Research Insights

Nano-Phytoremediation Technologies for Groundwater Contaminates

Emerging Research and Opportunities

Khushboo Chaudhary, Suphiya Khan,
and Pankaj Kumar Saraswat

IGI Global
DISSEMINATOR of KNOWLEDGE

Nano-Phytoremediation Technologies for Groundwater Contaminates:

Emerging Research and Opportunities

Khushboo Chaudhary
Banasthali University, Tonk Newai, India

Suphiya Khan
Banasthali University, Tonk Newai, India

Pankaj Kumar Saraswat
ICAR, Krishi Vigyan Kendra, Tamenglong District, Manipur, India

A volume in the Advances in Environmental Engineering and Green Technologies (AEEGT) Book Series

IGI Global
DISSEMINATOR OF KNOWLEDGE

Published in the United States of America by
IGI Global
Engineering Science Reference (an imprint of IGI Global)
701 E. Chocolate Avenue
Hershey PA, USA 17033
Tel: 717-533-8845
Fax: 717-533-8661
E-mail: cust@igi-global.com
Web site: http://www.igi-global.com

Library of Congress Cataloging-in-Publication Data

Names: Chaudhary, Khushboo, 1989- author. | Khan, Suphiya, 1976- author. | Saraswat, Pankaj Kumar, 1976- author.
Title: Nano-phytoremediation technologies for groundwater contaminates : emerging research and opportunities / by Khushboo Chaudhary, Suphiya Khan, and Pankaj Kumar Saraswat.
Description: First edition. | Hershey, PA : Engineering Science Reference, an imprint of IGI Global, [2020] | Includes bibliographical references.
Identifiers: LCCN 2019011811| ISBN 9781522590163 (hardcover) | ISBN 9781522590170 (ebook) | ISBN 9781522599012 (softcover)
Subjects: LCSH: Phytoremediation. | Groundwater--Purification. | Groundwater--Pollution. | Heavy metals--Environmental aspects.
Classification: LCC TD192.75 .C45 2020 | DDC 628.1/68--dc23 LC record available at https://lccn.loc.gov/2019011811

This book is published in the IGI Global book series Advances in Environmental Engineering and Green Technologies (AEEGT) (ISSN: 2326-9162; eISSN: 2326-9170)

British Cataloguing in Publication Data
A Cataloguing in Publication record for this book is available from the British Library.

All work contributed to this book is new, previously-unpublished material.
The views expressed in this book are those of the authors, but not necessarily of the publisher.

For electronic access to this publication, please contact: eresources@igi-global.com.

Advances in Environmental Engineering and Green Technologies (AEEGT) Book Series

ISSN:2326-9162
EISSN:2326-9170

Editor-in-Chief: Sang-Bing Tsai, University of Electronic Science and Technology of China Zhongshan Institute, China, Ming-Lang Tseng, Lunghwa University of Science and Technology, Taiwan, Yuchi Wang, University of Electronic Science and Technology of China Zhongshan Institute, China

MISSION

Growing awareness and an increased focus on environmental issues such as climate change, energy use, and loss of non-renewable resources have brought about a greater need for research that provides potential solutions to these problems. Research in environmental science and engineering continues to play a vital role in uncovering new opportunities for a “green” future.

The **Advances in Environmental Engineering and Green Technologies (AEEGT)** book series is a mouthpiece for research in all aspects of environmental science, earth science, and green initiatives. This series supports the ongoing research in this field through publishing books that discuss topics within environmental engineering or that deal with the interdisciplinary field of green technologies.

COVERAGE

- Waste Management
- Alternative Power Sources
- Electric Vehicles
- Radioactive Waste Treatment
- Contaminated Site Remediation
- Industrial Waste Management and Minimization
- Biofilters and Biofiltration
- Air Quality
- Water Supply and Treatment
- Sustainable Communities

IGI Global is currently accepting manuscripts for publication within this series. To submit a proposal for a volume in this series, please contact our Acquisition Editors at Acquisitions@igi-global.com or visit: http://www.igi-global.com/publish/.

The Advances in Environmental Engineering and Green Technologies (AEEGT) Book Series (ISSN 2326-9162) is published by IGI Global, 701 E. Chocolate Avenue, Hershey, PA 17033-1240, USA, www.igi-global.com. This series is composed of titles available for purchase individually; each title is edited to be contextually exclusive from any other title within the series. For pricing and ordering information please visit http://www.igi-global.com/book-series/advances-environmental-engineering-green-technologies/73679. Postmaster: Send all address changes to above address.

Titles in this Series

For a list of additional titles in this series, please visit:
https://www.igi-global.com/book-series/advances-environmental-engineering-green-technologies/73679

Implementation and Evaluation of Green Materials in Technology Development Emerging Research and Opportunities
M.T. Mastura (Universiti Teknikal Malaysia Melaka, Malaysia) and S. M. Sapuan (Universiti Putra Malaysia, Malaysia)
Engineering Science Reference • © 2020 • 215pp • H/C (ISBN: 9781799813743) • US $175.00

Modern Techniques for Agricultural Disease Management and Crop Yield Prediction
N. Pradeep (Bapuji Institute of Engineering and Technology, India) Sandeep Kautish (Lord Buddha Education Foundation, Nepal & Asia Pacific University, Malaysia) C.R. Nirmala (Bapuji Institute of Engineering and Technology, India) Vishal Goyal (Punjabi University, Patiala, India) and Sonia Abdellatif (Seoul National University, South Korea)
Engineering Science Reference • © 2020 • 291pp • H/C (ISBN: 9781522596325) • US $195.00

Novel Advancements in Electrical Power Planning and Performance
Smita Shandilya (Sagar Institute of Research, Technology and Science, India) Shishir Kumar Shandilya (Vellore Institute of Technology, India) Tripta Thakur (Maulana Azad National Institute of Technology, India) and Atulya K. Nagar (Liverpool Hope University, UK)
Engineering Science Reference • © 2020 • 388pp • H/C (ISBN: 9781522585510) • US $225.00

Global Food Politics and Approaches to Sustainable Consumption Emerging Research and Opportunities
Luke Amadi (University of Port Harcourt, Nigeria) and Fidelis Allen (University of Port Harcourt, Nigeria)
Engineering Science Reference • © 2020 • 242pp • H/C (ISBN: 9781799801252) • US $185.00

For an entire list of titles in this series, please visit:
https://www.igi-global.com/book-series/advances-environmental-engineering-green-technologies/73679

IGI Global
DISSEMINATOR OF KNOWLEDGE

701 East Chocolate Avenue, Hershey, PA 17033, USA
Tel: 717-533-8845 x100 • Fax: 717-533-8661
E-Mail: cust@igi-global.com • www.igi-global.com

Dedicated to my parents, my husband, and my loving kids Bhagyashree and Lavyansh

If an experiment doesn't work at right time, don't start changing things. Leave the experiment alone for a few hours and let your mind work things out.

Table of Contents

Preface

The present book entitled *Nano-Phytoremediation Technologies for Groundwater Contaminates: Emerging Research and Opportunities* is aimed at presenting precise information for undergraduate, post graduate student and also very helpful to research scholar. This book has updated information on phytoremediation and some experimental method used in life science laboratories. Plants are valuable resources for all living organisms which provide food, medicine, produce oxygen and regulate water cycle. Heavy metal stress has negative impact on environment. Its mean direct and indirect effect on human beings and drastic effect on crop yield. Heavy metals produced from industries and factories are non-biodegradable and have accumulated in soil and human body organ. Heavy metal toxicity has become a major threat to plant growth and crop yield. The mechanism of heavy metal accumulation, detoxification and tolerance have become the basis for using plants for remediating heavy metal contaminated soil and water. Phytoremdiation is the use of plants to uptake pollutants from the soil and water, sustainable environment cleanup technology is used worldwide. In this book are 11 chapters that will shed light on different heavy metals, non-metals, nanoparticles, and their effect on remedial approaches. All chapters is an overview on heavy metals and non-metal (Fluoride) toxicity, nutrient uptake, effect on plant growth, photosysnthesis, antioxidative properties, hormonal signaling, efficiency increase by plant growth promoting bacteria, biosynthesis of phytochelatins, molecular mechanism and at last their role in signaling. This book is a compilation of maximum information regarding phytoremdiation after our best efforts. I would like to thank Prof. Aditya Shastri and also to the funding support from "DST" Banasthali University, Newai (Rajasthan) India and Dr Naveen Kumar (Principal Scientist) NCVTC, National Research Center on Equines Hisar, Haryana India.

Khushboo Chaudhary
Banasthali University, Tonk Newai, India

Suphiya Khan
Banasthali University, Tonk Newai, India

Pankaj Kumar Saraswat
ICAR, Krishi Vigyan Kendra, Tamenglong District, Manipur, India

Acknowledgment

I acknowledge to the funding support from “MHRD project on center for excellence on Water and Energy”, under training and research in frontiers areas of science and technology (FAST 5-5/2014 TS VII) UGC and DST, Banasthali University, Newai (Rajasthan) India.

Chapter 1

Introduction to Phytoremediation

ABSTRACT

In this chapter, the authors describe phytoremediation technology, which is helpful for remediation of contaminated soil and groundwater. This information can be used for water and soil purification and may contribute to successful transfer of phytotechnologies to the agricultural or commercial sectors.

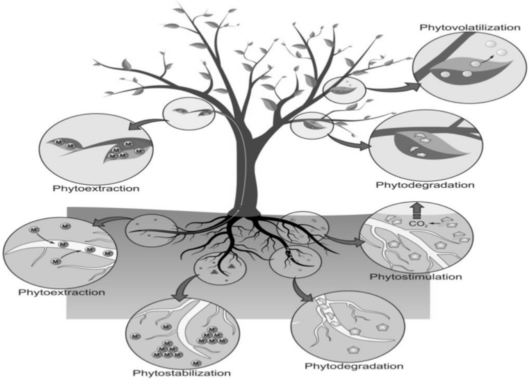
INTRODUCTION

Phytoremediation is a technology that uses plants for remediating soils and ground water. The processes of phytoremediation include number of techniques (phytodegradation, phytoextraction, phytostabilization, phytovolatilization and phytostimulation) showed in (figure 1). It is currently an exciting area of active research now to clean up the environment. A promising approach to low cost remediation technologies is phytoextraction, the use of plants to clean up polluted soils. Heavy metals are the most important inorganic pollutants, which are not degraded and progressively accumulate in the environment. Heavy metal pollutants are mostly resulting from industries such as; chemical fertilizers, chemical reagents, industry wastes and most important used in agriculture field herbicides and pesticids.

Pollution also comes from long sewage sludge, vehicle exhaust and several sources of waste water and it causes severly effects on plants, animal, soil, human beings and also on beneficial microbes which use for improvement of

DOI: 10.4018/978-1-5225-9016-3.ch001

Figure 1. Different kinds of phytoremediation used for cleaning polluted soil and water



crops. These contaminants accumulate in soils and after that crop uptake their contaminants and move into the food chain of humang being and also living organisms (Tak et al., 2013). The mainly general heavy metals (Cd, Cr, Hg, Se, Mn, Ni, Cu, Mg, Pb) significantly effect on environment and ecological evolutionary (Allen, 2014; Orisakwe, 2012). So, plants have the ability to translocate and accumulate metals in their organs and cells; i.e phytoextraction processs. There are different steps that involve in phytoextraction process (i) uptake and bioavalibity (ii) translocate of heavy metals (iii) sequestration of metals in leaves and vacuoles. High amount of heavy metals concentration accumulate in plant organs is not usually a naturally process for favoured reaction some how it's the plant capability to uptake more than other plants. Under conditions dependent mechanism are favoured reactions in specific plants to uptake the nutrients more than limits. In this condition plant defence system mechanism play a role for metabolic, physiological and expressional changes under stressful conditions caused by different pollutants. Studies can be required in details for known hyperaccumulators plants to enhance its phytoremdiation process.

PHYTOREMEDIATION TYPES

1. Phytoextraction: absorbed contaminates and store in above shoots and parts of roots.
2. Phytostabilization: immobilize contaminates through adsorption and preventing the spreading of contaminants in plants
3. Phytodegredation: enzymatic degradation of organic contaminates through the secreted enzymes
4. Phytostimulation: soil microbial communities break down the contaminants
5. Phytovolatilization: volatile through the stomata when gas exchange occurs

HYPERACCUMULATOR PLANTS

Hyperaccumulator term was proposed first time by Brooks et al. (1977) in reverence to those plants that can accumulate more than their natural favoured condition approximately 1000 mg kg^{-1} of heavy metals. Plants accumulate more and more contaminants and tolerate without showing any symptoms (Memon and Schroder, 2009). According to Baker and Brooks suggested that the minimum threshold tissue concentration for plants as 0.1% considered Ni, Cr, Cu, Co, and Pb hyperaccumulators but same as above the experiment was done in case of Mn, and Zn threshold value for plants established 1% (Baker and Brooks, 1989; Baker et al., 2000). Plants accumulate heavy metals in root to shoots which favoured that can allow translocation of minerals and sugars as they required a proper ratio maintained between the amounts of heavy metals specific in roots to shoots. These process names as translocation factor (TF). Hyperaccumulator plant need more than the TF value 1 (Tangahu et al., 2011). Same as above another factor discussed here about bioaccumulation factor (BF) value also is required more than 1 for hyperaccumulation (Ahmadpour et al., 2014).

Some different 450-500 plants have been identified as hyperaccumulator include *Thlaspi caerulescens* that accumulate (Pb, Ni, Cd and Zn), *Arabidopsis halleri* that can accumulate high levels og heavy metals (Cd and Zn but not Pb), *Alyssum bertolonii* can uptake (Ni and Co) and some other plants which belong to different family can also be participate to accumulate heavy metals such as *Caryophyllaceae, Fabaceae, Poaceae, Lamiaceae, Asteraceae, Cunoniaceae*

Table 1. Plants capable of hyperaccumulating metals

Plant species	Metal accumulated	Accumulated concentration mg/kg dry matter
Brassica juncea	Cu,Ni and Se	3,916
Brassica napus	Cd	7,800
Psychatria dauarrei	Ni	3,700
Pelargonium sp.	Cd	1,288
Lemnagibba	As	2666
Spartina plants	Hg	1,000
Rorippa globosa	Cd	2,189
Crotalaria juncea	Ni and Cr	1,000

and Cyperaceae and many others table 3.1 (Berti et al., 2002; Prasad, 2005; Padmavathiamma and Li, 2007; Maestri et al., 2010). Plants have specific properties that give us some specific advantages to remediate environment (Meagher, 2000; Meagher et al., 2000). Plants absorb metal particle through roots and roots hairs that generate surface area through which pollutants can be extracted form contaminated soil and water. Plants are autotrophs, it takes up nutrients directly from environment in gaseous form with the help of photosynthesis process. Heavy metals translocate in roots to shoots and also leaves it depend on plants which used for phytoremdiation purpose and called as hyperaccumulator (absorb more than required) and non-hyperaccumulator when they are not absorb as limit amount. Drawbacks also considered when significantly reduction of biomass of the plants. Different species were used for remove contaminants from the soil, and water but sometimes inability of plants mechanism to absorb insoluble form of heavy metals present in soil. This process is dependent on many circumtences like soil pH, water contents and also present of organic and inorganic substances. Naturally plants have the ability to uptake contaminants due to exist in soluble form iin soil and water. However, other types of reaction can also be taken up by the use of different amendments like plant growth promoting bacteria (PGPB) and also chelant-induce hyperaccumulation mechanisms around root (Abollino et al., 2006).

Figure 2. Photo view of Mariyan Oorini (right side) and laboratory setup of duckweed ecosystem (left side)

CASE STUDY

Phytoremediation of Polluted Pond Water by Duckweed

In this study, five artificial ecosystems has been studied using pond water taken from Mariyan oorini, Sattur, Virudhunagar district, Tamilnadu. An aquatic plant macrophyte (*Duckweed*) belonging to lemnacea family is used for this experimental study. A toxic herbicide (*Glyphosate*) is also used to test the toxicity effects on the artificial ecosystem. Mariyan Oorini is a part of Sattur town and is situated at 9º27´ North latitude and 77º46´ East latitude. figure 2 shows the image of Mariyan Oorini taken using digital camera as well as downloaded from Google Earth. From the experimental studies conducted, it was understood that the *Duckweed* plant efficiently removes 75% phosphate from pond water. Comparatively, the Duckweed growth rate enhancement is seen more in the pond water without toxicant *Glyphosate*. According to (Phewnil et al., 2012) there is no current set of standard in toxicity of Atrazine in surface water. In particular, the toxicity to the aquatic plants which are primary producers will cause an imbalance of the aquatic ecosystem. Similarly *Glyphosate* is being used in large quantities in India causing contamination of surface water. The result obtained in this study may be used to develop *Glyphosate* application standards for the surface waters of India.

CONCLUSION

Phytoremediation technique can be effectively used to purify contaminated water, soil and also increase biomass level. This technique is cost effective and easier approach for the removal of contamination. There is a lot of literature on phytoremdiation technology but unfortunately not many of them can be used for diagnosis of the phytoremdiation process. This chapter can be used for water and soil purification and may contribute to successful transfer of phytotechnologies to the agricultural or commercial sectors, which encourage exploration of ways to improve the economic viability of these technologies.

REFERENCES

Abollino, O., Giacomino, A., Malandrino, M., Mentasti, E., Aceto, M., & Barberis, R. (2006). Assesssment of metal availability in a contaminated soil by sequential extraction. *Water, Air, and Soil Pollution*, *173*(1-4), 315–338. doi:10.100711270-005-9006-9

Ahmadpour, P., Ahmadpour, F., Mahmud, T., Abdu, A., Soleimani, M., & Tayefeh, F. H. (2014). Phytoremediation of heavy metals: a green technology. In E. Asrari (Ed.), *Heavy metal contamination of water and Soil: Analysis, Assessment, and Remediation strategies* (p. 249). CRC Press, Taylor and Francis. doi:10.1201/b16566-15

Allen, L. H. (2014). Food safety: heavy metals. In Encyclopedia of human nutrition (3rd ed.; pp. 331-336). Academic Press.

Baker, A. J. M., & Brooks, R. R. (1989). Terrestrial higher plants which hyperaccumulate metallic elements– a review of their distribution, ecology and phytochemistry. *Biorecovery*, *1*, 81–126.

Baker, A. J. M., McGrath, S. P., Reeves, R. D., & Smith, J. A. C. (2000). Metal Hyperaccumulator Plants: A Review of the Ecology and Physiology of a Biological Resource for Phytoremediation of Metal Polluted Soils. In N. Terry & G. Bañuelos (Eds.), *Phytoremediation of Contaminated Soils and Waters* (pp. 85–107). Boca Raton, FL: CRC Press LLC.

Berti, W. R., & Cunningham, S. D. (2000). Phytostabilization of metals. In I. Raskin & B. D. Ensley (Eds.), *Phytoremediation of toxic metals - using plants to clean-up the environment* (pp. 71–88). New York: John Wiley & Sons, Inc.

Brooks, R., Lee, J., Reeves, R. D., & Jaffre, T. (1977). Detection nickeliferous rocks by analysis of herbarium specimens of indicator plants. *Journal of Geochemical Exploration*, *7*, 49–57. doi:10.1016/0375-6742(77)90074-7

Maestri, E., Marmiroli, M., Visioli, G., & Marmiroli, N. (2010). Metal tolerance and hyperaccumulation: Costs and trade-offs between traits and environment. *Environmental and Experimental Botany*, *68*(1), 1–13. doi:10.1016/j.envexpbot.2009.10.011

Meagher, R. B. (2000). Phytoremediation of toxic elemental and organic pollutants. *Current Opinion in Plant Biology*, *3*(2), 153–162. doi:10.1016/S1369-5266(99)00054-0 PMID:10712958

Meagher, R. B., Rugh, C. L., Kandasamy, M. K., Gragson, G., & Wang, N. J. (2000). Engineered phytoremediation of mercury pollution in soil and water using bacterial genes. In N. Terry & G. Banuelos (Eds.), *Phytoremediation of contaminated soil and water* (pp. 203–221). Lewis.

Memon, A. R., & Schroder, P. (2009). Implications of metal accumulations mechanisms to phytoremediation. *Environmental Science and Pollution Research International*, *16*(2), 162–175. doi:10.100711356-008-0079-z PMID:19067014

Orisakwe, O.E. (2012). *Other Heavy metals: Antimony, Cadmium, Chromium and Mercury*. Woodhead Publishing.

Padmavathiamma, P. K., & Li, L. Y. (2007). Phytoremediation technology: Hyperaccumulation metals in plants. *Water, Air, and Soil Pollution*, *184*(1-4), 105–126. doi:10.100711270-007-9401-5

Phewnil, A., Tungkananurak, N., Panichsakpatana, S., & Pitiyont, B. (2012). Phytotoxicity of atrazine herbicide to fresh water macrophyte Duckweed (*Lemna Perpusilla Torr.*) in Thailand. *Environ. Natur. Resour. J.*, *10*(1), 16–27.

Prasad, M. N. V. (2005). Nickel ophilous plants and their significance in phytotechnologies. *Brazilian Journal of Plant Physiology*, *17*(1), 113–128. doi:10.1590/S1677-04202005000100010

Tak, H. I., Ahmad, F., & Babalola, O. O. (2013). Advances in the application of plant growth-promoting rhizobacteria in phytoremediation of heavy metals. *Reviews of Environmental Contamination and Toxicology*, *223*, 33–52. doi:10.1007/978-1-4614-5577-6_2 PMID:23149811

Tangahu, B. V., Sheikh Abdullah, S. R., Basri, H., Idris, M., Anuar, N., & Mukhlisin, M. (2011). A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremdiation. *International Journal of Chemical Engineering*, *31*, 939161.

Chapter 2

Heavy Metal Pollution

ABSTRACT

In this chapter, the authors give information about heavy metal pollution in environmentally caused toxicity for plants and animals. The heavy metal contaminants polluting agricultural land reduce the crop productivity. So, the authors explore the process to work against these problems and reduce the contaminants.

INTRODUCTION

Heavy metals are non-biodegradable elements and progressively more accumulate in the environment and harmful to the human helath and animal also and toxicity cause in plants. Many conventional methods were used to remove contaminants but not successful due to high cost, least effective and remaining some waste after completion of process. Therefore, we focus on phytoremdiation technology in this method use of different kinds of plants for the removal of contaminants from the soil and water, so it provides some ecologically and environmentally sound and safe method for remediation (Chaudhary et al., 2015). However, a number of plant species have ability of hyperaccumulation of heavy metals but this approach is not applicable for all various contaminants to remove. Number of contaminants such as Benzene, toluene, ethylbenzene, xylene, chlorinated solvents (TCE, PCE), chlorinated pesicides, insecticides, petroleum hydrocarbones, oxides of sulfur, nitrogen, radionuclides (cesium, stronium, uranium) pollutants are

DOI: 10.4018/978-1-5225-9016-3.ch002

best suited for phytoremdiation. Phytoremdiation technology has been carried out commercially as field scale study in U.S.

The cultivation of plants in agriculture in the form of food, biofuels, fiber, medicinal and other products are used in human life. The study of "agriculture is known as agricultural sciences. The most coming up sectors in the "Indian economy and gross domestic product of the country is the biggest industry in several states can provide job opportunities throughout the nation. Thousands of years ago in agricultural history; there was agricultural development with the help of climates. Heavy metal pollutants are basically derived from industries in the form of waste, chemical reagents and agriculture practices such as pesticides, chemical fertilizers and most important herbicides". Heavy metal pollution also cause from vehicle exhaust; the long durability of sewage sludge; waste water of several sources to soils. These chemical have adverse effects on human helath, plants, soil, microflora, including beneficial microbes to the plants and also producing nitrogen for plant growth. These contaminants has been accumulate in the agricultural soils where they are destroyed crop and reach into the food chain through vegetables, harm to be creating at high risk to the living organisms and finally generous rise to the food scarceness. The contaminants reach plants through the process of absorption by roots from soil and disturb the balance of the food chain in the form contaminants (Tak et al., 2013). The pollutants is any substances in the environment that causes "unlikable effects to the human health, impairing the benefit of the environment to reducing the quality of life, and ultimately causing deaths of plants and animals at their higher concnetration of contaminants present. Heavy metals significantly effect on environmental things and their toxicity is a problem of increasing significance for ecological, evolutionary, and nurtional. The most common heavy metals contaminants are lead (Pb), Magnesium (Mg), copper, (Cu), nickel (Ni) manganese (Mn), selenium (Se), mercury (Hg), chromium (Cr) and cadmium (Cd) (Allen, 2014; Orisakwe, 2012). Some of these metals are micronutrients necessary for plant growth such as cobalt (Co), Ni, Mn, Fe, Zn," whereas other as unknown for biological functions such as As, Hg, Pb, and Cd (Gaur and Adholeya, 2004). They are stable and cannot be degraded; therefore they have a tendency to accumulate in soils and can be harmful to the aquatic life and water contaminated by toxic metals ions leave serious public health problem and destroyed environment where they are present at high amount and cross their limits.

Heavy metal toxicity in plant varies with plant species and apart from toxic and carcinogenic effects tend to be accumulated in living organisms. It is well known that heavy metals cannot be chemically degraded but need to be physically removed; or be transformed into nontoxic compounds (Gaur and Adholeya, 2004). The hazardous impact of the heavy metals was reported on the human body to correlate with the level of heavy metals in the environment. Heavy metals bioavailability in higher levels of the food chain may be due to water, house dust, honey, fish, vegetables and fruits (Gebrekidan et al., 2013). Heavy metals contamination causes cytotoxicity to human health and increasing level to antioxidant enzymes expression in plants which is harmful to the plants and also cause their toxicity in animals (Saidi et al., 2013). It can be evaluated by spectrophotometric detection of heavy metals present in soil at higher level, ground water, plants extracts, biosensors and through proteomics we can findout their presence at the higher limit (Asano et al., 2010; Soldatkin et al., 2012). Unlike; the organic contaminants remains in the soil for longer period of time and don't go through degradation for biological or chemical process (Bolan et al., 2014). Some heavy metals are very useful for plant growth in a very limit amount quantity and therefore on the other hand if it is present at beyond the level of amount are disturbing the environment.

LEAD CONTAMINATION

Lead contamination is soft metal that has been much application over the years and comes from automobile emmisions, pesticides; paint chips, betteries, some product of fertilizers and also from industries wastes. These contaminants spreades from major emission sources are mainly through airs (Rosman et al., 1993; Wang et al., 2015). The Pb-handling and health effects go back more than 20 centuries, which are remarkably repetitive record of Pb poisoning occurring well into this century. Lead (Pb) occurring naturally in the environment and exists in many forms such as (volcanoes, windblown dust and erosion) throughout the world and is now one of the most widely and evenly distributed heavy metals discusse in this chapter. The Soil can be contaminated by Pb from car exhaust, dust, and gases released from various industrial sources and move these contaminants into the plant organ. The Pb concentrations that are found in the environment are a result of human activities. It may be ingested by eating or drinking contaminated food and swallowing large particles. The cosmetics are also source of Pb contamination spread into the environment (Miller et al., 2004; Datko et al., 2014).

Lead may be may be transferred into the palnts directly through fall out or indirectly through uptake from the soil by air. Plant leaves take in carbon dioxide which is required for photosysnthesis and liberate oxygen due to presence of these conamination. Lead (Pb) adheres to the outer surfaces of the leaf and obstract light intensity which reaches into the inner wall. This will leads to the detection of palnt growth as well as its death by checking the velocity of photosynthesis, interruption in respiration and cause premature stages causes elongation in root cells, which affects its growth development. The skin is an important exposure route for Pb contamination which can easily penetrate (Miller et al., 2004). It can enter in human body through uptake of food such as (fruits, vegetables, crop products, meat, cold drink and alcohol). It can causes several health problems, such as (disturbance in the biosynthesis of hemoglobin and anemia, a rise in blood pressure, kidney damage, decline man fertility through sperm damage and can enter a fetus through placenta of the mother and cause serious damage to the nervous systems and also cause spontaneous abortions". So it is big problem to the environment.

ARSENIC CONTAMINATION

Arsenic compounds were first come from Chinese, grick and Egyptian civilization. They found its poisonous properties and also have several uses such as in bronzing, wood, preservation, pesticides and fireworks and was used in a variety of semi conductor applications. It is the naturally occurring element that is ubiquitous present within the earth crust. Arsenic is chemically classified as non-metallic but it is generally considered as a metal. The As combined with oxygen chlorine and sulfur, to make an inorganic arsenic compounds. The compounds are dangerous for human health and also somewhere in plants and animals. It cannot be destroyed within its surroundings and undergoes adjustment of its types or combined or separates from particles. The As toxicity in food occurred within the western areas of Japan in 1955. It is nonessential element and toxic to plants and animal also specially damaged in fish organs due to presence of higher concnetration of As in sea water. In plant roots are the first tissue to be showing where that metalloid As inhibits root extension and explosion. Plant first contact these contaminants by roots and after they move inside the plant and translocate into the shoots so it can severely inhibit the plant growth decrease the level of plant biomass and its accumulation as well as reproductive capacity through sufferers infertility, yield of crop and food production under these conatminants areas (Garg and Singla, 2011).

The sufficiently high concentration of As interference with dangerously metabolic process which can lead to the death of plants. The biological nitrogen (N)-fixation contribution was made by symbiosis in the root nodules legumes plants. Different microbes supply a large proportion of N in biological system and it's generally sensitive to As toxicity in respect to plants, animal and human being. Its toxicity even at low amount concentration has been caused black foot disease these contamination is also associated with coal mines (Dikshit et al., 2000; Lin et al., 1998; Manahan, 1997). In India human health are severely infected with As by contaminated drinking water and also its effect on plant like they were damaged physiologically and metabolically (Nordstrom, 2002). Arsenic enters into farming systems through geochemical process, mining operations and municipal solid wastes and also through groundwater (Meharg et al., 2009).

CADMIUM CONTAMINATION

Cadmium (Cd) is nonessential metal it attracts most consideration in soil science and plant nutrition for the reason that of its potential toxicity to humans and also its quality within the soil plant system mechanism. It is not naturally occurred but encourage as a visitor metal with Pb (Barker et al., 1990). It released into the surroundings by power stations, heating systems, metal operational industries or urban traffic areas. The important sources of Cd occur from domestic waste water; atmospheric deposition and industrial discharges (Benavides et al., 2005). Cadmium toxicity does not appear to be totally understood. Cd present in soil at high amount cause negatively affects on the plant growth and development mechanism (Aery and Rana, 2003). The most common symptoms of Cd toxicity in plants is chlorosis, stunning and leaf tissue damage (Sandalio et al., 2001) and it inhibit plant growth, transpiration reaction; reduces the absorption of nitrate and its transport from roots to shoot by inhibiting nitrate reductase activity in shoots or decreasing enzymatic and non-enzymatc antioxidant activity also decreased (Sandalio et al., 2001). The long term exposure of Cd leads to several morphological changes in the kidneys (Satarug and Moore, 2004). The major way of Cd contact to non-smoking population is via food and its detrimental effect on the central nervous system of human being. Tobacco is an important source of Cd uptake in smokers because it can accumulate from the soil into plant organ. Experimental data on animals and human have shown that absorption via the lungs is higher than gastrointestinal absorption (stomach). Up to 50% of the inhaled Cd may be absorbed, decreased attention and memory loss in humans being. The neurons are brain cells that communicate and transmit information, where they are affected brain function high blood pressure and cardiovascular disease also in human (Telisman et al., 2001).

MERCURY CONTAMINATION

Human activity has increased Hg levels in the environment over the past time. They emitted into the air, it falls to the earth and builds up in our water and soils where it is transformed into methyl mercury is highly toxic from that accumulate in the tissues of wildlife, plants, and animals. It is spread into the environmental and significantly accumulates in phytoplanktons and plant such as rice, all kind of fish and also move into the food chain (Hutchinson and Meema, 1987). It can releases from natural sources have remained comparatively stable in recent time, and rise in the environment. It salts and organomercury compounds are amongst the most poisonous substances into the environment. The toxicity depends strongly on the type of the compound and the redox state of Hg; terrestrial plants are generally not sensitive to the harmful effects of Hg compounds. It is also affect on the photosynthesis oxidative metabolism by interfering with the electron transport chain in mitochondria and chloroplast which inhibit the activity of aquaporins and reduces the plant water uptake (Sas-Nowosielska et al., 2008).

Some mushrooms, carrots, potatoes and aquatic plants were found to be good source of mercury accumulation. The Hg compounds was accumulate as toxins form and in small quantity per area units is dangerous to human health related to the exposure of extremely through food chain (Resaee et al., 2005). According to the United Nations, alkyl radical mercury within the human diet is nearly fully absorbed into the blood serum distributed to all tissue of the organs, accumulation occurring in brain cells, kidney and liver also affected these contaminants. It included symptoms like convulsions fits, extremely erratic movements shown among stock, particularly cats whose diets were high in food, affects the central system and therefore the areas related to the sensory, auditory, visual and cocoordinating functions in human being. In humans Hg inhaled through vapor form is absorbed via respiratory tract and spread all part of the body; symptoms such as a taxia, dysarthria, visual changes, hearing defects, coma, loss of speech and death.

METALS BIOAVAILIBILTY FOR UPTAKE PLANTS

Metal bioavailibity is low in soil system and are more bioavailable at acidic pH values. The fraction of soil metal is available for the plant uptake. In the soil organic matter and clay mineral content are important factors that can reduce metal bioavilability. The clay reduces metal bioavailability and toxicity just because of high caytion exchange capacities such as montmorillonite. Metals present in the soil is commonly as insoluble form and unavailable for transport into the plant roots. Metals, which are taken up by the plants are those which exist as soluble components in the soil solution and easily absorbed and solubilize by root exudates and solubilities of metals is depend only on the soil characterstics and strongly influenced by the soil pH and amount of soluble ligands present in soil.

CONCLUSION

Heavy metals contaminants are world wide problem and they are produced basically from industries effluents and also from agriculture wastes. These contaminants polluting agriculture land and nations whch simulataneously degraded of soil fertility and reduce the crop productivity. So find out the process to work against these problems to reduce the contaminants from the soil and water and increase the productivity of crop for agricultural production.

REFERENCES

Allen, L. H. (2014). Food safety: Heavy metals. In Encyclopedia of Human Nutrition (3rd ed.; pp. 331-336). Academic Press.

Arey, C., & Rana, D. K. (2003). Growth and cadmium uptake in barley under the cadmium stress. *Journal of Environmental Biology*, *24*, 117–123. PMID:12974451

Asano, T., Wang, P. C., & Iwasaki, A. (2010). Spectrophotometric detection of labile zinc 9ii) released from metal-Iothionein:a simple method to evaluate heavy metal toxicity. *Journal of Bioscience and Bioengineering*, *109*(6), 638–644. doi:10.1016/j.jbiosc.2009.11.016 PMID:20471607

Baker, A. J. M., Ewart, K., Hendry, G. A. F., Thorpe, P. C., & Walker, P. L. (1990). The evolutionary basis of cadmium tolerance in higher plants. *4th International Conference on Environmental Contamination*, 23-29.

Benavides, M. P., Susana, M., & Tomaro, M. (2005). Cadmium toxicity in plants. *Brazilian Journal of Plant Physiology*, *17*(1), 21–34. doi:10.1590/S1677-04202005000100003

Bolan, N., Kunhiikrishnan, A., Thangarajan, R., Kumpiene, J., Park, J., Makino, T., ... Schecker, K. (2014). Remediation of heavy metal (loid) contaminated soils-to mobilize to immobilize? *Journal of Hazardous Materials*, *266*, 141–166. doi:10.1016/j.jhazmat.2013.12.018 PMID:24394669

Chaudhary, K., Jan, S., & Khan, S. (2016). Heavy Metal ATPase (HMA2, HMA3 and HMA4) Genes in Hyperaccumulation Mechanism of Heavy Metals. In *Plant Metal Interaction*. Elsevier.

Datko, W. L., Wilkie, A., & Bryant, J. R. (2014). Analysis of U.S. soil lead (Pb) studies from 1970 to 2012. *The Science of the Total Environment*, *468-469*, 854–863. doi:10.1016/j.scitotenv.2013.08.089 PMID:24076506

Dikshi, A. K., Pallamreddy, K., Reddy, L. V. P., & Saha, J. C. (2000). Arsenic in ground water and its sorption by kimberlite tailings. *J. Environ. Sci. Health*, *35*(1), 65–85. doi:10.1080/10934520009376955

Garg, N., & Singla, P. (2011). Arsenic toxicity in crop plants: Physiological effects and tolerance mechanisms. *Environmental Chemistry Letters*, *9*(3), 303–321. doi:10.100710311-011-0313-7

Gaur, A., & Adholeya, A. (2004). Prospects of arbuscular mycorrhizal fungi in phytoremdiation of heavy metal contaminated soils. *Current Science*, *86*(4), 528–534.

Gebrekidan, A., Weldegebriel, Y., Hadera, A., & Bruggen, B. V. D. (2013). Toxicological assessment of heavy metals accumulated in vegetables and fruits grown in Ginfel river Sheba Tannery, Tigry, Northern Ethopia. *Ecotoxicology and Environmental Safety*, *95*, 171–178. doi:10.1016/j.ecoenv.2013.05.035 PMID:23790523

Hutchinson, T.C., & Meema, K.M. (1987). Lead, Mercury, Cadmium and Arsenic in the Environment. *Scientific Communities of Problems of the Environment (SCOPE), 31.*

Lin, T. H., Huang, Y. L., & Wang, M. Y. (1998). Arsenic species in drinking water, hair, fingernails, and urine of patients with black foot disease. *Journal of Toxicology and Environmental Health*, *53*(2), 85–93. doi:10.1080/009841098159376 PMID:9444313

Manahan, S. E. (1997). Article. *Environmental Science & Technology*, *11*, 383–394.

Meagher, A. A., Williams, P. N., Adomako, A., Lawgali, Y. Y., Deacon, C., Villada, A., ... Yanai, J. (2009). Geographical variation in total and inorganic arsenic content of polished (white) rice. *Environmental Science & Technology*, *43*(5), 1612–1617. doi:10.1021/es802612a PMID:19350943

Miller, R. L., Tegen, I., & Perlwitz, J. P. (2004). Surface radiative forcing by soil dust aerosols and the hydrologic cycle. *Journal of Geophysical Research*, *109*(D4), n/a. doi:10.1029/2003JD004085

Nordstrom, D. K. (2002). Public health-worldwide occurrences of arsenic in ground water. *Science*, *296*(5576), 2143–2145. doi:10.1126cience.1072375 PMID:12077387

Orisakwe, O.E. (2012). 1*1-Other Heavy metals: Antimony, Cadmium, Chromium and Mercury.* Woodhead Publishing.

Resaee, A., Deraya, J., Mortazavi, S. B., Yamini, Y., & Jafarzadeh, M. T. (2005). Removal of Mercury from chlor-alkali industry wastewater using Aceobacter xylinum cellulose. *American Journal of Environmental Sciences*, *1*(2), 102–105. doi:10.3844/ajessp.2005.102.105

Rosman, K. J. R., Chisholm, W., Boutron, C. F., Candelone, J. P., & Gorlach, U. (1993). Isotopic evidence for the source of lead in Greenland snows since the late 1960s. *Nature*, *362*(6418), 333–335. doi:10.1038/362333a0 PMID:29634020

Saidi, S. A., Azaza, M. S., Windmolders, P., Pelt, J. V., & Feki, A. E. (2013). Cytotoxicity evaluation and antioxidant enzyme expression related to heavy metals found in tuna by-products meal: An invitro study in human and rat liver cell lines. *Experimental and Toxicologic Pathology*, *65*(7-8), 1025–1033. doi:10.1016/j.etp.2013.03.001 PMID:23578882

Sandalio, L. M., Dalurzo, H. C., Gomez, M., Romero-Puertas, M. C., & del Río, L. A. (2001). Cadmium induced changes in the growth and oxidative metabolism of pea plants. *Journal of Experimental Botany*, *52*(364), 2115–2126. doi:10.1093/jexbot/52.364.2115 PMID:11604450

Sas-Nowosielska, A., Galimska-Stypa, R., Kucharski, R., Zielonka, U., Makowski, E., & Gray, L. (2008). Remediation aspect of microbial changes of plant rhizosphere in mercury contaminated soil. *Environmental Monitoring and Assessment*, *137*(1-3), 101–109. doi:10.100710661-007-9732-0 PMID:17492484

Satarug, S., & Moore, M. R. (2004). Adverse health effects of chronic exposure to low-level cadmium in foodstuffs and cigarette smoke. *Environmental Health Perspectives*, *112*(10), 1099–1103. doi:10.1289/ehp.6751 PMID:15238284

Soldatkin, O. O., Kucherenko, I. S., Pyashkova, V. M., Kukla, A. L., Jaffrezic, R. N., El'skaya, A. V., ... Soldatkin, A. P. (2012). Novel conductometric biosensor based on three-enzyme system for selective determination of heavy metal ions. *Bioelectrochemistry (Amsterdam, Netherlands)*, *83*, 25–30. doi:10.1016/j.bioelechem.2011.08.001 PMID:21903487

Tak, H. I., Ahmad, F., & Babalola, O. O. (2013). Advances in the application of plant growth-promoting rhizobacteria in phytoremediation of heavy metals. *Reviews of Environmental Contamination and Toxicology*, *223*, 33–52. doi:10.1007/978-1-4614-5577-6_2 PMID:23149811

Telisman, S., Jurasovic, J., Pizent, A., & Cvitkovic, P. (2001). Blood pressure in relation to biomarkers of lead, cadmium, copper, zinc, and selenium in men without occupational exposure to metals. *Environmental Research*, *87*(2), 57–68. doi:10.1006/enrs.2001.4292 PMID:11683589

Wang, J., Liu, G., Lu, L., Zhang, J., & Liu, H. (2015). Geochemical normalization and assessment of heavy metals (Cu,Pb, Zn, Ni) in sediments from the Huaihe River, Anhui, China. *Catena*, *129*, 30–38. doi:10.1016/j.catena.2015.02.008

Chapter 3
Non-Metal Pollution (Fluoride)

ABSTRACT

In this chapter, the authors describe Fluoride contamination spread in the environment. Fluoride in groundwater is a serious problem. Groundwater is the most valuable fresh water used for drinking purposes in different areas. Irrigation is one primordial sector in India where one-third of land surface falls under arid and semi-arid climate, and rainfall is seasonal and erratic. Semi-arid climate prevailing in Tonk district necessitates the characterization of groundwater quality for optimizing its use in irrigation as well as in domestic consumption. The majority of underground water contains a high concentration of salts, and their continuous use adversely affects soil, animal, and plant health, and thereby crop production. The plant-based phytoremediation approach to improve the quality of water and soil has become an area of importance to study regarding Fluoride.

INTRODUCTION

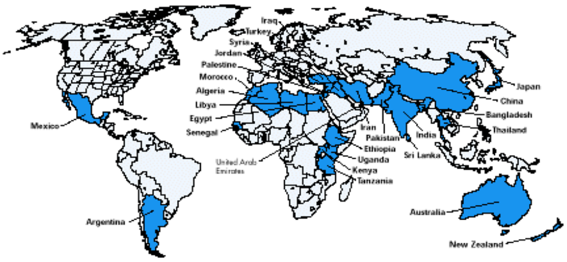
This chapter addresses about fluoride (F) in the soil, human being and plant parts affected when increased to a large extent with increasing fluoride concentration. Fluoride is as one of the most important environmental pollutant problems is responsible for soil and groundwater pollution causing dental and skeletal fulorosis and still no cure yet. The environment can largely be affected by the increase concentration of fluoride. Fluoride is one of the most important environmental macro pollutants responsible for the soil and groundwater pollution causing dental and skeletal fulorosis (Agarwal et al., 1997). Fluoride

DOI: 10.4018/978-1-5225-9016-3.ch003

(F) toxicity is a great cause of the concern in different lands, where it is found in high amount in the ground water. Elevated concentrations of fluoride in groundwater are responsible for serious health problem in many parts of the world. Worldwide, (Figure 1) more than 200 million people (including 70 million in India and 45 million in China) from 28 tropical countries are at the risk from dental, skeletal non-skeletal endemic fluorosis (Figure 2), i.e. the sign of fluoride poisoning (Yang et al., 2003). Fluoride pollution spread all over the world, India is severely suffering from its effects (Meenakshi and Maeshwari, 2006). Researchers investigate that F concentration in drinking water present at high amount has been found in Mexico, Holland, Itlay and Spain in South and North American countries (Mella et al., 1994). India has been critically affected by high F concentration and 17 out of 32 states were severely contaminated areas especially in Rajasthan (Vikas et al., 2013). In India, about 20% of F concentration were found in the household water supply; out of these 10% was only found in Rajasthan (Hussain et al. 2010). According to Saini et al. (2013) total F content in soil was higher level than normal ones KVK farm (127.56 $\mu g\ g^{-1}$) and Banasthali (679.63 $\mu g\ g^{-1}$). Fluoride concentration as high as 86 mgl^{-1} has been reported from Motipura village of Haryana, India (Garg et al., 2009). Application of fluoride-contaminated groundwater for the irrigation is common in many fluoride endemic areas, which can affect the crops considerably.

Fluoride is absorbed by plant roots and then transported via xylematic flow to different parts of the plant (Pant et al., 2008), where it can get accumulated. The effect of fluoride on germination, physiological and biochemical parameters in different plant species have been studied by many workers. These studies revealed that display to elevated fluoride can cause decreased germination, retarded plant growth (Miller et al., 1999), chlorosis (Mcnulty and Newman, 1961) and leaf necrosis (Elloumi et al., 2005). However, in fluorosis endemic areas, the fluoride content in plant parts have shown higher concentrations (Gupta et al., 2009). The risk for human health and the environment can largely be effected by the concentration of fluoride that occurs in groundwater and the rate by which fluoride transfer to groundwater as both these processes can be strongly persuade by the interaction of dissolved fluoride, with the soil solid phase via adsorption and desorption (Daniel et al., 1985). F- Present in soil is bound in complexes and is usually transported through the water cycle; F was estimated using Fluoride Ion Selective Electrode with TISAB (Rai et al., 2000).

Figure 1. Fluoride affected countries due to excess fluoride in driking water



Countries with endemic fluorosis due to excess fluoride in drinking water

Fluorine compound is industrially important and is widely used in semiconductors, fertilizers, metallurgical industries, phosphate production, glass manufacturing and nuclear applications" and also for plant growth at sufficient amount (Cheng et al., 2007). For instance, in phosphoric acid production, the fluoride concentration in waste water effluent could be reach up to 3000 mgL^{-1}. An appropriate concentration of fluoride in drinking water is necessary to prevent dental decay, but an excessive exposure to high concentrations of fluoride during tooth development can cause bone disease and mottling of the teeth also (Rango et al., 2012). Algae, aquatic plants, invertebrates and fishes in fresh water are also very sensitive by fluoride toxicity" (Camorgo et al., 2003). The acceptable amount of F is (1.5 mgl^{-1}) according to World Health Organization. Solution of this problem is used drinking water with low F level. There are many different methods for removed F like Donnan dialysis, electrodialysis, reverse osmosis, adsorption and biosorption, electro-coadgulation/flotation, nanofiltraton, and ion-exchange method (Kir et al., 2006; Sahli et al., 2007; Richards et al., 2010; Goswami et al., 2011; Emamjomeh et al., 2011; Chakrabortty et al., 2013; Guo et al., 2013). Above given methods have drawbacks are high cost efficiency, low regeneration power, low capacity to done and remain high amount of debris.

FLUORIDE ACCUMULATION PATHWAY IN PLANT

Field observations, Gupta et al. (2009) had detonation the mean F content in root, leaf, stem and seed of paddy plants to be 6.28, 5.85, 3.25 and 2.86 mg Kg^{-1}, respectively, when mean F concentration in paddy field soil was 2.6. These values are comparable with our result for 5 mg l^{-1} NaF exposure. They

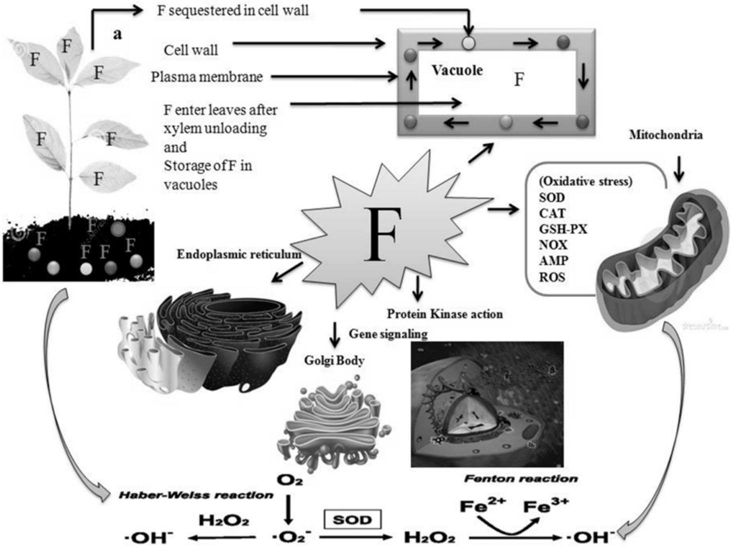
Figure 2. Fluoride problems in human being (dental fluorosis, skeletal endemic fluorosis and Foot-joint fuorosis)

concluded that F tends to hyper accumulate in paddy roots as soil to root TF is more than 1. There are only few studies on F concentrations in plant parts. From those studies, it can be said that, except in tea plants where more F is accumulated in the leaves (Fung et al., 1999), in general, F accumulation in the roots is higher than in leaves, stems and seeds (Arnesen, 1997; Weinstein and Davison, 2004; Gupta et al., 2009). Most F in the roots and their transport across the roots remain in the cell walls and intercellular spaces (apoplasts), rather than through the cell membranes and the endodermis (symplast) (Takmaz-Nisancioglu and Davison, 1988). The impermeable casparian strips in the wall of the endodermis act as barrier for F to enter the systems, which limit transport to the shoot and leaves. However, the casparian strip is discontinuous at the root tips and at sites of developing lateral roots. At these points, molecules can be cross into the xylem and can be conducted within the system (Figure 3).

FLUORIDE UPTAKE BY HYPERACCUMULATOR PLANTS

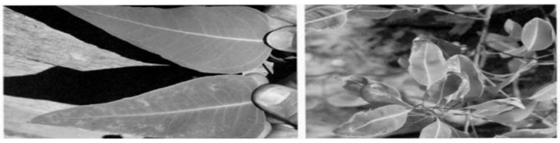
The mild washing procedure was most residues exchangeable and extractable from the root for uptake of F are passive (Pitman, 1965). Plants have different characterstics that can help to uptake of F from root to shoots and cell wall is the first barrier to the accumulation and translocation of F. Different plants have tolerate power towards contaminants but it depend on calcium (Ca) (present in the cell wall). Calcium acts as a buffer against F accumulation. Still were not known process about F mechanism, how they enter into the cell and cause toxicity, but since chloride deficiencies increase the uptake of F. According to Miller et al. (1985) suggest that being a halide for cellular uptake of F may be mediated by the channels of chloride. The most of the amount

Figure 3. Fluoride toxicity in plant organs, over production of reactive oxygen species in mitochondria and storage of F in vacuoles



of F in roots is in the apoplast cell walls, and intercellular spaces; and in the same way small movement through symplast cell membrane, plasmalemma, or tonoplast due to permanent negative charge of cell membrane support exclusion of negatively charged of F ions and also because of low permeability of cell membrane (Takmaz-Nisancioglu and Davison, 1988). The transport of shoots is limited due to the endodermis acts as an effective barrier to the vascular tissue. F contacts the vascular system by a non-selective route, that bypasses the endodermis and the concentration in individual leaves may be function of water flow (Davison et al., 1985). In soil the accumulation of F level in the different of plant parts as root>leaves>fruit>shoot (Gupta et al., 2011). The bioaccumulation of F in wheat plants treated with 20 mg/L NaF was found to be highest in roots (4.24 mg/g) and lowest in leaves (1.45 mg/g) (Bhargava and Bhardwaj, 2011). *Camellia sinensis* (Tea plants) accumulate large amounts of F in mature leaves from soil (Ruan et al., 2003), but the mechanism of F absorption by this plant species is not well understood yet. The F accumulation in leaves is mainly in form of free F anions or in connection with aluminum (Al), Ca, and magnesium (Mg) (Weinstein, 2004).

Figure 4. Fluoride causes chlorosis and necrosis in leave



Studies have revealed that there is a strong connection between F and Al as compared to other elements. If both are present in the medium, their uptake and translocation is better (Weinstein et al., 1982). Soluble fluorides are also accumulated by some aquatic biota. Aquatic plants and animals when exposed to (50 mg/kg F solution) reported bioconcentration factor >10 (Sloof et al., 1989). Among marine organism, red algae are known to accumulate the highest levels of F. At higher concentrations (>10 ppm), water hyacinth efficiently absorbed F from the intermediate though the affinity for accumulation decreased at lower levels (Rao et al., 1973). Studies conducted on the bioconcentration of F in two submerse (underwater) plant species viz. Milfoil (*Myriophyllum spicatum L.*) and Hornwort (*Ceratophyllum demersum L.*) showed that elevated F concentrations in water directly affected the F content of the submerged plants (Pinskwar et al., 2006). McNulty and Newman, (1957) suggested chlorosis and necrosis have long been recognized as the first visible symptoms of F injury to plants (Figure 4).

FLUORIDE REMOVES FROM SOIL

Phytoremediation is the use of green plants to remove contaminates pollutants from soil, water, and air (Greipsson et al., 2011). A search for F hyperaccumulators is an essential process for phytoremediation in F-endemic areas. Four important character used in defining a plant as a hyperaccumulator are (i) translocation factor (ii) bioconcentration factor (iii) tolerance (Yoon et al., 2006), and (iv) enrichment factor (Lorestani et al., 2011). A detailed knowledge about hyperaccumulator species that can accumulate F in significant amount is speedily growing high biomass crop with an extensive root system and yet showing least toxicity would be a safe, easy, and cheap approach. Such species can be raised to remediate F from soil. Similarly, the aquatic plant species can be easily utilized to remove F from polluted water bodies.

Toxicological exposure risk on humans in terms of estimated daily intake were assessed. It was found that the maximal F accumulation took place in roots (16.64–106.2 mg kg^{-1}) whereas in the edible part (fruit), it varied between 39.3 to 48.51mg kg^{-1} in the treatment range of 0-600 mg NaF kg^{-1} soil. The order of F accumulation in plant tissues followed root>leaf>fruit>shoot. Recently investigate the potential of eight tree species of semi-arid region for hyperaccumulation of F (Baunthiyal and Sharma, 2014). Their result suggested potential use of *Prosopis juliflora* in F removal from groundwater and soil. Plants tolerant and resistant to F are good candidate for remediating F from water and soil (Saini et al., 2012). F accumulation in the human body occurs through F-contaminated drinking water, stable amounts of F can also be ingested through crops and vegetables irrigated with F-contaminated water (Gupta and Banerjee, 2011). Fluoride uptake by plants is controlled by many factors including soil pH and organic compounds in the soil medium. Gupta et al. (2009) reported significant accumulation of F in paddy crops irrigated with F contaminated water from a village in West Bengal, India. Majority of F affected countries lie in the tropical belts where paddy is the major crop and rice is the staple food. Intake of F via food can be of significance with respect to human health, particularly in areas where F level in drinking water is already elevated. There are few field-based observations on F uptake by paddy, little information is available on the pattern of uptake and transfer of F in the paddy plant parts in a controlled condition. F accumulation in soil and vegetation in the vicinity of brick fields was previously reported (Jha et al., 2012). F uptake by different species of plants differs significantly based on their generic features and morphologically.

