

Phytoremediation of Cr(VI) in Wastewater Using the Vetiver Grass (*Chrysopogon zizanioides*)

Farai F. Masinire, Dorcas O. Adenuga, Shepherd M. Tichapondwa, Evans M. Nkhalambayausi Chirwa*

Water and Environmental Engineering Division, University of Pretoria, Pretoria 0002, South Africa
evans.chirwa@up.ac.za

ABSTRACT

In this study, the phytoremediation of Cr(VI) in wastewater using vetiver grass (*Chrysopogon zizanioides*) was investigated. The influence of parameters such as initial concentration, grass density, and solution pH was evaluated. The toxicity of Cr(VI) to the grass was observed at concentrations ≥ 30 ppm as demonstrated by the drying up of the aboveground biomass of the plants. High grass density (15 vetiver slips) resulted in faster and more efficient Cr(VI) removal regardless of the initial concentration. However, faster removal (100% in 26 d) was attained for the 5 ppm solution compared to 82% removal in 30 d for the 30 ppm Cr(VI) solution. Cr(VI) remediation was favored under acidic conditions with 100% removal being attained within 20 d for the 30 ppm solution. The distribution of Cr(VI) within the grass structure was also investigated. At low initial concentrations, most of the chromium was located in the subterranean component of the plants. However, as the concentration increased, the roots became saturated, and more Cr was accumulated in the leaf mass fraction (LMF). The results thus far have indicated that vetiver grass is a viable option for phytoextraction. Additionally, results showed that vetiver grass could be used in the phytostabilisation of heavy metal in aqueous solutions. The accumulation of the metal in the leaves indicates the possibility of utilising vetiver grass in the extraction and recovery of the metal 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: phytoremediation, Cr(VI) removal, heavy metal, plant uptake, plant density, phytostabilisation.

- Vetiver grass can be used in the phytoremediation of chromium polluted wastewater
- Cr(VI) uptake is favoured at low pH levels
- Plant density affects the rate of heavy metal uptake
- The root to shoot translocation of Cr is higher under alkaline conditions
- Cr(VI) uptake increased with an increase in initial concentration

1. Introduction

Heavy metal pollution in the environment emanates from both natural and anthropogenic sources. The main natural contributors are weathering of minerals, erosion, and volcanic eruptions (Ali et al., 2019; Gautam et al., 2014; Masindi and Muedi, 2018; Walker et al., 2012), whereas the main anthropogenic contributors include mining, agricultural activities, smelting, electroplating, sludge dumping, industrial effluents, etc (Defarge et al., 2018; Wuana and Okieimen, 2011). Heavy metals have detrimental health effects on humans and mammalian life forms, even at very low concentrations (Tchounwou et al., 2012). Among these deleterious effects are carcinogenicity, teratogenicity, and mutagenicity under low and subchronic exposure conditions (Bridgewater et al., 1994; Davies, 1984; Langård and Vigander, 1983; Singh et al., 1998; Xu et al., 1996). For this reason, great care is needed when disposing of wastes containing heavy metals to prevent contamination of the ecosystem. The heavy metals commonly found in wastewater include lead, chromium, cadmium, and copper (Gautam et al., 2014).

Chromium is widely used in the manufacture of paper, paint, steel and pigments and as well as in the wood, and leather processing industry (Saha et al., 2017). Chromium is commonly present in the environment as either Cr(VI) or Cr(III), these two oxidation states have very distinct physical, chemical, and biochemical properties (Dhal et al., 2013). Cr(III) precipitates as $\text{Cr}(\text{OH})_3$ at $\text{pH} \geq 6$, making it easy to remove from water (Gomes et al., 2017). Cr(VI) is the most toxic and bioavailable form of chromium owing to its high mobility (Gomes et al., 2017). Cr(VI) is mutagenic, carcinogenic and causes acute effects such as stomach bleeding, cramps, kidney damage, and liver damage because of its strong oxidizing capacity and ability to penetrate biological membranes (Kolomazník et al., 2008; Teklay, 2016). According to the WHO (2011), the recommended limits for discharging Cr(VI) into inland surface water is 0.1 ppm and 0.05 ppm for potable water.

Cr(VI) remediation techniques previously applied are for the reduction of Cr(VI) to the less-toxic Cr(III), followed by pH adjustment to form $\text{Cr}(\text{OH})_3$ precipitate (Lin and Huang, 2008; Pettine et al., 2002). Although this is easy to implement and quite efficient, this approach is not cost-effective when remediating trace amounts of Cr(VI), and the continuous use of reducing agents such as FeSO_4 and SO_2 results in high quantities of metallic sludge (Malaviya and Singh, 2011). The application of adsorption

materials like activated charcoals, clays, zeolites, and agricultural wastes, in the removal of Cr(VI) from industrial wastewaters is highly efficient and simple to operate (Mohan and Pittman, 2006). The downside of the adsorption techniques is that there is a need for the regeneration of the adsorption column and Cr(VI) is not reduced or detoxified to Cr(III) (Mohan and Pittman, 2006). Recently, there has been research into the development of efficient, cost-effective and environmentally friendly methods in the remediation of heavy metals (Li et al., 2019). One of these emerging green technologies is phytoremediation (Gratão et al., 2005).

Phytoremediation refers to the use of plants to degrade, extract, contain or immobilize pollutants from soil or water (Saha et al., 2017; Truong et al., 2010). Compared to conventional remediation techniques, phytoremediation is a cost-effective and environmentally friendly alternative, it has attracted widespread use in both the public and private sectors. Phytoremediation can be well applied in very large areas where the application of conventional technologies is not practicable and cost-effective (Garbisu and Alkorta, 2003). In situ bioremediation is considered in situations such as legacy sites (e.g. land affected by fallout and land contaminated from natural sources) where continuing contamination cannot be prevented (Bansal et al., 2019; Wang et al., 2017; Xu et al., 2019). The costs associated with installation and maintenance of phytoremediation are low in comparison to conventional remediation techniques (Mohanty, 2015; Van Aken, 2009), which can be less than 5% of conventional clean-up methods. Hyperaccumulators can be used in the remediation of heavy metal contaminated environments, as they are capable of absorbing, accumulating and detoxifying contaminants from soil and/or water through physical, chemical, or biological processes (Jabeen et al., 2009). For a plant to be classified as a hyperaccumulator, the concentration of heavy metal should reach the hyperaccumulator level, for Cr this level is 300 mg kg^{-1} (van der Ent et al., 2012). The heavy metals may be stored in the roots and/or transported to the stem and leaves (Baker et al., 1994). The absorption of contaminants may continue until the plant is harvested. The efficiency of phytoremediation depends on various factors such as the physicochemical properties of the environment, type of plant, heavy metal bioavailability, and the rate of metal uptake, translocation, accumulation, and detoxification by the plants (Hooda, 2007).

The mechanisms by which Cr is absorbed and distributed in the plant are not well understood (Hayat et al., 2012). Non-essential elements such as Cr do not have

a specific uptake mechanism, the carriers used for the uptake of essential ions such as sulfate are also used for Cr(VI) (Cervantes et al., 2001). At lower Cr concentration the uptake mechanism is active, while the passive mechanism prevails at higher toxic levels (when membrane selectivity has been lost) (Hayat et al., 2012). The uptake of metal ions can be through active absorption into root cells via plasmalemma, and adsorption onto cell walls via passive diffusion or through 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). Plants usually restrict the translocation of toxic Cr ions to the shoots, as a way of protecting the aerial parts responsible for important metabolic activities such as photosynthesis from the toxic effects of Cr (Malaviya and Singh, 2011). Higher concentrations of Cr are found in the roots of most plants than in the aerial parts because of the root's ability to reduce Cr(VI) to Cr(III) which accumulates in the roots and is poorly translocated to the above-ground plant parts (Paiva et al., 2009).

