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Nxumalo, Nokukhanya Nokuphila

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COMPARISON OF HEAVY METAL UPTAKE BETWEEN THE INDIGENOUS AND INVASIVE *TAMARIX* SPECIES IN SOUTH AFRICA

Nokukhanya Nokuphila Nxumalo

A research report submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the Degree of Master of Science by Coursework and Research Report

in

INTERDISCIPLINARY GLOBAL CHANGE STUDIES

Supervisor: Prof Solomon Newete

Co-Supervisor: Dr Samalesu Mayonde

June, 2022

Johannesburg

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DECLARATION

I, Nokukhanya Nxumalo, declare that this research report, apart from the contributions mentioned in the acknowledgements, is my own, unaided work. It is submitted for the Degree of Master of Science by coursework and research report to the University of the Witwatersrand. It has not been presented before for any degree or examination to any other University.



_____

(Signature of candidate)

8th day of June 2022

ABSTRACT

The main aim of this study was to investigate and compare the metal uptake efficiency between the indigenous *T. usneoides* and the exotic *T. chinensis* in South Africa and to assess the effect of mycorrhizal fungi in enhancing cadmium uptake and alleviating the plant metallotoxicity. A pot experiment was conducted with treatments of cadmium and cadmium with mycorrhizal fungi and controls with no cadmium treatments for each of the two *Tamarix* species (*Tamarix usneoides* and *T. chinensis*) for a 21-days period. The control plants however, were split as control (plants with no Cd or mycorrhizal fungi) and control with mycorrhizae (plants with mycorrhizal fungi but no Cd). Mycorrhizae was applied to the soil one week prior to the treatment of the soil with cadmium at a concentration of 100mg/kg of soil. At the end of the experiment dry plant biomass and chlorophyll content were recorded and the soil as well as the plant tissues (roots and shoots) were analysed for Cd content using the ICP-OES and ICP–MS methods. The bioconcentration factor (BFC) and translocation factor (TF) were calculated. The results showed decreased plant biomass and chlorophyll content in the presence of cadmium in both species. The plant biomass and chlorophyll content of *T. chinensis* was significantly greater than *T. usneoides* in all treatments. The shoot cadmium concentration of *T. chinensis* in the cadmium only treatment was 47% higher than in the *T. usneoides*, while in the roots this trend reversed with *T. usneoides* having 53% higher Cd content than the *T. chinensis*. The same trend was observed for the cadmium with mycorrhizal fungi treatments. Both *Tamarix* species showed BCF results of >1 and TF of < 1. The BCF of *T. usneoides* was, however, significantly 53% higher than that of the *T. chinensis*. The mycorrhizal fungi did not have an impact on increasing the uptake of cadmium or in improving the plant cadmium toxicity effect on both species. This study indicates that the *Tamarix* species are good candidates for phytoremediation, but the *T. usneoides* had a greater ability of Cd uptake from the contaminated soil than the exotic *T. chinensis*. Thus, considering that it is an indigenous species, the *T. usneoides* remains the preferred plant of choice for phytoremediation in mining sites of South Africa.

DEDICATION

I would like to dedicate this research report to my late father Daniel Nkosinathi Nxumalo, my mother Thokozile Pollet Nxumalo, my brother Philani Nxumalo and my sister Nokwanda Nxumalo for their great support throughout my studies. I also pay a special tribute to my husband Siphiwe Mtshali and my children Simakade and Sibani Mtshali for their love and support throughout my endeavour to complete this study.

ACKNOWLEDGEMENTS

I wish to convey much appreciation to Prof. Solomon Newete for his constructive supervision, and continuous support throughout the period of my research. A big thank you is also due to Dr. Samalesu Mayonde for his assistance and support for the research especially on the practical side.

This work is based on the research supported in part by the National Research Foundation of South Africa (Grant Numbers: 114345) under the lead investigator Prof Solomon Newete.

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ABBREVIATIONS

ARC:	Agricultural Research Council
AMF:	Arbuscular mycorrhizal fungi
BCF:	Bioconcentration factor
ICP– OES:	Inductively coupled plasma- optical emission spectrophotometry
ICP–MS:	Inductively coupled plasma mass spectrometry
NEM: BA:	National Environmental Management: Biodiversity Act, 2014
TF:	Translocation factor
SA:	South Africa
USA:	United States of America

CHAPTER ONE: INTRODUCTION

1.1 General Introduction

Contamination of soil with heavy metals results from mining and other anthropogenic activities including industrialization and smelting that cause serious environmental challenges globally (Chibuike & Obiora, 2014). Removing these heavy metal contaminants from the environment is often costly, difficult and is mainly achieved through physical and chemical methods. Such methods include soil washing, soil flushing, encapsulation, and electro-kinetics (Marques et al., 2009). These heavy metals bind closely to particles of the soil which makes it difficult for them to be mineralized (Marques et al., 2009; Lata et al., 2019). Although these methods are not without challenges, they do affect soil organic content, pH, the soil structure, chemical composition, and soil microorganisms (Cunningham & Ow, 1996; Thakur et al., 2016). This is further restricted by exorbitant costs required to decontaminate large, polluted areas. The cost associated with soil washing method is estimated to be between USD$46–250 per metric ton of contaminated soil in the United States of America (USA) (Tran et al., 2022). These high costs are often regarded as a financial liability to the mining companies, and this discourages them from addressing the heavy metal pollution caused by leaching from mine dumps (Alkorta et al., 2004; Newete & Byrne, 2016).

Tamarix usneoides E. Mey. ex Bunge, *T. ramosissima* Ledeb. and *T. chinensis* Lour. are the three *Tamarix* species that occur in South Africa. However, *T. usneoides* is the only *Tamarix* species indigenous to South Africa (Obermeyer, 1976; Henderson, 2001; Mayonde et al., 2015). In South Africa it is unclear how and when the alien *Tamarix* arrived, but it is thought to have been introduced as ornamentals or for phytoremediation purposes in the early years of 1900s (Marlin et al., 2017). *Tamarix* is mostly found in southwestern regions of the country, and this include Northern, Western and Eastern Cape provinces (Newete et al., 2019) with some populations occurring on mine dumps in the Gauteng, Free State, and North West provinces (Mayonde et al., 2015)

Tamarix outcompetes most co-occurring plants for water, light and nutrients and is very invasive in areas outside its native zone. It thrives in temperate and sub-tropical climates (Heywood et al., 2007; Marlin et al., 2017). It has high evapotranspiration rates, according to water use studies conducted in the USA. In areas near streams or rivers with heavy infestation, *Tamarix* consumes a lot of water (Johnson, 1987; Di Tomaso, 1998). Each day, a large tree can absorb up to 760 litres of water (Di Tomaso, 1998). In South Africa *Tamarix* species have been found to deplete water resources in areas where they have invaded (Le Maitre et al., 2016) and displace native biodiversity (Newete et al., 2019; Setshedi & Newete, 2020). However, exotic *Tamarix* species along with the indigenous species are growing on tailing storage facilities for phytoremediation of gold and uranium mines in South Africa (Weiersbye et al., 2006; Mayonde et al., 2015).

Tamarix chinensis and *T. ramosissima* have been declared as invasive species by the Alien Invasive Species regulations of the National Environmental Management: Biodiversity Act 2014 (NEM: BA, 2014) which classifies them as Category 1b invaders. This necessitates their removal and prohibition of propagation. Chemical and mechanical strategies are employed to control invasive *Tamarix* in South Africa; however, biological control has also been used, especially in the USA (Marlin et al., 2017). In the USA, a leaf beetle from the genus *Diorhabda* (Coleoptera: Chrysomelidae), specifically *D. carinulata* and *D. carinata* were identified (DeLoach et al., 2003), and approved for release in May 2001 for use as a biological control agent of *Tamarix* (DeLoach et al., 2011). Therefore, for phytoremediation the focus is on the indigenous *T. usneoides* since no exotic *Tamarix* species is permitted for planting in South Africa.

