The efficiency of phytoremediation using *Panicum maximum* and TiO_2 nanoparticles

By

Nozipho Sinenhlanhla Cibane

Student Number: 21143665

Dissertation in fulfilment of the requirement for the degree

Masters of Applied Sciences

In

Chemistry

in the

Faculty of Applied Sciences

at the

Durban University of Technology

Supervisor: Prof P.S. Mdluli

Co-supervisor: Prof K.G. Moodley

Co-supervisor: Prof G.D. Arthur

2021

Declaration

I Nozipho Sinenhlanhla Cibane, declare that:

This dissertation is wholly my work, and that all the references to the best of my knowledge, are accurately reported.

This work has not been submitted for a degree at any other university.

This thesis does not contain text, graphs, or tables copied from the internet unless specifically acknowledged, and the source is cited accordingly.

This thesis does not contain other people's information, diagrams, and pictures, or other data, unless particularly recognized as being sourced from different authors. Written sources have been cited, their words have been re-written yet the general data assigned to them has been referenced and their correct words have been utilized, their written work has been set inside quotes and referenced.

on this _24__ day of _September 2021_

Ms. NS Cibane (Candidate)

on this_24__ day of _ September 2021____

Dr. PS Mdluli (Supervisor)

on this_26__ day of __ September 2021___

Prof. KG Moodley (Co-supervisor)

on this__ day of _29_ September 2021___

Prof. D Arthur (Co-supervisor)

Acknowledgments

My sincerest gratitude goes to Professor Arthur for her unwavering encouragement and support, believing that it can be done and will be done! even when I thought it was impossible, you are a queen! Prof. Mdluli you have given me guidance and your expertise and for that, I am eternally grateful. Prof KG Moodley, I am grateful to have had you as a part of this work, your contribution has seen me to the finish line, thank you.

To Chemistry research group students particularly Mr. Stanley Onwubu for his dedication to excellence and unwavering support, I appreciate your assistance greatly. Mangosuthu University of Technology staff Mrs. X Msimango for use of the facilities and also my manager Dr. Chili who kept a keen interest in my studies from the beginning I am happy you were around to encourage me to do well.

I would also like to extend my appreciation to the Durban University of Technology for allowing me to pursue my studies. My family and friends who have supported my dreams and listened to me moan I thank you. To my children I thank you for your wonderful faces which reminded me that giving up was not an option, you make it all worth it!

To God, the creator of all things and enabler of all that is good

MAKUBENJALO!

Dedication

To my parents who took me to the library and my family at large, I hope you are inspired and proud!

Abstract

This study focused on the application of Panicum maximum (guinea grass) for evaluating the phytoremediation of titanium dioxide nanoparticles ($nTiO_2$). This study was done to explore the ability of *Panicum maximum* Jacq as a hyperaccumulator for phytoremediation of $nTiO_2$. Titanium dioxide has steadily become more abundant in our environment over the years due to human activities, and this could potentially harm the environment. *Panicum maximum* (guinea grass) is a non-vascular plant with a short life cycle. It is well adapted to a wide variety of conditions. It originated from Africa but is presently found and cultivated in almost all parts of the world with tropical climates. It is loosely to densely tufted, with short rhizomous rooting at the lower nodes. Leaf blades are linear to narrowly lanceolate.

Plant to metal oxide nanoparticle interaction was investigated by germination of seeds in the presence of titanium dioxide nanoparticles ($nTiO_2$). The uptake of $nTiO_2$ by *Panicum maximum* Jacq was evaluated after treatment of the seedlings with $nTiO_2$. The synthesized $nTiO_2$ was characterized, using Transmission Electron Microscope, Scanning Electron Microscope. Energy Dispersive Spectroscopy (EDX), and X-ray Diffraction (XRD). The average mean particle distribution was analyzed using Image J. The Image J analysis showed that the average particle distribution of $nTiO_2$ was 9 nm. The TEM and SEM results revealed that the particles in the $nTiO_2$ were spherical in shape. The XRD analysis revealed that the $nTiO_2$ was predominantly 67.1% and 32.9% of anatase and rutile forms, respectively. Metal uptake was analyzed using the Inductively Coupled Plasma – Optical Emission Spectrometer method (ICP-OES) after the plants

were digested using the wet digestion and microwave digestion methods. The ability of the plants to translocate the metals to the aerial parts of the plants (Translocation Factor - TF) was evaluated for the metal using concentration ranging from 5 ppm to 50 ppm. It was observed that the root had the highest concentration of $nTiO_2$ while the lowest uptake was found in the leaf. The TF was highest for the 5 ppm sample. The roots with the shortest length, which indicated stress/toxicity were that of the plants which were treated with 50 ppm of $nTiO_2$. These also had the highest accumulated nanoparticles which suggested that these plants were negatively impacted by a higher concentration of $nTiO_2$. The standard with 5 ppm treatment showed the highest value of the translocation factor which suggested that at this concentration the nanomaterial aided and catalyzed the movement of nanoparticles to the aerial parts of the plant. The results suggested that seed treated with nanoparticles before planting for phytoremediation purposes could increase the metal uptake selectivity.

Table of Contents

Title page

Declaration ii

Acknowledgments iii

Dedication iv

Abstract v

Table of Contents vii

List of Figures x

List of tables xii

List of Abbreviations xiii

1 Chapter 1: Introduction 15

1.1 Introduction 16

1.1.1 Environmental impact of Potentially Harmful Elements 16

1.1.2 Sources of environmental pollutants 17

1.1.3 Mining and its effect on the environment 18

1.1.4 Titanium Nanoparticles 19

1.1.5 Remediation strategies 20

1.1.6 Hyperaccumulator plants 22

1.2 Nano phytoremediation 24

1.3 Hypothesis 24

1.4 Aim of this project 25

1.5 Objectives 26

1.6 Chapter summary 27

2 Chapter 2: Literature Review 28

2.1.1 Phytostabilization 30

2.1.2 Phytoextraction 31

2.1.3 Phytovolatilization 33

2.1.4 Rhizofiltration 35

2.1.5 Rhizodegradation 36
2.2 Hyper-tolerance of plants 37
2.2.1 *Panicum maximum* Jacq 38
2.2.2 Interaction of the plant with nanoparticles 40
2.3 Nanotechnology applications in phytoremediation 42
2.3.1 Titanium dioxide Nanomaterials 43
2.3.2 Application of TiO_2 in Plant Germination and Growth 44
2.3.3 Semiconductor photocatalyst TiO_2 production 45
2.3.4 Chemical priming for enhanced abiotic stresses tolerance 46
3 Chapter 3: Research Design and Methodology 48
3.1 Methods of characterization of $nTiO_2$ 49
3.1.1 X-Ray Diffraction Analysis 49
3.1.2 Energy Dispersive X-Ray Spectroscopy and Scanning Electron Microscope Analysis 50
3.1.3 Transmission Electron Microscopic Analysis 50
3.1.4 Image J analysis 51
3.2 Seedling preparation 51
3.3 Modification of the cell walls of *Panicum maximum* with the synthesized nTiO2 53
3.3.1 Seed culture and exposure 53
3.4 Phytoremediation 54
3.5 Data analysis.. 54
3.5.1 Validity and Reliability 55
4 Chapter 4: Theoretical simulation of the interaction of titanium dioxide nanoparticle with the soil components and plants.. 56
4.1 Introduction 57
4.2 Interaction of $nTiO_2$ with NOM using Molecular Modelling 58
5 Chapter 5: Application of titanium nanoparticles to demonstrate the phytoremediation process.. 70
5.1 Introduction 71

5.2 Results and discussion 73

5.2.1 Structure, Crystallinity, and Mineral composition of the synthesized nano-TiO_2 73

5.2.2 Micro-Analysis and Visual characterization of particle size and shape of nTiO2 75

5.3 Demonstrating the nanophytoremediation process using statistics 79

5.3.1 Evaluating the region with the highest uptake 81

5.4 Phytotoxicity assessment of modified *Panicum maximum jacq* seedling with nanosized TiO_2 85

5.4.1 Effect of nanoparticles on growth and development 86

5.4.2 Effect of concentration and time on absorption of nanoparticle 86

5.4.3 Portal of storage of titanium nanoparticles in the plant 88

5.5 Impact of metal and metal oxide nanoparticles on plants. 88

5.6 Low-Temperature Synthesis of Anatase TiO_2 Nanoparticles for enhancing Photocatalytic Activity 90

5.7 Environmental aspects 91

5.8 Potential use of $nTiO_2$ as a Genetic modifier in food 92

6 Chapter 6 Conclusion and Recommendation 94

6.1 Conclusion 95

6.2 Recommendations 97

7 References 98

List of Figures

Figure 1.1: Schematic pathways of some emerging pollutants from sources to receptors (Stuart *et al.* 2012)........ 18

Figure 1.2: Schematic pathways of some emerging pollutants from sources to receptors (Mrozik and Piotrowska-Seget 2010). 21

Figure 1.3: Mechanisms involved in heavy metal hypertolerance and heavy metal. The spots are indicative of the inside region of the plant wherein the different mechanisms would happen distribution (Rascio and Navari-Izzo 2011). 23

Figure 2.1: Schematic diagram of phytoremediation (Mani and Kumar 2014)........ 30

Figure 2.2: Mechanism of phytostabilization (Galende *et al.* 2014) 31

Figure 2.3: Mechanism of phytoextraction (Sheoran and Poonia 2016) 33

Figure 2.4: Phytovolatilization mechanisms (Sheoran et al., 2016) 34

Figure 2.5: Diagram showing rhizofitration (Yadav et al., 2016) 36

Figure 2.6: Depiction of the Rhizodegradation process by plant (Limmer and Burken 2016) 37

Figure 2.7*panicum maximum* Jacq (http://pza.sanbi.org/panicum-maximum)........ 39

Figure 2.8: Basic plant-nanomaterial interaction (Jiamjitrpanich et al. 2013) 40

Figure 4.1 Schematic representation of uses and dispersion in the environment of nano-TiO_2 and interaction with the plants........ 63

Figure 4.2: Natural Organic models where (a) Citric acid (b) Fulvic acid and (c) Humic Acid 65

Figure 4.3: HOMO and LUMO diagram of the three NOMs........ 66

Figure 4.4: Models of titanium nanoparticles functionalized with Natural Organic models where (a) Citric acid (b) Fulvic acid and (c) Humic Acid 67

Figure 4.5: Radial distribution of titanium nanoparticles functionalized with Natural Organic models where (a) Citric acid (b) Fulvic acid and (c) Humic Acid 69

Figure 5.1: XRD pattern showing (a) crystallinity of $nTiO_2$ and (b) mineral content of $nTiO_2$. 74

Figure 5.2: Micrographs showing particle distribution of $nTiO_2$ where (a) SEM and (b) TEM images 76

Figure 5.3: Particle size distribution of $nTiO_2$ 77
Figure 5.4: Energy Dispersive Spectroscopy spectrum of $nTiO_2$.................................. 79
Figure 5.5: Mean concentration values of phytoremediation 82
Figure 5.6: Region with the highest mean uptake of titanium dioxide........................... 85
Figure 5.7: Relative seed germination inhibition properties.. 86
Figure 5.8: (a) comparison of plant growth (b) inhibited growth 87

List of tables

Table 2.1: Nanomaterial uses in phytoremediation 41

Table 4.1: Summary of studies about plants exposed to nano-TiO_2 in Petri dishes.. 59

Table 1.1: Energy Dispersive Spectroscopy elemental composition of nanoTiO_2 78

Table 1.2: ANOVA tests for different concentration of nTiO_2 in plant samples 80

Table 1.3: Multiple comparison test 81

Table 1.4: Descriptive statistics showing region with the highest amount of uptake of titanium nanoparticle by *Panicum maximum* Jacq region... 83

Table 1.5: Mixed factorial ANOVA test 84

List of Abbreviations

Cadnium	Cd
Degrees Celsius	ºC
Fourier Spectral Infrared Spectroscopy	FTIR
Grams	g
Iron	Fe
Iron oxide	Fe_2O_3
Kilo volt	kV
Liter	L
Meter	m
Milli liter	ml
Milli meter	mm
Nano Titanium dioxide	$nTiO_2$
Nano meter	nm
nanoparticles	NPs
Nitric acid	HNO3
Part per million	ppm
Reactive Oxygen Species	ROS
Scanning electron microscope	SEM
Silver	Ag
Sodium Hydroxide	NaOH
Sodium hypochlorite	NaClO
Titanium	Ti

Titanium Dioxide	TiO_2
Titanium Isopropoxide	$Ti\ (iso\text{-}OC_3H_7)_4$
Transmission Electron Microscope	TEM
Ultra violet	UV
Zinc	Zn
Zinc oxide	ZnO
X-ray diffraction	XRD

Chapter 1: Introduction

1.1 Introduction

The use of nanotechnology as a model to study phytoremediation is a promising solution. The impact of metal oxide nanomaterial in plants is highlighted as follows: nano-anatase TiO_2 has photocatalytic characteristics and improves the light absorbance and the transformation from light energy to electrical and chemical energy Nanoparticles can mediate growth stimulation, seed germination, and plant root growth/elongation. Nanomaterials can increase the proficiency of the uptake of trace and heavy metals in soil by hyperaccumulator plants.tttt

1.1.1 Environmental impact of Potentially Harmful Elements

Lately, there has been a significant concern with respect to urban soil contamination from potentially harmful elements (Wang *et al.* 2012). Reports had shown that the rapid industrialization and urbanization could release various metals and metalloids into the environment (Yuan *et al.* 2014). This has a negative/undesired impact, and puts at risk, the natural environment including the local community (Chang *et al.* 2014; Ingle *et al.* 2014). The pollution of soil and water is the primary pathway for the introduction of heavy elements which are harmful if they are exposed to living things. These heavy metals tend to also pass into the human body via consumption of contaminated food crops, potable water, or inhaling contaminated dust (Mahmood and Malik 2014; Kutty and Al-Mahaqeri 2016). The World Health Organization (WHO) and many researchers have noted that biophysical threats to agricultural food production, due to land pollution, could pose a global food insecurity to humanity (Sundström *et al.* 2014).

In developing countries, including those in Africa, a large proportion of the populace depends on agriculture for their livelihood. Hence, soil quality ought to be managed critically so as to strengthen and sustain ecosystem services (Lal 20,15). A study by Jaishankar and coworkers suggested that metals have essential, and biologically important functions, in living organisms namely animals and florae (Jaishankar *et al.* 2014). Occasionally, the chemical coordination and oxidation-reduction properties make it impossible for living organisms to exhibit mechanisms that are designed to control processes related to homeostasis, transport, compartmentalization, and binding to cell constituents. This results in the malfunctioning of cell after the removal of heavy metals from the sites exposed to the contaminant. The binding of these metals with protein active sites through the displacing original metals from their natural binding sites can result in a malfunctioning of cells (Jaishankar *et al.* 2014).

1.1.2 Sources of environmental pollutants

Pollution has various sources as depicted in Figure 1.1 below. The accumulation of trace metals can be attributed to mining industries, smelting processes, fertilizers, pesticides, atmospheric deposition, municipal and/or industrial dumping sites. The accumulation of toxic metals has a detrimental impact on the consumer. Potentially harmful elements gain entry into the environment through the anthropological activities. The impacts of the differing potentially harmful elements (PHEs) are not only limited to soil disintegration, natural weathering of the crust of the earth, mining industries, including associated industrial effluents, urban areas overflowing, the releasing of sewage, among other known sources (Lapworth *et al.* 2017).

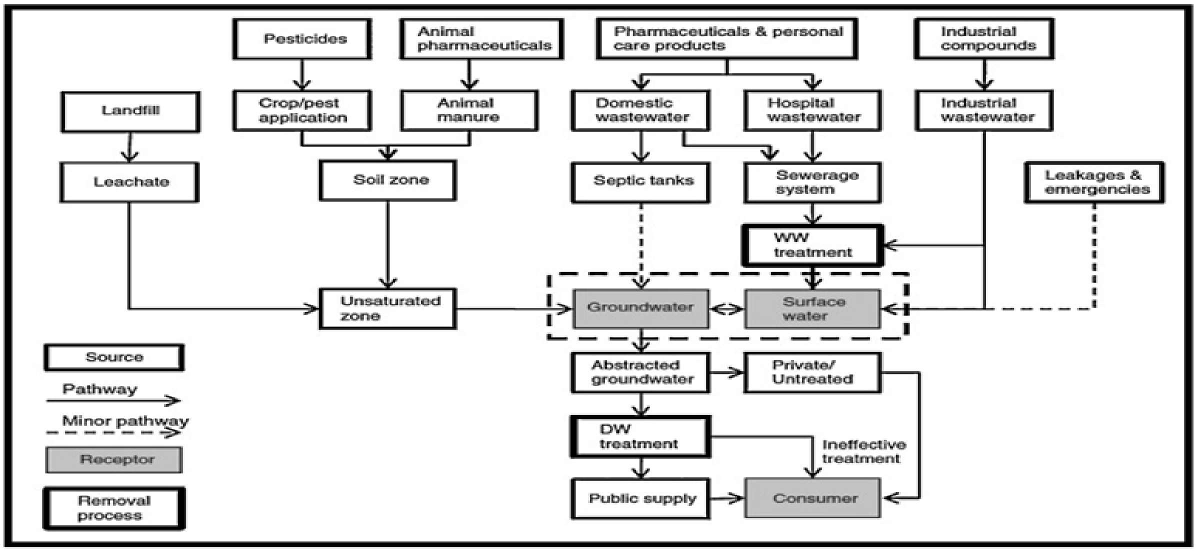


Figure 1.1: Schematic pathways of some emerging pollutants from sources to receptors (Stuart *et al.* 2012)

1.1.3 Mining and its effect on the environment

Mining processes cause leakage of chemicals which, eventually, contaminate soil, groundwater, and surface water (He *et al.* 2012; Fernández Rodríguez *et al.* 2014; Ingle *et al.* 2014; Surriya *et al.* 2015). Pollution caused by heavy metals which are discharged with untreated wastewaters from both urban and industrial uses and mining processes poses a significant threat to ecological welfare and human well-being. The effect of mining depends on numerous variables, specifically, the type of mining and the size of the activity. A standout amongst the most imperative effects of mining is subsidence which is noted in abandoned gold mine sites, which is common in Johannesburg, South Africa.

The topsoil is acidified and contaminated with potentially harmful elements which, often, are eventually leached into groundwater resulting in continued pollution (Naicker, Cukrowska and McCarthy 2003; Bell and Donnelly 2014) The discharge of acid mine drainage has low pH, high specific conductivity, high concentrations of aluminum, manganese, and iron, and the lowered concentration levels of toxic heavy metals (Akcil and Koldas 2006). Lima et al. (2011) found that harmful heavy metals may be transferred through the food chain where they can be ingested, and significantly contribute to an increase in neurodegenerative disorders in people living around these abandoned mines and industrial sites.

1.1.4 Titanium Nanoparticles

Titanium dioxide nanoparticles (TiO_2-NPs) are generally used as parts of commercial items, for example, sunscreens, toothpaste, paint, paper and, polish~~,~~ as well as for photocatalytic processes, for example, water treatment, As a result, TiO_2-NPs are unintentionally released into soils used for agricultural purposes through water system or sewage-sludge applications and become available as nanofertilizers or nanopesticides (Keller *et al.* 2013; Simonin *et al.* 2016).

The presence of TiO_2-NPs contribute to microbial processes which are assumed to play a significant role in plant productivity and biogeochemical processes, for example, the nitrogen (N) cycle, in which nitrification and denitrification processes regulate soil inorganic N accessibility and ensuing soil fertility (Falkowski, Fenchel and Delong 2008; Simonin *et al.* 2016). Diminishing microbial respiration was observed in some studies which were due to a general and decreased enzyme activity of soils exposed to elevated concentration of TiO_2-NPs (Du *et al.* 2011; Xu *et al.* 2015). In the study by Schaumann, it was also observed that, in non-organic matter, the efficiency as a stabilizing agent is increased in titanium dioxide nanoparticles when compared to the silver nanoparticles (Schaumann *et al.* 2015).

1.1.5 Remediation strategies

The remediation of heavy metals in the environment involves the use of conventional technologies namely: digging to excavate (physical removal of the contaminated material), stabilization of the metals in the soil on-site. These methods tend to be destructive and are often very costly to achieve. Hence, phytoremediation, involving the use of growing plants, to inhibit the spread of contaminants or to extract metals from within the soil (Fernández Rodríguez *et al.* 2014), is a potential strategy to increase the efficiency of bioremediation. Phytoremediation is a promising alternative process to recover soils polluted by harmful metals utilizing plants with a high prospect for extraction, referred to as hyperaccumulators (Lum *et al.* 2014).

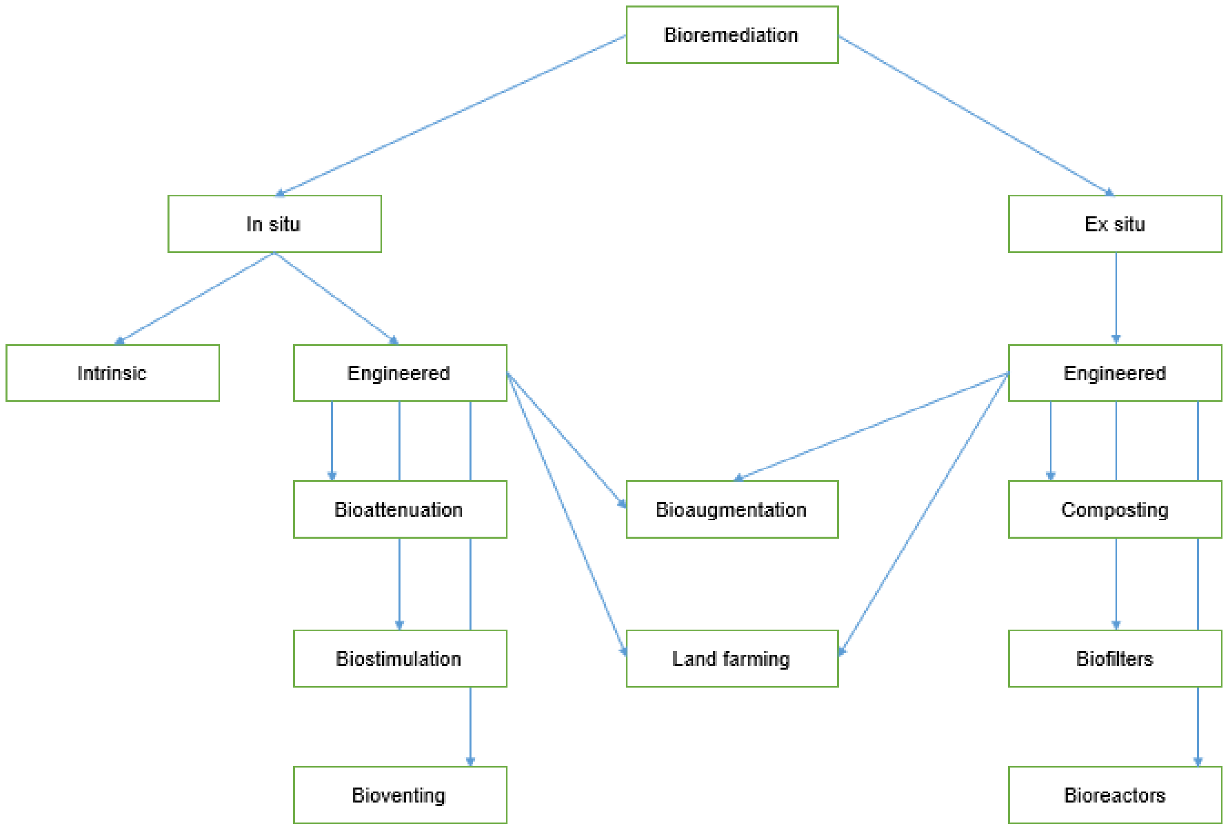


Figure 1.2: Schematic pathways of some emerging pollutants from sources to receptors (Mrozik and Piotrowska-Seget 2010).

Other methods to remediate soil involve the addition of nutrients to contaminated soil, with a specific goal of encouraging the natural occurrence of chemical degradation of microorganisms, termed as biostimulation. Bioattenuation is another technique that is dependent on regular / natural processes to dissipate contaminants through a biological change. Bioaugmentation, where genetically modified microorganisms are added to contaminated waste areas so as to fast track the removal of undesired compounds, can also be used. It was demonstrated that the bioaugmentation approach ought to be applied when the biostimulation and bioattenuation have not been successful (Mrozik and Piotrowska-Seget 2010).

1.1.6 Hyperaccumulator plants

Hyperaccumulators are plants that possess the ability to absorb unusually elevated quantities of heavy metals from soil. There are two classes of metals that are found in soil, the essential micronutrients (Fe, Zn, Mg, Mn, Cu, Mg, Ni, and Mo) which play a pivotal role in plant development and, the nonessential elements that have an unknown biological and/or physiological function (Cd, Pb, Sb, Ag, Cr, As, Co, Se, and Hg) (Rascio and Navari-Izzo 2011). Plants are in need of micronutrients in quantities that are minimal for their sustained growth, development, and metabolism. However, both the concentration of the essential and the non-essential metals is a factor that is important in the growing process. When plants are confronted with high concentrations of these heavy metals they employ a variety of both internal and external resistance strategies for either tolerance and/or detoxification (Emamverdian *et al.* 2015b). In environments of high levels of heavy metals, there is an increased regeneration of the reactive oxygen species (ROS) which can effect oxidative stress. This condition can result in programmed cell death in plants (Rascio and Navari-Izzo 2011). In the review article by Rascio, it was mentioned that the hyperaccumulation of heavy metals by plants is due to a defense mechanism that is utilized by plants to fight against natural enemies, namely, the herbivores, and there are many strategies used as tolerance mechanisms that are for detoxification and are often activated for metal sequestration and also for compartmentalization inside various intracellular compartments as in Figure 1.3 below.

