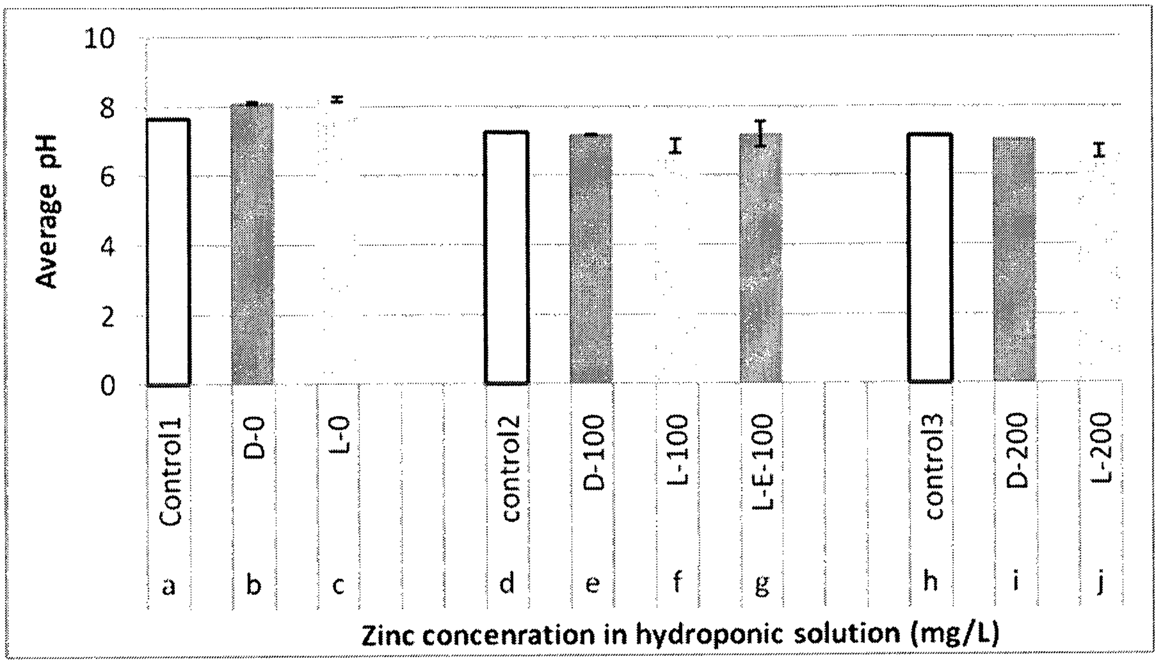


Fig. (5-2) Average tests' pH values in hydroponic systems without nutrients; D - dark, L - illumination, and E- electricity, numbers represent initial zinc concentrations (mg/L), (group 2).

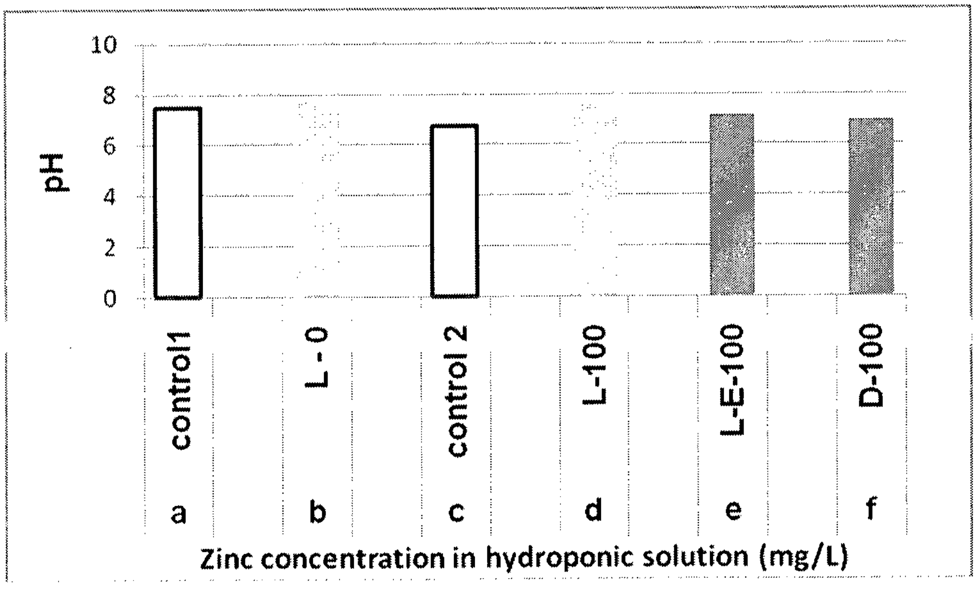


Fig. (5-3) Individual Test pH values in systems with nutrients; a) control 1 with nutrients b) plants with nutrients (no zinc) c) control2 with nutrients and Zn (no plants) d) plants with illumination, nutrients and zn e) plants with AC, illumination, nutrients and Zn f) plants in darkness and with nutrients and zinc.

Fig. 5-5 shows that the solution electrical conductivity (EC) showed a higher standard deviation under conditions of light than in the dark. This is logical, since plants are more active in the presence of light allowing variations caused by individual plants to be reflected in the conductivity results. Hydroponic solutions in which zinc was not added (Fig. 5-5 b, c) have a higher EC than the solution in the control 1 (Fig. 5-5 a) indicating that plants have exudated chemicals that change the solution properties. Likewise, at 100 mg/L zinc concentration, the average solution EC for the plants grown hydroponically (Fig. 5-5 e, f, g) was higher than that for the case of control 2 (zinc with no plant) where EC was 549μS/cm (Fig. 5-5 d). This increase in EC was less in comparison to the previous case when zinc has not been added to the solution. This less increase in EC might be due to zinc absorption by plant roots which decreases soluble zinc concentration in comparison to control 1. The same trend occurred at zinc concentration of 200mg/L. This might be due to the same reasons previously mentioned. As noticed later, zinc absorption in the illuminated condition was higher than that in the dark. From this, one can say that EC of hydroponic grown in the dark should be higher than that of hydroponic grown under the light. However, EC value was higher in illuminated conditions (Fig. 5-5 c, j) than in the dark (Fig. 5-5 b, i) at concentrations of 0 and 200mg/L respectively. This might indicate that the plants were induced to generate more root exudates in the light than under the dark at these concentrations. Meanwhile, pH measurements indicate that these exudates might be hydrogen ions.

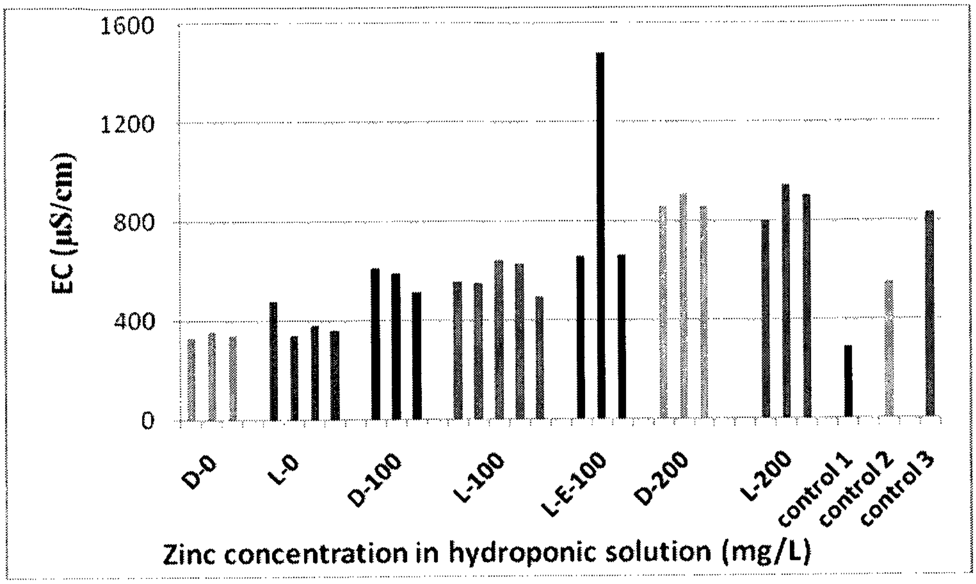


Fig. (5-4) Individual tests' EC values in hydroponic systems without nutrients; D- dark, L- illumination, and E- electricity, numbers represent initial zinc concentrations (mg/L), (group2).

