QATAR UNIVERSITY

COLLEGE OF ARTS AND SCIENCES

HEAVY METALS HYPERACCUMULATION AND THE ROLE OF STRESS INDUCED PROTEINS IN PHYTOREMEDIATION MECHANISM IN SELECTED QATARI PLANTS

BY

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A Dissertation Submitted to

the College of Arts and Sciences

in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy in Biological and Environmental Sciences

January 2020

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ABSTRACT

USMAN KAMAL, Doctor of Philosophy: January: 2020, Biological and Environmental Science

Title: Heavy Metals Hyperaccumulation and the Role of Stress-Induced Proteins in Phytoremediation Mechanism in Selected Qatari Plants

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Progressive pollution of the environment by trace and heavy metals pose a significant risk and causes diverse human diseases. Current world initiatives to remove these toxic substances from the environment are costly and might add to the pollution. New emerging technology is phytoremediation by diverse plant species, a friendly and less expensive approach as compared with traditional methods. Lead (Pb), the second most toxic heavy metal on earth, generally slow or inhibit growth in most plant species at concentrations higher than 30 mg/kg in the soil. Some plants demonstrate the capacity to accumulate Pb in high concentration. Current work in this area is invested in elucidating mechanisms that underpin metal uptake in plants to optimize the process for large-scale application in polluted environments. In this study, we screened eight (8) plants species for Cd, Cr, Cu, Ni, and Pb accumulation and showed that *T. qataranse*, an undershrub plant accumulates higher concentration of Cd, Cr, Cu, Ni and Pb than the soil. Pb treatment at varying levels (25, 50 and 100 mg/kg) in greenhouse conditions showed that the toxic metal stimulated seed germination and seedlings growth. It concentrates on the plant tissues by more

than 270 % than the metal hyperaccumulation threshold of 1000 mg/kg Pb in plants. Up to 2,784 and 1141.6 mg/kg Pb accumulates in the plant root and shoot, respectively. Evaluation of the activities of antioxidant enzymes (CAT, SOD, APX, GPX, and GR), and enrichment analysis of differentially expressed proteins due to Pb stress provides insight into the mechanism of Pb tolerance and uptake. The plant showed higher activities of these enzymes with increasing Pb concentration, suggesting the crucial role of the plant antioxidative system in scavenging ROS. A total of eighty-six (86) differentially expressed proteins, the majority of which functions in ion and protein binding, antioxidant activity, transport, and response to stress were identified. Essential stress regulating metabolic pathways, including glutathione metabolism, cellular response to stress, and regulation of HSF1-mediated heat shock response, were also enriched. Of the 86 identified proteins, enrichment analysis showed six (6) proteins with unknown function are potentially novel Pb chelators. Therefore, the antioxidative system, over-expressed stress response, and induced metal-binding proteins (phytochelatins and glycine-rich proteins), respectively, regulate Pb tolerance and detoxification in *T. qataranse*.

DEDICATION

This work is dedicated to my late father, Malam Usman Abdullahi, of blessed memory.

ACKNOWLEDGMENTS

First, praises and thanks be to Allah, the Almighty, for his guidance and showers of blessings throughout my study period. Nobody has been more important to me in the pursuit of this project than my family. I am incredibly grateful for their love, prayers, care, and sacrifices. They are the ultimate role models.

I want to express my deep and sincere gratitude to my research supervisors, Dr. Mohammed H. Abu-Dieyeh and Dr. Mohammad A. Al-Ghouti, for their invaluable support and guidance. Their friendship, empathy, and a great sense of humor had no doubt contributed to the successful completion of this work. Beyond research, our association has provided me with extensive personal and professional guidance and taught me a great deal about life in general.

This work would not have been possible without the scholarly contributions of my Dissertation Committee members, Dr. Nabil Zouari, Dr. Serhiy Souchelnytskyi, and Dr. Aishah Latiff. I am also indebted to Dr. Chaevien S. Clendinen of the Pacific Northwest National Laboratory (PNNL), Richland, Washington, USA. Who actively contributed to the successful completion of this manuscript by providing support in MALDI-TOF/MS data processing, and have been supportive of my career goals.

The support of the Department of Biological and Environmental Sciences and the Office of Graduate Studies are sincerely appreciated.

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Chapter 1: Introduction

1.1 Background of the Study

Heavy metals constitute some of the most dangerous contaminants from the pollution sources. Several studies report that increased anthropogenic activities, especially in construction and petrochemical works, results in the generation of high concentration of different heavy metal contaminants (Kushwaha et al., 2018; Nagajyoti et al., 2010). These contaminants end up in different environmental compartments through various processes both direct and accidental, and their accumulation in the environment has many negative impacts to the ecosystem and present a great danger to sustained development (Yasseen and Al-Thani, 2013b). For instance, lead (Pb), the subject heavy metal in this study was more than three times (48.89 µg/L) the recommended maximum allowable limit by United States Environmental Protection Agency (USEPA). (Usman et al., 2019a). Lead (Pb) is a non-degradable substance that cannot be used metabolically by living organisms; whether in it's free form or part of a complex, it can accumulate to toxic levels in the environment. This toxicity and the solubility of Pb is dependent upon the chemical nature of the complexes this material forms. Organic forms of Pb is thought to be more toxic than inorganic forms (complexes). Although Pb can be found naturally in the environment or introduced through natural incidents like volcanic eruptions, the toxic levels of Pb found in the environment is largely due to human activities. In addition, Toxic levels of lead in soil pose a serious risk to the ecosystem and thus can have a major impact on human health and the well-being of other organisms (Kumar and Prasad, 2018).

Traditional methods for the clean-up of polluted environments, such as

physical and chemical remediation strategies are limited in a number of ways. To say the least, they are laborious, expensive and often times compounds environmental pollution (Abu-Dieyeh et al., 2018; Sarma, 2011; Schiavon and Pilon-Smits, 2017). Therefore, the importance of alternative treatment strategies for Pb remediation cannot be over emphasized. Indeed, in recognition of the foregoing challenge, the state of Qatar listed research and development as one of its key objectives to realizing the ambitious vision 2030. In it, environment and sustainability constitute parts of the major development pillars. Against this backdrop, various alternative approaches to the conventional system of pollution management are constantly being explored. Among them is a more cost-effective, less laborious and environmentally friendly method, phytoremediation. It has many advantages over conventional treatment strategies and has since gained the attention of researchers and other stakeholders alike (Ali et al., 2013a; Schiavon and Pilon-Smits, 2017). Phytoremediation process utilizes plants species to sequestrate pollutants (Erakhrumen and Agbontalor, 2007; Frick et al., 1999; Ghosh and Singh, 2005; Jadia and Fulekar, 2009; Vidali, 2001) through various mechanisms of actions. For over a century, hundreds of plant species including halophytes are known to remediate heavy metal pollutants at varying degrees (Ahmadpour et al., 2012; Usman et al., 2018).

Although few studies demonstrate the phytoremediation potentials of plants species native and invasive to Qatar, such as T*ypha domingenesis* and *Phragmites australis* (Yasseen and Al-Thani, 2013a), to date, there is no known hyperaccumulator plant of some of the most common metal pollutants (e.g. Pb) in Qatari environment. In a critical review of heavy metals hyperaccumulation,

van der Ent et al. (2013) summarized several criteria and suggests hyperaccumulation threshold for different metals thus; Zn – 3000 μg/g, Mn – 10000 μg/g, Co, Cr and Cu – 300 μg/g, Pb and As – 1000 μg/g, Cd, Se, and Ti – 100 μg/g. There are several examples in which the specific nature of Pb and its bioavailability is dependent upon environmental factors such as temperature; climate and other plant stressors (Kumar and Prasad, 2018). The efficiency by which any plant species can uptake and accumulate Pb suggest that the efficiency is not only dependent upon plant species but also Pb speciation (Buscaroli, 2017; Li et al., 2017). For example, Vicia faba roots, in the presence of Ethylenediaminetetraacetic acid (EDTA) do not elicit a Pb-induced phytotoxic response. This is because EDTA can form complexes with Pb and thus reduce its mobility and plant uptake (Shahid et al., 2012). Therefore, it is imperative that native plant species growing in Qatari environment are being explored. One of the most common native plant found to be growing in polluted areas is *T. qataranse.*

Further, the mechanism by which Pb is remediated remains to be sufficiently documented, even in reported hyperaccumulator species in other environments (Kumar and Prasad, 2018). Advances in proteomic technologies potentially provide a suitable platform to further our understanding of the phytoremediation process (Jorrin-Novo et al., 2018). Although the living organism's genome may maintain stability through many generations, however, protein populations may change in the course of development, especially under stress conditions. These changes could be due to biotic, abiotic and in response to external stimuli, but yet, may not be proportionate. In plants, comparative proteomics is emerging as an alternative and complementary tool for the

elucidation of biochemical and molecular mechanisms governing heavy metal sequestration. There is an increasing number of studies on proteomics of plant metal hyperaccumulation. A sizable literature is reviewed on the physiology, biochemistry and molecular aspects of plants metal hyperaccumulation (da Conceição Gomes et al., 2016; Lima et al., 2018). Significant progress in the area of plant proteomics, especially on model plants such as *Oryza sativa* and *Arabidopsis thaliana* led to improved plant proteome analysis using high throughput technologies. Today, proteins are automatically identified by sequence homology. However, because of its complexity and dynamic nature, plant proteome generally requires the use of different technologies for proper pattern analysis and identification of individual proteins (Goodin, 2018). Of the available proteomics technology, tandem mass spectrometry (MS/MS) is fast becoming popular means of obtaining significant protein matches, statistically, from peptide mass fingerprint data (Naryzhny, 2019). Polymorphism, transcriptional regulation, and epigenetic control, all of which contribute to plant adaptation to environmental stress, post-transcriptional modification, as well as protein folding may mainly be responsible for hyperaccumulator phenotypic trait (Kumar and Prasad, 2018). Therefore, the importance of proteomic technologies in the elucidation of Pb tolerance, hyperaccumulation and sequestration mechanisms in plants cannot be over-emphasized.

Another important complementary mechanism by which plants withstand metal toxicity is the antioxidative system. When present in high concentration, Pb toxicity generates free radicals or reactive oxygen species (ROS), which negatively affects plants health and consequently leads to death (Kaur et al., 2015). However, the antioxidant system found in plants plays a major role in

shielding them against Pb toxicity. The activities of antioxidant enzymes are used to measure plants potentials towards oxidative stress. Prominent among such enzymes are superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX), all of which counteract ROS effects (Shakoor et al., 2014b). SOD is the first line of defense; it sequesters noxious superoxide ions and breaks it into less harmful hydrogen peroxide and oxygen molecules. CAT, APX, and GPX assist subsequent detoxification steps.

Meanwhile, GR combats oxidative stress by balancing reduced (GSH) and oxidized glutathione (GSSG) (Hasanuzzaman et al., 2018; Sidhu et al., 2016b). In a recent study, we found that wild *T. qataranse* tolerates and accumulates various heavy metals, including Pb in an oil and gas industrial area (Usman et al., 2019b). The present work assessed and evaluated Qatari native plant, *T. qataranse* Pb tolerance, and hyperaccumulation. Further, attempt is made to elucidate the mechanism of Pb uptake and detoxification by differential protein expression and antioxidative system through the activities of antioxidant enzymes SOD, CAT, APX, GPX, and GR.

1.1.1.1 Research scope

Although many studies reported hundreds of plants including desert species as good phytoremediation agents, however, selection of appropriate hyperaccumulators for efficient phytoremediation of toxic metals polluted environment is still a challenge. Hence, there remains a gap in the search for suitable plants to realize the huge potential phytoremediation present. Additionally, for the few known hyperaccumulators, their mechanism of heavy metal uptake remains poorly understood, which is limiting progress in this area.

This study was focused on how selected plants respond to lead stress. Several greenhouse and field experiments were conducted to investigate the potential of these plants to tolerate and accumulate varying concentrations of Pb. Its mechanism of tolerance and bioaccumulation is also investigated through the antioxidative system and differentially expressed proteins.

1.1.1.2 Research questions and hypothesis

The research hypothesis in this study as stated below, are set to answer several scientific questions that will further an understanding of phytoremediation mechanism, tolerance, and the fate of heavy metal contaminants in selected desert plants.

(i) Many Qatari plants are promising phytoremediators of lead (Pb)

(ii) The plants respond differently following Pb stress.

(iii) These species also accumulate high concentration of Pb when grown in media containing high concentration of the metal

(iv) The plants exhibit different strategies of phytoremediation inclusive of phytoextraction and phytostabilization.

(v) Exposure to lead stress triggers the accumulation of important metal responsive proteins.

(vi) Pb translocation and accumulation in different tissues of the plant will change the proteome profile composition, and other novel proteins will be induced following Pb exposure.

(vii) Metal-binding proteins, and others involved in response to stress and general metabolism provide the plant with the capacity to withstand the metal stress.

1.1.1.3 Research objectives

The objectives of this study are as stated below.

(i) To determine and identify common heavy metals with the highest concentration in Qatari soil environment.

(ii) To screen out native Qatari plants found growing in Ras-Laffan and Mesaieed industrial areas for their heavy metal accumulation capacity *in situ.*

(iii) To assess and evaluate candidate plant with the highest phytoremediation potential for Pb remediation and investigate its morphological response.

(iv) To evaluate and understand the appropriate phytoremediation strategy of the plant species as either Pb tolerant or hyperaccumulator.

(v) To identify differentially expressed proteins and understand their roles in Pb tolerance and detoxification.

1.1.4 Significance of the study

Although many studies reported hundreds of plants including desert species as good phytoremediation agents, however, selection of appropriate hyperaccumulators for efficient phytoremediation is still a challenge; this is caused by slow growth, above-ground biomass, root system, and harvest. Equally important, the mechanism differs between species and depending on the available heavy metals (HM) present. Hence, there remains a gap in the search for suitable plants to realize the huge potential phytoremediation present.

This study is different from many other similar ones conducted on desert plants and phytoremediation in Qatar and even beyond on non-desert

metallophytes. In most phytoremediation studies, often emphasis is given to reporting how much concentration of heavy metals plants can take up. Additionally, many of such are conducted in purely artificial conditions such as the addition of chemical metal chelating agents or pure hydroponic culture in which case it is very difficult to conclude on the phytoremediation potential in real-life scenario. Indeed, many studies reported higher accumulation of metal concentration in an induced phytoremediation condition, where metal complexing and mobilizing substances such as ethylenediaminetetraacetic acid (EDTA) and ethylenediamine-N,N'-di-succinic acid (EDDS) are used (Vassil, 1998; Salt, 1998; Kumar, 1995 & Chaney, 2007). Here, special attention will be given to PC's, and although they are known to be important in metal detoxification and hyper-accumulation in certain plants, the role of these small proteins remain to be determined in the majority of potential metal hyper-accumulators (van der Ent et al., 2013).

The study will provide the further potential to cheap means of phytoremediation of heavy metal contaminated soil, increase knowledge base and present an emerging dimension to studying phytoremediation, especially in this region. Additionally, with the focus on identification of mechanism of tolerance and evaluation of protective proteins against heavy metal stress, it will set the stage and open avenues for further exploration of native and invasive plants species with emphasis on metabolism and eco-physiology, as the search for heavy metal hyper-accumulators continues. These will immensely contribute to advancement of scientific research potential in solving problems through multiple scientific approaches, such as understanding the role of actors of metabolism and physiology in the phytoremediation of heavy

metals. Although there are no comprehensive reviews on the proteomics of hyperaccumulation, there exist sizable number of researches on the physiology, biochemistry, and molecular aspects of hyperaccumulation reported in recent reviews (DalCorso et al., 2013; Visioli and Marmiroli, 2012; Visioli and Marmiroli, 2013). It is suggested that phenotypic changes in hyperaccumulators are a factor of gene activity, protein function and interaction with the environment (Agrawal et al., 2012; Cramer et al., 2011). While it has been established that polymorphism, transcriptional regulation, and epigenetic control contribute to plants adaptation to environmental stress, post-transcriptional modification or regulation, as well as protein folding, may largely be responsible for hyperaccumulator phenotypic trait determination (Maestri et al., 2010).

1.1.5 Merit and contribution to science

This study will complement similar studies; add to the catalog of known Qatari heavy metal hyper-accumulator plants. It will increase knowledge base and present an emerging dimension to studying phytoremediation, especially in this region. Similarly, with the focus on identification of mechanism of tolerance and evaluation of protective proteins against metal stress, it will set the stage and open avenues for further exploration of native and invasive plants with emphasis on metabolism and eco-physiology to improve plants performance for large-scale phytoremediation. Additionally, this work, being the first published on native Qatari plants metal hyperaccumulation an internationally peer reviewed journal; it will enrich the literature on desert plants phytoremediation mechanism. Consequently, the benefits of cheap, efficient and environmentally friendly means of treating soil and water, polluted with

heavy metal contaminants for agricultural and construction purposes will be reaped.

Chapter 2: Literature Review

Progressive pollution of the environment by trace and heavy metals due to increased anthropogenic activities pose a significant risk and causes diverse human diseases. Current world initiatives to remove these toxic substances from the environment are costly and often add to the pollution. New emerging technologies like phytoremediation using diverse plant species are receiving increased attention. It is friendly and less expensive approach as compared with traditional methods. Current work in this area is invested in elucidating mechanisms that underpin metal uptake in plants and the optimization of large-scale application of the methods. Advanced proteomic technologies are useful in understanding the molecular mechanisms that underlie plant metal bioaccumulation. This section gives an overview of pollution in Qatar; discuss phytoremediation and comparative proteomics as choice methods for heavy metals remediation in contaminated soil and elucidation of toxic metals tolerance and bioaccumulation in plants, respectively.

2.1 Environmental Pollution

The environment is continuously threatened by the increased generation of wastes, largely owing to the inappropriate disposal and or management practices occasioned by the rapid growth of urban population (Singh et al. 2011). A major component of these is the municipal and industrial waste, which according to Shekdar (2009) is a function of gross national domestic product (GDP) and economic status of a given population. Globally, there exist an increasing trend of municipal solid waste generation among both high and low-income countries at 3.2–4.5% and 2–3% respectively. Approximately 17 billion tons of waste is said to be generated worldwide, which is projected to increase

by more than double come 2050 (Laurent et al. 2014). These therefore calls for appropriate and cost effective management strategy for efficient waste disposal, and although there are recent efforts to integrate waste management strategies to maximize efficiency and ensure safe waste disposal. However, waste management is yet critical, largely due to the lack of suitable treatment capacity, especially in urban areas (Sharholy et al., 2008). The composition is critical to the selection of suitable management and or disposal options, depending on several factors, characteristics may differ and may includes season, source (household or commercial), weather, topographical features, population and country's economic status (Al-Salem and Lettieri, 2009). Before oil discovery, the Gulf Cooperation Council (GCC) had one of the lowest waste generation per capita (Al-Maaded et al., 2012). However, today, the story is different, due to increased population among member states, occasioned by opportunities in various sectors of the economy that attracts a large number of expats from across the globe. A large chunk of the population is concentrated in the major cities and the surrounding environs, poses a great challenge in the efficient management of wastes generated, even in relatively more developed cities (Chung and Lo, 2008). An estimated 120 million tons of waste from the region, with Saudi Arabia accounting for more half, about 20% of the waste comes from the United Arab Emirates (UAE) and the rest from Bahrain, Qatar, Oman and Kuwait.

Solid waste is arguably one of the most important and complex human by-products. Its significance is by the obvious fact that it is something we deal with daily. The volumes of these wastes generated, and its complexity in constituent components makes it a subject of environmental, health, economic

and social concerns. The public is exposed to the dangers of pollution arising from solid waste, which poses a great health risk, largely owing to improper management practices. It is especially true in third world countries, where management practices are poor when compared to developed nations, and the detrimental effect of pollution is widely manifested in the air, water, and soil environmental compartments, thereby increasing the public health risk exposure (Calabro, 2009). Other problems associated with solid waste are social and economic, this true considering the fact the success of any management option is subject to public perception and acceptance or otherwise, on the other hand, economic factors such as costs associated to collection, final disposal and the potential of recycling (Ikhlayel and Nguyen, 2017).

Approximately 17 billion tons of solid waste is said to be generated worldwide, which is projected to increase by more than double come 2050 (Laurent et al., 2014). These, therefore, calls for an appropriate and cost-effective management strategy for efficient waste disposal, and although there are recent efforts to integrate waste management strategies to maximize efficiency and ensure safe waste disposal. However, waste management is yet critical, largely due to the lack of suitable treatment capacity, especially in urban areas (Sharholy et al. 2008). To protect the environment, reduce public health risks and ensure sustainable development, an integrated solid waste management practices became imperative. However, the complex nature of solid waste composition and management implications suggest that making it success also requires consistent regulatory frameworks. Indeed, traditional waste management practices are becoming unsuitable and inefficient, at least

independently, considering the pace at which modern societies are developing today. Traditionally, health concerns were the major problems identified with waste management strategies (McDougall et al. 2008). However, the realities of modern societies suggest the need for a more concerted effort to tackle environmental degradation, ensure social acceptance and economic viability.

2.1.1 Pollution in Qatar. Qatar lies in a semi-arid desert environment, soil is highly saline, and rainfall is very scarce (Yasseen and Al-Thani 2013). Endowed with abundant natural resources, especially oil and gas, revenues generated over the years have positively benefited the country and the world at large, leading to massive expansion of many sectors of the economy critical to building an urban and industrial nation. The aggressive drive of the state in its quest for development, evidenced by the massive infrastructural transformation of the city of Doha and its environs in recent years, has attracted a huge number of expat workers, leading to a dramatic increase in the country's population. As contained in the state's development planning document "Qatar Vision 2030", and in recognition of the social repercussions of urbanization and industrialization, one cardinal pillar in the state's development agenda and policy is "Environmental Development" The increase in human population presents many opportunities and challenges, one of which is the generation of tons wastes. These originate from households, commercial and industrial sources, and these may be in the form of remains of plant materials, paper, plastic, metals and so on. These waste materials are composed of many organic, inorganic, and even gaseous contaminants (Gracia 2009; Pappu et al. 2007; Smit and Nasr 1992), and if not properly managed, could spell doom for a country with limited land mass and harsh climatic conditions.

Ensuring a sustained solid waste management system is one of the many challenges to development in developing countries like the state of Qatar. Although sustainable waste management is one of the areas where most governments in the GCC, including Qatar are most active, landfilling is seen as the most economically viable option and leaves other management programs near infeasible (Hadidi and Omer 2017). With a projected annual growth of more than 4% Although efforts are being made to improve management strategies, only about 3% and 4% of the daily solid generated wastes are recycled and incinerated respectively, with the large chunk of heaps being buried in landfills (Ayoub et al. 2014). Contaminants from these wastes end up in different environmental compartments through various processes both direct and accidental (Khan et al. 2004; Riser-Roberts 1998), and their environmental accumulation poses a wide range of negative impacts, both short and long term to the ecosystem and present a great danger to sustainable development (Bhatt et al. 2012; Kinako 1981; Yasseen and Al-Thani 2013).

In the state of Qatar, the formal launch of environmental management dates back to 1981, with a committee whose main objective was to consult and discuss with all relevant stakeholders on the environment, information was gathered via formal and non-formal means such as baseline studies on the state of regulations guiding environmental protection. The committee that now transition to the current Ministry of Environment in 2008 is saddled with the responsibilities of policy formulation, implementation, monitoring, evaluation, documentation, and enforcement on issues relating to the environment such as pollution control and emergency response (Winkler and Bilitewski 2007). Municipal solid waste management involves many stages, from the collection,

storage, transport, processing and eventual disposal of generated waste from municipal sources by guiding technical principles, emphasizing on environment, health and economy to mention but a few (Daskalopoulos et al. 1997). Several other factors could influence solid waste management, including sources, enabling policies, and social, cultural and political factors among others. In order to ensure sustainable environment, both technical and non-technical factors remain to be addressed.

Other forms of pollution, including atmospheric pollutions, are also high, which obviously remains one of the most threatening, locally, and across the globe, it poses a greater health risk to the entire populace, and further contamination of the environment and inappropriate municipal solid waste disposal is further compounding the implications. Although some may argue on the inadequacy of clear-cut environmental laws and regulations, It should, however, be noted that enforcement of regulations on violation of any environmental law is in most cases the problem. For instance, the Gulf States had recently adopted unanimous legislation to tackle and deal with environmental problems including solid waste generation and disposal, however, years down the line, enforcement and implementation is still a concern (Al-Maaded, 2012). To manage waste, several approaches are available and are often complementary to each other. However, in Qatar and other developing nations, the cheaper and easier methods, which is the least preferred such as landfilling with no proper design and maintenance is the most widely used, resulting in harsh environmental consequences and depletion of natural resources (Tolba and Saab 2008).

Despite increased industrial activities across the GCC, households

garbage's constitute the majority of accumulated waste compared to industries and other commercial sources, a trend attributed to social and cultural variations with other listed countries such as the USA and UK (Sufian and Bala 2007). Handling and separation are key components of efficient waste management, however, in Qatar, although biodegradable plastic bags are widely used, mixed garbage's are mostly disposed of together, making it difficult to separate and only possible at the landfill site. Accordingly, and in response to this, the state development policy planned for an ambitious reduction in waste generation per capita, and improvement on the recycling share of generated solid waste to 38%. These are largely contained as recommendation for massive public enlightenment campaigns on the reduction of waste generation, step-up of recycling culture and strict compliance to environmental policy and regulations with a view to ensuring a healthy society and sustained development; others are energy conversion and reduction of the landfill share to 53% (Al–Muhannadi, 2013). The Domestic Solid Waste Management (DSWM) center for the management of generated waste established in 2011 at Um Said is indeed encouraging. It provides a solid foundation for achieving improved solid waste management, with an integrative approach and system with various components of waste management, such as waste to energy recovery, engineered landfill and incineration (Ayoub et al. 2014). The integrative system practice in the DSWMC is laudable; the center can treat 2,300 tons of domestic waste daily. It consists of pre-processing drum for sorting of recyclable and non-recyclable waste, anaerobic digestion and composting plants for the production of soil enhancers used in agriculture, landscaping, and energy as well as an incineration plant for steam and

electricity generation from non-composted waste. However, as at present, only 1,557 tons of mixed solid waste is treated daily, accounting to less than 1.5% of the total, and hence, far more is still expected, this could be by operations at full capacity, optimization of operational procedures and perhaps establishment of additional treatment plant. Current solid waste management, practice and the composition potentially provide room for further improvement. According to Al- Maaded et al. (2012), about 80% of the total solid waste can be decomposed and recycled. Indeed this present a promising potential to exploit and improve municipal solid waste management in the state of Qatar, and interestingly, the state's commitment, resources and recent regulatory frameworks on ensuring environmental sustainability are encouraging. DSWMC is an excellent legacy and foundation to build upon, ensuring proper monitoring and strict compliance to the plans, and targets set for improved solid waste management, as contained in the National vision to increase recycling of solid waste share to 38% and significantly reduce landfilling practice. This will no doubt remarkably help in improving waste management, healthy society and consequently ensure sustainable development.

2.1.2 Implications for the soil, agriculture and environment. One of the objectives of municipal solid waste management is to transform wastes in to useful materials. When treated, waste generates products such as mature compost; ashes, bio chars and others. These are useful for soil amendment and results in variable impacts by changing the physical, biological and chemical composition of the soil and by extension plants. This way, the general soil environment quality is improved; enhancing crop productivity for plants growing in agricultural fields and the overall activities of soil biota is also

stimulated (Hargreaves, 2008).

It is of great benefit to supplement a crop requirement for nutrients from organic fertilizers such as compost, which enhances soil fertility and improve crop productivity. Municipal solid waste is majorly composed of household garbage, but also partly commercial and industrial disposables. This is processed to a mature compost for use as organic fertilizer (Singh, 2010). This is a diversion from the usual practice of massive burial of solid waste made up of organic matter; it is cheap and efficient source of natural fertilizer useful in the agricultural fields for plant growth (Eriksen et al. 1999). The increasing demand for cost effective (legislation and transport) and environmentally friendly products favor the much-turned attention to composting as opposed to direct landfilling. The waste compost is very rich in organic matter with proven variable components of important soil organics, with highly beneficial effect on soil mineralization. Studies have proven that, among other things, mature waste compost is composed of humic and fulvic acid, with the more stable humic acid conferring on the soil buffering capacity (He et al. 1995). Regular soil treatment with waste compost significantly enriches the carbon to nitrogen ratio much better than untreated or un-amended soil. Mature compost of municipal origin is also linked to facilitating improved water holding capacity, structure, and makes aggregate amended soil stable (Hernando et al. 1989).

Studying the ecology of soil is fast becoming popular in the assessment of soil quality, and any change in the soil as an environment is said to affect microbial population the most (Crecchio et al. 2004), as highlighted in other sections, compost addition to soil dramatically improve fertility and productivity of crops resulting in an increased overall biomass. To monitor soil microbial

health, basal respiration rate, and nutrients enzymatic activity are determining the (Nannipieri, 2003). Studies indicated that continuous application of municipal solid waste compost on soil environment increase microbial activity, and accordingly, activities of some key enzymes involved in nutrients utilization such as phosphatase were shown to also be on the increase (Crecchio et al. 2004; Perucci 1990). Hence, it is safe to conclude that compost addition facilitates the transformation of phosphorous from organic to inorganic form (Diacono, 2010). Other enzymes activities including dehydrogenase also rise following the addition of mature compost to the soil (Jindo, 2012).

Similarly, the chemical composition of the soil environment changes in the presence of waste compost. Nutrients, minerals and parameters levels, such as pH, phosphorous, sulfur, nitrogen, calcium, magnesium, copper and zinc increases with an increased application of the compost. Thereby positively enriching the soil and promoting plant growth, while others such as iron, boron are only found to be present with no increase in terms of concentration following the addition the organic product (Hargreaves et al. 2008).

The combustion products of waste are useful for a soil amendment mainly to tackle or mitigate the effect of greenhouse emission arising from agriculture, while synergistically improving crop yield. Fly, and bottom ashes are finely powdered particles pulled in the flue gas steam and aggregates of small porous materials dropping into the boiler bottom respectively (Skousen et al. 2013). Though similar and subject to the burnt coal type, bottom and fly ash are physically different but are said to be typically composed of the same elements (Aluminum, iron, calcium, and silicon) in variable proportions and highly alkaline (Belyaeva, 2009).

However, trace elements differ considerably with fly ash richer in the most potentially hazardous type, such as cadmium, boron, arsenic, selenium, lead, and mercury (ASTM, 1998). Their alkaline property is harnessed in soil stabilization by adjusting acidic soil and liming material (Jiangjiang et al. 2010; Yang et al. 2011), enrichment of plants nutrients is another characteristic of this coal waste material, and can also bind to heavy metals in the soil (Stewart and Daniels 1992). Though most plants found in the heavy metal polluted soil are tolerant of the stress condition, however, at high concentrations, toxicity may affect general plant physiological process, especially for non-hyper-accumulators (Zhao, 2012). It is therefore indeed an important property, conferring on the surrounding growing plants, an alternative defense mechanism against the heavy metal negative effect on growth. This will allow for more energy utilization towards biomass production as opposed to dealing with stress condition (Abbasi, 2010). Additionally, fly ash is widely targeted for improving the productivity of saline soil since calcium in exchange for sodium will be needed in sodic soils, which is possible by the addition of calcium-containing products or materials (Skousen et al. 2013).

Bio-chars constitute another product of transformed solid waste, mainly used in farm practices to reduce the emission of hazardous gases into the atmosphere by reducing methane and nitrous oxide emission and increasing organic carbon in amended soil. It is widely acknowledged as an effective means of improving soil fertility and an important component of improved ecosystem management strategies (Zhang et al. 2012). As one key role of bio-chars in mitigating the effect of climate change, it reduces the emission of carbon dioxide while improving on carbon storage. However, variations were

observed on how bio-char amendment affects respiration in the soil and carbon dioxide emission (Zimmerman et al. 2011).

Although some of the most common municipal solid waste management practices in Qatar, including direct landfilling and soil disposal of raw waste appear to offer the best economic bargain and somewhat environmentally friendly when compared to others. Such may not be true in the long run and hence the need for more focused attention on the generation of waste reduction, re-usage, and recycling. In other words, an integrated system of waste management before final land disposal is imperative. Among many factors to consider when deciding particular waste management and or disposal strategy, economic and environmental consequences are at the forefront. Landfilling requires a sizable land area for waste burial. Transportation and storage facilities costs are cheap comparative to full pledge recycling plants. Accumulated and buried waste move in the soil, following land disposal could lead to negative impacts on the soil, especially in agriculture and the degradation of environment in general. For instance, when constructing landfills, suitability study before siting any landfill location is imminent to minimize negative consequences on agricultural fields and the environment from possible leachates. Compost addition to soil enhances fertility in many ways (Garcıa-Gil et al. 2000; Perucci 1990).

However, there are concerns in some quarters that, soil amended with solid waste, especially in its raw form, may be loaded with a high concentration of metals. Although heavy metals concentration beyond certain limits in the soil affects plant growth, some plants are known to thrive in metal contaminated soil with a varying response mechanism in dealing with the stress condition. Studies

have shown that accumulation of heavy metals in plant tissues results in a wide range of negative effects on growth, inclusive of seed germination, growth of seedlings and photosynthetic processes (Maheswari et al., 2012). Additionally, it also leads to the inhibition of the plant's important enzymatic activity (Sharma and Dubey 2005; Singh et al. 1997). However, variation exists in plants response to the accumulation of these metals in plants (Liu et al. 2000) and typically deal with heavy metal stress generally by phytoextraction, phytostabilization and phytovolatilization summarized in the figure below (Sharma and Pandey 2014). The root plays a critically important role in plant growth and development and hence can directly affect other tissues response to growth conditions (Biernacki and Lovett-Doust, 2002).

2.1.3 Heavy metal pollutants. Heavy metals and other organic compounds constitute the major environmental contaminants, and the trials of phytoremediation to free pollutants from wastewater and contaminated soil dates back to hundreds of years ago in plants such as the Thlaspi caerulescens and Viola calaminaria, which were reported to remediate high concentration of heavy metals (Lasat, 2002). Anthropogenic activities arising from industrialization largely contribute to the proliferation of these contaminants, either by direct leakage or accidents during transport of solid and liquid wastes from storage and industrial facilities (Lone et al., 2008; Yasseen and Al-Thani, 2013a). Strategies to clean up environmental contaminants, both organic and inorganic are either by physical, chemical and or biological treatments (Baldwin et al., 2015; Hasegawa et al., 2016). However, physical and chemical methods are recognized for some disadvantages or limitations such as high cost and labor intensiveness. Additionally, chemical processes create another pollution

and are especially costly since they generate heaps of sludge (Tangahu et al., 2011). Given this context, new and better approaches to clean up of metal contamination were thought up and became imperative, hence the exploration of various bio-based techniques. The use of biological agents is considered cheap, safer and has limited or no negative impact on the environment (Doble and Kumar, 2005). Bio-based remediation methods include bio-augmentation, bioremediation, bioventing, composting and phytoremediation. However, phytoremediation proves the most viable and useful alternative and has gained increasing attention in recent times (Ullah et al., 2015; Witters, 2011).

The adverse and negative effects associated with these elements make them targets for phytoremediation (Monica and Cremonini, 2009). Phytoremediation offers several advantages. It is cheap, promotes biodiversity, reduces erosion, less destructive and decreased energy consumption leading to reduced carbon dioxide emission (Ali-Zade et al., 2010). To date, about 400 plant species were suggested to be metal hyper-accumulators (Walliwalagedara et al., 2010). However, few studies reported the toxicity of several metals combined (Visioli and Marmiroli, 2013), and while hyper-accumulation of nickel (Ni), cadmium (Cd), manganese (Mn), zinc (Zn) and selenium (Se) have been well established, the same is yet be available or demonstrated beyond doubt in plant species for copper (Cu), chromium (Cr), lead (Pb), thallium (Th) and cobalt (Co) metals. For instance, Cu is an important element for growth and general plant physiology, owing to its role as a cofactor to various types of enzymes involved in the transfer of electrons during metabolic processes, such as the tricarboxylic acid (TCA) cycle (Hall, 2002; Himelblau and Amasino, 2000). However, at high concentrations, it is toxic to

plants signaled by stunted growth, and although there is some physiological insight to Cu stress in plants, the responses are still vague at the functional level (Lin et al., 2013).

The accumulation of heavy metals in plant tissues results in a wide range of negative effects on growth. Although it affects seed germination, growth of seedlings and photosynthetic processes, which generally leads to the inhibition of the plant's important enzymatic activity (Sharma and Dubey, 2005; Singh et al., 1997), however, plants responds differently (Liu et al., 2000). In dealing with the heavy metal stress, the root tissue is the first to be exposed to the associated toxins, its cell wall has a mechanism of exchange that fixes the heavy metal ions and thereby limiting the transmission of the toxins to other plant tissues (Allan and Jarrell, 1989; Branquinho et al., 1997). Several studies reported many plants, including desert species as good phytoremediation agents, however, few are metal hyperaccumulators, and their selection for efficient phytoremediation is still a challenge. It is demonstrated by slow growth, above-ground biomass, root system and harvest (van der Ent, 2013 and Sathya, 2016). Accordingly, successful heavy metal phytoremediation requirement of hyperaccumulation capacity in candidate plants position halophytes as suitable phytoremediators, due to their extensive stress tolerance mechanism, which enables them thrive in saline soil and other desert conditions.