The availability of Cr is affected by various factors such as solution pH, redox potential, organic content, and microbial community structure. In an environment with low oxidation-reduction potential, the most stable form of Cr is Cr(III). Cr(VI) is readily reduced to Cr(III) under acidic conditions and a high proportion of organic matter (Sinha et al., 2018). However, it has been hypothesised that the reduction of Cr(VI) to Cr(III) is incomplete as indicated by the presence of many Cr(VI) ions which are further translocated to the aerial parts (Malaviya and Singh, 2011).

The use of vetiver grass (*C. zizanioides*) as a hyperaccumulator has been under continuous investigation due to its high tolerance to harsh environmental conditions such as elevated heavy metal concentrations and varying pH conditions (Truong and Baker, 1998; Truong et al., 2010). It has been proven that vetiver is effective in the uptake of contaminants and nutrients, particularly phosphate (P) and nitrogen (N) (Truong and Baker, 1998). It also has high threshold levels of heavy metals in comparison to most vascular plants (Oshunsanya and Aliku, 2017). The use of vetiver grass in decontaminating agrochemicals has also been reported (Ramlee et al., 1996), this is due to its ability to retain these chemicals and preventing their accumulation in soil and crops (Truong, 2000). Vetiver grass has a dense root system capable of growing to as much as 7 m (Oshunsanya and Aliku, 2017). This enables heavy metal ions to be absorbed through the channels, transporters, and pores in the plasma membrane of the root.

Thus, vetiver grass (*C.zizanioides*) could be utilised with the multipurpose of decontaminating industrial wastewaters and phytoextraction of heavy metals, while delivering environment-improving greenspaces and addressing one of today's pressing concerns as a carbon sink. The present work aimed to investigate the effect of different initial Cr(VI) concentrations, solution pH levels and different plant densities on the removal, and accumulation of toxic hexavalent chromium under hydroponic conditions using vetiver grass.

2. Materials and methods

2.1. Material and reagents

Vetiver grass slips were obtained from Hydromulch (Pty) Ltd (Johannesburg, South Africa). Potassium chromate (K_2CrO_4) was used to prepare the synthetic Cr(VI) solutions. To prepare 1,5-diphenylcarbazide (DPC) solution, specific amounts of DPC were dissolved in HPLC grade acetone and stored in an aluminum foil covered brown bottle. The pH adjustments were done using hydrochloric acid and sodium hydroxide. Tap water was used for topping up the pots, and distilled water was used in the analysis of the water samples.

2.2. Vetiver grass batch studies

The grass originally planted in soil was transferred to a water medium. Special attention was given to ensure that the roots were not damaged. The grass was washed and left in water for 2 weeks for acclimatization. Essential macronutrients N, P, K were added to the water to ensure the growth of the roots and shoots to the desired length. Following acclimatization, the shoot length was adjusted to a height of 45 cm. The grass was transferred to 2 L Cr(VI) solution pots for the commencement of the phytoremediation studies. The decrease in water level in each pot over time was accounted to evaporation and uptake by the grass. With the assumption that the evaporating water contained no Cr(VI), water was topped to each pot before sampling. This was to ensure that the change in concentration was not due to evaporation. Water samples were collected every 2 d for analysis.

Grass samples were collected at 2-week (14 d) intervals. Roots and shoots were separated, washed with tap water, and rinsed with distilled water. The washed samples were dried at 70 °C until they were completely moisture free. The dried

samples were then ground using mortar and pestle. 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 resulting leachate 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 Cr using SPECTRO Analytical Instruments Genesis (ICP-OES) Spectrometer (Perkin-Elmer, Johannesburg).

The first part of the investigation sort to establish the effect of initial Cr(VI) concentrations on Cr(VI) uptake, and accumulation of chromium in the plant and its effect on the growth of vetiver grass. 2 L Cr(VI) solutions were set up at different initial concentrations of 5, 10, 30, and 70 ppm. Two vetiver slips were placed in each pot. The experiment was conducted over a duration of 7 weeks. All 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.

The second phase of the study investigated the effect of plant density on the Cr(VI) uptake rate. Experiments were conducted using the concentrations of 5, 10, and 30 ppm Cr(VI) in 2 L pots. The slip densities were grouped into three classes, namely low density (5 slips), medium density (10 slips), and high density (15 slips). Analysis of the Cr(VI) uptake was analyzed by following the changes in concentration in the pots as well as the accumulation in different parts of the plant.

The last phase of the investigation assessed the effect of solution pH on the uptake and accumulation of Cr in different parts of the grass. Four 2 L pots contained 30 ppm Cr(VI) adjusted to pH values of 3.5; 5; 7.5 and 10.5 were prepared. 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 vetiver grass.

2.3. Cr(VI) and Total Cr Measurement

Cr(VI) was measured using UV-Vis spectrophotometer (Biochrom WPA, Light Wave II, and Labotech, South Africa) at a wavelength of 540 nm after reaction of an acidified sample by 1 N H₂SO₄ with 0.5% solution of 1,5-diphenyl-carbazide (DPC) to produce a violet colour in response to the presence of Cr(VI) (APHA, 2005). Plant

samples were analysed for trace amounts of total Cr using SPECTRO Analytical Instruments Genesis (ICP-OES) Spectrometer (Perkin-Elmer, Johannesburg).

2.4. Removal efficiency

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

$$\text{Removal efficiency} = \frac{C_i - C_t}{C_i} \quad (1)$$

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

2.5. Bioaccumulation factor (BAF)

The BAF indicates the plant's ability to accumulate the metal relative to the metal concentration in the external solution. The BAF is useful in evaluating a plant's potential in the accumulation of heavy metals, it is calculated on a dry mass basis using equation 2 (Lu et al., 2004; Yoon et al., 2006; Zayed et al., 1998)

$$\text{BAF} = \frac{C_{\text{plant tissue}}}{C_{i,\text{wastewater}}} \quad (2)$$

where $C_{\text{plant tissue}}$ is the concentration of the metal in the harvested plant tissue and $C_{i,\text{wastewater}}$ is the initial concentration of Cr(VI) in the simulated wastewater.

2.6. Translocation factor (TF)

The TF is an indicator of the plant's ability to translocate the heavy metal from the roots to the aerial part, it is calculated using equation 3:

$$\text{TF} = \frac{C_{\text{shoot}}}{C_{\text{root}}} \quad (3)$$

where C_{shoot} is the concentration of the metal in the shoots, and C_{root} is the concentration of the metal in the roots of the plant (Ali et al., 2013).