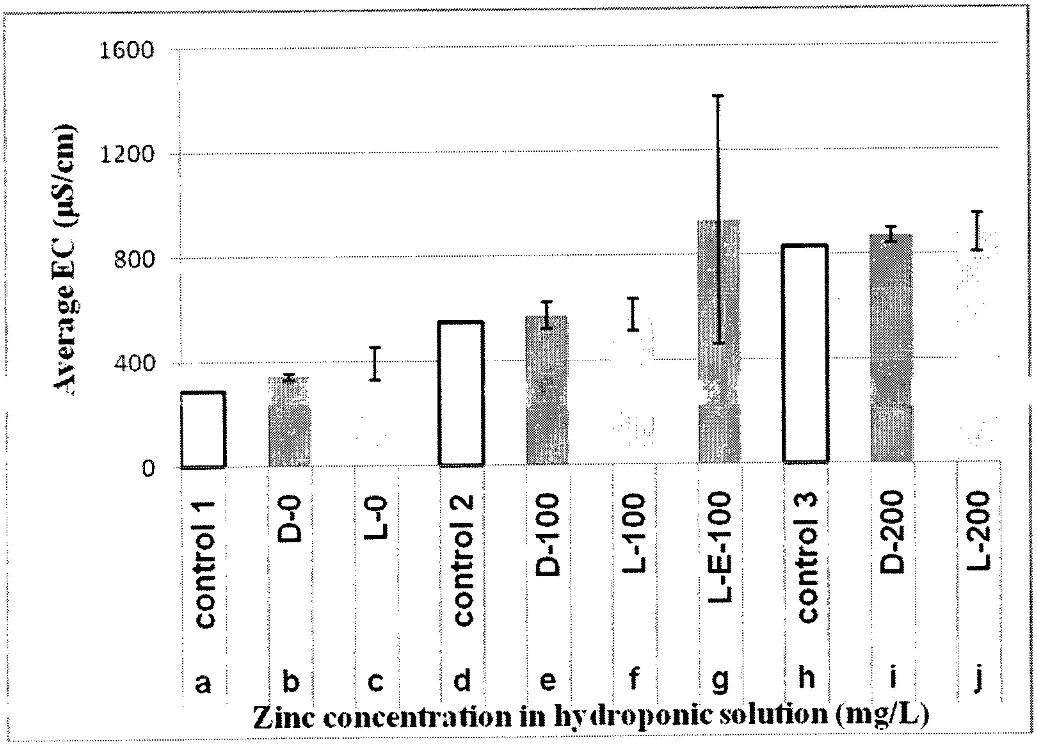


Fig.(5-5) Average tests' EC values in hydroponic systems without nutrients ; D- dark, L- illumination, and E- electricity, numbers represent initial zinc concentrations (mg/L),(group 2).

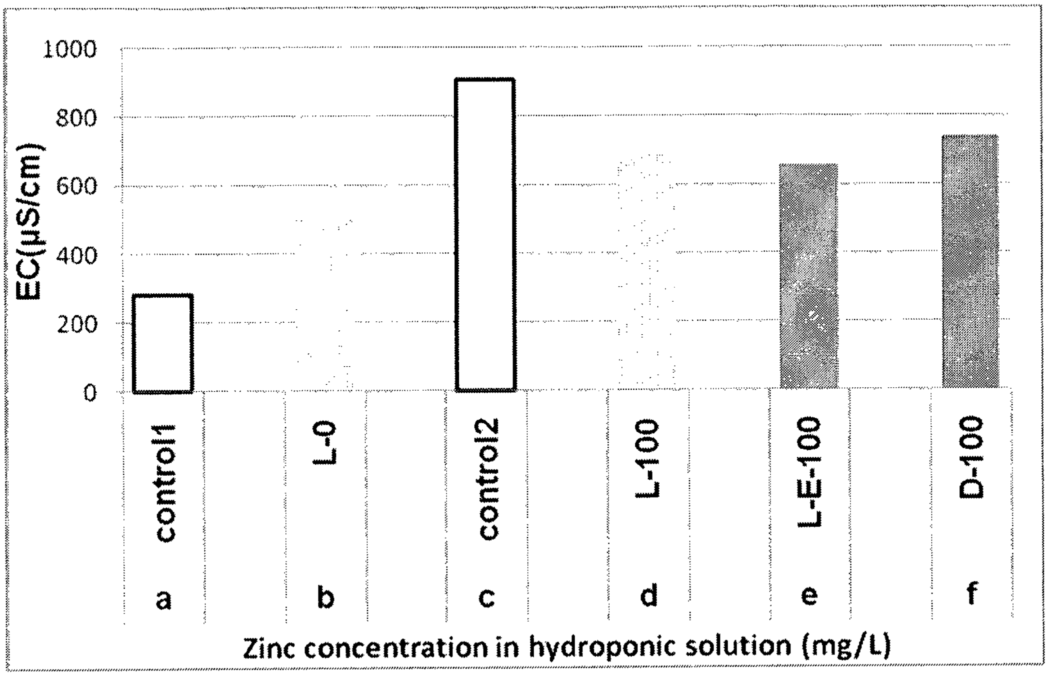


Fig. (5-6) Individual tests' EC values in hydroponic systems with nutrients; D- dark, L- illumination, and E- electricity, numbers represent initial zinc concentrations (mg/L).

Fig. 5-6 shows the interference brought in by nutrients in relation to the zinc uptake under various conditions. In the presence of nutrients, in control 2 (Fig. 5-6 c), the solution acquired a higher EC than the solution grown with a zinc concentration of 100mg/L (Fig. 5-6 d). This is partly a result of the nutrient uptake by the plants.

The application of AC (Fig. 5-5 g) generated an EC value higher than that in control 2 (Fig. 5-5 d). The EC of the solution subjected to illumination(Fig. 5-5 f) was less than that for the illuminated case with the AC (Fig. 5-5 g). This might linked to the fact that the plants absorb less zinc in the presence of AC as will be noticed in section 5.3.2.1.

5.3 ZINC ACCUMULATION IN PLANTS

5.3.1 Variation of zinc uptake in the presence of nutrients

The following section briefly describes some of the results related to nutrients. In the preliminary studies, macronutrients, such as N, P, and K were mixed with zinc in the hydroponic solution to know their effects on phytoremediation. However, to avoid synergetic effects that might blur the results caused by nutrients, they were excluded in the test proper.