Thus, this study investigated the metal uptake ability of the indigenous and exotic *Tamarix* species to determine if the indigenous *Tamarix* species is equally or more effective for phytoremediation of mines dumps in South Africa. The outcome of this research will help promulgate the indigenous *Tamarix* over the propagation of the invasive species for phytoremediation in South Africa. This is because the historical planted exotic *Tamarix* species have spread outside of mining sites and

are now invading riparian ecosystems across the country (Marlin et al., 2017). Proving that the indigenous *Tamarix* species is as good as the exotic *Tamarix* species in phytoremediation, the planting of the native *T. usneoides* will be recommended in future.

1.2. Research question

- Does the indigenous *T. usneoides* have greater potential to tolerate, uptake and accumulate heavy metals, compared to the invasive *T. chinensis*?
- Do mycorrhizal fungi play a role in enhancing tolerance, uptake, and accumulation of heavy metals in *Tamarix* species?

1.3 Research Aim and Objectives

1.3.1 Research aim

The main aim of the study is to compare the metal uptake capacity of the indigenous *T. usneoides* and the invasive *T. chinensis* in South Africa and to assess their tolerance to metal-induced plant stress in the presence of mycorrhizal fungi.

1.3.2 Research objectives

The objectives are:

- to measure the physiological responses of *T. usneoides* and *T. chinensis* to heavy metal-induced stress:
    - ✓ Plant biomass
    - ✓ Leaf chlorophyll content
- to measure the concentration of heavy metals in the roots and shoots of *T. usneoides* and *T. chinensis*
- to measure the final metal concentration in the soil after the treatments to confirm presence of cadmium and determine bioconcentration and translocation factors of *T. usneoides* and *T. chinensis*.
- to evaluate the effect of mycorrhizal fungi on heavy metal tolerance and uptake on *T. usneoides* and *T. chinensis*.

1.3.3 Null hypothesis:

- The uptake and accumulation of heavy metals from the soil between the indigenous *T. usneoides* and the invasive *T. chinensis* is the same.
- Both the indigenous *T. usneoides* and the invasive *T. chinensis* have the same tolerance to heavy metal-induced plant stress with and without mycorrhiza fungi.
- The indigenous *T. usneoides* and the invasive *T. chinensis* have the same bioconcentration and translocation factors.

1.3.4 Alternative hypothesis:

- The uptake and accumulation of heavy metals from the soil by the indigenous *T. usneoides* is higher than that of the invasive *T. chinensis*.
- The indigenous *T. usneoides* has higher tolerance to heavy metal-induced plant stress with and without mycorrhizal fungi compared to the invasive *T. chinensis*.
- The indigenous *T. usneoides* has higher bioconcentration and translocation factors compared to the invasive *T. chinensis*.

CHAPTER TWO: LITERATURE REVIEW

2.1 The taxonomic classification and geographic distribution of *Tamarix*

Tamarix species have naturalized and spread over the world outside their native regions (Baum, 1978; Henderson, 2001; Gaskin, 2003). *Tamarix* is represented by 55 species and is native to the Old-World Eurasian regions as well as some parts of the African continent (Baum, 1978; Villar et al., 2019). In South Africa, there are three species of *Tamarix* comprising of the indigenous *T. usneoides* and the invasive *T. chinensis* and *T. ramosissima* together with different hybrid combinations among them (Mayonde et a., 2016; Mayonde et al. 2019). *Tamarix* species are morphologically similar, with characteristics such as the leaf shape, leaf base, their nectar disk shape and arrangement of the stamens used to differentiate the different *Tamarix* species. Hybridization between the species also makes it difficult to distinguish the *Tamarix* species from each other (Baum, 1978; Gaskin & Schaal 2002, 2003; Mayonde et al., 2015).

2.2 Biology of *Tamarix*

Tamarix are multi-branched halophytic trees that may grow up to 20 metres in length and can withstand saline soils, therefore the common name is Saltcedar (Baum 1978). *Tamarix* plants are adaptable shrubs or trees with multiple stems and slender branches, that are red brown in colour when they are young. *Tamarix* leaves have unique salt glands that excrete salt crystals. *Tamarix* has a deep tap root, which allows the plant to directly access the underground water table. This allows *Tamarix* to thrive in a various habitats including arid areas (Brock, 1994; Le Maitre et al., 2016). *Tamarix* is able to reproduce both sexually and asexually (Brotherson & Field, 1987).

2.3 Effects of cadmium

Cadmium (Cd) is a silver-coloured metallic element which is soluble in acids and is non-corrosive. It has an atomic mass of 112.48g/mol (Lata et al., 2019) and its natural concentration in the soil is estimated to be 0.1-0.51mg/kg (Gallego et al., 2012). It is a non-biodegradable metal, and its persistence in the water and soil

poses a threat to plants and animals including humans (Kathal et al., 2016). It is toxic and has an adverse effect on plants by inhibiting metabolism and reducing photosynthesis, and water and mineral uptake (Di Toppi & Gabbrielli, 1999; Kathal et al., 2016; Lata et al., 2019). Cadmium is introduced to the environment through human activities and natural weathering of rocks (Zeng et al., 2009). Cadmium toxicity affects humans via the food chain and once exposed to even low concentrations over a long period, the heavy metal causes health problems such as reproductive system failure, brain and kidney damage and contributes to cancer development (Bernard, 2008; Zeng et al., 2009). To the plant, Cd is a readily bioavailable metal, along with zinc (Zn) nickel (Ni), selenium (Se) and other metals (Prasad, 2003). Cadmium concentrations in soils near anthropogenic activities and industrial areas are typically high (McLaughlin et al., 1999; Gallego et al., 2012). Even at low concentrations plants are still capable of taking up Cd (McLaughlin et al., 1999).

To be up taken by the plant, Cd enters plant cells using the same pathway for the uptake of macro and micronutrients (Khan et al., 2017) and it is absorbed by the roots and transported to the shoots, where it affects plant development and growth (Di Toppi & Gabrielli, 1999; Khan et al., 2017). Phytotoxicity caused by Cd could result in leaf chlorosis, browning of the leaves, premature leaf fall and the inhibition of growth. Cadmium toxicity affects plant chlorophyll content, which in turn decreases the photosynthetic activity (Basa et al., 2014). Plants have devised some defence mechanisms to combat heavy metal toxicity, such as reduced heavy metal uptake, metal sequestration within vacuoles, and antioxidant activation (Shahid et al., 2015). Different plants tolerate different concentrations of Cd and above a particular threshold, they succumb to metal toxicity. Cadmium uptake by plants is influenced by its concentration and bioavailability (Clarkson & Lüttge, 1989; Rivetta et al., 1997). Since certain plants can take up Cd from the soil, they are therefore used to remove Cd from soils, a process known as phytoremediation.

2.4 Phytoremediation

Remediation is a combination of physicochemical and biological procedures used to clean heavy metal polluted soils (Ali et al., 2013). These physicochemical

techniques are rapid but expensive due to the technical difficulties such sites with low soil permeability, soils with mixed contaminants and bonds between soil particles and metal contaminants. They also have an impact on the soil's properties which results in secondary pollution of the soil (Reddy, 2010; Wuana & Okieimen, 2011). Phytoremediation uses plants and their microbes to minimise environmental pollution to safer levels (Lorestani et al., 2012). These plants must have the ability to tolerate, extract, and accumulate heavy metals in their aerial biomass (Thakur et al, 2016). Phytoremediation techniques have low-cost inputs; can enhance the aesthetic value of an area; stabilize the soil; allow for harvesting of valuable metals from the plant biomass and are environmentally beneficial (Ghosh & Singh, 2005; Thakur et al., 2016).

Plants remediate heavy metals through different methods namely, phytostabilization, phytoextraction, phytovolatilization, and rhizofiltration as summarized in literature (Salt et al., 1998; Sarma, 2011; Tangahu et al., 2011; Ali et al., 2013). The criteria for a candidate phytoremediation plant species outlined below, as stated by Sarma. (2011), Ali et al. (2013) and Thakur et al. (2016), including references therein are used to choose ideal plant species suitable for phytoremediation.

