INVESTIGATING THE PHYTOREMEDIATION POTENTIAL OF SELECTED SAUDI PLANTS IN REMOVING POLLUTANTS FROM CONTAMINATED WATERS

BY

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To friends and family…

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ASE	Accelerated Solvent Extractor
ATSDR	Agency for Toxic Substances & Disease Registry
BCF	Biological Concentration Factor
CAS	Chemical Abstract System
DNA	Deoxyribonucleic Acid
EMB	Eosine Methylene Blue
DCM	Dichloromethane
GC/MS	Gas Chromatography/Mass Spectroscopy
ICP-OES	Inductively coupled plasma optical emission spectrometry
ITRC	Interstate Technology & Regulatory Council
MENA	Middle East and North Africa
NA	Nutrient Agar
PAHs	Polycyclic Aromatic Hydrocarbons
PBS	Phosphate Buffered Saline

PCBs	Polychlorinated biphenyls
PME	Presidency of Meteorology and Environment
SAHSS	South Australia Health Scientific Services
TDS	Total dissolved solids
TF	Translocation Factor
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

ABSTRACT

This study was carried out to demonstrate the phytoremediation potential of two Saudi plant species - *Bolboschoenus maritimus* and *Phragmites australis*, in removing selected organic and inorganic pollutants in water. Naphthalene was used as a target organic pollutant while cadmium (Cd), lead (Pb) and nickel (Ni) were used as target inorganic pollutants. The experiments were carried out in a hydroponic medium and 5ppm was used as concentration of each of the heavy metals whereas 10ppm was used as the concentration of naphthalene. The mechanisms employed by each of the plants to carry out the phytoremediation were also investigated as well as the microbial community present in the spiked water and rhizosphere of the plants. The results of the study showed that *B. maritimus* had a residual of 1% (99% removal) of cadmium, 6% (94% removal) of lead and 17% (83% removal) of nickel over a 6-week period. *P. australis* on the other hand, had a residual of 7% (93% removal) of cadmium, 5% (95% removal) of lead and 16% (84% removal) of nickel over a 6-week period. The results of the phytoremediation of naphthalene revealed a residual of 4% (96% removal) and 9% (91% removal) for *B. maritimus* and *P. australis* respectively over a period of 6 weeks. The major mechanisms employed by the two plants were probably phytostabilization and rhizodegradation and four distinct bacteria colonies were identified in planted media, namely: *Enterobacter spp., Staphylococcus spp., Pseudomonas spp. and Bacillus spp*.

ملخص الرسالة

الاسم الكامل: **حكيم أولاوالي بيلو**

عنوان الأطروحة: **دراسة إمكانية العلاج النباتي في نباتات سعودية مختارة على إزالة الملوثات من المياه الملوثة**

التخصص: **العلوم البيئية**

التاريخ: مايو 2015م

أجرِيَتْ هذه الدراسة لبيان إمكانيات العلاج النباتي الكامنة في اثنين من الأنواع النباتية السعودية لإزالة ملوِّثات مختارة من بين الحيوية وغير الحيوية في الماء، والنوعان هما: *Bolboschoenus maritimus* و *Phragmites australis*. وقد اسْتُخدِم النَّفْثَالِين كملوث حيوي مستهدف، بينما كانت الكادميوم والرصاص والنيكل مستخدمة كملوثات غير حيوية، وأجريت التجارب بواسطة زراعة النباتات في المائية حيث تم استعمال 5أجزاء لكل مليون (5 ppm) كتركيز لكلٍ من المعادن الثقيلة في حين استخدم 10 أجزاء لكل مليون (10 ppm) كتركيز للنفثالين، وقد تم التحقيق من الآليات التي استخدمتها كل من النباتات للقيام بالعلاج النباتي، وكذلك مجتمع الجراثيم الحاضر في الماء المستعمل والجذور التي تنتمي إليها. وأظهرت نتائج الدراسة أن *B. maritimus* كان لها نسبة متخلفة من 1٪ (أي إزالة نسبة 99٪) من الكادميوم، و6٪ متبقية (أي إزالة نسبة 94٪) من الرصاص، و17٪ (أي إزالة نسبة 83٪) من النيكل على مدى فترة 6 أسابيع؛ أما *P. australis* من ناحية أخرى، فكانت المتبقية 7٪ (إزالة 93٪) من الكادميوم، و5٪ (إزالة 95٪) من الرصاص، و16٪ (إزالة 84٪) من النيكل على مدى فترة 6 أسابيع؛ ونتائج العلاج النباتي من النفثالين كشفت عن المتبقي 4٪ (إزالة 96٪)، و9٪ (إزالة 91٪) لـ*B. maritimus* و*P. Australis* على التوالي خلال فترة ستة أسابيع، وربما كانت الآليتان الرئيسيتان التان استخدمها النباتان هما: الرسوخ النباتي والانحلال الجذري، وتم تحديد أربع جماعات متميزة من البكتيريا في الوسائل المزروعة، وهي: *Pseudomonas spp. Enterobacter spp., Staphylococcus spp.,* و*Bacillus spp.*

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Water is the fuel on which this world operates. More than 70% of the surface of the earth is covered by water and of this, 97.4% is saline – found in oceans and seas; of the 2.6% freshwater, only 0.6% is accessible to man with the rest found in ice caps and glaciers (US Geological Survey, 2014). This makes freshwater the most limiting, important and critical resource of the world upon which all socio-economic and environmental activities depend (UN World Water Assessment Programme, 2009). However, the global freshwater systems are under serious threat by human activities such as urbanization and industrialization (Vörösmarty et al., 2010), growing water demands and high levels of pollution (Radstake & Tuinhof, 2003). The global level of pollution is so high that it was estimated that about 2 million tons of human wastes is discharged into watercourses daily (UN World Water Assessment Programme, 2003).

On a regional scale, the Middle East and North Africa (MENA) region is one of most water-scarce regions in the world, having a regional average of 1,200 cubic meters per person per year as compared to the global average of about 7,000 cubic meters per person per year (Shetty, 2006). The MENA is characterized by arid to hyper-arid conditions, average precipitation of 56 mm/year, and evaporation that may be in excess of 4,000 mm/year

(Abu-Zeid, 2006). The Kingdom of Saudi Arabia is a member country of the region and is by far the largest on the Arabian Peninsula; it lies between the tropical and subtropical desert region with dry wind and great extremes of temperature (FAO-AQUASTAT, 2008). The Kingdom suffers from absolute water scarcity which is compounded by increase in water consumption as a result of population growth which leads to over-exploitation, increase in production activities and household water consumption patterns (Al-Zahrani & Baig, 2011) and contamination of freshwater resources by organic and inorganic pollutants are of serious concern (Freije, 2014). The major sources of water in the Kingdom are the aquifers which are large underground water reservoirs, responsible for more than 90% of water withdrawal with wastewater treatment and seawater desalination providing the rest of the water withdrawal (FAO-AQUASTAT, 2008; Al-Zahrani & Baig, 2011) as shown in *Figure 1*. The majority of the water withdrawn is used for agricultural activities, with municipal and industrial water consumption accounting for small usage percentage (FAO-AQUASTAT, 2008; Al-Zahrani & Baig, 2011) as shown in *Figure 2*.

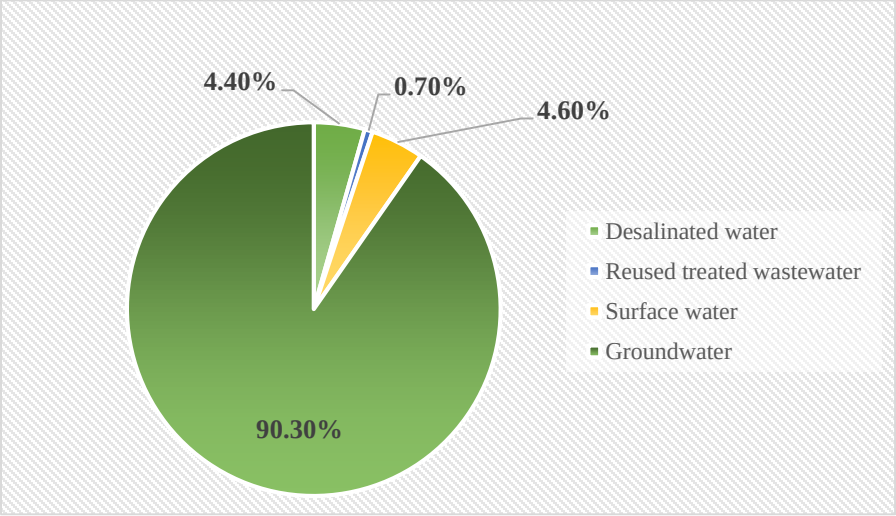


Figure 1: Water withdrawal by source in Saudi Arabia for 2006 (FAO-AQUASTAT, 2008)

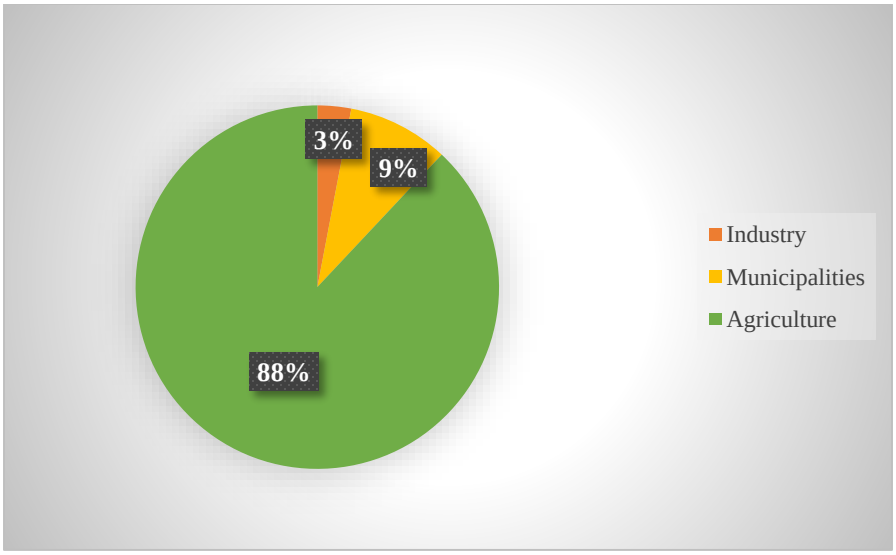


Figure 2: Water withdrawal by sector in Saudi Arabia for 2006 (FAO-AQUASTAT, 2008)

1.2 WATER POLLUTION

Over the past few decades, the global concern over environmental pollution and the resultant impacts it has on public health has increased. It is estimated by the World Health Organization (2005) that a quarter of the diseases affecting mankind today are a result of prolonged exposure to environmental pollution.

The greatest number of illnesses and deaths are attributed to pollution by heavy metals; consequently, heavy metal pollution is of great environmental concern to both developing and developed countries. Apart from heavy metals, which are inorganic pollutants, a number of organic pollutants can have profound effects on the environment and human health, and are thus receiving global attention. Polycyclic Aromatic Hydrocarbons (PAHs) are examples of such organic contaminants.

1.2.1 Heavy Metal

There are several definitions of heavy metal based on atomic number, toxicity or density; however density is used in most cases as the defining factor. Thus, heavy metal is a general name given to a group of metals and metalloids having a specific density in excess of $5g/cm^3$ (Jarup, 2003; Hashim et al., 2011). In all, there are 23 elements known as heavy metals - antimony, arsenic (not a metal but a metalloid), bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium and zinc (Fernández-luqueño et al., 2013); however, not all these elements are of health and environmental concerns.

The main threats to human health from heavy metals are attributable to exposure to lead, cadmium, mercury and arsenic (Jarup, 2003); however, a number of other heavy metals have been implicated in health effects. In order to protect water bodies from heavy metal pollution, there are enforceable regulation standards (as shown in *Table 1*). Heavy metals are hazardous to humans and other life forms, and their presence in the environment can cause soil and water pollution, deterioration of soil structure, destruction of ecological landscapes and decrease in biodiversity.

Table 1: Drinking water quality guidelines (mg/L) for selected heavy metals published by USEPA, WHO and PME

Heavy metal	USEPA[a]	WHO[b]	PME[c]
Arsenic	0.010	0.010	0.005
Cadmium	0.005	0.003	0.005
Chromium (total)	0.100	0.050	0.050
Copper	1.300	2.000	0.050
Lead	0.015	0.010	0.005
Mercury (inorganic)	0.002	0.006	0.001
Nickel	-	0.070	0.050

a: US Environmental Protection Agency, 2009; b: World Health Organization, 2011; c: KSA Presidency of Meteorology and Environment, 2011

1.2.2 Polycyclic Aromatic Hydrocarbons

Polycyclic Aromatic Hydrocarbons (PAHs) are a class of diverse organic compounds containing two or more fused benzene rings and which occur naturally in coal, crude oil and gasoline, and are also produced by incomplete combustion or high-pressure processing of organic products, which enables them to become ubiquitous in the environment (Agency for Toxic Substances & Disease Registry, 2009). Based on their sources, PAHs can be divided into three broad categories – biogenic, petrogenic and pyrogenic PAHs (Thorsen et al., 2004). Biogenic PAHs are produced from natural biological processes, petrogenic

PAHs are produced from petroleum while pyrogenic PAHs are produced as a result of incomplete combustion of fuels.

Certain PAH metabolites interact with DNA (Deoxyribonucleic Acid) and are genotoxic, thereby causing malignancies and heritable genetic damage in humans. Heavy occupational exposure to mixtures of PAHs poses a substantial risk of skin, lung or bladder cancer (Agency for Toxic Substances & Disease Registry, 2009). There are over 100 different chemicals that constitute PAHs, however 16 of them have been identified by the US EPA as target compounds (US Environmental Protection Agency, 1999). *Table 2* gives the name of the PAH target compounds and their physical and chemical properties.

Table 2: Summary of physical-chemical properties of target PAHs at 25°C including molecular formula (MF), Chemical Abstract System (CAS) number, molecular weight (MW) in g/mol, melting point (MP) in ^{0}C, boiling point (BP) in ^{0}C, solubility in water (S) in mg/L and octanol-water partition coefficient (LK)

PAHs	MF	CAS	MW	MP	BP	S	LK
Acenaphthene	$C_{12}H_{10}$	83-32-9	154.21	96.2	277.5	3.80	4.00
Acenaphthylene*	$C_{12}H_8$	208-96-8	152.2	92-93	265-275	3.93	4.07
Anthracene	$C_{14}H_{10}$	120-12-7	178.2	216.2	340	0.045	4.54
Benzo(a)anthracene	$C_{18}H_{12}$	56-55-3	228.3	160	435	0.011	5.91
Benzo(a)pyrene	$C_{20}H_{12}$	50-32-8	252.3	175	495	0.0038	6.04
Benzo(b)fluoranthene*	$C_{20}H_{12}$	205-99-2	252.3	168.3	-	0.0012	6.04
Benzo(g,h,i)perylene*	$C_{22}H_{12}$	191-24-2	276.3	273	550	0.0003	6.5
Benzo(k)fluoranthene*	$C_{20}H_{12}$	207-08-9	252.3	215.7	480	0.0008	6.06
Chrysene	$C_{18}H_{12}$	218-01-9	228.3	255	448	0.002	5.75

Dibenzo(a,h)anthracene	$C_{22}H_{14}$	53-70-1	278.35	267	524	0.0006	6.75
Fluoranthene	$C_{16}H_{10}$	206-44-0	202.3	111	375	0.26	5.22
Fluorene	$C_{13}H_{10}$	86-73-7	166.2	116	295	1.90	4.18
Indeno(1,2,3-cd)pyrene*	$C_{22}H_{12}$	193-39-5	276.3	163.6	530	0.062	6.58
Naphthalene	$C_{10}H_8$	91-20-3	128.19	80.5	218	31.0	3.37
Phenanthrene	$C_{14}H_{10}$	85-01-8	178.2	101	339	1.10	4.57
Pyrene	$C_{16}H_{10}$	129-00-0	202.3	156	360	0.132	5.18

N.B: All information obtained from Mackay & Callcott, (1998) except where otherwise stated

* Information obtained from Agency for Toxic Substances and Disease Registry, (1995)

1.3 TREATMENT OF HEAVY METAL AND PAH-POLLUTED WATER

Heavy metals and PAHs have been a focus of scientific research for decades because of the effects they have on human health and the environment. Since it is almost certain that these substances will be released into the environment, different methods of removal have been developed. Heavy metal pollution can be treated by physicochemical processes, such as precipitation and use of metal chelators, ion exchange, reverse osmosis, coagulation and flocculation (Matthew et al., 2002; Kurniawan et al., 2006; Johnson et al., 2008; Barakat, 2011; Fu & Wang, 2011) and biological processes, such as microbial metal uptake (Sierra-Alvarez et al., 2006), biosorption, activated sludge process, biofilter, anaerobic digestion, stabilization ponds (Dhokpande & Kaware, 2013) and phytoremediation.

PAH-polluted water can be treated using physico-chemical methods such as adsorption processes: e.g. activated carbon; electrochemical treatment; advanced oxidation process e.g. ozonation, ultrasound, fenton, etc.; thermal process (Rubio-Clemente et al., 2014); and biological process such as microbial degradation, e.g. activated sludge (Ayanda, 2014), and phytoremediation.

Though the choice of treatment option depends on the contaminated site, extent of contamination, and other factors, the physicochemical processes are generally more economically costly than the biological processes. The use of phytoremediation as an alternative treatment method offers much more advantages when compared to other methods (Interstate Technology & Regulatory Council, 2009; US Environmental Protection Agency, 2010).

1.4 PHYTOREMEDIATION

Hinchman et al. (1998) defined phytoremediation as "the engineered use of green plants, including grasses, forbs, and woody species, to remove, contain, or render harmless such environmental contaminants as heavy metals, trace elements, organic compounds, and radioactive compounds in soil or water". Cunningham & Ow (1996) recognized the importance of microorganisms in phytoremediation and modified their definition to include plant-associated microorganisms. Schwitzguébel (2001) succinctly defined phytoremediation as "the use of green plants and their associated microorganisms, soil amendments, and agronomic techniques to remove, contain, or render harmless environmental pollutants in soils, groundwater and waste water". Phytoremediation is an

innovative and cost-effective technique of using plants to extract, degrade, contain, or immobilize pollutants in the environment. It can be used for the treatment of a wide range of both organic and inorganic pollutants in air, soil, sediments, sludge, surface water, storm water, groundwater, wastewater, freshwater, salt marshes, and brackish water (Zhu et al., 2004; Interstate Technology & Regulatory Council, 2009; Nwoko, 2010). The organic contaminants that can be treated by phytoremediation include petroleum hydrocarbons, chlorinated compounds, crude oil, persistent organic pollutants, explosive compounds and pesticides while the inorganic contaminants include plant nutrients, heavy metals and metalloids, salinity and radioisotopes (Russell, 2005; Epps, 2006; Hamidov et al., 2007; Interstate Technology & Regulatory Council, 2009; Aisien et al., 2010; Nwoko, 2010; Hammad, 2011).

1.4.1 Mechanisms of Phytoremediation

In using plants for remediation, several mechanisms are often involved which eventually lead to the removal, containment, degradation, conversion or detoxification of the contaminants. These mechanisms include:

i. Phytodegradation/phytotransformation involves the breaking down of contaminants taken up by plants by the enzymes present within the plant tissues. The degradation of contaminants may also occur outside the plants by the release of chemicals by the plants. This mechanism is applicable for the treatment of organic contaminants and inorganic nutrients in soil, sediment, sludge, groundwater and surface water.

ii. Phytoextraction/phytoaccumulation involves the taking up of contaminants by the plant within the transpiration stream, followed by translocation within the plant, which results in the accumulation of such contaminants in the aboveground tissue. This mechanism is most often applied to metal contamination of soil, sediment, sludge, and, to a lesser extent, water.

iii. Rhizodegradation/plant-assisted degradation involves the breaking down of contaminants by microorganisms present in the rhizospheres of plants. These microbes release phytochemicals that enhance the breaking down of contaminants. It is applicable for the treatment of a wide range of organic contaminants in soil, sediment, sludge, and groundwater.

iv. Phytovolatilization involves the uptake and transpiration of contaminants, followed by the subsequent release of the contaminant or its modified form into the atmosphere. This mechanism can occur along with phytodegradation and can be used for the treatment of organic contaminants and inorganic contaminants that can volatilize, such as selenium, mercury and lead, in groundwater, soil, sediment and sludge.

v. Phytostabilization/phytosequestration involves the use of plants to prevent the migration, erosion, leaching and dispersion of contaminants by wind or water by immobilizing such contaminants through absorption and accumulation by roots, adsorption onto roots or precipitation within the rhizospheres of plants. This mechanism is applicable for the treatment of metals in soil, sludge, and sediment.

vi. Rhizofiltration involves the adsorption or precipitation of contaminants onto plant roots or the absorption of contaminants that are in solution into the roots of plant as

a result of biotic or abiotic process. This mechanism is applicable to low-concentration, high-water-content conditions of groundwater, surface water and wastewater and does not work well with soil. It is used for the treatment of metals and radionuclides.

vii. Phytohydraulics involves the use of plants to remove groundwater through consumption and uptake so as to contain the movement of contaminants. It is applicable for the treatment of water-soluble leachable organic and inorganic contaminants in groundwater, surface water and soil water.