Results of the preliminary experiments are demonstrated in Fig. 5-7 and Fig. 5-8. Figs 5-7 b and d show that, conditions of light, or the application of a 1V/cm voltage gradient and light to the hydroponic solution containing 100mg/L of zinc resulted in a similar accumulation of zinc in roots. However, application of AC in the presence of light rendered zinc concentration in the shoot (Fig. 5-8d) to be less than that for the application of light alone (Fig. 5-8 b). In roots, the plant that was grown in the zinc solution (100mg/L) under conditions of illumination (Fig. 5- 7 b) showed a large zinc concentration compared to the plant grow in darkness (Fig. 5-7 c). Similarly, in the shoots, the plant that was grown in the zinc solution (100mg/L) under conditions of illumination (Fig. 5- 8 b) showed a large zinc concentration compared to the plant grown in darkness (Fig. 5-8 c)

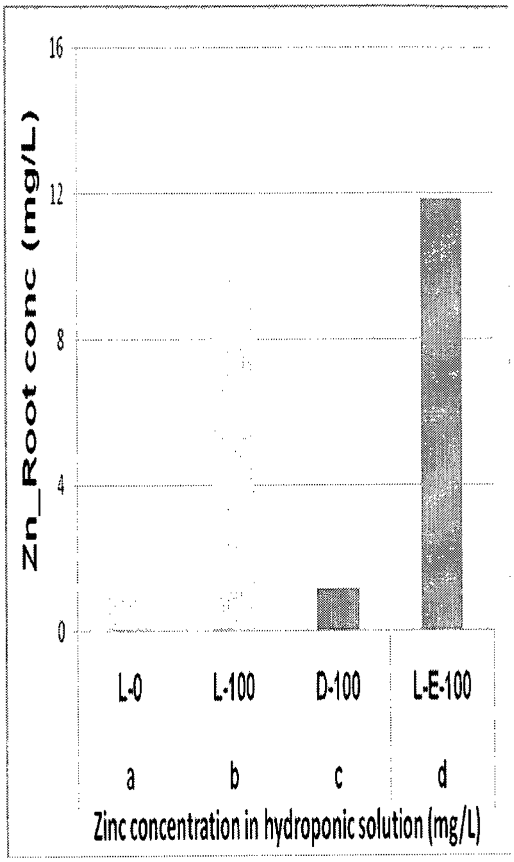


Fig. (5-7) Concentration of Zn in root DM with nutrients

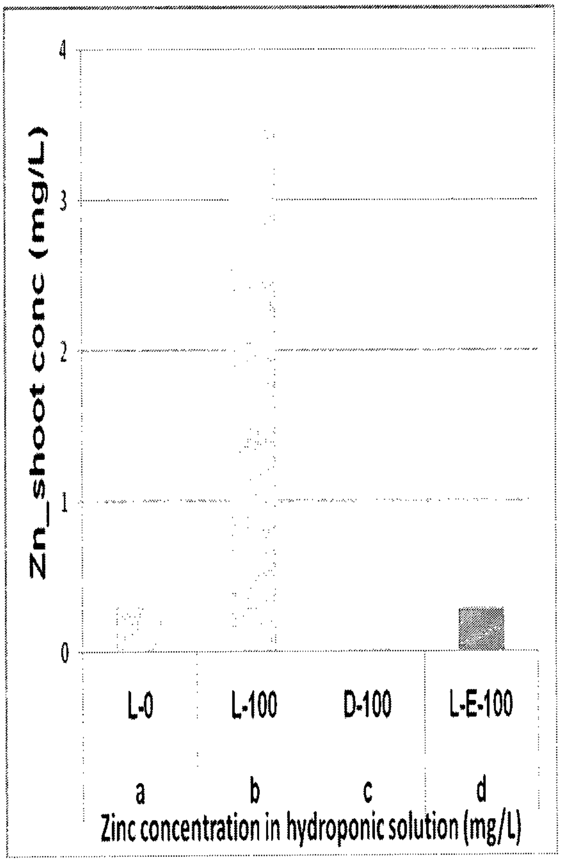


Fig.(5-8) Concentration of Zn in shoot DM with nutrients

5.3.2 Variation of zinc uptake in the absence of nutrients

5.3.2.1 Zinc accumulation in roots

In these tests, zinc sulphate was not mixed with the other nutrients. Figs 5-11, 5-12, 5-15, and 5-16 show zinc concentration in dry mass (DM) of shoots and roots for group 1 and group 2 tests. The standard deviations of test concentrations are also reported.

The results of these experiments demonstrated that the plant root system accumulated zinc up to a maximum concentration of about 20mg/g of DM of root tissue.

Hyperaccumulators are expected to accumulate at least 10 mg/g of zinc in their organs. Hence, the tested plants can be considered to be hyperaccumulators.

Zinc accumulation in tomato roots (Fig. 5-9 and 5-10) showed an appreciable variation demonstrating the intrinsic heterogeneity among individual plants. This heterogeneity can be attributed to the variability in the plant's gene structure, growing conditions, health status, as well as to experimental errors. Plants grew in the greenhouse in different times during the year, they were subjected to variable amount of sun light during summer and winter. As such, this might have affected their physiological growth.

Even though the data acquired showed variability, some trends could be noticed based on observing the average accumulated concentrations in each group of experiments. However, in order to avoid the effects of the unknown factors that might result from variability of plant physiological age, plants were classified into two groups, depending on the time of the year they were cultivated. Group 1 denoted plants grown in summer for preliminary tests that included the plants subjected to light without the effects of alternate current (AC). Group 2 denoted plants grown in winter to conduct the main tests involving the effects of plants exposed to darkness and light. In test with light, the effects of alternate current (AC) were also determined. In all test series, variation in zinc concentration was also determined. Figs 5-9 and 5-11 show the values of root zinc concentration for group 1. Figs 5-10 and 5-12 show the values of root zinc concentration for group 2.

The results showed that, for both plant groups, a higher zinc concentration in the hydroponic solution tends to cause a higher accumulation of zinc in plant roots (Figs 5-11 and 5-12). The sketches indicate that plant roots tend to be saturated with zinc at about 200mg/L. This fact confirms the hypothesis (page 7) that metal accumulation rate in plant roots will reach a specific limit. In group 2, increasing hydroponic zinc concentration in the dark environment from 0 to 200 mg/L demonstrated a higher accumulation of zinc in plants, which was in accordance to the results found under illuminated conditions (Fig. 5-12). For group 2, illumination seems to enhance metal uptake by plant roots which is supposed to be due to stimulation of active uptake in the presence of light. One notes that zinc absorption in the dark is also significant in comparison to the illuminated environment indicating that passive uptake mode was substantial during the tests. Furthermore, application of alternating current seems to decrease the amount of zinc uptake in plant roots. Zinc might be appreciably accumulated via the passive mode. The electrokinetic gradient might have hindered the free movement of zinc ions in the solution and thereby decrease their diffusion into plant roots. In other words, the carbon electrodes might cause the path of zinc ions to be more restrictive and thus decreasing the amount of zinc surrounding plant roots. In a previous study (Bi et al 2007), AC stimulation of plants grown in soil increased phytoextraction of Zn, Cu, Cd, and pb metals. Possibly, the AC stimulant in the soil matrix may be different from the aqueous matrix for plants. Furthermore, for a full investigation of AC stimulant on plant growth, one should conduct more tests in both matrices using a wide range of AC currents.