- high production of above-ground biomass and quick growth rateroot system that is extensive, branching, and capable of continuous development under harsh conditions ability to thrive outside of their natural environments
- increased rate of accumulation of the targeted heavy metal and translocation (and uptake) from roots to shoots
- high pH and salinity tolerance to the harmful effects of the targeted heavy metals
- good response capabilities to prevailing environmental and climatic circumstances; and
- the plant must be simple to grow and easy to harvest.

Plant uptake of heavy metals happens through the plant's root system, because of its large surface area to absorb and store water and important growth nutrients

(Newete and Byrne, 2016). Some metal ions in the soil are not easily available for plants to absorb and accumulate and their availability is influenced by metal concentrations, interactions between metals and organic matter in terms of iron exchange, temperature, nutrients, and soil salinity (Rieuwerts et al., 1998; Thakur et al., 2016). Plants need to withstand heavy metal toxicity for phytoremediation to be successful. Hence, to improve the efficiency of phytoremediation the plant's tolerance levels to heavy metal must be enhanced through plant breeding or genetic engineering to be able to metabolize heavy metals (Newman et al., 1997; Wuana & Okieimen, 2011). By assisting in the detoxification of contaminated soils, microbial organisms around plant roots such as the soil fungi, contributes to cadmium-induced plant stress tolerance (Sidhu et al., 2019).

Plants capable of tolerating and accumulating up to 1000mg/ kg of heavy metal in their tissues are called hyperaccumulators and are often used for phytoremediation depending on the soil contaminant levels, soil properties, and the type of heavy metal contaminant (Baker & Brooks 1989; Lorestani et al., 2012). Hyperaccumulators are limited to 400 vascular plant species worldwide occurring in about 45 different plant families and are often constrained by geographical barriers (Newete & Byrne; 2016). Using plants in conjunction with microorganisms to remediate polluted soils leads to more effective and faster heavy metal removal from polluted soils. Several studies have shown that using mycorrhizal fungi assists to improve plant tolerance and heavy metal accumulation (Jamal et al., 2002; Weyens et al., 2009).

2.5 The role of mycorrhizal fungi in phytoremediation

Microbes can consume soil contaminants and transform it into non-toxic compounds that could be used to enhance plant growth and tolerance to heavy metal stress (Thassitou & Arvanitoyannis, 2001). They can increase bioavailability of metals in the soil through processes such as acidification, chelation, and precipitation of the metals (Merdy et al., 2009; Mishra et al., 2017). Mycorrhizal fungi that associate with plants within soil assist plants to improve their ability to uptake nutrients and water. In return the plant produces soluble carbon sources for the fungi, thereby the plant's absorption capability is increased

by the fungus (Bago et al., 2000; Entry et al., 2002). This has a direct impact of enhancing biomass production by the plant and tolerance to heavy metals toxicity (Tiwari & Lama, 2018). Most plants have mycorrhizal fungi that are linked to specific and broad beneficial relationships, such as the usage of fungi as primary nutrient uptake structures for plants (Bago et al., 2000). Phytoremediation with plants and mycorrhizal fungi may improve efficiency in removing heavy metal contaminants found in the environment (Entry et al., 2002; Chibuike & Obiora, 2014).

Arbuscular mycorrhizal fungi (AMF) are microbes found in soil that form links between the soil and plant roots (Rothwell, 1984), to increase the surface area available for nutrients to be absorbed (Saxena et al., 2017). Fungal hyphae present within the root cells characterizes AMF, which also assists in reducing toxicity of heavy metals to the host plants (Leyval et al., 1997; Entry et al., 2002). The effectiveness of how AMF reduce metal toxicity within the host plants can be determined by several parameters, including the species of fungi (del Val et al., 1999), metal concentration and its bioavailability in the soil (El-Kherbawy, 1989; Guo et al., 1996), soil properties (Lambert & Weidensaul, 1991) and the density of the roots which is a requirement for plant growth (Pawlowska & Charvat, 2004). Since AMF can increase the bioavailability of metals in the soil, and improve plant-metal tolerance (Bago et al., 2000; Meier et al., 2012), this microbe was used in this investigation as an added factor to test *Tamarix*'s ability to uptake and tolerate heavy metal.

2.6 The use of *Tamarix* for phytoremediation in South Africa

Since the early 1930s, *Tamarix* species have been employed indiscriminately in South Africa for phytoremediation of gold mining tailings dams (Mayonde et al., 2015; Marlin et al., 2017). Using molecular genetics approaches, Mayonde (2013) confirmed that majority of *Tamarix* species planted in mining areas were the exotic *T. ramosissima* and *T. chinensis*. Due to their ability to tolerate salt and absorb and excrete contaminates, *Tamarix* species can grow in saline conditions and avoid salt-induced stress by excreting salt through foliar salt glands (Di

Tomaso, 1998). Therefore, *Tamarix* trees could be suitable for phytoremediation of saline and heavy metal contaminated soils. Newete et al. (2020) investigated salt excretion efficiency and salt-induced physiological response between the indigenous and exotic *Tamarix* species (*T. ramosissima* and *T. chinensis*) including their hybrids and discovered that *T. chinensis* and *T. usneoides* had faster growth rates than the other genotypes and showed less stress when exposed to high salt concentrations (180 mM) (Newete et al., 2020).

Manousaki et al. (2008) found that *Tamarix* can efficiently absorb Cd with increased levels of salinity. Salinity can change the characteristics of heavy metals, making them more readily available for absorption by plants and increases their translocation from roots to aerial parts of the plant (Greger et al., 1995; Fitzgerald et al., 2003). The presence of salt played a key role by enhancing the phytoremediation of Cd contaminated soils using *Tamarix* (Manousaki & Kalogerakis, 2011a; 2011b).

Manousaki et al. (2009) discovered that *T. smyrnensis* Bunge. might be used for phytoextraction due to its high biomass output and high lead and cadmium accumulation in the shoots. *Tamarix smyrnensis* roots were non-selective in up taking Cd and Pb ions from the root zone because the salts secreted by the salt gland were the same with the salt composition found in the root environment. *Tamarix usneoides* showed tolerance to high concentrations of Cd in soil, although reduction in plant biomass was observed (Mayonde et al., 2021). *Tamarix usneoides* showed the ability to survive 12mg/kg soil cadmium concentration which is 1.5 more than what is found in naturally cadmium contaminated soils (Mayonde et al., 2021).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Plant collection

The two *Tamarix* species used in this study were *T. usneoides* and *T. chinensis*. *Tamarix* cuttings were sampled in November 2019 from different naturally occurring *Tamarix* trees previously identified by Mayonde et al. (2016) using molecular analysis. The areas of collection were Noorspoort farm near Steytlerville (S33°19.350′; E24°20.410′ in Eastern Cape, and Dwyka (S33°05.217′; E21°04.783′ in Western Cape provinces.

3.2 Plant propagation and experimental design

Truncheons of 15 cm long were excised. A root growth hormone Dynaroot1 (PBR Trading International CC) was applied to enhance substantial root development before the cutting was planted in river sand to enhance root development. The cuttings were propagated in play sand for a period of nine weeks (14 November 2019 – 16 January 2020) and watered on a weekly basis. The rooted cuttings were transplanted into individual planting pots (plastic) with a height of 16.5cm and a diameter of 20cm. The pots had a substrate (1,5kg of dry weight) consisting of a mixture of organic compost, silica sand and topsoil. The transplanted cuttings were allowed to grow for a further eight weeks (17 January -13 March 2020) for sufficient biomass production and development of roots.

The plants were fertilized once with 100g of cadmium free fertilizer, which consists of 95g/kg of N, 14g/kg of P, 41g/kg of K, and 80g/kg of C (Wonder Vitalizer Lawn and Leaf) per 1.5 kg of the soil substrate. The experiment was conducted at the ‘Wits greenhouse’ in the Oppenheimer Life Science (OLS) Building, at the University of the Witwatersrand. Eight donor trees of *T. usneoides* and *T. chinensis* were sampled to yield three clones per tree to give a total sample size of n = 24 (Table 3.1). Plants from each *Tamarix* species were labelled according to their treatments. The controls were *Tamarix* planted in Cd-free soils and plants treated with mycorrhiza fungi only. Cadmium treatments

constituted of *Tamarix* plants treated with cadmium only and a combination of Cd with mycorrhizal fungi (Table 3.1).