3. Results and discussion

3.1. Effect of initial concentration

The phytoremediation performance of vetiver grass at different initial Cr(VI) concentrations was investigated. This was to evaluate the effect of initial concentration on the uptake of Cr(VI) and growth of vetiver grass. Fig. 1(a)–(d) shows Cr(VI) removal profiles at different initial concentrations over the duration of the experiment. Fig. 1a shows that the initial concentration dropped from 5 ppm to 0.65 ppm after 52 d, this represents an 87% removal of Cr(VI) (Fig. 1e). As the initial concentration increased, a decrease in removal efficiency was observed, at 10 ppm (Fig. 1b) a 52% Cr(VI) removal efficiency was observed. Concentrations 30 ppm and 70 ppm showed removal efficiencies of 28% (Fig. 1c) and 11.5% (Fig. 1d) respectively. This was in line with what was observed in previous studies, where the rate of removal increased with an increase in initial Cr(VI) concentration (Roongtanakiat and Chairaj, 2001; Suelee et al., 2017). Vetiver grass exposed to higher 30 and 70 ppm Cr(VI) concentrations showed elevated uptake of chromium. The increased uptake can be attributed to increased mobility of Cr ions from the highly concentrated Cr(VI) solution to the root cell walls by diffusion-driven nonmetabolic and passive process (Hayat et al., 2012). At lower concentrations (5 and 10 ppm), the removal of Cr(VI) is mainly through the active mechanism, which can be limited by the available carriers and also hindered by the presence of essential ions such as sulfate in the solution, with which Cr(VI) competes for binding sites on the plant (Cervantes et al., 2001).

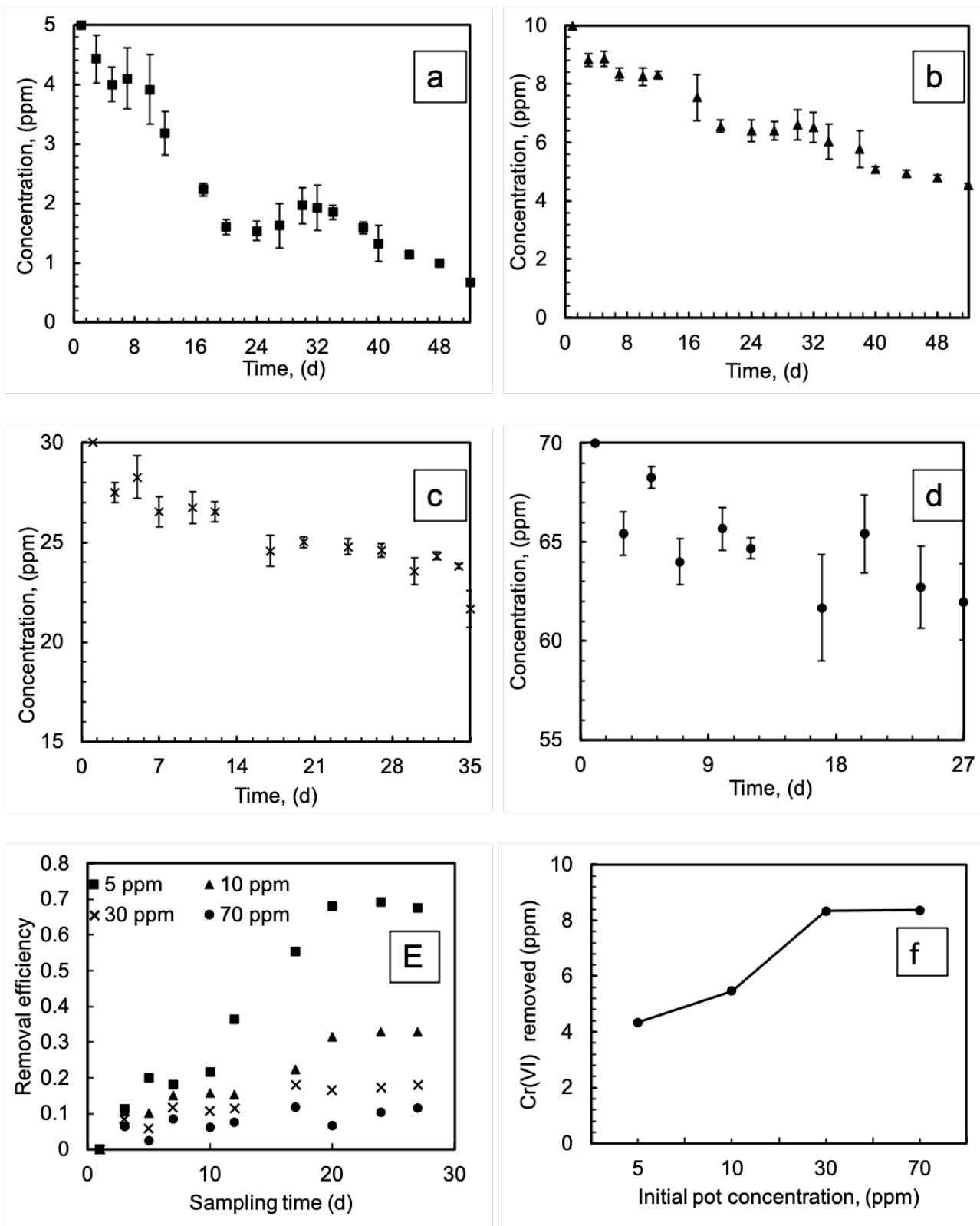


Fig. 1: Reduction of Cr(VI) from the solution at 5 ppm (a), 10 ppm (b), 30 ppm (c), and 70 ppm (d). Cr(VI) removal efficiency at different concentrations (e) and the correlation between the initial pot concentration and the amount of Cr(VI) removed (f).

Fig. 1f shows a correlation between the Cr(VI) removed and the initial concentration in the pot. The amount of Cr(VI) removed increased from 4 ppm to 8 ppm at initial pot concentrations of 5ppm to 30ppm and remained constant at 70 ppm initial concentration. This shows that at higher Cr(VI) concentrations, the toxic effects of chromium on vetiver grass lead to the early drying out of the plant and eventually stopping the uptake of Cr.

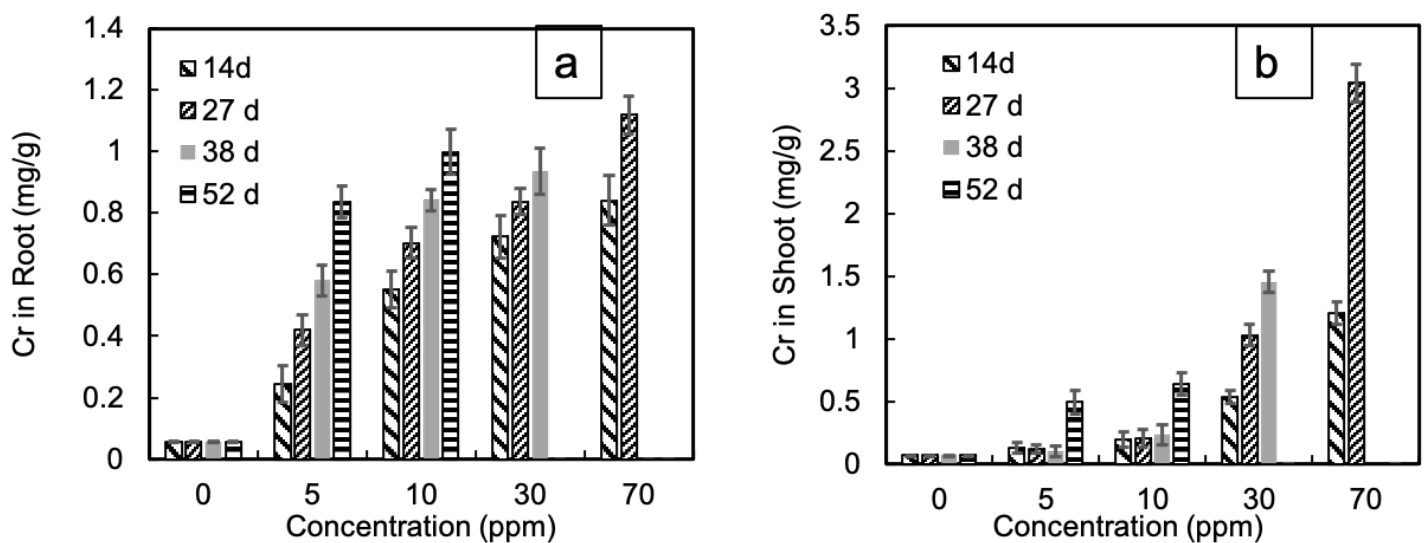


Fig. 2: Distribution of Cr in (a) the roots and (b) the shoots at different initial concentrations