Therefore, the adverse effects of F in different crops may also vary significantly in different forms. F in soil or more specifically the phyto-availability of F is predominantly governed by the types of soil in which the crop is grown under the contaminants. The effect of F on photosynthesis in different plants is variable and depends not only on species but also on variety in a study done by Kumar and Rao, (2008). Recent research generated the fluoride accumulation in the paddy plants follows the order root>leave>seed. A different variety of paddy has being different translocation efficiency. Organ-wise accumulation of F for bioaccumulation factor, translocation factor, growth ratio, and F tolerance index were examined for *P. juliflora* plants grown in F enriched soil (Saini et al., 2012). In another experiment, the direction of *Jerbi* variety of grapes to balance F accumulation by parallel Ca accumulation in its leaf margins was studied. The comparing two varieties of mulberry towards F sensitivity. Mulberry variety Kanva (M4) showed lesser inhibition in all of photosynthetic parameters like leaf area, chlorophyll-a and b as compared to Mulberry variety (S54), reflecting tolerance nature of M4 variety.

CONCLUSION

The chapter of the adverse effect of fluoride, fluoride has been reported to interaction with the mineral by adsorption or chemical interaction. The plant-based phytoremediation approach to improve the quality of water and soil has become an area of importance to intense about study regarding F. A detailed knowledge finding about hyperaccumulators, which can accumulate F in substantial amounts and yet show least toxicity to the plant, would be a safe, easy, and cheap approach towards the removing F from F rich soils and water. Therefore many of the different plant species have been identified so far, which are accumulating F to a thousand times increase concentration. So we have to conclude that, more aware about F heavy non-metal and focus on the research work going on phytoremediation, findings the information that not be much available still.

REFERENCES

Agarwal, V., Vaish, A. K., & Vaish, P. (1997). Groundwater quality on fluoride and fluorosis in Rajasthan. *Current Science*, *73*, 743–764.

Akcil, A. (2004). Potential bioleaching developments towards commercial reality: Turkish metal mining's future. *Minerals Engineering*, *17*, 477–480.

Arnesen, A. K. M. (1997). Availability of fluoride to plants grown in contaminated soils. *Plant and Soil*, *191*(1), 13–25. doi:10.1023/A:1004210713596

Baunthiyal, M., & Sharma, V. (2012). Phytoremediation of fluoride contaminated water and soil: A search for fluoride hyperaccumulators. *J. Agricul. Technol.*, *8*(6), 1965–1975.

Bhargava, D., & Bhardwaj, N. (2011). Phytotoxicity of Fluoride on a Wheat Variety (*Triticum aestivum var.* Raj. 4083) and Its Bioaccumulation at the Reproductive Phase. *Asian J. Exp. Sci.*, *25*(1), 37–40.

Camargo, J. A. (2003). Fluoride toxicity to aquatic organisms: A review. *Chemosphere*, *50*(3), 251–264. doi:10.1016/S0045-6535(02)00498-8 PMID:12656244

Chakrabrabortty, S., Roy, M., & Pal, P. (2013). Removal of fluoride from contaminated groundwater by cross flow nanoflitration: Transport modeling and economic evoluation. *Desolination, 313*, 115-124.

Cheng, K.K., Chalmers, I., & Sheldon, T.A. (2007). *Adding fluoride to water supplies*. Academic Press.

Conover, C. A., & Poole, R. T. (1971). Influence of Fluoride on Foliar Necrosis of Cordyline terminalis "Baby Doll". *Proceedings of the Florida State Horticultural Society, 84*, 380–383.

Daisy, S., Khan, T. I., & Saxena, R. (2006). Effect of sodium fluoride on cluster bean (*Cyamopsis Tetragonolaba*) seed germination and seedling growth. Research report. *Fluoride*, *39*(3), 228–230.

Daniel, C. P., & Volk, V. V. (1985). Fluoride sorption and desorption in soils. *Soil Science Society of America Journal*, *49*(3), 583–586. doi:10.2136ssaj1985.03615995004900030010x

Das, D. P., Das, J., & Parida, K. M. (2003). Physicochemical Characterization and Adsorption Behavior of Calcined Zn/Al Hydrotalcite-like Compound (HTlc) towards Removal of Fluoride from Aqueous Solution. *Journal of Colloid and Interface Science*, *261*(2), 213–220. doi:10.1016/S0021-9797(03)00082-1 PMID:16256525

Davison, A. W. (1982). The effects of fluorides on plant growth and forage quality. In M. H. Unsworth & D. P. Ornrod (Eds.), *Effects of Gaseous Pollutants in Agriculture and Horticulture* (pp. 267–291). London: Butterworth Scientific. doi:10.1016/B978-0-408-10705-1.50018-1

Davison, A. W., Takmaz-Nisancioglu, S., & Bailey, I. F. (1985). The dynamics of fluoride accumulation by vegetation. In A. K. Susheela (Ed.), *Fluoride Toxicity* (pp. 30–46). New Delhi: ISFR.

Elloumi, N., Abdallah, F. B., Mezghani, I., Rhouma, A., & Boukhris, M. (2005). Effect of fluoride on almond seedlings in culture solution. *Fluoride*, *38*(3), 193–198.

Emamjomeh, M. M., Sivakumar, M., & Varyani, A. S. (2011). Analysis and the understanding of fluoride removal mechanisms by an electro coagulation / flotation (ECF). *Process. Desolination*, *275*(1), 102–106. doi:10.1016/j.desal.2011.02.032

Fung, K. F., Zhang, Z. Q., Wong, J. W. C., & Wong, M. H. (1999). Fluoride contents in tea and soil from tea plantations and the release of fluoride into tea liquor during infusion. *Environmental Pollution*, *104*(2), 197–205. doi:10.1016/S0269-7491(98)00187-0

Garg, V. K., Suthar, S., Singh, S., Sheoran, A., Garima, Meenakshi, & Jain, S. (2009). Driking water quality in villages of southwestern Haryana, India: Assessing human health risks associated with hydrochemistry. *Environ. Geol.*, *58*(6), 1329–1340. doi:10.100700254-008-1636-y

Goswami, A., & Purkait, M.K. (2011). *Kinetic and equilibrium study for the fluoride adsorption using pyrophyllite separation science and technology*. Academic Press.

Greipsson, S. (2011). Phytoremediation. *Nat. Educ. Knowl.*, *3*(10), 7.

Guo, S., Zhao, Z. X., Zhou, Q., Chan, W. L. H., & Chay, C. L. (2013). High electrostriction and relax or ferroelectric behaviour in proton irradiated poly (vinlidene fluoride trifluoroethylene) copolymer. *Applied Physics Letters*, *84*(17), 3349–3358. doi:10.1063/1.1715151

Gupta, S., & Banerjee, S. (2009). Fluoride Accumulation in Paddy (Oriza sativa) Irrigated with Fluoride-contaminated Groundwater in an Endemic Area of the Birbhum District, West Bengal. *Fluoride*, *42*, 224–227.

Gupta, S., & Banerjee, S. (2011). Fluoride Accumulation in Crop and Vegetable and Dietary Intake in Fluoride Endemic Area of West Bengal. *Fluoride*, *44*(3), 153–157.

Gupta, S., Banerjee, S., & Mondal, S. (2009). Phytotoxicity of fluoride in the germination of paddy (*Oryza sativa*) and its effect on the physiology and biochemistry of germinated seedlings. *Fluoride*, *42*(2), 142–146.

Gupta, S., Banerjee, S., & Mondal, S. (2009). Phytotoxicity of Fluoride in the Germination of Paddy (*Oryza sativa*) and Its Effect on the Physiology and Biochemistry of Germinated Seedlings. *Fluoride*, *42*(2), 142–146.

Jha, S. K. (2012). Geochemical and spatial appraisal of Fluoride in the soils of indo- gangetic Plows of india using multivariate Analysis, clean Soil, Air. *Water (Basel)*, *40*, 1392–1400.

Kir, E., & Alkan, E. (2006). Fluoride removal by Donnon dialysis with plasma- modified and unmodified anion exchange membranes. *Desalination*, *197*(1), 217–224. doi:10.1016/j.desal.2006.01.018

Kumar, A., & Bhaskara, V. R. (2008). Physiological Responses to Fluoride in Two Cultivars of Mulberry. *World Journal of Agricultural Sciences*, *4*(4), 463–466.

Lorestani, B., Cheraghi, M., & Yousefi, N. (2011). Phytoremediation Potential of Native Plants Growing on a Heavy Metals Contaminated Soil of Copper Mine in Iran. *World Academy of Science, Engineering and Technology*, *77*, 377–382.

Miller, G. W., Pushnik, J. C., & Giannini, J. L. (1985). A model for the subcellular distribution of fluoride in higher plants: Relation to in vitro concentrations required for enzyme inhibition, in Fluoride Research, Studies in Environmental Science. *Proceedings of the 14th Conference of the International Society for Fluoride Research*, 2, 241-251.

Miller, G. W., Shupe, J. L., & Vedina, O. T. 1999. Accumulation of fluoride in plants exposed to geothermal and industrial water. International Society for Fluoride Research.

Pant, S., Pant, P., & Bhiravamurthy, P.V. (2008). Effect of fluoride on early root and shoot growth of typical crop plants of India. *Fluoride Journal, 41*(1), 57-60.

Pinskwar, P., Jezierska-Madziar, M., & Gołdyn, H., Arczyn_ska-Chudy, E., & Golski, J. (2006). Fluorine Content of Two Submerged Plant Species in Four Warta River Oxbow Lake Reservoirs near Poznan, Poland. *Fluoride*, *39*(4), 310–312.

Pitman, M. G. (1965). Fluoride Transport across Plant Roots. *Quarterly Reviews of Biophysics*, *15*(3), 481–554. doi:10.1017/S0033583500003437 PMID:6758018

Qiu, G., Li, Q., Yu, R., Sun, Z., Liu, Y., Chen, M., ... Liu, X. (2011). Column bioleaching of uranium embedded in granite porphyry by a mesophilic acidophilic consortium.'. *Bioresource Technology*, *102*(7), 4697–4702. doi:10.1016/j.biortech.2011.01.038 PMID:21316943

Rai, K., Agarwal, M., Dass, S., & Shrivastava, R. (2000). Fluoride diffusive mobility in soil and some remedial measures to control its plant uptake. *Current Science*, *79*(9), 1370–1373.

Rango, T., Kravchenko, J., Atlaw, B., McCarnick, P.G., Jeuland, M., Merola, B., & Vengosh, A. (2012). *Groundwater quality and its health impact an assessment of dental fluorosis in rural inhabitants of the Main Ethiopian Rift*. Academic Press.

Rao, K. V., Khandekar, A., & Vaidyanadham, K. (1973). Uptake of Fluoride by Water Hyacinth, Eichhornia crassipes. *Indian Journal of Experimental Biology*, *11*, 68–69. PMID:4779299

Richards, L.A., Vuachere, M., & Schafer, A.I. (2010). *Impact of p H on the removal of fluoride, nitrate and boron by nanofiltration / reverse osmosis*. Academic Press.

Ruan, J., Ma, L., Shi, Y., & Han, W. (2003). Uptake of Fluoride by Tea Plant (*Camellia sinensis L.*) and the Impact of Aluminium. *Journal of the Science of Food and Agriculture*, *83*(13), 1342–1348. doi:10.1002/jsfa.1546

Sahli, M. A., Annouar, A., Tahaikt, S., Mountadar, M., Soufiane, A., & Elmidaoui, A. (2007). Fluoride Removal for Underground Brackish Water by Adsorption on the Natural Chitosan and by Electrodialysis. *Desalination*, *212*(1-3), 37–45. doi:10.1016/j.desal.2006.09.018

Saini, P., Baunthiyal, M., & Sharma, V. (2012). Organ-Wise Accumulation of Fluoride in *Prosopis juliflora* and Its Potential for Phytoremediation of Fluoride Contaminated Soil. *Chemosphere*, *5*(5), 633–635. doi:10.1016/j.chemosphere.2012.05.034 PMID:22704972

Sloof, W., Eerens, H., Janus, J., & Ros, J. (1989). *Integrated criteria document: Fluorides*. Report No. 758474010, National Institute of Public Health and Environmental Protection, Bilthoven.

Takmaz-Nisancioglu, S., & Davison, A. W. (1988). Effects of Aluminium on Fluoride Uptake by Plants. *The New Phytologist*, *109*(2), 149–155. doi:10.1111/j.1469-8137.1988.tb03702.x

Weinstein, L. H., & Alscher-Herman, R. (1982). Physiological responses of plants to fluorine. In M. Unsworth & D. P. Ormrod (Eds.), *Effects of Gaseous Air Pollution in Agriculture and Horticulture* (pp. 139–167). London: Butterworth Scientific. doi:10.1016/B978-0-408-10705-1.50012-0

Weinstein, L. H., & Davison, A. W. (2004). *Fluorides in the Environment: Effects on Plants and Animals*. Wallingford, UK: CABI Publishing. doi:10.1079/9780851996837.0000

Yang, M. C., & Lu, T. Y. (2003). The permeation performance of polyacrylonitrile/ Polyvinylidene, fluoride blend memebranes. *Journal of Membrane Science*, *226*(1), 119–130. doi:10.1016/j.memsci.2003.08.013

Yoon, J., Cao, X., Zhou, Q., & Ma, L. Q. (2006). Accumulation of Pb, Cu, and Zn in Native Plants Growing on a Contaminated Florida Site. *The Science of the Total Environment*, *368*(2-3), 456–464. doi:10.1016/j.scitotenv.2006.01.016 PMID:16600337

Chapter 4

Fluoride Contaminated Groundwater

ABSTRACT

In this chapter, the authors explore Fluoride (F) in groundwater as a major issue of water pollution. Geo-statistical analysis of groundwater quality in Newai Tehsil (India) has been done in order to identify the possible spatial distribution of water quality parameters and to assess the spatial dependence of water properties with the help of principal component analysis (PCA) structure. Two types of maps (spatial map and principal component map) of groundwater quality have been developed. A field experiment was conducted to investigate the effect of different Fluoride (F) concentration combined with Pseudomonas fluorescens (P.F) on Prosopis juliflora plant. The field design was used as completely randomized block design with three replicates. The study revealed that parameters were found to be positively and highly correlated with principal component. Low and high values (with their acceptable limit) have also been displayed over each spatial map. Plants treated with P. fluorescens showed the highest F uptake in root, shoot, and leaves tissues were 33.14, 19.41, and 15.15 mg kg^{-1} after 120 days, respectively. Both total bioaccumulation factor (BF) and translocation factor (TF) were obtained above one (i.e., 1.06 and 1.04). This confirmed the high accumulation and translocation of F in plant tissues. The F uptake efficiency of plant was enhanced to 67.7%, and plant biomass was increased to 57.03%. The present study will be beneficial for researchers working towards further improvement of F phytoremediation technology.

DOI: 10.4018/978-1-5225-9016-3.ch004

INTRODUCTION

Fluoride (F) contamination is a worldwide problem and severely affected by high F concentration, including India (Singh et al., 2018). About 260 million people worldwide are affected by F contamination (Amini et al., 2008; Banerjee, 2015; Kumari and Khan, 2017; Chaudhary et al., 2019). In India, Rajasthan state is the most severely affected from high F contamination in water and soil (Hussain et al., 2010; Saini et al., 2013). Dental and skeletal fluorosis is the most common effects of F contaminated water on human health and still there is no cure reported for it. F contamination also affect fetus, cerebral function, reduced intelligence in children and damages neurotransmitters (Xiang et al., 2003). The permissible limit for F in drinking water is specified by the World Health Organization as 1.5 mg L^{-1} (WHO, 2008). Fluoride is present in groundwater basically in the form of fluorite-CaF_2, cryolite-Na_3AlF_6 and fluor-apatite-$Ca_5(PO_4)_3F$, (Rao, 2009; Raj and Shaji, 2017). The fluorite and fluorapatite (FAP) are generally formed which is considered as groundwater fluoride contamination (Reddy et al., 2010). The calcite precipitation can be an enhanced dissolution of fluorite and fluorapatite (FAP) by the following reaction $CaCO_3 + 2F^- + H^+ = CaF_2 + HCO^-_3$. Fluoride has low mobility in soils and does not accumulate in upper soil horizons, but in slightly acidic soils, it is more soluble and shows greater leaching (Nowak, 2002; Amini et al., 2008). The large amount of F tend to accumulate in soil, which has an unfavorable impact on agricultural production (Chaudhary and Khan, 2016; Thapa et al., 2017).

Fluoride in groundwater is a serious problem (Crevecoeur et al., 2011). Groundwater is the most valuable fresh water used for drinking purposes in different areas. Irrigation is one primordial sector in India where one third of land surface falls under arid and semi-arid climate and rainfall is seasonal and erratic. Semi-arid climate prevailing in Tonk district necessitates the characterization of groundwater quality for optimizing its use in irrigation as well as in domestic consumption. The majority of underground water contains high concentration of salts and their continuous use adversely affects soil, animal and plant health, thereby crop production (Shahid et al., 2008). Geo-informatics technologies help in achieving goals viz. mapping of groundwater contamination and its availability and encompassing the modern tools of remote sensing (RS), Geographic information system (GIS), and Global positioning system (GPS) (Marwah, 2003; Magesh et al., 2012). In India, GIS has been introduced in various fields like optimizing land use plans, characterization of water, soil quality, waste's land and management of salt affected soils. Combination of these technologies provides cost effective means of acquiring high resolution real time data through remote sensing, data management and analysis through GIS and geo-referencing the groundwater through data with GPS information for a specific purpose. Groundwater quality is an essential parameter to be studied for sustainable development of agriculture and human life. The advent of information technology has developed tools like GPS and GIS which help in spatial characterization of groundwater quality. The maps generated through GPS and GIS delineate homogenous units to decide on the size and collecting a systematic set of geo-referenced samples and generating spatial data about groundwater quality (Sood et al., 2004 and Sharma, 2004). A comprehensive understanding of spatial variability in groundwater quality has become essential in precision agriculture. Groundwater quality varies spatially from field to a large region scale and is influenced by geology, topography, climate as well as soil use (Quine and Zhang, 2002). Quantitative evaluation of ground water parameters to obtain quality indices classification using principal component analysis (PCA) has immense utility (Norris, 1971). PCA also known as factor analysis is a statistical tool useful to reduce the number of variables to a smaller number of indices. The transformation of raw data using PCA can result in new values which are often easier to interpret than the original data (Norris, 1972).

Several conventional methods have been developed for the removal of F such as ion-exchange, phytoremediation, excavation and landfilling (Zhu et al., 2009). Among the reported remediation techniques, phytoremediation has proved to be more advantageous as it is highly effective and inexpensive (Abdul and Schroder, 2009).

Phytoremediation benefits depend on the enhancement in plant biomass and its resistance to withstand in F contaminated soil (Ali et al., 2013). Microbes also improve the soil quality by increasing uptake and translocation of non-metals for the growth and development of the plant. The microbes present in root-soil area are capable of performing metabolic activities promote plant growth and heavy metal-bioavailability (Zhang et al., 2011; Sumi et al., 2015). Microbes can also control groundwater fluoride contamination by dissolving different minerals such as phosphorous as apatite inclusions for nutrients, biotite and apatite by field verification (Welch et al., 2006). Our previous study reported that application of *P. fluorescens* increased the F uptake efficiency of plant *Prosopis juliflora* in controlled pot experiments condition. In addition, *P. fluorescens* also enhanced the mineral content and biomass of plant (Chaudhary and Khan, 2016). So, it is necessary to carry out field trials in addition to laboratory tests (Dickinson et al., 2009). However, successful results obtained in lab experiments under controlled conditions do not assure reproducibility at the field level. The multivariate statistical techniques and integration of GIS-based approaches are valuable when performing such spatial studies.

CASE STUDY

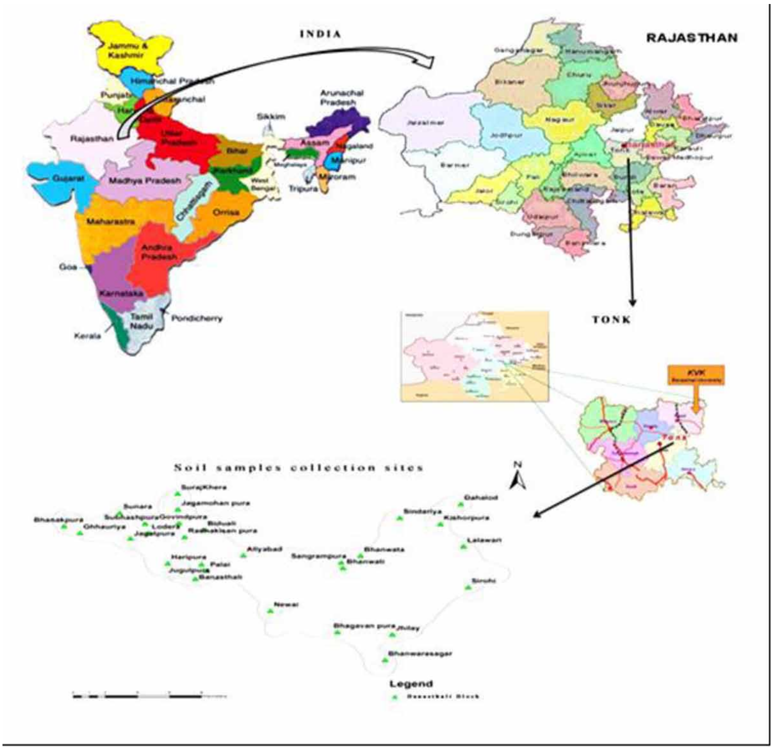
Bacterial Strains and Plant Materials

The strain *Pseudomonas fluorescens* MTCC 8904 was used as inoculums for the treatment. This strain was bought as discs of bacteria and obtained in lyophilized form from MTCC, Chandigarh, India. Seeds of *Prosopis juliflora* using in this study were collected from Central Arid Zone Research Institute (CAZRI), Jodhpur, Rajasthan (India).

SURVEY OF AREA AND GROUND WATER SAMPLING

Water samples were collected from twenty eight villages from Banasthali and Newai blocks for spatial characterization of groundwater quality using GPS (Trimble R_3) technique. The sixteen villages are taken for water samples such as namely Palai, Jugulpura, Bidauli, Aliyabad, Radhakishanpura, Govindpura, Jagmohanpura, Subhashpura, Sunara, Bhanakpura, Lodeda, Jagatpura, Surajkheda, Chhauriya, Banasthali and Haripura come under Banasthali block. Whereas twelve villages namely Bhanwati, Bhanwata, Saindariya, Kishorpura, Dahlod, Lalwadi, Sirohi, Jhilay, Sangrampura, Bhanwarsagar and Bhagwanpura under Newai block (Fig. 1). The water samples from 0 to 50 cm and 80-100 cm depth has been taken (Saby et al., 2006 and Inigo et al., 2011). Taking all protocols into account, a total number of 140 ground water samples of tube well, bore well and hand pump origin from twenty eight villages (5 from each) were collected in properly labeled neutral plastic bottles and brought to the Soil and Water testing laboratory for further chemical analysis. GPS points were recorded keeping in view that five water sample location are representing a single village. GPS survey helped to plot the latitudinal and longitudinal information in real world coordinate system. The GPS enabled data containing the positional location of the source of sample

Figure 1. Map showing the groundwater sampling site at Banasthali, Newai, Tehsil Tonk, Rajasthan, India



collection was further utilized to analyze the groundwater quality parameters in geo-spatial distribution pattern using geo statistical tools.

GROUND WATER QUALITY ANALYSIS

Collected water samples were employed for characterization of drinking and irrigation quality analysis. Groundwater samples were analyzed for pH, EC (ESEL Brand), cations (Ca^{+2}+Mg^{+2}), anions (Cl^{-}, CO_3 and HCO_3), residual sodium carbonate (RSC) and trace elements (Fe, Cu, Mn, Zn & F) using standard methods and procedures as outlined by Richards (1954), Tandon (2009) and Trivedy and Goel (1993). The pH and soluble salts in water samples were estimated by using pH and EC Meter. Cations and anions were analyzed by titration method and residual sodium carbonate (RSC) was calculated using formula as RSC (meq L^{-1}) = (CO_3+HCO_3) - (Ca^{+2} + Mg^{+2}). The presence of trace elements in groundwater was estimated by employing processed water samples on atomic absorption spectrophotometer (AAS Model No. 4129). Total soluble F content of soil samples was determined by alkali fusion-ion selective method (McQuaker and Gurney, 1977). The outcome of analysis of quality parameters of the samples (range and mean) are given in table 1a.

PRINCIPAL COMPONENT ANALYSIS AND GEOSPATIAL CHARACTERIZATION OF GROUNDWATER

Principal component analysis (PCA) is one of the multivariate analyses used as a method of data compression to remove data redundancy. Here the components of the variables are considered as spatial data set to i.e. in the form of geospatial image. The image of PCA data is independent and non-correlated, and also more interpretable than the quality parameters data. The process is easily explained graphically with an example of data in two quality parameters image/ bands. The two quality parameters shown by scatter plot, which shows the relationships of both the quality parameters in two axis bands. The values of one band are plotted against those of the other. If both bands have normal distributions, it forms an ellipse shape. The direction and length of the largest ellipse are calculated by using matrix algebra, which related to the longest ellipse axis, called the first principal component of data. It is also called first eigen vector and its length of value is first eigen. The points in the scatter plot are now given new coordinates, which corresponds to this new axis and new origin described. Therefore, it measures the highest variation within the data or as an axis in spectral space. The first eigen value is always greater than the range of the input quality bands, just as the hypotenuse of the right triangle must always be longer than the legs, the second principal component is the widest transect of the ellipse that is perpendicular or non-correlated to the first principal component. The analysis of the second principal component corresponds to the minor excess of the ellipse in a two dimensional way. Even though, there is an output in a PCA and high proportion of the variance in data in some cases almost 100%. Therefore, PCA is useful for compressing data into fewer bands. To compute the principal component image/band it is necessary to analyze the all water quality parameters in the geospatial model. In the present investigation there are twelve quality parameters from 140 geographical locations of the study area. The present study considered as non-spatial attributes of the water sample sources like tube well, bore well and hand pump.

The Arc-GIS environment the non-spatial data were attached to the spatial coordinate locations of all 140 sampling sites using GIS. As mentioned in PCA concept it requires a relationship among individual quality parameters. Using spatial interpolation each quality parameter converted into twelve water quality image/band in spatial domain. Now it is necessary to perform a spatial correlation among the parameters. Further using matrix algebra these twelve images of quality parameters were considered as row (m) and column (n) with quality bands (k) of images. All quality parameters considered as twelve bands and the variance matrix were generated. With the help of these matrices spatial correlation between these qualities parameters were identified. Further using transpose matrices and input of twelve quality parameters bands the twelve principal components were generated. The eigen value and eigen vector of all components were also calculated. Finally the relationship between component and quality parameters were also spatially evaluated using spatial correlation between component and quality parameter. With the help of Indian/WHO standards, all the quality parameters were explained in the form of spatial pattern and distribution of potable zone or threat zone. The raw data (Table 1 and 2) was interpolated to generate spatial surface map under principal factor analysis. Quantitative evaluation of groundwater quality indices using PCA has immense utility (Norris 1971).

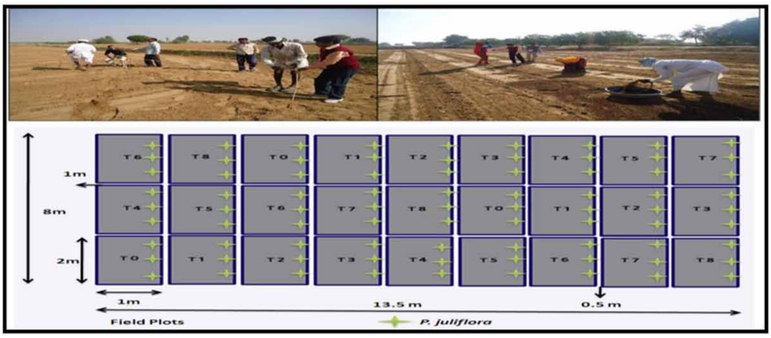
An attempt has been made to evaluate the groundwater quality of Banasthali and Newai Block area so as to make interpretation of groundwater quality by the use of principal component analysis. Principal factor analysis is a method or technique which provides a mean of identifying or measuring the relationship basic pattern in a data set. The principal component analysis of raw data of groundwater provided important statistical parameters viz. (i) correlation matrix (ii) variance-covariance matrix (iii) Eigen values and corresponding Eigen values. Eigen values explained the amount of variance contributed by each component. Eigen vectors are the coefficient of transformed equation of each component. Water quality of 28 village areas has been analyzed with respect to above mentioned quality parameters and further interpretation and inference have been drawn through spatial distribution considering their variability through GPS spatial map for all tewlve parameters (Table 1, 2, 3 and Spatial map 1-12). For the general discussion of groundwater quality parameters the range and mean of each five water samples collected from every village have been discussed in detail. Further the values of groundwater quality parameters have been put under factor analysis for making correlation estimation. The chemical analysis of all water samples is given in table 1, 2 and 3.

FIELD EXPERIMENT DESIGN

The field study was designed on the basis of phytoextraction experiments conducted at lab scale results of previous studies (Chaudhary and Khan, 2016). Phytoremediation experiments were conducted at Krashi Vigyan Kendra (KVK), Banasthali, Rajasthan, India during the period of February to June 2015 with temperatures ranging from 25 to 45°C. The study area lies between upper left 26°25'10.21' N latitude to 75°51'8.55' E longitude, mean elevation ranging from 304 m above mean sea level with mean annual rainfall of around 850 mm per annum. The field soil was sandy, loam and saline in nature. Firstly, the test area soil was excavated and immediately divided into twenty seven plots (length × width = $2m\times1m=2m^2$) (Figure 2), which were then bordered at the sides to prevent lateral flow between the plots. Soil wetland was constructed at experimental site of $2m^2$ (length×width) and 0.15 m depth (Figure 2). On the basis of pot experiments, *P. fluorescens* was inoculated in the amount of 10^8 cfu $mL^{-1}kg^{-1}$ and F concentrations for treatment were decided. Next, it was inoculated with test cultured and incubated at 37°C for 48 h. One ml of the culture was taken into a clean beaker and 9 ml of sterile water was added and mixed well in pot condition (1 ml for 1 kg of soil (total 10 ml per pot) and 1000 ml for 100 kg of soil in field condition = 100 ml culture and 900 ml distilled water). The amount of culture was taken for treatment according to the amount of soil present in field side. Before sowing, seeds were surface sterilized and soaked in distilled water for 12 hrs. Next day, seeds were washed and treated with tween 20 for 5 min.

Seeds again washed under tap water; sodium hypochlorite (2 ml) was applied for 5 min and finally rinsed under tap water for further use. Six seeds were sowed on one line bed at 1 cm depth in soil. Plants were artificially treated with different NaF concentrations of 25, 50, 75, 100 mg kg^{-1} in different bed sites alone and also further treated with *P. fluorescens.* The range of investigated F concentration added to the soil was screened with respect to the normal contents of F in soil. In case of 12 plots, we have supplied 12,000 ml (according to plot design) of *P. fluorescens* in field area (108 m) by spraying on soil surfaces after 10 days of germination (Marques et al., 2010), whereas the control group [12 plots with F concentration (added 12,000 ml of NaF) and 3 plots without F and P.F] were supplied with 1000 ml of

Figure 2. Overview of plot layout of field experiment at Krashi Vigyan Kendra (KVK) Banasthali, Tonk, Rajasthan, India. T0-Control; T1-25 mg kg^{-1} F; T2-50 mg kg^{-1} F, T3-75 mg kg^{-1} F, T4-100 mg kg^{-1} F; T5-25 mg kg^{-1} F+P.F; T6-50 mg kg^{-1} F+P.F; T7-75 mg kg^{-1} F+P.F; T8-100 mg kg^{-1} F+P.F



millipore water (three replications, Figure 2). The harvested time was 120 days. The F uptake efficiency of plant *P. juliflora* was detected at field scale through the method of Niu et al. (2007). The total F content in plant samples was measured through alkali fusion-ion selective technique through Thermo Scientific Orion Fluoride Ion Selective Electrodes (McQuaker and Gurney, 1977). Growth parameters and plant biomass were observed after 120 days. The wetland consisted of 27 plots, which included eight F treatment plots along with control group and all plots were in triplicates. Bioaccumulation factor and translocation factor were calculated by using method of (Zhao et al., 2003).

BF = [F concentration in shoot] / [F concentration in soil (1)

TF = [F concentration in shoot] / [F concentration in root] (2)

DATA ANALYSIS AND SOFTWARE

The statistical analysis was subjected to one-way ANOVA followed by LSD (Least Significant Difference) post hoc test at probability level 0.05 to separate means when ANOVA indicated a significant effect in accordance with the experimental design using SPSS (Software Programme for Social Science) software to quantify and evaluate the source of variation and the correlation between the different value were calculated (Steel and Torrie, 1981; Gerber et al., 1997). The principal component analysis (PCA) and multivariate statistical analysis were used for the following methods (Chabukdhara and

Nema, 2012a). Spatial distributions of F in groundwater were analyzed by an interpolation method (inverse distanced weighted (IDW) method). The graphs were prepared by Origin Pro software and analysis of the map image was carried out using ArcGIS (ver.10.2) software. The map interpretation was done using Geographic information system (GIS) based method for correctness spatial map decision.

RESULTS AND DISCUSSION

Chemical Characteristics of Banasthali Block Groundwater

The data given in table 1a reveals that irrespective of water sampling area in Banasthali block, the mean values of ground water reaction (pH) varied from 8.2-8.5 being lowest in Aliyabad and Jagmohanpura villages and highest in Govindpura village, but all water samples showed slightly alkalinity taste with respect to its pH. Water samples of Govindpura village area showed lowest electrical conductivity (3.46 dS m^{-1}) whereas Jagmohanpura area was of higher magnitude of EC values (14.8 dS m^{-1}) on the basis of its mean values. The Ca^{+2}+Mg^{+2} contents (1.08 meq L^{-1}) were found to be lowest in Surajkheda water samples whereas Banasthali water samples contained 3.76 meq L^{-1} (Ca^{+2}+Mg^{+2}) being highest compared to the rest of water samples. Jagatpura water samples registered lowest chloride (4.60 meq L^{-1}) and that of Radhakishanpura and Govindpura samples contained highest (19.80 meq L^{-1}) amount of chloride ions (table 1a). Carbonate (0.80 meq L^{-1}) and bicarbonate (5.42 meq L^{-1}) contents were of lowest magnitude in Surajpura and Jagatpura samples respectively.

Where as Jagmohanpura and Palai village water samples registered highest amount of carbonate and bicarbonate contents being 3.0 and 17.3 meq L^{-1} respectively (Table 1a). Residual sodium carbonate (RSC) showed positive correlation with CO_3+HCO_3 and Ca^{+2}+Mg^{+2}cations in water. RSC value was highest in Palai area (14.46) being lowest (5.56 meq L^{-1}) in Surajkheda village water samples. Although RSC showed a positive correlation with EC during study, but in Banasthali block RSC of ground water was found to be low in water samples having higher EC. These findings get a support from the work of Gupta (1981) who also found and reported that RSC of ground water decreases with increase in EC. Data pertaining to trace elements in groundwater, given in table 1a reveals that iron and manganese contents were comparatively higher than copper and zinc in groundwater irrespective of area under study. Fluoride content was found higher in Banasthali (69.22 mg L^{-1}) and low in Palai (18.29 mg L^{-1}). The present study is supported from the previous research (Saini et al., 2013; Chaudhary and khan, 2016). Bhanakpura area water samples registered higher amount of iron on the basis of mean values (table 1a). Manganese contents in water samples were lowest in Palai water and highest content of the same element was recorded in Bidauli and Chhauriya village water samples. Among copper and zinc, the copper contents were in general higher in Bidauli village (2.42 mg L^{-1}) and lowest in Jagmohanpura area. Whereas Zn content in groundwater was highest in Jagmohanpura and lowest in Bidauli village water samples.

CHEMICAL CHARACTERISTICS OF NEWAI BLOCK GROUNDWATER

The chemical analysis of groundwater samples of twelve village areas have been furnished in table 1a. A critical perusal of the data given in table 1a shows that irrespective of groundwater sampling area, the contents of chloride, carbonate and zinc were lowest in Bhagwanpura water samples where as Cl (14.60 meq L^{-1}), CO_3 (1.40 meq L^{-1}) and Zn (1.24 mg L^{-1}) were of higher magnitude in Saindariya and Dahlod area respectively (table 2b). Data further reveal that lowest values of HCO_3 (5.0) and RSC (1.72) were found in Saindariya and Dahlod respectively where as Bhagwanpura area showed the highest values of HCO_3 (9.60 meq L^{-1}) respectively.

The RSC was found (9.36 meq L^{-1}). Many evidences have been put forward for dominance of Cl, CO_3 and HCO_3 ions in groundwater of arid and semi-arid regions (Paliwal and Yadav, 1976; Minhas and Gupta, 1992). High depth of aquifer also contributes more cations thereby increasing salinity and hardness in groundwater (Cama et al., 2010). Deeper aquifers also show low resistivity in terms of longitudinal conductance than upper aquifers. Jacks et al., (2005) also reported that in semi-arid regions groundwater contains more salinity and presence of carbonate and bicarbonates which affect the irrigation and drinking quality of water. A critical perusal of the data with respect to trace elements (Fe, Cu, Mn and Zn) given in table 1a reveals that highest value of Fe (8.94 mg L^{-1}) and Mn (4.20 mg L^{-1}) were found in Kishorpura village where as Sangrampura and Bhanwarasagar contained lowest quantity of Fe

(5.30 mg L^{-1}) and Mn (0.68 mg L^{-1}) among all the area under study. Data further reveals that Newai and Dahlod showed highest contents of copper and Zinc respectively where as Dahlod and Bhagwanpura water samples area showed lowest Cu and Zn contents in them.

GEOSPATIAL CHARACTERISTICS OF GROUNDWATER QUALITY (GEO-STATISTICS OF INDIVIDUAL COMPONENT)

Groundwater sampling area covering (twenty eight villages) was divided in to Banasthali block (sixteen villages) and Newai block (twelve villages). Collected water samples were analyzed for eleven parameters (pH, Electrical conductivity (EC), calcium magnesium (Ca^{+2}+Mg^{+2}), carbonate (CO_3), bicarbonate (HCO_3), chloride (Cl^-), residual sodium carbonate (RSC) and trace elements including iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn). The general statistics of individual water quality parameter is furnished in table 1a. Data reveal that standard deviation values of all twelve layers are lower than the mean values, which indicate that the effect of abnormal data on sampling values were not great. Desirable or acceptable limits of these parameters have also been given within the table 1. Covariance matrix of individual quality parameters (Table 3) reveals that in manganese (-0.125), iron (-0.013), carbonate (-0.035), bicarbonate (-0.383) and residual sodium carbonate (-0.827) were found to be negatively correlated with Ca^{+2}+Mg^{+2} where as rest of all layers showed positive correlation with presence of Ca^{+2}+Mg^{+2} in groundwater sample. Manganese showed negative correlation (Mn -0.125) with Ca^{+2}+Mg^{+2}, copper (-0.185) and pH (-0.050) and rest all were found to positively correlated.

The iron showed negative correlation leaving only Mn, Cl, CO_3 and EC of groundwater. Leaving aside Ca+Mg, Zn and pH, the Cu had negative correlation with rest of all. Presence of Zn with Fe, Cl, HCO_3, pH and RSC showed negative correlation whereas rest of all parameters were found to be positively correlated with Zn. Chlorine showed positive correlation with most of the parameter barring Cu (-0.580), Zn (-0.030) and pH (-0.182). Presence of CO_3 in groundwater was found to be negatively correlated with Ca + Mg, copper and pH whereas HCO_3 amount had negative correlation with Ca+Mg, Mn, Cu, Zn and pH. The water reaction (pH) itself showed negative correlation leaving Ca+Mg, Cu, pH and EC of ground water. Further

EC was positively correlated with almost all layer barring only Cu whereas RSC showed positive association only with Mn, Cl, $CO_{3,}$ $HCO_{3,}$ EC and RSC of groundwater and rest parameters were highly positively correlated. Correlation matrix of these eleven layers has also been worked out and is given in table 2. Data reveals that RSC showed highly negative correlation among all the layers of groundwater quality parameter having negative value (-0.552) whereas manganese was highly positively correlated with Chlorine (0.422), Carbonate (0.558) and RSC (0.422) respectively. CO_3 and HCO_3 also showed strong correction with RSC layer. Presence of Zn and Fe did not show any strong correlation with any of the layer under study. Ca^{+2}+Mg^{+2} in groundwater were found to be highly and negatively correlated with RSC showing the presence of CO_3 and HCO_3 thereby higher pH in groundwater. The F has positive correlation with Fe, Mn, Zn and Cu and also with pH. Chloride showed highly positive correlation with Ca^{+2}+Mg^{+2}. Many saline sodic soils contain soluble carbonates besides the excess of soluble salts (Mamatha and Rao, 2010). In such soils, the topography, soil depth and amount of rainfall might have caused increase in sodicity and salinity of ground water. Nordstrom et al., 1989 also reported higher pH and EC of groundwater due to low recharging of wells and soils leaving soluble salts accumulated in subsurface layer. Higher EC in groundwater might be due to the fact that salts reach quickly in groundwater in shallow soils. Fluoride has better correlation with pH, EC, Ca^{+2}+Mg^{+2}, Fe, Mn, Zn, Cu and residual sodium biocarbonate. Declining water table in certain parts of Rajasthan and Gujarat has also been reported to be responsible for fluctuations in sodicity and salinity in groundwater (Welch et al., 2006; Zhang et al., 2006). The positive and negative correlation both were found in present study. After evaluating the present data of these all twelve layers suggested that groundwater of some villages was not suitable for drinking purposes. The most alarming condition was seen in some sites where F concentrations at high amount.

PRINCIPAL COMPONENT ANALYSIS OF INDIVIDUAL LAYERS

The results were found positively correlated of principal component analysis in case of F contamination in groundwater and total variance explained by the component of quality parameters in Table 1b, supplementary file. Eigen values with percent of eigen value and cumulative for each component are also

*Table 1. Geo-statistics of individual layers of groundwater quality parameters (*WHO/Indian Standard for Drinking Purpose) Banasthali, Tonk, Rajasthan, India.*

S. No.	Layer	Min.	Max	Mean	SD	Desirable Limit*	Units	Class	Value
1	$Ca^{+2}+Mg^{+2}$	0.00	7.63	2.76	1.05	15-40	meq/L		
2	Mn	0.32	4.85	1.99	1.11	5 .00	µg/L	High	0.90 to 0.70
3	Fe	4.18	9.79	6.55	0.92	100.0	µg/L	High	0.70 to 0.50
4	Cu	0.48	5.32	2.31	1.19	50.00	µg/L	Medium	0.50 to 0.35
5	Zn	0.38	2.12	1.01	0.20	5.00	µg/L	Low	Below 0.35
6	Cl	2.66	24.17	9.02	4.04	1.00	meq/L		
7	CO_3	0.00	3.33	1.10	0.57		meq/L		
8	HCO_3	1.83	19.60	7.12	3.11		meq/L		
9	pH	7.30	9.39	8.49	0.32	6.50-8.50			
10	EC	2.73	29.05	9.68	3.70	<3	dS/m		
11	RSC	0.00	20.82	5.63	3.73	1.25-2.50	meq/L		
12	F	5.89	40.45	23.17	3.78	1.5	mg/L	High	0.50 to 1.50

Note: Values are mean of three replicates, S.No. Serial number; SD-Standard deviation (Electrical conductivity-EC dSm^{-1}), (Calcium-Ca, Chloride-Cl, Carbonate- CO_3, Biocarbonate-HCO_3, Residual sodium carbonate-RSC, Magnesium-Mg, Sulfur-S, Phosphorus-P, Potassium-K, Iron-Fe, Manganese-Mn, Zinc-Zn, Copper-Cu, Fluoride-F).

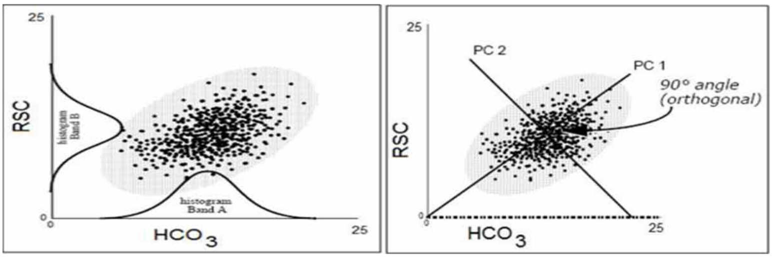
given in the table 1b. Data reveals that eigen values corresponding to the first four components are 44.925, 28.402, 18.594, and 3.136 in decreasing order and nearly 95.057% of variation is explained. Data further shows that eigen values corresponding to the first four components are 10.084, 6.375, 4.174 and 0.704, respectively (Fig. 3). The principal component analysis results of groundwater quality parameters reveal the correlation between these variables and the intensity of their affinity with the significant tests. In addition the factors contribution more in explaining the variations of the results are also studies by toposheet graph of each principal component (Aitchison, 1983; Tariq et al., 2008; Inigo et al., 2011).

Table 2. Covariance matrix of individual layer of F contamination in groundwater quality parameters Banasthali, Tonk, Rajasthan, India.

	1	2	3	4	5	6	7	8	9	10	11	12
Layer	**$Ca^{+2}+Mg^{+2}$**	**Mn^{+4}**	**Fe^{+3}**	**Cu^{+2}**	**Zn^{+2}**	**Cl^-**	**CO^-_3**	**HCO^-_3**	**pH**	**EC**	**RSC**	**F**
$Ca^{+2}+Mg^{+2}$	0.422	-0.125	-0.013	0.184	0.011	0.094	-0.035	-0.383	0.02	0.228	-0.827	0.351
Mn^{+4}	-0.125	0.475	0.120	-0.185	0.019	0.726	0.136	0.476	-0.05	0.308	0.671	0.152
Fe^{+3}	-0.013	0.120	0.327	-0.052	-0.004	0.242	0.037	-0.075	-0.00	0.025	-0.029	0.751
Cu^{+2}	0.184	-0.185	-0.052	0.540	0.000	-0.580	-0.034	-0.332	0.06	-0.207	-0.514	0.457
Zn^{+2}	0.011	0.019	-0.004	0.000	0.015	-0.030	0.003	-0.031	-0.00	0.034	-0.040	0.767
Cl^-	0.094	0.726	0.242	-0.580	-0.030	6.227	0.409	0.864	-0.18	1.512	0.985	0.135
CO^{-}_3	-0.035	0.136	0.037	-0.034	0.003	0.409	0.126	0.267	-0.02	0.241	0.425	0.212
HCO^-_3	-0.383	0.476	-0.075	-0.332	-0.031	0.864	0.267	3.710	-0.07	0.691	4.141	0.102
pH	0.022	-0.050	-0.007	0.065	-0.002	-0.182	-0.027	-0.079	0.04	0.047	-0.139	0.108
EC	0.228	0.308	0.025	-0.207	0.034	1.512	0.241	0.691	0.047	5.237	0.625	0.107
RSC	-0.827	0.671	-0.029	-0.514	-0.040	0.985	0.425	4.141	-0.13	0.625	5.328	0.678
F	0.523	0.455	0.275	0.345	0.256	0.962	0.232	0.197	0.68	0.478	0.655	0.265

Note: (Electrical conductivity-EC dSm^{-1}), (Calcium-Ca, Chloride-Cl, Carbonate-CO_3, Biocarbonate-HCO_3, Residual sodium carbonate-RSC, Magnesium-Mg, Sulfur-S, Phosphorus-P, Potassium-K, Iron-Fe, Manganese-Mn, Zinc-Zn, Copper-Cu, Fluoride-F).

Figure 3. Principal component analysis scatter plot of two quality parameters image/ bands and scatter plot of new coordinates Eigen values



MICROBE-ASSISTED PHYTOREMEDIATION EXPERIMENTS

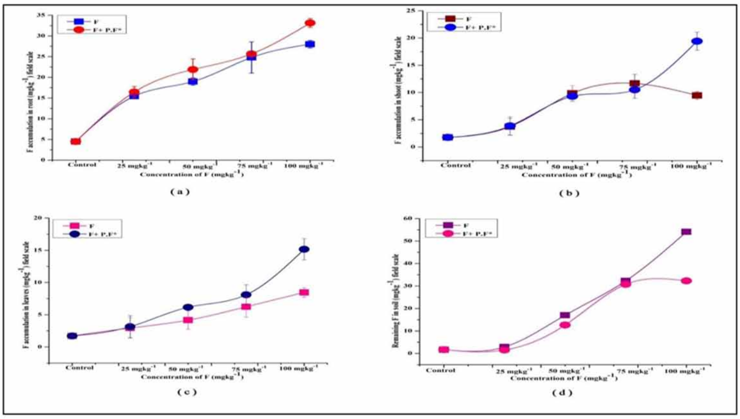
F concentrations in *P. juliflora* roots, shoot and leaves, as well as the total accumulation for single plants in treatments with *P. fluorescens* were higher than those in treatments with F only. *P. juliflora* accumulated 33.14 in root, 19.41 in shoot and 15.15 mg kg^{-1} of F in leave dry tissue, respectively and

Table 3. Covariance matrix of individual layer of F contamination in groundwater of Banasthali, Tonk, Rajasthan, India.

1	2	3	4	5	6	7	8	9	10	11	12
Ca^{+2}+Mg^{+2}	**Mn^{+4}**	**Fe^{+3}**	**Cu^{+2}**	**Zn^{+2}**	**Cl^-**	**CO^-_3**	**HCO^-_3**	**pH**	**EC**	**RSC**	**F**
1											
-0.278	1										
-0.034	0.303	1									
0.385	-0.366	-0.124	1								
0.132	0.227	-0.060	-0.005	1							
0.058	0.422	0.170	-0.316	-0.099	1						
-0.152	0.558	0.182	-0.129	0.065	0.463	1					
-0.306	0.359	-0.068	-0.234	-0.130	0.180	0.391	1				
0.174	-0.367	-0.063	0.444	-0.076	-0.366	-0.382	-0.207	1			
0.153	0.195	0.019	-0.123	0.121	0.265	0.297	0.157	0.103	1		
-0.552	0.422	-0.022	-0.303	-0.140	0.171	0.520	0.931	-0.302	0.118	1	
PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**	**PC10**	**PC11**	**PC12**
10.084	6.375	4.174	0.704	0.380	0.344	0.223	0.107	0.025	0.020	0.011	0.35
44.925	28.402	18.594	3.136	1.691	1.532	0.995	0.476	0.110	0.089	0.050	0.561
44.925	73.327	91.921	95.057	96.748	98.280	99.275	99.751	99.861	99.950	100.000	0.452

the remaining F detected in soil was 32.57 mg kg^{-1} at 100 mg kg^{-1} F (Figure 4). Translocation factor (TF) was calculated from the shoot and leave tissue and the bioaccumulation factor (BF) was obtained from the root, shoot and leaves at 100 mg kg^{-1} F. Present study calculated the BF as the ratio between the total F content in the root, shoot and leaves against total F in the soil. The total translocation (shoot and leaves) and bioaccumulation factor obtained from the shoot and leaves tissue were 1.04 and 1.06 at 100 mg kg^{-1} F. In this study, BF obtained was more than one which reflects the potential of *P. juliflora* for phytoremediation of F. As for efficient hyperaccumulator plant, it is essential that the BF is greater than 1 (Ahmadpour et al., 2014). Present study showed that *P. fluorescens* application significantly increased the B.F and T.F of F treated plants in comparison to control plants.

Figure 4. Effect of Pseudomonas fluorescence on P. juliflora plant F accumulation (a) root uptake, (b) shoot uptake (c) leaves uptake (d) remaining F in soil (P.F-Pseudomonas fluorescence, P<0.05)*



CONCLUSION

Present study reported that combination of geostatistical and multivariate statistical analysis to examine the spatial distribution of Fluoride contamination in groundwater is valuable and good for public health protection. Present study has been identified of groundwater quality parameters when performing spatial studies. The groundwater quality parameters were used to carve out spatial distribution maps of all water quality parameters of study area covering twenty eight villages in Newai Tehsil, Tonk, Rajasthan, India. Proper legends have also been developed over each spatial map to describe the relative distribution of each quality parameter/water property. From the present study, it can be concluded that the groundwater is not suitable for drinking purpose of the studied area. We reported F uptake efficiency increased of hyperaccumulator plant *P. juliflora* through the application of *P. fluorescens* at field scale. Application of *P. fluorescens* enhanced the F removal efficiency of *P. juliflora* by more than 50% in field condition. In addition, outputs in present study can be helpful or improve land management for agriculture purposes.

REFERENCES

Abdul, M. R., & Schroder, P. (2009). Implications of metal accumulation mechanisms to phytoremediation. *Environmental Science and Pollution Research International*, *16*(2), 162–175. doi:10.100711356-008-0079-z PMID:19067014

Ahmadpour, P., Ahmadpour, F., Mahmud, T., Abdu, A., Soleimani, M., & Tayefeh, F. H. (2014). Phytoremediation of heavy metals: a green technology. In E. Asrari (Ed.), *Heavy metal contamination of water and Soil: Analysis, Assessment, and Remediation strategies. CRC Press*. doi:10.1201/b16566-15

Aitchison, J. (1983). Principal component analysis of compositional data. *Biometrika*, *70*(1), 57–65. doi:10.1093/biomet/70.1.57

Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals-concepts and applications. *Chemosphere*, *91*(7), 869–881. doi:10.1016/j.chemosphere.2013.01.075 PMID:23466085

Amini, M., Mueller, K., Abbaspour, K. C., Rosenberg, T., Afyuni, M., Møller, K. N., ... Johnson, C. A. (2008). Statistical modeling of global geogenic fluoride contamination in groundwaters. *Environmental Science & Technology*, *42*(10), 3662–3668. doi:10.1021/es071958y PMID:18546705

Banerjee, A. (2015). Groundwater fluoride contamination: A reappraisal. *Geoscience Frontiers*, *6*(2), 277–284. doi:10.1016/j.gsf.2014.03.003

Cama, J., Zhang, L., Soler, J. M., De Giudici, G., Ardvison, R. S., & Luttge, A. (2010). Fluorite dissolution in acidic pH: In situ AFM and ex situ VSI experiments and Monte Carlo simulations. *Geochimica et Cosmochimica Acta*, *74*(15), 4298–4311. doi:10.1016/j.gca.2010.04.067

Chabukdhara, M., & Nema, A. K. (2012a). Assessment of heavy metal contamination in Hindon River sediments: A chemometric and geochemical approach. *Chemosphere*, *87*(8), 945–953. doi:10.1016/j.chemosphere.2012.01.055 PMID:22406241

Chaudhary, K., & Khan, S. (2016). Physiochemical characterization of Fluoride (F) contaminated soil and its microbe-assited bioremediation by *Prosopis juliflora. J. Plant Biol. States of Health*, *3*(2), 8.

