ASSESSMENT OF THE POTENTIAL UPTAKE OF CHROMIUM BY SELECTED PLANT SPECIES AND ITS CONCENTRATION ALONG THE KLEIN WINDHOEK RIVER FROM THE UJAMS INDUSTRIAL WASTE WATER TREATMENT PLANT IN NAMIBIA

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ABSTRACT

The aim of the study is in threefold: (i) to assess the potential uptake of Cr(VI) and its potential toxicological effects on the total Chlorophyll content of *Rumex lanceolatus* and *Cullen obtusifolia,* exposed to the treated industrial effluent from UWWTP. (ii) To assess the concentration of Cr(VI) discharged from UWWTP into the KWR, its compliance to NWQSE and its behavior in the KWR in relation to the increased distance from the discharge point. (iii) To assess the performance of the UWWTP and its compliance to the NWQSE with regard to the concentrations of solids, organics and nutrients in the effluent discharged into the KWR. Comparisons of the total chlorophyll content and Cr(VI) concentration in the leaves were made between plants growing in exposed sites and the control sites. The total chlorophyll content in the plant leaves was determined using a potable meter SPAD 502Plus in the field, validated against a conventional spectrophotometric method in the laboratory. The Cr(VI) in the leaves was determined by a Direct Spectrophotometric Method, after the leaves were digested with a di-acidic mixture of 4 ml nitric acid (HNO_3) and 1 ml hydrochloric acid (HCL) (4:1 v/v). The total chlorophyll content was significantly lower in the plants exposed to Cr(VI) containing effluent than those growing in the control site for both *R. lanceolatus* (Mann-Whitney U test, U =396, $p < 0.001$) and *C. obtusifolia* ($t(118) = 4.496$, $p < 0.001$). The results further revealed a significantly higher concentration of Cr(VI) in the plant leaves of *R. lanceolatus*; from the exposed site; t-test ($t(118) = 5.692$, $p < 0.001$), but the difference in the Cr(VI) concentration between the two groups was not significant for *C. obtusifolia*. Simple linear regression analysis revealed a highly significant negative relationship between the Cr(VI) concentration and the total chlorophyll concentration in the leaves of *R. lanceolatus* exposed to the effluent from UWWTP ($r(118) = 0.34$, $p < 0.001$). *R. lanceolatus* accumulated more Cr(VI) concentration in its leaves and also appeared to be sensitive to high

Cr(VI) concentration, as marked by the significant reduction in its chlorophyll content. Interestingly, there was no significant relationship between the Cr(VI) concentration and the total chlorophyll concentration in the leaves of the of *C. obtusifolia* exposed to the effluent from UWWTP. The study showed that *C. obtusifolia* use avoidance as a mechanism against Cr(VI) induced stress. Both species need to be investigated further to understand their response to Cr(VI) induced stress at the root level and determine their bio accumulation factors. The UWWTP showed high removal efficiency, and complied with NWQSE general guideline in terms of TSS, COD, TKN and PO_4. However, the concentrations of Cr(VI) and TDS discharged from the UWWTP were significantly higher than the allowable concentration limit of 0.05 mg/l Cr(VI) and 500 mg/l TDS as set by the NWQSE ($t(59) = 12.475$, $p < 0.001$ and ($t(124) = 37.778$, $p < 0.001$ respectively) and could be having negative effects on the environment. It was further shown that the concentration of Cr(VI) in the effluent discharged from UWWTP significantly decreased with the increase in the distance away from the UWWTP discharge point along the KWR ($r(5) = 0.88$, $p = 0.02$). This study has demonstrated the need for continually assessing the UWWTP discharged effluent to ensure that the concentrations of Cr(VI) are within the set guidelines as provided by the NWQSE and the WHO.

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ABBREVIATIONS

BOD = Biochemical Oxygen Demand

BOOT = Build, Own, Operate and Transfer

COD = Chemical Oxygen Demand

DOC = Dissolved Organic Carbon

CWSS = Central Water Supply Scheme

EPA = Environmental Protection Agency

KWR = Klein Windhoek River

IARC = International Agency for Research on Cancer

NWQSE = Namibian Water Quality Standards for Effluents

NWQSPW = Namibian Water Quality Standard for Potable Water

NAMWATER = Namibia Water Corporation Limited

TDS = Total Dissolved Solids

TKN = Total Kjeldahl Nitrogen

TSS = Total Suspended Solids

UNAM = University of Namibia

UOPS = Ujams Oxidation Ponds System

USEPA = United States Environmental Protection Agency

UV = Ultra Violet rays

UWWTC = Ujams Waste Water Treatment Company

UWWTP = Ujams Waste Water Treatment Plant

WHO = World Health Organization

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DEDICATION

I dedicate this achievement to my Grandmother, Maria Shilimonhulo Nkandi, Kuku gwa Nkandi ya Katjenye (12/06/1922 – 16/07/2019). May her soul rest in eternal peace. I am eternally grateful to her for her dedication in raising me up and most especially for instilling in me the values of gratitude, faith and strong work ethics, among others. For her lessons have given me a strong and long-lasting foundation.