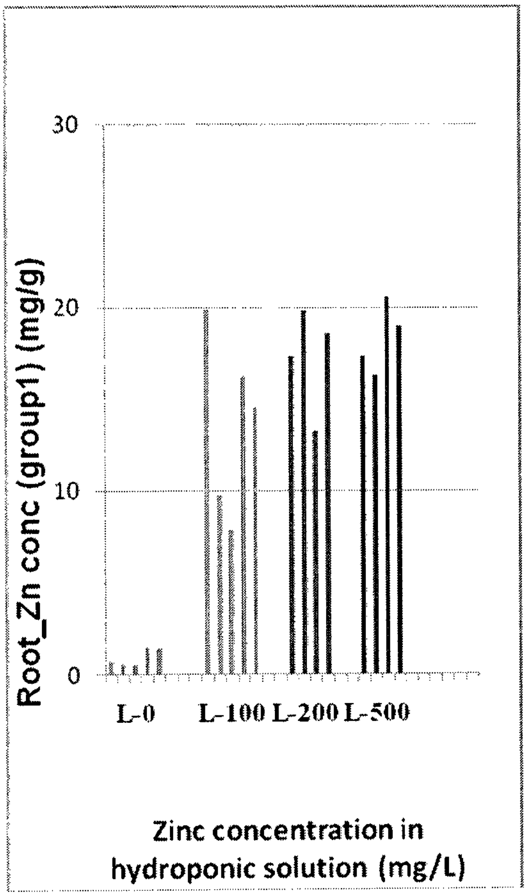


Fig. (5-9) Concentration of Zn in root DM For group 1 without nutrients

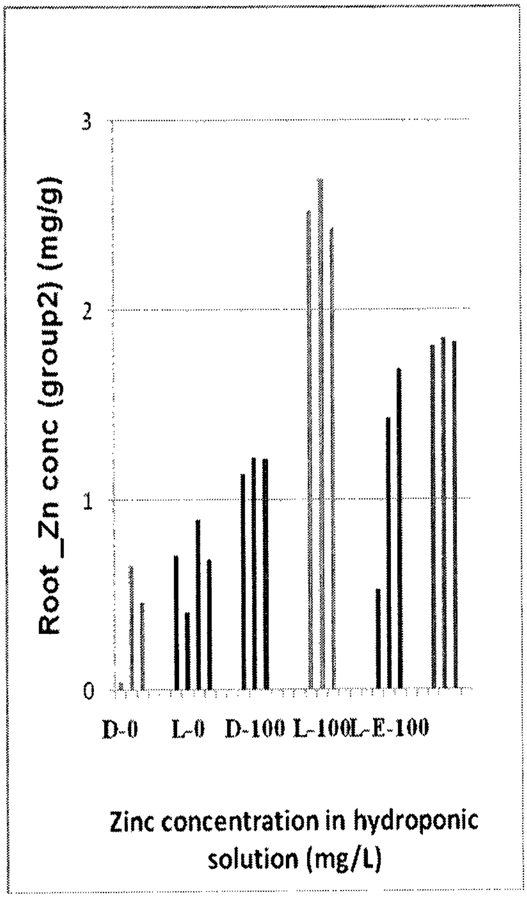
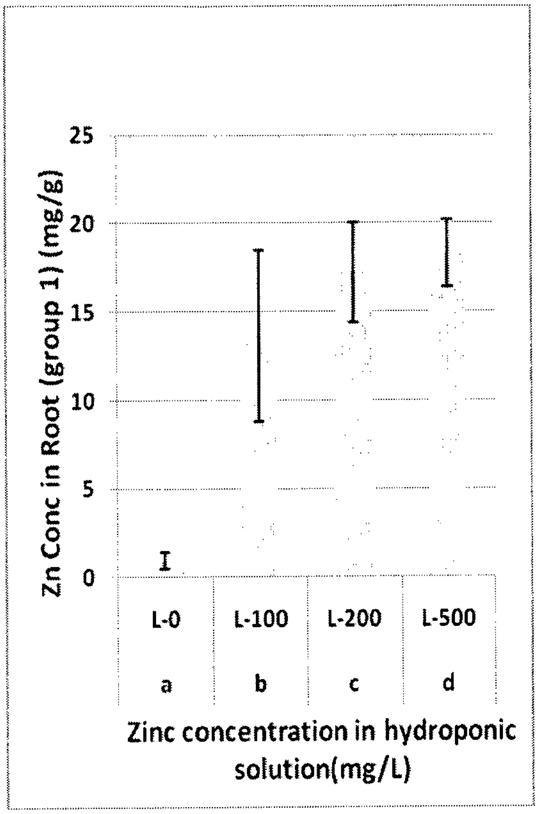


Fig.(5-10) Concentration of Zn in root DM for group 2 without nutrients



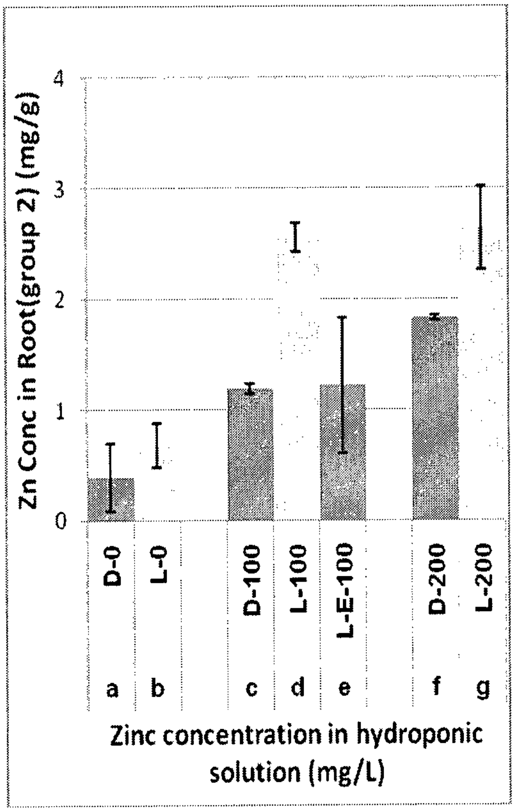


Fig. (5-11) Average Zinc concentration in roots for group1 without nutrients Condition-iluminated

Fig.(5-12) Average Zinc concentration in roots for group2 without nutrients

5.3.2.2 Zinc accumulation in shoots

Fig. 5-15 demonstrated that zinc has been accumulated in plant shoots up to a concentration of about 3 mg/g of its dry prime tissue. This signifies that the plant shoots act as accumulators. Moreover, for plant group 1, the results showed that Zn concentration in shoot Dry mass (DM) tends to increase with increasing its concentration of the metal in the hydroponic solution under illuminated condition. However, in group 2 (Fig. 5-16), the effect of changing hydroponic zinc concentration on metal translocation appears to have reached a saturation value without significant changes in metal concentration.

For group 1, Fig. 5-15 shows that a concentration of 200mg/L, in comparison to 100 mg/L, generated a slightly higher metal translocation into shoots under illuminated conditions, while for group2 no significant change was observed with increased concentration of zinc in the hydroponic solution.

