PHYTOTOXIC EFFECTS OF HEAVY METALS-ENRICHED WATER IRRIGATION ON FOOD CROPS USING A CLOSED HYDROPONIC SYSTEM

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by

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LIST OF ABBREVIATIONS

Abbreviation	**Description**
ABTS	2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)
$ABTS^{\bullet+}$	2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical cation
ANOVA	Analysis of variance
APX	Ascorbate peroxidase
B	Boron
BOD	Biological oxygen demand
BSA	Bovine serum albumin
Ca	Calcium
CAT	Catalase
Cd	Cadmium
CEC	Cation exchange capacity
Cl	Chlorine
Co	Cobalt
COD	Chemical oxygen demand
Cr	Chromium
Cr (III)	Trivalent chromium
Cr (VI)	Hexavalent chromium
$\left[Cr\left(H_2O\right)_6\right]^{3+}$	Hexa-aqua positively charged chromium complex
CrO_4^{2-}	Chromate
$Cr_2O_7^{2-}$	Dichromate

Cu	Copper
DID	Department of Irrigation and Drainage
DMEM-F12	Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
EC	Electrical conductivity
EDTA	Ethylenediaminetetraacetic acid
EPA	US Environmental Protection Agency
FBSi	Fetal bovine serum
Fe	Iron
Fe-EDTA	Ferric ethylenediaminetetraacetic acid
$FeCl_3 \cdot 6H_2O$	Ferric chloride hexahydrate
$FeSO_4 \cdot 7H_2O$	Iron (II) sulfate heptahydrate
FPG	Formamidopyrimidine-DNA glycosylase
FRAP	Ferric reducing antioxidant power
FW	Fresh weight
GDP	Gross Domestic Product
h	Hour
HCl	Hydrochloric acid
$HCrO^{4-}$	Hydrogenchromate
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid
HepG2	Hepatocellular carcinoma cell line
H_2O_2	Hydrogen peroxide

HoxN	High-affinity nickel transport protein
HRS	Hydroxyl radical scavenging
HRSA	Hydroxyl radical scavenging activity
IARC	International Agency for Research on Cancer
K	Potassium
LMP	Low melting point
MDA	Malondialdehyde
MDGs	Millennium Development Goals
Mg	Magnesium
Mn	Manganese
MTT	3-(4,5-dimethylthiasol-2-yl)-2,5,-diphenyltetrazolium bromide
N	Nitrogen
Na	Sodium
NaOH	Sodium hydroxide
Na_3PO_4	Sodium phosphate
NASA	National Aeronautics and Space Administration
NBT	Nitroblue tetrazolium
Ni	Nickel
NMP	Normal melting point
$N\text{-}NO_3$	Nitrate nitrogen
NO	Nitric oxide
NO_2	Nitrogen dioxide
NTU	Nephelometric turbidity
O_2^-	Superoxide anion

$^1O_2$	Singlet oxygen
OECD	Organisation for Economic Co-operation and Development
OH	Hydroxyl radical
ONOO-	Peroxynitrite
P	Phosphorus
Pb	Lead
PBS	Phosphate buffer saline
POD	Guaiacol peroxidase
RNS	Reactive oxygen species
RO	Lipid alkoxyl radical
ROO	Lipid peroxyl radical
ROOH	Lipid hydroperoxide
ROS	Reactive nitrogen species
SAR	Sodium absorption rate
SDGs	Sustainable Development Goals
SEM	Scanning electron microscope
SOD	Superoxide dismutase
SS	Suspended solids
TCA	Trichloroacetic acid
TNK	Total nitrogen Kjeldhal
TPTZ	2,4,6-Tri(2-pyridyl)-s-triazine
UV	Ultraviolet
UV-VIS	Ultraviolet and visible
WUE	Water use efficiency
Zn	Zinc

LIST OF SYMBOLS

Symbol	Description
A_0	Absorbance of the control reaction in ABTS assay
A_{663}	Absorbance at 663 nm
A_{645}	Absorbance at 645 nm
A_{470}	Absorbance at 470 nm
A_{600}	Absorbance at 600 nm
A_{532}	Absorbance at 532 nm
A_{734nm}	Absorbance at 734 nm
A_c	Absorbance of control in HRSA assay
A_s	Absorbance of extract in HRSA assay
A_t	Absorbance for the plant extract at time t in ABTS assay
C_a	Concentration of chlorophyll-*a*
C_b	Concentration of chlorophyll-*b*
°C	Degree celcius
%	Percentage
nm	Nanometre
rpm	Revolution per minute
v	Volume
w	Weight