DECLARATIONS

I, Gerhard Iipinge Iiputa, hereby declare that this study is my own work and is a true reflection of my research, and that this work, or any part thereof has not been submitted for a degree at any other institution.

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Gerhard Iipinge Iiputa **Date**

1 CHAPTER 1: INTRODUCTION

1.1 Background of the study

Windhoek, the capital City of Namibia, has practiced direct potable water reclamation of its domestic sewage since 1968 (Lahnsteiner & Lampert 2007). A new reclamation plant with an ultrafiltration membrane treatment process was commissioned in 2002 (Van der Merwe *et al*. 2008). As part of its multiple barrier strategy of attaining the highest possible safety levels from potential industrial pollution, industrial sewage derived mainly from five industries, including a chocolate producer, breweries, beverage, abattoir and tannery, has been treated at the Ujams Oxidation Ponds System (UOPS) since 1969 until 2014 (Van der Merwe *et al*. 2008).

This wastewater treatment facility became increasingly overburdened over the years and could no longer fulfill stricter discharge regulations. It was therefore replaced with a new and sustainable waste water treatment plant commissioned in October 2014. This new reclamation plant, comprises of a treatment train of fine screening, grit removal, dry weather buffer tank, fine sieving, membrane bioreactor (membrane filtration), UV disinfection, clear water tank and sludge treatment. It has a treatment capacity of 5,175 m^3/d and designed for reclaiming industrial wastewater reusable for the purpose of irrigation, industrial reuse and discharge into the nearby Klein Windhoek River (KWR). The Ujams Waste Water Treatment Plant (UWWTP) is operated by the Ujams Waste Water Treatment Company (UWWTC), under a 21 years agreement of Build, Own, Operate and Transfer (BOOT), with the City of Windhoek (WABAG 2017).

A tannery forms part of the sources of industrial effluent being treated at UWWTP (Oliveira 2012; Mishra & Bhargava 2016). As such, a tannery is one of the industries that produces effluent containing high concentrations of heavy metals including Chromium (Cr) which is a major source of Cr pollution in the environment. Chromium salts such as basic chromium (III) sulfate

($Cr(H_2O)_5(OH)SO_4$) are extensively used as tanning agents to convert hides into leathers (Ramteke *et al*. 2009; Mwinyihija 2012). The Cr concentrations in these effluents ranges between 1.07 – 7.80 mg/l Cr and the worldwide anthropogenic discharge of Cr in freshwater bodies has been estimated to be 3,550 mt (Nriagu 1990). Any possible discharge of wastewater from the tannery, without adequate treatment or disposal of the chromium contaminated sludge into the environment causes serious pollution to the environment, along with serious threats to animal, plant and human health (Chandra *et al*. 2011; Ramteke *et al*. 2009; Sundar *et al*. 2002).

Chromium occurs in different chemical forms, primarily as chromite (Cr(III)) and chromate (Cr(VI)) in soil and these vary markedly in terms of their biogeochemical behavior. It is a potentially toxic heavy metal which does not have essential metabolic functions in plants. Cr contamination alters the structure of soil and microbial communities, as well as reduces their growth (Shahid *et al*. 2017). Furthermore, Cr in its chromate (Cr(VI)) form is reported to be highly toxic in nature than any other Cr forms, as it reacts with nucleic acids and other cellular components to produce mutagenic, teratogenic and carcinogenic effects in biological systems (Mishra & Bharagava 2016). Cr(VI) is a very toxic, powerful epithelial irritant and proven human carcinogen, as established by the International Agency for Research on Cancer (IARC), the Environmental Protection Agency (EPA) of the United State of America and the World Health Organization (WHO 1988). It induces phytotoxicity by interfering with plant growth, nutrient uptake and photosynthesis, inducing enhanced generation of reactive oxygen species, causing lipid peroxidation and altering the antioxidant activities (Shahid *et al*. 2017). This Cr(VI) containing effluent as discharged into the KWR and flows to the Swakoppoort dam (Lehmann 2010). It infiltrates the underground and also support the riparian vegetation along the river. Among the various plants in this area, this study focuses on herbaceous plants such as *Rumex lanceolatus*

Thumb. from the Polygonaceae family and *Cullen obtusifolia* (DC.) C.H. Stirt. from the Fabaceae family, which inhabits the grassy areas, edges of pans, dams, rivers and various wet places (Hyde *et al*. 2019; Grimes 1997).