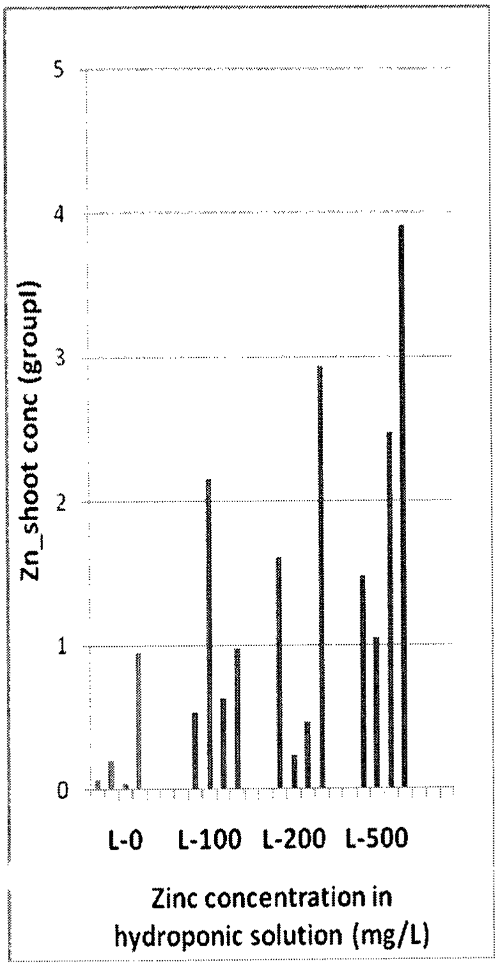


Fig.(5-13) Individual Zn concentration in shoots for group1 in the absence of nutrients

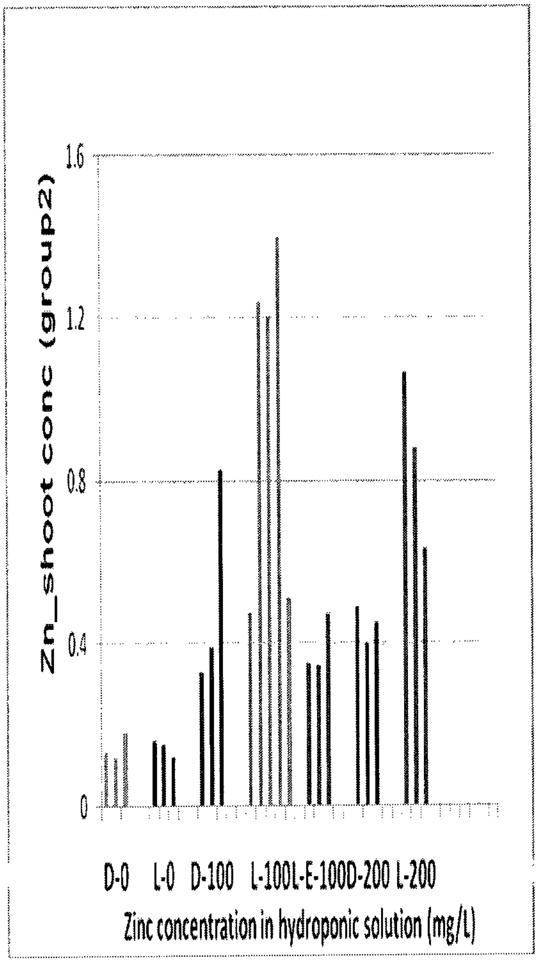


Fig.(5-14)Indiviual Zn concentration in shoots for group 2 in the absence of nutrients

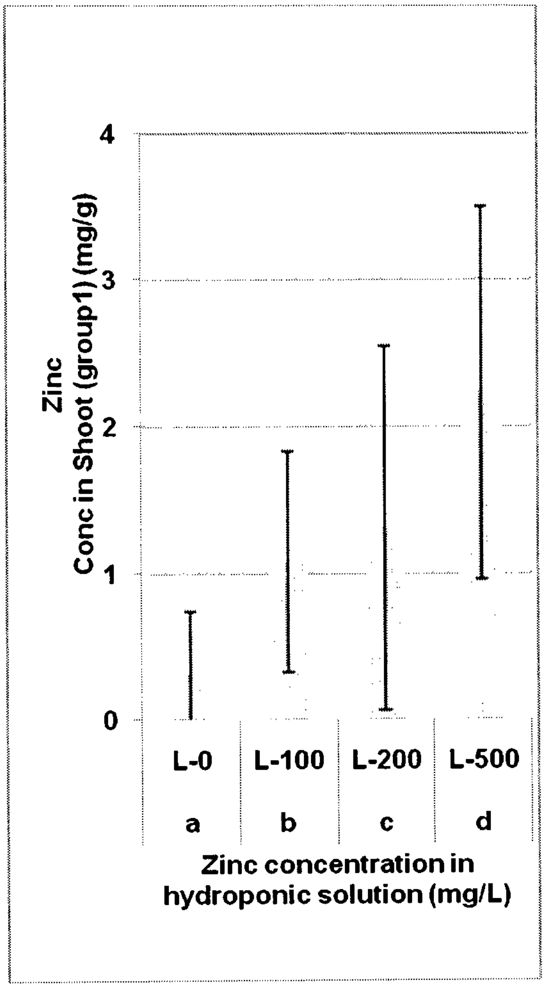


Fig. (5-15) AverageZn concentration in shoot DM for group1 without nutrient Condition-iluminated

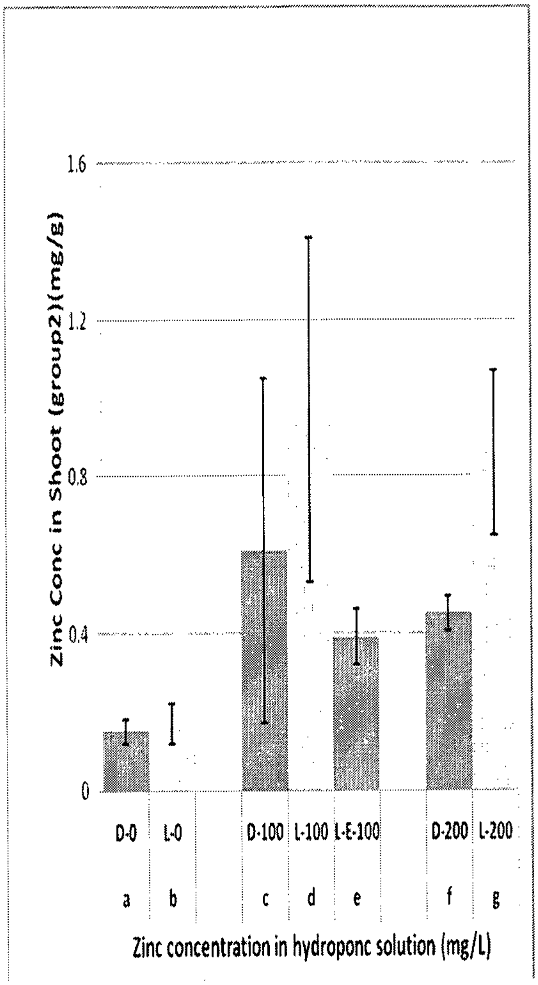


Fig. (5-16) Average Zn concentration in shoot DM for group2 without nutrient

In both group tests 1 and 2, when the solution concentration exceeded 100mg/L, toxicity appears to set in for the plants. This was indicated by the appearance of yellow leaves. Zinc can get transferred to the plant xylem by bypassing the plant cells in the apoplastic mode which is passive (Fig. 2-1). Further, this transport is mainly dependent on the gradient formed by concentration C_{soln} of zinc in the solution and the concentration C_o of zinc in the xylem. Consequently, increased hydroponic solution concentration results in increased apoplastic zinc transfer. This part of metal transfer is independent of the toxicity effect. Zinc in the solution can also be transferred through the cell membrane and enter the xylem by the symplastic mode which denotes both active and passive transport (Friotof and Griger, 2007). This mode gets affected by the effects of toxicity.

A difference appears to exist between the pattern of metal accumulation in the roots and in the shoots under illuminated conditions (Figs. 5-11 & 5-15). In Fig. 5-11, zinc concentration in root DM reaches a maximum value at a solution concentration of 100mg/L. On the other hand, the shoot concentration keeps increasing with increasing solution zinc concentration. The Part of this tendency may be attributed to the plant's different systems of control in the roots and in the shoots.