(Interstate Technology & Regulatory Council, 2009; US Environmental Protection Agency, 2000, 2010)

1.4.2 Advantages and Limitations of Phytoremediation

Phytoremediation, like all other remediation technologies, has its advantages as well as limitations. One of the major advantages of phytoremediation is that it can be used for a wide array of organic and inorganic contaminants in a broad range of environmental conditions and media (Interstate Technology & Regulatory Council, 2009). In addition, it is cost-effective, having low operation and maintenance cost; it is clean with minimal air emission and waste generation; and, it is sustainable and environmental-friendly. Also, it is solar-powered and does not require additional energy; it can be used in combination with other remediation approaches and can also be used to supplement them (Green & Hoffnagle, 2004; Suresh & Ravishankar, 2004).

However, phytoremediation is limited by depth, area and time. That is, it is limited by depth of penetration of the plant roots and consequently can be used for shallow soil, streams and groundwater; it is limited by area since it requires large surface area for remediation; and it generally takes a longer time than it would take physico-chemical methods and is affected by seasonal changes (Interstate Technology & Regulatory Council, 2009). Some of these limitations have been alleviated with advances in technology. For example, the use of genetic engineering has produced plants with deeper roots (Green & Hoffnagle, 2004).

1.4.3 Concept of Hydroponics and Phytoremediation

Hydroponics is the system of cultivating plants without the use of soil but using mineral solutions in an inert substrate, which include sand, gravel, perlite, vermiculite and rockwool (Hershey, 1994). Hydroponics is a very popular concept in agriculture and biology, and has a very colorful history of development (Hershey, 1994). It is of profound importance in plant biological studies (Asao, 2012) with emphasis on research relating to plant nutrition, plant diseases and plant breeding (Anderson et al., 1989), as well as plant-environment interactions (Mhadhbi, 2012).

The use of hydroponics in plant biological studies has many advantages over soil cultivation, which include (i) higher yields, which could be as much as ten times, (ii) less space requirement, (iii) mineral nutrient re-usability which leads to much lower water need, and (iv) ease of managing and adjusting nutrients to the best growing condition for plants (Anderson et al., 1989). Almost all studies involving phytoremediation of contaminated water are done in hydroponics because the use of hydroponics allows for better

understanding of the mechanisms of contaminant uptake since the potential interactions between the growth medium and elements of interest are eliminated or minimized (Baldwin & Butcher, 2007).

1.5 STATEMENT OF THE PROBLEM

PAHs and heavy metals represent two categories of contaminants that are of serious environmental concern. PAHs include over 100 different chemicals that have varied toxicity and behavior in the environment. They are ever-present in the environment and have both natural sources (forest fires and volcanoes) and anthropogenic sources (burning fuels, refuse, used tires and polystyrene; coke production; motor vehicle exhaust and tobacco smoke) (Valle et al., 2007; Agency for Toxic Substances and Disease Registry, 2009). They are persistent in the environment because they do not burn readily and most of them do not break down easily in water. Long-term exposure to PAHs can cause cataracts, kidney and liver damage, jaundice, increased risk of cancers of the skin, lung, bladder and gastrointestinal tract (South Australia Health Scientific Services, 2009).

Heavy metals are one of the most dangerous contaminants when present in high concentration. They are very persistent in the environment and are hazardous to humans and other biota. Some heavy metals are believed to be carcinogens, mutagens, teratogens and endocrine disruptors, while others are implicated for neurological and behavioral changes (Ali et al., 2013).

Different physical and chemical methods of remediation are available for PAHs and heavy metals. These methods have varied levels of removal and often are too costly. For example, the use of thermal, adsorption, photochemical and physicochemical processes for the treatment of PAHs are effective and fast (Nkansah, 2012), but the high energy demand of these processes are a concern. The physical and chemical methods used for remediation of heavy metals also have their limitations such as high cost, intensive use of labor, alteration of soil properties and disturbance of indigenous microorganisms, and, in some cases, secondary pollution (Ali et al., 2013). Consequently, there is a need for a more environmental-friendly and cost-effective technique for remediation of both organic and inorganic contaminants.

1.6 SIGNIFICANCE OF THE STUDY

Phytoremediation offers an effective, environmental-friendly and relatively cheap technique of contaminant remediation. However, the success of this technique depends on being able to use the ideal plant that has the potential and ability to take up, use or transform the contaminant. This makes the search for an ideal plant one of the most important steps in phytoremediation. Moreover, the application of phytoremediation is site- and plant-specific; that is, a plant that remediates a particular contaminant in a particular location might not perform well at other locations and on other contaminants. Hence, the need to evaluate indigenous plants that are well adapted to the particular location for their phytoremediation potential. This work will be the foundation upon which future field experiments will be based.

1.7 OBJECTIVES

The overall objective of this study is to evaluate the phytoremediation potential of two indigenous plants of Saudi Arabia for removal of selected organic and inorganic pollutants from water. In particular, the objectives of the study include:

- To identify and evaluate the phytoremediation potential of indigenous plants that are well adapted to the location;
- To identify the patterns of accumulation of contaminants in the organs (namely: roots, stems and leaves) of selected plants;
- To identify the mechanisms of contaminant uptake in the tested plants; and
- To identify the microbial communities in each of the tested plants and their roles in the phytoremediation of the contaminants.

CHAPTER 2

LITERATURE REVIEW

2.1 PHYTOREMEDIATION APPROACHES

A great amount of research work involving phytoremediation has been carried out since the 1990s when the phytoremediation-based company *Phytotech Inc.* published the results of its experiments on phytoremediation (Raskin et al., 1997). Schnoor (1997) compiled a list of 15 field locations where phytoremediation was successfully applied for the treatment of both organic and inorganic contaminants. In many of these locations, phytoremediation resulted in more than 90% reduction and removal of the treated contaminants; however, there was a location where more than 95% of the phytoremediation plants died. Gerhardt et al. (2009) were of the opinion that all the success stories of phytoremediation were mostly from the results of laboratory and greenhouse experiments. They maintained that there have been numerous attempts at phytoremediation in the field that were inconclusive and unsuccessful probably due to either (i) the presence of plant stress factors in the field, which are not observed in the laboratory and greenhouse experiments, or (ii) the inadequacy of the current methods of assessing phytoremediation for indicating whether the concentrations of contaminants are actually decreasing. Euliss et al. (2008) reported a similar inconclusive report for a field assessment of phytoremediation for petroleum contaminants and concluded that there might have been a continuous source of contamination during the field study.

When it comes to the field application of phytoremediation, it is evident that there is need for more research in the area of field application of phytoremediation so as to bridge the gap between what is obtained in the laboratory and what is obtained in the field (US Environmental Protection Agency, 2000; Euliss et al., 2008; Gerhardt et al., 2009).

2.2 PHYTOREMEDIATION OF ORGANIC CONTAMINANTS

Organic contaminants are major groups of contaminants that are amenable to treatment by phytoremediation. The uptake and transpiration of organic contaminants by plants depend on the log of octanol-water partition coefficient (log K_{ow}), which is a measure of the affinity of the contaminants for water against lipids. This must be in the optimum range of 0.5 – 3.0 for the contaminant to be transpired by plants (Yu & Gu, 2006). Among the organic contaminants reported to have been treated by phytoremediation are chlorinated solvents, hydrocarbon and other petroleum compounds, explosives and pesticides. Huesemann et al. (2009) reported the use of eelgrass for the removal of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). They claimed that there was about 73% and 60% reduction in the concentration of PAHs and PCBs respectively. O'Niell & Nzengung (2004) reported cases involving the phytoremediation of hydrocarbons and chlorinated solvents and claimed that there was about 85% removal. Oil sludge contaminated soil was reported to have been remediated using rye and alfalfa (Muratova et al., 2008). The organic contaminants of special interest in phytoremediation are PAHs because of their widespread distribution, persistence and effects on the environment and biotas.

The first specific application of grasses for the degradation of toxic and recalcitrant organic chemicals at low concentrations in soil was carried out by Aprill & Sims (1990). In their experiment, they used eight types of prairie grasses for the treatment of four PAHs and reported that the extent of disappearance of PAHs was significant in vegetated soil. Ever since then, many researchers have used grasses to remediate sites contaminated with PAHs (Huang et al., 2004; Lee et al., 2008). Apart from grasses, trees such as hybrid poplar have also been used for the remediation of PAHs (Widdowson et al., 2005). Also, mangroves have been investigated for their potential for phytoremediation of PAHs. Ke et al. (2003) evaluated the removal of pyrene using two mangrove species namely *Kandelia candel* and *Bruguiera gymnorrhiza* with more than 90% removal. In order to evaluate the potential of plants for phytoremediation of PAHs and other organics, it is important to be able to extract these compounds from the plants. The extraction of organic contaminants from different biological matrixes is one of the most important and complex steps in contaminant analysis; and for this purpose, soxhlet extraction, pressurized liquid extraction, supercritical fluid extraction, microwave-assisted extraction, ultrasonic extraction, dispersive liquid-liquid and dispersive solid phase extractions have been used (Pryček et al., 2004; Zitka et al., 2012).

2.3 PHYTOREMEDIATION OF INORGANIC CONTAMINANTS

Some inorganic pollutants are found naturally as elements in the earth's crust while some others are a result of human activities such as mining, smelting, military, agriculture, traffic and industrial activities which promote their release into the environment (Nwoko, 2010).

These inorganic contaminants include salinity (Negri et al., 2003), nutrients such as nitrates and phosphates (Lu et al., 2010), metals, metalloids and radionuclides (McGrath et al., 2002; Eapen et al., 2007; Nwoko, 2010).

Of all these inorganic contaminants, heavy metals are receiving most of the attention of phytoremediation studies. This is partly because of the toxicity of these metals and the threat they pose to humans and other biota as well as the abundance of plants with potential for the removals of these heavy metals (Aisien et al., 2010; Badr et al., 2012). Among the heavy metals that have been remediated by phytoremediation are Cadmium, Chromium, Cobalt, Manganese, Copper, Iron, Nickel, Lead and Zinc (Al-Qahtani, 2012; Al-Dhaibani et al., 2013; Muhammad et al., 2013).

The presence of heavy metals in the soil or water where the plants are growing is believed to be putting considerable pressure on such plants, which develop mechanisms to combat such pressure in return (Baker, 1987; Mganga et al., 2011). Owing to this, plants with the ability to grow on metal-contaminated soils have been classified into three categories namely (i) excluders (ii) indicators (iii) accumulators / hyper-accumulators (Baker, 1981, 1987; Baker & Walker, 1990; Baker et al., 1994). The excluders are those plants that limit within them, the levels of heavy metal movement from roots to shoots and leaves thereby maintaining a relatively low levels of heavy metals within the shoots, though, they can still contain huge amounts of heavy metals in their roots. The indicators are those plants that accumulate heavy metals in their shoots and leaves and these accumulated metals are reflective of the amounts present in the soils. The accumulators are those plants that

accumulate heavy metals in their shoots and leaves in great concentrations such that the amount of metals in their shoots is greater than what is present in the soil.

Different plants have been used for the phytoremediation of heavy metals. Giordani et al. (2005) used seven herbaceous crops for the remediation of soil polluted by nickel and found spinach to be the most efficient accumulator of nickel. Cullaj et al. (2004) also investigated the potential of several plants for phytoremediation of nickel-contaminated soil. They collected 145 different plants and investigated them for nickel phytoextraction; they discovered only 16 of them to be hyperaccumulators. Lorestani et al. (2011) evaluated the potential of indigenous plants for phytoextraction and phytostabilization of heavy metals. They concluded that none of the collected plants was suitable for phytoextraction of heavy metals; however, they found *Euphorbia macroclada* to be most efficient in phytostabilization of copper (Cu) and iron (Fe), whereas *Ziziphora clinopodioides*, *Cousinia sp.* and *Chenopodium botrys* were most suitable for phytostabilization of zinc (Zn) while *Chondrila juncea* and *Stipa barbata* were suitable for phytostabilization of manganese (Mn).

In a similar work, Nouri et al., (2011) examined the potential of twelve indigenous plants for phytoextraction and phytostabilization of heavy metals. They concluded that *Scrophularia scoparia* was most efficient for phytostabilization of lead (Pb) whereas *Centaurea virgata*, *Echinophora platyloba* and *Scariola orientalis* were suitable for phytostabilization of zinc (Zn) while *Centaurea virgata* and *Cirsium congestum* were the most efficient in phytostabilization of manganese (Mn). Nouri et al., (2011) based their decision on the measurement of Biological Concentration Factor (BCF) and Translocation

Factor (TF) and maintained that Plants with a high BCF and low TF could be suitable for phytostabilization whereas plants with BCF and TF values both greater than one could be used for phytoextraction. Eissa et al. (2011) studied the accumulation of Cd, Pb and Ni by three halophyte species namely *Atriplex amnicola, A. undulate* and *A. lentiformis* and found significant differences in heavy metals concentration and transportation from the roots to the shoots among the studied species. They concluded that *A. lentiformis* could be more effective in the phytoextraction of Cd from the contaminated soils. Mojiri (2011) investigated the potential of corn for phytoremediation of soil contaminated with Cd and Pb and indicated corn to be an effective accumulator of these metals.

2.4 THE USE OF NATIVE PLANTS IN PHYTOREMEDIATION

There has been an increasing global focus on biological invasions since the 1980s (Pyšek et al., 2004) with resultant growing concern over the invasion of natural habitats by non-native plant species as exemplified by increasing number of cases and studies on the topic of plant invasion (Pyšek et al., 1995). In fact, the impact of biological invasions on the global economy, native or indigenous species, the ecosystem and biodiversity has been very significant (Sharma et al., 2005; Charles & Dukes, 2007). Pejchar and Mooney (2009) were of the opinion that although the impacts of invasive species on native species are well documented, the various ways by which such invasive species affect ecosystem services are still unfolding.

There are many examples of invasive plant species that have profound negative effects on the native plants and ecosystems, such as Eurasian water milfoil and Kudzu (US

Environmental Protection Agency, 2000). Smith and Barko (1990) described Eurasian water milfoil as one of the most troublesome invasive plants in North America. Kudzu on the other hand has invaded the South-eastern United States, causing serious damage to the area's ecosystem. However, some invasive plants have positive effects; such as the case with rice, wheat, maize, potato, soybean, barley, cassava, oats and sugarcane that provide over 70% of the world's food and are grown outside of their native places (Sharma et al., 2005).

As a result of the impacts of invasive plants on native plants and ecosystems, native plants are most desirable in phytoremediation studies, though plants that are most effective for remediating a particular contaminant may or may not be native to that location. The use of non-native plant species for phytoremediation studies are encouraged only under certain circumstances, such as (i) the plants have already been introduced and are now common, such that their use would not cause any risk to the native ecosystem, (ii) it is not possible for the plants to be successfully propagated in the wild, and/or (iii) plants that have been genetically altered to be harmless are introduced (US Environmental Protection Agency, 2000).

Consequently, there have been continuous efforts in all parts of the world to find indigenous plant species that are most suitable for the remediation of contaminants of concern. For examples, Sasmaz and Sasmaz (2009) evaluated the potential of three indigenous plants of Turkey growing in a mining area. Nouri et al., (2011) examined the potential of 12 indigenous plants growing in the surrounding of Ahangaran mine in Iran while Lorestani et al., (2011) examined the potential of 17 native species growing around

Hame Kasi mine in Iran.. The phytoremediation potential of five native plants growing on mine tailings in Arizona, USA was also investigated (Haque, 2008) and that of another five native plants was investigated in California (Devinny et al., 2005). Anh et al., (2011) evaluated the potential of 33 indigenous plants in Thai Nguyen Province of Vietnam. In this regard, the kingdom of Saudi Arabia is not left behind. Al-Zahrani and Hajar (2014) examined the phytoremediation potential of five native plants growing at industrial area of Jeddah whereas Badr et al., (2012) and Al-Qahtani (2012) evaluated the potential of seven native plants from industrial city in Riyadh. Also, Al-Taisan (2009) examined the suitability of using two native plants for phytoremediation in an industrial area of Dammam.

2.4.1 Phytoremediation Potential of *Phragmites australis* (Cav.) Trin. ex Steud

Phragmites australis (common reed) is a large perennial grass (family Poaceae) that can grow to a height of 5 meters (15 feet), and has an extensive system of scaly rhizomes and stolons (Magee, 2005; Swearingen & Saltonstall, 2010; Tilley & St. John, 2012). It is extremely widespread and found abundantly almost throughout the world, with ability to exploit man-made habitats. It has a wide range distribution, occurring from north-west Europe through central and southern Europe to North Africa and Southern Africa through Russia and the Middle East to the Far East and South-east Asia to Australia, and it is native to over 260 countries including Saudi Arabia (Lansdown, 2013). Common reed is adapted to a wide range of soil conditions and can thrive in marshes, tidal and non-tidal wetlands, and along lakes and rivers. It can tolerate anaerobic conditions in soil, variety of nutrient conditions and can survive a pH range of 3.7 to 8.7 (Tilley & St. John, 2012).

Though, *P. australis* is considered a weed in some places, it has many uses which include (i) provision of high quality forage for cattle and horses (ii) used for lattices in constructing adobe houses and hunting blinds (iii) its stems have been used for making arrow shafts, weaving mats, baskets, carrying nets, flutes and rafts (iv) used medicinally to treat diarrhea, and made into a poultice to treat boils and has been used as antiasthmatic, antidote, antiemetic, antitussive, depurative, diuretic, febrifuge, lithontripic, refrigerant, sialagogue, stomachic, and styptic (v) used for erosion control and wetland restoration (Magee, 2005; Swearingen & Saltonstall, 2010; Tilley & St. John, 2012; Lansdown, 2013). Common reed has also been implicated to have potential for the treatment of different contaminants. Furthermore, *P. australis* provides important habitat for many native species including invertebrates, fish, birds and small mammals.

P. australis is one of the most commonly used plant species for the treatment of wastewater in constructed wetlands (Vymazal & Krőpfelová, 2005). A constructed wetland is a planted artificial swamp area and a complex biological system that utilizes the interaction of vascular plants, saturated substrates and microorganisms to treat wastewater (Todorovics et al., 2005; Mothes et al., 2010). Apart from its use in constructed wetland, *P. australis* is among the plant species that are commonly found growing naturally in a contaminated environment such as industrial and mining areas. Globally, many researchers have assessed common reeds growing in such environments for the presence of contaminants (Al-Taisan, 2009; Bonanno, 2011; Ebrahimi et al., 2011; Al-Qahtani, 2012; Badr et al., 2012; Ashraf et al., 2012, 2013; Bonanno, 2013; Ibrahim et al., 2013). Some researchers have also tried to assess the potential of common reed under greenhouse, pot and hydroponic conditions

and have suggested its use for phytoremediation in singly-contaminated media but not in co-contaminated media (Ait Ali et al., 2004; Fellet et al., 2007; Goudarzi & Afrous, 2012; Hechmi et al., 2014).

2.4.2 Phytoremediation Potential of *Bolboschoenus maritimus* (L.) Palla

Bolboschoenus maritimus (syn *Schoenoplectus maritimus* (L.) Lye; *Scirpus maritimus* L.) is a cosmopolitan, rhizomatous perennial sedge (family Cyperaceae) widely distributed in temperate and tropical regions and native to over 100 countries including Saudi Arabia (Kumar et al., 2013). It can grow to a height of 1.5 meters, with erect stems and flowers borne in sessile spikelets, densely clustered at the tip of the stems (Hroudová et al., 2007; Tilley, 2012). It is found growing in ponds, brackish and salt water lagoons, back mangroves and margins of salt marshes, and mud flats (Tilley, 2012; Kumar et al., 2013).

Among the uses to which *B. maritimus* is put include (i) source of food, e.g. seeds and rhizomes can be ground into flour and baked into bread (ii) the leaves are used for making mats (iii) the roots have been used as an astringent and diuretic (iv) erosion control, land rehabilitation and wastewater treatment by constructed wetlands (Tilley, 2012; Kumar et al., 2013). Owing to the presence of many bacteria, the plant has been implicated to have potential for phytoremediation.

B. maritimus has been used for wastewater treatment in a constructed wetland (Bragato et al., 2006; Ganjo & Khwakaram, 2010). It has also been evaluated for its potential to remediate both organic and inorganic contaminants (Couto et al., 2011, 2012; Fellet et al., 2007; Shuping et al., 2010; Marques et al., 2011; Goudarzi & Afrous, 2012).