2.1.3.1 Lead. Lead (Pb) is a non-degradable substance that cannot be used metabolically by living organisms; whether in its free form or part of a complex, it can accumulate to toxic levels in the environment. This toxicity and the solubility of Pb is dependent upon the chemical nature of the complexes this material forms. Organic forms of Pb is thought to be more toxic than

inorganic forms (complexes). Though Pb can be found naturally in the environment or introduced through natural incidents like volcanic eruptions, the toxic levels of Pb found in the environment is largely due to human activities like mining. Also, Pb is also used in many industrial products like batteries due to its stability and malleability. Toxic levels of lead in soil pose a serious risk to the ecosystem and thus can have a major impact on human health and the well-being of other organisms. Though technologies for soil remediation of toxic metals such as lead exist, these are often very expensive (Kumari et al., 2016; Kuppusamy et al., 2016; Peng et al., 2018). An alternative and low-cost method that has been investigated is phytoremediation. Phytoremediation is a technique by which living plants stabilize metals in the rhizosphere and extract these metals from the soil by absorbing them in specialized plant tissues. There are several examples in which the specific nature of Pb and its bioavailability is dependent upon environmental factors such as temperature, climate, lab conditions, and other plant stressors. The efficiency by which any plant species can uptake and accumulate Pb has been studied (Buscaroli, 2017; Ferreyroa et al., 2017; Kohli et al., 2018); this efficiency is not only dependent upon plant species but also Pb speciation. For example, *Vicia faba* roots, in the presence of EDTA, do not elicit a Pb-induced phytotoxic response, due to EDTA , thereby–Pb complex (Shahid et al., 2012).

2.1.3.1.1 Lead bioavailability. Pb levels can accumulate differently in different parts of the plant. Though there are some exceptions (Bi et al., 2009), Pb tends to accumulate more in the roots than the stem or the leaves. Finster et al. (2004), determined that among the different plants studied, the roots always accumulated more Pb and the fruits of the plant tended to have only

trace levels. Several factors contribute to Pb restriction to the root. (a) Immobilization or precipitation by lignin and pectins, (b) Binding to acids within the cell walls (Arias et al., 2010; Islam et al., 2007), (c) in intercellular spaces (Malecka et al., 2009), (d) sequestration in vacuoles of cortical and rhizodermal cells (Pourrut et al., 2011), and (e) physical restriction by the endoderm.

There are two common mechanisms by which plants deal with trace metals: metal avoidance and metal uptake. Metal avoidance can involve the active efflux of metal, metal precipitation, and redox barrier. Contrary to this, metal uptake and accumulation involves 3 main steps: uptake, transportation, and compartmentalization. Metals uptake involves selective mechanisms in response to concentration gradients. Before compartmentalization, Pb is translocated to a degree that can be described by the translocation factor (ref: Bhatti et al 2018.

The translocation factor is an index used to identify the level of transportation of Pb within plant tissue and is essentially the ratio between the levels of Pb in the aerial parts of the plant to the roots. Usually, the translocation factor is very low because Pb accumulate in the roots, and only small amounts translocate to the aerial parts of plants. In the roots, precipitated Pb is accumulated in the cell wall, while free Pb is transported to other parts of the cell. Transporters such as ATP-binding cassette (ABC) transporters play key roles in transportation of Pb across the cell membrane followed by sequestration into inactive organelles. The translocation of Pb from the roots to other parts of the plants is thought to be via the xylem and phloem cells.

2.1.3.1.2 Lead-induced toxicity. Under Pb stress, the growth of plants is severely decreased. This decrease in growth potential is proportional to the concentration of lead in which the plant is exposed. All parts of the plant, including root elongation, plant height, number and structure of leaves is affected by phytotoxicity. Reactive oxygen species serve an important role in Redox signaling essential for cellular homeostasis. Pb disrupts this balance by replacing essential cations and altering metal-containing enzyme activity. Chloroplasts, mitochondria, and peroxisomes are the main sources of ROS. Pb toxicity changes electron transport rates and increases the generation of ROS. Nearly every stage of the central dogma (DNA, RNA, protein) is affected by Pb toxicity. A known marker of ROS-mediated stress is the presence of lipid peroxidation. ROS alter the structure and thus the function of cell membranes by initiating the lipid peroxidation reaction. This reaction begins with the transfer of hydrogen from a methylene group of a polyunsaturated FA to hydroxyl radical. Enzymes, such as lipoxygenases and phospholipases, can cause lipid peroxidation.

Another type of damage is photo-oxidative damage. Pb affects the ability of the plant carry out photosynthesis by altering levels and structure of photosynthetic pigments (chlorophyll). Though there are a few exceptions, generally, chlorophyll, under Pb stress, can be significantly reduced; this results in a decrease in photosynthetic rate. Guard cells are also affected. Pb also affects the activity of some photosynthetic enzymes, including δ-aminolevulinic acid dehydratase via direct inhibition of activity or structural modification. Such interactions decrease the photosynthetic capacity of the plant. Pb is a mutagen that could damage genetic material resulting in DNA strand-breakage, damage

to the cytoskeleton, instability, and so on. Its genotoxic effects are largely due to the indirect or direct disruption of DNA and RNA via ROS. This also contributes to the mitotic toxicity due to the damage of mitotic spindles fibers. Some plants are more resistant to this damage, and such toxic potential is dependent on length of exposure and environmental condition. Proteins can be oxidized by the free radicals created during Pb stress. Free radicals interact with proteins and cause site-specific modifications and fragmentation via proteolysis. Some modifications are irreversible, but many stress-related proteins are synthesized to help the plant overcome the Pb stress.

2.2 Phytoremediation

During phytoremediation, plants growing on soil or water contaminated with trace or heavy metals could absorb or tolerate these elements differently, depending on the physiological means involved and the kinds of metals present (Pilon-Smits and Freeman, 2006). According to Halder and Ghosh (2014), phytoremediation techniques are categorized into five; phytoextraction, phytofiltration, phytovolatilization, phytostabilization, and phytotransformation.

2.2.1 Phytoextraction. Phytoextraction is a technique of phytoremediation where plants take up metals by translocation and accumulate them in a form that can be extracted on its tissue (Nwoko, 2010). It is one of the most common types of phytoremediation and the names; phyto-absorption, phytoaccumulation, and phyto-sequestration are often used interchangeably to refer to phytoextraction (Ali et al., 2013b). It is considered as the major phytoremediation technique among all others for the removal of metals from contaminated water, sediment, and soil. Though the efficiency of this remediation process depends on many factors from soil properties, metal

bioavailability, and speciation to the type of plant species. However, high concentration of absorbed metals usually ends up in the shoot biomass of the plant in harvestable form (Walliwalagedara et al., 2010). Many recent studies reported various plant species that demonstrate phytoextraction strategy from both water and soil media (Kacálková et al., 2015; Li et al., 2010; Shaheen and Rinklebe, 2015; Simmons et al., 2015).

Plants able to exhibit phytoextraction strategy in metal sequestration may potentially be hyperaccumulators, referring to plants that consistently accumulate certain threshold of metal concentration in their shoot tissue, which varies according to the metals (van der Ent et al., 2013). Generally, all hyperaccumulators should possess characteristics such as high growth rate, widely branched shoot, high bioaccumulation and translocation capacity, high above-ground biomass, easily cultivated and harvested (Sharma and Pandey, 2014; van der Ent et al., 2013). However, Ali et al. (2013b) demonstrated two methods or approaches for metal phytoextraction in different plants, one producing less above-ground biomass but significantly accumulate metals in high concentration and vice versa in the other plant species, with final metal accumulation in agreement with those of hyperaccumulators. Consequently, hyperaccumulation is more important in phytoremediation than volume of biomass produced, and suggests the use of hyperaccumulators as more acceptable since it has advantages such as safe disposal, cheap process and easy handling (Ali et al., 2013b).

2.2.2 Phytofiltration. Phytofiltration or rhizofiltration as used interchangeably refers to the absorption or adsorption of contaminants from surface wastewater by plant roots thereby preventing them from leaching to the

underground water (Sarma, 2011). It is a type of phytoremediation technique that can be demonstrated in situ by directly growing plants in the polluted water body (da Conceição Gomes et al., 2016). Although it is commonly applied using aquatic plant species (Olguín and Sánchez-Galván, 2012), there are suggestions that the process may be applied to terrestrial plants, which remediate metals to precipitate with the aid of microbes root biofilter (Salt et al., 1995). Indeed, root exudates cause metal precipitation which alters the rhizosphere pH level (Rai, 2008). Many terrestrial plants including grasses grown in a hydroponic culture were shown to effectively remove metals such via phytofiltration (Khilji, 2008). In the same study, Indian mustard was especially reported to accumulate higher fold of metal concentration far beyond the initial concentration, and the removal is by tissue-specific adsorption mediated by root metal concentration.

Several studies have shown many species of aquatic macrophytes that demonstrate phytofiltration potential. While experimenting for phytoremediation under different water conditions polluted with heavy metals, Liao and Chang (2004) found that *Eichhonia crassipes* absorb and accumulate metal contaminants, it has also exhibited high growth rate and increased biomass production and thus considered a good phytofiltration agent. This plant species absorb high concentrations of Pb, Ni, Zn and Cu which accumulates much higher in the root tissue than the shoot, suggesting the important role of fibrous and tap root system found in the plant, which is one of the key characteristics of potential phytofiltration agent. In a similar study, other aquatic plant species including *S. hergozii,* E. *crassipes, P. stratiotes* and *H. stolonifera* were shown to absorb high concentration of Cd. *P. stratiotes* accumulates higher

concentration and exhibited faster growth rate, a feature attributed to possible complementary mechanism for the enhanced metal uptake (Maine et al., 2001). Absorption of Cd in the root of all the plants relates to the added concentration. In another study by Thayaparan et al. (2013) also reported that *Azolla pinnata* had shown great potential in the removal of high Pb concentration by phytofiltration from polluted water. As in phytoextraction, potential phytofiltration agents should tolerate high metal concentration, exhibit fast and high growth rate as well as above-ground biomass, however, in contrast to phytoextraction, they are expected to show limited translocation capacity of absorbed metals from root to shoot tissues (da Conceição Gomes et al., 2016). For efficient phytofiltration, this is an advantage over phytoextraction, since low translocation of contaminants means reduced contamination of other parts of the plant.

2.2.3 Phytostabilization. In this technique, pollutants are converted into a less toxic or bioavailable form by the continuous precipitation of the plant rhizosphere. This is achieved either by surface runoff prevention, erosion or leaching (Nwoko, 2010). It is applicable in the stabilization of metals in contaminated soil, sediment or water environments, which ensures they are not transferred to the food chain from the soil by translocating to other parts of food crops or to the underground water. It is possible by sorption via the root, precipitation and subsequent metal reduction around the plant rhizosphere, for instance the toxic Cr^{6+} is converted to Cr^{3+}, which is less toxic (Ghosh and Singh, 2005; Wu et al., 2010). Variation exists as to how prone a metal is to phytostabilization and is subject to its chemical character. This is evidenced in a comparative study to evaluate metal accumulation capacity of two aquatic

macrophytes Phragmites australis and Typha domingensis, where both are found to stabilize As and Hg but inefficient in the phytostabilization of other metals (Bonanno, 2013).

Although phytostabilization offer some advantages over other phytoremediation techniques, it is however limited to temporary measure to deal with pollutants contamination since metals are only inactivated and their movement restricted, but remains in the contaminated environmental compartment (Vangronsveld et al., 2009). It is useful in emergencies since it can rapidly immobilize pollutants from soil, water or sediment. Equally important, it ensures that contaminants are not translocated to other plant tissues by trapping most of it in the plant root (Abreu et al., 2012). Considering the strategies employed in phytostabilization, plants that can appropriately fall under this mechanism is their ability to tolerate and immobilize metals and other contaminants, low translocation capacity from root to plant aerial parts and of course extensive and fibrous tap root system (Doble and Kumar, 2005). Among several studies that reported plants species with these characteristics (Andreazza et al., 2015; Najeeb et al., 2014; Zhang et al., 2016) demonstrating the phytostabilization of Zn, Pb, Cu and Cd by different plants in soil and sediment polluted environments.

2.2.4 Phytotransformation/Phytodegradation. Phytotransformation or phytodegradation is another technique of phytoremediation where contaminants and other nutrients are chemically modified through plant metabolism and render associated contaminants inactive in both plant root and shoot tissues (Tangahu et al., 2011). Plant metabolic enzymes act on the surrounding contaminants, thereby transforming them to a less toxic form,

plants rhizosphere microbes also aid in the transformation process of the compounds (Dobos and Carmen, 2009). Although this mechanism is mostly against organic contaminants, inorganic compounds such as metals were also suggested, in which case a strategy akin to phytostabilization is employed to convert toxic metals to less toxic form (Kobraee et al., 2011). However, this technique seems less efficient and reliable compared to others in that it requires longer period, strict soil characteristics such as depth and underground water availability and often require soil amendments.

2.2.5 Phytovolatilization. In phytovolatilization, contaminants are converted into a volatile form and released to the air via plants leaves stomata (Nwoko, 2010; Sarma, 2011). However, this mechanism merely transfers contaminants from one environmental compartment to another, which may somehow return back to the original source (soil) by precipitation and hence could be less popular to other phytoremediation techniques especially phytoextraction and phytofiltration (Nikolić and Stevović, 2015; Sarma, 2011). It is commonly employed when treating groups of highly volatile metals like Hg and As. Phytovolatilization of As involves the conversion of elemental As to selenoaminoacids, such as selenomethionine, which is modified by methylation to a volatile and less toxic form, di-methylselenide (Wang et al., 2012).

2.3 Metal Hyperaccumulator Plants

Several plants species are known to tolerate high concentration of toxic metals. Tolerant species are best described as excluders, where metal uptake and translocation to different tissue parts are limited. While others that are capable of accumulating higher concentrations with improved translocation from the root to shoot part of the plant, thereby significantly reducing its

availability in the soil, and they do so with no visible sign of toxicity effects. To date, heavy metals have no standard definition by recognized bodies in the area. Various researchers use different characteristics and levels in their description such as atomic mass and number, density, chemical character as well as their toxicity. However there appears no connection between such properties (Sathya et al., 2016). According to Wang and Chen (2006), three categories of heavy metals arising from both natural and artificial sources are of interest, these include valuable metals e.g. Ag, Au, Pd, Pt, harmful metals e.g. As, Cu, Co, Cd, Cr, Hg, Ni, Pb, and radionuclides such as Am, Th, and Ra. The non-biodegradable and stable nature of heavy metals suggest increased exposure to living species including humans (Sathya et al., 2016), periodic reviews of toxic metals effects are documented by many research groups (Duruibe et al., 2007; Nagajyoti et al., 2010; Wongsasuluk et al., 2014). When determining the hyperaccumulators of toxic metal, the most important factor is the concentration of the metal ion threshold.

Therefore, plants can be regarded as hyperaccumulators, when capable of accumulating toxic metals concentration to about 50 to 100 times more than non-hyperaccumulator plants (Baker and Brooks, 1989; Visioli and Marmiroli, 2013). For instance, the threshold for Zn and Mn hyperaccumulation in plant shoot is pegged at 1% of dry biomass, 0.01% for Cd and 0.1% respectively for Ti, Se, Sb, Pb, Ni, Cu, Cr, Co, and As (Krämer, 2010; Visioli and Marmiroli, 2013). To date, few plant species are classified as hyperaccumulators, the majority of them (3/4) are tolerant to Ni and belongs to the Brassicaceae family native to Western Asia and Southern Europe, with up to 48 species implicated in Ni accumulation of around 3% dry shoot mass (Baker et al., 2010; Krämer,

2010; Milner and Kochian, 2008). There is increasing interest in plant hyperaccumulators in recent times, owing to their potential use in metal contaminated soil and water detoxification (Chaney et al., 2007; Pilon-Smits and Freeman, 2006).

2.3.1 Bioconcentration and translocation factors. Bio-concentration (BCF) and Translocation (TF) factors represent the ratio of a given element concentration in the plant tissues at harvest to the concentration of the element in the external environment (BFC = P/E), and the concentration of a trace element accumulated in the root tissues by that accumulated in shoot tissues (TF= (As / Ar) respectively (Bose and Bhattacharyya, 2008). The Bioconcentration and translocation factors are useful in comparing plants grown in regular soil and hydroponic solutions. Translocation factor specifically represents root to shoot metal iron concentration ratio and should typically be greater than one in potential hyperaccumulator plants (van der Ent et al., 2013).

2.3.2 Metal-chelation and antioxidant enzymes. The phytoremediation of heavy metals involves many physiological, biochemical and molecular activities. In this process, especially phytoextraction which involves the accumulation and translocation of heavy metals to plant tissues. Plant metal chelators or phytochelatins (PCs) and metallothioneins (MTs) are the most common transporter proteins for heavy metal phytoremediation. MTs are cysteine-rich proteins that are famous for metal binding and greatly assist in the process of sequestration of metals in ionic form (Kärenlampi et al., 2000). PCs are glutathione synthase products, and they bind to heavy metals thereby forming central part of the phyto-detoxification mechanism (Fulekar et al., 2009; Yurekli and Kucukbay, 2003), and its general structure is as shown in the figure

below (Seth, 2012). Phytochelatins are induced by the activity of an enzyme, phytochelatins synthase, which is triggered by the activity of metal ions present (Cobbett, 2000; Sarma, 2011). In an experiment to demonstrate the role of synthases, mutants in model plant Arabidopsis thaliana were shown to be hypersensitive to Hg and Cd, which is attributed to their inability to produce PCs (Memon and Schröder, 2009).

On the other hand, MTs are genetically encoded metal-binding proteins and usually bear low molecular weight. Several studies demonstrate MT's role in the protection of plants against the toxicity of heavy metals in soil, sediment, and water (Fulekar et al., 2009; Jabeen et al., 2009; Sheoran et al., 2010). According to Wu et al. (2010), MTs, PCs expression alongside organic acid synthesis together functions in heavy metals uptake by plants and also their translocation to other tissue parts. The expression of these natural chelators can be enhanced to increase the efficiency of heavy metal accumulation and translocation. Currently, there are many ongoing studies aimed at characterizing and identifying biomolecules involved in the transport and detoxification of heavy metals. It will aid in understanding the whole detoxification process involved in plants (Ali et al., 2013b; Seth, 2012), and to achieve this, the importance of comparative proteomic studies cannot be over-emphasized.

Further, the plant's antioxidative enzymes rise with increased metal toxicity. When present in high concentration, heavy metal toxicity generates free radicals or reactive oxygen species (ROS), which negatively affects plants health and consequently leads to death (Kaur et al., 2015). However, the antioxidant system found in plants plays a major role in shielding them against

Pb toxicity. The activities of antioxidant enzymes are used to measure plants potentials towards oxidative stress. Prominent among such enzymes are superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX), all of which counteract ROS effects (Shakoor et al., 2014b). SOD is the first line of defense; it sequesters noxious superoxide ions and breaks it into less harmful hydrogen peroxide and oxygen molecules. CAT, APX, and GPX assist subsequent detoxification steps. Meanwhile, GR combats oxidative stress by balancing reduced (GSH) and oxidized glutathione (GSSG) (Hasanuzzaman et al., 2018; Sidhu et al., 2016b).

2.3.3 The Halophytes of Qatar. Some studies demonstrated the potential of several Qatari plants as good phytoremediation candidates, many among which are heavy metal hyperaccumulators. Examples includes species belonging to the genus *Tetraena* (Formerly *Zygophyllum*), which are as either metal tolerant or accumulators when tested on both polluted soil and wastewater media (De La Rosa et al., 2007; Hu et al., 2012; Lefèvre et al., 2005; Osman and Badawy, 2013; Rejeb et al., 2013). Others include *Typha domingenesis* and *Phragmites australis* (Yasseen and Al-Thani, 2013a). According to Carvalho and Martin (2001), *Typha domingensis* remediates heavy metals from industrial wastewater and solution cultures. Similarly, members of the halophytes plant family Brassicaceae were reported to be important phytoremediation agents (Baker and Brooks, 1989; Ebbs et al., 1997; Lone et al., 2008) and the tree plant *Prosopis juliflora* exhibited phytoremediation of heavy metals potential (Prasad; Shukla et al., 2011). Others include *Phragmites australis* (Nie et al., 2011), *Medicago sativa* and

Glycine max (Frick et al., 1999; Njoku et al., 2009). Some examples of other species tested for phytoremediation studies and their metal uptake capacity are summarized in Table 1. *T. qataranse* is one of the most common desert plants found to be growing all over Qatari environment. A desert undershrub, well adapted to rocky and sandy saline soil, it is characterized by fleshy terminal branches and succulent leaves (Fig. 1)

2.4 Plant proteomics

Proteomics involves the analysis of complex protein mixtures using various tools, specifically revealing information about individual proteins and their biological roles in a living system. Although living organism's genome may maintain stability through many generations, protein populations may, however, change in the course of development especially under stress conditions, which may be biotic, abiotic and even in response to external stimuli but these changes may not be proportionate (Kosová et al., 2011). Understanding plant proteomics requires the use of techniques such as polyacrylamide gel electrophoresis (PAGE), multidimensional protein identification techniques and the use of various bioinformatics tools to analyze the huge data generated (Perotti et al., 2011; Rabilloud et al., 2010).

The major progress in this area, especially on model plants such as *Oryza sativa* and *Arabidopsis thaliana* led to an improvement in the analysis of plant proteome using high throughput technologies, and today, proteins are automatically identified by sequence homology (Agrawal et al., 2012). As a consequence of its complexity and dynamic nature, plant proteome analysis generally requires the use of different technologies (Baerenfaller et al., 2008)

Table 1. Examples of Phytoremediation Studies Using Plants Found in Qatar

Plant species	**Metal type**	**Metal accumulation (mg/kg)**	**References**
A. halimus	Cadmium	606.51	(Nedjimi and Daoud, 2009)
A. halimus L.	Cadmium	830	(Lutts et al., 2004)
	Zinc	44	
Arthrocnemum macrostachyum	Lead	620	(Conesa et al., 2011)
Crucianella maritima	Zinc	390	(Conesa et al., 2011)
Dittrichia viscosa	Lead	270	(Conesa et al., 2011)
Tamarix smyrnensis Bunge	Lead	800	(Manousaki and Kalogerakis, 2009)
	Cadmium	800	
Paspalum conjugatum L.	Lead	150	(Paz-Alberto et al., 2007)
Prosopis laevigata			
Prosopis laevigata	Cadmium	21,437	(Buendía-González et al., 2010)
	Chromium	8090	

Figure 1. Naturally growing *Tetraena qataranse* at Ras Laffan Industrial area, Qatar.

One key and popular technique in proteomics is the technology known as tandem mass spectrometry (MS/MS); it was suggested as the most ideal in obtaining significant protein matches statistically from peptide mass fingerprint data (Haas et al., 2006; Xian et al., 2012)

2.4.1 The shoot proteome. Plant shoot is an important tissue in phytoremediation process; it is especially responsible for accumulating the highest metals concentration when the subject plant employs phytoextraction technique, which is subject to the type of metal elements and bioavailability. In recent times, there has been an increased interest in the proteomics study of plant hyperaccumulators acting in metal sequestration and detoxification (Visioli and Marmiroli, 2012). These are possible with the advancement in modern mass spectrometry techniques such as Two-dimensional Liquid Chromatography Matrix-assisted Daser desorption/ionization Time-of-Flight (2D-LC/TOFMS), Time of Fight/Mass Spectrometry (TOF/MS), two-dimensional polyacrylamide gel electrophoresis (2D PAGE), and Liquid

Chromatography- Tandem Mass Spectrometry (LC-MS/MS). For instance, the proteome of many plants species including *T. caerulescencs*, *P. vittata, H. annus,* and *Agrostis tenuis* were recently searched for heavy metals detoxifying proteins. Several key functional proteins were found that protect plants against oxidative stress, as well as those responsive to biotic and abiotic stress condition among others (Bona et al., 2010; Visioli et al., 2010; Walliwalagedara et al., 2010).

In the proteomics study of plant metal hyperaccumulators, a comparison could be made, even when these studies are from different plants and metals. In 2005, (Ingle et al.) found that prolonged exposure of *A. lesbiacum* to Ni in an optimized experimental condition induced only three proteins, and one of these proteins, Iron superoxide dismutase (Fe-SOD) was demonstrated to have antioxidant activity (Freeman et al., 2004). Two other proteins were identified as chloroplast phosphoglycerate kinase and a transketolase both having a role in the carbohydrates metabolism. In *A. halleri,* photosynthetic protein (chlorophyll a/b binding) and membrane protein (photosystem II) were constantly translated and upregulated when treated with Zn and Cd, which is linked to the improved metabolic energy demand in this metal hyperaccumulating plant (Farinati et al., 2009).

At high metal concentrations, increased proteins induction are involved in the defense against antioxidants and energy metabolism has been consistently observed; examples include Renal Epithelial Protein (APX), Superoxide dismutase (SOD), cytochrome P450 and glutathione S-transferase (GST). These suggest that, for the uptake, translocation, and accumulation of heavy metals concentration on the shoot tissue, plants require the functional

photosynthetic process as well as the activity of proteins that scavenge oxygen radical species (Visioli and Marmiroli, 2013). Metabolic energy active proteins were also suggested to have important roles in metal tolerance by plants. The proteomes of *T. caerulescens* with variable tolerance to Cd and Zn metals were compared and there was a higher accumulation of the extrinsic subunit of photosystem II protein, which led to its stabilization in the more metal tolerant variant as against the less tolerant accession. In addition to GST and cytochrome P450 earlier mentioned, other proteins such as aspartate aminotransferase and thioredoxin are commonly found, and linked to the sequestration of xenobiotics including metals. GSTs have particularly been demonstrated to be up-regulated in many other living species including bacteria and fungi treated with metals like Zn, Cu, and Cd (Waschke et al., 2006); hence GST was suggested to confer resistance to toxic genes in these cells.

2.4.2 The root proteome. In plants, the root tissue is the first to be exposed to all potential toxicants whether in the soil or surface water and hence serve as the gateway route through which they can subsequently be translocated to other tissue parts. Plants diversity as to hyperaccumulators and non-hyperaccumulators exist, this is because, while some species bear the complete mechanism of enhanced metal uptake and eventual translocation, others have limited sequestration capacity in their root vacuoles (Maestri et al., 2010). In non-hyperaccumulators plant roots, Zn transporters are only detectable in the absence of Zn, whereas in hyperaccumulators, there is constitutive expression of these proteins such as ZT1 even in Zn deficient condition (Assunção et al., 2001; Pence et al., 2000). In *T. caerulescens*, the iron transporter protein IRT1 was found to be involved in Zn and Cd uptake

(Lombi et al., 2002), similarly, root proteome study of this hyperaccumulator and A. lesbiacum were conducted by Tuomainen et al. (2010) to evaluate proteins involved in Zn and Cd hyperaccumulation. In these studies, various classes of proteins were identified, their availability and or abundance varies relative to metal exposure and accessions. As is the case with similar studies on shoot proteome of hyperaccumulators, ROS scavenging proteins were more abundant in the more metal tolerant accessions compared to the less metal tolerant species. It was concluded that the changes in the enzyme, superoxide dismutase (SOD) availability upon which Zn depends in the different accessions may be linked to ROS increase.

An important organelle, cell wall, in the plant root is also affected by its exposure to heavy metal stress. The putative protein, glycosyl hydrolase family 18, involved in the formation of cell wall structure was shown to be regulated in accordance to treatment conditions and accession. These proteins, which are particularly known to be involved in cell wall expansion differ in terms of abundance between the root proteome of two accessions, which in turn also affect the capacity of metals uptake; higher Ni and Cd accumulation was observed in the variant with more protein abundance (Visioli and Marmiroli, 2013). Despite the recent advancement in proteomics technology, root protein transporters are yet to be differentially identified. Indeed, this is in agreement with transcriptomic studies, with analyzed data suggesting the constitutive expression of metal genes transporters in plant metal hyperaccumulators (Assunção et al., 2001; Hammond et al., 2006; Visioli and Marmiroli, 2013).

2.4.3 Methods in plants proteomics

2.4.3.1 Protein extraction and sample preparation. Generally, tissues of plants are commonly composed of elevated level of proteases and stubborn metabolites, which greatly hinders the extraction of proteins, inclusive of resolution and subsequent identification. Therefore, quality samples preparation of plant tissue protein is a difficult venture (Wang et al., 2008). Given this, protein profiling in plants and other living species require modern sophisticated equipment utilized in many techniques. Gel separation of protein samples is mostly employed to check protein profiles, and one of the most widely used is the two-dimensional form (2-D) (Peltier et al., 2004). Despite its limitations, the 2-D have so far remains the most suitable method for the expression of large and complex protein profiles (Agrawal et al., 2005; Görg et al., 2004). Recent advances in proteomics became possible with improved technologies including mass spectrometry (MS) and bioinformatics (Gygi et al., 2000; Plomion et al., 2007), and hence, to ensure optimized plant protein extraction, many separation techniques are currently being used. The outermost layer of plant tissue is composed of complex polysaccharides which are hard to disrupt (Lerouxel et al., 2006).

One of the most famous technique is pulverizing the sample material using mortar and pestle in liquid nitrogen, and this method is advantageous in that it minimizes protein loss and degradation that potentially can occur during disruption process because of protease activity. Another means is homogenizing a tissue material in TCA/acetone using glass homogenizer. It generates high and strong shear force such as the which can similarly produce a finely powdered starting sample (Phogat et al., 2010), and fine powder is

usually obtained from fiber-rich tissues such as leaf and stem of a plant (Phogat et al., 2010; Sheoran et al., 2009). Equally important, is the yield of a total protein obtained from the plant tissue using this practice, which usually depends on the powdered sample, and finer powder results in greater protein yield (Phogat et al., 2010). Hence, the use of a fine powder has long been a choice as starting sample material and is now used a standard practice in plant proteomics sample preparation (Saravanan and Rose, 2004).

Vascular plants contain high amount of secondary metabolites, and these are organic compounds with roles such as support for structure and protection (Stalikas, 2007). There exist diversity in terms of secondary metabolites composition in different tissues and can vary as these tissues develop, they are commonly accumulated in the vacuoles with varying solubility and in much more abundance in older plants (Granier, 1988). Phenolic compounds usually constitute the majority, with about 8000 forms of identified compounds belonging to the plant phenolic family such as lignin's, flavonoids, tannins, and phenols, all of which bear an aromatic ring with one or more hydroxyl group attached (Nelson et al., 2008; Stalikas, 2007). These organic compounds can affect both the quality of extracted protein as well as resolution on 2-D gels severely.

For instance, phenol irreversibly complex with proteins and cause their oxidation, which may lead to streaking and generate in artefactual gel spots (Nelson et al., 2008); the interfering substances are however removed routinely either before or after protein extraction (Sheoran et al., 2009). Removal of these secondary metabolites before protein extraction involves subjecting finely ground tissue powder to a 10% TCA/acetone followed by precipitation using

0.07% 2-mercaptoethanol (2-ME). Following a thorough organic clean-up, pellets appear very light or white colored which indicates the removal of major secondary metabolites such as the phenolic (Wang et al., 2006).

Total protein extraction requires ideal and reproducible protocols, this is to ensure limited contamination of sample protein by other molecules, and in most cases, and different protocols are employed for optimization, to get all the protein content. (Wang et al., 2008). For good plant proteomic analysis, many protocols are being developed including commercial kits (Carpentier et al., 2005; Vincent et al., 2006; Watson et al., 2003). However, two of these are most frequently applied (Saravanan and Rose, 2004). The most commonly used method involves precipitation using classical TCA/acetone, developed under protein denaturation condition in acidic or hydrophobic conditions, which helps in concentrating the protein and removing potential contaminants. The other is phenol-based extraction; here, phenol dissolves proteins and lipids which leaves behind water-soluble substances such as polysaccharides and nucleic acids in the aqueous phase (Hurkman and Tanaka, 1986). A combination of both is often explored in order to obtain adequate protein from recalcitrant tissues (Sheoran et al., 2009; Wang et al., 2006). A diagrammatic representation of the three protocols is shown in the figure below. It should, however, be noted that these protocols can be modified for optimum results depending on the type of the plant tissue used (Rose et al., 2004; Song et al., 2006).

2.4.3.2 Protein analysis. Protein expression can be quantitatively analyzed to provide an insight into the dynamics of plant proteome. Recently, comparative 2-D electrophoresis proves the most famous and widely used for

comparative proteomics serving as a preparatory procedure to mass spectrometric analysis of the separated proteins (Chen and Harmon, 2006; Visioli and Marmiroli, 2013). It is simple and straightforward visual method which involves protein separation, staining, and examining patterns, as well as intensities of the spots with the aid of advanced software for image analysis to assess varied protein expression patterns between samples. Advancement in proteomic technologies allows reproducibility of highly sensitive stained proteins (Görg et al., 2004; Schulenberg and Patton, 2004), indeed, comparative analysis expression of protein by 2-D separation method maintained its viability in plant proteomics (Islam et al., 2003; Khan et al., 2005; Sheffield et al., 2006; Watson et al., 2003).

However, it is important to emphasize that 2-D gel technology may limit quantitative comparisons (gel-to-gel) occasioned by variations in gel and automation complexities. However, this can be reduced using two-dimensional different gel electrophoresis (2D-DIGE) technology which allows two protein samples to be compared in one gel (Karp et al., 2004). Several quantification methods not limited to the above currently exist for the quantitative analysis of protein, an example of these methods include Kjeldahl (Shyong et al., 1998) which is not protein specific. Others that are more specific to proteins, and with a great potential for higher concentration output and low contaminants level were primarily developed for animal tissue samples (Van Raamsdonk et al., 2007). Plant proteins are usually found in low concentrations (about 1-4%) but highly rich in secondary metabolites such as phenolic, which usually affects the protein assays (Lattanzio et al., 2006; Markwell et al., 1978); other methods that use the protein precipitation or hydrolysis require multiple stages and

laborious and often tedious (Harborne, 1984; Jaiswal et al., 2015; Jones et al., 1989).

2.4.3.2.1 Bradford assay. In order to estimate the yields of plant proteins from the extraction methods, the ideal procedure should be highly sensitive to the proteins with little or no sensitivity to interfering molecules. The Bradford assay has long been ascertained to fulfill these requirements (Kruger, 2009), it is also widely used in animal proteins as well. This quantitative procedure is relatively cheap and simple (Trötschel and Poetsch, 2015). It relies on Coomassie brilliant blue G-250 dye-binding capacity resulting in a complex that absorbs at 595nm. The three forms of the dye; cations, anions and neutral exist, with the proteins binding only to the anionic form.

2.4.3.2.2 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). One dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (1-D SDS-PAGE) remains widely used as a tool for resolving protein extracts obtained from various pre-fractionation processes for several years (Chivasa et al., 2002; Dugo et al., 2008). It is especially even more popular in the proteomics study of membrane proteins (Brugière et al., 2004; Luche et al., 2003; Peltier et al., 2004). However, if highly resolved proteins are required, 2-D gel separation is the most preferred alternative, it received attention and acceptance since the early 1970s, and may remain the most popular shortly (Görg et al., 2004; Molloy et al., 1998). Recent advancements in immobilized pH gradient (IPG) further improve the simplicity, reproducible, and resolving capacity of 2-D gels (Boguth et al., 2000). Particularly, various IPG strips of different specifications such as pH range and lengths are available. Specifically the smaller pH 1 permits the proteome in-depth scrutiny

(Görg et al., 2004; Pang et al., 2010). Following the separation of proteins by gel electrophoresis, bound proteins are stained and analyzed to visualize and quantify each protein based on size. In recent times, highly sensitive staining agents are available that works with mass spectroscopy to allow multiple complex staining (Elliott et al., 2009; Schulenberg and Patton, 2004). Multiplex stain enables different proteins or sub-proteomes staining and visualization in sequence on one gel and enhance the correlation of various spots by removing differences arising from gel-to-gel runs (Carpentier et al., 2005).

2.4.3.2.3 Protein digestion. As soon as proteins are isolated from interfering compounds, and analyzed by 1-D or 2-D PAGE subject to the complex nature of the sample. Protein digestion is the next in sequence, which is an important component just before protein identification by mass spectrometry. However, in certain conditions digestion may proceed with no electrophoretic separation step, for example, when a general survey of protein components are required or minimizing loss following polyacrylamide gel binding (Kinter and Sherman, 2005).

Various proteolytic agents can be used to digest protein, these include trypsin enzymes in which proteases (Lys-C, Arg-C, Asp-N, Glu-C), or chymotrypsin and other chemicals. The reagents are highly specific to the cleavage bonds, which facilitates their spectral analysis. Most common and popular enzyme in this process is trypsin; it is relatively cheap and in highly pure form. Trypsin chops off amide bonds at Lysine and Arginine residues C-terminal except when linked to Proline. Aside of this selective potential, Arginine and Lysine are the most abundant residues found throughout most proteome, and hence cryptic cleavages result in protein bonds bearing a strong basic

residue at the C-terminal end (Martínez-Maqueda et al., 2013).

To digest proteins in any proteomic studies, there exist two mainly options: (i) In-gel digestion and (ii) In-solution digestion. However, in-gel digestion is the most widely practiced for proteins separated on both 1-D and 2-D PAGE, and the identification of proteins by acrylamide gels presents many obvious benefits relative to non-gel approaches (Visioli and Marmiroli, 2013; Wang et al., 2007). Gel digestion was first developed in 1992 and since then there has been no major modification of the established procedure, even though there are little variations incorporated to improve its output (Imai and Mische, 1999). Notwithstanding its widespread application, trypsin digestion proves laborious, which presents the challenge in obtaining high throughputs in proteomic analysis. However, in recent times, many researchers have been focused on establishing fast and efficient protein digestion method. (Martínez-Maqueda et al., 2013).

2.4.3.2.3 Protein identification using mass spectrometry. Proteins are identified based on the concepts of chromatography and spectrometry, meaning that it comprises the physical separation abilities with the mass analysis capabilities of different chemical compounds. This technique employed to look for different protein profiles in the extracted plant sample. Therefore, following standard procedure of the analysis, the masses of the resulting proteins obtained from trypsin-digestion will be determined by LC-MS analysis for mass profiling of the protein contained in the plant (DalCorso et al., 2013; Niessen, 2012). Typical steps in plant proteomics are shown in (Fig. 2). The early objectives of proteomics were to identify large and complex protein mixtures. Today, advancements in this field have seen protein identified based

on MALDI-TOF/MS and HPLC ESI-MS/MS peptide mass fingerprint (PMF). Since the invention of “mass spectrometers,” MALDI-TOF PMF became the most favored means of protein identification, owing to its high throughput, salt tolerance and cost-effectiveness (Hajduch et al., 2005; Li et al., 2013; Saravanan and Rose, 2004; Watson et al., 2003). By using tandem mass spectrometry techniques, proteins are identifiable by two means. Raw mass spectra may be queried against a given database such as the www. matrixscience.com. The output is homologs with correct statistical identity (Cottrell and London, 1999). With strict adherence to statistics, homology matches result in significant correct identifications which may be proceeded with biochemical evidence to establish actual protein identity (Rampitsch and Srinivasan, 2006).