Table 3.2: The experimental design for the uptake of cadmium by *T. usneoides* and *T. chinensis* and the number of plants per treatment

***Tamarix* species & Treatment groups**	**Treatment codes**	***Total replicates**
T. usneoides control	Tu	3
T. chinensis control	Tc	3
T. usneoides control with mycorrhiza	Tu+Myco	3
T. chinensis control with mycorrhiza	Tc+Myco	3
T. usneoides cadmium	Tu+Cd	3
T. chinensis cadmium	Tc+Cd	3
T. usneoides with cadmium & mycorrhiza fungi	Tu+Cd+Myco	3
T. chinensis with cadmium & mycorrhiza fungi	Tc+Cd+Myco	3
Total Plants		**24**

*The number of replicates for the initial experiment was n= 6, but due to plant mortality during the Covid-19 related national lockdown, n was reduced to 3 replicates (n=3).

3.3 Inoculation with mycorrhizal fungi

Mycoroot^{TM} Super Booster for trees and shrubs (Mycoroot™ (Pty) Ltd 2010, South Africa), was administered to the transplanted cuttings of the indigenous and exotic *Tamarix* species (Table 3.1), according to the manufacturer's manual. The soil was gently loosened for 50g of Mycoroot^{TM} granules to be applied around the base of the stem of the plant followed by gentle watering based on the manufacture's manual. Mycoroot^{TM} was applied to all the experimental plants seven days prior to the Cd treatment for establishment of the mycorrhiza in the soil.

3.4 Cadmium treatment of *Tamarix*

The experimental *Tamarix* plants were treated with 200ml of Cadmium [$Cd(NO_3)_2.4H_2O$] (cadmium nitrate tetrahydrate, Merck Schuchardt, Germany) diluted in distilled water. The concentration of Cd was 100mg/L which was applied once off as per Manousaki et al. (2009). A volume of 200ml Cd-free distilled water was applied to the two control treatments. The plants were monitored and watered with 200ml of de-ionized water twice a week for a period of 21 days. The potted plants were placed in trays to prevent leaching of cadmium contaminated water. The experiment was conducted in a glass house with 78% diffused light penetration and mean temperatures for day of 36.7°C and night temperature of 19°C measured using an ibutton temperature logger. The position of the individual pots in the green house were randomized to reduce bias caused by environmental effects.

3.5 Measurement of plant physiological parameters

3.5.1 Chlorophyll content

Following the end of 21 days, the relative leaf chlorophyll content (nmol/cm) of the Cd-treated *Tamarix*, as well as the control plants with and without mycorrhizal fungi, was measured. A non-destructive Konica Minolta SPAD-502 Plus (KONICA MINOLTA, INC Made in Japan) chlorophyll meter was used. The measurements of the plants' relative chlorophyll content (nmol/cm) were taken at the second shoot starting at the top of each branch (newest growing twig). Three branches were randomly selected from each plant and the averaged measurements were recorded.

3.5.2 Plant biomass

During the experiment, plants were observed for leaf chlorosis and wilting of the shoots/twigs. The plant biomass was measured after the experiment as an indicator of growth. On the last day of the experiment, 24 *Tamarix* plants were harvested and gently rinsed using water that is deionized to remove soil particles. Plant roots and shoots were separated, then they were placed into labelled brown paper bags to be dried. Harvested material was oven dried at a temperature of

75°C for three days and left to cool before weighing to three decimal places using a laboratory scale.

3.6 Plant cadmium concentration analysis

The dried plant material from the cadmium and Cd with mycorrhiza treatment groups for each species were grinded into fine powder using a mortar and pestle. Control and control with mycorrhiza treatments were not analysed for Cd concentration because the aim was to compare the difference in Cd concentration between the two *Tamarix* species. Grinded samples were sent to Agricultural Research Council (ARC) – Institute for Soil, Climate and Water for determining the concentration of Cd in the roots and shoots. The dried plant material was analysed using Inductively Coupled Plasma- Optical Emission Spectrophotometry (ICP– OES) with a Spectro Ciros CCD from Spectro Analytical Instruments (manufactured in German) according to Steenkamp et al. (2006) protocol.

3.7 Soil cadmium concentration analysis

At the end of the experiment, the soil from all the experimental plants was analysed at the ARC – Institute for Soil, Climate and Water, to determine the presence and concentration of cadmium. To estimate the ability of *Tamarix* species in absorbing Cd, soil samples from the controls and Cd treated soil were analysed. The soil samples were dried in the oven at a temperature of 75°C for three days and sieved (2mm sieve) to remove course soil particles prior to Cd analysis. The concentration of Cd was measured by Inductively Coupled Plasma Mass Spectrometry (ICP–MS) according to a method from Sposito (1989).

3.8 Bioconcentration and Translocation factors

To estimate a plant's potential for phytoremediation, both bioconcentration factors (BCF) and translocation factors (TF) can be used (Yoon et al., 2006). The BCF, is defined as the ratio of heavy metal concentration of the roots to that in the soil, can be used to estimate a plant's ability to accumulate metals from soils (Yoon et al., 2006). The TF, is defined as the ratio of metal concentration found in the shoots to that in the roots, it is used to assess a plant's ability to transport metals in

the roots to the aerial parts of the plant (shoots) (Yoon et al., 2006; Nazareno & Buot, 2015).The BCF and TF of Cd for *T. usneoides* and *T. chinensis* were calculated according to Sidhu et al. (2017a; 2017b) as shown in the formulas below

Bioconcentration factor (BCF) formula

$$\text{BCF} = \frac{concentration\ in\ the\ root}{concentration\ in\ the\ soil}$$

Translocation factor (TF) formula

$$\text{TF} = \frac{concentration\ in\ the\ shoot}{concentration\ in\ the\ root}$$

3.9 Statistical Analysis

Data collected was first analysed for normality and homogeneity. Plant biomass, chlorophyll content, cadmium content in the roots and shoots; BCF and TF and soil Cd concentration parameters measured were compared between the different treatments as well as the *Tamarix* species. One-way analysis of variance (ANOVA) was used and Fisher's least significant difference (LSD) *post hoc* test (StatSoft Inc, 2014). The statistical significance of $p < 0.05$ was used to test differences between the treatments.

3.10 Disposal of contaminated soil samples

The remaining soil, the plastic pots and collecting trays used for the Cd treatment were placed in black 25litre containers for safe collection and disposal by Averda Waste Management, which is a registered waste management company which is contracted to the University of the Witwatersrand, School of Animal Plants and Environmental Sciences (APES) for safe disposal.

CHAPTER FOUR: RESULTS

4.1 Effect of cadmium and mycorrhizal fungi on *Tamarix* plant biomass

The effect of Cd and the role of mycorrhizal fungi on the plant biomass was investigated in *T. usneoides* and *T. chinensis*. The *T. usneoides* control plants were compared to the Cd only and Cd with mycorrhizal fungi treatments. The presence of Cd to the soil results in a decrease of the biomass of *T. usneoides*, although the decrease was not significant for both treatments. The same pattern was observed for *T. chinensis* when the control plant was compared to Cd only and Cd with mycorrhizal fungal treatments. There was a significant decrease ($F_{7,16} = 19.5857$, $P < 0.005$) (Fig. 4.1) in plant biomass of *T. chinensis* with 18% reduction obtained for the Cd only and 27% for Cd with mycorrhizal fungi. The plant biomass of the Cd treated plants was compared to Cd with mycorrhizal fungi compared to the control. The results showed that the Cd treatment with mycorrhizal fungi had a lower plant biomass in both *Tamarix* species compared to the Cd only treatment and no significance differences were observed.