Fig. 2 shows the concentration of Cr in different parts of vetiver grass as a function of time. The concentrations in both shoots and roots increased over the duration of the experiment in all Cr(VI) concentrations tested. Furthermore, the accumulation within the different parts of vetiver grass also increased along with the initial Cr(VI) concentration. At low concentrations (5 ppm and 10 ppm), more Cr concentration was found in the roots than in the shoots. The maximum recorded concentration in the roots and shoots at 5 ppm was 0.84 mg.g⁻¹ and 0.49 mg.g⁻¹ respectively and at 10 ppm the recorded concentrations were 0.64 mg.g⁻¹ in the shoot and 0.998 mg.g⁻¹ in the roots. The observed lesser concentration of Cr in the shoots is because Cr ions are immobilized in the vacuoles of the root cells (Shanker et al.,

2004). Zhang et al., (2009) reported that plants reduce the translocation of Cr to aerial parts as a mechanism by which they protect shoots from Cr phytotoxicity.

At a higher initial concentration of 30 ppm and 70 ppm, more Cr was translocated to the shoots as the experiments proceeded. At 30 ppm the concentrations recorded after 14 d showed slightly more Cr of 0.55 mg.g^{-1} in the roots than 0.54 mg.g^{-1} in the leaves. After 27 d more Cr was detected in the leaves than in the roots, the same was also confirmed at the end of 38 d at which 0.93 mg.g^{-1} was obtained in the roots and 1.45 mg.g^{-1} was obtained in the shoots. At 70 ppm more Cr was detected in the leaves than in the roots at 14 d and 27 d. At an initial concentration of 70 ppm, the shoots accumulated about 3 times the amount of Cr in the roots (Fig. 2). After 27 d the shoots had accumulated a total of 3.04 mg.g^{-1} and the roots a total of 1.12 mg.g^{-1} . As the concentration of Cr in the roots approached 1 mg.g^{-1} more Cr was translocated to the shoots. To illustrate this observation clearly, the accumulation in the roots and leaves at different initial concentrations was plotted in Fig. 3.

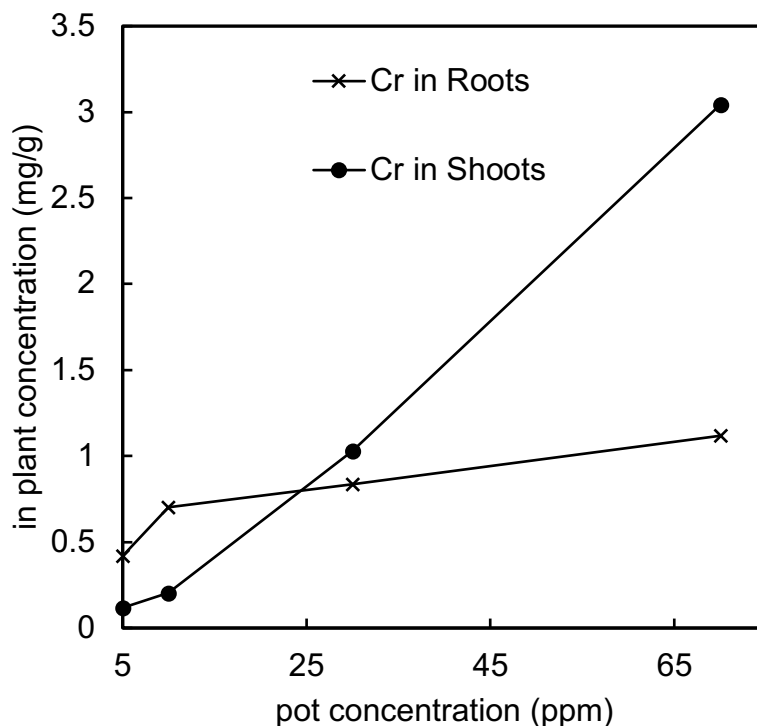


Fig. 3: Correlation between the amount of Cr in the roots, shoots of vetiver grass, and initial pot concentration, after 27 d.

Fig. 3 shows the relationship between the accumulated Cr in the roots and the shoots at different initial solution concentrations after 27 d. A saturation phenomenon

was noticed in the roots at approximately 1 mg.g^{-1} (Fig. 3). After the Cr in the roots reached a concentration of 1 mg.g^{-1} the accumulation of Cr within the shoots increased beyond that of the roots.

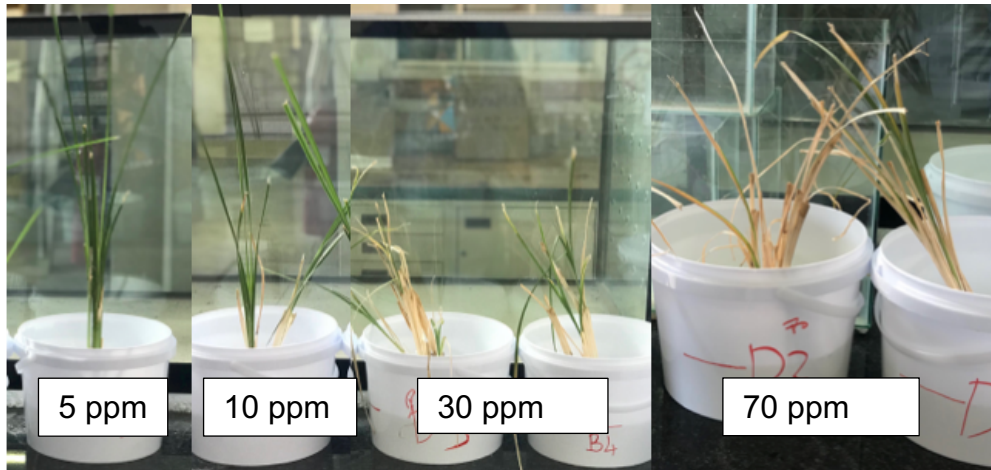


Fig. 4: Vetiver grass in 5, 10, 30, and 70 ppm after 27 d

Fig. 4 shows that at Cr(VI) concentrations of 5 and 10 ppm, vetiver grass thrived up to the end of the experiment. On the other hand, at 30 ppm and 70 ppm, the grass showed restricted growth and it dried up after 38 and 27 d respectively. The grass at higher Cr(VI) dried because of increased toxicity, according to Hegemeyer (1999), plants under heavy metal stress usually have growth related changes as the early sign of heavy metal poisonousness. When plants are exposed to Cr it alters the uptake activity in the plasma membrane in root cells limiting the uptake of essential elements, such as N, P, K, Fe, Mg, Zn, Ca, etc. (Shanker et al., 2004). This results in the disruption of some essential metabolic processes and eventually drying out of the plant as observed at 30 ppm and 70 ppm.