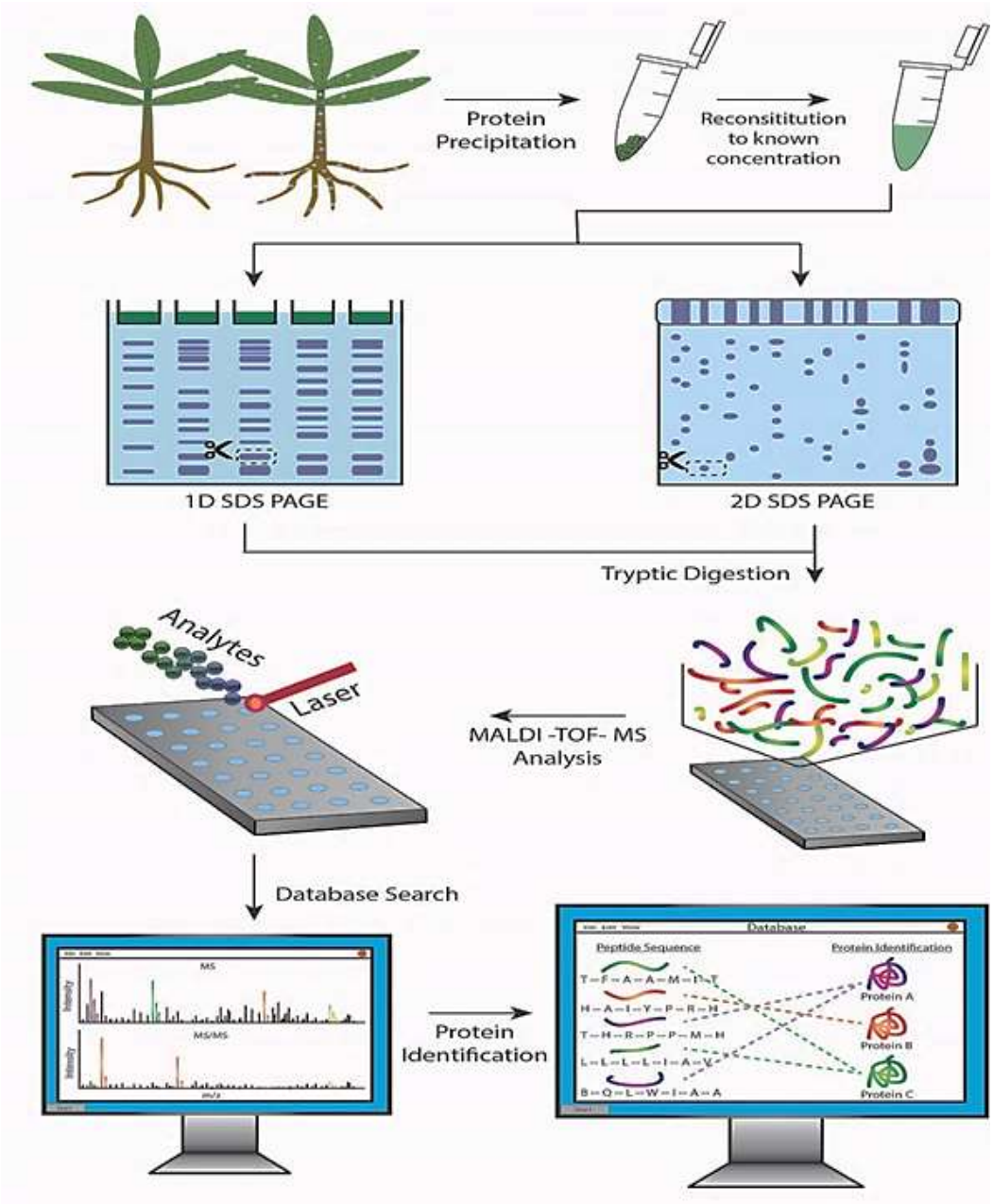


Figure 2. Summary of typical steps in plants total protein profiling.

Chapter 3: Methodology

3.1 Field Assessment of Heavy Metals and Screening of Plants Phytoremediation Potential

3.1.1 Sample collection and processing in the laboratory. All plants and soil samples collected from Ras Laffan and Mesaieed industrial areas, located approximately 80 and 40 kilometers from Doha city center, respectively. Sampling performed in a grid-like pattern at every 4 meters spacing; comprising of a whole plant and rhizosphere soil at approximately 20 cm deep from the surface. At Ras-Laffan (25°50'30.43"N51°34'34.31"E), along with the soil sample, major plants growing in the area and collected for the study were *Atriplex leucoclada*, *Salsola vermiculata*, *Sueada aegyptiaca,* and Tet*raena qataranse.* While for Mesaieed, along with the soil samples, major plants growing in the area (24°58'03.2"N51°34'26.6"E), and collected for the study were Atriplex halimus, Salsola vermiculata, *Tamarix aphylla,* and *Phragmites australis*. Both plants and soil samples were collected using shovel and or auger. In order to minimize soil contamination, a plastic knife used to trim down soil from either of the samples tools. Placed in acid-treated plastic bags, both samples transported to the laboratory. Air-dried soil samples crushed into smaller particles, after which plant materials, such as roots and visible residues removed with tweezers. Each sample was ground to a fine powder using agate mortar and pestle prior to digestion. Plants samples were washed with tap water to remove excess soil, particularly in the root, followed by acid washing in 0.01% HCl and thoroughly rinsed with deionized water as described by Reuter D J (1988). Following sterilization, individual plants were then separated into two parts; foliar (above the ground biomass) and root (below the ground

biomass). Tissue samples were later air and oven-dried for 48 and 48-72 hours at room and 80^{o}C temperature respectively. Dried shoot and root tissues were later ground to powder using mechanical stainless steel grinder, and a wooden mortar and pestle respectively. A fine powdered sample starting materials were then obtained by sieving with a 2 mm diameter mesh utensil.

3.1.2 Analysis of soil physicochemical parameters. Soil analysis carried out according to Soil analysis methods described by Anderson (1982). Soil pH using a portable digital pH meter (Mettler Toledo FE20 ATC). In order to determine the total concentration of soluble salts in the soil, electrical conductivity (YSC Environmental EC-30) was also measured in dS m^{-1} using an inductive electromagnetic device (Mettler Toledo S230 SevenCompact) (Rhoades and Corwin, 1981). Total inorganic carbon, nitrogen, and hydrogen determined using CHNS-O Analyzer (Flash 200 – Thermo Fischer Scientific). The estimation of total organic carbon (TOC) was using auto-titrator according to Walkley (1947). All analysis samples carried out in three replicates, and the average reported.

3.2 Phytoremediation Simulation in the Laboratory

3.2.1 Seed collection and storage. Seeds were collected from mature plants in Qatar University campus (25°22'21.60"N 51°29'45.28"E) Doha-Qatar, during March and April 2018. Following cleaning and drying, seeds were then stored in the dark at 4^{o}C for subsequent germination experiment.

3.2.2 Seed treatment. The seeds were prepared by washing with tap water, and later soaked in concentrated sulfuric acid (H_2SO_4) for 15 min. with occasional stirring. Afterward, seeds were soaked in 6% sodium hypochlorite for 5min, and subsequently rinsed with deionized water for 10 min.

3.2.3 Germination condition. Metal phytotoxicity tests are more sensitive in agar media than filter paper (Di Salvatore et al. 2008). Metal treatment solutions in this study were solidified in 0.8% (w/v) nutrient-based Bacto agar (DIFCO) in 500 mL mason jars containing about 250 ml each. The nutrient medium was made up of modified Hoagland solution according to Peralta et al. (2001). Since agar solidification is pH-dependent and said to be optimal between 5.4 - 5.8 (Bae et al. 2016), all-metal treatment solutions pH were adjusted before autoclaving. Three jars were spiked with the metal solutions 0, 5, 10 and 20 mg/L of Cd, 0, 20, 40 and 80 mg/L of Cr or 0, 25, 50 and 100 mg/L of Pb obtained from $CdCl_2$, $K2Cr_2O_7$, and $PbCl_2$.

3.2.4 Metal treatments. Three different concentrations of Cd, Cr, and Pb, were prepared and used for the phytotoxicity test. Treatments were 5, 10 and 50 mg/L for Cd. 20, 40 and 80 mg/L for Cr. 25, 50 and 100 mg/L for Pb. All metal treatments were chosen based on a preliminary study on the background concentrations in select industrial areas of Qatar (Unpublished data, 2017), and hyperaccumulation threshold (van der Ent et al. 2013). Metal salts dissolved in deionized water further diluted to prepare treatment solutions, while deionized water only was the control.

3.2.5 Seed germination and seedlings growth. Five seeds were aseptically planted into each of the jars (3 replicates per treatment) in a completely randomized design and transferred to a growth chamber at a temperature of 25^{o}C, 12 h photoperiod and photon irradiance of 39 µmol m^{-2} s^{-1} (3000 lux). Seed germination experiment ran for seven (7) days. Seed germination was recorded every 24 h. Radicle emergence of at least 2 mm long was considered for seed germination (Bae et al., 2016). Subsequently,

germination variables; final germination percentage (TG), germination rate (T_{50}), which is the time taken to reach 50% germination and germination index (seed vigor) were computed as described by Farooq et al. (2005).

At the end of the germination experiment, Cd and Cr treated plants showed severe signs of physiological stress, including wilting and chlorosis. Hence, only Pb treatments were extended to seedlings growth stage for another twenty-five (25) days, after which plant biomass (Root and shoot) morphological, Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and Fourier Transformed Infrared Spectroscopy (FTIR) analysis was performed to evaluate Pb tolerance and hyperaccumulation capacity.

Before biomass preparation for metal analysis, growth parameters such as fresh weight, shoot, and root length as well as chlorophyll contents were all computed using weighing balance (Intell-Lab PBW-3200), electronic digital caliper (Titan 23175) and chlorophyll meter (SPAD 502 Plus), respectively. For this purpose, three plants per replicate were chosen at random from each treatment. Measurements of shoot and root lengths were from the crown to main shoot apex and from root apex to the crown, respectively. Total chlorophyll measurement taken from three leaves per replicate, a total of nine per treatment and average values reported.

3.3 Phytoremediation Simulation in Greenhouse

3.3.1 Seedlings collection and pre-treatment. Young seedlings of approximately one week old were collected from Qatar University campus (25°22'21.60"N 51°29'45.28"E) Doha-Qatar, during October and November 2018. The young seedlings were planted in a pot containing regular soil amended with peat moss in a ratio of 3:1 and irrigated with distilled water for

two weeks before Pb treatment in a greenhouse. A summary of Pb phytotoxicity is shown in Fig. 3.

3.3.2 Pb treatments. For the treated plots, seedlings were irrigated with varying Pb concentrations of 25, 50, and 100 mg/L Pb. While the control plot was irrigated with only water containing 0 mg/L Pb. Metal salt ($PbCl_2$) dissolved in a modified Hoagland solution was used in the preparation of treatments. While modified Hoagland nutrient solution only served as the control (Peralta et al., 2001). Treatments were chosen based on Pb soil concentrations in Qatar and hyperaccumulation threshold (van der Ent et al. 2013).

3.3.3 Seedlings growth. For the experiment proper, three weeks old seedlings of similar height and weight selected. Five seedlings were planted (three replicates per treatment) in a randomized manner and watered for seven weeks in a greenhouse. After seven weeks, growth parameters were evaluated as described above.

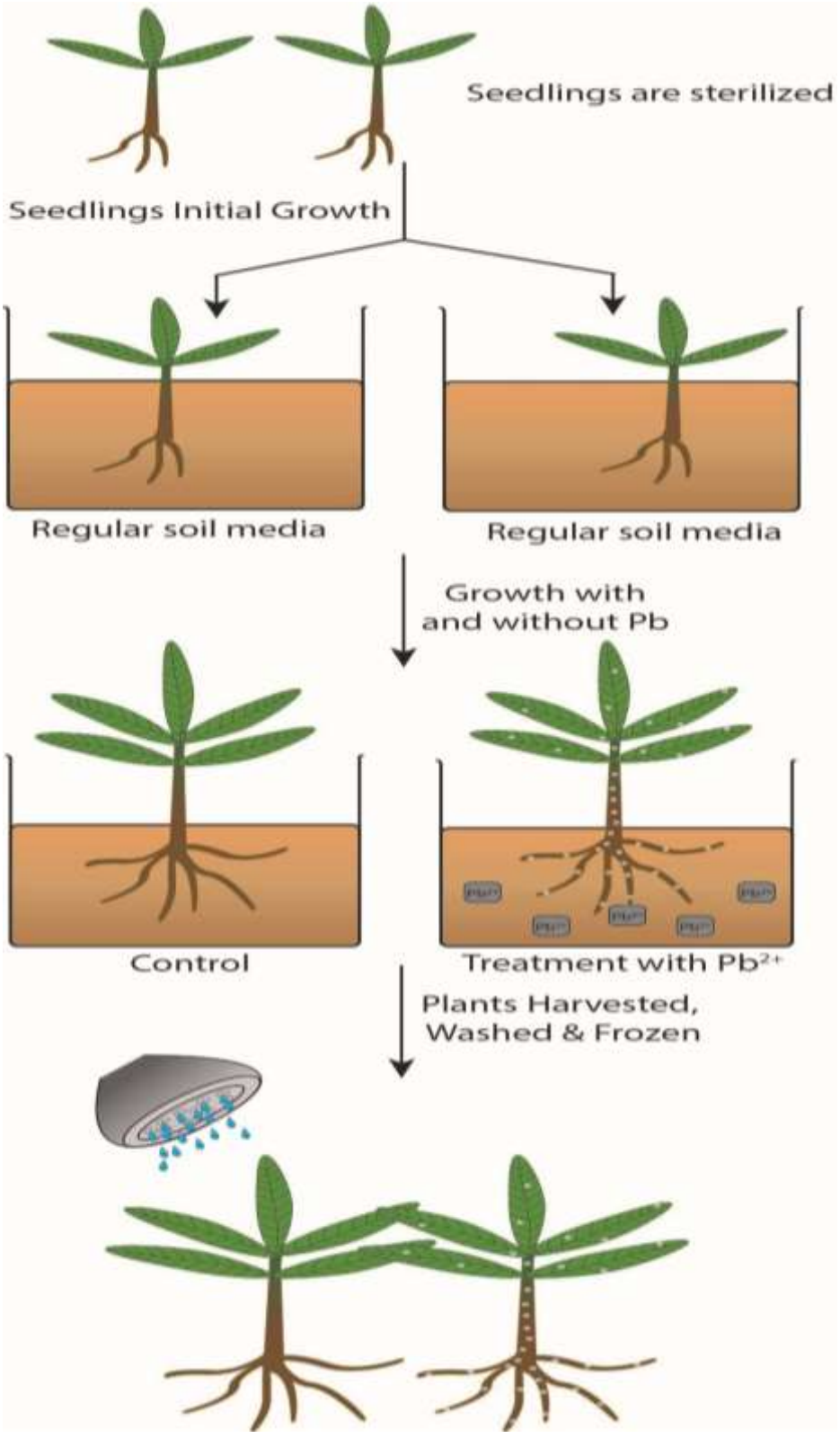


Figure 3. Schematic representation of summarized *T. qataranse* lead (Pb) phytotoxicity test. Seedlings are washed with deionized water before initial growth. Pb treatment and control contains 50 mg/L Pb, and deionized H2O solidified in the growth media, respectively.

3.3.4 Evaluation of phytoremediation potential

3.3.4.1 Nitric acid digestion. Samples digestion were carried out using nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) or hydrogen fluoride (HF) for plant tissues and soil, respectively. A large capacity Environmental Express SC154 HotBlock, ® digestion system, was used as an alternating temperature following the Environmental Protection Agency (EPA) method 3050 (Edgell, 1989). Before digestion, all vessels and glassware were acid-washed and water rinsed. The analytical weighing balance was used to weigh samples to approximately 0.5g for plant tissues (Root and shoot) and 0.25g for soil samples, which proceeded as follows. Soil: About 9 mL 65% nitric acid added to each sample containing digestion vessel, gently swirled and placed onto a Hotblock system at 95^{o}C for 30 min.

After 30 min, while samples were still on the Hotblock digester, 3 mL HF added and further digest at 95^{o}C for another 30 minutes. Afterward, the temperature increased to 135^{o}C for 1 hour and later raised to 155^{o}C to evaporate samples to almost dryness. Subsequently, about 3 mL HNO_3 followed by 40 mL of deionized water was added and boiled until clear. Clear solutions quantitatively transferred to a 150 ml volumetric flask. After cooling, all samples made to a final volume with deionized water. Plant tissues: Root and shoot, samples were digested as follows; 10 mL 65% HNO_3 and 2 mL 30% H_2O_2 were added gently swirled and placed onto the HotBlock digestion system. Samples heating follows at an alternating temperature of 95^{o}C –135°C until clear. After cooling, clear solutions were quantitatively transferred into a 100mL volumetric flask and made up to final volume with deionized water.

3.3.4.2 Metal analysis. Following nitric acid digestion, samples analyzed by direct injection into Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The concentration of six heavy metals (Ba, Pb, Ni, Cu, Cd, and Cr) quantified against National Institute of Standards and Technology (NIST) multi-element Standard Reference Materials (SRM's); Soil 2709a and Apple leaves 1515. The choice of these metals is because of their public health risk, and evidence of occurrence in the Qatari environment documented in the literature. The metal analysis was performed on samples collected at Ras Laffan, and for FTIR analysis, soil and *T. qataranse* samples collected from Qatar University to be used as controls were also analyzed in ICP-OES.

3.3.4.3 Bioconcentration factor (BCF) and Translocation factor (TF). The Bioconcentration and translocation factors are useful in comparing plants grown in regular soil and hydroponic solutions. Translocation factor specifically represents root to shoot metal iron concentration ratio and should typically be greater than one in potential hyperaccumulator plants (van der Ent et al., 2013).

Bioconcentration factor (BCF) computed as heavy metal accumulated in each plant tissue to that dissolved in the soil medium as shown below.

Root bioconcentration factor: $BCF^r = C_{root}/C_{soil}$ 1

Foliar bioconcentration factor: $BCF^f = C_{root}/C_{soil}$ 2

Translocation factor (TF) of examined heavy metals were computed using the above equations as follows

$TF = BCF^r / BCF^f$ 3

(Bose and Bhattacharyya, 2008).

3.3.4.4 Fourier transformed infrared spectroscopy (FTIR). FTIR analysis was performed according to Naumann et al. (1991). Soil and *T. qataranse* tissues collected from Ras Laffan were the treatments, while soil and *T. qataranse* tissues collected from Qatar University (An area with the studied metals below detection limit) are the controls. Samples dispersed in dry KBr pellets were analyzed using FTS-135 (Bio-Rad) spectrometer; spectra data recorded within 400-4000 Cm-1 range. Codes were assigned to both treatments and controls as follows; Soil treatment (SoT); Root treatment (RoT); Shoot treatment (ShT); Soil control (SoC); Root control (RoC) and Shoot control (ShC).

3.3.5 Statistical analysis. One and two ways (as the case may be) analysis of variance (ANOVA) and least-significant-difference test were performed to evaluate statistical significance using Sigma Plot 13 software. Statistical significance was considered at $P<0.05$. For critical analysis of *T. qataranse* metals hyperaccumulation capacity, the correlation coefficients (r) of various metals were computed using Pearson's regression equation at two levels (i) between metal concentration in the soil and different plant parts (root and shoot), and (ii) between metal concentration in the root and shoot. All results are averages of five and three replicates as the case may be.

3.4 Proteomics Analysis

3.4.1 Preparation of plant starting materials. For this proteomic analysis, harvested biomass of Pb treated *T. qataranse* were grounded to a fine powder and subjected to various extraction protocols at initial stage to optimize the protein extraction method. Subsequent quantification and quality check of extracted protein pattern performed by various plant methods described below.

3.4.2 Total protein extraction. The total proteins were extracted using different extraction methods namely; (i) Phenol/SDS buffer with three preliminary washed steps (ii) Phenol/SDS buffer without preliminary washed steps and (iii) Commercial kit. Following which Bradford Assay will be employed to quantify the total protein and quality checked by subjecting it to a 12% (w/v) SDS-PAGE gel. All tissue protein extraction were as described by (Gammulla et al., 2010; Jagadish et al., 2010) with minor modifications for the Trichloroacetic acid (TCA)/acetone extraction, and according to manufacturer's protocols where commercial kits are used.

3.4.2.1 Phenol/SDS buffer with three preliminary wash steps. Approximately 330mg of leaf/root tissues ground to a fine powder with liquid nitrogen in a mortar and pestle. Powdered tissue collected in a 2mL micro centrifuge tubes, 1mL of 10% (v/v) TCA/acetone added vortexed and centrifuged at 4^{o}C and 14,000 rpm for 3 min. Following the first centrifugation, tissue supernatant was discarded, and 1mL of 80% (v/v) methanol and 0.1M ammonium acetate acetone added vortexed and centrifuged for another 3 min at 4^{o}C and 14,000 rpm. Supernatant from the second centrifugation was again discarded and 1 mL of 80% (v/v) acetone added and vortexed until the pellets are fully dispersed. Finally, centrifuged at 4^{o}C and 14,000 rpm for 3 min and supernatant discarded. The settled pellets at the bottom of the tubes will then be air-dried at room temperature to remove residual acetone. About 400µL each for phenol and 10% (w/v) SDS buffer in 5% (v/v) of β-mercaptoethanol added, thoroughly mixed and centrifuged for 3 min at 14,000 rpm following 5 minutes ice incubation. The upper phenol phases transferred to a new 2mL micro centrifuge tubes were, 1.2 mL of 0.1M ammonium acetate added and

incubated overnight at -20ºC. The incubated mixture of the above centrifuged at 4ºC and 14,000 rpm for 5 min and supernatant discarded. 1mL of 100% methanol added, vortexed, centrifuged and the supernatant discarded. Extracted protein sample will be air-dried at room temperature, re-suspended in 200µL sample buffer (2x Laemmli) with 5% (v/v) β-mercaptoethanol and kept at -80ºC.

3.4.2.2 Phenol/SDS buffer without preliminary wash steps. Approximately, 250mg of tissue ground to a fine powder using liquid nitrogen in a mortar and pestle, powdered tissue were collected in a 2mL micro centrifuge tube and re-suspended with 0.8mL of Tris-buffered phenol (p.H 8.0) and 0.8ml SDS buffer in 5% (v/v) β-mercaptoethanol; vortexed for 10 minutes and centrifuged at 4ºC and 14,000 rpm for 5 minutes. Upper phenol phase transferred to a new 2mL micro centrifuge tube and 1.5mL of pre-cooled 0.1M-ammonium acetate in absolute methanol added. Centrifuged again for 5 min at 4ºC and 14,000 rpm with supernatant discarded after 2 hours incubation at -20ºC. This was treated with pre-cooled ammonium acetate in absolute methanol and 80% (v/v) pre-cooled acetone, vortexed, centrifuged and supernatant discarded in each. Extracted protein samples air-dried at room temperature and dissolved in 200µL sample buffer with 5% (v/v) β-mercaptoethanol (Li et al., 2013).

3.4.2.3 Commercial kit (Thermo Fischer Scientific). About 100mg of root and leaf tissues were ground to a fine powder using liquid nitrogen in a mortar and pestle. Powdered tissues transferred to a 2mL micro centrifuge tube. 1.5mL of methanol solution added, briefly vortexed and kept at -20oC to incubate for 5 min. The mixture will be centrifuged at 16, 000 x g for 5 min at

4ºC to pellet protein and supernatant will be discarded. Methanol addition, vortexing, incubation and centrifuged repeatedly for two more times. Following the removal of the supernatant in the last extraction with methanol solution, tubes inverted over a clean towel to remove any visible methanol. 1.5 mL of pre-chilled acetone is to be added, briefly vortexed and placed at -20oC for 5 minutes to allow mixture incubation and centrifuged at 16, 000 x g for 5 min at 4ºC to pellet proteins and plant tissue debris. Supernatant was discarded and the pellet air-dried for 10 min at room temperature to remove residual acetone. Dried samples weighed and the pre-determined mass of the tubes subtracted to determine the plant tissue mass. 4uL of the reagent type 4 working solution were added to each mg of plant tissue, and the tissue completely broken by vortexing and solution allowed to incubate for 15 min with continuous gently mixing at ambient temperature. After incubation, the solution was centrifuged at 16, 000 x g for 30 min at 4ºC to pellet plant tissue debris. The supernatant, which contains the protein sample, will carefully remove and place in a clean 2mL micro centrifuge tube and kept at -80oC (Gammulla et al., 2010).

3.4.3 Determination of protein concentration by Bradford assay.

Assay was performed according to Bio-Rad protocols as described in Li et al. (2013) to measure the quantity of the protein extracted from the various extraction methods above in terms of concentration and yield. Assay carried out using Bovine Serum Albumin (BSA) as standard. Series of BSA concentrations 0, 1, 2, 4, 6, 8 and 10 µg/mL will be prepared from the stock and top up with different volumes of distilled water to a volume of 0.5mL. At the same time, protein samples will be diluted approximately 100x in the same volume containing 10µL of protein samples and 490µL of distilled water. The

same amount of 0.5mL of Bradford reagent added to both the standards and sample to bring the total volume of each to 1mL and mixed by shaking the tubes. The mixtures of both allowed incubating for 20 min and triggering the reaction of the Bradford reagent to both the protein standard and sample. The absorbance of the reactions measured at 595nm using spectrophotometer (Genesys UV VIS spectrophotometer) against the blank. The measure of absorbance taken from the standard plotted against each BSA concentration used to determine protein samples concentrations and yields using the equation of the standard curve.

3.4.4 Protein separation and identification

3.4.4.1 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). One dimension SDS-PAGE carried out in this investigation to separate proteins based on size. Approximately 20 µg of extracted proteins are resolved using NuPAGE 4-12% Bis-Tris Protein Gels (Invitrogen) and separated by SDS-PAGE according to Laemli (1970) on a Bio-Rad Protean II system. Loaded samples run for 15 min at 100V, and later for 70 min at 150V. Subsequently, gels stained with Coomassie Stain G-250 and de-stained overnight for further protein pattern analysis according to bands intensity.

3.4.4.2 Trypsin digestion. To prepare the extracted samples for MALDI-TOF MS and the eventual generation of mass proteins data for protein identification, in-solution digestion was carried out with sequencing grade trypsin (Shevchenko et al., 2000). The protein concentration of 50ug will be prepared in 100uL total volume with 50mM NH_4HCO_3. About 5µL of 200 mM DTT (in 100mM NH_4HCO_3) will then be added to reduce the sample by boiling for 10 min followed by incubation for 50 min at room temperature. To alkylate

the sample, 4µL 1M iodoacetamide added, vortexed briefly spun and incubated for 50 min at room temperature. Iodoacetamide neutralized by the addition of 20µL 200mM DTT, vortexed, spun and incubated at room temperature for 50 min. Digestion was carried out in a ratio of 1:20 of trypsin to sample, samples were later be vortexed and briefly spun prior to overnight incubation at 37^{o}C. The pH neutralized by the addition of 2% formic to the sample repeatedly and monitored until it reaches 6.0 using pH paper indicators. Finally, digested protein samples cleaned with C18 Zip tip and kept at -80^{o}C for MALDI-TOF MS analysis.

3.4.4.3 Protein analysis by MALDI-TOF/MS. Proteins were eluted in 50% acetonitrile containing α-cyano-4-hydroxycinnamic acid directly applied onto the target metal plate and analyzed by MALDI-TOF MS on a Bucker Ultraflex (Brucker Daltonics, Bremen, Germany). Peptide mass spectra were analyzed using embedded Flex analysis software. Calibration of peptide spectra were internally performed using trypsin autolytic proteins (842.51, 1045.56 and 2211.10 Da). Protein identity searches were performed using Mascot protein identification server via http://www.matrixscience.com/ using the model plant "*Arabidopsis*" protein database. Set parameters included one miscut, alkylation and partial oxidation of methionine. Others were *Arabidopsis thaliana* as taxonomy, pI and Mr determined from gel migration spots position and mass tolerance of 0.1 Da. The statistical significance of all identified proteins was evaluated according to Z-value and sequence coverage based on Mascot algorithms. Subsequent gene ontology and enrichment analysis were performed using Gene Ontology Resource (http://geneontology.org/) and Uniprot database via https://www.uniprot.org/.

3.5 Antioxidant Enzymes Assay

Enzyme extraction followed the method of Mishra et al. (2006) Briefly, 0.2g of the fresh tissue sample is homogenized in an ice-cooled mortar using 5mL 10mM potassium phosphate buffer (p.H 7.0), 1% (w/v) polyvinylpyrrolidone and 0.1 mM Ethylenediaminetetraacetic acid (EDTA). Centrifugation of homogenate followed under 4^{o}C for 15 min at 15 000g. Enzymatic activities were determined and expressed as Unit/mg of protein.

3.5.1 Superoxide dismutase (SOD). To measure SOD (EC 1.15.1.1) activity, nitrobluetetrazolium (NBT) photochemical reduction inhibition is determined by Beauchamp and Fridovich (1971). A total of 3 mL assay mixture was used. After 15 min under illumination, absorbance was recorded. The non-illuminated mixture was used as the control.

3.5.2 Catalase (CAT). CAT (EC 1.11.1.6) assay was performed, according to Zhang et al. (2009). A total reaction mixture of 3 mL was prepared and absorbance recorded at 240nm for 4 min.

3.5.3 Ascorbate peroxidase (APX). The determination of APX (EC 1.11.1.7) activity followed the ascorbic acid oxidation rate as described in Nakano and Asada (1981).

3.5.4 Guaiacol peroxidase (GPX). GPX (EC 1.11.1.9) activity measured following guaiacol oxidation as described in Egley et al. (1983).

3.5.5 Glutathione reductase (GR). GR activity was determined following the method of Rao et al. (1996).

Chapter 4: Results and Discussion

4.1 Assessment of Heavy Metals Pollution at Ras Laffan and Mesaieed

The first major objective of this research work was to determine common heavy metals in Qatari environment with substantial anthropogenic activities, and subsequently evaluate the capacity of plants found growing in these areas for the accumulation of metals species present. To achieve these tasks, two famous industrial areas; Ras Laffan and Mesaieed were selected. This subsection therefore presents the results from the sites assessments and heavy metals evaluation.

4.1.1 Ras Laffan. Ras Laffan Industrial area is located north of Doha, about 80 km from the city center. Ras Laffan is home to the largest gas processing plant and houses many petrochemical companies. Along with the soil sample, major plants growing in the area and collected for the study were *A. leucoclada*, *S. vermiculata*, *S. aegyptiaca,* and *T. qataranse.* Sample collection site sits at 25°50'30.43"N51°34'34.31"E. Characterized by low elevation and sandy soil; it is the largest site for the production of liquefied natural gas and gas to liquid. In addition to existing oil and gas refineries, Ras Laffan is home to three power generation and water desalination plants. It harbors the largest artificial port with an enclosed water area of about 4,500 hectares.

4.1.1.1 Soil physicochemical properties. The soil collected from Ras Laffan Industrial area was analyzed for pH, salinity, total inorganic carbon, nitrogen and hydrogen, electrical conductivity (EC) and total organic carbon (TOC). The results are as presented in Table 2. Qatari soil is indeed mostly alkaline with high calcium-magnesium carbonates composition. It has relatively

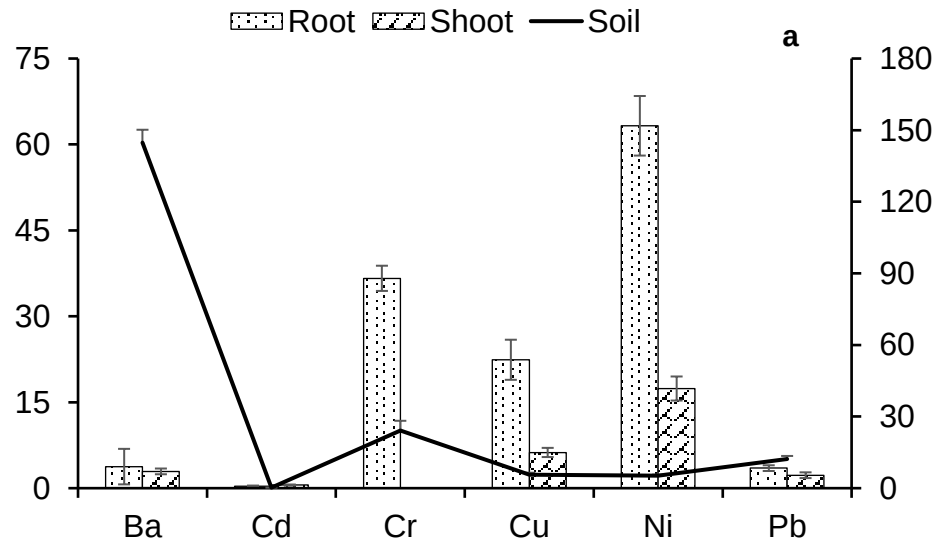
low organic matter contents as well as significant iron and clay constituents (Peng et al., 2016).

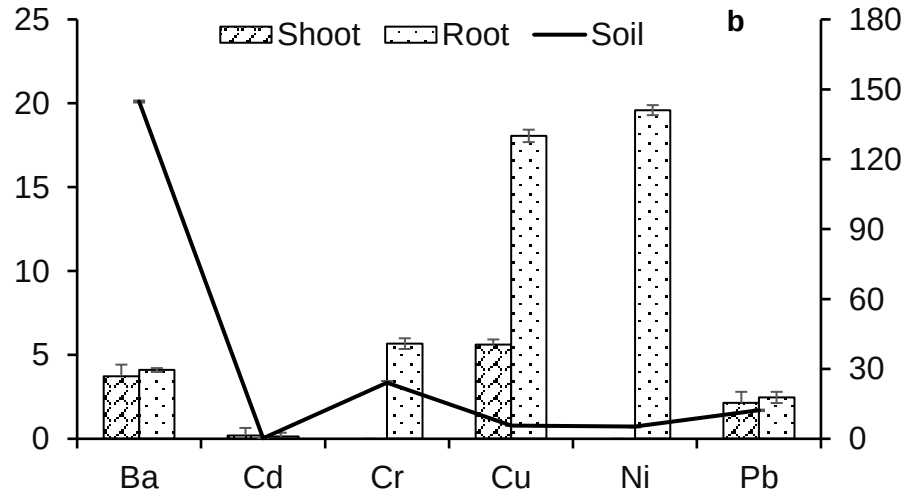
Table 2. Soil Physicochemical Properties at Ras Laffan

pH	**EC (mS/m)**	**TOC (%)**	**Total Carbon; C, Nitrogen; N and Hydrogen; H (%)**	**Salinity (ppt)**
8.05 ± 0.12	4.25 ± 0.44	1.1 ± 0.03	C: 9.8 ± 0.3 N: 0.006 ± 0.08 H: 0.27 ± 0.01	2.4 ± 0.2

4.1.1.2 Heavy metals concentration. Metals concentration in the soil and plants tissues (mg/kg) is shown in Fig. 5. Soil metals concentration were in the order Ba (144.8) > Cr (24.1) > Pb (12.2) > Cu (5.7) > Ni (5.2) > Cd (0.2). With respect to the plants, accumulation follows the trends; (i) *T. qataranse* (Fig. 5a): Ni (63.3) > Cr (36.6) > Cu (22.4) > Ba (3.8) > Pb (3.5) > Cd (0.4) and Ni (17.4) > Cu (6.2) > Ba (2.9) > Pb (2.3) > Cd (0.5) > Cr (0), for root and shoot, respectively. (ii) *A. leucoclada* (Fig. 5b): Ni (19.5) > Cu (18.1) > Cr (5.6) > Ba (4.1) > Pb (2.4) > Cd (0.1) and Cu (5.6) > Ba (3.7) > Pb (2.1) > Cd (0.2) > Ni (0)

> Cr (0), for root and shoot, respectively. (iii) *S. aegyptiaca* (Fig. 5c): Ni (63.2) > Cu (23.9) > Ba (5.1) > Cr (4.5) > Pb (2.2) > Cd (0.1) and Cu (6.4) > Pb (1.4) > Ba (1.2) > Cd (0.1) > Ni (0) > Cr (0), for root and shoot, respectively. (iv) *S. vermiculata* (Fig. 5d): Cu (17.3) > Ba (7.0) > Cr (5.1) > Pb (2.3) > Ni (1.6) > Cd (0.2) and Cu (8.3) > Ba (2.9) > Pb (2.1) > Cd (0.2) > Ni (0) > Cr (0), for root and shoot, respectively.