KESAN FITOTOSIK AIR PENGAIRAN DIPERKAYAKAN DENGAN LOGAM BERAT TERHADAP TANAMAN MAKANAN MENGGUNAKAN SISTEM HIDROPONIK TERTUTUP

ABSTRAK

Isu keselamatan makanan dan air telah diutarakan secara konvensional dan bebas, dan kini, perhubungan tersebut telah muncul sebagai agenda global yang rumit. Hari ini, pengairan air sisa telah muncul sebagai satu strategi meluas untuk memenuhi keperluan mendesak terhadap sumber air bukan-konvensional. Sumber buangan tersebut mengandungi unsur toksik, melebihi had maksimum yang dibenarkan, dan merupakan penanda risiko terhadap tanaman makanan dan rantaian makanan. Logam berat merupakan juzuk karsinogenik, teratogenik dan mutagenik, yang terkumpul dalam air pengairan. Kromium trivalen [Cr (III)], plumbum [Pb (II)] dan nikel [Ni (II)] merupakan bahan pencemar yang paling berbahaya, berasaskan sifat kimia elektronik yang kompleks, ketakterbiodegradan, kelarutan dan mobiliti. Kajian ini bertujuan untuk menyiasat salinghubungan kompleks antara pengairan air yang diperkayakan dengan logam terhadap *Vigna radiata*, *Ipomoea aquatica* dan *Brassica chinensis*, dalam perhubungannya dengan pertumbuhan fizikal; ciri-ciri fisiologi: pigmen fotosintesis, proline, tindak balas anti-oksidatif [*guaiacol peroxidase* (POD), *catalase* (CAT) dan *ascorbate peroxidase* (APX)], dan keupayaan antioksidan; serta ciri morfologi. Implikasi toksik telah dinilai dengan pengujian *in vitro*. Pengairan air yang diperkayakan dengan logam berat menginduksikan perencatan permanjangan akar dan pucuk secara ketara berasaskan kepekatan dan tempoh pengairan. Perawatan Cr (III) mencatatkan kadar perencatan tertinggi dalam *Vigna radiata* dan *Brassica chinensis*,

sebanyak 68.04% untuk akar dan 75.69% untuk pucuk. *Ipomoea aquatica* adalah amat sensitif terhadap perawatan Ni (II), dengan catatan perencatan pertumbuhan sebanyak 83.78% dan 87.33% masing-masing pada akar dan pucuk. Kandungan klorofil-a, klorofil-b, dan karotenoid berkurangan pada 83.92%, 75.86%, dan 63%. Lipid peroxidasi yang ketara (170.30%) dan peningkatan proline (201.08%), dikesan selaras dengan perubahan aktiviti POD, APX dan CAT yang nyata. Penindasan aktiviti antioksidan yang ketara melebihi paras ambang kepekatan 0.50 mM Pb (II), 0.20 mM Cr (III) dan 0.05 mM Ni (II) dalam ketiga-tiga model tumbuhan, menunjukkan bahawa mekanisme pertahanan antioksidan tidak dapat melindungi sistem tumbuhan daripada kerosakan oksidatif. Gangguan ini disokong dengan herotan xilem dan floem, serta gangguan ke atas stomata. Kesan sitotoksik yang ketara dengan penurunan 50% dalam keupayaan sel HepG2 dapat diperhatikan, pada kepekatan 0.20 mM Pb (II), 0.40 mM Ni (II) dan 1.20 mM Cr (III). Kesan genotoksik pada momen ekor dan kerosakan ekor DNA dicatatkan pada kepekatan terendah, iaitu 0.04 μg/mL dalam ketiga-tiga ion logam berat. Kesimpulannya, ancaman kesihatan yang berkait rapat dengan tekanan oksidatif dan kemerosotan terhadap tanaman makanan akan berleluasa, dengan pengamalan pengairan air sisa tercemar dengan logam berat secara tidak terkawal, yang memberikan tamparan hebat terhadap kelestarian hubungan nexus makanan-air.