As stated earlier, the leaves turn yellow at a solution zinc concentration of 200mg/L indicating that toxicity sets in at this stage. Even in the dark environment (Fig. 5-16 a, c, f), shoot zinc translocation did not increase at a higher concentration (Fig. 5-16c). This is possibly due to toxic effects at higher zinc concentrations. This zinc toxicity might be reflected in modifying plant processes responsible for mobilizing zinc from root to root xylem, and finally to shoot.

Compared to Fig. 5-16 a (dark conditions), in Fig. 5-16 b (illuminated conditions), the small increase in concentration of zinc in the shoots for the plant under illuminated conditions can be traced to the transfer of the slight amount of residual zinc. Since plants were transplanted into the hydroponics from the peatmoss soil where it was previously grown, a small amount of residual zinc is present in the roots. A very small part of this residual zinc in the roots appears to have been translocated to the shoots in the presence of light. However, Fig. 5-16 d and g indicate that toxicity at higher solution zinc concentration (Figs 5-16 d and g) may have prevented further increase in shoot zinc concentration, under illuminated conditions.

To a certain degree, metal translocation is enhanced by evapotranspiration (Takeda et al, 2005). As such, one should expect a higher zinc concentration in shoot DM under illuminated conditions as observed in the tests (Fig. 5-16 a, b, c, d, f and g). Application of electrokinetic gradient seems to decrease appreciably the translocated amount of zinc in plant shoots even though it seems to induce a higher evapotranspiration rates. On the other hand, in a separate test, when additional nutrients have been added to the hydroponic solution, the electrokinetic gradient appreciably enhanced zinc translocation to a value comparable to that occurred in plants which has not been subjected to electrokinetic gradient. This can be due to a synergetic effect as a result of presence of nutrients.

It is conceivable that the shoot zinc concentration may change with the strength of the applied AC voltages and frequencies. In the present tests, an AC voltage gradient of 1V/cm at the frequency of 16HZ was applied to the system (Fig. 4-1a). In the presence of this electric field, a good part of zinc ions may be constrained to follow a specific path between the electrodes and thus fail to reach the xylem and hence the shoots. Hence, in comparison to the two other conditions of illumination and darkness, a drop in the shoot zinc concentration (Fig. 5-16 e) was noticed in these tests. Further, the drop in shoot zinc concentration under the applied voltage is far more than the increase attributable to illumination conditions (Figs 5-16 d and e).

Next section will demonstrate the effect of environmental controls on plant evapotranspiration. Meanwhile, to gain more insight on how this factor might affect zinc translocation in plant shoots, translocation efficiency and its relation to evapotranspiration has been described in the subsequent section

5.4 TOTAL HYDROPONIC SOLUTION UPTAKE (THSU)

The total hydroponic solution uptake (THSU) mainly measures the evapotranspiration as well as the amount of water absorbed by the plant roots. In Fig. 5-17, THSU is higher while applying an electro kinetic gradient. Contrary to what has been expected (SAPS 2010), there was little difference between THSU under dark and under illuminated conditions. This might signify that plants were too mature so that stomata opening did not vary appreciably between dark and illuminated environments.

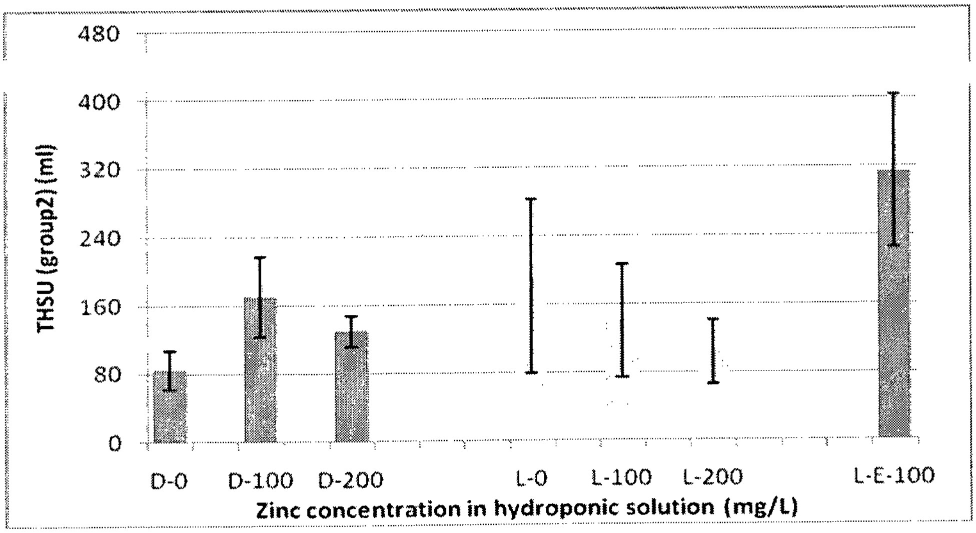


Fig. (5-17) Average THSU for group 2

5.5 TRANSLOCATION EFFICIENCY

Translocation effeceincy (TF) denotes the ratio of the zinc concentration in shoot DM to that in the root DM. Fig. 5-18 c, f and d, g, shows that TF is more for conditions of light than for darkness. This is a result of higher zinc transport to shoots during conditions of light that promote higher evapotranspiration as stated earlier. However, no correlation has been found between translocation effeciency and THSU, (Appix I), indicating that other factors might contribute to metal translcoation to the shoot. For the test that was conducted under conditions of AC current, TF value was the same as that for conditions of light. This result is fortuitous. It may be noted that for a plant that has a high TF value, frequent harvesting may be practiced to provide an effective phytoremediation.

Next chapter will summarize the derived conclusions as well as it will suggest some recommendations for future experiments.

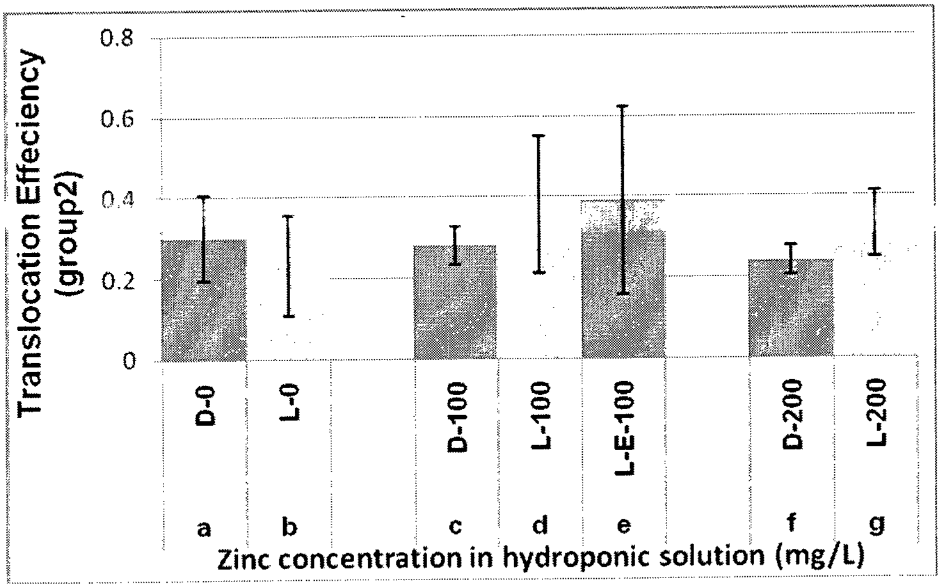


Fig. (5-18) Translocation effeciency for group 2. (D-0 &L-0 , control)

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

- Toxicity symptoms begin to appear, when zinc concentration in the hydroponic solution was 200mg/L and more. However, alternate current by itself did not bring in toxicity effects on plants.
- Illuminating the plants stimulated their active uptake of zinc, since they exudated more hydrogen ions, as noticed in the lowering of the pH.
- The accumulated amount of zinc in the plant roots increases with the increase in the zinc concentration of the hydroponic solution.
- Accumulated amount of zinc in the shoots was not well correlated with the zinc concentration in the hydroponic solution. This confirms the fact that plants control translocation of ions into the shoots more than their accumulation into the roots.
- Translocation of zinc in plant shoot seems to be independent of evapotranspiration, indicating that there are other dominating factors controlling this process.
- Application of AC with a voltage gradient (1V/cm and a frequency of 16HZ) seems to reduce plant capability to extract zinc in both shoot and root fragments.