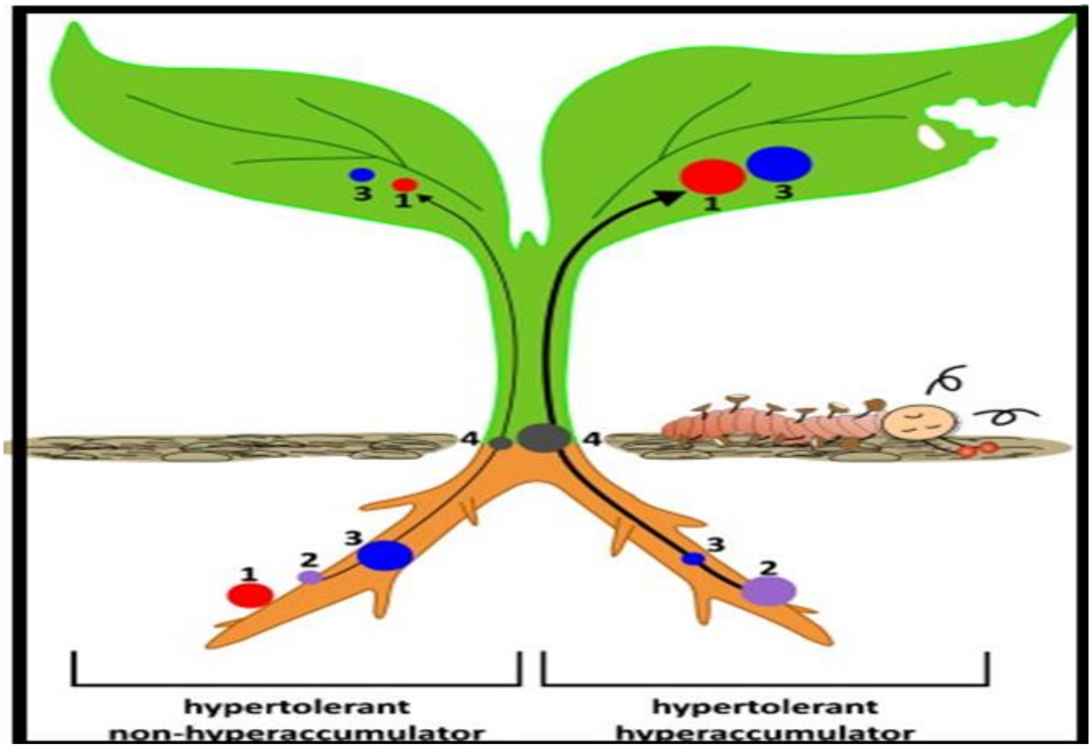


Figure 1.3: Mechanisms involved in heavy metal hypertolerance and heavy metal. The spots are indicative of the inside region of the plant wherein the different mechanisms would happen distribution (Rascio and Navari-Izzo 2011).

Figure 1.3 depicts the processes of hypertolerance, including distribution of heavy metals by either non-hyperaccumulator or hyperaccumulator plants. Firstly, the heavy metal binds within walls of the cell and/or the cell exudates.This is followed by root uptake, then by the chelation inside the cytosol and/or sequestration in the vacuoles, finally translocation from root-to-shoot takes place. The spots are indicative of the inside region of the plant where the different mechanisms take place. The elemental defense hypothesis is that the elevated heavy metal level concentrations render the hyperaccumulator leaves poisonous to the herbivores (Rascio and Navari-Izzo 2011).

1.2 Nano phytoremediation

Nanotechnology has been suggested as a remediation strategy of the environment. It could enable the protection of the environment by removing long-existing hazardous waste (Fernández Rodríguez *et al.* 2014). The innovation of using both nanotechnology and phytoremediation is a novel tactic that will create and actualize cleanup methodologies and advancements that will bring about better, less expensive, and quicker site cleanups. Nanotechnology will broaden the scope of further understanding in this area, and its applications in the fields of chemistry, biotechnology, agriculture, and botany (Arif *et al.* 2016; Hussain *et al.* 2016)

1.3 Hypothesis

Nanotechnology affords the ability to enable the manipulation of matter at the molecular level. It can then be used in developing remediation tools that will be specific for any metal pollutant. It, therefore, can increase the affinity, selectivity, and sensitivity of the selected technique. In the past, cleanup methods have generally been laborious and time-consuming. There is a need for the uptake of the metal to be efficient and/or optimised, as well as need to catalyse the process of phytoremediation. This research project, therefore, aims to improve the phytoremediation traits of *Panicum maximum* Jacq and to provide a better understanding of possible alterations caused by the exposure of seeds to nanoparticles of Titanium dioxide, nanoparticle-assisted phytoremediation, and also to simulate the mechanism of absorptionof $nTiO_2$ by plants.

It is hypothesized that

- Nano-anatase TiO_2 can be absorbed by the plant and be translocated to leaves and other plant parts.
- Nanoparticles can mediate growth stimulation, seed germination, and plant root growth/elongation.
- Nanomaterials can increase the selectivity and proficiency of the uptake of metal in soil by hyperaccumulator plants.

1.4 Aim of this project

To investigate the efficiency of nanophytoremediation using *Panicum maximum* Jacq as the hyperaccumulator plant and titanium dioxide nanoparticles. The study was divided into two parts. The impact of a metal oxide nanomaterial in a grass species was studied. Initially, experiments were designed to evaluate the growth potential of *Panicum maximum* Jacq under stress from a metal oxide, which is a trait hyperaccumulator plants exhibit for efficient phytoremediation.

1.5 Objectives

The main objectives of this project are:

- To determine the germination of *Panicum maximum* Jacq seeds in different concentrations of synthesized Titanium dioxide nanoparticles in respect of germination rate, germination percentage, germination index, relative seed germination inhibition, and root length as evaluated plant growth parameters.

- To investigate the strategy used by the plant for metal accumulation, a factor that would affect bioremediation.

The above aims were achieved by fulfilling the following objectives:

- Cultivating *Panicum maximum* Jacq in a growth chamber under controlled conditions
- Treating seeds with different concentrations of Titanium dioxide heavy metal nanoparticles, and then exposing them to a constant amount of nanoparticles in soil.
- Using ICP-OES to determine the concentration of Titanium in the different parts of the plant.

1.6 Chapter summary

Research and development of engineered nanomaterials have improved the understanding of applying engineered nanomaterials to plants. Nanophytoremediation is the result of the improvement associated with a variety of economical applications for contaminated site remediation initiatives. An application of engineered nanomaterials is a novel technique worth exploring. The behavior of a nanomaterial is reflective and dependent on the different environments and conditions it is exposed to. According to the review article by Aslani and colleagues, surface functionalization of the material, and non-covalent modification of the metal are among some of the studied traits of engineered nanoparticles (Aslani *et al.* 2014). The work, presented herein, sought to use $nTiO_2$ because of its optical properties which would affect the photosynthetic properties of the plant used.

This dissertation is submitted as six chapters. The first chapter is the introduction, followed by the second chapter which is a literature review giving an overview of phytoremediation and nanotechnology applications. Background of the current knowledge regarding the metals used in the study is given in chapter two. The third chapter outlines the methodology used. The fourth chapter demonstrates the molecular modeling of the interaction of $nTiO_2$ with natural organic material that is found in soil. The fifth and sixth chapters are the results and discussion followed by the conclusion and recommendations chapters respectively.

Chapter 2: Literature Review

2.1. Phytoremediation

Phytoremediation has been defined by the United Nations Environment Programme, as 'the use of living green plants for in situ removal, degradation, and containment of contaminants in soils, surface waters, and groundwater.' Contaminated soil with heavy metals is a genuine source of concern in many nations. Ecologically rehabilitating these spoiled soils in the agricultural, industrial, horticultural and urban territories has proven to be challenging in the recent decades as a result of anthropogenic activities, as noted by researchers (Mani and Kumar 2014) and in the review article of Mahar (Mahar *et al.* 2016). In contrast to pollutants that are organic, simple biodegradation of heavy metals is somewhat out of the question. Therefore, over time, we find these persistently accumulating in our environment. The modern-day technologies of phytoremediation are particularly based on the different uptake mechanisms of plants as illustrated in Figure 2.1 below. These include phytostabilization, rhizofiltration, phytoevaporation, phytoextraction and rhizodegradation.

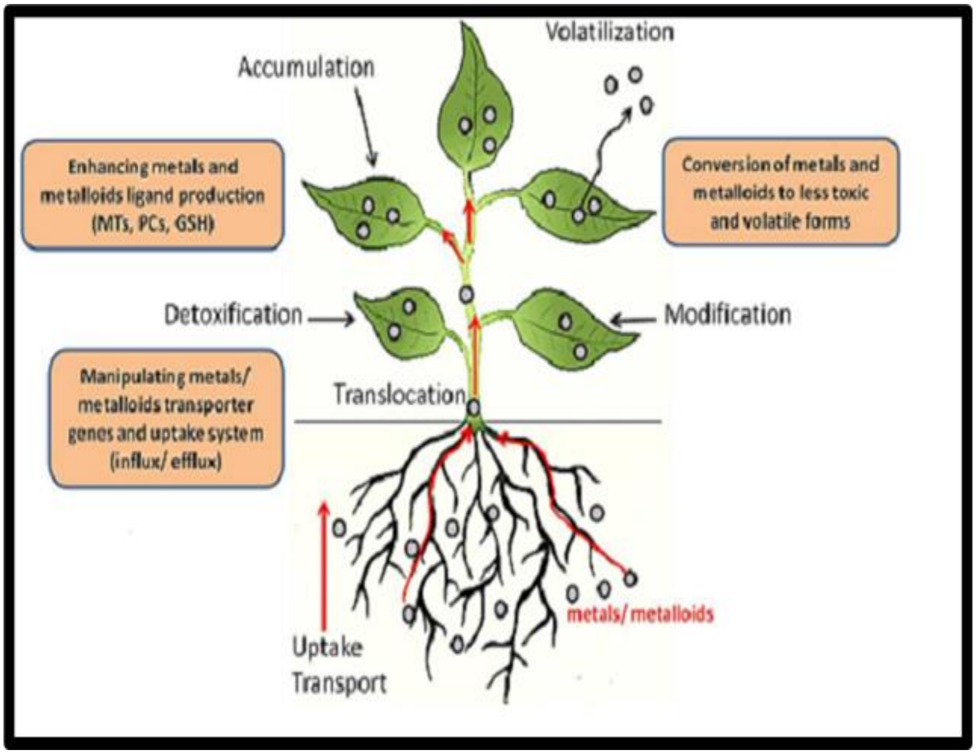


Figure 2.1: Schematic diagram of phytoremediation (Mani and Kumar 2014)

2.1.1 Phytostabilization

Phytostabilization results in a decrease of heavy metal mobility in soil. It involves microorganisms in plants as a way to reduce soil metal bioavailability (Galende et al., 2014. Amendments to the soil can diminish the dissolvability of metals within the soil and limit filtering into groundwater (Galende *et al.* 2014; Mahar *et al.* 2016). The mobility of the contaminants is lessened by an accumulation of contaminants by roots, adsorption onto roots, and precipitation inside the root region. These amendments of the plant parts due to the increased exposure can directly alter soil conditions influencing the mobility of metal contaminants. Assisted phytostabilization permits the reuse of agricultural and industrial spaces (Sarwar *et al.* 2017). The associated costs are significantly lesser with this technology though the site continuously needs to be reworked to keep the conditions stable. Therefore, this solution is not permanent, because the metals remain within the soil only with a restricted/limited movement (Galende *et al.* 2014).

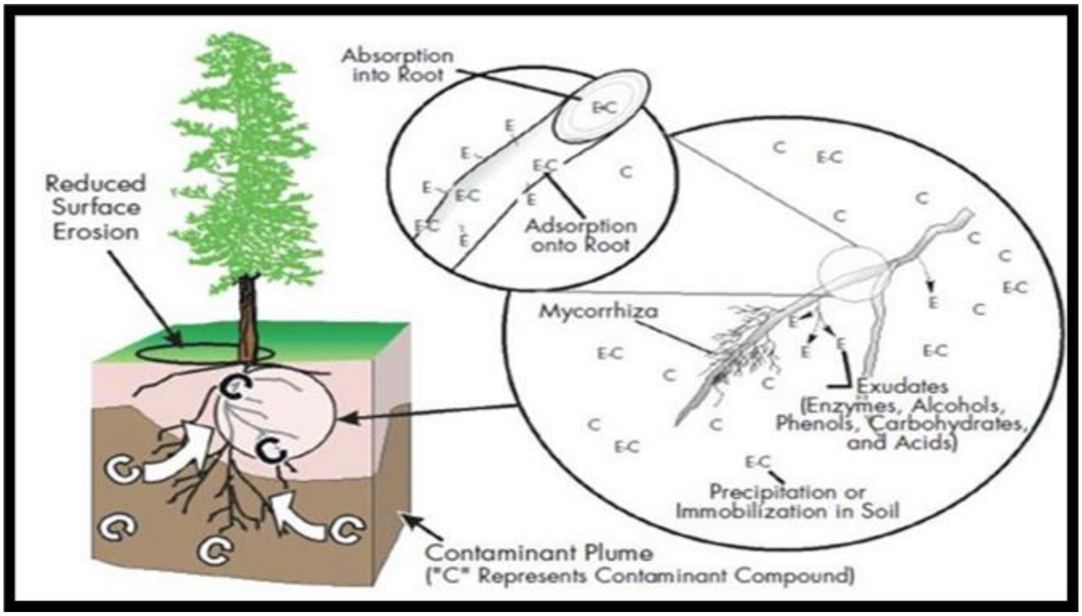


Figure 2.2: Mechanism of phytostabilization (Galende *et al.* 2014)

2.1.2 Phytoextraction

Phytoextraction is the utilization of plants including related microorganisms to remove metals in the soil so that they can be accumulated on the part above-the-ground into the harvestable parts as shown in Figure 2.3 below. Phytoextraction is a promising technology that makes use of the part of the plant that is harvestable to remove pollutants. This technology represents a green and environmentally conscious instrument for remediating metal-polluted waters and soil (Sheoran, Sheoran and Poonia 2016; Sarwar *et al.* 2017). The afore mentioned researchers noted that, apart from soil cleaning by phytoextraction, it can also prove invaluable for collecting precious metals such as gold, thallium, platinum, and nickel, which may be recovered through phytomining for commercial purposes. The desirable qualities of a plant species/variety for consideration as an efficient one for phytoextraction are quick development including high biomass, an extension of the root system, great resilience to a higher concentration of metals in plant tissue, higher translocation factor, adaptability into particular condition/site, and easy agricultural management (Sheoran, Sheoran and Poonia 2016). Generally, the order for the affinity for metal cations in complex by organic matter is: Cu^{2+} > Cd^{2+} > Fe^{2+} > Pb^{2+} > Ni^{2+} > Mn^{2+} > Co^{2+} > Mn^{2+} > Zn^{2+}. Organic components of soil constituents containing a higher affinity for metal cations due to the presence of ligands or groups that act as chelating agents. As the pH is increased, carboxyl, the phenolic, the alcoholic, and the carbonyl functional group in the organic matter dissociates, there is an increase in the affinity of ligand ions for the metal cation. (Niaounakis 2013). The utilizing of genetic engineering as a way to improve phytoextraction aims at controlling the plant's ability to be tolerant, to accumulate, and metabolize pollutants. Accumulation and tolerance are to a great extent exclusive/independent properties. With these in mind; the engineering ought to exploit the plant for the metal extraction (Niaounakis 2013; Sheoran, Sheoran and Poonia 2016).

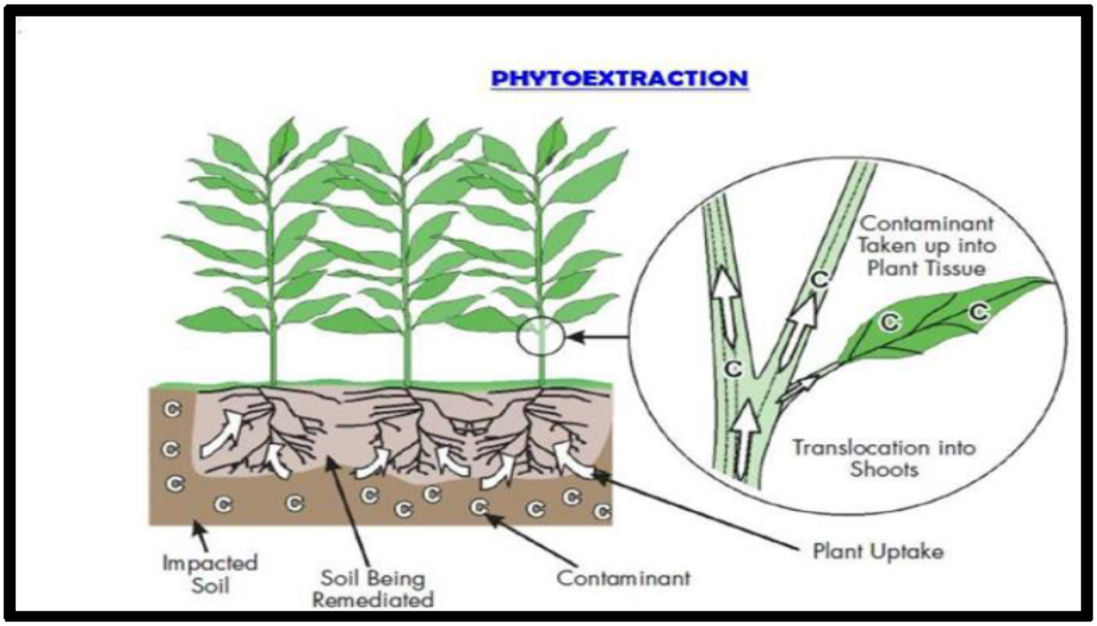


Figure 2.3: Mechanism of phytoextraction (Sheoran and Poonia 2016)

2.1.3 Phytovolatilization

Phytovolatilization involves the dividing of a contaminant into the vacant air spaces within a plant, followed by the ensuing release into the air around it. This assumes that the ambient air is significantly less polluted. Phytovolatilization is regularly viewed as being advantageous since phytovolatilization of the contaminant results in a decrease in concentration and photochemical decay in the environment. As an alternative, phytovolatilization might be seen as a hazard in urban zoned areas, where it may present potential air quality-associated risks (Sheoran, Sheoran and Poonia 2016). Phytovolatilization is in two unique forms: that is, direct and indirect phytovolatilization. The direct form is the more examined form, coming about because of plant take-up and translocation of contaminants; eventually at the end prompting volatilization of a compound from stem/trunk and leaves. The direct pathway of phytovolatilization frequently differs from transpiration. Compounds phytovolatilized are reasonably hydrophobic, ready to be diffused over hydrophobic boundaries such as a cutting in the epidermis. It was noted that for direct phytovolatilization to take place the plant needs to take-up, then translocate and volatilize the compound. Thus volatilization of compounds made and/or changed by plants are not viewed as specifically phytovolatilized. Indirect phytovolatilization increases contaminant flux removal from the subsurface arising from root activities.

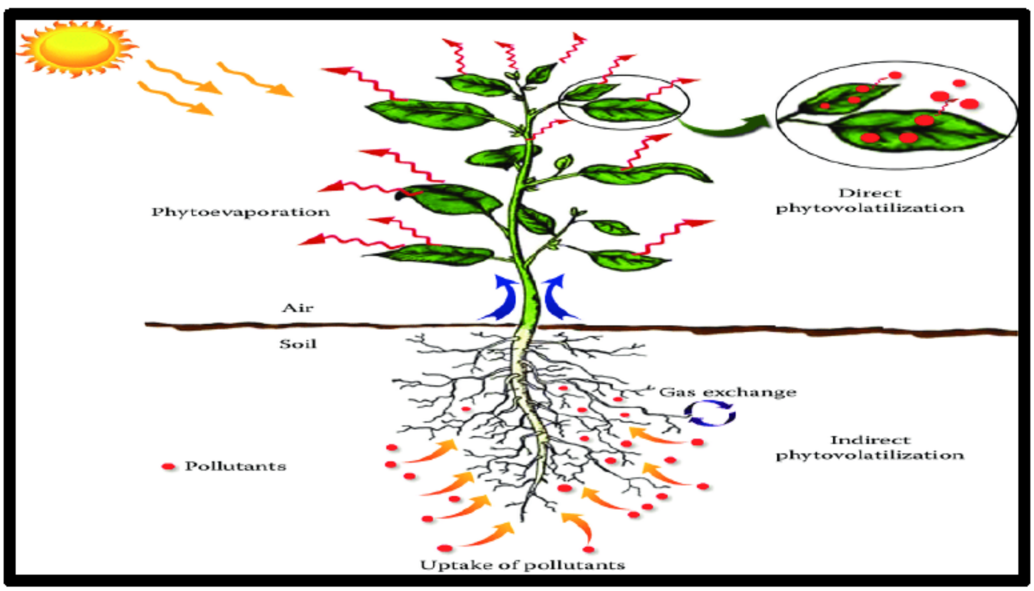


Figure 2.4: Phytovolatilization mechanisms (Sheoran et al., 2016)

2.1.4 Rhizofiltration

Rhizofiltration includes using plants as absorbent, as well concentration, and precipitation of low-concentration contaminants from the roots as shown in Figure 2.5 below. This procedure may be applied for both *in situ* and *ex-situ* of other species other than hyperaccumulators. In the work of Limmer and Burken, they noted that, in rhizofiltration, the terrestrial plants, instead of aquatic are utilized in light of the fact that they form extensive roots systems which are secured with root hairs, and in this way have more surface area than the others. Ideally, the characteristics of a plant that is utilized for rhizofiltration must absorb high amounts of metals and ought to be easy to handle, have low maintaining cost, and deliver insignificant waste that may require easy disposal. These plants must produce significant root biomass or have an extensive root surface area (Limmer and Burken 2016). In the review by Yadav and coworkers, it was noted that initially, plants that are in direct contact with the contamination, would assimilate contaminants up through the root system, resulting in the storage in the root biomass. The plants would retain the contaminants until the time when they are gathered for harvesting (Yadav et al., 2016). The procedure has been found to be more suitable for concentrating and precipitating heavy metals than organic contaminants.

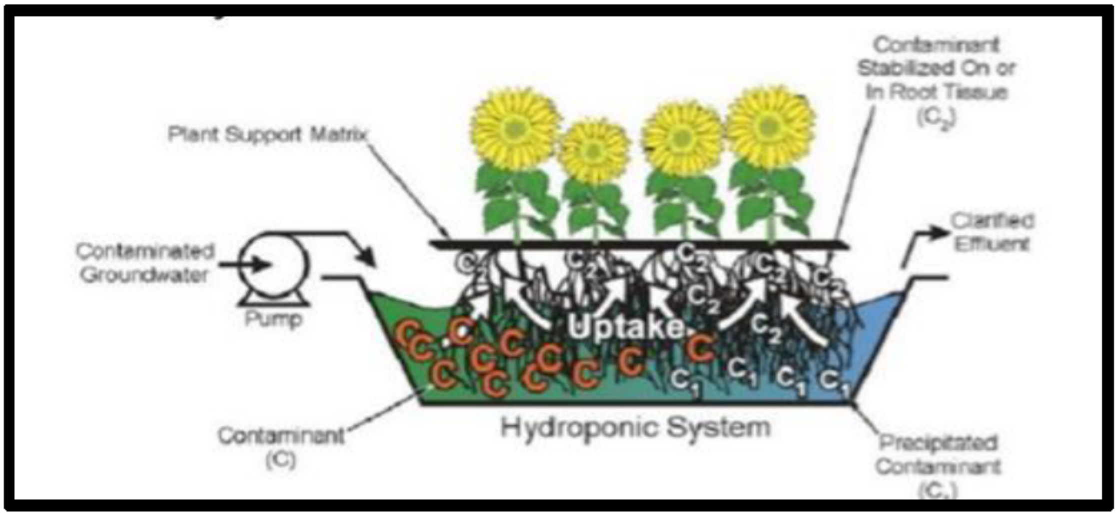


Figure 2.5: Diagram showing rhizofitration (Yadav et al., 2016)

2.1.5 Rhizodegradation

Rhizodegradation which is also known as phyto-stimulation is the breaking down of toxins in the rhizosphere (soil around the roots) through microbial activity that is improved by the presence of plant roots as shown below in Figure 2.6. This process is a much slower process than phytodegradation. In this case, micro-organisms are able to process natural substances, namely fuels or solvents that are a hazard to humans and convert them into harmless products in a procedure called biodegradation. In this process, it is conceivable to create transgenic plants with enhanced plant-organism interaction. The plant would be enhanced in its capacity to discharge natural substances, which invigorate microbial action.