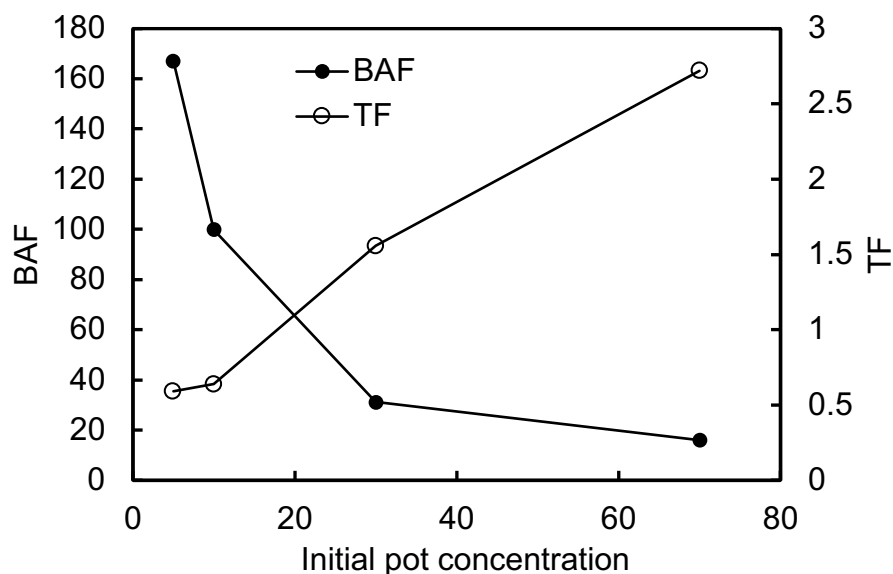


Fig. 5: Bioaccumulation factor (BAF) and translocation factor (TF) at different initial concentrations 5, 10, 30, and 70 ppm Cr(VI).

Bioaccumulation factor is the quotient of heavy metal content in the dried root biomass (mg.kg^{-1}) to heavy initial metal content in wastewater (ppm). Translocation factor (TF) is the ratio of heavy metal content in dried shoot biomass (mg.kg^{-1}) to the heavy metal content in dried root biomass (mg.kg^{-1}). For application in phytoextraction a plant should have both BAF and TF greater than 1 (Yoon et al., 2006), and plants with $\text{BAF} > 1$ and $\text{TF} < 1$ are more suitable for phytostabilisation (Lorestani et al., 2013). Similar to the results of Cr(VI) removal, the highest BAF values 100 and 167 were obtained when the initial Cr(VI) concentration was 10 ppm and 5 ppm respectively. This indicates the ability of vetiver grass to accumulate over a hundred times the Cr concentration in the solution at such low concentrations. Thus, proving the phytoremediation potential of vetiver grass (Yoon et al., 2006). At Cr(VI) concentration 30 ppm and 70 ppm the BAF decreased to 31 and 16 respectively Fig. 5. At different initial concentrations, the BAF was found to be above one. The opposite observation was found for the translocation factor which increased with increased initial Cr(VI) concentration. Initial Cr(VI) concentrations of 5 and 10 ppm had $\text{TF} < 1$, and $\text{TF} > 1$ at 30 and 70 ppm. This indicates that the translocation of Cr from roots to shoots is effective at concentrations above 30 ppm. Phytoextraction usually requires the metal to be translocated to the aerial parts of the plants which can be easily harvested (Yoon et al., 2006). The translocation rate of metal to the shoots may depend on the concentration of the metal in the roots.

It is postulated that when the amount of Cr accumulated in the roots is high, it interferes with the metabolic activities which are responsible for immobilizing, detoxifying, and limiting the translocation of toxic metals to the aerial parts. When these defense mechanisms are disrupted, simple acropetal movement of Cr to the shoots becomes responsible for the increased TF at higher concentrations. Both the bioaccumulation and translocation factor values obtained in the present study were greater than one at concentrations above 30 ppm. The results obtained from these experiments provide evidence for the phytoextraction potential of vetiver grass at concentrations above 30 ppm. However, at lower concentrations vetiver grass proves to be more effective for phytostabilisation.

3.2. Effect of plant density

Chromium removal at different plant densities was studied at 5, 10, and 30 ppm Cr(VI) initial concentrations. The general trend showed that the removal of Cr(VI) increased over the duration of the study for all different plant densities (Fig. 6a). At an initial Cr(VI) concentration of 30 ppm, the highest removal efficiency was 82% using high density vetiver grass. This was observed after 30 d. The medium and low density grass resulted in the removal of 47% and 29%, respectively (Fig. 6b). The findings were in line with those in previous studies, where metal removal efficiency increased with an increase in plant density (Suelee et al., 2017). An increase in plant density results in an increase in contact surface area for metal absorption by the roots (Darajeh et al., 2014). An increase in plant density results in more plants for heavy metal absorption from the solution because more plants increase the rate of water uptake. This was confirmed by the fact that the reduction of water in the pots over time was proportional to an increase in plants density. In a study by Chomchalow (2003), it was concluded that higher plant density would absorb much effluent and heavy metal concentration in that area.

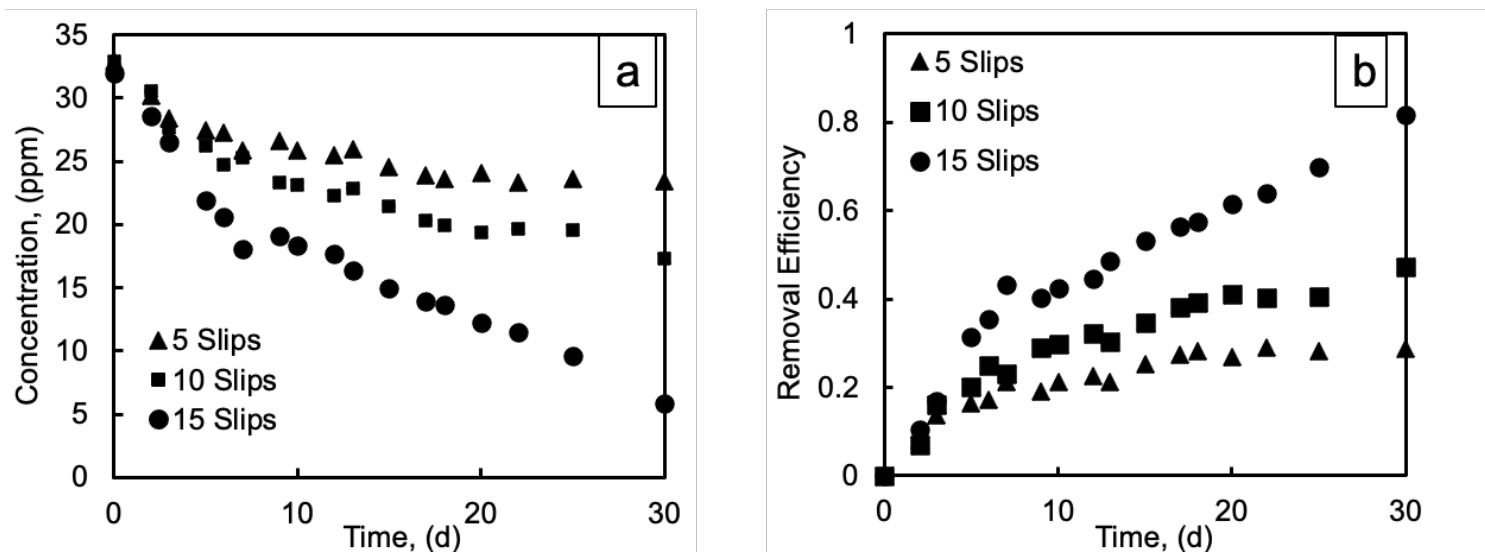


Fig. 6: Effect of plant density on the uptake of Cr(VI) using low density (5 Slips), medium density (10 Slips), and high density (15 Slips) (Chromium concentration 30 ppm)