PHYTOTOXIC EFFECTS OF HEAVY METALS-ENRICHED WATER IRRIGATION ON FOOD CROPS USING A CLOSED HYDROPONIC SYSTEM

ABSTRACT

The issues of food and water security have conventionally been addressed independently, and to date, their nexus has emerged to be the most intricate global agenda. Today, wastewater irrigation has emerged to be a widespread strategy to fulfil the pressing need of non-conventional water resources. These discharges carry toxic elements, exceeding the maximum allowable limits, suggesting the possible risks to food crops and food chain. Heavy metals are the most carcinogenic, teratogenic and mutagenic constituents, accumulating in the irrigation water. Trivalent chromium [Cr (III)], lead [Pb (II)] and nickel [Ni (II)] are the most insidious pollutants, ascribed to their complex electronic chemistry, non-biodegradability, solubility and mobility. This study aimed to investigate the complex interconnection of metals-enriched water irrigation on *Vigna radiata*, *Ipomoea aquatica* and *Brassica chinensis* in relation to the physical growth; physiological characteristics: photosynthetic pigments, proline, antioxidative response [guaiacol peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX)], antioxidant capacities and morphological characteristics. The toxicity implications were evaluated using *in vitro* bioassays. Metals-enriched water irrigation adversely induced significant concentration- and duration-dependent reductions in the elongation of roots and shoots; with Cr (III) exerted the highest inhibition in *Vigna radiata* and *Brassica chinensis*, denoted the reduction of 68.04% and 75.69% for root and shoot. *Ipomoea aquatica* was specifically sensitive to Ni (II), with the highest root and shoot inhibition of 83.78% and 87.33%. Chlorophyll-*a*, chlorophyll-*b*, and carotenoid contents were significantly reduced, up to 83.92%, 75.86%, and 63%. Profound lipid peroxidation (170.30%) and rising proline content (201.08%) were complementary with the pronounced alterations for POD, APX and CAT activities. The marked suppressions of antioxidant activities above the threshold levels of 0.50 mM Pb (II), 0.20 mM Cr (III) and 0.05 mM Ni (II) in the plant models indicated that the antioxidant defense machinery could no longer protect the plant systems against the oxidative damages. These disturbances corroborated with the xylem and phloem distortion, and stomata disruption. Significant cytotoxic effect with 50% reduction in HepG2 cell viability was noted at 0.20 mM Pb (II), 0.40 mM Ni (II) and 1.20 mM Cr (III). Genotoxic effects on DNA tail moment and tail damage were observed at the lowest concentration of 0.04 µg/mL. Conclusively, the oxidative stress-related health threats associated with the deteriorating effects on the food crops could be exacerbated by a wide scale unregulated metals-polluted wastewater irrigation practice, and place alarming threats to the sustainability of water-food nexus.

CHAPTER ONE

INTRODUCTION

This chapter provides an overview on the food-water nexus, and highlights the global key challenges related to food and water security. The problem statements, research objectives, scope of study, significance of study and the organization of this thesis are outlined.

1.1 Overview of food-water nexus

Today, addressing the food, water and climate nexus is increasingly important to transparently meet with the rising global demand (Lele *et al*., 2013). In 2011, the World Economic Forum emphasized that global food and water resources were experiencing significant stress or shortfalls, and they were expected to be exacerbated for the next 20 years (Waughray, 2011). The Bonn Nexus conference predicted that world growing population, and economic development may lead to the resource depletion, degraded ecosystem services, and irreversible societal and environmental changes, that eventually threaten the overall sustainable development (Hoff, 2011). In parallel with this, the sixth World Water Forum, World Water Week 2012, the Rio + 20 Summit 2012 and the German Federal Government have urged an integrated approach to the food-water-climate challenges (Rasul, 2014).

A nexus strategy recognizes the interdependencies of water, food production and climate change, and aims to systemize a transparent framework for the effective use of resources, and to manage trade-offs and synergies, without compromising the sustainability (Hussey *et al*., 2012). The relationship between these systems goes beyond simply water-footprinting food production, carbon-footprinting water supply chains or new climate adaptation strategies in relation to water consumption, or the impact on land availability and food prices. At heart of the relationship is the interdependence of resources, that is interpreted in such a way that how demand for one resource can drive demand for another, and similarly, how the cost of one resource can determine the efficiency of production of the others (Gulati *et al*., 2013). Food, water and climate are inextricably interlinked in a nexus. Food production requires water, and relies on climate and weather conditions; climate and temperature alterations could affect water availability; and agricultural practices and food choices would influence the global climatic pattern and water usage. The relationships are dynamic (Figure 1.1). By ignoring the underlying interdependence of the three elements, policies sometimes have unintended consequence of shifting a crisis from one element to another (Tomain, 2011); that policies and actions taken in isolation, without considering their impact on other sectors, can aggravate resource constraints (Meerow *et al*., 2016).

