Phytoremediation of Cr(VI) and Pd(II) using vetiver grass (*Chrysopogon Zizanioides)*

by

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ABSTRACT

Heavy metal contamination from industrial effluents is one of the leading causes of concern. Chromium(VI) is amongst the top chemical pollutants that are released into the environment from serval industries. Chromium(VI) has devastating health effects on living organisms. Despite their scarcity, platinum group metals (PGMs) are also finding their way into the environment. Their health effects and short supply are the main causes of concern given the continuous growth in demand and continuous disposal into the environment. There is a need for environmentally friendly technologies that can remediate these metals from wastewaters and the environment without breaking the bank. Phytoremediation is an emerging cost-effective and environmentally friendly technology compared to conventional remediation techniques. Phytoremediation make use of plants to take up metals from the environment. The exact mechanisms by which plants take up and distribute toxic metals is not well understood. Several factors such as metal concentration, physicochemical properties of the environment, and the presence of other ions in the roots zone, affect the bioavailability of metals. It has been reported that carriers responsible for the uptake of essential ions are also involved in the uptake of nonessential metals. Plants restrict the translocation of toxic metals to the aerial parts to combat their detrimental effects.

The present study aimed to assess the phytoremediation potential of *Chrysopogon zizanioides* (vetiver grass) for chromium(VI) and palladium(II) from water. The study was conducted in four phases of batch experiments. The first phase was to access the effect Cr(VI) initial concentration on the growth of vetiver grass (VG), uptake of Cr(VI), and accumulation of chromium in the plant. Using different Cr(VI) concentrations from 5 ppm to 70 ppm. From this study it was found that the initial concentration had an effect on the uptake and translocation of chromium in vetiver grass. The grass exposed to 70 ppm managed to accumulate 1.12 mg g^{-1} in the roots and 3.04 mg g^{-1} in the leaves. The toxicity of chromium was visible at concentrations above 30 ppm, resulting in withering of the grass.

The second phase investigated the effect of plant density on the uptake of chromium at 30 ppm initial concentration. The densities were grouped in the following classes, low density (5 slips), medium density (10 slips), high density (15 slips). The uptake of Cr(VI) increased with an increase in plant density, with the high density pot achieving a removal of 26.1 ppm Cr(VI) from initial concentration of 31.9 ppm. The accumulation of chromium in the roots increased with the plant density. While the accumulation in the leaves decreased with an increase in plant density.

The third phase focused on the effect of pH on the uptake and accumulation of chromium. The pH levels ranged from 3.5 to 10.5 at initial Cr(VI) concentration of 30 ppm. The grass exposed to pH of 3.5 achieved 100 % Cr(VI) removal. The accumulation of Cr decreased in the roots and increased in the leaves as the solution pH increased. The translocation to the leaves was minimal under different pH levels.

The last phase was to evaluate the phytoextraction of palladium(II) a precious metal at different initial concentrations. The concentrations ranged from 10 ppm to 120 ppm. The uptake of Pd(II) increased with the initial concentration, the grass exposed to 120 ppm managed to reduce the pot concentration by 24 ppm, while accumulating the most palladium of 0.4 mg g^{-1} in the roots and 0.15 mg g^{-1} in the leaves. The toxicity of palladium was mainly visible at concentrations above 40 ppm resulting in the drying up of the exposed grass.

The results from the current study proved the potential of VG in phytoremediation of Cr(VI) and Pd(II). Phytoextraction potential of chromium proved possible at elevated concentrations and extended periods as demonstrated in the first experiments, while more research is required to access vetiver grass's potential in phytoextraction of palladium. The ability of VG to accumulate metal in its aerial parts showed the potential of applying VG in extracting and recovering metals for reuse by burning and reprocessing of ash. The latter is common practice in other industries such as the mining and refining of platinum and other platinum group metals (PGMs).

Keywords: bioremediation, phytoextraction, PGM, palladium, chromium, bioaccumulation, heavy metals

DECLARATION

I, **Farai Masinire**, hereby declare that this dissertation is my own original work (citation is provided where people’s work has been used) and has never been submitted for another degree at this or other institutions.

Signature:
Student name: Farai Masinire
Date:

DEDICATION

To my parents

Vongai M. Kwirihwiri and Timitia K. Masinire

For their sacrifices, love, prayers, and always believing in me,

To my brothers

Panashe and Tafadzwa

For their prayers and support

and to

Cornelis C. Scheepers

For his support and believing in me

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LIST OF ABBREVIATIONS

BAF	Bioaccumulation factor
C_i	Initial solution concentration
$C_{\text{Plant tissue}}$	Metal concentration in the plant
C_{root}	Metal concentration in the root
C_{shoot}	Metal concentration in the shoot
C_t	Solution concentration at time t
d	Day(s)
dw	Dry weight
g mol^{-1}	Gram per mole
IX	Ion Exchange
mg g^{-1}	Milligram per gram
mg kg^{-1}	Milligram per kilogram
nm	Nanometre
PGE	Platinum group elements
PGM	Platinum group metals
ppm	Parts per million
TF	Translocation factor
UV-Vis	Ultraviolet-visible spectrometry
VG	Vetiver grass
WHO	World Health Organisation

RESEARCH OUTPUTS

Journal articles

1. Masinire, F., Adenuga, D. O., Tichapondwa, S. M., Chirwa, E. M. N. (2021). Phytoremediation of Cr(VI) in Wastewater Using the Vetiver Grass (*Chrysopogon zizanioides*). Minerals Engineering. 172. 107141. https://doi.org/10.1016/j.mineng.2021.107141.

2. Masinire, F., Adenuga, D. O., Tichapondwa, S. M., Chirwa, E. M. N. (2020). Remediation of Chromium(VI) Containing Wastewater Using *Chrysopogon zizanioides* (Vetiver Grass). Chemical Engineering Transactions. 79. 385-390. https://doi.org/10.3303/CET2079065.

3. Palladium phytoremediation and phytomining potentiation of Vetiver Grass (Chrysopogon zizanioides). Chemical Engineering Transactions. (Accepted)

Conference presentations

1. Phytoremediation of Chromium(VI) in wastewater using *Chrysopogon zizanioides* (vetiver grass). 6th International Symposium on Sustainable Minerals (Sustainable Minerals '21), 21-23 June 2021, Online. Cornwall, UK (oral presentation)

2. Palladium phytoremediation and phytomining potentiation of Vetiver Grass (Chrysopogon zizanioides). Chemical Engineering Transactions. 7th International Conference on Industrial Biotechnology (IBIC 2022), 5-8 June, Napoli, Italy (oral presentation)

CHAPTER 1: INTRODUCTION

1.1 Background

Industrialisation has greatly improved our living conditions at the expense of the environment. Some areas in which the impact of industrialisation on the environmental is notable includes overexploitation of natural resources and pollution of soil, air, and fresh water sources. Until recent years it was believed that natural recourses such as water and minerals are in unlimited supply. To think about it, about 71 % of our planet is covered in water, however, only about 3 % of it is portable. Despite the knowledge we now have about the short supply of water, toxic wastes from anthropogenic activities continue to contaminate fresh water sources. High heavy metal concentrations and depletion of precious metals in the environment are a legacy left mainly by many industrial processes (Hogan, 2011).

Chromium finds its way into fresh water sources because poor monitoring and negligence of industrial effluents from industries such as; leather tanning, stainless steel manufacturing, ferrochrome (FeCr) production, dye and pigment production, mining and electroplating (Bhalerao and Sharma, 2015, Saha et al., 2017). This makes Cr(VI) one of the most abundant chemical pollutant from anthropogenic sources (Bhalerao and Sharma, 2015). Chromium exists in different forms ranging from Cr(-VI) to Cr(VI), however, its trivalent Cr(III) and the hexavalent Cr(VI) species are the most stable. Chromium(VI), the most toxic form of chromium generally exists with oxygen as chromate or dichromate oxyanions (Becquer et al., 2003, Gomes et al., 2017). Trivalent chromium usually exists in the environment bound to organic matter, it is less toxic, and less mobile in comparison to Cr(VI) (Becquer et al., 2003).

Hexavalent chromium is mutagenic, carcinogenic because it has a strong oxidizing capacity and it is able to go through biological membranes (Kolomazník et al., 2008, Teklay, 2016). Because of this, stringent regulations are imposed on its discharge into fresh water sources and the environment. According to the World Health Organisation (WHO), the concentration of Cr(VI) discharged into inland surface water should not be above 0.1 ppm and should not be above 0.05 ppm for drinking water (WHO, 2011). While Cr(III) is often considered as a non-toxic essential trace element which is part of a balanced nutritional intake (Kim et al., 2007, Stern, 2010).

There is a need for cost effective and green remediation techniques because of the deleterious effects of Cr(VI) upon living organisms. Some of the previously applied techniques include the removal of heavy metals through ion exchange (IX), reverse osmosis, coagulation, precipitation, and electrolysis (Kim et al., 2007). Most of the current applied techniques have several drawbacks including high capital and operational costs, and they often leave large amounts of toxic sludges (Malaviya and Singh, 2011).

Precious metals such as palladium(Pd) and platinum(Pt) are in low supply and yet have extensive industrial applications in automobiles, agriculture, electronics, medicine, and energy industries (Yong et al., 2002, Gunn, 2014). Despite their short supply these precious metals continue to find their way into the environment directly and indirectly through several anthropogenic activities. The attention of many researchers has shifted towards the recovery of precious metals from industrial effluents, mine leachates, and urban environments, as it serves a dual purpose of recovering metals and remediating the environment (Okibe et al., 2017). The downside of the conventional recovery methods such as pyrometallurgical and hydrometallurgical processes is that they are not cost effective when dealing with low pollutant concentrations such as those found in the environment and not environmentally friendly, as their large quantities of secondary wastes also contaminates the environment (Das, 2010).

In attempt to find cost effective and green technologies to remediate and recover these metals, there has been a growing interest in the `application of living plants to take up the metals from the surrounding environment. This technology is referred to as phytoremediation. Phytoremediation is a green and cost-effective alternative, in comparison to other clean-up technologies that may involve excavation or pumping of contaminated groundwater. Living plants use their roots to absorb water, essential minerals, and metals from the environment, and translocate them to the aboveground parts through the vascular system where they can be easily harvested and processed (Raskin et al., 1994, Gratão et al., 2005, Golubev, 2011). Phytoremediation involves two uptake processes, the first is biosorption, a fast and reversible process whereby metals bind to the surface of the plant roots. The second process is bioaccumulation, which is the slow metal-sequestration process (Kumar et al., 2013). This green technology is not limited to the extraction of metals only, it is currently being used around the world to remediate sites contaminated by pesticides, explosives, oils, and solvents (Greipsson, 2011).

Phytoremediation is a broad term for various ways by which plants are employed to clean up the environment. Some of the ways that are currently under research include: **Phytoextraction**; which is mainly for the recovery of metals from the aerial parts of plants through harvesting and processing (Jarvis and Leung, 2001, Sas-Nowosielska et al., 2004). **Phytostabilisation**; this process is most suitable when the end goal is to create a clean environmental with less intention to recover the metals. **Rhizofiltration**; this process similar is to phytostabilisation, however, it is mostly used for water with low pollutant concentration. (Eapen et al., 2003, Dary et al., 2010). **Phytovolatization**; this process involves the volatilisation of pollutants.

Besides its advantages as a green, and cheap technology, phytoremediation also has aesthetic benefits, as it gives rise to green lands and revegetation of areas with barren vegetation. The downside of this technology is that it requires extended periods of time as the process of phytoremediation is depended on various factors. These factors include, growth rate of the plant, metal-accumulation capacity, pH, weather, salinity, metal uptake rate, and metal tolerance (Salt et al., 1995).

Hyperaccumulator plants are able to accumulate high amounts of toxic metals and transport them to their aerial parts. The characteristics of plants suitable for phytoremediation include, high biomass, high tolerance to harsh environmental conditions, ability to take up and translocate metals/contaminants from the substrate to the aerial parts of the plant.

A lot of hyperaccumulator plants have been studied, some of the plants are bahia grass (*Paspalum notatum* Flugge), common reed (*Phragmites austr alis*), water hyacinth (*Eichhornia crassipes*), and water lettuce (*Pistia stratiotes L.*) (Gupta et al., 2012). Vetiver grass (*C. zizanioides*) is one of the promising hyperaccumulator plants, with a deep and dense root system that can grow up to 7 m and stiff and erect leaves that can grow up to 2 m (Oshunsanya and Aliku, 2017). Its use as a hyperaccumulator has been under continuous study because of its excellent characteristics such as its ability to survive under harsh environmental conditions including under high heavy metal content, and varying pH levels (Truong and Baker, 1998, Truong et al., 2010). Vetiver grass has all the characteristics of plants that can be used for phytoremediation. For a long time vetiver grass has been used for soil conservation (Roongtanakiat et al., 2008, Darajeh et al., 2014, Singh et al., 2015b). Its tolerance towards high concentrations of metals indicates its potential in phytoremediation (Chomchalow, 2003, Truong et al., 2010). Phytoremediation using VG has been successfully applied in serval countries (Truong, 2000).

1.2 Aim and objectives

The current study focuses on evaluating the phytoremediation potential of vetiver grass (VG) grown in Cr(VI) solution and Pd(II) solution under different conditions. The objectives that had to be met to achieve this were:

- Develop the techniques for planting vetiver grass and harvesting.
- Determine the effect of different initial concentrations on the uptake of chromium.
- Determine the effect of concentration of the uptake of palladium.
- Assess the effect of pH on the uptake of chromium.
- Assess the effect of plant density on the uptake of chromium.

- Evaluate the removal of chromium from the solution, and its accumulation in the roots and leaves of vetiver grass.
- Determine the removal of palladium from the solution and its accumulation within the roots and leaves of vetiver grass.
- Evaluate the effect of chromium and palladium on the growth of vetiver grass.
- Examine the effect of seasonal changes on vetiver grass.

1.3 Outline of the dissertation

This dissertation is outlined as follows:
Chapter 1: Introduction- highlights the background and objectives of the study. This section discusses current trends and major concerns of metal pollution, current applied remediation technologies, and the advantages of phytoremediation as an alternative process to remove and recover chromium and palladium.

Chapter 2: Literature review- a detailed background of the study is done. The information found in this section focusses on the occurrence of chromium and palladium in the environment, their uses, and health effects, and current remediation techniques. A detailed discussion of phytoremediation, its pros and cons, plants adaption to metal contamination and vetiver grass as a potential candidate for the technology is also included.

Chapter 3: Material and Methods- describes the materials and methods utilised during the study to meet the objectives.

Chapter: 4 Results and Discussion- presents the findings from the experiments and their interpretation, while comparing with previous findings.

Chapter 5: Conclusion and Recommendations- in this section a conclusion is deduced from the major findings and recommendations are made for future studies.

1.4 Significance of the study

South Africa is a huge producer of chromium and platinum group metals. During the mining, processing, and usage of these metals, large amounts of metals escape into the environment affecting the quality of water and the environment's ability to support life. The conventional remediation techniques are often costly, necessitate extensive labour, and produces substantial amounts of secondary wastes. On the other hand the phytoremediation approach is quite economic, does not require skilled workers, and requires less maintenance. It can be applied for in-situ bioremediation in situations such as legacy sites where continuing contamination cannot be prevented. The use of vetiver grass floating systems at constructed wetlands for the recovery of heavy metals and precious metals could be a viable option (Figure 1.1). Phytoremediation can be a huge step towards sustainability in areas with limited skilled workforce and limited budget.

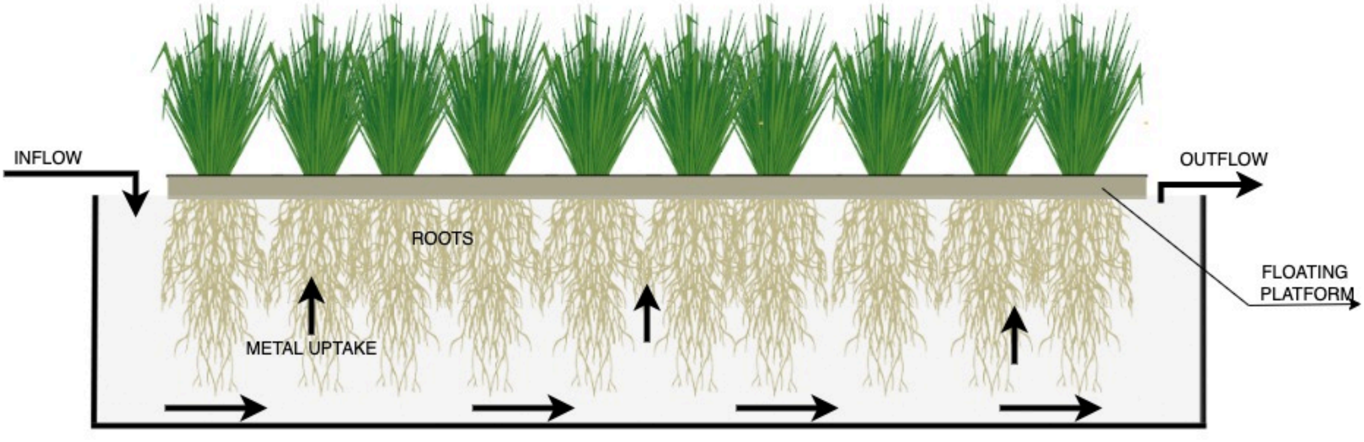

Figure 1.1: Schematic of vetiver grass floating system at a constructed wetland

CHAPTER 2: LITERATURE REVIEW

2.1 Heavy metals

The factor used to determine if a metal can be classified as a heavy metal is that it should have a density greater than 5 g/cm^3 (Jarup, 2003). Another definition by Singh et al. (2011)Singh et al. (2011)Singh et al. (2011)Singh et al. (2011)Singh et al. (2011)Singh et al. (2011) states that, a heavy metal is any metal which is hazardous to life irrespective of the atomic weight and density. Heavy metals make up a great deal of pollution in groundwater, industrial effluents, and sometimes in wastewater (Hogan, 2011). Heavy metals such as, cadmium (Cd), mercury (Hg), arsenic (As), chromium (Cr), zinc (Zn), and lead (Pb), find their way to waterbodies through industrial wastewater (Agarwal, 2009). Heavy metals are naturally found in the environment, however, their geochemical and biochemical balance has been altered by anthropogenic activities. The major natural contributors are weathering of minerals, erosion, and volcanic eruptions (Walker et al., 2012, Gautam et al., 2014, Masindi and Muedi, 2018, Ali et al., 2019), while the main sources as a result of human activities are industrial effluents, mining and agricultural activities, smelting, electroplating, and sludge dumping (Wuana and Okieimen, 2011, Defarge et al., 2018). Even at trace amounts, heavy metals have devastating health effects on living organisms (Tchounwou et al., 2012). Among these deleterious effects are carcinogenicity, teratogenicity, and mutagenicity under low and subchronic exposure conditions (Langård and Vigander, 1983, Davies, 1984, Bridgewater et al., 1994, Xu et al., 1996, Singh et al., 1998). For these reasons, the disposal of heavy metal contaminated wastes should be done in a regulated manor to avoid the contamination of the ecosystem.

2.2 Chromium

Heavy metal chromium(Cr) is a transition element located in the group VI-B of the periodic table. It was discovered by Vaquelin in 1798. It has an atomic weight of 51.99 g mol^{-1}; a specific gravity of 7.18 – 7.20; melting and boiling point of 1857 °C and 2672 °C respectively. Chromium is the 7th most abundant element on earth, however most of it is found in the core and mantle (Barnhart, 1997a). On earth's crust, chromium ranks 21st in natural abundance and it makes up around 0.037 % of the earth's crust (Shanker et al., 2004, Saha et al., 2011).

It is not surprising that it is one of the leading chemical pollutants of concern and the United States Environmental Protection agency has labelled it as a priority contaminant (Smith et al., 2002). Chromium is found in the environment in different oxidation states from Cr(-IV) to Cr(+VI). However, its most abundant forms are trivalent Cr(III) and hexavalent Cr(VI) which are the most stable forms of all chromium's oxidation states (Fendorf, 1995, Dhal et al., 2013). Chromium(VI) and Chromium(III) have very distinct biochemical and physiochemical properties in the environment. Cr(VI) contaminates the environment easily, penetrating the soil, water and groundwater environments due to its high mobility compared to Cr(III) (Cervantes et al., 2001, Dhal et al., 2013).

2.2.1 Sources of chromium

Chromium is a familiar pollutant in both surface and groundwater, it occurs naturally in the environment at elevated concentrations in ultramafic rocks (Barnhart, 1997b). The concentrations of Cr within the continental crust has been estimated to be ranging from 80 mg kg^{-1} to 200 mg kg^{-1} (NAS, 1974). Anthropogenic activities are a leading cause of Cr pollution in freshwater sources (Sedman et al., 2006). A larger portion of the world's chromite exploitable reserves are found in the southern part of Africa. The reserves in South Africa accounts for over 72-80 % (430 million tons of chromium metal) of the known world's reserves (Ober, 2017). The Great Dyke region of southern Zimbabwe has over 63 million tons of chromium metal (Stowe, 1994).

2.2.2 Natural sources of chromium

Chromium(VI) levels up to 73 ppm from natural sources have been recorded in surface and ground water in Mexico, Italy, California, and New Caledonia (Robles-Camacho and Armienta, 2000, Fantoni et al., 2002, Becquer et al., 2003, Oze et al., 2007). Chromite which is the main geological source of chromium is known to have high resistance towards weathering, digenesis, and low-grade metamorphic reactions (Oze et al., 2007). The means by which Cr(III) is released from chromite to the solution are perplexing. Manganese minerals within the chromium rich strata may function as potential oxidising agents of Cr(III) (Robles-Camacho and Armienta, 2000, Oze et al., 2007).

The concentration of Cr within the earth's crust is greater than 200 mg kg^{-1} in ultramafic rocks (Oze et al., 2007). Trivalent chromium discharged through weathering adsorbs on to clay minerals where precipitation occurs to form homogeneous solids or with aluminium/ion(III) oxide-hydroxides (Bartlett and James, 1988). The solubility of chromite is minimal under most natural and industrial processing conditions (Bialowolska and Salacinski, 1985, Ball and Nordstrom, 1998). Naturally existing oxidants for Chromium(III) are not a lot, the only naturally existing oxidising agents of aqueous Cr(III) at pH lower than 9 are Mn(IV) and Mn(III) oxides (Johnson and Xyla, 1991, Banerjee and Nesbitt, 1999), and hydrogen peroxide (H_2O_2) (Rock et al., 2001). The natural oxidation of the naturally found Cr(III) can lead to high concentrations of Cr(VI) in water (Oze et al., 2007). The natural oxidation of Cr(III) happens through catalytic reaction of Mn-bearing minerals as shown in Figure 2.1, via the reaction Equation 2.1, which has a half-life of 0.58 to 37.2 years. The natural reduction of Cr(VI) to Cr(III) follows reaction Equation 2, which has a much shorter half-life ranging from 15 min to 53 days (Loyaux-Lawniczak et al., 2001).