Interestingly, the control of *T. usneoides* and *T. chinensis* with mycorrhizal fungi (Tu+Myco and Tc+Myco) had a higher plant biomass in comparison to the *Tamarix* control plants (Tu and Tc). The significant increase in plant biomass for *T. usneoides* and *T. chinensis* was by 33% and 22% respectively in the presence of mycorrhizal fungi ($F_{7,16} = 19.5857$, $P < 0.005$) (Fig. 4.1). Overall, the exotic *T. chinensis* had a greater plant biomass compared to the indigenous *T. usneoides*. The plant biomass of the Cd with mycorrhizal fungi treated plants for both *Tamarix* species were also significantly reduced by 46% for *T. usneoides* and 43% for *T. chinensis* ($F_{7,16} = 19.5857$, $P < 0.00000$) (Fig. 4.1) when compared to the control with mycorrhizal fungi. The addition of mycorrhizal did not enhance plant biomass in the presence of Cd. The plant biomass between the two species was significantly different ($F_{7,16} = 19.5857$, $P < 0.005$) (Fig. 4.1) for the control and the control with mycorrhizal fungi. *Tamarix chinensis* control with mycorrhizal fungi recorded the highest plant biomass of 17.4g and *T. usneoides* Cd with

mycorrhizal fungi was the lowest at 7.9g when compared to other treatment groups.

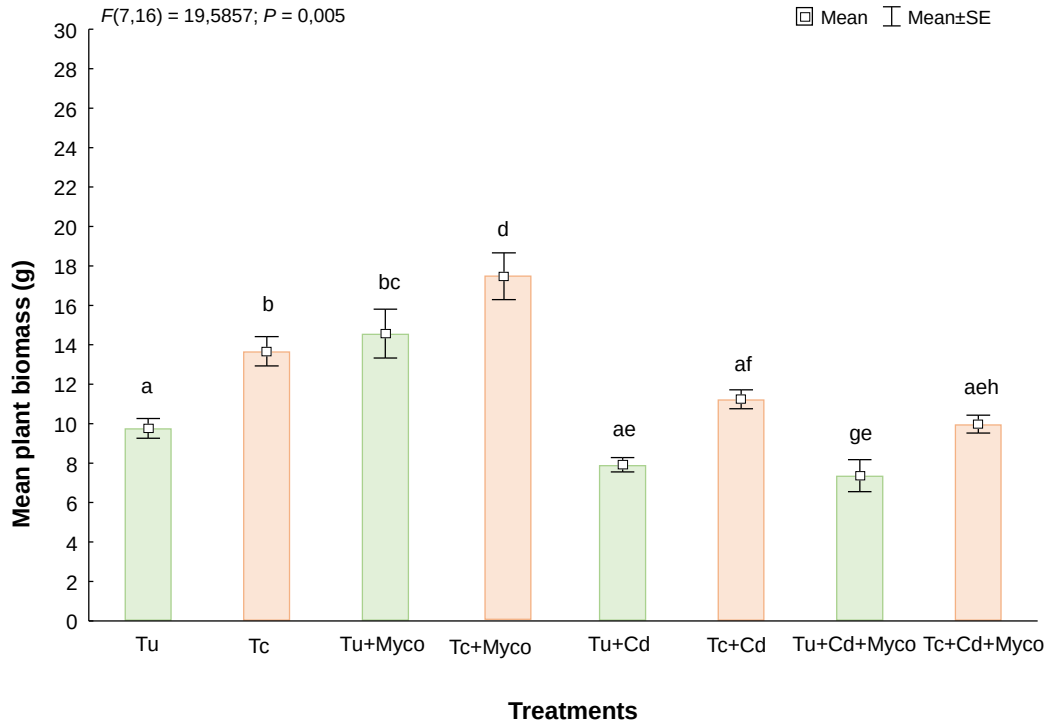

Figure 4.1: Overall effects of cadmium toxicity and mycorrhizal fungi on the mean plant biomass of *T. usneoides* and *T. chinensis*. Measurements were taken at the end of the experiment after three weeks of cadmium treatment. Different lowercase letters represent significant differences between the treatment means at the end of the experiment (n=3).

4.2 Effect of cadmium and mycorrhizal fungi on *Tamarix* chlorophyll content

The effect of Cd and the role of mycorrhizal fungi on the chlorophyll content was investigated in *T. usneoides* and *T. chinensis*. When *T. usneoides* control (Tu) was compared against the Cd only and Cd with mycorrhizal fungi treatments, the results showed that the presence of Cd results in a significant decrease in chlorophyll content by a magnitude of 17% (for Cd only) and 46% (for Cd+Myco) ($F_{7,16}$ = 23.2566, $P < 0.005$) (Fig. 4.2). *Tamarix chinensis* also displayed the same pattern

when the control (Tc) was compared to Cd only and Cd with mycorrhizal fungi treatments and shows a significant decrease in chlorophyll content was observed by 20% (for Cd only) and 48% (for Cd+Myco) ($F_{7,16} = 23.2566$, $P < 0.005$) (Fig. 4.2). The chlorophyll contents of Cd treated plants were also compared between Cd only and Cd with mycorrhizal fungi treatment groups. The results showed that the Cd treatment with mycorrhizal fungi had a significantly lower chlorophyll content than the Cd only treatment for both *Tamarix* species ($F_{7,16} = 23.2566$, $P < 0.005$) (Fig. 4.2). The decrease in chlorophyll content in the presence of mycorrhizal fungal was 37% for *T. usneoides* and 35% for *T. chinensis*.

The Cd with mycorrhizal fungi treated plants for both *Tamarix* species were also significantly lower than the control with mycorrhizal fungi by 49% for *T. usneoides* and 58% for *T. chinensis* ($F_{7,16} = 23.2566$, $P < 0.005$) (Fig. 4.2). The controls with mycorrhizal fungi plant showed higher chlorophyll content compared to the control plants for both species (Tu and Tc). A significant difference ($F_{7,16} = 23.2566$, $P < 0.005$) (Fig. 4.2) increase of 15% for *T. usneoides* and 19% for *T. chinensis* in chlorophyll content was observed in the presence of mycorrhizal fungi. Overall *T. chinensis* had greater chlorophyll content compared to the indigenous *T. usneoides*. The chlorophyll content between the two species were significantly different for the control, Cd only and Cd with mycorrhizal fungi treatments ($F_{7,16} = 23.2566$, $P < 0.005$) (Fig. 4.2). The *T. chinensis* control with mycorrhizal fungi recorded the highest chlorophyll content of 25.4 nmol/cm and *T. usneoides* had 8.6 nmol/cm, which was the lowest.

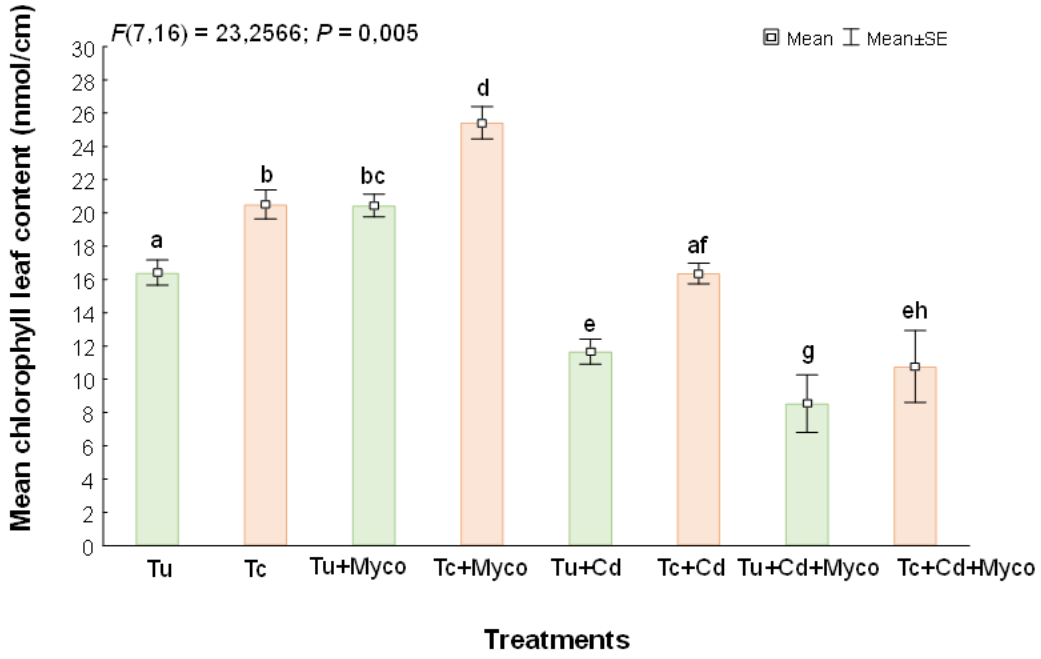

Figure 4.2: Overall effects of cadmium toxicity and mycorrhizal fungi on the mean chlorophyll content of *T. usneoides* and *T. chinensis* after their exposure to the treatments for three weeks. Different lowercase letters represent significant differences between the treatments means (n=3).