Chaudhary, K., Saraswat, P. K., & Khan, S. (2019). Improvement in fluoride remediation technology using GIS based mapping of fluoride contaminated groundwater and microbe assisted phytoremediation. *Ecotoxicology and Environmental Safety*, *168*, 164–176. doi:10.1016/j.ecoenv.2018.10.007 PMID:30388533

Crévecoeur, S., Debacker, V., Joaquim-Justo, C., Gobert, S., Scippo, M.-L., Dejonghe, W., ... Thomé, J.-P. (2011). Groundwater quality assessment of one former industrial site in Belgium using a TRIAD-like approach. *Environ. Pollut.*, *159*(10), 2461–2466. doi:10.1016/j.envpol.2011.06.026 PMID:21764196

Dickinson, N. M., Baker, A. J. M., Doronila, A., Laidlaw, S., & Reeves, R. D. (2009). Phytoremediation of inorganics: Realism and synergies. *International Journal of Phytoremediation*, *11*(2), 97–114. doi:10.1080/15226510802378368 PMID:28133994

Dobbelaere, S., Vanderleyden, J., & Okon, Y. (2003). Plant growth-promoting effects of diazotrophs in the rhizosphere. *CRC Crit. Rev. Plant Sci.*, *22*(2), 107–149. doi:10.1080/713610853

Fahad, S., Hussain, S., Bano, A., Saud, S., Hassan, S., Shan, D., ... Tabassum, M. A. (2015). Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: Consequences for changing environment. *Environmental Science and Pollution Research International*, *22*(7), 4907–4921. doi:10.100711356-014-3754-2 PMID:25369916

Gerber, S. B., Kristin, E., Voelki, Andrson, T.W., & Finn, J. D. (1997). SPSS Guide to New Statistical Analysis of Data. Springer.

Gupta, I. C. (1981). *Use of saline water in agriculture: A study of arid and semi-arid zones of India. Oxford & IBH Publication Co* Pvt. Ltd.

Hazotte, A. A., Peron, O., Abdelouas, A., Montavon, G., & Lebeau, T. (2016). Microbial mobilization of cesium from illite: The role of organic acids and siderophores. *Chemical Geology*, *428*, 8–14. doi:10.1016/j.chemgeo.2016.02.024

Hussain, J., Hussain, I., & Sharma, K. C. (2010). Fluoride and health hazards: community perception in a fluorotic area of central Rajasthan (India): an arid environment. *Environmental Monitoring and Assessment*, *162*(1-4), 1–14. doi:10.100710661-009-0771-6 PMID:19266303

Inigo, V., Andrades, M., Alonso-Martirena, J., Marín, A., & Jiménez-Ballesta, R. (2011). Multivariate statistical and GIS-based approach for the identification of Mn and Ni concentrations and spatial variability in soils of a humid Mediterranean environment: La Rioja, Spain. *Water, Air, and Soil Pollution*, *222*(1-4), 271–284. doi:10.100711270-011-0822-9

Jacks, G., Bhattacharya, P., Chaudhary, V., & Singh, K. P. (2005). Controls on the genesis of some high-fluoride groundwaters in India. *Applied Geochemistry*, *20*(2), 221–228. doi:10.1016/j.apgeochem.2004.07.002

Kheir, R. B., Greve, M. H., Abdallah, C., & Dalgaard, T. (2010). Spatial soil zinc content distribution from terrain parameters: A GIS-based decision-tree model in Lebanon. *Environ. Pollut.*, *158*(2), 520–528. doi:10.1016/j.envpol.2009.08.009 PMID:19773104

Kumari, S., & Khan, S. (2017). Defluoridation technology for drinking water and tea by green synthesized Fe_3O_4/Al_2O_3 nanoparticles coated polyurethane foams for rural communities. *Scientific Reports*, *7*(1), 8070. doi:10.103841598-017-08594-7 PMID:28808309

Lasat, M. M. (2002). Phytoextraction of toxic metals. *Journal of Environmental Quality*, *31*(1), 109–120. doi:10.2134/jeq2002.1090 PMID:11837415

Magesh, N. S., Chandrasekar, N., & Soundranayagam, J. P. (2012). Delineation of groundwater potential zones in Theni district, Tamil Nadu, using remote sensing, GIS and MIF techniques. *Geoscience Frontiers*, *3*(2), 189–196. doi:10.1016/j.gsf.2011.10.007

Mamatha, P., & Rao, S. M. (2010). Geochemistry of fluoride rich groundwater in Kolar and Tumkur districts of Karnataka. *Environmental Earth Sciences*, *61*(1), 131–142. doi:10.100712665-009-0331-y

Marques, A. P. G. C., Pires, C., Moreira, H., Rangel, A. O. S. S., & Castro, P. M. L. (2010). Assessment of the plant growth promotion abilities of six bacterial species using *Zea mays* as indicator plant. *Soil Biology & Biochemistry*, *42*(8), 1229–1235. doi:10.1016/j.soilbio.2010.04.014

Marwah, B. R. (2003). GIS applications for soil resources and its importance for country. *Journal of the Indian Society of Soil Science*, *51*, 466–472.

McQuaker, N. R., & Gurney, M. (1977). Determination of total fluoride in soil and vegetation using an alkali fusion-selective ion electrode technique. *Analytical Chemistry*, *49*(1), 53–56. doi:10.1021/ac50009a022

Mihailovi, C. A. (2015). Spatial distribution of metals in urban soil of Novi Sad, Serbia: GIS based approach. *Journal of Geochemical Exploration*, *150*, 104–114. doi:10.1016/j.gexplo.2014.12.017

Minhas, P. S., & Gupta, R. K. (1992). *Quality of irrigation water: Assessment and Management. Information and Publication division*. New Delhi: ICAR.

Niu, Z. X., Sun, L. N., Sun, T. H., Li, Y. S., & Wang, H. (2007). Evaluation of phytoextracting cadmium and lead by sunflower, ricinus, alfalfa and mustard in hydroponic culture. *Journal of Environmental Sciences (China)*, *19*(8), 961–967. doi:10.1016/S1001-0742(07)60158-2 PMID:17966853

Nordstrom, D. K., Ball, J. W., Donahoe, R. J., & Whittemore, D. (1989). Groundwater chemistry and watererock interactions at Stripa. *Geochimica et Cosmochimica Acta*, *53*(8), 1727–1740. doi:10.1016/0016-7037(89)90294-9

Norris, J. N. (1971). The application of multivariate analysis to soil studies. I. Grouping of soils using different properties. *Journal of Soil Science*, *22*(1), 69–80. doi:10.1111/j.1365-2389.1971.tb01594.x

Norris, J. N. (1972). The application of multivariate analysis to soil studies. II. soil variation. *Journal of Soil Science*, *23*(1), 62–75. doi:10.1111/j.1365-2389.1972.tb01642.x

Nowak, J., Kuran, B., & Smolik, B. (2000). Dynamics of fluorine conversion from soluble forms into insoluble water compounds in various soil. *Folia Univ. Agric. Stetin., 209*(83), 125-130.

Paliwal, K.V., & Yadav, B.R. (1976). Irrigation water quality and crop production management in Union territory of Delhi. *Research Bulletin I.A.R.I., 166*.

Quine, T. A., & Zhang, Y. (2002). An investigation of spatial variation in soil erosion, soil properties and crop production within an agricultural field in Devon U.K. *Journal of Soil and Water Conservation*, *57*, 50–60.

Radosław, S., & Ciecko, Z. (2017). Effect of soil contamination with fluorine on the yield and content of nitrogen forms in the biomass of crops. *Environmental Science and Pollution Research International*, *24*(9), 8588–8860. doi:10.100711356-017-8523-6 PMID:28194675

Raj, D., & Shaij, E. (2017). Fluoride contamination in groundwater resources of alleppey, southern India. *Geoscience Frontiers*, *8*(1), 117–124. doi:10.1016/j.gsf.2016.01.002

Rao, S. (2009). Fluoride in groundwater, Varaha River basin, Visakhapatnam district, Andhra Pradesh, India. *Environmental Monitoring and Assessment*, *152*(1-4), 47–60. doi:10.100710661-008-0295-5 PMID:18509735

Reddy, D. V., Nagabhushanam, P., Sukhija, B. S., Reddy, A. G. S., & Smedley, P. L. (2010). Fluoride dynamics in the granitic aquifer of the Wailapally watershed, Nalgonda district, India. *Chemical Geology*, *269*(3-4), 278–289. doi:10.1016/j.chemgeo.2009.10.003

Richard, L. A. (1954). *Diagnosis and Improvements of Saline Alkali Soils. Hand Book No. 60*. USDA.

Saby, N., Arrouays, D., Boulonne, L., Jolivet, C., & Pochot, A. (2006). Geostatistical assessment of Pb in soil around Paris, France. *The Science of the Total Environment*, *367*(1), 212–221. doi:10.1016/j.scitotenv.2005.11.028 PMID:16442151

Saini, P., Khan, S., Mamta, B., & Sharma, V. (2012). Organ-wise accumulation of fluoride in *Prosopis juliflora* And its potential for phytoremediation of fluoride contaminated soil. *Chemosphere*, *89*(5), 633–635. doi:10.1016/j.chemosphere.2012.05.034 PMID:22704972

Saini, P., Khan, S., Mamta, B., & Sharma, V. (2013). Mapping of Fluoride endemic area and assessment of F-1 accumulation in soil and vegetation. *Environmental Monitoring and Assessment*, *185*(2), 2001–2008. doi:10.100710661-012-2683-0 PMID:22638723

Shahid, M., Singh, A. P., Bhandari, D. K., & Intjar, A. (2008). Ground water quality appraisal and categorization in Julana block of Jind district, Haryana. *Journal of the Indian Society of Soil Science*, *1*, 1232–125.

Shan, Y., Tysklind, M., Hao, F., Ouyang, W., Chen, S., & Lin, C. (2013). Identification of sources of heavy metals in agricultural soils using multivariate analysis and GIS. *J. Soils Sediments, 13*, 720-729.

Sharma, P. K. (2004). Emerging technologies of remote sensing and GIS for the development of spatial data in fracture. J. Indian Soci. *Soil Science*, *52*, 384–406.

Singh, A. K., Singh, R. S., & Shyampura, R. (2008). Soil and water resources of changeri watershed- Udaipur, Rajasthan. J. Indian Soci. *Soil Science*, *56*, 106–108.

Singh, G., Kumari, B., Sinam, G. G., Kriti, Kumar, N., & Mallick, S. (2018). Fluoride distribution and contamination in the water, soil and plants continuum and its remedial technologies, an Indian perspective: A review. *Environ. Pollut.*, *239*, 95–108. doi:10.1016/j.envpol.2018.04.002 PMID:29649763

Sood, A., Setia, R. K., Bansal, R. L., Sharma, P. K., & Nayyar, V. K. (2004). Spatial distribution of micronutrients in soils of Amritsar district using frontier technologies. *Proceedings of 7th Punjab Science Congress* (Abstract vol.).

Steel, R. G., & Torrie, J. H. (1981). *Principles and Procedures of Statistics* (2nd ed.). McGraw Hill Book Comp.

Sumi, H., Kunito, T., Ishikawa, Y., Sato, T., Park, H. D., Nagaoka, K., & Aikawa, Y. (2015). Plant roots influence microbial activities as well as cadmium and zinc fractions in metal-contaminated soil. *Chemistry and Ecology*, *31*(2), 105–110. doi:10.1080/02757540.2014.950570

Tandon, H. L. S. (2009). Methods of Analysis of Soils, Plants, Waters and Fertilizers. Fertilizer Development and Consultation Organization.

Tariq, S. R., Shah, M. H., Shaheen, N., Jaffar, M., & Khalique, A. (2008). Statistical source identification of metals in groundwater exposed to industrial contamination. *Environmental Monitoring and Assessment*, *138*(1-3), 159–165. doi:10.100710661-007-9753-8 PMID:17492482

Thapa, R., Gupta, S., & Reddy, D. V. (2017). Application of geospatial modelling technique in delineation of fluoride contamination zones within Dwarka Basin, Birbhum, India. *Geoscience Frontiers*, *8*(5), 1105–1114. doi:10.1016/j.gsf.2016.11.006

Trivedy, R.K., & Goel, P.K. (1984). Chemical and biological methods for waste water studies. *Environmen. Publish. Karad India, 104*.

Van Der Heijden, M. G., Bardgett, R. D., & Van Straalen, N. M. (2008). The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, *11*(3), 296–310. doi:10.1111/j.1461-0248.2007.01139.x PMID:18047587

Welch, S. A., Taunton, A. E., & Banfield, J. F. (2006). Effect of microorganisms and microbial metabolites on apatite dissolution. *Geomicrobiology Journal*, *19*(3), 343–367. doi:10.1080/01490450290098414

WHO. (2008). Guidelines fo drinking-water quality (3rd ed.). WHO.

Xia, B., Wang, L., Nie, L., Zhou, Q., & Huang, X. (2017). A pathway of bisphenol a affecting mineral element contents in plant roots at different growth stages. *Ecotoxic. Enviorn. Safet.*, *135*, 115–122. doi:10.1016/j.ecoenv.2016.09.028 PMID:27723463

Xiang, Q., Liang, Y., Chen, L., Wang, C., Chen, B., Chen, X., ... Shanghai, P. R. (2003). Effect of fluoride in drinking water on children's intelligence. *Fluoride*, *36*(2), 84–94.

Zhang, L., Li, Q., Ma, L., & Ruan, J. (2013). Characterization of fluoride uptake by roots of tea plants (Camellia sinensis L. O. Kuntze). *Plant and Soil*, *366*(1-2), 659–669. doi:10.100711104-012-1466-2

Zhang, R., Hu, S., & Zhang, X. (2006). Experimental study of dissolution rates of fluorite in HCl-H_2O solutions. *Aquatic Geochemistry*, *12*(2), 123–159. doi:10.100710498-005-3658-3

Zhang, Y. F., He, L. Y., Chen, Z. J., Wang, Q. Y., Qian, M., & Sheng, X. F. (2011). Characterization of ACC deaminase-producing endophytic bacteria isolated from coppertolerant plants and their potential in promoting the growth and copper accumulation of *Brassica napus. Chemosphere*, *83*(1), 57–62. doi:10.1016/j.chemosphere.2011.01.041 PMID:21315404

Zhu, S., Zhang, J., & Dong, T. (2009). Removal of fluorine from contaminated field soil by anolyte enhanced electrokinetic remediation. *Environmental Earth Sciences*, *59*(2), 379–384. doi:10.100712665-009-0036-2

Chapter 5

Effect of Plant Growth Promoting Bacteria (PGPB) on Phytoremdiation Technology

ABSTRACT

In this chapter, the authors describe how plant-growth-promoting bacteria is helpful for removing soil contaminants and also increasing the efficiency of phytoremediation technology. The plant growth bacteria seem almost good for removal of soil contaminants, and they can adsorb and accumulate metals in their cells and are being used in microbial leaching and also as agents of cleaning the environment.

INTRODUCTION

Phytoremediation has gained increased attention as a cost-effective method for the remediation of heavy metal-contaminated sites. Phytoremediation is the use of plants to remediate contaminated soil and water; it is a low cost effective technique (Singh et al., 2003). Phytoremediation techniques involve such as, phytofiltration, rhizofiltration, phytoextraction, phytostabilization, phytoimmobilisation and phytodegradation, and rhizodegradation (Ali et al., 2013). A primary termed of phytoremediation is phytofiltration. Phytofiltration is based on the adsorption and absorption of heavy metal contaminants from water with the help of plant roots (Mukhopadhyay and Maiti, 2010). Another form of involves phytovolatilization is the conversion

DOI: 10.4018/978-1-5225-9016-3.ch005

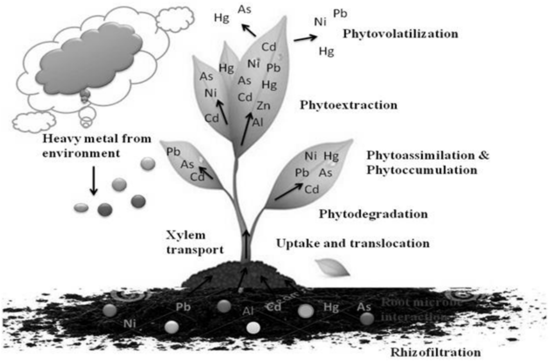
of the pollutant into volatile form which allowing its escape from the soil into the atmosphere (Prasad and Freitas, 2003). Phytodegradation is also the category of phytoremidiation, metals pollutant are degraded into the small particles which easily uptake by the roots but yet does not apply to heavy metals because it is time consuming (Dixit et al., 2015). Phytostabilization is used for highly polluted areas to provide a complete capable of producing extensive and dense root systems covering compressed soils (figure 1) and also the restricting the pollutants to the soil zone near their roots by preventing their movement or leaching with a direct implication of the plant being more tolerant of pollutants (Salt et al., 1998; Yao et al., 2012; Dixit et al., 2015).

After high levels of heavy metal uptake in the plant organs which are harvested after drying, pollutant containing material dupmed separately and also use to make nanoparticle from this concentration mass material (Yao et al., 2012).The treatment of soil using plants as heavy metal uptake and storage by phytoremdiation approach can be distinguished (Yao et al., 2012; Dixit et al., 2015). The depth of soil which can be cleaned or stabilized is restricted to the root zone of the plants being used. Heavy metals are non-biodegrdable and they are very toxic to human health, plant and animals and also very affected to the microorganisms when they are present at higher quantity in the soil. As a result at higher concentration of heavy metals increase toxicity by generating reactive oxygen species which can lead to degradation of macromolecule, DNA damages, cell damage and also affected to ion uptake molecules (Ahmad et al., 2008, 2010, 2011, 2015). They also affected the process of photosynthesis by interfering with electron transport chain, water relations, enzymatic and biochemical activities (Rascio and Navari-Izzo, 2011; Ahmad et al., 2008, 2010, 2011, 2015; Qadir et al., 2014). They are also affecting biomass yield and somewhat soil fertility under the major condition present of heavy metals accumulation in soils (Bhargava et al., 2012). During the past few decades phytoremediation technologies has fastly grown and many new hyperaccumulator plants were identified.

ROLE OF HYPERACCUMULATOR PLANT

Hyperaccumulator term was proposed first time by Brooks et al. (1977) in reverence to those plants that can accumulate more than their natural favored condition approximately 1000 mg kg^{-1} of heavy metals. Plants accumulate more and more contaminants and tolerate without showing any symptoms (Memon and Schroder, 2009). According to Baker and Brooks suggested that

Figure 1. Mechanism of phytoremdiation process



the minimum threshold tissue concentration for plants as 0.1% considered Ni, Cr, Cu, Co, and Pb hyperaccumulators but same as above the experiment was done in case of Mn, and Zn threshold value for plants established 1% (Baker and Brooks, 1989; Baker et al., 2000). Plants accumulate heavy metals particularly in those organs that can allow translocation of sugars and minerals as they need a proper ratio maintained between the concentrations of heavy metals particularly in roots to shoots. This is termed a translocation factor (TF). For hyperaccumulator it is necessary that the TF is greater than 1 (Tangahu et al., 2011). Another factor that bioconcentration factor (BC) ratio should also be greater than 1 for hyperaccumulator (Ahmadpour et al., 2014). Some different 450-500 plants have been identified as hyperaccumulator include *Thlaspi caerulescens* that accumulate (Pb, Ni, Cd and Zn), *Arabidopsis halleri* that can accumulate high levels og heavy metals (Cd and Zn but not Pb), *Alyssum bertolonii* can uptake (Ni and Co) and some other plants which belong to different family can also be participate to accumulate heavy metals such as *Caryophyllaceae, Fabaceae, Poaceae, Lamiaceae, Asteraceae, Cunoniaceae and Cyperaceae* and many others table.1 (Berti et al., 2002; Prasad, 2005; Maestri et al., 2010; Padmavathiamma and Li, 2007).

Plants have specific properties that give us some specific advantages to remediate environment (Meagher, 2000; Meagher et al., 2000). Plants absorb metal particle through roots and root hairs that generate surface area through which pollutants can be extracted form contaminated soil and water. Plants are autotrophs, it takes up nutrients directly from environment in gaseous form with the help of photosynthesis process. Study of four crop plants which belong to *Ipomea alpine, Centella asiatica, Eichlarnia*

Table 1. Metal hyperaccumulating plants

Metal	Plant	Reference
Arsenic	*Pteris vittata, Arabidopsis bisulcatus,*	Dong 2005, Mehdawi et al., 2011
Cadmium	*Sesbania drummondi, Sedum alfredii,*	Israr and Sahi, 2006, Jin et al., 2008
Copper	*Ipomea alpine,* *Centella asiatica and Eichhornia crassipes* *Euphorbia macroclada*	Baker and Walker, 1989 Mokhtar et al., 2011 Nematian and Kazemeini, 2013
Chromium	*Phragmites australis, Zea mays L. Cv Ganga 5*	Calheiros et al., 2008, Sharma et al., 2003
Manganese	*Phytolacca americana*	Pollard et al., 2009
Nickel	*Berkheya coddii* *Alyssum* and *Thlaspi*	Robinson et al., 1997 Bani et al., 2010
Selenium	*Astragalus racemosus,* *Cardamine hupingshanesis*	Beath et al., 1937;Tong et al., 2014
Titanium	*Iberis intermedia*	Leblanc et al., 1999
Zinc	*Sedum alfredii* *Euphorbia macroclada*	Nematian and Kazemeini, 2013

crassipes, Euphorbia maeleocloela, Berkheya coddii, Alyssum and Thlaspi, Zea mays, Pteris vittata, Arabidopsis bisulcatus, Sesbaniia drumnondia, Sedum alfredei, Euphorbia marcoleida Phragnites australis Phytolacca Americana, Astragolosus, craenosus, Cardanine hupingshas and Iberis intermedia reported as hyperaccumulators for phytoextraction particularly of Cu, Ni, Cd, Zn, Cr, As, Mg, Se and Ti accumulation in shoots" (Beath et al., 1937; Baker and walker, 1989; Robinson et al., 1997; Leblanc et al., 1999; Sharma et al., 2003; Meers et al., 2005; Dong, 2005; Israr and Sahi, 2006; Jin et al., 2008; Colheiros et al., 2008; Pollard et al., 2009; Jin & Liu, 2009; Bani et al., 2010; Mokhtar et al., 2011; Mehdami et al., 2011 Namatian & Kazemein, 2013; Tong et al., 2014).

Plant accumulate inside the organs have multiple steps involve from roots to shoots from where they are translocate and accumulate of the plant figure 2 (Mahmood, 2010). Firstly heavy metals accumulate around the roots and favored reaction occur by plants to help for movement; second one is translocate metals from roots to shoots through xylem loading process and last is sequestration of metals in leaves specially in vacuoles of plant cell (Bhargava et al., 2012). In nonhyperaccumulators of plants do not have efficiency to translocate from roots to shoots and stored metals in roots. Heavy metals present in soil in different forms so they are either as free ions aor present as in complexes form which bound to inorganic and organic matter (Salt and Rauser, 1995). Naturally plants accumulate metals due to the present of these ions in the form of soluble or low complexes so heavy metals can easily bind up to other metals where ther have high affinity. Any other type of can also be taken up by the use of different soil amendments to induce hyperaccumulation mechanism (Abollino et al., 2006).

The pH of the soil is maintained by the release of number of protons, which are controlled by proton pumps located in the outer membranes of the plant and can acidify the soil, thus lowering its pH. Previous findings on *Alyssium murale* showed the increase in Ni accumulation due to changes in pH (Bernal et al., 1994). Heavy metals transfer from soil to root; root to shoot through xylem tissues and need to be transfer from roots to leaves and vice versa. It is transfer through symplast pathway where they are energy consuming process that allows all non essential heavy metals. This is due to normal transportation intracellular movement pathway block by casparian strip in the endodermis. Hence the movement of heavy metals through apoplast pathway gets blocked which means that the only way to enter xylem vessels is by taking the symplast pathway (Mahmood, 2010).

PLANT GROWTH PROMOTING BACTERIA

The presence of a rhizopheric microbial population in the contaminated sites, greatly increased the accumulation of metals in shoots of the hyperaccumulator *Arabidopsis halleri* (Farnati et al., 2009). Microorganism's uptake metal either actively bioaccumulation and/or passively biosorption (Farnati et al., 2009). Different biochemical processes such as bioleaching involving *Thiobacillus spp.* bacteria and *Aspergillus niger*, Fungus, biosportion of low concentrations of metals in water by algal or bacterial cells, bio-oxidation or bioreduction of metal accumulation by *Baccillus subtilis Berknolderia sp., Methylobacterium, Baccillus megaterium, Pseudomonas sp., Kluyvera ciscorbata, Brevibaccillus, Pseudomonas fluoreseus, Flavobacterium, Baccillus Cereus, Bacillus sp., Berk molderica sp.* and sulfate-reducing bacteria and biomethylation of heavy metals such as Ni, Pb, Zn, Cr IV, Hg, Cd, Cu, (table 2). Heavy metals in soil that adversely affect microbial population and also affected soil properties which leading to loss yield of crops (Linger et al., 2002; Emamverdian et al., 2015). Most of the heavy metals are not degraded properly due to less mobiltty, hence persist in the environment. Plant growth promoting rhizobacteria promote the growth of plants by activate mechanisms such as production of growth promoting substances, siderophores and detoxification/removal of contaminates" (Linger et al., 2002).

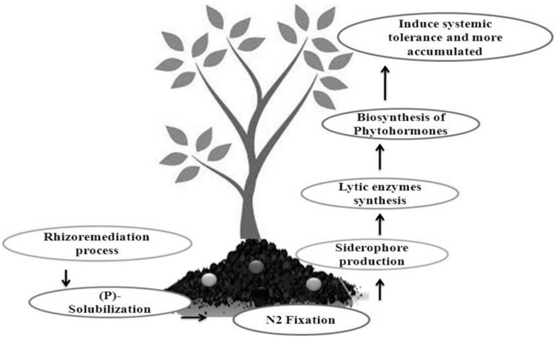
Under heavy metal stress endogenously production of ethylene enhances which adversely affects root growth and also plant growth (Saleem et al., 2007). PGPR utilizing 1-aminocyclopropane-1-carboxylate (ACC) to prevent plant growth when inhibited by ethylene through decrease in ACC content and demonstrated to removal of ethylene (Saleem et al., 2007). Another way to ACC deaminase that hydrolyzes ethylene precursor (ACC) which stimulates plant growth and promising opportunity to enhance the crop yield. Degradation of ACC to ammonium and a-ketobutyrate for use as carbon and nitrogen sources when bacteria occurring on the root surface. In leguminous plants symbiotic relationship with bacteria that provide fixed nitrogen e.g of nitrogen-fixing bacteria ae *Azotobacter vinelandii*, *Beijerinckia derxii* and *Zoogloea* strain Ky1 (Meunchang et al., 2006).

Microorganisms are able to survive in extreme cold/heat and desert/salt conditions due to their potentially produced enzymatic activities, which allow to converting hazardous compounds into harmless compounds (Ali et al., 2013; Singh et al., 2014). Different microorganism evolved to different mechanism for resistance to metal toxicity and adapted to live in the presence of soil pollutants (Nie, 2003; Kuffner et al., 2010). Soil bacteria that were resistant to metals and found that a P-solubilizer (*Baccilllus megaterium* HKP-1) and a K-solublizer (*Bacillus mucilaginosus* HKK-1) had a high resistance to heavy metal toxicity Zn, Cu, and Pb: 250, 100, and 300 mg/L respectively due to formation of endospores which are capable to live extreme condition (Wu et al., 2006). *Pseudomonas aeruginosa* strain Wl-1 could tolerate up to 0.6 mM lead nitrate and also accumulated lead at 26.5 mg/g of dry cell biomass (Naik et al., 2012). Isolated phosphate solubilizing bacteria *Burkholderia metalliresistences sp.* Nov D414T from heavy metals such as "Pb (800 mg/L), Cd (2000 mg/L), Cu (150 mg/L) and Zn (2500 mg/L) polluted paddy soils of china" (Guo et al., 2015). Finding suggested that the metal chelating molecules synthesized by bacteria may contribute in heavy metal accumulation for help to phytoremdiation technology (figure 2).

Table 2. Efficiency enhanced by plant growth promoting bacteria (PGPB) in phytoremediation

Bacteria	Plant	Heavy Metal	References
Berknolderia sp.	Lycopersicon	Ni, Cd	Madhaiyan et al., 2007
Methylobacterium	Lycopersicon	Ni, Cd	Madhaiyan et al., 2007
Bacillus megaterium HKP-1	Brassica Juncea	Pb, Zn	Wu et al., 2006
Pseudomonas sp.	Mustard	Cr IV	Rajkumar et al., 2006
Kluyvera ascorbata SUD165	Indian Mustard, Canola, Tamato	Ni, Pb, Zn	Burd et al. 2000
Brevibacillus	Trifolium repens	Zn	Vivas et al., 2006
Pseudomonas fluoresecens	Soybean	Hg	Gupta et al., 2006
Flavobacterium	Brassica juncea	Cd	Belimov et al., 2005
Bacillus Cereus	Mungbean	Cr VI	Faisal and Hasnain 2006
Bacillus sp.	Mustard	Cr VI	Rajkumar et al., 2006
Berknolderica sp.	Lycopersicon esculentom	Ni, Cd	Madhaiyan et al., 2007
Acineotobacter sp.	Brassica napus	Pb	Zhang et al., 2011
Bacillus subtilis	Chineses violet cress	Cd	Liang et al., 2014
Burkholderia sp. J62	Zea mays	Cd, Pb	Jiang et al., 2008
Enterobacter	White mustard	Zn	Ptociniczak et al., 2013
Staphyllococcus arlettae NBRIEAG-6	Brassica juncea	As	Srivastava et al., 2013
Pseudomonas eeruginosa	Triticum aestivum	Zn	Islam et al., 2014
Pseudomonas putida	Cicuta virosa L.	Zn	Nagata et al., 2015
Pseudomonas sp. Lk9	Solanum nigrum L.	Cd	Chen et al., 2014
Mesorhizobium amorphae	Robinia pseudoacacia	Cr, Zn, and Cu	Hao et al., 2014
Photobacterium spp.	Phragmites australis	Hg	Mathew et al., 2015

Figure 2. Diagram of rhizoremediation process



CONCLUSION

Heavy metals are the most important inorganic pollutants, which are not degraded and progressively accumulate in the environment. Although a number of plant species are capable of hyper-accumulation of heavy metals, however, this approach is not applicable for remediating sites with multiple contaminants. The biogeochemical capacities of microorganisms seem almost limitless and they can adsorb and accumulate metals in their cells and are being used in microbial leaching and also as agents of cleaning the environment. The best approach would be to combine the advantages of plant-microbe interactions within the plant rhizosphere into an effective cleanup technology.

REFERENCES

Abollino, O., Giacomino, A., Malandrino, M., Mentasti, E., Aceto, M., & Barberis, R. (2006). Assesssment of metal availability in a contaminated soil by sequential extraction. *Water, Air, and Soil Pollution*, *173*(1-4), 315–338. doi:10.100711270-005-9006-9

Ahmad, P., Jaleel, C. A., Salem, M. A., Nabi, G., & Sharma, S. (2010). Roles of enzymatic and non-enzymatic antioxidants in plants during abiotic stress. *Critical Reviews in Biotechnology*, *30*(3), 161–175. doi:10.3109/07388550903524243 PMID:20214435

Ahmad, P., Nabi, G., & Ashraf, M. (2011). Cadmium-induced oxidative damage in mustard (*Brassica juncea* (L.) Czern. & Coss.] plants can be alleviated by salicylic acid. *South African Journal of Botany*, *77*(1), 36–44. doi:10.1016/j.sajb.2010.05.003

Ahmad, P., Sarwat, M., Bhat, N. A., Wani, M. R., Kazi, A. G., & Tran, L. S. P. (2015). Alleviation of cadmium toxicity in *Brassica juncea L.* (Czern & Coss.) by calcium application involves various physiological and biochemical strategies. *PLoS One*, *10*(1), e0114571. doi:10.1371/journal.pone.0114571 PMID:25629695

Ahmad, P., Sarwat, M., & Sharma, S. (2008). Reactive oxygen species, antioxidants and signaling in plants. *Journal of Plant Biology*, *51*(3), 167–173. doi:10.1007/BF03030694

Ahmadpour, P., Ahmadpour, F., Mahmud, T., Abdu, A., Soleimani, M., & Tayefeh, F. H. (2014). Phytoremediation of heavy metals: a green technology. In E. Asrari (Ed.), *Heavy metal contamination of water and Soil: Analysis, Assessment, and Remediation strategies* (p. 249). CRC Press, Taylor and Francis. doi:10.1201/b16566-15

Ahmed, M., & Malik, A. (2011). Bioaccumlation of heavy metals by zinc resistant bacteria isolated from agri-cultural soils irrigated with wastewater. *Bacteriol. J.*, *2*(1), 12–21. doi:10.3923/bj.2012.12.21

Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals- concepts and applications. *Chemosphere*, *91*(7), 869–881. doi:10.1016/j.chemosphere.2013.01.075 PMID:23466085

Baker, A. J. M., & Brooks, R. R. (1989). Terrestrial higher plants which hyperaccumulate metallic elements– a review of their distribution, ecology and phytochemistry. *Biorecovery*, *1*, 81–126.

Baker, A. J. M., McGrath, S. P., Reeves, R. D., & Smith, J. A. C. (2000). Metal Hyperaccumulator Plants: A Review of the Ecology and Physiology of a Biological Resource for Phytoremediation of Metal Polluted Soils. In N. Terry & G. Bañuelos (Eds.), *Phytoremediation of Contaminated Soils and Waters* (pp. 85–107). Boca Raton, FL: CRC Press LLC.

Baker, A. J. M., & Walker, P. L. (1989). Ecophysiology of metal uptake by tolerant plants. In *Heavy metal tolerance in plants: Evolution aspects* (pp. 155–177). CRC Publishers.

Bani, A., Pavlova, D., Echevarria, G., Mullaj, A., Reeves, R. D., Morel, J. L., & Sulc¸e, S. (2010). Nickel hyperaccumulation by the species of Alyssum and Thlaspi (Brassicaceae) from the ultramafic soils of the Balkans. *Botanica Serbica*, *34*(1), 3–14.

Beath, Q. A., Eppsom, H. F., & Gilbert, G. S. (1937). Selenium distribution in and seasonal variation of vegetation type occurring on seleniferous soils. *Journal of the American Pharmaceutical Association*, *26*, 394–405.

Belimov, A. A., Hontzeas, N., Safronova, V. I., Demchinskaya, S. V., Piluzza, G., Bullitta, S., & Glick, B. R. (2005). Cadmium-tolerant plant growth promoting rhizobacteria associated with the roots of Indian mustard (*Brassica juncea L. Czern.*). *Soil Biology & Biochemistry*, *37*(2), 241–250. doi:10.1016/j.soilbio.2004.07.033

Bernal, M., McGrath, S., Miller, A., & Baker, A. (1994). Comparision of the chemical changes in the rhizosphere of the nickel hyperaccumulator Alyssum murale with the non-accumulator *Raphanus sativus. Plant and Soil*, *164*(2), 251–259. doi:10.1007/BF00010077

Berti, W. R., & Cunningham, S. D. (2000). Phytostabilization of metals. In I. Raskin & B. D. Ensley (Eds.), *Phytoremediation of toxic metals - using plants to clean-up the environment* (pp. 71–88). New York: John Wiley & Sons, Inc.

Bhargava, A., Carmona, F. F., Bhargava, M., & Srivastava, S. (2012). Approaches for enhanced phytoextraction of heavy metals. *Journal of Environmental Management*, *105*, 103–120. doi:10.1016/j.jenvman.2012.04.002 PMID:22542973

Brooks, R., Lee, J., Reeves, R. D., & Jaffre, T. (1977). Detection nickeliferous rocks by analysis of herbarium specimens of indicator plants. *Journal of Geochemical Exploration*, *7*, 49–57. doi:10.1016/0375-6742(77)90074-7

Burd, G. I., Dixon, D. G., & Glick, B. R. (2000). Plant growth promoting bacteria that decrease heavy metal toxicity in plants. *Canadian Journal of Microbiology*, *46*(3), 237–245. doi:10.1139/w99-143 PMID:10749537

Calheiros, C. S. C., Rangel, A. O. S. S., & Castro, P. M. L. (2008). The effects of tannery waste water on the development of different plant species and chromium accumulation in *Phragmites australis. Archives of Environmental Contamination and Toxicology*, *55*(3), 404–414. doi:10.100700244-007-9087-0 PMID:18214580

Chen, L., Luo, S. L., Li, X. J., Wan, Y., Chen, J. L., & Liu, C. B. (2014a). Interaction of Cd hyperaccumulator Solanum nigrum L. and functional endophyte *Pseudomonas sp.* Lk9 on soil heavy metals uptake. *Soil Biology & Biochemistry*, *68*, 300–308. doi:10.1016/j.soilbio.2013.10.021

Dixit, R., Malaviya, D., Pandiyan, K., Singh, U. B., & Sahu, A. (2015). Bioremediation of heavy metals from soil and aquatic environment: An overview of principles and criteria of fundamental processes. *Sustainability*, *7*(2), 2189–2212. doi:10.3390u7022189

Dong, R., Formentin, E., Losseso, C., Carimi, F., Benedetti, P., Terzi, M., & Schiavo, F. L. (2005). Molecular cloning and characterization of a phytochelatin synthase gene, *PvPCS1*, from *Pteris vittata* L. J. *Ind. Microbiol. Biol.*, *32*(11-12), 527–533. doi:10.100710295-005-0234-1 PMID:15918023

Emamverdian, A., Ding, Y., Mokhberdoran, F., & Xie, Y. (2015). Heavy metals stress and some mechanisms of plant defense response. *Sci. World J., 18.*

Faisal, M., & Hasnain, S. (2006). Growth stimulatory effect of *Ochrobactrum intermedium* and *Bacillus cereus* on *Vigna radiata* plants. *Letters in Applied Microbiology*, *43*(4), 461–466. doi:10.1111/j.1472-765X.2006.01977.x PMID:16965380

Farinati, S., DalCorso, G., Bona, E., Corbella, M., Lampis, S., Cecconi, D., ... Furini, A. (2009). Proteomic analysis of *Arabidopsis halleri* shoots in response to the heavy metals cadmium and zinc and rhizosphere microorganisms. *Proteomics*, *9*(21), 4837–4850. doi:10.1002/pmic.200900036 PMID:19810031

Guo, J. K., Ding, Y. Z., Feng, R. W., Wang, R. G., Xu, Y. M., Chen, C., ... Chen, Y. M. (2015). *Burkholderia metalliresistens sp.* Nov. a multiple metal resistant and phosphate solubilising species isolated from heavy metal polluted soil in Southeast China. Anton. *Van Leeuwen.*, *107*(6), 1591–1598. doi:10.100710482-015-0453-z PMID:25896306

Hao, X., Mohamad, O. A., Xie, P., Rensing, C., & Wei, G. (2014). Removal of zinc from aqueous solution by metal resistant symbiotic bacterium *Mesorhzobium Amorphae. Separation Science and Technology*, *49*(3), 376–387. doi:10.1080/01496395.2013.843195

Islam, F., Yasmeen, T., Ali, Q., Ali, S., Arifa, M. S., Hussain, S., & Rizvi, H. (2014). Influence of Pseudomonas aeruginosa as PGPR on oxidative stress tolerance in wheat under Zn stress. *Ecotoxicology and Environmental Safety*, *104*, 285–293. doi:10.1016/j.ecoenv.2014.03.008 PMID:24726941

Israr, M., Sahi, S. V., & Jain, J. (2006). Cadmium accumulation and antioxidative responses in the *Sesbania drummondii* callus. *Archives of Environmental Contamination and Toxicology*, *50*(1), 121–127. doi:10.100700244-005-5029-x PMID:16247566

Jiang, C. Y., Sheng, X. F., Qian, M., & Wang, Q. Y. (2008). Isolation and characterization of a heavy metal-resitant Burkholderia sp. from heavy metal-contaminated paddy filed soil and its potential in promoting plant growth and heavy metal accumulation in metal polluted soil. *Chemosphere*, *72*(2), 157–164. doi:10.1016/j.chemosphere.2008.02.006 PMID:18348897

Jin, X., Yang, X., Islam, E., Liu, D., & Mahmood, Q. (2008). Effects of cadmium on ultrastructure and antioxidative defense system in hyperaccumulator and nonhyperaccumulator ecotypes of *Sedum alfredii* Hance. *Journal of Hazardous Materials*, *156*(1-3), 387–397. doi:10.1016/j.jhazmat.2007.12.064 PMID:18242844

Jin, X. F., Liu, D., Islam, E., Mahmood, Q., Yang, X.-E., He, Z.-L., & Stoffella, P. J. (2009). Effects of zinc on root morphology and antioxidant adaptations of cadmium-treated *Sedum alfredii H. Journal of Plant Nutrition*, *32*(10), 1642–1656. doi:10.1080/01904160903150909

Kuffner, M., DeMaria, S., Puschenreiter, M., Fallmann, K., Wieshammer, G., Gorfer, M., ... Sessitsch, A. (2010). Culturable bacteria from Zn- and Cd-accumulating Salix caprea with differential effects on plant growth and heavy metal availability. *Journal of Applied Microbiology*, *108*(4), 1471–1484. doi:10.1111/j.1365-2672.2010.04670.x PMID:20132372

Leblanc, M., Petit, D., Deram, A., Robinson, B., & Brooks, R. R. (1999). The phytomining and environmental significance of hyperaccumulation of thallium by Iberisintermedia from southern France. *Economic Geology and the Bulletin of the Society of Economic Geologists*, *94*(1), 109–113. doi:10.2113/gsecongeo.94.1.109

Liang, X., He, C. Q., Ni, G., Tang, G. I., Chen, X. P., & Lei, Y. R. (2014). Growth and Cd accumulation of Orychophragmus violaceus as affected by inoculation of Cd-tolerant bacterial strains. *Pedosphere*, *24*(3), 322–329. doi:10.1016/S1002-0160(14)60018-7

Linger, P., Mussig, J., Fishcher, H., & Kobert, J. (2002). Industrial hemp (*Cannabis Sativa L.*) growing on heavy metal contaminated soil: Fibre quality and phytoremediation Potential. *Industrial Crops and Products*, *16*(1), 33–42. doi:10.1016/S0926-6690(02)00005-5

Madhaiyan, M., Poonguzhali, S., & Sa, T. (2007). Metal tolerating methylotrophic bacteria reduces nickel and cadmium toxicity and promotes plant growth of tomato (*Lycopersicon esculentum L.*). *Chemosphere*, *69*(2), 220–228. doi:10.1016/j.chemosphere.2007.04.017 PMID:17512031

Maestri, E., Marmiroli, M., Visioli, G., & Marmiroli, N. (2010). Metal tolerance and hyperaccumulation: Costs and trade-offs between traits and environment. *Environmental and Experimental Botany*, *68*(1), 1–13. doi:10.1016/j.envexpbot.2009.10.011

Mahmood, T. (2010). Phytoextraction of heavy metals the process and scope for remediation of contaminated soils. *Soil & Environment*, *29*(2), 91–109.

Mathew, D.C., Ho, Y.N., Gicana, R.G., Mathew, G.M., Chien, M.C., & Huang, C.C. (2015). A rhizosphere associated symbiont, photobacterium spp. strain MELD1, and its targeted synergistic activity for phytoprotection against mercury. *PLoS One, 10*(3). .pone.0121178 doi:10.1371/journal

Meers, E., Ruttens, A., Hopgood, M., Lesage, E., & Tack, F. M. (2005). Potential of Brassic rapa, Cannabis sativa, Helianthus annuus and Zea mays for phytoextraction of heavy metals from calcareous dredged sediment derived soils. *Chemosphere*, *61*(4), 561–572. doi:10.1016/j.chemosphere.2005.02.026 PMID:16202810

Mehdawi, E. (2011). Selenium hyperaccumulators facilitate selenium-tolerant neighbors via phytoenrichment and reduced herbivory curt. *Current Biology*, *21*(17), 1440–1449. doi:10.1016/j.cub.2011.07.033 PMID:21856156

Memon, A. R., & Schroder, P. (2009). Implications of metal accumulations mechanisms to phytoremediation. *Environmental Science and Pollution Research International*, *16*(2), 162–175. doi:10.100711356-008-0079-z PMID:19067014

Meunchang, S., Panichsakpatana, S., & Weaver, R. W. (2006). Tomato growth in soil amended with sugar mill by-products compost. *Plant and Soil*, *280*(1–2), 171–176. doi:10.100711104-005-2949-1

Mokhtar, H., Morad, N., & Fizri, F. F. A. (2011). Phytoaccumulation of copper from aqueous solutions using *Eichhornia Crassipes* and *Centella asiatica. International Journal of Environmental Sciences and Development*, *3*(1), 89–95.

Mukhopadhyay, S., & Maiti, S. K. (2010). Phytoremediation of metal enrhiched mine wate: A review. *Global Environmental Research*, *4*, 135–150.

Nagata, S., Yamaji, K., Normura, N., & Ishimoto, H. (2015). Root endophytes enhance stress-tolerance of Cicuta virosa L. growing in a mining pond of eastern Japan. *Plant Species Biology*, *30*(2), 116–125. doi:10.1111/1442-1984.12039

Naik, M. M., Pandey, A., & Dubey, S. K. (2012). Pseudomonas aeruginosa strain WI-1 from Mandovi estuary possesses metallothione in to alleviate lead toxicity and promotes plant growth. *Ecotoxicology and Environmental Safety*, *79*, 129–133. doi:10.1016/j.ecoenv.2011.12.015 PMID:22284824

Nematian, M.A., & Kazemeini, F. (2013). Accumulation of Pb, Zn, Cu and Fe in plants and hyperaccumulator choice in Galali iron mine area. *Iran. Inter. J. Agricult. Crop Sci., 5*(4), 426–432.

Padmavathiamma, P. K., & Li, L. Y. (2007). Phytoremediation technology: Hyperaccumulation metals in plants. *Water, Air, and Soil Pollution*, *184*(1-4), 105–126. doi:10.100711270-007-9401-5

Pollard, A. J., Stewart, H. L., & Roberson, C. B. (2009). Manganese hyperaccumulation in Phytolacca americana L. from the southeastern United States. *Northeastern Naturalist*, *16*(sp5), 155–162. doi:10.1656/045.016.0513

Prasad, M. N. V. (2005). Nickel ophilous plants and their significance in phytotechnologies. *Brazilian Journal of Plant Physiology*, *17*(1), 113–128. doi:10.1590/S1677-04202005000100010

Prasad, M. N. V., & Freitas, H. (2003). Metal hyperaccumulation in plants: Biodiversity prospecting for phytoremediation technology. *Electronic Journal of Biotechnology*, *6*(3), 275–321. doi:10.2225/vol6-issue3-fulltext-6

Ptocinicezak, T., Sinkkonen, A., Romantschuk, M., & Piotrowska-Seget, Z. (2013). Charcaterization of Enterobacter intermedius MH8b and its use for the enhancement of heavy metals uptake by *Sinapis alba L. Applied Soil Ecology*, *63*, 1–7. doi:10.1016/j.apsoil.2012.09.009

Qadir, S., Jamshieed, S., Rasool, S., Ashraf, M., Akram, N. A., & Ahmad, P. (2014). Modulation of plant growth and metabolism in cadmium enriched environments. *Reviews of Environmental Contamination and Toxicology*, *229*, 51–88. doi:10.1007/978-3-319-03777-6_4 PMID:24515810

Rajkumar, M., Nagendran, R., Lee, K. J., Lee, W. H., & Kim, S. Z. (2006). Influence of plant growth promoting bacteria and Cr6+ on the growth of Indian mustard. *Chemosphere*, *62*(5), 741–748. doi:10.1016/j.chemosphere.2005.04.117 PMID:15982703

Rascio, N., & Navari-Izzo, F. (2011). Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? *Plant Science*, *180*(2), 169–181. doi:10.1016/j.plantsci.2010.08.016 PMID:21421358

Robinson, B., Fernandez, J. E., Madejon, P., Maranon, T., Murillo, J., Green, S., & Clothier, B. (2003). Phytoextraction: An assessment of biogeochemical and economic viability. *Plant and Soil*, *249*(1), 117–125. doi:10.1023/A:1022586524971

Saleemm, M., Arshad, M., Hussain, S., & Bhatti, A. S. (2007). Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. *Journal of Industrial Microbiology & Biotechnology*, *34*(10), 635–648. doi:10.100710295-007-0240-6 PMID:17665234

Salt, D. E., & Rauser, W. E. (1995). MgATP-dependent transport of phytochelatins across the tonoplast of oat roots. *Plant Physiology*, *107*(4), 1293–1301. doi:10.1104/pp.107.4.1293 PMID:12228436

Salt, D. E., Smith, R. D., & Raskin, I. (1998). Phytoremediation. *Annual Review of Plant Physiology and Plant Molecular Biology*, *49*(1), 643–668. doi:10.1146/annurev.arplant.49.1.643 PMID:15012249

Sharma, D. M., Sharma, C. P., & Tripathi, R. D. (2003). Phytotoxic lesions of chromium in maize. *Chemosph*, *51*(1), 63–68. doi:10.1016/S0045-6535(01)00325-3 PMID:12586157

Singh, A. K., Dhanjal, S., & Cameotra, S. S. (2014). Surfactin restore and enhances swarming motility under heavy metal stress. *Colloids and Surfaces. B, Biointerfaces*, *116*(1), 26–31. doi:10.1016/j.colsurfb.2013.12.035 PMID:24441179

Singh, O. V., Labana, S., Pandey, G., Budhiraja, R., & Jain, R. K. (2003). Phytoremediation: An overview of metallic ion decontamination from soil. *Applied Microbiology and Biotechnology*, *61*(5-6), 405–412. doi:10.100700253-003-1244-4 PMID:12764555

Srivastava, S., Verma, P. C., Chaudhary, V., Singh, N., Abhilash, P. C., Kumar, K. V., ... Singh, N. (2013). Influence of inoculation of arsenic resistant Staphylococcus arlettae on growth and arsenic uptake in *Brassica juncea (L). Czern. Var. R-46. Journal of Hazardous Materials*, *262*, 1039–1047. doi:10.1016/j.jhazmat.2012.08.019 PMID:22939092

Tangahu, B. V., Sheikh Abdullah, S. R., Basri, H., Idris, M., Anuar, N., & Mukhlisin, M. (2011). A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremdiation. *International Journal of Chemical Engineering*, *31*, 939161.

Tong, X., Yuan, L., Luo, L., & Yin, X. (2014). Characterization of a Selenium-Tolerant Rhizosphere Strain from a Novel Se-Hyperaccumulating Plant *Cardamine hupingshanesis*. *Scientific World J, 1*(8).

Vivas, A., Biro, B., Nemth, T., Barea, J. M., & Azcon, R. (2006). Nickel-tolerant *Brevibacillus brevis* and arbuscular mycorrhizal fungus can reduce metal acquisition and nickel toxicity effects in plant growing in nickel supplemented soil. *Soil Biology & Biochemistry*, *38*(9), 2694–2704. doi:10.1016/j.soilbio.2006.04.020

Wu, S. C., Cheung, K. C., Luo, Y. M., & Wong, H. M. (2006). Effects of inoculation of plant growth-promoting rhi-zobacteria on metal uptake by *Brassica juncea. Environ. Pollut.*, *140*(1), 124–135. doi:10.1016/j.envpol.2005.06.023 PMID:16150522

Yao, Z., Li, J., Xie, H., & Yu, C. (2012). Review on remediation technologies soil contaminated by heavy metals. *Procedia Environmental Sciences*, *16*, 722–729. doi:10.1016/j.proenv.2012.10.099

Zhang, Y., He, L., Chen, Z., Zhang, W., Wang, Q., Qian, M., & Sheng, X. (2011). Characterization of lead-resistant and ACC deaminase-producing endophytic bacteria and their potential in promoting lead accumulation of rape. *Journal of Hazardous Materials*, *186*(2-3), 1720–1725. doi:10.1016/j.jhazmat.2010.12.069 PMID:21227577

Chapter 6

Role of Plant Growth Promoting Rhizosphere (PGPR) on Molecular Mechanisms Transporters Under Heavy Metal Stress

ABSTRACT

In this chapter, the authors discuss the molecular mechanisms transporters used for the removal of heavy metals contaminants from soil and water. The bioremediation method used for soil remediation render the land useless as a medium for plant growth as they also remove other contaminants that harm microbes and maintaining soil fertility with the help generating heat shok protein and metallothiones and also molecular transporters.

INTRODUCTION

Bacteria have ability to tolerance of metals using different mechanism which maintain metal homeostasis with keeping concentration of essential metals (Nies, 2003). Microorganisms involve both actively and passively for metal uptake, remaining or sequestering. Effects of different bacteria on metal uptake, depends on the basis of chromosomally or extrachromosomally which controlled detoxification of metals (Ehrlich, 1997). Several type sequestration

DOI: 10.4018/978-1-5225-9016-3.ch006

mechanism of metal resistant system including efflux pumps to remove metals from the cell and to bind inside the cell. Metals pump out by using adenosine triphosphates two efflux system and through antiports proton generate protein gradient across the cell membrane (Nies, 2003). Researchers indicated about another mechanism about metals resistance in cyanobacteria is sequestration by metallothioneins signaling. Metallothioneins compounds bind to different metals on receptor of plasmamembrane to sufhydryl group of cysteine residue with phosphate groups and activate the channels for the expression of gene to tolerate under stress. More cadmium (Cd) accumulation and tolerance by Stenotrophomonas maltophilia and also found Cd efflux pump. Some bacteria produce more extracellular polymeric susbstances (EPS) which bind to metal and create environment around the plant root for less toxic.

Hyperaccumulation mechanism in pant described briefly that hyperaccumulation is not only depends on plant but also interaction between microbes and present amount available of metals and non netals in soil. Bacterial communities present in soil with tolerable metals like zinc, lead, coper and nickel. According to Idris et al. (2004) were reported that rhizosphere of hyperaccumulating plants such as *Arabidopsis murale* and *Thlaspigoesingense* has an increased proportion of metal resistant bacteria. Many bacteria have tendency that can alter heavy metal mobility for uptake plant easily (Rajkumar and Freitas, 2006). Isolated Ni mobilizing bacteria from Ni rich soil and check their efficiency to promoting plant growth with the using Brassica species (Abou-Shanab et al., 2003). Soil bacteria produce such compounds biosurfactants, siderphores and organic acids which stimulate metal bioavailability in soil and increase root absorption of various ions like (Mn^{2+}, Fe^{2+} and Cd^{2+}).

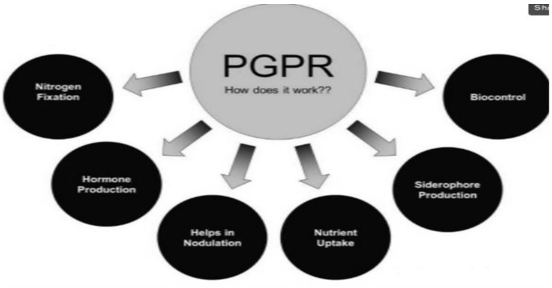
PLANT GROWTH PROMOTING RHIZOSPHERE

Rhizobium leguminosarum of different strains was showed Cd tolerant and increased the levels of glutathione which indicating about tripeptide allows bacterium to deal under heavy metals stress rather than efflux systems activate (Ma et al., 2013). Metal toxicity occured in cell or plant organs at that condition important glutathione antioxidant generate to protect against stress. Apart from other mechanism plants also dealing with toxic metals which involve polyphosphates and long chains of thiophosphates for metals sequester (Lv et al., 2012). Biofilm formation from E.coli under nickel stress which may serve as as tolerance mechanism and involved in adherence by inducing genes encoding curli in transcription (Carpene et al., 2007). Root associated bacteria suggests EPS protective mechanism for specific bacteria might be replace the process of root zones with synthetic cross-linked polyacrylates and production of hydrogels to safe roots from harm metal toxicity (Blaylock et al., 1997). A recent study about proteomics that the "presence of a rhizosphere microbial population, adapted to heavy metal-polluted sites and enhanced the accumulation of metals in shoot of the hyperaccumualtor Arabidopsis halleri. The algae present in aquatic environment that may interact with microbes to remove contaminants from the environment. Artifically system generated by dried mixture of algae with bacteria to remove contaminants from water and soil (Loutseti et al., 2009). Combination of Ralstonia basilensis bacteria and microalga chlorella sorokiniana were successfully adsorption of Cd, Ni, Cu and Zn (Ma et al., 2013).