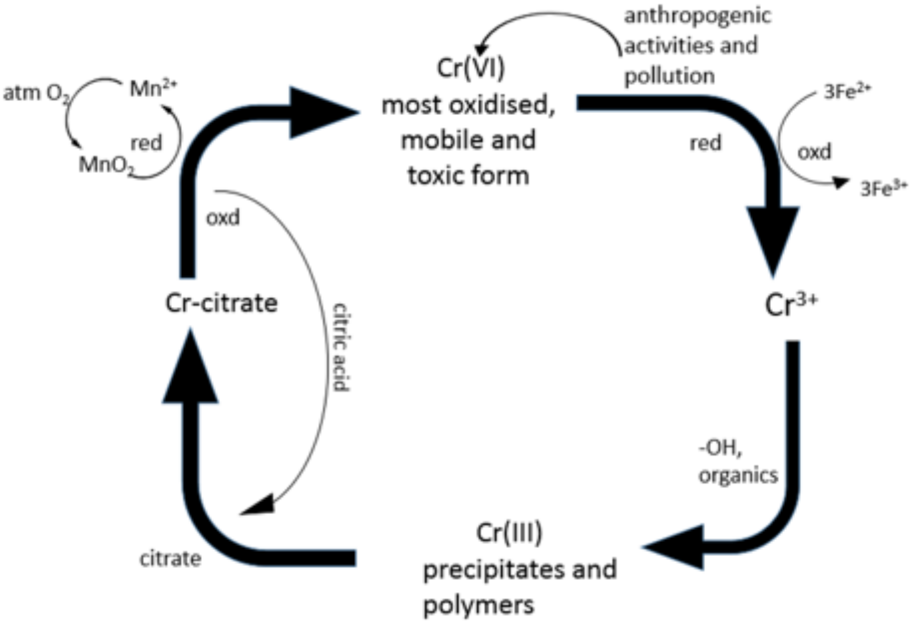

Figure 2.1: Environmental natural cycle of Cr (Coetzee et al., 2020)

$$Cr^{3+} + 1.5\ MnO_2 + H_2O \rightarrow HCrO_4^- + 1.5Mn^{2+} + H^+ \qquad (1)$$

$$C_6H_6O_2 + Cr_4^{2-} + 2H_2O \rightarrow 0.5Cr_2O_3 + 1.5C_6H_4O_2 + 2.5H_2O + 2OH^- \qquad (2)$$

The concentration of chromium in freshwater usually ranges from 0.1 to 117 μg L^{-1}, and seawater chromium ranges from 0.2 to 50 μg L^{-1} (Bowen, 1979). Chromium commonly occur naturally as Cr(III) (Choppala et al., 2013), which is largely immobile and is only found in trace amounts in freshwater, unless under low pH conditions. Chromium can be found as Cr(VI) under strong oxidizing conditions. The presence of Cr in the atmosphere is mainly as a contribution of burning of fossil fuels, dust from chromate mining and processing industries, and incineration of wastes, veld fires, volcanic eruptions, and meteoric dust (Nriagu and Nieboer, 1988, Tian et al., 2012). In aquatic systems, some of the natural sources of chromium includes natural weathering of rock formations and surface soil run-offs (Kotaś and Stasicka, 2000). Natural sources contribute approximately 54,000 tons of Cr (Kieber et al., 2002).

2.2.3 Anthropogenic chromium sources

The major source of chromium contamination is through anthropogenic activities. There are several ways through which chromium from industrial activities infiltrate the environment, such as leachate from landfills and mining wastes, sludge from sewage systems, seepage from industrial lagoons, spills, and leaks from industrial metal processing, wood preservation facilities, leather turning industries, and several other industries. Approximately 80 % of the mined chromium is used in metallurgical industries (Choppala et al., 2013). It is estimated that between 4.35×10^5 and 1.18×10^6 tons of Cr is deposited into soils every year (Nriagu and Nieboer, 1988). According to Gadd and White (1993), the global chromium waste disposed into the environment every year from industrial and manufacturing activities is approximately 170 000 tons.

Within South Africa, the mining of chromate and other mining activities has had a positive impact on the economy of the country (Hamann, 2004). The Merensky Reef houses the largest concentration of accessible chromite (Bansal et al., 2019). About three quarters of viable chromite ore reserves in the world are found in South Africa. South Africa’s western Bushveld complex produces large amounts of the world’s total ferrochromium (Bansal et al., 2019). In the years 2019 and 2020 despite the effects of Covid19, unreliable electricity supply, increased labour costs, South Africa was the biggest producer of chromite in the world. In terms of chromium-consumption, stainless steel and ferrochromium production, China was leading, followed by South Africa worldwide (MCS, 2021).

The ferrochrome industry in South Africa alone was responsible for the production of 3,590,000 ton of ferrochrome in the year 2015 (Koleli and Demir, 2016). Not only does the mining of chromite and the ferrochrome industries pollute the soil and water sources, but these industries are also culprits for causing air pollution. Studies have shown that approximately all atmospheric chromium eventually comes back with rain, adding to the pollution of soil and freshwater sources (Bansal et al., 2019)

2.2.4 Uses of chromium

Chromium is extensively used in various industries such as the stainless steel manufacturing, leather turning industry, FeCr production, dye and pigment production, and for electroplating. The chromium consumed globally every year can be distributed in the following three principal industries: 69 % for metallurgical, 18 % for refractory, and 13 % for chemical applications (Figure 2.2) (DeYoung et al., 1984, Saha et al., 2011).

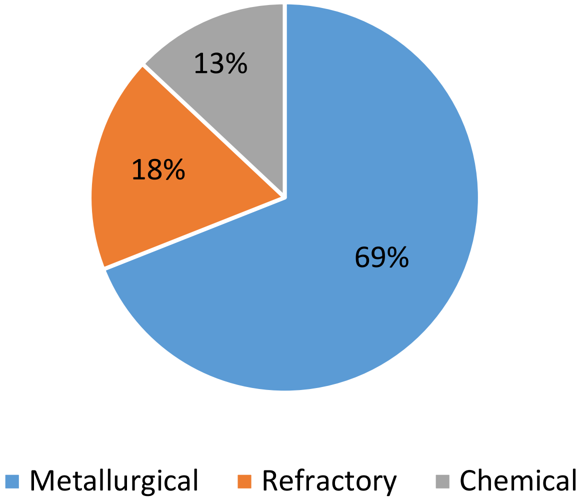

Figure 2.2: Chromium usage per industry

Leather tanning- The use of chromium in the leather tanning industry started back in 1858. The process uses Cr(III) in the formation of stable complexes with the proteins in the hide or synthetic polymers, creating a bacteria resistant leather and enhancing stability in the ambient environment (Nriagu and Nieboer, 1988).

Ferroalloys- The chrome for the production of steel has to have the following specifications: Cr_2O_3 > 45 %, Cr:Fe ratio >3.1, and SiO_2 less than 8 % (Dunham, 1986). Chromium is added to steal in order to increase its resistance towards corrosion, and to improve its mechanical properties. Steels with chromium can be separated into three groups based on the fraction of chromium: steel with < 3 %

chromium is low-chromium engineering steels, the tool steels has 3 – 12 % chromium, and stainless steels has > 12 % chromium (Farrer, 2004).

Refractories- Chromite has a high melting point (2040 °C) and it is considered to be highly chemically inert which enables it to resist attacks from acid and bases at high temperatures (Nriagu and Nieboer, 1988). For these reasons chromite is used in the manufacturing of refractory bricks, making of mortars, castable, ramming gunning mixes, and in joining of bricks within furnaces. (Nriagu and Nieboer, 1988)

Metal finishing and corrosion control- Chromium compounds are utilised in the treatment of metal surfaces to protect them against corrosion, enhancement of durability, and improving the retention of paints and other final finishes (Nriagu and Nieboer, 1988). Chromium is used to give a hard and smooth surface to machine parts, such as crankshafts, in a process referred to as 'hard" chromium plating.

Pigments- The production of pigments has an increasing share of the chromite ores. The pigments consist of two broad groups: chromite colour pigments and corrosion inhibition pigments (Yassi and Nieboer, 1988). These pigments are used in residential and industrial structures, machinery and industrial equipment, etc (Nriagu and Nieboer, 1988).

2.2.5 Toxicity of chromium

Trivalent Cr(III) is the most dominant species of chromium found in nature. Factors such as the pH of the environment affect the ratio of Cr(VI) and Cr(III) species (Kotaś and Stasicka, 2000). Chromium(VI) has the highest mobility of all forms of chromium, which makes it the most toxic and the most bioavailable form (Gomes et al., 2017). Chromium(VI) can cause severe effects such as liver damage, camps, kidney damage, and stomach bleeding, due to its ability to penetrate biological membranes and its strong oxidizing capacity (Kolomazník et al., 2008, Teklay, 2016). In 1932 Lehmann suggested the first studies of the relationship between work-related exposures to chromium and the risk of getting lung cancer (Yassi and Nieboer, 1988), there after several other researchers reported the relationship between exposure to chromates and increased cancer risk (Enterline, 1974, Collins et al., 2006). Chromium(VI) is predominant in alkaline conditions pH > 7 and in high oxidation potential environments (Zink et al., 2010). It is toxic to plants at very low concentrations of 0.5 mg L^{-1} in water and 5 mg kg^{-1} in soil (Fendorf, 1995).

2.2.6 Chromium in water

Different species of chromium behave differently in aqueous mediums. Cr(III) inclines towards the formation of hydroxy-complexes, which is usually the dominant C(III) species in water (Rai et al., 1987). Figure 2.3 shows the various chromium oxidation states available at equilibrium at different Eh and pH values. It can be seen that the most thermodynamically stable form under reducing conditions is Cr(III). Chromium(III) can be easily removed from water under basic conditions, because it precipitates as $Cr(OH)_3$ at pH ≥ 6 (Gomes et al., 2017), while Cr(III) is predominantly present at pH values less than 3.

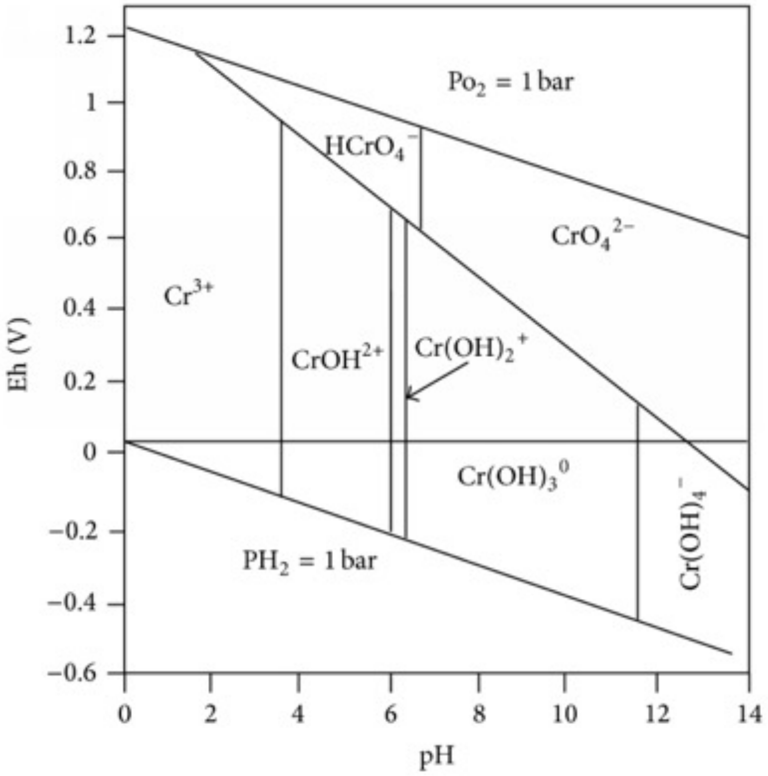

Figure 2.3: Chromium phase diagram showing the thermodynamic stability of different Cr aqueous species, over a typical natural Eh and pH range (Sueker, 1964)

Within aqueous mediums, Cr(VI) can exist in different species as shown in Figure 2.4. At pH < 1 Cr(VI) exists as chromic acid, and at pH > 6, Cr(VI) exists mainly as chromate ions (CrO_4^{2-}) (Mohan and Pittman, 2006).

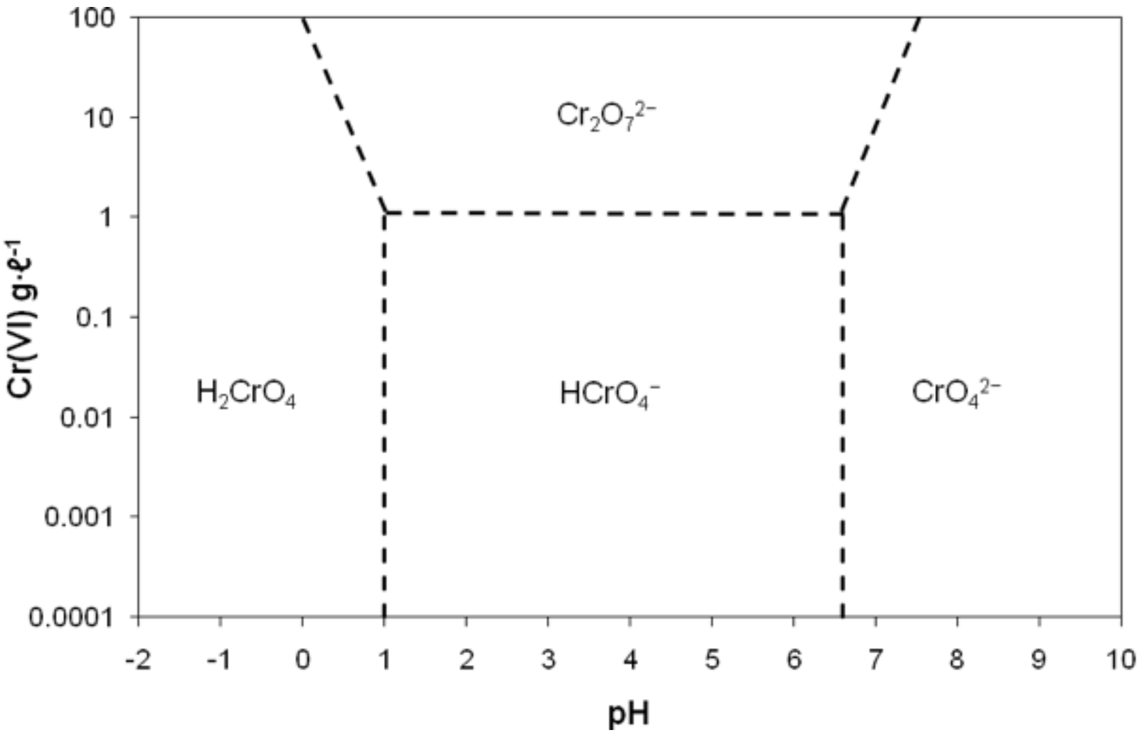

Figure 2.4: The distribution of different species of Cr(VI) in aqueous solution (Mohan and Pittman, 2006)

2.2.7 Bioavailability of chromium

Bioavailable metals are available in the environment as free metal ions and soluble metal complexes. The metals that are not bioavailable are present in the environment in forms that are bound to organic matter, and also as hydroxide, oxide, and carbonate precipitates which cannot be absorbed easily by plants (Sheoran et al., 2016). The bio-available and unavailable metals in a system are usually at equilibrium, when there is a reduction of available metals due to uptake by plants, a fraction of the unavailable metals become available in order to maintain the equilibrium, the same happens when there is a shift due to the external factors such as metal concentration, organic content, pH, and temperature (Wei et al., 2008, Sheoran et al., 2016).

The total metal content in the environment does not necessarily indicate the bioavailability of metal as this is dependent on several other factors such as the pH of the environment, redox potential, organic content, competing ions, metal form, and microbial community structure. Under low oxidation-reduction potential environments, the most stable state of chromium is Cr(III). In the presents of high organic matter and under low pH levels Cr(VI) can be easily reduced to Cr(III) (Sinha et al., 2018). However, the reduction of Cr(VI) to Cr(III) may not be complete as many Cr(VI) ions can still be found further translocated to the aerial parts by plants (Malaviya and Singh, 2011).

The ion composition of the environment can also enhance or inhibit the bioavailability of Cr(VI). The presence of SO_4^{2-} and other group VI anions presents a competitive inhibition to the uptake of Cr(VI) because of the chemical similarity. This results in low uptake of chromium in the presence of the anions as reported by Shewry and Peterson (1974). On the contrary the presence of calcium (Ca) enhances the uptake of Cr(VI) by plants (Ertani et al., 2017, Malaviya et al., 2020).

2.3 Palladium

Palladium(Pd) is a group VIII silvery-white metal that was discovered in 1803 by William Hyde Wollaston. It falls under the six chemical elements collectively referred to as platinum group metals (PGM) or platinum-group elements (PGE). In its elemental state, palladium is relatively inert and is more plastic than platinum (Lebeau, 2015). PGMs in include; ruthenium (Ru), rhodium (Rh), palladium (Pd), osmium (Os), iridium (Ir), and platinum (Pt). These are rare precious metals, with an extended range of industrial applications including the making of jewellery (Gunn, 2014). Palladium and platinum are the most commercially important of the six PGMs, they are mainly used to combat toxic emission from vehicles in catalytic converters in the automotive industry (Yong et al., 2002). These metals are extremely rare on the Earth's crust with the concentration of palladium and platinum both around 5 ppb (Lorand et al., 2008).

2.3.1 Sources of palladium

Palladium occurs usually in a form of a wide range of alloys with other PGMs, or with other metals such as iron(Fe), tin(Sn), copper(Cu), lead (Pb), mercury(Hg) and silver(Ag). Together with other PGMs it is naturally available in very low concentrations in the environment as shown in Table 2.1, palladium makes about 20 % of the PGMs (Renner and Schmuckler, 1991).

Table 2.1: Distribution of PGMs in the environment (Renner and Schmuckler, 1991)

Region	Estimated concentration of PGMs
Earth	~ 30 mg kg^{-1}
Mantle (siliceous lithosphere)	~0.05 mg kg^{-1}
Earth's crust (attainable by mining)	~0.01 mg kg^{-1}
Hydrosphere	< 10^{-6} mg L^{-1}
Biomass (dry matter)	< 10^{-7} mg kg^{-1}

The supply of palladium in the world is mainly from extraction of the reserves in South Africa, Canada, Russia, and USA (Table 2.2) (Melber et al., 2002, Thethwayo, 2018). The larger part of Pd is retrieved as a by-product during the refining of copper and nickel sulfide ore (Canada and Russia) or from PGM deposits as alloys (South Africa and USA) (Melber et al., 2002). Because of the high costs involved in the mining and purification of PGMs, they are usually recycled by the users and producers. The recycling practices mainly focusses on platinum and palladium and it accounts for about 20% of primary production (Fornalczyk and Saternus, 2009). In 1995 recycling led to the recovery of about 60 tons of PGMs in the USA (Fornalczyk and Saternus, 2009). In 1998 scrapped automobile catalysts had a contribution of 5.4 tons to the world Pd demand of 72 tons in the automotive industry (Cowley, 1999). Some of the automobile catalysts end up at the waste sites or incineration ashes.

Table 2.2: PGMs reserves around the world (Thethwayo, 2018)

Country	PGM reserves (kg)
Canada	310 000
United States	900 000
Russia	1 100 000
South Africa	63 000 000
Other countries	800 000

2.3.2 Sources of palladium in the environment.

Studies have indicated that anthropogenic activities have resulted in the elevation of PGMs concentrations in the sewage sludge, surface water, and ditch sediments (Rauch et al., 2000, Kalavrouziotis and Koukoulakis, 2008, Jackson et al., 2010). Recent studies in the United States of America and in Germany have shown that the levels of palladium in the environment are drastically increasing, with concentrations reaching 770 ± 208 ng g^{-1} in tunnel dust, 311 ng g^{-1} in the road dust, and 193 ng g^{-1} in roadside soil samples (Spada et al., 2012, Leopold et al., 2017). Palladium has been used as a substitute of platinum in catalytic converters (Merget and Rosner, 2001). Because of its similar properties and lower prices it has also become a useful replacement for gold. (Spada et al., 2012, Leopold et al., 2017)

The largest anthropogenic contribution of palladium to the environment is from catalytic converters in vehicle. Three way catalytic convertors (TWCs) are used to control and minimize the emissions from automotives, they work by catalysing the conversion of exhaust emissions such as, nitrogen oxides, carbon monoxide, and hydrocarbons, into compounds like carbon dioxide, water and nitrogen. PGMs, Pd, Pt, and Rh are used as the catalytic active component (Zimmermann et al., 2005). These precious metals are constantly being released to the environment during the operations of the automobiles (Zereini and Alt, 2011). Various studies have shown that fine PGM particles are also emitted with the exhaust fumes from the vehicles.

These particles have potential to accumulate in environments such as, soils, dust, silt, and waters along major roads (áMilagros Gˇmez and áAntonia Palacios, 1999, Zereini and Urban, 2000, Ravindra et al., 2004). Whiteley and Murray (2003) discovered that the amount of PGMs in the roadside soil samples were elevated to averages above the crust values, with the following maximum concentrations being reported 420 ng g^{-1}, 440 ng g^{-1}, and 91 ng g^{-1} for Pt, Pd and Rh respectively.

2.3.3 Uses of palladium

Automotive industry-The largest consumer of palladium is the automotive industry, PGMs are used in the production of catalytic converters, which are used in the conversion of noxious exhaust emissions to harmless non-toxic products. There has been a remarkable increase in the demand for PGMs in autocatalysis since the 1970s following the introduction of legislations setting standards for motor vehicle emissions (Gunn, 2014). Palladium is currently the main constituent of catalytic converters used in petrol operated motor vehicles, while Pt is the main active component of autocatalysis and particulate filters used in diesel operated vehicles.

Electronic components- The second biggest consumer of palladium is the electronics industry, where it has a cost advantage over other precious metals like gold (Gunn, 2014). PGMs have exceptional chemical resistance, high hardness, high melting points and high conductivity. For this reason, they are quite suitable for application as anti-corrosion coatings. PGM coatings are important in the electronic industry. Palladium coatings were often used as alternatives to hard gold layers (Böck, 2006). A substantial portion of this Pd is applied in multi-layer ceramic capacitors (MLCC), and other notable uses of palladium are in hybrid integrated circuits, mainly used in the automotive industry (Gunn, 2014). Other uses of PGMs are listed in Table 2.3.

Table 2.3: Applications of palladium and usage by sector in 2011 (Johnson Matthey, 2012).

Sector	Application	Usage (thousand kg)
Autocatalyst	Catalytic convectors	188
Investment	Exchange traded funds, coins	18
Jewellery	Production of jewellery, white gold.	16
Chemical	Catalyst for nitric acid production and other bulk and speciality chemicals.	14
Electrical	Multi-layer ceramic chip capacitors	43
Dental, medical, and biomedical	Alloying agent, mainly with minor platinum, gold, silver	17
Other	Emission control catalysts in stationary applications.	3
	Total global consumption	263

2.3.4 Environmental issues

PGMs are considered as non-toxic, and inert in their metallic form. However, some PGM-chlorinated salts are highly toxic, allergic, and there has been reports of damaged DNA due to exposure to platinum group metals (Gagnon et al., 2006). Some researchers have suggested that palladium is the most toxic of the PGMs (Farago and Parsons, 1994, Havelkova et al., 2014). The high risk posed by palladium is accounted to its rapid mobility and high bioavailability, which is comparable to that of zinc (Schäfer et al., 1998, Ek et al., 2004). The continuous increase of PGMs in the environment poses a great public health and biosphere threat as the toxicological and ecotoxicological danger of these metals is not yet known (Botre et al., 2007). Rauch et al. (2000), reported an increase in PGM concentration of the snow in Greenland. Although these concentrations were very low, they were a significant increase from those obtained from the sample of ice aged 7000 years. Due to the noted PGM pollution problems in the environment, their behaviour in aquatic environments, soil, and living organisms is currently being studied with the aim to assess their effects on these systems and more importantly on human health (Caroli et al., 2001, Zimmermann et al., 2005, Dubiella-Jackowska et al., 2009, Linde et al., 2017).