A review of the available literature on *P. australis* and *B. maritimus* revealed a common trend in most of the phytoremediation studies carried out in Saudi Arabia. Most studies were conducted to assess the potential of plants already growing on contaminated areas. There is therefore the need to evaluate the potential of these plants when freshly grown in contaminated water. In this study, the potential of *P. australis* and *B. maritimus* for phytoremediation in contaminated water was investigated.

CHAPTER 3

METHODOLOGY

3.1 SITE DESCRIPTION AND SAMPLE LOCATION

This study was conducted on a bench-scale at the ground floor of *Building 26* in King Fahd University of Petroleum and Minerals (KFUPM), Dhahran, Saudi Arabia. This location was selected because it provided protection against heavy sandstorm that was prominent at the time of the experiment and at the same time, it also afforded the plants sufficient sunlight. Two selected indigenous plant species, namely (i) *Phragmites australis* (Cav.) Trin. ex Steud, a large perennial grass; and (ii) *Bolboschoenus maritimus* (L.) Palla (syn *Schoenoplectus maritimus* (L.) Lye; *Scirpus maritimus* L.), a rhizomatous perennial sedge, were collected from Al-Asfar Lake, Al-Hassa in the Eastern Province of the Kingdom (N 25.53637^{o} E 49.79899^{o}, N25.53938^{0} E49.82228^{0}).

Lake Al-Asfar (*Figure 3*), located in Al-Hassa oasis, is one of the most important wetlands in Saudi Arabia and it is a large man-made freshwater habitat that is formed by run-off from Al-Hassa oasis and sewage effluent from Al-Hofouf, Abqaiq and other neighboring small towns (Youssef et al., 2009). The lake is characterized by many vegetation communities such as wetland vegetation, sabkha vegetation and dune vegetation (Al-Sheikh & Fathi, 2010). Consequently, Birdlife International designates the lake as one of Saudi Arabia's 39 Important Bird Areas.

Figure 3: Lake Al-Asfar showing the locations from which samples were collected

3.2 PLANT ESTABLISHMENT AND HYDROPONIC SET UP

The two plant species were removed from Al-Asfar Lake by uprooting them using a stainless steel shovel and sickle to avoid contamination. Once removed, plant samples were

put in clean plastic bags for transportation. Water samples from the lake were collected using clean 2-L polyethylene bottles for analysis to determine the amount of the contaminants present in the growing media. The plants were first potted for a period of two weeks so as to establish them in the new environment before being transplanted into prepared hydroponic media. The hydroponic system was manually designed and set up as shown in *Figure 4*.

Figure 4: A deep-water culture hydroponic set up showing the air pump, net pot, air tube, growing media and transplanted growing plants

The type of hydroponic system used was a Deep Water Culture, which consisted of the following components:

i. A container that held nutrient solution
ii. A net pot that held the plants and allowed the plants' roots to go into the nutrient solution
iii. An air pump for aeration
iv. An air tube for connecting the air pump to the container
v. Gravel used for the growing medium for supporting the plants
vi. Nutrient solution constituted using inorganic salts

3.3 PREPARATION OF NUTRIENT SOLUTIONS

The nutrient solution for the hydroponic system was constituted based on Hoagland's solution (Hoagland & Arnon, 1950) and was modified by replacing iron tartrate, which was not available, with iron nitrate. The solution contained all the essential elements needed for plant growth and was a mixture of macroelements and microelements. The macroelement stocks were prepared as follows:

a. 1.0 M KH_2PO_4 and using 1 ml/liter of nutrient solution
b. 1.0 M KNO_3 and using 6 ml/liter of nutrient solution
c. 1.0 M $Ca(NO_3)_2$ and using 4 ml/liter of nutrient solution
d. 1.0 M $MgSO_4$ and using 2 ml/liter of nutrient solution

The microelement stocks were prepared by mixing the following:

a. 0.286% H_3B0_3,
b. 0.181% $MnCl_2.4H_20$,
c. 0.022 % $ZnSO_4.7H_20$,

d. 0.008% $CuSO_4.5H_20$,

e. 0.002% $H_2MoO_4.H_2O$,

f. 0.5% $Fe(NO_3)_2$; and using 1 ml/liter of the entire micronutrient mixture.

3.4 PREPARATION OF CONTAMINANT SOLUTIONS

Naphthalene solution was prepared by dissolving naphthalene crystals in n-Hexane. To prepare 10 ppm, 0.1 gm was dissolved in 10 ml of n-hexane. The resulting solution was diluted by taking 1 ml and making it up to 10 ml using n-hexane. This was further diluted two more times to get the final concentration. Cadmium, lead and nickel solutions were prepared by dissolving the respective solid salts in water. To prepare 5 ppm, 5 mg of each salt ($CdCl_2$, $PbCl_2$ and $NiCl_2$) was dissolved in one liter of water. The water used for all sample preparation was obtained from a Milli-Q Direct water purification system by Millipore Corporation.

3.5 TREATMENT OF PAH- AND HEAVY METAL-SPIKED WATER

The experiment was carried out in a locally-constructed hydroponic system. Each hydroponic system consisted of a 2.25 liter bottle which served as the container for holding the nutrient solution, a net pot for holding the plants, an air pump and connecting air tube for aeration, gravel which served as the growing medium and nutrient solution. In each container was put one liter of water to which macronutrients, micronutrients and contaminants were added. Inside the container was placed an air tube which was connected to an air pump and on the container was placed the net pot which held the plants. The plants

were supported by the addition of gravel. Each experiment was set up as described for both the *P. australis* and *B. maritimus*. Also set up was a control experiment which had all the experimental variables except the plants.

After the completion of the setup, samples were taken from the solution on that same day to represent time t_0. After this, the experiment was left to run for a period of 6 weeks with samples being taken from the solution every two weeks to represent times t_1, t_2, and t_3. The solution containing the nutrients and contaminants was replenished as at when due by adding already prepared stock solutions of nutrients plus contaminants. Samples for heavy metal experiment was put in plastic vials whereas the samples for the PAH experiment were kept in glass vials. The samples were kept at 4^0C until analysis.

3.6 MICROBIOLOGICAL INVESTIGATION

In order to understand the roles played by bacteria, both bacteria found in the growing media and endophytic bacteria (bacteria found within the plant intracellular tissues) were studied. The bacteria growth medium used for culturing was Nutrient Agar (NA), which was prepared by dissolving 28 gm of powder NA in 1 litre of distilled water, followed by stirring and mixing until all powder was completely dissolved. After that, the prepared medium was autoclaved in Astell® Autoclave for 4 hours at a chamber temperature of 120^0C and pressure of 0.03 Bar. At the end of the 4 hours, the medium was brought out and aseptically poured into sterile petri dishes in the presence of a flame. The petri dishes were then allowed to cool overnight before they were used for inoculation.

3.6.1 Inoculation and identification of bacteria in the growing media

Samples of the growing media which contain water, plant nutrients and the various contaminants were taken on regular bases to be used for inoculation and identification of bacteria. 0.1 ml of the sample was aseptically spread onto NA medium plates in the presence of a flame. Serial dilution of the sample was also done by putting 1 ml of the sample into 9 ml of prepared Phosphate Buffered Saline (PBS) solution to make a dilution factor of 10. From this dilution was taken another 1 ml which was made to 10 ml by PBS and a dilution factor of 10^2. This approach was repeated up to the dilution factor of 10^3 was reached. From each of the dilution tubes of 10, 10^2, 10^3, 0.1 ml of the aliquot was spread onto the NA medium plates. At the end of the inoculation, all petri plates were put in an incubator at 37^0C for 24 hours (Figure 5), after which they were taken out and the bacteria colonies were counted using Stuart Scientific Colony Counter (Figure 6).

Figure 5: Incubator set at 37^0C and showing the inoculated petri dishes

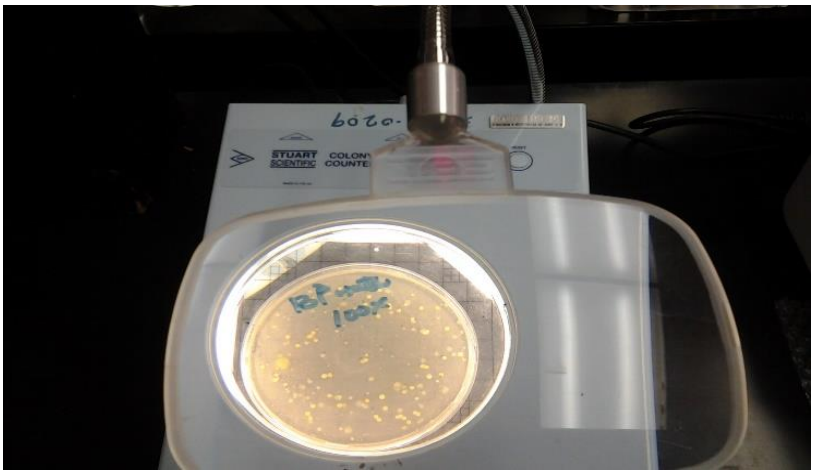


Figure 6: Stuart Scientific Bacteria Colony Counter showing the bacteria colonies to be counted

Each colony was then pure cultured on NA plates by quadrant streak method. The morphological characteristics such as color, size and edge of the colonies were determined on three different agar medium plates, namely: NA, MacConkey agar and Eosine Methylene Blue (EMB) agar plates.

The pure isolated colonies were streaked on the MacConkey agar and EMB agar plates for the determination of lactose fermentation and biochemical tests were carried out to distinguish the biochemically related bacterial genera from other group of bacteria based on their abilities to produce various biochemical products and utilize different energy source. The overall tests carried out are Indole, Methyl Red, Voges-Proskauer, Carbohydrate fermentation, catalase and oxidase tests.

3.6.2 Inoculation and identification of endophytic bacteria

In order to inoculate the endophytic bacteria, the plant materials were treated differently compared to the way the growing medium was treated. Small parts from each of the plants' roots (Figure 7) were taken in preparation for the isolation of endophytic bacteria. The collected root parts were surfaced sterilized using 70% ethanol for 30 seconds and 2% sodium hypochlorite for 5 minutes. They were then washed two times with sterilized distilled water and cut aseptically into smaller sections (Zinniel et al., 2002). These were then crushed and homogenized and put in PBS to make a solution. The resulting solution was treated like the samples taken from the growing plant media and appropriate dilutions were made for inoculation, which was followed by identification.

Figure 7: A net pot showing the plant's root used for the isolation of endophytic bacteria

3.7 EXTRACTION AND ANALYSIS OF PAHs

At the end of each cycle of sample collection, samples containing naphthalene collected from the growing water media were extracted and concentrated in preparation for analysis.

3.7.1 Extraction of naphthalene from growing media

Naphthalene was extracted from the liquid media using liquid-liquid extraction. The extraction was done using a mixture of *n*-hexane and dichloromethane (DCM) in the ratio of 1:1, and the whole extraction process was as follows: 40 ml of the sample was put in a 125 ml separatory funnel and 5 ml of 1:1 *n*-hexane-DCM mixture was added. The funnel was rigorously shaken for 2 minutes and then was left for 5 minutes. Two different layers were observed, with organic layer on top of the water layer. The water layer was drained into a beaker and the organic layer was collected. The collected water was later put back into the funnel and the whole process was repeated two more times. The whole extraction process was done in a fume hood and the organic extract collected was evaporated to about 1 ml.

3.7.2 Extraction of naphthalene from plant materials

The harvested plants collected at the end of the experiment was also subjected to an extraction process. This was done to assess the amount of the naphthalene absorbed by the plants. The plants were first separated into roots, shoots and leaves and these parts were then left in a fume hood for about a week to dry. After having dried, each part was separately ground using a mortal and a pestle, and this was followed by sieving using a

size 500 µm mesh opening sieve. The sieved plant parts were then extracted using an Accelerated Solvent Extractor (ASE 200) by DIONEX as shown in Figure 8.

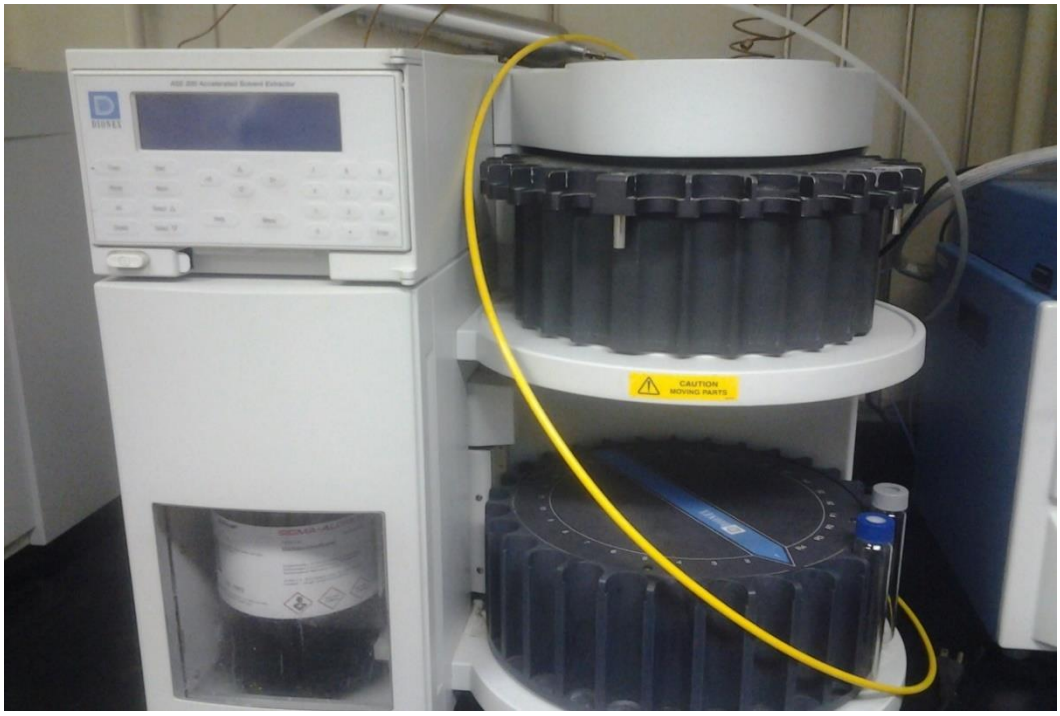


Figure 8: ASE 200 used for extraction of PAHs from plant materials

3.7.3 Analysis of Naphthalene

The extracted naphthalene was analyzed using a Thermo Scientific Trace GC Ultra Gas Chromatography/Mass Spectroscopy (GC/MS) system (Figure 9). This GC/MS was coupled with an ISQ single quadrupole mass spectrometer and combined with a TriPlus Autosampler and Head Space. The column used was the Supelco® low bleed SLB-5ms having a length of 30 meters, internal diameter of 0.25 mm and thickness of 0.25 µm. the carrier gas was helium at a constant rate of 0.9 ml/min. the column temperature was

programmed at 50 ^{0}C and held for 1 minute; then it was ramped at 10^{0}C/minute to 325 ^{0}C and held for 5 minutes. The injection temperature was 300 ^{0}C and sample was injected at 1 µl splitless.

Figure 9: Thermo Scientific Trace GC Ultra Gas Chromatography/Mass Spectroscopy

3.8 ANALYSIS OF CADMIUM, LEAD AND NICKEL

The samples collected from the growing water media were analyzed for cadmium, lead and nickel. Since the types of water used were distilled water and groundwater, there was no need for sample digestion. However in the case of harvested plants, there was the requirement of acid digestion.

3.8.1 Digestion of Plant Materials

Harvested plant samples were separated into leaves, shoots and roots before they were oven-dried at 75^{o}C for 48 hours. After the thorough drying, the separate plant parts were ground using mortar and pestle and each part was put in a plain zipper bag. Plant digestion was done using aqua regia hot plate digestion method (Chen & Ma, 2001). Aqua regia is an acid mixture formed by adding concentrated HNO_3 to concentrated HCl in a ratio of 1:3 by volume. In this method, the digestion was performed in a 150 ml beaker covered with watch glass for refluxing. 0.5 gm of each ground sample was digested in 12 ml of aqua regia which was placed on a hotplate for 3 hours at 110 ^{0}C. After the sample had been evaporated to near dryness, it was diluted with 20 ml of 2% v/v HNO_3 solution; filtered using Whatman 42 filter paper; transferred into a 100 ml flask and diluted to 50 ml with distilled water.

3.8.2 Analysis of cadmium, lead and nickel

Analysis of cadmium, lead and nickel was done using Perkin Elmer Optima™ 8000 inductively coupled plasma optical emission spectrometry (ICP-OES) (*Figure 10*). The experimental conditions used are shown in *Table 3*.

Figure 10: Perking Elmer Optima 8000 ICP-OES for analysis of cadmium, lead and nickel

Table 3: Experimental conditions of Optima™ 8000 ICP-OES

Parameters	**Values**
Plasma gas flow	8 L/min
Auxiliary gas flow	0.4 L/min
Nebulizer gas flow	0.6 L/min
Peristaltic pump flow rate	1.5 mL/min
RF power	1500 Watts
Viewing height	15 mm
Purge Flow	High
Read parameters	Auto
Processing Peak	Area/Height
Calibration	Linear Calculated Intercept
Plasma view	Axial
Spray Chamber	Cyclonic Glass
Nebulizer	Concentric Glass, MEINHAR
Injector	Alumina 2.0 mm i.d.
Quartz torch	1-slot

3.9 SHORT DURATION PHYTOREMEDIATION EXPERIMENT

Another phytoremediation experiment was carried out using one of the two selected plants, *P. australis*, to investigate the pattern of removal of the selected heavy metal within a period of two weeks. All experimental procedures that were carried out during the first experiment were repeated for this experiment. However, samples were taken every three days over a period of two weeks and only heavy metals were studied. At the end of the experiment, the collected samples were analyzed for cadmium, lead and nickel.

3.9.1 Assessment of Heavy metal concentration in plant's parts

This activity was carried out in order to assess how much of each contaminant was present in each of the root, shoot and leaf. Also, Biological Concentration Factor (BCF) and Translocation Factor (TF) were calculated using the following formulae:

$$BCF = \frac{Total\ metal\ concentration\ in\ plant}{metal\ concentration\ in\ solution} \qquad \text{..... Equation 1}$$

$$TF = \frac{metal\ concentration\ in\ shoot}{metal\ concentration\ in\ root} \qquad \text{..... Equation 2}$$

3.9.2 Statistical Analysis

The data obtained from the various experiments were subjected to Analysis of Variance (ANOVA) using the Microsoft Excel 2013 Data Analysis Plug-in and SigmaPlot version 11.0. A two-factor ANOVA was run at confidence level ($\alpha = 0.05$) of 95% to (i) ascertain

if there was significant difference between the phytoremediation ability of *B. maritimus* and *P. australis*, (ii) ascertain if there was significant difference between the contaminants, and (iii) determine if there was significant interaction between each of these plants and the contaminants.

CHAPTER 4

RESULTS AND DISCUSSION

4. 1 PHYTOREMEDIATION OF HEAVY METAL

The remediation of cadmium, lead and nickel by *B. maritimus* and *P. australis* as well as the effects of both pH and salinity on the remediation of these metals was studied.

4.1.1 Remediation of cadmium, lead and nickel by *B. maritimus* and *P. australis*

This experiment was carried out to determine the ability of *B. maritimus* and *P. australis* to remove cadmium, lead and nickel from spiked distilled water.

Figure 11 shows the results of the experiment involving *B. maritimus* and *P. australis* for the remediation of cadmium. A residual of 6% (94% removal) of cadmium remained in just 2 weeks in *B. maritimus* experiment and this was reduced to 1% (99% removal) by the end of the experiment. In *P. australis* experiment, a residual of 8% (92% removal) remained in 2 weeks and this was maintained at 7% (93% removal) for 6 weeks. In the control experiment, a maximum removal of 4% was observed over 6 weeks.

The results of the experiment for the removal of lead by the two plants are shown in *Figure 12*. A residual of 6% lead remained after 2 weeks in *B. maritimus* experiment which was maintained till the end of the experiment. In *P. australis* experiment, a residual of 6% lead

remained in 2 weeks and this was reduced to 5% by the end of 6 weeks. The control experiment revealed a residual of 96% lead by the end of the experiment.

The results of the experiment for the removal of nickel by the two plants are shown in *Figure 13*. A residual of 43% nickel remained after 2 weeks in *B. maritimus* experiment and this was reduced to 17% by the end of the experiment. In *P. australis* experiment, a residual of 43% lead remained in 2 weeks and this was reduced to 16% by the end of 6 weeks. The control experiment revealed a residual of 80% nickel by the end of the experiment.