The planned Sustainable Development Goals (SDGs) of zero poverty (SDG 1), ending hunger and food insecurity (SDG 2), ensuring water security (SDG 6), sustainable economic growth (SDG 8), sustainable consumption and production (SDG 12), and conservation, protection, and sustainable use of marine and terrestrial resources and ecosystems (SDG 14 and SDG 15) are closely interlinked, and the successful establishment of these goals would rely heavily on the sustainable use and management of water, land (food), and other natural resources. These factors are not only interdependent, but they also both reinforce and impose constraints on one another (Rasul, 2014; Weitz *et al*., 2014).

paradigm shift in farming and food production that offers suitable and efficient methods for urban agriculture, with an effective use of water and land, to maximize crop yield and provides a sustainable food-production model that supplies crop year-round, without interference by climate change, season, or adverse natural events.

1.6 Organization of thesis

This thesis consists of five major chapters. Chapter one provides an overview on the interlinkages of food-water nexus. The global agenda of heavy metals-enriched water irrigation practice and the possible deterioration on the plant physiology, food chain, human health, and the ecosystem equilibrium were highlighted. The subsequent section outlines the research objectives, scope and significant of study. In chapter two, the literature findings on the food security management, and wastewater irrigation practice were presented, mainly focusing on the trivalent chromium, lead, and nickel. To develop a better understanding within this research, a deep insight of phytotoxicity, in relation to the physical growth, physiological properties, oxidative stress, enzymatic and non-enzymatic antioxidants machinery, structural and morphological characteristics were concisely defined. The possible cytotoxic and genotoxic implications of heavy metals contamination, and the fundamental concept of hydroponic culture, hydroponic culture systems, with the specific features of hydroponic cultivation were described.

Chapter three summarizes the lists of chemicals and materials for the accomplishment of this study. The unique features of the hydroponic setups, plant models, and nutrient solution for plant cultivation were provided. The working principals of the analytical systems, subsequent by the experimental design, including descriptions on the standard protocols for (1) physical assessment on roots and shoots growth; (2) physiological profile, notably photosynthetic pigmentation, proline, and lipid peroxidation; (3) antioxidative responses of guaiacol peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD); (4) antioxidant capacities: ferric reducing antioxidant property (FRAP) assay, ABTS radical scavenging activity assay, and hydroxyl radical scavenging activity (HRSA) assay; (5) cytotoxicity assay; (6) comet assay; and (7) morphological study were outlined. The applications of statistical analysis, and a flowchart summarizing the research activities was clearly explained. Chapter four presents the results and discussions on the macroscopic symptoms, alterations in photosynthetic pigments, degree of lipid peroxidation, and accumulation of stress biomarker, proline content. The underlying mechanisms for these disruptions, and the antioxidative stress and antioxidant capacities in response to heavy metals-induced stress were deeply discussed. Additionally, the possible cytotoxicity and genotoxicity effects, supported by the morphological changes were highlighted. Chapter five summarizes the major findings of this research, to reflect the accomplishment of the listed objectives. The recommendations and future prospects suggested are in view of their significance to the present research.

CHAPTER TWO

LITERATURE REVIEW

This chapter presents the literature findings on the food security management and global wastewater irrigation practice. The heavy metals-enriched water irrigation primarily focusing on Cr (III), Pb (II) and Ni (II) are discussed. The heavy metals-induced phytotoxic effects are summarized in relation to the physical growth, physiological properties, oxidative stress, morphological characteristics, and toxicity effects on human cell lines. To provide a better understanding, the hydroponic system is defined in terms of the varieties, specific features, and advantages as compared to the conventional agricultural practice.

2.1 Food security management

Food security is a condition at individual, household, national, regional, and global levels, when all people at all times, have physical and economic access to sufficient, safe and nutritious food that meets with their dietary needs and food preferences for an active and healthy life (World Food Summit, 1996). This definition of food and nutrition security reflects four key dimensions of food, including bioavailability, access, utilization, and stability. Physical availability is determined by food production, distribution, stock levels and net trade, while stability is governed by the weather conditions, political stability, and economic factors. Food availability and food stability are achieved when sufficient quantities of food are provided at all times. Food access is achieved when individuals have the adequate resources to obtain appropriate food for a safe and nutritious diet. Access to foods for the landless is a function of food prices, which are interplayed between different factors, including supply shortfalls owing to crop failures caused by such phenomena as droughts, floods and diseases, and changing food consumption patterns (Chowdhury, 2011). Food utilization reflects the ability to use food efficiently to assure that all physiological needs are met through a nutritious and safe diet, clean water, adequate sanitation, and proper health care. Individuals might have access to adequate food, but due to bad health, they may be unable to absorb the micronutrients (Pieters and Swinnen, 2016). Food insecurity could present, if all four major dimensions are not met simultaneously.