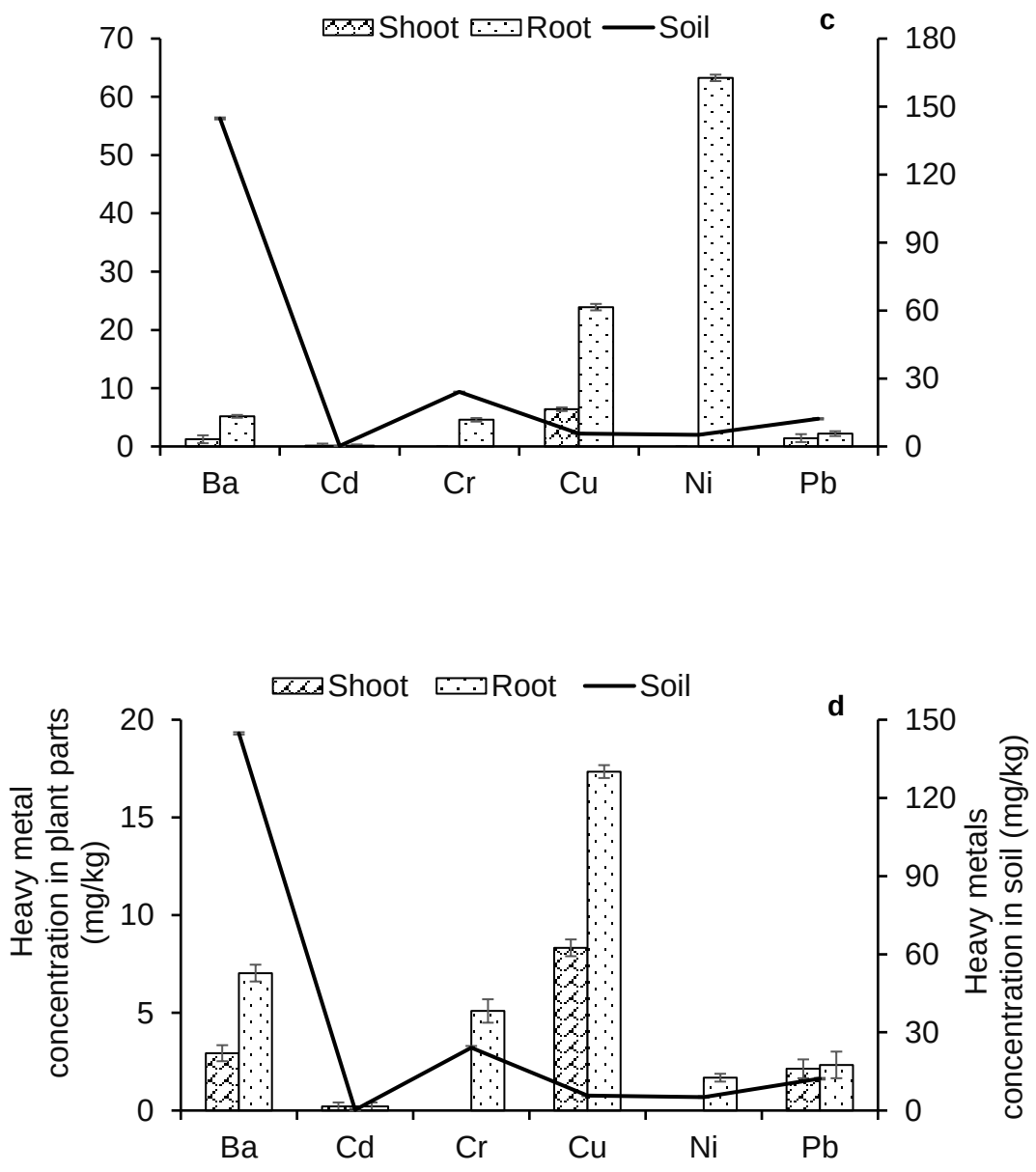


Figure 4. Heavy metals concentration in roots, shoot and soil - Ras Laffan **(a)** *T. qataranse* **(b)** *A. leucoclada* **(c)** *S. aegyptiaca* and **(d)** *S. vermiculata*. Mean concentrations are averages of five replicates (n=5) ± SEM with statistically significant differences between soil and plant parts at P<0.05 level (ANOVA-TUKEY).

4.1.1.2.1 Bioconcentration (BCF) and translocation factor (TF). Metals BCF computed based on soil and plant tissues accumulation are shown in Fig. 9. While Fig. 6 show metals TF based on plant tissues BCF.

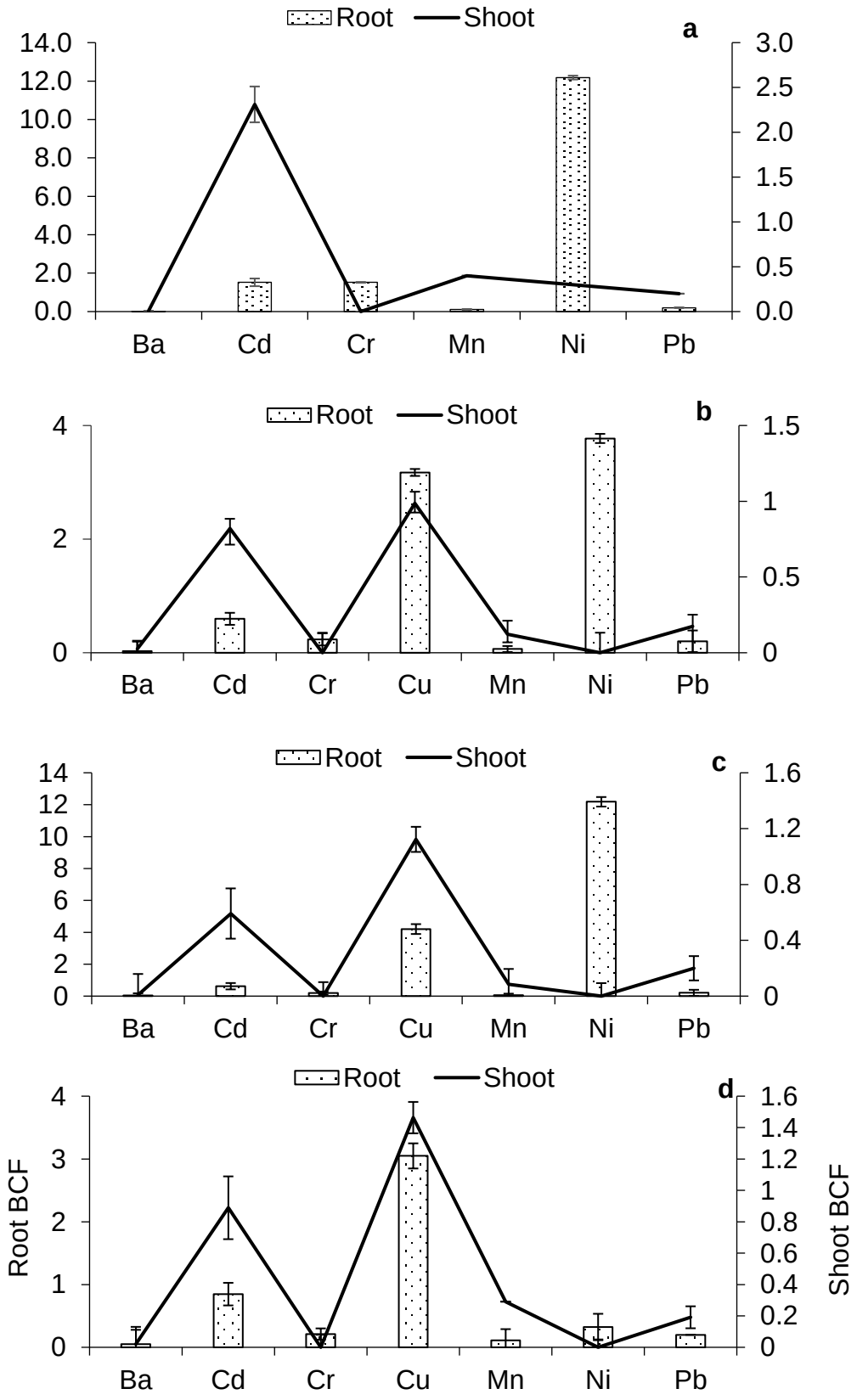


Figure 5. Root and shoot metals BCF - Ras Laffan ***(a)*** *T. qataranse* ***(b)*** *Atriplex leucoclada* ***(c)*** *S. aegyptiaca and* ***(d)*** *S. vermiculata.* Means are averages of five BCF values (n=5).

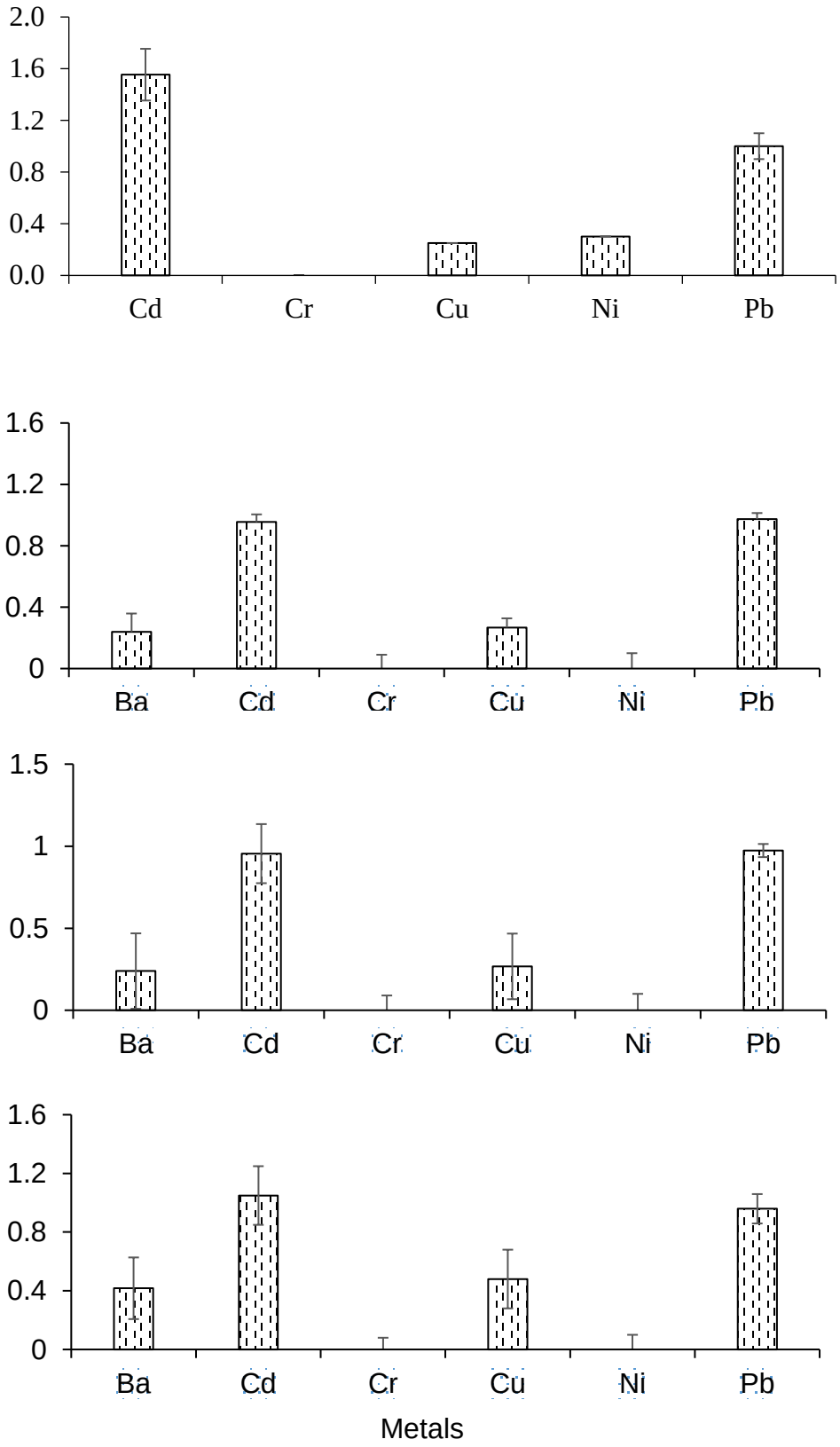


Figure 6. Metals translocation factor (TF) - Ras Laffan in **(a)** *T. qataranse* **(b)** *A. leucoclada* **(c)** *S. aegyptiaca* and **(d)** *S vermiculata.* Means are averages of five TF values (n=5) ± SEM.

4.1.2 Mesaieed. An industrial area located south of Doha is approximately 40 km from the city center. Major petrochemical, chemical, and Aluminum companies are located in Mesaieed. Along with the soil samples, major plants growing in the area and collected for the study were *A. halimus*, *S. vermiculata*, *T. aphylla*, and *P. australis*. Sample collection site sits at 24º58'03.2"N51º34'26.6"E.

4.1.2.1 Soil physicochemical properties. The soil collected from Mesaieed Industrial area was also analyzed for pH, salinity, total inorganic carbon, nitrogen and hydrogen, electrical conductivity (EC) and total organic carbon (TOC). The results are as presented in Table 3.

Table 3. Soil Physicochemical Properties at Mesaieed

pH	**EC (mS/m)**	**TOC (%)**	**Total Carbon; C, Nitrogen; N and Hydrogen; H (%)**	**Salinity (ppt)**
9.03 ± 0.2	3.89 ± 0.24	4.1 ± 0.08	C: 8.8 ± 0.4 N: 0.06 ± 0.33 H: 0.48 ± 0.05	2.1 ± 0.3

4.1.2.2 Heavy metals concentration. Metals concentration in the soil and plants tissues (mg/kg) at Mesaieed shown in Fig. 7. Soil metals concentration is in the order Ba (147.8) > Cr (111.8) > Ni (60.5) > Cu (29.4) > Pb (2.5) > Cd (0.04). With respect to the plants, accumulation follows the trends; (i) *A. halimus* (Fig. 7a): Cu (32.4) > Pb (6.7) > Cr (6.5) > Ba (4.9) > Ni (4.3) > Cd (0.7) and Cu (45.0) > Ba (6.7) > Ni (1.8) > Cr (1.7) > Cd (0.6) > Pb (0.3), for root and shoot, respectively. (ii) *T. aphylla* (Fig. 7b): Cu (65.7) > Ni (8.0) > Cr (7.8) > Ba (3.5) > Cd (1.7) > Pb (1.0) and Cu (55.7) > Ni (6.8) > Cr (6.1) > Ba (2.3) > Cd (2.1) > Pb (0.9), for root and shoot, respectively. (iii) *S. imbricata* (Fig. 7c): Cu (31.0) > Cr (12.3) > Ni (13.1) > Cd (3.5) > Ba (2.8) > Pb (0.4) and Cu (20.4) > Ni (7.6) > Cd (4.3) > Cr (0.8) > Ba (0.7) > Pb (0.5), for root and shoot, respectively. (iv) *P. australis* (Fig. 7d): Cu (67.4) > Ba (6.7) > Cr (5.1) > Ni (4.9) > Pb (1.8) > Cd (0.2) and Cu (24.1) > Ni (11.4) > Ba (2.2) > Pb (1.5) > Cr (1.2) > Cr (0.4), for root and shoot, respectively.

4.1.2.2.1 Bioconcentration (BCF) and translocation factor (TF). Metals BCF computed based on soil, and plant tissues accumulation are shown in Fig. 8**.** While Fig. 4.6 show TF based on plant tissues BCF.

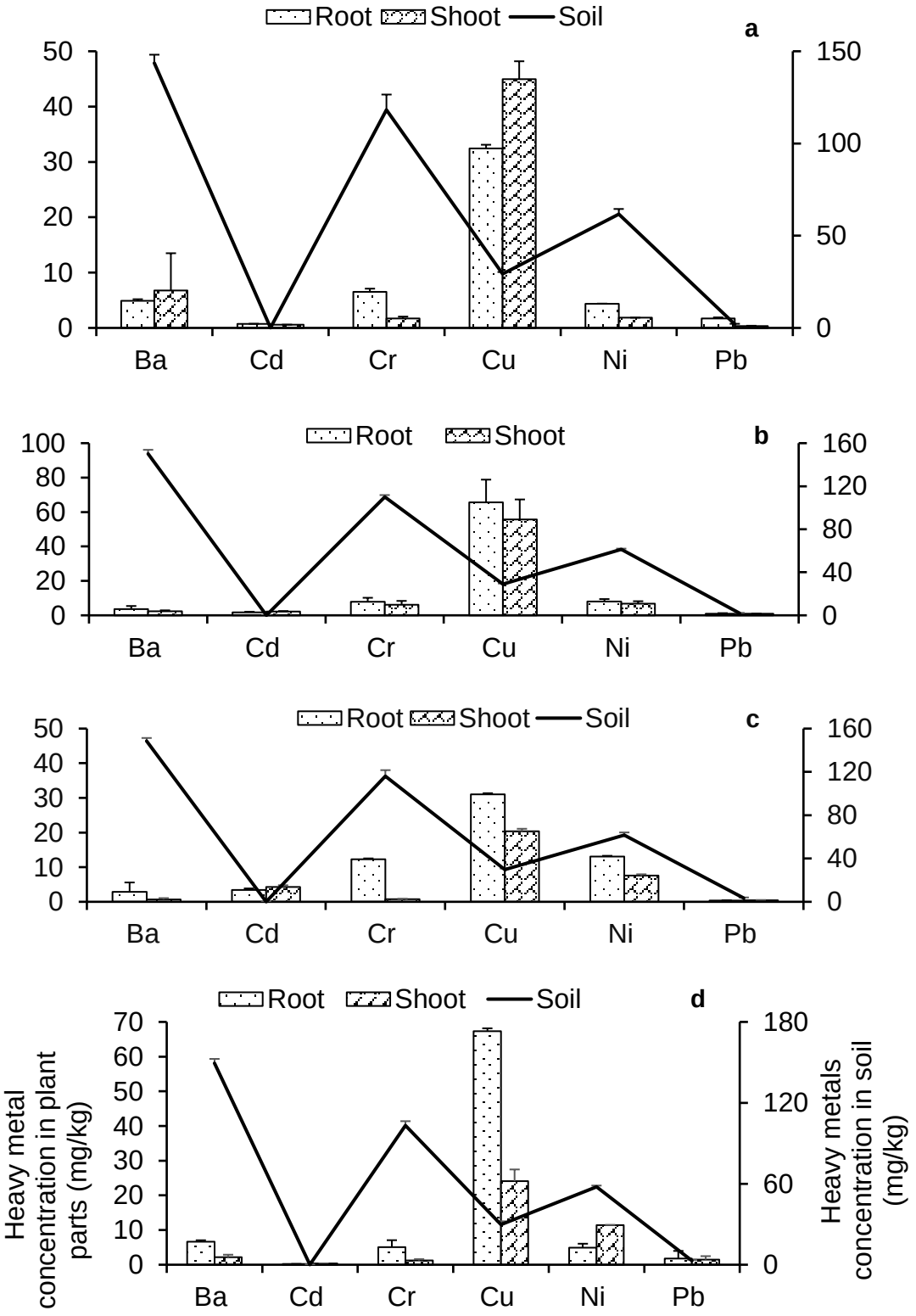


Figure 7. Heavy metals concentration in the root, shoot and soil - Mesaieed **(a)** *A. halimus* **(b)** *T. aphylla* **(c)** *S. imbricata and* **(d)** *P. australis.* Mean are averages of five replicates (n=5) ± SEM with statistically significant differences between soil and plant parts at P<0.05 level (ANOVA-TUKEY).

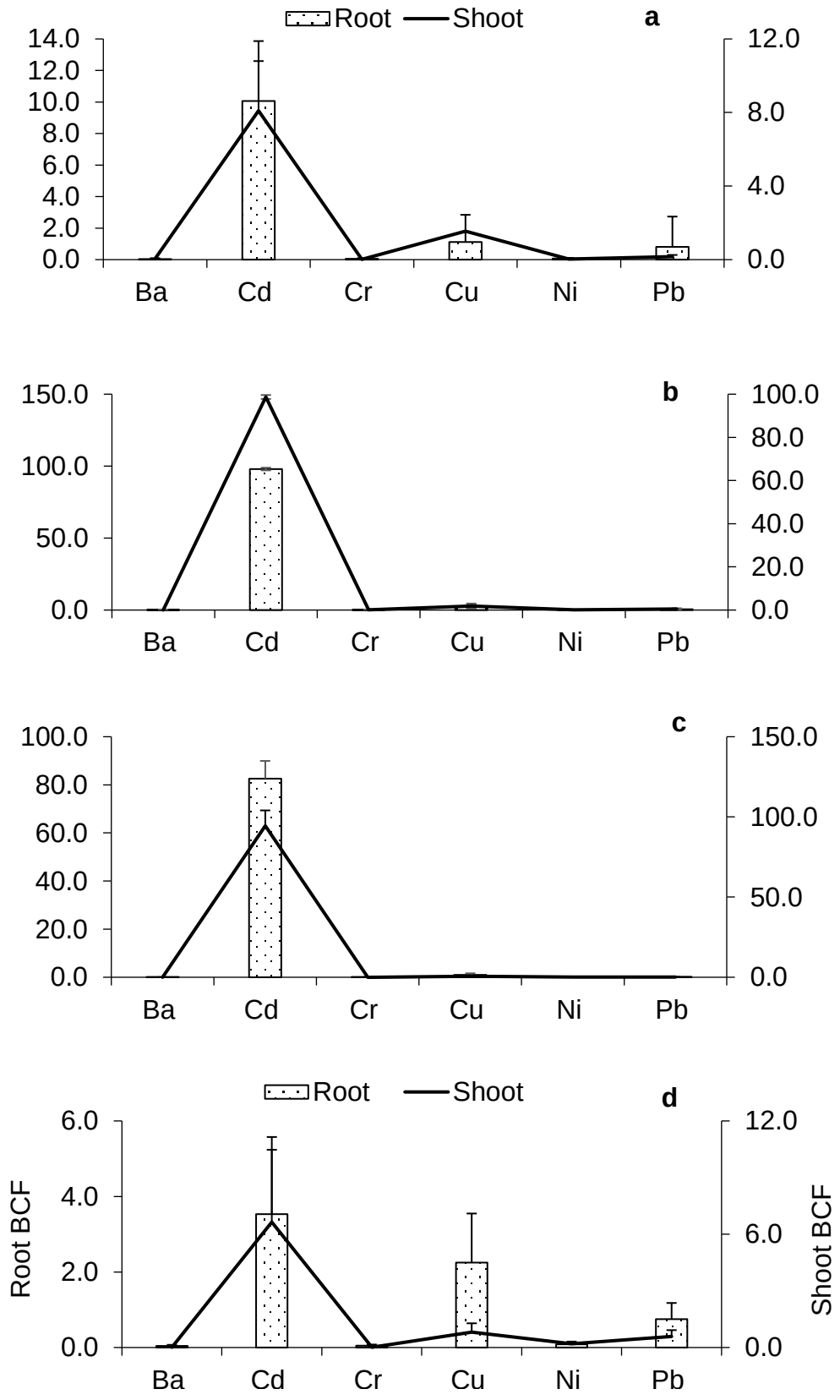


Figure 8. Root and shoot metals BCF - Mesaieed (**a)** *A. halimus* **(b)** *T. aphylla* **(c)** *S. imbricata and* **(d)** *P. australis.* Mean concentrations are averages of five replicates (n=5) ± SEM.

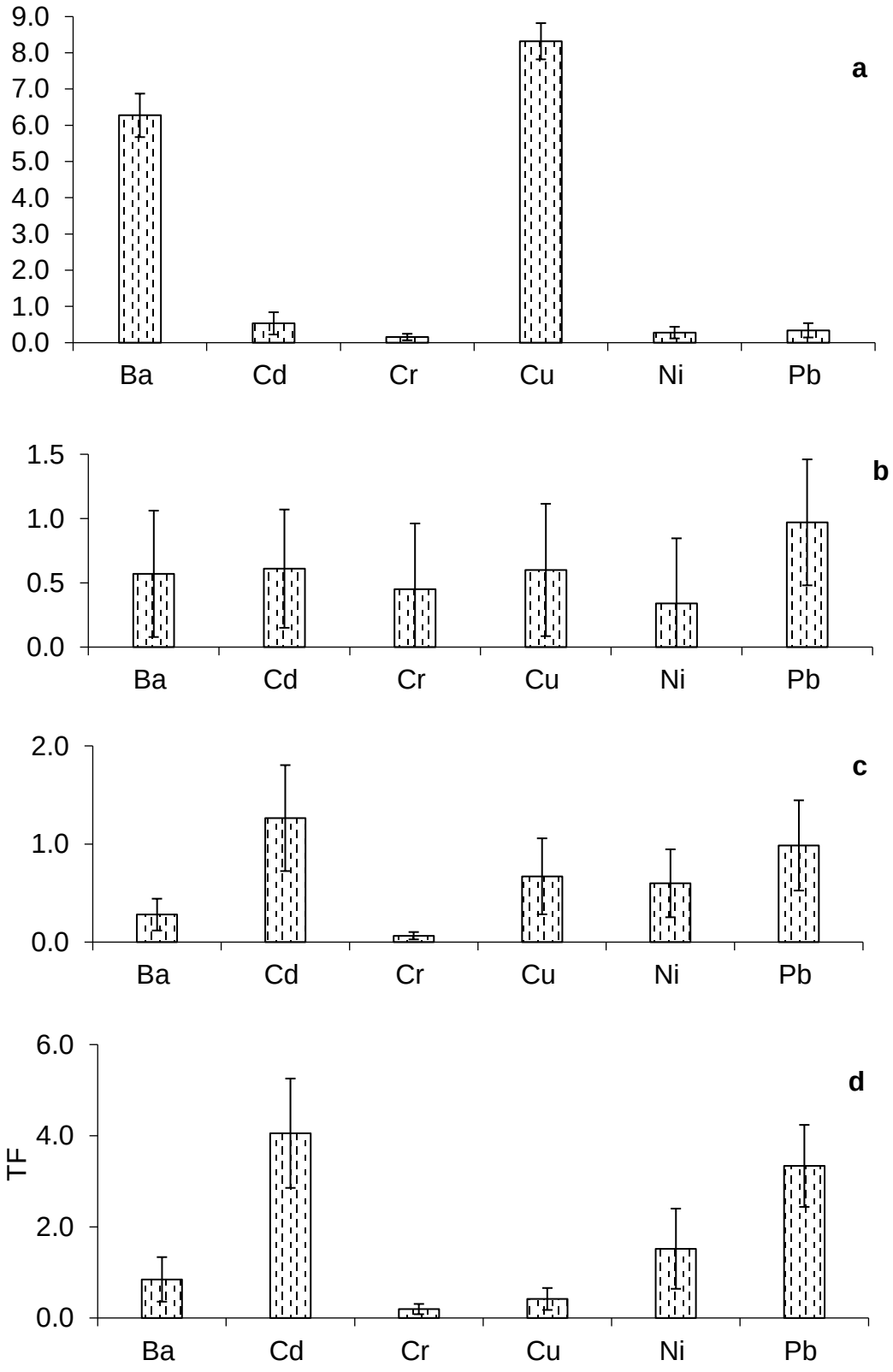


Figure 9. Metals TF - Mesaieed in **(a)** *A. halimus* **(b)** *T. aphylla* **(c)** *S. imbricata and* **(d)** *P. australis.* Mean concentrations are averages of five replicates (n=5) ± SEM.

4.1.3 Comparison between Ras Laffan and Mesaieed. Comparison between the two study sites (Fig. 10) was made in terms of their physicochemical properties, heavy metals concentration in the soil and Pb accumulation in plant tissues.

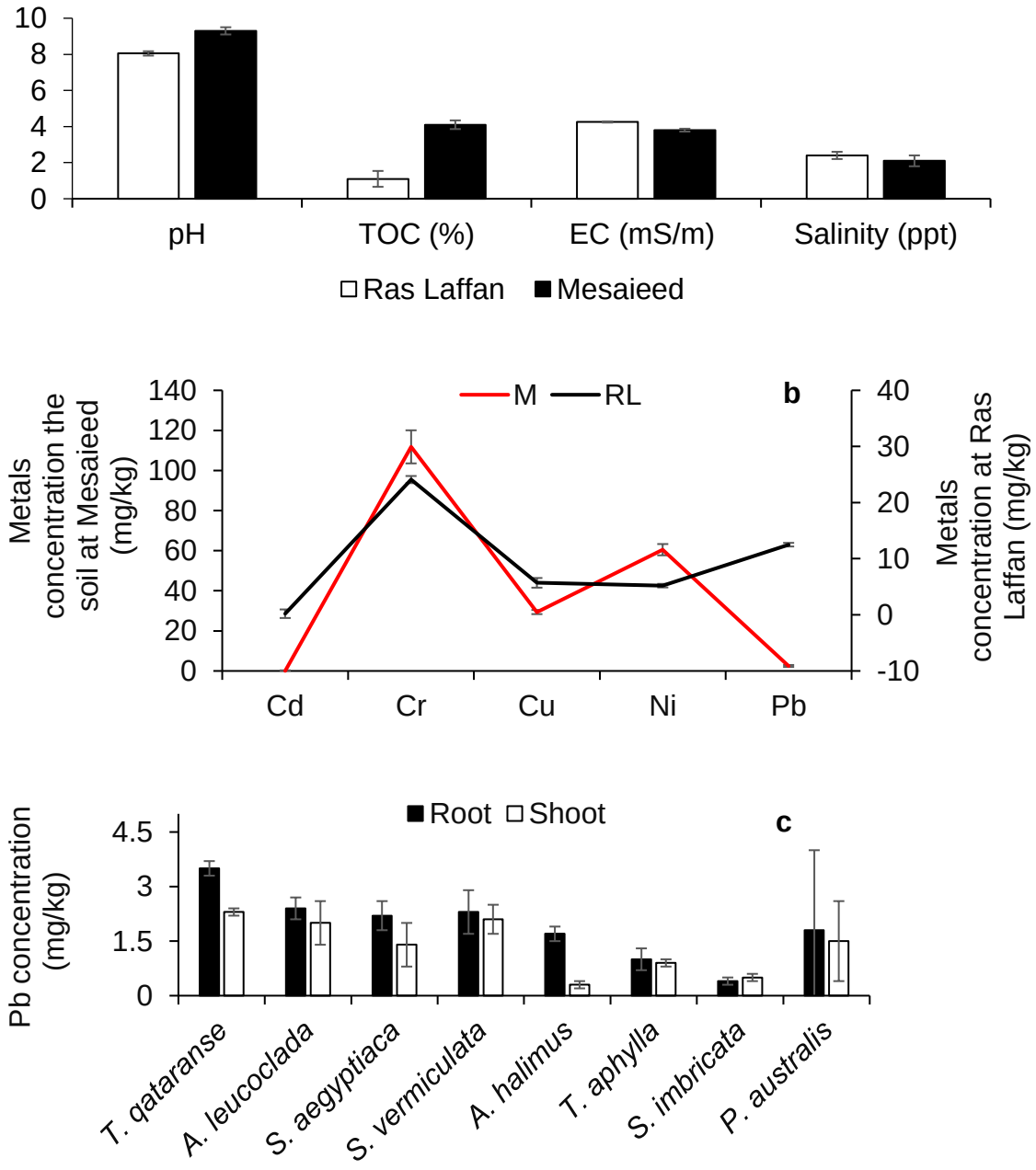


Figure 10. Comparison between Ras Laffan; RL and Mesaieed; M **(a)** Soil pH, TOC (%), EC (mS/m) and Salinity (ppt) **(b)** Heavy metals concentration in the soil and **(c)** Pb accumulation in plants.

4.2 Evaluation of Cadmium (Cd), Chromium (Cr), Copper (Cu), Nickel (Ni) and Lead (Pb) Accumulation and Adsorption Mechanism in *T. qataranse*

The comparison between all plants from the two study sites revealed that *T. qataranse* collected from Ras Laffan had the highest Pb accumulation (Fig. 11C), and therefore chosen for further analysis. In this section, we critically assessed the accumulation of the metal and alongside their adsorption mechanism. For this purpose, Qatar University's Department of Biological and Environmental Sciences field (25°22'21.60"N 51°29'45.28"E) is the control site for FTIR result comparison. It is a protected field, characterized by compact soil. It supports more moisture and organic matters. Vegetation type includes shrub trees, grasses, and herbs, among which includes *Acacia* spp., *P. juliflora, Z. nummularia, S. imbricata, C. imbricata, and L. shawii.* The site is used as control to compare FTIR data only. All metals in the soil and *T. qataranse* samples from the control site were below detection limit following ICP-OES analysis.

4.2.1 Soil properties. Except for the toxic metals, the soil properties at Ras Laffan is generally similar to that of Qatar University, hence its choice as the control area to compare FTIR data. Analysis of Ras Laffan soil gives a pH of 8.05 ± 0.12, electrical conductivity of 4.25 ± 0.44 mS/m and 1.1± 0.03 % TOC level, revealing its alkaline and saline nature. As for the control site, pH level stood at pH of 8.31 ± 0.36, electrical conductivity of 4.63 ± 0.61 mS/m and 0.57± 0.01 % TOC. No differences in the pH and EC in the soil from the control site (Qatar University). Qatari soil is indeed mostly alkaline with high calcium-magnesium carbonates composition. It has relatively low organic matter

contents as well as significant iron and clay constituents (Peng et al., 2016).

4.2.2 Heavy metal bioaccumulation. Several desert plants are tolerant to heavy metals stress. Some of these are commonly found growing on Qatari soil; examples include *P. australis, T. domingensis, P. juliflora, Tamarix spp., M. polymorpha* and others (Baker et al., 2010; Yasseen and Al-Thani, 2013b). Although we previously reported that *T. qataranse* grows when irrigated with crude produced water containing difference heavy metals at a varying concentration (Usman et al., 2016). However, to the best of our knowledge, this is the first study examining *T. qataranse* specific metal tolerance and tissue accumulation capacity relative to background metal concentration in the soil.

Metal concentration in the soil and plant tissue parts (Root and shoot) is as shown in Fig 4.1a. Soil concentration (mg/kg) dry weight are in the following order Ba (144.8) > Cr (24.1) > Pb(12.2) > Cu (5.7) > Ni (5.19) > Cd (0.2). Although industrial activities increase toxic metals contamination, soil leaching or wearing reduces metal concentration in affected areas (Pandey and Singh, 2010). For the plant tissue parts (root and shoot), metal accumulation follows the trend Ni (63.3) > Cr (36.6) > Cu (22.4) > Ba (3.8) > Pb (3.5) > Cd (0.4) and Ni (17.4) > Cu (6.2) > Ba (2.9) > Pb (2.3) > Cd (0.5) > Cr (0) respectively. It is important to note that Ba, Cr and Pb concentrations in the soil are much higher that of Cd, Cu, and Ni. However, accumulation of these metals in *T. qataranse* tissues was not impressive, except for Cr with up to 36.6 mg/kg in the root, but was also below the detection limit in the shooting part. Soil properties, which in turn determines metal bioavailability for plant uptake is partly responsible for the observed discrepancies. Other vital factors include metal behavior and toxicity to the plant species in question (Usman et al., 2018).

4.2.2.1 Correlation between metals in the soil and T. qataranse tissues. The phytoavailability of heavy metals in the soil can be determined by establishing a correlation between metal concentration in the soil and accumulation in plant tissues (Chen et al., 2014), subject to plant type and soil properties, especially elemental composition (Maiti and Nandhini, 2006). Single regression analysis is used to assess the phytoavailability of all analyzed metals in this study. The correlation coefficients (r) of various metals were computed using Pearson's regression equation at two levels (i) between metal concentration in the soil and different plant parts (root and shoot), and (ii) between metal concentration in the root and shoot. The correlation coefficient was used to determine positive or negative correlations, indicating the suitability or unsuitability of metal accumulation in plants. Table 4 shows the relationship between metals in the soil and root/shoot metal concentration in *T. qataranse* grown naturally in the soil. The correlation coefficient between metals in *T. qataranse* tissue parts (root and shoot) are as shown in Table 5.

Table 4. Correlation between Metals in the Soil and *T. qataranse* Plant Parts

	Plant parts	
Metals (*n* = 5)	Root	Shoot
Ba	-0.66	-0.98*
Cd	0.02	0.28
Cr	0.60	n.a
Cu	-0.11	-0.75
Ni	0.96*	0.45
Pb	0.76	-1.00*

*Correlations is significant at 0.05 level
n.a = Correlation data not applicable (No metal accumulation in the shoot)

Table 5. Correlation Coefficient (r) Between Root and Shoot Metals Concentration in *T. qataranse* (n = 5)

	Root					
Shoot	Ba	Cd	Cr	Cu	Ni	Pb
Ba	0.50	-0.88	-0.71	-1.00*	-0.86	-0.50
Cd	-0.28	-0.95	-1.00*	-0.68	-0.96	0.28
Cr	n.a	n.a	n.a	n.a	n.a	n.a
Cu	-0.39	-0.91	-0.99*	-0.58	-0.93	0.39
Ni	-0.98*	0.21	-0.07	0.67	0.18	0.98*
Pb	0.79	0.60	0.80	0.12	0.63	-0.79

*Correlations are at 0.05 significance level
n.a = Correlation data not applicable (No metal accumulation in the shoot)

4.2.2.2 Bioconcentration factor (BCF) and translocation factors (TF).

Bioconcentration (BCF) and translocation (TF) factors are important parameters used in the feasibility study of heavy metals plants remediation potential, phytoremediation (Radziemska, 2018). The evaluation of *T. qataranse* accumulation of Ba, Cd, Cr, Cu, Ni, and Pb by BCF and TF are shown in Fig. 9a and Fig. 6a respectively. The BCF values for Cd, Cr, Cu, and Ni are greater than one but lower than one for Ba and Pb. Root BCF were in the order; Ni (12.2) > Cu (3.9) > Cd (1.5) > Cr (1.5) > Pb (0.2) > Ba (0.02), shoot BCF are Ni (3.3) > Cd (2.3) > Cu (1.1) > Pb (0.2) > Ba (0.02) > Cr (0.0). Together, the BCF values indicate Cd, Cr, Cu and Ni phytostabilization by *T. qataranse.* On the other hand, only Cd had a TF greater than one at 1.6 (Fig. 6a), while the TF of all other metals (Ba, Cr, Cu, Ni, and Pb) is less than one, suggesting *T. qataranse* Cd phytoextraction.