1.2 Statement of the problem

Although the City of Windhoek regularly monitors the effluent quality released from the Ujams Waste Water Treatment Plant (UWWTP), the actual concentration of Cr(VI) released into this environment through the discharge of the treated effluent is not yet documented (Lahnsteiner *et al*. 2017). The disposal of large quantity of Cr(VI) may overcome the reducing capacity of the environment and thus persist for long periods as a pollutant (Mishra & Bharagava 2016). Cr(VI) may eventually accumulate in plants from contaminated soils, and cause severe health risks in humans via food chain contamination (Broadway *et al*. 2010). In view of this, there is a lack of knowledge on how plants species in the environment exposed to the effluent from UWWTP are being affected by the potential presence of Cr(VI).

Furthermore, the effluent discharged from UWWTP into the Kleine Windhoek River (KWR) will eventually reach the Swakoppoort dam which is one of the sources of potable drinking water for the Central region, treated and supplied by Namwater. According to Lehmann (2010), the Swakoppoort dam suffers from severe level of anthropogenic pollution emanating from the catchment area, particularly from the Klein Windhoek River (KWR) and Otjiseru River, with high levels of heavy metals such as Chromium, Cadmium, Nickel as well as high loads of Phenol, Formaldehyde, ammonia and nutrients like phosphates and Dissolved Organic Carbon (DOC). Consequently, the high concentrations of Cr(VI) as well as other pollutants in this effluent present a new industrial waste cycle and would negatively affect the waste water reclamation of Windhoek.

1.3 Aims of the study

The study had three aims as follows: (i) to assess the potential uptake of Cr(VI) and its potential toxicological effects on the total Chlorophyll content of selected plant species exposed to the treated industrial effluent from UWWTP. (ii) To assess the concentration of Cr(VI) discharged from UWWTP into the KWR, its compliance to NWQSE and its behavior in the KWR in relation to the increased distance from the discharge point. (iii) To assess the performance of the UWWTP and its compliance to the NWQSE with regard to the concentrations of solids, organics and nutrients in the effluent discharged into the KWR.

The specific objectives of the study were:

a. To determine and compare the total chlorophyll content in the leaves of *Rumex lanceolatus* and *Cullen obtusifolia* exposed to the effluent from UWWTP and those not exposed to the effluent from UWWTP.
b. To determine and compare the concentration of Cr(VI) in the leaves of *Rumex lanceolatus* and *Cullen obtusifolia* exposed to the effluent from UWWTP and those not exposed to the effluent from UWWTP.
c. To determine the effects of Cr(VI) concentrations in the leaves on the total chlorophyll content of *Rumex lanceolatus* and *Cullen obtusifolia*
d. To assess the effect of distance from the discharge point on the concentration of Cr(VI) in the KWR water.
e. To determine whether the concentration of Cr(VI) in the effluent discharged from the UWWTP into the KWR complies with the Namibia Water Quality Standards for Effluents (NWQSE).

f. To compare the performance of the Ujams Oxidation Ponds System (UOPS) and the UWWTP in terms of their removal efficiencies for solids, organics and nutrients.

g. To determine whether the concentrations of solids, organics and nutrients in the effluent discharged from the UWWTP into the KWR complies with the NWQSE.

1.4 The Null hypotheses

a. There is no significant difference between the total chlorophyll content in the leaves of plants exposed to the effluent from the UWWTP and those not exposed to the effluent from the UWWTP.

b. There is no significant difference between the concentration of Cr(VI) in the leaves of plants exposed to the effluent from the UWWTP and those not exposed to the effluent from the UWWTP.

c. There is no significant relationship between the total chlorophyll content in the leaves of the plants and the concentration of Cr(VI) in the leaves of the same plants.

d. There is no significant relationship between the concentration of Cr(VI) and the distance from the discharge point.

e. There is no significant difference between the concentration of Cr(VI) in the effluent discharged from the UWWTP and the allowable concentration limits for Cr(VI) set by the NWQSE.

f. There is no significant difference between the performance of the UOPS and the UWWTP in terms of their removal efficiencies for solids, organics and nutrients.

g. There is no significant difference between the concentrations of solids, organics and nutrients in the effluent discharged from the UWWTP and the allowable concentration limits for solids, organics and nutrients set by the NWQSE.