Overall, it can be inferred that the two plants were effective for the remediation of cadmium and lead in just 2 weeks (94% removal of Cd and 92% removal of Pb by *B. maritimus*; 94% removal of both Cd and Pb by *P. australis*); however, the two plants were not that effective for nickel (57% removal in 2 weeks and 83% removal in 6 weeks). This implies that the two plants are excellent candidates for phytoremediation of cadmium and lead in hydroponic system. This enhanced performance is probably due to the abundance of air supplied to the roots of the plants, which allowed them to perform optimally. Though the two plants showed a slight difference in performance for the three contaminants, the results of analysis of variance (*Table 4*) however revealed that there was no significant difference between the performances of *B. maritimus* and *P. australis* for the remediation of cadmium, lead and nickel and that the interaction between the plants and the contaminants was also not significant. In order words, the two plants behaved exactly the same way for the remediation of the three contaminants.

Table 4: Analysis of variance table showing the significant difference between B. maritimus and P. australis for the remediation of cadmium, lead and nickel

Parameters	Mean values of percent residual		
	Cd	Pb	Ni
B. maritimus	4.5	4.9	9.1
P. australis	7.4	4.6	13.4
Control	92.5**	92.5**	81.0**

** means highly significant difference between any two rows

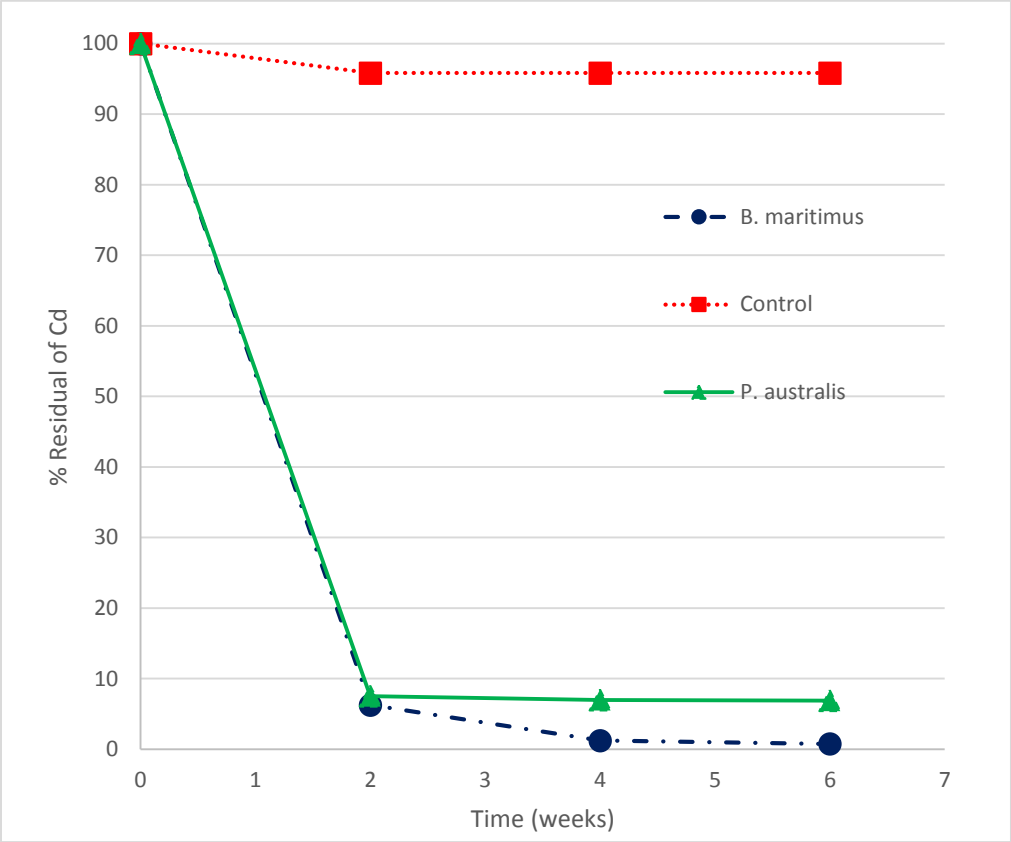


Figure 11: Removal of Cadmium in distilled water experiment at pH7 by B. maritimus and P. australis over a 6-week period

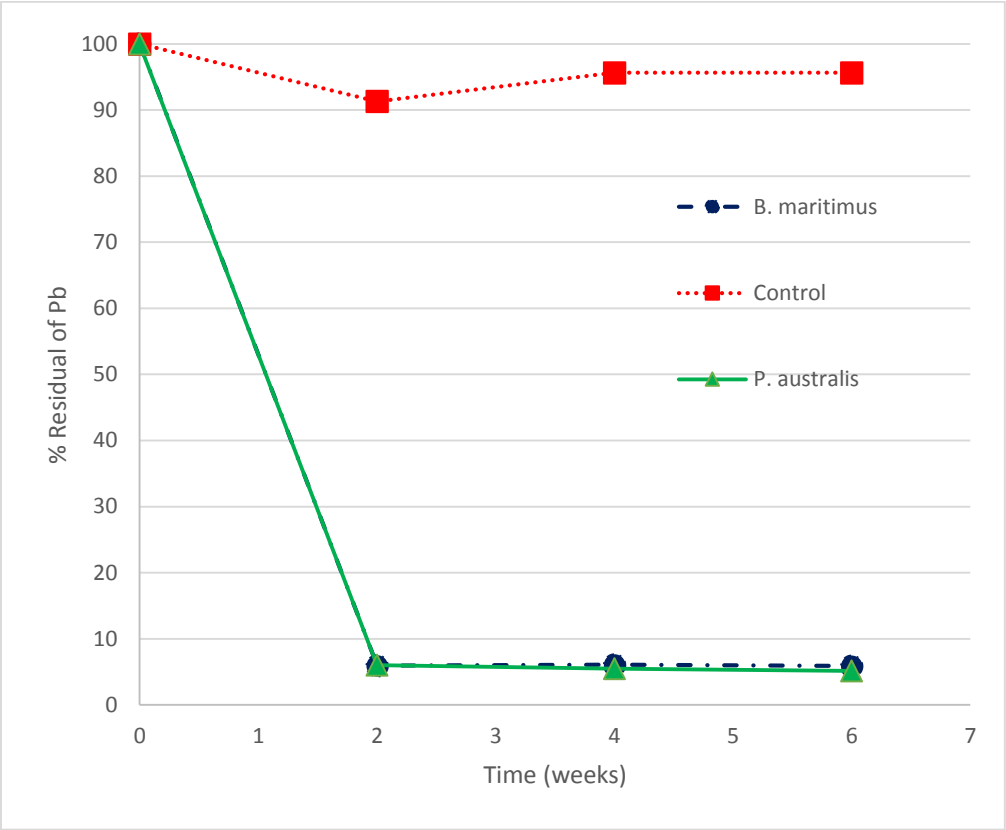


Figure 12: Removal of Lead in distilled water experiment at pH7 by B. maritimus and P. australis over a 6-week period

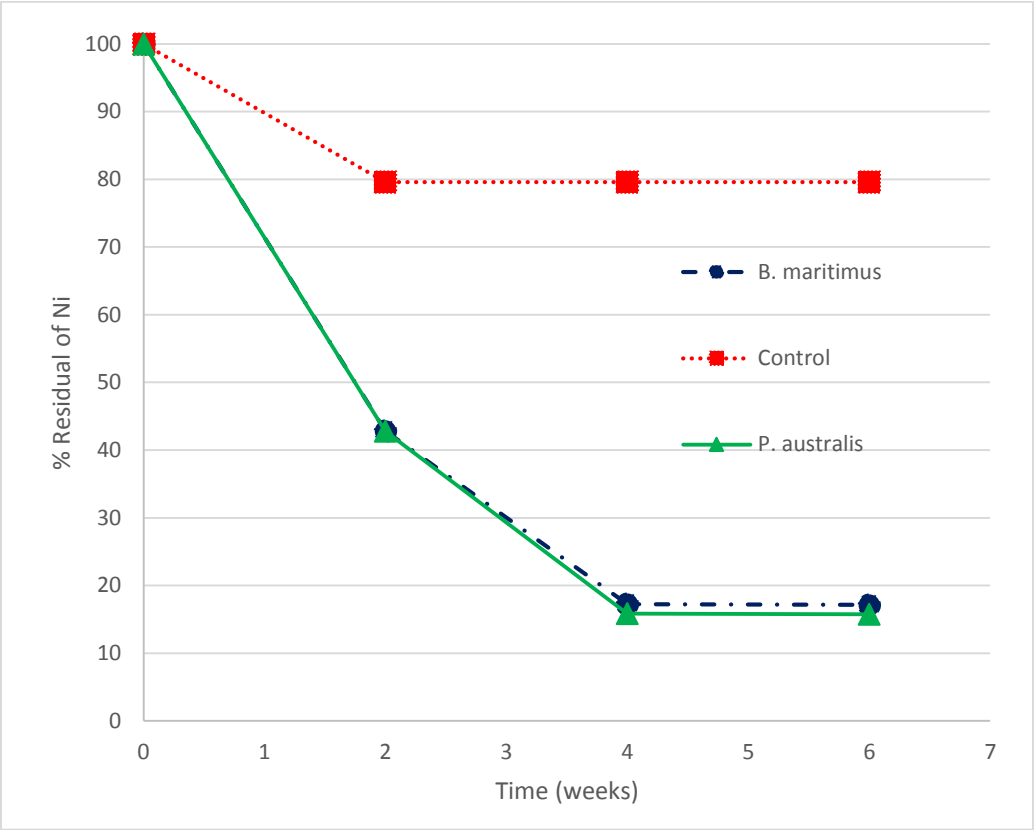


Figure 13: Removal of Nickel in distilled water experiment at pH7 by B. maritimus and P. australis over a 6-week period

4.1.2 Effect of pH on the phytoremediation of cadmium, lead and nickel

This experiment was carried out to determine how pH affected the remediation of cadmium, lead and nickel by *B. maritimus* and *P. australis*. Three different pHs of 4, 7 and 10 were used for this study.

Figure 14 and *Figure 15* show the effect of pH on the remediation of cadmium by *B. maritimus* and *P. australis* respectively. At all pH conditions for the two plants, there was excellent remediation of cadmium in the planted experiment as against the control experiment. For the effect of pH on the removal of cadmium by *B. maritimus* (*Figure 14*), there was a residual of 1% (99% removal) at pH4; a residual of 6% (94% removal) at pH7; and a residual of 11% (89% removal) at pH10 in two weeks. Also for the effect of pH on the removal of cadmium by *P. australis* experiment (*Figure 15*), there was a residual of 8% (92% removal) at pH4; a residual of 8% (92% removal) at pH7; and a residual of 11% (89% removal) at pH10. These results show that at pH10, there was slightly less removal of cadmium when compared to pH4 and pH7. This implies that the two plants preferred an acidic to neutral condition for cadmium remediation.

The effect of pH on the remediation of lead by *B. maritimus and P. australis* is shown in *Figure 16* and *Figure 17* respectively. At pH 4, a residual of 12% lead was achieved by *B. maritimus* in two weeks while a residual of 6% lead was achieved by *P. australis* in two weeks. The final residual left by the end of the 6 weeks at pH4 by *B. maritimus* and *P. australis* were 6% and 4% respectively. At pH7, a residual of 6% lead was left in two weeks by both *B. maritimus* and *P. australis*. The final residual lead that remained at pH7

by 6 weeks was maintained at 6% by *B. maritimus* and 5% by *P. australis*. At pH10, a residual of 4% lead was left by *B. maritimus* in two weeks whereas a residual of 6% lead was left by *P. australis* in two weeks. The final residual left at pH10 by the end of the 6 weeks period was 2% and 5% for *B. maritimus* and *P. australis* respectively. These results show that at pH4, *P. australis* performed slightly better than *B. maritimus* in terms of percent lead removal, however at pH10, *B. maritimus* performed slightly better. The two plants performed at the same level at pH7.

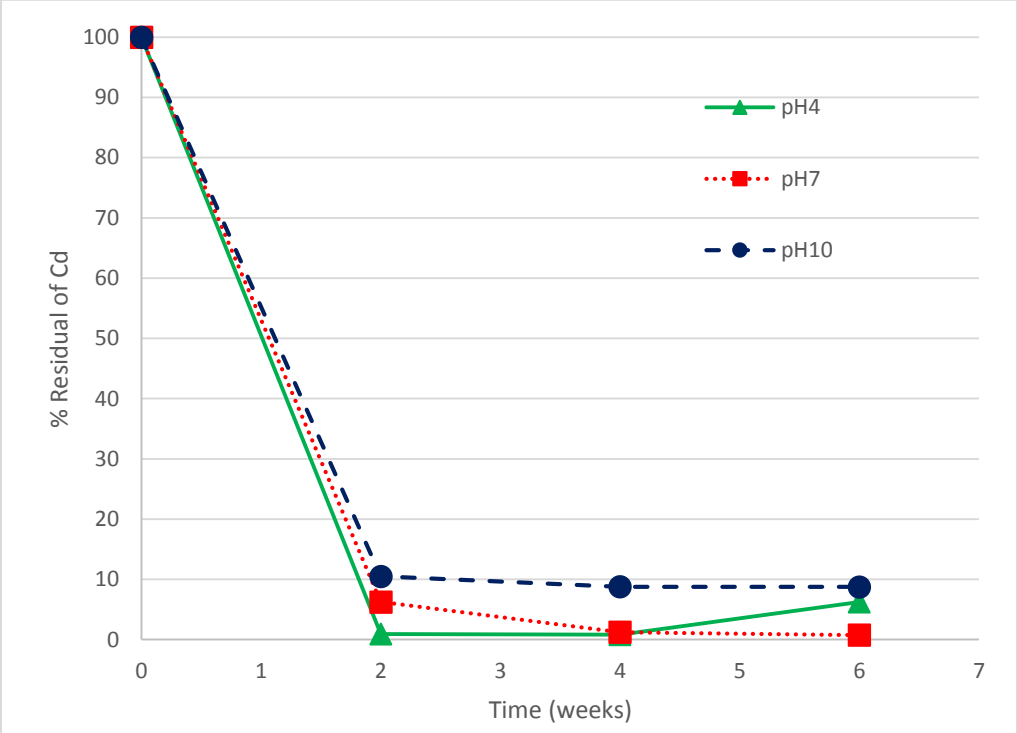


Figure 14: Effect of pH on the remediation of cadmium by B. maritimus

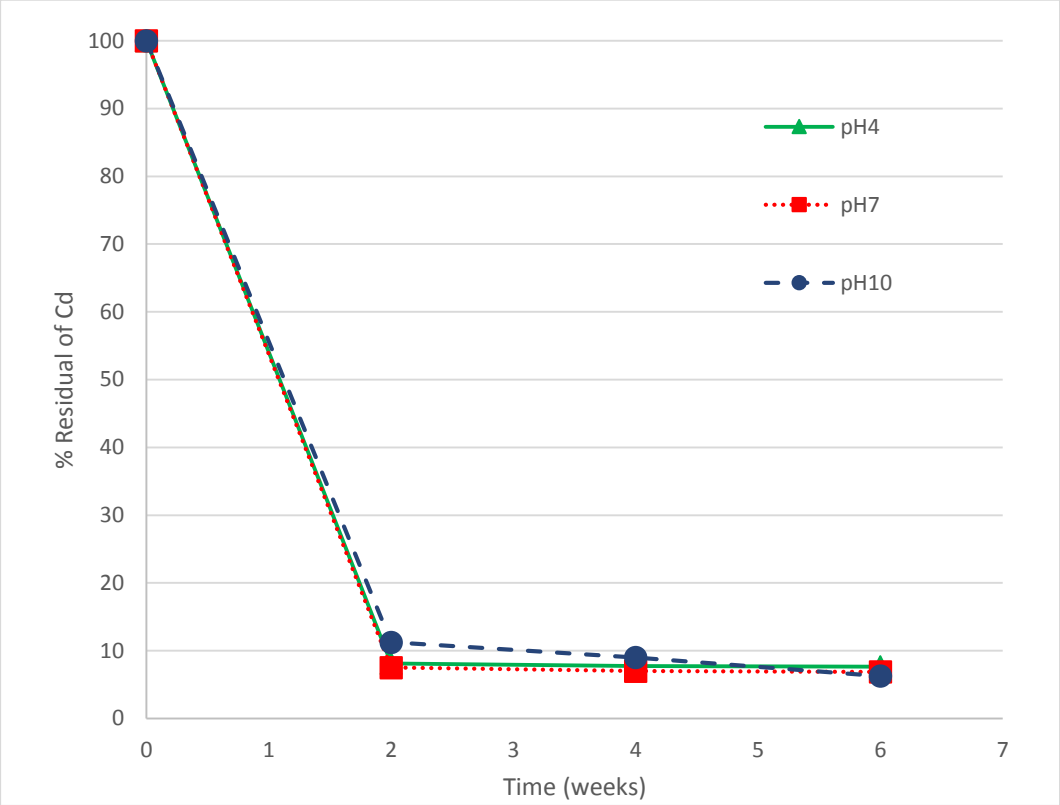


Figure 15: Effect of pH on the remediation of cadmium by P. australis

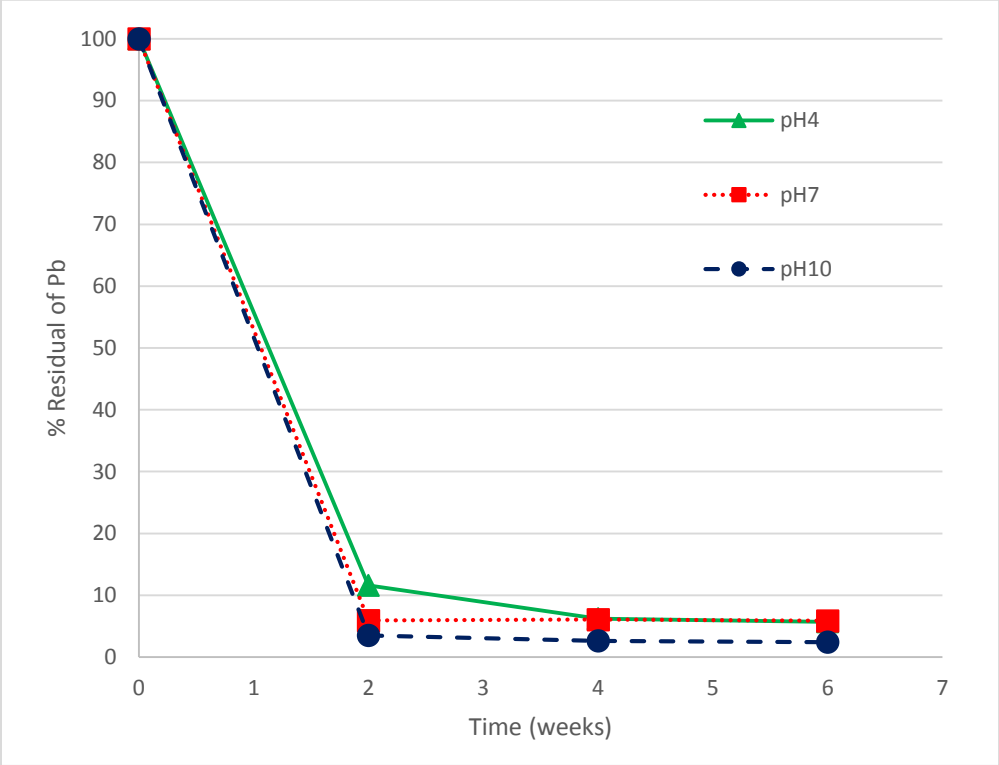


Figure 16: Effect of pH on the remediation of lead by B. maritimus

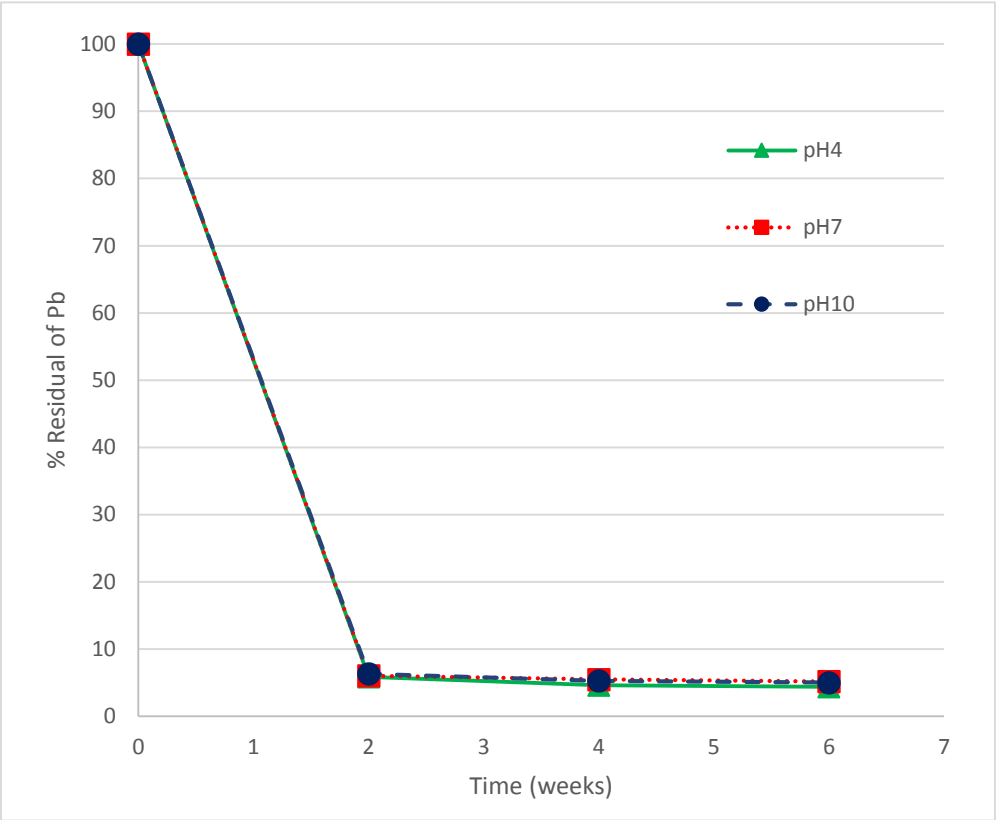


Figure 17: Effect of pH on the remediation of lead by P. australis

The effect of pH on the remediation of nickel by *B. maritimus* and *P. australis* is shown in *Figure 18* and *Figure 19* respectively. At pH4, a residual of 15% nickel was observed in two weeks and a total residual of 13% nickel was recorded over 6 weeks period in *B. maritimus* experiment. At the same pH4 in *P. australis* experiment, a residual of 59% and 7% nickel was attained in two weeks and six weeks respectively. At pH7, a residual of 43% and 17% nickel was achieved by *B. maritimus* in two weeks and 6 weeks respectively. At this pH7, a residual of 43% and 16% was achieved by *P. australis* in two weeks and 6 weeks respectively. At pH10, a residual of 16% and 7% nickel was achieved by *B. maritimus* in two weeks and 6 weeks respectively. At this pH10, a residual of 9% and 3% was achieved by *P. australis* in two weeks and 6 weeks respectively. These results imply that *P. australis* performed better at pH10 for nickel remediation.