The Global Water Partnership (2012) has defined water security as, "*Ensuring the availability of adequate and reliable water resources of acceptable quality, to underpin water service provision for all social and economic activities in a manner that is environmentally sustainable; mitigating water-related risks, including floods, droughts and pollution; addressing the conflicts that may arise from disputes over shared waters, particularly in situations of growing stress, and turning them into win-win solutions*." In other words, water security is referred to "access to safe drinking water and sanitation". This emphasizes on three key elements: water access, water safety, and water affordability. As a whole, water security is the capacity of a population to safeguard sustainable access to adequate quantities of and acceptable quality water to sustain livelihoods, human well-being, and socio-economic development, ensure protection against water-borne pollution and water-related disasters, and preserve ecosystems in a climate of peace and political stability (UN-Water Task Force on Water Security, 2013).

In recent years, profiling water and food as a nexus has been strongly promoted as a global research agenda and emerging development paradigm. At the core of nexus debates are natural resource scarcities, and the recognition that water and food are interlinked in a web of complex relations, where resource use and availability are interdependent (Dupar and Oates, 2012). Food and water are interrelated to each other in such a way that, food security depends on the supply of water with adequate quality and quantity; while agriculture has a major influence on both water supply and water quality. As a whole, new water cannot be created, but it can be reused. Rainfall supplies new freshwater locally, but the benefits of the new water can be destroyed by pollution. The water courses, however could be too polluted to be applied for irrigation purposes (Olsson, 2013).

During the last century, research focus has been devoted to the food productivity and "Green Revolution" (1966–1985) has been witnessed, parallel with the technological advancement for better yields, and food production (Ingram *et al*., 2013); mainly driven by the radical improvement of fertilizers and pesticides use, agricultural machinery, and irrigation systems. Between 1990 and 2010, the production of food (+ 56%) grew at a faster rate than the world population (+ 30%) (FAO 2013). Current food production systems continue to deplete natural resources and pollute ecosystems to compromise the capacity for the future generations, exacerbated by the effects of climate change, and associated mitigation and adaptation requirements (Godfray *et al*., 2010). The key trends and issues in food, water, and energy security in South Asia are summarized in Table 2.1.

Food security concern is embedded in Malaysia agriculture policy since the inception before Independence, with modifications over time. Agriculture remains an important sector in Malaysia, and has been the backbone of Malaysian economy by producing agricultural products for domestic consumption, and as the earner of foreign exchange. According to Ahmad (2010), the Malaysian economy witnessed a transformation of its economic structure rapidly from the late 1950s, when agriculture, mainly rubber and timber contributed approximately 50% of the country's Gross Domestic Products (GDP). A programme was initiated to diversify agricultural output in the 1960s, leading to a successful diversification into palm oil and cocoa. The government introduced the National Agricultural Policy to liberalize the sector and enhance the productivity, efficiency and competitiveness of the sector. Agriculture was, however, relegated to the backseat as the country began the drive to industrialize, particularly in the late 1980s and early 1990s. The sharp rise in domestic food prices in the aftermath of the 1997-98 Asian Financial Crisis, which led to the re-prioritization of agriculture and food security on the domestic agenda, has contributed to renewed growth in Malaysian agriculture. Malaysia has registered high growth in agricultural research and development spending since the late 1990s.

The population in Malaysia is projected to continue to grow linearly, and is expected to reach approximately 43 million people in 2050. To date, there is no specific policy on food security, but it has been embedded into the theme of self-sufficiency level, primarily referred to paddy or rice, which is the staple diet of majority of the population. However, the scope has been expanded to other food items, particularly fruits and vegetables. Nonetheless, Malaysia is still dependent on imports of food, notably vegetables including tomatoes, chilies, onions, ginger and potatoes. The 1997 Asian financial crisis affected Malaysia in a serious way due to the high imports of food and food products (Razak *et al*., 2013).

Additionally, the crops productivity in Malaysia has been related to the climate change impacts. Under current climate change scenario, temperature above 25°C may decline grain mass of 4.4% per 1°C rise and the grain yield may reduce as much as

Table 2.1. Key challenges in food and water security in South Asia region.