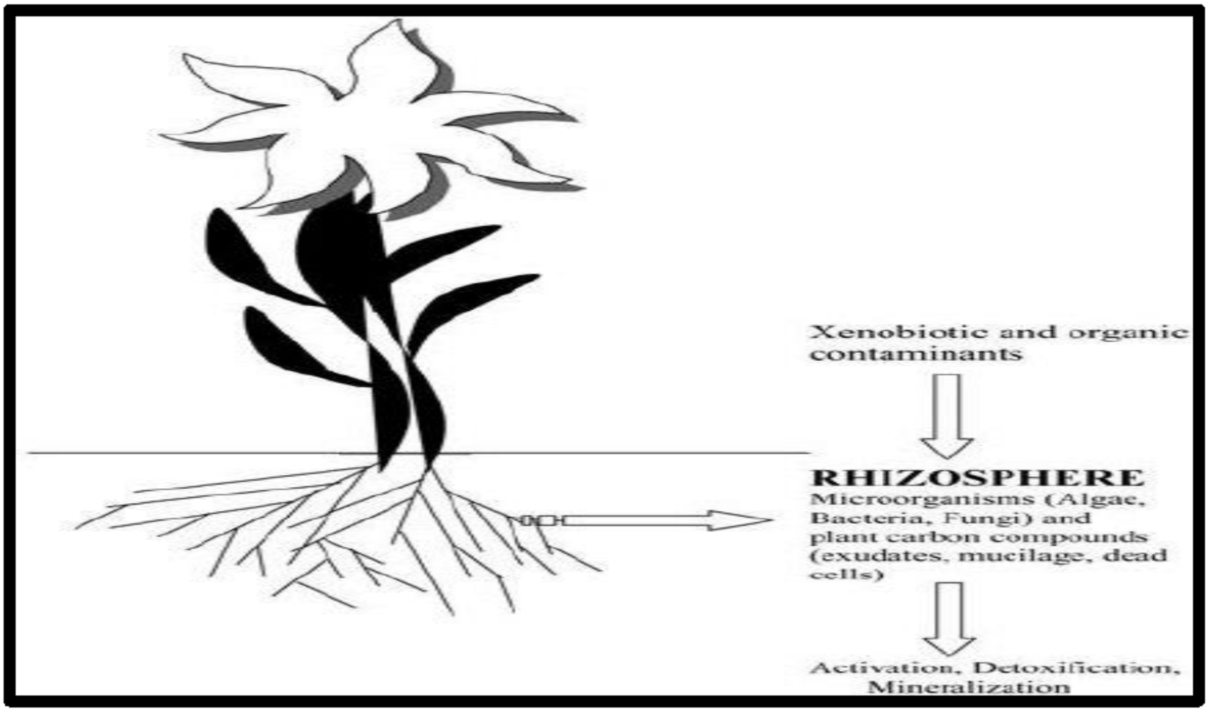


Figure 2.6: Depiction of the Rhizodegradation process by plant (Limmer and Burken 2016)

2.2 Hyper-tolerance of plants

There are three procedures that make a major contribution to the ability of a plant to hyperaccumulate/hypertolerate metals namely, upgraded root uptake and stacking into the xylem, better root-than shoot translocation; and productive detoxification via chelation and sequestration, dominantly inside leaf cell vacuoles. The productivity and time it takes to effect clean-up via phytoremediation are determined by the type of the plants and population on the contaminated site, and the concentration of contaminants. Other factors that may contribute to the proper phytoremediation process is the degree of contamination in soil. However, the prevailing soil conditions may differ with different locations and soil class (Yadav *et al*., 2017). In a report by Messou *et al*. (2013), it was found that root exudate of a plant due to overwhelming metal exposure is important to ensure the effective application of phytoremediation. Root exudates can activate heavy metal in soil, as a result, they enhance the phytoavailability through dissolving, chelating, deoxidizing, and diminishing the portability of heavy metals through inactivation and fixation by some plant species; apparently as a result of adjusting mechanisms that may be likely to be engaged with general homeostasis (Yazdanpanah and Rajaei, 2016).

2.2.1 *Panicum maximum* Jacq

The South African Institute of biodiversity (http://pza.sanbi.org/panicum-maximum, accessesd 2019) reports that *Panicum maximum* Jacq is a perennial and tufted grass consisting of a short and crawling rhizome. The stems of this strong grass are able to achieve a height of up to 2 meters. Leaf-sheaths that are found at the base of the stem are protected in fine hairs. *Panicum maximum* Jacq retains the green colour until late into the winter season. The leaves, having sharp edges, can be up to 35 mm wide and decrease into a long and fine point. The inflorescence is a substantial multiple-fanned, with open panicle, free and flexuous branches. Generally, the lower floret is typically male consisting of a well-developed palea upper bract enclosing flower http://pza.sanbi.org/panicum-maximum, accessesd 2019.

Figure 2.7*panicum maximum* Jacq (http://pza.sanbi.org/panicum-maximum)

It is viewed as one of the most significant fodder plants in the area where it is distributed. The production of seed and leaf is very high and is exceptionally tasteful to game and also domesticated animals. It is generally cultivated as a pasture grass and used particularly to make a great quality feed. In the event that it gets satisfactory water, it develops quickly and occurs in abundance in veld that is maintained in a decent condition. It is a plant whch is naturally abundant in urban gardens to give nourishment source to little birds. In an urban setting, the short-life cycle of this grass can be exploited in relation to the rapid and efficient phyto-procedures upon optimization over several successive remediation sites of contaminated areas (Jiamjitrpanich et al., 2013).

The processes of phytoremediation of this grass involve phytostabilization wherein the contaminants inside the soil are immobilized by means of the plant roots (Limmer and Burken 2016). In phytofiltration, the plants uptake the contaminants by binding them in their roots and shoots and also through phytodegredation or rhizodegradation where the contaminants are degraded and transformed to its metabolites within the plant or rhizosphere and finally through phytoextraction where the contaminants are taken up and stored in the aerial part of the plants.

2.2.2 Interaction of the plant with nanoparticles

Nanoparticles interfere with the plants' metabolism in a few different ways; for example, by giving the micronutrients needed, by regulating genes, or even meddling with various oxidative processes in plants which may result in oxidative burst as shown in Figure 2.8 (Jiamjitrpanich et al. 2013). Nanoparticles give rise to ROS generation and interfere with the oxidative mechanism. However, there is still much work required to comprehend alternate pathways (Messou *et al.* 2013).

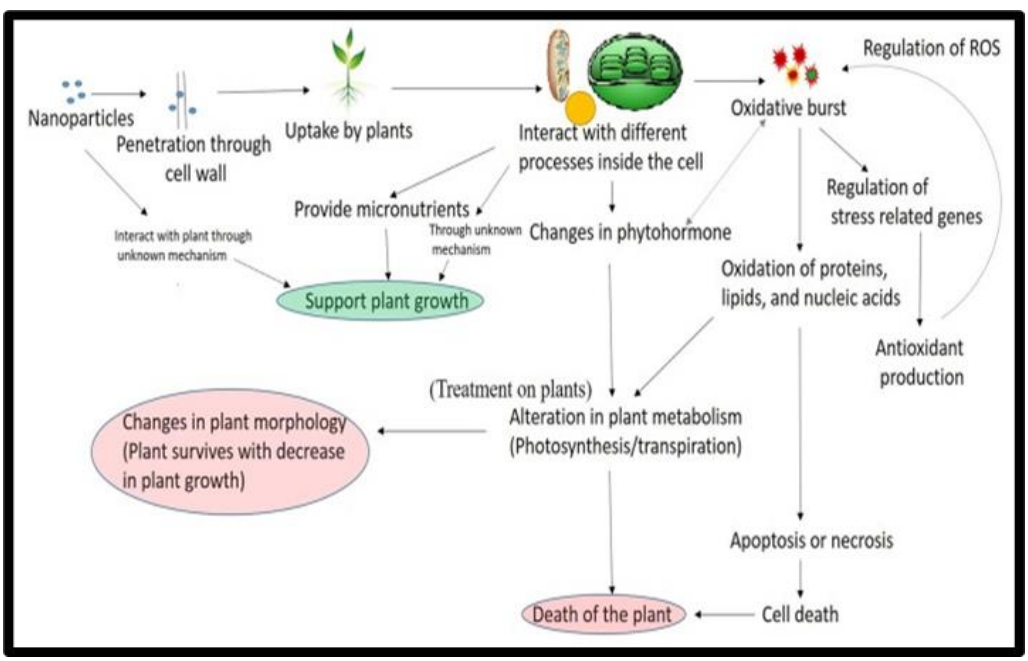


Figure 2.8: Basic plant-nanomaterial interaction (Jiamjitrpanich et al. 2013)

Table 2.1: Nanomaterial uses in phytoremediation (Rastogi et al., 2017a)

	Compounds targeted	**Nanomaterial used**	**Novel properties**
Photocatalysis	Organic pollutants, VOC, Congo red and azo dye, PAH, NOX 4*chlorophenol, and Azo dye	Oxides of titanium and zinc, Species of iron oxides	Solar spectrum photocatalytic activity, the toxicity toward humans is low, selectivity and the stability is high, with low costs.
Adsorption	Organic compounds, arsenic, mercury, PAHs, phosphate, dioxin, heavy metals, DDT	Oxides of iron, C-based nanomaterial e.g dendrimers, polymer and carbon nanotubes	Specific surface area is higher and assessable sites of adsorption, they also more selective. Short intra-particle diffusing distance, easier reuse, and tuneable surface chemistry
Membranes	1,2*dichlorobenzene, Chlorinated compounds, inorganic/organic solutes (including halogenated)	TiO_2/Magnetite/ NanoAg/CNT and Zeolites	Antimicrobial activity is strong, hydrophilicity, high mechanical/chemical stability, low toxicity to humans, photocatalytic activity
Disinfection	Phenol, H_2O_2, silver ions, glutaraldehyde, formaldehyde, diamines	nanosized titanium/silver dioxide and CNTs	low toxicity to humans, low costs, strong antimicrobial activity, highly chemically stable
Redox reactions			

metals, arsenate, PAH, nitrate, PCB, Halogenated organic compounds	nanosized zero-valent iron, nanoscale peroxide of calcium	Antimicrobial activity is strong, hydrophilicity, high mechanical/chemical stability, low toxicity to humans, photocatalytic activity

2.3 Nanotechnology applications in phytoremediation

Nanotechnology involves the utilization of very small particles (<100 nm), called nanoparticles (NPs). Nanoparticles are molecular aggregates with measurements somewhere in the range of 1 and 100 nm that can be drastically changed with regard to the physico-chemical properties when compared to their bulk material. Various researchers (Khan *et al.* 2014; Olatunji *et al.* 2014; Rastogi *et al.* 2017a; Rastogi *et al.* 2017b) reported that new applications of nanomaterials are being discovered by the treatment of existing environmental contaminants and counteracting new contamination NPs can be utilized in the treatment of different polluted media by synthetically changing contaminants or as a super adsorbent (Liu and Lal 2015). NPs have an additional ability to provide a fundamental role in improving quick and precise environmental sensors which can be utilized in the location of contaminants at atomic levels and to inactivate destructive microorganisms. NPs can be produced using an assortment of bulk materials depending upon the composition, size, and/or shape of the particles desired. Their reactivity and mobility are greater in nature. There are two chemical groupings for NPs, namely, organic and inorganic nanoparticles. The former comprise carbon nanoparticles (fullerenes) while some of the inorganic nanoparticles will include magnetic nanoparticles, noble metal nanoparticles (e.g. gold and silver), and semiconductor nanoparticles (e.g. titanium dioxide and zinc oxide) (Khan *et al.* 2014; Olatunji *et al.* 2014; Rastogi *et al.* 2017a; Rastogi *et al.* 2017b).

2.3.1 Titanium dioxide nanomaterials

The $nTiO_2$ has been reported to be influencing the electron transfer and water absorption, in plants. $nTiO_2$ also affects the photosynthetic processes. The impacts on photosynthesis can be ascribed to the photocatalytic and the thermo-conductivity properties of this material, which was reported to be nanosized TiO_2 (Liu and Lal 2015; Rastogi *et al.* 2017b).

$nTiO_2$ possesses numerous applications across several fields that include photocatalysis, agribusiness, dye-sensitized solar cells, and biomedical gadgets (Rastogi *et al.* 2017b). In the farming sector, the utilization of $nTiO_2$ is moderately novel and requires some further investigation. The properties of nanosized TiO_2 as photo semiconductors have kept on attracting the interest of agricultural researchers and analysts; due to its good physical/chemical properties, cost-effectiveness, and higher stability (Khan *et al.* 2014).

2.3.2 Application of TiO_2 in plant germination and growth

Lately, a number of researchers have investigated the effects that nanomaterials have on plant germination and growth as means of promoting the usage of nanomaterials for agricultural-based applications (Rastogi *et al.* 2017b; Yadav *et al.* 2017). TiO_2 nanomaterials can induce the production of reactive oxygen, including superoxide and hydroxide anions. This reactive oxygen aid in photocatalytic processes for primarily building the seed stress resistance. Zheng and coworkers have detailed the impacts of TiO_2 photocatalysts on the development of spinach seeds Zheng *et al.* 2005). There are reports that have indicated that the plants that are obtained from the nano-TiO_2-treated seeds contained a higher dry weight, also a higher photosynthetic rate (Khot *et al.* 2012), and an improved chlorophyll development (Rastogi *et al.* 2017b). This research on titanium dioxide nanoparticles showed that TiO_2 nanomaterials promoted the ingestion of inorganic micronutrients and increased the photosynthetic rate (Zheng *et al.* 2005). The results of the experiments by Song and co-workers demonstrated that the impact of plant development was somewhat more articulated with nanoparticles of TiO_2 than those with bulk TiO_2 material (Song *et al.* 2012). Research on the introduction of TiO_2 nanoparticles into plants found that these nanomaterials invigorated plant development (Ali Mansoori *et al.* 2008; Suresh 2013). It was demonstrated that leaves of spinach were able to remain green utilizing nano-anatase TiO_2 treatment because of the N_2 fixation (Zheng *et al.* 2005). Some other researchers have shown that the dry weight, the fresh weight, chlorophyll, the total nitrogen content NH_4^+, and protein in spinach were increased due to $nTiO_2$ (Ali Mansoori *et al.* 2008), Raliya *et al.* (2015) and Keller *et al.* (2013), undertook research on the physiological impacts of TiO_2 nanoparticles in mung bean (Keller *et al.* 2013). The outcomes showed a noteworthy increase in plant development for mung bean plants which were treated with TiO_2 nanoparticles. In the control, untreated plants exposed to TiO_2 nanoparticles demonstrated noteworthy results and improvements in the shoot length, root area and the length, and root nodules (Keller *et al.* 2013). It was reported by researchers including Feizi *et al.* 2012a that $nTiO_2$ in a reasonable fixation could advance the seed germination of wheat in contrast with bulk TiO_2, however, in higher concentrations, it had an inhibitory impact on wheat (Ali Mansoori *et al.* 2008; Song *et al.* 2012). The $nTiO_2$ that is present in plant cells or its accumulation on root cell walls of wheat was reported to alter the biochemical conditions at the contact site therefore positively affecting the accessibility of nutrients and subsequently, the growth of the plant (Feizi *et al.* 2012a; Raliya *et al.* 2015).

2.3.3 Semiconductor photocatalyst TiO_2 production

Sol-gel materials as used in this study to synthesize $nTiO_2$ can be used in a varying range of applications, namely; environmental protection, solar cells, etc (Feizi *et al.* 2012a). anatase and rutile phases are both present in the synthesized crystals of $nTiO_2$. In the synthesized sample, if anatase is predominant, the photocatalytic activity will be higher as reported by many researchers (Feizi *et al.* 2012b). The advantage of the sol-gel technology is its ability to control the mechanisms and kinetics of the chemical reactions as reported by Suresh in (Feizi *et al.* 2012a) and the works by Feizi *et al.* (2012b). The large band-gaps of semiconductor titanium dioxide (TiO_2) and zinc oxide (ZnO) are constantly being studied for their ability to remove contaminants from several media. These nanoparticles are activated by light (Suresh 2013). With the photocatalytic oxidation process, the elimination of pesticides in the presence of TiO_2 photocatalyst and a UV light source is possible. The TiO_2 photocatalytic degradation of pesticides relies on generating highly reactive OH radicals, capable of converting pesticides into relatively harmless end-products as reported by Tabaei et al . (2012).

2.3.4 Chemical priming for enhanced abiotic stresses tolerance

Chemical seed priming is the pre-sowing treatment of seeds with a synthetic or organic compound to increase the vigor and uniformity of seedlings and thereby enhancing their tolerance of stress. Pretreating plants at various stages of development, for example, at the germinating stage, or reproductive stage is all aimed at increasing particular or multiple stress tolerance of plants, and there are multiple mechanistic pathways used by plants for this (Suresh 2013). It was noted that some plants display systemic acquired tolerance, and the impact of exposure to stress, on their physiology and growth is lessened in comparison to non-treated plants (Suresh 2013). Chemically pretreating plants can initiate a form of stress cue, inducing an acclimation response that could potentially enhance the tolerance levels of the plant to that stress (Ali Mansoori *et al.* 2008). As with all biotic and abiotic stress in plants, the stress is accompanied by oxidative stress and develops due to reactive oxygen species production. Plants pre-treated with a chemical have also shown enhanced tolerance to over-accumulation of heavy metals such as cadmium amongst others, as in the work of Tabaei, Kazemeini, and Fattahi (2012). The plants have also changed their distribution of the metals within themselves (Savvides *et al.* 2016)

Chapter 3: Research Design and Methodology

3.1 Synthesis of titanium dioxide nanoparticles

The $nTiO_2$ was synthesized following the method described (Tian, 2015) via acid-catalyzed sol-gel formation: to 30 mL of 1 M HNO_3 aqueous solution, 7.4 mL of titanium isopropoxide was added dropwise, and then agitated for 2 hrs to give a transparent sol, which contains 2.0 g of TiO_2. The pH of the colloidal solution was adjusted to pH 3, with the addition of 1M NaOH solution after diluting the colloid with 100 mL of water, resulting in a turbid TiO_2 colloid. The suspension was agitated at room temperature, centrifuged, and then washed with distilled water. The isolated TiO_2 was dried for 1hr at 100 ˚C. The resulting powder was then calcined at 450 ˚C for 3 hrs.

$$Ti\,(iso\text{-}OC_3H_7)_4 + 4H_2O \rightarrow Ti\,(OH)_4 + 4C_3H_7OH$$

The XRD spectrum was obtained using a PANalytical-Empyrean instrument; Co radiation 1.54056 A^o was used to analyse the powder between 0-90^o (2 theta).

3.1 Methods of characterization of $nTiO_2$

3.1.1 X-Ray diffraction analysis

X-ray diffraction (XRD) analysis was performed to determine the crystallinity and phases of the synthesized $nTiO_2$ powder. The XRD patterns were recorded using a diffractometer (PANalytical- Empyrean instrument; Co radiation 1.54056 Å) and analysed between 0-90^o. The voltage, current and pass time used were 40 kV, 40 mA, and 1 s, respectively.

3.1.2 Energy dispersive X-ray spectroscopy and scanning electron microscope analysis

Energy dispersive x-ray spectroscopy was used in conjunction with a scanning electron microscope (Field Emission-Carl Zeiss) operating at controlled atmospheric conditions at 20 kV to examine the surface morphology and the elemental composition of the $nTiO_2$. Prior to SEM observation, the surface was coated with a thin, electric conductive gold film to prevent a build-up of electrostatic charge.

3.1.3 Transmission electron microscopic analysis

A transmission electron microscope was used to observe the particle size, shape, and distribution of $nTiO_2$. Very small quantities of the powder were dispersed in 10ml ethanol and sonicated at 10kv for 10 min. Subsequently, thin cross-sections of cryo-microtomed specimens were prepared using a Leica microtome (South Africa) and placed on carbon copper grids. The analysis was conducted using a transmission electron microscope (TEM-Philips CM 120 model) at 120 kV.

3.1.4 Image J analysis

The particle size distribution of $nTiO_2$ was further analyzed using Image J software. The area of the particle was measured while the diameter of the particle was calculated with the equation below.

$$A = \pi r^2 \qquad \text{equation 1}$$

Where: A= area, π= a constant proportionality given as 3.142, *r* = calculated radius

The diameter was then calculated from the radius with the following equation:

$$D = 2\,r \qquad \text{equation 2}$$

Where D = diameter, *r* = calculated radius

The calculated values were plotted in the histogram using OrginPro software (version 11).

3.2 Seedling preparation

Seedlings were grown in seedling mix in a ATC 40 Conviron plant growth chamber illuminated with ultra-violet light. The average temperature of the growth chamber was

20 ^{o}C in the daytime and 23 ^{o}C at night. Moisture and nutrients were provided by watering the plants with a standard hydroponic nutrient solution. The HydroGro solution from House and Home store was diluted by adding 1 L in distilled water to 1.5 mL of the HydroGro solution from House and Home. Seeds of *Panicum maximum* Jacq sourced from McDonald seeds were soaked in concentrations of 5 ppm, 10 ppm, 20 ppm, and 50 ppm of the synthesized $nTiO_2$ powder for 30 minutes under sonication; then rinsed with distilled water. Plants were then grown in a 12 g seedling mix spiked with 1.5 g $nTiO_2$ at 25 °C and watered daily with nutrient solution (photoperiod 12 h day/light). Seeds soaked pure distilled water served as control. Metal content was determined in root, stem, and leaves of 35-day old seedlings using Inductively Coupled Plasma (Agilent 5100). In addition, using a statistical package (SPSS v24; IBM Corp), the mean metal uptake was evaluated with mixed factorial ANOVA (α =.05).

3.3 Modification of the cell walls of *Panicum maximum* with the synthesized $nTiO_2$

3.3.1 Seed culture and exposure

Experiments were used to evaluate the effect of different concentrations of nanosized TiO_2 on *Panicum maximum jacq* seed germination in a randomized design with four replications. The treatments in the experiment were done at four concentrations (5 ppm, 10 ppm, 20 ppm, and 50 ppm) and untreated control (no TiO_2 treatment). The experiment was performed in a germinator with an average temperature of 25^{o}C ±2 for 12/12 hrs (day/light) at MUT. Seeds of similar size were randomly selected and sterilized using NaClO (3%) for 10 minutes and then carefully washed with distilled water three times. An ultra-sonication treatment was applied to the seed-nanoparticle treatments in distilled water for 30 min. The seeds were placed on paper in four groups of 25 seeds in Petri dishes. Thereafter, 5 mL of each concentration treatment was added. For the control/blank only distilled water was added to the Petri dishes. Germination tests were performed according to the rule issued by the International Seed Testing Association. All concentrations of TiO_2 and control were tested at the same time to make sure uniform conditions of light and temperature across all tests. The number of germinated seeds was noted at 7, 14, and 28 days. Seeds were considered germinated when the radicle showed at least 5 mm length.

3.4 Phytoremediation

Seedlings were grown in potting mix in a growth chamber illuminated with ultra-violet light. The average temperature of the growth chamber was approximately 20 ˚C in the daytime and 23 ˚C at night. Moisture and nutrients were provided by watering the plants with a standard hydroponic nutrient solution. The HydroGro solution was mixed at 1.5 mL to 1 L in distilled water. Seeds of *Panicum maximum* Jacq sourced from McDonald seeds were soaked in treatments of 5 ppm, 10 ppm, 20 ppm, and 50 ppm of nanoTitanium dioxide powder for 30 minutes under sonication then rinsed with distilled water. Plants were then grown in a 12 g potting mix spiked with 1.5 g nanotitanium powder at 25 ˚C and watered daily with nutrient solution (photoperiod 12 hr day/light). Seeds soaked in pure distilled water served as control. Metal content in root, stem, and leaves of 35-day old seedlings was determined after digestion by an Agilent 5100 Inductively Coupled Plasma. Statistical analysis using a mixed factorial ANOVA was used to compare and determine the region that had the highest uptake. The statistical analysis was performed between treatments in a randomized design in four replicates.

3.5 Data analysis

Using software (IBM SPSS Statistics v 24; IBM Corp), One-way analysis of variance (ANOVA) was used to compare the mean value of phytoremediation, followed by the

Bonferroni tests (α =0.05). In addition, a mixed factorial ANOVA was used to compare the region that had the highest uptake.

3.5.1 Validity and reliability

The two most important aspects of precision are reliability and validity. The validity is the extent to which a test or instrument measures what it is supposed to measure and that particular measure is free from systematic random errors. In the context of this study, previous research conducted on phytoremediation was used to ensure the accuracy of the phytoremediation process. With reference to reliability, it is pointed out that the reliability of an experimental procedure is determined through reproducibility and repeatability tests. While reproducibility has been described as the variability of the average values obtained by several observers, repeatability is the variability of the measurement obtained by one person while measuring the same item repeatedly. In terms of the reliability of this study, the experimental process was carried out in triplicate and the standard deviation value given confirmed the repeatability of the phytoremediation tests.

Chapter 4: Theoretical simulation of the interaction of titanium dioxide nanoparticle with the soil components and plants

4.1 Introduction

Titanium nanoparticles remain one of the most produced nanomaterials. Studies have indicated that these nanomaterials are mainly used in pigments, photocatalysis, food additives, and personal care products (Hu *et al.* 2016; Savvides *et al.* 2016). This variety of applications have contributed to their widespread distribution in different environmental sectors. There have been many experimental techniques that have been used to demonstrate their interaction with plants as shown in Table 4.1 below. However, there are a few studies that have demonstrated the mechanism of the interaction with soil leading to their translocation as well as the transgenerational effects in plant cells which could contribute to their alteration at agronomical and physiological levels (Li, Wang and Shen 2012). This chapter will demonstrate their interaction with natural organic matters (NOM). These NOM consist of humic and non-humic fractions, such as fulvic and citric acid. There are few studies that have shown that NOM is contributing to the transportation of $nTiO_2$ by changing the surface (Hu *et al.* 2009). Studies have indicated that the mechanism of this interaction involved the adsorption of NOM onto the uncoated $nTiO_2$ (Keller and Lazareva 2014). It is therefore very important to demonstrate this interaction at a molecular level. This chapter will demonstrate the application of molecular modeling to study the interaction of $nTiO_2$ with NOM.