6.2 RECOMMENDATIONS

- Testing more than one plant variety as well as more than one metal solution would provide more understanding of hydroponic plant interaction with its solution.
- The rate constants for the various phytoremediation mechanisms may be determined to assist the development of a model.
- It may be interesting to have a range of voltages and frequencies of AC current to determine their effect on phytoremediation.
- It will be more interesting to have test under conditions of darkness, illumination and also illumination with AC voltage for a range zinc concentrations to get a more comprehensive idea of the effects of each of these factors on phytoremediation.

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Appendix I
Experimental Data

The following tables provide information related to the results displayed in the sketches of previous chapters

Table 1: Average (μ) and standard deviation (σ) for pH and electrical conductivity (EC) of group 1 tests
Conditions: D = Dark, L = Light (illuminated) and E = Sample subject to AC voltage gradient

Zinc Concentration in hydroponic (mg/L)	pH	μ pH	σ pH	EC (μS/cm)	μ EC (μS/cm)	σ EC (μS/cm)
L-0	8.28	8.25	0.04	364	366.25	29.03
	8.18			346		
	8.26			408		
	8.26			347		
L-100	6.47	6.91	0.15	533	564.67	69.17
	6.99			644		
	7.00			517		
L-200	6.86	6.73	0.15	857	932	139.42
	6.86			1141		
	6.58			861		
	6.63			869		
L - 500	6.54	6.56	0.02	1655	1677	183
	6.55			1890		
	6.58			1717		
	6.55			1446		
Control1 (Tap Water)	7.66	7.66	0	288	288	0
Control 2-100 mg/L	7.24	7.24	0	549	549	0
Control3-200 mg/L	7.13	7.13	0	831	831	0

Table 2: Average (μ) and standard deviation (σ) for pH and electrical conductivity (EC) of group 2 tests
Conditions: D = Dark, L = Light (illuminated) and E = Sample subject to AC voltage gradient

Zinc Concentration in hydroponic (mg/L)	pH	μ_{pH}	σ_{pH}	EC (μS/cm)	μ_{EC} (μS/cm)	σ_{EC} (μS/cm)
D – 0	8.14	8.11	0.03	331	342	12
	8.08			355		
	8.1			339		
L - 0	8.24	8.22	0.06	480	390	62
	8.29			340		
	8.19			378		
	8.16			362		
D – 100	7.18	7.19	0.01	610	571	51
	7.20			590		
	7.19			514		
L-100	7.12	6.85	0.19	555	573	61
	6.60			549		
	6.75			641		
	6.84			628		
	6.92			492		
L - E - 100	7.63	7.20	0.38	655	932	475
	7.07			1481		
	6.91			660		
D - 200	7.05	7.05	0	855	872	28
	7.05			905		
	7.05			857		
L - 200	6.89	6.69	0.18	801	882	74
	6.55			945		
	6.64			900		
Control1 (Tap Water)	7.66	7.66	0	288	288	0
Control 2-100 mg/L	7.24	7.24	0	549	549	0
Control3-200 mg/L	7.13	7.13	0	831	831	0

Table 3: Zn concentration in group 1 roots and shoots.
Conditions: L = Light (illuminated) for all cases of group1.

Zinc concn in hydroponic solution (mg/L)	Zn_Root concn (mg/g) (individual tests)	Zn_Root concn (mg/g) (average for tests)	Zn_Root concn (mg/g) (standard deviation)	Zn_Shoot concn (mg/g) (individual tests)	Zn_Shoot concn (mg/g) (average for tests)	Zn_Shoot concn (mg/g) (standard deviation)
L-0	0.752	0.83	0.44	0.065	0.31	0.43
	0.55			0.200		
	0.54			0.039		
	1.475			0.950		
L-100	19.86	13.65	4.85	1.028	1.07	0.65
	9.76			0.535		
	7.877			2.160		
	16.2			0.636		
	14.54			0.979		
L-200	17.360	17.25	2.85	1.610	1.310	1.24
	19.840			0.237		
	13.260			0.462		
	18.550			2.930		
L-500	17.350	18.31	1.87	1.48	2.23	1.27
	16.320			1.047		
	20.580			2.47		
	19.000			3.91		

Table 4: Zn concentration in group 2 roots and shoots.
Conditions: D = Dark, L = Light (illuminated) and E = Sample subject to AC voltage gradient

Zinc concn in hydroponic solution (mg/L)	Zn_Root concn (mg/g) (individual tests)	Zn_Root concn (mg/g) (average for tests)	Zn_Root concn (mg/g) (standard deviation)	Zn_Shoot concn (mg/g) (individual tests)	Zn_Shoot concn (mg/g) (average for tests)	Zn_Shoot concn (mg/g) (standard deviation)
D-0 (group 2)	0.45	0.52	0.12	0.135	0.15	0.03
	0.659			0.12		
	0.463			0.18		
L-0(group 2)	0.71	0.67	0.25	0.16	0.17	0.05
	0.412			0.15		
	0.899			0.12		
L-100 (group2)	2.522	2.55	0.13	0.478	0.97	0.44
	2.690			1.24		
	2.431			1.205		
	2.680			1.40		
	2.440			0.51		
D-100(group2)	1.139	1.19	0.05	0.33	0.34	0.05
	1.222			0.39		
	1.220			0.29		
L-E-100 (group2)	0.53	1.22	0.61	0.35	0.39	0.07
	1.43			0.346		
	1.69			0.47		
L-200(group2)	2.52	2.64	0.38	1.064	0.86	0.21
	3.06			0.88		
	2.33			0.63		
D-200 (group2)	1.81	1.83	0.02	0.49	0.45	0.05
	1.85			0.40		
	1.83			0.45		

Table 5: Zn concentration in Roots and shoots for experiments conducted with hydroponics mixed with nutrients (N, P, K)
Conditions: D = Dark, L = Light (illuminated) and E = Sample subject to AC voltage gradient

Hydroponic Zinc concn (mg/L)	Dry weight of root W(g)	Zn concn in digestion tubes (mg/L)	Final zinc concn in root X/W (mg/g)	Hydroponic Zinc concn (mg/L)	Dry weight of shoot W(g)	Zn concn in digestion tubes (mg/L)	Final zinc concn in shoot X/W(mg/g)
D-100	0.52	39.00	1.13	D-100	0.52	0.43	0.01
L-E-100	0.48	377.50	11.83	L-E-100	0.65	12.10	0.28
L-100	0.45	335.00	11.12	L-100	0.47	113.4	3.59
L-0	0.53	33.6.00	0.95	L-0	0.52	10.50	0.30

$X = C^* V$

Where:

C: Concentration of zinc in 15ml digestion solution (mg/L)

V: Total volume of digestion solution (L)