Meanwhile mycorrhizal fungi associated with plants can enhance uptake of metals both in case of high and low amount of metals concentrations (Banni and Faituri, 2013). *Prosopis juliflora* is a legume species was used to revegetation trail with fly ash ameliorated of different amendements like (blue green algal biofertilizer and rhizobium) significantly increased of plant biomass, chlorophyll pigments, photosynthesis pigments, protein content and also increased the level of antioxidant. According to Lodewyckx et al. (2001) reported that inoulation of Lupinus luteus grown on a nickel enriched substrate with the engineered nickel-resistant bacterium Burkholderia cepacia and nickel concentration in roots increased significantly (30%). Significantly total lead uptake and biomass production of B. nappus increase after inoculation with microbacterium sp. G16, a bacterial strain that can produce indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase and siderophore (Nies et al., 2003).

Under heavy metals toxicity bacteria produced siderophores for protection, enhancing plant growth for providing iron at sufficient amount and pass them to rise above the toxic condition. Another factor for plant growth is ACC deaminase has been studied in respect to heavy metals stress, produced ethylene as an intermediate in same favored reaction. Ali et al. (2013) reported that ACC deaminase can produce by bacteria at lower levels of ethylene in plants for promoting plant growth. Both plants and bacteria produce the auxin IAA, which increasing plant biomass and metal accumulation (Sing et al., 2014). Cr-resistant bacteria were enhancing plant growth by reinoculation and less accumulation of Cr compare to uninoculated sunflower plants (Faisal and Hasnain, 2005). Plant growth promoting rhizobacteria play role to stimulate root growth, increased soil nutrients for obtainable to the plant, fix nitrogen,

Figure 1. Plant growth promoting rhizosphere synthesis of many enzymatic activities



improve soil fertility, suppress soil-borne pathogens, hormone production, nodule formation, nutrietent uptake, siderophore production and protect from different diseases to important role in crop yield under various environmental stresses like heavy metals, drought, salinity and temperature Figure 1 (Naik et al., 2012). Bacteria have ability to produce some enzymatic activities which allowing to covert hazardous compounds to hamless froms to survive extreme heat, cold, and heavy metals stress conditions (Ali et al., 2013; Singh et al., 2014). Nickel (Ni)-resistant bacteria have been found in the rhizosphere of the N-hyperaccumulator Thlaspi goesingense and Alyssum serpyllifolium subsp. (Idris et al., 2004; Becerra-Castro et al., 2009) Figure 1. Bacterial species Arthrobacter spp. and Streptomyces spp. were dominant and could tolerate as much as 10 mM Ni in plate assays. Pseudomonas aeruginosa strain WI-1 could torate up to 0.6 mM lead nitrate and also accumulated lead at 26.5 mg/g of dry cell biomass (Naik et al., 2012). Phosphate solubilizing bacterium Burkholderia metalliresistens sp. nov., D414T from heavy metal was able to tolerate (Cd-2000 mg/L; Pb-800 mg/L; Cu-150 mg/L and Zn- 2500 mg/L).

The inoculation of wheat with Zn-toerant *P. aeruginosa* improved plant biomass, the N and P uptake, increased leaf chlorophyll, and total soluble protein in plants grown in contaminated soil containing 1000 mg/kg Zn (Islam et al., 2014). The root-associated microbes could also improve the chemical and biological activites of heavy metal-contaminated soils. For example, inoculation of *Brassica juncea* with As-resistant *Staphylococcus arlettae* strain increased soil dehydrogenase, phosphate, microbial biomass carbon, organic carbon and available phosphorus in arsenic spiked 5, 10, 15 mg/kg in soil (Srivastava et al., 2013). The legume-rhizobia sysmbioses have been reported as one of the important approaches for heavy metal remediation (Teng et al., 2015). The symbiotic performance was hampered by heavy metals, reducing rhizobial colonization in the root and nitrogenase activity and also inhibition of nitrogen fixation has been observed for peas (Pisum sativum-366 mg/kg Zn) grown under Zn-contaminated soil (Chaudhary et al., 2004). Rhizosphere microbes with high colonization ability and enzymatic activity may improve metal solubility and also reducing the soil ph or by producing chelators and siderophores (Islam et al. 2014). Hyperaccumulators plant grow under heavy metal stress in soil microbes improve plant growth through the phytohormone production and phosphate solubilization abilities (Sessitsch et al., 2013).

The root-associated microbes could also improve the chemical and biological activites of heavy metal-contaminated soils. For example, inoculation of Brassica juncea with As-resistant Staphylococcus arlettae strain increased soil dehydrogenase, phosphate, microbial biomass carbon, organic carbon and available phosphorus.

MECHANISM BY WHICH MICROBES INFLUENCE HEAVY METAL ACCUMULATION

Root associated microbes can use various mechanisms to stimulate plant growth and development to protect plant from soil borne diseases to increase plant stress tolerance and degrade heavy metals or organic pollutants (Nadeem et al., 2014). Different mechanism include: (1) production of phytohormones such as cytokinins, gibberellic acid, indole acetic acid (2) antifungal metabolites and lytic enzymes (3) production of ACC deaminase to reduce the level of ethylene in roots of developing plants (4) solubilization of minerals such as potassium and phosphorus (5) production of exopolysaccharides and osmoprotects (6) immobilization of heavy metals (Egamberdieva, 2009; Tu et al., 2013; Glick, 2014; Erkovan et al., 2010; Ma et al. 2013). Heavy metal stress reduces the endogenous levels of phytohormones (Iqbal and Ashraf, 2010).

Plant growth promoting rhizosphere synthesized phytohormones (secondary metabolites) associated with different plants for the supply of rich substrate exuded from the roots and reaction occurred with the help of nitrogen fixation process (Eganberdieva et al., 2014). Plant growth regulators synthesize with the help of microbial activity such as (auxins, gibberellins and cytokinins) can enchancing the root surface area, root branching, greater absorption and increase the ability of phytoremdiation of hyperaccumulating plants. Different strains of *Pseudomonas* species like putida TSAU produced IAA (5.3 mgmL^{-1}) and aureantiaca TSAU22 (7.1 mgmL^{-1}) when exposed to 1.5%NaCl apart from this tryptophan increased IAA by (29. 5 and 31.2 mgmL^{-1}) respectively (Egamberdieva and Kucharova, 2009). Burkholderia sp. J62 of cadmium resistant bacteria was able to produce IAA (3.8 mgmL^{-1}) and 234 mgmL^{-1} solubilize of inorganic phosphate as compared to control (32.4 mgmL^{-1}) and meanwhile Pb and Cd contents in the shoots of maize (38-192%) compared with control plants. IAA (3.95 mgmL^{-1}) produce by *Brevibacillus sp*. B-I was shown and 5.6% of Zn accumulate in *Trifolium repens*, and enhanced plant growth (Vivas et al., 2006).

Inoculation of *B. napus* with a Pb-resistant strain *Pseudomonas fluorescence* G10 significantly increased the root length of seedlings by 21-35% in the presence of 5 mg/L Pb (Sheng et al., 2008a), Whereas Cr-resistant Microbacterium arborescens “HU33” stimulated the root length of ryegrass (*Prosopis juliflora L.*) by 39% in the presence of 2243 mg/kg Cr in soil (Khan et al., 2015). Mercury (Hg)-tolerant “bacterial strains isolated from the rhizosphere of reed (P. australis) photobacterium spp. strain that increased root length, uptake was found to produce from 77 to 81 mg/mL IAA and showed higher reductase activity (Mathew et al., 2015). Some bacterial strain contain ACC deaminase, which can cleave the plant ethylene precursor and low level of ethylene in stressed plants grown in metal contaminated soils, thus preventing the inhibition of root growth by ethylene (Migocka et al., 2014). Heavy metal resistant strain *P. myrsinacearum* RC6b has the potential to use ACC as the sole source of nitrogen by producing the enzyme ACC deaminase (Ma et al., 2013). Root-assoiated microbes and metal resistant bacteria may solublize insoluble phosphates is dependent on the different low molecular mass of organic acids such as (2-ketogluconic, gluconic acids and acetic acid) production for enhancing the plant capability to tolerate toxicity (Muleta et al., 2013). Chelates induce organic acid and inorganic acid ions decrease the pH activity to take Phosphorus from the soil and solubilizing the metal ions to accumulate has been documented (Marra et al., 2012). *Pseudomonas myrsinacearum* RC6b, was found phosphates solubilization 105 mg of P mgL^{-1} after 72 h of incubation along with decrease pH significantly from 4.88 to 3.73. Inoculation of *Pseudomonas aeruginosa* was found to enhanced antioxidative enzyme such as peroxidase (180%), Catalase (83%), Superoxidase dismutase 9161%) and also the non-enzymatic components such as total phenolics (10%) and ascorbic acid (65%) compared with treated plants under Zn stress. Phytoremdiation process successfully depends not only on the plants roots interaction with bacteria but also depends on the bioavailability of metals in soils. Plants roots produced some enzymatic activites to easily absorb contaminants of metals and mobilze metals ions for the bioavailable fraction so plants can not easily absorb metals without help of enzymes produce around the roots (Li et al., 2009). *Pseudomonas koreensis* AGB-1 strain increased SOD and CAT activities by 33% and 42% respectively (Babu et al., 2015).

The composition of the plant root-soil microbal interference can assist significantly under heavy metal-contaminated soils through mediating nutrient mineralization to uptake by different plant species. Explaination about beneficial bacteria effect on the production of hormones, secondary metabolites under toxicity in plant cell, antioxidant enzymes, cell-wall degrading enzymes, ACC deaminase, siderophores, induced systemic tolerance, mineral solubilization and biosurfactants of metal chelating compounds in different mechanism. Mechanism of phytoremdiation technology and metal accumulation some how depends on the chelating molecules synthesized by bacteria. The combination of bacteria interaction with some chelating compounds in specific plants may give more benefits to the plants for better accumulation of contaminants from the soil with different process (bioaccumulation, bioleaching, biotransformation, biodegradation and biosorption) Figure 2.

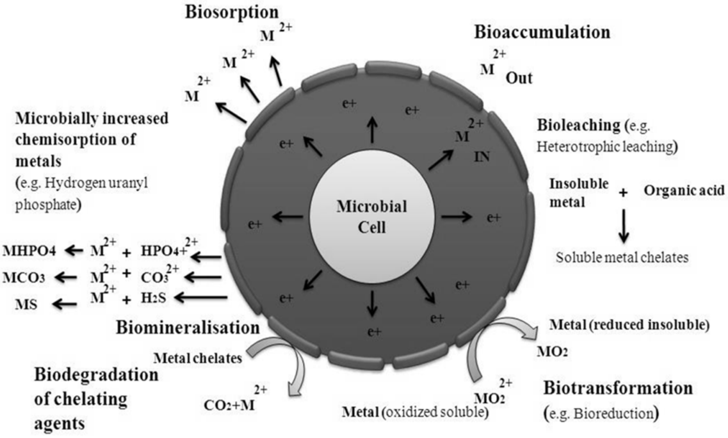
MOLECULAR MECHANISMS

Plants to the array of metal toxicity and elucidating of different traits in plants differentiate (Migocka et al., 2014; Gustin et al., 2011). Different mechanism of metal detoxification to identify genes in metal tolerance has been discussed as well. The assembly of metal transporters, metallothionein, heat shock protein and other metal binding proteins to enhances tolerance power against heavy metals (Lv et al., 2012).

METAL TRANSPORTERS

Different mechanism about molecular tools was found recently about understanding of metal hyperaccumulation physiology of plants (Verbregg et al., 2009). The production of metals detoxifying, enzymes of sulphur metabolism, phytochelatins and metallo thionesins has been improved (Kotrba and Najmanova, 2009). CPx-type of ATPases involved in metal ion of homeostasis, tolerance in plants, cation diffusion facilitator-CDF

Figure 2. Microbial cell-interactions with metal and process of (bioaccumulation, bioleaching, biotransformation, biodegradation and biosorption; M^{2+}- metal ions)



family; natural resistance-associated macrophage protein-Nramp family proteins; and zinc-iron permease-ZIP family protein involved under heavy metals stress (Guerinot, 2000; Williems, 2000). Many plants have different transporters remain to be identified at the molecular scale. In case of Cu, Zn, Cd and Pb CPx-type of heavy metal ATPases have been identified in different range of organisms and also have been drawn in the movement of ions (Williams, 2000). Ethylene signaling pathway in plants has been done by some responsive to antagonist 1 RNA1, and functional (CPx-ATPase). Protein Zinc (Zn) transporters (ZAT1) of *Arabidopsis thaliana* may have a role in sequestration of Zn (Zaal et al., 1999). Under high concentration of Zn was observed that ZAT1 overexpressing in transgenic plants and showed resistant at high content of zinc in roots.

METALLOTHIONEINS

Cysteine (Cys) rich metallothioneins are low molecular weight protein, nonenzymatic and efficient in complexing the metals by affinity with sulfur present in the cysteine. It was classified based on the arrangement of Cysteine 1) have more than 20 conserved Cys are common in mammals and vertebrates and known to confirm tolerance to Cadmium 2) without specific arrangement of Cys include all those found plants, fungi, vertebrates (Carpene et al., 2007). The diversity of metallothioneins in plants suggests that they may not only differ in amino acid sequence, but also in function and specificity of a particular metal. However there is still no information about the true function of each metallothioneins in plants due to difficulty obtaining to purify Metallothioneins. The metallothionenins has a tendency to hydrolyze itself, particulary in the region between Cys in the protein sequences.

HEAT SHOCK PROTEINS

Plants grow above their optimal growth temperature due to the presence of heat shock proteins (HSPs) and increased the expression in reaction to the growth of different organisms. Heat shock protein are found in all living organisms and can be classified according to molecular size, and expressed in different stress like heavy metal toxicity, drought, cold and heat (Lewis, 1999). These proteins act as molecular chaperones in normal protein folding activity and assemble to function in the repair of proteins and protection under stress condition. Today, combine reports have been dicussed about HSP expression in plants under heavy metal stress condition and HSP17 over expressed in the roots of *Armeria maritime* plants grown on Cu-rich soil were observed by (Neumann et al., 1995).

CONCLUSION

Higher plants have evolved many genes which have potentials to metabolize or degrade different kinds of xenobiotic compounds. Transgenic plants so far have been developed for the hyperaccumulation of toxic heavy metals such as Cu, As, Cd, Pb, Hg, Zn, Ni and Co. Furthermore about the bioremdiation method used for soil remediation render the land useless as a medium for plant growth as they also remove other contaminants which harm to microbes and maintaining soil fertility. Through bioremediation process can enhance the phytoremediation efficiency with gentic combination of high tolerance, fast growing plants and high biomass yielding for best suited for the purpose of phytoremdiation.

REFERENCES

Ali, S. Z., Sandhaya, V., & Rao, L. V. (2013). Isolation and characterization of drought tolerant ACC deaminase and exopolysaccharide-producing fluorescent *Pseudomonas sp. Annals of Microbiology*, *64*(2), 493–502. doi:10.100713213-013-0680-3

Babu, A. G., Shea, P. J., Sudhakar, D., Jung, I. B., & Oh, B. T. (2015). Potential use of *Pseudomonas koreensis AGB-1* in association with *Miscanthus sinensis* to remediate heavy metal (loid) contaminated mining site soil. *Journal of Environmental Management*, *151*, 160–166. doi:10.1016/j.jenvman.2014.12.045 PMID:25575343

Bani, A., Pavlova, D., Echevarria, G., Mullaj, A., Reeves, R. D., Morel, J. L., & Sulc¸e, S. (2010). Nickel hyperaccumulation by the species of *Alyssum and Thlaspi* (*Brassicaceae*) from the ultramafic soils of the Balkans. *Botanica Serbica*, *34*(1), 3–14.

Becerra-Castro, C., Monterroso, C., Garcia-Leston, M., Prieto-Fernandez, A., Acea, M. J., & Kidd, P. S. (2009). Rhizosphere microbial densities and trace metal tolerance of the nickel hyperaccumulator Alyssum serpylli-folium subsp. *Iusitanicum. Intern. J. Phytorem.*, *11*(6), 525–541. doi:10.1080/15226510902717549 PMID:19810353

Blaylock, M. J., Salt, D. E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., ... Raskin, I. (1997). Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environmental Science & Technology*, *31*(3), 860–865. doi:10.1021/es960552a

Carpene, E., Andreani, G., & Isani, G. (2007). Metallothionein functions and structural charcateristics. *Journal of Trace Elements in Medicine and Biology*, *21*(Suppl. 1), 35–39. doi:10.1016/j.jtemb.2007.09.011 PMID:18039494

Chaudhary, P., Dudeja, S. S., & Kapoor, K. K. (2004). Efectivity of host-Rhizobium leguminosarum symbiosis in soils receiving sewage water containing heavy metals. *Microbial. Res.*, *159*(2), 121–127. doi:10.1016/j.micres.2004.01.009 PMID:15293945

Egamberdieva, D., & Kucharova, Z. (2009). Selection for root colonizing bacteria stimulating wheat growth in saline soils. *Biology and Fertility of Soils*, *45*(6), 561–573. doi:10.100700374-009-0366-y

Egamberdieva, D., & Lugtenberg, B. (2014). PGPR to alleviate salinity stress on plant growth. In M. Miransari (Ed.), *Use of microbes for the alleviation of soil stresses* (Vol. 1, pp. 73–96). New York: Springer. doi:10.1007/978-1-4614-9466-9_4

Ehrlich, H. L. (1990). *Geomicrobiology* (2nd ed.). New York: Dekker.

Erkovan, I., Gullap, M. K., Dasci, M., & Koc, A. (2010). Effects of phosphates fertilizers and phosphorus solubilizing bacteria application on clover dominant meadow: Yield and botanical composition. *Turkish Journal of Field Crops*, *15*(1), 12–17.

Faraldo-Gomez, J. D., & Sansom, M. S. P. (2003). Acquisition of siderophores in gram-negative bacteria. *Nature Reviews. Molecular Cell Biology*, *4*(2), 105–116. doi:10.1038/nrm1015 PMID:12563288

Glick, B. (2014). Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiological Research*, *169*(1), 30–39. doi:10.1016/j.micres.2013.09.009 PMID:24095256

Guerinot, M. L. (2000). The ZIP family of metal transporters. *Bioch Biophys Acta Biomembr*, *1465*(1-2), 190–198. doi:10.1016/S0005-2736(00)00138-3 PMID:10748254

Gustin, J. L., Zanis, M. J., & Salt, D. E. (2011). Structure and evolution of the plant cation diffusion facilitate or family of ion transporters. *BMC Evolutionary Biology*, *11*(1), 76. doi:10.1186/1471-2148-11-76 PMID:21435223

Idris, R., Trifonova, R., Puschenreiter, W., Wenzel, W. W., & Sessitsch, A. (2004). Bacterial communities associated with flowering plants of the Ni hyperaccumulator Thlaspi goesingense. *Applied and Environmental Microbiology*, *70*(5), 2667–2677. doi:10.1128/AEM.70.5.2667-2677.2004 PMID:15128517

Iqbal, M., & Ashraf, M. (2010). Gibberellic acid mediated induction of salts tolerance in wheat plants:growth, ionic partitioning, photosynthesis, yield and hormonal homeostasis. *Environmental and Experimental Botany*, *86*, 76–85. doi:10.1016/j.envexpbot.2010.06.002

Islam, F., Yasmeen, T., Ali, Q., Ali, S., Arifa, M. S., Hussain, S., & Rizvi, H. (2014). Influence of Pseudomonas aeruginosa as PGPR on oxidative stress tolerance in wheat under Zn stress. *Ecotoxicology and Environmental Safety*, *104*, 285–293. doi:10.1016/j.ecoenv.2014.03.008 PMID:24726941

Khan, M.U., Sessitsch, A., Harris, M.M., Fatima, K., Imran, A., Arslan., M., … Afzal, M. (2015). Cr-resistant rhizo-and endophytic bacteria associated with *Prosopis juliflora* and their potential as phytoremediation enhancing agents in metal-degraded soils. *Front. Plant Sci.* .2014.00755 doi:10.3389/fpls

Kotrba, P., Najmanova, J., Macek, T., Ruml, T., & Mackova, M. (2009). Genetically modified plants in phytoremedaition of heavy metal and metalloid soil and sediment pollution. *Biotechnology Advances*, *27*(6), 799–810. doi:10.1016/j.biotechadv.2009.06.003 PMID:19567265

Lewis, S., Handy, R. D., Cordi, B., Billinghurst, Z., & Depledge, M. H. (1999). Stress proteins (HSPs): Methods of detection and their use as an environmental biomarker. *Ecotoxicol.*, *8*(5), 351–368. doi:10.1023/A:1008982421299

Li, W., Ye, Z., & Wong, M. (2009). Metal mobilization and production of short chain organis acids by rhizosphere bacteria associated with a Cd/Zn hyperaccumulating plant, Sedum alfredii. *Plant and Soil*, *326*(1-2), 453–467. doi:10.100711104-009-0025-y

Lv, Y., Deng, X., Quan, L., Xia, Y., & Shen, Z. (2012). Metallothioneinses BcMT1 and BcMT2 from *Brassica campestris* enhance tolerance to cadmium and cooper and decrease production of reactive oxygen species in *Arabidopsis thaliana. Plant and Soil*, *367*(1-2), 507–519. doi:10.100711104-012-1486-y

Ma, Y., Rajkumar, M., Luo, Y., & Freitas, H. (2013). Phytoextraction of heavy metal polluted soils using *Sedum plumbizincicola* inoculated with metal mobilizing *Phyllobacterium myrsinacearum RC6b. Chemosphere*, *93*(7), 1386–1392. doi:10.1016/j.chemosphere.2013.06.077 PMID:23890964

Marra, L. M., Soares, C. R. F. S. S., de Oliveira, S. M., Ferreira, P. A. A. A., Soares, B. L., Carvalho, R. F., ... Moreira, F. M. (2012). Biological nitrogen fixation and phosphate solubilization by bacteria isolated from tropical soils. *Plant and Soil*, *357*(1-2), 289–307. doi:10.100711104-012-1157-z

Mathew, D.C., Ho, Y.N., Gicana, R.G., Mathew, G.M., Chien, M.C., & Huang, C.C. (2015). A rhizosphere associated symbiont, photobacterium spp. strain MELD1, and its targeted synergistic activity for phytoprotection against mercury. *PLoS One, 10*(3). .pone.0121178 doi:10.1371/journal

Migocka, M., Papierniak, A., Maciaszczyk-Dziubinska, E., Pozdzik, P., & Posyniak, E., Garbiec, & Filleur, S. (2014). Cumcumber metal transport protein MTP8 confers increased tolerance to manganese when expressed in yeast and *Arabidopsis thaliana. Journal of Experimental Botany*, 295.

Muleta, D., Assefa, F., Borjesson, E., & Granhall, U. (2013). Phosphate solubilising rhizobacteria associated with *Coffea Arabica L.* in natural coffee forests of southwestern Ethiopia. *Journal of the Saudi Society of Agricultural Sciences*, *12*(1), 73–84. doi:10.1016/j.jssas.2012.07.002

Nadeem, S. M., Ahmad, M., Zahir, Z. A., Javaid, A., & Ashraf, M. (2014). The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnology Advances*, *32*(2), 429–448. doi:10.1016/j.biotechadv.2013.12.005 PMID:24380797

Naik, M. M., Pandey, A., & Dubey, S. K. (2012). *Pseudomonas aeruginosa* strain Wl-1 from Mandovi estuary possesses metallothione into alleviate lead toxicity and promote plant growth. *Ecotoxicology and Environmental Safety*, *79*, 129–133. doi:10.1016/j.ecoenv.2011.12.015 PMID:22284824

Neumann, D., Nieden, U. Z., Lichtenberger, O., & Leopold, I. (1995). How does *Armeria maritime* tolerate high heavy metal concentrations? *Journal of Plant Physiology*, *146*(5-6), 704–717. doi:10.1016/S0176-1617(11)81937-1

Nies, D. H. (2003). Efflux-mediated heavy metal resistance in prokaryotes. *FEMS Microbiology Reviews*, *27*(2-3), 313–339. doi:10.1016/S0168-6445(03)00048-2 PMID:12829273

Sessitch, A., Kuffner, M., Kidd, P., Vangronsveld, J., Wenzel, W. W., Fallmann, K., & Puschenreiter, M. (2013). The role plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. *Soil Biology & Biochemistry*, *60*, 182–194. doi:10.1016/j.soilbio.2013.01.012 PMID:23645938

Sheng, X. F., Xia, J. J., Jiang, C. Y., He, L. Y., & Qian, M. (2008a). Characterization of heavy metal resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. *Environ. Pollut.*, *156*(3), 1164–1170. doi:10.1016/j.envpol.2008.04.007 PMID:18490091

Singh, A. K., Dhanjal, S., & Cameotra, S. S. (2014). Surfactin restores and enhances swarming motility under heavy metal stress. *Colloids and Surfaces. B, Biointerfaces*, *116*(1), 26–31. doi:10.1016/j.colsurfb.2013.12.035 PMID:24441179

Srivastava, S., Verma, P. C., Chaudhary, V., Singh, N., Abhilash, P. C., Kumar, K. V., ... Singh, N. (2013). Influence of inoculation of arsenic resistant Staphylococcus arlettae on growth and arsenic uptake in *Brassica juncea* (L). Czern. Var. R-46. *Journal of Hazardous Materials*, *262*, 1039–1047. doi:10.1016/j.jhazmat.2012.08.019 PMID:22939092

Teng, Y., Wang, X., Li, L. Z., & Luo, Y. (2015). Rhizobia and their biopartners as novel divers for functional remediation in contaminated soils. *Frontiers in Plant Science*, *6*. doi:10.3389/fpls.2015.00032

Tu, Q., Chen, J., & Guo, J. (2013). Screening and identification of antagonistic bacteria with potential for biological control of Penicillium italicum of citrus fruits. *Scientia Horticulturae*, *150*(4), 125–129. doi:10.1016/j.scienta.2012.10.018

Verbruggen, N., Hermans, C., & Sachat, H. (2009). Molecular mechanisms of metal hyperaccumulation in plants. *The New Phytologist*, *181*(4), 759–776. doi:10.1111/j.1469-8137.2008.02748.x PMID:19192189

Vivas, A., Biro, B., Nemth, T., Barea, J. M., & Azcon, R. (2006). Nickel-tolerant *Brevibacillus brevis* and arbuscular mycorrhizal fungus can reduce metal acquisition and nickel toxicity effects in plant growing in nickel supplemented soil. *Soil Biology & Biochemistry*, *38*(9), 2694–2704. doi:10.1016/j.soilbio.2006.04.020

Williams, L. E., Pittman, J. K., & Hall, J. L. (2000). Emerging mechanisms for heavy metal transport in plants. *Biochim. Biophys. Acta Biomembr.*, *1465*(1-2), 104–126. doi:10.1016/S0005-2736(00)00133-4 PMID:10748249

Chapter 7

Phytormediation Efficiency Increased by Using Plant Growth Promoting Bacteria (PGPB) and Chelates

ABSTRACT

In this chapter, the authors give information about the plant-growth-promoting bacteria and chelating agents removing high number of contaminants with the help of phytoremdiation technology. To the best of the authors' knowledge, this is the first chapter about heavy metal contamination in groundwater and soil removing by microbes and chelates.

INTRODUCTION

Phytoremediation is a cost-effective method for the remediation contaminated sites. In the present review, our major objective is to concisely evaluate the progress made so far in improvement of phytoremediation efficiency using different tools. The current discussion is about plant growth promoting bacteria (PGPB), chelating agents for improvement of phytoremediation efficiency. This chapter discusses about the background, concepts and current future trends in phytoremdiation technology. Soil is one of the most important elements necessary for human survival and development with the increased industrilization and urbanization source. Heavy metals contaminants have

DOI: 10.4018/978-1-5225-9016-3.ch007

happen to a severe problem to human health, and extensively severly affected to the environment that face very vast problem in world. Soil contamination basically most affected to the agriculture and increase with the urbanization problem; so it is well invested that all country will focus to solve these problems very soon otherwise it will pay extensively very high cost with human health. In 1980 many efforts were undertaken by European countries and also for US Congress Passed the Comprehensive Environment Response and Compensation and Liability. In the 1990s, Britain also passed the Environmental Protection Act for the same purpose here we discuss but compared with the developed countries, research and investments are not encouraged in developing countries such as India and China". Several remediation technologies are used such as land fill, excavation, thermal treatment, retrieval by means of electricity and leaching of acids but those are not suitable due to their high cost and very less effective, and less reliability on the specific metal contamination with site and its properties. The technology basically employs physical, chemical and biological remediation for removing contaminants.

PHYSICAL REMEDIATION

Physically remediation is the enhancement process for soil without addition of any other compounds such as 'chemicals. This method is useful for minute contaminated soil and done by surrogating contaminated soil and by thermal desorption method. The replacement of polluted soil aims to reduce the concentration of a pollutant in a particular area and to the improve soil quality by physical remediation method (Zhang et al., 2004). The soil replacement is divided into three steps: replacement, importing, spading. In soil replacement method, the polluted rhizosphere is replaced by new soil, applicable only in a very small area; new soil should be treated properly for remediation. In soil spading (the area of unhygienic soil is dug deeply and the noxious wastes) are spread completely into the subterranean sites to achieve dilution and natural remediation process. A great deal of clean and pure soil is imported and mixed properly into the polluted soil to cover it completely and the concentration of pollution is ultimately reduced. However this method have a large functioning capacity to remove contaminants, second one is cost deal method; suitable for small area pollution (Zhou et al., 2004). Heavy metal can be removed from the surface and remain in a very minute amount for thermal desorption which is based on the instability of noxious wastes. Thermal desorption can be further classified into high temperature desorption range from 320 to 560 °C, and low temperature from 90 to 320 °C. This is an advantageous technique is simple procedure for tools can be transferred from one place to another and the remediation soil can be reused is most significantly. This method was carried out in United States as a measure of in situ remediation, limiting facts such as high cost long process duration; so restricted its uses for soil remediation (Aresta et al., 2008). A field experiment was conducted in West Bengal India for As remediation reactor provides hope for the removal of As along the temperature from w 266 to <5mg/L in real groundwater at a low cost amount. The new technology applied for remediation was nanotechnology also opens opportunities in the form of magnetic nanoadsorbents for the removal of Cd from the environment (Sharma et al., 2014).

CHEMICAL REMEDIATION

Chemical remediation is the treatment of the rhizosphere by applying chemical agents in soil and water. The impure soil is washed by hygienic water, some reagents and some other chemical reagents or gases that filter the noxious waste from the rhizosphere (Tampouris et al., 2001). The inorganic compounds are different surfactants used in the form of leachate for soil remediation. The effects of hydrogen fluoride, nitric acid, phosphoric acid and hydrogen chloride for removal of contaminants from the rhizosphere at different concentrations. The yellow brown forest soil was collected of contaminated with As and then used as the standard soil for the experiment and that potassium and phosphorus was the most efficient for removing As as compared with many potassium and sodium salts. The As contamination can be also removed by a soil washing process; which was successfully done in Korea (Lee et al., 2007). Chemical agents used for chelation i.e ethylene-di-amine-tetra-acetic acid is more effective in biodegradability and alo for remediation of heavy metals from soil (Kos and Domen, 2003). The effect of saponin obtained from tea on contaminant elimination and observed more specificity for (Pb, Zn, Cd, and Cu) to reduce the environmental risk by removing both soluble as well as reductive metals. Chemical obsession is the process of addition of reagents into the rhizosphere to make them scarcely movable and less toxic in the soluble form and immigration of noxious waste could be decreased" in the soil (Zhou et al., 2004). Calcium chloride, sodium bentonite, diatoms in the earth and diethylene triamine pentaacetic acid can reduce the presence of contaminants in soil (Lv et al., 2009). *Attapulgite terracotta* is also used in many places for contaminated soil remediation and supports the concepts in the form of superb findings that state that an addition of attapulgite soil in ample amounts, 40-60% Cd contamination could be reduced with an increase in the efficacy of crop production (Fan et al., 2007).

The method were applying electric field gradient for soil purification at both sides of the rhizosphere (Luo et al., 2004). The heavy metals are passed from one pole to another pole through the passage electricity. It is feasible only for soil of meager permeability with an advantage of unproblematic installation that can be operated at very low cost, not destructing the nature, can easily make the rhizosphere pure and protect the environment (Zhang et al., 2001; Virkutyte et al., 2002; Xu et al., 2006). The nature of the rhizosphere i.e acidic or basic could not be changed by instant electrokinetics in low management efficiency. Morever, there are other techniques to eliminate heavy metals pollutants from the rhizosphere such as electrokinetic oxidation and reduction-mediated remediation; one of the most important soil remdiation methods is microbe-mediated remedition (Yu et al., 2009). Vitrification technology used for remediation was reported that heating the rhizosphre from (1400 to nearly 2000 °C) to either volatized or fester the natural means organic matters. The product formed after the heat productiona are collected by an off gas management systems thease pyrolyzed materials from rock-shaped glass like structure called vitreous and sourrodings the heavy metals which are finally not allowed to move. So it can be concluded that by using this technique contaminants can be removed with higher efficiency. Moreover all given above discussion on the application of these methods has some negative affect as several complications in operating and low cost benefit for this method (Fu, 2008).

BIOLOGICAL REMEDIATION

Biological remediation methods are used for heavy metal-contaminated soils. Biologically involve of various groups of (plants, mushrooms, algae, and plant growth-promoting bacteria) showed that the potential of accumulating heavy metals from the environment. The remediation is mainly done by green plant and bacteria to absorb metal conatminants and reduce their risk by cleaning the pollutant from their surroundings (Shen and Chen, 20001; Ullah et al., 2015). Phytoremediation is divided into parts: phytostabilization, phytovolatalization, and phytoextraction and phytodegradation (Shen and Chen, 2000). The amount of heavy metal contaminants and their efficiency from the rhizosphere and ground water up to the food chain are reduced by phytostabilization where, the pollutants are adsorbed, precipitated and finally reduced in their concentration (Wang et al., 2009). Phytovolatilization is the process of transforming the stages of heavy metals contaminants, either in a volatile stage or in gaseous one by root secreted chemicals. The waste materials attached to the underground part of plants having greater capacity of resistance, adsorption and accumulated in harvested parts of used plants (Bhargava et al., 2012). This technology includes having massive uptake power as a key characteristics feature, at low pollutant concentration and the accumulating efficiency should be high (Xiong et al., 2013). The heavy metal pollutants could not be removed or harmed by microbes but can affect their migration and change by changing their shape and size along with their chemical properties. Microbial leaching is one of the most promising technique by which the important metals are absorbed from substances ore and mineral concentration. The resistant plant growth promoting bacteria assist the phytoextraction" of heavy metal in *Helianthus annuus* (Prapagdee et al., 2013).

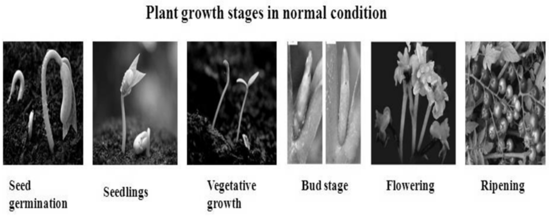
METAL DETOXIFICATION

Most of the metals are essential for plant growth and their nutrition level such as Cu, Zn, Mn, and Ni, but they become toxic when present at high amount inside the plant organ. To overcome this problem several plants show diverse type of mechanism to maintain their metabolic activity (Seth et al., 2007). Some plants have accumulating heavy metals contaminants up to the level of thousands of parts permillion and are capable of eliminating toxicity of metals (Pollard et al., 2002). Proteomics provides us a tool for examining the toxicity of heavy metals in plants and animals (Luque-Garcia et al., 2011). Along the same line another secondary metabolites of plants, metallothionein, and a protein has the capacity of heavy metal contamination binding properties (Tomsett and Thurman, 1988), along with numerous plants and bacteria (Kagi, 1991). The role of phytochelatins and metallothionein and their mechanism in heavy metals detoxification was clearly reported and explained by Cobbett (2000). Plantshave developed some other mechanism other than phytochelatins and metallothionein such as increased tolerance on inoculation of PGPR of plants, aerobic microbe generated reactive oxygen species in which H_2O_2 plays a crucial role (Mishra et al., 2006).

PLANT GROWTH PROMOTING BACTERIA (PGPB)

Plant growth promoting rhizobacteria are playing an important role in sustainable development by improving the crop production and quality of grains, ultimately performing land reformation. Common PGPR are *Pseudomonas fluorescence*, *Pseudomonas putida*, *Bacillus subtilis*, *Paenibacillus polymyxa*, *Bacillus licheniformis* and *Paenibacillus elgii* which are playing a very important role in sustainable development as well as in land reformation. There are several functions of PGPR in the improvement of soil are as phytohormone production, nitrogen fixation, phosphate solubilization, siderophores production, induced systemic resistance. Normally phytohormones have both effects i.e., beneficial and harmful to the plant. There are five major groups of hormones such as abscisic acid, cytokinins, gibberellins, ethylene and auxins. A recent study shows that auxins can also be an important factor involved in the signaling pathways of bacteria and consequently can affect the microbe's physiology (Spaepen et al., 2007). There is soil microbes involved in the synthesis of auxins in pure culture. The "potential for auxins biosynthesis by rhizobacteria, can be used as an implement for the selection of effective PGPR strain (Khalid et al., 2004). The evidence shows that PGPR influences plant growth and development by the production of phytohormone such as (auxins, gibberellins, and cytokinins). The strain that produce the highest amount of auxinsdindole acetic acid (IAA) and indole acetamidedin nonsterilized soil, causes maximum increase in growth and consent of the wheat crop. Even the strains, which create a low amount of IAA, liberate it constantly, thus improving plant growth (Tsavkelova et al., 2007). The best studied example of signal exchange is the Rhizobium legume symbiosis, in which the plant releases flavonoid compounds that act as signals for the bacterium to secrete Nod factors. Nod factors are perceived by plant root hairs and function in a hormone-like fashion to induce root nodules in which the Rhizobium bacterium can fix atmospheric Nitrogen. The bacterium grows at the expense of carbohydrates from the host but provided fixed N for amino acid biosynthesis in return (Gray and Smith, 2005). This symbiosis is aprime example of an intimate relationship between a soil bacterium and its host plant and illustrates PGPR. The rhizobium bacterium promotes legume plant growth by providing a limiting nutrient. Iron is important nutrient for almost all forms of life. Normally iron occurs primarily in the form of Fe^{3p} is known to form insoluble hydroxides and oxyhydroxides, which makes it unreachable for both plants and animals (Rajkumar et al., 2010). Siderophores

Figure 1. Normal plant growth stages



are easily soluble in water and on the basis of their occurrence in the cell can be divided into two forms: extracellular and intracellular. Actually, siderophores are useful in the solubilization of iron from organic compounds under some conditions like iron deficiency (Indiragandhi et al., 2008).

Plants absorb iron from PGPR siderophores by diverse mechanisms such as chelation and secretion (Schmidt, 1999). Several studies have supported the siderophores-mediated iron absorption as a result of rhizobacterial inoculation in the rhizosphere. *Pseudomonas fluorescence* an important member of PGPR is characterized by the production of yellow-green pigments termed pyoverdines, which fluoresence under ultraviolet light function as siderophores (Kloepper et al., 1981; Vansuyt et al., 2007). Phosphorus (P) is one of the chief macronutrients for plant growth and development. Phosphorus exists in two forms in soil: organic and inorganic phosphates. To convert insoluble phosphates both organic and inorganic, compounds in a form accessible to the plants is an important trait for a PGPR in increasing yield of crops (Igual et al., 2001; Rodriguez et al., 2006). The biological N fixation is very important in enhancing soil fertility. In addition to biological N fixation, phosphate solubilization is similarly important. The capacity of different microorganisms was to convert insoluble P to a reachable form, like orthophosphates is an important trait in a PGPR for increasing plant yield. The normal plant growth stages discussed in diagram Figure 1 without add any amendments, naturally it was done but if we provide some plant growth promoting bacteria amendments at appropriate amount so that will help to increase growth of plant.

The phosphates-solublizing microorganism, included bacteria have provided a substitute biotechnological solution in sustainable agriculture to meet the P demands for plant. The production of organic acids specially gluconic acids, seems to be the most frequent agent of minerals phosphates solubilization by bacteria such as *Pseudomonas sp., Pseumonas capacia, Burkhoderia cepacia and Erwinia herbicola* (Rodriguez and Fraga, 1999). Nitrogen is an essential component for plant development as well as their yield, but due to a non-utilizing form, it is unavailable to the green lungs. The unavailable N is converted into the utilizable form using PGPR by process biological N_2 fixation by converting N in the ammonia. In the whole process, nitrogenase, important, complex enzymes, responsible for N_2 fixations plays a very crucial role around the plant (Kim and Rees, 1994). Biological N fixation, an economical and ecofriendly process, improve the soil contamination by reducing the demands, needs of chemicals fertilizers and pesticides which leave toxic compounds ultimately converting arable land into barren land (Ladha et al., 1997).

CHELATES

Heavy metals naturally found in the soil because of weathering and other pedogenic processes on rocks and the source of the material of soil. This is the one of the environmental problem, keeping in mind that metals reach the soil and end up depreciating the whole area (underground water, fauna, flora and air). Heavy metals were present at high amount than damage plants and organisms, affecting their organs, changing their biochemical process, cellular membranes, cell organelles and also health problem (Seth et al., 2007). Phytoremdiation is low cost and effective technology use for degradation of contaminants (Lu et al., 2009). The more efficient and very useful technique is phytoextraction but it is more difficult process to others. In this technology growth of tolerant plants in contaminants soils which uptake large amounts of heavy metals from the soil and translocation into the aerial parts of the plant (Lu et al., 2009). Solubility of heavy metals in soil increased positively by chelating agents and significantly increased accumulation process by plants (Luo et al., 2015). Chelating compounds is a chemical substance that composed with metal ions whose molecules can form several bonds with single metal ions. Chelating agent used as a micronutrient fertilizers and maintains their solubility with metal (Socha and Guerinot, 2014).

Chelating assisted phytoremediaton demonstrated as a possible treatment for remediation of heavy metal contaminated soil and sediment. EDTA enrichment could be an appropriate technique for phytostabilization of Pb-contaminated environments (Ullah et al., 2014). Previous reports suggest that a great number of chelating agents have been used for enhanced phytoextraction

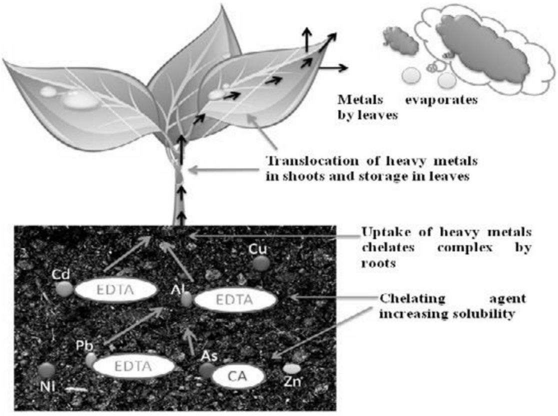
(EDTA, citric acid and malic acid). The introduction of EDTA and citric acid as a chelator is considered as important in chelation therapy (Komarek et al., 2007). Ethylene diamine tetra acetic acid was reported as a chelating agent for the enhancement in phytoextraction process (Blaylock et al. 1997). EDTA and citric acid is the well-organized and successful chelating agents for increasing the solubility of heavy metals in soils and has been widely used for the extraction of heavy metals from contaminated soils.

Certain chelating agents present in soil increases the translocation of metals from soil into the shoots has opened a wide range of possibilities for metal phytoextraction (Blaylock et al., 1997). If the toxic heavy metals exceeds with high amount of concentration than it certain threshold inside the cells to active metabolic process contricute to the production of chelating substances (Akhter et al., 2014; Oliva et al., 2012; Viehweger, 2014). Chelate agent contributes to metal degradation or detoxification by reducing the concentration of free metal in cytosol (Soudek et al., 2014). The cost for conventional procedures estimated such as, vitrification and soil washing, as between US$ 100000 and 10,00000 per ha (Russel et al., 1991), whereas the cost of phytoremediation was judged between US$ 60000 and 100000 per ha (Salt et al., 1995). The current markets outside the US, however, are believed to be small. In 1999 Canadian phytoremediation revenues were estimated to be at US $1–2 million, and European revenues at $2–5 million. Calculations for the US phytoremediation market have been put between $100-150 million.

Ethylenediaminetetraacetic acid (EDTA) was recognized as the most efficient that increases metal mobility to uptake by plants for large scale field application (Tandy et al., 2004; Luo et al., 2005). Nitrilotriacetate (NTA), [S,S]-EDDS (S,S-ethylenediaminedisuccinic acid), and others has shifted to some more biodegradable chelants for improving the uptake of heavy metals by plants and leaching of metals from soil with the studies comparing the previous EDTA results in metal uptake efficiencies (Meers et al., 2005, 2007; Ghnaya et al., 2013). The uptake of metals by the roots of plants, and their transport upwards to the shoots of the plants, and shoots to leaves should be based on the full understanding of the processes involved for metal solubilization from the application of chelants (Blaylock et al., 1997; Evangelou et al., 2007; Luo et al., 2007). Firstly, chelant agent can adsorb metals from the soil matrix the mobilized metals uptake by plant roots with the help of mobilizing activity of microorganism (figure 2).

Applying chelant agent in low amount of dose can enhanced phytoextraction of Pb (Shen et al., 2002). Combination of chelating agent can also be very effective to improve the metal phytoextraction efficiency by lowering the pH

Figure 2. Bioavailability of heavy metals increase in soil by forming chelates complexes (Ethylene diamine tetra acetate; EDTA and citric acid; CA)



of the soil with one type of combination of two chelants chemical (Blaylock et al., 1997). The interaction between metals and chelants in which the solubility of metals by a chelant can be increased by another chelant through the reduction of competition from other metals in soil occur second type of combination. The combination of EDTA and (S,S)-EDDS led to a higher level of efficiency that could be obtained by the application of either chelant alone performed in the phytoextraction of Zn, Cu, Pb and Cd (Luo et al., 2005).

There are two reason for this result that EDTA and (S,S)-EDDS have different levels of efficiency in soil and also decrease in the competitive cations for trace metals with EDTA because of the addition of (S,S)-EDDS (Tandy et al., 2004). Utilization of one chemical to destroy the plant root structure to facilitate the direct uptake of metal-chelants and their translocation into shoots occur in third type of combination, it was found that glyphosate increase the Pb accumulation of the tested crops (Mathis and Kayser, 2001). Chelating agents are the most prevalent removal agents used in soil washing and desirable plant species are those that are fast- growing which have a high biomass production and are easily harvested. According to Nowack, (2002) formation of complexes in soils is controlled by the kinetic of all complexation reactions. Interestingly applying one large dose of chelant often result that extraction of more toxic metals (Finzgar and Lestan, 2007). Plants can remove between 180 and 530 kgha^{-1} of Pb per year, with the hep of applying EDTA, making remediation of sites contaminated with up to 2500 mg kg^{-1}) Pb possible under 10 years. (Huang & Cunningham 1996; Blaylock et al., 1997). According to Salido et al., (2003) reported that when apply 10 mmol EDTA of 1kg soil is used to remove Pb from a soil containing 338 mg Pb 1kg soil, *B. juncea* plants extract approximately 32 mg of this metal (Gleba et al., 1999)". Shen et al., (2002) studied the effect of the application of 3.0 mmol EDTA per kg of soil on the uptake of Pb by cabbage (*Brassica rapa*), mung bean (*Vigna radiata*), and wheat (*Triticum aestivum*).

All the plants showed increased concentrations of Pb in shoots and roots, reaching Pb concentrations in cabbage shoots of 5010 and 4620 mg/kg) dry matter on days 7 and 14 after EDTA application, respectively. Concerning field demonstrations of this technology, an important decrease in soil Pb concentration over two years at two sites in the United States using a combination of *B. juncea* and EDTA but, unfortunately, the mass balance of Pb was not shown for that operation and, then, it is uncertain how much Pb the plants removed and, most importantly, how much Pb and/or EDTA leached through the soil profile (Robinson et al., 2003). Lead (Pb) appears to move from roots to shoots as a metal-complex in the xylem (Huang et al., 1997) via the transpiration stream (Blaylock et al., 1997). Plants are capable of immobilizing metal in soil by forming insoluble compounds as results of the interaction of plant exudates in the biostimulation consortia. Chelation of heavy metal is a ubiquitous detoxification strategy described in a wide variety of plants. Inside the cell heavy metals ions that are not immediately required metabolically may reach toxic concentrations and plant cells have evolved various mechanisms to store excess metals to prevent their participation in unwanted toxic reactions (Ali et al., 2013). If the toxic metal concentration exceeds a certain threshold inside the cell and metabolic active to the production of chelating compounds (Akhter et al., 2014).

CONCLUSION

Plant growth promoting and chelates have the ability to increase metal solubility to such an extent that leaching of metals can occur. In addition, they can allow soil microbes to absorb these heavy metals. Both ones are more environmentally friendly and are effective in metal removal. Phytoremediation technologies are based on the plants how much contaminants remove and very cost effective, economically viable and socially accepted method for management of polluted waters as compared to the physical and chemical methods.

Table 1. Phytoremdiation efficency enhanced by chelating agent

Chelating Agent	Heavy Metal	References
EDTA:"ethylenediaminetetraacetic acid"	Pb, Cd	Luo et al., 2005
HEDTA:N-"hydroxyethylethylenediaminetriacetic	Pb	Luo et al., 2005
acid"	Pb, Cd	Grcman et al., 2003
DTPA:"diethylenetriaminopentaacetic acid"	-	Huang, 1996
CDTA:"trans-1,2-diaminocyclohexane-N,N,N¢,N¢-	-	Cooper,1999
tetraacetic"	-	Khan, 2000
acid	-	Salt, 1998
EGTA:"ethylenebis(oxyethylenetrinitrilo")-	-	-
N,N,N¢,N¢-"tetraacetic	-	-
Acid"	Pb, As	Luo et al., 2005
EDDHA:"ethylenediamine-di"	-	Tandy et al., 2004
(o-hydroxyphenylacetic acid")	-	-
HEIDA:N-(2-hydroxyethyl)iminodiacetic acid	-	-
EDDS:ethylenediaminesuccinate	Cd	Luo et al., 2005
NTA:nitrilotriacetic acid	Pb	Khan, 2000

REFERENCES

Akhter, M. F., Omelon, C. R., Gordon, R., Moser, D., & Macfie, S. M. (2014). Localization and chemical sp eciation of cadmium in the roots of barley and lettuce. *Environmental and Experimental Botany*, *100*, 10–19. doi:10.1016/j.envexpbot.2013.12.005

Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremdiation of heavy metals-concepts and applications. *Chemosphere*, *91*(7), 869–881. doi:10.1016/j.chemosphere.2013.01.075 PMID:23466085

Aresta, M., Dibenedetto, A., Fragale, C., Giannoccaro, P., Pastore, C., Zammiello, D., & Ferragina, C. (2008). Thermal desorption of polychlorobiphenyls from contaminated soils and their hydrodechlorination using Pd and Rh supported catalysts. *Chemoasphere*, *70*(6), 1052–1058. doi:10.1016/j.chemosphere.2007.07.074 PMID:17850843

Bhargava, A., Francisco Carmona, F., Bhargava, M., & Srivastava, S. (2012). Approaches for enhanced phytoextraction of heavy metals. *Journal of Environmental Management*, *105*, 103–120. doi:10.1016/j.jenvman.2012.04.002 PMID:22542973

Blaylock, M. J., Salt, D. E., Dushenkov, S., Zakarova, O., Gussman, C., Kapulnik, Y., ... Raskin, I. (1997). Enhanced Accumulation of Pb in indian mustard by soil- applied chelating Agents. *Environmental Science & Technology*, *31*(3), 860–865. doi:10.1021/es960552a

Brencic, A., & Winans, S. C. (2005). Detection of and response to signals involved in host-microbe interations by plant associated bacteria. *Microbiology and Molecular Biology Reviews*, *69*(1), 155–194. doi:10.1128/MMBR.69.1.155-194.2005 PMID:15755957

Cobbett, C. S. (2000). Phytochelatins and their role in heavy metal detoxification. *Plant Physiology*, *123*(3), 825–832. doi:10.1104/pp.123.3.825 PMID:10889232

Cooper, E. M., Sims, J. T., Cunningham, S. D., Huang, J. W., & Berti, W. R. (1999). Chelate-assisted phytoextraction of lead from contaminated soils. *Journal of Environmental Quality*, *28*(6), 1709–1719. doi:10.2134/jeq1999.00472425002800060004x

Evangelou, M. W. H., Ebel, M., & Schaeffer, A. (2007). Chelate assisted phytoextraction of heavy 594 metals from soils. Effect, mechanism, toxicity, and fate of chelating agents. *Chemosphere*, *595*(68), 989–1003. doi:10.1016/j.chemosphere.2007.01.062 PMID:17349677

Fan, D. F., Huang, S. S., & Liao, Q. L. (2007). Restoring experiment on cadmium polluted vegetables lands with attapulgite of varied dose. *Jiangsu Geol.*, *31*(4), 323–328.

Finzgar, N., & Leštan, D. (2007). Multi-step leaching of Pb and Zn contaminated soils with 605 EDTA. *Chemosphere*, *66*(5), 824–832. doi:10.1016/j.chemosphere.2006.06.029 PMID:16879858

Fu, J. H. (2008). The research status of soil remediation in China. *Annual meeting of Chinese Society for Environmental Sciences*, 1056-1060.

Ghnaya, T., Zaier, H., Baioui, R., Sghaier, S., Lucchini, G., Sacchi, G. A., & Abdelly, C. (2013). Implication of organic acids in the long-distance transport and the accumulation of lead in *Sesusvium portulacastrum* and *Brassica juncea. Chemosphere*, *90*(4), 1449–1454. doi:10.1016/j.chemosphere.2012.08.061 PMID:23026160

Gleba, D., Borisjuk, N. V., Borisjuk, L. G., Kneer, R., Poulev, A., Skarzhinskaya, M., ... Raskin, I. (1999). Use of plant roots for phytoremediation and molecular farming. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(11), 5973–5977. doi:10.1073/pnas.96.11.5973 PMID:10339526

Gray, E. J., & Smith, D. L. (2005). Intracellular and extracellular PGPR: Commonalities and distinctions in the plant bacterium signaling process. *Soil Biology & Biochemistry*, *37*(3), 395–412. doi:10.1016/j.soilbio.2004.08.030

Grcman, H., Vodnik, D., Velikonja-Bolta, S., & Lestan, D. (2003). Ethylenediaminedissuccinate as 615 a new chelate for environmentally safe enhanced lead phytoextraction. J. 616 Environ. *Qual.*, *32*(2), 500–506. doi:10.2134/jeq2003.5000 PMID:12708673

Huang, J. W., Chen, J., Berti, W. R., & Cunningham, S. D. (1997). Phytoremediation of lead-contaminated soils: Role of synthetic chelates in lead phytoextraction. *Environmental Science & Technology*, *31*(3), 800–805. doi:10.1021/es9604828

Huang, J. W., & Cunningham, S. D. (1996). Lead phytoextraction: Species variation in lead uptake and translocation. *The New Phytologist*, *134*(1), 75–84. doi:10.1111/j.1469-8137.1996.tb01147.x

Igual, J.M., Valverde, A., Cervantes, E., & Velazquez, E. (2001). Phosphate-solubilizing bacteria as inoculants for agriculture:use of updated molecular techniques in their study. *Agronomies, 2196*(7), 561-568.