4.2 Interaction of nTiO2 with NOM using molecular modelling

Soil is one of the most complex systems on earth for plant growth. In soil, plants interact with microbial systems, soil minerals as well as natural organic matter. As a result, after the $nTiO_2$ enters the soil, the components of the soil affect the nanomaterial behavior, mobility, and bioavailability (Keller *et al.* 2013). The other factors that critically contribute to this interaction are pH which contributes to the stability of $nTiO_2$ in the soil (Fang *et al.* 2009; Lin *et al.* 2010; Peralta-Videa *et al.* 2011; Han *et al.* 2014; Pachapur *et al.* 2016; Tan, Peralta-Videa and Gardea-Torresdey 2018). After $nTiO_2$ has entered the soil, it has been demonstrated that they interact with NOM as depicted in Figure 4.1. These figures demonstrate several aspects of the release and the adsorption of $nTiO_2$ from the soil. In this case, as illustrated in Figure 1b, plants are primarily exposed to $nTiO_2$ through the soil in different proportions. There has been a report that the main primary source of $nTiO_2$ that contributes to the uptake by plant are personal care products, pigments, and also some food additives (Peralta-Videa *et al.* 2011; Sheoran, Sheoran and Poonia 2016).

Table 4.1: Summary of studies about plants exposed to nano-TiO_2 in Petri dishes**.**

Particle size (nm)	phase	concentration	Plant species	Exposure pathway	Detection Method	Effects on plants	reference
2.8 ±1.4	Anatase, surface coated with alizarin red S	10 µM	Thale cress	Seed, root, and foliar	Bright-field and fluorescent microscopy	· NPs accumulated in nuclei and vacuoles of root and leaf stomata · No effects on cell viability, oxidative stress, and morphology	(Aiken, Hsu-Kim and Ryan 2011)
2.8 ± 1.4	Anatase, surface coated with alizarin red S	20 µM	Thale cress	Root	Fluorescence microscopy, immunoblotting	Severely disrupted epidermal and stomatal guard cells and induced tubulin aggregation	(Han *et al.* 2014)
< 100	Mixture of anatase and rutile	0.2, 1.0, 2.0, 4.0 %	Corn, carbon bean	Seed	Karyotype	· Inhibit germination rate after 24 h exposure · Reduced root elongation at 4 % NPs · Lower mitotic index and higher aberration index	(Keller *et al.* 2013; Keller and Lazareva 2014)
12, 25 ± 7	Mixture of anatase (75%) and rutile	10, 100, 1000 mg/L	Wheat, oilseed rape, thale cress	Seed	µX-ray fluorescent microscopy, µ - XANES	12 nm anatase NPs were taken up by wheat roots and accumulated in	(Kurepa *et al.* 2010)

	(25%), anatase, rutile					parenchymal and vascular cylinder	
21	Mixture of anatase and rutile	1, 2, 10, 100, 500 mg/L	Wheat	Seed	N/A	· Lowest germination time at 10 mg/L · Higher shoot length at 2 and 10 mg/L	(Wang, Kurepa and Smalle 2011)
27 ± 4	Mixture of anatase (82%) and rutile (18%)	50, 100, 1000, 2500, 5000 mg/L	Tomato	Seed	ICP-OES	· Significantly increased Ti uptake · no effects on germination	(Castiglione *et al.* 2014)
< 25	Anatase	12.5, 25, 50, 100 mg/L	Onion	Root	Fluorescent microscopy, optical, and confocal laser scanning microscopy	Concentration-dependent decreased in mitotic index and increased chromosomal aberrations	(Castiglione *et al.* 2011)
< 100	Mixture of anatase and rutile	0.2, 1.0, 2.0, 4.0 %	Narbon bean	Seed	UV/vis, DNA fragmentation	· Significantly high H_2O_2 production showed in 1‰ treatment · Proline, glutathione and vigor index was affected at different degrees	(Larue *et al.* 2011)

< 25	Anatase	500, 1000, 2000 mg/L	Barley	Seed	Karyotype, Random Amplified Polymorphic DNA, TEM-EDS	· NPs clusters were present in root cortical parenchymal and xylem	(Feizi *et al.* 2012a)
50	Anatase	0.01, 1, 10 mg/L	Rice	Seed	UV/vis	· Reduced mean germination time at all concentrations · Dose-dependent change in vigor index	
5-15	Anatase	20, 200, 2000 mg/L	Corn	Seed	UV/vis, ICP-OES	Reduced germination rate, root length, shoot length	(Song *et al.* 2013)
33	Anatase, mixture of anatase (82%) and rutile (18%)	100 mg/L	Thale cress	Seed	qRT-PCR	· DNA and hormone metabolism, tetrapyrrole synthesis and photosynthesis were affected · Increased the percentages of seeds with emergent radicles, development of hypocotyls and cotyledons of seed	(Pakrashi *et al.* 2014)
5-25	Anatase	0.5, 1, 2.5, 10, 20	Barley	Seed	UV/vis	· Increased shoot biomass at 1 mg/kg · Concentration-dependent	(Castiglione *et al.* 2011)

						reductions in shoot and root elongations.	
21,< 25	Anatase, mixture of anatase (82%) and rutile (18%)	5, 10, 50, 100 mg/L	Lettuce, basil	Seed	Flow cytometry, micronucleus assays	· Basil was more sensitive to cytostatic effects · Lettuce was more sensitive to genotoxic effects	(Mattiello *et al.* 2015)

As a result of the availability and the use of these products more than 13.8% of $nTiO_2$ is discharged into soil absorbed by the plant (Tumburu *et al.* 2015; Fellmann and Eichert 2017). Although the wastewater treatment plant can remove some traces of $nTiO_2$ from water, some have been demonstrated to escape into the soil. As indicated in the illustration in Figure 4.1 below some $nTiO_2$ may penetrate root cells, translocate from roots to stems or fruits depending on the type of plant that is exposed to the nanomaterial. There have been studies that have demonstrated that the xylem of the cucumber plants accumulated the same forms of $nTiO_2$ added to the soil. However, most studies have indicated that most of the $nTiO_2$ would remain in the root cell. Moreover, there have been fewer studies that have demonstrated the mechanism of the absorption of $nTiO_2$ with soil and NOM.

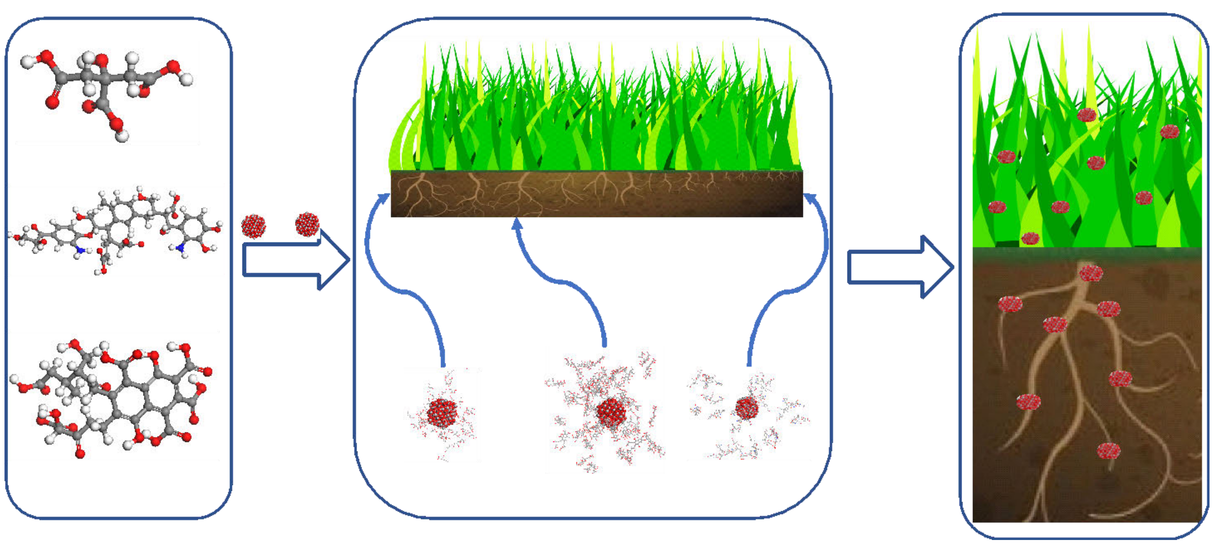


Figure 4.1 Schematic representation of uses and dispersion in the environment of nano-TiO_2 and interaction with the plants.

In Figure 4.2 below which were generated models of the three NOM using a molecular modeling program. The main functional group present in these NOM is the carbonyl group mainly due to the carboxylic end. These functional groups are susceptible to the interaction with the surface of $nTiO_2$. It was noted during the development of these NOM that they possess chiral centers which may be the indication that there may be different diasteromers in soil. However, to simplify the molecular modeling these different diasteromers were not considered in this project. This project was mainly focussing on understanding the adsorption of NOM on the surface of $nTiO_2$, thus giving us a better underastanding of the stability of nanomaterial, and the translocation process into the other parts of the plant. The interaction of NOM with the surface of $nTiO_2$ is mainly due absolute value of the zeta potential which contributes to the stability of $nTiO_2$. Generally, the $nTiO_2$ with zeta potential between 0 and 5 positive or negative are stable, however, they tend to aggregate faster. The stability of nanomaterial will increase by increasing zeta potential. The charges of the zeta potential depend mainly on the functional groups due to the surface coatings. It is well known that function groups such as –COOH and –NH_2 will produce negative and positive charges respectively.

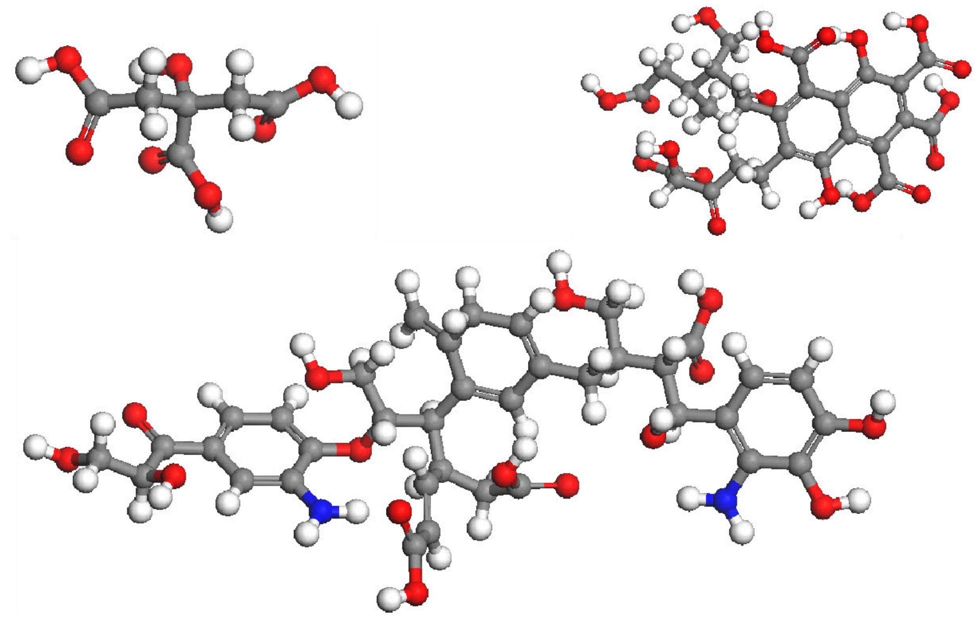


Figure 4.2: Natural Organic models where (a) Citric acid (b) Fulvic acid and (c) Humic Acid

To investigate the role of molecular orbitals in determining the interaction of NOM with $nTiO_2$, the highest occupied molecular orbitals (HOMOs), and the lowest unoccupied molecular orbitals (LUMO) and Energy gap (ΔE) for each NOM were calculated using Dmol module of Material Studio and are shown in Figure 4.3 below. It is very important to note that all HOMOs for fulvic and humic acids are centered on the phenyl ring. This is due to the fact that there are more resonance electrons in these rings. However, we observed that the HOMO for citric acid occupies the whole skeletal structure, especially in the carbonyl functional group. Moreover, it is interesting to note that LUMOs for all NOMs are centered in a similar pattern as HOMOs, however, it was observed that there were increased electron densities in carbonyl functional groups for fulvic and Humic acid. It was further observed that there is a low electron densities on the LUMO for humic acids as shown in Figure 4.3 which may have contributed to less reaction with the surface of $nTiO_2$. As a result of high intensity on the carbonyl group for LUMOs, it was concluded that the main functional group that is involved in the interaction of NOM with the surface of $nTiO_2$ is the carbonyl group. The HOMO-LUMO gap defines the chemical stability, reactivity and electron conductivity. The HOMO-LUMO gap is 5.083 eV, 2.032 eV and 1.865 eV for citric acid, fulvic acid and humic acid, respectively, suggesting its high reactivity towards bonding with metals. Furthemore, the calculated radial distribution which is given below were generated using the carbonyl group of each NOM.

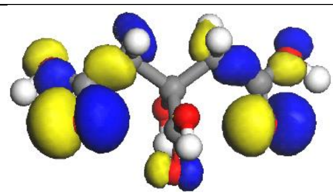
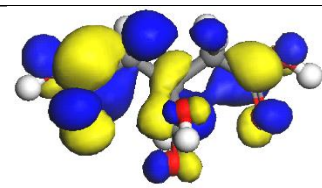
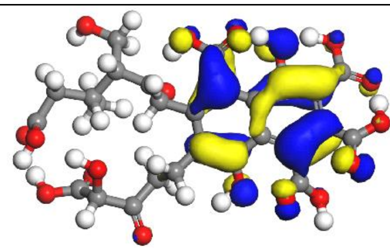
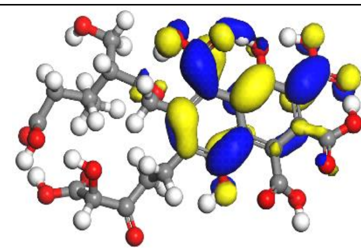
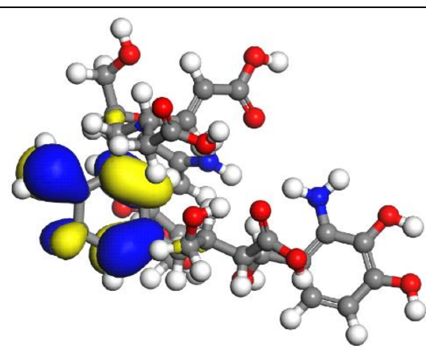
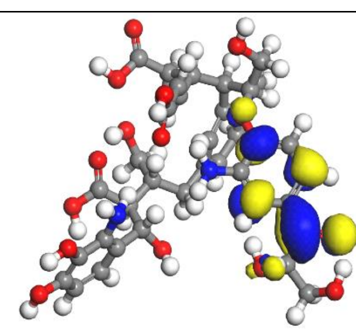
NOM	**HOMO**	**LUMO**	**∆E**
Citric Acid	 **-7.046eV**	 **-1.963eV**	**5.083 eV**
Fulvic Acid	 **-6.194eV**	 **-4.162eV**	**2.032**
Humic Acid	 **-4.877eV**	 **-3.010eV**	**1.865 eV**

Figure 4.3: HOMO and LUMO diagram of the three NOMs

The model for the interaction of $nTiO_2$ with different NOM is shown in Figure 4.4 below. It can be noted in Figure 4.4a that the citric acid-coated $nTiO_2$ model has all citric acids which are very closer to the surface followed by those that are coated by fulvic (Figure 4.4b) and lastly those that are coated by fumic acid (Figure 4.4c).

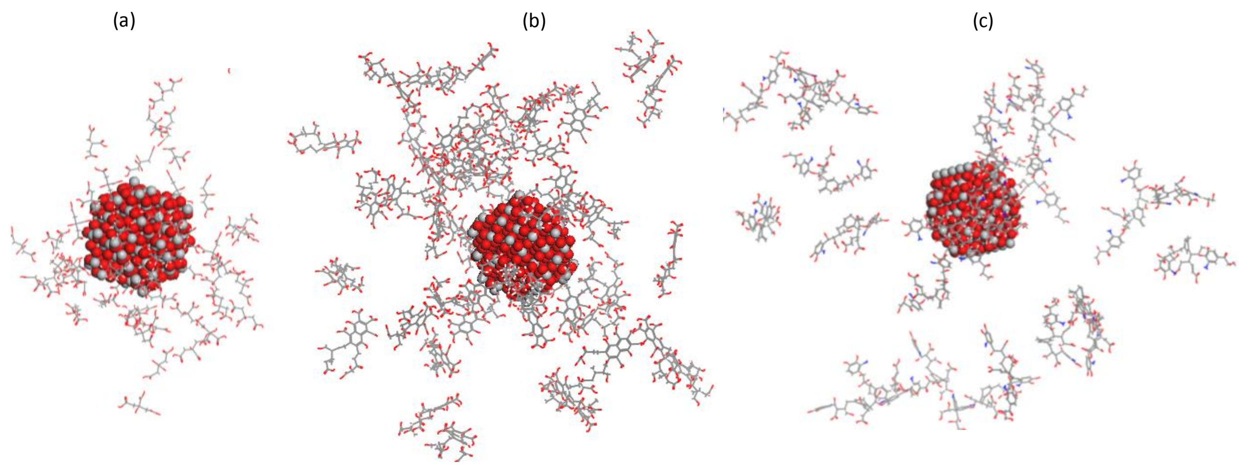


Figure 4.4: Models of titanium nanoparticles functionalized with Natural Organic models where (a) Citric acid (b) Fulvic acid and (c) Humic Acid

Interestingly, it can be observed that steric hindrance as a result of the bigger size of humic acid contributed to less coverage, such interaction may have limited the possibility of humic acid contributing to the stability and translocation of $nTiO_2$. The strong interaction of NOM (citric and fumic acids) with the surface of $nTiO_2$ is mainly due to the dissociation of H^+ from carboxylic as result NOMs with the negative charge have high hydrophilic coatings, this was attributed to the generated negative charge due to dissociation, these NOMs shall attract positive charge $nTiO_2$ via the electrostatic interaction. There has been a report that supports that particles with higher negative charges on the surface would exhibit better and uniform distribution in the soil matrix as a result of the enhanced steric repulsion between them and soil particles. There has been a report as well that showed that the presence of some of these NOMs can stabilize the dielectric environment of nanomaterial, regardless of the ionic environment by altering the surface from hydrophobicity to hydrophilicity. It is also very important to note that the aggregation of $nTiO_2$ would decrease by increasing the coating with NOM. This was demonstrated in the study regarding the surface coating of anatase $nTiO_2$ with citric acid at different pHs (Fellmann and Eichert 2017). It is thus clear that pH is the main driver for plant growth since most plants will grow at an optimum pH of between 5.5 and 7. There has been supporting evidence of study that has indicated the relationship of pH and NOM as a contributor to plant growth dynamics, which was reported to be mainly driven by the surface area of $nTiO_2$ (Silva *et al.* 2017).

Radial distribution functions (RDFs) were calculated to evaluate the quantitatively structural and dynamics effects of $nTiO_2$ complexation with NOM. In this case, the $nTiO_2$-NOM simulations are shown in Figure 4.5 a,b, and c for citric, fulvic, and humic acids, respectively. The results show that $nTiO_2$ interacted very weakly with humic acid which is in agreement with the optimized models in Figure 4.4 c. This was indicated with radii of the first and second coordination shells of humic acid starting from 4.5 Å whereas for citric and fulvic acids the radii of the first and second coordination shells were both starting from 2.5 Å. This agreed very well with the optimized models in Figure 3 above where it was noticed that the two NOMs formed a strong compact layer on the surface of $nTiO_2$. This simulated result confirmed that the main NOM driving the translocation of $nTiO_2$ in the soil is citric and fulvic acids, though humic acid may have contributed less to this phenomenon, some small tracing may also have an impact. The application of radial distribution has been studied elsewhere for other metal ions complexation with NOMs (Keller and Lazareva 2014).

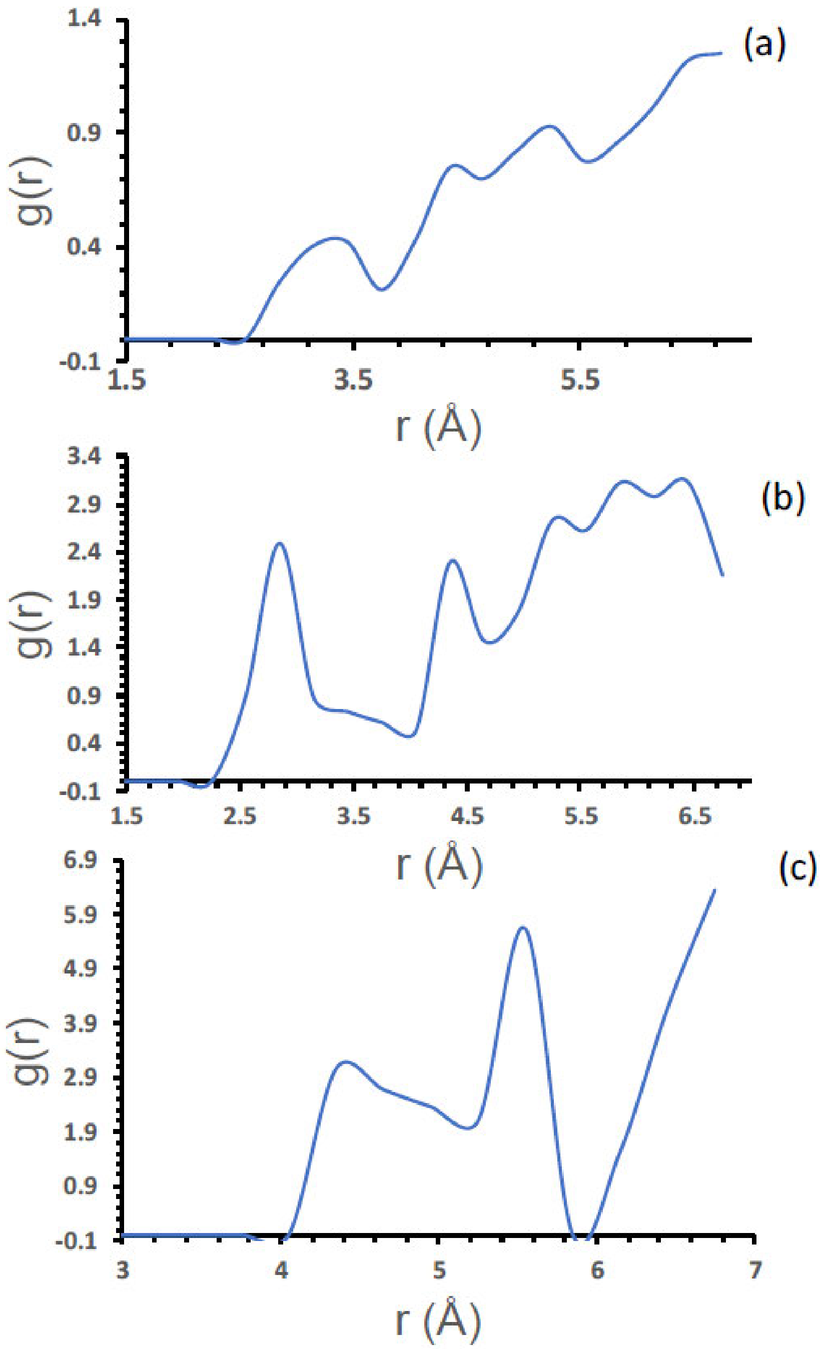


Figure 4.5: Radial distribution of titanium dioxide nanoparticles functionalized with natural organic models where (a) citric acid (b) fulvic acid and (c) humic Acid

Chapter 5: Application of titanium nanoparticles to demonstrate the phytoremediation process

5.1 Introduction

Bioaccumulation as a tool for the remediation of contaminated environments has been the subject of several reviews. Some of them cover the following aspects: the exploitation of invasive plants to control contamination of the environment by Mani and Kumar (2014); phytoremediation of metal-contaminated land using field crops by Vamerali, Bandiera, and Mosca (2010) and probing the mechanisms of phytoremediation by (Bora and Sarma 2020.

Since nanotechnology has proven to be beneficial in many applications involving technology in general, it is not surprising that nanotechnology is attracting the interest of researchers who are striving to find strategies to clean up polluted air, water, and soil. The studies on the effects of nanomaterials on plants while and the use of nanomaterials in aiding phytoremediation of contaminated soil has been the subject of a review by Song *et al.* (2019). These reviews also revealed that 'plant biomass and growth rate' were an important factors affecting the efficiency of a bio-accumulator. Genetic engineering has been used to cultivate plants with high biomass and high tolerance for metal pollutants (Vamerali, Bandiera, and Mosca, 2010).