W: Weight of sample after drying at 105°C (g)

Table 6: Translocation efficiency for group 2 experiments
Conditions: D = Dark, L = Light (illuminated) and E = Sample subject to AC voltage gradient

Zinc concn in hydroponic solution(mg/L)	Zn_Shoot/Zn_Root (concn) (individual tests)	Zn_Shoot/Zn_Root (concn) (average of tests)	Zn_Shoot/Zn_Root (concn) (Standard deviation)
D-0	0.3	0.3	0.11
	0.19		
	0.40		
L-0	0.38	0.23	0.12
	0.16		
	0.17		
D-100	0.23	0.28	0.05
	0.29		
	0.32		
L-100	0.51	0.38	0.17
	0.18		
	0.48		
	0.52		
	0.21		
L-E-100	0.66	0.39	0.23
	0.24		
	0.28		
D-200	0.27	0.24	0.04
	0.20		
	0.25		
L-200	0.42	0.33	0.08
	0.29		
	0.27		

Note: A rough estimate of evapotranspiration was obtained by determining the weight loss of the hydroponic solution during the tests. Strictly speaking, the increase in mass due to biosynthesis as a result of photosynthesis was not considered to be highly significant

Table 7: Evapotranspiration rate, and the ratio of dry shoot mass to dry root mass for group 2 experiments
Conditions: D = Dark, L = Light (illuminated) and E = Sample subject to AC voltage gradient

Zinc concn in hydroponic solution(mg/L)	THSU (ml) (individual tests)	THSU (ml) (average of tests)	THSU (ml) (standard deviation)	Shoot Mass/Root Mass
D-0	60	84	23.1	1.63
	106			2.70
	86			5.77
L-0	138	181	102.1	2.26
	298			1.34
	108			2.53
D-100	96	170	47.5	5.2
	159			5.5
	66			6.38
L-100	256	140	66.4	2.45
	130			3.39
	96			2
	123			1.71
	97			3.4
L-E-100	299	314	89.0	5.43
	234			4.66
	410			5.13
D-200	119	129	18.2	3.98
	150			6.29
	118			5.23
L-200	131	103	37.2	1.75
	61			1.53
	118			0.11

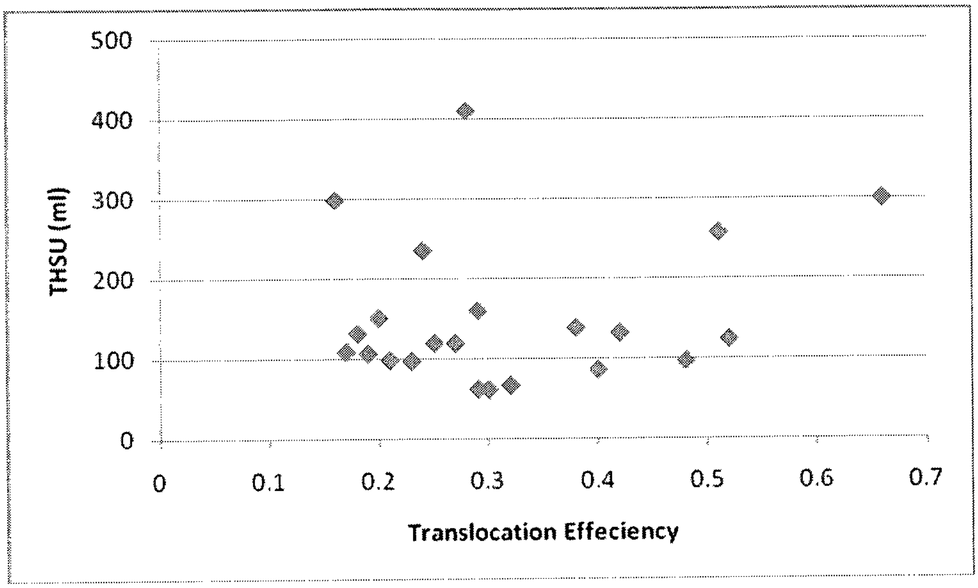

Fig. (A-1) Raltionship between THSU and TF for group 2 tests.

Appendix II

Zinc Accumulation in Fruits

As can be noticed in Fig (A-2) and Table 8, the results showed that zinc content in fruits is highly variable, and independent of other factors whether it is illumination, alternate current, or zinc solution concentration. The fruit data are combined for both groups. Only few plants yielded fruits. No general conclusion could be reached based on these results. The contaminants cannot easily get into the fruits of plants as the plant restricts the entrance of metals into fruits than the roots and the shoots.

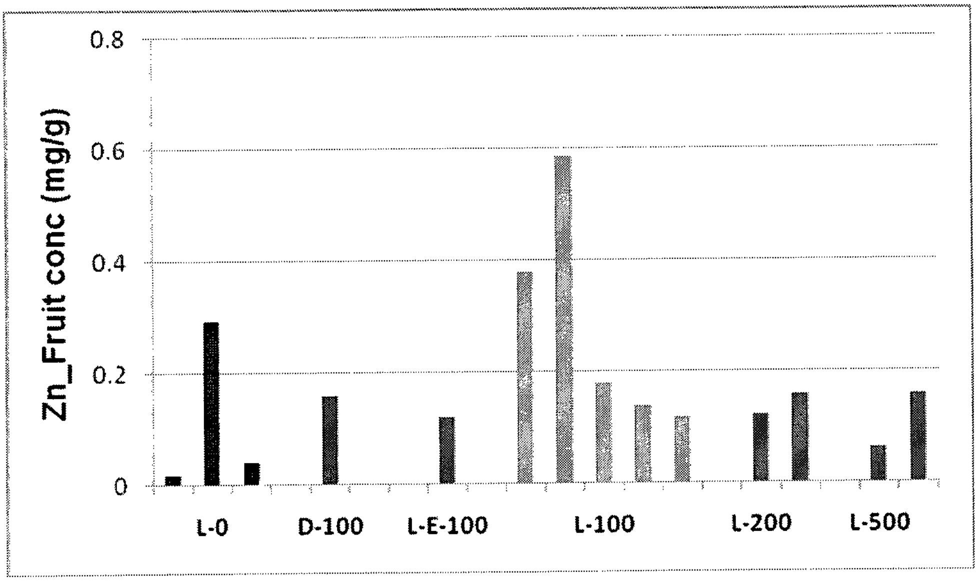

Fig. (A-2) Zn concentration in fruit DM

Table 8: Zn concentration in fruits for both plant groups 1 and 2.

Zinc concn in hydroponic solution (mg/L)	Zn concn in fruit(mg/g)
L -0	0.02
L -0	0.29
L -0	0.04
D-100	0.16
L -E-100	0.12
L-100	0.38
L-100	0.59
L-100	0.18
L-100	0.14
L-100	0.12
L-200	0.12
L-200	0.16
L-500	0.06
L-500	0.16

Experimental Conditions:
D = Dark
L = Light (illuminated)
E = Sample subjected to AC voltage gradient

Glossary:

Active Accumulation: Selective transport of heavy metal into plant roots while consuming energy.

Passive Accumulation: The diffusion of metal from bulk solution into plant roots as a result of metal concentration gradient between these two phases.

Symplast: The pathway through which metals transfer from bulk solution into root xylem when it passes through root cells.

Apoplast: The pathway through which metals transfer from bulk solution into root xylem when it passes around root cells.

Action Potential: An electrical signal generated inside a plant cell as a result of non damaging stimuli, like light or an electromagnetic gradient which is self propagated from a cell to another. This potential has constant velocity and amplitude (Davies 2006).