Indiragandhi, P., Anandham, R., Madhiyan, M., & Sa, T. M. (2008). Characterization of plant growth promoting traits of bacteria isolated from larval guts of diamond back moth *Plutella xylostella* (*Lepidoptera: Plutellidae*). *Current Microbiology*, *56*(4), 327–333. doi:10.100700284-007-9086-4 PMID:18172718

Khalid, A., Arshad, M., & Zahir, Z. A. (2004). Screening of plant growth promoting rhizobacteria for improving growth and yield of wheat. *Journal of Applied Microbiology*, *96*(3), 473–480. doi:10.1046/j.1365-2672.2003.02161.x PMID:14962127

Khan, M. S., Zaidi, A., & Aamil, M. (2002). Biocontrol of fungal pathogens by the use of plant growth promoting rhizobacteria and nitrogen fixing microorganisms. *Ind. J. Bot. Soc.*, *81*, 255–263.

Kim, J., & Rees, D. C. (1994). Nitrogenase and biological nitrogen fixation. *Biochemistry*, *33*(2), 389–397. doi:10.1021/bi00168a001 PMID:8286368

Kloepper, J. W., Scher, F. M., Laliberte, M., & Tipping, B. (1981). *Emergence-promoting rhizobacteria: description and implications for agriculture* (Vol. 117). Iron, Siderophores, and Plant Diseases.

Kos, B., & Domen, L. (2003). Induced phytoextraction/soil washing of lead using biodegradable chelate and permeable barriers. *Environmental Science & Technology*, *37*(3), 624–629. doi:10.1021/es0200793 PMID:12630481

Ladha, J. K., de Bruijn, F. J., & Malik, K. A. (1997). Introduction assessing opportunities for nitrogen fixation in rice-a frontier project. *Plant and Soil*, *12*(1/2), 1–10. doi:10.1023/A:1004264423436

Lee, M., Paik, I. S., Do, W., Kim, I., Lee, Y., & Lee, S. (2007). Soil washing of As-contaminated stream sediments in the vicinity of an abandoned mine in Korea. *Environmental Geochemistry and Health*, *29*(4), 319–329. doi:10.100710653-007-9093-1 PMID:17492479

Lu, L., Tian, S. K., Yang, X. E., Li, T. Q., & He, Z. I. (2009). Cadmiumuptake and xylem loading are active processes in the hyperaccumulator Sedum alfredii. *Journal of Plant Physiology*, *166*(6), 579–587. doi:10.1016/j.jplph.2008.09.001 PMID:18937997

Luo, C. L., Shen, Z. G., & Li, X. D. (2005). Enhanced phytoextraction of Cu, Pb, Zn and Cd with 680 EDTA and EDDS. *Chemosphere*, *59*(1), 1–11. doi:10.1016/j.chemosphere.2004.09.100 PMID:15698638

Luo, Q., Sun, L. N., Wang, H., & Hu, X. M. (2015). Metabolic profiling analysis of root exudates from the Cd hyperaccumulator Sedum alfredii under different Cd exposure concentration and times. *Analytical Methods*, *7*(9), 3793–3800. doi:10.1039/C5AY00159E

Luo, Q. S., Zhang, X. H., & Wang, H. (2004). Influence of nonuniform electro-kinetic remediation technology on soil properties. *Chin. J. Environ. Eng.*, *5*(4), 40–45.

Luque-Garcia, J. L., Cabezas-Sanchez, P., & Camara, C. (2011). Proeteomics as a tool for examining the heavy metals. *Trends in Analytical Chemistry*, *30*(5), 703–716. doi:10.1016/j.trac.2011.01.014

Lv, L. L., Jin, M. Y., & Li, B. W. (2009). Study on remediation of the soil contaminated with cadmium by applying four minerals. *J. Agri. Univ. Hebei*, *32*(1), 1–5.

Mathis, P., & Kayser, A. 2001. Plant uptake of heavy metals following glyphosate treatment. *Proc. 6th Int. Conf. on the 703 Geochemistry of Trace Elements (ICOBTE).*

Meers, E., Ruttens, A., Hopgood, M., Lesage, E., & Tack, F. M. (2005). Potential of *Brassic rapa, Cannabis sativa, Helianthus annuus* and *Zea mays* for phytoextraction of heavy metals from calcareous dredged sediment derived soils. *Chemosphere*, *61*(4), 561–572. doi:10.1016/j.chemosphere.2005.02.026 PMID:16202810

Mishra, S., Srivastava, S., Tripathi, R. D., Kumar, R., Seth, C. S., & Gupta, K. (2006). Lead detoxification by coontail (*Ceratophyllum demersum L.*) involves induction of phytochelatins and antioxidant system in response to its accumulation. *Chemosphere*, *65*(6), 1027–1039. doi:10.1016/j.chemosphere.2006.03.033 PMID:16682069

Nowack, B. (2002). Environmental chemistry of aminopolycarboxylate chelating agents. 722. *Environmental Science & Technology*, *36*(19), 4009–4016. doi:10.1021/es025683s PMID:12380068

Oliva, S. R., Mingorance, M. D., & Leidi, E. O. (2012). Tolerance to high Zn in the metallophyte Erica and evalensis Cabezudo & Rivera. *Ecotoxicol.*, *21*(7), 2012–2021. doi:10.100710646-012-0953-9

Pollard, J. A., Powell, K. D., Harper, F. A., & Smith, J. A. C. (2002). The genetic basis of metal hyperaccumulation in plants. *Critical Reviews in Plant Sciences*, *21*(6), 539–566. doi:10.1080/0735-260291044359

Prapagdee, B., Chanprasert, M., & Mongkolsuk, S. (2013). Bioagumentation with Cadmium-resistant plant growth-promoting rhizobacteria to assist cadmium phytoextraction by *Helianthus annus. Chemosphere*, *92*(6), 659–666. doi:10.1016/j.chemosphere.2013.01.082 PMID:23478127

Rajkumar, M., Ae, N., Prasad, M. N. V., & Freitas, H. (2010). Potential of siderophores-producing bacteria for improving heavy metal phytoextraction. *Trends in Biotechnology*, *28*(3), 142–149. doi:10.1016/j.tibtech.2009.12.002 PMID:20044160

Robinson, B., Fernandez, J. E., Madejon, P., Maranon, T., Murillo, J., Green, S., & Clothier, B. (2003). Phytoextraction: An assessment of biogeochemical and economic viability. *Plant and Soil*, *249*(1), 117–125. doi:10.1023/A:1022586524971

Rodrigeuz, H., Fraga, R., Gonzalez, T., & Basan, T. (2006). Genetics of phosphates solubilization and its potential applications for improving plant growth promoting bacteria. *Plant and Soil*, *287*(1-2), 15–21. doi:10.100711104-006-9056-9

Rodriguez, H., & Fraga, R. (1999). Phosphate solubilising bacteria and their role in plant growth promotion. *Biotechnology Advances*, *17*(4-5), 319–339. doi:10.1016/S0734-9750(99)00014-2 PMID:14538133

Salido, A. L., Hasty, K. L., Lim, J. M., & Butcher, D. J. (2003). Phytoremediation of arsenic and 750 lead in contaminated soil using Chinese brake ferns (*Pteris vittata*) and Indian mustard 751 (*Brassica juncea*). *Internat. J. Phytoreme.*, *5*(2), 89–103. doi:10.1080/713610173 PMID:12929493

Salt, D. E., Blaylock, M., Kumar, P. B. A. N., Dushenkov, V., Ensley, B. D., Chet, I., & Raskin, I. (1995). Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. *Biotechnol.*, *13*, 468–475. PMID:9634787

Salt, D. E., Smith, R. D., & Raskin, I. (1998). Phytoremediation. *Annual Review of Plant Physiology and Plant Molecular Biology*, *49*(1), 643–668. doi:10.1146/annurev.arplant.49.1.643 PMID:15012249

Schmidt, W. (1999). Mechanisms and regulation of reduction-based iron uptake in plants. *The New Phytologist*, *141*(1), 1–26. doi:10.1046/j.1469-8137.1999.00331.x

Seth, C. S., Chaturvedi, P. K., & Misra, V. (2007). Toxic effect of arsenate and cadmium alone and in combination on giant ducweed (*Spirodela polyrriziza L.*) in response to its accumulation. *Environmental Toxicology*, *22*(6), 539–549. doi:10.1002/tox.20292 PMID:18000854

Sharma, R. K., Aditi, P., Yukti, M., & Alok, A. (2014). Newly modified silica-based magnetically driven nano-adsorbent:a sustainable and Versatile platform for efficient and selective recovery of cadmium from water and fly-ash ameliorated soil. *Separation and Purification Technology*, *127*, 121–130. doi:10.1016/j.seppur.2014.02.030

Shen, Z. G., & Chen, H. M. (2000). Bioremediation of heavy metal polluted soils. *Rural Eco-Environ.*, *16*(2), 39–44.

Shen, Z. G., Li, X. D., Wang, C. C., Chen, H. M., & Chua, H. (2002). Lead phytoextraction from 758 contaminated soil with high-biomass plant species. *Journal of Environmental Quality*, *31*(759), 1893–1900. doi:10.2134/jeq2002.1893 PMID:12469839

Soudek, P., Petrova, S., Vankova, R., Song, J., & Vanek, T. (2014). Accumulation of heavy metals using Sorghum sp. *Chemosphere*, *104*, 15–24. doi:10.1016/j.chemosphere.2013.09.079 PMID:24268752

Spaepen, S., Vanderleyden, J., & Remans, R. (2007). Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiology Reviews*, *31*(4), 425–448. doi:10.1111/j.1574-6976.2007.00072.x PMID:17509086

Tampouris, S., Papassiopi, N., & Paspaliaris, I. (2001). Removal of contaminants metals from fine grained soils, using agglomeration, chloride solutions and pile leaching techniques. *Journal of Hazardous Materials*, *84*(2-3), 297–319. doi:10.1016/S0304-3894(01)00233-3 PMID:11406313

Tandy, S., Bossart, K., Mueller, R., Ritschel, J., Hauser, L., Schulin, R., & Nowack, B. (2004). 768 Extraction of heavy metals from soils using biodegradable chelating agents. *Environmental Science & Technology*, *38*(3), 937–944. doi:10.1021/es0348750 PMID:14968886

Tomsett, A. B., & Thurman, D. A. (1988). Molecular biology of metal tolerance of plants. *The Plant Cell*, *11*(5), 383–394. doi:10.1111/j.1365-3040.1988.tb01362.x

Tsavkelova, E. A., Cherdyntseva, T. A., Klimova, S. Y., Shestakov, A. I., Botina, S. G., & Netrusov, A. I. (2007). Orchid-associated bacteria produce indole-3-acetic acid, promote seed germination, and increase their microbial yield in response to exogenous auxins. *Archives of Microbiology*, *188*(6), 655–664. doi:10.100700203-007-0286-x PMID:17687544

Ullah, A., Heng, S., Munis, M. F. H., Fahad, S., & Yang, X. (2015). Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: A review. *Environmental and Experimental Botany*, *117*, 28–40. doi:10.1016/j.envexpbot.2015.05.001

Ullah, N., Waqar, A., Munir, H. Z., Zaffar, M., & Weijun, Z. (2014). Enhancing the lead phytostabilization in wetland plant *Juncus effusus L.* through somaclonal manipulation and EDTA enrichment. *Arabian Journal of Chemistry*, 1878–5352.

Vansuyt, G., Robin, A., Briat, J. F., Curie, C., & Lemanceau, P. (2007). Iron acquisition from Fe-pyoverdine by Arabidopsis thaliana. *Molecular Plant-Microbe Interactions*, *20*(4), 441–447. doi:10.1094/MPMI-20-4-0441 PMID:17427814

Viehweger, K. (2014). How plant cope with heavy metals. *Botanical Studies (Taipei, Taiwan)*, *55*(1), 35. doi:10.1186/1999-3110-55-35 PMID:28510963

Virkutute, J., Sillanpaa, M., & Latostemaa, P. (2002). Electrokinetic soil remediation-critical overview. *The Science of the Total Environment*, *289*(113), 97–121. doi:10.1016/S0048-9697(01)01027-0 PMID:12049409

Wang, H. F., Zhao, B. W., & Xu, J. (2009). Technology and research progress on remediation of soils contaminated by heavy metals. *Environ. Sci. Manage.*, *34*(11), 15–20.

Watanable, M. E. (1997). Phytoremdiation on the brink of commercialization. *Environmental Science & Technology*, *31*(4), 182–186. doi:10.1021/es972219s

Xiong, C., Zhang, Y., Xu, X., Lu, Y., Ouyang, B., Ye, Z., & Li, H. (2013). Lotus roots accumulate heavy metals independently from soil in main production regions of China. *Scientia Horticulturae*, *164*, 295–302. doi:10.1016/j.scienta.2013.09.013

Xu, Q., Huang, X. F., & Cheng, J. J. (2006). Progress on electro kinetic remediation and its combined methods for POPs from contaminated soils. *Environmental Sciences*, *27*(11), 2363–2368. PMID:17326456

Yu, Y. T., Tian, G. M., & He, M. M. (2009). Comparision of two different combined bioleaching-electrokinetics remediation processes. *Acta Sci. Circumstantiae*, *29*(1), 163–168.

Zhang, X. H., Wang, H., & Luo, Q. S. (2001). Electrokinetics in remdiation of contaminated groundwater and soils. *Adv. Water Sci.*, *12*(2), 249–255.

Zhang, Y. F., Sheng, J. C., & Lu, Q. Y. (2004). Review on the soil remediation technologies. *Gansu Agric. Sci. Technol.*, *10*, 36–38.

Zhou, D. M., Hao, X. Z., & Xue, Y. (2004). Advances in remediation technologies of contaminated soils. Ecol. *Environmental Sciences*, *13*(2), 234–242.

Chapter 8

Mechanism of Heavy Metal ATPase (HMA2, HMA3 and HMA4) Genes

ABSTRACT

Heavy metals are the most important pollutants that are non-biodegradable and increasingly accumulate in the environment. Phytoremediation can be defined as the use of plants for the extraction, immobilization, containment, or degradation of contaminants. It provides an ecologically, environmentally sound and safe method for restoration and remediation of contaminated land. Plant species vary in their capacity of hyper-accumulation of heavy metals. The chapter reviews the current findings on the molecular mechanism involved in heavy metals tolerance, which is a valuable tool for phytoremediation. The heavy metal tolerance genes help in the hyper-accumulation trait of a plant. Heavy metal transporter ATPases (HMAs) genes help in the refluxing of heavy metal ions from the cytosol, either into the apoplast, the vacuole, or other organelles, which help in the hyperaccumulation of metal. Understanding the signaling mechanism of transporter genes will be an important tool to understand the genetics of hyperaccumulation.

DOI: 10.4018/978-1-5225-9016-3.ch008

INTRODUCTION

Heavy metal contamination is world wide problem to human and animal health. Using hyperaccumulator plants for specific metals cleanup from the environment over the last 20 years and recently it thunder to be used phytoremdiation technology (Rocca et al., 2009). Some heavy metals like (Se, As, Cd, Hg and Pb) are not essential element for plant growth and anyother function. Meanwhile such as Zn, Ni, Mn, Mo, Cu, Co, and Fe are essential elements required for normal plant growth and physiological and metabolism function in plants. At low amount of these metals use as micronutrients for plant and if their concnetration increase to high values can easily go ahead to poisoning (Park et al., 2014). Numerous physiological processes and metabolism activity in plants as they caused molecular and cellular level were toxicity by heavy metals and also inactivating of some enzymes, blocking functional groups of metabolically important molecules for normal plant growth. Some where they were generating reaction against displacing or substituting for essential elements and distrupt integrity of plasmamembrane and increase antioxidant acitivity (Quartacci et al., 2001). However, phytoremediation can be considered for the decontaminated land.

Phytoremediation is the most popular advantage is that, its low cost and effectiveness technique for decontaminated land (Memon and Schroder, 2009). The processes of phytoremediation include the number of techniques i.e, phytodegradation, phytoaccumulation, phytostabilization, phytovolatilization and rhizofiltration (Salt et al., 1995). The basis of the genetics of metal hyper accumulation in woody plant species is largely still unknown but molecular mechanisms of the adaptation to metals in model plants viz *Arabidopsis* are well understood (Becher et al., 2004). Broad spectrum of plant yield is controlled by the involvement of different genes and it's very difficult to tell about to promote by single gene insertion in specific plants. This capter focus on the function of different gene involvement in diverse plant species to metals tolerance and translocation in plant organs". Rascio and Navari-Izzp, (2011), suggested about the specific metal localization through different metal transporters in various plants are essential for the developing genetically modified plants to acumulate heavy mtals in plants organs with the following uses in either phytoremediation to decontaminated or to improve human nutrition by biofortification process. Remove contaminats from soil and water followed phytoremediation process are more appropriate for agriculture applications and enhancement of phytoremediation efficiency has been done with the help of (PGPR) plant growth promoting bacteria (Wang et al., 2003; Huang et al., 2005). Plant-microbe interaction has promoted the successful activity of microbes for the plant growth development. Khan et al., 2012 find out about the inoculation of mycorrhizae to some plants might be efficiency enhanced for uptake, translocation and accumulation of soil metals.

The focus of this chapter to sustainable development by helping to protect soil by using phytoremediation natural resources for preventing the spread of pollution into the environment. Similarly, about phytoremediation systems apply for accumulate different heavy metals are still poorly understood and also their molecular mechanisms not known perfectly. The contaminants of heavy metals Cd, As, Cr, Zn,, Cu, Ni, Mn, Se that take may be a more time to give a effective results by applying phytoremediation such as microbe interaction with plant and also associated with chelating compounds to accumulate contaminants. Heavy metal toxicity induces the production of antioxidant activity due to the involvement with electron transport activities particulary in chloroplast membranes (Rocca et al., 2009). These enhance of ROS exposes into the cells to oxidative stress leading for biological macromolecule deterioration; lipid peroxidation; ion leakage, DNA-strand cleavage and membrane dismantling" (Quartacci et al., 2001).

Plants developed various cellular and molecular mechanism for tolerate the heavy metal stress. Specific genes have been contribute to heavy metal hyperaccumulation and more highly expressed in the hyperaccumulators plants as compared to non hyperaccumulators plants (Iqbal et al., 2013). Significant development has been made in the identification and understanding the role of key components like hyper accumulation, *HMA2, HMA3 and HMA4* that ensure heavy metal tolerance to plants. Overexpression of genes responsible for heavy metal uptake, translocation, and sequestration may allow the production of plants that can be successfully exploited in phytoremediation. During the past few decades phytoremediation technologies has fastly grown and many new hyperaccumulator plants were identified. Present study chapter on the heavy metal transporters genes (*HMAs 2, HMAs 3 and HMAs 4*) helps in the phytoremediation approach in agriculture filed and this information help to many researchers for coming information in this book.

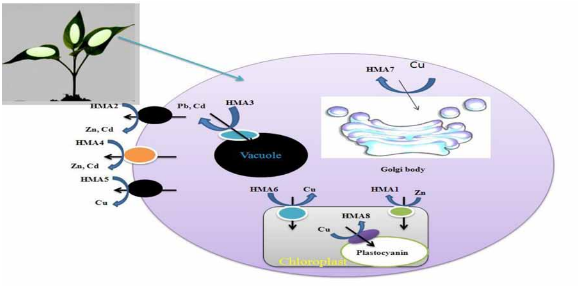
Table 1. Metal hyperaccumulating plants

Metal	Plant	Reference
Arsenic	*"Pteris vittata, Arabidopsis bisulcatus"*	Dong, 2005, Mehdawi et al., 2011
Cadmium	*"Sesbania drummondi, Sedum alfredii"*	Israr and Sahi, 2006,
Copper	*"Ipomea alpine,* *Centella asiatica and Eichhornia crassipes* *Euphorbia macroclada"*	Baker and Walker, 1989 Mokhtar et al., 2011
Chromium	*"Phragmites australis, Zea mays L. Cv Ganga 5"*	Calheiros et al., 2008
Manganese	*"Phytolacca Americana"*	Pollard et al., 2009
Nickel	*"Berkheya coddii* *Alyssum* and *Thlaspi"*	Robinson et al., 1997 Bani et al., 2010

HYPERACCUMULATING PLANTS

Improve the hyperaccumulator plant efficiency through selective breeding and by the transfer of different metal hyperaccumulation genes in plants for producing high plant biomass species. "Hyperaccumulation depends on the plant species, soil physiochemical properties (pH, cation exchange capacity, organic matter content, E.C) and different types of heavy metals (Barman et al., 2001). In hyperaccumulators, there is a rapid and capable translocation of the heavy metals to the shoot via the xylem, which could probably be driven by transpiration (Salt et al., 1995). Most of the heavy metals that do enter the plant are then kept in root cells; where they are detoxified by complexation with amino acids, organic acids or metal-binding peptides and sequestered into vacuoles (Hall, 2002). This greatly restricts translocation to the above-ground organs, thus protecting the leaf tissues, and particularly the metabolically active photosynthetic cells from heavy metal damage. The high metal tolerance may also be due to highly efficient intracellular compartmentalization and chelation process (Pilon-Smits and Pilon, 2002). Uptake of metal ions from the xylem apoplast into the shoot symplast is mediated by metal transporters in the shoot cell membrane. A list of hyperaccumulating plants are shown in (Table 1).

Figure 1. Heavy metal ATPase gene contributes in hyperaccumulation of heavy metals (Chaudhary et al., 2016)



HEAVY METALS TOLERANCE TRANSPORTERS GENE

Heavy metals are non-biodegradable pollutants and they effect on human health and environment. The progress in the molecular mechanism of plant stress response to heavy metals have made, especially in herbaceous plants such as *Arabidopsis halleri, Arabidopsis thaliana and Triticum caerulescens* (Meyer et al., 2012). *A. thaliana* has always played a very important role in uncovering the molecular mechanism of plant response to pollutants as its genome information is available. The most closely connected to members of the *A. thaliana* is P1B-type. HMA2 is also involved in the translocation of Zn and Cd in *A. thaliana*, barley, rice, and wheat (Table 2). In *Arabidopsis*, the cellular and subcellular patterns of *AtHMA2* expression were similar to those of *AtHMA4*. *HMA2p-GUS* expression was observed primarily in the vascular tissues of the leaf, root, stem and *HMA2- GFP* protein were also localized in the plasma membrane. Recently, findings on the characterization of the *HMAs 2* gene from the different plants for possible application in phytoremediation approach. The *ATPase* families of integral membrane transporter proteins that help to uptake transition metals are involved in mediating metal-resistant and metal-hyperaccumulating traits.

The Dose-dependence of metal root/shoot distribution, different in transgenic and wild-type has also been reported for plants expressing 35S promoter *AtHMA4* as well also the metal transporters such as *HvHMA2* (Barabasz et al., 2013). While *HMA3* may involve to metal detoxification by sequestering Cd into the vacuole (figure 1) *HMA4* acts as a physiological master switch during the process of hyperaccumulation metal, and *HMA2* and *HMA4* play roles in root to shoot metal translocation. It is hypothesized that description of the roles of several types of these metal transporters in plants will be essential for the development to genetically modify plants that accumulate specific metals with subsequent use in either phytoremediation or in improving human nutrition.

The efficiency increased of *HMA3* and *HMA4* is a prerequisite for hyperaccumulation and hyper resistance in hyperaccumulators plant. Through genetic engineering, these genes are able to generate non toxic food. Park et al., 2014 reported that overexpression of *CsHMA3* might increase Pb and Zn tolerance and uptake. Also, the transgenic lines showed a wider leaf shape compared with wild-type plant due to stimulation of genes, related to leaf growth and displayed a greater total seed production compared to the wild type species under heavy metal contamination. These include the *HMA3* and *HMA4* encoded proteins that belong to the *P1B*-type *ATPase* family which have been characterized in various organisms (Table 2). *HMA4* was by far the most highly expressed metal transporter in the three *Noccaea caerulescens* accessions (Halimaa et al., 2014) *Arabidopsis* has eight *P1B-ATPases*, including *AtHMA1–AtHMA4* which transportZn2^+, Cd2^+, Pb2^+, and Co2^+, and *AtHMA5-AtHMA8* which transport Cu^+ and Ag^+ Fig. 8.1 (Baekgaard et al., 2010). *AtHMA1* has also been described as a copper transporter" (Seigneurin-Berny et al., 2006).

Many genes from different organisms have been identified and characterized that are involved in acquisition, allocation and decontamination of metals. It is well known that *HMA3* is a tonoplast-localized transporter that involve to metal detoxification by sequestering specific metals such as Cadmium into the vacuole, and that *HMA2 and HMA4* are plasma-membrane proteins contribute in translocating Zn and Cd ions from the root to shoot and detoxifying these metals in above ground tissues. Although the sequences suggest similarities in function, *HMA2, HMA3*, and *HMA4* do not function same depending on the species. Many species of the plant have the ability to adapt harsh environmental conditions. Gene functions may develop due to selection from local climates and environments. In particular, these gene alterations may increase *HMA2, HMA3, and HMA4* substrate affinity depending on the type of heavy metal contamination. We recommend the co-overexpression of *HMA3 with HMA4* with an *HMA4* overexpressing plant using a root-specific promoter and *HMA3* overexpressing plant using a shoot-specific promoter for improving the rate of translocation, compartmentation, and detoxification of toxic metals in aboveground tissues.

Table 2. Heavy metal ATPase (HMA2, HMA3 and HMA4) transporters in different plants

Genes	Plants	Metals	References
HMA2	*"Arabidopsis thaliana"*	"HMA2 has N and C terminal domains that can bind to Zn ions with high affinity"	Wong et al., 2009
	"Hordeum vulgare"	"HMA2 functions as a Zn and Cd pump and play a role in root to shoot Zn and Cd transport"	Mills et al., 2012
	"Oryza sativa"	"HMA2 is a major transporter of Zn and Cd from root to shoot at the vegetative stage and "the role of HMA2 in the nodes is to preferentially transport Zn and Cd to the grain at the reproductive stage".	Satoh-Nagasawa et al., 2012; Takahashi et al., 2012;
	"Triticum aestivum"	"TaHMA2 overexpression improved root-shoot Cd and Zn translocation in rice"	Tan et al., 2013
HMA3	*"Arabidopsis thaliana*	"Sequestration of Cd into vacuoles to limit Cd transport to the xylem"	Morel et al., 2009
	"Arabidopsis thaliana"	"No function, efficient root to shoot translocation of Cd"	Park et al., 2012
	"Glycine max (AC Hime; a high Cd accumulator) *Glycine max (Westag 97; a low Cd accumulator)"*	"Single nucleotide change from G to A at nucleotide position 1823 in GmHMA3, high seed Cd levels glycine (G) at position 608 is critical for the function of GmHMA3, low seed Cd levels."	Wang et al., 2012
	"Oryza sativa (Choko-koku, Jarjan, Anjana Dhan) *O. sativa (Nipponbare, Koshihikari, Sasanishiki)"*	"High Cd concentrations in grains and shoots low Cd concentrations in grains and shoots"	Ueno et al., 2010; Miyadate et al., 2011

continued on following page

Table 2. Continued

Genes	Plants	Metals	References
HMA4	*"Arabidopsis thaliana (Wassilewskija and Columbia) Arabidopsis thaliana (Columbia)"*	"The HMA4 expression level in Col-0 was induced, but those of Ws tended to decrease as compared to the control". "C-terminal domain of AtHMA4 serves a dual role as Zn2$^+$ and Cd2$^+$ chelator (sensor) and as a regulator for the efficient export of Zn2$^+$ and Cd2$^+$.	Hussain et al., 2004; Park et al., 2012; Chao et al.,,2012. Baekgaard et al., 2010
	"Arabidopsis halleri"	"Cis-regulatory changes and tandem triplication play a major role in Zn and Cd resistance and hyperaccumulation"	Hanikenne et al., 2008
	"Thlaspi caerulescens"	"Key role in the extreme Cd tolerance. Cd tolerance seems to derive from lower uptake and efficient translocation to the shoots, perhaps by HMA4"	Lochlainn et al., 2011; Craciun et al., 2012 Ueno et al., 2011
	"Populus trichocarpa"	"At early stages of development, HMA4 relieves some negative effects of Zn"	Adams et al., 2011
	"Lycopersicon esculentum"	"Between transgenic and wild-type plants exposed to low and high Zn (leaves and roots)".	Barabasz et al., 2012
	"Noccaea caerulescens"	"Zn and Cd tolerance gene in root".	Halimaa et al. 2014

CONCLUSION

Present study accomplished that by overexpressing *HMAs 3* and *HMAs 4* genes, heavy metal stress tolerance can be imparted to plants. The heterologous overexpression of *HMAs 3* and *HMAs 4* in plants is probably a useful approach to engineer altered metal supply in tissues for phytoremediation purposes. As these transporter gene increases the power to tolerant heavy metal stress to clean up the environment successfully. Numbers of companies have started their business in phytoremediation approach to apply for clean up the environment and also increase the biomass production. This information will also be help to improve the productivity of bioenergy crops. However, many plants lack these properties. Thus, the possibility to produce new plants through transgenic methods is a good choice for excellent phytoremediation and on molecular approaches.

REFERENCES

Adams, J. P., Adeli, A., Hsu, C. Y., Harkess, R. L., Page, G. P., dePamphilis, C. W., ... Yuceer, C. (2011). Poplar maintains zinc homeostasis with heavy metal genes HMA4 and PCS1. *Journal of Experimental Botany*, *62*(11), 3737–3752. doi:10.1093/jxb/err025 PMID:21504875

Baekgaard, L., Mikkelsen, M. D., Sorensen, D. M., Hegelund, J. N., Persson, D. P., Mills, R. F., ... Palmgren, M. G. (2010). A combined zinc/cadmium sensor and zinc/cadmium export regulator in a heavy metal pump. *The Journal of Biological Chemistry*, *285*(4), 31243–31252. doi:10.1074/jbc.M110.111260 PMID:20650903

Baker, A. J. M., & Walker, P. L. (1989). Ecophysiology of metal uptake by tolerant plants. In *Heavy metal tolerance in plants: Evolution aspects* (pp. 155–177). CRC Publishers.

Bani, A., Pavlova, D., Echevarria, G., Mullaj, A., Reeves, R. D., Morel, J. L., & Sulçe, S. (2010). Nickel hyperaccumulation by the species of *Alyssum* and *Thlaspi* (Brassicaceae) from the ultramafic soils of the Balkans. *Botanica Serbica*, *34*(1), 3–14.

Barabasz, A., Krämer, U., Hanikenne, M., Rudzka, J., & Antosiewicz, D. M. (2010). Metal a ccumulation into bacco expressing *Arabidopsis halleri* metal hyperaccumulation gene depends on external supply. *Journal of Experimental Botany*, *61*(11), 3057–3067. doi:10.1093/jxb/erq129 PMID:20484319

Barabasz, A., Wilkowska, A., Ruszczyñska, A., Bulska, E., Hanikenne, M., Czarny, M., ... Antosiewicz, D. M. (2012). Metal response of transgenic tomato plants expressing P1B-ATPase. *Physiologia Plantarum*, *145*(2), 315–331. doi:10.1111/j.1399-3054.2012.01584.x PMID:22283486

Barabasz, A., Wilkowska, A., Tracz, K., Ruszczyñska, A., Bulska, E., Mills, R. F., ... Antosiewicz, D. M. (2013). Expression of HvHMA2 in tobacco modifies Zn-Fe-Cd homeostasis. *Journal of Plant Physiology*, *170*(13), 1176–1186. doi:10.1016/j.jplph.2013.03.018 PMID:23664582

Barman, S. C., Kisku, G. C., Salve, P. R., Misra, D., Sahu, R. K., Ramteke, P. W., & Bhargava, S. K. (2001). Assessment of industrial effluent and its impact on soil and plants. *Journal of Environmental Biology*, *22*, 251–256. PMID:12018593

Becher, M., Talke, I. N., Kral, I. L., & Kramer, U. (2004). Cross species microarray transcript profiling levels high constitutive expression of metal hameastasis gene in shoots of the zinc hyperaccumulation *Arabidopsis halleri. Plant J. Biol.*, *7*, 251–268.

Calheiros, C. S. C., Rangel, A. O. S. S., & Castro, P. M. L. (2008). The effects of tannery waste water on the development of different plant species and chromium accumulation in *Phragmites australis. Archives of Environmental Contamination and Toxicology*, *55*(3), 404–414. doi:10.100700244-007-9087-0 PMID:18214580

Chao, D. Y., Silva, A., Baxter, I., Huang, Y. S., Nordborg, M., Danku, J., ... Salt, D. E. (2012). Genome-wide association studies identify heavy metal ATPase3 as the primary determinant of natural variation in leaf cadmium in *Arabidopsis thaliana. PLOS Genetics*, *8*(9), 1002923. doi:10.1371/journal.pgen.1002923 PMID:22969436

Chaudhary, K., Jan, S., & Khan, S. (2016). Heavy Metal ATPase (HMA2, HMA3 and HMA4) Genes in Hyperaccumulation Mechanism of Heavy Metals. In *Plant Metal Interaction*. Elsevier.

Craciun, A. R., Meyer, C. L., Chen, J., Roosens, N., DeGroodt, R., Hilson, P., & Verbruggen, N. (2012). Variation in HMA4 gene copy number and expression among *Noccaea caerulescens* populations presenting different levels of Cd tolerance and accumulation. *Journal of Experimental Botany*, *63*(11), 4179–4189. doi:10.1093/jxb/ers104 PMID:22581842

Dong, R., Formentin, E., Losseso, C., Carimi, F., Benedetti, P., Terzi, M., & Schiavo, F. L. (2005). Molecular cloning and characterization of a phytochelatin synthase gene, *PvPCS1*, from *Pteris vittata* L. J. *Ind. Microbiol. Biol.*, *32*(11-12), 527–533. doi:10.100710295-005-0234-1 PMID:15918023

Halimaa, P., Lin, Y. F., Ahonen, V. H., Blande, D., Clemens, S., Gyenesei, A., ... Tervahauta, A. I. (2014). Gene Expression Differences between *Noccaea caerulescens* Ecotypes Help to Identify Candidate Genes for Metal Phytoremediation. *Environmental Science & Technology*, *48*(6), 3344–3353. doi:10.1021/es4042995 PMID:24559272

Hall, J. L. (2002). Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany*, *53*(366), 1–11. doi:10.1093/jexbot/53.366.1 PMID:11741035

Hanikenne, M., Talke, I. N., Haydon, M. J., Lanz, C., Nolte, A., Motte, P., ... Kramer, U. (2008). Evolution of metal hyperaccumulation required cis-regulatory changes and triplication of HMA4. *Nature*, *453*(7193), 391–395. doi:10.1038/nature06877 PMID:18425111

Huang, X. D., El-Alawi, Y., Gurska, J., Glick, B. R., & Greenberg, B. M. (2005). A multiprocess phytoremediation system for decontamination of persistant total petroleum hydrocarbons (TPHs) from soils. *Microchemical Journal*, *81*(1), 139–147. doi:10.1016/j.microc.2005.01.009

Hussain, D., Haydon, M. J., Wang, Y., Wong, E., Sherson, S. M., Young, J., ... Cobbett, C. S. (2004). P-type ATPase heavy metal transporters with roles in essential zinc homeostasis in *Arabidopsis. The Plant Cell*, *16*(5), 327–1339. doi:10.1105/tpc.020487 PMID:15100400

Iqbal, M., Nawaz, I., Hassan, Z., Hakvoort, H. W. J., Bliek, M., Aarts, M. G. A., & Schat, H. (2013). Expression of HMA4 cDNAs of the zinc hyperaccumulator *Noccaea caerulescens* from endogenous NcHMA4 promoters does not complement the zinc deficiency phenotype of the *Arabidopsis thaliana* HMA2 and HMA4 double mutant. *Plant Science*, *4*(404), 1–12.

Israr, M., Sahi, S. V., & Jain, J. (2006). Cadmium accumulation and antioxidative responses in the *Sesbania drummondii* callus. *Archives of Environmental Contamination and Toxicology*, *50*(1), 121–127. doi:10.100700244-005-5029-x PMID:16247566

Khan, A. G., Kuek, T. M., Chaudhary, C. S., & Khoo Hayes, W. J. (2012). Role of plants mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere*, *41*(1-2), 197–207. doi:10.1016/S0045-6535(99)00412-9 PMID:10819202

Lochlainn, S. O., Bowen, H. C., Fray, R. G., Hammond, J. P., King, G. J., White, P. J., ... Broadley, M. R. (2011). Tandem quadruplication of HMA4 in the zinc (Zn) and cadmium (Cd) hyper- accumulator *Noccaea caerulescens. PLoS One*, *6*(3), 1–9. doi:10.1371/journal.pone.0017814

Mehdawi, E.F.A., Quinn, C.F., & Pilon-Smits, E.A.H. (2011). Selenium Hyperaccumulators Facilitate Selenium-Tolerant Neighbors via Phytoenrichment and Reduced Herbivory Curt. *Biol.*, *21*, 1440–1449. PMID:21856156

Memon, A. R., & Schroder, P. (2009). Implications of metal accumulation mechanisms to phytoremediation. *Environmental Science and Pollution Research International*, *16*(2), 162–175. doi:10.100711356-008-0079-z PMID:19067014

Meyer, C., & Verbruggen, N. (2012). The use of the modal species *Arabidopsis halleri* towards phytoextraction of cadmium polluted soils. *New Biotechnology*, *30*(1), 9–14. doi:10.1016/j.nbt.2012.07.009 PMID:22850245

Mills, R. F., Peaston, K. A., Runions, J., & Williams, L. E. (2012). HvHMA2, a P1B-ATPase from barley, is highly conserved among cereals and functions in Zn and Cd transport. *PLoS One*, *7*(8), 1–14. doi:10.1371/journal.pone.0042640 PMID:22880063

Miyadate, H., Adachi, S., Hiraizumi, A., Tezuka, K., Nakazawa, N., Kawamoto, T., ... Akagi, H. (2011). OsHMA3, a P1B-type of ATPase affects root-to-shoot cadmium translocation in rice by mediating efflux into vacuoles. *The New Phytologist*, *189*(1), 190–199. doi:10.1111/j.1469-8137.2010.03459.x PMID:20840506

Mokhtar, H., Morad, N., & Fizri, F. F. A. (2011). Phytoaccumulation of Copper from Aqueous Solutions Using *Eichhornia Crassipes* and *Centella Asiatica. International Journal of Environmental Sciences and Development*, *3*(1), 89–95.

Morel, M., Crouzet, J., Gravot, A., Auroy, P., Leonhardt, N., Vavasseur, A., & Richaud, P. (2009). AtHMA3, a P1B-ATPase allowing Cd/Zn/Co/Pb vacuolar storage in *Arabidopsis. Plant Physiology*, *149*(2), 894–904. doi:10.1104/pp.108.130294 PMID:19036834

Park, W., Feng, Y., & Ahn, S. J. U. (2014). Alteration of leaf shape, improved metal tolerance, and productivity of seed by overexpression of CsHMA3 in *Camelina sativa. Biotech. Biol.*, *7*, 9. PMID:25018780

Park, W., Han, K. H., & Ahn, S. J. (2012). Differences in root-to-shoot Cd and Zn translocation and by HMA3 and 4 could influence chlorophyll and anthocyanin content in Arabidopsis Ws and Col-0 ecotypes under excess metals. *Soil Science and Plant Nutrition*, *58*(3), 334–348. doi:10.1080/00380768.2012.684643

Pilon-Smits, E., & Pilon, M. (2002). Phytoremediation of metals using transgenic plants. *Critical Reviews in Plant Sciences*, *21*(5), 439–456. doi:10.1080/0735-260291044313

Pollard, A. J., Stewart, H. L., & Roberson, C. B. (2009). Manganese hyper accumulation in *Phytolacca americana L.* from the southeastern United States. *Northeastern Naturalist*, *16*(sp5), 155–162. doi:10.1656/045.016.0513

Quartacci, M. F., Cosi, E., & Navari-Izzo, F. (2001). Lipids and NADPH-dependent superoxide production in plasma membrane vesicles from roots of wheat grown under copper deficiency and excess. *Journal of Experimental Botany*, *152*, 67–75. PMID:11181715

Rascio, N., & Navari-Izzo, F. (2011). Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? *Plant Science*, *180*(2), 169–181. doi:10.1016/j.plantsci.2010.08.016 PMID:21421358

Robinson, B. H., Brooks, R. R., Howes, A. W., Kirkman, J. H., & Gregg, P. E. H. (1997). The potential of the high biomass nickel hyperaccumulator *Berkheya coddii* for phytoremediation and phytomining. *Journal of Geochemical Exploration*, *60*(2), 115–126. doi:10.1016/S0375-6742(97)00036-8

Rocca, L. (2009). Responses of the Antarctic microalga Koliella antartica (*Trebouxiophyceae, Chlorophyta*) to cadmium contamination. *Photosyn.*, *47*(3), 471–479. doi:10.100711099-009-0071-y

Salt, D. E., Blaylock, M., Kumar, N. P., Dushenkov, V., Ensley, B. D., Chet, I., & Raskin, I. (1995). Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. *Nature Biotechnology*, *13*(5), 468–474. doi:10.1038/nbt0595-468 PMID:9634787

Satoh-Nagasawa, N., Mori, M., Nakazawa, N., Kawamoto, T., Nagato, Y., Sakurai, K., ... Akagi, H. (2012). Mutations in rice (*Oryza sativa*) heavy metal ATPase 2 (OsHMA2) restrict the translocation of zinc and cadmium. *Plant & Cell Physiology*, *53*(1), 213–224. doi:10.1093/pcp/pcr166 PMID:22123790

Seigneurin-Berny, D., Gravot, A., Auroy, P., Mazard, C., Kraut, A., Finazzi, G., ... Rolland, N. (2006). HMA1, a new Cu-ATPase of the chloroplast envelope, is essential for growth under adverse light conditions. *The Journal of Biological Chemistry*, *281*(5), 2882–2892. doi:10.1074/jbc.M508333200 PMID:16282320

Takahashi, R., Ishimaru, Y., Shimo, H., Ogo, Y., Senoura, T., Nishizawa, N. K., & Nakanishi, H. (2012). The OsHMA2 transporter is involved in root-to-shoot translocation of Zn and Cd in rice. *Plant, Cell & Environment*, *35*(11), 1948–1957. doi:10.1111/j.1365-3040.2012.02527.x PMID:22548273

Tan, J., Wang, J., Chai, T., Zhang, Y., Feng, S., Li, Y., ... Chai, X. (2013). Functional analyses of TaHMA2, a P1B-type ATPase in wheat. *Plant Biotechnology Journal*, *11*(4), 420–431. doi:10.1111/pbi.12027 PMID:23294838

Ueno, D., Milner, M. J., Yamaji, N., Yokosho, K., Koyama, E., Clemencia Zambrano, M., ... Ma, J. F. (2011). Elevated expression of TcHMA3 plays a key role in the extreme Cd tolerance in a Cd-hyperaccumulating ecotype of *Thlaspi caerulescens. The Plant Journal*, *66*(5), 852–862. doi:10.1111/j.1365-313X.2011.04548.x PMID:21457363

Ueno, D., Yamaji, N., Kono, I., Huang, C. F., Ando, T., Yano, M., & Ma, J. F. (2010). Gene limiting cadmium accumulation in rice. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(38), 16500–16505. doi:10.1073/pnas.1005396107 PMID:20823253

Wang, X. Z., Li, F. X., Okajaki, M., & Sugisaki, M. (2003). Phytoremdiation of contaminated soil. *Annual Report CESS*, *3*, 114–123.

Wang, Y., Yu, K. F., Poysa, V., Shi, C., & Zhou, Y. H. (2012). A single point mutation in GmHMA3 affects cadimum (Cd) translocation and accumulation in soybean seeds. *Molecular Plant*, *5*(5), 1154–1156. doi:10.1093/mpss069 PMID:22778157

Wong, C. K. E., & Cobbett, C. S. (2009). HMA P-type ATPases are the major mechanism for root-to-shoot Cd translocation in *Arabidopsis thaliana. The New Phytologist*, *181*(1), 71–78. doi:10.1111/j.1469-8137.2008.02638.x PMID:19076718

Wong, C. K. E., Jarvis, R. S., Sherson, S. M., & Cobbett, C. S. (2009). Functional analysis of the heavy metal binding domains of the Zn/ Cd-transporting ATPase, HMA2, in *Arabidopsis thaliana. The New Phytologist*, *181*(1), 79–88. doi:10.1111/j.1469-8137.2008.02637.x PMID:19076719

Chapter 9

Role of Phytochelatines (PCs) and Metallothionines (MTs) Genes Approaches in Plant Signalling

ABSTRACT

In this chapter, the authors reported that phytochelatines (PCs) and metallothionies (MTs) are actively involved in metal binding and detoxification as observed more in hyperaccumulation plant species. Also, most reports have explained single metal/metalloid detoxification via PCs and MTs; hence, it remains to be seen how plants use these metal ligands at the time of multiple metal stress and generate at the time of defence system against heavy metal stress condition.

INTRODUCTION

Heavy metal pollution is a growing concern all over the world and chemicals released in the in a soil in the form of cadmium (Cd), copper (Cu), cobalt (Co), lead (Pb), zinc (Zn), chromium (Cr), nickel (Ni), barium (Ba), argon (Ag), cobalt (Co), mercury (Hg) and antimony (Sb) and some of these elements are essential for many physiological function in living beings whereas no other known as biological function as required level (Fassler et al., 2010). These elements in (fungicides, fertilizers, urban trash, animal waste, sewage sludge

DOI: 10.4018/978-1-5225-9016-3.ch009

in soil) and deposits of industrial dust can increase the concentration in soil for making them toxic (Fassler et al., 2010). Phytoremediation technique is used because the biological property and physical structure of the soil is maintain and unexpensive and ecofriendly for the environment (Ali et al., 2013). Plants are capable of immobilizing metal in soil by forming insoluble compounds as result of the interaction of plant exudates in the rhizosphere or by adsorption (Kidd et al., 2009). Some species of plants are capable of accumulating heavy metals in their tissue so that contamination removed by harvesting the plant and some plants show toxicity if more amount of metals translocate and accumulate into the tissue (Maestri et al., 2000; Van Nevel et al., 2007). Different plants can present at different tolerance mechanisms in response to the excess of heavy metals including a reduction in the transport through the membrane for the metallothionein (MT) formation, exclusion, phytochelatins (PCs), chelation by organic acids and amino acids and metal compartmentalization in subcellular structure"(Ovecka and Takac, 2014).

PHYTOCHELATINS

Phytochelatins (PCs) are low molecular weight cysteine rich small polypeptide with a general structure (g-Glu-Cys)nGly, where n $^{1/4}$2-11 and are not only reported in plants but also have found in fungi and other organisms (Yadav et al., 2010; Mirza et al., 2014). Phytochelatins are one of the most important classes of metal chelators that respond to the harmful effects of a variety of toxic metals. Phytochelatins are known to be synthesized in the cytosol in response to the heavy metal toxicity". Phytochelatins-metal and Phytochelatins metalloid complexes are very stable in nature and are formed and "sequestration in the vacuolar compartments where the toxic effect metals is of less concern (Shen et al., 2010; Dago et al., 2014). The biosynthesis of PCs is catalyzed by the key enzyme of phytochelatins synthase PCs (Kutrowska and Szelag, 2014).

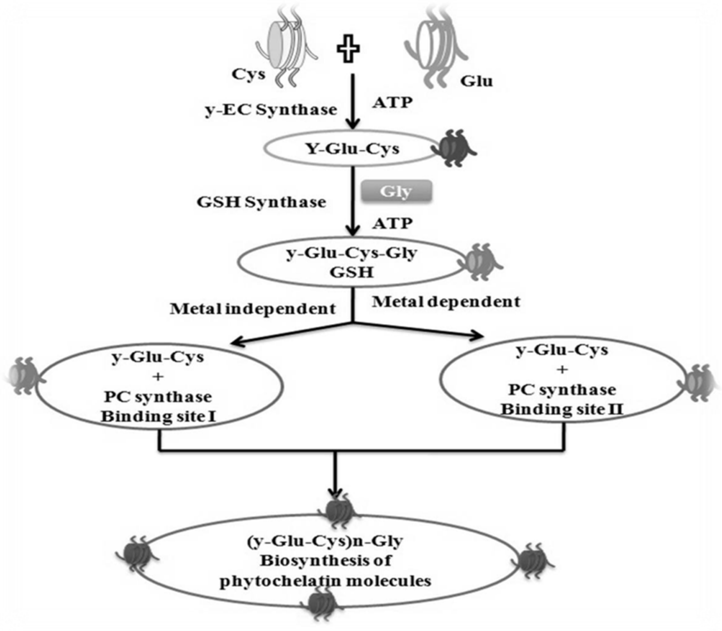
Phytochelatins belong to a family of peptides which were first discovered as cadmium (Cd)-binding complexes in *Schizosaccharomyces* pombe exposed to Cd and were named as cadystins (Inouhe, 2005). The amino acids required for the synthesis of this peptide are L-glutamate (Glu), L-cysteine (Cys), and glycine (Gly). Phytochelatins are syntheisized from GSH; therefore, the biosynthetic pathway overlaps with GSH biosynthesis. The general structure of PC oligomer is (g-Glu-Cys)n-Gly where n usually range from (2-5); but has been reported as high as 11 in some species (Sharma et al., 2015; Cobbett, 2000). During the PCs synthesis enzyme of g-glutamylcysteine synthase catalyzes the formation of g-glutamylcysteine from L-glutamate and L-cysteine. Further enzymes glutathione synthase adds glycine to g-glutamylcysteine to form GSH in both these reactions the presence of adenosines triphosphates (ATP) is required (Meister, 1988; Sharma et al., 2015). After the formation of GSH PCs are synthesized in presence of enzyme PC synthase" (Figure 1). The enzyme was normally named g-Glu-Cys dipeptididyl transpeptidase due to transpeptidation of g-Glu-Cys moiety of GSH (Cobbett, 2000; Sharma et al., 2015). However, before transpeptidation, glycine is cleaved from GSH and then in the next step, during transpeptidation, the resulting g-glutamylcysteine dipeptide forms a peptide bond with either GSH to form PC2 or another PC molecule that acts as an acceptor molecule to produce in np 1 oligomer (Clemens, 2006; Sharma et al., 2016).

PHYTOCHELATINS TOLERANCE STRATEGIES UNDER METAL STRESS

Phytochelatins are bind to multiple metals as well as metalloids. Several PCs-metal complexes have been discovered from various plants, fungi and microorganisms. PCs-metal complexes help plants to tolerate even high levels of toxic metals by lowering the binding capacity of metal to the cell wall, and detoxification of compartmentalization (Sunitha et al., 2013; Sharma et al., 2016). In comparision to free metal ions, the PCs metal complexes are more stable and less toxic. A large number of studies on detoxification of metal via PCs suggest the important role of PCs in heavy metals detoxification. PCs are also involved in degradation of various GSH conjugates as well. Under natural conditions for other metalloids are less evident (Franchia et al., 2015; Sharma et al., 2016).

The involvement of PCs in Zn metal complexation and compartmentalization was able to entirely overcome the toxic effects of metal in *Arabidopsis thaliana* plants to a great extent (Tennstedt et al., 2009). It has been reported recently that PCs is induced in several plant (non-hyperaccumulator) even at a low concentrations of "metals and metalloids (Sharma et al., 2016). There are several report suggest lack of Cad1 mutants (PCs mutant) expression resulting in an excalation of Cd sensitivity (Andresen et al., 2013). PC-metal complexes have also been reported in copper hyperaccumulating plants such as *Nicotiana*

Figure 1. Biosynthesis pathway of phytochelatines



caerulescenses. PCs genes are expressed that catalyzed he formation of metal-PC complexation. When molecules of GSH ligate with a heavy metals they form a thiolate leading to the formation of metal-PC complex (Sharma et al., 2015). These metals PC-complexes are then transported to vacuolar compartments to form more stable and less toxic form of heavy metals ions called the Mr (PC-metal)-complexes (Yadav, 2010). PC-mediated has been reported in *Ceratophyllum demersum L.* under lead toxicity and other heavy metal tolerance. The optimal exposure to Pb metal stress leads to an elevation in the contents of "glutathione and cysteine" (Mishra et al., 2006; Yadav et al., 2010; Sharma et al., 2016). The role of "PCs in *Avicennia germinans* under Cu and Cd stress at transcriptional level also seemed to suggest an increase the level of AvMt2 table. 1 (Yadav et al., 2010).

METALLOTHIONEINS

Metallothioneins (MTs) were first reported in "horse kidney cells in 1957 as metal-binding proteins (Thirumoorthy et al., 2011). Cysteine (Cys) rich

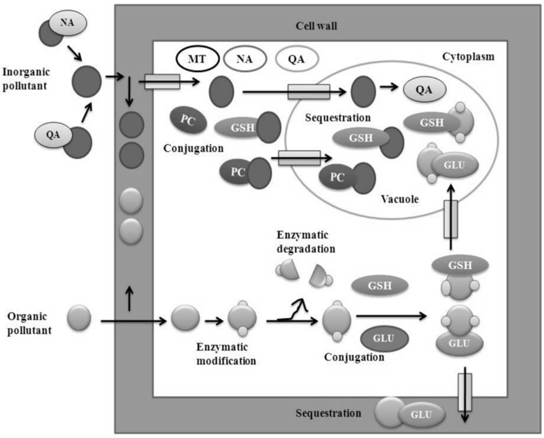
Table 1. Phytochelatines (PCs) genes expressed in various plants under heavy metal stress

S.No	PCs genes	Heavy metals and effect	Plant	References
1.	AtBCC3	Cd tolerance	Arabidopsis thaliana	Brunetti et al., 2015
2.	PCS1	Cd tolerance	Schizosaccharomyces pombe	Sine et al., 2015
3.	ACR2, ACR3	As hypersensitivity Zn hyperaccumulation	Petris vittata, Arabidopsis halleri	Indriolo et al., 2010; Barabasz et al., 2010
4.	SpHMT1	Cd, Zn, As tolerance	Schizosaccharomyces pombe	Lee, 2014
5.	HMA4, PCS1	Zn homeotosis	Liriodendron tulipfera	Adams et al., 2011
6.	CdPCS1	As and Cd assimilation	Ceratophyllum demersum	Shri et al., 2014
7.	HMA4	Cd, Zn hypersensitivity	Noccaea caerulescens	O Lochlainn et al., 2011
8.	AtPCS2	PCs synthesis	Arabidopsis thaliana	Kuhnlenz et al., 2014
9.	TcHMA3	Cd tolerance	Thlaspi caerulescens	Ueno et al., 2011
10.	TaPCS1	Detoxification of heavy metals	Triticum vulgaris	Liu et al., 2011

metallothioneins are low molecular weight protein, nonenzymatic and efficient in complexing the metals by affinity with sulfur present in the cysteine. It was classified based on the arrangement of Cysteine 1) have more than 20 conserved Cys are common in mammals, and vertebrates and known to confirm tolerance to Cadmium 2) without specific arrangement of Cys include all those found plants, fungi, vertebrates (Carpene et al., 2007). Metallothioneins bind to different metals via thiol group of cysteine and exert a major role in detoxification and metabolism of HMs (Jia et al., 2012).