Key challenges	**Socio-economic, environmental, and development implications and challenges**	**Interdependence of food and water resources**
Food security		
Huge chronically undernourished population - (Approximately 50% of the world poor population live in South Asia)	- To meet with the nutritional needs of all, food production needs to double in the next 25 years	- Provision of food and water to large malnourished population without degrading natural resource base and environment
Declining cropland per person - Very low per capita arable land area, declining continually owing to population growth and urbanization	- Limited options for growing more food grain by expanding crop area - Increased pressure on land and water	- Further intensification of food production needed with more external inputs of water resource
Increased water consumption in intensive food production	- Food production requires more water	- Agricultural growth constrained by shortage of water
Adverse weather condition and climate change (Temperature rise, accelerated glacial melting, increased evapo-transpiration)	- Uncertainty of water availability due to rapid glacier melting in the Himalaya	- Climate change is likely to be a critical factor in increasing water and land demand for food production
Water security		
Growing water stress - Growing water demand for agriculture, food industry, human and livestock usage: Annual water demand is predicted to increase by 55% in 2030	- Increased water pollution and water-borne diseases, poor human health and high child mortality	- Balancing water demand for food production, industrial growth and environment
Increased dependency on groundwater for food production - About 80% of agricultural production depends on groundwater irrigation	- Declination of water tables, posing threats to the sustainability of agriculture, food production, health and environment - Growing pressure on water resources	- Irrigation and other major economic activities rely mainly on river water during dry seasons

9.6 to 10.0% per 1°C rise (Tashiro and Wardlaw, 1989). Alam *et al*. (2011) reported that total yearly rainfall in Malaysia is increasing, but the monthly variation is too high. In Malaysia, the effect of lower rainfall is almost possible to check through proper irrigation system, but the opposite phenomenon of over rainfall for any particular time, especially at the end of the crop cycle or at the maturity period, causes serious damages of crops, that is absolutely uncontrollable for now.

Malaysia has made vigorous attempts to ensure that food security in the country can be achieved, including to provide adequate incentives and income to producers to produce more food, and to ensure adequate safe and quality food for consumers, with the major focus on four commodities including vegetables and rice. There are some of the adjustments recommended for the country food policy to ensure growth, and therefore food security: (i) conceptualizing food security in the bigger context, by taking into account the commercial imports as possible sources of commodity supply, brings in elements of stability of supply, access to food by the local population, food safety and dietary requirement for a healthy life; and (ii) increase public funding for agriculture and food, that could not only make Malaysia the major suppliers of agricultural raw materials but also uplifting food production that has been lagging behind in all fronts (Arshad and Hameed, 2010).

2.2 Wastewater irrigation

2.2.1 Historical background of wastewater irrigation practice

Agriculture is the largest user of water, accounting for about 75% of the world available freshwater. In some countries, irrigation covers as much as 90% of the total available of water resources (FAO, 2005). The issues of rising water scarcity and fertilizers, pesticides and additives in pigments and gasoline (Huang *et al*., 2012; Shahid *et al*., 2012). A significant increase of the Pb content has been observed in the motor vehicles and industrial plants, with an estimated annual emission of 500,000 tones/year. Being a non-redox active metal, Pb is a general protoplasmic poison, which can be easily absorbed, transformed, and accumulated in plants tissues, where root is the primary site of accumulation (Kumar *et al*., 2012).

The absorption of Pb by roots occurs via the apoplastic pathway or via calcium ion (Ca^{2+})- permeable channels. The behaviour of Pb in soil, and uptake by plants, is controlled by its speciation and by the soil pH and particle size, cation exchange capacity, root surface area, root exudation, and degree of mycorrhizal transpiration. Pb would primarily accumulate in the root cells of plants, due to the blockage by Casparian strips within the endodermis. Pb is also trapped by the negative charges that exist on roots' cell walls (Pourrut *et al*., 2011). The pattern of distribution of Pb in the roots may considerably differ according to the changing concentrations of Pb. At lower concentrations, Pb ions could predominantly flow in the apoplast; while at the higher concentrations, the barrier function of plasmalemma is damaged and a greater amount of Pb could enter into the cells. Generally, the apparent concentration of Pb in the aerial parts of the plant decreases as the distance from the root increases. This occurs due to the greater localization of Pb in cell walls of the root than in other parts of plant (Sharma and Dubey, 2005).