1.5 Significance of the study

This study contributes to the understanding of the toxicological effects of chromium on the chlorophyll content of selected plant species. Furthermore, the study will add to the number of known plant species that are capable of hyper-accumulating Cr in their shoot tissues without showing significant signs of defects and consequently suitable for phytoremediation applications in Cr(VI) polluted soil habitats.

The KWR discharges into the Swakoppoort Dam, which forms part of the three surface dams system and provides for over 60% of the potable to over 300 000 inhabitants of the central region of Namibia. There is therefore a great need to protect this important source from any potential pollution. Furthermore, the presence of water in the KWR is an essential resource that can help relieve pressure on the water supply to Windhoek, as it can be utilized informally for light industrial and domestic activities along the KWR. However, this also requires that the water quality be continuously profiled, especially with regard to heavy metals to ensure human health.

Lastly, given its extreme toxicity to living organisms and further consideration as a “human carcinogen” by the IARC, USEPA and WHO (Yadav 2010), it is therefore imperative that any Cr(VI) related operation in any land is given rigorous monitoring and scientific evaluation, especially in the soil-plant system, as a first step towards ensuring environmental safety and human health.

2 CHAPTER 2: LITERATURE REVIEW

2.1 General information about chromium

According to Shahid *et al*. (2017), Chromium (Cr) with atomic number 24, molecular weight 51.1 and density 7.19 g/cm,^{3} is a silver color hard metal. It is the 7th most abundant element (Nriagu 1988) and 21st most abundant metal (Sinha *et al*. 2005) of the earth's crust. It has been ranked 7th among top 20 hazardous substances by the Agency for Toxic Substances and Diseases Registry (Mishra & Bharagava 2016). This metal is also ranked 5th among the heavy metals in the Comprehensive Environmental Response, Compensation and Liability Act (Ma *et al*. 2007).

2.2 Chemistry of chromium

Chromium has a complex electronic and valence shell chemistry owing to its high potential to easily convert from one oxidation state to another (Mishra & Bharagava 2016). It has several oxidation states (-2 to +6), but chromite (Cr(III)) and chromate (Cr(VI)) forms are the most common and stable in the natural environment (Shahid *et al.* 2017). The Cr (VI) state is thermodynamically stable as the anions (CrO_4^{2-}) and dichromate (CrO_7^{2-}), which are soluble in aqueous solutions. The Cr(III) state is however insoluble in water. Therefore, treatment of aqueous waste streams usually involves reduction of Cr(VI) to Cr(III) prior to precipitation and sedimentation (Watts 1997). Both these forms have chemical, epidemiological and toxicological features; they are separately regulated by Environmental Protection Agency (EPA), which presents a distinctive feature of Cr among the heavy metals (Shahid *at al*. 2017).

Almost all naturally occurring chromium exist in trivalent form, while Cr(VI) is mostly of industrial origin (Mishra & Bharagava 2016). Both the species of Cr (Cr(III) and Cr(VI)) differ greatly with respect to their sorption and bioavailability in soil, absorption and translocation to aerial parts, and toxicity inside plants (Oliveira 2012). Cr (III), being necessary for lipid and sugar

metabolisms (Bassi *et al.* 1990), is an essential trace element for human and animal health (Swartz & Mertz 1959; Eskin 2016). However, it is not required by plants (Shanker *et al*. 2005). Cr(III) is the dominating Cr species in the environment as it is the most stable oxidation state in which Cr is found in living organisms, for instance in humans, it is an essential nutrient required for normal energy metabolism (Mishra & Bharagava 2016). Cr(VI) is the second most stable form of chromium, and a strong oxidizing agent, especially in acidic medium. In solution, Cr(VI) exist as hydro-chromate ($HCrO_4^-$) and dichromate ($Cr_2O_7^{2-}$) ionic species. The proportion of each ion in solution is pH dependent, such that at basic and neutral pH, the chromate form predominates, but at lower pHs (6.0 to 6.2), the hydro-chromate concentration increases. It is a strong oxidizing agent and exists only in the oxygenated form, which is highly soluble in aqueous media (Mishra & Bharagava 2016). It has been further confirmed that due to its very complex chemistry, its solubility, mobility and bioavailability in soil strongly depends on the various oxidation states, from -2 to + 6 (Oliveira 2012). Depending on its oxidation state and concentration, Cr metal acts as a toxic or essential element for living organisms (Amin *et al*. 2013; Oliveira 2012).