4.3 Phytoremediation of Pb in Controlled Environment

4.3.1 Evaluation of Pb tolerance and uptake in *T. qataranse.*

Environmental deterioration due to toxic metals presents a great danger to animals, plants, and human health. Lead (Pb), a heavy metal, is a non-essential element with no known biological role in plants. It is toxic and capable of causing morphological, biochemical, and physiological changes (Shahid et al., 2012). Several classifications suggest Pb as the second most toxic heavy metal. In 2015, Pb was listed as the number one heavy metal contaminant. Plants response to Pb varies; however, in general, a concentration higher than 30 mg/kg in the soil is toxic to most species (Sidhu et al., 2016a). Although some studies reported several Pb tolerant plants, few known hyperaccumulators exist, and poor understanding on its uptake and detoxification mechanism in

promising species is limiting scale-up and large application of phytoremediation at industrial level (Usman et al., 2018). In section 4.3, we showed that *T. qataranse* growing under field conditions accumulates various heavy metals, including Pb in an oil and gas industrial area. In this section, the effects of increasing Pb concentration (0, 25, 50, and 100 mg/kg) on tolerance and bioaccumulation capacity in *T. qataranse* in greenhouse environment is evaluated.

4.3.1.1 Effect of Pb on seedlings growth. Plants tend to be more susceptible to metal toxicity after germination (Lefèvre et al., 2009). Therefore, the evaluation of seedling growth is important in assessing metal toxicity in plants. The plants seedlings stage, development before and biomass accumulation before after Pb treatment are shown in Fig. 11 and 4.10, respectively. The effects of Pb treatment on total chlorophyll contents, fresh weight, root, and shoot length were evaluated (Fig. 13). A general increase ($p \leq 0.05$) in *T. qataranse* fresh weight observed (Fig. 11a). Furthermore, the highest biomass accumulation (~1.5 g) was observed under 50 mg/kg treatment condition; this suggests growth stimulation by Pb at this concentration. In terms of the length, *T. qataranse* tissues response varies with increasing Pb concentration (Fig. 11b). Both shoot and root length were highest ($p \leq 0.05$) under 25 and 50 mg/kg Pb treated samples, respectively, and shortest under 100 mg/kg Pb treatment condition. These data suggest that at 100 mg/kg, Pb disturbed normal growth and interfered with root development in *T. qataranse.* In *Z. fabago*, it was previously reported that Pb negatively affects root development (Ferrer et al., 2018). Additionally, a significant reduction on an important parameter in the assessment of plants health, total chlorophyll

content was observed (Fig. 11c).

a

b

Figure 11. *T. qataranse* seedlings **(a)** before and **(b)** after Pb treatment.

a

b

Figure 12. *T. qataranse* biomass after 7 weeks (a) Control (0 mg/L Pb) and (b) Pb treated (50 mg/L Pb)

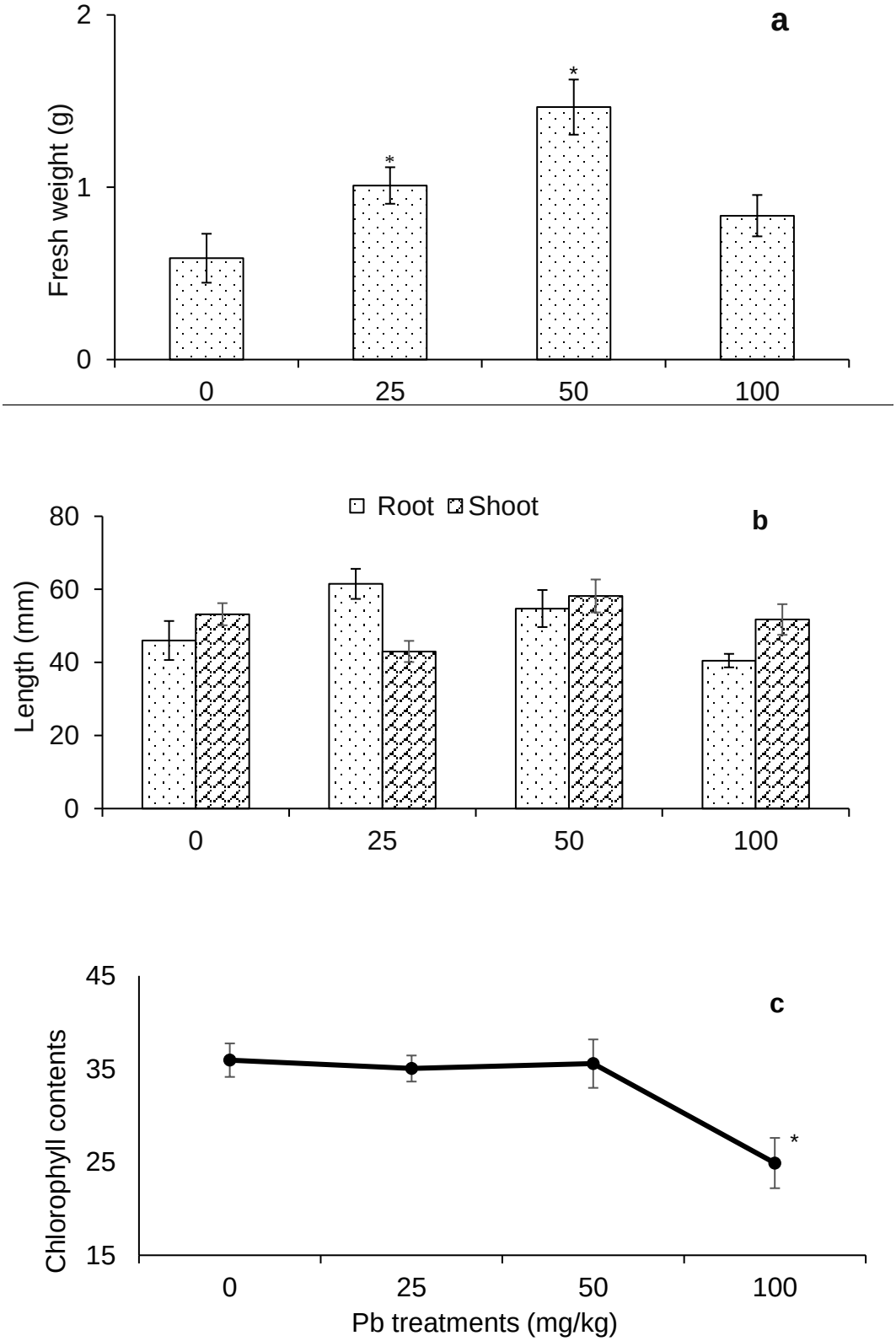


Figure 13. *T. qataranse* response to Pb treatments **(a)** Fresh weight **(b)** Root and shoot length and **(c)** Chlorophyll contents. Means are averages from five replicates (n=5) ± SEM. *Differences are significant at P<0.05 level (ANOVA-TUKEY).

4.3.1.2 Pb bioaccumulation in the shoot and root. In section 4.3 above, we found some Pb accumulation on *T. qataranse* obtained from the field (Ras Laffan) (Usman et al., 2019b). However, that study was based on field conditions, and in the presence of other metals. Here, we report Pb accumulation *T. qataranse* grown in a greenhouse setting (Fig. 14a). At 50 mg/kg Pb treatment, the root accumulates 2,784 mg/kg, while 1141.6 mg/kg concentrate in the shoot Lowest Pb accumulation was recorded under 100 mg/kg treatment concentration, with 1,732 and 817 mg/kg Pb for root and shoot, respectively. Overall data across all treatments indicates that Pb preferentially concentrates in the root in *T. qataranse.*

4.3.1.2.1 Bioconcentration and translocation factor. Bio-concentration (BCF) and translocation factor (TF) are important indices in the evaluation of metal bioaccumulation and translocation in plant tissues (Yoon et al., 2006). BCF estimates metal concentration against concentration in the treatment medium while TF determines whether or not plants translocate metals to their aerial parts. Fig. 14b and c show the BCF and TF values. In increasing order of concentration of Pb treatments, the root BCF were 36.53, 22.3, and 7.20, whereas the shoot records 66.44, 46.35 and 16.32. The root BCF across all treatments tend to be higher than that of the shoot. The TF under all treatments were greater than one (>1), indicating that T. qataranse sufficiently transfers Pb to the aerial part. The highest TF was for 100 mg/kg Pb.

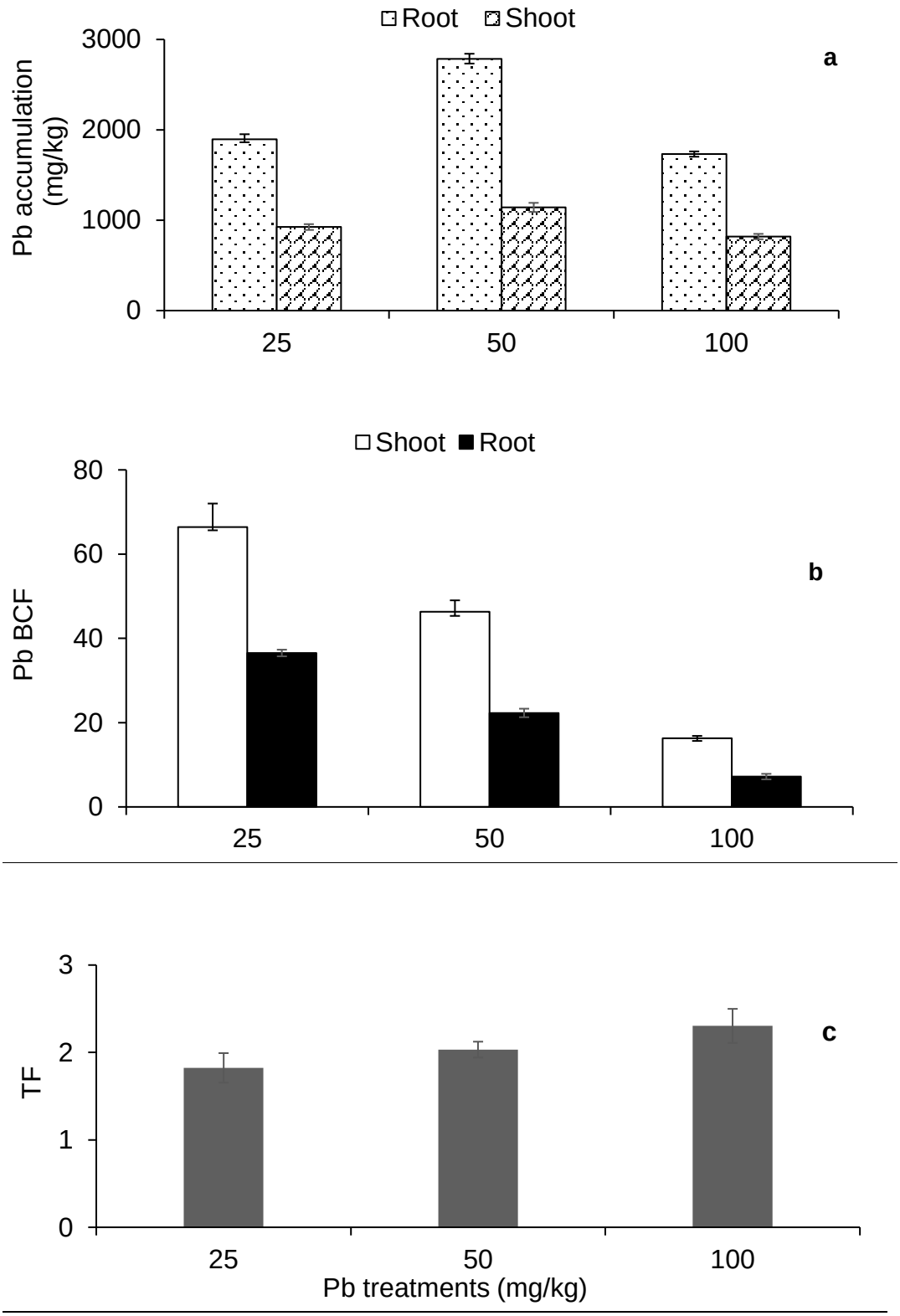


Figure 14. **(a)** *T. qataranse* root and shoot Pb accumulation **(b)** shoot and root BCF, and **(c)** TF. Means represent averages of three BCF and TF values (n = 3) ± SEM. Differences in Pb accumulation between tissues and treatments are significant at P ≤ 0.05 level (ANOVA-TUKEY).

4.3.2 Evaluation of Pb bioaccumulation in *Prosopis juliflora*. The desert plant, *P. juliflora*, commonly known as honey mesquite is widely distributed in arid and semi-arid environments. It is an important fuel and timbers source. A native of South and Central America, *P. juliflora* was introduced to many desert environments including the Gulf countries mainly to mitigate the effect of desertification. However, in many countries where the mesquite was introduced, it is fast becoming the most invasive plant and commonly found in managed environments including agricultural fields.

4.3.2.1 Germination response. Plant seeds imbibition and softening increase its permeability to various stress conditions. At this stage, the first exchange with the immediate environment occurs and is responsive to variable changes (Solanki and Dhankhar, 2011). In the present study, all-metal treatments recorded a final germination percentage (TG) of 80% and above (Fig. 15a). Generally, heavy metals exert a varying degree of toxicity to plants; however, much of these is a function of metal types and plant species. Similarly, while plants exposure to certain metal concentration may result in the manifestations of its toxicity, the same concentration may be tolerated by some species (Kranner and Colville, 2011). Germination rate (T50) and index (GI), which together demonstrates the speed of germination and seedlings vigor are important parameters in the assessment of heavy metal toxicity to plants (Ranal and Santana, 2006).

Accordingly, considering T50 and GI, we found *P. juliflora* to be highly tolerant to Pb. The T50 and GI demonstrated an inverse relationship between increasing concentration of all metals. At 25 mg/kg concentration, Pb demonstrates an interesting scenario by stimulating *P. juliflora* germination

compared to control with a T50 of 0.3 days and GI of 13.9, compared to the control's 0.4 days and 12.8, respectively (Fig. 15a). Together, the results of germination experiment suggest that the effects of metal treatments were in a dose-dependent manner. Although 100% TG were recorded by all Pb treatments, higher T50 and lower GI for 50 and 100 mg/L compared to the control (Fig. 15b) suggest that, at higher concentration, Pb negatively affects *P. juliflora* germination. Accordingly, Mishra and Choudhuri (1998) reported that at 36 mg/kg, Pb negatively impacted on rice seed germination by up to one third, and inhibited further seedlings development by approximately 50% (Verma and Dubey, 2003). Similarly, it reduces seed germination and caused stunted growth in roots of another South American native plant *Lupinus*, belonging to the same family, Fabaceae.

4.3.2.2 Seedlings growth. Plants are more vulnerable to metal toxicity at seedlings stage (Lefèvre et al., 2009). The assessment of seedlings growth is important in assessing metal toxicity. Fig. 16 shows *P. juliflora* germination and harvested biomass after Pb treatment. On physical inspection at harvest, though all treatments generally appear healthy with no major physical damage, such as wilting, chlorosis or root darkening, partial leaf chlorosis began to manifest for 100 mg/L Pb treatment. The results of the analysis of fresh weight, chlorophyll content as well as root, and shoot length are shown in Fig. 17. At 100 mg/L Pb, root length significantly differs (Fig. 17a) compared to the control and other treatments, suggesting that at this concentration, Pb caused root stunted growth.

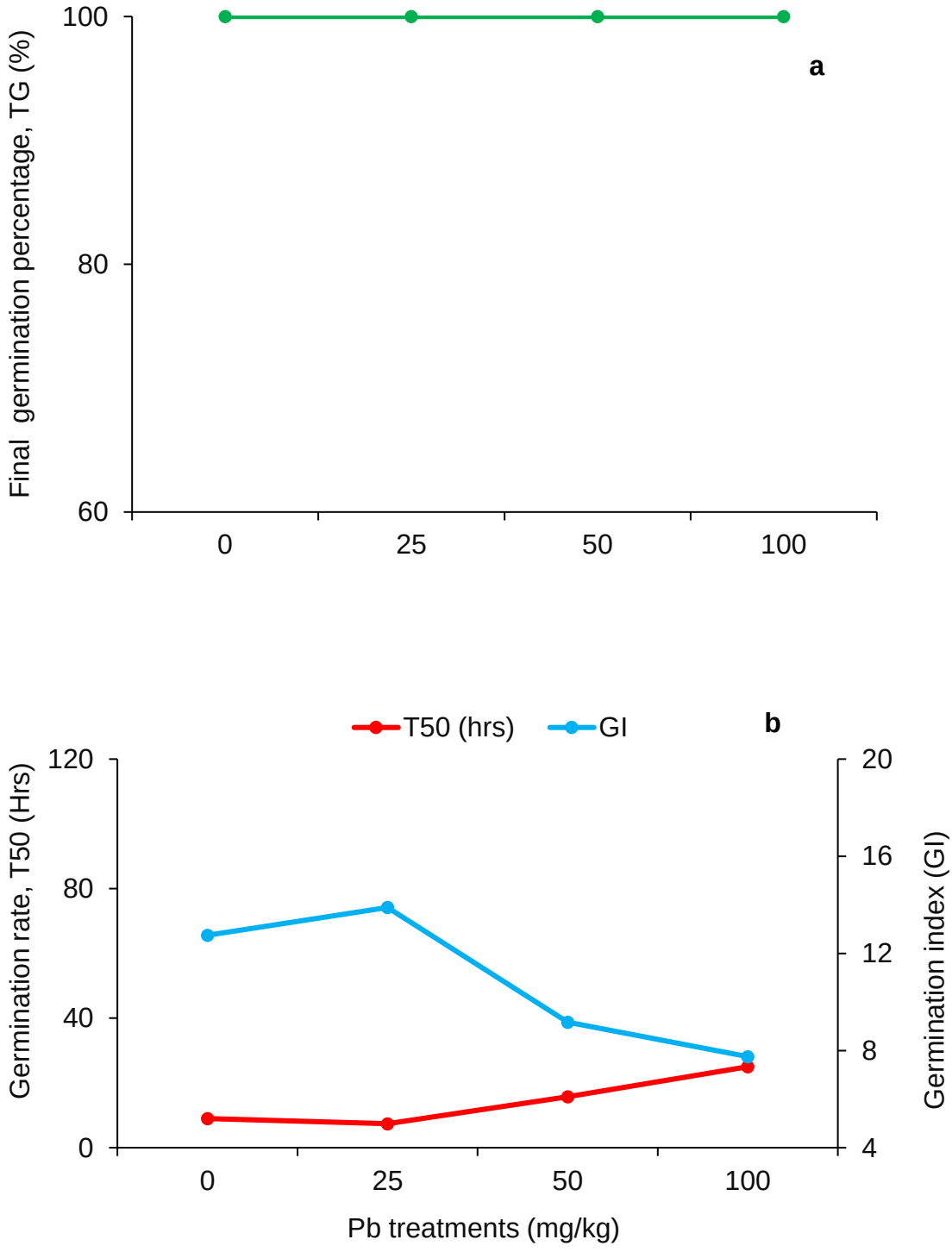
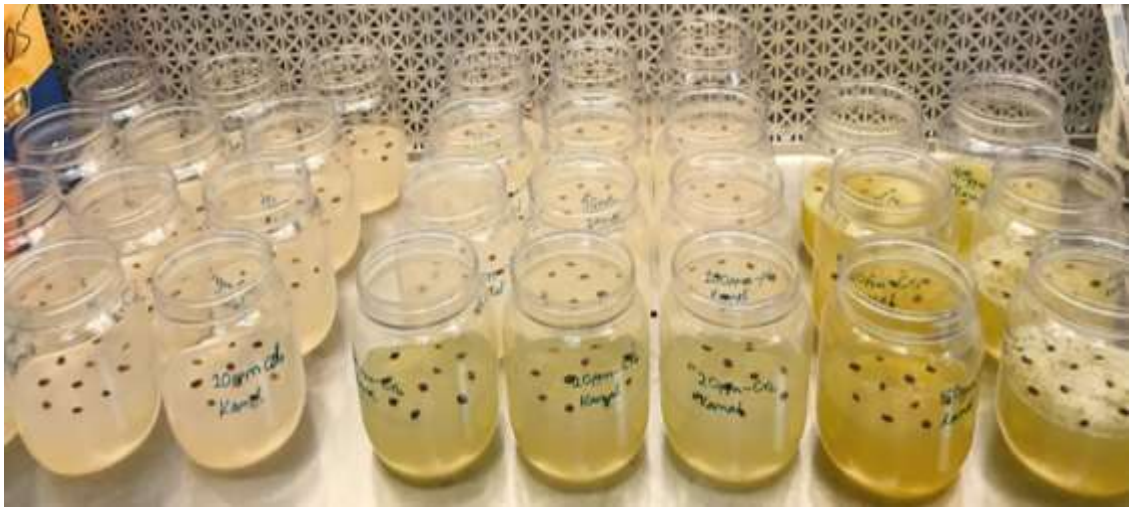


Figure 15. *P. juliflora* germination parameters **(a)** Germination % (TG) **(b)** Germination index (GI) and germination rate (T50)

a



b

c

Figure 16. *P. juliflora* **(a)** Seed germination in a modified Hoagland nutrient solution solidified in agar, **(b)** seedlings after Pb treatment and **(c)** harvested biomass for tissue metal analysis.

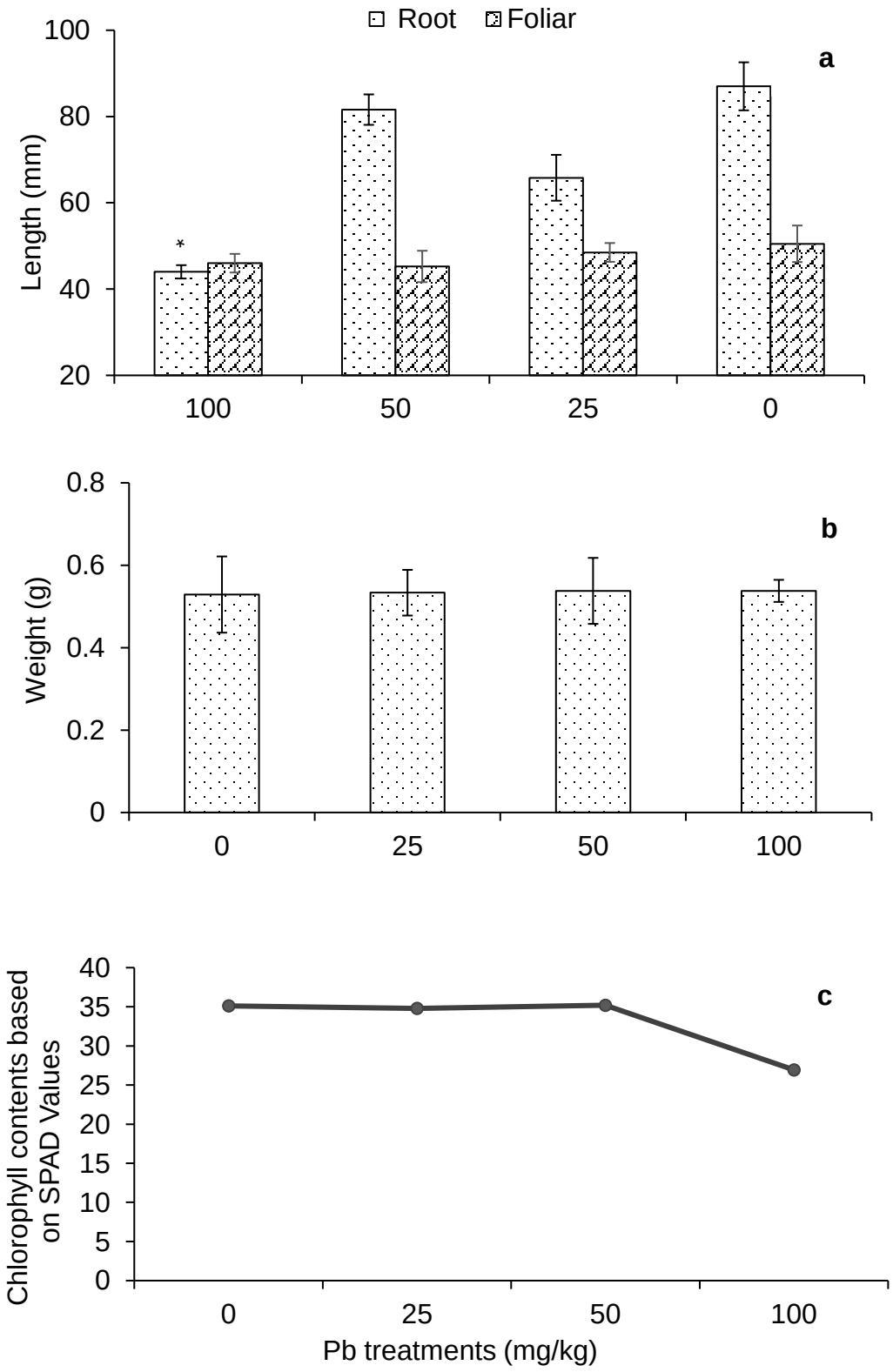


Figure 17. *P. juliflora* response to Pb treatment (**a)** Root and shoot length. Mean length are averages of nine plants from three replicates (n=9) ± SEM. *Mean difference between treatments in root length statistically significant at P<0.05 (ANOVA-TUKEY) **(b)** Fresh weight and **(c)** Total chlorophyll contents based on SPAD.

In sharp agreement, Arias et al. (2010) noted that root elongation was negatively affected in arbuscular mycorrhizal fungi associated *Prosopis* sp treated with 100 mg/L of Pb. Indeed, Pb toxicity is implicated in various physiological, biochemical and morphological stress symptom in plants, including chlorosis, root darkening, disturbed photosynthesis, and general growth inhibition. Several workers noted root growth inhibition by Pb at a concentration below 1 mg/L in other plant species (Pourrut et al., 2011).

Additionally, at higher concentration, not only does Pb inhibit root development but also that of other tissues, and subsequently affecting the general plant biomass accumulation (Gupta et al., 2009). However, we observed no significant differences in fresh weight and shoot length on Pb treated *P. juliflora* compared to control (Fig. 17a and b). It is probably due to low Pb accumulation in the plant shoot relative to the root. The root has an important role in plant growth and development, which directly affects how other tissues respond to growth conditions (Biernacki & Lovett-Doust, 2002). Under heavy metal stress, it suffers the first exposure. However, its cell wall has a mechanism of exchange that fixes heavy metal ions, thereby limiting transport to other plant tissues (Pourrut et al., 2011), which may in part also be responsible for the significant effect observed in *P. juliflora* treated with 100 mg/kg of Pb, but not in fresh weight and shoot length.

The chlorophyll contents is an important parameter used to assess plants health (Chaerle and Van Der Straeten, 2001), in the present study, SPAD values indicate a significant reduction in chlorophyll contents in *P. juliflora* treated with 100 mg/L Pb compared to the control (Fig. 17c). Accordingly, Pb increases the activities of chlorophyllase enzymes in plants, which facilitates

the degradation of chlorophyll (Hu et al., 2012) Strong evidence also suggests a direct relationship between reduced photosynthetic activity and high Pb presence in plants. At high concentration, it also inhibits cell division and partly impacts on metabolic activities at the cellular level, all of which are responsible for the decreased growth of plants. Therefore, the behavior of mesquite growth under Pb toxicity can be likened to that of other crop plants such as *Triticum aestivum* (Arias et al., 2010).

4.3.2.3 Pb bioaccumulation in the root and shoot. The amounts of Pb accumulated in the root and shoot tissues of *P. juliflora* cultivated in agar media containing 25, 50 and 100 mg/L of Pb are as shown in Fig. 18a. For all treatments, Pb accumulation is generally higher in the root than shoot. The accumulation of Pb by *P. juliflora* were (a) 25 mg/L of Pb: 2884 and 1142 mg/kg. (b) 50 mg/L of Pb: 3366.3 and 1228.6 mg/kg and (c) 100 mg/L of Pb: 2778 and 1410.4 mg/kg for root and shoot, respectively. Although Pb does translocate to the aerial part of plants, similar studies found that it preferentially accumulates in the root (Langley-Turnbaugh and Belanger, 2010). Other similar studies are reviewed by Pourrut et al. (2011) in a critical review of Pb toxicity to plants. Understandably, translocation of metal to the aerial parts in plants is restricted in some species including Pb courtesy of many factors. It is largely regulated when the metal species is entering the root using apoplast via water streams and into the inner endodermis region (Pourrut et al., 2011). In the course of transport, negatively charged molecules in the cell wall such as the pectin can immobilize Pb ions; others are plasma membrane accumulation or precipitation of Pb insoluble salts (Islam et al., 2007; Nie et al., 2011). Even more convincing is the fact that, since Pb may be trapped in the endodermis by the Casparian

strip, it can resort to symplastic transport by which most of the isolated Pb can be excreted out of the plant (Pourrut et al., 2011).

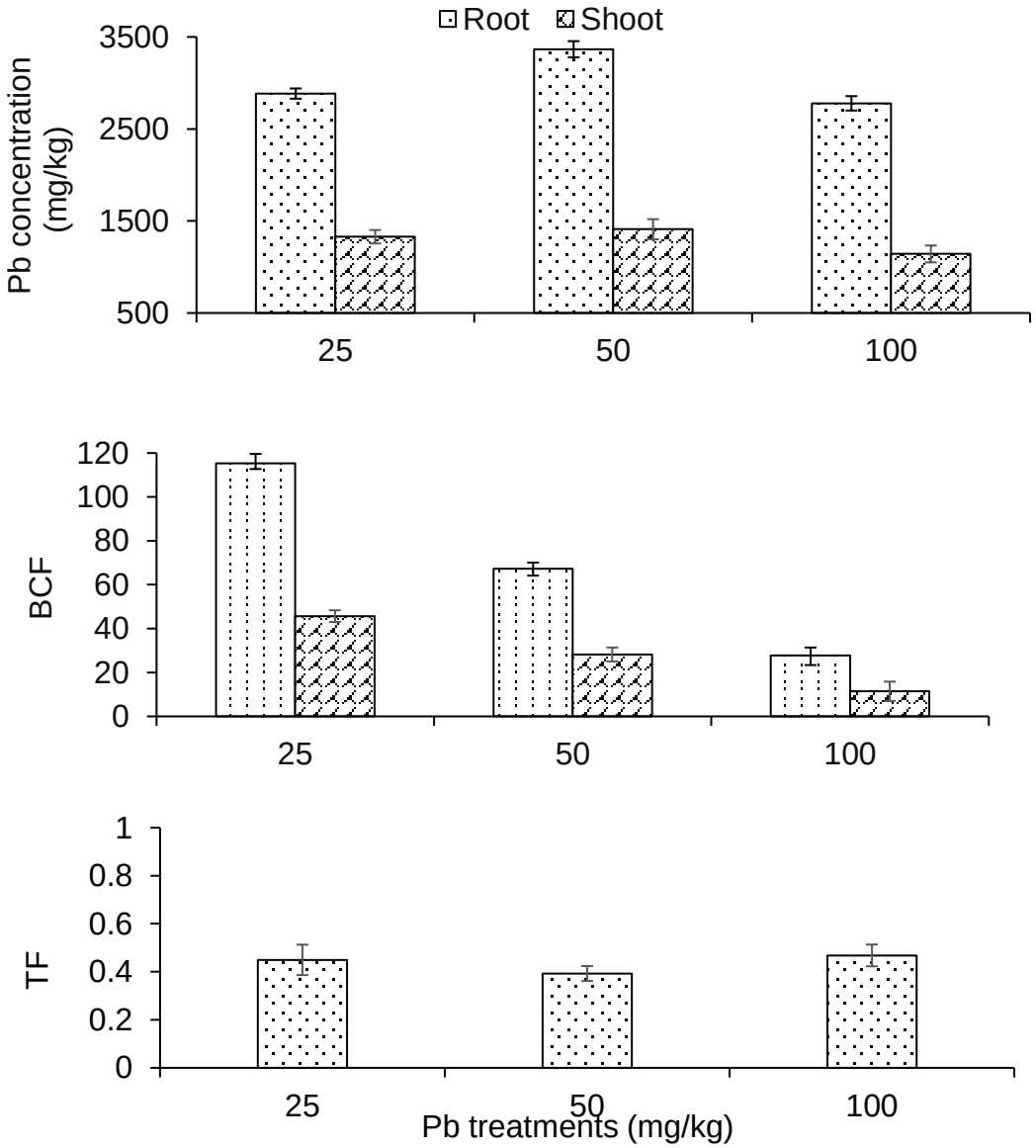


Figure 18. *P. juliflora* Pb accumulation. **(a)** Root and shoot Pb concentration **(b)** Root and shoot BCF **(c)** TF. Means are averages of three replicates (n=3) ± SEM. Differences in (a) are significant at P ≤ 0.05 level (ANOVA-TUKEY).

Our results suggest that for the root, *P. juliflora* absorbed the most Pb when treated with 50 mg/L of Pb. However, shoot recorded the highest Pb accumulation under 100 mg/L treatment. Supporting our findings, other workers report similar Pb accumulation pattern in *P. juliflora* tissues. For instance, Arias et al. (2010) observed that *P. juliflora* root accumulated higher Pb concentration when treated with 50 mg/L of Pb. The same observation was made by Aldrich et al. (2004), where at 50 mg/L of Pb treatment, the plant root tend to accumulate up to 63,396 mg/kg of the metal concentration in a hydroponic media amended with ethylenediaminetetraacetic acid (EDTA).

4.3.2.3.1 Bioconcentration and translocation factors. Other important parameters considered in the evaluation of metal bioaccumulation and plants phytoremediation potentials are bio-concentration (BCF) and translocation factor (TF) (Yoon et al., 2006). While BCF estimate metal accumulation in the tissues relative to that of the treatment medium, TF is useful in the determination of plants efficiency in heavy metals translocation right from the root and on to the shooting part. A BCF value greater than one (>1) and less than one (<1) indicates that a plant is a “hyperaccumulator” and “excluder” respectively (Maiti and Jaiswal, 2008). According to (Srivastava et al., 2005) a plant is considered effective in metal translocation from root to shoot when the TF is greater than one (>1), which is due to the efficient plants metal transport system. However, TF value less than one (<1) indicate ineffective metal transfer and therefore suggest that the plant species accumulate more metals in roots than the shoot.

The BCF and TF of *P. juliflora* treated with 25, 50, and 100mg/kg of Pb are as shown in Fig. 3b and 3c respectively. The root tissue recorded the most,

and so is the case with the bioconcentration factor. The root BCF were 115.4, 67.3 and 27.8 for 25, 50 and 100 mg/L of Pb treatments, respectively. While that of the shoot are as follows: 45.7, 28.2 and 11.4 for 25, 50 and 100 mg/L of Pb treatments, respectively. As a factor of metal concentration in the medium, it can be seen that the BCF decreases with increasing Pb concentration. Despite the relative higher root BCF across all treatments, the shoot BCF also indicates the capacity of *P. juliflora* to translocate reasonable concentration of Pb onto the shoot part, especially at 25 mg/L concentration, where the BCF is highest for the shoot at 45.7 (Fig. 18b). Indeed, hyperaccumulator plants such as *Brassica pekinensis* and *Pelargonium* were reported to accumulate higher Pb concentration to their aerial parts (Arshad et al., 2008).

Regarding the TF, all treatments were less than one, indicating that *P. juliflora* does not translocate Pb to its aerial parts (Fig. 18c). However, a different trend to that of BCF is evident in that the least TF value was under 50 mg/L, whereas the highest is that of 100 mg/L of Pb treatment. Pb ability to break the Casparian crisp barrier in *P. juliflora* endoderm at 100 mg/L concentration, to enable further cationic transport across tissues and onto the aerial part may in part be responsible. Numerous plants are known for reduced metal uptake to aerial parts, which preferentially bioconcentrate in the root (Pourrut et al., 2011). Plant species including *Nerium oleander L.* and *Brassica juncea* are known to accumulate higher Pb accumulation in the root with no visible signs of stress (Manousaki and Kalogerakis, 2009). Notwithstanding, however, here, *P. juliflora* accumulates more than 1000 mg/kg Pb in both roots and shoot under all treatments (Fig. 18a). A comparison of highest reported BCF and TF of some terrestrial plants is shown in Table 6.

Table 6. Comparison of Bioconcentration and Translocation Factor in Some Pb Accumulating Plants

Plants species	BCF	TF	References
Acacia mangium Willd	0.479 (Shoot)	0.312	(Ng et al., 2018)
Alternanthera philoxeroides	0.06 (Shoot)	0.62	(Yang et al., 2014)
Artemisia princeps	0.15 (Shoot)	0.57	(Yang et al., 2014)
B. pilosa	0.03 (Shoot)	0.75	(Yang et al. 2014)
Cynodon dactylon	0.05 (Shoot)	0.55	(Yang et al. 2014)
Digitaria sanguinalis	0.07 (Shoot)	0.79	(Yang et al. 2014)
Erigeron canadensis	0.02 (Shoot)	0.35	(Yang et al. 2014)
Phytolacca acinosa	0.02 (Shoot)	2.02	(Yang et al., 2014)
Raphanus sativus	0.03 (Seed) 0.55 (Shoot) 6.14 (Root)	0.6	(Hladun et al., 2015)
Prosopis juliflora	115.4 (Root) 45.7 (Shoot)	0.5	(This study)
Oryza sativa	0.01 (Whole plant)	3.29	(Kumar and Maiti, 2014)
Mucuna bracteata DC. ex Kurz	1.231 (Shoot)	0.299	(Ng et al., 2018)
Vetiveria zizanioides L. Nash	0.395 (Shoot)	0.661	(Ng et al., 2018)
Zea mays	0.022 (Whole plant)	3.95	(Kumar and Maiti, 2014)

Phytoextraction is a form of metal phytoremediation, where plants translocate metals to other parts can be demonstrated by BCF and TF. In this

context, alongside TF, above the ground metal BCF in plants tissues (leaf, shoot or stem) are considered. Table 1 shows the shoot BCF in this study to be higher at all treatment levels with 45.7, 28.2 and 11.4. Further, as found in this study, except for *Arabis paniculata* Franch, *Bidens frondosa, Setaria plicata, Phytolacca acinosa, Oryza sativa,* and *Zea mays,* majority of the plants reported as good candidates for the remediation of Pb polluted environment had TF < 1. However, a TF > 1 in the above species could in part be due to the occurrence of Pb alongside other metals in the studied areas, which influences uptake behavior across the plant tissues (Kumar and Maiti, 2014). Together, our results suggest that *P. juliflora* is a hyperaccumulator of Pb (Maestri et al., 2010), and therefore a suitable candidate for the phytostabilisation of Pb contaminated areas.