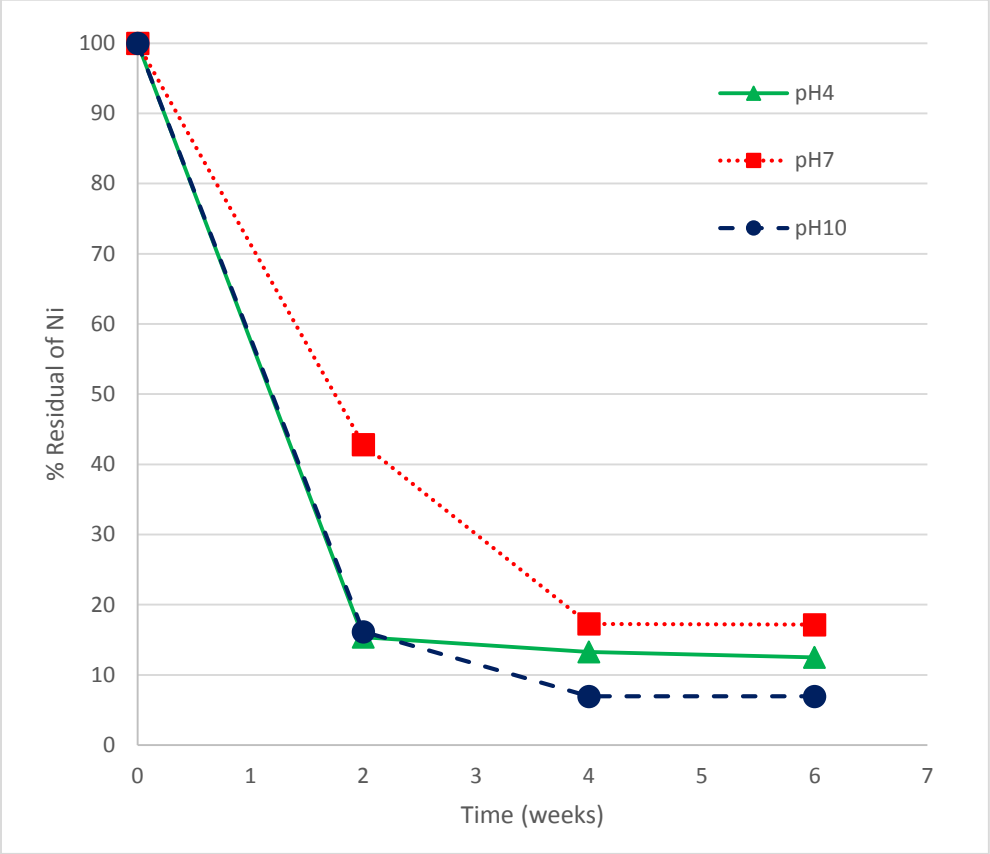


Figure 18: Effect of pH on the remediation of nickel by B. maritimus

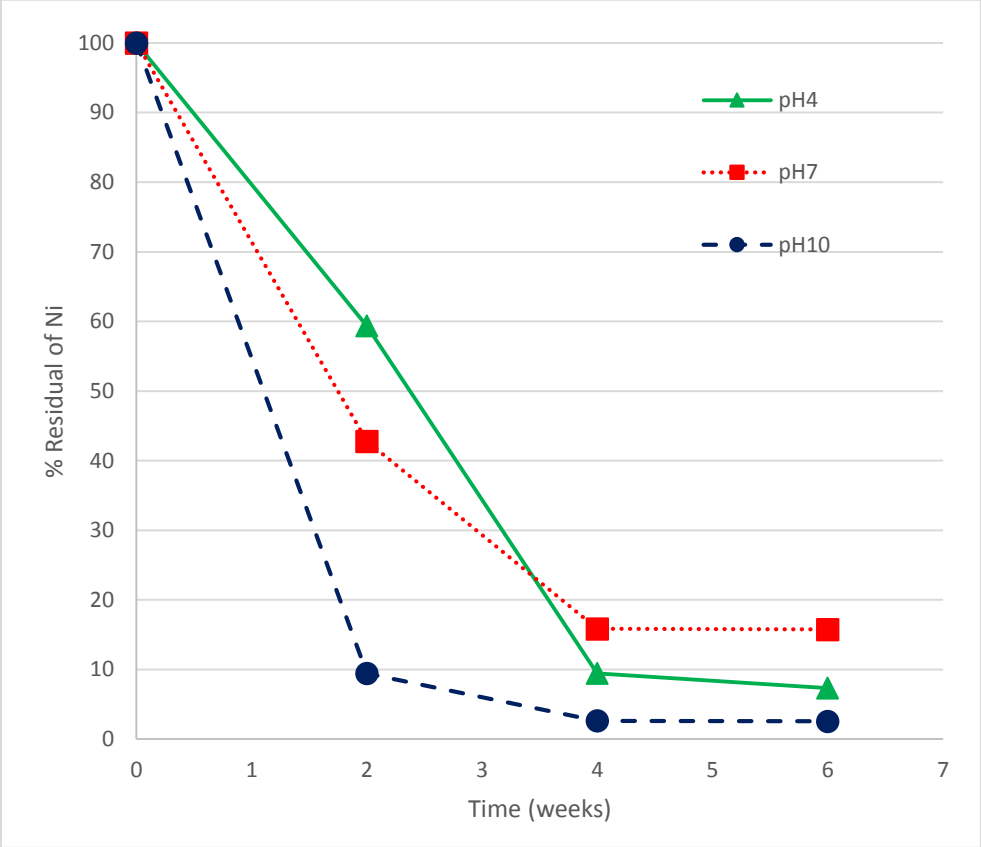


Figure 19: Effect of pH on the remediation of nickel by P. australis

4.1.3 Effect of salinity on the phytoremediation of cadmium, lead and nickel

This experiment was carried out to study the effect of three different salinity levels on the remediation of cadmium, lead and nickel by *B. maritimus* and *P. australis*. The salinity levels used as demonstrated by total dissolved solids (TDS), was 0ppm (represented by distilled water), 3645ppm (represented by groundwater) and 1822.5ppm (represented by a mixture of 50% groundwater and 50% distilled water). *Table 5* shows the characteristics of the groundwater sample.

Table 5: Characteristics of groundwater sample

Parameters	**Value**
pH	7.09
TDS, mg/L	3645
D.O., mg/L	5.78
Turbidity, NTU	0.1
TSS mg/L	5.0
TOC mg/L	5.36
Alkalinity-T ($CaCO_3$)	433.5
Cl^- mg/L	1220.4
NO_3^-, mg/L	3.809
Br^- mg/L	4.212
SO_4^{2-},mg/L	545.7
Ca^{2+} mg/L	255.1
Fe^{2+} mg/L	<0.009
K^+ mg/L	34.2

Mg^{2+} mg/L	82.5
Na^{+} mg/L	631.2

Source: (Tawabini, 2014)

The effect of salinity on the remediation of cadmium by *B. maritimus* and *P. australis* is shown in *Figure 20* and *Figure 21* respectively. In the *B. maritimus* experiment (*Figure 20*), a residual of 6% cadmium was left after two weeks at TDS of 0ppm and by the end of six weeks, the residual was reduced to 1% cadmium. At TDS of 1823ppm, a residual of 9% and 8% cadmium was achieved by two weeks and six weeks respectively. At TDS of 3645ppm, a residual of 10% and 8% cadmium was left by two weeks and six weeks respectively. The enhanced removal of cadmium (99%) demonstrated by *B. maritimus* is probably because it is a freshwater plant and thus performs optimally at minimal salinity level. In *P. australis* experiment (*Figure 21*), a residual of 8% cadmium remained after two weeks while a residual of 7% cadmium remained after six weeks at TDS of 0ppm. At TDS of 1823ppm, a residual of 8% and 6% cadmium remained after two weeks and six weeks respectively. At TDS of 3645ppm, a residual of 11% and 8% cadmium remained after 2 weeks and 6 weeks respectively. The slight difference in the removal of cadmium demonstrated by *P. australis* also points to its preference of freshwater over groundwater.

The effect of salinity on the remediation of lead by *B. maritimus* is shown in *Figure 22*. In distilled water sample, a residual of 6% lead was left after 2 weeks and this was maintained until the end of the experiment. In 50% groundwater sample, a residual of 6% lead was achieved in two weeks and this was further reduced to 3% by the end of the experiment. In full groundwater sample, a residual of 6% lead was left after two weeks and this was further

reduced to 4% by the end of the experiment. These results show that *B. maritimus* was able to remediate lead at the three tested salinity levels. The effect of salinity on the remediation of lead by *P. australis* is shown in *Figure 23.* After two weeks of the experiment, a residual of 6% lead remained at TDS of 0ppm and 1823ppm whereas, a residual of 53% lead remained at TDS of 3645ppm. However, at the end of the 6 weeks period of the experiment, a residual of 5%, 4% and 4% lead remained for TDS of 0ppm, 1823ppm and 3645ppm respectively. These results indicate that *P. australis* performed in the same way in the three salinity levels, though it was slower at TDS of 3645ppm (residual of 53% lead after 2 weeks) compared to 0ppm and 1823ppm (residual of 6% lead after 2 weeks).

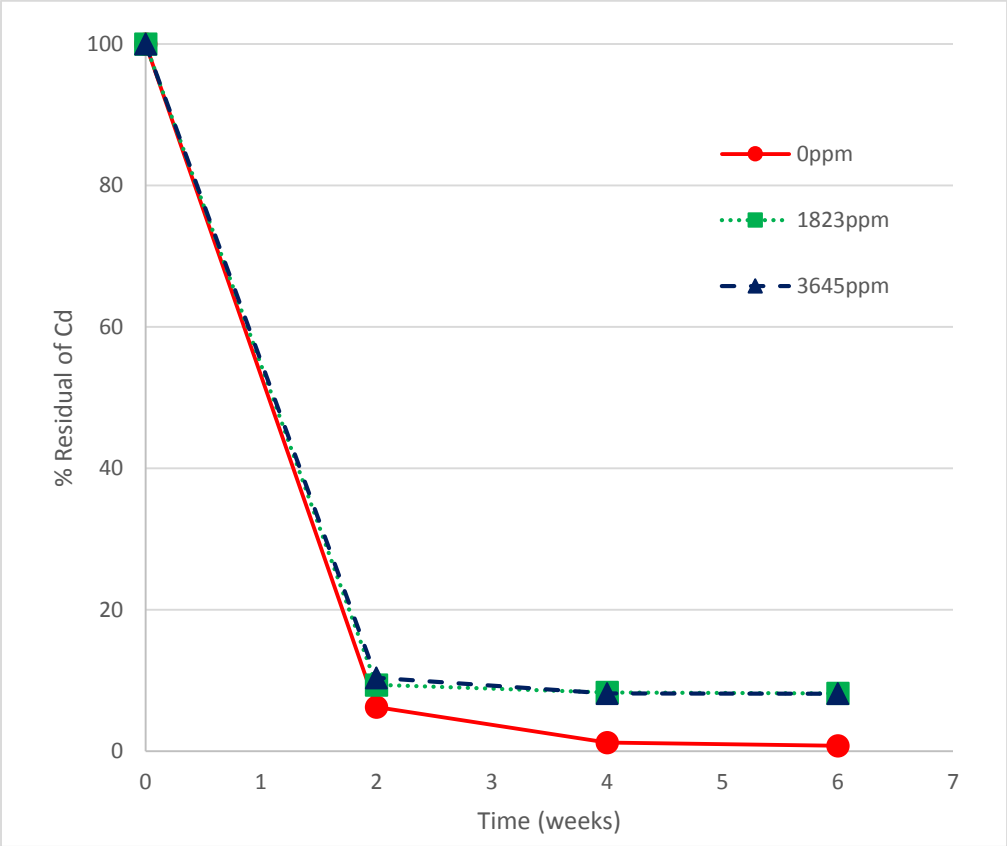


Figure 20: Effect of salinity on the remediation of cadmium by B. maritimus

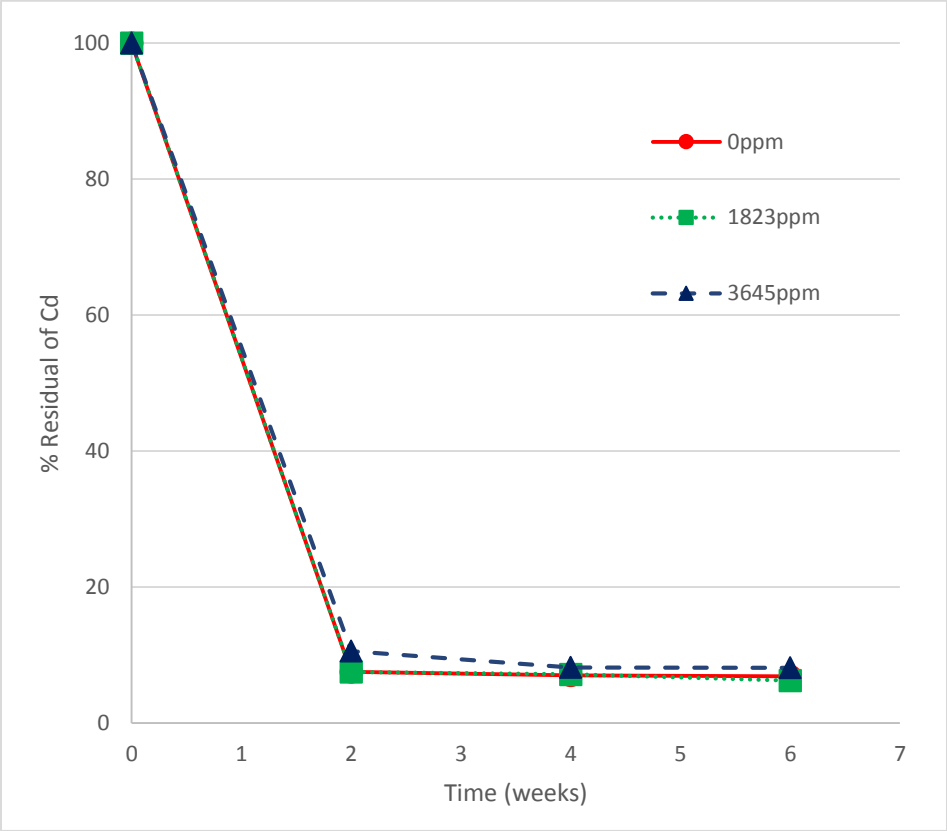


Figure 21: Effect of salinity on the remediation of cadmium by P. australis

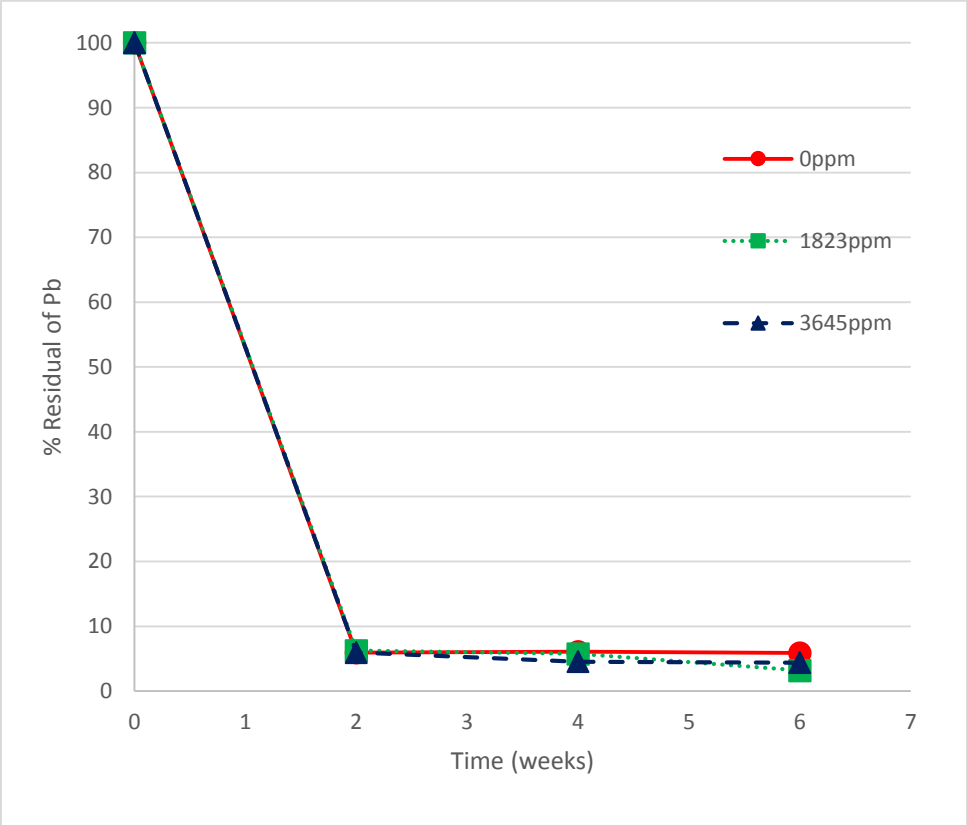


Figure 22: Effect of salinity on the remediation of lead by B. maritimus

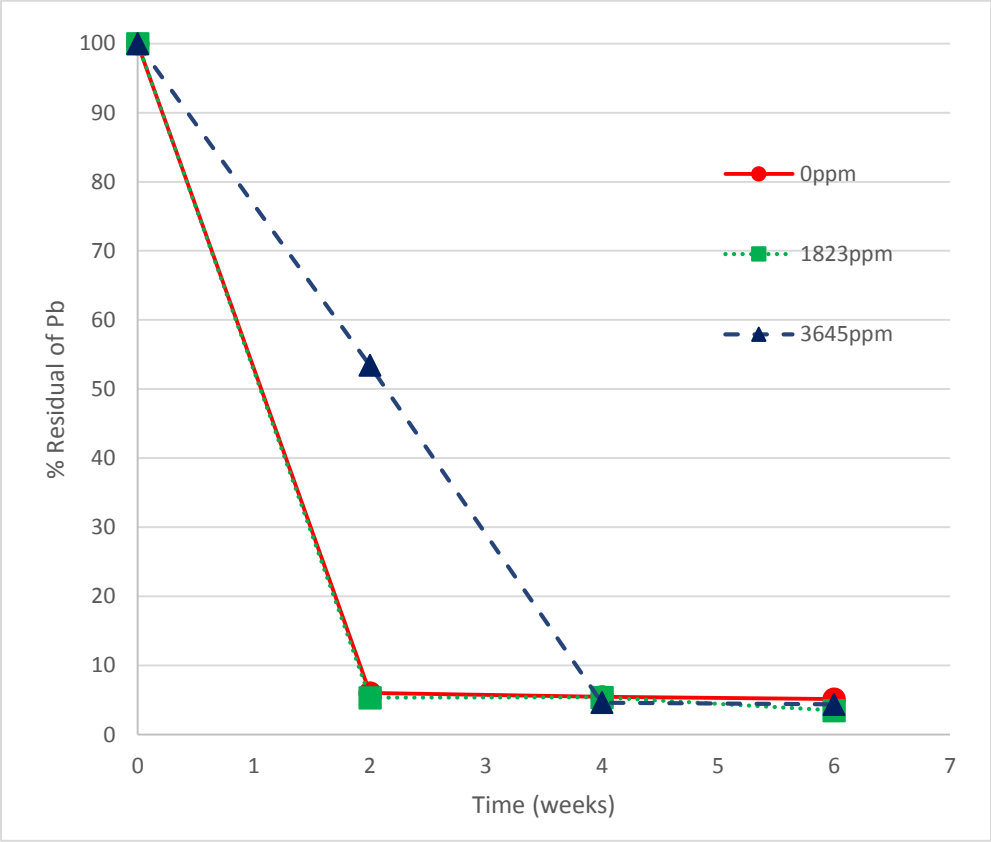


Figure 23: Effect of salinity on the remediation of lead by P. australis

Figure 24 shows the effect of salinity on the remediation of nickel by *B. maritimus*. There was a residual of 42% nickel after 2 weeks and a total residual of 17% nickel left by *B. maritimus* after 6 weeks in distilled water sample. In 50% groundwater, a residual of 15% nickel was left by *B. maritimus* after 2 weeks and a total residual of 9% nickel was left after 6 weeks. In groundwater sample, a residual of 26% nickel remained after 2 weeks of treatment and a residual of 1% nickel remained after 6 weeks of treatment. These results showed a better removal of nickel by *B. maritimus* in 50% groundwater sample after 2 weeks but a much better removal in groundwater sample after 6 weeks. *B. maritimus* remediated nickel better in both 50% groundwater and groundwater samples.