2.3.3 Nickel (Ni)

Nickel, a transition metal found in natural soils at trace concentrations except in ultramafic or serpentinic soils, is a micronutrient that is required by both higher and lower plants in very small amounts (Shafeeq *et al*., 2012). Among all, nickel toxicity has received aesthetic great concern, due to its excessive wide scale applications in different industries and anthropogenic activities, particularly metal mining, smelting, fossil fuel burning, vehicle emissions, disposal of household, municipal and industrial wastes, fertilizer application and organic manures. Nickel is primarily used as a raw material in the metallurgical and electroplating in the industries, as a catalyst chemical and food industry, and as a component of electrical batteries (Seregin and Kozhevnikova, 2006). In recent years, nickel pollution has been reported across the world, including Asia, Europe and North America (Papadopoulos *et al*., 2007; Zhao *et al*., 2008). Ni ions concentrations may reach 26,000 ppm in the polluted soils and 0.2 mg/L in polluted surface water; three times higher than that found in unpolluted areas, leaving a global problem of soil and water pollution (Zwolsman and Bokhoven, 2007).

Although the role of nickel in metabolic processes of plants has not been identified as extensively as other elements, it is a key factor in the activation of enzyme urease required for nitrogen metabolism (Bai *et al*., 2013), and plays a part in seed germination and iron uptake (Poonkothai *et al*., 2012). The uptake of Ni in plants is carried out mainly by root systems via passive diffusion and active transport (Seregin and Kozhevnikova, 2006). Soluble Ni could be absorbed via the Mg ion transport system, due to the similar charge and size ratio of the two ions while secondary active transport of chelated Ni^{2+} is possible, and specifically binded with HoxN (high-affinity nickel transport protein, a permease), metallothionein and metallochaperones. It is transported from roots to shoots and leaves through the transpiration stream via the xylem (Peralta-Videa *et al*., 2002). This essential element is supplied to the meristematic by retranslocation from old to young leaves, and to buds, fruits and seeds, via phloem (Page *et al*., 2006). Exceeding 50% of the Ni absorbed by plants would be retained in the roots, due to sequestration in the cation exchange sites of the walls of xylem parenchyma cells, and immobilization in the vacuoles of roots (Seregin and Kozhevnikova, 2006). However, over 80% of Ni in the roots would present in the vascular cylinder, while less than 20% would present in the cortex. The consensus is that Ni in stems and leaves are mainly located in the vacuoles, cell walls and epidermal trichomes associated with citrate, malate and malonate (Gendre *et al*., 2007), and within cells, the Ni contents of different organelles and cytoplasm may differ substantially.

2.4 Phytotoxicity

2.4.1 Heavy metals-induced phytotoxic in plants

Phytotoxicity of heavy metals, induced by the surpassing critical concentrations of metals ions, is the implication of the imbalance between the uptake of an element and the incapability of the metabolism to cope with the cellular, particular cytosolic concentration. The phytotoxic effects could occur via direct interference on the plant metabolic processes, or indirectly through the substitution of another metal in metalloproteins. These deterioration effects are interplayed between the environmental concentration and chemical speciation of metals, that would govern the uptake, translocation, incorporation, and cellular compartmentation process; as well as the genotypic and species specific features of plants (Ernst, 1996). Phytotoxicity of heavy metals in plants is sequentially defined in the cellular and plant from plant cultivation by submerging the roots in water in 1666, to the first application of mineral nutrient solutions by the German botanists, Julius von Sachs and Wilhelm Knop in 1842 and 1895. The water culture system was firstly proposed for commercialization in 1929, and the term "hydroponics" was coined by Professor William Frederick Gericke to describe the growing of crops with the roots in a liquid medium. However, since 1938, two of the plant nutritionists from the University of California named Dennis R. Hoagland and Daniel I. Arnon had developed nutrition solution named as Hoagland solution, that has been apply for hydroponics cultivation until today. In the 1960s, Allen Cooper from England had successfully developed the Nutrient Film Technique. In recent decades, many companies are strongly working in soilless agriculture globally, including National Aeronautics and Space Administration (NASA), who have conducted extensive hydroponics research for their Controlled Ecological Life Support System (Gruda *et al*., 2004; Savvas *et al*., 2014).

2.6.2 Definition of hydroponic system

The soilless culture is a new cultivation technology that applies nutrient solutions without the soil substrates as the rooting medium, with or without the presence of artificial or natural supporting medium (Bhattarai *et al*., 2008). The specific function of soilless cultivating method is to stimulate the optimum growth of plants, with the provision of well-adjusted supply of irrigation water or nutrient solution, precise compositions of mineral salts, and to the utmost importance, dissolved oxygen, in a well-controlled agricultural environment. The basic concept of soilless application is simple, in such a way that the plant roots are suspended in the moving water with highly saturated oxygen, as enable the absorption of nutrient and oxygen simultaneously to achieve acceleration in growth and development.