These metallo-protein defend plants against heavy metals through different mechanisms such as scavenging of the ROS, sequestration (Huang and Wang, 2010). Regulations activity are metalloenzymes, activation of the transcription genes and metabololism of metallo-drugs" under stress conditions (Bratic et al., 2009; Gautam et al., 2012). Metallothioneins are classified into four subfamilies. For instance, MT1 of Cicer arietinum and MT1a and MT1c of *A. thaliana* are "class I MTs, MT2 of *C.arietinum* and MT2a and MT2b of *A. thaliana* are class II MTs, MT3 of *A. thaliana* and *Musa* acuminate are class III MTs, and the plant PEC/pec subfamily includes MT4a-Ec-2 and MT4b-as Ec-1 of *A. thaliana* and *Triticum aestivum* fig. 9.2 (Hassinen et al.,

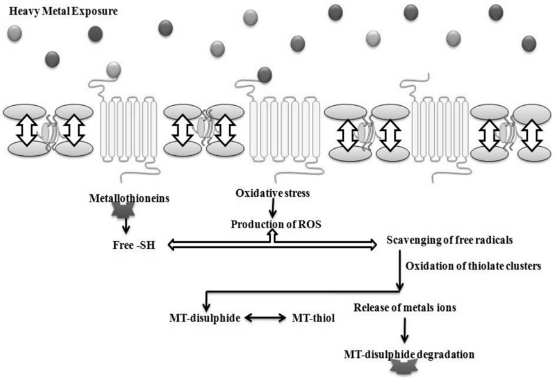
Figure 2. Diagram of detoxification, conjugation and sequestration in the vacuole where the pollutant can do harm to the cell. (Chelators shown are GSH: glutathione; MT: metallothioneins; and PCs: phytochelatins).



2011). There is strong similarity between the structures of GSH and class III MTs" which indicates that these metal binding polypeptides are synthesized from "thiol-rich" tripeptides or their precursors. The buthionine sulfoxamine" which is a powerful inhibitor of "g-glutamylcysteine synthetase" decrease the cellular concentrations of "g-glutamylcysteine and GSH". There is various alternatives mechanisms that may synthesize "class III MTs from GSH or g-glutamylcysteine. In Silene cucubalus, "class III MTs" are synthesized from "GSH" involving "g-glutamylcysteine dipeptidyl transpeptidase figure 2 (Grill et al., 1989).

The expression of MT genes in ontogenetically regulated and changes with the type of plant tissue (Yuan et al., 2008). These genes are activated when plant under abiotic stress such as cold, heat, salt, drought, heavy metal and oxidative stress" (Usha et al., 2007, 2009; Singh et al., 2011; Gautam et al., 2012). The stress busting properties of plant "MTs" are still largely unknown in comparision to those belonging to the mammals as there is difficulty in purifying of plant "MTs" because of their instability in the presence of oxygen (Guo et al., 2008, Sharma et al., 2016). The "MT" genes are expressed in a specific tissue or organs; for instance, "class MT1" genes are expressed in the root region, the "class MT2" genes in the leaves "class MT3" in ripened fruit, and "PEC/pec MT" genes in the growing seeds (Huang et al., 2011). Metal-responsive transcription factor-1 activates metal response elements

Figure 3. Basic mode of action in Metallothioneines under heavy metal stress



after heavy metal ions bind to the MTs. Through "Cytokinin signaling" the signal transducers and activators of transcription proteins are activated and similarly, the antioxidant response element proteins are triggered on by associated redox imbalance figure 3 and table 2 (Sharma et al., 2016).

CONCLUSION

The physiological and mechanistic roles played by PCs, yet MTs remain still elusive in the plant system. It is factually clear that both PCs and MTs are derived from amino acid-rich precursors and are actively involved in metal binding and detoxification as observed more in hyperaccumulation plant species. Also most reports have explained single metal/metalloid detoxification via PCs and MTs hence, it remains to be seen how plants use these metal ligands at the time of multiple metal stress. The metals ions are detoxified by PCs synthesized by GSH upon plant exposure to heavy metals. However, we need a better understanding of the pathway inclusive of PCs and MTs in respect to heavy metals.

Table 2. Metallothioneins (MTs) genes expressed in plant under heavy metal stress

S.No	MTs genes	Plant	Effect	References
1.	IIMT2b	Iris lactea	Cu concentration increased and reduced H_2O_2 production	Gu et al., 2015
2.	OsMT2c	Oryza sativa	Increased ROS activity and more tolerance to Cu	Liu et al., 2015
3.	ScMT2-1-3	Saccharum spp. L	Increased the host cells tolerance to Cd	Guo et al., 2013
4.	TaMT3	Tamarix androssowii	Tolerance to Cd and increased activity of ROS	Zhou et al., 2014
5.	BcMT1 and BcMT2	Brassica campestris	Enhance tolerance to Cu and Cd and decresed production reactive oxygen species	Lv et al., 2013
6.	SaMT2	Sedum alfredii	Cd accumulation and tolerance	Zhang et al., 2014
7.	MT2	Populus alba L.	Cd, Zn, Cu ions increased tolerance	Macovei et al., 2010
8.	PcMT3	Porteresia coarctata	Cd, Zn, Cu tolerance	Usha et al., 2011
9.	MT3 and MT4	Hordeum vulgare	Zn tolerance	Hegelund et al., 2012
10.	ThMT3	Tamarix hispida	Zn, Cd, Cu enhances	Yang et al., 2011
11.	Am MT2	Avicennia marina	Cd, Pb, Cu and Zn	Huang and Wang, 2010
12.	HbMT2	Hevea brasiliensis	Zn and Cu ions tolerance	Macovei et al., 2010

REFERENCES

Adams, J. P., Adeli, A., Hsu, C. Y., Harkess, R. L., Page, G. P., Schultz, E. B., & Yuceer, C. (2011). Poplar maintains zinc homeostasis with heavy metal genes HMA4 and PCS1. *Journal of Experimental Botany*, *62*(11), 3737–3752. doi:10.1093/jxb/err025 PMID:21504875

Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremdiation of heavy metals-concepts and applications. *Chemosphere*, *91*(7), 869–881. doi:10.1016/j.chemosphere.2013.01.075 PMID:23466085

Andresen, E., Mattusch, J., Wellenreuther, G., Thomas, G., Arroyo Abad, U., & Kupper, H. (2013). Different strategies of cadmium detoxification in the submerged macrophyte *Ceratophyllum demersum L. Metallomics*, *5*(10), 1377; Epub ahead of print. doi:10.1039/c3mt00088e PMID:23868355

Bractic, A. M., Majic, D. B., Samardzc, J. T., & Maksimovic, V. R. (2009). Functional analysis of the buckwheat metallothionein promoter: Tissue specificity pattern and up-regulation under complexes stress stimuli. *Journal of Plant Physiology*, *166*(9), 996–1000. doi:10.1016/j.jplph.2008.12.002 PMID:19185389

Brunetti, P., Zanella, L., & De Paolis, A., Di, Litta, D., Cecchetti, V., Falasca, G., … Cardarelli, M. (2015). Cadmium-inducible expression of the ABC-type transporters AtABCC3 increases phytochelatins-mediated cadmium tolerance in Arabidopsis. *Journal of Experimental Botany*. doi:10.1093/jxb/erv185

Clemens, S. (2006). Evolution and function of phytochelatins synthases. *Journal of Plant Physiology*, *163*(3), 319–332. doi:10.1016/j.jplph.2005.11.010 PMID:16384624

Cobbett, C. (2000). Phytochelatins and their roles in heavy metal detoxification. *Plant Physiology*, *123*(3), 825–833. doi:10.1104/pp.123.3.825 PMID:10889232

Dago, A., Gonzalez, I., Arino, C., Martinez-Coronado, A., Higueras, P., Diaz-Cruz, J. M., & Esteban, M. (2014). Evaluation of mercury stress in plants from the Almaden mining distric by analysis of phytochelatins and their Hg complexes. *Environmental Science & Technology*. PMID:24793970

Fassler, E., Robinson, B. H., Gupta, S. K., & Schulin, R. (2010). Uptake and allocation of plant nutrients and Cd in maize, sunflower and tobacco growing on contaminated soil and the effect of soil conditioners under field conditions. Nutr. *Cycl. Agroecosys.*, *87*(3), 339–352. doi:10.100710705-009-9342-z

Franchia, N., Piccinnia, E., Ferroa, D., Bassod, G., Spolaoree, B., Santovitoa, G., & Ballarin, L. (2015). Characterization and transcription studies of a phytochelatin synthase gene from the solitary tunicate Ciona intestinalis exposed to cadmium. *Aquatic Toxicology (Amsterdam, Netherlands)*, *152*, 47–56. doi:10.1016/j.aquatox.2014.03.019 PMID:24727215

Gautam, N., Verma, P. K., Verma, S., Tripathi, R. D., Trivedi, P. K., Adhikari, B., & Chakrabarty, D. (2012). Genome wide identification of rice class I metallothionein gene: Tissue expression patterns and induction in response to heavy metal stress. *Functional & Integrative Genomics*, *12*(4), 635–647. doi:10.100710142-012-0297-9 PMID:23053198

Grill, E., Loeffler, S., Winnacker, E. L., & Zenk, M. H. (1989). Phytochelatins the heavy metals binding peptides of plants are synthesized from glutathione by a specific g-glutamylcysteine dipeptidyl transpeptidase (phytochelatin synthase). *Proceedings of the National Academy of Sciences of the United States of America*, *86*(18), 6838–6842. doi:10.1073/pnas.86.18.6838 PMID:16594069

Gu, C. S., Liu, L. Q., Deng, Y. M., Zhu, X. D., Huang, S. Z., & Lu, X. Q. (2015). The heterologus expression of the Iris lacteal var. chinensis type 2 metallothionenin IIMT2b, gene enhances copper tolerance in *Arabidopsis thaliana. Bulletin of Environmental Contamination and Toxicology*, *94*(2), 247–253. doi:10.100700128-014-1444-x PMID:25533567

Guo, J., & Xu, L., Su, L., Wang, H., Gao, S., Xu, J., & Que, Y. (2013). ScMT2-1-3, a metallothionein gene of sugarcane, plays important role in the reguclation of heavy metal tolerance accumulation. *BioMed Research International*. doi:10.1155/2013/904769

Guo, W. J., Meetam, M., & Goldsbrough, P. B. (2008). Examining the specific contributions of individual *Arabidopsis* metallothioneins to copper distribution and metal tolerance. *Plant Physiology*, *146*(4), 1697–1706. doi:10.1104/pp.108.115782 PMID:18287486

Hassinen, V. H., Tervahauta, A. I., Schat, H., & Ka, S. O. (2011). Plant-mettallothioneins-metal chelators with ROS scavenging activity? *Plant Biology*, 13225–13232. PMID:21309968

Hegelund, J. N., Schiller, M., Kichey, T., Hansen, T. H., Pedas, P., Hustard, S., & Schjoerring, J. K. (2012). Barley metallothioneins: MT3 and MT4 are localized in the grain aleurone layer and show differential zinc binding. *Plant Physiology*, *159*(3), 1125–1137. doi:10.1104/pp.112.197798 PMID:22582132

Huang, G. Y., & Wang, Y. S. (2010). Expression and characterization analysis of type 2 metallothionein from grey mangrove species (*Avicennia marina*) in responses to metal stress. *Aquatic Toxicology (Amsterdam, Netherlands)*, *99*(1), 86–92. doi:10.1016/j.aquatox.2010.04.004 PMID:20435357

Huang, G. Y., Wang, Y. S., & Ying, G. G. (2011). Cadmium inducible BgMT2, a type 2 metallothionein gene from mangrove species (*Bruguiera gymnorrhiza*), its encoding protein shows metal-binding ability. *Journal of Experimental Marine Biology and Ecology*, *405*(1-2), 128–132. doi:10.1016/j.jembe.2011.05.034

Indriolo, E., Na, G., Ellis, D., Salt, D. E., & Banks, J. A. (2010). A vacuolar arsenite transporter necessary for arsenic tolerance in the arsenic hyperaccumulating fern pteris vittta is missing in flowering plants. *The Plant Cell*, *22*(6), 2045–2057. doi:10.1105/tpc.109.069773 PMID:20530755

Inhouhe, M. (2005). Phytohelatins. *Brazilian Journal of Plant Physiology*, *17*(1), 65–78. doi:10.1590/S1677-04202005000100006

Jia, D. U., Jing-Li, Y., & Cheng-Hao, L. I. (2012). Advances in metallotionein studies in forest trees. *China POJ*, *5*, 46–51.

Kidd, P., Barcelo, J., Bernal, M. P., Navari-Izzo, F., Poschenrieder, C., Shilev, S., ... Monterroso, C. (2009). Trace elements behavior at the root-soil interface: Implications in phytoremediation. *Environmental and Experimental Botany*, *67*(1), 243–259. doi:10.1016/j.envexpbot.2009.06.013

Kuhnlenz, T., Schmidt, H., Uraguchi, S., & Clemens, S. (2014). *Arabidopsis thaliana* phytochelatins synthase 2 is constitutively active in vivo and can rescue the growth defect of the PCS1-deficient cad1-3 mutant on Cd-contaminated soil. *Journal of Experimental Botany*, *65*(15), 4241–4253. doi:10.1093/jxb/eru195 PMID:24821959

Kutrowska, A., & Szelag, M. (2014). *Low molecular weight organic acids and peptides involved in the long-distance transport of trace metals*. Acta Physiol. Plan. doi:10.100711738-014-1576-y

Lee, S. (2014). Artifical induction of cadmium tolerance and its further enhancement via heterologus co-expression of SpHMT1 and AtPCS1 in the yeast cells. *J. Korean Soc. Appl. Biol. Chem.*, *57*(3), 307–310. doi:10.100713765-014-4092-2

Liu, G. Y., Zhang, Y. X., & Chai, T. Y. (2011). Phytochelatins synthase of *Thlaspi caerulescens* enhanced tolerance and accumulation of heavy metals when expressed in yeast and tobacco. *Plant Cell Reports*, *30*(6), 1067–1076. doi:10.100700299-011-1013-2 PMID:21327392

Liu, J., Shi, X., Qian, M., Zheng, L., Lian, C., Xia, Y., & Shen, Z. (2015). Copper induced hydrogen peroxide upregulation of a metallothioneins gene, OsMT2c, from *Oryza sativa L.* confers copper tolerance in Arabidopsis thaliana. *Journal of Hazardous Materials*, *294*, 99–108. doi:10.1016/j.jhazmat.2015.03.060 PMID:25867584

Lv, Y., Deng, X., Quan, L., Xia, Y., & Shen, Z. (2013). Mettallothioneins BcMT1 and BcMT2 from *Brassica campestris* enhance tolerance to cadmium and copper decrease production of reactive oxygen species in Arabidopsis thaliana. *Plant and Soil*, *367*(1-2), 507–519. doi:10.100711104-012-1486-y

Maestri, E., Marmiroli, M., Visioli, G., & Marmiroli, N. (2010). Metal tolerance and hyperaccumulation: Costs and trade-offs between traits and environment. *Environmental and Experimental Botany*, *68*(1), 1–13. doi:10.1016/j.envexpbot.2009.10.011

Meister, A. (1988). Glutathione metabolism and its selective modification. *The Journal of Biological Chemistry*, *263*, 17205–17208. PMID:3053703

Mirza, N., Mahmood, Q., Shah, M.M., Pervez, A., & Sultan, S. (2014). Plants as useful vectors to reduce environmental toxic arsenic content. *Sci. World J.*, 1-11.

Mishra, S., Shrivastava, S., Tripathi, R. D., Kumar, R., Seth, C. S., & Gupta, D. K. (2006). Lead detoxification by coontail (*Ceratophyllum demersum L.*) involves induction of phytochelatins and antioxidant system in response to its accumulation. *Chemosphere*, *65*(6), 1027–1039. doi:10.1016/j.chemosphere.2006.03.033 PMID:16682069

O'Lochlainn, S. O., Bowen, H. C., Fray, R. G., Hammond, J. P., King, G. J., White, P. J., ... Broadly, M. R. (2011). Tandem quadruplications of HMA4 in the zinc and cadmium hyperaccumulator *Noc-cae caerulescens*. *PLoS One*, *6*(3), e17814. doi:10.1371/journal.pone.0017814 PMID:21423774

Ovecka, M., & Takac, T. (2014). Managing heavy metal toxicity stress in plants: Biological and biotechnological tools. *Biotechnology Advances*, *32*(1), 73–86. doi:10.1016/j.biotechadv.2013.11.011 PMID:24333465

Pilon-Smits, E., & Pilon, M. (2002). Phytoremediation of metals using transgenic plants. *Critical Reviews in Plant Sciences*, *21*(5), 439–456. doi:10.1080/0735-260291044313

Prapagdee, B., Chanprasert, M., & Mongkolsuk, S. (2013). Bioagumentation with Cadmium-resistant plant growth-promoting rhizobacteria to assist cadmium phytoextraction by *Helianthus annus*. *Chemosphere*, *92*(6), 659–666. doi:10.1016/j.chemosphere.2013.01.082 PMID:23478127

Quartacci, M. F., Cosi, E., & Navari-Izzo, F. (2001). Lipids and NADPH-dependent superoxide production in plasma membrane vesicles from roots of wheat grown under copper deficiency and excess. *Journal of Experimental Botany*, *152*, 67–75. PMID:11181715

Rascio, N., & Navari-Izzo, F. (2011). Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? *Plant Science*, *180*(2), 169–181. doi:10.1016/j.plantsci.2010.08.016 PMID:21421358

Rocca, L. (2009). Responses of the Antarctic microalga Koliella antartica (*Trebouxiophyceae, Chlorophyta*) to cadmium contamination. *Photosyn.*, *47*(3), 471–479. doi:10.100711099-009-0071-y

Sharma, R., Bhardwaj, R., Handa, N., Gautam, V., Kohli, S.K., Bali, S., … Vig, A.P. (2016). Responses of phytochelatins and metallothiones in alleviation of heavy metal stress in plants: An overview. In *Plant metal interaction*. Academic Press.

Shen, G. H., Zhu, C., & Du, Q. Z. (2010). Genome wide identifications of phytochelatins and phytoch-synth domain-containing phytochelatins family from rice. *Electron. J. Biol.*, *6*, 73–79.

Shri, M., Dave, R., Diwedi, S., Shukla, D., Kesari, R., Tripathi, R. D., ... Chakrabarty, D. (2014). Heterollogus expression of *Ceratophyllum demersum* phytochelatin synthase, CdPCS1, in rice leads to lower arsenic accumulation in grain. *Scientific Reports*, *4*(1), 5784. doi:10.1038rep05784 PMID:25048298

Sine, AM., Shakya, V.P., & Idnum, A. (2015). Phytochelatin synthase is required for tolerating metal toxicity in a basidiomycete yeast and is a conserved factor involve din metal homeostasis in fungi. *Fungal Biol. Biotechnol., 2*(3).

Singh, R. K., Anandhan, S., Singh, S., Patade, V. Y., Ahmed, Z., & Pande, V. (2011). Metallothione-like gene from *Cicer microphyllum* is regulated by multiple abiotic stresses. *Protoplas*, *248*(4), 839–847. doi:10.100700709-010-0249-y PMID:21161305

Sunitha, M. S. L., Prashant, S., Kumar, S. A., Rao, S., Narasu, M., & Kishor, P. B. K. (2013). Cellular and molecular mechanisms of heavy metal tolerance in plants: A brief overvie of transgenic plants over-expressing phytochelatins synthase and metallothionein genes. *Plant Cell Biotech. Mol. Biol.*, *13*, 33–48.

Tennstedt, P., Peisker, D., Bottcher, C., Trampczynska, A., & Clemens, S. (2009). Phytochelatins synthase is essential for the detoxification of excess zinc and contributes significantly to the accumulation of zinc. *Plant Physiology*, *149*(2), 938–948. doi:10.1104/pp.108.127472 PMID:19074629

Thirumoorthy, N., Sunder, A. S., Kumar, K. T. M., Kumar, M. S., Ganesh, G. N. K., & Chatterjee, M. (2011). A review of metallothionein isoforms and their role in pathophysiology. *World Journal of Surgical Oncology*, *9*(1), 54–60. doi:10.1186/1477-7819-9-54 PMID:21599891

Ueno, D., Koyama, E., Yamaji, N., & Ma, J. F. (2011). Physiological, genetic, and molecular characterization of a high-Cd-accumulating rice cultivar. *Jarjan. J. Exp. Bot.*, *62*(7), 2265–2272. doi:10.1093/jxb/erq383 PMID:21127026

Usha, B., Keeran, N. S., Harikrishnan, M., Kavitha, K., & Parida, A. (2011). Characterization of type 3 metallothionein isolated from *Porteresia coarctata. Biologia Plantarum*, *55*(1), 119–124. doi:10.100710535-011-0016-8

Usha, B., Prashanth, S. R., & Parida, A. (2007). Differential expression of two metallothionein encoding genes during heavy metal stress in the mangrove species, *Avicennia marina* (Forsk.). *Vierh. Curr. Sci.*, *93*, 1215–1219.

Usha, B., Venkataraman, G., & Parida, A. (2009). Heavy metal and abiotic stress inducible metallothionein isoforms from *Prosopis juliflora* (SW) D.C. show differences in binding to heavy metals invitro. *Molecular Genetics and Genomics*, *281*(1), 99–108. doi:10.100700438-008-0398-2 PMID:19015881

Van Nevel, L., Mertens, J., Oorts, K., & Verheyen, K. (2007). Phytoextraction of metals from soils: How far from practice? *Environ. Pollut.*, *150*(1), 34–40. doi:10.1016/j.envpol.2007.05.024 PMID:17604889

Yadav, S. K. (2010). Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *South African Journal of Botany*, *76*(2), 167–179. doi:10.1016/j.sajb.2009.10.007

Yuan, J., Chen, D., Ren, Y., Zhang, X., & Zhao, J. (2008). Characteristic and expression analysis of a metallothionein gene, OsMT2b, down regulated by cytokinin suggests functions in root development and seed embryo germination of rice. *Plant Physiology*, *146*(4), 1637–1650. doi:10.1104/pp.107.110304 PMID:18258694

Zhang, J., Zhang, M., Tian, S., Lu, L., Shohag, M. J. I., & Yang, X. (2014). Metallothionein 2 (SaMT2) from *Sedum alfredii* confers increased Cd tolerance and accumulation in yeast and tobacco. *PLoS One*, *9*(7), e102750. doi:10.1371/journal.pone.0102750 PMID:25032704

Zhou, B., Yao, W., Wang, S., Wang, X., & Jiang, T. (2014). The metallothionein gene, TaMT3, from *Tamarix androssowii* confers Cd2p tolerance in tobacco. *International Journal of Molecular Sciences*, *15*(6), 10398–10409. doi:10.3390/ijms150610398 PMID:24918294

Zhu, J., Zhang, Q., Wu, R., & Zhang, Z. (2010). HbMT2, an ethephon induced metallothionein gene from *Hevea brasiliensis* responds to H2O2 stress. *Plant Physiology and Biochemistry*, *48*(8), 710–715. doi:10.1016/j.plaphy.2010.04.004 PMID:20471279

Chapter 10

Heavy Metal Stress Mechanism by Signaling Cascades in Plants

ABSTRACT

This chapter highlights the role of cascade for remediation of heavy metals, their mechanism of action, and their applications approach of hyperaccumulation. Further, it also highlights the role of uptake and detoxification of metals by cellular mechanisms that facilitate the bioremediation of heavy metals from contaminated areas.

INTRODUCTION

Heavy metal is a big problem in most parts of the world; but it also responsible for the loss of agricultural productivity and loss of farmers. Under physiological conditions based on their solubility, 17 heavy metals may be available for living cells and are important for organisms and ecosystem in the environment (Schutzendubel and Polle, 2002; Hameed et al., 2016). Heavy metals such as Mn, Mo, and Fe are important as micronutrients, whereas Vu, Ni, Zn, Co, W, V, and Cr are trace elements but present at higher amount so they are toxic to other metals like Ag, Hg, As, Sb, Cd, U and Pd have no function and seems to be toxic to the plants, animals and microorganisms (Nazar et al., 2012; Hammed et al., 2016). Heavy metal pollutants are basically derived from industries in the form of waste, chemical reagents and agriculture practices

DOI: 10.4018/978-1-5225-9016-3.ch010

such as pesticides, chemical fertilizers and most important herbicides. Chemicals have adverse effects on the "human beings, plants and, soil, microflora, including beneficial microbes. These contaminants accumulate in the agricultural soils where they are released and therefore reach to the food chain, creating a high risk to the living organisms and ultimately giving rise to the food scarcity. They reach plants by the process of absorption by roots from soil and disturb the balance of the food chain in the form of particulate contaminants because of their regular presence (Tak et al., 2013)

Many different industries and agricultural activities contribute to heavy metal contamination in urban areas. Heavy metals can directly influence growth, senescence and energy synthesis processes because of their high reactivity. They adversely affect the absorption and transport of the essential elements, thereby distributing the metabolism and having an impact on the growth of plants and reproduction (Xu et al., 2008; Hameed et al., 2016). More than 30 mg of lead Pb leads to a reduction of plants growth and decline in the chlorophyll synthesis in leaf reduction in growth as well as crop production, yellowing of young leaves, reduction in the absorption of essential elements such as Fe and decline in rate of photosynthesis. When plants are subjected to heavy metal stress production of reactive oxygen species (ROS) were found imbalanced in plants and disturb the metabolic activity (Hameed et al., 2016). The toxicity and tolerance to heavy metals differ from arrangement to the plants in the environment, and a number of efforts to their tolerance mechanism at molecular level have not been fully understood yet (Thapa et al., 2012).

EFFECT ON PLANT GROWTH UNDER STRESS

Heavy metals can affect on the growth of plants in a number of points and toxic effect at different levels of cell structure and function of the plants. Inhibition of the germination and root extension can be result of the interference with the cell division or with the cell elongation. It has been observed that the main pressure of heavy metals such as Zn, Cu, Co, Cd, Hg and Pb is the inhibition of the germination rate, root elongation and shoot and leaf growth (Munzurog and Geckil, 2002; Rascio et al., 2008; Hameed et al., 2016). The inhibition of root elongation in many instances is the most sensitive parameter of heavy metal toxicity (Schutzendubel et al., 2001; Hameed at al., 2015). In crop production Al toxicity is one of the major growth-limiting factors in acidic mineral soils (Panda et al., 2009). The root systems become stubby as a result of the inhibition of elongation to main axis points and lateral roots due to restricted cell division. The root become stunted and brittle and apices become swollen and damaged (Panda et al., 2009). It causes extensive root injury, especially in root cap region and hampers the mineral and water uptake; severity inhibition of root growth is a suitable indicator of genotypical differences in Al toxicity (reviewed by Rout et al., 2001; Hameed at al., 2016). Heavy metals interfere with cell division and therby reduce the growth of both root and shoot meristem. There Inhibition of mobilization of nitrogen and phosphorus during seedling growth has been observed in sinach on exposure to mercury (Hg) (Gothberg et al., 2004; Hameed et al., 2016). Under higher concentration of heavy metal exposure root and shoot growth declines and low pigment content (reviewed by Nazar et al., 2012; Hameed et al., 2016).

SIGNALING IN PLANT

Plants have evolved different mechanisms to maintain the physiological concentration of the essential metal ions and to reduce the contact to non-essential heavy metals. Some mechanisms are required for homoeostasis and are ubiquitous, whereas other mechanisms target individual metal ion for the exclusion of particular metals from the intracellular environment. When these two mechanisms are exhausted, then the plants activate oxidative stress defense mechanisms and synthesis of stress-related proteins and signaling molecules such as heat shock proteins, hormones and ROS and finally the transcriptional activation of specific metal responsive genes to work against the stress (Maksymiec, 2007; Hameed et al., 2016). However, different signaling pathways may be used to respond different heavy metals. The signal pathways comprise the calcium calmodulin system hormones, ROS signaling, and the mitogen-activated protein kinase (MAPK) phosphorylation cascade which congregate by activating the previously mentioned stress-related genes.

CALCIUM CALMODULIN SYSTEM SIGNALING IN PLANT

Calcium is an essential macronutrient taken by the plants through the roots and is delivered to the shoots by xylem loading pathway and regulate when plant defense against environment stresses (Tuteja et al., 2009; Kader and Lindberg, 2010). They function as the central node in overall signaling web and have a promising role in stress tolerance. The stability of Ca channels by increasing the calcium flux into the cell. Intracellular Ca is a secondary messenger that interacts with calmodulin to transmit the signal and regulate the downstream flux genes involved in HM transport metabolism, tolerance (Yang and Pooaiah, 2003; Choong et al., 2014). Calmodulin an important Ca^{2p} binding protein, is a small acidic protein and is responsible for the regulation of intracellular Ca^{2p} levels (Hameed et al., 2016). Increased Ca2p concentration activates calmodulin that then induces specific kinases. Calmodulin is a very important calcium-binding protein in Ca2p signaling and has been found to be involved in the biotic and abiotic stress (Tuteja and Sopory, 2008).

HORMONE SIGNALING PATHWAY IN PLANT

Plant hormone play a vital role in the adaptation to abiotic, biotic stress as shown by the regulation of hormone synthesis in the presence of heavy metals stress and some harmful microbes also affetced (reviewed by Ahmad et al., 2011; Hameed et al., 2016). Ethylene biosynthesis increase in reaction to Cd stress has been observed in mustard, pea and soybean (Masood et al. 2012; Rodriguez-Serrano et al., 2009; Chmielowska-Bak et al., 2013). The greatest amount of the ethylene production by *Arabidopsis* plants in respect to the Cd, and Cu and except for Zn (Arteca and Arteca, 2007). Ethylene may be involved in the inhibitory action of copper on roots and leaves of dicotyledonous plants. Some researchers have reported that the plants exposed to Cd, Cu, Zn, and Fe stresses produce the higher levels of ethylene, but the copper may not have same effect (reviewed by Hameed et al., 2016). Cadmium (Cd) and copper (Cu) induce the accumulation of jasmonic acid in *Phaseolus coccineus*, rice and *Arabidopsis thaliana* (Maksymiec et al., 2005; Maksymiec, 2011). However, a decrease in jasmonic acid content in Cd-stressed roots of the soybean leads to an increase in abscisic acid (ABA) and metabolites at different times of Cd stress (Perez Chaca et al., 2014). Pea seedling showed increase in salicyclic acid (SA) under Cd-stress and its exogenous capability helps to defend leaves from the lipid peroxidation (Popova et al., 2009).

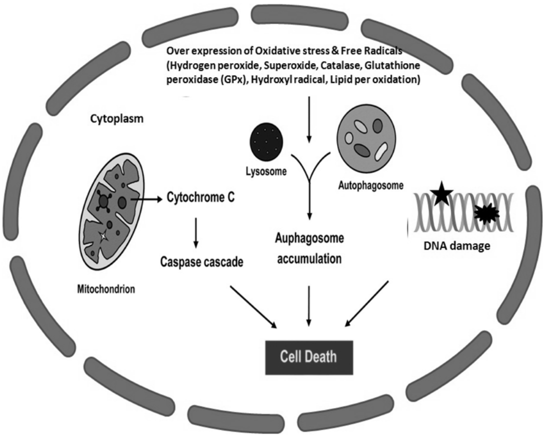
REACTIVE OXYGEN SPECIES (ROS) PRODUCTION UNDER HEAVY METALS STRESS

Hydrogen peroxide (H_2O_2) acts a signaling molecule in response to the heavy metals and other stresses also activate these signalling (Dat et al., 2000). Copper (Cu) and cadmium (Cd) increases the level of H_2O_2 in *A. thaliana* (Maksymiec and Krupa, 2006). Thus, H_2O_2 accumulation changes the redox status of the cell, and induces the production of antioxidant and also the mechanism of antioxidant (Foyer and Nnoctor, 2005). Hydorgen peroxidation level increases the plant tolerance to the heavy metals stress (reviewed by Hameed et al., 2016). Nevertheless with the treatment given at 100 mM H_2O_2 for 1 day mitigated of Cd stress by inducing the antioxidant enzymes activity of (SOD, CAT, GST, GPX, and APX) and prominent the contents of GSH and AsA. Increased the activity of SOD, POD, GSH, GPX, APX. AsA, MDHAR, and DHAR content and their of redox state in the response to the Aluminium stress. Therefore H_2O_2 pretreatment in Cd and Al respect makes the plant more tolerant and inducing “GSH”, and AsA, NPT, PCs contents as well as the GST activity in root tissue fig. 10.1 (reviewed by Hameed et al., 2016; Bai et al., 2011). Cadmium have been reported to foundation of lipid peroxidation and PCD in many plants species because of the perturbation in the chloroplast and mitochondria metabolism (Cho and Seo, 2005; Bi et al., 2009; Metwally et al., 2005; Kumar and Trivedi, 2016). Alumunium toxicity also disturbs the cellular redox homeostasis and causes the oxidative burst of “mitochondrial dysfunction and PCD in plants (Li and Xing, 2010). Mercury (Hg) exposure to induces of Ca^{2p} accumulation; reduces the ROS production, and activates MAPKs which contribute to the plant defense system against the Hg stress (Chen et al., 2014; reviewed by Kumar and Trivedi, 2016). Intensive research on the ROS generation under heavy metals stress has been conducted and the distinct signaling involved in stress response has been explored (reviewed by Kumar and Trivedi, 2016).

MITOGEN ACTIVATED PROTEIN KINASE CASCADE

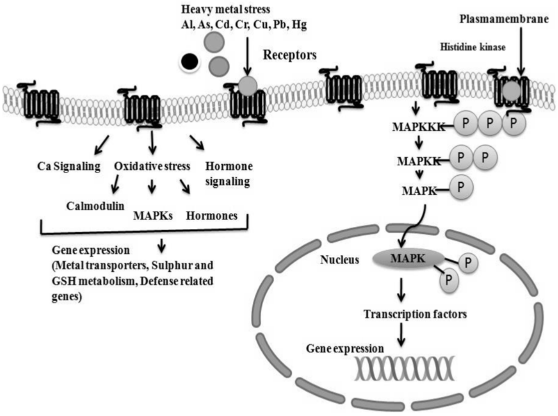
Heavy metal quick to respond changes to Cd, and Cu have shown to code for signal transduction components in rice, maize and *Arabidopsis* mitogen-activated protein kinase (MAPK), stress inducing protein, transcriptional factors, proteins participating in protein folding and sulfur and GSH metabolism

Figure 1. Reactive oxygen species signalling activate under heavy metal stress



(Yeh et al., 2007; Wang et al., 2010). The MAPK cascade consists of three kinases consecutively activated by phosphorylation (MAPKKK, MAPKK, MAPK). MAPKs phosphorylate substrate in different cellular compartments including transcription factor in the nucleus. The MAPKKK is a Ser/Thr protein kinase that phosphorylates and thereby activate the MAPKKs. These MAPKKs are dual-specific kinases that phosphorylate MAPKs on a Thr and Tyr residue (Hameed et al., 2016). However, all these pathways are regulated of transcription factors that activate genes under stress condition and also biosynthesis of chelating compounds. Different regulation of MAPKs under drought, cold, heat and salinity have been observed in rice suggesting the involvement of gene family in stress signaling as well as cross-talk synthesis (Kumar et al., 2008; Zhang et al., 2012; Kumar and Trivedi, 2016). Excess amount of heavy metals such as Cd, Cu As (III) activate the MAPKs in "*Medicago sativa*", rice and *Brassica juncea* which leads to the tolerance against the stress fig. 10.2 (Jonak et al., 2004; Yeh et al., 2004; Gupta et al., 2009).

Figure 2. Representation of mitogen activated protein kinase (MAPK) signaling cascades under stress of heavy metals



CONCLUSION

Heavy metal exposure is serious concern global problem for the environment. The understanding of the mechanisms that help to different plants to manage with heavy metal stress which help in making new tools that are used in phytoremediation technology. Recent findings have reveled besides other than mechanisms in plants that protect themselves from the heavy metal toxicity through the enzymatic and nonenzymatic activity. Number of signaling are not known properly so we will focus to identifying the more mechanism involve to uptake and transport of heavy metals in plants like ROS reacting, PCs biosynthesis, calcium-calmodulin kinases and mitogen activate protein kinase and also cross talk with other signaling molecules. Apart from the above given information about these protein kinase is still required more information to develop a better understanding and role of uncharacterized MAPKs involved in plant defense system.

REFERENCES

Aeteca, R. N., & Arteca, J. M. (2007). Heavy metal-induced ethylene production in *Arabidopsis thaliana. Journal of Plant Physiology*, *164*(11), 1480–1488. doi:10.1016/j.jplph.2006.09.006 PMID:17215058

Ahmad, P., Nabi, G., & Ashraf, M. (2011). Cadmium induced oxidative damage in mustard [*Brassica juncea (L) Czern. Coss*.] plants can be alleviated by salicylic acid. *South African Journal of Botany*, *77*(1), 36–44. doi:10.1016/j.sajb.2010.05.003

Bai, X. J., Liu, L. J., Zhang, C. H., Ge, Y., & Cheng, W. (2011). Effect of H_2O_2 pretreament on Cd tolerance of different rice cultivars. *Rice Science*, *18*(1), 29–35. doi:10.1016/S1672-6308(11)60005-X

Chen, Y. A., Chi, W. C., Trinh, N. N., Huang, L. Y., Chen, Y.-C., Cheng, K.-T., ... Huang, H.-J. (2014). Transcriptome profiling and physiological studies reveal a major role for aromatic amino acids in mercury stress tolerance in rice seedlings. *PLoS One*, *9*(5), e95163. doi:10.1371/journal.pone.0095163 PMID:24840062

Chmielowska-Bak, J., Lefevre, I., Lutts, S., & Deckert, J. (2013). Short term signalling responses in roots of young soybean seedlings exposed to cadmium stress. *Journal of Plant Physiology*, *170*(18), 1585–1594. doi:10.1016/j.jplph.2013.06.019 PMID:23942356

Cho, U. H., & Seo, N. H. (2005). Oxidative stress in Arabidopsis thaliana exposed to cadmium is due to hydrogen peroxide accumulation. *Plant Science*, *168*(1), 113–120. doi:10.1016/j.plantsci.2004.07.021

Choong, G., Liu, Y., & Templetion, A. M. (2014). Interplay of cadmium and cadmium in mediating cadmium toxicity. *Chemico-Biological Interactions*, *211*, 54–65. doi:10.1016/j.cbi.2014.01.007 PMID:24463198

Dat, J., Vandenabeele, S., Vranova, E., Van Montagu, M., Inze, D., & Van Breusegem, F. (2000). Dual action of the active oxygen species during plant stress responses. *Cellular and Molecular Life Sciences*, *57*, 779–795. doi:10.1007000180050041 PMID:10892343

Foyer, C. H., & Noctor, G. (2005). Redox homeostasis and oxidant signaling:a metabolic interface between stress perception and physiological responses. *The Plant Cell*, *17*(7), 1866–1875. doi:10.1105/tpc.105.033589 PMID:15987996

Gothberg, A., Greger, M., Holm, K., & Bengstsson, B. E. (2004). Influence of nutrient levels on uptake and effects of mercury, cadmium and lead in water spinach. *Journal of Environmental Quality*, *33*(4), 1247–1255. doi:10.2134/jeq2004.1247 PMID:15254106

Gupta, M., Sharma, P., Sarin, N. B., & Sinha, A. K. (2009). Differential response of arsenic stress in two varieties of *Brassica juncea L. Chemosphere*, *74*(9), 1201–1208. doi:10.1016/j.chemosphere.2008.11.023 PMID:19101007

Hameed, A., Rasool, S., Azooz, M.M., Hossain, M.A., Ahanger, M.A, & Ahmad, P. (2016). Heavy metal stress: Plant responses and signalling. In *Plant metal interaction*. Academic Press.

Jonak, C., Nakagami, H., & Hirt, H. (2004). Heavy metal stress. Activation of distinct mitogen-activated protein kinase pathways by copper and cadmium. *Plant Physiology*, *136*(2), 3276–3283. doi:10.1104/pp.104.045724 PMID:15448198

Kader, M.A., & Lindberg, S. (2010). Cytosolic calcium and pH signaling in plant under salinity stress. *Plant signal behave., 5*(3), 233-238.

Kumar, K., Rao, K. P., Sharma, P., & Sinha, A. K. (2008). Differential regulation of rice mitogen activated protein kinase kinase (MKK) by abiotic stress. *Plant Physiology and Biochemistry*, *46*(10), 891–897. doi:10.1016/j.plaphy.2008.05.014 PMID:18619847

Kumar S., & Trivedi, P.K. (2016). Heavy metal stress signalling in plants. In *Plant metal interaction*. Academic Press.

Li, Z., & Xing, D. (2010). Mitochondrial pathway leading to programmed cell death induced by aluminum phyto-toxicity in Arabidopsis. *Plant Signaling & Behavior*, *5*(12), 1660–1662. doi:10.4161/psb.5.12.14014 PMID:21512328

Maksymiec, W. (2011). Effects of jasmonate qnd some other signaling factors on bean and onion growth during the initial phase of cadmium action. *Biologia Plantarum*, *55*(1), 112–118. doi:10.100710535-011-0015-9

Maksymiec, W., & Krupa, Z. (2006). The effect of short term exposition to Cd, excess Cu ions and jasmonate on oxidative stress appearing in Arabidopsis thaliana. *Environmental and Experimental Botany*, *57*(1-2), 187–194. doi:10.1016/j.envexpbot.2005.05.006

Maksymiec, W., Wianowska, D., Dawidowicz, A. L., Radkiewicz, S., Mardarowicz, M., & Krupa, Z. (2005). The level of jasmonic acid in Arabidopsis thaliana and Phaseolus coccineus plants under heavy metal stress. *Journal of Plant Physiology*, *162*(12), 1338–1346. doi:10.1016/j.jplph.2005.01.013 PMID:16425452

Maksymiec. (2007). Signalling responses in plants to heavy metal stress. *Acta. Physiol. Plant, 29*, 177-187.

Masood, A., Iqbal, N., & Khan, N. A. (2012). Role of ethylene in alleviation of cadmium induced photosynthetic capacity inhibition by sulphur in mustard. *Plant, Cell & Environment*, *35*(3), 524–533. doi:10.1111/j.1365-3040.2011.02432.x PMID:21950968

Metwally, A., Safronova, V. I., Belimov, A. A., & Dietz, K. J. (2005). Genotypic variation of the response to cadmium toxicity in Pisum sativum L. *Journal of Experimental Botany*, *56*, 167–178. PMID:15533881

Munzuroglu, O., & Geckil, H. (2002). Effects of metals on seed germination, root elongation, and coleoptiles and hypocotyls growth in Triticum aestivum and Cucumis sativus. *Archives of Environmental Contamination and Toxicology*, *43*(2), 203–213. doi:10.100700244-002-1116-4 PMID:12115046

Nazar, R., Iqbal, N., Masood, A., Khan, M. I. R., Syeed, S., & Khan, N. A. (2012). Cadmium toxicity in plants and role of mineral nutrients in its alleviation. *American Journal of Plant Sciences*, *3*(10), 1476–1489. doi:10.4236/ajps.2012.310178

Panda, S. K., Baluska, F., & Matsumoto, H. (2009). Aluminum stress signaling in plants. *Plant Signaling & Behavior*, *4*(7), 592–597. doi:10.4161/psb.4.7.8903 PMID:19820334

Perez Chaca, M. V., Viligocco, A., Reinoso, H., Molina, A., Abdala, G., Zirulnik, F., & Pedranzani, H. (2014). Effects of cadmium on growth, anatomy and hormone contents in *Glycine max* (L.). *Merr. Acta Physiol. Plant.*, *36*(10), 2815–2826. doi:10.100711738-014-1656-z

Popova, L. P., Maslenkova, L. T., Yordanova, R. Y., Ivanova, A. P., Szalai, G., & Janda, T. (2009). Exogenous treatment with salicylic acid attenuates cadmium toxicity in pea seedlings. *Plant Physiology and Biochemistry*, *47*(3), 224–231. doi:10.1016/j.plaphy.2008.11.007 PMID:19091585

Rascio, N., Vecchia, F. D., La Rocca, N., Barbato, R., Pagliano, C., Raviolo, M., ... Gabbrielli, R. (2008). Metal accumulation and damage in rice (CV. Vialone nano) seedlings exposed to cadmium. *Environmental and Experimental Botany*, *62*(3), 267–278. doi:10.1016/j.envexpbot.2007.09.002

Rodrigeuz, M. C., Petersen, M., & Mundy, J. (2010). Mitogen-activated protein kinase signalling in plants. *Annual Review of Plant Biology*, *61*(1), 621–649. doi:10.1146/annurev-arplant-042809-112252 PMID:20441529

Rout, G. R., Samantaray, S., & Das, P. (2001). Aluminium toxicity in plants: A review. *Agronomie*, *21*(1), 3–21. doi:10.1051/agro:2001105

Schutzendubel, A., & Polle, A. (2002). Plant responses to abiotic stresses: Heavy metal induced oxidative stress and protection by mycorrhization. *Journal of Experimental Botany*, *53*(372), 1351–1365. PMID:11997381

Tak, H. I., Ahmad, F., & Babalola, O. O. (2013). Advances in the application of plant growth-promoting rhizobacteria in phytoremediation of heavy metals. *Reviews of Environmental Contamination and Toxicology*, *223*, 33–52. doi:10.1007/978-1-4614-5577-6_2 PMID:23149811

Thapa, S. S., Paudyal, I., Khanal, S., Paudel, N., Mansberger, S. L., & van Rens, G. H. (2012). Central corneal thickness and intraocular pressure in a Nepalese population: The Bhaktapur Glaucoma Sudy. *Journal of Glaucoma*, *21*(7), 481–485. doi:10.1097/IJG.0b013e3182182c0f PMID:21499121

Tuteja, N., Ahmad, P., Panda, B. B., & Tuteja, R. (2009). Genotoxic stress in plants: Shedding light on DNA damage, repair and DNA repair helicases. *Mutation Research*, *681*(2-3), 134–149. doi:10.1016/j.mrrev.2008.06.004 PMID:18652913

Tuteja, N., & Sopory, S. K. (2008). Chemical signaling unde abiotic stress environment in plants. *Plant Signaling & Behavior*, *3*(8), 525–536. doi:10.4161/psb.3.8.6186 PMID:19513246

Xu, Y., Feng, L., Jeffrey, P.D., Shi, Y.G., & Morel, F.M.M. (2008). Structure and Metal exchange in the cadmium carbonic anhydrase of marine diatoms. *Nature, 452*, 56-61.

Yang, T., & Poovaiah, B. W. (2003). Calcium /calmodulin-mediated signal network in plants. *Trends in Plant Science*, *8*(10), 505–512. doi:10.1016/j.tplants.2003.09.004 PMID:14557048

Yeh, C. M., Chien, P. S., & Huang, H. J. (2007). Distinct signaling pathways for induction of MAP kinase activities by cadmium and cooper in rice roots. *Journal of Experimental Botany*, *58*(3), 659–671. doi:10.1093/jxb/erl240 PMID:17259646

Yeh, C. M., Hsiao, L. J., & Huang, H. J. (2004). Cadmium activate a Mitogen-activated protein kinase gene and MBP kinases in rice. *Plant & Cell Physiology*, *45*(9), 1306–1312. doi:10.1093/pcp/pch135 PMID:15509854

Zhang, M., Pan, J., Kong, X., Zhou, Y., Liu, Y., Sun, L., & Li, D. (2012). ZmMKK3, a novel maize group B mitogen-activated protein kinase kinase gene, mediated osmotic stress and ABA signal responses. *Journal of Plant Physiology*, *169*(15), 1501–1510. doi:10.1016/j.jplph.2012.06.008 PMID:22835533

Chapter 11

Newer Approaches in Phytoremediation:

An Overview

ABSTRACT

The heavy metal pollution problem is all over the world. Plant-growth-promoting bacteria (PGPB) has transformed heavy metals present in the soil, which removes and minimizes their toxic effects. This chapter highlights the role of plant-growth-promoting bacteria, chelating agents, and nanoparticles for remediation of heavy metals; their mechanism of action; and their applications approach of hyperaccumulation. Therefore, this chapter focuses on the mechanisms by which microorganisms, chelating agents, and nanoparticles can mobilize or immobilize metals in soils and the nano-phytoremediation strategies are addressed for the improvement of phytoextraction as an innovative process for enhancement of heavy metals removal from soil.

INTRODUCTION

Heavy metals is a serious environmental problem which affecting human health and plant (Aldoobie and Beltagi, 2013; Park et al., 2014). The use of large amount of fertilizers has been produced heavy metals into non-pollluted sites which affect dramtically to agriculture (Saba et al., 2015). One of the most toxic heavy metal involved in many fertilizers is lead (Pb), which can be

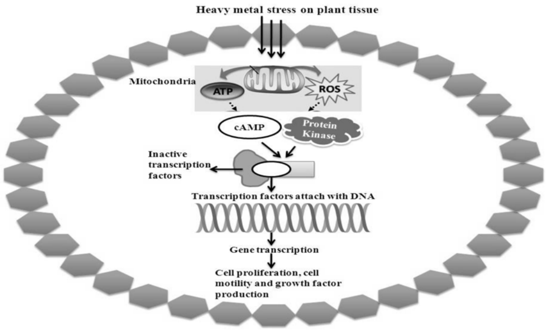
DOI: 10.4018/978-1-5225-9016-3.ch011

absorbed and translocate into plants and easily enter into food chain. Heavy metal contaminants movement from soil to the ground water is very slow because of less mobility so it is not easily absorbed by the plant (Rodriguez et al., 2011; Lori et al., 2015). Therefore heavy metals are low in the upper parts such as leaves, seeds and fruits because of high amount of accumulation in roots (Mishra and dubey, 2005; Ullah et al., 2015). High concentration in soil can also affect to microbial activity with soil fertility (Gao et al., 2010; Yuan et al., 2015). Heavy metals such as Zn, Cu and Mn, Al, Mg are necessary as micronutrients, but high amount of these heavy metals can be caused toxicity and negative effect on human health (Langer et al., 2009; Ali et al., 2013). Some other heavy metals such as Cd, As, Pb, Hg and Ni, are non-nutritional and toxic elements present in soil (Jourand et al., 2010).

Plants under heavy metal stress condition it produces a high level of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), hydroxyl radicals (OH), superoxide radicals (O_2) catalse, which result in damages to plant cell or tissue (Wang et al., 2015; Migocka et al., 2014). Reactive oxygen species produced continuously in different compartments with antioxidant molecule under as by-product (Reddy et al., 2005; Kwankua et al., 2012; Girisha and Ragavendra, 2009; Palma et al., 2013; Dubey et al., 2014). However the critical imbalance and excess amount of production ROS, antioxidant molecule in plant which is depleted and creates disorders in plant enzymatic activity, all type of biochemical molecules including cell wall, membrane lipids, protein, amino acid chain and carbohydrates figure 1 (Kaur et al., 2015; Qiao et al., 2015; Gratao et al., 2005). They also damage cell membrane, loss of cell metabolites, reduction in cell growth and impaired metabolic functions (Goncalves et al., 2007a,b; Lee et al., 2007; Merlot et al., 2014; Morel et al., 2009).

The conventional remediation technologies are cost effective more expensive and some of the techniques did not remove heavy metal effectively. Therefore, it is important to develop economically practical and more effective method to decontaminate soils from heavy metal contamination. The most popular advantage of phytoremediation is low cost effectiveness (Abdul and Schroder, 2009). It can be up to 1000-fold cheaper compared to conventional method such as (flotation-filtration, evaporation, ion-exchange, electrodialysis and ultrafiltration). It has been estimated to clean up one acre of sandy loam soil to a depth of 55 cm will cost 60,000-100,000$ compare to 400,000$ for conventional using traditional soil removal methods (Ali et al., 2013).

Figure 1. Diagramatically representation of inactive transcription factors under heavy metal stress



Microbe assisted phytoremediation technology has gain attention in recent years. Plant-growth promoting bacteria (PGPB) could be very suitable for the phytoextraction processes of heavy metals as well as for growth and development of plant under contaminated soils (Burd et al., 2000; Ullah et al., 2015). Hyper-accumulator plants tolerating or accumulating heavy metal contents in their rhizosphere can be use for cleanup of soils, sediments and water (Linger et al., 2002). Plant-microbe interaction has been promoted the activity of the microbes on the plant growth. It is also suggested that inoculation of mycorrhizae to some plants may promotes the uptake, translocation, accumulation of soil metals (Belliturk et al. 2015; Chanda et al. 2014). Enhancement of phytoremediation efficiency could be done with the help of (PGPR) plant growth promoting bacteria (Huang et al., 2005; (Khan et al., 2012).

Chelating assisted phytoremediaton demonstrated as a possible treatment for remediation of heavy metal contaminated soil and sediment. EDTA enrichment could be an appropriate technique for phytostabilization of Pb-contaminated environments (Ullah et al., 2014). Previous reports suggest that a great number of chelating agents have been used for enhanced phytoextraction (EDTA, citric acid and malic acid). The introduction of EDTA and citric acid as a chelator is considered as important in chelation therapy (Komarek et al., 2007). Ethylene diamine tetra acetic acid was reported as a chelating agent for the enhancement in phytoextraction process (Blaylock et al., 1997). EDTA and citric acid is the well-organized and successful chelating agents for increasing the solubility of heavy metals in soils and has been widely used for the extraction of heavy metals from contaminated soils. EDTA is identified as the superlative chelator to improve the metal mobilization in soils (Xie et al., 2012). Chelates especially EDTA can remove Cu, Zn, Pb and Cd with high efficiency when they use in low amount because drawback of EDTA is that it has poor biodegradability, which can cause leaching of soil (Ortega et al., 2008).

Nanoparticles role to metal uptake by plant with regard to phytoremdiation technology and also distinguished between metal hyperaccumulation. *Brassica juncea* cultivated under given solution with different salts to form silver nanoparticles $AgNO_3$, $Na_3Ag(S_2O_3)_2$, and $Ag(NH_3)_2NO_3$ (Haverkamp and Marshall, 2009). *Cucurbita pepo* plants which were grown in hydroponic solutions on the effect of Ag, Cu, ZnO, and Si nanoparticles on root elongation, seed germination and biomass production (Stampoulis et al., 2009). Cu nanoparticles reduced root length by 77% and 64% relative to untreated controls. Ag nanoparticle increases root length, seed germination and also biomass production (Stampoulis et al., 2009).

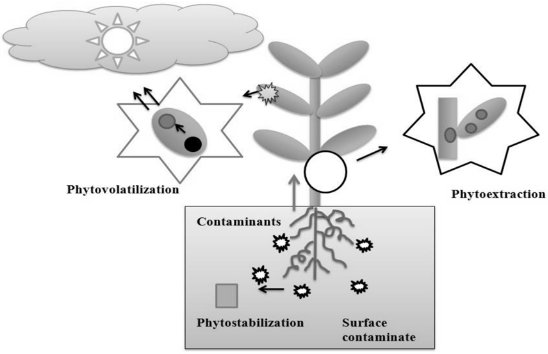
Genes that contribute to metal hyperaccumulation and more highly expressed in the hyperaccumulators as compared to non hyperaccumulators (Iqbal et al., 2013). Significantly progress has been made in the recognition and considerate the role of key components like hyper accumulation of "*HMA2, HMA3, HMA4, MerA MerB*, (*CPx-type*) ATPases, *ZAT1* transpoter that ensure the heavy metal tolerance to the plants (Chaudhary et al., 2016) . The overexpression of the genes is responsible for heavy metal uptake, translocation, and sequestration" may allow the production of plants that can be successfully exploited in phytoremediation. The cloning of different transporters genes are the foundation of genetically engineering a plant via phytoremediation approach. The goal of this review to sustainable development by helping to protect soil and water by using new approaches of phytoremediation for preventing the spread of pollution into the environment. Similarly, about phytoremediation systems apply for accumulate different heavy metals are still poorly understood and also their mechanisms not known perfectly. The contaminants of heavy metals such as Cd, As, Cr, Zn,, Cu, Ni, Mn, Se to be more effective results by applying new phytoremediation approaches involve like Plant growth promoting bacteria (PGPR), chelating compounds, nanoparticle and tolerable genes to accumulate contaminants and also for increasing plant biomass.