The effect of salinity on the remediation of nickel by *P. australis* is shown in *Figure 25*. A residual of 43% nickel remained after 2 weeks of experiment in distilled water sample whereas a residual of 6% and 18% nickel was left in 50% groundwater and groundwater samples respectively after two weeks. The residual nickel in distilled water sample was reduced to 16% after 6 weeks while that of 50% groundwater increased to 8% and that of groundwater reduced to 11% after 6 weeks. This implied that *P. australis* removed nickel better in 50% groundwater, followed by full groundwater.

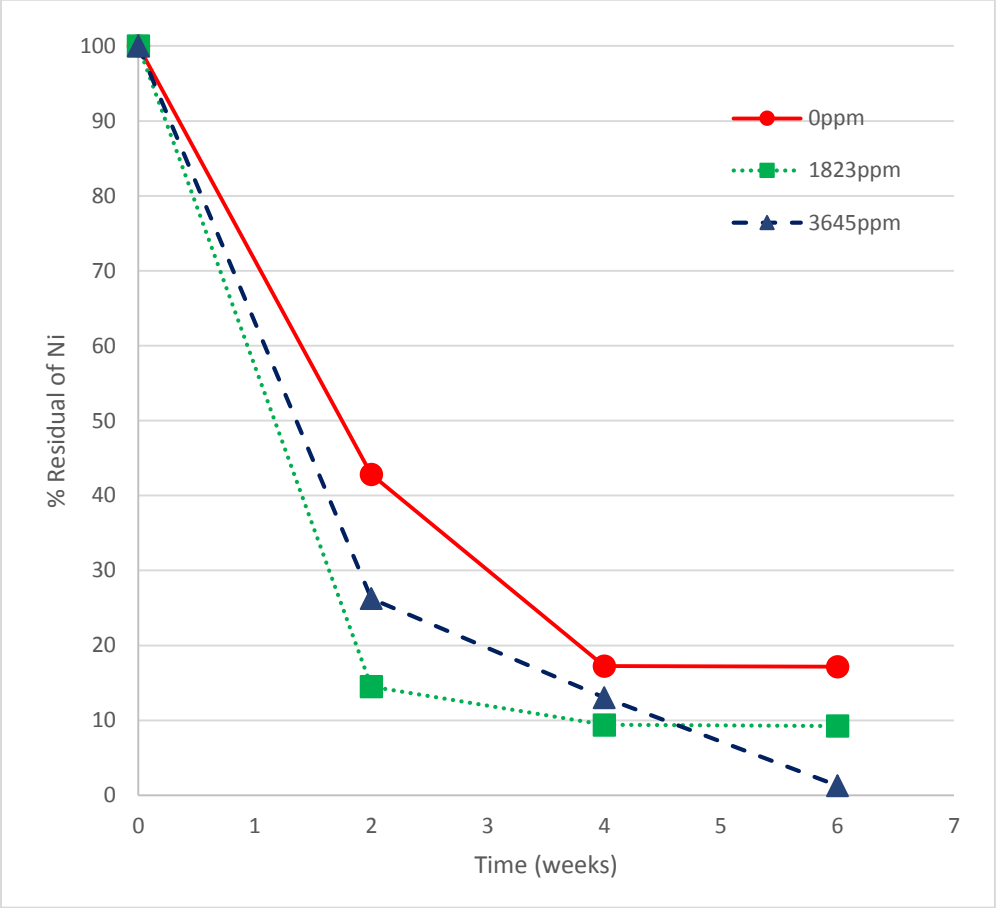


Figure 24: Effect of salinity on the remediation of nickel by B. maritimus

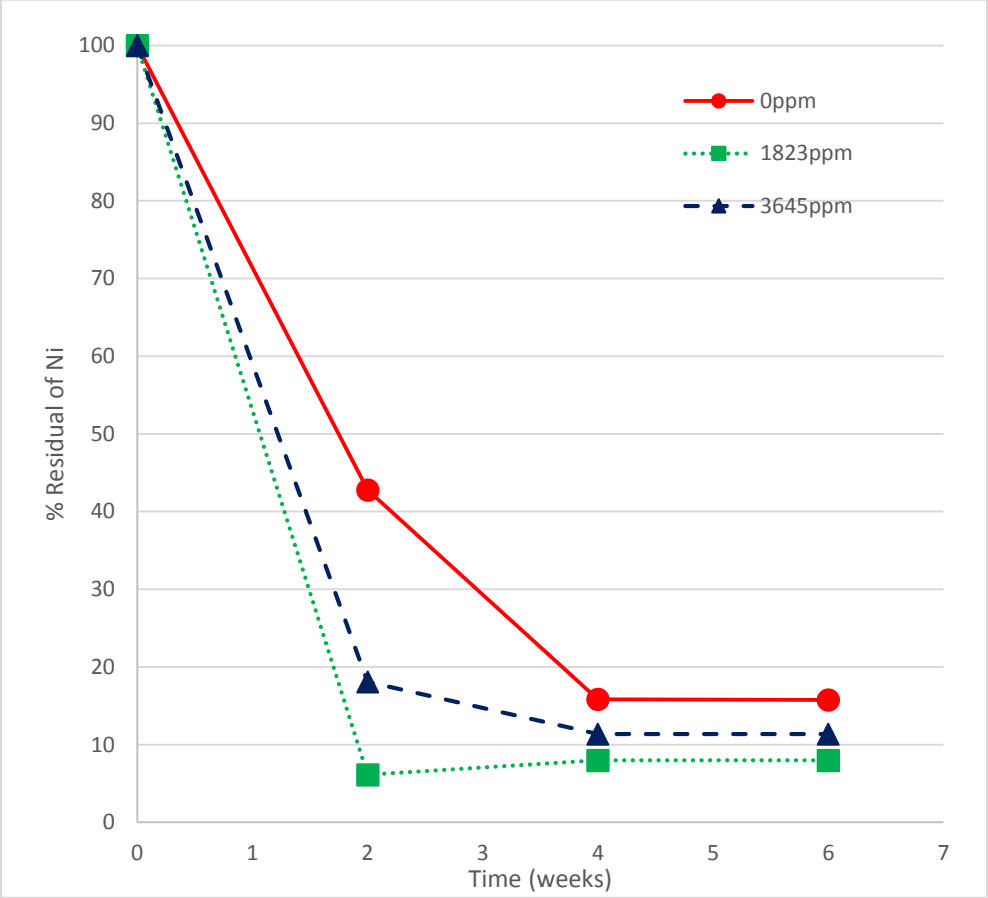


Figure 25: Effect of salinity on the remediation of nickel by P. australis

4.1.4 Assessing removal at shorter duration

This experiment was carried out to investigate the pattern of removal of cadmium, lead and nickel by *P. australis* in distilled water and groundwater samples within a 2-week period. This was necessary because the results obtained from previous experiments (*Figure 11*, *Figure 12* & *Figure 13*) revealed high removal within the first two weeks of the experiment. For this experiment, one of the selected plants (*P. australis*) was chosen and its performance in two water conditions (distilled water and groundwater) was evaluated.

Figure 26 shows the result of the remediation of cadmium by *P. australis* over a period of two weeks. There was gradual removal of cadmium in the two water samples as was revealed by their respective percent residual. In distilled water, there was a residual of 82%, 76%, 59%, 41% and 18% in 3, 6, 9, 12, and 15 days respectively while there was a residual of 60%, 54%, 30%, 27% and 22% in groundwater sample in 3, 6, 9, 12 and 15 days respectively. These results clearly showed that *P. australis* performed overall better at the end of the two weeks experiment. The results of the control experiment revealed a residual of 89% cadmium at the end of the experiment. These results are in agreement with the results of the previous experiment (*Figure 12*) which showed exceptional removal of cadmium in two weeks.

The results of the remediation of lead by *P. australis* over a two-week period (*Figure 27*) revealed a similar trend to what was observed in cadmium removal. A residual of 60%, 52%, 26%, 20% and 14% remained in 3, 6, 9, 12 and 15 days respectively in distilled water planted sample. In groundwater planted with *P. australis*, a residual of 58%, 49%, 25%,

24% and 20% remained in 3, 6, 9, 12 and 15 days respectively. The results of the control experiment were the same as what were observed for cadmium removal.

The results of the phytoremediation of nickel is shown in *Figure 28*. A residual of 50% nickel remained at the end of distilled water experiment and a residual of 55% remained at the end of the groundwater experiment. These results show that *P. australis* had a mid-level ability for phytoremediation of nickel in the two environments.

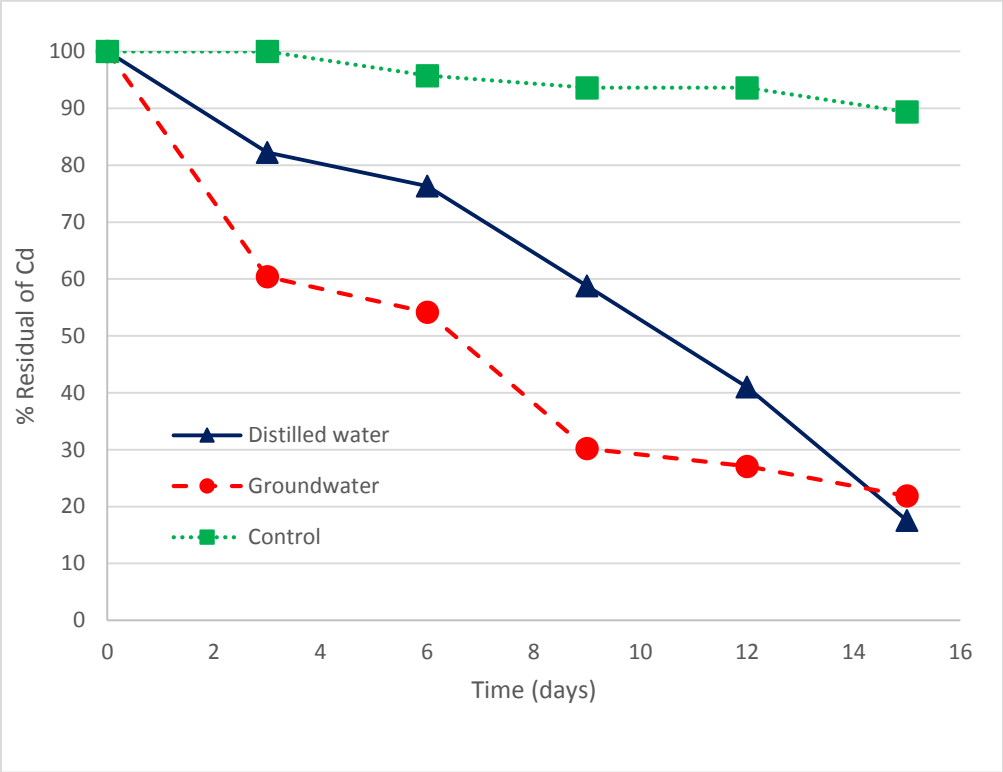


Figure 26: Phytoremediation of cadmium by P. australis over a two-week period

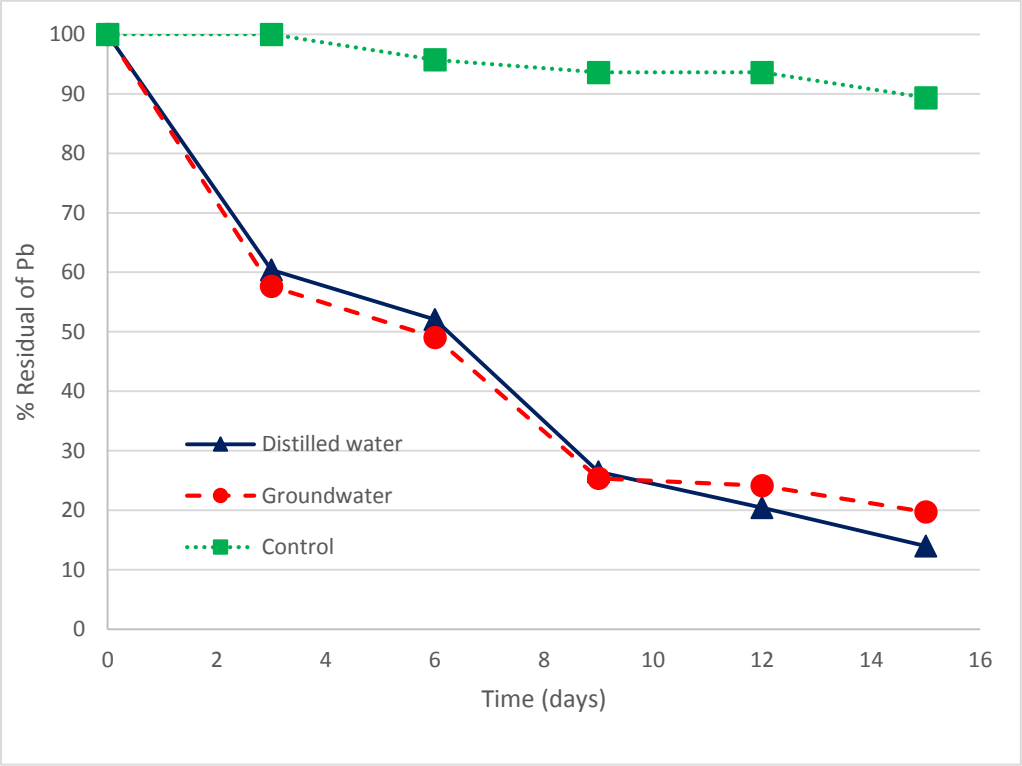


Figure 27: Phytoremediation of lead by P. australis over a two-week period

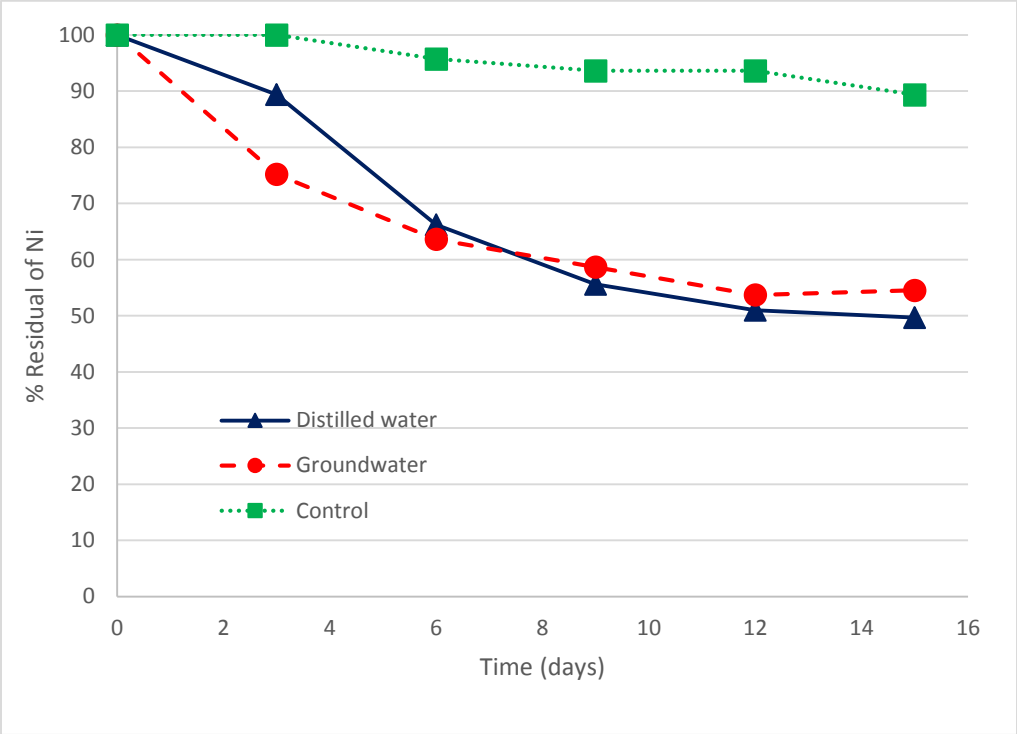


Figure 28: Phytoremediation of nickel by P. australis over a two-week period

4.1.5 Assessment of heavy metal in plant parts

Different parts of *B. maritimus* and *P. asutralis* were assessed at the end of the experiment to determine how much of each of cadmium, lead and nickel was retained in the roots / rhizomes, shoots and leaves, and to calculate the Biological Concentration Factor (BCF) and Translocation Factor (TF) which would be used to ascertain the mechanisms of contaminant uptake.

4.1.5.1 Background concentration of cadmium, nickel and lead in plant

Figure 29 shows the average background concentrations of cadmium, nickel and lead found in the various parts of *P. australis* before the plant was used for the phytoremediation experiment. Cadmium concentrations of 0.01ppm, 0.03ppm and 0.27ppm were found in the leaves, shoots and roots respectively. Nickel concentrations of 0.15ppm, 0.90ppm and 0.77ppm were found in the leaves, shoots and roots respectively. The concentrations of lead found in the leaves, shoots and roots were 0.15ppm, 0.12ppm and 0.15ppm respectively. The background analysis for these plants results showed a total concentration of 0.31ppm cadmium, 1.82ppm nickel and 0.42ppm lead. The background concentration of nickel in the plant can be considered high relative to cadmium and lead. The high nickel content could probably have come from the fertilizers and other natural sources. Since nickel is ranked the 24th most abundant metal on earth (Grandjean, 1984) and it is believed that some plants could utilize nickel as a micronutrient (Chen, et al. 2009), this could explain the reason for high nickel content.

4.1.5.2 Comparing the concentrations of cadmium, nickel and lead in plant parts before and after the experiments

Figure 30 shows the average concentrations of cadmium found in the various parts of *P. australis* before and after the experiment. The average concentration of cadmium found in the leaves before the experiment was 0.009ppm and this increased to 0.66ppm by the end of the experiment. In the shoots, the average concentrations of 0.03ppm and 1.62ppm were found before and after the experiment respectively. The same trend was also observed for the roots, with average concentrations of 0.27ppm and 3.74ppm before and after the experiment respectively. The average concentrations of lead in the leaves, shoots and roots before the experiment were 0.15ppm, 0.12ppm and 0.15ppm respectively and this increased to 1.32ppm, 1.89ppm and 5.21ppm accordingly (*Figure 31*). The average concentrations of nickel (*Figure 32*) found in the leaves were 0.15ppm before the experiment and 0.77ppm after the experiment. 0.9ppm nickel was found in the shoots before the experiment and this increased to 1.43ppm by the end of the experiment. The average concentration of nickel in the roots before the experiment was 0.77ppm and this increased to 3.9ppm by the end of the experiment. In all cases, the highest concentration of contaminant was found in the roots, followed by the shoots and finally the leaves. Removing the background concentrations from each of the contaminants will give a total concentration of 8ppm for lead, 5.7ppm for cadmium and 4.3ppm for nickel. This further confirms that the removal of cadmium, lead and nickel observed in *Figure 11*, *Figure 12* and *Figure 13* is a direct result of plant uptake of these contaminants.