2.3.5 Health effects

Until recently PGMs were not regarded as toxic metals because they were thought to be naturally inert and unreactive. Also people were rarely exposed to Pd and Rh, and were considered as being less toxic. Occupational Pd exposure is mainly through the following major categories: refining, mining, processing, and product use (Nygren, 2006). However, it must be emphasised that all PGMs are extremely dangerous, and that palladium and rhodium are carcinogenic (Kalavrouziotis and Koukoulakis, 2008). Pt emitted from catalytic converters is generally seen as low risk threat to the general population. However the potential for Pd emissions to cause harmful responses in the environment is looked at more critically because of its higher solubility and bioavailability. A study by Gagnon et al., (2006), where rats and plants were exposed to PGMs under controlled environmental conditions reported enlargement of white pulp in the spleen, shrinkage of glomerul in kidneys, eosinophil inclusion bodies in adrenal glands, and vacuolization. Some DNA damage was also reported. Some palladium salts causes severe irritations when they are in conduct with the skin, while some are known to have minimum irritancy such as palladium chloride (Campbell et al., 1975). Individuals occupationally exposed to palladium may suffer from primary skin and eye irritation (Orion and Wolf, 2006, Nigam et al., 2020).

2.3.6 Bioavailability of PGMs

PGMs can be readily taken up by plants providing the potential hazardousness of the metals to the ecosystem (Pallas and Jones, 1978, Alloway and Ayres, 1997).

Grass samples collected along the highway by Helmers and Mergel (1998) were found to have Pt concentrations between of 1.2 to 30 ng g^{-1} dw while the grass samples collected further away from the highway had Pt concentrations less than 0.03 ng g^{-1} dw. A study by Ban-Badjo et al (2007) found that Pd and Rh accumulation within the plants increased with an increase in exposure time.

It has been suspected that the mobility of the PGMs increases under acidic environments, thus increasing their uptake by the plants (Kalavrouziotis and Koukoulakis, 2008). Palladium is readily taken up by plants when it occurs in soluble forms. PGMs emitted from catalytic convertors may undergo rapid transformation in the environment, and the resultant behaviour becomes similar to that of soluble PGM salts (Whiteley and Murray, 2003). Thus far there hasn't been extensive research into the bioavailability of PGMs, however the fact that they undergo various transformations in the environment, these transformations leads to an increased chance of increased bioavailability of PGMs to the plants and to the biosphere (Kalavrouziotis and Koukoulakis, 2008)

2.4 Conventional treatment and recovery methods

Ever since the realisation of the effect of heavy metal pollution on the environment and living organisms several remediation technologies have been proposed and tested. Many of these technologies are based on ion exchange, precipitation, electrolysis, reverse osmosis, membrane, biosorption and solvent extraction (Tiravanti et al., 1997, Baciocchi et al., 2005, Moussavi and Barikbin, 2010). These technologies have unique approaches and often times they have certain advantages over each other depending on the pertaining situation (Kumar et al., 2013). When facing large amount of contaminated wastewater, it is ideal to choose a technique that result in the best results, minimum environmental impact, and cost-effective. Some of the conventional treatment methods are described in the following sections.

2.4.1 Ion Exchange

Ion exchange (IX) process involves a reversible exchange of ions between the ions of a species on the exchanger and the ions of another species in the solution. During the ion exchange process, a chromium/palladium containing solution is pressurised through the ion exchange resin, at which the Cr/Pd ions are removed from the solution and replaced by an equal amount of different ions (Sahu et al., 2009). This exchange is facilitated by the positive and negative excess charge within the matrix of the exchanger material. When the resin bed gets saturated, regeneration using acid or alkali is required. The resins have selectivity for specific ions, however if the solution to be treated has huge amounts of competing ions, the efficiency of the of the ion exchange process is affected (Kumar et al., 2013). This is one of the downsides of the ion exchange process, which results in incomplete removal of contaminants. Natural and synthetic clays, zeolites, and resins have been applied in ion exchange process (Simate and Ndlovu, 2014, Nleya et al., 2016).

Many studies have reported the use of different IX resin to achieve the removal of Cr(VI) from wastewater; Dowex 2-X4 (Sapari et al., 1996), Ambersep 132 (Lin and Kiang, 2003), and Solvent impregnated resin with aliquat 336 (Kabay et al., 2003). Sapari et al. (1996) achieved the total removal of Cr(VI) from plating wastewater using synthetic Dowex 2-X4 resin. Chromic acid was recovered from a synthetic platting solution using Ambersep 132, a synthetic IX resin (Lin and Kiang, 2003).

IX resins such as Amberlyst A 29 and A 21 (Hubicki and Wołowicz, 2009), Purolite A-400TL (Wołowicz and Hubicki, 2014), have been used in the recovery of palladium and other PGMs. Purolite A-400TL was used in the removal of Pd(II), Pt(VI) ,and Au(III) ions from water, the results obtained from this study showed that Purolite A-400TL was successful in the removal of the precious metals, the desorption study of the resin showed that not quantitative desorption of palladium was observed (Wołowicz and Hubicki, 2014).

2.4.2 Chemical reduction

During chemical reduction, certain ions which are not readily precipitated are reduced to a form that can be easily precipitated. Palladium(II) can be reduced to Pd(0) (McLaughlin and Verkade, 1998). Hexavalent chromium can be reduced to trivalent chromium using electrochemical reduction precipitate, which employs iron electrodes and electrical current (Pettine et al., 2002, Lin and Huang, 2008). Cr(III) precipitates as $Cr(OH)_3$ at pH ≥ 6, making it easy to remove from water (Gomes et al., 2017).

2.4.3 Membrane process

Membrane process such as reverse osmosis, ultrafiltration, nano-filtration, and microfiltration are used in remediating heavy metal contaminated water. In reverse osmosis, the contaminated water is fed under pressure through a semi-permeable membrane, resulting into purified and concentrated streams (Park et al., 2015, Nleya et al., 2016). The removal of chromium using nano-filtration achieved 98.8 % using NF270 and 76.5 % using NF90 as reported by (Giagnorio et al., 2018), which also overcame the typical problems such as fouling.

In a study by Muthukrishnan and Guha (2008) the removal of Cr(VI) at varying feed solution pH levels and concentrations using nanofiltration composite polyamide membranes was investigated. The study concluded that the pH of the solution had an influence on the percent rejection of Cr(VI) and solution concentration had low influence on the rejection rate. Some of the membranes which have been used in the removal of Cr(VI) include: carbon membrane, where a 96 % rejection from 1000 mg L^{-1} was recorded (Pugazhenthi et al., 2005), polymer-enhanced ultrafiltration, where 30 % rejection rate was obtained from 10 mg L^{-1} (Aroua et al., 2007).

2.4.4 Disadvantages of conventional treatment processes

The major drawbacks of many conventional treatment methods include:

- high capital and operational costs,

- incomplete metal removal,
- large quantities of toxic sludge
- requires skilled operators
- high energy requirements

2.5 Phytoremediation

Phytoremediation is the application of plants and associated microorganisms to extract, immobilize, degrade, or contain water and soil contaminants (Raskin et al., 1994). Plants are capable of extracting heavy metals, many manufactured chemicals, hydrocarbon and natural aromatic compounds (Saier and Trevors, 2010). Phytoremediation is considered to be a green and cost effective technology. It is sometimes used together with traditional microbial based bioremediation processes (Alkorta and Garbisu, 2001, Abhilash et al., 2009).The application of phytoremediation for metal, organic, and nutrient pollutants is most suitable for shallow environments (Golubev, 2011). It is also applicable for the remediation of large areas where conventional remediation techniques are not costly or practicable (Schnoor et al., 1995, McCutcheon and Schnoor, 2004).

The different processes of phytoremediation include; phytoextraction, phytostabilization, phytodegradation, rhizodegradation, rhizofilteration, and phytovolatalisation (Figure 2.5) (Cristina, 2014, Rani et al., 2020). The well-known mechanisms by which plants decontaminate heavy metals from the environment are phytoextraction and phytostabilisation (Laghlimi et al., 2015).

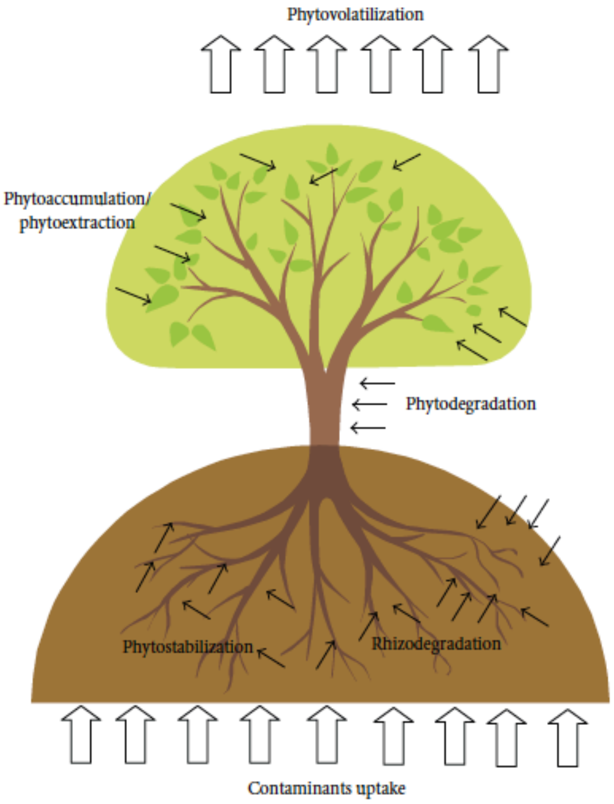

Figure 2.5: Different processes of phytoremediation (Rani et al., 2020)

Phytoextraction is the uptake of metals by plants into the root structure and further translocation to the aerial parts. The translocation of metals from the roots to easily harvestable parts of the plants is part of the major aims of phytoextraction research (Jarvis and Leung, 2001). Plants with the ability to translocate large amounts of metals to the aerial parts are more suitable for phytoextraction (Marques et al., 2009, Laghlimi et al., 2015). The recovery of metals in an economical way is also an important issue in phytoextraction. Several recovery and disposal methods like liquid-liquid extraction, incineration, direct disposal at a hazardous waste site have been studied and selection of the technique depends on the financial feasibility and availability of the relevant technology to perform the processes (Sas-Nowosielska et al., 2004).

Phytostabilisation is the immobilization or reduction in the mobility of metals, during this process metals could precipitate around the root zone, and/or be adsorbed on to the root surface, and/or accumulate in the roots system (Dary et al., 2010). Phytostabilisation is usually applied in heavily contaminated environments in which the uptake of metals by plants would require large amounts of time. In this case fast growing plants which have high tolerance to metal contamination can be used to immobilize heavy metals through absorption and accumulation by the roots or precipitation within the root zone, with minimal translocation to the aboveground parts (Wong, 2003, Kumpiene et al., 2009). It is usually applied to revegetate mine tailings in order to reduce wind and water erosion of tailings in a cost-effective way. Plants with extensive roots systems are more suitable for this process as the roots can function as a home to a wide range of microorganisms, that may assist in stabilising the toxic contaminants (Marques et al., 2009, Laghlimi et al., 2015).

Rhizofiltration is mainly for the decontamination of wastewater, surface water, and extracted ground water with low metal content. It is the use of plants to take-up, accumulate, and precipitate pollutants from contaminated water in the plant roots (Eapen et al., 2003, Peng et al., 2008). In rhizofiltration the contaminants do not have to be translocated to the aboveground parts of the plants, thus non hyperaccumulator plants can be utilised in this process.

Phytovolatization involves the uptake of contaminants by the plants, then transformation of the contaminants into volatile forms and transpiration of them into the atmosphere (Di Lonardo et al., 2011). Some natural occurring and genetically modified plants can grow in mercury contaminated environments and transfer Hg^{2+} to Hg^0 (Kališová-Špirochová et al., 2003).

2.5.1 Advantages of phytoremediation

The use of plants gives the chance to recover the absorbed metals (Cristina, 2014), it is considered cheaper by 50 – 80% compared to the other remediation techniques (Salt et al., 1995). Economic value through the recovery of metals, by making phytoremediation a financial self-supporting approach of environmental remediation (Cristina, 2014). Phytoremediation can be used for a wide range of pollutants, including many metals and hydrocarbons, consequently, plants can be used to remediate areas with multiple contaminants (Bauddh et al., 2017). It results in less secondary wastes in air and water compared to the traditionally used techniques.

Through phytovolatization toxic organic contaminants can be broken down and returned to the environment as non-toxic CO_2 and H_2O (Mojiri, 2012). The soil and water are left at the site after decontamination is done. It doesn't require the use of heavy machinery which often damages the soil and pollute the environment. The use of plants results in increased vegetation, thus, enhancing the appearance of the treated area, while functioning as a carbon sink (Laghlimi et al., 2015).

2.5.2 Disadvantages of phytoremediiation

Just like many other remediation techniques, phytoremediation has its own limitations. It is a slow process, and requires several seasons for plant growth, some plants are seasonal and dry out during cold seasons which affects the remediation (Salt et al., 1995). The plant biomass (fruit and other edible parts) enriched with heavy metals are a potential threat in the food chain (Farraji et al., 2016). Handling and disposal of the contaminated plant biomass is a major challenge of phytoremediation (Ahalya et al., 2003), to effectively remove metals from the environment, the metals should be recovered from the plant biomass (Keller et al., 2005). The process is usually applicable to surface contamination and ground water within a short distance from the surface. The growth of the plants depend on a lot of factors (Ahalya et al., 2003).

2.5.3 Plant selection for phytoremediation

Many different plant species show potential in phytoremediation however, grass species are more favourable due to fast growing, high biomass, and well-developed root system (Ondo Zue Abaga et al., 2014). Hyperaccumulators are plants with the ability to accumulate high amounts of heavy metals, they can accumulate 50 – 100 times more metal than normal plants. Hyperaccumulator plants can accumulate metals concentrations above the levels required for normal development and growth. The term “hyperaccumulator” was first used by Brooks et al. (1977), when describing plants that were capable of accumulating high levels of nickel. These plants are able to accumulate elevated amounts of metals more than metal concentration in the environment into their aerial parts. One definition suggested that a plant is a hyperaccumulator if it can accumulate more than 0.1 % (dw) of Cr, Cu, Co, Ni, and Pb or 1 % (dw) of Zn in its aerial parts, irrespective of metal content of the surrounding environment (Raskin et al., 1994). However, there are some plants which have high metals content in nature, but the metal concentration are nowhere close to the concentrations in the surrounding environment. For this reason an additional requirement for a plant to be classified as a hyperaccumulator has to be met, which is, the plant has to accumulate metal above the concentration in the surrounding area (Yang et al., 2005).

The selection of a plant suitable for phytoremediation varies depending of factors such as, climatic and environmental conditions, the type of pollutant, the soil and water (Laghlimi et al., 2015). The characteristics of plants suitable for phytoremediation usually includes:

- Deep and extensive root system.
- Translocate large quantities of metals (~1000 mg kg^{-1}) in their aerial biomass (Schnoor et al., 1995).
- Tolerance to elevated amounts of heavy metals.
- Tolerance to harsh environmental conditions.
- High aerial biomass.

2.5.4 Plant adaptation to metal stress

Elevated amounts of metals in the environment can be very harmful to plants and disturbs plant's metabolism (Golubev, 2011). The toxicity of metals in plants is visible when plants translocate the metals to the various parts of the shoots, and the effects differ depending on the contaminating metal (Ansari et al., 2016). In the event that plants are exposed to high levels of toxic metals, they do not have the option to move away from the contamination, however, they develop relevant mechanisms to adapt to metal stress. The plants' responses to metal stress can be separated into two types, metal resistance, and metal sensitivity. Metal sensitivity can lead to plant injuries or deaths, on the other hand metal resistance means that in the presence of metal stress the plant react in such a way that allows for its survival (Golubev, 2011). Metal resistance includes avoidance, which is a mechanism by which plants externally protect themselves from metal stress and develop tolerance in order to withstand internal stress caused by elevated metal concentrations (Orcutt and Nilsen, 2000).

When exposed to heavy metals, plants deploy several heavy metal copying mechanisms. They can isolate the metals into their cell wall (sequestration), use organic compounds to chelate the metals in the soil/water rendering them inactive or store the metals in dedicated cells and cell partitions. They can also store these metals in vacuoles, where they are safe from sensitive functions of the plant, protecting the important metabolic functions (Saier and Trevors, 2010). Plants develop proteins like phytochelatins and metallothioneins which function as transporters of metals while protecting the sensitive plant processes. Plants through phytovolatisation can also volatize toxic metals such as mercury (Hg^{2+}) by reducing them into less toxic and volatile form Hg^0 (Saier and Trevors, 2010).

2.5.5 Mechanisms of metal accumulation by plants.

The mechanisms by which plants take-up and distribute the metals are not well understood (Hayat et al., 2012). The first contact metals have with the plants is usually through the roots (Clemens et al., 2002). The bioavailability of metals relies on the physicochemical properties of the environment and the metal, as well as the metal concentration, and the presence of other ions in the root zone (Yang et al., 2005). Low bioavailability of metals can be a major limiting factor for phytoremediation. The metal oxidation state also affects the mechanisms of metal uptake by plants. Plants can actively contribute to metal availability by exuding the carboxylates and acidifying the rhizosphere (Clemens et al., 2002). Acidification of the rhizosphere has been observed in many plants taking up Cu, Ni, Zn, and Cd (Yang et al., 2005).

Metals such as Pd(II) and Cr(VI) are not essential to plants, thus, plants lack a direct uptake mechanism. However, these metals are taken up by plants through the carriers that are utilised in the uptake of essential ions like sulfates (Cervantes et al., 2001). The mechanism is influenced by the metal concentration, active uptake mechanism is dominant under low metal concentration, while the uptake mechanism is passive at higher metal levels (when the plant's membrane loses its selectivity) (Hayat et al., 2012). Active mechanism is when the root cells actively absorb the metals via plasmalemma, and the passive mechanism involves the adsorption of metals onto cell walls through passive diffusion or via acropetal movement into roots of aquatic plants (Denny, 2008). Acropetal transport (in contrast to passive diffusion) is crucial in the transportation of metal ions in the roots of submerged plants (Denny, 2008). As a way to protect their aerial parts that houses crucial metabolic functions such as photosynthesis from the toxic effects of toxic metals such as Cr and Pd, plants usually restrict the translocation of these metals to the shoots (Malaviya and Singh, 2011). Most plants manage to contain higher concentrations of chromium in their roots and restricting its translocation by their ability to reduce Cr(VI) to Cr(III) which has low mobility and is poorly translocated to the aerial parts of plants (Paiva et al., 2009).

2.6 Vetiver Grass

Vetiver grass (*Chrysopogon zizanioides*) is part of the Poaceae family, subfamily of Paniocoidae, tribe Andropogone and subtribe Sorghina (Bertea and Camusso, 2002). The name *zizanioides* was first given by a Swedish taxonomist Carolus Linnaeus in 1771, which means ‘’by the riverside’’ because it is commonly found along waterways in India. Vetiver grass (VG) is believed to be native to India, however, it is also commonly found in the tropical and subtropical regions of Africa, Australia, America, Asia, and Mediterranean Europe (Maffei, 2002). Countries where vetiver grass is known to be found are listed in Table 2.4.

Table 2.4: Countries where vetiver grass is found (Oshunsanya and Aliku, 2017)

Africa	Asia	Caribbean	America	Pacific	Others
Algeria	Bangladesh	Antigua	Argentina	American Samoa	France
Angola	Burma	Barbados	Brazil	Cook Islands	Italy
Burundi	China	Cuba	Colomnbia	Fiji	Spain
Comoro	India	DR	Costa Rica	New Caledonia	USA
CAR	Indonesia	Haiti	FG	New Guinea	USSR
Ethiopia	Japan	Jamaica	Guatemala	Tonga	
Gabon	Malaysia	Martinique	Guyana	Western Samoa	
Ghana	Nepal	Puerto Rico	Honduras		
Kenya	Pakistan	St. Lucia	Paraguay		
Madagascar	Philippines	St. Vincent	Suriname		
Malawi	Singapore	Trinidad			
Mauritius	Sri Lanka	Virgin Islands			
Nigeria	Thailand				
Rwanda					
Reunion					
Seychelles					
Somalia					
South Africa					
Tanzania					
Tunisia					
Uganda					
Zaire					
Zambia					
Zimbabwe					

2.6.1 Characteristics of vetiver grass

Researchers have managed to find the distinctive attributes, which makes vetiver grass suitable for its uses, through understanding its exceptional physiological and morphological features and its role in environmental conservation (Truong, 2000). Vetiver grass is a tall, scented, perennial, tufted grass, with a stiff and straight stem that grows up to 2 m in length, with long and narrow leaves and a complex root system (Figure 2.6), that can reach up to 7 m in length in a period of 36 months (Chomchalow, 2003). The deep and complex root system helps vetiver grass to survive during drought seasons as it can penetrate and utilise the deep soil moisture and also it helps the grass to remain firm during high velocity water flow (Truong et al., 2008, Stokes et al., 2014). VG is a non-inversive grass so it poses no risk of becoming a weed. When planted close to each other vetiver slips form a thick hedge (Figure 2.6), which can withstand water flow to the depth of 0.6 m (Carlin et al., 2002).

Figure 2.6: Vetiver grass mature level deep and dense root system and thick hedge formation

2.6.2 Uses of vetiver grass

The use of VG for water and soil conservation was started by the World Bank back in the 1980s in India (Truong, 2000). The technology includes the use of VG to control soil erosion and sediments on slope farmlands and floodplains (Oshunsanya, 2013, Oshunsanya and Aliku, 2017), restoration of acid sulphate and saline soils (Truong and Baker, 1998), phytoremediation of agro-chemicals (Pinthong et al., 1998); pest control, and the control of heavy metal pollution on and off-site. The essential oils from vetiver grass roots have been used in the medicine, perfume, and other industries (Danh et al., 2009).

Erosion control- Soil erosion is amongst the most devastating environmental disasters in developing countries (Truong, 2000). It is the washing away of soil by water or winds which results in the removal of top fertile soil thus affecting crop yield. Conventional management methods used for erosion could be costly and often less effective. Research done by Truong (2000), found that the use of VG technology in the treatment of surface runoff and soil loss resulted in lower soil loss and improved crop yields. The technology has been reported to result in a reduction of soil loss from 11 to 3 t ha^{-1} (Oshunsanya and Aliku, 2017). When vetiver grass is planted on the contour, it develops into a protective hedge across the slope, reducing the speed of the runoff water, resulting in deposition of the sediment (Truong and Loch, 2004). The tensile strength of the roots of vetiver grass also increase the strength of the soil against surface runoff and erosion.

Restoration of acid sulphate and saline soils- Agriculture especially in semi-arid areas suffers from salinity. Because of its high tolerance to salinity, VG has been applied in the rehabilitation of saline areas. According to Truong (2000), the salinity threshold level of vetiver grass is EC_{se} = 8 d/Sm. VG has been used successfully in the stabilisation and rehabilitation of soil where the pH was about 3.5 and oxidized pH was as low as 2.8 (Loch et al., 2000). Vetiver grass rehabilitate these environments by absorbing bioavailable metals and sodium contributing to the salinity or acidity of the soil (Truong and Baker, 1997).

2.6.3 Potential of vetiver grass in phytoremediation

Vetiver grass (*C. zizanioides*) has been under continuous investigation for its potential application as a hyperaccumulator. This is because it possesses all the characteristics of hyperaccumulators, which includes its high tolerance towards elevated heavy metal content, wide range of pH levels, and other harsh environmental conditions (Truong and Baker, 1998, Truong et al., 2010). Vetiver grass can thrive in environments with pH range (3.3 and 9.5) with enough supply of macronutrients. It has been reported that VG is effectual in the absorption of pollutants and nutrients particularly nitrogen (N) and phosphate (P) (Truong and Baker, 1998). VG has higher threshold levels of heavy metals compared to most vascular plants (Table 2.5) (Oshunsanya and Aliku, 2017). VG has also been used in the removal of agrochemicals as reported by Ramlee et al. (1996), because of its capacity to take-up these chemicals and stopping them from accumulating in soil and crops (Truong, 2000).