In the light of the foregoing, seedlings have been targeted for experiments to increase growth rate. Research and development of engineered nanomaterials have improved the understanding of applying engineered nanomaterials to plants. These reports also noted that nano-phytoremediation is the result of the improvement associated with a variety of economical applications for contaminated site remediation initiatives (Rastogi *et al.* 2017b; Song *et al.* 2019; Bora and Sarma 2020). The use of engineered nanomaterials is a novel application worth exploring. Nano remediation methods have been used for the exploitation of reactive nanomaterials for the transformation and detoxification of polluted sites (Pilon-Smits and Pilon 2002). These nanomaterials, due to their high surface / volume ratio, are extremely reactive and catalytic (Chen and Mao 2007). The interaction of nanoparticles can be evaluated from different perspectives. The effect of the nanoparticle on plant growth/development e.g shoots and roots etc., and also concerning the uptake of the heavy metals, were the targets of accumulation and translocation experiments.

Many synthetic methods have been developed, using the anatase phase of TiO_2 nanoparticles. Several approaches may be used to synthesize TiO_2 for the purposes of exploiting its photocatalytic properties. They are, inter alia, ultrasonic irradiation, chemical vapor deposition, and the sol-gel method; the latter being one of the methods used to prepare titanium nanoparticles (Cheng et al. 1995; Eapen and D'souza 2005; Karn, Kuiken and Otto 2009; Chen et al. 2012; Stamm, Gibson and Anklam 2012). This method is based on the mixing, polymerizing, hydrolyzing, aging, and calcining of various titanium molecular precursors in aqueous solutions or organic solvents (Litter and Navio 1996). $nTiO_2$ nanoparticles have shown an increase in nitrate reductase in soybean (*Glycine max*), which increases its capability to absorb water and stimulate the antioxidant system (which is the plant's defense system in reaction to metal contaminants in its growing environment). TiO_2 nanoparticles treated seeds produced plants that had 73 % more dry weight, three times higher photosynthetic rates, and a

45 % rise in chlorophyll when compared to the use of spring water over the germination period of 30 days (Palmisano *et al.* 1988). The selection of promising plants is an important approach to successfully phytoremediate water or soil contaminated by the presence of titanium dioxide nanoparticles, which is used on an extremely large scale in several domestically-used products such as toothpaste and paints. To the best of our knowledge of the literature, there were no reports on the use of *Panicum maximum* Jacq as a bioaccumulator for titanium dioxide nanoparticles. This chapter describes and discusses the results obtained from using *Panicum maximum* Jacq, as a bio-accumulator for nanosized titanium dioxide.

5.2 Results and discussion

5.2.1 Structure, crystallinity, and mineral composition of the synthesized nano-TiO_2

From Figure 5.1 a, the XRD pattern of $nTiO_2$ showed characteristic diffraction peaks with diffraction angles at 29.5°, 34.5°, 42.5°, 46.5°, 50.5°, 56°, 57°, 67°, 68°, and 72°. The XRD pattern of $nTiO_2$ showed a characteristic diffraction peak with values lying at $2\theta = 29.5°$ corresponds to anatase and was confirmed with International Centre for Diffraction Data (ICDD Ref: 98-009-6946). On the other hand, a diffraction peak with values lying at $2\theta = 30.0°$ corresponds to the rutile form of titanium dioxide. This suggests that the synthesized $nTiO_2$ comprised anatase and rutile crystalline forms of titanium dioxide.

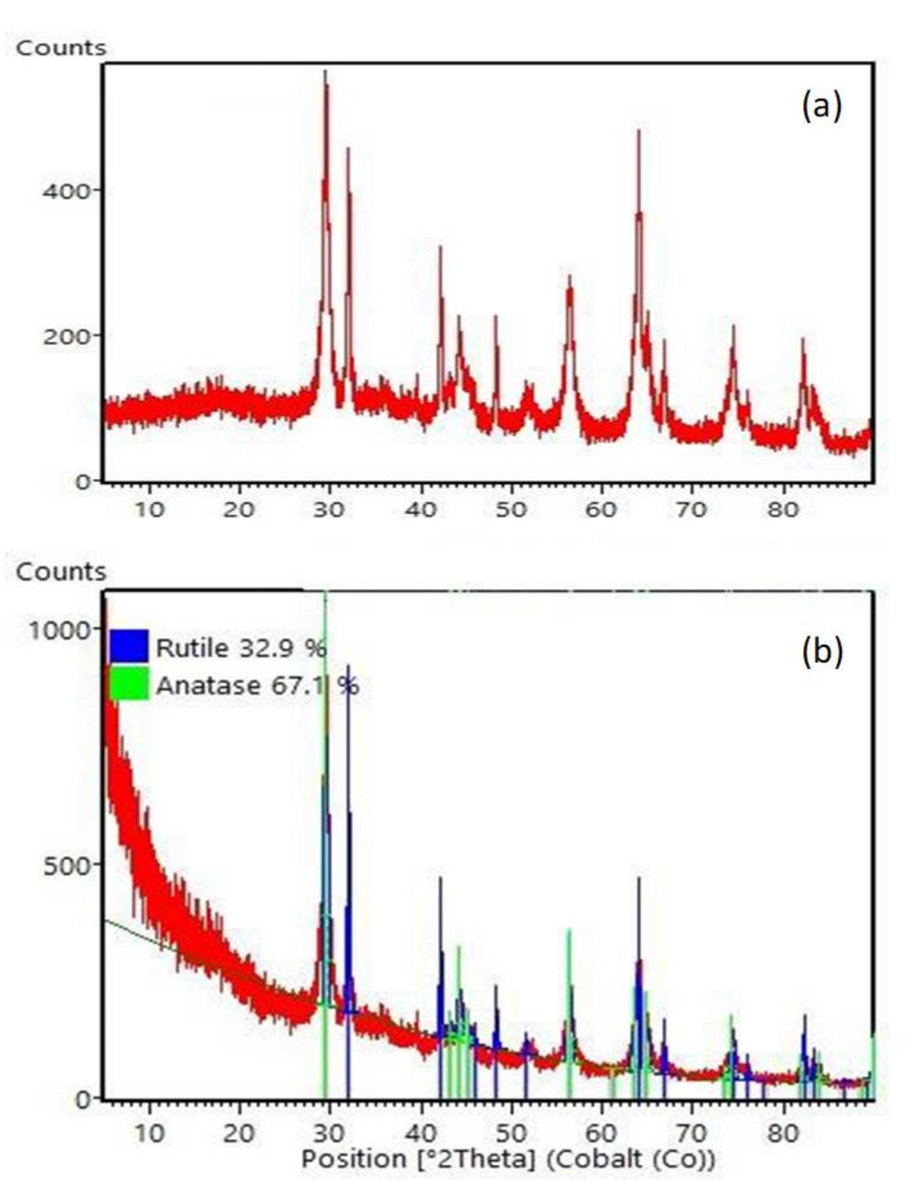


Figure 5.1: XRD pattern showing (a) crystallinity of $nTiO_2$ and (b) mineral content of nTiO2.

As shown in Figure 5.1 b, the presence of both the anatase and rutile phase of titanium dioxide was observed in the synthesized $nTiO_2$. In terms of the mineral content of $nTiO_2$, anatase consists of 67.1% while 32.9% was rutile. The presence of both forms of titanium dioxide in the synthesized nano-TiO_2 could be attributed to the calcination temperature. Consistent with report of Fagan and co-workers the anatase titanium dioxide has lower thermal stability and will begin to change to a more stable form of titanium dioxide (rutile), as the calcination temperature increases with time. The higher percentage of anatase in the $nTiO_2$ could be associated with the calcination during synthesis.

5.2.2 Micro-Analysis and Visual characterization of particle size and shape of nTiO2

The SEM images of $nTiO_2$ are shown in Figure 5.2 a. The presence of compact and spherical particles in the $nTiO_2$ can be observed. The characterization of particle size and shape of nanosized TiO_2 using SEM images of $nTiO_2$ is given in Figure 5.2a below. The SEM image shows that the particles are more compact due to the agglomeration of the nanoparticles. It can also be observed that the morphology of the $nTiO_2$ particles is in a spherical shape. This confirms the trend observed of spherical shaped particles being a common attribute of synthesized nanosized titanium dioxide.

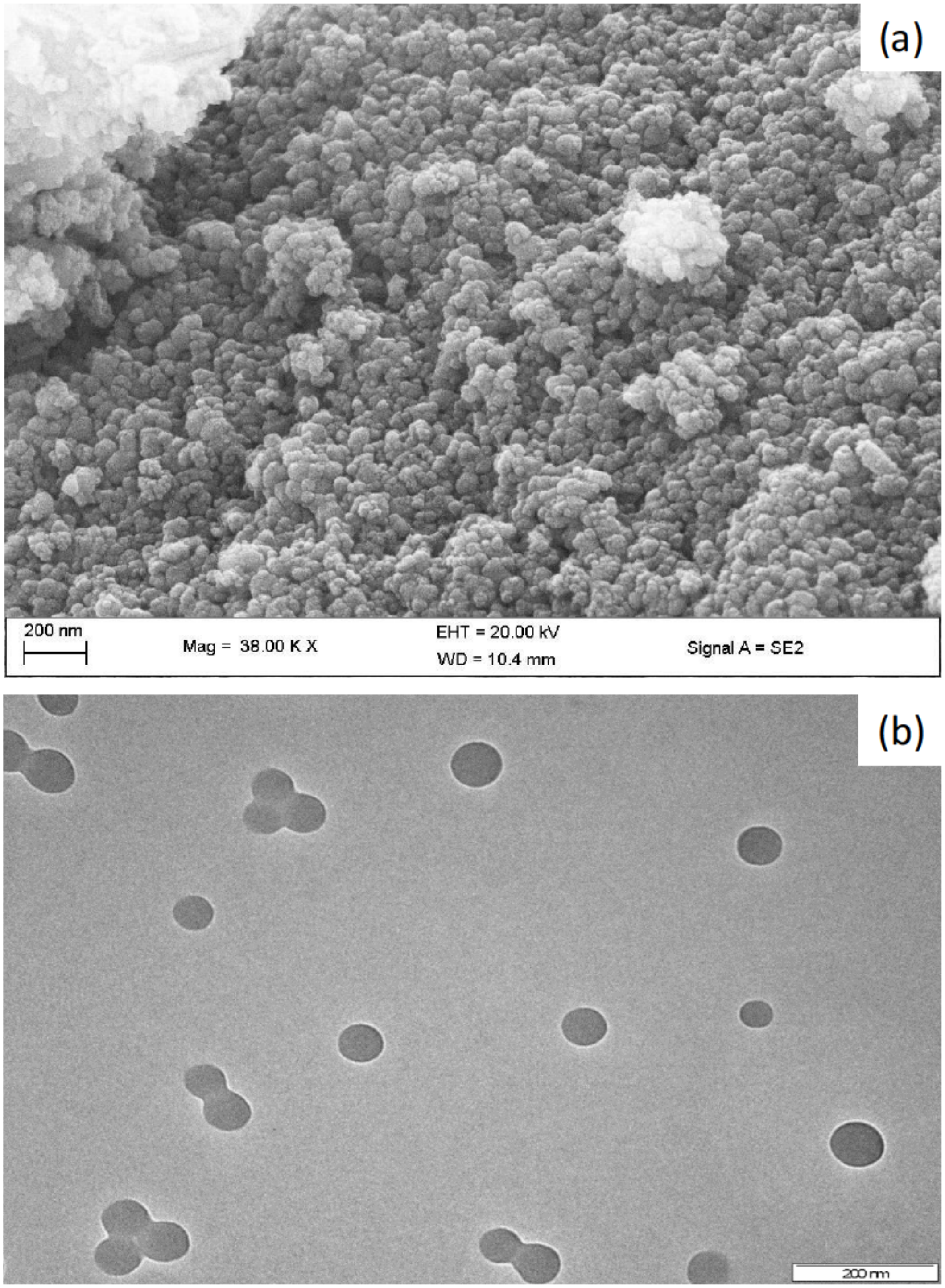


Figure 5.2: Micrographs showing particle distribution of $nTiO_2$ where (a) SEM and (b) TEM images

The TEM micrograph is shown in Figure 5.2 b indicates the presence of compact and spherical particles in the $nTiO_2$. Figure 5.2 b shows a more uniform spherical-shaped distribution of $nTiO_2$. This correlates with report of Gupta, 2010, who indicates that spherical shaped particles are a common attribute of synthesized $nTiO_2$.

The normal distribution curve in Figure 5.3 represents the particle size distributions of the synthesized $nTiO_2$. The average mean size of $nTiO_2$ was revealed to be 9 nm. This confirms that the synthesized titanium dioxide is nanosized.

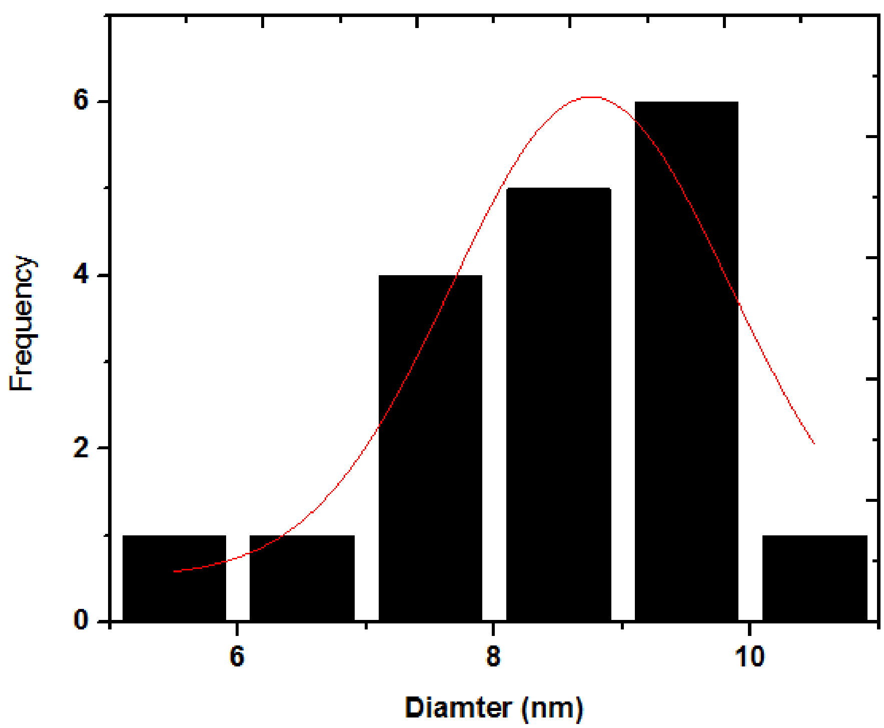


Figure 5.3: Particle size distribution of $nTiO_2$

Table 5.1 below shows the elemental composition of the synthesized $nTiO_2$. High levels of oxygen and titanium were evident. This is attributed to the main component of titanium dioxide.

Table 5.1: Energy Dispersive Spectroscopy elemental composition of nanosized TiO_2

Element	**Weight (%)**
Oxygen (O)	53.46
Titanium (Ti)	44.77
Sodium (Na)	1.77

The presence of sodium (Na) was also observed. The presence of sodium as evident in Figure 5.4 below could be attributed to the use of sodium hydroxide in the adjustment of pH during the preparation of $nTiO_2$.

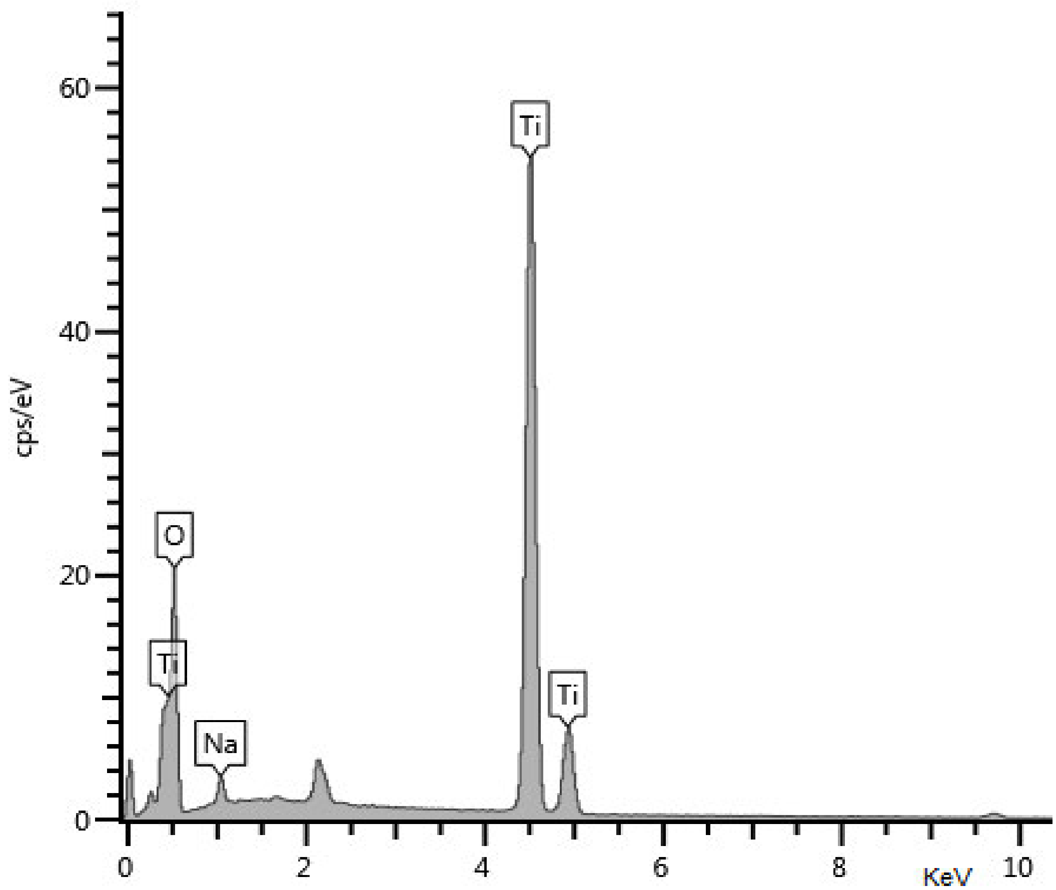


Figure 5.4: Energy Dispersive Spectroscopy spectrum of $nTiO_2$

5.3 Demonstrating the nanophytoremediation process using statistics

The one-way ANOVA, mean standard deviation and standard error results are shown in Table 5.2. As indicated by the levels of significance, the one-way ANOVA tests revealed that the phytoremediation values for the samples exhibited statistically significant differences ($p<0.01$). It can be observed that the control group had the lowest phytoremediation mean values (0.27±0.18), while the group modified with 50 ppm $nTiO_2$ had the highest mean phytoremediation values (0.98±0.62).

Table 5.2: ANOVA tests for different concentration of $nTiO_2$ in plant samples

	N	Mean	Std. Deviation	Std. Error	95%Confidence Interval for Mean		F	P-value
					Lower Bound	Upper Bound		
Control	9	0.2744	0.18338	0.06113	0.1335	0.4154	4.241	0.006
5 ppm	9	0.3567	0.17734	0.05911	0.2203	0.4930		
10 ppm	9	0.7411	0.52193	0.17398	0.3399	1.1423		
20 ppm	9	0.8878	0.57974	0.19325	0.4422	1.3334		
50 ppm	9	0.9756	0.61796	0.20599	0.5005	1.4506		
Total	45	0.6471	0.52277	0.07793	0.4901	0.8042		

Table 5.3 illustrates the multiple comparisons of phytoremediation properties of the samples. The mean values of the control were not significantly different from 5 ppm, 10 ppm, and 20 ppm, respectively ($p > 0.05$). There was a statistically significant difference between the control and 50 ppm sample ($p < 0.05$). Table 5.3 further illustrates the differences in the phytoremediation value of the various samples. A difference in the uptake value is evident amongst all samples.

Table 5.3: Multiple comparison test

Standards	Concentration (ppm)	Sig.
Control	5	1.000
	10	0.373
	20	0.072
	50	0.024
5ppm	10	0.835
	20	0.187
	50	0.067
10ppm	20	1.000
	50	1.000
20ppm	50	1.000

5.3.1 Evaluating the region with the highest uptake

Figure 5.5 shows the region with the highest uptake of titanium dioxide. From the table below, it was observed that the root had the highest uptake for all the concentrations while the lowest uptake was found in the leaf. This suggests that the root of the *Panicum maximum* Jacq plant is the suitable part of the plant for phytoremediation as it showed the least strain.

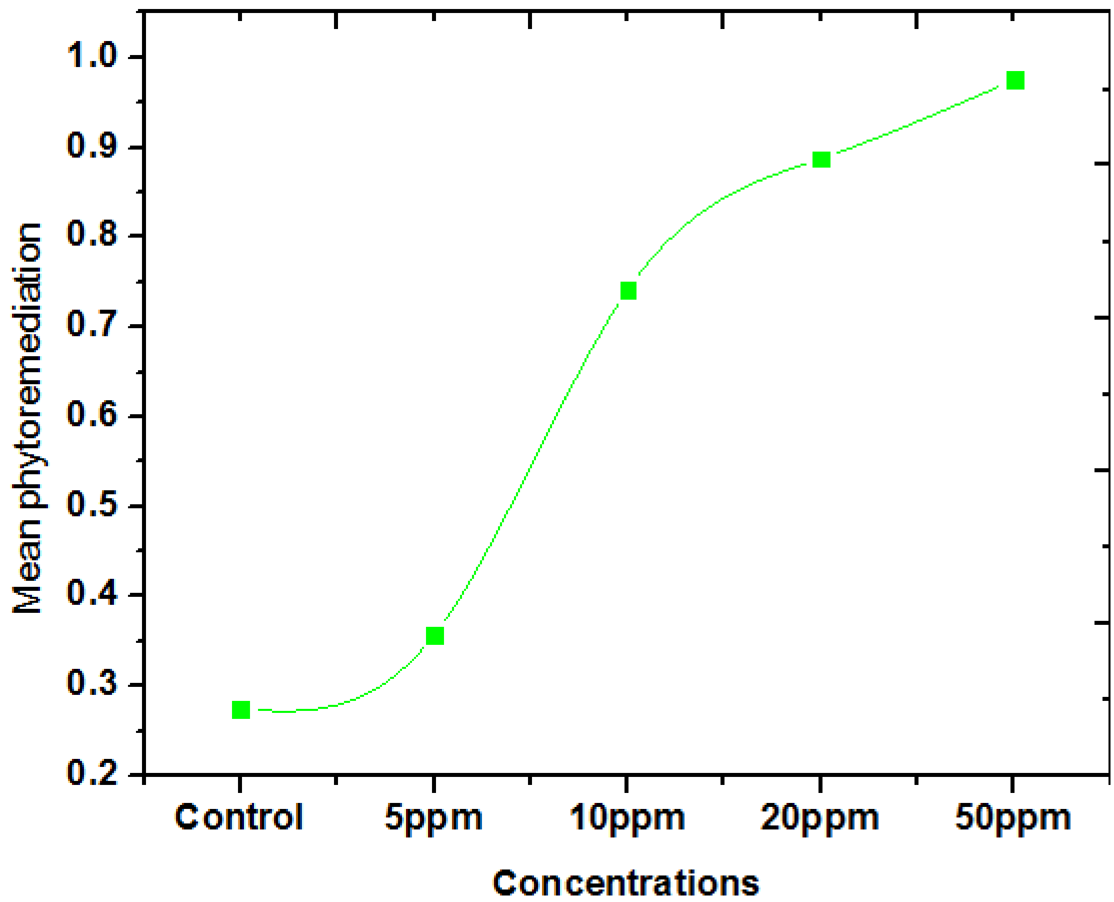


Figure 5.5: Mean concentration values of phytoremediation

As shown in Table 5.4, the mean uptake by concentration showed significant differences beyond the 0.01 level: F (1.202, 7.213) = 43.500; $p<0.01$. Partial eta squared = 0.879 representing a medium effect. This suggests that there was a large difference between the uptake of titanium dioxide in the stem, leaf, and root of the plant.

Table 5.4: Descriptive statistics showing region with the highest amount of uptake of titanium nanoparticle by *Panicum maximum jacq* region

		Mean	Std.	N
Control	Root	0.4267	0.24420	3
	Stem	0.2567	0.09713	3
	Leaf	0.1400	0.05568	3
5 ppm	Root	0.5300	0.17088	3
	Stem	0.3500	0.05568	3
	Leaf	0.1900	0.08185	3
10 ppm	Root	1.4100	0.11136	3
	Stem	0.5467	0.10017	3
	Leaf	0.2667	0.04163	3
20 ppm	Root	1.6167	0.17243	3
	Stem	0.6933	0.17559	3
	Leaf	0.3533	0.04163	3
50 ppm	Root	1.7600	0.21633	3
	Stem	0.7467	0.12220	3
	Leaf	0.4200	0.03464	3

As shown in Table 5.4, the mean uptake by concentration showed significant differences beyond the 0.01 level: $F(1.202, 7.213) = 43.500$; $p<0.01$. Partial eta squared = 0.879 representing a medium effect. This suggests that there was a large difference between the uptake of titanium dioxide in the stem, leaf, and root of the plant.

Table 5.5: Mixed factorial ANOVA test

Source	Type III Sum of Squares	df	Mean Square	F	Sig.		Partial Eta Squared
Concentration	Greenhouse-Geisser	3.581	1.202	2.979	43.500	0.000	0.879
Total Concentration	Greenhouse-Geisser	0.494	7.213	0.068			

Figure 5.6 further confirms the region with the highest uptake. The difference in the uptake by concentration is evident amongst the uptake. For example, it can be observed from the graph that the 50 ppm had the highest uptake of titanium dioxide while the control group showed the lowest overall uptake. It can also be seen that the uptake decrease as the concentration decrease from 50 ppm to 5 ppm.