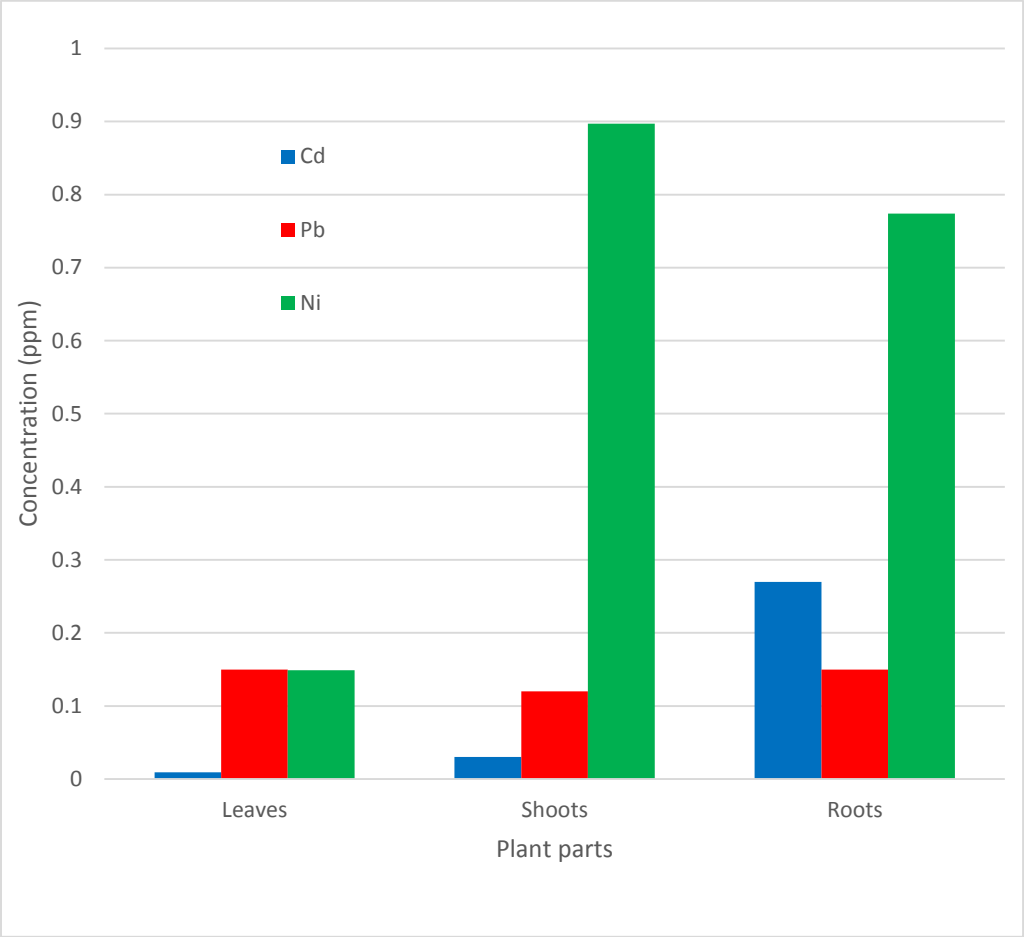


Figure 29: Background concentrations of cadmium, lead and nickel in the leaves, shoots and roots of P. australis before the experiment

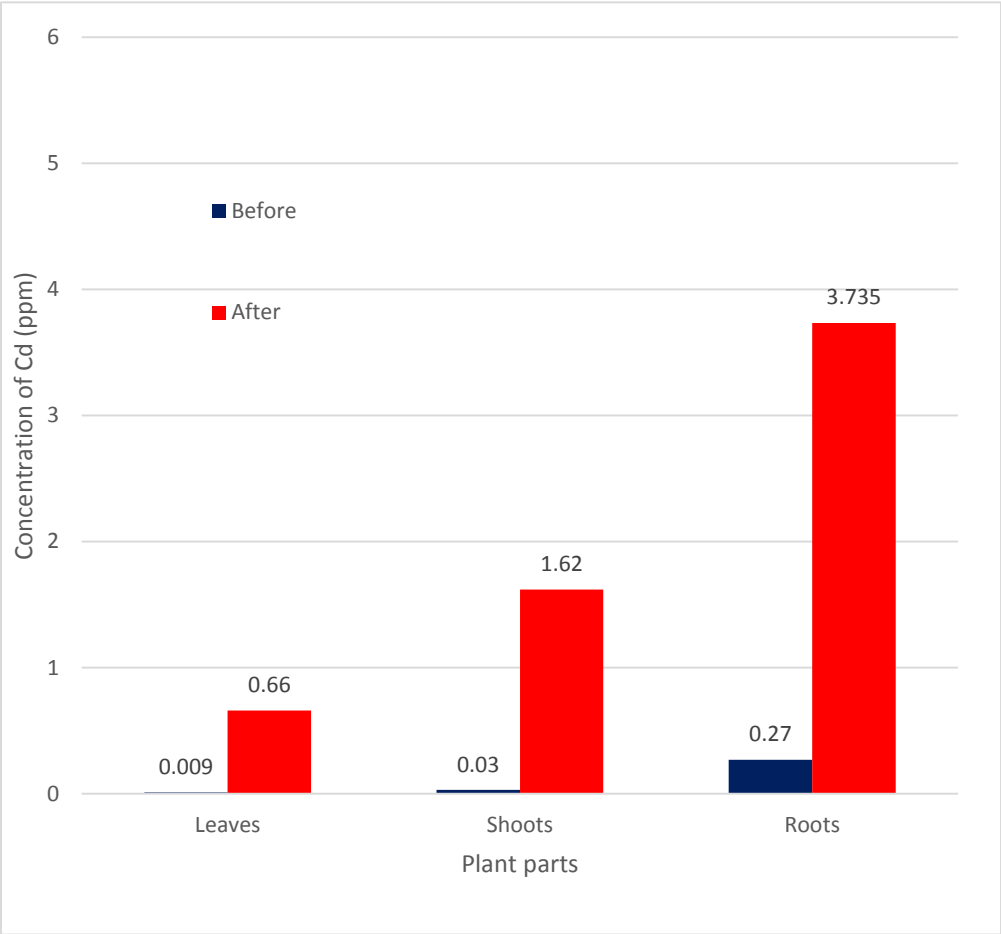


Figure 30: Concentrations of cadmium in the leaves, shoots and roots before and after the experiment

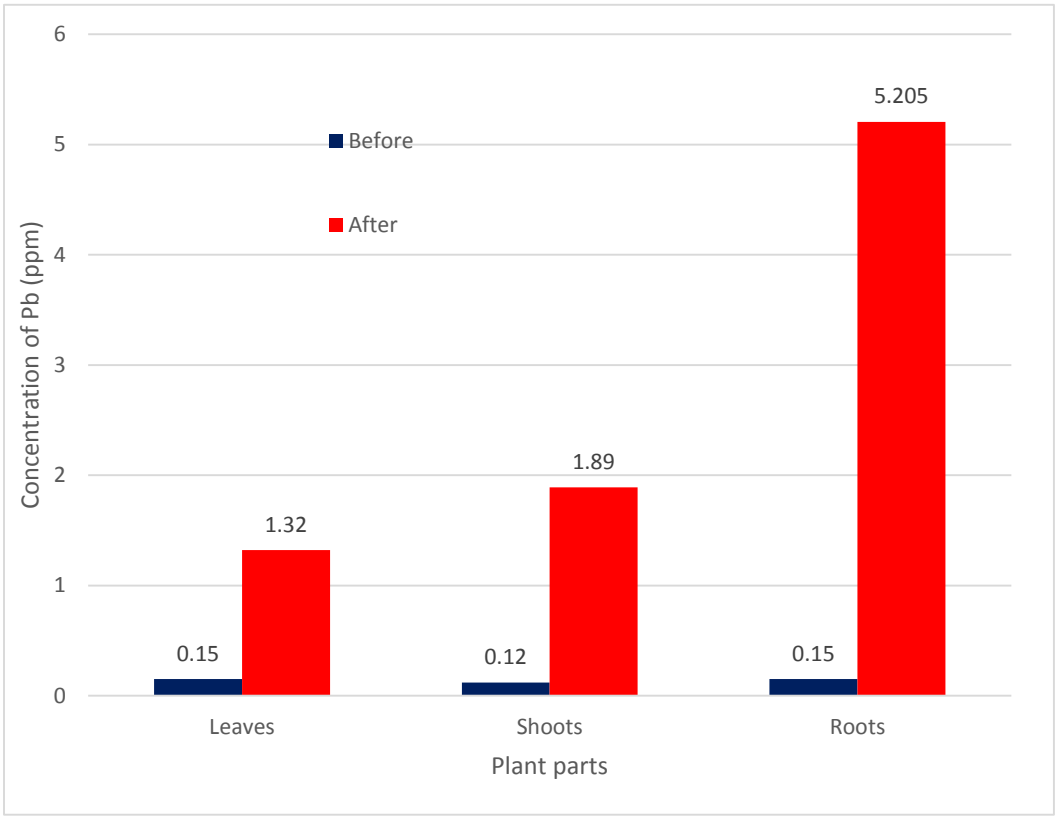


Figure 31: Concentrations of lead in the leaves, shoots and roots before and after the experiment

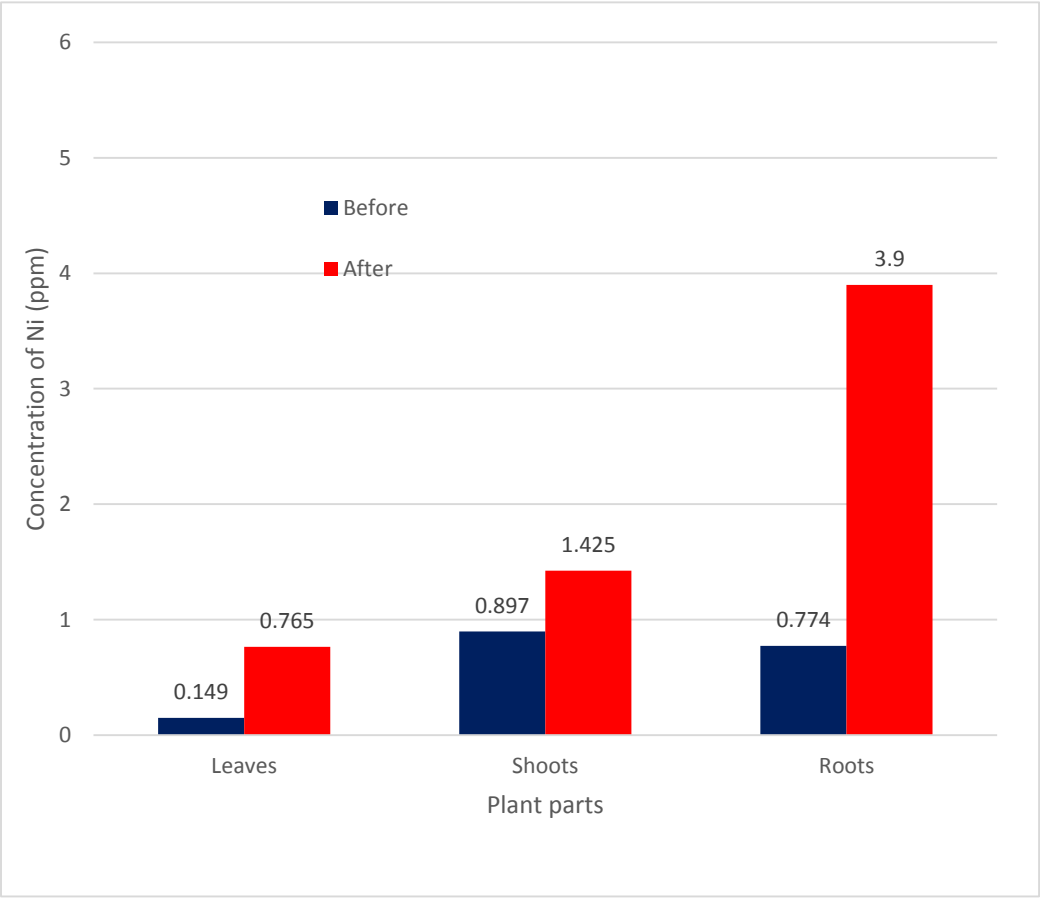


Figure 32: Concentrations of nickel in the leaves, shoots and roots before and after the experiment

4.1.5.3 Determination of mechanisms of plant uptake of contaminants

In order to determine the mechanisms of contaminant uptake, Biological Concentration Factor (BCF) and Translocation Factor (TF) are calculated. BCF measures the ability of plant to accumulate metals with respect to metals present in the substrate while TF measures the ability of plant to transport extracted metals from the root to the shoot. They are calculated as follows:

$$BCF = \frac{Total\ metal\ concentration\ in\ plant}{metal\ concentration\ in\ solution} \qquad \text{…….. Equation 1}$$

$$TF = \frac{metal\ concentration\ in\ shoot}{metal\ concentration\ in\ root} \qquad \text{…….. Equation 2}$$

BCF for Cd

$$BCF = \frac{5.7ppm}{5.0ppm}$$

$$= 1.14$$

BCF for Ni

$$BCF = \frac{4.3ppm}{5.0ppm}$$

$$= 0.86$$

BCF for Pb

$$BCF = \frac{8.0ppm}{5.0ppm}$$

$$= 1.6$$

TF for Cd

$$TF = \frac{1.6ppm}{3.7ppm}$$

$$= 0.43$$

TF for Ni

$$TF = \frac{1.4ppm}{3.9ppm}$$

$$= 0.36$$

TF for Pb

$$TF = \frac{1.9ppm}{5.2ppm}$$

$$= 0.37$$

As stated by Nouri et al., (2011), Plants with a high BCF value and low TF value could be suitable for phytostabilization whereas plants with BCF and TF values both greater than one could be used for phytoextraction. Since the BCF values for cadmium and lead are high and their TF values are low, *P australis* could be used for Phytostabilization of Cadmium and Lead. As indicated from Figure 28, the BCF and TF values, *P australis* might not be effective for nickel remediation.

4.2 PHYTOREMEDIATION OF PAHs

The remediation of naphthalene by *B. maritimus* and *P. australis* as well as the effects of both pH and salinity on its remediation was studied.

4.2.1 Remediation of naphthalene by *B. maritimus* and *P. australis*

This experiment was carried out to determine the ability of *B. maritimus* and *P. australis* to remove naphthalene from spiked distilled water. *Figure 33* shows the percent residual of naphthalene in distilled water over a 6-week period by *B. maritimus* and *P. australis*. In *B. maritimus* experiment, there was residual of 52%, 23% and 4% after 2, 4 and 6 weeks respectively. In *P. australis* experiment, there was a residual of 70%, 11% and 8% after 2, 4 and 6 weeks respectively. In control experiment, there was a residual of 58%, 55% and 58% after 2, 4 and 6 weeks respectively. These results showed that both *B. maritimus* and *P. australis* were effectively able to enhance the removal of naphthalene after 6 weeks.

4.2.2 Effect of pH on the phytoremediation of naphthalene

This experiment was carried out to investigate the effect of pH on the remediation of naphthalene by *B. maritimus* and *P. australis*.

Figure 34 shows the effect of pH on the remediation of naphthalene by *B. maritimus*. At pH4, an overall residual of 26% was left by *B. maritimus* after 6 weeks whereas at pH10, a residual of 46%, 14% and 4% remained after 2, 4 and 6 weeks respectively. For the effect of pH on the remediation of naphthalene by *P. australis* (*Figure 35*), an overall residual of

41% was left after 6 weeks at pH4 whereas a residual of 56%, 24% and 8% remained after 2, 4 and 6 weeks at pH10.

Comparing these results to the results obtained in distilled water of pH7 (*Figure 33*), then one can say that both *B. maritimus* and *P. australis* were not that effective at pH4. However, both plants behaved in a similar way to the way they behaved in the distilled water experiment at pH10. This implies that pH did not enhance the remediation ability of both plants.

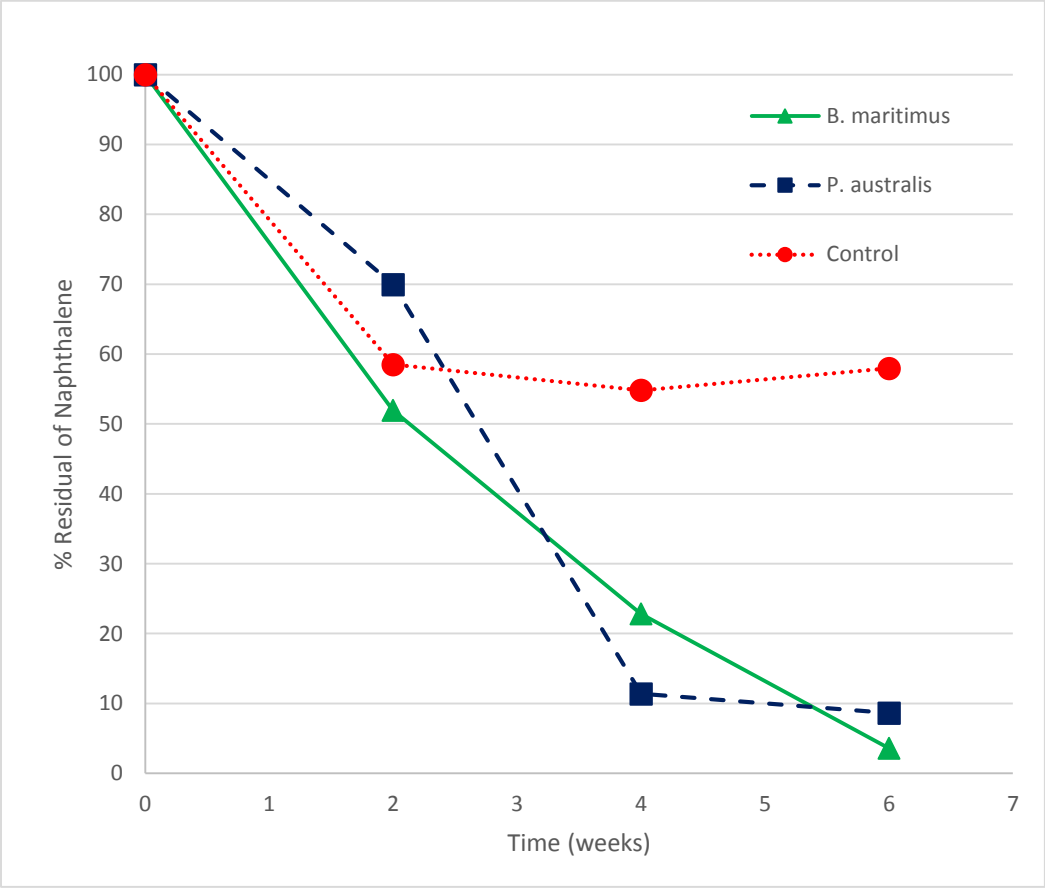


Figure 33: Remediation of Naphthalene by B. maritimus and P. australis in distilled water over a-6 week period