The dense root system of VG provides the necessary surface area for the absorption of metal ions through the transporters, channels, and pores in the plasma membrane of the root. It is also favorable for phytoremediation because of its high biomass (Reeves and Baker, 2000). According to Truong (2003), vetiver grass grows very fast producing high amounts of biomass under tropical hot and wet conditions more than 100 tons of dry biomass per hectare every year. According to a study by Xia et al., (2003), vetiver grass can stay under submergence for more than 120 days. It can also survive under a wide range of temperatures from -15 °C to 55 °C (Xia et al 1999; Xu and Zhang, 1999).

Table 2.5: Heavy metal concentration in roots and shoots of vetiver grass (Truong et al., 2010)

Heavy metals	Soil (mg kg^{-1}dw)	Shoot (mg kg^{-1}dw)	Roots (mg kg^{-1}dw)
Arsenic (As)	620	11.2	268
Cadmium (Cd)	1.66	0.31	14.2
Copper (Cu)	50	13	68
Chromium (Cr)	600	18	1750
Lead (Pb)	730	78.2	87.8
Mercury (Hg)	6.17	0.12	10.8
Nickel (Ni)	300	448	1040
Zinc (Zn)	750	880	1030
Selenium (Se)	74.3	11.3	24.8

Transpiration is also among the top indicators of a hyperaccumulator plant, as plants have to transpire large amounts of water in order to effectively take up the contaminants (Pivetz, 2001). In a study by Danh et al (2009), vetiver grass had the fastest uptake rate of water in comparison to other wetland plants such as *iris pseudacorus, Typha spp., and Schoenoplectus Validus,* on average vetiver grass had an uptake rate of 600 ml day^{-1} pot^{-1}(Danh et al., 2009).

CHAPTER 3: MATERIALS AND METHODS

3.1 Vetiver grass

The vetiver grass (*V. Chysopogon*) used in this study was obtained from Hydromulch (Pty) Ltd (Johannesburg, South Africa). Upon collection the grass was cut short for ease of transportation. The grass was transferred to a water medium from the soil pots in which it was planted. Great care was given when detaching the grass from soil to avoid damaging the roots. The grass was washed and left to acclimatize in water for 2 weeks (Figure 3.1). Essential macronutrients nitrogen (N), phosphorus (P), potassium (K) were added to facilitate the growth of the roots and shoots to the desired length.

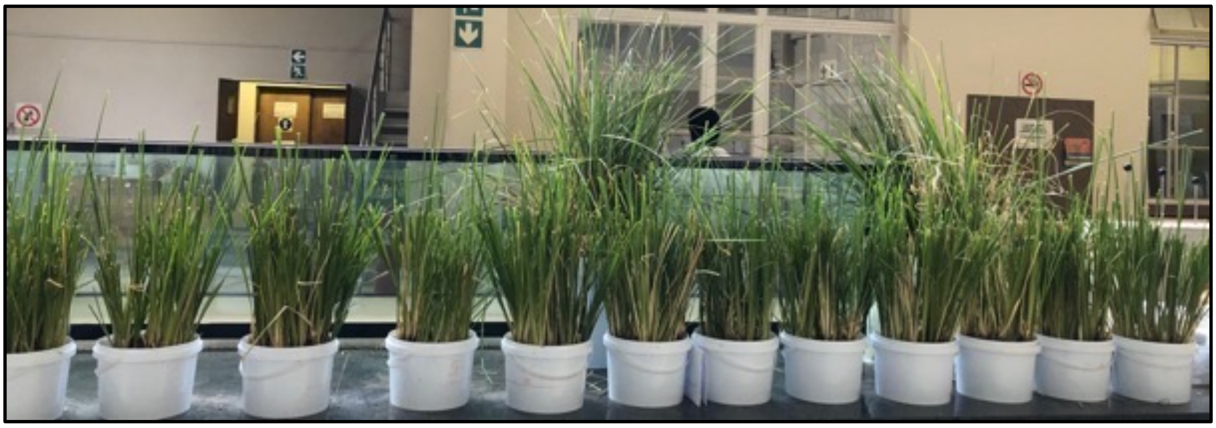

Figure 3.1: Vetiver grass acclimatization

After the grass had acclimatized, the shoots were trimmed to a height of 45 cm. The grass was transferred to 2 L pots containing metal solutions for the commencement of the phytoremediation studies. Each pot was marked at 2 L solution level. The reduction in water level in the pots was due to evaporation and uptake by the grass. It was assumed that the water vapor was pure water with no metals, and tap water was used to replace the evaporated water back to the 2 L mark before sampling. This was to ensure that the change in concentration was not due to evaporation, but solely to the uptake by plants.

3.2 Reagents

3.2.1 Cr(VI) and Pd(II) standard solution

Cr(VI) stock solution was made by dissolving 3.74 g of 99% pure K_2CrO_4 (Analytical grade) in 1 L of deionized water to form a 1000 ppm Cr(VI) solution. Pd(II) stock solution was purchased as 1000 ppm Pd(II) solution. The stock solutions were used throughout the experiments. The standard solutions of Cr(VI) and Pd(II) were prepared by diluting the stock solution in deionized water to make the target concentrations, from these concentrations a calibration curve (absorbance vs concentration linear graph) was obtained.

3.2.2 DPC solution

Diphrnylcarbozide (DPC) solution was prepared for the analysis of Cr(VI) by dissolving 0.5 g of 1,5 diphenylcarbozide in 100 mL of HPCL grade acetone it was store in a cold room inside a brown bottle covered by aluminum foil.

3.3 Cr(VI) removal at different initial concentration

The first phase of the study investigated the effect of different Cr(VI) concentrations on the uptake and accumulation of Cr in vetiver grass. After acclimatisation the grass was transferred to 2 L Cr(VI) solutions at different initial concentrations. The synthetic 1000 ppm Cr(VI) stock solution was diluted to make pots of 5, 10, 30, and 70 ppm concentration. Two control pots were also prepared with water containing 0 ppm Cr(VI). Two vetiver slips were added to each pot. The experiments were conducted fourfold, this was primarily to eliminate the effect harvesting plants would have on the rate of Cr(VI) uptake in each pot, as harvesting meant removing both plants from the pot. Figure 3.2 shows the experimental setup. The grass was supported by a polystyrene slotting platform.

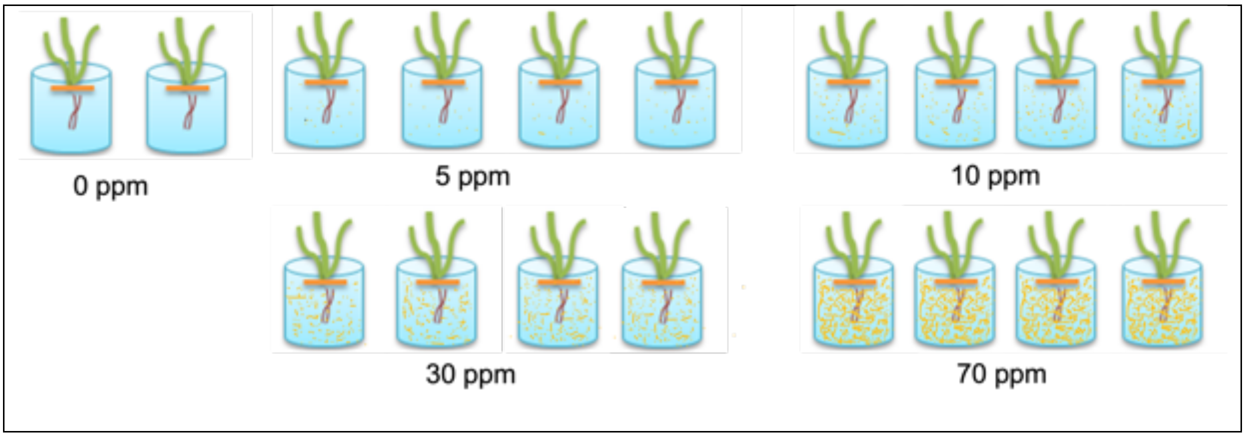

Figure 3.2: Different initial concentration experimental setup

The experiment was conducted over an extended period of 52 d. Solution samples were taken at an interval of 2 d. Plant samples were harvested at an interval of 14 d.

3.4 Cr(VI) removal at different plant densities

The second phase of the study focused on the influence of different plant density on the uptake rate of Cr(VI). Chromium(VI) solutions with concentrations of 5, 10, and 30 ppm in 2 L pots were used. Vetiver grass slip densities were classified into three groups namely low density (5 slips), medium density (10 slips), and high density (15 slips), Figure 3.3 shows the experimental setup. Analysis of Cr(VI) uptake were done by following the changes in concentration in the pots as well as the accumulation in different parts of the plant.

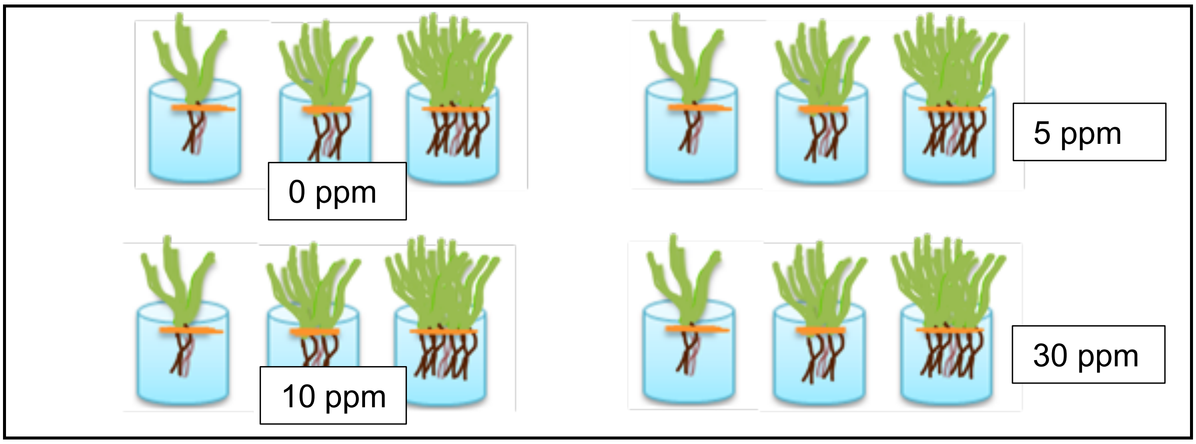

Figure 3.3: Density experimental set up

The experiment was conducted over a period of 30 d. Solution samplings were done at an interval of 2 d, and plant harvesting was done at an interval of 14 d. Unlike at initial concentration experiments where only 2 vetiver slips were used, plant harvesting during density experiments involved carefully picking roots and slips of different vetiver slips.

3.5 Cr(VI) removal at different solution pH

The third phase of the investigation assessed the effect of different pH levels on the reduction of Cr(VI) concentration and accumulation of Cr in the roots and shoots of the grass. For this investigation, 30 ppm of Cr(VI) was added to four 2 L pots, in which the pH of the solution was adjusted to pH values of 3.5; 5; 7.5 and 10.5. These pH values were selected to represent acid, neutral and alkaline conditions. A control pot consisting of unadjusted tap water at pH 8-8.4 was also prepared. Periodic checks of the solution pH were carried out to ensure the pH values were maintained. The experiments were conducted using medium-density (10 slips) vetiver grass.

3.6 Pd(II) removal at different initial concentration

The last phase of the experiments focused on the phytoextraction potential of vetiver grass in recovering precious metals. Vetiver grass was exposed to different concentration of Pd(II). Synthetic solutions of Pd(II) containing 10, 20, 40, 80 and 120 ppm concentrations were prepared. These experiments were conducted during the winter season during which the grass had weak slips. Two vetiver slips were added to 1 L palladium solutions of different concentrations. Figure 3.5 shows the experimental setup.

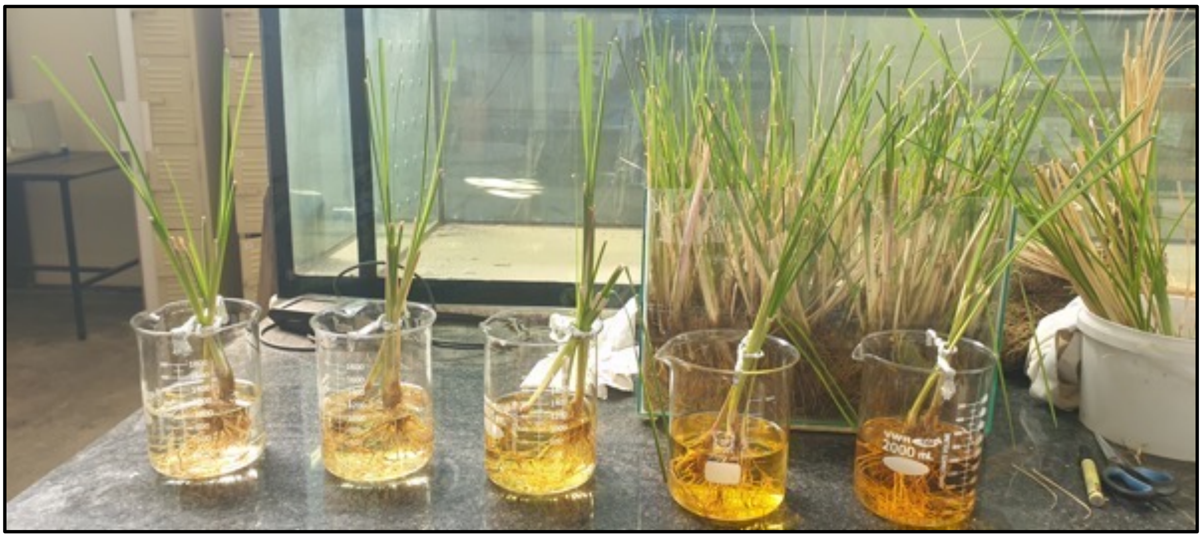

Figure 3.4: Pd experimental setup

The experiment was conducted over a period of 20 d. Solution samples were analysed at an interval of 2 d and the plants samples were only harvested at the end of the experiment.

3.7 Analytical methods

3.7.1 Cr(VI) concentration measurements

The concentration of Cr(VI) in the solution was determined using the DPC method (APHA, 2005). Solution samples were taken at an interval of 2 d, using syringe filters to extract 1 mL from each pot, 0.2 mL of the filtered solution was pipetted into a 10 mL volumetric flask for analysis. The sample was then acidified by 1 ml of 1 N H_2SO_4, followed by the addition of distilled water to the calibration mark of the volumetric flask and a reaction with 0.2 mL of 1,5-diphenyl-carbazide (DPC) to produce a violet colour in response to the presence of Cr(VI) (APHA, 2005). The DPC solution was measured for Cr(VI) content using UV-Vis spectrophotometer (Biochrom WPA, Light Wave II, and Labtech, South Africa) at a wavelength of 540 µm.

3.7.2 Pd(II) concentration measurements

Pd(II) content in the pots was measured at an interval of 2 d. Using syringe filters, 1 ml solution was extracted from each pot, followed by relevant dilutions using deionized water making sure the concentration stayed within the detection limit. Palladium levels were determined using Atomic Absorption Spectrometry, AAnalyst 400 spectrometer fitted with S/N 201S8070301 Autosampler Model 510. It used an air-acetylene flame, PerkinElmer Lumina Pd hollow cathode lamp at a wavelength of 244.79 nm, with a corresponding energy of 79.

3.7.3 Plant sample harvesting

Plant samples were harvested at different intervals as stated in the preceding sections. They were obtained by cutting the leaves and shoots of the grass in the density and pH experiments, while the full slips were harvested in the initial Cr(VI) and Pd(II) concentration studies. The harvested grass was washed under running tap water and rinsed with distilled water to wash off any residual metals of the surface of the plant. The grass was separated into roots and shoots, the roots crown was discarded as it was assumed that it does not accumulate metals (Ladislas et al., 2013, Suelee et al., 2017). The grass samples were oven dried at 70 °C until they were completely moisture free. The dry samples were ground using mortar and pestle (Figure 3.6). The extraction of metals from the grass sample was done using acid leaching, 0.1 g of each sample was digested using 10 mL nitric acid and 2 drops of hydrogen peroxide for 48 h (Zarcinas et al., 1987). The leachate from the digestion process was recovered by filtering the mixture through 0.45 μm syringe filters. The filtrate was subsequently mixed with 10 mL distilled water before analyzing for metal presence (Figure 3.6).

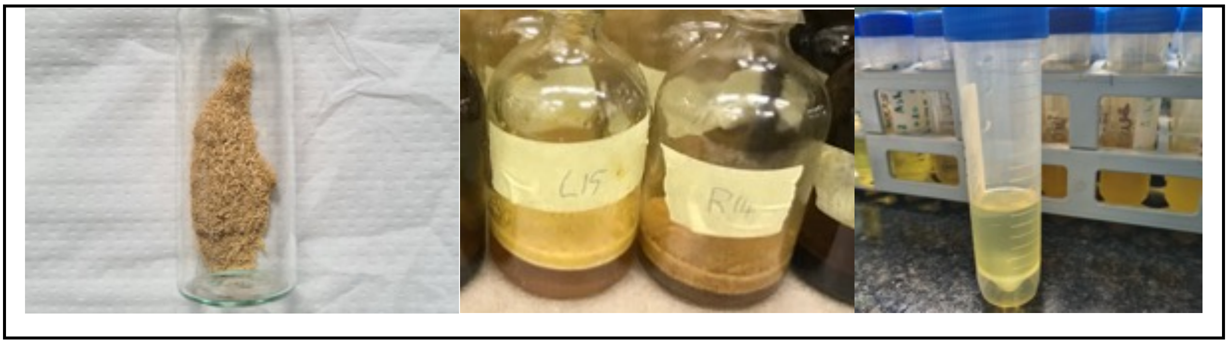

Figure 3.5: Acid leaching of metals from plant samples

The total Cr and Pd content of the plant samples was analysed using SPECTRO Analytical Instruments Genesis (ICP-OES) Spectrometer (Perkin-Elmer, Johannesburg).

3.7.4 pH measurements and adjustments

The solution pH was measured every time before sampling using a Metrohm pH sensor (Metrohm, Herisau, Switzerland). pH adjustments were done using NaOH and HCL (Glassworld, Gauteng, South Africa).

3.7.5 Removal efficiency

Removal efficiency is the measure of the amount of metal removed from the solution. It was calculated as follows:

$$\text{Removal efficiency} = \frac{C_i - C_t}{C_i} \tag{3.1}$$

where C_i is the initial metal concentration of solution and C_t is the metal concentration of solution at time t.

3.7.6 Bioaccumulation factor (BAF)

Bioaccumulation factor (BAF) relates the concentration of metal accumulated in the roots to the concentration of metal in the substrate. It indicates the plant's ability to take up metals from the external environment. The BAF is a useful parameter in accessing the potential of a plant in accumulating metals, it is calculated on a dry weight basis using Equation 3.2 (Zayed et al., 1998, Lu et al., 2004, Yoon et al., 2006)

$$\mathrm{BAF} = \frac{\mathrm{C}_{\mathrm{plant\ tissue}}}{\mathrm{C}_{\mathrm{i,wastewater}}} \tag{3.2}$$

where $\mathrm{C}_{\mathrm{plant\ tissue}}$ is the metal concentration in the harvested plant tissue and $\mathrm{C}_{\mathrm{i,wastewater}}$ is the initial concentration of metal in the simulated wastewater.

3.7.7 Translocation factor (TF)

The translocation factor is a ratio of the amount of metals translocated to aerial parts, to the amount of metals in the roots. The TF is an indicator of the plant's ability to translocate the metal from the roots to the aboveground parts, it is calculated using Equation 3.3:

$$\mathrm{TF} = \frac{\mathrm{C}_{\mathrm{shoot}}}{\mathrm{C}_{\mathrm{root}}} \tag{3.3}$$

where $\mathrm{C}_{\mathrm{shoot}}$ is the concentration of the metal in the shoots, and $\mathrm{C}_{\mathrm{root}}$ is the concentration of the metal in the roots of the plant (Ali et al., 2013).

CHAPTER 4: RESULTS AND DISCUSSION

The work reported here has been publish in the journals listed in the publications section.

1 Effect of initial Cr(VI) concentration

4.1.1 Removal at 5 ppm

The phytoremediation potential of VG was investigated at different initial Cr(VI) concentrations. This was to assess the effect of initial concentration on the growth of vetiver grass, uptake of Cr(VI), and bioaccumulation of chromium. Figure 4.1a shows the results obtained from water samples and Figure 4.1b shows the results from plant samples. From Figure 4.1a, a fast reduction in Cr(VI) concentration was observed in the first 20 d, and thereafter the reduction was gradual. The average rate of reduction in the first 20 d is 0.22 ppm d^{-1}, and thereafter, the rate reduced to 0.094 ppm d^{-1}. Figure 4.1a shows that the initial concentration dropped from 5 ppm to 0.65 ppm after 52 d.

From the plant samples, an increase in Cr(VI) concentration was observed over the experimental period. On day 1, vetiver samples were analysed for Cr(VI) concentration before the commencement of the experiment. More Cr(VI) was found in the roots than in the leaves throughout the study (Figure 4.1b), as a result of restricted heavy metal translocation (Roongtanakiat et al., 2008). A total of 0.49 mg g^{-1} and 0.84 mg g^{-1}, was accumulated in the leaves and roots respectively of vetiver planted in 5 ppm pots after 52 d.

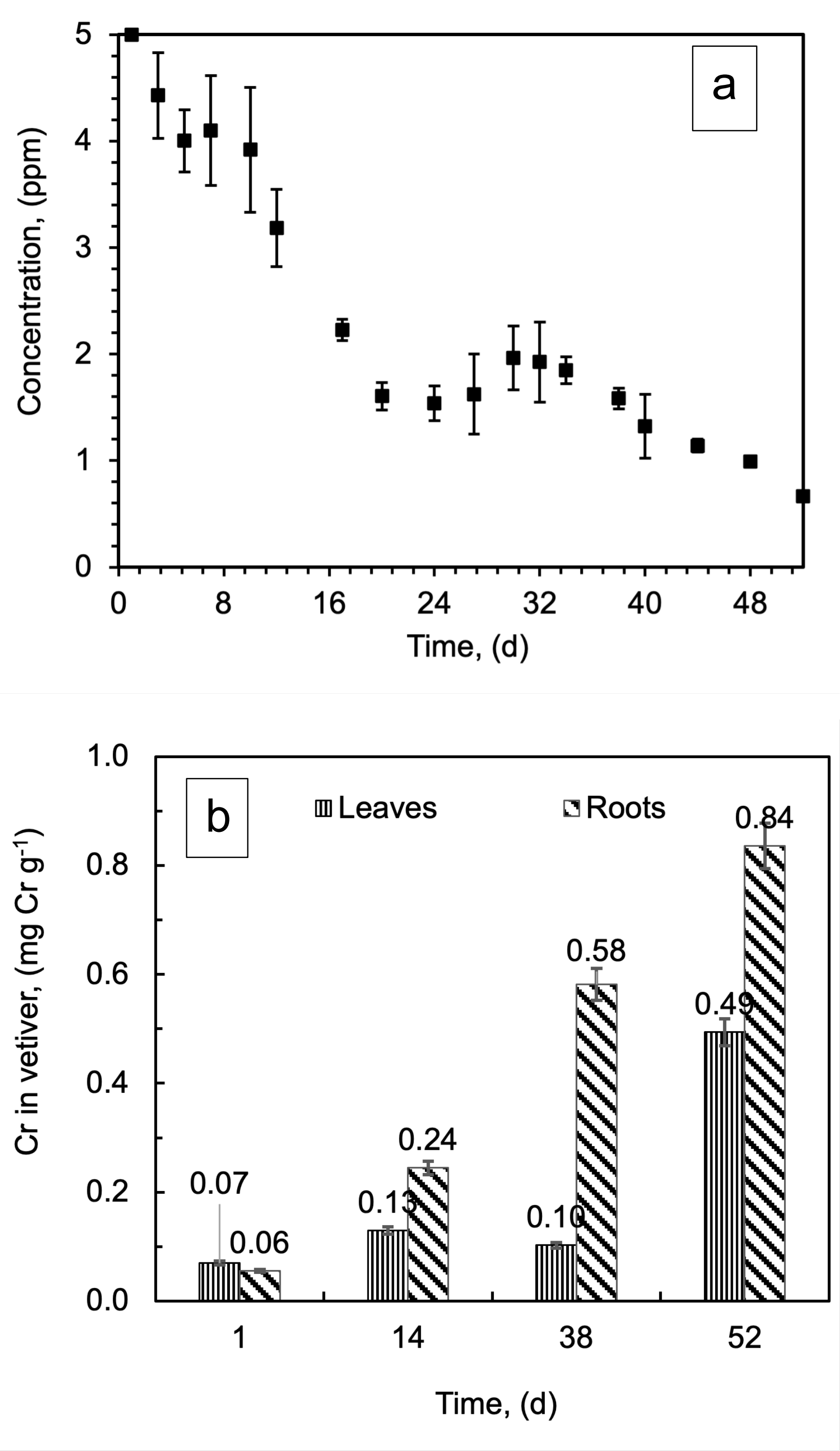

Figure 4.1: (a) Cr(VI) reduction in the 5 ppm solutions, (b) The concentration of Cr(VI) in the root and leaves of vetiver

4.1.2 Cr(VI) removal at 10ppm

At 10 ppm initial concentration the rate of Cr(VI) uptake was faster than at 5 ppm initial concentration. The concentration of Cr(VI) was reduced from 10 ppm to 4.5 ppm after 52 d. Which correspond to a reduction in Cr(VI) content of the pots by 5.2 ppm. Around day 24, a reverse trend to appeared indicating a slight increase in Cr(VI) content for both the 5 ppm pot (Figure 4.1a) and 10 ppm pot (Figure 4.2a), this is because the fast reduction in Cr(VI) concentration observed in the first 20 d, was mainly due to biosorption of chromium on to the roots of vetiver, which is a reversible process (Kumar et al., 2013). When the rate of bioaccumulation becomes slow because of high metal content in the plant, the metals bind to the roots start to return to the solution because of the concentration difference between the roots and the solution.