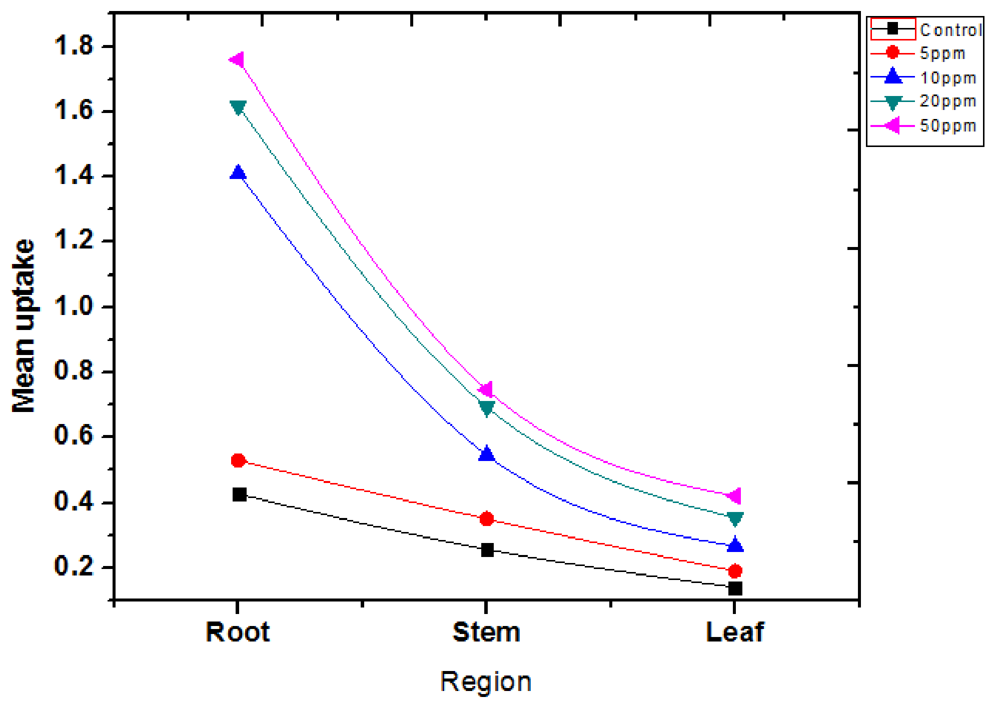


Figure 5.6: Region with the highest mean uptake of titanium dioxide nanoparticles

5.4 Phytotoxicity assessment of modified *Panicum maximum jacq* seedling with nanosized TiO_2

Figure 5.7 illustrates the phytotoxicity effect of $nTiO_2$ in the germination rate of *Panicum maximum* Jacq seedlings. It can be observed that 20 ppm $nTiO_2$ gave the best growth for the entire period, which is consistent with the findings of a study by Wang, Zhang, and Ying (1997). In contrast, the growth rate was highly inhibited with a concentration of 50 ppm.

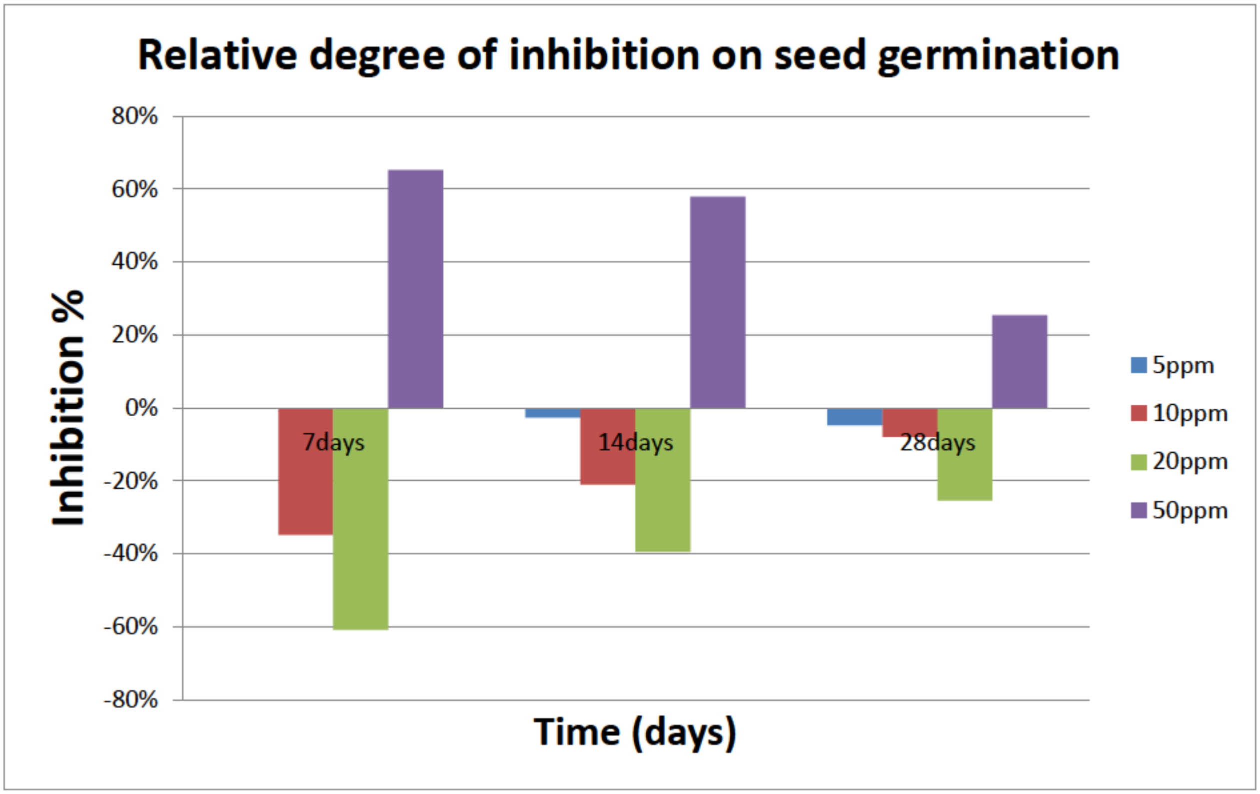


Figure 5.7: Relative seed germination inhibition properties

5.4.1 Effect of nanoparticles on growth and development

Plants that had exposure to the highest concentration treatment showed detectible phenotypical changes, which included wilting. The samples that had been exposed to the other different concentrations of titanium nanoparticle treatment of (5, 10, and 20 ppm) did show a uniform growth rate.

5.4.2 Effect of concentration and time on absorption of nanoparticle

The overall effect of the exposed plants to 5 ppm, 20 ppm, and 10 ppm Titanium nanoparticles over a 16-day period showed an increase in absorption respectively, this was then analysed by measurement of the root lengths. Simple linear regression analysis between the change in the root length over a period of time and with increased concentrations showed that the relation between dosage and root growth is not linear, at 100 ppm there is a retardation of growth. Figure 5.8 below shows the comparison with the control sample also indicated that its growth was slightly inhibited as they were not as green or longer.

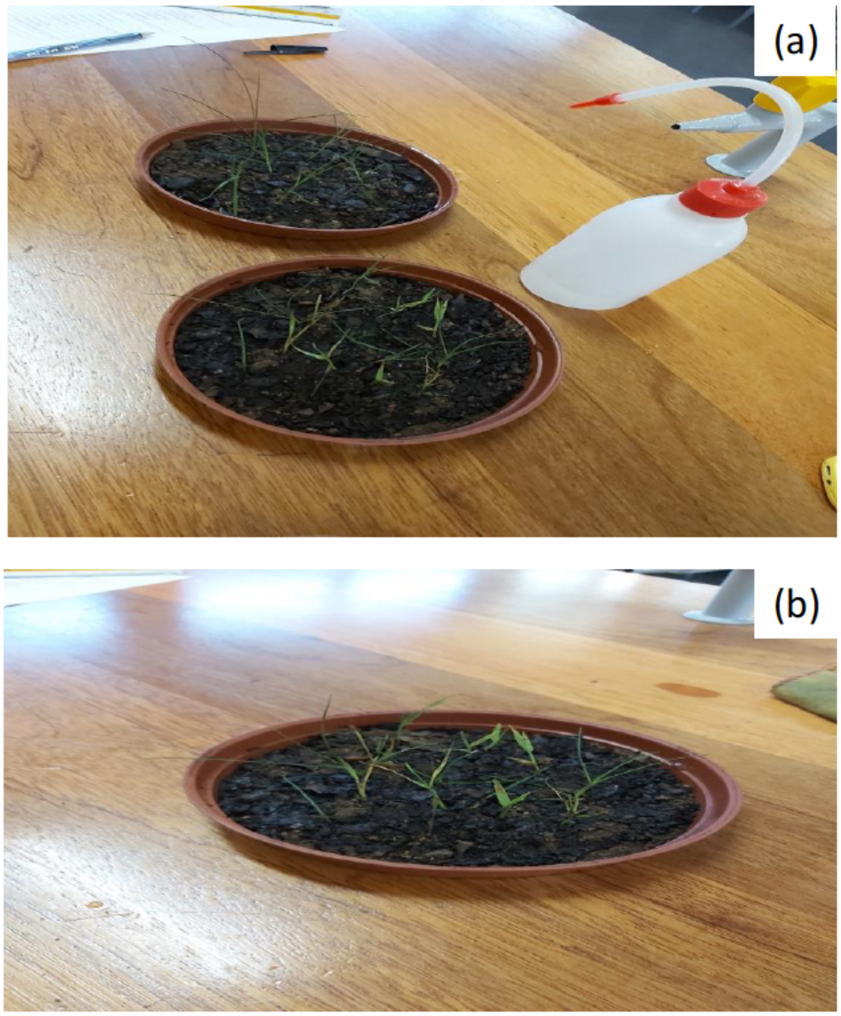


Figure 5.8: Comparison of (a) plant growth (b) inhibited growth

5.4.3 Portal of storage of titanium nanoparticles in the plant

The concentrations of TiO_2 accumulated in the range from 0.14 ppm up to 1.76 ppm. The root system accumulating the highest concentration. The levels of $nTiO_2$ absorbed in the different plant portals of stem, leaf, and root were significantly different. Most of the nTi was stored in the roots system, where at an increasing Cr treatment concentration (5 ppm, 10 ppm, 20 ppm, and 100 ppm) plant roots absorbed more titanium dioxide.

5.5 Impact of metal and metal oxide nanoparticles on plants

Engineered nanoparticles are manufactured to possess properties that are not present in bulk samples of similar material. In respect of their physicochemical properties, as well as surface, thermal, optical, and electrical properties are influenced by specific size and shapes. With the manipulation of species at a nanoscale level it was possible to demonstrate phytoremediation results of seedling grown from modified seeds to that of unmodified seeds, as would be a typical field environment that would require plant assisted clean-up of metals from soils as well as water, in places for domestic and\or agricultural and commercial usage.

Although phytoremediation is well established and nanotechnology has been applied in various fields, nano-phytoremediation by titanium dioxide nanoparticles is scarcely reported in the literature. Phytoremediation can be assisted by a synthesized nano-metal oxide in the plant. Nano-phytoremediation using synthesized nano-metal species and plants grown under controlled conditions would be a viable method in the open veld. In this study no field studies were done, nano-phytoremediation of titanium dioxide has been currently confined to phytoremediation assisted by a nanomaterial of the same material that would be found in a site selected for rehabilitation.

The results show the biocompatible nature of titanium dioxide nanoparticles but at higher concentrations it inhibited germination rate. There are conflicting reports on the effects of titanium dioxide nanoparticles in different plants. Synthesized titanium dioxide nanoparticles demonstrated an affinity with the region; with the highest concentration being in the root system. Some were also taken to the aerial parts of the plant. This suggests that the plant, *Panicum maximum* Jacq, may not be the ultimate hyperaccumulator of titanium dioxide nanoparticles. It has demonstrated a nano-metal accumulation tolerance which is higher than that for untreated seedlings. *Panicum maximum* Jacq is thus able to phytostabilize a metal in a soil environment. This is a realitively simple but cost-effective method for removal of heavy metals from contaminated soil.

It is worth noting that *Panicum maximum* Jacq is able to remove heavy metals from different soils types by phyto-accumulation. Furthermore the efficiency of the the plant to bioaccumulate titanium dioxide nanoparticles materials is greater when *Panicum maximum* Jacq is grown in the media in which the heavy metal is embedded. This is a very desirable property which would be beneficial in the clean-up of veld contaminated by heavy metals. The fast growth of the grass makes it a desirable plant for clean-up missions. It does not require much attention and grows readilyto maturity in the veld.

5.6 Low-Temperature Synthesis of Anatase TiO_2 Nanoparticles for enhancing Photocatalytic Activity

The anatase form of titanium dioxide typically demonstrates higher photocatalytic activity compared to rutile and the brookite crystalline forms. Colloidal titanium dioxide nanoparticles finds uses in the degradation of toxic chemicals in the water. Physicochemical methods such as microemulsion, chemical precipitation, chemical vapor deposition, hydrothermal crystallization, and sol-gel methods are used to produce Titanium dioxide nanomaterials. The sol-gel method has been developed to synthesise different mesoporous materials which exhibit high specific surface area and adjustable/narrow pore size distribution. The sol-gel synthesis is an environmentally-friendly approach, which is of significance considering the extensive applications of titanium dioxide nanomaterial. Using this method there is a shift in the optical responsive region range into the visible light range.Tthis trait has been exploited in visible light photocatalysis. Photocatalytic degradation has proven to be an effective and economical solution for decomposing organic contaminants into substances that are non-threatening.

5.7 Nano-anatase TiO_2 Treatment

Chemical priming with nanoscale nutrients is one of the ways nanotechnology seeks to manipulate matter at its molecular level a bottom-up approach to applying this would be for purposes of furthering agriculture and enhancing the growth potential of seeds. Nanotechnology can assist crop production by minimizing nutrient loss potential and helps to increase the yield of production (Jiamjitrpanich et al., 2013). The germination results from the experiment suggest that the synthesized nanosized TiO_2 at the 20 ppm concentration catalyses seed germination which can be of particular application in food crops. Researchers have shown interest in using nanosized titanium for its photocatalytic properties, and this is an opportunity to further study the metal and its applications; as a way to have an impact on malnutrition, as food can be available even when not in the best soil. The use of titanium dioxide to prime seeds is a promising vehicle for creating sustainable food reserves, after consideration of all relevant parameters.

5.8 Environmental aspects

The results show that it would be possible to carry out environmental impact studies using *Panicum Maximum* Jacq as a hyperaccumulator plant. Studies such as this one will assist to restore abandoned mining sites and industrial effluent sites thus benefiting communities in the area where they are located. The South African government in 2010-2011 launched a campaign to teach and encourage communities to plant vegetables and trees as a measure to curb poverty, combat diseases, and also help in the fight against climate change. For agricultural or urban garden projects to thrive, the residents of Ethekwini require the soil around them to be rehabilitated in a relatively cheap manner. Phytoremediation strategies can be used to rehabilitate current residential/domestic areas where elemental contamination is a problem. Gardens established in such rehabilitated areas will to improve food security and engage the communities in beneficial activities. South Africa has many informal settlements in most urbanized localities where contamination by metals is quite probable.. Thus here is good chance that slum dewllers may encounter increased exposure to titanium. It will be helpful if local authorities adopt such remediating approaches in their environmental cleansing policy to solve the contamination issue. *Panicum maximum* Jacq is able to reduce metal concentrations in the soil within the surrounding environment. The seeds are not expensive and the life cycle is that of the usual grass that we are familiar with. The important point is that the metals of concern can be collected using the plant so they can be repurposed or disposed off more safely. This is a green, and efficient strategy to improve the aesthetics of neighborhoods if well maintained, and also assist in remediating an environment that is polluted by an array of potentially harmful elements

5.9 Potential use of nTiO_2 as a genetic modifier in food

World Health Organisation (WHO) 2010 describes genetically modified organisms (GMOs) as plants or animals in which the genetic material has been modified such that it behaves in a manner that is not naturally occurring. Food security in South Africa is under stress from human interactions and the changing climate; for example, drought which, among other factors which negatively affect our food source. This has necessitated the need to consider genetically modifying organisms in order to produce plants that have improved resistance and crops with better yields. Local authorities regulate the safety assessment of genetically modified foods. Titanium-containing products are in abundance in our everyday lives, as titanium dioxide is used in numerous household and industrial products and people, are daily exposed to titanium containing products that they consume. Titanium dioxide nanoparticles ($nTiO_2$) are in use as they are considered non-toxic, chemically inert, and also a biocompatible pigment. Therefore, using $nTiO_2$ to enhance phytoremediation will not pose a problem to the environment.

Chapter 6 Conclusion and Recommendation

6.1 Conclusion

Seed priming is widely used in agricultural systems to enhance seed vigor in terms of germination potential and increases instress tolerance**.** In this project, it was found that soaking the seeds of *Panicum maximum* Jacq in nanosized titanium dioxide solution increased the affinity of the seedlings of *Panicum maximum* Jacq to take up titanium from the soil compared to the seedlings of the control. On the premise that the relatively small size (9 nm) of the metal particles and spherical shape of the particles used in this project would lead to a significant increase in the amount of metal uptake as well as a high translocation factor, the results for both these processes suggest that other factors are at play in the uptake and translocation of metal particles by seedlings. The soaking of the seeds in a solution containing titanium dioxide nanoparticles (5 ppm and 10ppm) and growth promoters resulted in a sturdy seedling. This property of the seedling could affect the uptake and translocation in at least two ways: it could help the seedling to withstand the possible toxicity of metals and could also strengthen the cell walls to the extent that fewer metal nanoparticles can penetrate the cell walls of panicum maximum Jacq. The latter prospect could explain the lower than expected uptake by the root and a low translocation factor.

According our literature search, there have not been any recorded investigations based on the efficiency of nanophytoremediation using *Panicum maximum* Jacq as the hyperaccumulator plant for titanium dioxide from soil. The impact of this metal oxide nanomaterial in a grass species has been studied and has revealed that the ability of

Panicum maximum Jacq to translocate the metals to the aerial parts of the plants (Translocation Factor - TF) is greater in the 5 ppm pre-treated seeds

Many synthetic methods have been developed, using the anatase phase of TiO_2 nanoparticles. Several approaches may be used to synthesize $nTiO_2$ for the purposes of exploiting its photocatalytic properties. Anatase form which is considered to be photocatalytic was successfully synthesized and the obtained powder was used to prime seeds. The performed germination tests revealed low mean germination time representing earlier seed germination, and in the study, it was found that the 20 ppm seed treatment had the earliest germination time. The 50 ppm treatment had an early mean germination time relative to the control but it had the highest inhibitory effects over the course of the study which suggests the exhibition of phytotoxic effects of this treatment. . The 50 ppm treatment also showed plant stress that was observed on the leaves. The comparison of the 50 ppm treatment with the control sample also indicated that its growth was slightly inhibited, as they were not as green or longer. The 5 ppm treatment had the least germination inhibition when compared to the control sample, of all the treatments across the study period which suggests that this treatment has very little enhancement effect on the germination parameter.

6.2 Recommendations

The pathways of potentially harmful elements in the soil are not fully understood yet, particularly with regard to *Panicum maximum* Jacq as a unique hyperaccumulator plant. Its applications in field experiments on contaminated sites are worth exploring as it is easily handled, fast-growing grass that can also benefit the environment in phytoremediation initiatives. Titanium can be considered a beneficial element for plant growth. It has been documented to improve crop performance by stimulating photosynthesis and increasing chlorophyll content (Jaishankar *et al.* 2014). Commercial fertilisers, which contain titanium as 'tytant', are in use as biostimulants. As a proposal, several trials on basic food that can be grown in a home garden, e.g. herbs for cooking, tomatoes, spinach, and onions can be experimented with $nTiO_2$; to evaluate if the seedling vigor/yield and quality of the product would be significantly affected using various methods of introduction of the titanium nano oxide. It is noteworthy that the mechanisms of action underlying the beneficial effects of titanium dioxide on plants are still to be fully understood and collaboration with scientists from specialized fields would prove invaluable in bridging the existing gap.

References

.

Aiken, G. R., Hsu-Kim, H. and Ryan, J. N. 2011. Influence of dissolved organic matter on the environmental fate of metals, nanoparticles, and colloids: *Environmental Science and Technology*, 45 (8): 3196–3201

Akcil, A. and Koldas, S. 2006. Acid Mine Drainage (AMD): Causes, treatment and case studies. *Journal of Cleaner Production*, 14 (12): 1139-1145.

Ali Mansoori, G., Bastami, T. R., Ahmadpour, A. and Eshaghi, Z. 2008. Environmental application of nanotechnology. In: *Annual Review of Nano Research*. World Scientific, 439-493.

Arif, N., Yadav, V., Singh, S., Singh, S., Mishra, R. and Sharma, S. 2016. Current Trends of Engineered Nanoparticles (ENPs) in Sustainable Agriculture: An Overview. J*ournal of Environmental and Analytical Toxicology*, 6: 1-5.

Aslani, F., Bagheri, S., Muhd Julkapli, N., Juraimi, A. S., Hashemi, F. S. and Baghdadi, A. 2014. Effects of engineered nanomaterials on plants growth: an overview. *Scientific World Journal*, 2014: 641759.

Bell, F. G. and Donnelly, L. J. 2014. *Mining and its Impact on the Environment*. ISBN 9780367390792 Published September 23, by CRC Press

Bora, M. S. and Sarma, K. P. 2020. Phytoremediation of Heavy Metals/Metalloids by Native Herbaceous Macrophytes of Wetlands: Current Research and Perspectives. In: *Emerging Issues in the Water Environment during Anthropocene*. Springer, 261-284.

Castiglione, M. R., Giorgetti, L., Cremonini, R., Bottega, S. and Spanò, C. 2014. Impact of TiO_2 nanoparticles on Vicia narbonensis L.: potential toxicity effects. *Protoplasma*, 251 (6): 1471-1479.

Castiglione, M. R., Giorgetti, L., Geri, C. and Cremonini, R. 2011. The effects of nano-TiO_2 on seed germination, development and mitosis of root tip cells of Vicia narbonensis L. and Zea mays L. *Journal of Nanoparticle Research*, 13 (6): 2443-2449.

Chang, C. Y., Yu, H. Y., Chen, J. J., Li, F. B., Zhang, H. H. and Liu, C. P. 2014. Accumulation of heavy metals in leaf vegetables from agricultural soils and associated potential health risks in the Pearl River Delta, South China. *Environmental Monitoring and Assessment*, 186 (3): 1547-1560.

Chen, G.-C., Liu, Z., Zhang, J. and Owens, G. 2012. Phytoaccumulation of copper in willow seedlings under different hydrological regimes. *Ecological Engineering*, 44: 285-289.

Chen, X. and Mao, S. S. 2007. Titanium dioxide nanomaterials: synthesis, properties, modifications, and applications. *Chemical Reviews*, 107 (7): 2891-2959.

Cheng, H., Ma, J., Zhao, Z. and Qi, L. 1995. Hydrothermal preparation of uniform nanosize rutile and anatase particles. *Chemistry of Materials*, 7 (4): 663-671.

Du, W., Sun, Y., Ji, R., Zhu, J., Wu, J. and Guo, H. 2011. TiO_2 and ZnO nanoparticles negatively affect wheat growth and soil enzyme activities in agricultural soil. *Journal of Environmental Monitoring*, 13 (4): 822-828.

Eapen, S. and D'souza, S. 2005. Prospects of genetic engineering of plants for phytoremediation of toxic metals. *Biotechnology Advances*, 23 (2): 97-114.

Emamverdian, A., Ding, Y., Mokhberdoran, F. and Xie, Y. 2015b. Heavy metal stress and some mechanisms of plant defense response. *The Scientific World Journal*, 756120.

Falkowski, P. G., Fenchel, T. and Delong, E. F. 2008. The microbial engines that drive Earth's biogeochemical cycles. *science*, 320 (5879): 1034-1039.

Fang, J., Shan, X.-q., Wen, B., Lin, J.-m. and Owens, G. 2009. Stability of titania nanoparticles in soil suspensions and transport in saturated homogeneous soil columns. *Environmental Pollution*, 157 (4): 1101-1109.

Feizi, H., Moghaddam, P. R., Shahtahmassebi, N. and Fotovat, A. 2012a. Impact of bulk and nanosized titanium dioxide (TiO_2) on wheat seed germination and seedling growth. *Biological Trace Element Research*, 146 (1): 101-106.

Feizi, H., Moghaddam, P. R., Shahtahmassebi, N. and Fotovat, A. 2012b. Impact of bulk and nanosized titanium dioxide (TiO_2) on wheat seed germination and seedling growth. *Biological trace element research*, 146 (1): 101-106.

Fellmann, S. and Eichert, T. 2017. Acute Effects of Engineered Nanoparticles on the Growth and Gas Exchange of Zea mays L. What are the Underlying Causes? *Water, Air, & Soil Pollution*, 228 (5): 1-13.

Fernández Rodríguez, M. D., García Gómez, M. C., Alonso Blazquez, N. and Tarazona, J. V. 2014. *Soil Pollution Remediation.* In Encyclopedia of Toxicology; Wexler, P., Ed.; Academic Press Inc.: Oxford, UK, 344–355.