The high density grass provided the highest removal efficiency even at different initial Cr(VI) concentrations (Fig. 7). Comparing the removal efficiency of medium and high density at different Cr(VI) initial concentrations, it was found that at lower concentrations, the difference between high density and medium density's removal efficiency was small. At 5 ppm both high and medium density grass achieved 100% removal efficiency after 30 d. Based on the current study, it would be more effective to apply medium density treatment when treating Cr(VI) concentrations lower or equal to 5 ppm, as this would reduce intraspecific competition for nutrients, and improve the efficiency of vetiver grass. With an increase in Cr(VI) initial concentration, the difference between the removal efficiency of Cr(VI) at high and medium grass density also increased. At 30 ppm, the removal efficiency of high-density grass was found to be 82 % and 47 % for the medium density grass (Fig. 7). At higher concentrations, the use of high density grass is more effective in removing Cr(VI) from the solution.

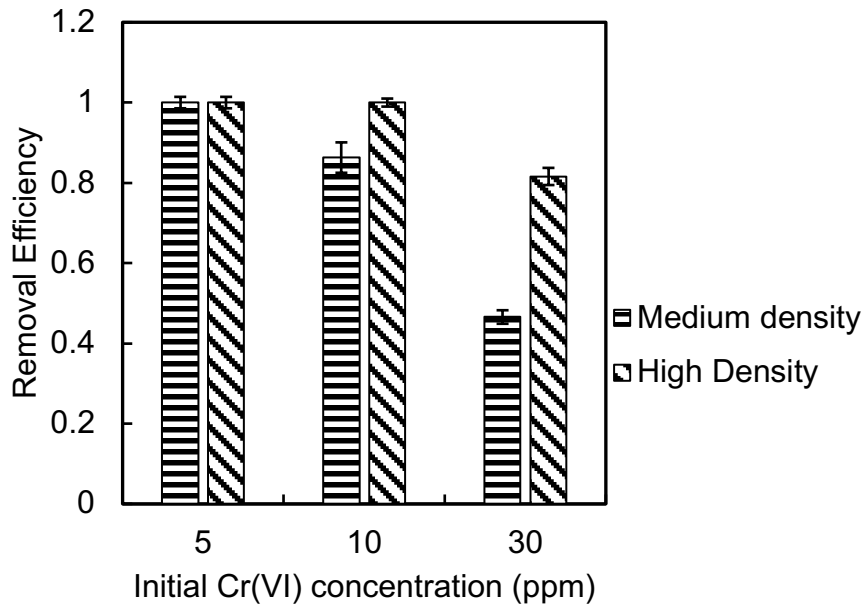


Fig. 7: Comparison between medium density (10 slips) and high density (15 slips) at 5 ppm, 10 ppm, and 30 ppm Cr(VI) concentrations after 30 d.

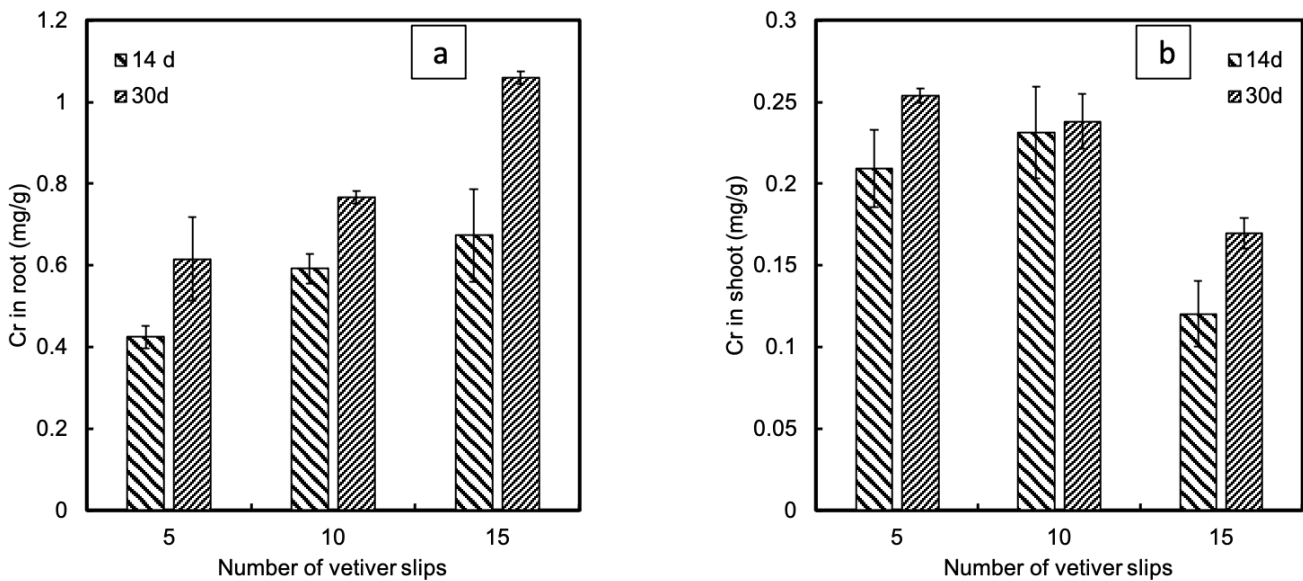


Fig. 8: Distribution of Cr in the roots (a) and shoots (b) of vetiver grass at different plant densities and initial Cr(VI) concentration of 30 ppm.'

The distribution of Cr within the leaves and roots of vetiver grass at different grass densities and initial Cr(VI) concentration of 30 ppm is shown in Fig. 8. It can be seen from Fig. 8a that the amount of Cr accumulated in the roots increased with an increase in plant density. The amount of Cr accumulated within the roots at 5, 10, and

15 vetiver slips was 0.616, 0.76, and 1.06 mg.g⁻¹ respectively. This observation is contrary to what is expected. As the density increases so does the biomass and this should lead to a reduction in the amount of Cr distributed per gram of the grass.

The translocation and accumulation of Cr depend on the type of the plant, the oxidation state of the Cr ions, and its concentration in the solution (Ertani et al., 2017). As mentioned earlier, Cr(VI) can be reduced to a less toxic Cr(III) in the presence of high organic matter and an environment with low redox potential (Sinha et al., 2002). 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) within the roots of the grass at high density. Within the plant Cr(III) tends to bind to cell walls, which further hinders the translocation of Cr within the plant tissue (Sharma et al., 2020). As a result, the Cr at high density was mainly restricted to the roots, whereas at low density much Cr was not reduced to Cr(III) and it was actively translocated to the aerial parts. After 30 d the concentration found in the leaves at 5, 10, and 15 vetiver slips were 0.254, 0.238, and 0.170 mg.g⁻¹. It can be seen that much more Cr was accumulated in the roots than in the shoots (Fig. 8). The concentration difference is a result of poor translocation of Cr from the roots to the shoots. Many researchers have reported poor translocation of Cr in different plant species (Malaviya and Singh, 2011).