A similar trend as that obtained from the grass planted in 5 ppm solution was observed from grass planted in 10 ppm pots. There was an increase in plant chromium content over the duration of the study, and more Cr(VI) was accumulated in the roots than in the leaves (Figure 4.2b). Vetiver grass in the 10 ppm pots accumulated more Cr(VI) than vetiver grass in the 5 ppm buckets. A total of around 0.64 mg g^{-1} and 1.00 mg g^{-1} was accumulated in the leaves and roots respectively of the grass (Figure 4.2b).

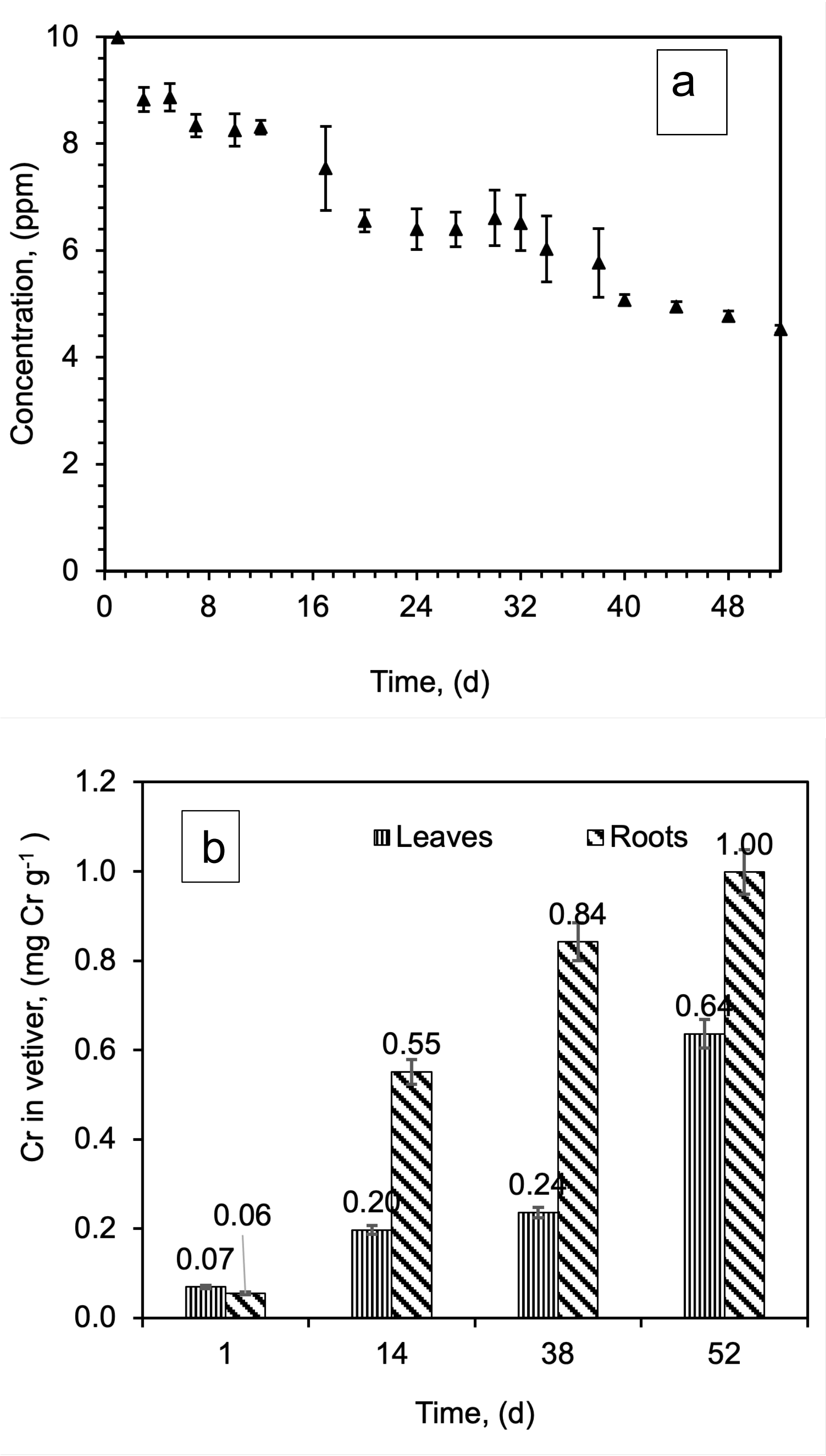

Figure 4.2: (a) Cr(VI) reduction in the 10 ppm solutions, (b)The concentration of Cr(VI) in the root and leaves of vetiver

4.1.3 Removal at 30 ppm

The amount of Cr(VI) removed from the pots under 30 ppm initial concentration was higher compared to the amount removed at 5 and 10 ppm. A total reduction of Cr(VI) concentration of 8.4 ppm from 30 ppm to 21.4 ppm was observed (Figure 4.3a), which correspond to a removal efficiency of 28 %.

The amount of Cr accumulated in vetiver grass after 14 d showed slightly more Cr concentration of 0.72 mg g^{-1} in the roots than 0.53 mg g^{-1} in the leaves. By the end of the experiment after 38 d, much more chromium was detected in the leaves of vetiver grass than in the roots. The roots had accumulated 0.93 mg g^{-1} while 1.45 mg g^{-1} was detected in the leaves (Figure 4.3b).

4.1.4 Removal at 70 ppm

At a higher Cr(VI) concentration of 70 ppm the obtained results from the water samples were oscillatory. Multiple samplings were performed to confirm the obtained results. The average results obtained at 70 ppm are shown in Figure 4.4a. The removal efficiency was low, at only 11.5 %. Vetiver reduced Cr(VI) concentration by 8.05 ppm, which is lower than the reduction found in 30 ppm pots.

From grass samples (Figure 4.4b), more Cr(VI) was recorded in the leaves than in the root which corresponds to the results at 30 ppm. The amount of chromium accumulated in the leaves in 70 ppm pots surpassed the amount in the roots at 14 d and 27 d. The accumulated chromium in the leaves was threefold that in the roots by the end of the experiment (Figure 4.4b). At day 27 the concentration of chromium was 3.04 mg g^{-1} and the accumulation in the roots was 1.12 mg g^{-1}.

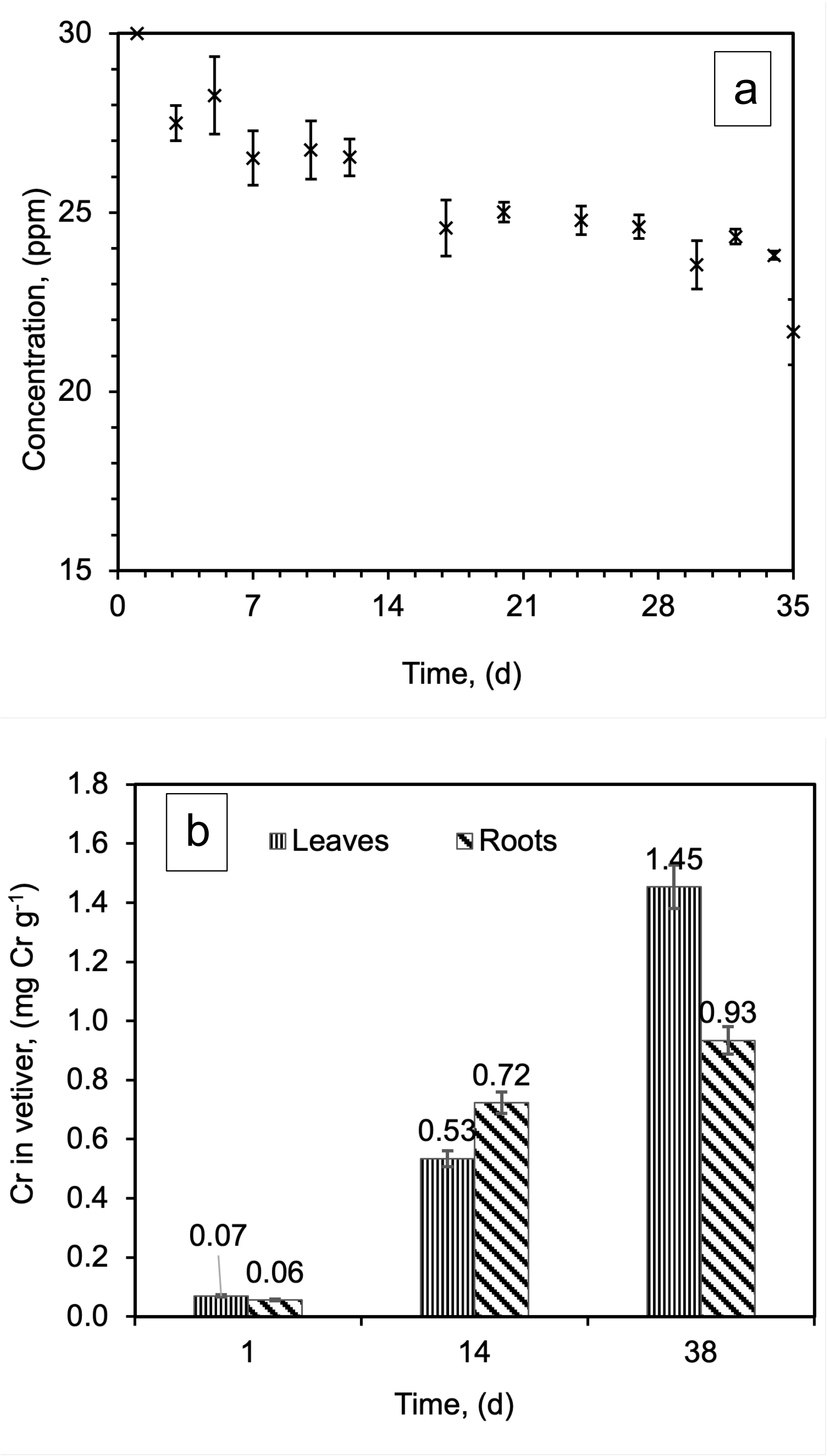

Figure 4.3: (a) Cr(VI) reduction in the 30 ppm solutions, (b)The concentration of Cr(VI) in the root and leaves of vetiver

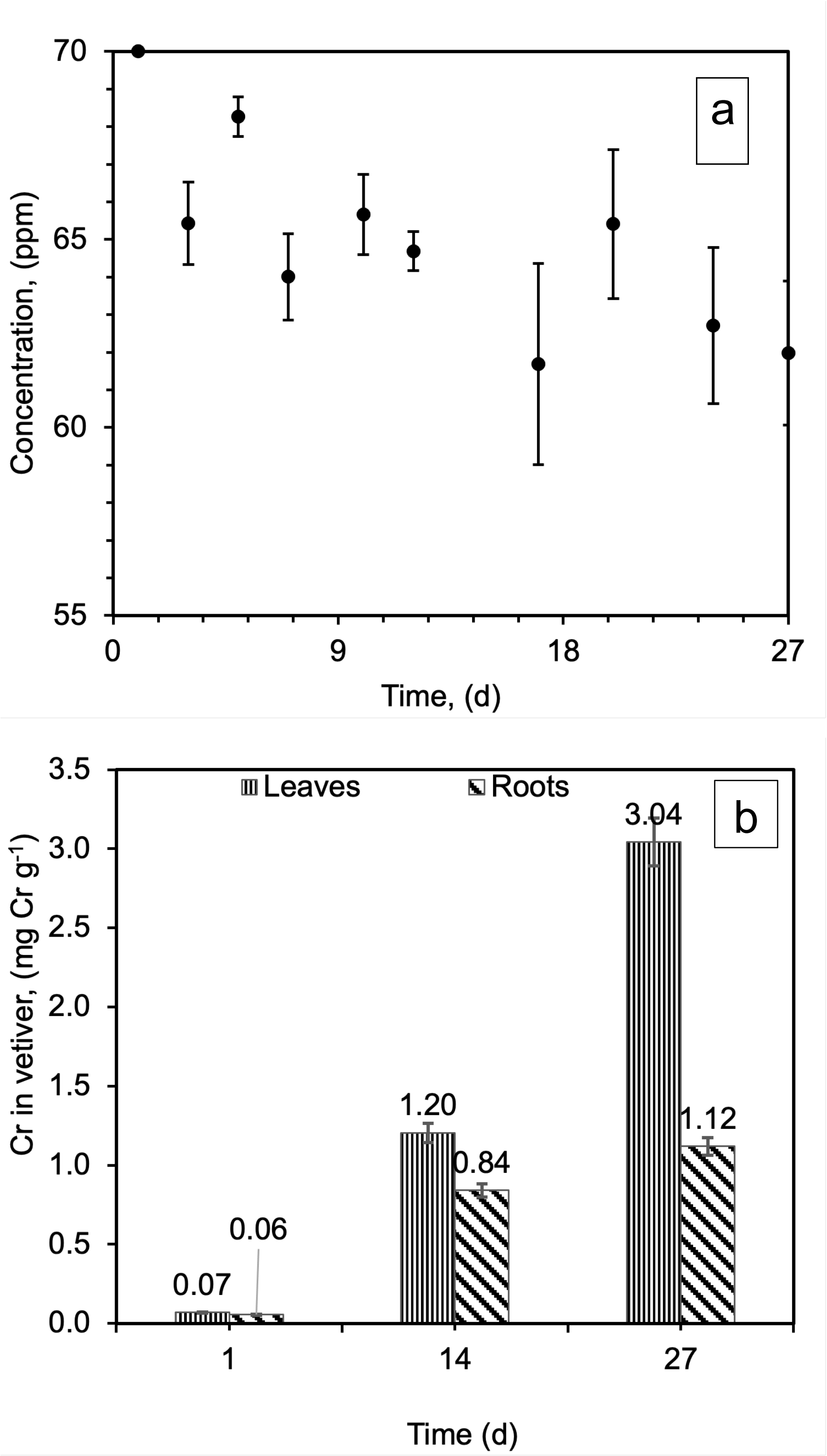

Figure 4.4: (a) Cr(VI) reduction in the 70 ppm solutions, (b)The concentration of Cr(VI) in the root and leaves of vetiver

4.1.5 Discussion of Cr(VI) initial concentration experiments

The amount of Cr(VI) removed at different initial concentrations increased together with initial Cr(VI) pot concentration, as shown in Figure 4.5. The rate of Cr(VI) removal increased with an increase in initial pot concentration, a similar observation was done by previous researchers (Roongtanakiat and Chairoj, 2001, Suelee et al., 2017). The increment in Cr(VI) uptake rate observed at elevated concentrations can be as a result of the diffusion driven nonmetabolic mechanism where the Cr ions were moving from the highly concentrated solution to the root surface where they were absorbed (Hayat et al., 2012). At lower concentrations (5 and 10 ppm), the dominant uptake mechanism of chromium is the active mechanism, which is usually limited by the availability of relevant carriers and restricted by the presence of essential ions in the solution. The presence of essential ions such as sulfate in the solution hinders the uptake because they compete for the same binding sites as Cr(VI) on plant roots (Cervantes et al., 2001).

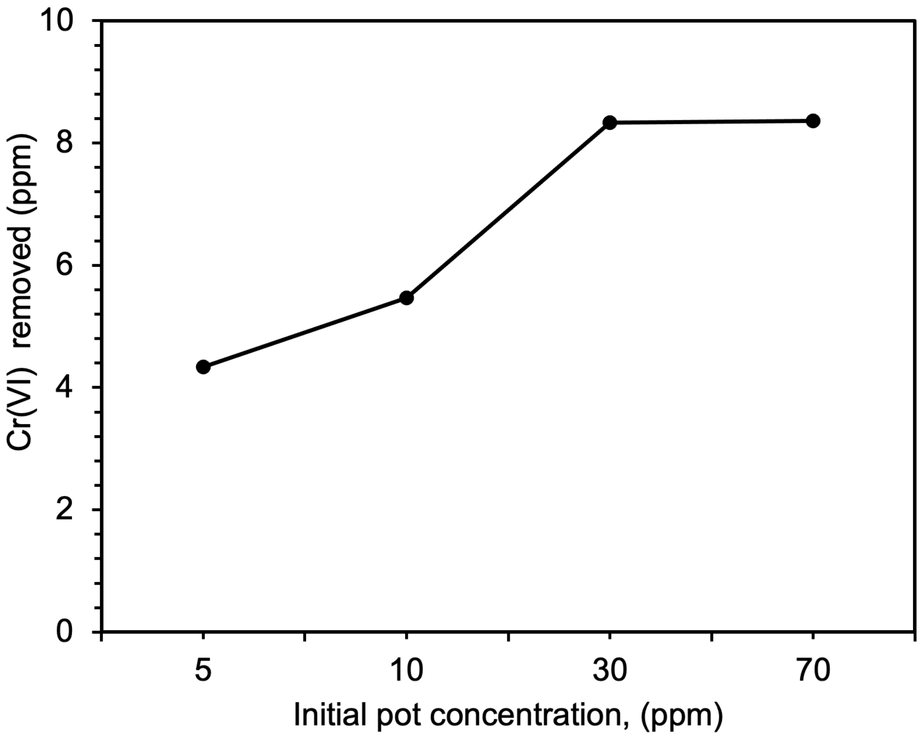

Figure 4.5: Total removed Cr(VI) at different initial concentration

Figure 4.5 illustrates a correlation between the amount of Cr(VI) removed and the initial pot concentration. As the initial concentration increased the amount of Cr(VI) removed from the pots also increased, from 4 ppm reduction at 5 ppm initial pot concentration to 8 ppm reduction at 30 ppm initial pot concentration. However, the removed Cr(VI) remained constant between 30 ppm and 70 ppm initial concentration. At higher concentrations the toxic effects of chromium led to the early (27 d for 70 ppm) drying out of the grass and eventually stopping the uptake of chromium, leaving the plants at lower concentrations with more time to reduce an equivalent amount of Cr(VI) before drying out (38 d at 30 ppm).

Figure 4.6 shows the comparison of Cr concentration in the roots and leaves of vetiver grass at different initial pot concentrations. The amount of chromium accumulated in the roots and leaves of VG increased with time and it also increased with the initial Cr(VI) concentration (Figure 4.6). At lower initial concentrations (5 ppm and 10 ppm), the amount of Cr accumulated in the roots remained higher than the amount accumulated in the leaves throughout the study, as a result of immobilization of Cr ions in the vacuoles of root cells leading to less Cr translocation to the leaves (Shanker et al., 2004). Plants reduce the translocation of Cr to the leaves as a way to avoid the toxic effects of Cr from affecting the important metabolic activities (Zhang et al., 2009). However at elevated Cr(VI) substrate concentration (30 ppm and 70 ppm) the amount of Cr recorded in the shoots increased to surpass the concentration in the roots as the experiments progressed.

As the concentration of Cr in the roots approached 1 mg g^{-1}, the concentration of Cr in the shoots started to increase beyond that of the roots. A saturation phenomenon was noticed in the roots at approximately 1 mg g^{-1} (Figure 4.7).