In soilless culture, the optimum concentrations and combination of nutrients, consisting of nitrogen, potassium, phosphorus, calcium, magnesium, sulphur, iron, manganese, copper, zinc, molybdenum, boron, chloride, and vitamins in general, is particularly necessary to maximize the crops yield and quality. Furthermore, the basic necessity for the normal survival of plants including water, light, carbon dioxide and oxygen play an important role this new technique of cultivation. As a whole, the art of soilless cultivation is interplayed between the important parameters: temperature, humidity, carbon dioxide levels, light intensity, ventilation, pH of growing medium, and plants genetic make-up (El-Kazzaz and El-Kazzaz, 2017).

Most of the soilless agricultural systems operate automatically to control the amount and supply of water, nutrients and lighting time, based on the requirements of different plants (Hochmuth and Hochmuth, 2011; Resh, 2013). Likewise, different natural or artificial media or substrate provide various particle sizes, shapes, and penetrability; each medium affects the plants and roots differently by retaining water, supporting plants, and making pore space at different rates. The selection of the supporting medium depends on the nature of the plants, species to be cultivated, cultivation phase including germination, rooting or cuttings, plant production, plant breeding, environment, cost and the type of cultivation technique employed (Jones, 1997).

In soilless culture, the substrate replaces the soil as the natural soil is often poorly suited to cultivation, due to chemical constraints of reactions and nutrient availability; physical properties specifically density, structure and water retention capacity; or biological aspects including the presence of pathogens and exhaustion of soil contents, to govern the plant growth in a better way. In order to ensure the plants grown are maximized, the substrate applied must be (i) capable to support the plant by providing adequate air, water and nutrients to the roots; (ii) does not contain pathogens; and (iii) not phytotoxic. A good culture medium should be able to offer the plant with the highest availability of water, or possess a good water retention capacity, but at the same time could ensure sufficient aeration to the roots. There should be a balanced ratio between the microporosity, justified by the substrates that are constituted by pores and able to retain the water at the end of the drainage after it reach to the complete saturation; and the macroporosity, that is porosity free, and all the pores will not retain water and can be filled with air (Di Lorenzo *et al*., 2013).

All the soilless cultivation systems, with or without substrate, have the common features of the distribution of essential nutrients (except carbon) by using a nutrient solution (El-Kazzaz1 and El-Kazzaz, 2017). The principles of mineral nutrition of plants cultivated using soilless cultivation technique are in no difference from those of plants grown on soil. However, the main difference between these two systems is represented by the reduced volume of the substrate and nutrient solution available to each plant, and this justifies the particular management of the nutrient solution in the soilless agriculture. Additionally, one of the most relevant characteristics of the nutrient solutions is the ionic concentration of nutrients, which is usually much greater than that of the circulating solution of the soil culture.

Such high concentrations in soilless cultivation are used to ensure a good nutrient reserve and to simplify the preparation, the control of the electrical conductivity, and the reintegration of the nutrient solution. The composition of the nutrient solution reflects the chemical composition of the cultivated plants rather than that of the circulating solution of the soil. Under these conditions, the plants require less energy to actively remove nutrients. Moreover, the nutrient solutions are generally more concentrated than the circulating solution of the soil as smaller concentrations could cause a situation of deficit, especially in the case of infrequent renewal of the nutrient solution. However, the removal ratios between nutrients and water vary widely in response to different climatic conditions, even on the same day. Hence, higher concentrations of nutrients than those provided by the removal ratio between nutrients and water are often suggested to ensure sufficient availability of all nutrients. The solutions of soil should better buffered by reactions of ion exchange, absorption-desorption, dissociation and precipitation, as well as by the cycle of nutrients and mineralisation of organic matter. The absence of a similar buffering capacity in soilless culture systems requires the use of high concentrations of nutrients. Finally, the nutrient solutions are very often easily prepared with four or five salts to meet the needs of the macroelements; this reduces the possibility of obtaining wider ratios among the concentrations of the elements.

2.6.3 Types of hydroponic cultures

Soilless cultures are divided into two categories, closed soilless culture that involves the framework consisting of recycled dissolved supplements. The concentrations of supplements are monitored and balanced with the adjustment conducted on a weekly basis; and open soilless culture where new dissolved supplements are supplied for every irrigation cycle using dripping irrigation system. In general, open hydroponic system may be less sensitive to salinity of the water than closed systems, but the closed systems are more cost-effective (El-Kazzaz1 and El-Kazzaz, 2017). Currently, with the new evolution of media, tubes, connectors, valves,