4.4 Mechanisms of Pb Tolerance, Uptake, and Bioaccumulation in *T. qataranse*

4.4.1 Pb adsorption in *T. qataranse* tissues. Transition metals found in biological samples interact with the functional groups of biomolecules, the composition of which can be determined by analyzing their infrared light adsorption (Jackson and Mantsch, 2000). Schematic representation of Pb accumulation on *T. qataranse* tissues is shown in Fig. 19. FTIR data can be used to study metal cation binding in biological samples (Morikawa et al., 1974). For FTIR analysis, soil and *T. qataranse* samples collected from a non-metal polluted area located at Qatar University as controls.

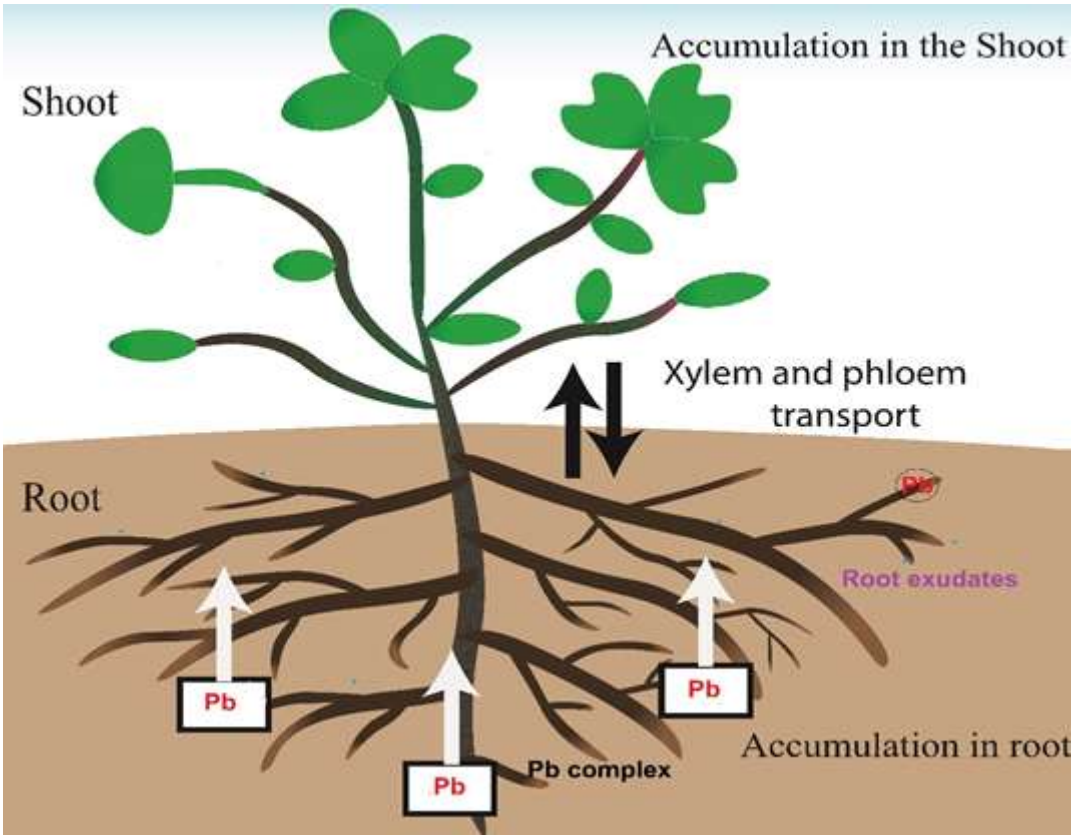


Figure 19. Schematic representation of Pb accumulation on *T. qataranse* tissues.

All metals in the control sites were below the detection limit when analyzed using ICP-OES. Although up to five major bands were found to correspond to metal interaction in the soil (Fig. 20a), only three of these peaks, and their corresponding shifts were consistent in comparison to *T. qataranse* tissues, and therefore critically examined (Fig. 20b-d). FTIR results showed that dry biomass has different functional groups available for binding of heavy metal ions, such as carboxyl, phosphate, amide, and hydroxide. Broad and robust IR spectra regions spanning 3600-3200 cm^{-1} characterize O-H and N-H stretch (Panda et al., 2007).

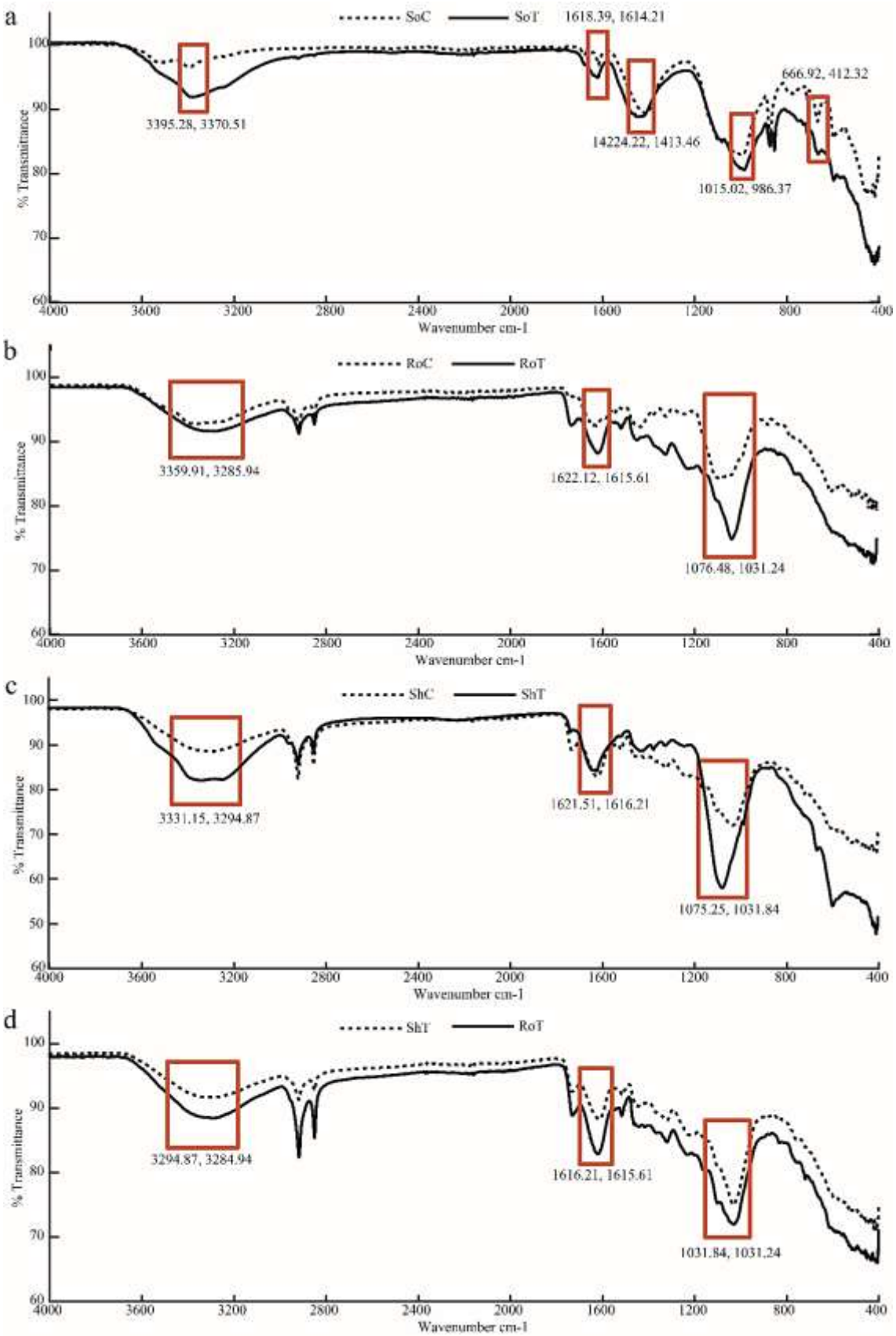


Figure 20. FTIR spectra for soil **(a)** and *T. qataranse* root and shoot **(b-d)**. **(a)** Soil control (SoC) vs Soil treatment (SoT). **(b)** Root control (RoC) vs Root treatment (RoT). **(c)** Soot control (ShC) Shoot treatment (ShT). **(d)** Root treatment (RoT) vs Shoot treatment (ShT).

All bands at 1618.39 and 1614.21 cm^{-1} (Fig. 20a), 1622.12 and 1615.61 cm^{-1} (Fig. 20b), 1621.51 and 1615.21 cm^{-1} (Fig. 20c) as well as 1616.184 and 1615.61 cm^{-1} (Fig. 20d) corresponds to specific amide groups due to C=O stretch (Dumas and Miller, 2003). The regions from 1200 to 900 cm-1 signify C-C, C-O, and C-O-P stretch overlaps (Wolkers et al., 2004) occurring mainly in cellular polysaccharides.

4.4.2 Protein expression in response to Pb treatment

4.4.2.1 Optimized protein extraction and quantitation. To optimize total protein extraction, protein samples were subjected to three extraction protocols; Phenol/SDS with three prewashed steps, Phenol/SDS without prewashed steps, and a commercial kit (Thermo Fischer Scientific). Subsequent quantification and quality check were performed by Bradford assay and 12% (w/v) SDS-PAGE. The result from both is as shown in Table 7 indicating the protein concentration and yield based from the three extraction protocols of both varieties, calculated based on Protein Bovine Serum Albumin (BSA) standard curve (Fig. 21). Based on protein concentration and yield (Table 7) it is clear that Phenol/SDS with three pre-washed steps was the best method. Better electrophoretic separation was also observed for proteins obtained from this method. Variations in protein quantity and quantity may be caused by many factors, such as high rate of contaminants, extraction buffer used and sample preparation among others. In this study, Phenol/SDS with pre-wash steps proved to be the best among all. Extraction buffer is one obvious factor that influences such outcome. The different extraction methods are composed of different buffers and components. Considering the Phenol/SDS with pre-wash steps buffers, it is possible that both phenol and SDS buffers played a vital role

in the better purification and separation of protein extracted using this method as reported by Wang et al. (2006). When extracting protein using the above protocol, some important points must be noted; (i) samples should be kept at low temperature, and (ii) carefully recovering phenol phase following centrifugation (Wang et al., 2008) all of which were strictly adhered observed in this study. Most of the time, the pH of phenol solution were adjusted before use in order to meet the basic condition, where the distribution coefficients of proteins are usually greater than 100 (Pusztai, 1966). Buffered phenol solution (pH 8.0) and bromophenol blue used in this method were found to be compatible with phenol with a pH indicator blue at greater than 7.0.

Table 7. Total Protein Concentration and Yield Based on Three Extraction Methods. Means are Averages of Three Replicates (n = 3) ± SEM

Method	Concentration (µg/µL)	Yield (µg/mg)
SDS/Phenol (Pre-washed)	47.04±15.1	28.5±9.1
SDS/Phenol	19.9±2.3	15.9±1.9
Commercial kit	27.4±16.1	18.2±12.1

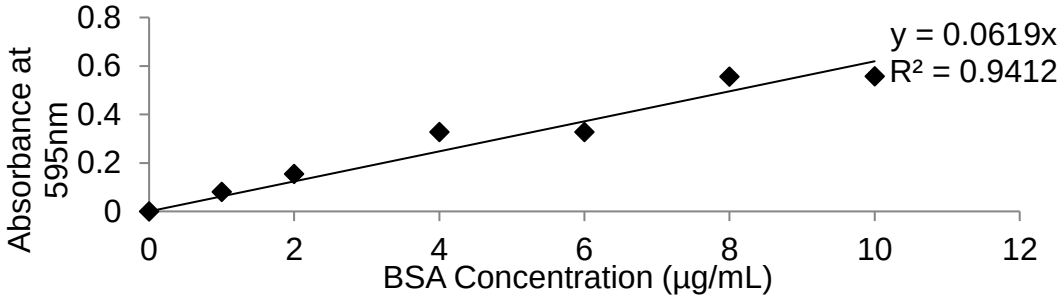


Figure 21. BSA Standard Curve.

4.4.2.2 Separated proteins using SDS-PAGE. SDS/Phenol (pre-washed) Pb treated *T. qataranse* (whole plant) extracted proteins (Fig. 22a), were resolved using NuPAGE 4-12% Bis-Tris Protein Gels (Invitrogen) and visualized by Imperial™ Protein Stain (Thermo Fischer Scientific) (Fig.4.20b). The green arrow indicates probable induction of catalase (CAT) (Romero-Puertas et al., 2002), glutathione reductase (GR) (Romero-Puertas et al., 2006) or phytochelatins synthase (PCS) (Filiz et al., 2019) at approximately 52 kDa while the red arrow shows prominent polypeptide Rubisco (large subunit), a characteristic feature of plant tissue protein extracts at 55kDa (Walliwalagedara et al., 2010).

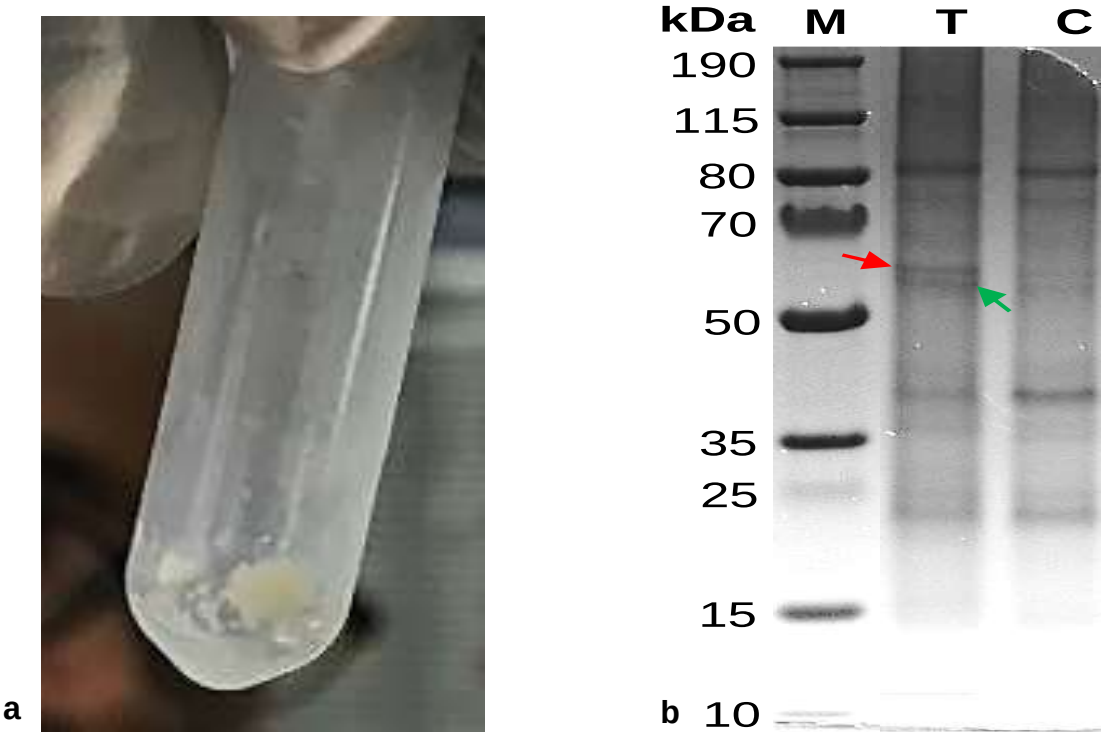


Figure 22. Pb treated *T. qataranse* total proteins (whole plant) **(a)** Protein pellets (47 µg/mL) and **(b)** SDS-PAGE separation. Lane 1, Protein marker; Lanes 2, 4 and 6, Treatments (50 mg/L Pb); Lanes 3, 5 and 7, Control condition (without Pb). Approximately 20 µg of proteins are resolved using NuPAGE 4-12% Bis-Tris Gels (Invitrogen) and visualized by Imperial™ Protein Stain.

4.4.2.2 The identified protein. In this section, we show that Pb treatment-induced metal-binding proteins expression. Before matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis, the gel containing separated proteins were image analyzed to determine band intensity and sizes of separated proteins using GelAnalyzer 2010a software (Bourven et al., 2012), and tryptic digested. Both treatment and control lanes were cut into 1mm sizes on adjacent areas covering ~15 to 115kDa. MALDI TOF/MS analysis was performed following Kwiecińska et al. (2018). Before bioinformatics analysis for proteins identification, preliminary inspection was carried out for differential mass lists. As expected, raw peptide mass lists obtained showed higher and differential spectra peaks between treatment and the control (Fig.4.21a and b) suggesting obvious differences in protein expression. Following MALDI-TOF/MS analysis, mass list searches were performed in Mascot for peptide matches and protein identification in NCBI and Uniprot databases. Only proteins with significant scores according to Mascot algorithms are reported. Sample search result page is shown in Fig. 23c. List of the identified proteins with wide a spectrum of functions are shown in Table 8, 86 proteins were identified, of these, six proteins (Proteins AXX17_AT2G26660, AXX17_AT4G36160, AXX17_AT2G13500, AXX17_AT5G33340, AXX17_AT5G16980 and AXX17_AT1G74880) were of unknown function.

Overall, the majority of the identified proteins showed a large number of molecules were involved in metabolism, and response to stress, including heavy metals. In the context of this study, these are some of the most important categories, as they contain numerous metal binding and antioxidative proteins.

For instance, Heat shock 70kDA 80-3kDA and 90kDa, which are chloroplastic and function in both protein and ATP binding, are typical heat and other abiotic stress-responsive proteins (Ford et al., 2011, Majoul et al., 2003). Other ATPase transporting chloroplastic proteins have also repeatedly appeared in varying molecular weight (MW) and isoelectric point (pI) as does Rubisco, Heat shock, and Chepronin. Interestingly, some quite irregular proteins such as Transketolase, Flavin containing monooxygenase family protein, Pumilio homolog 6 and 5 and 14-3-3-like protein GF14 psi were also detected. In addition to the many heat shock proteins, others such as carbonic anhydrase and Flavin containing monooxygenase family were shown to respond to cadmium stress and play important roles in auxin biosynthesis, respectively. Carbonic anhydrase was involved in glycophytes assisted phytoremediation of zinc, lead, and cadmium (Abdu et al., 2011), while Zhao et al. (2001) noted that Flavin containing monooxygenase family protein, which regulates auxin biosynthesis also mediates the translocation of these metals across plant tissues. Flavin containing monooxygenase family protein was also shown to play vital role in xenobiotic detoxification mechanism by directing the correct folding of protein-containing sulfide bonds (Naumann et al., 2002), and hence, may have similar role in Pb detoxification in *T. qataranse.*

Further, the two Pumilio homolog proteins were both chloroplastic and cytosolic with binding function were also suggested to be emerging regulators for plants response to environmental constraints (Ambrosone et al., 2012). The results of gene ontology in Table 8 provides useful information for the general overview of *T. qataranse* total proteome in response to Pb stress. However, a further gene enrichment analysis by protein groups will provide a more

comprehensive interpretation.

Table 8. Identified Proteins and Their Gene Ontology. Accession Number was obtained from NCBI based on Search against *Arabidopsis* Database. MW = Molecular Weight; pI = Isoelectric Point; GO = Gene Ontology; Araiy = *Arabidopsis iyrata; Arath* = *Arabidopsis thaliana;* B = Biological Process; C = Cellular Component; M = Molecular Function

Accession	**MW(Da)**	**pI**	**Homology**	**Protein Name**	**GO**
17367307	16707.8	5.46	Arath	Actin-depolymerizing factor 6	B: Regulation of actin depolymerizing activity
297316109	70755.3	5.58	Arath	Adenosylhomocysteinase	B: Carbon metabolism M: Adenosylhomocysteinase activity.
20140328	71364.2	9	Arath	Asparagine--tRNA ligase	B: Protein biosynthesis C: Cytoplasm M: Aminoacyl-tRNA synthase
18404975	91170.2	9.38	Arath	ATH subfamily protein ATH8	B: Regulation of Ribosome binding C: Cytoplasm M: Ester bond activity

Accession	MW(Da)	pI	Homology	Protein Name	GO
14423416	63809.5	6.21	Arath	ATP synthase subunit beta-3	B: ATP Synthesis C: Chloroplast M: ATP binding
5881679	55328.5	5.19	Arath	ATPase subunit	B: ATP Hydrolysis C: Chloroplast. M: Catalysing transmembrane movement
15226092	85933.8	5.44	Arath	ATPase F1 complex alpha subunit protein	B: ATP Hydrolysis C: Chloroplast M: Poly(U) RNA and zinc ion binding
297318710	43408.6	4.45	Arath	Calreticulin 2	B: Metabolic process C: Mitochondrion M: Calcium ion binding
3249100	50581.9	5.46	Arath	Carbonic anhydrase	B: Carbon utilization

Accession	MW(Da)	pI	Homology	Protein Name	GO
38503395	37450.1	5.74	Arath	Carbonic anhydrase	C: Chloroplast M: Zinc ion binding B: Carbon utilization
21554572	62130.4	5.04	Arath	Chaperonin-60 alpha	C: Chloroplast M: Metal ion binding B: Cellular protein metabolic process
115385	27733.7	6.22	Araiy	Chlorophyll a-b binding protein 4	C: Chloroplast. M: ATP binding B: Photosynthesis
116831194	28063.2	6.53	Araiy	Chlorophyll a/b-binding protein	C: Chloroplast B: Photosynthesis C: Chloroplast

Accession	MW(Da)	pI	Homology	Protein Name	GO
					M: Chlorophyll and metal ion binding.
30697525	30008.9	8.24	Arath	D-ribulose-5-phosphate-3-epimerase	B: Carbohydrate metabolic process C: Chloroplast M: Ribulose-phosphate 3-epimerase activity
15225693	25921.5	5.25	Arath	Dienelactone hydrolase domain-containing protein	B: Nucleoside metabolic process C: Chloroplast M: Hydrolase and transferase activity
75161476	54120.1	9.07	Arath	Endoglucanase 16	B: Carbohydrate metabolism C: Mitochondrion M: Protein and single-stranded DNA

Accession	MW(Da)	pI	Homology	Protein Name	GO
					binding
7433553	59304.7	5.89	Araiy	Enolase	B: Glycolysis M: Phosphopyruvate hydratase activity
10086473	51654.2	6.13	Araiy	Flavin-containing monooxygenase family protein	B: Auxin biosynthesis M: Flavin adenine dinucleotide binding
297326214	43059.2	6.27	Araiy	Fructose biphosphate adolase	B: Glucose catabolic process C: Cytoplast M: Fructose-biphosphate adolase activity
297338561	42703.9	7.62	Araiy	GAPA	B: Protein modification C: Chloroplast

Accession	MW(Da)	pI	Homology	Protein Name	GO
					M: NAD, NADP and nucleotide binding
8778823	107175.8	9.1	Arath	GTP binding Elongation factor Tu family protein	B: Response to cadmium ion C: Chloroplast M: GTP binding
15222111	42847	8.16	Arath	Glyceraldehyde 3-phosphate dehydrogenase (GAPA2)	B: Glycolysis C: Chloroplast M: NAD and NADP binding
297326214	43059.2	6.18	Araiy	Glyceraldehyde-3-phosphate dehydrogenase	B: Protein modification M: Oxidoreductase activity
9758815	40548.1	8.79	Arath	Glycolate oxidase	B: Defence response to bacterium C: Chloroplast M: Catalytic and oxidoreductase

Accession	MW(Da)	pI	Homology	Protein Name	GO
					activity
591401946	60991.4	5.66	Arath	Glycosyltransferase	B: Glycosylation
297310259	78479.1	5.01	Araiy	Heat shock protein 81-3	B: Stress response
					C: Nucleolus
					M: ATP binding
297318892	71428.2	5.06	Araiy	Heat shock protein 70	B: Response to cadmium ion
					C: Cytoplasm.
					M: ATP binding
219766617	77106	5.13	Arath	Heat shock protein 70	B: Response to cadmium ion
					C: Chloroplast
					M: ATP binding
1032296797	7540	9.52	Arath	Protein AXX17_AT2G26660	Unknown
1032282636	304279	4.72	Arath	Protein AXX17_AT4G36160	Unknown

Accession	MW(Da)	pI	Homology	Protein Name	GO
OAP09027	61847	7.53	Arath	Protein AXX17_AT2G13500	Unknown
1032280307	49017	5.77	Arath	Protein AXX17_AT5G33340	Unknown
1032277271	66677	8.77	Arath	Protein AXX17_AT5G16980	Unknown
OAP18313	35666	5.74	Arath	Protein AXX17_AT1G74880	Unknown
297328438	43331.5	8.63	Araiy	Kinase family protein	B: Chloroplast relocation C: Chloroplast M: ATP binding
15237622	108115.3	8.77	Arath	Kinesin-like protein	B: DNA methylation M: Nucleic acid-binding
297322140	27214.2	5.88	Arath	L-ascorbate peroxidase 2	B: Stress response C: Chloroplast M: Metal ion binding
118572828	28006.2	5.88	Arath	L-ascorbate peroxidase 2	B: Stress response M: Heme binding and peroxidase

Accession	MW(Da)	pI	Homology	Protein Name	GO
					activity
297334190	52966.4	5.96	Araiy	Large subunit of RuBisco	B: Protein modification C: Chloroplast M: Magnesium ion binding
297332730	52973.4	6	Araiy	Large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase	B: Carbamylation of the active site M: Magnesium ion binding
15239602	28706.8	4.96	Arath	Light-harvesting chlorophyll B-binding protein 3	B: Photosynthesis C: Membrane
75330960	29766.5	4.97	Arath	Methyl-esterase 1	B: Fatty acid catabolic process C: Extracellular region
15221728	25845	9.82	Arath	Non-intrinsic ABC protein 10_AT1G63270	B: C: M:

Accession	MW(Da)	pI	Homology	Protein Name	GO
67460972	82079.5	9.06	Arath	Oligopeptide transporter 8	B: Oligopeptide transport C: Nucleus M:NAD+ ADP-ribosyltransferase activity
19883896	53397.9	5.55	Arath	Oxygen-evolving enhancer protein 1-1	B: Photosynthesis C: Chloroplast
12323399	128658.4	4.97	Arath	P-loop containing nucleoside triphosphate hydrolases superfamily protein	B: Protein modification C: Nucleus M: Nucleotide-binding
297330766	50007.9	6.08	Araiy	Phosphoglycerate kinase	B: Glycolysis M: Phosphoglycerate kinase activity.
297312819	35189.2	6.1	Araiy	Photosystem II oxygen-evolving complex protein 1	B: Photosynthesis C: Chloroplast M: Calcium ion binding

Accession	MW(Da)	pI	Homology	Protein Name	GO
15232249	28802.9	5.62	Araiy	Photosystem II light-harvesting complex protein 2.3	B: Cellular response to water deprivation C: Golgi apparatus M: Chlorophyll binding
297334193	39547.8	5.46	Araiy	Photosystem II protein D2	B: Electron transporter C: Chloroplast
297337134	28054	9.24	Araiy	Photosystem II subunit S	B: Cysteine biosynthesis C: Plastid thylakoid
15235490	23051.8	9.85	Arath	Photosystem I subunit L	B: Cellular cation homeostasis C: Chloroplast
297332701	82475.7	6.89	Ara	Photosystem I P700 chlorophyll a Apo protein A2	B: Chlorophyll biosynthesis C: Chloroplast M: Chlorophyll binding
297334183	56053.4	6.4	Araiy	Photosystem II 47kDa protein	B: Electron transport

Accession	MW(Da)	pI	Homology	Protein Name	GO
					C: Chloroplast M: Chlorophyll binding
15235478	15686.3	5.01	Arath	Photosystem II manganese-stabilizing protein (PsbO)	B: Photosynthesis C: Chloroplast M: Calcium ion binding
17380270	28007.9	9.25	Arath	Photosystem II 22 kDa protein	B: Photosystem II stabilization C: Chloroplast M: Xanthophyll binding
75163506	74006.6	8.17	Arath	Probable inactive receptor kinase At5g67200	B: Protein phosphorylation C: Plasma membrane ATP binding and protein kinase activity
75170207	38585.6	5.07	Arath	Probable UDP-arabinopyranose	B: Response to salt stress

Accession	MW(Da)	pI	Homology	Protein Name	GO
				mutase 5	M: Nucleic acid and zinc ion binding
1709740	72176	5.92	Arath	Poly [ADP-ribose] polymerase 2	B: Protein ADP-ribosylation C: Chloroplast
42570340	144479.7	8.25	Arath	Protein helicase in vascular tissue and tapetum	B: Cytokinesis M: ATP binding
334187718	110499	5.64	Arath	Protein embryo defective 2247_AT5G16715	B: C: M:
42572779	40737.6	9.44	Arath	Protein FORKED 1	C: Cytoplasm M: Actin binding
297334210	83199.1	6.6	Araiy	psi P700 Apo protein A1	B: Electron transport C: Thylakoid
297332727	51868.1	6.7	Araiy	PS II 43kDa protein	B: Electron transporter

Accession	MW(Da)	pI	Homology	Protein Name	GO
					C: Membrane M: Chlorophyll binding
313471415	106995.7	5.61	Arath	Pumilio homolog 5	B: Translation Regulation C: Cytoplasm M: RNA binding
75168940	96105.5	6.96	Arath	Pumilio homolog 6	B: Translation regulation C: Chloroplast
75182934	29474.5	9.2	Arath	Putative cysteine-rich repeat secretory protein 61	C: Chloroplast M: mRNA binding
42558968	12664.6	9.14	Arath	Putative uncharacterized mitochondrial protein AtMg00280	B: Carbon fixation C: Chloroplast M: ATP binding
3914541	52955.3	5.88	Arath	Ribulose biphosphate carboxylase	B: Carbon fixation

Accession	MW(Da)	pI	Homology	Protein Name	GO
				large chain	C: Membrane
15229244	52434.6	5.53	Arath/Araiy	RING/FYVE/PHD zinc finger-containing protein	B: Transferring phosphorus-containing groups M: DNA binding
79314769	27748.6	8.94	Arath	RNA recognition motif-containing protein	B: RNA processing C: Chloroplast M: RNA binding
297337086	47782.9	8.99	Arath	Serine hydroxymethyltransferase	B: L-serine metabolic process C: Mitochondrion M: Pyridoxal phosphate binding
544602156	158724.2	5.76	Arath	SNF2 domain-containing protein CLASSY 3	M: ATP binding
79587640	100474.8	7.25	Arath	Transducin/WD40 domain-containing	C: Cell wall

Accession	MW(Da)	pI	Homology	Protein Name	GO
				protein-like protein	M: Protein binding
7329685	81475.4	5.8	Arath	Transketolase	B: Acetyl-coA metabolic process C: Chloroplast. M: Transketolase activity
297322418	79851.5	5.85	Araiy	Transketolase	B: Acetyl-coA metabolic process C: Chloroplast M: Metal ion binding and transketolase activity
8778823	107175.8	9.1	Arath	Translation elongation factor eEF-1 alpha chain	B: Protein biosynthesis M: Translation elongation factor activity
13431953	33345.9	5.39	Araiy	Triosephosphate isomerase	B: Golgi organization C: Chloroplast

Accession	MW(Da)	pI	Homology	Protein Name	GO
13432260	27169.2	5.39	Arath	Triosephosphate isomerase	M: Catalytic activity B: Golgi organization C: Cell wall
75170045	51654.2	8.22	Arath	Tryptophan aminotransferase-related protein 3	M: Copper ion binding B: Auxin biosynthesis C: Extracellular region M: Catalytic and pyridoxal phosphate binding
297335760	53397.9	6.45	Arath	Tyrosyl-tRNA synthetase - like	B: Chloroplast organization C: Chloroplast M: ATP binding
75172681	80713.7	5.38	Arath	Vacuolar protein sorting-associated protein 52 B	C: Chloroplast M: NAD binding

Accession	MW(Da)	pI	Homology	Protein Name	GO
332641995	40934.4	6.91	Arath	2-Cys peroxiredoxin (2-Cys PrxA)	B: Cuticle development
					C: Chloroplast
14916972	29092.2	6.91	Arath	2-Cys peroxiredoxin BAS1	C: Chloroplast
					M: Protein binding
1702987	30194.1	4.79	Arath	14-3-3-like protein GF14 phi	B: Response to cadmium ion
					C: Cytoplasm
					M: FK506 binding
110740990	36144.5	5.55	Arath	33 kDa polypeptide of oxygen-evolving	B: Photosynthesis
				complex	C: Chloroplast
73919362	61453.2	5.24	Arath	70 kDa peptidyl-prolyl isomerase ROF1	C: Chloroplast
					M: Phosphatidylinositol binding

4.4.2.2.1 Gene ontology and enrichment analysis. Gene ontology and enrichment platforms provide useful tools for the functional annotation of gene products. Gene products are categorized into groups to understand their roles in a living system. Protein interaction network (PPI) provides an additional tool that use statistical algorithms to validate peptide matches obtained using mass list data. One of the most popular tools for GO is the QuickGO server (https://www.ebi.ac.uk/QuickGO/). It builds a relationship tree by grouping individual proteins into known GO terms from curated databases (e.g. Uniprot), according to their molecular function, biological process, and cellular component. In this subsection, we present protein interaction network (Fig. 24) using STRING via https://string-db.org/, gene enrichment analysis based on molecular function, cellular process and cellular component (Fig. 25) from Uniprot database via https://www.uniprot.org/, and generated GO trees using QuickGO server (https://www.ebi.ac.uk/QuickGO/) (Appendix B). Protein interaction network or PPI enrichment reveal a significant interaction between proteins ($P \leq 0.01$) (Fig. 24). It indicates that the proteins have more interaction than would be expected for a random set of proteins (Fig. 24a), and that binding and catalytic networks dominates (Fig. 24b). With respect to the overall enrichment analysis (Fig. 25), though other biological processes and cellular component GO are important, the molecular function, which is the most critical to the objectives of this study showed that binding function dominates with 79 proteins, followed by, catalytic (66), transporter (7) and antioxidant activities (6) (Fig. 25a).

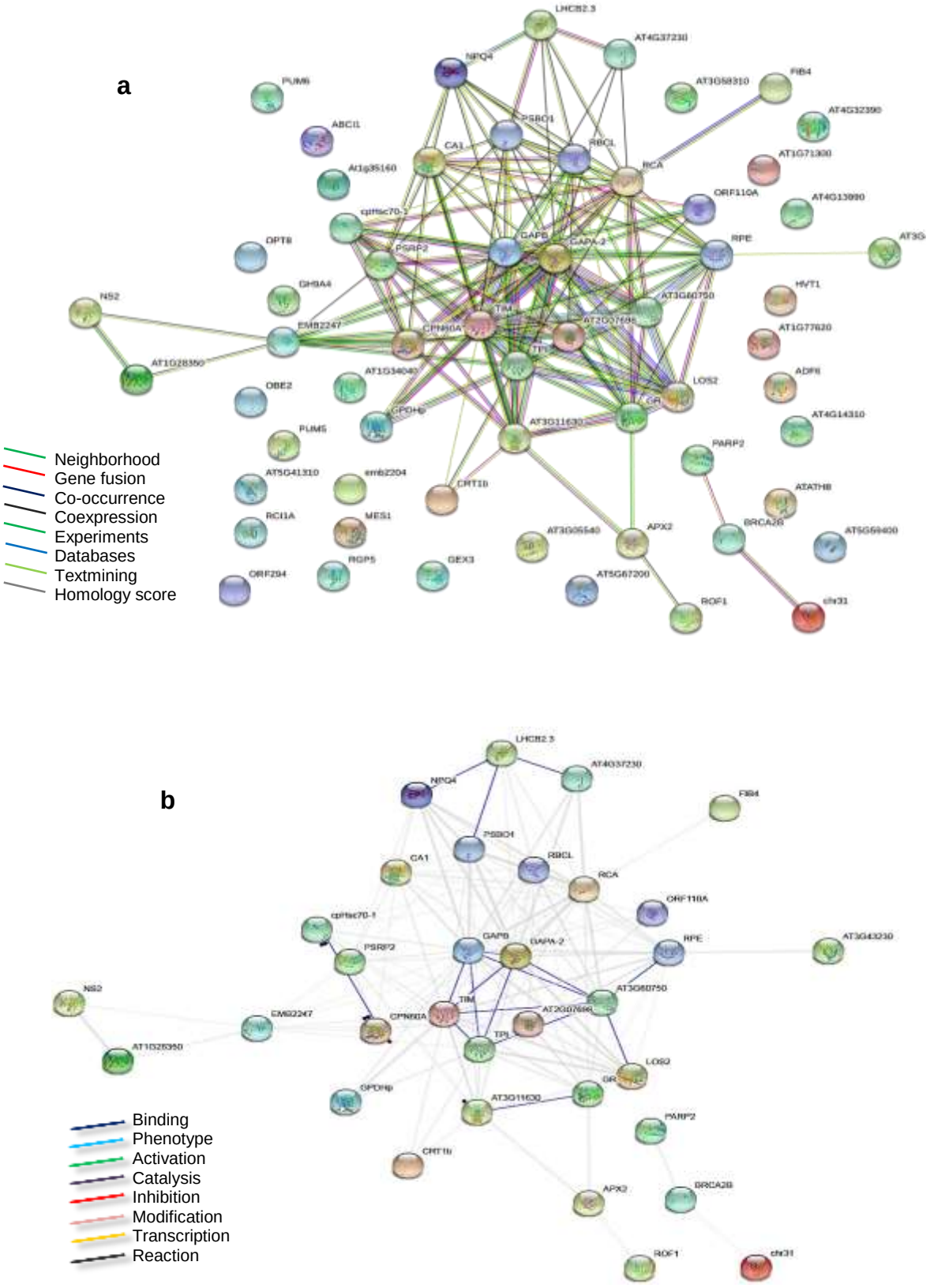


Figure 24. Protein interaction network (PPI) **(a)** Interaction evidence **(b)** Molecular action. Analysis performed using STRING (https://string-db.org/cgi/network). PPS enrichment is significant (P ≤ 0.01).