4.3 Cadmium concentration in the shoots and roots of *Tamarix*

Cadmium concentration of the shoots showed an increase when Cd with mycorrhizal fungi was compared to the Cd only treatment in both species. The Cd concentration (Tc+Cd) in the shoots of *T. chinensis* increased significantly by 19% ($F_{3,8} = 35.1723$, $P < 0.00006$) (Fig. 4.3A) when compared to Cd with mycorrhizal fungi (Tc+Cd+Myco). The Cd concentration in the shoots was significantly greater in *T. chinensis* than in the indigenous *T. usneoides* ($F_{3,8} = 35.1723$, $P < 0.00006$) (Fig. 4.3A) for both Cd only and Cd with mycorrhizal fungi treatments by 47% and 49%, respectively. Overall, the mycorrhizal fungi significantly improved Cd concentration in *T. chinensis* but no improvement was seen in *T. usneoides*.

In contrast the pattern of the root Cd concentration was different from that of the shoots with the highest Cd concentration of 74.6 mg/kg recorded in the

indigenous *T. usneoides* treated with Cd only. There was a significance difference between *T. usneoides* treated with Cd only and all other treatment ($F_{3, 8} = 93.6049$, $P < 0.0000$) (Fig. 4.3B). For both the Cd only and Cd with mycorrhizal fungi treatments *T. usneoides* had a higher Cd concentration in the root compared to those in *T. chinensis*. In the Cd only treatment, *T. usneoides* had significantly higher the Cd than in *T. chinensis* by 53 % ($F_{3, 8} = 93.6049$, $P < 0.0000$) (Fig. 4.3B). Cadmium concentration in the Cd with mycorrhizal fungi treatment group was significantly lower by 47% when compared to Cd only treatment for *T. usneoides* ($F_{3, 8} = 93.6049$, $P < 0.0000$) (Fig. 4.3B).

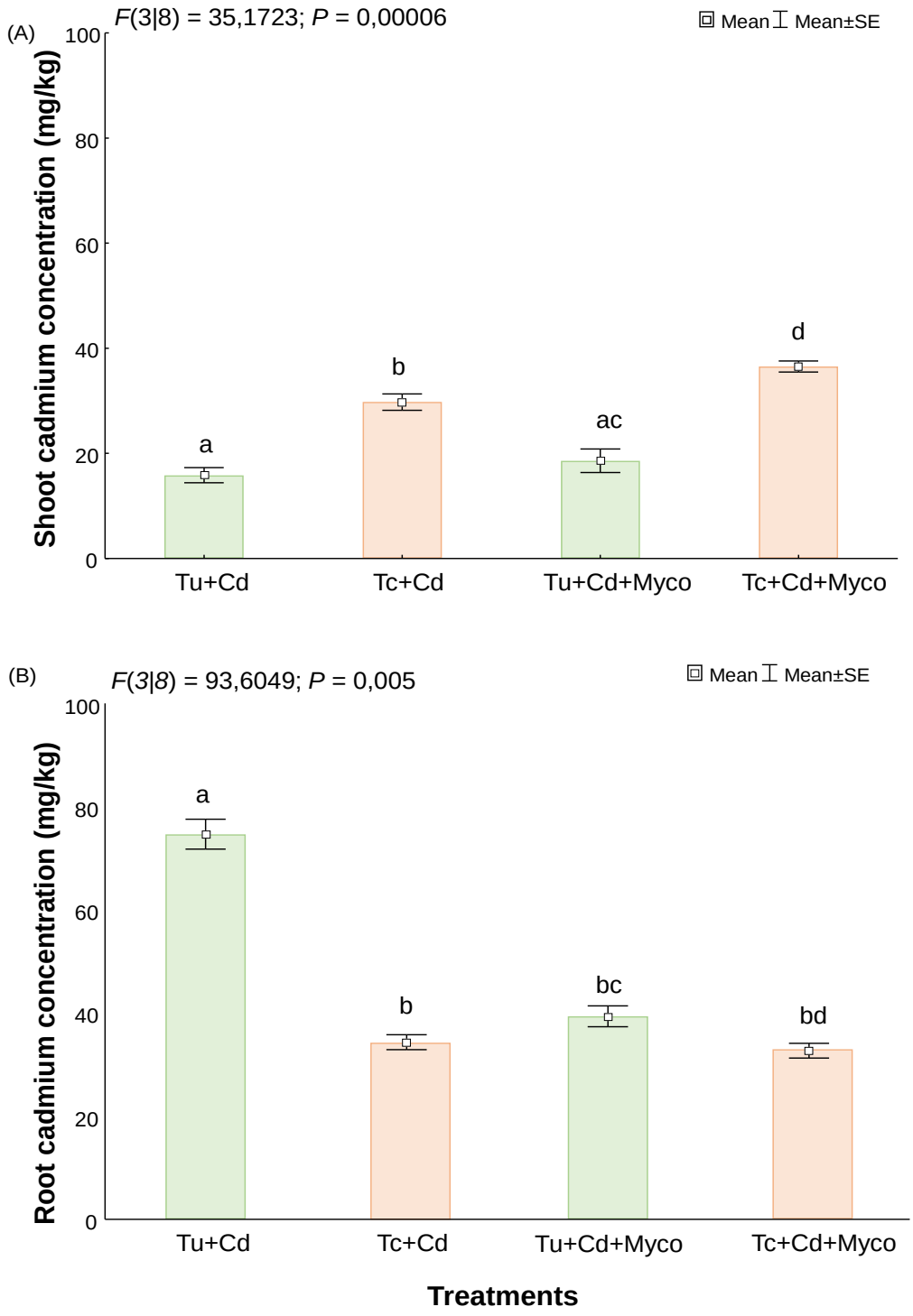

Figure 4.3: Mean shoot and root cadmium concentration for *T. usneoides* and *T. chinensis*. Measurements taken after the twenty-one-day cadmium treatment. Different lowercase letters represent significant differences between the treatments means at the end of the experiment (n=3).

4.4 The Bioconcentration and Translocation factors values for *T. usneoides* and *T. chinensis*

The Bioconcentration Factor (BCF) results showed that the plants with cadmium only treatment had BCF values of > 1 for both *Tamarix* species, while in the cadmium with mycorrhiza treatment the BCF value was < 1 for both species. The BCF value of *T. usneoides* with cadmium only treatment was 55% significantly higher than the *T. chinensis* ($F_{3, 8}$ = 101, 4581; P < 0.001) (Table 2). For the Translocation Factor (TF), results however showed TF values < 1 for both species in the Cd only and Cd with mycorrhiza treatment groups ($F_{3, 8}$ = 75, 2000; P < 0.001) (Table 4.4).

Table 4.4: The Bioconcentration Factor (BCF) and Translocation Factor (TF) of *Tamarix usneoides* and *Tamarix chinensis* plants treated with cadmium only and cadmium with mycorrhiza**l** fungi. **Values > 1 are represented in bold.**

***Tamarix* species & Treatments**	**Treatment codes**	***BCF**	****TF**
T. usneoides cadmium	Tu+Cd	**2.28**a	0.11a
T. chinensis cadmium	Tc+Cd	**1.02**b	0.63b
T. usneoides with cadmium & mycorrhiza fungi	Tu+Cd+Myco	0.66c	0.33c
T. chinensis with cadmium & mycorrhiza fungi	Tc+Cd+Myco	0.57cd	0.73bd

*BCF= metal concentration ratio of plant roots to soil. ($F_{3, 8}$ = 101, 4581; P < 0.005)

**TF= metal concentration of plant shoots to roots. ($F_{3, 8}$ = 75, 2000; P < 0.005) Values > 1 are represented in bold.