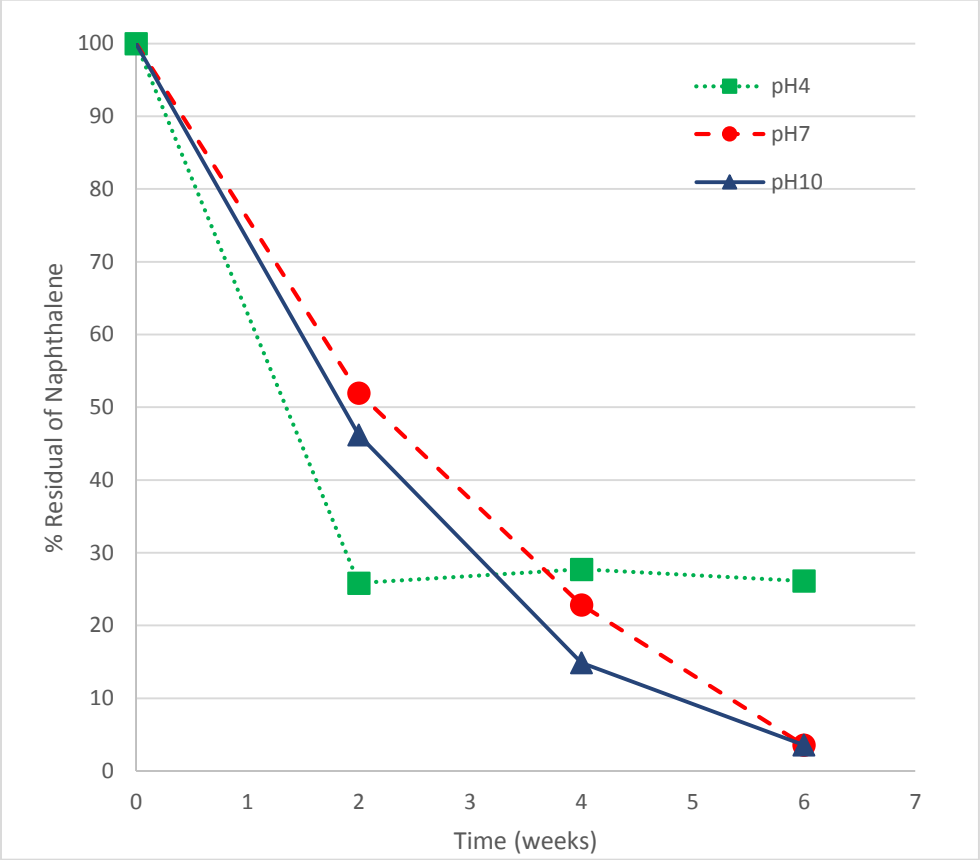


Figure 34: Effect of pH on the remediation of Naphthalene by B. maritimus

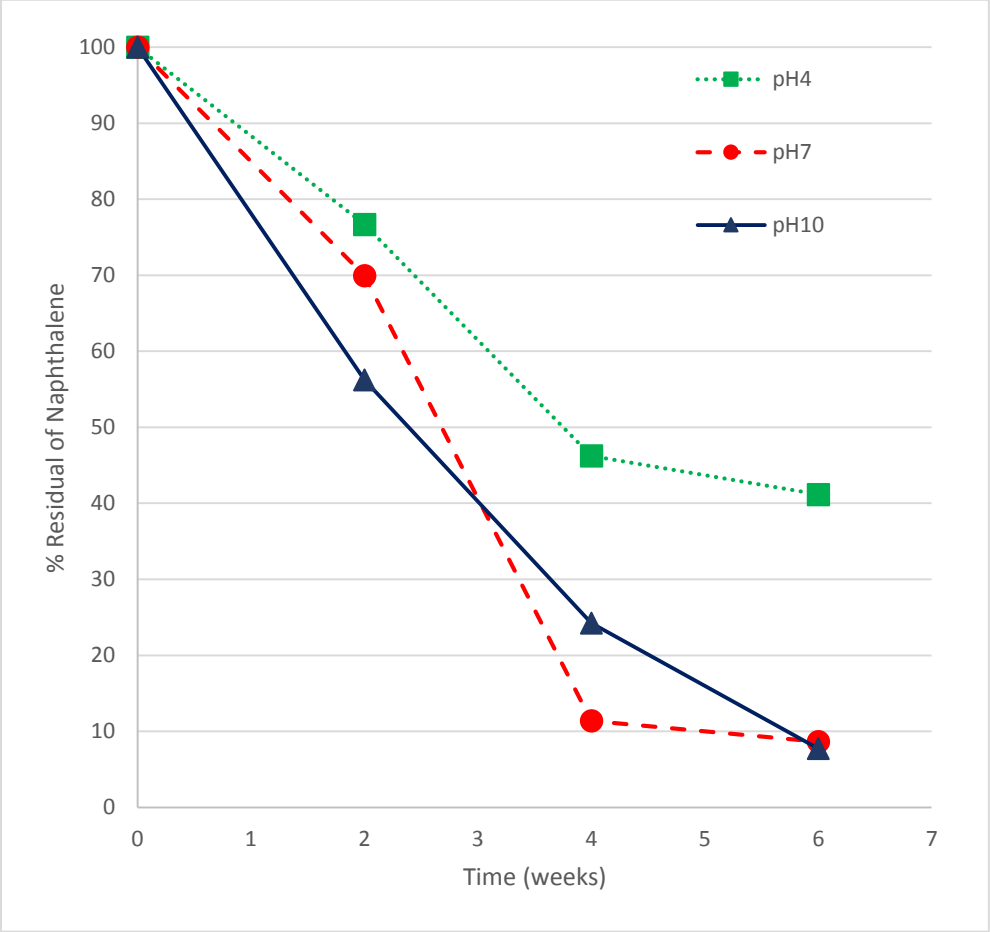


Figure 35: Effect of pH on the remediation of Naphthalene by P. australis

4.2.3 Effect of salinity on the phytoremediation of naphthalene

This experiment was carried out to investigate the effect that salinity, as measured by TDS, would have on the remediation of naphthalene by *B. maritimus* and *P. asutralis*.

Figure 36 shows the effect of salinity on the remediation of naphthalene by *B. maritimus.* The results of the experiment carried out using groundwater sample (TDS of 3645ppm) show a residual of 70%, 37% and 28% after 2, 4 and 6 weeks respectively. The results of the experiment using 50% groundwater sample (TDS of 1823ppm) show a residual of 73%, 38% and 31% after 2, 4 and 6 weeks respectively.

The effect of salinity on the remediation of naphthalene by *P. australis* is shown in *Figure 37*. A residual of 87%, 22% and 10% after 2, 4 and 6 weeks respectively for *P. australis* in groundwater sample whereas the residual of 62%, 55% and 36% after 2, 4 and 6 weeks respectively for was observed for 50% groundwater.

From these results, it can be inferred that salinity did not improve the performance of the two plants. In contrast, the experiment conducted in the distilled water sample performed better.

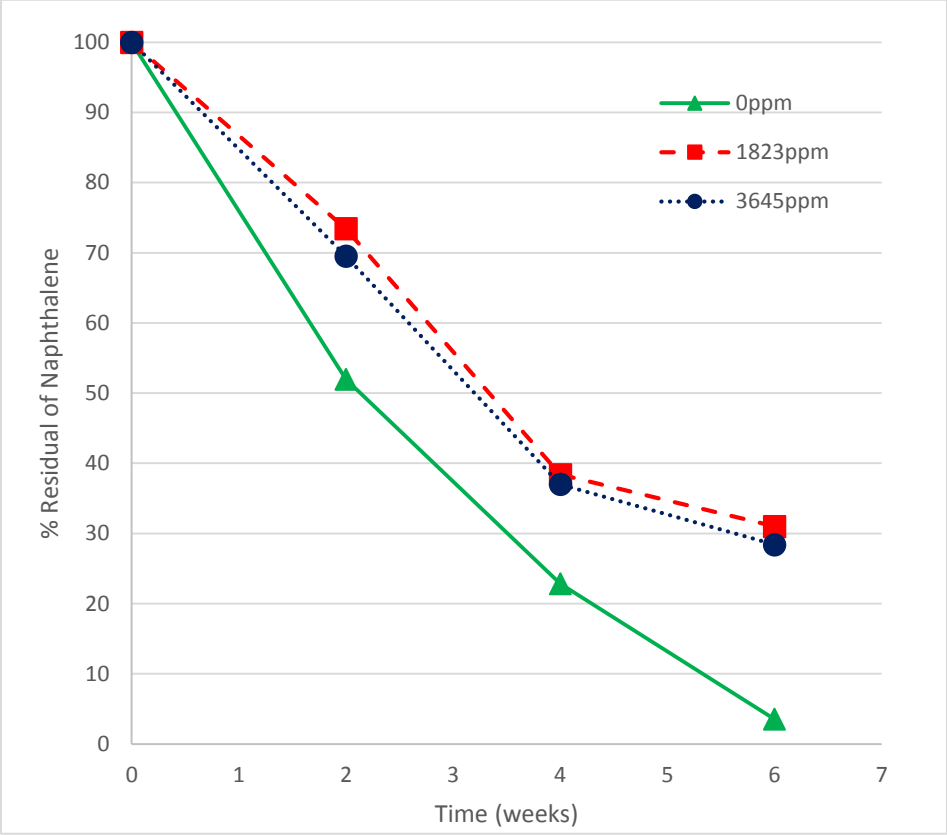


Figure 36: Effect of salinity of the remediation of Naphthalene by B. maritimus

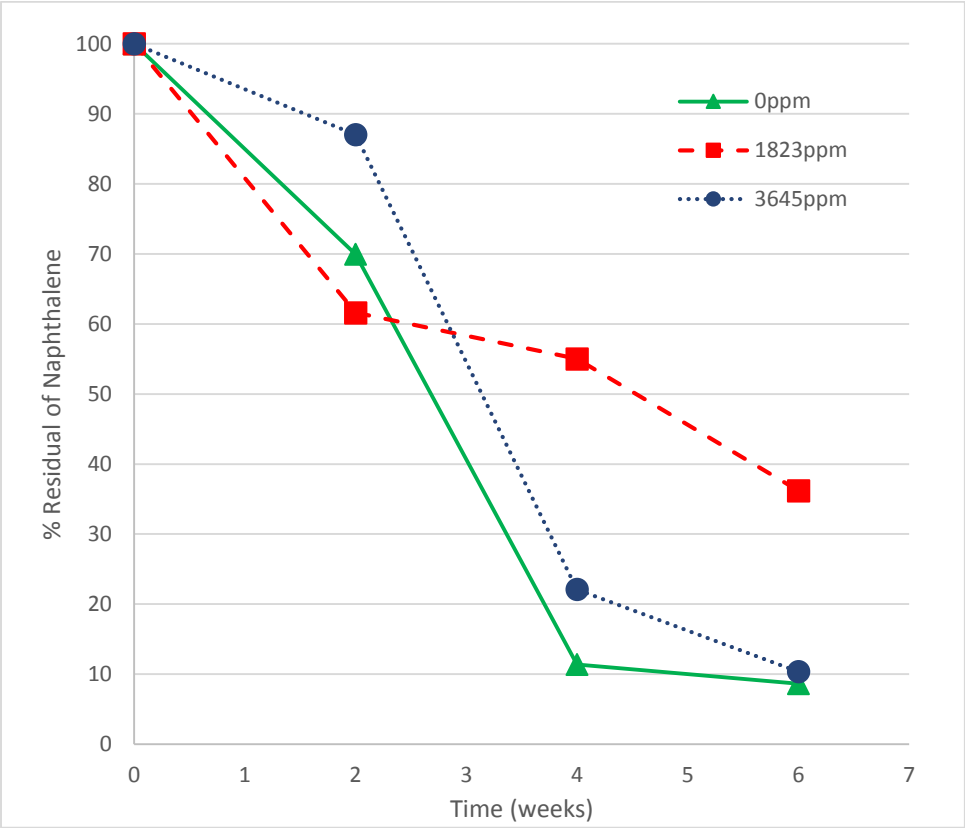


Figure 37: Effect of salinity on the remediation of Naphthalene by P. australis

4.3 MICROBIOLOGICAL INVESTIGATION

This experiment was carried out to investigate the role of bacteria in the phytoremediation of naphthalene as well as to identify the type of bacteria present.

4.3.1 Bacteria population in naphthalene-spiked water samples

The number of bacteria colonies present in naphthalene-spiked water were investigated as a measure of the role of bacteria in phytoremediation.

Figure 38 revealed the pattern of growth adopted by the bacteria colonies in naphthalene-spiked water. The initial $\log_{10}$ CFU/ml in *B. maritimus* planted water was 5.9 as against the 5.6 found in *P. australis* planted water. After 2 weeks, the $\log_{10}$ CFU/ml in *B. maritimus* planted water rose to 6.7 as against the 7.0 in *P. australis* planted water and this period coincided with the period of initiation of naphthalene removal by both plants. This is probably because the bacteria were utilizing naphthalene as carbon source. After this time, the bacteria population dropped to their lowest level of 4.3 and 5.0 for *B. maritimus* and *P. australis* respectively, which was probably because many bacteria colonies were killed. After this period, the bacteria population rose up again to 6.3 CFU/ml for *B. maritimus* and 6.0 CFU/ml for *P. australis*. This pattern of growth continued until the end of the experiment. However, in control experiment, after the initial rise of bacteria population from 5.0 CFU/ml to 5.6 CFU/ml in week 2, the number became more or less constant until the end of the experiment.

The overall implication is that the growth pattern of the bacteria colonies in the two planted water samples suggest a correlation between their rise and fall and the observed remediation in planted media as against the control

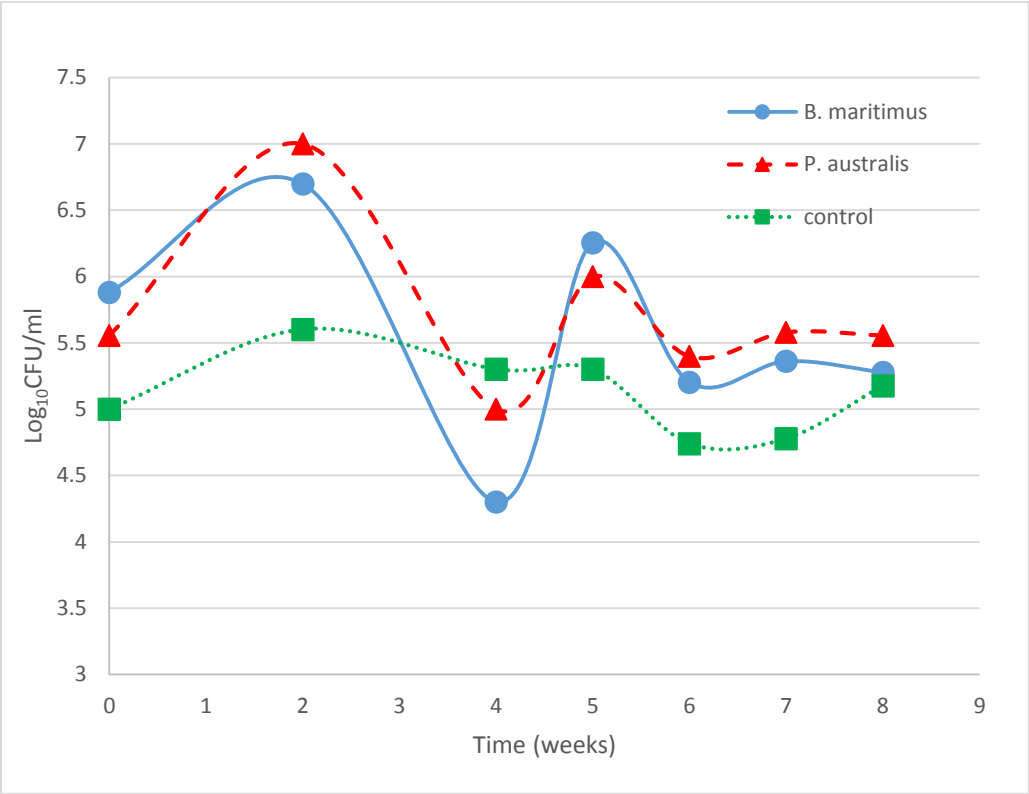
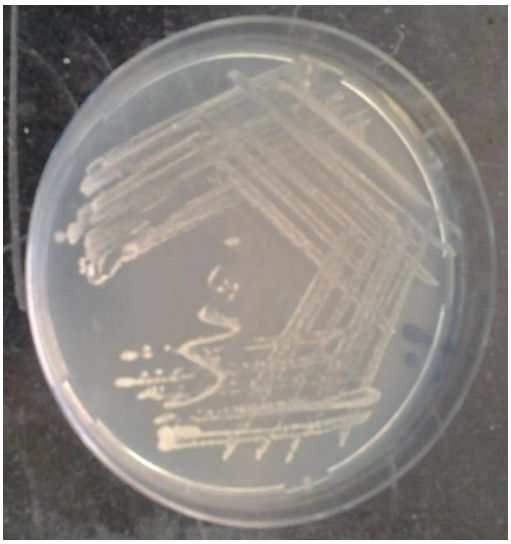


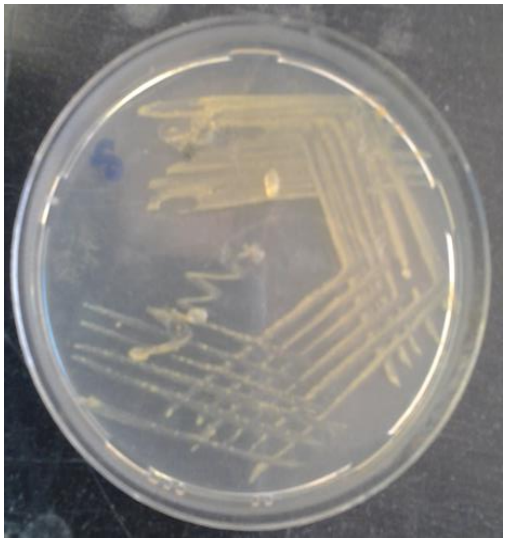
Figure 38: Population of bacteria in naphthalene-spiked water

4.3.2 Identification of isolated bacteria colonies

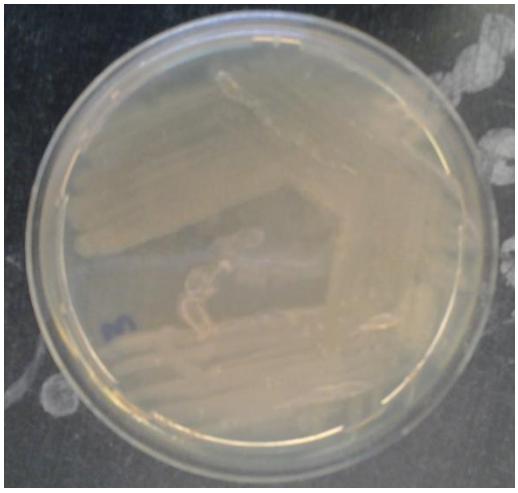
Four distinct bacteria colonies (*Figure 39*) were identified using a combination of colony morphology and biochemical tests. These bacteria were identified using Bergey's Manual of Determinative Bacteriology (Breed et al., 1957). The different colony morphology, the biochemical tests as well as the names of the colonies are presented in *Table 6*



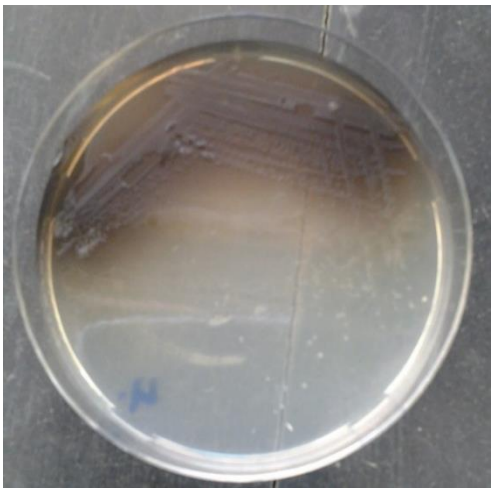
Colony 1



Colony 2



Colony 3



Colony 4

Figure 39: Distinct isolated bacteria colonies

Table 6: Determinative tests for the identification of bacteria colonies

Organisms	Colony Morphology		Gram Stain	Bio Chemical Test			Carbohydrate Fermentation Test			Oxidase	Catalase
				Indole	Methyl Red	Voges Prausker	Glucose	Lactose	Sucrose		
Enterobacter spp.	Nutrient Agar	White circular colonies	-	-	-	+	AG	A	A	-	-
	MacConkey Agar	Colony growth with lactose fermentation									
	EMB Agar	Dark centered brown colonies									
Staphylococcus spp.	Nutrient Agar	Yellow color colonies with central curvature	+	-	-	-	A	A	A	-	+
	MacConkey Agar	No growth									
	EMB Agar	No growth									
Pseudomonas Spp.	Nutrient Agar	Dirty white colonies with light green pigments	-	-	-	-	-	-	-	+	-
	MacConkey Agar	Good growth non lactose fermenting colonies									
	EMB Agar	Good growth and no fermentation of lactose									
Bacillus Spp.	Nutrient Agar	Colorless colonies with brown pigments	+	-	-	-	A	V	A	-	+
	MacConkey Agar	No growth									
	EMB Agar	No growth									

AG: Acid/Gas **A: Acid** **V: Variable** **+: Positive** **-: Negative**

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The aim of this study was to investigate the phytoremediation potential of two indigenous Saudi plants (*B. maritimus* and *P. australis*) in removing selected heavy metals and selected PAHs from contaminated waters. The goal was to see how much cadmium, lead and nickel the two plants could remove from spiked water. The phytoremediation potential of these plants was investigated under hydroponic conditions over a period of six weeks. 5ppm was selected as the concentration of each of the heavy metal while a concentration of 10ppm was selected for PAHs.

The phytoremediation potential was estimated by measuring the residual concentration in water as well as the amount of these contaminants present in different parts of the plants. It was observed that the two plants were excellent for the remediation of cadmium, lead and nickel but not that effective for the remediation of naphthalene. The effects of pH and salinity were observed not to be significant for the remediation of the contaminants. Also, the plants accumulated more heavy metals in their roots, followed by their shoots and small amount in their leaves. The presence of plant bacteria was believed to enhance the phytoremediation of these contaminants. By using the BCF and TCF as well as the effect of bacteria, rhizodegradation and phytostabilization are believed to be the mechanisms of

contaminant removal. Four distinct bacteria colonies were identified in planted media, namely: *Enterobacter spp., Staphylococcus spp., Pseudomonas spp. and Bacillus spp*.

5.2 RECOMMENDATIONS

In order to build upon the findings in this study and to better understand the utilization of the indigenous plants for phytoremediation, the following recommendations are proposed:

1. Different concentrations of cadmium and lead should be tried;
2. The phytoremediation potential of the plants used in this study should be evaluated for other heavy metals;
3. The phytoremediation potential of the plants used in this study should be evaluated for composites of lead and cadmium at different concentrations; and
4. The performances of other types of hydroponic system should be evaluated for phytoremediation.

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