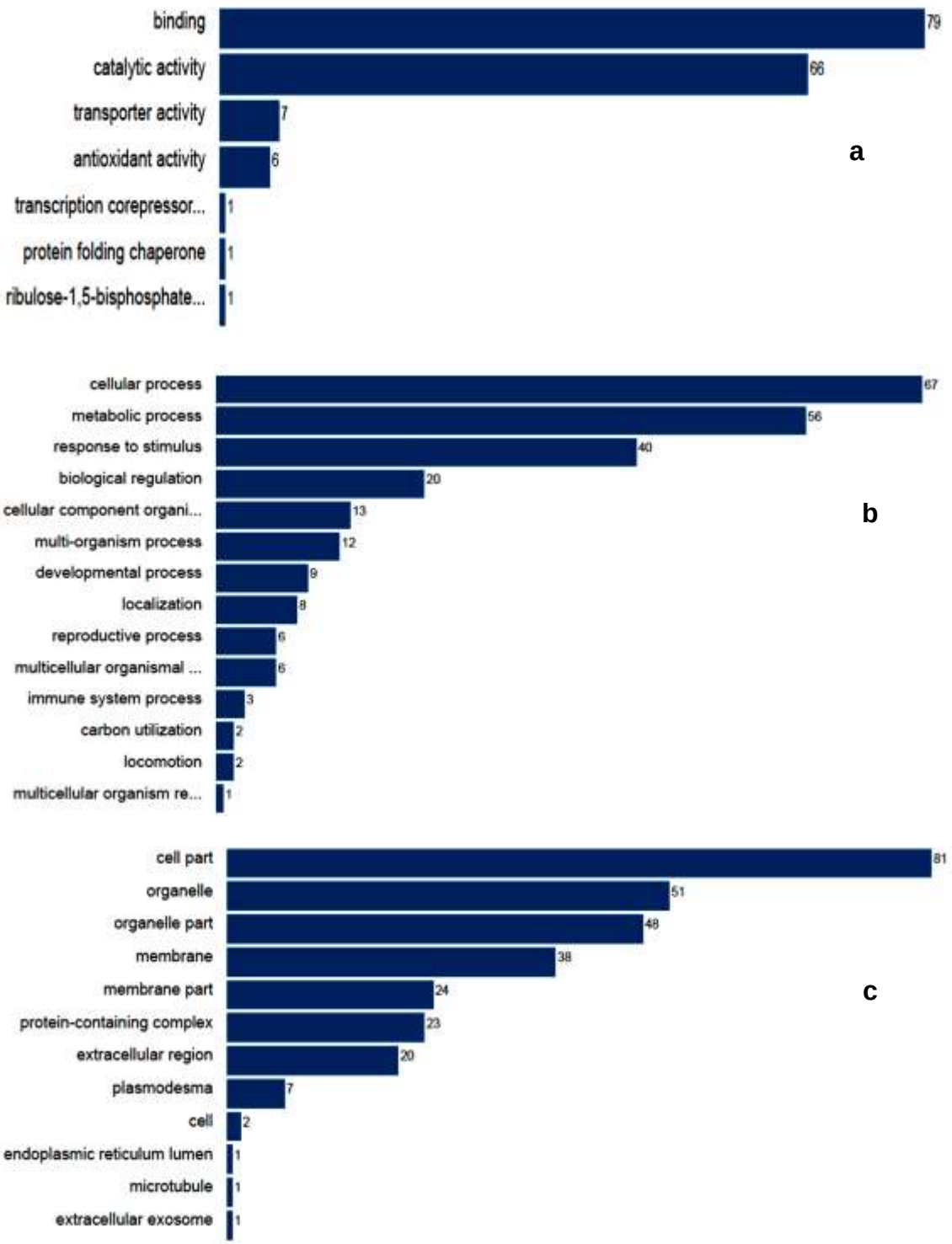


Figure 25. Gene enrichment analysis **(a)** Molecular function **(b)** Biological process and **(c)** Cellular component. All enrichment analysis was performed via Uniprot (https://www.uniprot.org).

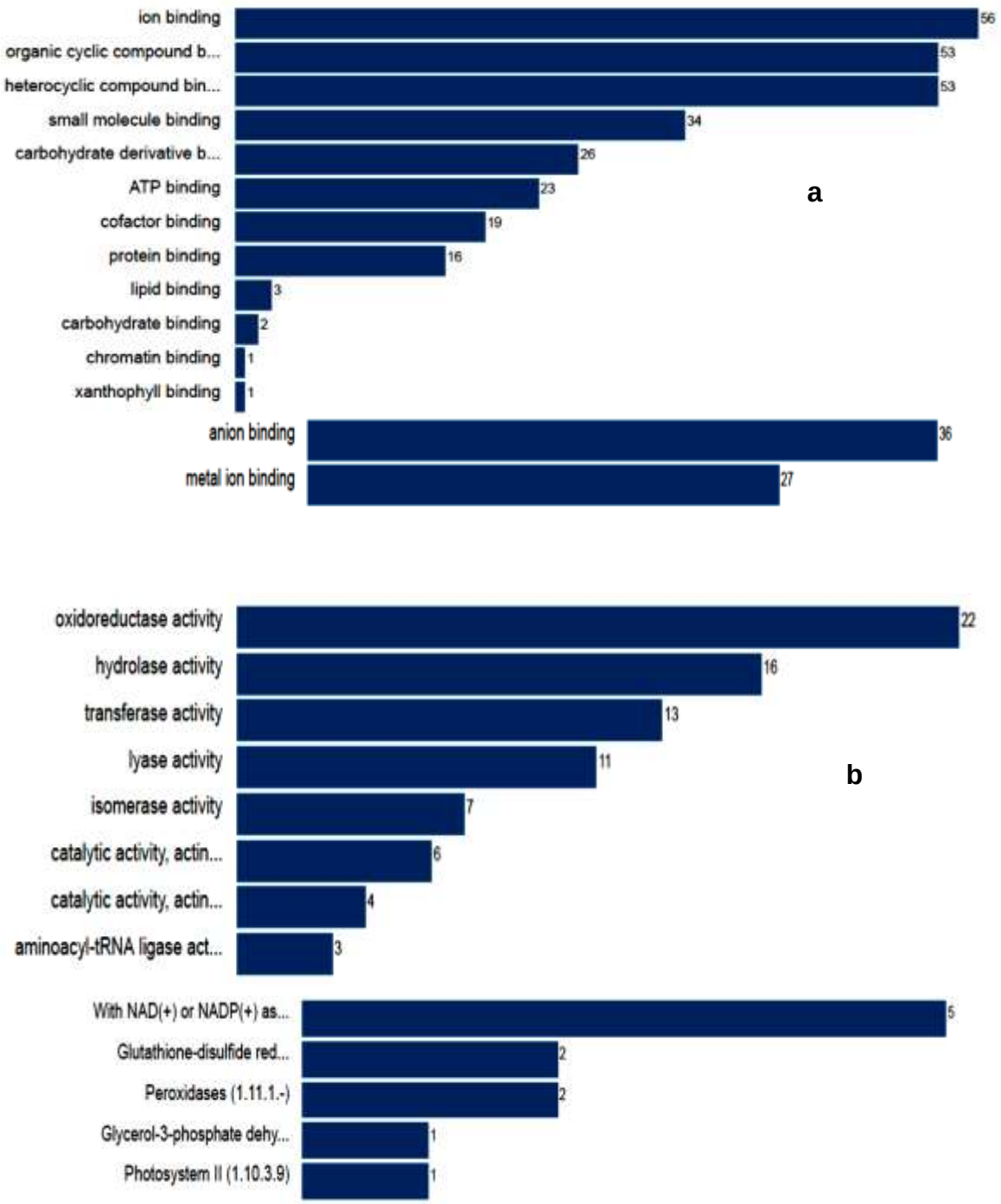


Figure 26. Molecular GO **(a)** shows major binding functions is to anions and metals, while **(b)** show oxidoreductase activity oxidoreductase dominance in catalytic function.

It is interesting to note that considering the binding annotation, all enriched proteins demonstrate ionic and anionic binding functions (Fig. 26a), indicating that identified proteins constitutes probable Pb binding protein domains.

Meanwhile, oxidoreductases tops in catalytic binding function, which is also majorly composed of glutathione di-sulfide reductase and peroxides (Fig. 26b), suggesting the roles of antioxidative enzymes.

Further, pathways enrichment analysis obtained from model plant pathways database, “Plant Reactome” accessible via https://plantreactome.gramene.org/ (Table 9) showed the enrichment of metabolic processes, regulation of HSF1-mediated heat shock response, cellular responses to stress, HSF1 activation and glutathione metabolism, all of which have documented evidence in regulating tolerance to heavy metal stress in plants (Kumar and Prasad, 2018). So far, proteomic analysis is suggesting that gene coded metal-binding proteins (metallothioneins) and antioxidative system play critical roles in Pb tolerance and bioaccumulation in T. qataranse. However, to conclude the mechanism, it is important to show the activities of key antioxidant enzymes involved in GST-ROS mediated Pb detoxification.

Meanwhile, as earlier observed, our enrichment analysis reports no known function of all the identified proteins with unknown functions (AXX17_AT2G26660, AXX17_AT4G36160, AXX17_AT2G13500, AXX17_AT5G33340, AXX17_AT5G16980, and AXX17_AT1G74880), suggesting their probable novelty. These proteins were obtained from ~52 and ~49 kDa MW proteins on the gel. The overplayed spectra of mass lists corresponding to the ~52 and ~49 kDa MW proteins for treatment (T52 and 49) and control (C52 and 49) are shown in Fig. 27. The dominance of binding and redox related proteins from gene enrichment analysis of the total proteome is an indication that the proteins belong to the stress response glycine-rich proteins (GRPs) family or metallothioneins, and potentially chelates Pb ions.

The repeated glycine residues e.g., (K)GGSGSGGGKGGGGGGSGGGR(G) for protein AXX17_AT2G26660 (Appendix) strongly supports such conclusion. GRPs are involved in cellular response to stress and are characterized by high glycine content and the presence of conserved segments including glycine-containing structural motifs (Czolpinska and Rurek, 2018). To explore their role in Pb tolerance, a sample in silico modeling of protein AXX17_AT2G26660 was performed using I-TASSER Server, of the Department of Computational Medicine and Bioinformatics, University of Michigan (http://zhanglab.ccmb.med.umich.edu/I-TASSER), and the result confirmed it to be involved in ion binding.

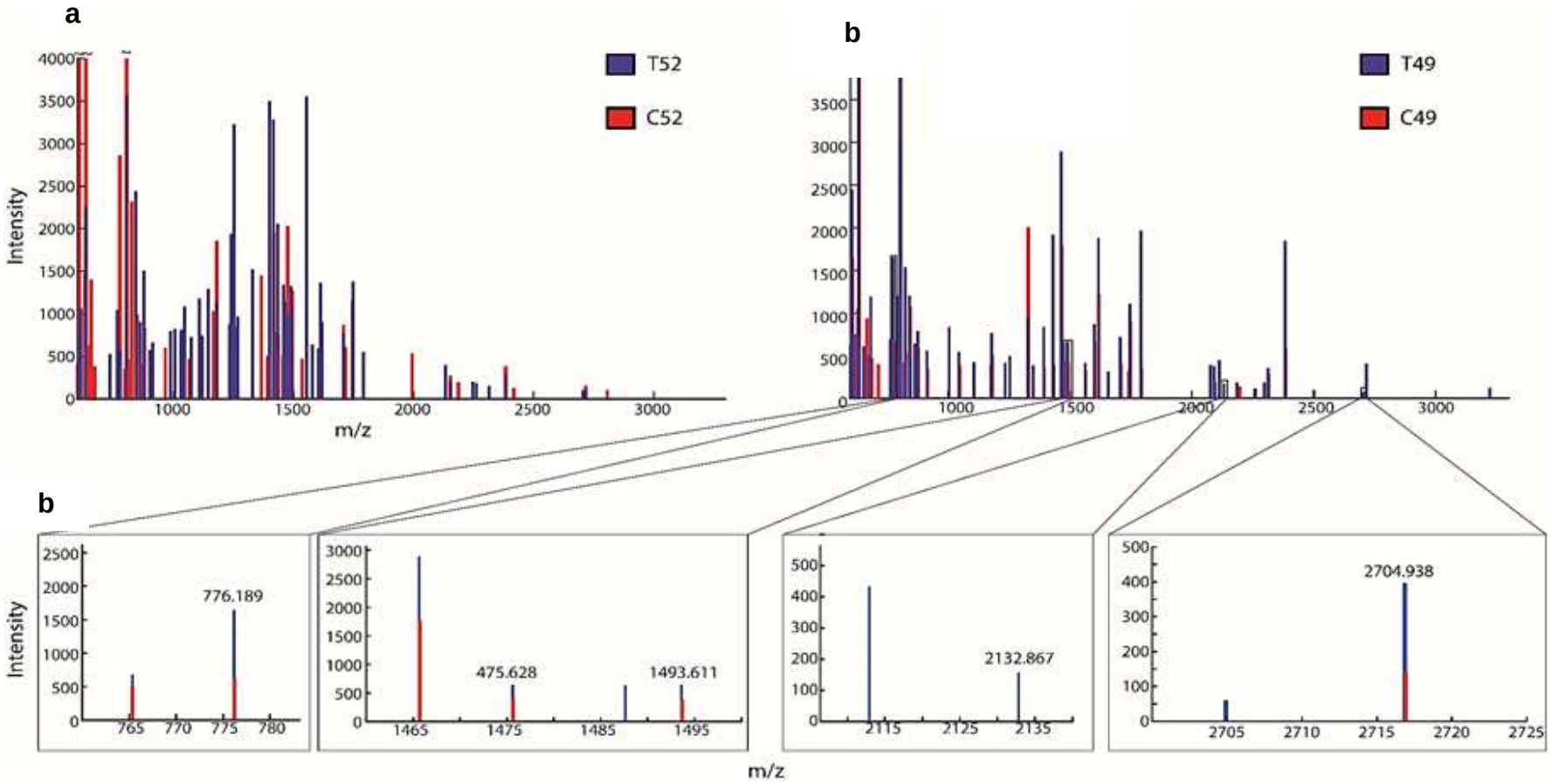


Figure 27. Overplayed MALDI-TOF/TOF mass lists spectra obtained from **(a)** ~52 kDa MW and **(b)** ~49 kDa MW proteins, and (c) mass lists corresponding to protein AXX17_AT2G26660. T = Treatment; C = Control.

Table 9. Enriched Pathways based on Reactome Plant Pathways Database (http://plantreactome.gramene.org/PathwayBrowser/)

Term ID	Term description	Gene count	False discovery rate	Matching proteins in your network (IDs)
ATH-5628897	TP53 Regulates Metabolic Genes	2	0.0049	AT3G11630.1, AT3G54660.1
ATH-70171	Glycolysis	3	0.0049	AT2G21170.1, AT2G36530.1, AT3G55440.1
ATH-70263	Gluconeogenesis	3	0.0049	AT2G21170.1, AT2G36530.1, AT3G55440.1
ATH-71387	Metabolism of carbohydrates	4	0.0049	AT2G21170.1, AT2G36530.1,AT3G55440.1, AT3G60750.1
ATH-1445148	Translocation of SLC2A4 (GLUT4)	2	0.005	AT1G35160.2, AT5G38480.1

Term ID	Term description	Gene count	False discovery rate	Matching proteins in your network (IDs)
	to the plasma membrane			
ATH-3371511	HSF1 activation	2	0.0183	AT1G35160.2,AT5G38480.1
ATH-2262752	Cellular responses to stress	3	0.048	AT1G35160.2,AT3G54660.1,AT5G38480.1
ATH-3371453	Regulation of HSF1-mediated heat shock response	2	0.048	AT1G35160.2,AT5G38480.1
ATH-00480	Glutathione metabolism	2	0.0467	AT3G09640.1,AT3G54660.1

4.4.2.2.1 The antioxidant enzymes activities. The activities of antioxidant enzymes measure plants potentials towards oxidative stress. Prominent among such enzymes are superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione reductase (GR), all of which counteract ROS effects (Shakoor et al., 2014b). SOD is the first line of defense; it sequesters noxious superoxide ions and breaks it into less harmful hydrogen peroxide and oxygen molecules. CAT, APX, and GPX assist subsequent detoxification steps. GR combats oxidative stress by balancing reduced (GSH) and oxidized glutathione (GSSG) (Hasanuzzaman et al., 2018; Sidhu et al., 2016).

In the preceding subsection, gene enrichment analysis indicates the strong presence of antioxidant proteins. In order to support previous proteomic data in elucidating the mechanism of Pb tolerance and detoxification, the activities of key antioxidants enzymes are evaluated and the results are shown in Fig. 28 Our results showed that the activities of antioxidant enzymes exhibited a concentration-dependent increase in Pb treated *T. qataranse tissues* compared to the control (Fig. 4a, b, c). This agrees with the findings of Sidhu et al., 2016, where a similar pattern was observed in Pb treated *C. didymus*. The literature is filled with abundant examples of such (Kumar and Prasad, 2018). ROS play a critical role in Redox signaling and is essential for cellular homeostasis. Pb disrupts such balance by replacing essential cations and altering metal-containing enzyme activity. The main sources of ROS are chloroplasts, mitochondria, and peroxisomes. Pb toxicity alters the electron transport chains, which increases ROS accumulation. Nearly every stage of the central dogma (DNA, RNA, protein) is affected by Pb toxicity (Nouet et al.,

2011).

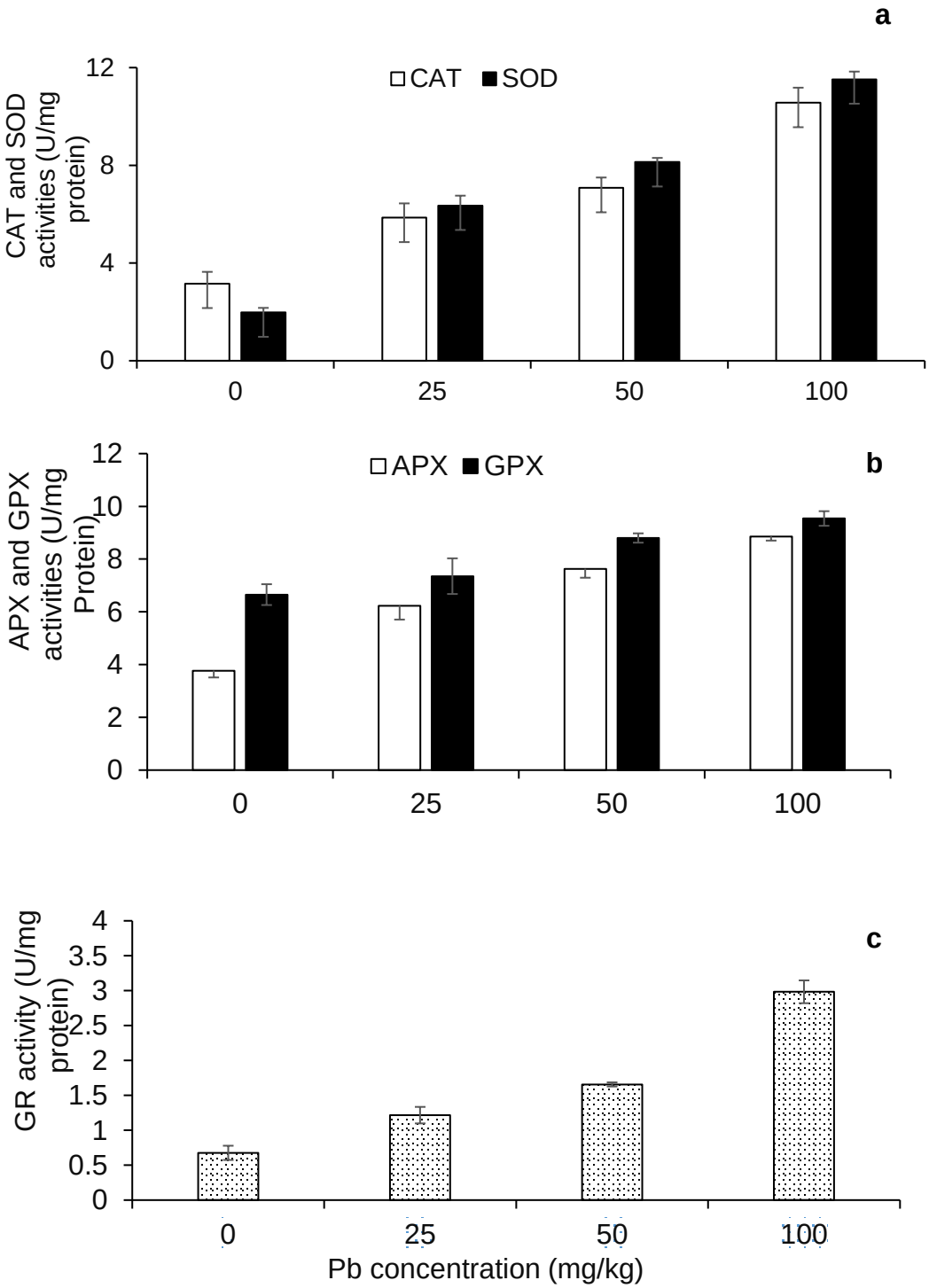


Figure 28. Antioxidant enzymes **(a)** CAT and SOD **(b)** APX and GPX, and **(c)** GR activities. Means represent the average of three replicates (n=3) ± SEM and are significantly different at P<0.05 level (ANOVA-TUKEY).

4.4 Further Discussion and Perspectives

Although few studies are available on soil heavy metal pollution in the state, Peng et al. (2016) noted the spatial distribution of Cr, Cu, Ni and Pb on Qatari soil. The effects of toxic metals accumulation in the soil are not limited to inland environment and biota. Subject to their properties, metals travel across the soil environment, and subsequently deposits into water bodies. In Qatar, desalination is the main source of household water supply. A recent survey of household water quality in some Doha residences by Nriagu et al. (2018) found the concentration of some toxic metals (Cd, Cr, Ni, and Pb) to be significantly lower than the set limits for water quality in the state. Transport, accumulation, and bioconcentration of toxic metals in the sea, presents a potential health risk to both humans, and aquatic life. Several factors including soil pH, organic, metal type, concentrations in the soil, plant type and age affects plant metal uptake (Abu-Dieyeh et al., 2018; da Conceição Gomes et al., 2016; Maiti and Jaiswal, 2008; Sharma and Pandey, 2014; Zeng et al., 2011).

Although Ba is the metal with the highest concentration (144.8 mg/kg) in the soil at Ras Laffan (Fig. 5a), however, poor accumulation in the plant tissue parts was observed, particularly in the shoot, where only 2.9 mg/kg translocated. These could partly be due to the metal unavailability for *T. qataranse* uptake. Consistent with these findings, Kowalska et al. (2012) found that less than 1% of Ba was bioavailable for plant uptake from the soil with 300 mg/kg Ba concentration. Although some plants, including *T. domingensis* and *C. papyrus* tolerate Ba at low to medium concentration. It is toxic to plants at high concentration. Few known plants including *I. cordifolia* and *V. densiflora* (Kamachi et al., 2015) can tolerate relatively high concentrations. Information

on Ba toxicity to humans remains to be adequately established. However, Kowalska et al. (2012) noted high mortality rate of people aged 65 in communities with elevated Ba concentration, due to high cardiovascular and heart disease incidence (de Castro Ribeiro et al., 2018; Kamachi et al., 2015).

Cr records a higher concentration in the soil after Ba, at 24.1 mg/kg (Fig. 5a). Although undetected in the shoot, it preferentially accumulates in the root, with a total concentration of 36.6 mg/kg. Cr rarely occurs naturally, and only exist in trace amount even when produced by anthropogenic activities (Zayed et al., 1998). Despite accumulation in the root, limited translocation to the shoot is expected, irrespective of its available form. The soil at Ras Laffan is alkaline at 8.05, and therefore likely to have more of bioavailable Cr^{6+}. Cr^{6+} is highly poisonous with strong oxidation potential, more mobile than Cr^{3+} and mostly associated with oxygen as chromate or dichromate ions. In plants, it easily translocates to the aerial parts than Cr^{3+}, due to its water solubility, ability to penetrate complex physiological barriers and transformation capacity. The most common form of naturally occurring Cr is Cr^{3+}, which complexes with organic matter in the soil environment and largely innocuous. It exists as either chromic oxides (Cr_2O_3), hydroxides (Cr (OH_3)) or sulfates ($Cr_2 (SO_4)_3.12(H_2O)$. Cr^{3+} has a higher affinity to cation exchange sites of the cell wall, and hence less likely to move across plant tissues in substantial quantity. (Sinha et al., 2018)

With Pb, both root and shoot accumulates lower concentrations of 2.5 and 2.3 mg/kg, respectively, compared to the soil 12.2 mg/kg (Fig. 5a). Although Pb does translocate to the aerial part of plants, several studies found that it preferentially accumulates in the root (Kushwaha et al., 2018; Pourrut et al.,

2011). Translocation of metal to the aerial parts in plants is restricted in some species including Pb, courtesy of many factors noted above. Another limiting factor for Pb is the complex transport mechanisms involved. Regulation begins when the metal enters the root using apoplast via water streams and into the inner endodermis region. In the course of transport, negatively charged molecules in the cell wall such as pectin can immobilize Pb ions; others are plasma membrane accumulation or precipitation of Pb insoluble salts. Even more convincing is the fact that, since Pb may be trapped in the endodermis by the Casparian strip, it can resort to symplastic transport by which most of the isolated Pb is excreted out of the plant (Kushwaha et al., 2018).

On the other hand, Cu and Ni exhibit similar accumulation pattern. They accumulate higher tissue concentration relative to that of the soil, respectively (Fig. 5a). Additionally, both translocate even higher concentrations to the shoot than the soil, suggesting *T. qataranse* phytoextraction potential. Of all the metals reported in this study, Ni showed higher accumulation in *T. qataranse* tissues, with a concentration of 63.3 and 17.4 mg/kg in the root and shoot, respectively. Although it occurs in several forms, under natural environmental conditions, Ni is most commonly present in the oxidized state as N^{2+}. Unlike Cd and Pb for instance, it is highly mobile and therefore easily transported from the soil unto the root and across other tissue parts. An extensive review of Ni accumulation and transport in plants, Amari et al. (2017) noted several studies in which many plants demonstrate the capacity to uptake Ni^{2+} to different tissue parts, particularly at an alkaline pH level of 8 and above. Another important factor in plant metal uptake is the soil organic matter.

Interestingly, plants root system influences soil organic matter, which in turn determines how it affects the mobility of the surrounding metals. In a recent study, Nguyen et al. (2017) found a positive relationship between Ni and Cu availability and organic content level in plants rhizosphere. Cu is essential to plants growth and development. However, it can be toxic at a concentration of more than 20 mg/kg (Nguyen et al., 2017). The same study found *P. arundinacea*, *T. repens* and *P. virgatum* to accumulate a higher concentration of Cu at 55.8, 41.8 and 29.4 mg/kg, respectively.

As regards to Cd, it is the least concentrated in the soil at 0.2 mg/kg. However, *T. qataranse* root and shoot Cd accumulation are higher relative to that of the soil with 0.4 and 0.5 mg/kg, respectively. Although not covered here, Fe and Zn are two elements known to have adverse effects on Cd uptake (Hart et al., 2005). Zn inhibits Cd uptake by its higher affinity to a common transporter molecule across the root plasma membrane (Yang et al., 2004). In both *T. qataranse* root and shoot, Cd concentrations are higher than that of the soil. An explanation to this could be that, either Fe or Zn was not available in considerable amount to deter Cd uptake, or their inhibition effect suppressed by other metals present. Equally important is the soil pH, which is one of the most critical factors affecting metal bioavailability in the soil. Many studies reported that Cd becomes less bioavailable in the soil with an increase in pH level (Adams et al., 2004; Tudoreanu and Phillips, 2004). The soil at Ras Laffan is alkaline, with a pH level of 8.05 ± 0.12, and may be responsible for the low bioavailable Cd in the soil. Indeed, several studies reviewed by Kirkham (2006) suggest pH level above 7.0 significantly reduce Cd bioavailability.

Correlation analysis between metals concentration in the soil, and *T. qataranse* root and shoot indicates a significant positive correlation between Ni concentration in the soil and that of the root part ($r = 0.96$, $p < 0.05$) (Table 4), implying that the root easily accumulates Ni. There is a highly significant, but negative correlation between the soil and shoot against Ba ($r = -0.98$, $p < 0.05$) and Pb concentration ($r = -1.00$, $p < 0.05$), respectively. The same significant correlation between metals concentration in *T. qataranse* root and shoot also exist (Table 5). There is a significant positive relationship between Pb concentration in the root against Ni concentration in the shoot ($r = 0.98$, $p < 0.05$).

Contrarily, similar but negative correlation coefficient ($r = -0.98$, $p < 0.05$) exist between Ba concentration in the root against Ni shoot concentration. It is a known fact that certain elements like Cu^{2+} are essential to the metabolic process in plants, but toxic when present in excess; it is, however, less toxic compared to non-essential elements like Cd and Pb (Kučera et al., 2008). There is no correlation between Cu concentrations in the soil and *T. qataranse*. A highly significant but negative correlation exists between root Cu concentration against Ba in the shoot ($r = -1.00$, $p < 0.05$). The same observation is made between Cr concentrations in the root and Cu ($r = -0.99$, $p < 0.05$), and Cd ($r = -1.00$, $p < 0.05$) concentrations in the shoot. BCF values indicate that the root accumulates more metals (Cd, Cr, Cu and Ni) than the shoot (Cd and Cu) (Fig. 9a). The translocation factor of these metals (Fig. 6a) showed that only Cd was transferred into the shoot. Indeed, numerous plants are known for reduced metal uptake to aerial parts, which preferentially accumulate in the root (Pourrut

et al., 2011). The BCF and TF suggest that *T. qataranse* is tolerant to Cd, Cr, Cu, and Ni.

Under Pb stress, the growth of plants is severely decreased. This decrease in growth potential is proportional to the concentration of lead in which the plant is exposed. All parts of the plant, including root elongation, plant height, number and structure of leaves is affected by phytotoxicity. In the present work, our controlled phytoremediation experiment involving Pb showed that it stimulates growth and accumulates in high concentration in *T. qataranse.* Though Pb toxicity symptoms, such as leaf chlorosis and root darkening were not apparent across all treatments. However, reduced total chlorophyll and shorter root length under 100 mg/kg treatment were observed. The root plays a vital role in plant health and development, which influences the response of other tissues to stress conditions. Indeed, Pb accumulation in plants raises chlorophyllase level, an enzyme which negatively affects chlorophyll (Hu et al., 2012). It slows down photosynthesis, and therefore, affects general growth and development. Consequently, cell division will also be negatively affected due to slow metabolic activities; thereby inhibiting normal growth.

Two common mechanisms by which plants deal with trace metals are avoidance and uptake. Metal avoidance can involve the active efflux of metal, metal precipitation, and redox barrier. Contrary to this, metal uptake and accumulation involves three main steps: uptake, transportation, and compartmentalization. Metals uptake involves selective mechanisms in response to concentration gradients (Kumar and Prasad, 2018). Before compartmentalization, Pb is translocated to the degree that can be described by the TF (Bhatti et al., 2018). The translocation factor is an important

parameter in understanding Pb transport within plant tissues and is essentially the ratio between Pb concentration in above the ground biomass to that of the roots. Usually, the translocation factor is very low because Pb accumulates more in the roots and only small amounts translocate to the shoot (Chandra et al., 2018). In the roots, precipitated Pb concentrates in the cell wall, while free Pb is transported to other parts of the cell. The transfer of Pb from the roots to other parts of the plants is thought to be via the xylem and phloem cells (Rascio and Navari-Izzo, 2011).

With regards to Pb accumulation in *T. qataranse* tissues, our result is also consistent with the findings of Langley-Turnbaugh and Belanger (2010). Further, Kumar et al. (2017a); Pourrut et al. (2011) reported several other studies to support our findings in separate critical reviews of Pb toxicity to plants. Other known plants, such as *Nerium oleander* L. and *Brassica juncea* accumulates higher concentration of Pb in the root (Manousaki and Kalogerakis, 2009). Although there are some exceptions (Bi et al 2009), Pb levels can accumulate differently in different parts of plants. It accumulates higher in the root than stem or the leaves. Finster et al. (2004) determined that among different plants studied, the roots always accumulated more Pb and the fruits of the plant had only trace level of Pb. Several factors that contribute to Pb restriction to the root such as precipitation, binding, and restriction by the endoderm were observed (Arias et al., 2010; Pourrut et al., 2011). The ability of *T. qataranse* to accumulates more than 1000 mg/kg Pb suggests that it is Pb hyperaccumulator (van der Ent et al., 2013), and therefore suitable for the metal phytostabilization in polluted areas. The BCF and TF values indicates that *T. qataranse* sequestrate Pb from a medium containing up to 100 mg/kg

Pb. The plant's ability to tolerate such degree of Pb toxicity suggest the existence of an efficient response mechanism to deal with Pb stress.

In the present work, FTIR results confirm metals adsorption unto *T. qataranse* tissues. With reference to Fig. 20, SoC showed a band at 3395.28 cm^{-1}, which is higher than that of SoT at 3370.51 cm^{-1} (Fig. 20a). Similar trends were also observed for RoC at 3359.91 cm^{-1} shifting to 3285.94 cm^{-1} for RoT (Fig. 20b), from 3331.15 cm^{-1} for ShC to 3294.87 cm^{-1} for ShT (Fig. 20c), and finally, RoC at 3294.87 cm^{-1} decreasing to 3285.94 for RoT cm^{-1} (Fig. 20d). These band shifts are due to $Cd^{2+,}$ Cr^{2+}, Cu^{2+}, or Ni^{2+} cationic interaction with the hydroxyl group for metal-oxygen binding. D'Souza et al. (2008) noted a similar trend when *P. tetrastromatica* was treated with and without Cd. A similar pattern was observed by Panda et al. (2007) following Cd and Ni adsorption by *L. sativus* biomass, and conclude that this binding interaction is between amide group and Ni^{2+} via nitrogen atom. Consistent with Al-Ghouti et al. (2010), the relatively higher band shift in RoT to 1615.61 from 1622.12 cm^{-1} for RoC (Fig. 20b) is due to Cu^{2+} binding to a lignocellulose material in *T. qataranse* root biomass. There were sharp decrease in bands intensity for SoC at 1015.02 lowering to 986.47 cm^{-1} for SoT (Fig. 20a), RoC at 1076.48 to 1030.23 cm^{-1} for RoT (Fig. 20b) and ShC at 1075.25 to 1030.23 cm-1 for ShT (Fig. 20c). However, a narrower drop in intensity from 1031.84 to 1031.24 cm^{-1} for RoT against ShT (Fig. 20d) is observed. Both indicate shifts can be attributed to strong metal binding involving Cu^{2+}, Cd^{2+} or Ni^{2+} (Sheng et al., 2004). Finally, with a consistent decrease in band intensity of RoT when compared to ShT (Fig. 20d), the FTIR further confirmed our ICP-OES result that *T. qataranse*

adsorb more metals (Cr, Cu, and Ni) in the root, with Cd further translocating to the shoot.

Binding interaction exists with amide, hydroxyl, phosphate, and carboxyl groups. The mechanism of heavy metals removal is mostly due to ion exchange via the carboxyl groups present on the plant's surface. For transition metals, such as Cd, Cr, Cu and Ni, interaction with plant biomass largely via amino sugars (Panda et al., 2007). Primarily, these sites are for H^+, Na^+, K^+, Ca^{2+}, Mg^{2+} and Fe^+ cations. However, in the presence of metals such as Cd^{2+}, Cr^{3+}, $Cu^{2+,}$ and Ni^{2+} there is the tendency for substitution between metals (Schneider et al., 2001). The affinity of different plant tissues towards specific metal ions depends on the available binding sites (Hawari and Mulligan, 2007).

Numerous plants are known for reduced metal uptake to aerial parts (Pourrut et al., 2011). The root has a vital role in plant growth and development, and therefore dictate other tissue response (Biernacki and Lovett-Doust, 2002). Under heavy metal stress in the soil, the root tissue suffers first exposure. The plant cell wall has a mechanism of exchange that fixes the heavy metal ions, thereby limiting transmission to other tissues (Allan and Jarrell, 1989). According to the Pearson classification, Cr, Cu and Ni are on the borderline of polarizable and non-polarizable metals, whereas Cd belongs to the polarizable or soft category (Sengupta, 2001). The translocation of Cd to the shoot may be due to its soft cationic nature. It is more likely to form stable complexes with like donors, the soft ligands, such as the amino and sulfhydryl groups. Whereas Cr, Cu and Ni affinity to root is due to their more stable complex formation with hard ligands; hydroxyl, carboxylate, carbonate, and phosphate groups. Considering Cd elemental properties and mechanism of uptake in plants, it is

readily bioavailable and efficiently translocate from the roots to other parts. Substantial evidence suggests that it enters the plant via essential elements (Ca, Fe and Zn) uptake system, and that even guard cell Ca^{2+} channels are permeable to Cd^{2+} (Perfus-Barbeoch et al., 2002). Dalton et al. (2005) refer to Cd^{2+} as "opportunistic hitchhiker."

Other metal properties presented in Table 10 contributes to their binding behavior and strength, which in turn influence the adsorption mechanism in plant biomass. The hydrated ion radius determines hydration effects, and it is important to note that, for all metal ions, the hydrated radius is larger than the crystal radius. Generally, an increase in hydrated radius indicates strong cationic hydration energy.