CONCEPTS OF PHYTOREMEDIATION

Phytoremediation is the use of plants to remediate contaminated soil and water; it is a low cost effective technique (Singh et al., 2003). Phytoremediation techniques involve such as, phytofiltration, rhizofiltration, phytoextraction, phytostabilization, phytoimmobilisation and phytodegradation, and rhizodegradation figure 2 (Ali et al., 2013). A primary termed of phytoremediation is phytofiltration. Phytofiltration is based on the adsorption and absorption of heavy metal contaminants from water with the help of plant roots (Mukhopadhyay and Maiti, 2010). Another form of involves phytovolatilization is the conversion of the pollutant into volatile form which allowing its escape from the soil into the atmosphere (Prasad and Freitas, 2003). Phytodegradation is also the category of phytoremidiation, metals pollutant are degraded into the small particles which easily uptake by the roots but yet does not apply to heavy metals because it is time consuming (Dixit et al., 2015). Phytostabilization is used for highly polluted areas to provide a complete capable of producing extensive and dense root systems covering compressed soils (Figure 1) and also the restricting the pollutants to the soil zone near their roots by preventing their movement or leaching with a direct implication of the plant being more tolerant of pollutants (Salt et al., 1998; Yao et al., 2012; Dixit et al., 2015). The more efficient and very useful technique is phytoextraction but it is more difficult process to others. In this technology growth of tolerant plants in contaminants soils which uptake large amounts of heavy metals from the soil and translocating into the aerial parts of the plant.

After high levels of heavy metal uptake in the plant organs which are harvested after drying, pollutant containing material dupmed separately and also use to make nanoparticle from this concentration mass material (Dixit et al., 2015; Yao et al., 2012). The treatment of soil using plants as heavy metal uptake and storage by phytoremdiation approach can be distinguished (Yao et al., 2012; Dixit et al., 2015). The depth of soil which can be cleaned or stabilized is restricted to the root zone of the plants being used. Heavy metals are non-biodegrdable and they are very toxic to human health, plant and animals and also very affected to the microorganisms when they are present at higher quantity in the soil. As a result at higher concentration of heavy metals increase toxicity by generating reactive oxygen species which can lead to degradation of macromolecule, DNA damages, cell damage and also affected to ion uptake molecules (Ahmad et al., 2008, 2010, 2011, 2015).

Figure 2. Diagram of Phytoremediation Technology



They also affected the process of photosynthesis by interfering with electron transport chain, water relations, enzymatic and biochemical activities (Rascio and Navari-Izzo, 2011; Ahmad et al., 2008, 2010, 2011, 2015; Qadir et al., 2014).

Heavy metal concentration increases is a consequence of natural activities such as weathering and volcanic eruptions and soil erosion, antropogenic activities, burning, fossil fuels, transport and industrial wastes, smelting and also use some fertilizers which effect on agriculture (Bhargava et al., 2012). They are also affecting biomass yield and somewhat soil fertility under the major condition present of heavy metals accumulation in soils (Bhargava et al., 2012). Phytoremdiation is economically effective againsty metal heavy toxicity (Dixit et al., 2015; Yao et al., 2012). Based on their nature remediation technology can be divided into two main types (ex situ and in situ) method. *Ex situ* method are those which require physical removal or immobilization of contaminated soil included increasing the soil pH to decrease the heavy metal solubility in the soil (Dixit et al., 2015). *Insitu* methods require biological mechanisms at the contaminated site and do not involve soil leaving its original site. However more promising insitu methods to remove toxic metals and chemicals from soil than ex situ method due to insitu method is low cost, easy usage and environmental friendly (Dixit et al., 2015; Salla et al., 2011; John et al., 2009). During the past few decades phytoremediation technologies has fastly grown and many new hyperaccumulator plants were identified.

Hyperaccumulator plants that more uptake and tolerate without visible symptoms a hundred times or greater metal concentrations in shoots than those usually found in non-accumulators (Memon and Schroder, 2009). Some different 450-500 plants have been identified as hyperaccumulator include

Thlaspi caerulescens that accumulate (Pb, Ni, Cd and Zn), *Arabidopsis halleri* that can accumulate high levels og heavy metals (Cd and Zn but not Pb), *Alyssum bertolonii* can uptake (Ni and Co) and some other plants which belong to different family can also be participate to accumulate heavy metals such as *Caryophyllaceae, Fabaceae, Poaceae, Lamiaceae, Asteraceae, Cunoniaceae and Cyperaceae* and many others (Berti et al., 2002; Prasad, 2005; Maestri et al., 2010; Padmavathiamma and Li, 2007). Plants have specific properties that give us some specific advantages to remediate environment (Meagher, 2000; Meagher et al., 2000). Plants absorb metal particle through roots and root hairs that generate surface area through which pollutants can be extracted form contaminated soil and water. Plants are autotrophs, it takes up nutrients directly from environment" in gaseous form with the help of photosynthesis process. Study of four crop plants which belong to *Ipomea alpine, Centella asiatica, Eichlarnia crassipes, Euphorbia maeleocloela, Berkheya coddii, Alyssum and Thlaspi, Zea mays, Pteris vittata, Arabidopsis bisulcatus, Sesbaniia drumnondia, Sedum alfredei, Euphorbia marcoleida Phragnites australis Phytolacca Americana, Astragolosus, craenosus, Cardanine hupingshas and Iberis intermedia* reported as hyperaccumulators for phytoextraction particularly of Cu, Ni, Cd, Zn, Cr, As, Mg, Se and Ti accumulation in shoots (Beath et al., 1937; Baker and walker 1989; Robinson et al., 1997; Leblanc et al., 1999; Sharma et al., 2003; Meers et al., 2005; Dong, 2005; Israr and Sahi, 2006 Colheiros et al., 2008; Jin et al., 2008; Pollard et al., 2009; Jin & Liu, 2009 Bani et al., 2010; Mehdami et al., 2011; Mokhtar et al., 2011; Namatian & Kazemein 2013; Tong et al., 2014).

Same as above were studied for their phytoextraction ability on five willow tree species *Salix triandra* (Noir de villaines), *Salix fragilis* (Belgisch Rood), *Salix purpurea –Salix daphnoides* (Bleu), *Salix schwerinii* (Christina) *and Salix dasyclados* (Loden) results indicated that there is no significant accumulation of Ni, Cr, Pb and Cu in the shoots. Among all five species under study *S. fragilis, S. schwerinii* and *S. dasyclados* Zn and Cd accumulate at higher concentration in the shoots and more potential for phytoextraction (Meers et al., 2007). Recently finding about *Lolium multiflorum* is also used for phytoremdiation of Cu, Mn, Zn and Pb at La Concha (Mugica-Alvarez et al., 2015). The aquatic plant *Hydrilla verticillata* has high uptake capacity and also consider a promising accumulator of Cd and Cr in water reservoirs (Phunkan et al., 2015). *Sauropus androgynus* is perennial plant has the ability to accumulate more toxic metals from the soils and highly effective plant for the removal of heavy metals and also significantly did not decrease by the accumulation of heavy metals and can be used commercially (Xia et al., 2013). Another study reported on *Pannicum virgatum* (Switchgrass) as a metal accumulator for Cd, Cr and Zn and also with high biomass" (Chen et al., 2012). Apart from those mentioned previously there are many other studies that has been demonstrate the potential of plants for accumulation at high amount table. 1 (Ali et al., 2013; Bhargava et al., 2012; Pollard et al., 2014).

INTERFERENCE OF NUTRIENTS

Different kinds of nutrients were involvement including calcium, zinc, magnesium, phosphorus, potassium, iron and cooper (Ribeiro et al., 2013). Heavy metals such as Cu, Zn, Al, Cd, Ni, Hg and As has negative effects on macronutrient and micronutrients (Mariano and Keltjens, 2005; Baligar et al., 1993). Calcium ions serves as secondary messenger in signal transduction and movement in plants is unidirectional. Metal ions uptake from roots and translocated into meristematic zones, young tissue. Calcium is unable to recycle after deposited in the leaves (Hanger, 1979). Heavy metals bind to all calcium binding sites on the cell surface, at low pH (4.5) it work with calcium absorption and uptake calcium by roots (Roy et al., 1988, Hossain et al., 2014). High amount of heavy metals expose to the inhibition of calcium (Hossain et al. 2014; Ribeiro et al., 2013). Inhibit the influx of calcium ion at 100 mM aluminum (Al) (Nichol et al., 1993). Aluminum ions interfere with the action of Guanosine 5' triphosphate binding protein as well as inhibit calcium ion uptake by binding verapamil-specific channel (Rengel and Elliott, 1992). The uptake of calcium ions dcreased in beech plants due to the combination of nitrogen and aluminum at high concentration" (Bengtsson et al., 1994). Under aluminum treatment the concentration of calcium is unaffected in shoots because aluminum enters the plant cells through calcium channel (Hakan et al., 1988; Liu and Luan, 2001).

Potassium channel influx inhibit due to the toxicity of heavy metals like Aluminium. Active pathway involvement of uptake potassium is also inhibited by high concentration of aluminum (Ribeiro et al., 2013; Hossain et al., 2014). At low pH (4.5) concentration of potassium decreases, when they are treated with heavy metals (Moustakes et al., 1995). Potassium ions "decrease in the roots cell as well as guard cell due to the aluminum toxicity which cause by blocking the channels at cytoplasmic site of plamsmembrane (Liu and Luan, 2001). Somewhat significantly increase potassium ion from barley roots under aluminum stress (Kasai et al., 1992). Potassium ions increase in root and shoots with increase in aluminum concentration in pine trees (Huang and Bachelard, 1993). Durum wheat is more tolerant against aluminum toxicity and also decrease in potassium ion concentration with winter wheat (Zsoldos et al., 2000).

Magnesium affected by heavy metals stress more than other nutrient parameters of plant (Wheeler and Follett, 1991). Plant treated with different concentration of heavy metals and the level of magnesium decreases at pH (4.5). Magnesium uptake trough roots much stronger than calcium uptake (Bose et al., 2011; Bose et al., 2013). Concentration decreased of magnesium ions in roots and shoots by increasing the concentration of heavy metals (Huang and Bachelard, 1993). Iron concentration decreases at a pH of 4.5 when they are treated with different concentration of heavy metals and also significantly much affected root growth (Moustakas et al., 1995). Some researchers focus on nutrition level value which really affected by heavy metals. The amount of nitrogen accumulated in roots decreases as a result of higher amount of aluminum exposure as well as translocate to aerial parts of plants (Purcino et al., 2003; Gomes et al., 1985). Plants exposed to higher amount of heavy metals results to decrease the amount of lipooxgenase enzyme, ROS, antioxidant enzymes and also decline physiological effect (Wang and Yang, 2005).

Soils have high amount of heavy metals stress than it have low amount of phosphorus and positively correlated with aluminum (Liao et al., 2006). The concentration of metals is present in low amount than it has been increase such micronutrients and also macronutrients but if it is present more than requirement of plant result to cause toxicity and also decreases the level of phosphorus (Nichol et al., 1993, Cumming et al., 1986).

HEAVY METAL TRANSPORATION PATHWAY

Modern lifestyle and industrial contaminants have effect on environmental problem due to generate different kinds of wastes and dumping out without given any proper treatment. They have most crucial lethal effects on living entities, environment and normal metabolic activities of the organisms (Ahmad et al., 2011, 2015; Qadir et al., 2014). The liberate of these heavy metals in the environment which imbalance of the natural environment. The most effective treatment to the contaminated water and soil on the basis of cost or energy ways need to be discovered and brought into use on the economic way also has been found to be phytoremediation (Dixit et al., 2015; Ma et al., 2011; Greipsson, 2011; Kamran et al., 2014). Plants have the ability to store metals in their cells and organs. There are multiple steps involve to uptake of heavy metals from the soil into roots, from where there are translocated through different transporters" to aerial parts of the plant (fig. 11.2) (Mahmood, 2010). There are "three major steps" by which plants accumulate the heavy metals from the soil through roots, "second one is translocation of heavy metals from roots to shoots through xylem loading and third is the sequestration of heavy metals in leaves particularly in vacuoles" (Bhargava et al., 2012). The second process is the translocation from roots to shoots, non-hyperaccumulators do not have the ability to translocate "heavy metals to above-ground parts, so this process in nonhyperaccumulators does not occur in them and heavy metals are stored in roots. Transport of heavy metals from "roots to shoots occurs by the movement of metals into xylem channels" through the process known as xylem loading figure 2 (Bhargava et al., 2012).

The pH of the soil is maintained by the release of number of protons, which are controlled by proton pumps located in the outer membranes of the plant and can acidify the soil, thus lowering its pH. Previous findings on Alyssium murale showed the increase in Ni accumulation due to changes in pH (Bernal et al., 1994; Abollino et al., 2006). Heavy metals transfer from soil to root; root to shoot through xylem tissues and need to be transfer from roots to leaves and vice versa. It is transfer through symplast pathway where they are energy consuming process that allows all non essential heavy metals. This is due to normal transportation intracellular movement pathway block by casparian strip in the endodermis. Hence the movement of heavy metals through apoplast pathway gets blocked which means that the only way to enter xylem vessels is by taking the symplast pathway figure 2 (Mahmood, 2010).

The soil microbes can increases the bioavailability of metals in the soil be secreting such ions, organic acids and other types of natural chelants. Microorganisms in rhizosphere such as bacteria and and mycorrhizal fungi are involved in increasing the solubility of heavy metals in soil (Yang et al., 2005). Different plants have different mechanisms for the uptake of different heavy metals and variations in the transport pathways are observerd. There are two pathways by which they can enter into the roots. First is apoplastic pathway and second one is the symplastic pathway. The apoplastic pathway allows the soluble metals to travel without entering the cells and also through intracellular spaces (figure 2). On the other hand symplast pathway allows the movement of nonessential metals like Ni, Cd, and Pb through the cytoplasm by consuming energy (Lombi et al., 2002; Lu et al., 2009). This paper need for phytoremdiation based selctions, how plants can accumulate metals in roots, shoots, leaves, seeds and fruits which are assisted phytoextraction that uses plant growth promoting bacteria (PGPB), chelanting agent, nanoparticles to increase the plants accumulation capacity". Furthermore the mechanism of genes to identify to enhance the process of accumulation with the involvement of biotechnology approaches.

NANOPARTICLE

Nanoparticle formation in modern nanotechnology is one of the most exciting areas of research in the biological invention. Plants have naturally capacity to uptake metal through various metabolic pathways. Apart from this naturally activity silver nanoparticle produces *invitro* condition through seedlings of *Brassica juncea*" (Shekhawat and Arya, 2009). Two weeks old seedlings dip into nutrient solution with silver nitrate, after seven days plants were "harvested and analyzed under UV-VIS spectrophotometer and also silver nanoparticles confirmed by transmission electron microscopy. Reduction of metals ions Ag^+ and $Au3^+$ to Ag^0 and Au^0 metal nanoparticle in *Brassica juncea* (Beattie and Haverkamp, 2011). Total metal content by atomic absorption spectroscopy and through X-ray absorption spectroscopy chemical state of the both metal was analysed. At concentration of 0.40% Ag and 0.44% Au naoparticle in the roots, stem, leaves and cell walls of the plants. Metal salts to nanoparticle were chloroplasts, regions of high reducing sugars like glucose and fructose content is the most abundant reduction at that sites. Above statement given by authors that sugar are responsible for the reduction of heavy metals". Biosynthesis of gold nanoparticles in aqueous medium by using *Terminalia catappa* leaf extract (Ankamwar, 2010). Rapid reduction of chloroaurate ions (with leaf extract) was observed to the formation of highly stable gold nanoparticle in solution with the treating chloroauric acid. Gold nanoparticles ranged in size from 10 to 35 nm with mean size of 21.9 nm.

Biosynthesis processes for nanoparticle would be useful when nanoparticle were produced using plants according to their size, dispersity and shape (Kumar and Yadav, 2009). Plants can use for the synthesis of nanoparticles upto large-scale suitablely. Different known plant species which can form different silver and gold nanoparticles such as *Medicago sativa, Cicer arietinum, Avena sativa, Triticum aestivum, Pelargonium graveolens, Emblica officinalis, Tamarindus indica, Azadirachta indica, Cinnamommum camphora, Cymbopogon flexuosus, Aloe vera* etc (Kumar and Yadav, 2009). Nanoparticles role to metal uptake by plant with regard to phytoremdiation technology and also distinguished between metal hyperaccumulation. *Brassica juncea* cultivated under given solution with different salts to form silver nanoparticles $AgNO_3$, $Na_3Ag(S2O3)2$, and $Ag(NH_3)_2NO_3$ (Haverkamp and Marshall, 2009). High levels of silver nanoparticle obtained only by the concentration of metal salts within the plant not to deposition of metal and there is a limit on the amount of metal nanoparticle that may be deposited about (0.35 wt%) Ag to dry plant. Metal nanoparticle accumulation on the range of metals present in plant and controlled by the reducing capacity of the plant and also reactions occurring at an electrochemical potential greater than zero volts. Silver nanoparticles have high electrochemical reduction potential and they exhibit good catalytic properties. On the other hand, silver is used as a model compound due to this metal not only can form metal nanoparticles in plants but high levels of silver have been achieved in plants and they also have useful properties (Li and Xing, 2007). *Cucurbita pepo* plants which were grown in hydroponic solutions on the effect of Ag, Cu, ZnO, and Si nanoparticles on root elongation, seed germination and biomass production (Stampoulis et al. 2009). Cu nanoparticles reduced root length by 77% and 64% relative to untreated controls an seed germination was unaffected by given any of the treatment respectively.

Biomass of plants reduced to Cu (90%) to compare with control, Ag reduced by (75%)and they also observed phytotoxicity with treatment of nanoparticle (Stampoulis et al., 2009). Some other effect of metal nanoparticle such as Al, ZnO, Zn on the root growth of six plant species radish, lettuce, corn, rape, cucumber and rye-grass and also seed germination (Li and Xing, 2007). Inhibition of root growth of plants greatly varied among different nanoparticle. Seeds germination was affected only in case of ryegrass otherwise it was not affected for the inhibition of nanoscale of zinc or zinc oxide on corn at 2000 mg/L. Fifty percent inhibitory concentrations (IC50) of nano-Zn and nano-ZnO were estimated to be near 50 mg/L for radish, and about 20 mg/L for rape and ryegrass. The inhibition occurred during the seed incubation process rather than seed soaking stage (Li and Xing, 2007). Core-shell copper oxide nanoparticles effect on *Chlamydomonas reinhardtii* green alga on the change of primary photochemistry of photosystem II, cellular population structure and the formation of reactive oxygen species (Saison et al., 2010). Algal cultures were exposed to 0.004, 0.01 and 0.02 g/L of core-shell copper oxide nanoparticles for 6 h. It was found induced cellular aggregation processes and had a deteriorative effect on chlorophyll by inducing the photo inhibition of photosystem II and also the inhibition of photosynthetic electron transport pathways. However, "reactive oxygen species was not formed when *C. reinhardtii* was exposed to the core without the shell or to the shell only. It should be noticed, that not only vascular plants but also microorganisms such as bacteria, yeasts, algae, fungi and actinomycetes can be used for the biosynthesis of nanoparticles (Sastry et al. 2003). However there is a remarkable lack of information on some key aspects, which prevents a better understanding and assessment of the toxicity and ecotoxicity of nanoparticles, especially engineered nanoparticles to living organism's vascular as well as non-vascular plants (Navarro et al., 2008).

MOLECULAR APPROACHES GENES

Genes to be selected for manipulation is based on the involvement in three aspects (metal ion transporation, protein involved in metal chelation and last one is metabolic enzymes) involved in detoxifying of metal contaminant (Chaturvedi et al., 2014). Manipulation of several genes will be required to increase phytoremdiation potential (Singh et al., 2015). Transgenic plants improved to metal uptake have been developed for Zn, Cd, Hg, As, Pb and Se and exceptional gene tank for novel insights into molecular mechanisms towards environment (Cracium et al., 2012). Cloning of different metallothionein (MT) genes and introduce into suitable plant species can result to elevated metal uptake. Tobacco and oil seeds enhanced Cd tolerance were involved through transfer of human MT-2 gene, whereas Mt-gene transfer from pea resulted in copper (Cu) tolerance in *A. thaliana* and tobacco (Kumar et al., 2012). Glutathione (GSH) synthesis has been correlated with copper and cadmium tolerance in *A. thaliana* under expression of MT gene and elevated of transcription of genes. Genes transferred in *Brassica sp. Arabidopsis thaliana and Nicotiana tabacum* to elevated of Cd tolerance demonstrated in constitutive expression of various MT genes like MTI, human MTIA, MTII, pea PsMTA, yeast CUPI and TaMT3. Recently studies that overexpressing BcMT1 and BcMT2 gene from *Brasssica* campestris in *Arabiodopsis*; transgenic tobacco plants SbMT-2 gene enhances Cd and Cu under heavy metal stress and accumulated reactive oxygen species (Chaturvedi et al., 2014).

Transgenic plant technology utilized to improve the performance of metal uptake from contaminated soil (Kopp et al. 2001; Pilon-Smit and Pilon, 2002; Berken et al., 2002). Insertion of DNA into plant genome to produced a transgenic plant with desired trait with the help of genetic engineering. Biotechnology has been performed best role to illuminate biochemical and genetic mechanisms for the process of phytoremdiation to make use natural characterstics or enhance plants with novel character from other organisms (Hirschi et al., 2000; Pilon-Smits and Pilon, 2002; Cohen, Gravin and

Kochain, 2004). Transgenic plants have novel character from contaminated resistant microorganisms to enhanced growth and degradation of organic and inorganic pollutants. Transgenic plants that convert hazardous organomercurial compounds and toxic ionic mercury (Hg(II)) into less toxic and volatile elemental mercury Hg through degradation pathways that occur naturally in some bacteria not in all plants was demonstrated a successful engineering strategy (Heaton et al., 1998; Kramer and Chardonnens, 2001; Rugh, 2001). Isolate microorganisms have mercury resistance from mercury contaminated soil, which is genetically encoded by the *mer* operon containing a cluster of genes involved in the mobilization, and enzymatic detoxification of mercury, and specifically contains the *merB* and *merA* genes that code for the mercury processing enzymes organomercurial lyase and mercuric ion reductase" respectively (Summers, 1986; Rugh et al., 1998).

Subsequent research further improved the efficiency of this process in transgenic plants by modifying the bacterial *merB* gene to target the MerB protein for accumulation in the endoplasmic reticulum and for secretion to the cell wall (Bizily et al., 1999). The integration of both genes (*merA* and *merB*) into chloroplast genome of tobacco plants (*Nicotiana tabacum*) produced transgenic plants capable to tolerate high amount of phenylmercuric acetate, an organomercurial compound and more uptake heavy metals (Ruiz et al., 2003). The molecular mechanism conferring the differential tolerance in plants to metal toxicity and several tools are being used for genomic approach (Migocka et al., 2014; Gustin et al., 2011). In *A. thaliana expression* of GSH synthesis genes GSH1 and GSH2 under Cd stress (Semane et al., 2007; Wojcik and Tukiendorf, 2011). Different number of genes has been reports to signifying that overexpression of phytochelatin (PC) synthase gene to improve heavy metals tolerance under stress (Liu et al., 2011). Genes encoding bivalent cation transporters belonging to HMAs (among which HMA4) are overexpressed in roots and shoots of Zn and Cd" hyperaccumulators *T. caerulescens* and *A. halleri* (Papoyan and Kochian, 2004; Mils et al., 2003; Hanikenne, et al., 2008).

Heavy metals are the main groups of pollutants and they effect on human health and progress in the molecular mechanism of plant stress response to heavy metals have made, especially in herbaceous plants such as *Arabidopsis halleri*, *Arabidopsis thaliana* and *Triticum caerulescens*. *A. thaliana* has always played a very important role in uncovering the molecular mechanism of plant response to pollutants as its genome information is available. The most closely connected to members of the *A. thaliana* is P1B-type". *HMA2* is also involved in the translocation of "Zn and Cd in *A. thaliana*, barley, rice, and wheat. In *Arabidopsis*, the cellular and subcellular patterns of *AtHMA2* expression were similar to those of *AtHMA4*. *HMA2p-GUS* expression was observed primarily in the vascular tissues of the leaf, root, stem and *HMA2*-GFP proteins were also localized in the plasma membrane. Recently, findings on the characterization of the *HMAs* 2 gene from the different plants for possible application in phytoremediation approach (Chaudhary et al., 2016).

ADVANTAGES OF PHYTOREMEDIATION TECHNOLOGY

Phytoremediation have few advantages are Variety of organic and inorganic compounds is willing to the phytoremediation process. Phytoremediation can be used either as an *in situ* or *ex situ* application. It is a green technology, and environmentally friendly to the public. Phytoremediation does not require expensive equipment and it is relatively easy to implement. The greatest advantage of phytoremediation is its low cost compared to conventional clean-up technologies was estimated at $60,000-$100,000 compared to $400,000 for the conventional method. Disposal sites are not needed. It is more likely to be accepted by the public. It avoids excavation and transport of polluted media.

DRAWBACK AND LIMITATIONS OF PHYTOREMEDIATION

Phytoremediation does have a few disadvantages and limitations that are: It is restricted to the rooting depth of plants. Remediation with plants is a lengthy process, thus it may take several years or longer to clean up a hazardous waste. It can affect biodiversity. The consumption of contaminated plants by wildlife is also of concern. Unfavorable climate is another important consideration because it can limit plant growth and phytomass production.

CONCLUSION

Phytoremdiation is a cost effective and attractive technique that has emerged over recent years. This technology helps for the development of plants with the higher potentially to clean our environment by giving favorable condition. Fast growing technology focus on molecular biology to design such transgenic plant species to more accumulate contaminants and to identify gene for resistance against such toxicable compounds. As these transporter gene increases the power to tolerant heavy metal stress to clean up the environment successfully. Numbers of companies have started their business in phytoremediation approach to apply for clean up the environment and also increase the biomass production. Understanding the “signaling mechanism of transporter genes will be an important tool” to understand the genetics of hyperaccumulation. Genetic engineering should focus more on vaculor sequestration which is an important component of metal hypertolerance, this mechanism necessary for hyperaccumulation” and also efforts for improving root uptake, vacuolar transport, xylem loading and mobilization of heavy metals. This information will also be help to improve the productivity of bioenergy crops. However, many plants lack these properties. Thus, the possibility to produce new plants through transgenic methods is a good choice for excellent phytoremediation.

REFERENCES

Abdul, M. R., & Schroder, P. (2009). Implications of metal accumulation mechanisms to phytoremediation. *Environ. Sci.Pollution Resear.*, *16*(2), 162–175. doi:10.100711356-008-0079-z PMID:19067014

Abollino, O., Giacomino, A., Malandrino, M., Mentasti, E., Aceto, M., & Barberis, R. (2006). Assesssment of metal availability in a contaminated soil by sequential extraction. *Water, Air, and Soil Pollution*, *173*(1-4), 315–338. doi:10.100711270-005-9006-9

Ahmad, P., Jaleel, C. A., Salem, M. A., Nabi, G., & Sharma, S. (2010). Roles of enzymatic and non-enzymatic antioxidants in plants during abiotic stress. *Critical Reviews in Biotechnology*, *30*(3), 161–175. doi:10.3109/07388550903524243 PMID:20214435

Ahmad, P., Nabi, G., & Ashraf, M. (2011). Cadmium-induced oxidative damage in mustard [*Brassica juncea (L.) Czern. & Coss.*] plants can be alleviated by salicylic acid. *South African Journal of Botany*, *77*(1), 36–44. doi:10.1016/j.sajb.2010.05.003

Ahmad, P., Sarwat, M., Bhat, N. A., Wani, M. R., Kazi, A. G., & Tran, L. S. P. (2015). Alleviation of cadmium toxicity in *Brassica juncea L.* (Czern & Coss.) by calcium application involves various physiological and biochemical strategies. *PLoS One*, *10*(1), e0114571. doi:10.1371/journal.pone.0114571 PMID:25629695

Ahmad, P., Sarwat, M., & Sharma, S. (2008). Reactive oxygen species, antioxidants and signaling in plants. *Journal of Plant Biology*, *51*(3), 167–173. doi:10.1007/BF03030694

Ahmed, M., & Malik, A. (2011). Bioaccumlation of heavy metals by zinc resistant bacteria isolated from agri-cultural soils irrigated with wastewater. *Bacteriol. J.*, *2*(1), 12–21. doi:10.3923/bj.2012.12.21

Akhter, M. F., Omelon, C. R., Gordon, R., Moser, D., & Macfie, S. M. (2014). Localization and chemical sp eciation of cadmium in the roots of barley and lettuce. *Environmental and Experimental Botany*, *100*, 10–19. doi:10.1016/j.envexpbot.2013.12.005

Aldoobie, N. F., & Beltagi, M. S. (2013). Physiological, biochemical and molecular responses of common bean (*Phaseolus vulgaris L.*) plants to heavy metals stress. *African Journal of Biotechnology*, *12*(29), 4614–4622. doi:10.5897/AJB2013.12387

Alexandre, G., Greer, S. E., & Zhulin, I. B. (2000). Energy taxis is the dominant behavior in Azospirillum brasilense. *Journal of Bacteriology*, *182*(21), 6042–6048. doi:10.1128/JB.182.21.6042-6048.2000 PMID:11029423

Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals-concepts and applications. *Chemosphere*, *91*(7), 869–881. doi:10.1016/j.chemosphere.2013.01.075 PMID:23466085

Ankamwar, B. (2010). Biosynthesis of gold nanoparticles (green-gold) using leaf extract of *Terminalia catappa. E-Journal of Chemistry*, *7*(4), 1334–1339. doi:10.1155/2010/745120

Baker, A. J. M., & Brooks, R. R. (1989). Terrestrial higher plants which hyperaccumulate metallic elements– a review of their distribution, ecology and phytochemistry. *Biorecovery*, *1*, 81–126.

Baker, A. J. M., & Walker, P. L. (1989). Ecophysiology of metal uptake by tolerant plants. In *Heavy metal tolerance in plants: Evolution aspects* (pp. 155–177). CRC Publishers.

Baligar, V. C., Shaffert, R. E., Dos Santos, H. L., Pitta, G. V. E., Bahia Filho, A. F., & De, C. (1993). Soil aluminium effects on uptake influx and transport of nutrients in sorghum genotypes. *Plant and Soil*, *150*(2), 271–277. doi:10.1007/BF00013024

Bani, A., Pavlova, D., Echevarria, G., Mullaj, A., Reeves, R. D., Morel, J. L., & Sulc¸e, S. (2010). Nickel hyperaccumulation by the species of *Alyssum and Thlaspi* (Brassicaceae) from the ultramafic soils of the Balkans. *Botanica Serbica*, *34*(1), 3–14.

Barabasz, A., Wilkowska, A., Tracz, K., Ruszczyñska, A., Bulska, E., Mills, R. F., ... Antosiewicz, D. M. (2013). Expression of HvHMA2 in tobacco modifies Zn-Fe-Cd homeostasis. *Journal of Plant Physiology*, *170*(13), 1176–1186. doi:10.1016/j.jplph.2013.03.018 PMID:23664582

Beath, Q. A., Eppsom, H. F., & Gilbert, G. S. (1937). Selenium distribution in and seasonal variation of vegetation type occurring on seleniferous soils. *Journal of the American Pharmaceutical Association*, *26*, 394–405.

Beattie, I. R., & Haverkamp, R. G. (2011). Silver and gold nanoparticles in plants: Sites for their reduction to metal. *Metallomics*, *6*(6), 628–632. doi:10.1039/c1mt00044f PMID:21611658

Beattie, I. R., & Haverkamp, R. G. (2011). Silver and gold nanoparticles in plants: Sites for their reduction to metal. *Metallomics*, *6*(6), 628–632. doi:10.1039/c1mt00044f PMID:21611658

Belimov, A. A., Hontzeas, N., Safronova, V. I., Demchinskaya, S. V., Piluzza, G., Bullitta, S., & Glick, B. R. (2005). Cadmium-tolerant plant growth promoting rhizobacteria associated with the roots of Indian mustard (*Brassica juncea L. Czern.*). *Soil Biology & Biochemistry*, *37*(2), 241–250. doi:10.1016/j.soilbio.2004.07.033

Belliturk, K., Shrestha, P., & Gorres, J. H. (2015). The importance of phytoremdition of heavy metal contaminated soil using vermicompost for sustainable agriculture. *J. Rice Res.*, *3*, 114.

Bendtsson, B., Asp, H., & Jensn, P. (1994). Uptake and distribution of calcium and phosphorus in beech (*Fagus sylvatica*) as influences by aluminum and nitrogen. *Tree Physiology*, *14*(1), 63–73. doi:10.1093/treephys/14.1.63 PMID:14967634

Berken, A., Mulholland, M., LeDuc, D. L., & Terry, N. (2002). Genetic engineering of plants to enhance selenium phytoremediation. *Critical Reviews in Plant Sciences*, *21*(6), 567–582. doi:10.1080/0735-260291044368

Berken, A., Mulholland, M., LeDuc, D. L., & Terry, N. (2002). Genetic engineering of plants to enhance selenium phytoremediation. *Critical Reviews in Plant Sciences*, *21*(6), 567–582. doi:10.1080/0735-260291044368

Bernal, M., McGrath, S., Miller, A., & Baker, A. (1994). Comparision of the chemical changes in the rhizosphere of the nickel hyperaccumulator Alyssum murale with the non-accumulator *Raphanus sativus. Plant and Soil*, *164*(2), 251–259. doi:10.1007/BF00010077

Berti, W. R., & Cunningham, S. D. (2000). Phytostabilization of metals. In I. Raskin & B. D. Ensley (Eds.), *Phytoremediation of toxic metals - using plants to clean-up the environment* (pp. 71–88). New York: John Wiley & Sons, Inc.

Bhargava, A., Carmona, F. F., Bhargava, M., & Srivastava, S. (2012). Approaches for enhanced phytoextraction of heavy metals. *Journal of Environmental Management*, *105*, 103–120. doi:10.1016/j.jenvman.2012.04.002 PMID:22542973

Bizily, S. P., Rugh, C. L., Summers, A. O., & Meagher, R. B. (1999). Phytoremediation of methylmercury pollution: *merB* expression in *Arabidopsis thaliana* confers resistance to organomercurials. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(12), 6808–6813. doi:10.1073/pnas.96.12.6808 PMID:10359794

Blaylock, M. J., Salt, D. E., Dushenkov, S., Zakarova, O., Gussman, C., Kapulnik, Y., ... Raskin, I. (1997). Enhanced Accumulation of Pb in indian mustard by soil- applied chelating Agents. *Environmental Science & Technology*, *31*(3), 860–865. doi:10.1021/es960552a

Bose, J., Babourina, O., & Rengel, Z. (2011). Role of magnesium in alleviation of aluminium toxicity in plants. *Journal of Experimental Botany*, *62*(7), 2251–2264. doi:10.1093/jxb/erq456 PMID:21273333

Bose, J., Babourina, O., Shabala, S., & Rengel, Z. (2013). Low-ph and aluminium resistance in *Arabidopsis* correlates with high cytosolic magnesium content and increase magnesium uptake by plant roots. *Plant & Cell Physiology*, *54*(7), 1093–1104. doi:10.1093/pcp/pct064 PMID:23620479

Burd, G. I., Dixon, D. G., & Glick, B. R. (2000). Plant growth promoting bacteria that decrease heavy metal toxicity in plants. *Canadian Journal of Microbiology*, *46*(3), 237–245. doi:10.1139/w99-143 PMID:10749537

Calheiros, C. S. C., Rangel, A. O. S. S., & Castro, P. M. L. (2008). The effects of tannery waste water on the development of different plant species and chromium accumulation in *Phragmites australis. Archives of Environmental Contamination and Toxicology*, *55*(3), 404–414. doi:10.100700244-007-9087-0 PMID:18214580

Chaturvedi, R., & Archana, G. (2014). Cytosolic expression of synthetic phytochelatin and bacterial metallothionein genes in Deinococcus radiodurans R1 for enhanced tolerance and bioaccumulation of cadmium. *Biometals*, *27*(3), 471–182. doi:10.100710534-014-9721-z PMID:24578153

Chaudhary, K., Sumira, J., & Khan, S. (2016). Heavy metal ATPase (HMA2, HMA3 & HMA4) genes in hyperaccumulation mechanism of heavy metals. *Plant metal interaction (Emerging Remediation Techniques)*, 545-552.

Chen, B. C., Lai, H. Y., & Juang, K. W. (2012). Model evaluation of plant metal content and biomass yield for the phytoextraction of heavy metals by switchgrass. *Ecotoxicology and Environmental Safety*, *80*, 393–400. doi:10.1016/j.ecoenv.2012.04.011 PMID:22541831

Cohen, C. K., Garvin, D. F., & Kochian, L. V. (2004). Kinetic properties of a micronutrient transporter from *Pisum sativum* indicate a primary function in Fe uptake from the soil. *Planta*, *218*(5), 784–792. doi:10.100700425-003-1156-7 PMID:14648120

Cracium, A. R., Meyer, C. L., Chen, J., Roosens, N., DeGroodt, R., & Hilson, P. (2012). Varaiations in HMA4gene copy number and expression among *Noccaea caerulescens* populations presentating different levels of Cd tolerance and accumulation. *Journal of Experimental Botany*, *63*(11), 4179–4189. doi:10.1093/jxb/ers104 PMID:22581842

Cumming, J. R., Eckert, R. T., & Evans, L. S. (1986). Effect of aluminium on ^{32}P uptake and translocation by red spruce seedlings. *Canadian Journal of Forest Research*, *16*(4), 864–867. doi:10.1139/x86-152

Dixit, R., Malaviya, D., Pandiyan, K., Singh, U. B., & Sahu, A. (2015). Bioremediation of heavy metals from soil and aquatic environment: An overview of principles and criteria of fundamental processes. *Sustainability*, *7*(2), 2189–2212. doi:10.3390u7022189

Dong, R., Formentin, E., Losseso, C., Carimi, F., Benedetti, P., Terzi, M., & Schiavo, F. L. (2005). Molecular cloning and characterization of a phytochelatin synthase gene, *PvPCS1*, from *Pteris vittata* L. J. *Ind. Microbiol. Biol.*, *32*(11-12), 527–533. doi:10.100710295-005-0234-1 PMID:15918023

Dubey, S., Shri, M., Misra, P., Lakhwani, D., Bag, S. K., Asif, M. H., & Chakrabarty, D. (2014). Heavy metals induce oxidative stress and genome-wide modulation in transcriptome of rice root. *Functional & Integrative Genomics*, *14*(2), 401–417. doi:10.100710142-014-0361-8 PMID:24553786

Gao, S., Ou-yang, C., Tanga, L., Zhub, J. Q., Xua, Y., Wang, S. H., & Chen, F. (2010). Growth and antioxidant responses in Jatropha curcas seedling exposed to mercury toxicity. *Journal of Hazardous Materials*, *182*(1-3), 591–597. doi:10.1016/j.jhazmat.2010.06.073 PMID:20655143

Girisha, S. T., & Ragavendra, V. B. (2009). Accumulation of heavy metals in leafy vegetables grown in urban areas by using sewage water and its effect. *Arc. Phytopathol. Plant Protect.*, *42*(10), 956–959. doi:10.1080/03235400701543806

Glick, B. R. (1995). The enhancement of plant growth by freelivingbacteria. *Canadian Journal of Microbiology*, *41*(2), 109–117. doi:10.1139/m95-015

Gomes, M. M. S., Cambraia, J., Sant'anna, R., & Estevão, M. M. (1985). Aluminium effects on uptake and translocation of nitrogen in sorghum (*Sorghum bicolor, L. Moench*). *Journal of Plant Nutrition*, *8*(6), 457–465. doi:10.1080/01904168509363360

Goncalves, A. C., Antas, S. E., & Nunes, M. L. (2007a). Freshness and quality criteria of iced farmed Senegalese sole (*Solea senegalensis*). *Journal of Agricultural and Food Chemistry*, *55*(9), 3452–3461. doi:10.1021/jf0632942 PMID:17417866

Goncalves, J. F., Becker, A. G., Cargnelutti, D., Tabaldi, L. A., Pereira, L. B., Battisti, V., ... Schetinger, M. R. C. (2007b). Cadmium toxicity causes oxidative stress and induces responses of the antioxidant system in cucumber seedlings. *Brazilian Journal of Plant Physiology*, *19*(3), 223–232. doi:10.1590/S1677-04202007000300006

Gratao, P. L., Polle, A., Lea, P. J., & Azevedo, R. A. (2005). Making the life of heavy metal-stressed plant a little easier. *Functional Plant Biology*, *32*(6), 481–494. doi:10.1071/FP05016

Greipsson, S. (2011). Phytoremediation. *Nat. Educ. Knowl.*, *3*(10), 7.

Gustin, J. L., Zanis, M. J., & Salt, D. E. (2011). Structure and evolution of the plant cation diffusion facilitate or family of ion transporters. *BMC Evolutionary Biology*, *11*(1), 76. doi:10.1186/1471-2148-11-76 PMID:21435223

Hakan, A. S. P., Bengtsson, B., & Jensn, P. (1988). Growth and cation uptake in spruce (Picea abies Karst.) grown in sand culture with various aluminium contents. *Plant and Soil*, *111*(1), 127–133. doi:10.1007/BF02182046

Hanger, B. C. (1979). The movement of calcium in plants. *Communications in Soil Science and Plant Analysis*, *10*(1-2), 171–193. doi:10.1080/00103627909366887

Heaton, A. C. P., Rugh, C. L., Wang, N.-J., & Meagher, R. B. (1998). Phytoremedation of mercury- and methylmercury-polluted soils using genetically engineered plants. *J. Soil Contam.*, *7*(4), 497–509. doi:10.1080/10588339891334384

Heverkamp, R. G., & Marshall, A. T. (2009). The mechanism of metal nanoparticle formation in plants: Limits on accumulation. *Journal of Nanoparticle Research*, *6*(6), 1453–1463. doi:10.100711051-008-9533-6

Heverkamp, R. G., & Marshall, A. T. (2009). The mechanism of metal nanoparticle formation in plants: Limits on accumulation. *Journal of Nanoparticle Research*, *6*(6), 1453–1463. doi:10.100711051-008-9533-6

Hirschi, K. D., Korenkov, V. D., Wilganowski, N. L., & Wagner, G. J. (2000). Expression of Arabidopsis CAX2 in tobacco: Altered metal accumulation and increased manganese tolerance. *Plant Physiology*, *124*(1), 125–133. doi:10.1104/pp.124.1.125 PMID:10982428

Hossain, M. A., Ashrafuzzaman, M., Hossain, A. K. M. Z., Ismail, M. R., & Koyama, H. (2014). Role of accumulated calcium in alleviating aluminum injury in wheat plants. *The Scientific World Journal*, 457187. PMID:24701169

Huang, J., & Bachelard, E. P. (1993). Effects of aluminium on growth and cation uptake in seedlings of Eucalyptus mannifera and Pinus radiate. *Plant and Soil*, *149*(1), 121–127. doi:10.1007/BF00010769

Huang, X. D., El-Alawi, Y., Gurska, J., Glick, B. R., & Greenberg, B. M. (2005). A multiprocess phytoremediation system for decontamination of persistant total petroleum hydrocarbons (TPHs) from soils. *Microchemical Journal*, *81*(1), 139–147. doi:10.1016/j.microc.2005.01.009

Israr, M., Sahi, S. V., & Jain, J. (2006). Cadmium accumulation and antioxidative responses in the *Sesbania drummondii* callus. *Archives of Environmental Contamination and Toxicology*, *50*(1), 121–127. doi:10.100700244-005-5029-x PMID:16247566

Jin, X., Yang, X., Islam, E., Liu, D., & Mahmood, Q. (2008). Effects of cadmium on ultrastructure and antioxidative defense system in hyperaccumulator and nonhyperaccumulator ecotypes of *Sedum alfredii* Hance. *Journal of Hazardous Materials*, *156*(1-3), 387–397. doi:10.1016/j.jhazmat.2007.12.064 PMID:18242844

Jourand, P., Ducousso, M., Reid, R., Majorel, C., Richert, C., Riss, J., & Lebrun, M. (2010). Nickel-tolerant ectomycorrhizal Pisolithus albus ultramafic ecotype iso-lated from nickel mines in New Caledonia strongly enhance growth of thehost plant Eucalyptus globulus at toxic nickel concentrations. *Tree Physiology*, *30*(10), 1311–1319. doi:10.1093/treephys/tpq070 PMID:20688880

Kamran, M. A., Amma Mufti, R., Mubariz, N., Syed, J. H., Bano, A., Javed, M. T., ... Chaudhary, H. J. (2014). The potential of the flora from different regions of Pakistan in phytoremeidtaion: A review. *Environmental Science and Pollution Research International*, *21*(2), 801–812. doi:10.100711356-013-2187-7 PMID:24091528

Kasai, M., Sasaki, M., Yamamoto, Y., & Matsumoto, H. (1992). Aluminum stress increases Kp efflux and activities of ATP- and PPi dependent Hp pumps of tonoplast-enriched membrane vesicles from barley roots. *Plant & Cell Physiology*, *33*(7), 1035–1039.

Kaur, G., Singh, H. P., Batish, D. R., & Kohli, R. K. (2015). Adaptations to oxidative stress in Zea mays roots under short-term Pb exposure. *Biologia.*, *70*(2), 190–197. doi:10.1515/biolog-2015-0023

Khan, A. G., Kuek, T. M., & Chaudhary, C. S. (2012). Role of plants mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere*, *41*(1-2), 197–207. doi:10.1016/S0045-6535(99)00412-9 PMID:10819202

Komarek, M., Tlustos, P., Szakova, J., Chrastny, V., & Ettler, V. (2007). The use of maize and poplar in chelant-enhanced phytoextraction of lead from contaminated agricultural soils. *Chemosphere*, *67*(4), 640–651. doi:10.1016/j.chemosphere.2006.11.010 PMID:17184814

Kopp, R. F., Smart, L. B., Maynard, C. A., Isebrands, J. G., Tuskan, G. A., & Abrahamson, L. P. (2001). The development of improved willow clones for eastern north America. *Forestry Chronicle*, *77*(2), 287–292. doi:10.5558/tfc77287-2

Kramer, U., & Chardonnens, A. N. (2001). The use of transgenic plants in the bioremediation of soils contaminated with trace elements. *Applied Microbiology and Biotechnology*, *55*(6), 661–672. doi:10.1007002530100631 PMID:11525612

Kumar, G., Kushwaha, H. R., Panjabi-Sabharwal, V., Kumari, S., Joshi, R., Karan, R., ... Pareek, A. (2012). Clustered metallothionein genes are co-regulated in rice and ectopic expression of OsMT1e-P confers multiple abiotic stress tolerance in tobacco via ROS scavenging. *BMC Plant Biology*, *12*(1), 107. doi:10.1186/1471-2229-12-107 PMID:22780875

Kumar, V., & Yadav, S. K. (2009). Plant-mediated synthesis of silver and gold nanoparticles and their application. *Journal of Chemical Technology and Biotechnology (Oxford, Oxfordshire)*, *84*(2), 151–157. doi:10.1002/jctb.2023

Kwankua, W., Sengsai, S., Muangphra, P., & Euawong, N. (2012). Screening for plants sensitive to heavy metals using cytotoxic and genotoxic biomarkers. *Witthayasan Kasetsat Witthayasat*, *46*, 10–23.

Langer, I., Krpata, D., Fitz, W. J., Wenzel, W. W., & Schweiger, P. F. (2009). Zinc accumu-lation potential and toxicity threshold determined for a metal-accumulating *Populus canescens* clone in a dose-response study. *Environ. Pollut.*, *157*(10), 2871–2877. doi:10.1016/j.envpol.2009.04.003 PMID:19446384

Leblanc, M., Petit, D., Deram, A., Robinson, B., & Brooks, R. R. (1999). The phytomining and environmental significance of hyperaccumulation of thallium by Iberisintermedia from southern France. *Economic Geology and the Bulletin of the Society of Economic Geologists*, *94*(1), 109–113. doi:10.2113/gsecongeo.94.1.109

Lee, S., Ryu, J. Y., Kim, S. Y., Jeon, J. H., Song, J. Y., Cho, H. T., ... Park, Y. I. (2007). Transcriptional regulational of the respiratory genes in the *Cyanobacterium Synechocystis sp.* PCC 6803 during the early response to glucose feeding. *Plant Physiology*, *145*(3), 1018–1030. doi:10.1104/pp.107.105023 PMID:17827271

Li, W., Ye, Z., & Wong, M. (2009). Metal mobilization and production of short-chain organic acids by rhizosphere bacteria associated with a Cd/Zn hyperaccumulating plant, *Sedum alfredii. Plant and Soil*, *326*(1-2), 453–467. doi:10.100711104-009-0025-y

Liao, H., Wan, H., Shaff, J., Wang, X., Yan, X., & Kochian, L. V. (2006). Phosphorus and aluminium interactions in soybean in relation to alumunium tolerance. Exudation of specific organic acids from different regions of the intact root system. *Plant Physiology*, *141*(2), 674–684. doi:10.1104/pp.105.076497 PMID:16648222

Lin, D., & Xing, B. (2007). Phytotoxicity of nanoprticles: Inhibition of seed germination and root growth. *Environ. Pollut.*, *150*(2), 243–250. doi:10.1016/j.envpol.2007.01.016 PMID:17374428

Lin, D., & Xing, B. (2007). Phytotoxicity of nanoprticles: Inhibition of seed germination and root growth. *Environ. Pollut.*, *150*(2), 243–250. doi:10.1016/j.envpol.2007.01.016 PMID:17374428

Linger, P., Mussig, J., Fishcher, H., & Kobert, J. (2002). Industrial hemp (*Cannabis Sativa L.*) growing on heavy metal contaminated soil: Fibre quality and phytoremediation Potential. *Industrial Crops and Products*, *16*(1), 33–42. doi:10.1016/S0926-6690(02)00005-5

Liu, G. Y., Zhang, Y. X., & Chai, T. Y. (2011). Phytochelatin synthase of *Thlaspi caerulescens* enhanced tolerance and accumulation of heavy metals when expressed in yeast and tobacco. *Plant Cell Reports*, *30*(6), 1067–1076. doi:10.100700299-011-1013-2 PMID:21327392

Liu, K., & Luan, S. (2001). Internal aluminum block of plant Inward K^{p} channels. *The Plant Cell*, *13*(6), 1453–1466. doi:10.1105/TPC.010016 PMID:11402172

Lombi, E., Terall, K. L., Howarth, J. R., Zhao, F. J., Hawkesford, M. J., & McGrath, S. P. (2002). Influence of iron status on cadmium and zinc uptake by different ecotypes of the hyperaccumulator *Thlaspi caerulescens. Plant Physiology*, *128*(4), 1359–1367. doi:10.1104/pp.010731 PMID:11950984

Lori, V., Pietrini, F., Massacci, A., & Zacchini, M. (2015). *Morphophysiological responses, Heavy metal accumulation and phytoremoval ability in four Willow clones exposed to cadmium under hydroponics*. Phytoremediation Springer International Publishing.

Lu, L., Tian, S. K., Yang, X. E., Li, T. Q., & He, Z. I. (2009). Cadmiumuptake and xylem loading are active processes in the hyperaccumulator *Sedum alfredii. Journal of Plant Physiology*, *166*(6), 579–587. doi:10.1016/j.jplph.2008.09.001 PMID:18937997

Ma, Y., Rajkumar, M., Luo, Y., & Freitas, H. (2011). Inoculation of endophytic bacteria on host and non-host plants-effects on plant growth and Ni uptake. *Journal of Hazardous Materials*, *195*, 230–237. doi:10.1016/j.jhazmat.2011.08.034 PMID:21872991

Maestri, E., Marmiroli, M., Visioli, G., & Marmiroli, N. (2010). Metal tolerance and hyperaccumulation: Costs and trade-offs between traits and environment. *Environmental and Experimental Botany*, *68*(1), 1–13. doi:10.1016/j.envexpbot.2009.10.011

Mahmood, T. (2010). Phytoextraction of heavy metals the process and scope for remediation of contaminated soils. *Soil & Environment*, *29*(2), 91–109.

Mariano, E. D., & Keltjens, W. G. (2005). Long term effects of aluminum exposure on nutrient uptake by maize genotypes differing in aluminum resistance. *Journal of Plant Nutrition*, *28*(2), 323–333. doi:10.1081/PLN-200047625

Meagher, R. B. (2000). Phytoremediation of toxic elemental and organic pollutants. *Current Opinion in Plant Biology*, *3*(2), 153–162. doi:10.1016/S1369-5266(99)00054-0 PMID:10712958

Meagher, R. B., Rugh, C. L., Kandasamy, M. K., Gragson, G., & Wang, N. J. (2000). Engineered phytoremediation of mercury pollution in soil and water using bacterial genes. In N. Terry & G. Banuelos (Eds.), *Phytoremediation of contaminated soil and water* (pp. 203–221). Lewis.

Meers, E., Ruttens, A., Hopgood, M., Lesage, E., & Tack, F. M. (2005). Potential of Brassic rapa, Cannabis sativa, Helianthus annuus and Zea mays for phytoextraction of heavy metals from calcareous dredged sediment derived soils. *Chemosphere*, *61*(4), 561–572. doi:10.1016/j.chemosphere.2005.02.026 PMID:16202810

Meers, E., Vandecasteele, B., Ruttens, A., Vangronsveld, J., & Tack, F. M. G. (2007). Potential of five willow species (*Salix spp.*) for phytoextraction of heavy metals. *Environmental and Experimental Botany*, *60*(1), 57–68. doi:10.1016/j.envexpbot.2006.06.008

Mehdawi, E. (2011). Selenium hyperaccumulators facilitate selenium-tolerant neighbors via phytoenrichment and reduced herbivory curt. *Current Biology*, *21*(17), 1440–1449. doi:10.1016/j.cub.2011.07.033 PMID:21856156

Memon, A. R., & Schroder, P. (2009). Implications of metal accumulations mechanisms to phytoremediation. *Environmental Science and Pollution Research International*, *16*(2), 162–175. doi:10.100711356-008-0079-z PMID:19067014

Merlot, S., Hannibal, L., Martins, S., Martinelli, L., Amir, H., Lebrum, M., Thomine, S., 2014. The metal transporters PgIREG1 from the hyperaccumulator *Psychotria gabriellae* is a candidate gene for nickel tolerance and accumulation. *J. Exp. Bot., 25*.