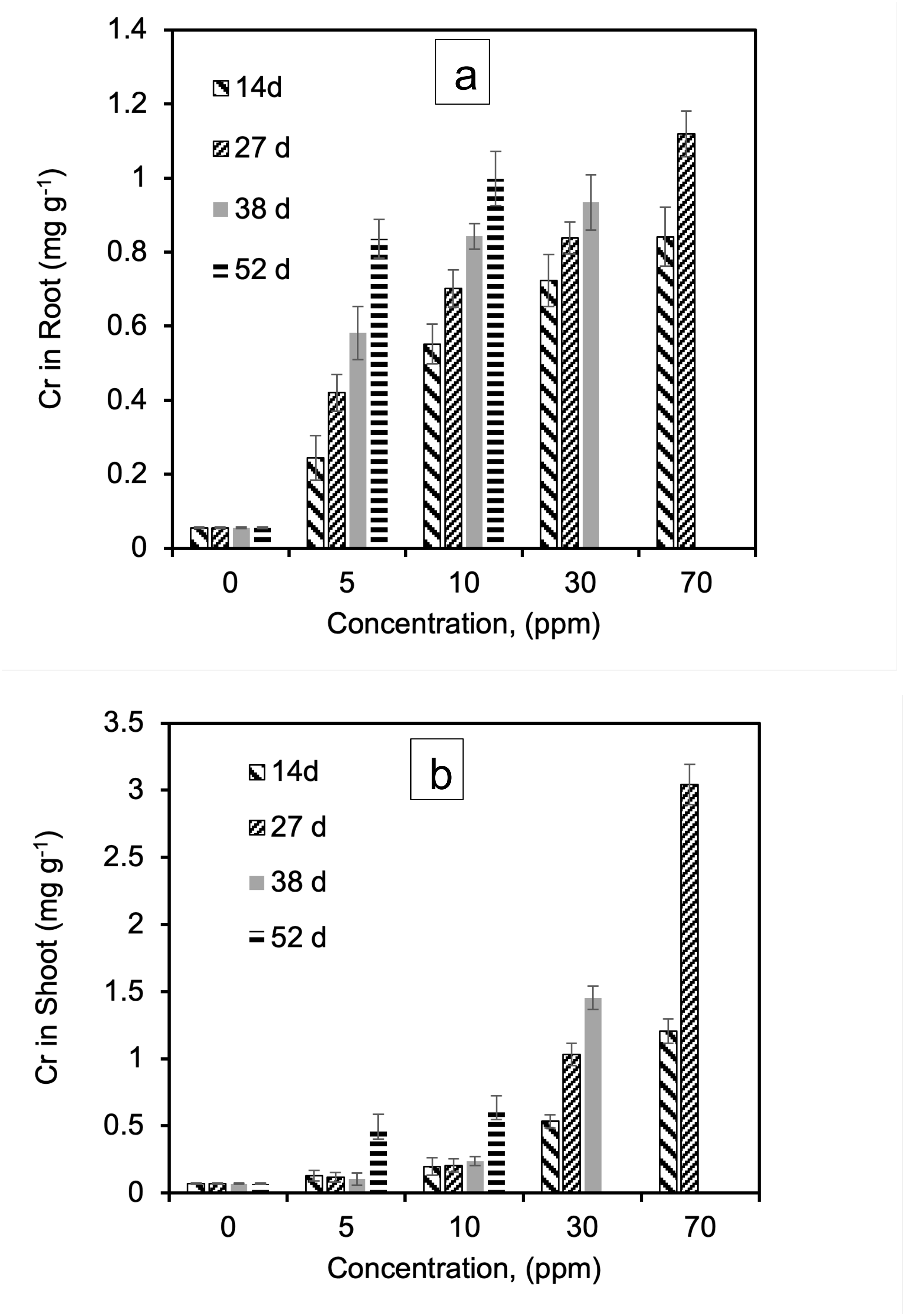

Figure 4.6: Accumulation of chromium at different initial concentration in (a). roots and (b) leaves

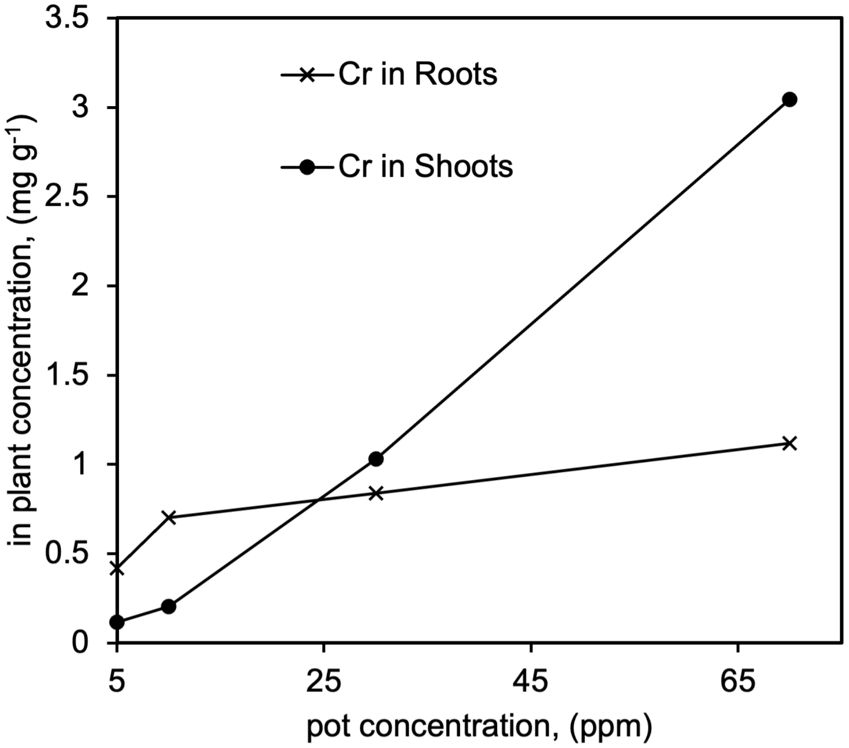

Figure 4.7: Correlation between the amount of Cr in the roots, shoots of vetiver grass, and initial pot concentration, after 27 d

The response of vetiver grass to different Cr(VI) concentrations can be seen in Figure 4.8. According to Hegemeyer (1999), the early signs of heavy metal toxicity are growth related changes. Heavy metal exposure changes the plant's uptake activity in the plasma membrane in root cells limiting the uptake of essential elements such as N, P, K, Ca, Zn, Mg Fe, etc. (Shanker et al., 2004). The resulting effects of this is the disruption essential metabolic processed and ultimately the withering of the grass. Little to no effects of chromium toxicity were observed from grass planted at pot concentrations of 5 and 10 ppm, under these concentrations vetiver grass thrived throughout the study with no signs of heavy metal stress. At higher initial concentrations vetiver grass showed restricted growth followed by complete withering of the aerial parts after 27 d for the grass at 70 ppm and 38 d for plants under 30 ppm initial concentration. The grass at higher Cr(VI) dried because of elevated Cr(VI) toxicity.

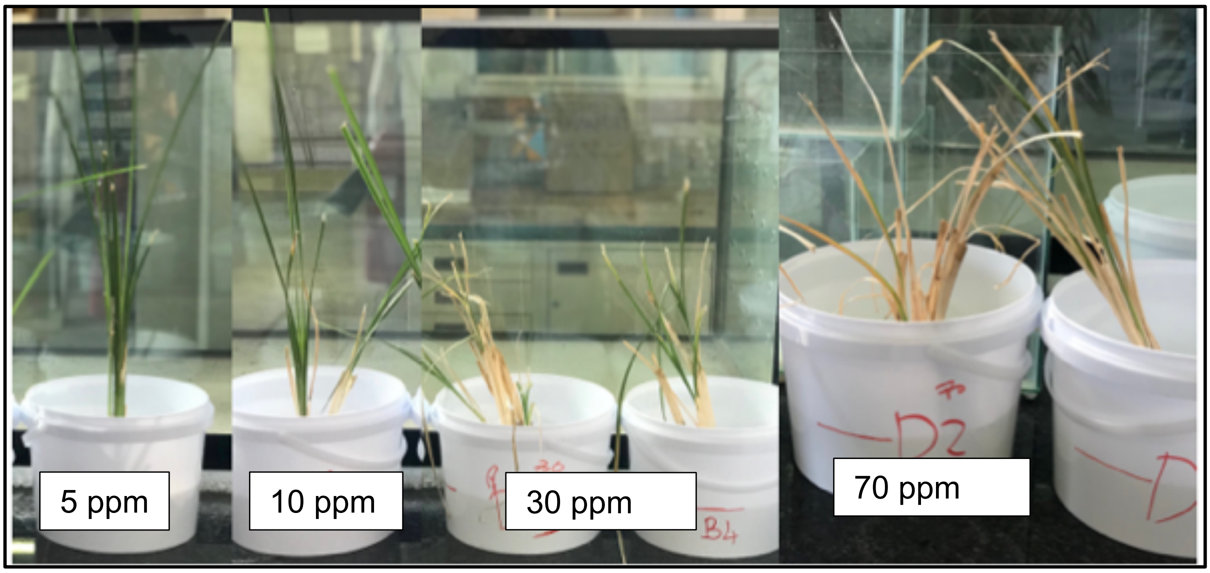

Figure 4.8:Vetiver grass in 5, 10, 30, and 70 ppm after 27 d

Translocation factor (TF) and bioaccumulation factor (BAF) are important parameters in phytoremediation studies. BAF is the quotient of heavy metal content in the dried root biomass (mg kg^{-1} dw) to initial heavy metal concentration in the substrate (ppm). TF is the quotient of heavy metal concentration in shoot (mg kg^{-1} dw) to the heavy metal concentration in the roots (mg kg^{-1} dw). For application in phytoremediation a plant should have the ability to accumulate more metals in its roots than the metal content in the substrate, thus BAF should be greater than 1. For phytoextraction a plant should be able accumulate more heavy metals in the aboveground parts than in the roots (Yoon et al., 2006, Ghazaryan et al., 2021), thus both the BAF and TF should be greater than 1. Plants with BAF > 1 and TF < 1 are more suitable for phytostabilisation (Lorestani et al., 2013), as this process doesn’t necessarily require the translocation of the metals to the above ground parts.

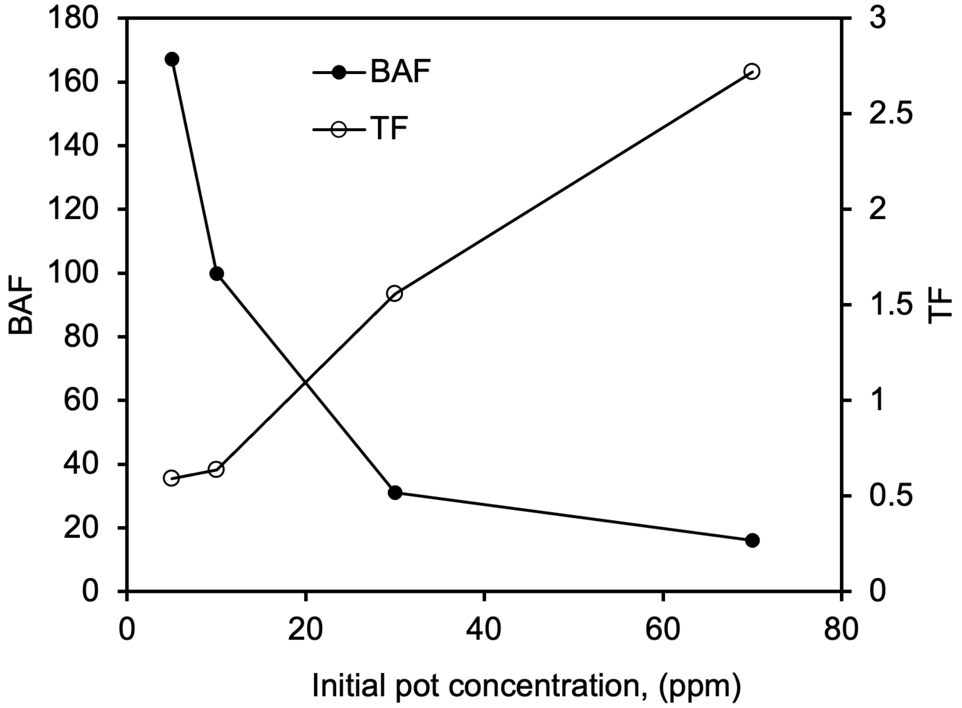

Figure 4.9:Bioaccumulation factor (BAF) and translocation factor (TF) at different initial concentrations 5, 10, 30, and 70 ppm Cr(VI)

The BAF decreased with the increase in initial concentration. The highest bioaccumulation factor of 167 was calculated at initial Cr(VI) concentration of 5 ppm, while a BAF of 100 was calculated at 10 ppm (Figure 4.9). This shows the capacity of vetiver to accumulate Cr in its roots at concentration hundred times more than the concentration in the solution at such low concentrations. At higher concentration 30 ppm and 70 ppm the BAF decreased to 31 and 16 respectively (Figure 4.9). The BAF at all different initial concentrations was above one, proving the phytoremediation potential of vetiver grass (Yoon et al., 2006, Ghazaryan et al., 2021).

The reverse trend was observed for the translocation factor which increased with increased initial Cr(VI) concentration. The TF at Initial Cr(VI) concentrations of 5 and 10 ppm was below one, while it was above one at 30 and 70 ppm. Indicating that the translocation of Cr from roots to shoots is effective at concentrations above 30 ppm. The successes of phytoextraction relies on the ability of a plant to translocated the metals to easily harvestable parts of the (Yoon et al., 2006). The translocation rate of metal to the shoots is dependent on the root metal content.

It is postulated that higher Cr content in the roots, disrupts the metabolic activities responsible for immobilizing, detoxifying, and limiting the translocation of toxic metals to the aboveground parts. When these defense mechanisms are disrupted, simple acropetal movement of Cr to the shoots becomes responsible for the increased translocation at higher concentrations. At initial concentrations above 30 ppm the BAF and TF values obtained were greater than one. Thus, vetiver grass has potential in phytoextraction of Cr at concentrations above 30 ppm. However, at lower concentrations vetiver grass proves to be more effective for phytostabilisation.

4.2 Effect of plant density

4.2.1 Cr(VI) removal from solution

The removal of chromium(VI) at varying plant densities was studied using low density (5 vetiver slips), medium density (10 vetiver slips) and high density (15 vetiver slips). Initial Cr(VI) concentrations of 5, 10, and 30 ppm were chosen for the study in 2 L pots. The duration of this experiment was 30 d. The high-density vetiver grass at initial concentration of 30 ppm achieved the highest removal efficiency of 82 %, by reducing the amount of Cr(VI) in the pot from 31.9 ppm to 5.8 ppm after 30 d (Figure 4.10). The medium-density grass achieved a removal efficiency of 42 % (from 33 ppm to 19.7 ppm) and low-density grass achieved a removal efficiency of 29 % (Figure 4.10). For the different plant densities, the general trend showed an increase in the removal of Cr(VI) over the duration of the study.

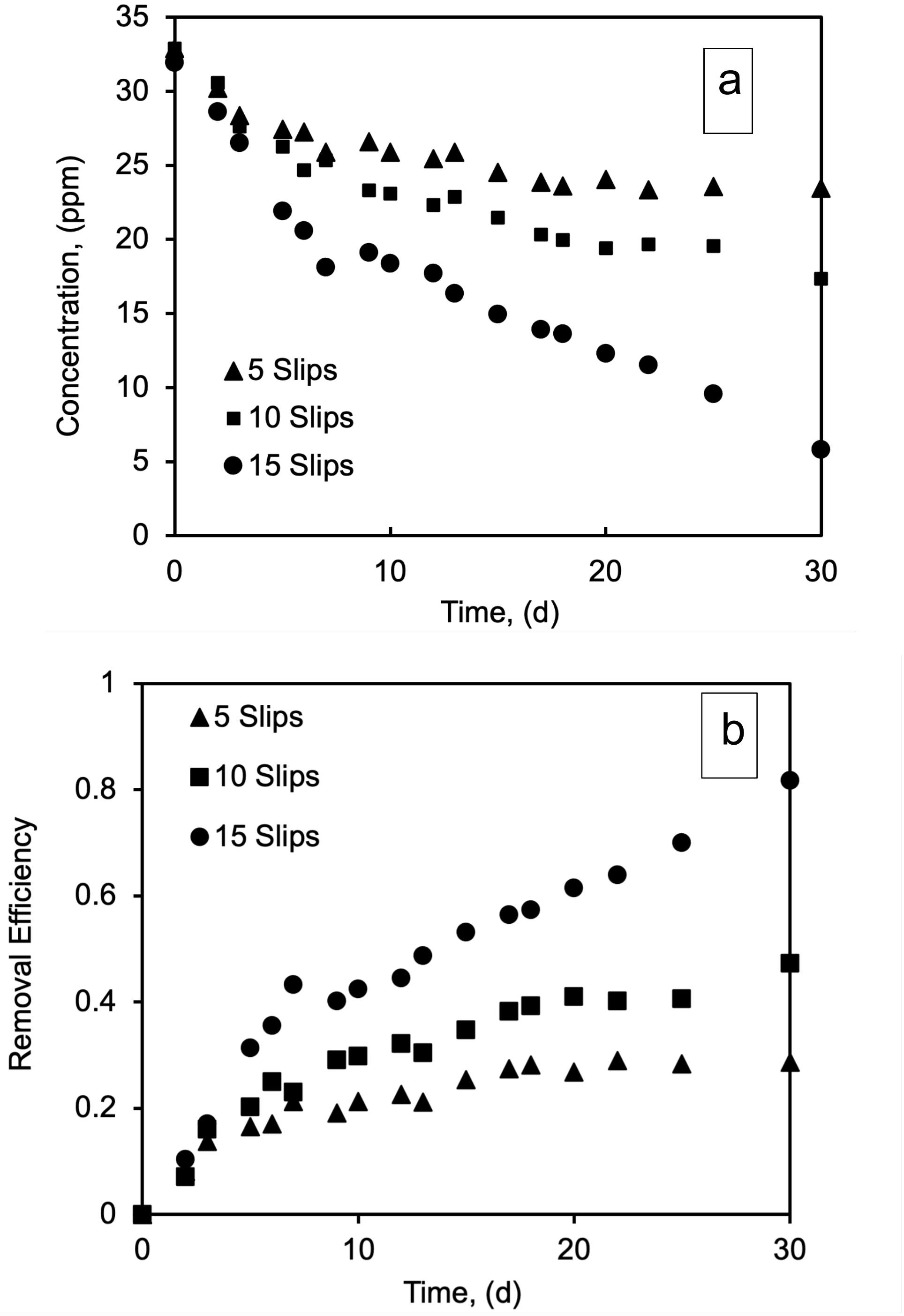

Figure 4.10: Effect of plant density on the uptake of Cr(VI) using low density (5 slips), medium density (10 slips) and high density (15 slips) at 30 ppm Cr(VI) concentration. Change in Cr(VI) concentration (a), removal efficiency (b)

Some previous studies have reported that the uptake of Cr(VI) increases with an increase in plant density (Suelee et al., 2017). The similar observation was obtained in the current study. At higher plant density, the contact surface area for metal absorption by the roots is higher in comparison to lower plant densities (Darajeh et al., 2014). Higher plant density also results in increased water loss through perspiration, increased uptake rate of water, and consequently increase absorption of metals. This was observed over the duration of the study, where water level reduction in descending order was high-density pots, medium-density pots, and low-density pots. Chomchalow (2003), reported that higher plant density would absorb much effluent and heavy metal concentration in that area.

Regardless of the initial Cr(VI) concentration, the high-density grass had the highest removal efficiency (Figure 4.11). At low initial concentration, the difference in removal efficiency achieved by medium and high-density grass was small. At 5 ppm initial concentration both densities achieved 100 % removal efficiency by the end of 30 d. Based on the current study, it would be more efficient to apply medium density treatment when remediating Cr(VI) concentrations lower or equal to 5 ppm, as this would reduce intraspecific competition for nutrients, improve the efficiency of vetiver grass, and reduce capital, maintenance, and harvesting costs.

However, as the initial Cr(VI) concentration increased, so did the difference between the removal efficiency achieved by the medium and high density grass. At 10 ppm, the removal efficiency achieved by the high density and medium density grass were 100 % and 86 % respectively, and at 30 ppm the removal efficiency achieved by high-density grass was 82 % and 47 % for medium density grass (Figure 4.11). Therefore, it would be more effective to apply high density grass when remediating elevated Cr(VI) concentrations.

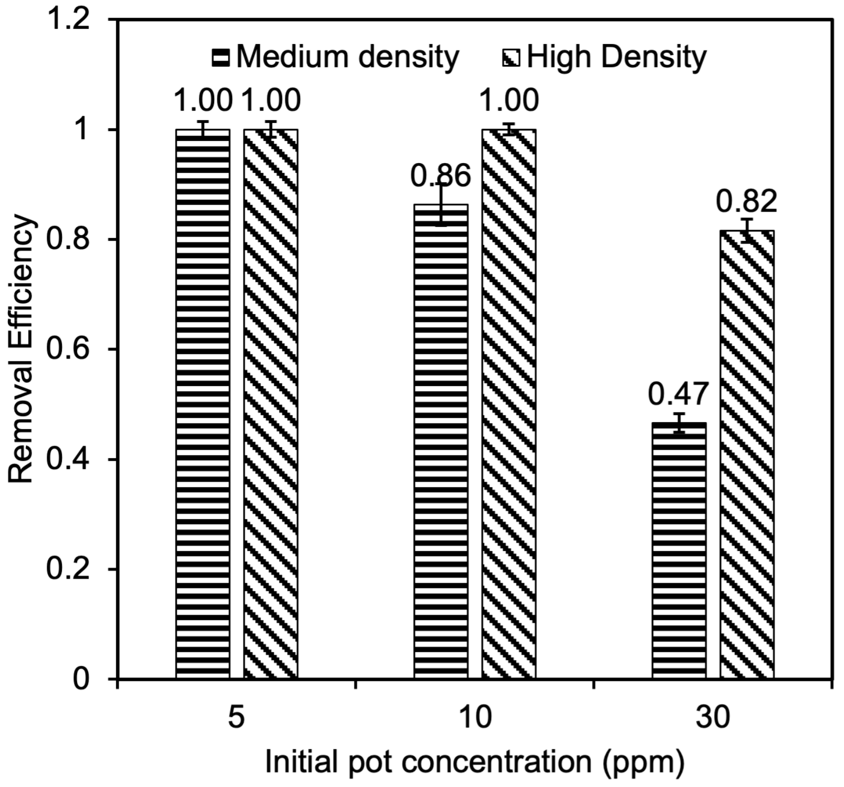

Figure 4.11: Comparison between medium-density (10 slips) and high-density (15 slips) removal efficiencies at 5, 10, and 30 ppm Cr(VI) concentrations after 30 d

4.2.2 Chromium accumulation at different densities

The accumulation of chromium in the roots and leaves of vetiver grass at initial Cr(VI) concentration of 30 ppm is shown in Figure 4.12. Contrary to what is expected, the concentration of Cr in the roots of VG increased with the plant density (Figure 4.12a). The concentration of Cr in the roots was 0.616, 0.76, and 1.06 mg g^{-1} at low, medium, and high grass density respectively. Increasing the plant density results in an increment of the biomass, which should reduce the amount of Cr distributed per mass of the grass.

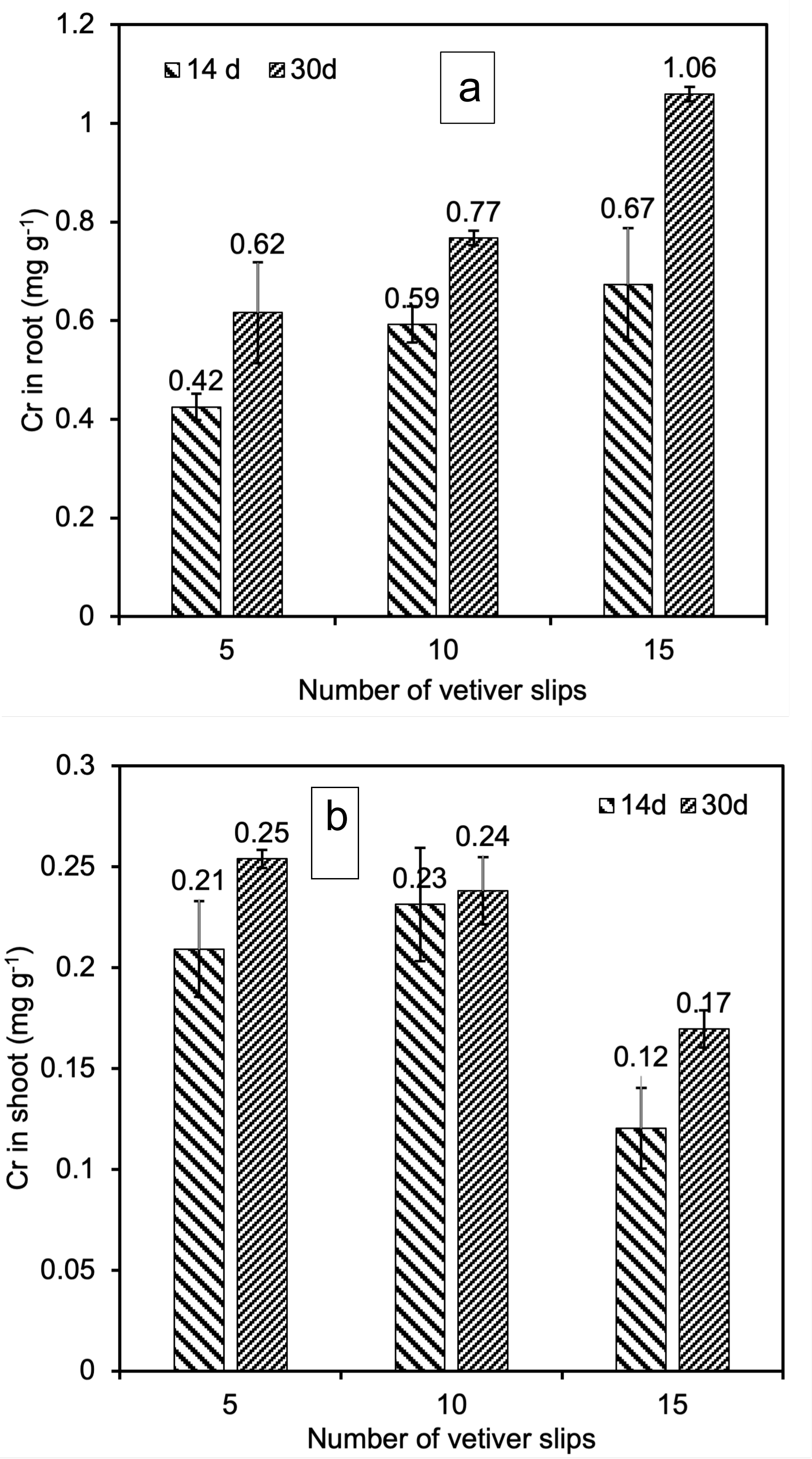

Figure 4.12: Distribution of Cr in the roots (a) and shoots (b) of vetiver grass at different plant densities and initial Cr(VI) concentration of 30 ppm

The oxidation state of Cr ions in the solution has an influence on the accumulation and translocation of chromium in the plant (Ertani et al., 2017). As stated in the preceding sections, in the presence of high organic matter and under low redox potential hexavalent Cr(VI) can be reduced to a less toxic trivalent Cr(III) (Sinha et al., 2002). Chromium(III) has a tendency to bind to cell walls in the roots, and its translocation to the aerial parts is very low (Sharma et al., 2020). Increasing plant density results in increased organic matter, and in this study, the increase in plant density might have also resulted in an environment with low redox potential. As a result, the absorbed Cr(VI) may have been reduced to Cr(III) which was mainly restricted to the roots of the grass at high density. Whereas at low density the dominant Cr(VI) was actively translocated to the aerial parts reducing the accumulation in the roots. After 30 d the concentration found in the leaves at low, medium, and high-density grass was 0.254, 0.238, and 0.170 mg g^{-1} respectively. Much more Cr was accumulated in the roots than in the shoots (Figure 4.12). Many studies reported poor translocation of toxic Cr to the areal parts of different plants species (Malaviya and Singh, 2011).