4.5 The concentration of cadmium in the soil

Soil Cd concentration results showed that the control and control with mycorrhizal fungi were below detectable limits. The soil samples from the Cd treatments had an average of 30.1mg/kg of Cd after the 21-day experiment. This indicated that the Cd concentration of the soil was significantly greater by 96% in the Cd treatment soils compared to the control soil samples (no Cd) ($F_{7, 16}$ = 99.479; P < 0.005) (Fig. 4.5). The differences were significant between Cd only and Cd with mycorrhizal fungi treated soils in the *T. usneoides* ($F_{7, 16}$ = 99.479; P < 0.005) (Fig. 4.5). *Tamarix usneoides* in the Cd only treatment showed the lowest Cd concentration of the soil with 11mg/kg while in the Cd with mycorrhizal fungi treatment group, the *T. usneoides* had the highest Cd concentration with 43mg/kg. This proportionally correlates to the high Cd concentration observed for the roots of the same treatment by *T. usneoides*.

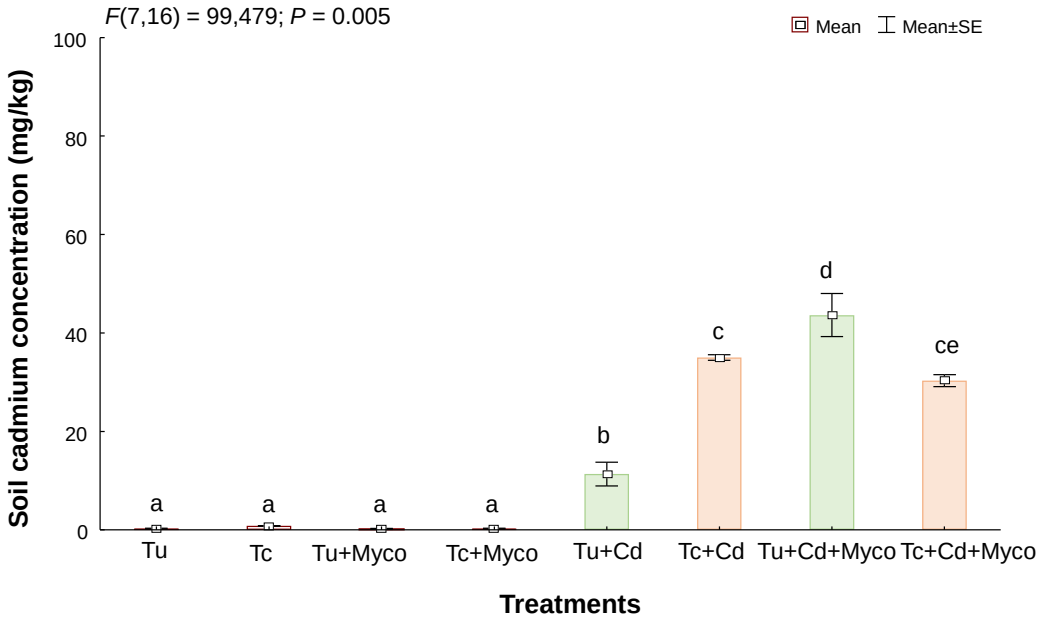

Figure 4.5: Mean cadmium soil concentration for *T. usneoides* and *T. chinensis*. Measurements taken after 21 days of cadmium treatment. Different lowercase letters represent significant differences between the treatment means (n=3).

CHAPTER FIVE: DISCUSSION

The absorption of cadmium by plants disrupts metabolic processes that are involved in normal plant development such as protein synthesis (Stiborova et al., 1987), the functioning of photosystems and transportation of electrons within plants (Murthy et al., 1990). For the current study, the indigenous *T. usneoides* and exotic *T. chinensis* were subjected to Cd treatment to determine what role is played by mycorrhizal fungi in Cd uptake and alleviation of Cd-induced plant stress. Cadmium presence affected biomass production with significant reductions observed in both *T. usneoides* and *T. chinensis* when compared to the controls. A decrease in plant biomass production is commonly observed when exposed to high concentrations of heavy metals due to the decrease in carbon assimilation and the reduction in photochemical efficiency of photosystem II resulting in growth disorders (Panich-Pat et al., 2010; Asgher et al., 2015). Therefore, the reduction in *Tamarix* biomass production could be due to the disruption of metabolic and physiological processes caused by Cd toxicity (Leng et al., 2018). The biomass of the halophyte *Thellungiella halophila* (saltywater cress; Brassicaceae) was also reduced due to Cd-induced stress (Leng et al., 2018). Our study showed that the exotic *T. chinensis* had a greater biomass production compared to the indigenous *T. usneoides* when exposed to Cd contaminated soils. Plant biomass production is usually a good indicator for plants health when subjected to abiotic stress (Malar et al., 2016). The high biomass production observed in the exotic *T. chinensis* could be attributed to the plant ability to adapt and tolerate adverse environmental conditions (Friedman & Rubin, 2015).

Cadmium impacts on the synthesis of chlorophyll by limiting the activity of protochlorophyllide reductase, which explains the reduction in chlorophyll (Stobart et al., 1985; Rascio et al. 1993). A significant decrease in chlorophyll content was observed in the indigenous *T. usneoides* and the exotic *T. chinensis* because of Cd toxicity. Similarly, Mayonde et al. (2021) observed a reduction in chlorophyll content of *T. usneoides* as a result of Cd-induced stress. Cadmium toxicity is known to antagonize metabolic functions of the chloroplast by causing changes in the ultrastructure of the cell organelle (Kamran et al., 2015). The

changes caused to the chloroplast by Cd-induced stress affects photosynthetic activity and the biosynthesis of chlorophyll (Li et al., 2015).

Overall, the chlorophyll content of *T. chinensis* was significantly higher than *T. usneoides* across all the treatment classes. In the absence of Cd, mycorrhizal fungi had the greatest effect by increasing the chlorophyll content the highest in *T. chinensis*. The exotic *T. chinensis* had higher plant biomass and chlorophyll content compared to the indigenous *T. usneoides*. *Tamarix chinensis* has been found to adapt to salt and drought stress by changing its normal growth pattern; alternating soluble sugars, proline content, enzyme activities and other physiochemical process to improve its ability to adapt (Liu at al., 2014). Such abilities ensured that *T. chinensis* was able to tolerate Cd toxicity better than *T. usneoides* since biomass production and chlorophyll content levels were higher in the exotic *T. chinensis* than in the indigenous *T. usneoides*. A recent study showed that Cd toxicity significantly reduced chlorophyll content and biomass production in *T. usneoides* (Mayonde et al., 2021). Similarly, Manousaki et al. (2009) found that *T. smyrnensis* treated with 100mg Cd/kg of soil did not have a significant effect on the plant biomass and chlorophyll content was decreased compared to the control.

Plants absorb heavy metals from the soil through the roots before they are translocated into the above ground biomass. Cadmium uptake by plants and confining the metal in the root system is a strategy used to adapt and circumvent heavy metal-induced plant stress (Cataldo et al., 1983; Benavides et al., 2005). Many plants have adapted in excluding toxic heavy metals from their sensitive aerial parts which house the photosynthetic system, and storing them in the intercellular walls, cell walls, and cell vacuoles of the roots (Shahid., et al 2015). Hence lower Cd concentration was found in the shoots. The results showed that Cd accumulation in *T. usneoides* and *T. chinensis* was greater in the roots compared to the shoots. Our results corroborated with the finding by Manousaki

et al. (2009) who also found the concentration of Cd to be greater in the roots than the shoots of *T. smyrnensis*. Similarly, Leng et al. (2018) found that the Cd concentration was greater in the roots than the shoots of *Thellungiella halophila*.