Meyer, C., & Verbruggen, N. (2012). The use of the modal species *Arabidopsis halleri* towards phytoextraction of cadmium polluted soils. *New Biotechnology*, *30*(1), 9–14. doi:10.1016/j.nbt.2012.07.009 PMID:22850245

Migocka, M., Kosieradzka, A., Papierniak, A., Maciaszczyk-Dziubinska, E., Posyniak, E., Garbiec, A., & Filleur, S. (2014). Two metal tolerance proteins, MTP1 and MTP4 are involved in Zn homeostasis and Cd sequestration in cucumber cells. *Journal of Experimental Botany*, 459. PMID:25422498

Migocka, M., Posyniak, E., Maciaszczyk-Dziubinska, E., Papierniak, A., & Kosieradzka, A. (2015). Functional and biochemical characterization of cucumber genes encoding two copper ATPaes CsHMA5.1 and CsHMA5.2. *J. Biol. Chem. Jbc-M, 114*.

Mils, R. F., Krjiger, G. C., Baccarini, P. J., Hall, J. L., & Williams, L. E. (2003). Functional expression of AtHMA4, a P-1B-type ATPase of the Zn/Co/Cd/Pb subclass. *The Plant Journal*, *35*(2), 164–176. doi:10.1046/j.1365-313X.2003.01790.x PMID:12848823

Mishra, S., & Dubey, R. (2005). Heay metal toxicity induced alteration. In M. Pessaraki (Ed.), *Handbook of Photosynthesis* (Vol. 28, pp. 827–844). Boca Raton: CRC Press.

Mokhtar, H., Morad, N., & Fizri, F. F. A. (2011). Phytoaccumulation of copper from aqueous solutions using *Eichhornia Crassipes* and *Centella asiatica. International Journal of Environmental Sciences and Development*, *3*(1), 89–95.

Morel, M., Crouzet, J., Gravot, A., Auroy, P., Leonhardt, N., Vavasseur, A., & Richaud, P. (2009). AtHMA3, a P1B-ATPase allowing Cd/Zn/Co/Pb vacuolar storage in *Arabidopsis. Plant Physiology*, *149*(2), 894–904. doi:10.1104/pp.108.130294 PMID:19036834

Moustakas, M., Ouzounidou, G., & Lannoye, R. (1995). Aluminum effects on photosynthesis and elemental uptake in an aluminium-tolerant and non-tolerant wheat cultivar. *Journal of Plant Nutrition*, *18*(4), 669–683. doi:10.1080/01904169509364930

Mugica-Alvarez, V., Cortes-Jimenez, V., Vaca-Mier, M., & Dommguez-Soria, V. (2015). Phytoremediation of mine tailings using *Lolium multiflorum. International Journal of Environmental Sciences and Development*, *6*(4), 246–251. doi:10.7763/IJESD.2015.V6.599

Mukhopadhyay, S., & Maiti, S. K. (2010). Phytoremediation of metal enrhiched mine wate: A review. *Global Environmental Research*, *4*, 135–150.

Navarro, E., Baun, A., Behra, R., Hartmann, N. B., Fisler, J., Miao, A. J., ... Sigg, L. (2008). Environmental behaviour and ecotoxicity of engineered nanoparticles to algae, plants and fungi. *Ecotoxicol.*, *17*(5), 372–386. doi:10.100710646-008-0214-0 PMID:18461442

Nematian, M.A., & Kazemeini, F. (2013). Accumulation of Pb, Zn, Cu and Fe in plants and hyperaccumulator choice in Galali iron mine area. *Iran. Inter. J. Agricult. Crop Sci.,* *5*(4), 426–432.

Nichol, B. E., Oliveira, L. A., Glass, A. D. M., & Saiddiqi, M. Y. (1993). The effect of aluminum on the influx of calcium, potassium, ammonium nitrate and phosphate in an aluminum sensitive cultivar of barley (Hordeum vulgare L.). *Plant Physiology*, *101*(4), 1263–1266. doi:10.1104/pp.101.4.1263 PMID:12231781

Nies, D. H. (1999). Microbial heavy metal resistance. *Applied Microbiology and Biotechnology*, *51*(6), 730–750. doi:10.1007002530051457 PMID:10422221

Nowack, B. (2002). Environmental chemistry of aminopolycarboxylate chelating agents. 722. *Environmental Science & Technology*, *36*(19), 4009–4016. doi:10.1021/es025683s PMID:12380068

Padmavathiamma, P. K., & Li, L. Y. (2007). Phytoremediation technology: Hyperaccumulation metals in plants. *Water, Air, and Soil Pollution*, *184*(1-4), 105–126. doi:10.100711270-007-9401-5

Palma, J. M., Gupta, D. K., & Corpas, F. J. (2013). Metalloenzymes involved in the metabolism of reactive oxygen species and heavy metal stress. In *Heavy Metal Stress in Plants* (pp. 1–17). Springer Berlin Heidelberg. doi:10.1007/978-3-642-38469-1_1

Papoyan, A., & Kochian, L. V. (2004). Identification of Thlaspi caerulescens genes that may be involved in heavy metal hyperaccumulation and tolerance. Characterization of a novel heavy metal transporting ATPase. *Plant Physiology*, *136*(3), 3814–3823. doi:10.1104/pp.104.044503 PMID:15516513

Park, W., Feng, Y., & Ahn, S. J. U. (2014). Alteration of leaf shape, improved metal tolerance, and productivity of seed by overexpression of CsHMA3 in *Camelina sativa. Biotech. Biol.*, *7*, 9. PMID:25018780

Pence, N. S., Larsen, P. B., Ebbs, S. D., Letham, D. L. D., Lasat, M. M., Garvin, D. F., ... Kochian, L. V. (2000). The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(9), 4956–4960. doi:10.1073/pnas.97.9.4956 PMID:10781104

Peters, R. W. (1999). Chelant extraction of heavy metals from contaminated soils. Journal of 729 Hazard. *Mater.*, *66*, 151–210. PMID:10379036

Phukan, P., Phukan, R., & Phukan, S. N. (2015). Heavy metal uptake capacity of *Hydrilla verticillata*: A commonly available aquatic plant. *Int. Res. J. Environ. Sci.*, *4*(3), 35–40.

Pilon-Smits, E., & Pilon, M. (2002). Phytoremediation of metals using transgenic plants. *Critical Reviews in Plant Sciences*, *21*(5), 439–456. doi:10.1080/0735-260291044313

Pollard, A. J., Stewart, H. L., & Roberson, C. B. (2009). Manganese hyperaccumulation in Phytolacca americana L. from the southeastern United States. *Northeastern Naturalist*, *16*(sp5), 155–162. doi:10.1656/045.016.0513

Prasad, M. N. V. (2005). Nickel ophilous plants and their significance in phytotechnologies. *Brazilian Journal of Plant Physiology*, *17*(1), 113–128. doi:10.1590/S1677-04202005000100010

Prasad, M. N. V., & Freitas, H. (2003). Metal hyperaccumulation in plants: Biodiversity prospecting for phytoremediation technology. *Electronic Journal of Biotechnology*, *6*(3), 275–321. doi:10.2225/vol6-issue3-fulltext-6

Purcino, A. A. C., Alves, V. M. C., Parentoni, S. N., Belele, C. L., & Loguercio, L. L. (2003). Aluminum effects on nitrogen uptake and nitrogen assimilating enzymes in maize genotypes with contrasting tolerance to aluminum toxicity. *Journal of Plant Nutrition*, *26*(1), 31–61. doi:10.1081/PLN-120016496

Qadir, S., Jamshieed, S., Rasool, S., Ashraf, M., Akram, N. A., & Ahmad, P. (2014). Modulation of plant growth and metabolism in cadmium enriched environments. *Reviews of Environmental Contamination and Toxicology*, *229*, 51–88. doi:10.1007/978-3-319-03777-6_4 PMID:24515810

Qiao, X. Q., Zheng, Z. Z., Zhang, L. F., Wang, J. H., Shi, G. X., & Xu, X. Y. (2015). Lead tolerance mechanism in sterilized seedlings of Potamogeton crispus L. Subcellular distribution, poluamine and proline. *Chemosphere*, *120*, 179–187. doi:10.1016/j.chemosphere.2014.06.055 PMID:25033323

Rajkumar, M., Nagendran, R., Lee, K. J., Lee, W. H., & Kim, S. Z. (2006). Influence of plant growth promoting bacteria and Cr^{6+} on the growth of Indian mustard. *Chemosphere*, *62*(5), 741–748. doi:10.1016/j.chemosphere.2005.04.117 PMID:15982703

Rascio, N., & Navari-Izzo, F. (2011). Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? *Plant Science*, *180*(2), 169–181. doi:10.1016/j.plantsci.2010.08.016 PMID:21421358

Reddy, A. M., Kumar, S. G., Jyothsnakumari, G., Thimmanaik, S., & Sudhakar, C. (2005). Lead induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) and bengal gram (*Cicer arietinum L.*). *Chemosphere*, *60*(1), 97–104. doi:10.1016/j.chemosphere.2004.11.092 PMID:15910908

Rengel, Z., & Elliott, D. C. (1992). Mechanism of aluminium inhibirion of net ^{45}Ca2p Uptake by Amaranths Protoplasts. *Plant Physiology*, *98*(2), 632–638. doi:10.1104/pp.98.2.632 PMID:16668688

Ribeiro, M. A. Q., de Almeida, A. A. F., Mielke, M. S., Gomes, F. P., Pires, M. V., & Baligar, V. C. (2013). Aluminum effects on growth, photosynthesis, and mineral nutrition of cacao genotypes. *Journal of Plant Nutrition*, *36*(8), 1161–1179. doi:10.1080/01904167.2013.766889

Robinson, B., Fernandez, J. E., Madejon, P., Maranon, T., Murillo, J., Green, S., & Clothier, B. (2003). Phytoextraction: An assessment of biogeochemical and economic viability. *Plant and Soil*, *249*(1), 117–125. doi:10.1023/A:1022586524971

Rodriguez, E., Azevedo, R., Fernandes, P., & Santos, C. (2011). Cr (VI) induces DNA damage, cell cycle arrest and polyploidization: A flow cytometric and comet assay study in Pisum sativum. *Chemical Research in Toxicology*, *24*(7), 1040–1047. doi:10.1021/tx2001465 PMID:21667992

Roy, A. K., Sharma, A., & Talukder, G. (1988). Some aspects of aluminum toxicity in plants. *Botanical Review*, *54*(2), 145–178. doi:10.1007/BF02858527

Rugh, C. L., Gragson, G. M., Meagher, R. B., & Merkle, S. A. (1998). Toxic mercury reduction and remediation using transgenic plants with a modified bacterial gene. *HortScience*, *33*(4), 618–621.

Ruiz, O. N., Hussein, H. S., Terry, N., & Daniell, H. (2003). Phytoremediation of organomercurial compounds via chloroplast genetic engineering. *Plant Physiology*, *132*(3), 1344–1352. doi:10.1104/pp.103.020958 PMID:12857816

Saba, G., Parizanganeh, A. H., Zamani, A., & Saba, J. (2015). Phytoremdiation of heavy metals contaminated environments: Screening for native accumulator plants in Zanjan-Iran. *International Journal of Environmental of Research*, *9*(1), 309–316.

Saison, C., Perreault, F., Daigle, J. C., Fortin, C., Claverie, J., Morin, M., & Popovic, R. (2010). Effect of core-shell copper oxide nanoparticles on cell culture morphology and photosynthesis (photosystem II energy distribution) in the green alga, *Chlamydomonas reinhardtii. Aquatic Toxicology (Amsterdam, Netherlands)*, *96*(2), 109–114. doi:10.1016/j.aquatox.2009.10.002 PMID:19883948

Salido, A. L., Hasty, K. L., Lim, J. M., & Butcher, D. J. (2003). Phytoremediation of arsenic and 750 lead in contaminated soil using Chinese brake ferns (*Pteris vittata*) and Indian mustard 751 (*Brassica juncea*). *International Journal of Phytoremediation*, *5*(2), 89–103. doi:10.1080/713610173 PMID:12929493

Salla, V., Hardaway, C. J., & Sneddon, J. (2011). Preliminary investigation of *Spartina alterniflora* for phytoextraction of selected heavy metals in soil from Southwest Louisiana. *Microchemical Journal*, *97*(2), 207–212. doi:10.1016/j.microc.2010.09.005

Salt, D. E., Smith, R. D., & Raskin, I. (1998). Phytoremediation. *Annual Review of Plant Physiology and Plant Molecular Biology*, *49*(1), 643–668. doi:10.1146/annurev.arplant.49.1.643 PMID:15012249

Sastry, M., Ahmad, A., Khan, M. I., & Kumar, R. (2003). Biosynthesis of metal nanoparticles using fungi and actinomycete. *Current Science*, *85*, 162–170.

Semane, B., Cuypers, A., Smeets, K., Van Belleghem, F., Horemans, N., Schat, H., & Vangronsveld, J. (2007). Cadmium responses in *Arabidopsis thaliana*: Glutathione metabolism and antioxidative defenc system. *Physiologia Plantarum*, *129*(3), 519–528. doi:10.1111/j.1399-3054.2006.00822.x

Sharma, D. M., Sharma, C. P., & Tripathi, R. D. (2003). Phytotoxic lesions of chromium in maize. *Chemosphere*, *51*(1), 63–68. doi:10.1016/S0045-6535(01)00325-3 PMID:12586157

Shekhawat, G. S., & Arya, V. (2009). Biological synthesis of Ag nanoparticles through i*n vitro* cultures of *Brassica juncea* Czern. Nanomaterials and Devices: Processing and Applications. Book Series. *Advanced Materials Research*, *67*, 295–299. doi:10.4028/www.scientific.net/AMR.67.295

Singh, O. V., Labana, S., Pandey, G., Budhiraja, R., & Jain, R. K. (2003). Phytoremediation: An overview of metallic ion decontamination from soil. *Applied Microbiology and Biotechnology*, *61*(5-6), 405–412. doi:10.100700253-003-1244-4 PMID:12764555

Socha, A. L., & Guerinot, M. L. (2014). Mn-euvering manganese: The role of transporter gene family members in manganese uptake and mobilization in plants. Fron. *Plant Science*, 5.

Stampoulis, D., Sinha, S. K., & White, J. C. (2009). Assay-dependent phytotoxicity of nanoparticles to plants. *Environmental Science & Technology*, *24*(24), 9473–9479. doi:10.1021/es901695c PMID:19924897

Summers, A. O. (1986). Organization, expression, and evolution of genes for mercury resistance. *Annual Review of Microbiology*, *40*(1), 607–634. doi:10.1146/annurev.mi.40.100186.003135 PMID:3535655

Tong, X., Yuan, L., Luo, L., & Yin, X. (2014). Characterization of a Selenium-Tolerant Rhizosphere Strain from a Novel Se-Hyperaccumulating Plant *Cardamine hupingshanesis*. *Scientific World J*, 1-8.

Ullah, A., Heng, S., Munis, M. F. H., Fahad, S., & Yang, X. (2015). Phytoremdiation of heavy metal assisted by plant growth promoting (PGP) bacteria: A review. *Environmental and Experimental Botany*, *117*, 28–40. doi:10.1016/j.envexpbot.2015.05.001

Vassil, A. D., Kapulnik, Y., Raskin, I., & Salt, D. E. (1998). The role of EDTA in lead transport and accumulation by Indian mustard. *Plant Physiology*, *117*(2), 447–453. doi:10.1104/pp.117.2.447 PMID:9625697

Viehweger, K. (2014). How plant cope with heavy metals. *Botanical Studies (Taipei, Taiwan)*, *55*(1), 35. doi:10.1186/1999-3110-55-35 PMID:28510963

Wang, Q. Q., Wang, L. H., Han, R. B., Yang, L. Y., Zhou, Q., & Huang, X. H. (2015). Effects of bisphenol A on antioxidant system in soybean seedling roots. *Environmental Toxicology and Chemistry*, *34*(5), 1127–1133. doi:10.1002/etc.2904 PMID:25651304

Wang, Y. S., & Yang, Z. M. (2005). Nitric oxide reduces aluminum toxicity by preventing oxidative stress in the roots of *Cassia tora L. Plant & Cell Physiology*, *46*(12), 1915–1923. doi:10.1093/pcp/pci202 PMID:16179356

Wheeler, D. M., & Follett, J. M. (1991). Effect of aluminium on onions, Asparagus and Squash. *Journal of Plant Nutrition*, *14*(9), 897–912. doi:10.1080/01904169109364251

Xia, B., Shen, S., & Xue, F. (2013). Phytoextraction of heavy metals from highly contaminated soils using *Sauropus androgynus*. Soil Sed. *Contamin.*, *22*(6), 631–640.

Xie, Z., Wu, L., Chen, N., Liu, C., Zheng, Y., Xu, S., ... Xu, Y. (2012). Phytoextraction of Pb and Cu contaminated soil with maize and microencapsulated EDTA. *International Journal of Phytoremediation*, *14*(8), 727–740. doi:10.1080/15226510903390452 PMID:22908640

Yan, Z., Li, X., Chen, J., & Tam, N. F. Y. (2015). Combined toxicity of cadmium and copper in Avicennia marina seedlings and the regulation of exogenous jasmonic acid. *Ecotoxicology and Environmental Safety*, *113*, 124–132. doi:10.1016/j.ecoenv.2014.11.031 PMID:25497768

Yang, X., Feng, Y., He, Z., & Stoffella, P. J. (2005). Molecular mechanism of heavy metal hyperaccumulation and phytoremdiation. *Journal of Trace Elements in Medicine and Biology*, *18*(4), 339–353. doi:10.1016/j.jtemb.2005.02.007 PMID:16028496

Yao, Z., Li, J., Xie, H., & Yu, C. (2012). Review on remediation technologies soil contaminated by heavy metals. *Procedia Environmental Sciences*, *16*, 722–729. doi:10.1016/j.proenv.2012.10.099

Yuan, L., Zhi, W., Liu, Y. S., Karyala, S., Vikesland, P. J., Chen, X., & Zhang, H. S. (2015). Lead toxicity to the performance, viability, and community composition of activated sludge microorganisms. *Environmental Science & Technology*, *49*(2), 824–830. doi:10.1021/es504207c PMID:25536278

Zaidi, A., Khan, M. S., & Aamil, M. (2004). Bioassociative effect of rhizospheric microorganisms on growth, yield and nutrient uptake of greengram. *Journal of Plant Nutrition*, *27*(4), 599–610. doi:10.1081/PLN-120030370

Zhao, D., Li, T., Wang, J., & Zhao, Z. (2015). Diverse strategies conferring extreme cadmium (Cd) tolerance in the dark septate endophyte (DSE), *Exophiala pisciphila*: Evidence from RNA-seq data. *Microbio. Res.*, *170*, 27–35. doi:10.1016/j.micres.2014.09.005 PMID:25294257

Zhao, F. J., Hamon, R. E., Lombi, E., McLaughlin, M. J., & McGrath, S. P. (2002). Characteristics of cadmium uptake in two contrasting ecotypes of the hyperaccumulator *Thlaspi caerulescens*. *Journal of Experimental Botany*, *53*(368), 535–543. doi:10.1093/jexbot/53.368.535 PMID:11847252

Zsoldos, F., Vashegyi, A., Bona, L., Pecsvaradi, A., & Szegletes, Z. (2000). Growth of and potassium transport in winter wheat and durum wheat as affectes by various aluminium exposure times. *Journal of Plant Nutrition*, *23*(7), 913–926. doi:10.1080/01904160009382070

Related Readings

To continue IGI Global's long-standing tradition of advancing innovation through emerging research, please find below a compiled list of recommended IGI Global book chapters and journal articles in the areas of wastewater treatment, environmental sustainability, and nanotechnology. These related readings will provide additional information and guidance to further enrich your knowledge and assist you with your own research.

Affam, A. C., & Chaudhuri, M. (2019). Comparative Study of Advance Oxidation Processes for Treatment of Pesticide Wastewater. In H. Aziz & S. Abu Amr (Eds.), *Advanced Oxidation Processes (AOPs) in Water and Wastewater Treatment* (pp. 261–323). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5766-1.ch011

Ahmedi, F., Ahmedi, L., O'Flynn, B., Kurti, A., Tahirsylaj, S., Bytyçi, E., ... Salihu, A. (2018). InWaterSense: An Intelligent Wireless Sensor Network for Monitoring Surface Water Quality to a River in Kosovo. *International Journal of Agricultural and Environmental Information Systems*, *9*(1), 39–61. doi:10.4018/IJAEIS.2018010103

Akash, P. V. M., Babu, N., & Navneet, (2018). Bioremediation of Environmental Pollutants. In V. Pathak, & Navneet (Eds.), Handbook of Research on Microbial Tools for Environmental Waste Management (pp. 80-104). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-3540-9.ch005

Akbar, N. A., Matsin, Z. B., & Ramli, S. F. (2019). Groundwater Treatment via Ozonation. In H. Aziz & S. Abu Amr (Eds.), *Advanced Oxidation Processes (AOPs) in Water and Wastewater Treatment* (pp. 200–225). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5766-1.ch009

Ali, D. A., Palaniandy, P., & Feroz, S. (2019). Advanced Oxidation Processes (AOPs) to Treat the Petroleum Wastewater. In H. Aziz & S. Abu Amr (Eds.), *Advanced Oxidation Processes (AOPs) in Water and Wastewater Treatment* (pp. 99–122). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5766-1.ch005

Amorim, C. L., Moreira, I. S., Duque, A. F., van Loosdrecht, M. C., & Castro, P. M. (2017). Aerobic Granular Sludge: Treatment of Wastewaters Containing Toxic Compounds. In Á. Val del Río, J. Campos Gómez, & A. Mosquera Corral (Eds.), *Technologies for the Treatment and Recovery of Nutrients from Industrial Wastewater* (pp. 231–263). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-1037-6.ch009

Anderson, S., & Rahman, P. K. (2018). Bioprocessing Requirements for Bioethanol: Sugarcane vs. Sugarcane Bagasse. In V. Pathak, & Navneet (Eds.), Handbook of Research on Microbial Tools for Environmental Waste Management(pp. 48-56). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-3540-9.ch003

Ara, T., Bashir, R., Chisti, H., & Rangreez, T. A. (2019). Wastewater Pollution From the Industries. In A. Hussain & S. Ahmed (Eds.), *Advanced Treatment Techniques for Industrial Wastewater* (pp. 98–113). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5754-8.ch007

Aris, A., Jusoh, M. N., & Wahab, N. S. (2019). Applications of Advanced Oxidation Processes in Palm Oil Mill Effluent Treatment. In H. Aziz & S. Abu Amr (Eds.), *Advanced Oxidation Processes (AOPs) in Water and Wastewater Treatment* (pp. 123–149). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5766-1.ch006

Aziz, N. A., & Palaniandy, P. (2019). Photocatalysis (TiO2/Solar) in Water and Wastewater Treatment. In H. Aziz & S. Abu Amr (Eds.), *Advanced Oxidation Processes (AOPs) in Water and Wastewater Treatment* (pp. 171–199). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5766-1.ch008

Babu, N., & Pathak, V. M. Akash, & Navneet, (2018). Biosorption of Heavy Metals: Biological Approach to Control the Industrial Waste. In V. Pathak, & Navneet (Eds.), Handbook of Research on Microbial Tools for Environmental Waste Management (pp. 270-281). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-3540-9.ch013

Bagheri, S., & Julkapli, N. M. (2017). Biomass-Derived Activated Carbon: Synthesis, Functionalized, and Photocatalysis Application. In T. Saleh (Ed.), *Advanced Nanomaterials for Water Engineering, Treatment, and Hydraulics* (pp. 162–199). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-2136-5.ch007

Banerjee, P., Mukhopadhayay, A., & Das, P. (2016). Advances in Bioremediation for Removal of Toxic Dye from Different Streams of Wastewater. In A. Rathoure & V. Dhatwalia (Eds.), *Toxicity and Waste Management Using Bioremediation* (pp. 266–278). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9734-8.ch013

Basak, B., & Dey, A. (2016). Bioremediation Approaches for Recalcitrant Pollutants: Potentiality, Successes and Limitation. In A. Rathoure & V. Dhatwalia (Eds.), *Toxicity and Waste Management Using Bioremediation* (pp. 178–197). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9734-8.ch009

Basheer, T., & Umesh, M. (2018). Valorization of Tannery Solid Waste Materials Using Microbial Techniques: Microbes in Tannery Solid Waste Management. In V. Pathak, & Navneet (Eds.), Handbook of Research on Microbial Tools for Environmental Waste Management (pp. 127-145). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-3540-9.ch007

Becker, J. A., & Stefanakis, A. I. (2016). Pharmaceuticals and Personal Care Products as Emerging Water Contaminants. In A. McKeown & G. Bugyi (Eds.), *Impact of Water Pollution on Human Health and Environmental Sustainability* (pp. 81–100). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9559-7.ch004

Belmonte, M., Fajardo, C., Toledo-Alarcón, J. B., Heredia, D. V., Jorquera, L., Méndez, R., ... Ruiz-Filippi, G. (2017). Autotrophic Denitrification Processes. In Á. Val del Río, J. Campos Gómez, & A. Mosquera Corral (Eds.), *Technologies for the Treatment and Recovery of Nutrients from Industrial Wastewater* (pp. 147–173). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-1037-6.ch006

Bender, S. R. (2016). Floodplain Infrastructure and the Toxic Tide. In A. McKeown & G. Bugyi (Eds.), *Impact of Water Pollution on Human Health and Environmental Sustainability* (pp. 150–173). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9559-7.ch007

Benjamin, S. R., de Lima, F., & Rathoure, A. K. (2016). Genetically Engineered Microorganisms for Bioremediation Processes: GEMs for Bioremediaton. In A. Rathoure & V. Dhatwalia (Eds.), *Toxicity and Waste Management Using Bioremediation* (pp. 113–140). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9734-8.ch006

Bhakta, J. N. (2016). Microbial Response against Metal Toxicity. In A. Rathoure & V. Dhatwalia (Eds.), *Toxicity and Waste Management Using Bioremediation* (pp. 75–96). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9734-8.ch004

Bhatt, P. (2018). Insilico Tools to Study the Bioremediation in Microorganisms. In V. Pathak, & Navneet (Eds.), Handbook of Research on Microbial Tools for Environmental Waste Management (pp. 389-395). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-3540-9.ch018

Bogatu, C., & Leszczynska, D. (2016). Transformation of Nanomaterials in Environment: Surface Activation of SWCNTs during Disinfection of Water with Chlorine. *Journal of Nanotoxicology and Nanomedicine*, *1*(1), 45–57. doi:10.4018/JNN.2016010104

Bonmatí-Blasi, A., Cerrillo-Moreno, M., & Riau-Arenas, V. (2017). Systems Based on Physical-Chemical Processes: Nutrient Recovery for Cycle Closure. In Á. Val del Río, J. Campos Gómez, & A. Mosquera Corral (Eds.), *Technologies for the Treatment and Recovery of Nutrients from Industrial Wastewater* (pp. 43–75). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-1037-6.ch003

Cema, G., & Sochacki, A. (2017). Treatment of Landfill Leachate by Anammox Process. In Á. Val del Río, J. Campos Gómez, & A. Mosquera Corral (Eds.), *Technologies for the Treatment and Recovery of Nutrients from Industrial Wastewater* (pp. 290–311). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-1037-6.ch011

Cervantes, F. J., Cuervo-López, F., & Hernández, J. G. (2017). Fundamentals of the Biological Processes for Nitrogen Removal. In Á. Val del Río, J. Campos Gómez, & A. Mosquera Corral (Eds.), *Technologies for the Treatment and Recovery of Nutrients from Industrial Wastewater* (pp. 112–146). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-1037-6.ch005

Chaudhry, S., & Garg, S. (2019). Industrial Wastewater Pollution and Advanced Treatment Techniques. In A. Hussain & S. Ahmed (Eds.), *Advanced Treatment Techniques for Industrial Wastewater* (pp. 74–97). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5754-8.ch006

Chaurasia, P. K., Bharati, S. L., & Mani, A. (2018). Enzymatic Treatment of Petroleum-Based Hydrocarbons. In V. Pathak, & Navneet (Eds.), Handbook of Research on Microbial Tools for Environmental Waste Management (pp. 396-408). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-3540-9.ch019

Cook, C., & Gude, V. G. (2017). Characteristics of Chitosan Nanoparticles for Water and Wastewater Treatment: Chitosan for Water Treatment. In T. Saleh (Ed.), *Advanced Nanomaterials for Water Engineering, Treatment, and Hydraulics* (pp. 223–261). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-2136-5.ch009

Cuker, B., & Bugyi, G. (2016). The Fundamentals of Water and Natural Waters. In A. McKeown & G. Bugyi (Eds.), *Impact of Water Pollution on Human Health and Environmental Sustainability* (pp. 1–28). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9559-7.ch001

de la Varga, D., Soto, M., Arias, C. A., van Oirschot, D., Kilian, R., Pascual, A., & Álvarez, J. A. (2017). Constructed Wetlands for Industrial Wastewater Treatment and Removal of Nutrients. In Á. Val del Río, J. Campos Gómez, & A. Mosquera Corral (Eds.), *Technologies for the Treatment and Recovery of Nutrients from Industrial Wastewater* (pp. 202–230). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-1037-6.ch008

Dhatwalia, V. K., & Nanda, M. (2016). Biodegradation of Phenol: Mechanisms and Applications. In A. Rathoure & V. Dhatwalia (Eds.), *Toxicity and Waste Management Using Bioremediation* (pp. 198–214). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9734-8.ch010

Di Paola, N., Spanò, R., Caldarelli, A., & Vona, R. (2016). Fighting Ecomafias: The Role of Biotech Networks in Achieving Sustainability. In A. Rathoure & V. Dhatwalia (Eds.), *Toxicity and Waste Management Using Bioremediation* (pp. 295–311). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9734-8.ch015

Dima, J. B., & Zaritzky, N. (2017). Performance of Chitosan Micro/ Nanoparticles to Remove Hexavalent Chromium From Residual Water. In T. Saleh (Ed.), *Advanced Nanomaterials for Water Engineering, Treatment, and Hydraulics* (pp. 262–288). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-2136-5.ch010

Dubey, S. K., Hussain, A., & Ajnavi, S. (2019). Environmental Recycling System (ERS): An Emerging Approach to Solid Waste Management. In A. Hussain & S. Ahmed (Eds.), *Advanced Treatment Techniques for Industrial Wastewater* (pp. 225–237). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5754-8.ch013

Farraji, H. (2018). Phytoremediation of Nitrogen and Phosphorus in Municipal Wastewater by Cyperus alternifolius Planted Constructed Wetland. In V. Pathak, & Navneet (Eds.), Handbook of Research on Microbial Tools for Environmental Waste Management (pp. 146-163). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-3540-9.ch008

Farraji, H., Zaman, N. Q., Vakili, M., & Faraji, H. (2016). Overpopulation and Sustainable Waste Management. *International Journal of Sustainable Economies Management*, *5*(3), 13–36. doi:10.4018/IJSEM.2016070102

Fra-Vázquez, A., & Valenzuela-Heredia, D. (2017). Biogeochemical Cycles of Nitrogen and Phosphorus: Implications of Anthropogenic Activities. In Á. Val del Río, J. Campos Gómez, & A. Mosquera Corral (Eds.), *Technologies for the Treatment and Recovery of Nutrients from Industrial Wastewater* (pp. 1–20). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-1037-6.ch001

Gatt, K. (2016). Youths' Social Traits in Water Management as a Precursor for Good Water Governance. *International Journal of Information Systems and Social Change*, *7*(3), 16–26. doi:10.4018/IJISSC.2016070102

Gupta, C., & Prakash, D. (2016). Novel Bioremediation Methods in Waste Management: Novel Bioremediation Methods. In A. Rathoure & V. Dhatwalia (Eds.), *Toxicity and Waste Management Using Bioremediation* (pp. 141–157). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9734-8.ch007

Hassan, S. H., & Halim, A. A. (2019). Water Quality Legislation and Regulation. In H. Aziz & S. Abu Amr (Eds.), *Advanced Oxidation Processes (AOPs) in Water and Wastewater Treatment* (pp. 30–45). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5766-1.ch002

Hilles, A. H., Abu Amr, S. S., Aziz, H. A., & Bashir, M. J. (2019). Advanced Oxidation Processes for Water and Wastewater Treatment: An Introduction. In H. Aziz & S. Abu Amr (Eds.), *Advanced Oxidation Processes (AOPs) in Water and Wastewater Treatment* (pp. 46–69). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5766-1.ch003

Hivrale, A. U., Pawar, P. K., Rane, N. R., & Govindwar, S. P. (2016). Application of Genomics and Proteomics in Bioremediation. In A. Rathoure & V. Dhatwalia (Eds.), *Toxicity and Waste Management Using Bioremediation* (pp. 97–112). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9734-8.ch005

Hussain, A., Bhattacharya, A., & Ahmed, A. (2019). Plastic Waste Pollution and Its Management in India: A Review. In A. Hussain & S. Ahmed (Eds.), *Advanced Treatment Techniques for Industrial Wastewater* (pp. 62–73). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5754-8.ch005

Ibrahim, N., Zainal, S. F., & Aziz, H. A. (2019). Application of UV-Based Advanced Oxidation Processes in Water and Wastewater Treatment. In H. Aziz & S. Abu Amr (Eds.), *Advanced Oxidation Processes (AOPs) in Water and Wastewater Treatment* (pp. 384–414). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5766-1.ch014

Jagdale, S., & Chabukswar, A. (2016). Phyto-Remediation: Using Plants to Clean Up Soils: Phyto-Remediation. In A. Rathoure & V. Dhatwalia (Eds.), *Toxicity and Waste Management Using Bioremediation* (pp. 215–235). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9734-8.ch011

Kamaruddin, M. A. (2019). Advanced Oxidation Processes (AOPs) in Landfill Leachate Treatment. In H. Aziz & S. Abu Amr (Eds.), *Advanced Oxidation Processes (AOPs) in Water and Wastewater Treatment* (pp. 355–383). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5766-1.ch013

Kaur, A. (2017). Applications of Nanomaterials for Water Treatment: A Future Avenue. In T. Saleh (Ed.), *Advanced Nanomaterials for Water Engineering, Treatment, and Hydraulics* (pp. 289–304). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-2136-5.ch011

Kehinde, F. O., Abu Amr, S. S., & Aziz, H. A. (2019). Application of Persulfate in Textile Wastewater Treatment. In H. Aziz & S. Abu Amr (Eds.), *Advanced Oxidation Processes (AOPs) in Water and Wastewater Treatment* (pp. 70–98). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5766-1.ch004

Khelfi, A. (2018). Sources of Air Pollution. In V. Pathak, & Navneet (Eds.), Handbook of Research on Microbial Tools for Environmental Waste Management(pp. 220-258). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-3540-9.ch011

Khelfi, A. (2018). Sources of Soil Pollution. In V. Pathak, & Navneet (Eds.), Handbook of Research on Microbial Tools for Environmental Waste Management(pp. 190-218). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-3540-9.ch010

Khelfi, A. (2019). Sources of Groundwater Pollution. In A. Hussain & S. Ahmed (Eds.), *Advanced Treatment Techniques for Industrial Wastewater* (pp. 177–210). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5754-8.ch011

Koli, S. K., & Hussain, A. (2019). Status of Electronic Waste Management in India: A Review. In A. Hussain & S. Ahmed (Eds.), *Advanced Treatment Techniques for Industrial Wastewater* (pp. 238–250). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5754-8.ch014

Kulkarni, A. G., & Vedamurthy, A. B. (2018). Cellulolytic Microorganisms: A Review. In V. Pathak, & Navneet (Eds.), Handbook of Research on Microbial Tools for Environmental Waste Management (pp. 34-47). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-3540-9.ch002

Kumar, P. S., & C., F. C. (2019). Soil Bioremediation Techniques. In A. Hussain, & S. Ahmed (Eds.), *Advanced Treatment Techniques for Industrial Wastewater* (pp. 35-50). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5754-8.ch003

Kumar, P. S., & Saravanan, A. (2019). Water Pollutants and Their Removal Techniques. In A. Hussain & S. Ahmed (Eds.), *Advanced Treatment Techniques for Industrial Wastewater* (pp. 114–133). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5754-8.ch008

Kumar, P. S., & Yaashikaa, P. R. (2019). Agriculture Pollution. In A. Hussain & S. Ahmed (Eds.), *Advanced Treatment Techniques for Industrial Wastewater* (pp. 134–154). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5754-8.ch009

Laha, S. D., Dutta, K., & Kundu, P. P. (2018). Biodegradation of Low Density Polyethylene Films. In V. Pathak, & Navneet (Eds.), Handbook of Research on Microbial Tools for Environmental Waste Management (pp. 282-318). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-3540-9.ch014

Manyuchi, M. M., Muzenda, E., & Mbohwa, C. (2018). Design and Development of a Sanitary Landfill for Low Income Countries for Optimal Waste Management. In V. Pathak, & Navneet (Eds.), Handbook of Research on Microbial Tools for Environmental Waste Management (pp. 373-388). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-3540-9.ch017

Mbuh, M. J., Houser, P. R., & Heidari, A. (2016). Water Quality Estimation Using Combined Water Chemistry and Field Spectroscopy in the Shenandoah River, Virginia. *International Journal of Applied Geospatial Research*, *7*(2), 14–37. doi:10.4018/ijagr.2016040102

McKeown, A. E. (2016). Nurses, Healthcare, and Environmental Pollution and Solutions: Breaking the Cycle of Harm. In A. McKeown & G. Bugyi (Eds.), *Impact of Water Pollution on Human Health and Environmental Sustainability* (pp. 174–197). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9559-7.ch008

Mobin, R., Chisti, H., Rangreez, T. A., Bashir, R., & Najar, A. A. (2019). Pesticidal Pollution. In A. Hussain & S. Ahmed (Eds.), *Advanced Treatment Techniques for Industrial Wastewater* (pp. 155–176). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5754-8.ch010

Mohamed, Z. (2016). Cyanobacterial Toxins in Water Sources and Their Impacts on Human Health. In A. McKeown & G. Bugyi (Eds.), *Impact of Water Pollution on Human Health and Environmental Sustainability* (pp. 120–149). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9559-7.ch006

Mojiri, A., Ziyang, L., Hui, W., & Gholami, A. (2019). Concentrated Landfill Leachate Treatment by Electro-Ozonation. In H. Aziz & S. Abu Amr (Eds.), *Advanced Oxidation Processes (AOPs) in Water and Wastewater Treatment* (pp. 150–170). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5766-1.ch007

Mujere, N. (2016). Water Hyacinth: Characteristics, Problems, Control Options, and Beneficial Uses. In A. McKeown & G. Bugyi (Eds.), *Impact of Water Pollution on Human Health and Environmental Sustainability* (pp. 343–361). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9559-7.ch015

Mujere, N., & Isidro, M. (2016). Impacts of Artisanal and Small-Scale Gold Mining on Water Quality in Mozambique and Zimbabwe. In A. McKeown & G. Bugyi (Eds.), *Impact of Water Pollution on Human Health and Environmental Sustainability* (pp. 101–119). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9559-7.ch005

Nazir, R. (2017). Advanced Nanomaterials for Water Engineering and Treatment: Nano-Metal Oxides and Their Nanocomposites. In T. Saleh (Ed.), *Advanced Nanomaterials for Water Engineering, Treatment, and Hydraulics* (pp. 84–126). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-2136-5.ch005

Obeng, P. A., Oduro-Kwarteng, S., Keraita, B., Bregnhøj, H., Abaidoo, R. C., Awuah, E., & Konradsen, F. (2016). On-Site Sanitation Systems for Low-Income Countries: Technical Guidelines for Groundwater Pollution and Nuisance Control. In A. McKeown & G. Bugyi (Eds.), *Impact of Water Pollution on Human Health and Environmental Sustainability* (pp. 319–342). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9559-7.ch014

Olajubu, E. A., Ajayi, G., Oke, I., & Asahiah, F. O. (2017). An ANN Model for Predicting the Quantity of Lead and Cadmium Ions in Industrial Wastewater. *International Journal of Information Communication Technologies and Human Development*, *9*(4), 32–44. doi:10.4018/IJICTHD.2017100103

Paris, R. S. (2016). Issues with Water Quality: How Do We Get Our Fellow Citizens to Care? In A. McKeown & G. Bugyi (Eds.), *Impact of Water Pollution on Human Health and Environmental Sustainability* (pp. 199–214). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9559-7.ch009

Pedemonte, D. C., Frison, N., Tayà, C., Ponsa, S., & Fatone, F. (2017). Chemical and Biological Processes for Nutrients Removal and Recovery. In Á. Val del Río, J. Campos Gómez, & A. Mosquera Corral (Eds.), *Technologies for the Treatment and Recovery of Nutrients from Industrial Wastewater* (pp. 76–111). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-1037-6.ch004

Prabu, S. L., Suriyaprakash, T., Kandasamy, R., & Rathinasabapathy, T. (2016). Effective Waste Water Treatment and its Management. In A. Rathoure & V. Dhatwalia (Eds.), *Toxicity and Waste Management Using Bioremediation* (pp. 312–334). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9734-8.ch016

Priyanka, M., Kumar, D., Shankar, U., Yadav, A., & Yadav, K. (2018). Agricultural Waste Management for Bioethanol Production. In V. Pathak, & Navneet (Eds.), Handbook of Research on Microbial Tools for Environmental Waste Management(pp. 1-33). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-3540-9.ch001

Rajasekaran, R. N., Puddoo, H., & Nguyenhuynh, T. N. (2019). An Overview of Treatment of Antibiotics Using Advanced Oxidation Process. In H. Aziz & S. Abu Amr (Eds.), *Advanced Oxidation Processes (AOPs) in Water and Wastewater Treatment* (pp. 226–260). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5766-1.ch010

Rangreez, T. A., Mobin, R., Chisti, H., Bashir, R., & Ara, T. (2019). Advanced Water Treatment Systems and Their Applications. In A. Hussain & S. Ahmed (Eds.), *Advanced Treatment Techniques for Industrial Wastewater* (pp. 1–18). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5754-8.ch001

Rastogi, M., & Nandal, M. (2018). Composting an Emerging Technology for Solid Waste Management in India. In V. Pathak, & Navneet (Eds.), Handbook of Research on Microbial Tools for Environmental Waste Management (pp. 105-126). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-3540-9.ch006

Rathoure, A. K. (2016). Heavy Metal Pollution and its Management: Bioremediation of Heavy Metal. In A. Rathoure & V. Dhatwalia (Eds.), *Toxicity and Waste Management Using Bioremediation* (pp. 27–50). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9734-8.ch002

Sadegh, H., & Ali, G. A. (2019). Potential Applications of Nanomaterials in Wastewater Treatment: Nanoadsorbents Performance. In A. Hussain & S. Ahmed (Eds.), *Advanced Treatment Techniques for Industrial Wastewater* (pp. 51–61). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5754-8.ch004

Said, S., Hussain, A., & Sharma, G. (2018). Water Quality Mapping of Yamuna River Stretch Passing Through Delhi State Using High Resolution Geoeye-2 Imagery. *International Journal of Applied Geospatial Research*, *9*(4), 23–35. doi:10.4018/IJAGR.2018100102

Saleh, T. A. (2017). An Overview of Nanomaterials for Water Technology. In T. Saleh (Ed.), *Advanced Nanomaterials for Water Engineering, Treatment, and Hydraulics* (pp. 1–12). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-2136-5.ch001

Saleh, T. A., Adio, S. O., Parthasarathy, P., & Danmaliki, G. I. (2017). Scientific Insights Into Modified and Non-Modified Biomaterials for Sorption of Heavy Metals From Water. In T. Saleh (Ed.), *Advanced Nanomaterials for Water Engineering, Treatment, and Hydraulics* (pp. 13–39). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-2136-5.ch002

Saleh, T. A., & Danmaliki, G. I. (2017). Polymer Consumption, Environmental Concerns, Possible Disposal Options, and Recycling for Water Treatment. In T. Saleh (Ed.), *Advanced Nanomaterials for Water Engineering, Treatment, and Hydraulics* (pp. 200–222). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-2136-5.ch008

Saleh, T. A., Majeed, S., Nayak, A., & Bhushan, B. (2017). Principles and Advantages of Microwave-Assisted Methods for the Synthesis of Nanomaterials for Water Purification. In T. Saleh (Ed.), *Advanced Nanomaterials for Water Engineering, Treatment, and Hydraulics* (pp. 40–57). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-2136-5.ch003

Satpathy, R. (2018). Microbial Bioreactor Systems for Dehalogenation of Organic Pollutants. In V. Pathak, & Navneet (Eds.), Handbook of Research on Microbial Tools for Environmental Waste Management (pp. 319-340). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-3540-9.ch015

Shankara, S., Dupadahalli, K., Vijayakumar, M. H., & Gaddad, S. M. (2016). Decolorization of Direct Blue: 14 Dye by Thermoalkalophilic Aerobic Bacillus sp. In A. Rathoure & V. Dhatwalia (Eds.), *Toxicity and Waste Management Using Bioremediation* (pp. 279–294). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9734-8.ch014

Sharma, A., Kaur, M., Katnoria, J. K., & Nagpal, A. K. (2016). Heavy Metal Pollution: A Global Pollutant of Rising Concern. In A. Rathoure & V. Dhatwalia (Eds.), *Toxicity and Waste Management Using Bioremediation* (pp. 1–26). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9734-8.ch001

Shikuku, V. O., & Achieng’, G. O. (2019). Occurrence and Fate of Selected Heavy Metals in a Conventional Municipal Wastewater Treatment Plant in Kisumu City, Kenya: A Case Study. In A. Hussain & S. Ahmed (Eds.), *Advanced Treatment Techniques for Industrial Wastewater* (pp. 211–224). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5754-8.ch012

Shikuku, V. O., Kowenje, C. O., & Nyairo, W. N. (2017). Fundamentals and Sources of Magnetic Nanocomposites and Their Sorption Properties. In T. Saleh (Ed.), *Advanced Nanomaterials for Water Engineering, Treatment, and Hydraulics* (pp. 58–83). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-2136-5.ch004

Shikuku, V. O., Nyairo, W. N., & Kowenje, C. O. (2019). Preparation and Application of Biochars for Organic and Microbial Control in Wastewater Treatment Regimes. In A. Hussain & S. Ahmed (Eds.), *Advanced Treatment Techniques for Industrial Wastewater* (pp. 19–34). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5754-8.ch002

Shrivas, K., Ghosale, A., & Maji, P. (2017). Advanced Nanomaterials for the Removal of Chemical Substances and Microbes From Contaminated and Waste Water. In T. Saleh (Ed.), *Advanced Nanomaterials for Water Engineering, Treatment, and Hydraulics* (pp. 127–161). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-2136-5.ch006

Simmonds, J., Gómez, J. A., & Ledezma, A. (2018). Statistical and Data Mining Techniques for Understanding Water Quality Profiles in a Mining-Affected River Basin. *International Journal of Agricultural and Environmental Information Systems*, *9*(2), 1–19. doi:10.4018/IJAEIS.2018040101

Singh, R., & Chauhan, M. (2016). Effective Management of Agro-Industrial Residues as Composting in Mushroom Industry and Utilization of Spent Mushroom Substrate for Bioremediation. In A. Rathoure & V. Dhatwalia (Eds.), *Toxicity and Waste Management Using Bioremediation* (pp. 158–177). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9734-8.ch008

Smith, M. C. (2016). Design of the Education Campaign: Creating a Motivated, Environmentally Concerned Public through Improved Scientific Literacy. In A. McKeown & G. Bugyi (Eds.), *Impact of Water Pollution on Human Health and Environmental Sustainability* (pp. 215–239). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9559-7.ch010

Starkl, M., Brunner, N., Hauser, A. W., Feil, M., & Kasan, H. (2018). Addressing Sustainability of Sanitation Systems: Can it be Standardized? *International Journal of Standardization Research*, *16*(1), 39–51. doi:10.4018/IJSR.2018010103

Stefanakis, A. I. (2016). Constructed Wetlands: Description and Benefits of an Eco-Tech Water Treatment System. In A. McKeown & G. Bugyi (Eds.), *Impact of Water Pollution on Human Health and Environmental Sustainability* (pp. 281–303). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9559-7.ch012

Stefanakis, A. I., & Becker, J. A. (2016). A Review of Emerging Contaminants in Water: Classification, Sources, and Potential Risks. In A. McKeown & G. Bugyi (Eds.), *Impact of Water Pollution on Human Health and Environmental Sustainability* (pp. 55–80). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9559-7.ch003

Stratigea, A., Leka, A., & Panagiotopoulou, M. (2017). In Search of Indicators for Assessing Smart and Sustainable Cities and Communities' Performance. *International Journal of E-Planning Research*, *6*(1), 43–73. doi:10.4018/IJEPR.2017010103

Tajarudin, H. A., Makhtar, M. M., Azmi, M. S., Zainuddin, N. I., & Ali, D. H. (2019). Introduction to Water and Wastewater Treatment. In H. Aziz & S. Abu Amr (Eds.), *Advanced Oxidation Processes (AOPs) in Water and Wastewater Treatment* (pp. 1–29). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5766-1.ch001

Tischler, D., Qi, J., Ngo, A. C., & Schlömann, M. (2018). Microbial Degradation of Azo Dyes: The Role of Azoreductase to Initiate Degradation. In V. Pathak, & Navneet (Eds.), Handbook of Research on Microbial Tools for Environmental Waste Management (pp. 341-371). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-3540-9.ch016

Turcea, V. C., & Mihai, M. M. (2019). The Impact of CO2 Emissions From Waste on Human Health: A Consequence of Globalization. *International Journal of Sustainable Economies Management*, *8*(1), 17–28. doi:10.4018/IJSEM.2019010102

Umesh, M., & Basheer, T. (2018). Microbe Mediated Bioconversion of Fruit Waste Into Value Added Products: Microbes in Fruit Waste Management. In V. Pathak, & Navneet (Eds.), Handbook of Research on Microbial Tools for Environmental Waste Management (pp. 57-78). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-3540-9.ch004

Val del Río, Á., Fernández, P. C., Gómez, J. L., & Mosquera-Corral, A. (2017). Nutrients Pollution in Water Bodies: Related Legislation in Europe and the United States. In Á. Val del Río, J. Campos Gómez, & A. Mosquera Corral (Eds.), *Technologies for the Treatment and Recovery of Nutrients from Industrial Wastewater* (pp. 21–42). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-1037-6.ch002

Val del Río, Á., Fuentes, A. P., Giustinianovich, E. A., Gomez, J. L., & Mosquera-Corral, A. (2017). Anammox Process: Technologies and Application to Industrial Effluents. In Á. Val del Río, J. Campos Gómez, & A. Mosquera Corral (Eds.), *Technologies for the Treatment and Recovery of Nutrients from Industrial Wastewater* (pp. 264–289). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-1037-6.ch010

Valverde-Pérez, B., Flores-Alsina, X., Vangsgaard, A. K., & Mauricio-Iglesias, M. (2017). Modelling and Control of Nitrogen and Phosphorus Removing Systems. In Á. Val del Río, J. Campos Gómez, & A. Mosquera Corral (Eds.), *Technologies for the Treatment and Recovery of Nutrients from Industrial Wastewater* (pp. 174–201). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-1037-6.ch007

Verma, A., & Shukla, P. K. (2016). A Prospective Study on Emerging Role of Phytoremediation by Endophytic Microorganisms. In A. Rathoure & V. Dhatwalia (Eds.), *Toxicity and Waste Management Using Bioremediation* (pp. 236–265). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9734-8.ch012

Vipond, E., & Rahman, P. K. (2018). Anaerobic Digestion Enhancement With Microbial Electrolysis Cells: Is Biomethane Production the Direction to Go for Commercialization? In V. Pathak, & Navneet (Eds.), Handbook of Research on Microbial Tools for Environmental Waste Management (pp. 259-269). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-3540-9.ch012

Voulvoulis, N., & Georges, K. (2016). Industrial and Agricultural Sources and Pathways of Aquatic Pollution. In A. McKeown & G. Bugyi (Eds.), *Impact of Water Pollution on Human Health and Environmental Sustainability* (pp. 29–54). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9559-7.ch002

Wahla, V., & Shukla, S. (2018). Role of Microorganisms in Bioremediation of Pesticides. In V. Pathak, & Navneet (Eds.), Handbook of Research on Microbial Tools for Environmental Waste Management (pp. 164-189). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-3540-9.ch009

Willems, R. C., & MacDonald, S. A. (2016). Access to Safe Drinking Water: A Low Income Sustainable Water Purification System. In A. McKeown & G. Bugyi (Eds.), *Impact of Water Pollution on Human Health and Environmental Sustainability* (pp. 304–318). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9559-7.ch013

Zakaria, S. N. (2019). Ozonation With Catalyst in Landfill Leachate Treatment. In H. Aziz & S. Abu Amr (Eds.), *Advanced Oxidation Processes (AOPs) in Water and Wastewater Treatment* (pp. 324–354). Hershey, PA: IGI Global. doi:10.4018/978-1-5225-5766-1.ch012

Zaman, A., Das, P., & Banerjee, P. (2016). Biosorption of Dye Molecules. In A. Rathoure & V. Dhatwalia (Eds.), *Toxicity and Waste Management Using Bioremediation* (pp. 51–74). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9734-8.ch003

Ziemba, R., Cuker, B. E., Stein, J., Meuninck, R., & Wan, J. A. (2016). Health Professionals Can Protect Water Quality: Tools for Educators, Advocates, and Practitioners. In A. McKeown & G. Bugyi (Eds.), *Impact of Water Pollution on Human Health and Environmental Sustainability* (pp. 240–280). Hershey, PA: IGI Global. doi:10.4018/978-1-4666-9559-7.ch011

About the Authors

Dr Khushboo Chaudhary is presently working as a Research Associate in NRCE, Hisar Haryana, India and having 1 year of teaching experience. Previously, she worked on "Improvement of Phytoremediation efficiency of Fluoride". She has published several research papers in international and national journals. She has got best paper and poster presentation award by Indian Society of Genetics and Biotechnology Research and Development and received the president appreciation awards also in the International conference. She has got best poster award by ISSGPU Central Institute Research on Goats, Makhdoom. She has published several gene banks in NCBI Pubmed. She has published research article in virology journal. She is likely to be a co-author in several of the publications and have submitted/likely to submit. Besides, she has been a co-author in 5 of the abstracts likely to present in various conferences of National and International scientific societies such as Indian Society of Virology, American Society of Virology, Indian Society of Sheep and Goat Production and Utilization etc.

Dr Suphiya Khan is presently an Associate Professor of Biotechnology in Banasthali Vidyapith (Raj) and having 12 years of teaching and research experience. Her expertise include DNA fingerprinting and phytoremediation of Fluoride. She has published several research papers in international and national journals of repute and one book on DNA fingerprinting also in her credit. Her work has been appreciated in various international and national conferences that she has attended. She has got Young scientist award by Indian Society of Genetics and Biotechnology Research and Development and received the best oral presentation and recognition awards also in the

International conference. She has been the recipient of merit scholarship in secondary education and won various prizes for extracurricular activities at the school and college level. Department of Biotechnology Research Associate ship (DBT-RA) has also been awarded to her for three years. She has successfully run the DNA fingerprinting lab as she got two major projects from the funding agencies viz UGC and MHRD, under the Center for Excellence "Water and Energy" programme of 11.50 laks and 2.25 crores respectively. She is the co Pi MHRD project and working on water and soil purification of Fluoride contaminated soil.

Dr Pankaj Saraswat graduated from RBS College Bichpuri Agra. He completed PhD from Banaras Hindu University. Presently, he has been working for agricultural technology assessment also working as PI in one of the NEC-Shillong funded demonstration-based project. He published international and national research article. Previously, he completed many projects from DST and DBT. He is presently working as Senior Scientist and Head of the KVK Tamenglong ICAR-RC Manipur Centre, India.

Index