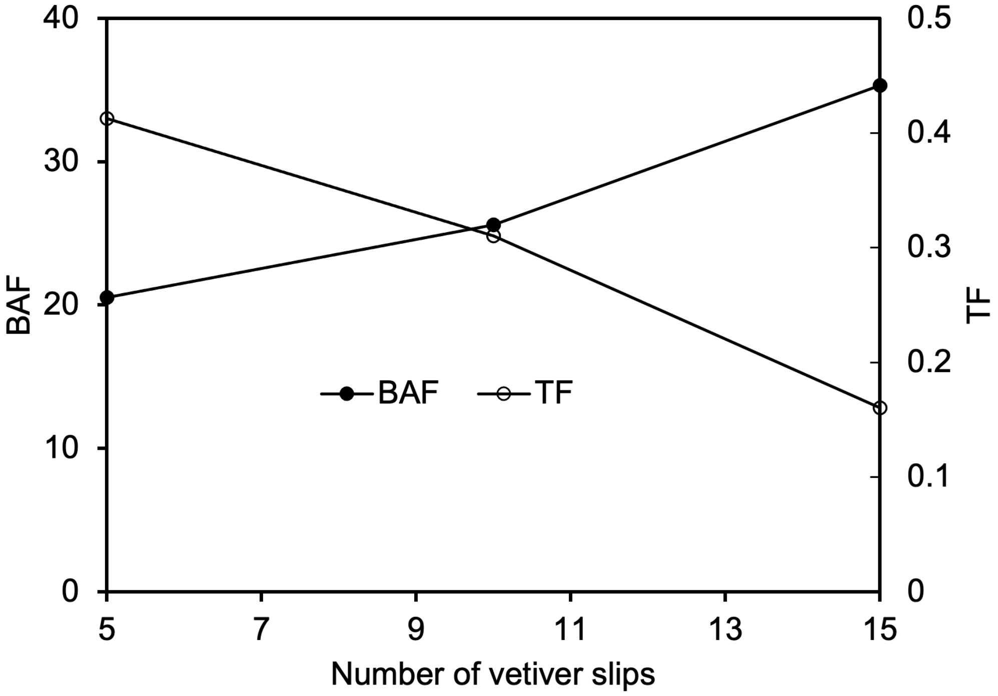

Figure 4.13: Bioaccumulation factor (BAF) and translocation factor (TF) at different vetiver grass densities, low (5 slips), medium (10 slips), and high (15 slips)

Figure 4.13 shows the BAF and TF of different grass densities. The calculated BAF values increased with the plant density ranging from 20.5 to 35.32. However, the translocation factors decreased as the plant density increased. This is because of the reduced translocation of Cr ions at higher density as they get reduced to Cr(III) and an increase in biomass result in low metal concentration as it gets distributed over a larger mass. The TF values calculated were, 0.41, 0,31, and 0.16 at 5, 10 and 15 vetiver slips respectively.

Vetiver grass proves to be a good candidate for phytstabilisation based on the BAF and TF values obtained from this study, as all the BAF values were above one (Yoon et al., 2006). The TF values obtained were below one. The difference in the BAF and TF values found in this study suggests that Cr is largely retained in the roots and its translocation to the above ground parts is minimum.

4.2.3 Plant response at different densities

Plants develop a response due to the conditions around which they are planted. Vetiver grass at different plant densities under Cr(VI) concentration of 30 ppm is shown in Figure 4.14 at the end of 30 d. The grass at different plant densities did not have any visible change in height meaning the grass didn’t grow under these conditions. More slips in the high-density group dried up. Increased intraspecific competition for sunlight, water, and nutrients possibly influenced the observed high mortality at high density (Marzinelli, 2012, Awan and Chauhan, 2016). Slight signs of stress were visible at low density, this could be because of the toxicity of Cr.

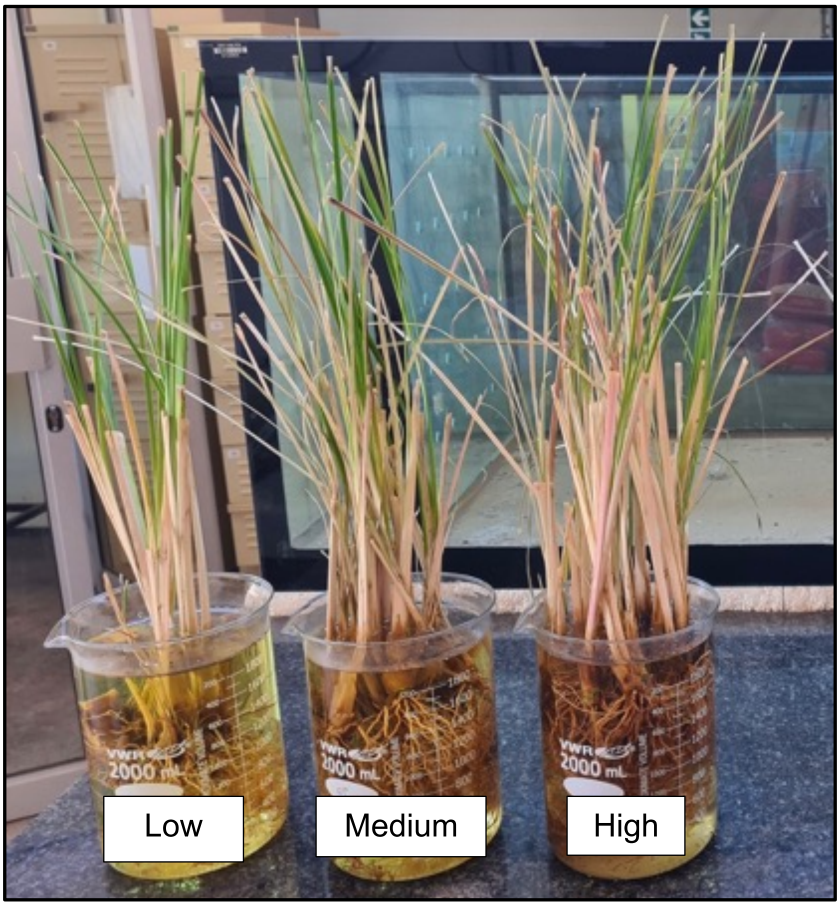

Figure 4.14: low (5 slips), medium (10 slips), and high (15 slips) dense vetiver grass in 30 ppm solution after 30 d

4.3 Effect of solution pH

4.3.1 Cr(VI) removal at different pH levels

The effect of pH on the uptake of Cr(VI) by vetiver grass at 30 ppm initial Cr(VI) concentration was investigated at different pH levels (3.5, 5, 7.5, 10.5). Medium grass density (10 vetiver slips) was used for this experiment. Complete Cr(VI) removal from the 3.5 pH pot was recorded after 20 d (Figure 4.15). The removal efficiency decreased as the pH level increased. At the end of the study (20 d), the grass in pH 5, 7.5, and 10.5 achieved removal efficiency of 83 %, 47.3 %, and 52.4 % respectively (Figure 4.15a). This study presented different results from those reported by Singh et al. (2015b), in which the highest removal of Cr(VI) was reported under neutral conditions. They reported the removal of Cr(VI) and Pb(II) by VG at initial Cr and Pb concentration of 20 ppm for 15 days which resulted in final Cr(VI) concentrations of 3.90, 2.88, 4.59, 4.32, and 6.10 ppm at pH 3, 6, 7, 9 and 12 respectively. It was then concluded that the pH range of 6-9 was more suitable for heavy metal uptake by vetiver grass. However, Kumar (2013) reported that acidic conditions favored the uptake of Cr(VI) by VG, the recorded removal efficiencies at pH 2.5 and 3.5 were 97% and 55% respectively. Kumar (2013) reported that there was no observed effect of pH on the uptake of Cr(VI) in the pH range (4-8). In the current study the removal of Cr(VI) at pH levels (7.5-10) was the same (Figure 4.15).

Different Cr(VI) species are dominant at different pH levels (Figure 2.3 and 2.4), these different Cr(VI) species affect the rate at which Cr is absorbed by vetiver grass. Under acidic conditions when pH level is below 5, $HCrO_4^-$ becomes the dominant species, while at pH levels above 6 the dominant species is CrO_4^{2-}. The pH level of the solution can influence the protonation or deprotonation of the binding sites on the plant roots, leading to a positively or negatively charged biosorbent surface (Miretzky and Cirelli, 2010). Under acidic conditions the cell wall binding sites of the roots are protonated, increasing anion binding sites which often favours the biosorption of metal anions (Fomina and Gadd, 2014). This results in the observed fast Cr(VI) uptake rates under acidic conditions. Conversely, alkaline conditions results in the deprotonation of metal binding sites, thus reducing the availability of anionic binding sites (Elangovan et al., 2008, Viviani et al., 2018), leading to the reduced Cr(VI) uptake at higher pH levels.

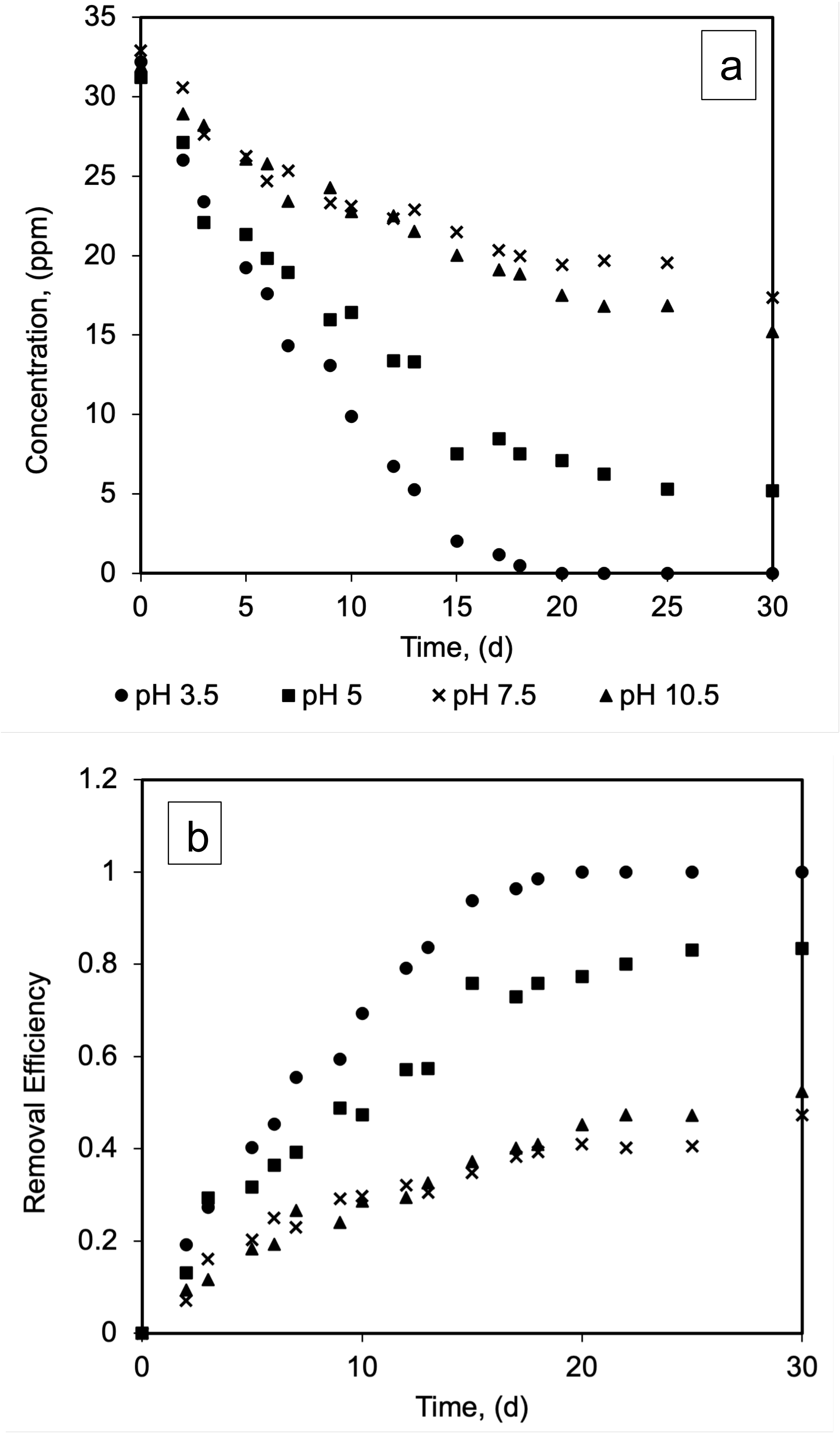

Figure 4.15: Effect of different pH levels on Cr(VI) removal (a), removal efficiency (b) at 30 ppm Cr(VI) concentration

4.3.2 Chromium accumulation at different pH levels

Figure 4.16 shows the concentration of Cr in the roots and shoots of vetiver grass at different pH levels. The amount of Cr accumulated in the roots was higher than the accumulation in the leaves. Under acidic conditions, the roots accumulated high amounts of Cr. The concentration in the roots decreased as the pH level increased from 2.77 mg g^{-1} at pH 3.5 to 0.78 mg g^{-1} at pH 7.5 after 30 d, a slight increase in the amount of Cr was observed at pH 10.5 (Figure 4.16a).

The difference between the amount of Cr accumulated in the shoots from pH 3.5 to pH 7.5 was small, however, a rapid increase in chromium content is seen at pH 10.5 (Figure 4.16b). The amount of Cr in the leaves increased with the pH level. The recorded Cr concentration in the leaves at pH of 3.5, 5, 7.5, and 10.5 after 30 d were 0.22, 0.20, 0.25, and 0.46 mg g^{-1} respectively. Under strong acidic conditions, hexavalent Cr(VI) can be readily reduced to trivalent Cr(III) in the presence of biomaterials due to its high redox potential (+1.33V at pH 1.5 – 3.7) (Park et al., 2006). Therefore, Cr(VI) was reduced to Cr(III) under acidic condition, which was highly retained in the roots. At high pH levels Cr(VI) is weakly reduced because of the low redox potential (Park et al., 2006, Yang et al., 2021), and it is translocated to the aerial parts.

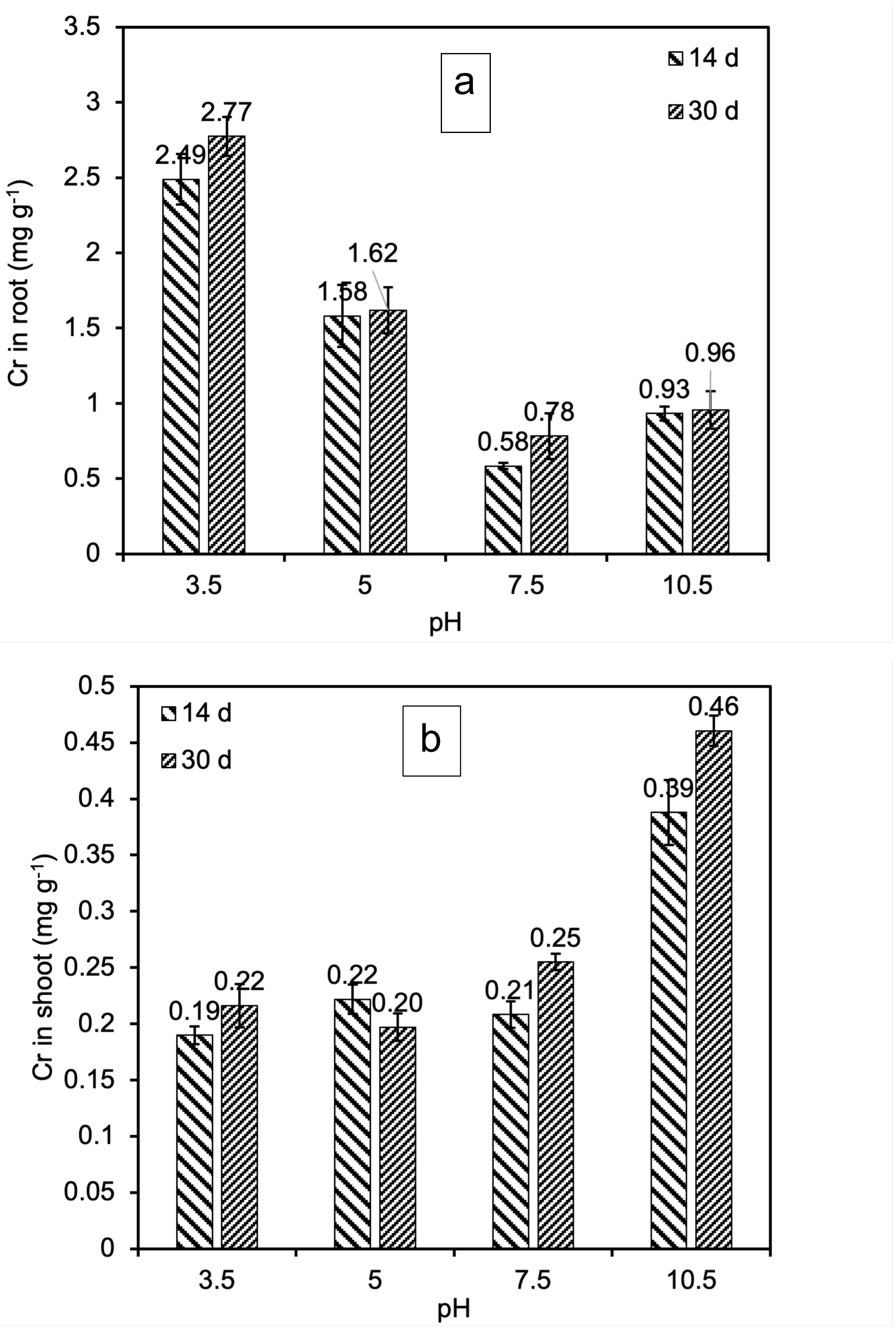

Figure 4.16: The effect of different solution pH on the accumulation of Cr in roots (a), and shoot (b)

Figure 4.17 shows the bioaccumulation and translocation factors of vetiver grass at different pH levels. The BAF values increased with the increase in pH levels. The recorded BAF and TF values are listed in Table 4.1. The translocation of Cr increased with the pH level (Figure 4.17). Different pH levels affected the translocation and bioaccumulation of Cr because of their influence of the oxidation state of the accumulated chromium.

As mentioned in the preceding sections different chromium species are dominant at different pH levels, the mechanisms by which these chromium species are taken up and translocated by the grass are different. The uptake of Cr(VI) is active, while the mechanism of Cr(III) uptake is passive (Shewry and Peterson, 1974, Malaviya et al., 2020). Plants detoxify toxic Cr(VI) by reducing it to a less toxic form Cr(III) which poorly penetrates biological cell membranes (Ertani et al., 2017). Thus, plants concentration toxic metals like Cr in the roots and reduce their translocation to the leaves as a way to protect important aerial functions of the plant. In a study by Skeffington (1976), plants exposed to Cr(VI) translocated much Cr to the shoots than plants exposed to Cr(III).

Based on the results obtained from this study, where BAF values are greater than one and TF values are lower than one, vetiver grass is more suitable for phytostabilisation of chromium. However, because of vetiver grass’s extensive root system and its ability to accumulate exceptionally high amounts of chromium in the roots at low pH levels, phytoextraction of chromium could be attained by constructing floating systems for the treatment of contaminated water, thus, making plant roots readily harvestable.

Table 4.1: Bioaccumulation factor (BAF) and Translocation factor (TF) at different pH levels

pH	**BAF**	**TF**
3.5	92.47	0.08
5	53.92	0.12
7.5	26.08	0.33
10.5	32.82	0.48

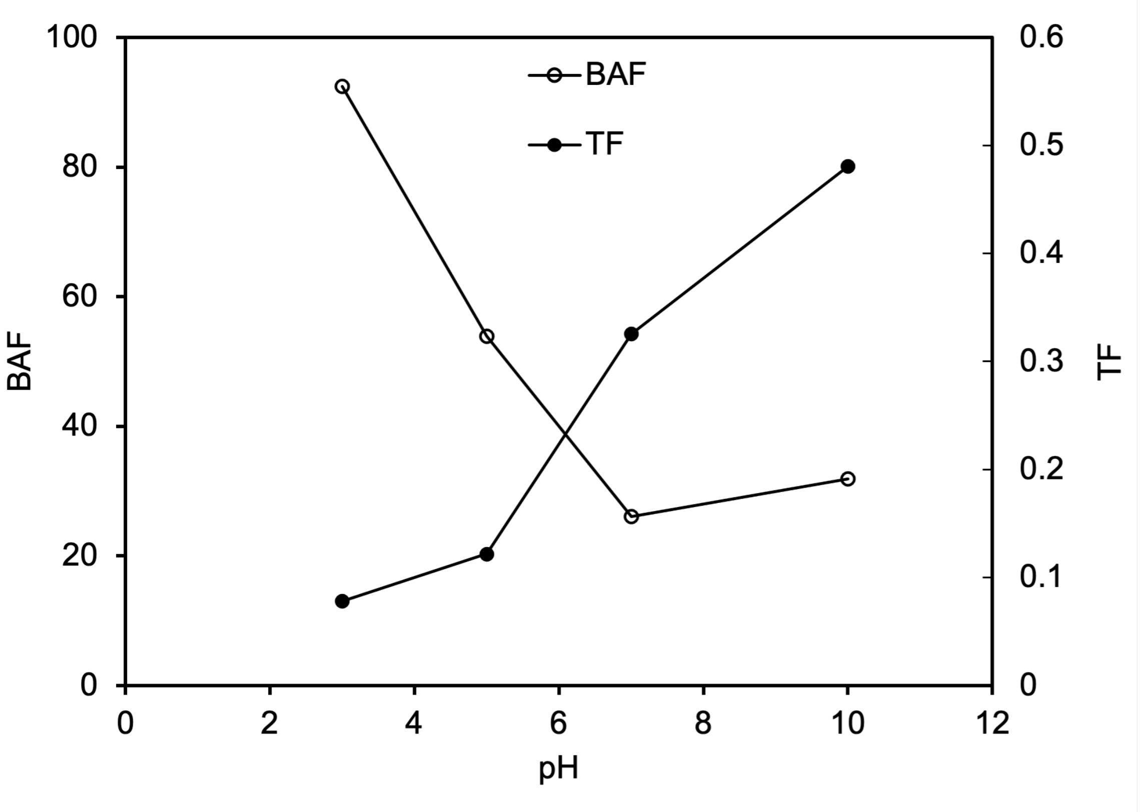

Figure 4.17: Bioaccumulation factor (BAF) and translocation factor (TF) at different pH levels

4.4 The effect of pd(II) initial concentration

The phytoremediation and phyto-mining potential of vetiver grass was studied under different initial concentrations of palladium. This was to evaluate the effect of initial Pd(II) concentration on the growth, remediation, and accumulation potential of vetiver grass. The chosen concentrations were 10, 20, 40, 80, and 120 ppm Pd(II), over a period of 20 d.