Fig. 9: low (5 slips), medium (10 slips), and high (15 slips) dense vetiver grass in 30 ppm solution after 30 d

Fig. 9 shows vetiver grass at different densities in 30 ppm solution after 30 d. There was no visible change in the height of vetiver grass at all the different densities, however, more slips dried at higher vetiver density. The high mortality at high density is due to increased intraspecific competition for nutrients, water, and sunlight, (Awan and Chauhan 2016; Marzinelli et al., 2012). Slight signs of stress were visible at low density, this could be because of the toxicity of Cr.

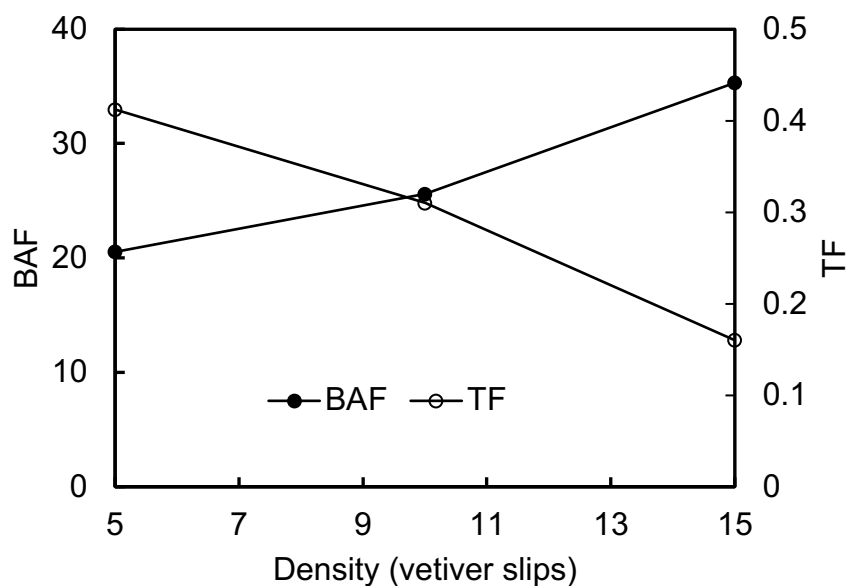


Fig. 10: Bioaccumulation factor (BAF) and translocation factor (TF) at different vetiver grass densities, low (5 slips), medium (10 slips), and high (15 slips)

Fig. 10 shows the bioaccumulation and translocation factors at different vetiver grass densities. The recorded BAF values increased with an increase in plant density in the range of 20.5 to 35.32. This shows that vetiver grass is a good candidate for phytostabilisation as all BAF values were above 1 (Yoon et al., 2006). The TF values decreased with an increase in plant density. This is because increasing the plant density results in high biomass and thus low metal concentration. The TF values obtained were, 0.41, 0.31, and 0.16 at 5, 10 and 15 vetiver slips respectively. All the values of TF obtained were less than one. The difference in the BAF and TF found in this experiment suggests that Cr is largely retained in the roots and translocation is minimum.

3.4. Effect of pollutant pH

The effect of pH on the uptake of Cr(VI) by vetiver grass at an initial Cr(VI) concentration of 30 ppm was investigated at varying pH conditions. Higher Cr(VI) removal was recorded at acidic conditions, with pH 3.5 resulting in the removal of 30 ppm Cr(VI) after 20 d (Fig. 12a). At pH of 5, the maximum removal of Cr(VI) was found to be 83% after 30 d. After 30 d the removal efficiency at pH 7 and 10 were 47.3% and 52.4%, respectively (Fig. 12b). These findings contradict those obtained by (Singh et al., 2015) in which the most removal of Cr(VI) took place at neutral conditions. In a study by Singh (2015), the removal of Cr(VI) by vetiver grass at initial Cr(VI) and Pb concentration of 20 ppm for 15 days 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 further concluded that the removal of heavy metals is much efficient for pH values of 6-9. The change in pH affected the rate of uptake of Cr(VI) due to the influence of the dominant type of Cr(VI) species at a given pH value. At acidic conditions ($\text{pH} < 5$), HCrO_4^- becomes the dominant species, whereas at $\text{pH} > 6$ the dominant species is CrO_4^{2-} . However, in a study by Kumar (2013), the removal of Cr(VI) by vetiver grass was more effective at pH levels below 3.5, the removal efficiencies at pH 3.5 and 2.5 were 55% and 97% respectively. At high pH (4-8), Kumar (2013) reported that there was no observed effect of pH on the uptake of Cr(VI), this is in line with what was observed in the current study where there was no significant difference in the removal efficiency at pH 7.5 and 10.

The solution pH can either result in 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). The observed fast Cr(VI) removal rates under acidic conditions are a result of increased protonation of cell wall binding sites, thus creating an increase in anion binding sites. Which often favours the biosorption of metal anions (Fomina and Gadd, 2014). Conversely, an increase in solution pH results in the deprotonation of metal binding sites, thus reducing the availability of anionic binding sites (Elangovan et al., 2008), leading to the reduce Cr(VI) uptake at higher pH levels.