Table 10. Characteristics of Metal Binding Strength

Metal	**Charge, *z*[a]**	**Crystal radius, $r_{crystal}$ (A˚)**	**Hydrated ion radius, r_{hyd}[c] (A˚)**	**Pauling electronegativity[d]**	**Parameter for covalent binding, x^2 (r_{cryst} + 0.85)[e](A˚)**
Cu	2	0.73	4.19	2	6.41
Ni	2	0.69	4.04	1.8	5.73
Cd	2	0.95	4.26	1.7	5.51
Cr	3	0.75	4.13	1.6	4.10

a (Russell, 1980), b (Dean, 1985), c (Marcus and Kertes, 1969), d (Dean, 1985), e (Nieboer and McBryde, 1973)

Upon weak binding, hydration effect is most dominant and weakly hydrated larger ions preferentially accumulates in the interface (Russell, 1980). It shows that Ni^{2+} has the lowest hydration radius, while Cd has the highest hydration radius.

Although intra-particular mechanism may control adsorption, however, the structure and size of metal ions will most certainly influence ion mobility and consequently the adsorption mechanism. Hence, considering the ionic radius of Cd^{2+}, Cr^{3+}, Cu^{2+} and Ni^{2+} and other properties, their adsorption means may have evolved. Indeed, a similar observation was made by Hawari et al., it, therefore, make sense to assume that, Ni^{2+} easily migrate and adsorb onto *T. qataranse* biomass. High electronegativity increases metal ions adsorption capacity. Cu^{2+} has a comparatively higher electronegativity. Additionally, considering the parameter for covalent binding, which is a product of crystal radius and electronegativity Cu^{2+} has the highest value. These indicate that like Ni^{2+}, compared to other metals, Cu^{2+} also readily adsorb and has a strong binding strength to *T. qataranse* biomass.

Therefore, metals interaction with *T. qataranse* biomass is via carboxyl and amino group. Following cationic exchange for metals via the functional groups, alteration of lignin, cellulose and protein structural moieties leads to changes in the plant's growth pattern, photosynthetic activity and antioxidant system (Kumar et al., 2017b; Panda et al., 2007). Indeed, several studies reported single and combined effects of these metals (Cd, Cr, Cu, Ni and Pb) (Israr et al., 2011; Kutrowska et al., 2017; Rizwan et al., 2017; Shen et al., 2018) on photosynthetic activity, proline content and soluble sugars. Others are increased activities of total glutathione, ascorbate, peroxidase, superoxide

dismutase and catalase in plant species including *B. juncea, K. obovata, O. sativa, S. drummondii.* Therefore, *T. qataranse* response to Ba, Cd, Cr, Cu, Ni and Pb, and bioaccumulation pattern in this study is partly due to the metals antagonistic effects and antioxidant enzymatic activity.

Reactive oxygen species serve an important role in Redox signaling essential for cellular homeostasis. Pb disrupts this balance by replacing essential cations and altering metal-containing enzyme activity. Chloroplasts, mitochondria, and peroxisomes are the main sources of ROS. Pb toxicity changes electron transport rates and increases the generation of ROS. Nearly every stage of the central dogma (DNA, RNA, protein) is affected by Pb toxicity. Pb is a mutagen that could damage genetic material resulting in DNA strand-breakage, damage to the cytoskeleton and instability. Its genotoxic effects are largely due to the indirect or direct disruption of DNA and RNA via ROS. Some plants are more resistant to this damage, and such toxic potential is dependent on length of exposure and environmental condition. Proteins can be oxidized by the free radicals created during Pb stress. Free radicals interact with proteins and cause site-specific modifications and fragmentation via proteolysis. Some modifications are irreversible, but many stress-related proteins are synthesized to help the plant overcome the Pb stress.

Increased protein synthesis due to Pb stress in plants is one of the major cellular metabolic processes (Kohli et al., 2018). The mitogen-activated protein (MAP) kinase pathways (Fig. 29) regulate such processes, which serves as signaling system against oxidative stress (Mapanda et al., 2005). The signaling occurs through multiple stages of reaction, which modify gene expression and ultimately protein synthesis (Sidhu et al., 2016a). Studying the differential

expression pattern of such proteins provides insight into the mechanism of plant-metal interaction, which is useful in the development of transgenic species of plants with enhanced metal tolerance and detoxification system for phytoremediation. For instance, Wang et al. (2015) identified 16,246 uniquely expressed genes in *Platanus acerifolia* due to Pb exposure. Of the differentially identified unigenes, antioxidant proteins, metal chelators and transporters dominates. While glutathione and other metabolic pathways were found to play role in the defense and detoxification of Pb.

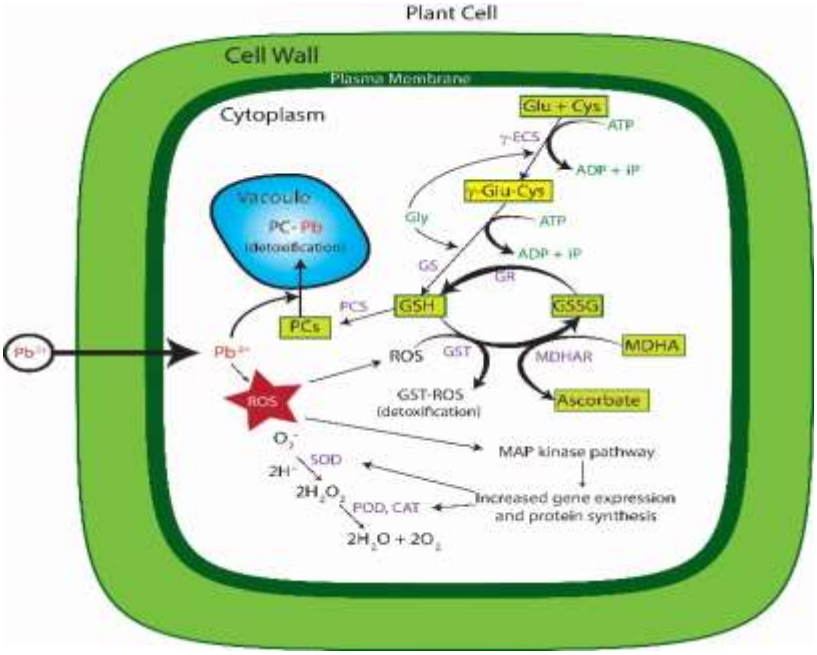


Figure 29. Pb tolerance mechanism in plants (Adapted from Kumar and Prasad, 2018).

Accordingly, in this study, protein identification and gene enrichment analysis reveal several differentially expressed molecules due to Pb stress. Of these, the majority are for binding, antioxidant activity, transport, and stress

response. Additionally, key stress-regulated metabolic pathways including glutathione metabolism, cellular response to stress and regulation of HSF1-mediated heat shock response among others were identified. Of the stress-responsive proteins, the heat shock proteins (HSPs) is one of the most abundant, suggesting it's key role in Pb detoxification. Indeed, HSPs induction has proven to play critical protective role, confer organisms with eco-physiological adaptation and genetically conserved response to environmental stress. In a similar study involving Pb exposed *Acalypha indica*, Venkatachalam et al. (2017) found differentially expressed proteins to contain heat shock proteins (HSP). These functions in plants defense against oxidative stress and maintain cellular homeostasis (Kumar and Majeti, 2014). Additionally, HSP is involved in translocation, degradation and prevents protein aggregation during transport in stressed environments (Wang et al., 2011). Furthermore, in a comparative proteomic study of Pb stress in a related halophyte *S. salsa*, Liu et al. (2016) reported significant differential expressions of proteins. Majority of the identified proteins are involved in defense related metabolic pathways. Some of the proteins include carbonic anhydrase, ribulose 1,5 bisphosphate, chlorophyll a-*b* binding protein and glutathione peroxidase, all of which were also identified in the present study.

In a critical review of Pb induced stress in plants, Kumar and Prasad (2018) showed the critical roles of ROS in the metal tolerance, uptake, and detoxification mechanism (Fig. 97). In addition to binding and stress response proteins, our proteomic data enrichment analysis showed major oxidoreductases. Accordingly, progressive increase in the activities of antioxidant enzymes was observed in the present study, which further supports

the result of our gene enrichment analysis and strongly indicates the critical role of the *T. qataranse* antioxidative system in Pb detoxification.

Changes in enzymatic activities account for the elimination of ROS and improvement of stress condition (Shakoor et al., 2014a). SOD prevents the accumulation of oxygen radicals through the formation of H_2O_2 and O_2. It initiates the reduction and formation of highly reactive hydroxyl radical in the presence of metals; high Pb concentration seems to lead to a reduction of redox-active enzymes. Peroxidases, which are involved in H_2O_2 signaling, can break H_2O_2 into H2O and O_2. APX is crucial to the Halliwell–Asada cycle. Increased APX enzyme activity often correlates to increased ascorbic acid, which promotes effective antioxidative mechanism (Potters et al., 2010).

Furthermore, genetic studies show that exposure to Pb increased antioxidative genes (e.g., APX) expression (Hattab et al., 2016). Further, enzymes involved in glutathione metabolism mediate metals detoxification (Anjum et al., 2012). Glutathione-S-transferases (GSTs) are a major phase II GSH-dependent ROS scavenging enzymes. They play important roles in GSH conjugation with exogenous and endogenous species found during oxidative stress, including H_2O_2 and lipid peroxides (Kumar and Majeti, 2014). Glutathione metabolism regulates the biosynthesis of phytochelatins (PC), which bind Pb and transports it to vacuoles where detoxification can occur. GSH and Phytochelatins (PCS) related genes are actively involved in GSH-dependent PCs synthesis (Fan et al., 2016). In this work, the enriched glutathione catalytic enzymes and metabolic pathway suggest PCs induction. The modelled protein AXX17_AT2G26660, which showed it to bind to ions, suggest that other hypothetical proteins with unknown functions also belongs

to the GRP's family , and therefore play similar role to PCs in Pb chelation in *T. qataranse*. Though GRPs have recently emerged as important biomarkers of stress, little information is available on their metal chelating capacity in plants (Czolpinska and Rurek, 2018). However, several studies on the proteomics of toxic metals including Pb reviewed in Jain et al. (2018); Jalmi et al. (2018) have demonstrated the role of other gene coded metal binding proteins such as metallothioneins in this regard.

CONCLUSION

Anthropogenic activities, mainly arising from industrialization, contributes to the continued accumulation of toxic heavy metals in the environment. Although background metal concentrations in the soil from the present study is low, however, it is a known fact that once deposited in the soil; metal species are transported and could persist for an extended period in different environmental compartments. Often, these pollutants end up in the sea, river, or a pond, thereby endangering aquatic and by extension, human life via the food chain. Therefore, the need for efficient remediation strategy, even for trace amounts is imperative. In this study, we provide evidence of some heavy metals contamination at Ras Laffan and Mesaieed industrial areas, and further show *T. qataranse*, an undershrub plant accumulates higher concentration of Cd, Cr, Cu and Ni than the soil. Results suggest that *T. qataranse* remediate Cd by phytoextraction, and Ba, Cr, Cu, Ni and Pb by phytostabilization. *T. qataranse* is edible to animals in an arid environment; its ability to stabilize toxic metals in the root and limited translocation to other plant parts restrict soil transport, prevent animal's ingestion and transmission across the food chain.

Additionally, we showed that Pb stimulates growth and accumulates in high concentration in *T. qataranse* and *P. juliflora* grew in controlled environments. The plants exhibited high Pb tolerance in the root and shoot, with preferential accumulation in the root. More than 1000 mg/kg Pb (Pb hyperaccumulation threshold) accumulate in the plant and high translocation across tissues observed. The BCF excellently reveal the capacity of the plant to be a hyperaccumulator, and hence suitable for Pb phytostabilization in the

metalliferous soil. FTIR demonstrate the adsorption of Pb ions onto the plant tissue biomass, and different functional groups e.g., carboxyl and amino group were associated with the metal adsorption. Increased protein synthesis due to Pb stress in plants is one of the most critical cellular metabolic processes. Antioxidant enzymes stimulate increased proteins synthesis and induce the accumulation of metal-binding proteins.

Evaluation of the activities of antioxidant enzymes (CAT, SOD, APX, GPX, and GR), and enrichment analysis of differentially expressed proteins due to Pb stress provides insight into the mechanism of Pb tolerance and uptake. The plant showed higher activities of these enzymes with increasing Pb concentration, suggesting the crucial role of the plant antioxidative system in scavenging ROS. A total of eighty-six (86) differentially expressed proteins, the majority of which functions in ion and protein binding, antioxidant activity, transport, and abiotic response stress. Essential stress regulating metabolic pathways, including glutathione metabolism, cellular response to stress, and regulation of HSF1-mediated heat shock response, were also enriched. Indeed, HSPs induction has proven to play critical protective role, confer organisms with eco-physiological adaptation and genetically conserved response to environmental stress. Further, enrichment analysis showed six (6) proteins with unknown function are potentially novel Pb chelators. Therefore, the antioxidative system increased the synthesis of stress-responsive proteins, and induction of known and unknown metal-binding proteins, phytochelatins (PCs) and glycine-rich proteins (GRPs), respectively, regulates Pb tolerance and detoxification in *T. qataranse*.

FUTURE WORK

According to the findings of this study, and the conclusions made. At least two immediate future works can be proposed. First, though the optimized protein extraction method is comparatively more efficient, it is rather long and laborious. Therefore, it will be of interest to modify existing protocols that are shorter with the same or better reproducibility for total protein extraction in recalcitrant tissues. Second, the novel glycine-rich proteins with predicted binding function can be functionally validated *in vivo* using Pb sensitive model plant, *Arabidopsis.* No doubt, such these steps will further our mechanistic understanding of the complex process, and contribute towards the development of transgenic plants with efficient metabolic machinery, for large scale and long-term phytoremediation of polluted soil, and water environments.

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Appendices

Appendix A. T. qataranse Identified Proteins and Their Peptide Sequences. Accession no was obtained from NCBI based on Search against Arabidopsis Database. MW = Molecular Weight; pI = Isoelectric Point; Araiy = Arabidopsis iyrata; Arath = Arabidopsis thaliana;

Accession	MW(Da)	Pi	Homology	Protein Name	Peptide Sequence(s)
17367307	16707.8	5.46	Arath	Actin-depolymerizing factor 6	(K)TTFLELQRK(K) (K)DIQPWQER(G)
297316109	70755.3	5.58	Arath	Adenosylhomocysteinase	(K)VIITAPGK(G) (R)LVGVSEETTTGVK(R) (K)IVLPVPAFNVINGGSHAGNK(L) (K)VLQDALTR(R)
20140328	71364.2	9	Arath	Asparagine--tRNA ligase	(R)VTTLLGKTDDK(E)
18404975	91170.2	9.38	Arath	ATH subfamily protein ATH8	(K)LINGIPDIEFTK(V)
14423416	63809.5	6.21	Arath	ATP synthase subunit beta-3	(R)APGFGER(K) (K)VVDLLAPYQR(G) (K)VAKGKSPR(K) (K)IGLFGGAGVGK(T)
5881679	55328.5	5.19	Arath	ATPase subunit	(R)VGSAAQIK(A) (K)SAPAFIQLDTK(L)

Accession	MW(Da)	Pi	Homology	Protein Name	Peptide Sequence(s)
15226092	85933.8	5.44	Arath	ATPase F1 complex alpha subunit protein	(K)LSIFETGIK(V) (R)EAYPGDVFYLHSR(L) (R)LIESPAPGIISR(R) (K)AVDSLVPIGR(G) (K)IINSDNVQEAAR(E) (R)VGSAAQIK(A)
297318710	43408.6	4.45	Arath	Calreticulin 2	(K)GGYYDFVK(G) (R)LVGQIAK(R) (K)LSEVESENRKMK(V)
3249100	50581.9	5.46	Arath	Carbonic anhydrase	(R)GGSTGYDNAVALPAGGR(G)
38503395	37450.1	5.74	Arath	Carbonic anhydrase	(K)GGYYDFVK(G)
21554572	62130.4	5.04	Arath	Chaperonin-60 alpha	(K)DIIPILEK(T) (K)VGAATETELEDR(K) (K)VVNDGVTIAR(A) (K)VGAATETELEDR(K) (K)DIIPILEK(T) (K)ILVTDQK(I)
115385	27733.7	6.22	Araiy	Chlorophyll a-b binding protein 4	(K)NPGSVNQDPIFK(Q) (K)NPGSVNQDPIFK(Q)

Accession	MW(Da)	Pi	Homology	Protein Name	Peptide Sequence(s)
116831194	28063.2	6.53	Araiy	Chlorophyll a/b-binding protein	(R)IPDLFK(D) (K)FGEAVWFK(A) (R)ELEVIHSR(W)
30697525	30008.9	8.24	Arath	D-ribulose-5-phosphate-3-epimerase	(R)VPDFIK(A)
15225693	25921.5	5.25	Arath	Dienelactone hydrolase domain-containing protein	(K)ALIPDLYR(G)
75161476	54120.1	9.07	Arath	Endoglucanase 16	(R)HVDHKYAR(R)
7433553	59304.7	5.89	Araiy	Enolase	(K)IVLPVPAFNVINGGSHAGNK(L) (K)VQIVGDDLLVTNPK(R)
10086473	51654.2	6.13	Araiy	Flavin-containing monoxygenase family protein	(K)ILQPVR(D) (R)IPDLFK(D)
297326214	43059.2	6.27	Araiy	Fructose biphosphate adolase	(R)FLAIDAVEK(A) (R)APWLEPLR(G) (R)ATPEQVAAYTLK(L) (R)STNLDWYK(G) (R)LASIGLENTEANR(Q) (R)LASIGLENTEANR(Q)

Accession	MW(Da)	Pi	Homology	Protein Name	Peptide Sequence(s)
297338561	42703.9	7.62	Araiy	GAPA	(K)LLSGDVDQK(K) (K)VIITAPGK(G)
8778823	107175.8	9.1	Arath	GTP binding Elongation factor Tu family protein	(R)STNLDWYK(G) (K)VGETVDLVGLR(E)
15222111	42847	8.16	Arath	Glyceraldehyde 3-phosphate dehydrogenase (GAPA2)	(K)IVDNETISVDGK(L) (R)AAALNIVPTSTGAAK(A) (K)AVSLVLPQLK(G) (R)IEFVR(L)
297326214	43059.2	6.18	Araiy	Glyceraldehyde-3-phosphate dehydrogenase	(K)KYPEEAAELK(S) (R)ALEAFR(L) (R)AAALNIVPTSTGAAK(A)
9758815	40548.1	8.79	Arath	Glycolate oxidase	(R)GLFIIDK(E) (R)ALSIVQGR(A)
591401946	60991.4	5.66	Arath	Glycosyltransferase, partial	(R)IAWDFR(R) (R)LDLAGR(D) (R)FTLATAGANQYEKTK(D)
297318892	71428.2	5.06	Araiy	Heat shock protein 70	(R)LVGQIAK(R) (R)IAGLEVLR(I) (R)TTPSVVAYTK(S) (R)TVVSIPNGPSALAVK(E)

Accession	MW(Da)	Pi	Homology	Protein Name	Peptide Sequence(s)
219766617	77106	5.13	Arath	Heat shock protein 70	(R)DSAAVFAWK(G) (K)AHGGVSVFGGVGER(T) (R)VEIIANDQGNR(T)
297328438	43331.5	8.63	Araiy	Kinase family protein	(R)LMASILFK(V) (R)IGLFEQQR(L) (R)IEFIR(R)
15237622	108115.3	8.77	Arath	Kinesin-like protein	(K)LNAQKAK(V) (R)EVAARAEEIR(R)
297322140	27214.2	5.88	Arath	L-ascorbate peroxidase 2	(R)NRELEVIHSR(W) (K)EGLLQLPTDK(A)
118572828	28006.2	5.88	Arath	L-ascorbate peroxidase 2	(K)EGLLQLPTDK(A)
297334190	52966.4	5.96	Araiy	Large subunit of RuBisco	(K)TFQGPPHGIQVER(D)
297332730	52973.4	6	Araiy	Large subunit of riblose-1,5-bisphosphate carboxylase/oxygenase	(K)FYWAPTR(E)
15239602	28706.8	4.96	Arath	Light-harvesting chlorophyll B-binding protein 3	(K)EPVWFK(A)
75330960	29766.5	4.97	Arath	Methyl-esterase 1	(K)LLTSLPNDEK(V)

Accession	MW(Da)	Pi	Homology	Protein Name	Peptide Sequence(s)
15221728	25845	9.82	Arath	Non-intrinsic ABC protein 10_AT1G63270	(R)NAQQILRHVNVSLHDGGALVLTGTNGSGK(S)
67460972	82079.5	9.06	Arath	Oligopeptide transporter 8	(K)LGTYMKIPPR(T)
19883896	53397.9	5.55	Arath	Oxygen-evolving enhancer protein 1-1	(R)GGSTGYDNAVALPAGGR(G) (R)GSSFLDPK(G)
12323399	128658.4	4.97	Arath	P-loop containing nucleoside triphosphate hydrolases superfamily protein	(K)VVDILK(E) (K)LAELSGK(G) (K)VAKGKSPR(K)
297330766	50007.9	6.08	Araiy	Phosphoglycerate kinase	(K)ALPTYTPDSPGDATR(N) (K)GVTTIIGGGDSVAAVEK(V) (K)IGVIESLLEK(C) (K)FAPDANSK(I) (R)ADVPFR(R) (K)SVNTIR(F) (K)RPFAAIVGGSK(V) (R)KIAEEKK(T) (R)IVGATPPTPK(L)

Accession	MW(Da)	Pi	Homology	Protein Name	Peptide Sequence(s)
297312819	35189.2	6.1	Araiy	Photosystem II oxygen-evolving complex protein 1	(K)EPVWFK(A)
15232249	28802.9	5.62	Araiy	Photosystem II light harvesting complex protein 2.3	(R)AAEDPEFETFYTK(N)
297334193	39547.8	5.46	Araiy	Photosystem II protein D2	(K)LAFYDYIGNNPAK(G) (R)AAEDPEFETFYTK(N) (R)AYDFVSQEIR(A) (K)NILLNEGIR(A)
297337134	28054	9.24	Araiy	Photosystem II subunit S	(R)FSQGLAQDPTTR(R)
15235490	23051.8	9.85	Arath	Photosystem I subunit L	(R)TVVSIPNGPSALAVK(E) (R)TAVNPLLR(G)
297332701	82475.7	6.89	Ara	Photosystem I P700 chlorophyll a Apo protein A2	(R)ADVPFR(K) (R)FSQGLAQDPTTR(R)
297334183	56053.4	6.4	Araiy	Photosystem II 47kDa protein	(R)APWLEPLR(G) (K)LAFYDYIGNNPAK(G) (K)EAAWGLAR(Y) (R)AQLGEIFELDR(A)

Accession	MW(Da)	Pi	Homology	Protein Name	Peptide Sequence(s)
15235478	15686.3	5.01	Arath	Photosystem II manganese-stabilising protein (PsbO)	(K)ITLSVTK(S)
17380270	28007.9	9.25	Arath	Photosystem II 22 kDa protein	(K)VEDGIFGTSGGIGFTK(A)
75163506	74006.6	8.17	Arath	Probable inactive receptor kinase At5g67200	(K)LYVYYVLVK(C) (K)LLYSLTER(Y)
75170207	38585.6	5.07	Arath	Probable UDP-arabinopyranose mutase 5	(K)SVPFFDSLK(L) (R)EAGLIK(G)
1709740	72176	5.92	Arath	Poly [ADP-ribose] polymerase 2	(K)RISEVIDR(Y)
42570340	144479.7	8.25	Arath	Protein helicase in vascular tissue and tapetum	(K)LNAQKAK(V)
42572779	40737.6	9.44	Arath	Protein FORKED 1	(R)LLAIAAEK(K)

Accession	MW(Da)	Pi	Homology	Protein Name	Peptide Sequence(s)
334187718	110499	5.64	Arath	Protein embryo defective 2247_AT5G16715	(M)NGRPTLWLPGTDHAGIATQLVVE(K)
75174175	73340	5.34	Arath	Protein gamete expressed 3	(R)VIFPRNGTK(S)
1032296797	7540	9.52	Arath	Protein AXX17_AT2G26660	(K)GGCGGGKSGGGGGYMVAPGSNGSSYIRS(D)
					(K)SGGGGGGGGYMVAPGSNGSSYIRS(D)
					(K)GGSGSGGGGKGGGGGGSGGGR(G)
					(K)GGGGGGSGGGR(G)
1032282636	304279	4.72	Arath	Protein AXX17_AT4G36160	(K)REEPESENALT(D)
OAP09027	61847	7.53	Arath	Protein AXX17_AT2G13500	(K)RRSSKVMSVEIFEDEH(D)
					(K)VDGDNYPETLNRMF(I)
1032280307	49017	5.77	Arath	Protein AXX17_AT5G33340	(K)VGGVECYEYGVKIGTQNQFTINYPYEC(I)
					(K)FEYEDASEVVVG(D)
1032277271	66677	8.77	Arath	Protein AXX17_AT5G16980	(K)QYGGHSMHNVEATPYLH(G)
					(K)TFSRVIYEQLH(I)

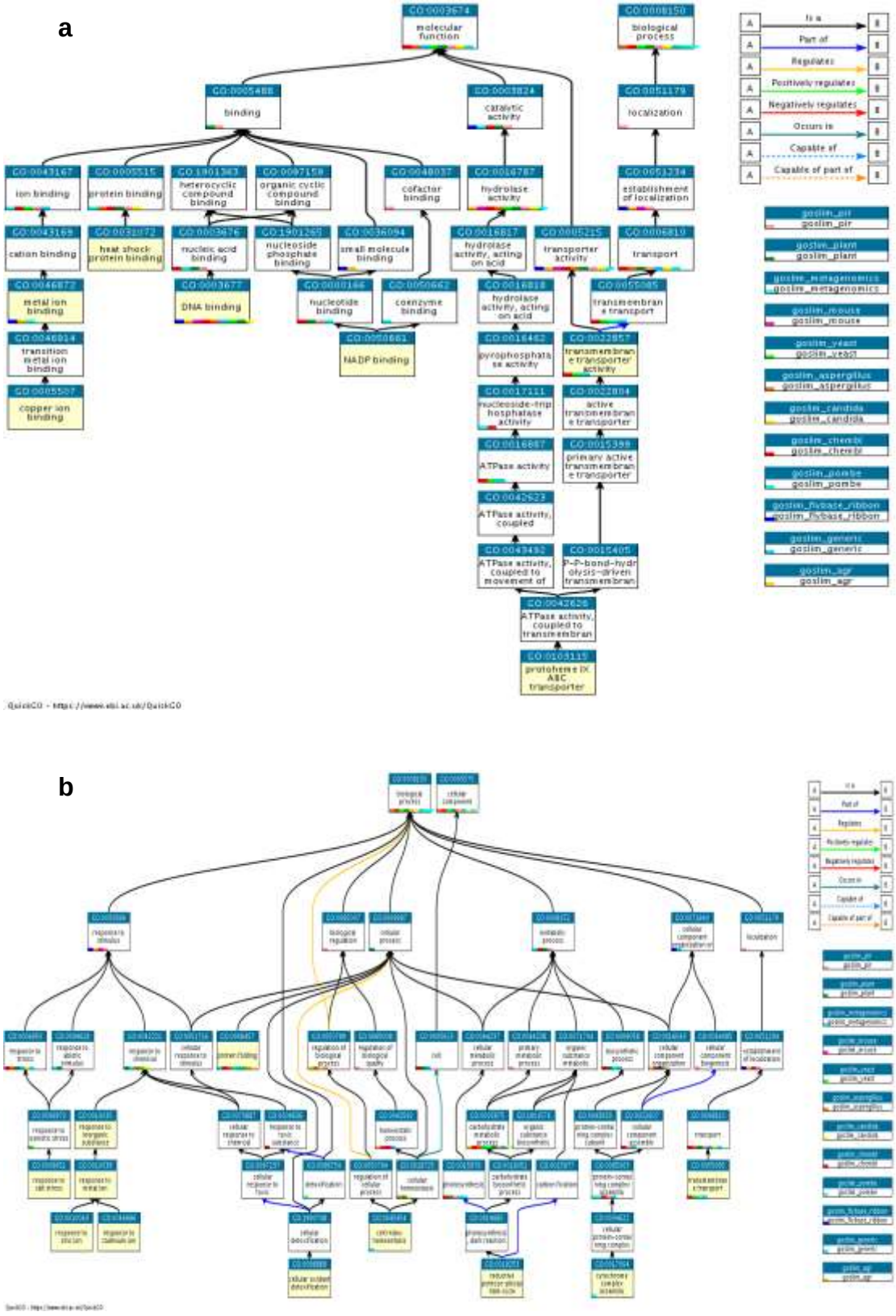
Accession	MW(Da)	Pi	Homology	Protein Name	Peptide Sequence(s)
OAP18313	35666	5.74	Arath	Protein AXX17_AT1G74880	(K)GEDLYDITLELSEAIFGSEK(E) (K)IPPGVSAGSILRVAGEGDSGPR(G) (K)VKTVEGDTELQIPPGTQPGDVLVLAK(K) (R)TRAKPQQPSTLSTAPSGSENK(K) (K)VKIPPGVSAGSILR(V) (K)CSGEGRVR(I)
297334210	83199.1	6.6	Araiy	psi P700 Apo protein A1	(R)LTFDEIQSK(T) (K)EIPLPHEFILNR(D)
297332727	51868.1	6.7	Araiy	PS II 43kDa protein	(R)FVQAGSEVSALLGR(M) (R)LGANVGSAQGPTGLGK(Y) (R)EAADLIK(K) (K)LLYSLTER(Y)
313471415	106995.7	5.61	Arath	Pumilio homolog 5	(K)LELSDIAGR(V)
75168940	96105.5	6.96	Arath	Pumilio homolog 6	(K)ALDVIEPDQR(V)
75182934	29474.5	9.2	Arath	Putative cysteine-rich repeat secretory protein 61	(K)ATRRSDK(L)

Accession	MW(Da)	Pi	Homology	Protein Name	Peptide Sequence(s)
42558968	12664.6	9.14	Arath	Putative uncharacterized mitochondrial protein AtMg00280	(K)GHYLNATAGTCEEMIKR(A) (R)AVYECLR(G) (R)AVYECLR(G) (K)DDENVNSQPFMR(W) (K)DDENVNSQPFMR(W)
12643259	51981.5	5.87	Arath	Ribulose biphosphate carboxylase/oxygenase activase	(K)TFQGPPHGIQVER(D)
15229244	52434.6	5.53	Arath/Araiy	RING/FYVE/PHD zinc finger-containing protein	(R)GWLNLPVGLSMEDEIYK(A)
79314769	27748.6	8.94	Arath	RNA recognition motif-containing protein	(K)VYVGNLAK(T)
297337086	47782.9	8.99	Arath	Serine hydroxymethyltransferase	(K)VQIVGDDLLVTNP(K) (K)KYPEEAAELK(S) (K)QFPTIGFEK(G) (K)AIQLHLRHGWK(K) (R)AAIPTIK(F)

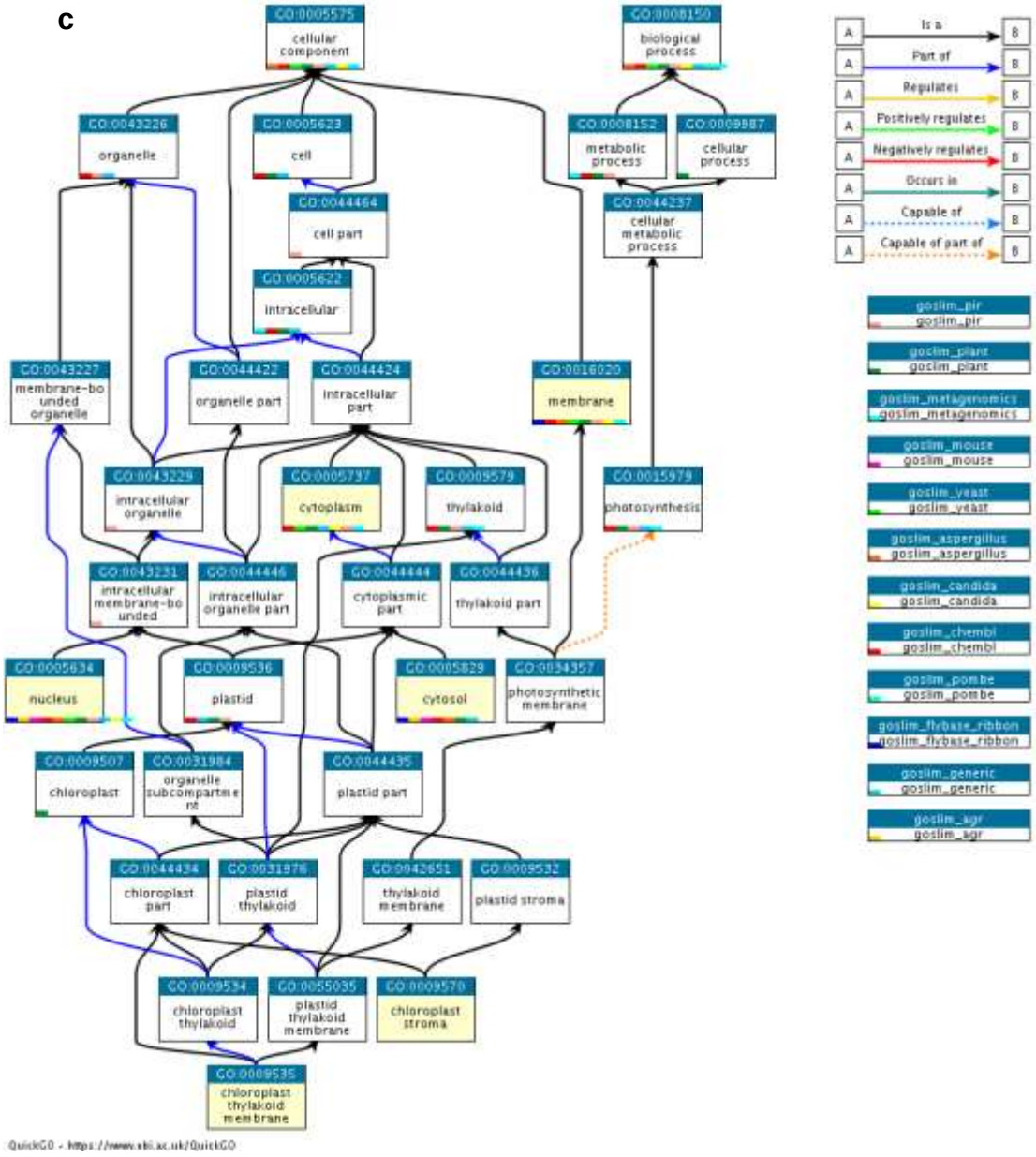
Accession	MW(Da)	Pi	Homology	Protein Name	Peptide Sequence(s)
544602156	158724.2	5.76	Arath	SNF2 domain-containing protein CLASSY 3	(R)VSDLGVEK(K)
79587640	100474.8	7.25	Arath	Transducin/WD40 domain-containing protein-like protein	(R)NESRVIGEK(G) (K)VTCQFSLK(D)
7329685	81475.4	5.8	Arath	Transketolase	(K)VGETVDLVGLR(E) (K)ALPTYTPESPGDATR(N) (R)SPDYGYGR(S)
297322418	79851.5	5.85	Araiy	Transketolase	(R)IEFVR(L) (K)ALPTYTPDSPGDATR(N) (K)IEDLR(L) (K)IVRNVEK(L) (R)FLAIDAVEK(A)
8778823	107175.8	9.1	Arath	Translation elongation factor eEF-1 alpha chain	(K)VAKGKSPR(K) (K)IGGIGTVPVGR(V) (R)LPLQDVYK(I) (R)VGLTALTMAEYFR(D) (R)AVDAVTAHFLR(I) (K)IGGIGTVPVGR(V)
13431953	33345.9	5.39	Araiy	Triosephosphate isomerase	(K)FFVGGNWK(C) (R)IIYGGSVNGGNSAELAK(E)
13432260	27169.2	5.39	Arath	Triosephosphate isomerase	(K)VAYALAQGLK(V) (K)FFVGGNWK(C)

Accession	MW(Da)	Pi	Homology	Protein Name	Peptide Sequence(s)
75170045	51654.2	8.22	Arath	Tryptophan aminotransferase-related protein 3	(R)FGWALVK(E)
297335760	53397.9	6.45	Arath	Tyrosyl-tRNA synthetase - like	(K)VAYALAQGLK(V)
75172681	80713.7	5.38	Arath	Vacuolar protein sorting-associated protein 52 B	(R)IQIIK(E)
332641995	40934.4	6.91	Arath	2-Cys peroxiredoxin (2-Cys PrxA)	(K)LERALGAK(L)
14916972	29092.2	6.91	Arath	2-Cys peroxiredoxin BAS1	(R)GLFIIDK(E)
1702987	30194.1	4.79	Arath	14-3-3-like protein GF14 phi	(R)NLLSVAYK(N) (K)VFYLK(M)
110740990	36144.5	5.55	Arath	33 kDa polypeptide of oxygen-evolving complex	(R)VPVFLDGGVR(R)

Accession	MW(Da)	Pi	Homology	Protein Name	Peptide Sequence(s)
73919362	61453.2	5.24	Arath	70 kDa peptidyl-prolyl isomerase ROF1	(K)LQDGTVFLK(K)



Appendix B. Gene ontology trees *based on* ***(a)*** *Molecular Function,* ***(b)*** *Biological process trees generated from QuickGO Via* https://www.ebi.ac.uk/QuickGO/.



*Appendix B. Gene ontology trees based on **(c)** Cellular component. GO Trees generated from QuickGO Via* https://www.ebi.ac.uk/QuickGO/.