4.4.1 Pd(II) removal from solution

Palladium removal from the solution at different initial concentrations is shown in Figure 4.18. Vetiver grass exposed to 10 ppm Pd(II) achieved the highest removal efficiency of 80 % after 20 d, followed by removal at 20 ppm which achieved removal efficiency of 63 %, 42 % at 40 ppm, 28 % at 80 ppm and 20 % at 120 ppm initial Pd(II) concentration (Table 4.2). The removal efficiency of palladium from the solution decreased as the initial concentration increased, however the amount of palladium removed per pot increased with the increased in concentration (Table 4.2). This is illustrated in Figure 4.18. At higher concentrations there is more plant-metal contact which results in higher metal uptake.

Table 4.2: Results obtained from solution samples at day 20.

Target Initial concentration (ppm)	Recorded initial concentration (ppm)	Final concentration (ppm)	Removal efficiency (%)	Removed Pd (ppm)
10	9.78	1.63	83.3	8.33
20	19.2	7.02	63.4	12.2
40	37.5	22.3	42.7	16.0
80	79.4	57.1	28.1	22.3
120	119.5	95.9	20	24.0

Several researchers have indicated that palladium is the most bioavailable of compared to platinum and rhodium (Akinbile et al., 2021). The amount of PGMs removed increased with an increase in pot concentrations. In a study by (Bali et al., 2010) the uptake of Pt(II) by two different plant species increased with an increase in substrate concentration. Lesniewska et al. (2004) studied the uptake of PGMs (Pd, Pt and Rh), in their study the uptake of all the PGMs increased with the increase in initial concentration. They recorded a Pd(II) concentration reduction of 1.38 ppm from 4.41 ppm, and a reduction of 6.5 ppm from 21.6 ppm initial concentration. However, they reported that these changes were not only attributed to the uptake by the plants, but also to the precipitation or adsorption of the studied metals to the walls of the pots (Lesniewska et al., 2004).

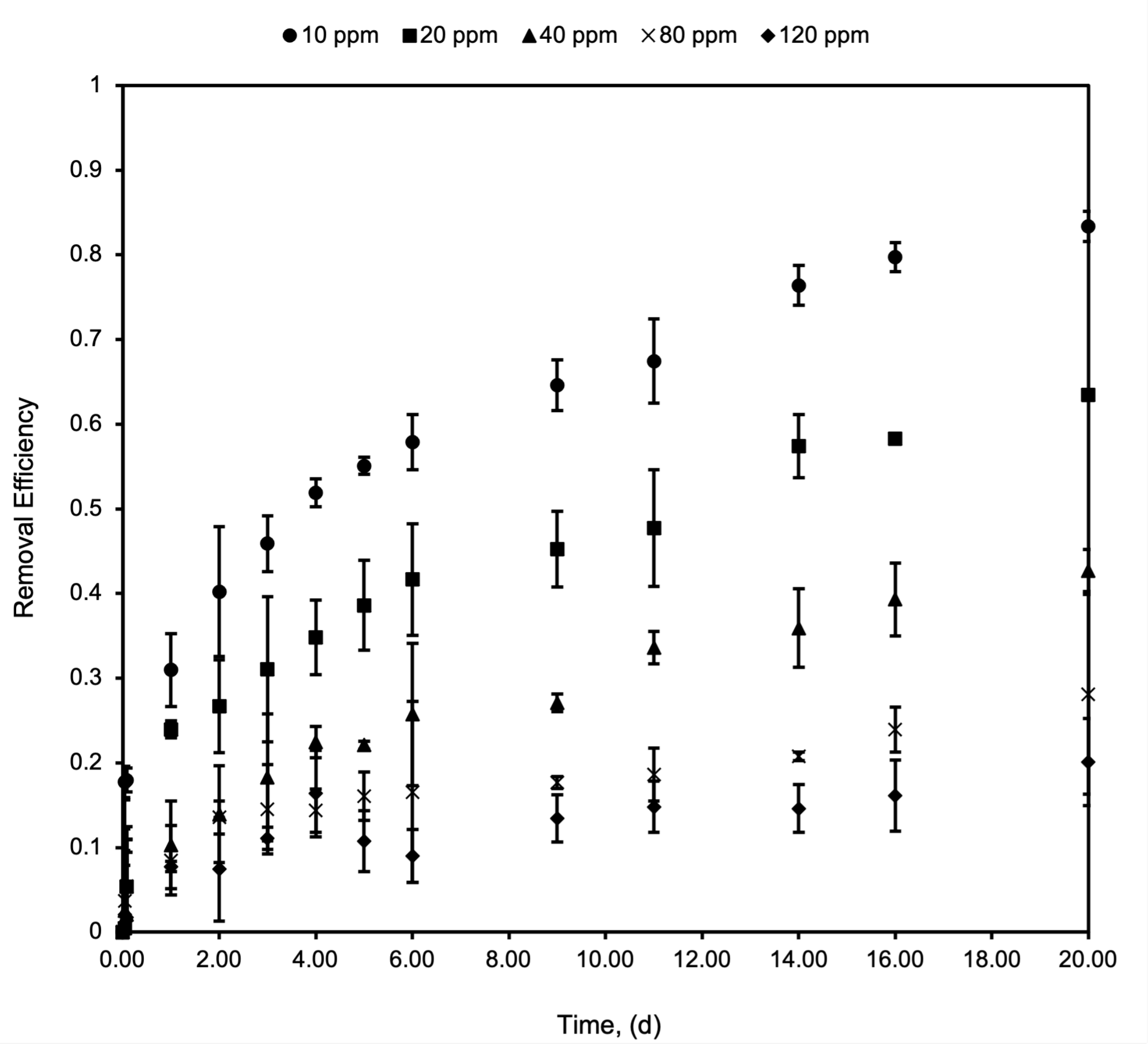

Figure 4.18: Palladium removal efficiency at different initial concentrations 10 ppm (a), 20 ppm (b), 40 ppm (c) 80 ppm (d) and 120 ppm (e)

4.4.2 Pd(II) accumulation at different initial concentrations

Palladium naturally has low solubility, it has been suggested that the concentration of Pd in plants worldwide is less than 10 ng g^{-1} (Kabata-Pendias, 2010). The concentration of Pd(II) in vetiver increased as the initial concentration increased in both the roots and the leaves. Much more Pd(II) was accumulated in the roots of

VG compared to the amount of Pd(II) in the leaves (Figure 4.19). The amount of Pd accumulated in the roots significantly increased with initial concentration, with the Pd in vetiver roots at 120 ppm (0.4 mg g^{-1}), reaching twice the value at 80 ppm (0.21 mg g^{-1}). According to Aquan (2015), the threshold level of Pd hyperaccumulation in plants is expected to be 1000 ng g^{-1}, compared to 10 ng g^{-1} in normal plants, since Pd is poorly soluble in soil and it is not naturally available to plant.

The results obtained confirm the Pd hyperaccumulation capacity of vetiver grass, because the accumulated Pd in both the roots and leaves was significantly higher than 1000 ng g^{-1}. The accumulation of Pd in the shoots over the different initial concentration was very gradual and almost insignificant. The roots immobilized larger amounts of Pd restricting their movement to the aerial parts, this was consistent to what was reported by other researchers regarding other PGMs like Pt (Farago and Parsons, 1986, Aquan, 2015). The concentration of Pd(II) in the shoots was very low compared to the concentration in the roots, this may be as a result of the binding of Pd to pectin and the protein fraction of the root cell walls leading to minimum translocation to the aerial parts (Verkleij et al., 1991). Similar findings were reported by Lesniewska et al. (2004) where the leaves of *Lolium mutiflorum* only accumulated about 0.05% of the Pd accumulated in the roots, which they suggested that the majority of the Pd was only adsorbed on the surface of the roots and only a little amount was available for translocation to the aerial parts of the plant.

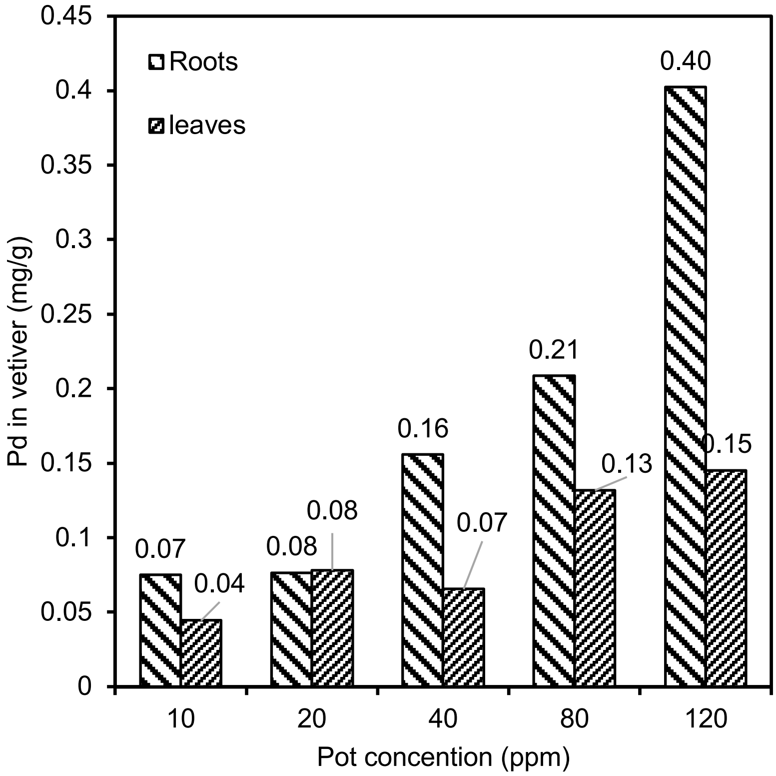

Figure 4.19: Distribution of palladium (Pd) in the roots and shoots of vetiver grass at different initial concentrations.

Figure 4.20 shows the BAF and TF of palladium in vetiver grass at different initial Pd concentrations. Both the TF and BAF decreased as the initial concentration increased. The bioaccumulation factor (BAF) was above one throughout the different initial concentrations suggesting that vetiver grass is able to accumulate Pd to levels above the substrate concentration. However, lower TF values indicates that the larger part of the accumulated Pd is retained in the roots of vetiver grass. Plant roots have been known to counteract Pd exposure stress through mechanisms including restricting Pd uptake by the synthesis and deposition of callose (Singh et al., 2015a, Egorova et al., 2019). It has been reported that longer exposure time, result in more Pd being translocated to the aerial parts of the plants (Kińska et al., 2018). From the recorded results vetiver grass isn't well suitable for the phyto-mining/phytoextraction of Pd, as the process requires the plant to accumulate higher amounts of the metal to aerial parts that can be harvested easily.

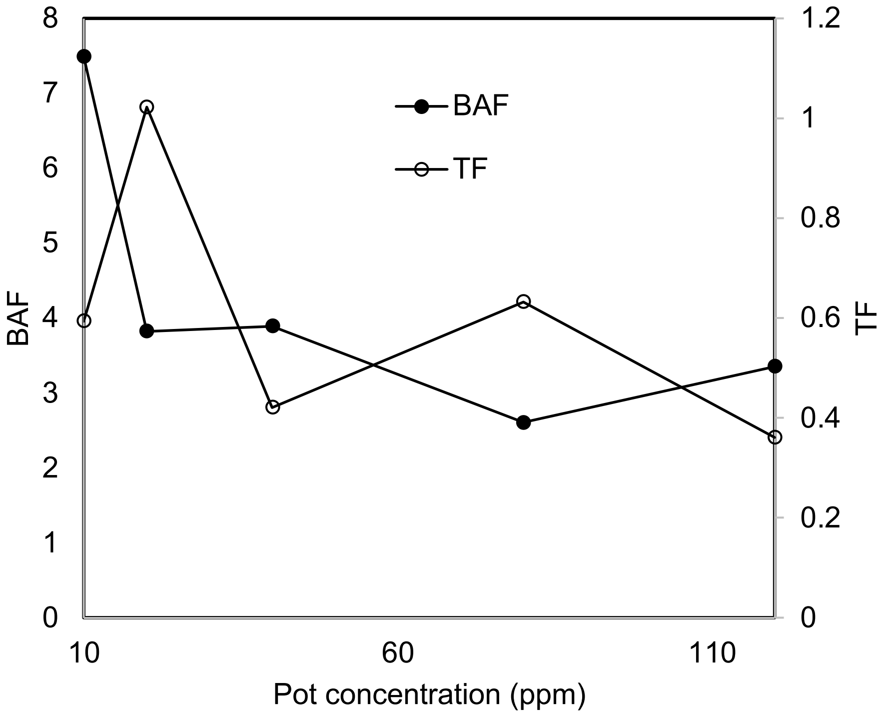

Figure 4.20: Bioaccumulation (BAF) and Translocation factor (TF) of palladium at different initial concentration.

4.4.3 Plant reaction to palladium

The different concentrations of Pd affected the growth of vetiver grass differently (Figure 4.21). Although adverse toxic effects of Pd(II) were experienced by the grass at concentrations above 40 ppm, vetiver grass showed the ability to tolerate Pd in its aerial parts. After 20 d the aerial parts of vetiver grass planted in 40, 80 and 120 ppm had dried out completely. Pd affected the growth of vetiver grass at all the different initial concentrations. At the beginning of the experiment the leaf length of the grass was trimmed to 45 cm, by the end of the experiment the grass submerged to 80 and 120 ppm Pd(II) had a final length of 45 cm, indicating that the was no growth at these high Pd(II) concentrations. At 20 and 40 ppm initial concentration the grass grew by 3 cm to a final length of 48 cm. At 10 ppm the grass had a final length of 52 cm. The control grass which was planted in a pot with 0 ppm initial concentration had a length 78 cm. Thus, the growth of vetiver grass was inhibited at all the different concentration levels, but mostly at higher concentrations.

The content of chlorophyll is a representation of a plant’s photosynthesis capacity. The stress of nonessential can directly affect the chlorophyll content, affecting the photosynthetic activity, and subsequently the growth of the plant (Maksymiec et al., 2007). After a period of one week the leaves at 120 and 80 ppm started to wilt, showing signs of chlorosis, and eventually dried up by the end of the second week. However, the roots remained active up to the end of the experiment as indicated by the continuous reduction of Pd concentration in the pots and much higher accumulation in the roots. The same effects were observed in plants exposed to different concentrations of heavy metals (Olkhovych et al., 2016, Kińska et al., 2018).

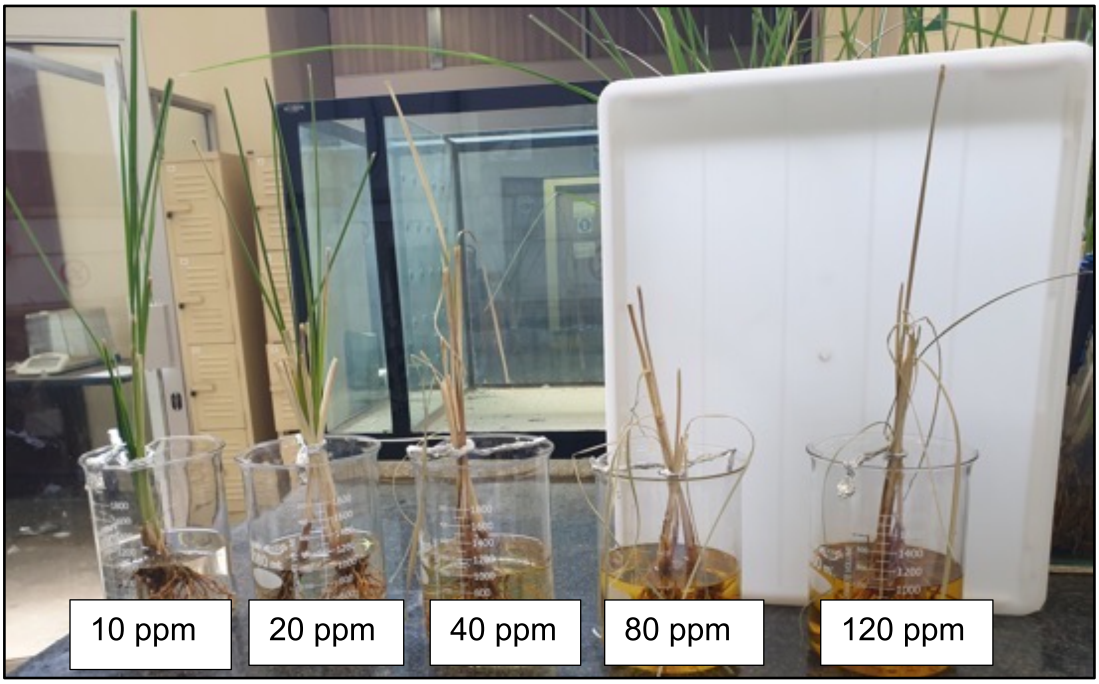

Figure 4.21: Vetiver grass response to different Pd initial concentrations**.**

4.5 Response of vetiver grass to seasonal changes

Phytoremediation requires long periods to successfully remediate the environment, because of this perennial plants are best suitable for phytoremediation (Koptsik, 2014). During cold and dry winter days, seasonal plants tend to shed their leaves and reducing their metabolic activities (Gardea et al., 2008), which also reduces their phytoremediation capacity.

Many researchers have identified vetiver grass as a perennial plant (Truong, 1994, Dalton et al., 1996, Panja et al., 2020). However, during this study vetiver grass thrived throughout the warm seasons (Figure 4.22a) and withered during the cold and dry winter days (Figure 4.22b). The following factors might have led to the withering observed in this study:

- Although nutrients were occasionally added and the water was changed, the buckets that were used to store the grass were a bit small to support such large quantities of grass (the grass shown in the figure was trimmed). Because of this they might have been intraspecific competition for sunlight, and nutrients (Marzinelli, 2012, Awan and Chauhan, 2016).
- The grass was planted inside a building with a glass roof top. The sunlight reached the grass for only few hours a day. It has been reported that vetiver grass's growth is greatly inhibited by the absence of light can completely dry out in shade (Truong, 2002).
- In March 2020, South Africa went through a total lockdown, limiting the maintenance time and reducing the frequency at with the nutrients and fresh water was added to the grass.

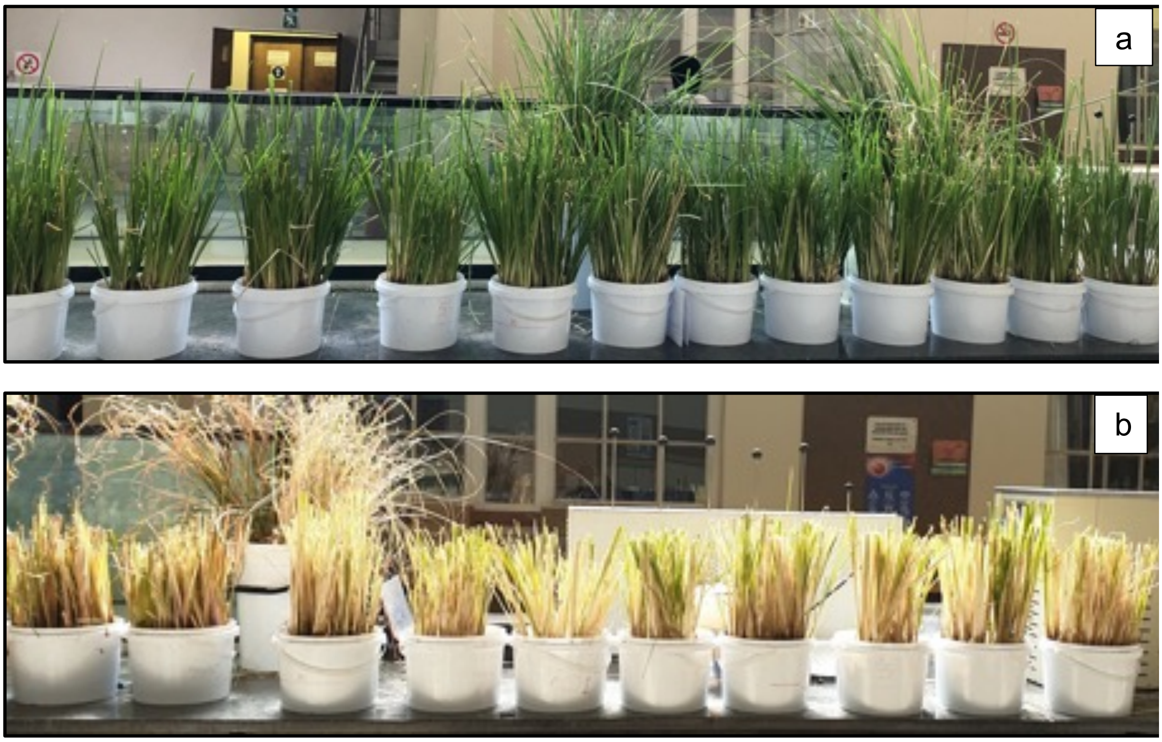

Figure 4.22: vetiver grass’s response to seasonal changes, during summer warm season (a), during winter cold season (b)

The observations made here are inconclusive as a lot of other external factors were involved.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The current work succeeded in confirming the phytoremediation potential of vetiver grass. At different Cr(VI) initial concentrations VG showed the capacity to take up high amounts of heavy metal Cr and translocate them to the aerial parts. The phytoextraction potential of VG was established when the translocation factors were above 1 and the concentration of Cr in VG leaves reached up to 3.04 mg g^{-1} at initial concentration of 70 ppm. Varying the grass density had an effect on the uptake and accumulation of Cr in vetiver grass. The uptake rate increased with an increase in plant density. While the phytoremediation potential was also confirmed at different densities, the phytoextraction capacity of vetiver grass could not be confirmed as not much chromium was translocated to the aerial parts. At different pH levels, acidic conditions resulted in higher chromium uptake, despite the poor translocation of chromium at the different pH levels, at pH 3.5 vetiver grass managed to accumulate up to 2.77 mg g^{-1} in its roots, without completely drying up.

Palladium studies served to shed light on vetiver grass's potential in recovering precious PMGs. The reduction in Pd(II) concentration increased with an increase in initial concentration. However, not much palladium was recovered within the plant. The results obtained confirmed the Pd hyperaccumulation capacity of VG because the accumulated Pd in both the roots and leaves was higher than 1000 ng g^{-1}. However, it is not favourable for phytoextraction because of the poor translocation of Pd to aerial parts.

The toxic effects were mainly visible at concentrations above 30 ppm for Cr(VI) and above 40 ppm for Pd(II). The phytoremediation potential of vetiver grass was confirmed, because the BF values of Cr and Pd were greater than 1 in all the experiments. The rate of metal removal from the solution, and metal accumulation in the plants is depended on the initial concentration, plant density, and solution pH. Most of the accumulated metal is concentrated in the roots with minimum translocation. To effectively recover these metals, wetlands floating systems can be applied so that the roots can be easily accessible, without having to destroy the plant.

5.2 Recommendations

- Phytoremediation processes requires long periods of time, conducting these experiments over a longer period may eventually lead to higher accumulation of metals in the leaves.
- Analysing the speciation of the accumulated metals might show the actual form the metals take within the plant.
- For real-life application, the study should be conducted using industrial effluents as this will help to understand the reaction of vetiver grass in the presence of multiple pollutants.
- Optimisation studies should be conducted to investigate the conditions that would deliver the optimum uptake of metals for real-life application.

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