Galende, M. A., Becerril, J. M., Barrutia, O., Artetxe, U., Garbisu, C. and Hernández, A. 2014. Field assessment of the effectiveness of organic amendments for aided phytostabilization of a Pb–Zn contaminated mine soil. *Journal of Geochemical Exploration*, 145: 181-189.

Han, P., Wang, X., Cai, L., Tong, M. and Kim, H. 2014. Transport and retention behaviors of titanium dioxide nanoparticles in iron oxide-coated quartz sand: effects of pH, ionic strength, and humic acid. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 454: 119-127.

He, S., He, Z., Yang, X. and Baligar, V. C. 2012. Mechanisms of Nickel Uptake and Hyperaccumulation by Plants and Implications for Soil Remediation. *Advances in Agronomy*, 117: 117-189.

Hu, Y., Ge, Y., Zhang, C., Ju, T. and Cheng, W. 2009. Cadmium toxicity and translocation in rice seedlings are reduced by hydrogen peroxide pretreatment. *Plant Growth Regulation*, 59 (1): 51.

Hu, Z., Fan, J., Xie, Y., Amombo, E., Liu, A., Gitau, M. M., Khaldun, A., Chen, L. and Fu, J. 2016. Comparative photosynthetic and metabolic analyses reveal mechanism of improved cold stress tolerance in bermudagrass by exogenous melatonin. *Plant Physiology and Biochemistry*, 100: 94-104.

Hussain, I., Singh, N., Singh, A., Singh, H. and Singh, S. 2016. Green synthesis of nanoparticles and its potential application. *Biotechnology Letters*, 38 (4): 545-560.

Ingle, A. P., Duran, N. and Rai, M. 2014. Bioactivity, mechanism of action, and cytotoxicity of copper-based nanoparticles: a review. *Applied microbiology and biotechnology,* 98 (3), 1001-1009

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew Blessy, B. and Beeregowda Krishnamurthy, N. 2014. *Toxicity, mechanism and health effects of some heavy metals*. Available: https://www.degruyter.com/view/j/intox.2014.7.issue-2/intox-2014-0009/intox-2014-0009.xml (Accessed 2018-03-10t14:04:11.463+01:00).

Jiamjitrpanich, W., Parkpian, P., Polprasert, C. and Kosanlavit, R. 2013. Trinitrotoluene and Its Metabolites in Shoots and Roots of Panicum maximum in Nano-Phytoremediation. *International Journal of Environmental Science and Development*: 7-10.

Kalinichev, A. and Kirkpatrick, R. 2007. Molecular dynamics simulation of cationic complexation with natural organic matter. *European Journal of Soil Science*, 58 (4): 909-917.

Karn, B., Kuiken, T. and Otto, M. 2009. Nanotechnology and in situ remediation: a review of the benefits and potential risks. *Environmental Health Perspectives*, 117 (12): 1813-1831.

Keller, A. A. and Lazareva, A. 2014. Predicted releases of engineered nanomaterials: from global to regional to local. *Environmental Science & Technology Letters*, 1 (1): 65-70.

Keller, A. A., McFerran, S., Lazareva, A. and Suh, S. 2013. Global life cycle releases of engineered nanomaterials. *Journal of Nanoparticle Research*, 15 (6): 1692.

Khan, I., Farhan, M., Singh, P. and Thiagarajan, P. 2014. Nanotechnology for Environmental Remediation. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 5(3): 1916.

Khot, L. R., Sankaran, S., Maja, J. M., Ehsani, R. and Schuster, E. W. 2012. Applications of nanomaterials in agricultural production and crop protection: a review. *Crop Protection*, 35: 64-70.

Kulkarni, M., Mazare, A., Gongadze, E., Perutkova, Š., Kralj-Iglič, V., Milošev, I., Schmuki, P., Iglič, A. and Mozetič, M. 2015. Titanium nanostructures for biomedical applications. *Nanotechnology*, 26 (6): 062002.

Kumar Rai, P. and Singh, J. S. 2020. Invasive alien plant species: Their impact on environment, ecosystem services and human health. *Ecological indicators*, 111: 106020-106020.

Kurepa, J., Paunesku, T., Vogt, S., Arora, H., Rabatic, B. M., Lu, J., Wanzer, M. B., Woloschak, G. E. and Smalle, J. A. 2010. Uptake and Distribution of Ultrasmall Anatase TiO_2 Alizarin Red S Nanoconjugates in Arabidopsis thaliana. *Nano Letters*, 10 (7): 2296-2302.

Kutty, A. A. and Al-Mahaqeri, S. A. 2016. An Investigation of the Levels and Distribution of Selected Heavy Metals in Sediments and Plant Species within the Vicinity of Ex-Iron Mine in Bukit Besi. *Journal of Chemistry*, 1-12.

Lal, R. 2015. Restoring Soil Quality to Mitigate Soil Degradation. *Sustainability*, 7(5), 5875-5895

Lapworth, D., Nkhuwa, D., Okotto-Okotto, J., Pedley, S., Stuart, M., Tijani, M. and Wright, J. 2017. Urban groundwater quality in sub-Saharan Africa: current status and implications for water security and public health. *Hydrogeology Journal*, 25 (4): 1093-1116.

Larue, C., Khodja, H., Herlin-Boime, N., Brisset, F., Flank, A., Fayard, B., Chaillou, S. and Carrière, M. 2011. Investigation of titanium dioxide nanoparticles toxicity and uptake by plants. In: Proceedings of *Journal of Physics: Conference Series*. IOP Publishing, 012057.

Li, L., Wang, Y. and Shen, W. 2012. Roles of hydrogen sulfide and nitric oxide in the alleviation of cadmium-induced oxidative damage in alfalfa seedling roots. *Biometals*, 25 (3): 617-631.

Lima, P., Vasconcellos, M., Montenegro, R., Bahia, M., Costa, E., Antunes, L. and Burbano, R. 2011. Genotoxic effects of aluminum, iron and manganese in human cells and experimental systems: a review of the literature. *Human & Experimental Toxicology*, 30 (10): 1435-1444.

Limmer, M. and Burken, J. 2016. Phytovolatilization of organic contaminants. *Environmental Science & Technology*, 50 (13): 6632-6643.

Lin, D., Tian, X., Wu, F. and Xing, B. 2010. Fate and transport of engineered nanomaterials in the environment. *Journal of Environmental Quality*, 39 (6): 1896-1908.

Litter, M. and Navio, J. A. 1996. Photocatalytic properties of iron-doped titania semiconductors. *Journal of Photochemistry and Photobiology A: Chemistry*, 98 (3): 171-181.

Liu, R. and Lal, R. 2015. Potentials of engineered nanoparticles as fertilizers for increasing agronomic productions. *Science of The Total Environment*, 514: 131-139.

Lum, A. F., Ngwa, E., Chikoye, D. and Suh, C. 2014. Phytoremediation potential of weeds in heavy metal contaminated soils of the Bassa Industrial Zone of Douala, Cameroon. *International Journal of Phytoremediation*, 16 (3): 302-319.

Mahar, A., Wang, P., Ali, A., Awasthi, M. K., Lahori, A. H., Wang, Q., Li, R. and Zhang, Z. 2016. Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: a review. *Ecotoxicology and Environmental Safety*, 126: 111-121.

Mahmood, A. and Malik, R. N. 2014. Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. *Arabian Journal of Chemistry*, 7 (1): 91-99.

Mani, D. and Kumar, C. 2014. Biotechnological advances in bioremediation of heavy metals contaminated ecosystems: an overview with special reference to phytoremediation. *International Journal of Environmental Science and Technology*, 11 (3): 843-872.

Mattiello, A., Filippi, A., Pošćić, F., Musetti, R., Salvatici, M. C., Giordano, C., Vischi, M., Bertolini, A. and Marchiol, L. 2015. Evidence of phytotoxicity and genotoxicity in Hordeum vulgare L. exposed to CeO_2 and TiO_2 nanoparticles. *Frontiers in Plant Science*, 6: 1043.

Messou, A., Coulibaly, L., Doumbia, L. and Gourene, G. 2013. Plants diversity and phytoaccumulators identification on the Akouedo landfill (Abidjan, Côte d'Ivoire). *African Journal of Biotechnology*, 12 (3): 253-264

Mrozik, A. and Piotrowska-Seget, Z. 2010. Bioaugmentation as a strategy for cleaning up of soils contaminated with aromatic compounds. *Microbiological Research*, 165 (5): 363-375.

Mudunkotuwa, I. A. and Grassian, V. H. 2010. Citric acid adsorption on TiO_2 nanoparticles in aqueous suspensions at acidic and circumneutral pH: surface coverage, surface speciation, and its impact on nanoparticle− nanoparticle interactions. *Journal of the American Chemical Society*, 132 (42): 14986-14994.

Nadeem, M., Tungmunnithum, D., Hano, C., Abbasi, B.H., Hashmi, S.S., Ahmad, W. and Zahir, A., 2018. The current trends in the green syntheses of titanium oxide nanoparticles and their applications. *Green Chemistry Letters and Reviews*, 11(4), 492-502.

Naicker, K., Cukrowska, E. and McCarthy, T. 2003. Acid mine drainage arising from gold mining activity in Johannesburg, South Africa and environs. *Environmental pollution*, 122 (1): 29-40.

Niaounakis, M. 2013. 4 - Disposal. In: Niaounakis, M. ed. Biopolymers Reuse, Recycling, and Disposal. Oxford: William Andrew Publishing, 107-150.

Olatunji, S. O., Ximba, J. B., Fatoki, S. O. and Opeolu, O. B. 2014. Assessment of the phytoremediation potential of Panicum maximum (guinea grass) for selected heavy metal removal from contaminated soils. *African Journal of Biotechnology*, 13 (19): 1979-1984.

Pachapur, V. L., Larios, A. D., Cledón, M., Brar, S. K., Verma, M. and Surampalli, R. Y. 2016. Behavior and characterization of titanium dioxide and silver nanoparticles in soils. *Science of The Total Environment*, 563: 933-943.

Pakrashi, S., Jain, N., Dalai, S., Jayakumar, J., Chandrasekaran, P. T., Raichur, A. M., Chandrasekaran, N. and Mukherjee, A. 2014. In vivo genotoxicity assessment of titanium dioxide nanoparticles by Allium cepa root tip assay at high exposure concentrations. *PloS One*, 9 (2): e87789.

Palmisano, L., Augugliaro, V., Sclafani, A. and Schiavello, M. 1988. Activity of chromium-ion-doped titania for the dinitrogen photoreduction to ammonia and for the phenol photodegradation. *The Journal of Physical Chemistry*, 92 (23): 6710-6713.

Peralta-Videa, J. R., Zhao, L., Lopez-Moreno, M. L., de la Rosa, G., Hong, J. and Gardea-Torresdey, J. L. 2011. Nanomaterials and the environment: a review for the biennium 2008–2010. *Journal of Hazardous Materials*, 186 (1): 1-15.

Pilon-Smits, E. and Pilon, M. 2002. Phytoremediation of metals using transgenic plants. *Critical Reviews in Plant Sciences*, 21 (5): 439-456.

Raliya, R., Nair, R., Chavalmane, S., Wang, W. N. and Biswas, P. 2015. Mechanistic evaluation of translocation and physiological impact of titanium dioxide and zinc oxide nanoparticles on the tomato (Solanum lycopersicum L.) plant. *Metallomics*, 7 (12): 1584-1594.

Rascio, N. and Navari-Izzo, F. 2011. Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? *Plant Science*, 180 (2): 169-181.

Rastogi, A., Zivcak, M., Sytar, O., Kalaji, H. M., He, X., Mbarki, S. and Brestic, M. 2017a. Impact of Metal and Metal Oxide Nanoparticles on Plant: A Critical Review. *Frontiers in Chemistry*, 5: 78-78.

Rastogi, A., Zivcak, M., Sytar, O., Kalaji, H. M., He, X., Mbarki, S. and Brestic, M. 2017b. Impact of Metal and Metal Oxide Nanoparticles on Plant: A Critical Review. *Front Chem*, 5: 78.

Sarwar, N., Imran, M., Shaheen, M. R., Ishaque, W., Kamran, M. A., Matloob, A., Rehim, A. and Hussain, S. 2017. Phytoremediation strategies for soils contaminated with heavy metals: Modifications and future perspectives. *Chemosphere*, 171: 710-721.

Savvides, A., Ali, S., Tester, M. and Fotopoulos, V. 2016. Chemical Priming of Plants Against Multiple Abiotic Stresses: Mission Possible? *Trends in Plant Science*, 21 (4): 329-340.

Schaumann, G. E., Philippe, A., Bundschuh, M., Metreveli, G., Klitzke, S., Rakcheev, D., Grün, A., Kumahor, S. K., Kühn, M., Baumann, T., Lang, F., Manz, W., Schulz, R. and Vogel, H.-J. 2015. Understanding the fate and biological effects of Ag- and TiO_2-nanoparticles in the environment: The quest for advanced analytics and interdisciplinary concepts. *Science of The Total Environment*, 535: 3-19.

Sheoran, V., Sheoran, A. S. and Poonia, P. 2016. Factors affecting phytoextraction: a review. *Pedosphere*, 26 (2): 148-166.

Silva, S., Oliveira, H., Silva, A. and Santos, C. 2017. The cytotoxic targets of anatase or rutile+ anatase nanoparticles depend on the plant species. *Biologia Plantarum*, 61 (4): 717-725.

Simonin, M., Richaume, A., Guyonnet, J. P., Dubost, A., Martins, J. M. F. and Pommier, T. 2016. Titanium dioxide nanoparticles strongly impact soil microbial function by affecting archaeal nitrifiers. *Scientific Reports*, 6: 33643.

Song, B., Xu, P., Chen, M., Tang, W., Zeng, G., Gong, J., Zhang, P. and Ye, S. 2019. Using nanomaterials to facilitate the phytoremediation of contaminated soil. *Critical Reviews in Environmental Science and Technology*, 49 (9): 791-824.

Song, G., Gao, Y., Wu, H., Hou, W., Zhang, C. and Ma, H. 2012. Physiological effect of anatase TiO_2 nanoparticles on Lemna minor. *Environmental Toxicology and Chemistry*, 31 (9): 2147-2152.

Song, U., Shin, M., Lee, G., Roh, J., Kim, Y. and Lee, E. J. 2013. Functional analysis of TiO_2 nanoparticle toxicity in three plant species. *Biological Trace Element Research*, 155 (1): 93-103.

Stamm, H., Gibson, N. and Anklam, E. 2012. Detection of nanomaterials in food and consumer products: bridging the gap from legislation to enforcement. *Food Additives & Contaminants: Part A*, 29 (8): 1175-1182.

Stuart, M., Lapworth, D., Crane, E. and Hart, A. 2012. Review of risk from potential emerging contaminants in UK groundwater. *Science of The Total Environment*, 416: 1-21.

Sundström, J. F., Albihn, A., Boqvist, S., Ljungvall, K., Marstorp, H., Martiin, C., Nyberg, K., Vågsholm, I., Yuen, J. and Magnusson, U. 2014. Future threats to agricultural food production posed by environmental degradation, climate change, and animal and plant diseases – a risk analysis in three economic and climate settings. *Food Security*, 6 (2): 201-215.

Suresh, S. 2013. Semiconductor nanomaterials, methods and applications: a review. *Nanoscience and Nanotechnology*, 3 (3): 62-74.

Surriya, O., Sarah Saleem, S., Waqar, K. and Gul Kazi, A. 2015. Phytoremediation of Soils. 1-36.

Tabaei, H. S. M., Kazemeini, M. and Fattahi, M. 2012. Preparation and characterization of visible light sensitive nano titanium dioxide photocatalyst. *Scientia Iranica*, 19 (6): 1626-1631.

Tan, W., Peralta-Videa, J. R. and Gardea-Torresdey, J. L. 2018. Interaction of titanium dioxide nanoparticles with soil components and plants: current knowledge and future research needs–a critical review. *Environmental Science: Nano*, 5 (2): 257-278.

Tumburu, L., Andersen, C. P., Rygiewicz, P. T. and Reichman, J. R. 2015. Phenotypic and genomic responses to titanium dioxide and cerium oxide nanoparticles in Arabidopsis germinants. *Environmental Toxicology and Chemistry*, 34 (1): 70-83.

Vamerali, T., Bandiera, M. and Mosca, G. 2010. Field crops for phytoremediation of metal-contaminated land. A review. *Environmental Chemistry Letters*, 8 (1): 1-17.

Wang, C.-C., Zhang, Z. and Ying, J. Y. 1997. Photocatalytic decomposition of halogenated organics over nanocrystalline titania. *Nanostructured Materials*, 9 (1-8): 583-586.

Wang, J., Feng, X., Anderson, C. W., Xing, Y. and Shang, L. 2012. Remediation of mercury contaminated sites–a review. *Journal of Hazardous Materials*, 221: 1-18.

Wang, S., Kurepa, J. and Smalle, J. A. 2011. Ultra-small TiO_2 nanoparticles disrupt microtubular networks in Arabidopsis thaliana. *Plant, Cell & Environment*, 34 (5): 811-820.

Xu, C., Peng, C., Sun, L., Zhang, S., Huang, H., Chen, Y. and Shi, J. 2015. Distinctive effects of TiO2 and CuO nanoparticles on soil microbes and their community structures in flooded paddy soil. *Soil Biology and Biochemistry*, 86: 24-33.

Yadav, K., Singh, J., Gupta, N. and Kumar, V. 2017. A review of nanobioremediation technologies for environmental cleanup: A novel biological approach. *Journal of Material and Environmental Science*, 8: 740-757.

Yuan, G.-L., Sun, T.-H., Han, P., Li, J. and Lang, X.-X. 2014. Source identification and ecological risk assessment of heavy metals in topsoil using environmental geochemical mapping: Typical urban renewal area in Beijing, China. *Journal of Geochemical Exploration*, 136: 40-47.

Zheng, L., Hong, F., Lu, S. and Liu, C. 2005. Effect of nano-TiO2 on strength of naturally aged seeds and growth of spinach. *Biological Trace Element Research*, 104 (1): 83-91.

Sheoran, V., Sheoran, A. S. and Poonia, P. 2016. Factors affecting phytoextraction: a review. *Pedosphere*, 26 (2): 148-166.

Silva, S., Oliveira, H., Silva, A. and Santos, C. 2017. The cytotoxic targets of anatase or rutile+ anatase nanoparticles depend on the plant species. *Biologia Plantarum*, 61 (4): 717-725.

Song, U., Shin, M., Lee, G., Roh, J., Kim, Y. and Lee, E. J. 2013. Functional analysis of TiO_2 nanoparticle toxicity in three plant species. *Biological Trace Element Research*, 155 (1): 93-103.

Tan, W., Peralta-Videa, J. R. and Gardea-Torresdey, J. L. 2018. Interaction of titanium dioxide nanoparticles with soil components and plants: current knowledge and future research needs–a critical review. *Environmental Science: Nano*, 5 (2): 257-278.

Tumburu, L., Andersen, C. P., Rygiewicz, P. T. and Reichman, J. R. 2015. Phenotypic and genomic responses to titanium dioxide and cerium oxide nanoparticles in Arabidopsis germinants. *Environmental Toxicology and Chemistry*, 34 (1): 70-83.

Wang, S., Kurepa, J. and Smalle, J. A. 2011. Ultra-small TiO2 nanoparticles disrupt microtubular networks in Arabidopsis thaliana. *Plant, Cell & Environment*, 34 (5): 811-820.

Appendix: Coordinates

Citric Acid

```
N_atoms =   21     N_atom_types =  3
```

```
INPUT_DMOL keywords (for archive):
______________________________________________________________________>8

#Warning: no global confinement specs in BASFILE
# Task parameters
Calculate                      optimize
Opt_energy_convergence         2.0000e-005
Opt_gradient_convergence       4.0000e-003 A
Opt_displacement_convergence   5.0000e-003 A
Opt_iterations                 50
Opt_max_displacement           0.3000 A
Initial_hessian                improved
Symmetry                       on
Max_memory                     2048
File_usage                     smart
Scf_density_convergence        1.000000e-005
Scf_charge_mixing              2.000000e-001
Scf_spin_mixing                5.000000e-001
Scf_diis                       6 pulay
Scf_iterations                 50
# Electronic parameters
Spin_polarization              unrestricted
Charge                         0
Basis                          dnd
Pseudopotential                none
Functional                     pwc
Aux_density                    hexadecapole
Integration_grid               medium
Occupation                     thermal 0.0500
Cutoff_Global                  3.3000 angstrom
# Calculated properties
Print_eigval_window            -1.d9
Plot                           homo
Plot                           lumo
Grid                           msbox  3 0.2500 0.2500 0.2500 3.0000
```

ATOM		X	Y	Z
1	O	-3.306655	-0.890189	1.336440
2	C	-2.293751	0.194143	1.054773
3	C	-0.804147	-0.049242	1.358802
4	C	-0.043992	-0.209338	0.030668
5	C	1.447993	-0.465284	0.314348
6	C	2.217594	-0.655161	1.007756
7	O	3.466152	-1.503775	1.036617
8	C	-0.201869	1.084376	0.787275
9	O	0.805491	2.198477	0.630508
10	O	-1.254979	1.166932	1.865472
11	O	-0.624521	-1.371642	0.736340
12	O	1.641515	-0.130037	2.300842
13	O	-2.754383	1.554635	0.588624
14	H	-0.379171	0.838961	1.933325

15	H	-0.691049	-0.997783	1.980982
16	H	1.879664	0.423042	0.883528
17	H	1.552937	-1.407655	0.947242
18	H	-1.699920	-1.177840	0.932080
19	H	-4.301336	-0.432878	1.519548
20	H	4.360558	-0.846158	1.035111
21	H	0.983866	2.676414	1.616281

Fulvic Acid

ATOM		X	Y	Z
1	C	-3.155337	-4.219905	-1.971990
2	C	-2.102220	-4.767346	-2.736476
3	C	-0.869031	-4.073429	-2.837585
4	C	-0.700612	-2.806183	-2.193155
5	C	-1.825661	-2.191721	-1.591354
6	C	-2.999649	-2.962636	-1.367098
7	C	-1.767038	-0.812112	-1.258200
8	C	-0.510118	-0.157553	-1.149779
9	C	0.672024	-0.861433	-1.517866
10	C	0.555196	-2.141453	-2.123365
11	C	-0.461115	1.300134	-0.685079
12	C	-0.756075	1.463578	0.790889
13	C	-0.905485	2.854663	1.378940
14	C	0.427738	3.314384	2.055646
15	C	0.513135	4.855964	2.177937
16	C	1.918377	5.322669	2.424811
17	C	0.677480	2.646441	3.421641
18	C	-2.155392	2.919071	2.284802
19	O	-2.403844	4.234829	2.694248
20	O	-0.844749	0.481279	1.515789
21	C	2.063821	-0.276158	-1.263258
22	C	2.413852	-0.256845	0.232242
23	C	3.870682	0.078755	0.433895
24	C	4.307923	1.456932	0.878975
25	C	4.032635	1.692777	2.338659
26	O	3.652747	2.437967	0.119626
27	O	4.717591	-0.767535	0.188555
28	O	1.703823	-2.802275	-2.566438
29	C	0.201658	-4.668814	-3.671374
30	O	0.591530	-4.075720	-4.725367
31	O	0.713835	-5.787178	-3.353406
32	C	-2.270295	-6.094010	-3.385946
33	O	-2.646805	-7.097650	-2.703677
34	O	-2.034756	-6.236331	-4.626450
35	C	-4.450371	-4.932781	-1.822254
36	O	-5.000355	-5.034278	-0.681367
37	O	-5.027271	-5.436713	-2.836107
38	O	-4.005268	-2.531335	-0.490953
39	C	-3.026266	-0.017957	-1.245563
40	O	3.499919	0.794578	3.064311
41	O	4.358183	2.802399	2.863489
42	O	2.808705	5.170669	1.531062
43	O	2.220110	5.886726	3.522230
44	O	-3.831310	-0.099678	-2.225285
45	O	-3.331727	0.710946	-0.252628
46	H	-1.222816	1.902819	-1.281887
47	H	0.578570	1.725878	-0.878461
48	H	-1.093826	3.563321	0.506053
49	H	1.277038	2.989392	1.368136
50	H	-0.149449	5.198081	3.040224
51	H	0.143249	5.334871	1.211794

52	H	1.610799	3.094584	3.898801
53	H	0.826369	1.525747	3.275087
54	H	-0.219966	2.824837	4.101615
55	H	-1.988260	2.264707	3.203211
56	H	-3.061748	2.535318	1.709605
57	H	-1.444030	4.730193	2.950124
58	H	2.099161	0.789179	-1.667496
59	H	2.845471	-0.905516	-1.804120
60	H	2.198071	-1.281734	0.682399
61	H	1.778141	0.527804	0.761202
62	H	5.430074	1.553961	0.703013
63	H	3.723474	3.416381	0.639053
64	H	2.596196	-2.365215	-2.071716
65	H	-3.562430	-1.872723	0.285078
66	H	1.638946	-5.616336	-2.764269
67	H	-3.297208	-6.756350	-1.871457
68	H	-4.322621	-4.620472	0.094224
69	H	3.445715	3.326332	3.217039
70	H	3.736596	5.695672	1.840077
71	H	-2.405726	1.103535	0.216941