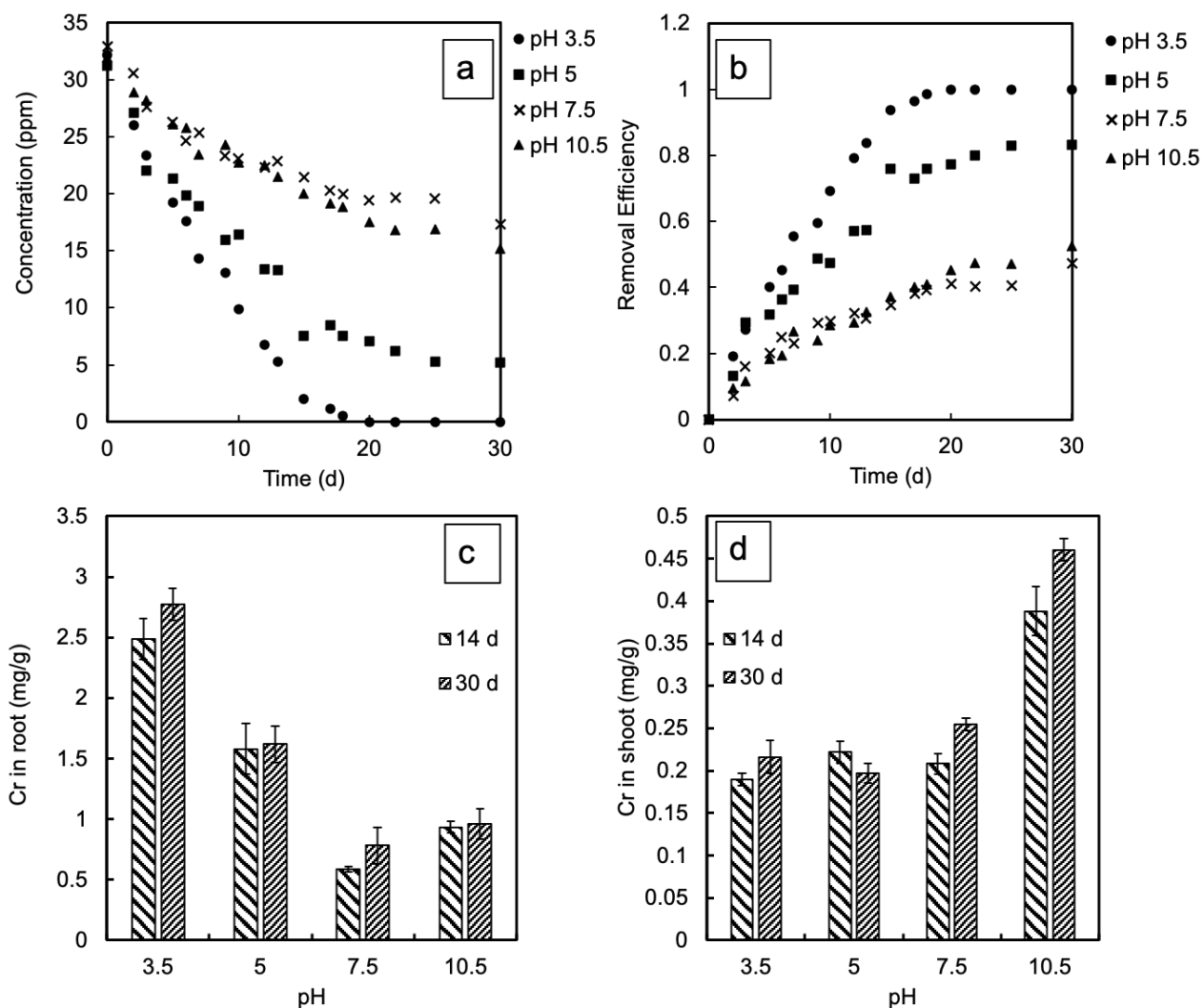


Fig. 12: Influence of varying solution pH on Cr(VI) removal (a), removal efficiency (b), root Cr accumulation (c), and shoot Cr accumulation

Fig. 12 c and d shows the distribution of Cr within the roots and shoots of vetiver grass. More Cr accumulated in the roots than in the shoots at the different pH levels. At acidic conditions, the roots showed the ability to accumulate high levels of Cr. As the pH increased, the root Cr concentration decreased from 2.77 mg.g^{-1} at pH 3.5 to 0.78 mg.g^{-1} at pH 7 after 30 d, a slight increase in the accumulated Cr was observed at pH 10.5 (Fig. 12c). Within the shoots, there was no substantial difference in accumulated Cr at pH 3.5 and 5, however, a sudden increase in Cr concentration can be seen at pH 10.5 (Fig. 12d). The concentration of Cr in the shoots increased as the pH increased. 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. It has been proven

that in strong acidic conditions Cr(VI) can be readily reduced to Cr(III) when it comes in contact with biomaterials owing to its high oxidation-reduction potential (+1.33V at pH 1.5 – 3.7) (Park et al., 2006). Thus, in acidic conditions, more Cr(VI) is reduced to Cr(III), which is accumulated in the roots and lowly translocated to the shoots. At high pH levels Cr(VI) is poorly reduced because of the low oxidation-reduction potential (Park et al., 2006).

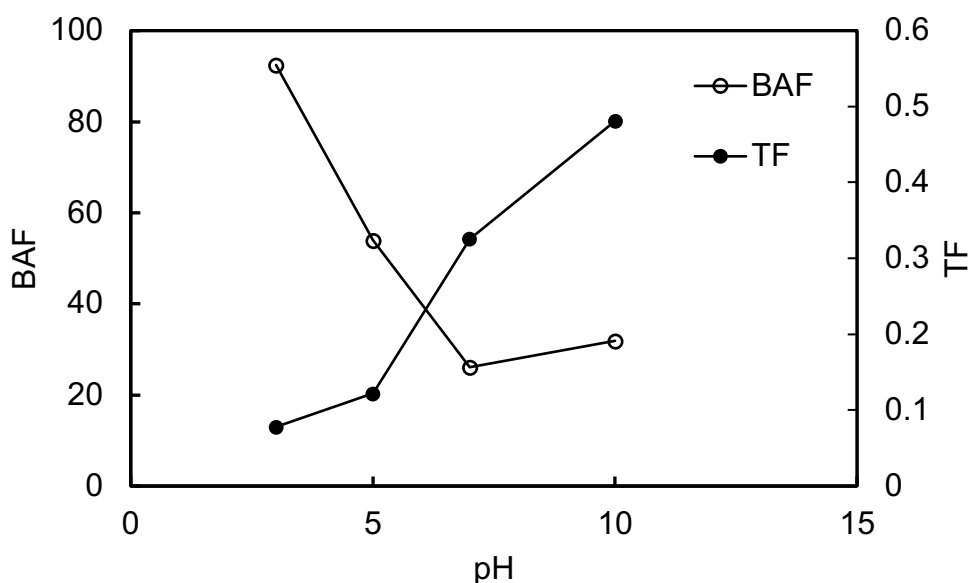


Fig. 13: Bioaccumulation factor (BAF) and translocation factor (TF) at different pH levels

Fig. 13 shows the BAF and TF of vetiver at different pH values. It can be seen that as the pH increased, the BAF also increased. The recorded BAF at pH values of 3.5, 5, 7.5, and 10.5 are 92.47, 53.92, 26.08, and 32.82 respectively. The TF values increased with an increase in pH values ranging from 0.08 at pH 3.5 to 0.48 at pH 10.5. The effect of pH on the bioaccumulation and translocation factors can be mainly attributed to the oxidative state of accumulated Cr present at the different pH levels. As mentioned earlier the mechanism of chromium uptake and translocation is dependent on the Cr species present. The uptake of Cr(VI) is active, while the mechanism of Cr(III) uptake is passive (Shewry and Peterson, 1974). At low pH, the reduction of Cr(VI) to Cr(III) results in low mobility of Cr to the aerial parts of the grass (Sinha et al., 2002), this is proven by the observed low TF at low pH in Fig. 13. Plants detoxify Cr(VI) by converting it to Cr(III), which can poorly penetrate biological cell membranes (Ertani et al., 2017). Thus, plants immobilize Cr in order to protect

important aerial functions of the plant. Skeffington (1976) observed that Cr was translocated to the shoot when the plant was exposed to Cr(VI) rather than Cr(III). The results obtained show that vetiver grass is more suitable for phytostabilisation as all the recorded BAF values were above one and the recorded TF values are below one. However, because vetiver grass can accumulate exceptionally high Cr concentration in the roots under acidic conditions, phytoextraction can be achieved through the use of floating systems when treating contaminated water, thus, making plant roots readily harvestable.

4. Conclusion

Vetiver grass was found to be effective in the removal of Cr(VI) from water. It has the ability to reduce Cr(VI) concentrations to lower than the recommended limits. The rate of removal depends on the initial concentration, plant density, and pH of the medium. Increasing plant density resulted in increased Cr(VI) uptake. Changing the pH of the medium greatly improved the removal efficiency under acidic conditions. No significant differences were obtained on Cr(VI) removal efficiency at neutral to basic conditions, however, basic conditions resulted in a higher translocation factor (TF). The bioaccumulation factor and translocation factor obtained in the concentration studies proved vetiver grass to have potential in phytoextraction. Although the bioaccumulation factor was above one throughout all the experiments, the translocation factor was below one at density and pH experiments. This suggests that vetiver grass is much more suitable for phytostabilisation. Vetiver grass showed its great potential in phytoremediation of Cr(VI) contaminated water throughout the experiments. Experiments over longer periods may show better results and may result in further translocation of Cr from the roots to the shoots.

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