In the roots, *T. usneoides* had more Cd concentration than *T. chinensis* both with and without mycorrhizal fungi. This indicates that the roots of indigenous *Tamarix* displayed the most potential for Cd accumulation. The Cd concentration in the roots of *T. usneoides* in the Cd only treatment was higher than in the *T. chinensis*, suggesting that *T. usneoides* had a higher uptake accumulating ability of Cd into the roots. Plant species can tolerate heavy metal toxicity by preventing metal translocation from the roots to the shoots (El-Gamal & Tantawy, 2010). In the roots of both *Tamarix* species, the mycorrhizal fungi did not show any impact in increasing the uptake of Cd because the uptake of Cd was lower when mycorrhizae fungi was applied to the soil. Joner and Leyval. (1997) have indicated that it may be due to a selective fungal transport mechanism which impairs the transport of non-essential elements. This selective fungal transport mechanism may reduce the transfer of metal at different stages such as during uptake of metal from the soil and translocation to the ariel part of the plant. Joner and Leyval. (1997) have indicated that it may be due to a selective fungal transport mechanism which impairs the transport of non-essential elements. This selective fungal transport mechanism may reduce the transfer of metal at different stages such as during uptake of metal from the soil and translocation to the ariel part of the plant.

Mycorrhizal fungi are soil microbes associated with plants to improve their ability to absorb water and nutrients from the soil which enhance plant growth, in this case biomass production and chlorophyll content (Pawlowska & Charvat, 2004; Saxena et al., 2017). Mycorrhizal fungi were added to enhance biomass and chlorophyll content in *Tamarix* with the aim of improving the plant growth. As expected, our study showed a significant improvement in biomass production and chlorophyll content in *Tamarix* as a result of mycorrhizal fungi when comparing between the

controls (without any Cd). Contrary, the presence of mycorrhizal fungi in the Cd treated *Tamarix* did not play any role in alleviating Cd toxicity by enhancing the plant biomass production and chlorophyll content as a significant reduction in both physiological parameters were observed for both *Tamarix* species. Therefore, our investigation indicates that mycorrhizal fungi did not play any role in enhancing biomass production and chlorophyll content in the presence of Cd. There has been report that heavy metal presence in the soil can cause delays, decreases (Joner & Leyval, 1997; Leyval et al., 1997, Repetto et al., 2003) and the removal of AMF colonization within the soil when present in high levels. This contributes to a disturbance or possibly a restriction of the mycorrhizal and plant beneficial association (Lingua et al, 2008).

The addition of mycorrhizal fungi into the Cd treatment seems to have no effect on increasing the the uptake of the Cd into the roots of *Tamarix* as there was a reduction in Cd concentration in the roots of *Tamarix* in the presence of mycorrhizal fungi. In addition, the highest Cd concentration was obtained in the roots of *T. usneoides* in the Cd only treatment group. The presence of microorganisms in the soil rhizosphere may play a role in decreasing metal uptake by plants through a process of microbial biosorption, thus reducing the transfer of heavy metal to the plant roots and shoots (Joner & Leyval, 1997; Joner et al., 2000). However, the mechanism of the decrease in uptake is not well understood (Joner & Leyval, 1997). Similarly, a study by Huang et al. (2005) found that accumulation of Cd in the roots and shoots of plants inoculated with mycorrhizal fungi had significantly lower Cd concentration than plants without mycorrhizal fungi when treated with Cd. In their study they showed that mycorrhizal fungi may be effective in restricting excessive uptake of heavy metal by host plants. In contrast, our study found that the addition of mycorrhizal fungi had a positive effect in the Cd accumulation in the shoots of *Tamarix*. Additionally, the exotic *T. chinensis* had higher Cd concentration in the shoots than the indigenous *T. usneoides*. While *T. usneoides* showed a greater ability to accumulate Cd in the roots which could be a mechanism to tolerate heavy metals, on the contrary *T.*

chinensis displayed a greater translocation ability of the metal from the root to shoots. This indicate *T. usneoides* was able to uptake remove Cd from the soil better than *T. chinensis* and it is better suited for use in phytoremediation

The BCF and TF ratios assist with evaluating the potential plants have in accumulating metals (Sidhu et al., 2018). Both *Tamarix* species resulted in BCF values >1 showing their positive ability to take up Cd from the soil in the absence of mycorrhizal fungi. However, the BCF value of *T. usneoides* showed significantly greater and was double that of *T. chinensis*. However, the addition of the mycorrhizal fungi did not improve or enhance Cd uptake and accumulation in the roots of *Tamarix* as the BCF <1. Plant accumulated high Cd concentration in the roots and reduced cadmium translocation in the shoots (Sruthi & Puthur, 2021). Benavides et al. (2005) found that the uptake of Cd in most plant species is normally confined to the roots as a strategy to manage heavy metal induced stress. When *Bruguiera Cylindrica* (L.) Blume plantlets were exposed to cadmium chloride and sodium chloride in a hydroponic experiment, the TF results were low (0.009) as the plant accumulated high Cd in the roots and reduced cadmium translocation to the shoots (Sruthi & Puthur, 2021).

The TF values of *T. usneoides* and *T. chinensis* grown in the Cd only and Cd with mycorrhizal fungi treatments were <1 indicating that the plants were not effective in the translocating of Cd from the roots to the shoots. Our findings suggest that Cd was sequestrated more in the roots than in the shoots of *Tamarix* which could be the strategy for adaptation to avoid the effects of metallotoxicity by the plants (Newete et al., 2016; Sruthi & Puthur, 2021). Du Laing et al. (2009) reported plants with the ability to accumulate heavy metals in the roots are most likely to transfer less heavy metal into the shoots. The low TF observed in both species indicates that these plants have adapted to compartmentalize the Cd elements safely the cell walls and intercellular spaces in the roots (Sruthi & Puthur, 2021). In plants, the roots are the first to encounter Cd in the soil. Cadmium is retained in the roots and less Cd is translocated to the shoots (Padmaja et al., 1990).

Conclusion

All *Tamarix* plants are generally considered very good plants for phytoremediation in mining areas (Weiersbye et al., 2006; Mayonde, 2013). The study shows that although, Cd resulted in a decrease in plant biomass and chlorophyll production, *Tamarix* plants were able to tolerate and uptake Cd from the soil. *T. usneoides* showed greater ability to uptake Cd from the soil into its roots compared to *T. chinensis* which had more Cd in the shoots than in the roots. The addition of mycorrhizal fungi did not enhance tolerance to Cd toxicity and did not play a role in increasing the uptake of Cd both species. This study indicates that Cd had an impact on the physiology of both *Tamarix* plants, for more so on *T. usneoides* than *T. chinensis*. This did not hinder their capabilities to uptake and accumulate Cd from the soil into their roots and shoots. In the absence of mycorrhizal fungi, *T. usneoides* displayed greater capabilities of removing cadmium from soil compared to *T. chinensis* hence it is better candidate for use in phytoremediation.

Recommendations

The remediation of contaminated soil using plants is more cost effective compared to all other conventional methods and is sustainable (Pandey et al., 2019). Any future research on this topic may focus on the measurement of more physiological characteristics such as relative water content, leaf gas exchange, root and shoot biomass that are affected by of cadmium at different cadmium concentration and over a longer period. Optimise the growth condition of *Tamarix* to ensure optimum plant biomass is achieved for both plant species before treatment of the plants with cadmium. Selection of mycorrhizal fungi that is associated with *Tamarix* species in South Africa and allow for longer time period of root colonization prior to the application of Cd to the soil and to check mycorrhizal fungi colonization at the end of the experiment to justify their effect. To increase the number of replicates used in the study so that plants with optimum biomass can be used to conduct the cadmium experiment.

Project Limitations

A National Lockdown due to Covid-19 pandemic from the 26 March 2020 in South Africa was declared nationally and regular access to the facilities where the experiment conducted at University of the Witwatersrand was restricted. Thus, cadmium treatment of the plants was delayed due to the lockdown. The plants received water once a week during lockdown up to July 2020 when National Lockdown restrictions were eased and limited accessed was permitted at University of the Witwatersrand. Some plants had died, the surviving plants did not have big biomass and did not grow well because of pests after not being unattended to for weeks because of the National Lockdown due to Covid-19. The after plants were monitored for the development of new shoots and biomass growth, but some continued to die. The number of plants for the experiment was further reduced.

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