Humic Acid

ATOM		X	Y	Z
1	O	-7.926378	0.956264	2.799981
2	C	-6.722212	1.185989	3.478914
3	C	-5.528284	0.875256	2.559371
4	C	-5.490496	1.745831	1.316291
5	C	-4.483923	1.529096	0.236083
6	C	-4.472832	2.365009	-0.894197
7	C	-3.508572	2.206606	-1.890657
8	C	-2.500627	1.228169	-1.768407
9	C	-2.568428	0.323171	-0.690490
10	C	-3.536968	0.490746	0.310095
11	O	-1.459012	1.092185	-2.713974
12	C	-0.927289	2.286152	-3.321987
13	C	-1.244059	2.296899	-4.827191
14	O	-0.921116	1.069457	-5.414656
15	C	0.587922	2.460958	-3.006582
16	C	1.564948	1.371717	-3.591282
17	C	1.118519	-0.070914	-3.435934
18	C	1.080046	-0.909041	-4.479740
19	C	0.704693	-2.325969	-4.346739
20	C	3.000733	1.526531	-3.040229
21	C	4.006258	0.809177	-3.897878
22	O	4.211514	1.189068	-5.041924

23	O	4.702738	-0.286359	-3.381737
24	O	0.319165	-2.777050	-3.279450
25	O	0.796196	-3.153791	-5.464142
26	C	0.783140	2.823164	-1.523017
27	C	0.970766	4.240019	-1.063392
28	C	1.422469	4.524384	0.353537
29	C	1.389756	3.326802	1.257932
30	C	1.058139	2.104288	0.829378
31	C	0.745491	1.862675	-0.590855
32	C	1.090882	0.921890	1.771018
33	C	2.277236	-0.043361	1.482325
34	C	3.628621	0.709601	1.632907
35	O	3.843980	1.561955	0.541361
36	C	2.268973	-1.318835	2.400942
37	C	0.886869	-2.059014	2.473465
38	O	-0.044097	-1.334723	3.239960
39	C	3.341860	-2.287985	1.914302
40	O	4.436683	-2.626219	2.716321
41	O	3.248462	-2.788046	0.803132
42	C	0.983931	-3.484661	3.025982
43	C	0.480186	-4.611315	2.322317
44	C	0.736103	-5.912372	2.805066
45	C	1.449521	-6.094164	4.006192
46	C	1.881238	-4.977196	4.730037
47	C	1.643687	-3.691382	4.251646
48	O	0.238035	-7.008387	2.090641
49	O	1.711523	-7.386104	4.474800
50	N	-0.335767	-4.454738	1.150277
51	O	-4.334379	1.059856	3.273771
52	O	-6.294182	2.657652	1.201348
53	C	0.670414	5.298599	-1.830886
54	N	-1.649521	-0.769561	-0.592897
55	H	-8.649568	1.109519	3.461368
56	H	-6.674898	0.528253	4.377165
57	H	-6.674020	2.244750	3.818666
58	H	-5.619072	-0.186960	2.234135
59	H	-5.207186	3.153333	-1.009796
60	H	-3.559375	2.852778	-2.756740
61	H	-3.542494	-0.202705	1.140423

62	H	-1.400679	3.203377	-2.908767
63	H	-0.691045	3.120056	-5.331514
64	H	-2.330731	2.485683	-4.958858
65	H	-1.146732	1.154224	-6.377240
66	H	0.897544	3.356990	-3.579687
67	H	1.620760	1.594115	-4.682948
68	H	0.927857	-0.465369	-2.454242
69	H	1.351147	-0.554067	-5.467086
70	H	3.053498	1.119979	-2.008175
71	H	3.284011	2.601050	-2.989814
72	H	0.746504	5.283646	0.806623
73	H	2.454060	4.936170	0.333339
74	H	1.661966	3.478892	2.295946
75	H	0.523323	0.854353	-0.868139
76	H	0.124699	0.400416	1.622786
77	H	1.131802	1.252551	2.832935
78	H	2.187143	-0.387374	0.427150
79	H	4.481205	-0.001101	1.681233
80	H	3.628362	1.295448	2.580015
81	H	4.673524	2.068555	0.741524
82	H	2.528324	-0.984040	3.428828
83	H	0.513443	-2.082484	1.432886
84	H	0.305928	-1.261401	4.164621
85	H	2.413521	-5.103191	5.664415
86	H	2.006798	-2.850125	4.829734
87	H	0.471104	-7.911668	2.484923
88	H	2.194452	-7.412646	5.363998
89	H	-0.507081	-5.254264	0.502836
90	H	-0.939636	-3.615060	1.016029
91	H	-4.239014	0.280817	3.883573
92	H	0.247087	5.216945	-2.820093
93	H	0.806441	6.305576	-1.453487
94	H	-1.613806	-1.358564	0.267590
95	H	-1.036851	-1.037732	-1.392503
96	H	5.360209	-0.677082	-4.047704
97	H	0.483882	-4.100065	-5.271940
98	H	4.421039	-2.179555	3.624842

$nTiO_2$

ATOM	X	Y	Z
Ti	-1.77724100	-3.73984022	-10.83440300
O	-0.37724100	-5.13984022	-10.83440300
Ti	2.81675900	-3.73984022	-10.83440300
O	1.41675900	-2.33984022	-10.83440300
O	4.21675900	-5.13984022	-10.83440300
Ti	7.41075900	-3.73984022	-10.83440300
O	6.01075900	-2.33984022	-10.83440300
Ti	2.81675900	-12.92784022	-7.87540300
O	1.41675900	-11.52784022	-7.87540300
Ti	-1.77724100	-8.33384022	-7.87540300
O	-0.88024100	-7.43684022	-9.35540300
O	-3.17724100	-6.93384022	-7.87540300
O	-0.37724100	-9.73384022	-7.87540300
Ti	2.81675900	-8.33384022	-7.87540300
O	1.91975900	-9.23084022	-9.35540300
O	3.71375900	-7.43684022	-9.35540300
O	1.41675900	-6.93384022	-7.87540300
O	4.21675900	-9.73384022	-7.87540300
Ti	7.41075900	-8.33384022	-7.87540300
O	6.01075900	-6.93384022	-7.87540300
Ti	-1.77724100	-3.73984022	-7.87540300
O	-2.67424100	-4.63684022	-9.35540300
O	-0.88024100	-2.84284022	-9.35540300
O	-3.17724100	-2.33984022	-7.87540300
O	-0.37724100	-5.13984022	-7.87540300
Ti	0.51975900	-6.03684022	-9.35540300
Ti	2.81675900	-3.73984022	-7.87540300
O	1.91975900	-4.63684022	-9.35540300
O	3.71375900	-2.84284022	-9.35540300
O	1.41675900	-2.33984022	-7.87540300
O	4.21675900	-5.13984022	-7.87540300
Ti	5.11375900	-6.03684022	-9.35540300
Ti	7.41075900	-3.73984022	-7.87540300
O	6.51375900	-4.63684022	-9.35540300
O	8.30775900	-2.84284022	-9.35540300
O	6.01075900	-2.33984022	-7.87540300
O	8.81075900	-5.13984022	-7.87540300
Ti	-1.77724100	0.85415978	-7.87540300
O	-0.37724100	-0.54584022	-7.87540300
Ti	0.51975900	-1.44284022	-9.35540300
Ti	2.81675900	0.85415978	-7.87540300
O	1.91975900	-0.04284022	-9.35540300
O	3.71375900	1.75115978	-9.35540300
O	1.41675900	2.25415978	-7.87540300
O	4.21675900	-0.54584022	-7.87540300
Ti	5.11375900	-1.44284022	-9.35540300
Ti	7.41075900	0.85415978	-7.87540300

O	6.51375900	-0.04284022	-9.35540300
O	6.01075900	2.25415978	-7.87540300
O	8.81075900	-0.54584022	-7.87540300
Ti	2.81675900	5.44815978	-7.87540300
O	4.21675900	4.04815978	-7.87540300
Ti	2.81675900	-12.92784022	-4.91640300
O	3.71375900	-12.03084022	-6.39640300
O	1.41675900	-11.52784022	-4.91640300
O	6.01075900	-11.52784022	-4.91640300
Ti	-1.77724100	-8.33384022	-4.91640300
O	-2.67424100	-9.23084022	-6.39640300
O	-0.88024100	-7.43684022	-6.39640300
O	-3.17724100	-6.93384022	-4.91640300
O	-0.37724100	-9.73384022	-4.91640300
Ti	0.51975900	-10.63084022	-6.39640300
Ti	2.81675900	-8.33384022	-4.91640300
O	1.91975900	-9.23084022	-6.39640300
O	3.71375900	-7.43684022	-6.39640300
O	1.41675900	-6.93384022	-4.91640300
O	4.21675900	-9.73384022	-4.91640300
Ti	5.11375900	-10.63084022	-6.39640300
Ti	7.41075900	-8.33384022	-4.91640300
O	6.51375900	-9.23084022	-6.39640300
O	8.30775900	-7.43684022	-6.39640300
O	6.01075900	-6.93384022	-4.91640300
O	8.81075900	-9.73384022	-4.91640300
O	10.60475900	-6.93384022	-4.91640300
O	-5.47424100	-2.84284022	-6.39640300
O	-4.97124100	-5.13984022	-4.91640300
Ti	-4.07424100	-6.03684022	-6.39640300
Ti	-1.77724100	-3.73984022	-4.91640300
O	-2.67424100	-4.63684022	-6.39640300
O	-0.88024100	-2.84284022	-6.39640300
O	-3.17724100	-2.33984022	-4.91640300
O	-0.37724100	-5.13984022	-4.91640300
Ti	0.51975900	-6.03684022	-6.39640300
Ti	2.81675900	-3.73984022	-4.91640300
O	1.91975900	-4.63684022	-6.39640300
O	3.71375900	-2.84284022	-6.39640300
O	1.41675900	-2.33984022	-4.91640300
O	4.21675900	-5.13984022	-4.91640300
Ti	5.11375900	-6.03684022	-6.39640300
Ti	7.41075900	-3.73984022	-4.91640300
O	6.51375900	-4.63684022	-6.39640300
O	8.30775900	-2.84284022	-6.39640300
O	6.01075900	-2.33984022	-4.91640300
O	8.81075900	-5.13984022	-4.91640300
Ti	9.70775900	-6.03684022	-6.39640300
O	11.10775900	-4.63684022	-6.39640300
O	10.60475900	-2.33984022	-4.91640300
O	-4.97124100	-0.54584022	-4.91640300
Ti	-4.07424100	-1.44284022	-6.39640300

Ti	-1.77724100	0.85415978	-4.91640300
O	-2.67424100	-0.04284022	-6.39640300
O	-0.88024100	1.75115978	-6.39640300
O	-3.17724100	2.25415978	-4.91640300
O	-0.37724100	-0.54584022	-4.91640300
Ti	0.51975900	-1.44284022	-6.39640300
Ti	2.81675900	0.85415978	-4.91640300
O	1.91975900	-0.04284022	-6.39640300
O	3.71375900	1.75115978	-6.39640300
O	1.41675900	2.25415978	-4.91640300
O	4.21675900	-0.54584022	-4.91640300
Ti	5.11375900	-1.44284022	-6.39640300
Ti	7.41075900	0.85415978	-4.91640300
O	6.51375900	-0.04284022	-6.39640300
O	8.30775900	1.75115978	-6.39640300
O	6.01075900	2.25415978	-4.91640300
O	8.81075900	-0.54584022	-4.91640300
Ti	9.70775900	-1.44284022	-6.39640300
O	-0.37724100	4.04815978	-4.91640300
Ti	0.51975900	3.15115978	-6.39640300
Ti	2.81675900	5.44815978	-4.91640300
O	1.91975900	4.55115978	-6.39640300
O	4.21675900	4.04815978	-4.91640300
Ti	5.11375900	3.15115978	-6.39640300
O	-0.88024100	-12.03084022	-3.43740300
Ti	2.81675900	-12.92784022	-1.95740300
O	3.71375900	-12.03084022	-3.43740300
O	1.41675900	-11.52784022	-1.95740300
O	6.01075900	-11.52784022	-1.95740300
Ti	-6.37124100	-8.33384022	-1.95740300
O	-5.47424100	-7.43684022	-3.43740300
O	-4.97124100	-9.73384022	-1.95740300
Ti	-4.07424100	-10.63084022	-3.43740300
Ti	-1.77724100	-8.33384022	-1.95740300
O	-2.67424100	-9.23084022	-3.43740300
O	-0.88024100	-7.43684022	-3.43740300
O	-3.17724100	-6.93384022	-1.95740300
O	-0.37724100	-9.73384022	-1.95740300
Ti	0.51975900	-10.63084022	-3.43740300
Ti	2.81675900	-8.33384022	-1.95740300
O	1.91975900	-9.23084022	-3.43740300
O	3.71375900	-7.43684022	-3.43740300
O	1.41675900	-6.93384022	-1.95740300
O	4.21675900	-9.73384022	-1.95740300
Ti	5.11375900	-10.63084022	-3.43740300
Ti	7.41075900	-8.33384022	-1.95740300
O	6.51375900	-9.23084022	-3.43740300
O	8.30775900	-7.43684022	-3.43740300
O	6.01075900	-6.93384022	-1.95740300
O	8.81075900	-9.73384022	-1.95740300
Ti	9.70775900	-10.63084022	-3.43740300
Ti	12.00475900	-8.33384022	-1.95740300

O	11.10775900	-9.23084022	-3.43740300
O	10.60475900	-6.93384022	-1.95740300
Ti	-6.37124100	-3.73984022	-1.95740300
O	-5.47424100	-2.84284022	-3.43740300
O	-4.97124100	-5.13984022	-1.95740300
Ti	-4.07424100	-6.03684022	-3.43740300
Ti	-1.77724100	-3.73984022	-1.95740300
O	-2.67424100	-4.63684022	-3.43740300
O	-0.88024100	-2.84284022	-3.43740300
O	-3.17724100	-2.33984022	-1.95740300
O	-0.37724100	-5.13984022	-1.95740300
Ti	0.51975900	-6.03684022	-3.43740300
Ti	2.81675900	-3.73984022	-1.95740300
O	1.91975900	-4.63684022	-3.43740300
O	3.71375900	-2.84284022	-3.43740300
O	1.41675900	-2.33984022	-1.95740300
O	4.21675900	-5.13984022	-1.95740300
Ti	5.11375900	-6.03684022	-3.43740300
Ti	7.41075900	-3.73984022	-1.95740300
O	6.51375900	-4.63684022	-3.43740300
O	8.30775900	-2.84284022	-3.43740300
O	6.01075900	-2.33984022	-1.95740300
O	8.81075900	-5.13984022	-1.95740300
Ti	9.70775900	-6.03684022	-3.43740300
Ti	12.00475900	-3.73984022	-1.95740300
O	11.10775900	-4.63684022	-3.43740300
O	10.60475900	-2.33984022	-1.95740300
Ti	-6.37124100	0.85415978	-1.95740300
O	-5.47424100	1.75115978	-3.43740300
O	-4.97124100	-0.54584022	-1.95740300
Ti	-4.07424100	-1.44284022	-3.43740300
Ti	-1.77724100	0.85415978	-1.95740300
O	-2.67424100	-0.04284022	-3.43740300
O	-0.88024100	1.75115978	-3.43740300
O	-3.17724100	2.25415978	-1.95740300
O	-0.37724100	-0.54584022	-1.95740300
Ti	0.51975900	-1.44284022	-3.43740300
Ti	2.81675900	0.85415978	-1.95740300
O	1.91975900	-0.04284022	-3.43740300
O	3.71375900	1.75115978	-3.43740300
O	1.41675900	2.25415978	-1.95740300
O	4.21675900	-0.54584022	-1.95740300
Ti	5.11375900	-1.44284022	-3.43740300
Ti	7.41075900	0.85415978	-1.95740300
O	6.51375900	-0.04284022	-3.43740300
O	8.30775900	1.75115978	-3.43740300
O	6.01075900	2.25415978	-1.95740300
O	8.81075900	-0.54584022	-1.95740300
Ti	9.70775900	-1.44284022	-3.43740300
Ti	12.00475900	0.85415978	-1.95740300
O	11.10775900	-0.04284022	-3.43740300
O	10.60475900	2.25415978	-1.95740300

Ti	-4.07424100	3.15115978	-3.43740300
O	-0.37724100	4.04815978	-1.95740300
Ti	0.51975900	3.15115978	-3.43740300
Ti	2.81675900	5.44815978	-1.95740300
O	1.91975900	4.55115978	-3.43740300
O	4.21675900	4.04815978	-1.95740300
Ti	5.11375900	3.15115978	-3.43740300
O	6.51375900	4.55115978	-3.43740300
Ti	9.70775900	3.15115978	-3.43740300
O	-0.88024100	-12.03084022	-0.47840300
Ti	2.81675900	-12.92784022	1.00159700
O	3.71375900	-12.03084022	-0.47840300
O	1.41675900	-11.52784022	1.00159700
O	6.01075900	-11.52784022	1.00159700
O	-5.47424100	-7.43684022	-0.47840300
Ti	-4.07424100	-10.63084022	-0.47840300
Ti	-1.77724100	-8.33384022	1.00159700
O	-2.67424100	-9.23084022	-0.47840300
O	-0.88024100	-7.43684022	-0.47840300
O	-3.17724100	-6.93384022	1.00159700
O	-0.37724100	-9.73384022	1.00159700
Ti	0.51975900	-10.63084022	-0.47840300
Ti	2.81675900	-8.33384022	1.00159700
O	1.91975900	-9.23084022	-0.47840300
O	3.71375900	-7.43684022	-0.47840300
O	1.41675900	-6.93384022	1.00159700
O	4.21675900	-9.73384022	1.00159700
Ti	5.11375900	-10.63084022	-0.47840300
Ti	7.41075900	-8.33384022	1.00159700
O	6.51375900	-9.23084022	-0.47840300
O	8.30775900	-7.43684022	-0.47840300
O	6.01075900	-6.93384022	1.00159700
O	8.81075900	-9.73384022	1.00159700
Ti	9.70775900	-10.63084022	-0.47840300
O	11.10775900	-9.23084022	-0.47840300
O	10.60475900	-6.93384022	1.00159700
O	-5.47424100	-2.84284022	-0.47840300
O	-4.97124100	-5.13984022	1.00159700
Ti	-4.07424100	-6.03684022	-0.47840300
Ti	-1.77724100	-3.73984022	1.00159700
O	-2.67424100	-4.63684022	-0.47840300
O	-0.88024100	-2.84284022	-0.47840300
O	-3.17724100	-2.33984022	1.00159700
O	-0.37724100	-5.13984022	1.00159700
Ti	0.51975900	-6.03684022	-0.47840300
Ti	2.81675900	-3.73984022	1.00159700
O	1.91975900	-4.63684022	-0.47840300
O	3.71375900	-2.84284022	-0.47840300
O	1.41675900	-2.33984022	1.00159700
O	4.21675900	-5.13984022	1.00159700
Ti	5.11375900	-6.03684022	-0.47840300
Ti	7.41075900	-3.73984022	1.00159700

O	6.51375900	-4.63684022	-0.47840300
O	8.30775900	-2.84284022	-0.47840300
O	6.01075900	-2.33984022	1.00159700
O	8.81075900	-5.13984022	1.00159700
Ti	9.70775900	-6.03684022	-0.47840300
O	11.10775900	-4.63684022	-0.47840300
O	10.60475900	-2.33984022	1.00159700
O	-5.47424100	1.75115978	-0.47840300
O	-4.97124100	-0.54584022	1.00159700
Ti	-4.07424100	-1.44284022	-0.47840300
Ti	-1.77724100	0.85415978	1.00159700
O	-2.67424100	-0.04284022	-0.47840300
O	-0.88024100	1.75115978	-0.47840300
O	-3.17724100	2.25415978	1.00159700
O	-0.37724100	-0.54584022	1.00159700
Ti	0.51975900	-1.44284022	-0.47840300
Ti	2.81675900	0.85415978	1.00159700
O	1.91975900	-0.04284022	-0.47840300
O	3.71375900	1.75115978	-0.47840300
O	1.41675900	2.25415978	1.00159700
O	4.21675900	-0.54584022	1.00159700
Ti	5.11375900	-1.44284022	-0.47840300
Ti	7.41075900	0.85415978	1.00159700
O	6.51375900	-0.04284022	-0.47840300
O	8.30775900	1.75115978	-0.47840300
O	6.01075900	2.25415978	1.00159700
O	8.81075900	-0.54584022	1.00159700
Ti	9.70775900	-1.44284022	-0.47840300
O	11.10775900	-0.04284022	-0.47840300
Ti	-4.07424100	3.15115978	-0.47840300
O	-0.37724100	4.04815978	1.00159700
Ti	0.51975900	3.15115978	-0.47840300
Ti	2.81675900	5.44815978	1.00159700
O	1.91975900	4.55115978	-0.47840300
O	4.21675900	4.04815978	1.00159700
Ti	5.11375900	3.15115978	-0.47840300
O	6.51375900	4.55115978	-0.47840300
Ti	9.70775900	3.15115978	-0.47840300
Ti	2.81675900	-12.92784022	3.96059700
O	3.71375900	-12.03084022	2.48059700
O	1.41675900	-11.52784022	3.96059700
Ti	-1.77724100	-8.33384022	3.96059700
O	-2.67424100	-9.23084022	2.48059700
O	-0.88024100	-7.43684022	2.48059700
O	-3.17724100	-6.93384022	3.96059700
O	-0.37724100	-9.73384022	3.96059700
Ti	0.51975900	-10.63084022	2.48059700
Ti	2.81675900	-8.33384022	3.96059700
O	1.91975900	-9.23084022	2.48059700
O	3.71375900	-7.43684022	2.48059700
O	1.41675900	-6.93384022	3.96059700
O	4.21675900	-9.73384022	3.96059700

Ti	5.11375900	-10.63084022	2.48059700
Ti	7.41075900	-8.33384022	3.96059700
O	6.51375900	-9.23084022	2.48059700
O	8.30775900	-7.43684022	2.48059700
O	6.01075900	-6.93384022	3.96059700
O	-5.47424100	-2.84284022	2.48059700
Ti	-4.07424100	-6.03684022	2.48059700
Ti	-1.77724100	-3.73984022	3.96059700
O	-2.67424100	-4.63684022	2.48059700
O	-0.88024100	-2.84284022	2.48059700
O	-3.17724100	-2.33984022	3.96059700
O	-0.37724100	-5.13984022	3.96059700
Ti	0.51975900	-6.03684022	2.48059700
Ti	2.81675900	-3.73984022	3.96059700
O	1.91975900	-4.63684022	2.48059700
O	3.71375900	-2.84284022	2.48059700
O	1.41675900	-2.33984022	3.96059700
O	4.21675900	-5.13984022	3.96059700
Ti	5.11375900	-6.03684022	2.48059700
Ti	7.41075900	-3.73984022	3.96059700
O	6.51375900	-4.63684022	2.48059700
O	8.30775900	-2.84284022	2.48059700
O	6.01075900	-2.33984022	3.96059700
O	8.81075900	-5.13984022	3.96059700
Ti	9.70775900	-6.03684022	2.48059700
O	11.10775900	-4.63684022	2.48059700
Ti	-4.07424100	-1.44284022	2.48059700
Ti	-1.77724100	0.85415978	3.96059700
O	-2.67424100	-0.04284022	2.48059700
O	-0.88024100	1.75115978	2.48059700
O	-0.37724100	-0.54584022	3.96059700
Ti	0.51975900	-1.44284022	2.48059700
Ti	2.81675900	0.85415978	3.96059700
O	1.91975900	-0.04284022	2.48059700
O	3.71375900	1.75115978	2.48059700
O	1.41675900	2.25415978	3.96059700
O	4.21675900	-0.54584022	3.96059700
Ti	5.11375900	-1.44284022	2.48059700
Ti	7.41075900	0.85415978	3.96059700
O	6.51375900	-0.04284022	2.48059700
O	8.30775900	1.75115978	2.48059700
O	6.01075900	2.25415978	3.96059700
O	8.81075900	-0.54584022	3.96059700
Ti	9.70775900	-1.44284022	2.48059700
Ti	0.51975900	3.15115978	2.48059700
Ti	2.81675900	5.44815978	3.96059700
O	1.91975900	4.55115978	2.48059700
O	4.21675900	4.04815978	3.96059700
Ti	5.11375900	3.15115978	2.48059700
O	-0.88024100	-7.43684022	5.43959700
O	1.91975900	-9.23084022	5.43959700
O	3.71375900	-7.43684022	5.43959700

Ti	-1.77724100	-3.73984022	6.91959700
O	-2.67424100	-4.63684022	5.43959700
O	-0.88024100	-2.84284022	5.43959700
O	-0.37724100	-5.13984022	6.91959700
Ti	0.51975900	-6.03684022	5.43959700
Ti	2.81675900	-3.73984022	6.91959700
O	1.91975900	-4.63684022	5.43959700
O	3.71375900	-2.84284022	5.43959700
O	1.41675900	-2.33984022	6.91959700
O	4.21675900	-5.13984022	6.91959700
Ti	5.11375900	-6.03684022	5.43959700
Ti	7.41075900	-3.73984022	6.91959700
O	6.51375900	-4.63684022	5.43959700
O	8.30775900	-2.84284022	5.43959700
O	6.01075900	-2.33984022	6.91959700
Ti	0.51975900	-1.44284022	5.43959700
O	1.91975900	-0.04284022	5.43959700
O	3.71375900	1.75115978	5.43959700
Ti	5.11375900	-1.44284022	5.43959700
O	6.51375900	-0.04284022	5.43959700