2.3 Sources and environmental mobility of chromium

According to Mishra & Bharagava (2016), the presence of chromium in the environmental has been widely reported. However, rapid industrialization has played an important role in polluting the environment and causing deterioration in the quality of hydrosphere and atmosphere. Industrial use of large quantity of water during conversion of raw materials into products, generates huge volumes of wastewater, which contains high concentrations of various organic and inorganic pollutants, such as chromium. Mishra & Bharagava (2016) listed some of the industrial applications or sources chromium, which use different types of chromium compounds and result in its release into the environment such as pigments in paints and plastics, anticorrosion coatings,

stainless steel, wood preservation, leather tanning, electroplating, printing, metallurgy, catalysts, textile preservatives, refractories, photographic emulsions. Although anthropogenic sources also release significant amount of Cr to soils and sediments indirectly via atmospheric deposition, Cr released as a result of dumping of Cr contaminated solid wastes and liquid is a major source of Cr in the environment (Shahid *et al*. 2017)

In Windhoek, the major source of chromium contamination is the tannery, which is one of the industrial effluent sources supplying the UWWTP (WABAG 2017). The soluble Cr(VI) in the waste water stream is firstly reduced to Cr(III), precipitation, then followed by sedimentation. The sludge is then removed and dewatered by using belt presses, before it is transported to the hazardous waste cell of the Kupferberg landfill site, located in the south western side of the Windhoek the CBD. Through adequate treatment optimization and good operational management of this treatment, the potential release of chromium into the environment is minimized. Therefore, there are only two possible ways that chromium can get into environment from the UWWTP. The first way is through poor handling of the sludge and secondly, through the discharged effluent. Effluent must be adequately monitored to ensure compliance with the limits as set by the NWQSE.

2.4 Toxicological effects of chromium in Humans

The Cr(VI) is 100 times more toxic than Cr(III) and penetrates biological membranes more easily than Cr(III) (Katz & Salem 1993). After crossing the cell membrane, it may be reduced to Cr(III) via a number of hypothesizes reactions. During the reduction process, several intermediates, such as pentavalent and tetravalent chromium species, are produced with the generation of reactive oxygen species, which easily combines with DNA-protein complexes, leading to the development of a large number of health abnormalities in human as well as in animals (Mishra & Bharagava

2016). Mishra & Bharagava (2016), further listed some of the major health effects related with chromium toxicity as follows: Carcinogenicity, Genotoxicity and mutagenicity, Respiratory, Cardiovascular, Reproductive and Developmental, Dermatotoxicity and Gastrointestinal. As reported by Levis & Bianchi (1982), Cr(VI) may enter the human and animal body by inhalation, ingestion and dermal absorption. Occupational exposure generally occurs through inhalation and dermal contacts, whereas the general population gets exposed most often by ingestion of chromium-contaminated food and water. It can act directly at the site of contact or be transported into other parts of the human or animal tissues. Cr(VI) is therefore classified as human carcinogen by the WHO (WHO 1988) and USEPA (USEPA 1998). This has been further supported by epidemiological studies by various countries, with results showing close relationship between chromium exposure and lung cancer (Mishra & Bharagava 2016; Welling *et al*. 2015).

2.5 Toxicological effects of chromium in plants

According to Shanker *et al*. (2005), chromium compounds are highly toxic and detrimental to plant growth and development. Although some crops are not affected by low Cr concentration (3.8×10^{-4} *u*g/l) (Huffman & Allaway 1973a). Davies *et al*. (2002), concluded that chromium is mostly toxic to higher plants at 100 *u*g/kg dry weights.

Cervantes *et al*. (2001) recorded that chromium compounds are highly toxic to plants, retarding their growth and development. Cr(VI) produces more damage in plants, due to its solubility and permeability to cross the cell membrane, which is relatively innocuous and less toxic because of its extremely low solubility and therefore prevents its leaching into ground water and its uptake by plants. However, the toxicity of Cr(VI) and Cr(VI) content in plants is species-specific (Cervantes *et al.* 2001).

Shanker *et al*. (2005) further reported that toxicological effects of chromium in plants can be classified in three categories; (1) Chromium uptake, translocation and accumulation, (2) growth and development and (3) physiological processes.

2.5.1 Chromium uptake, translocation and accumulation

Shanker *et al*. (2005), reported that the first interaction Cr has with a plant is during its uptake process. Cr is toxic, non-essential element to plants, hence they do not possess specific mechanisms for its uptake. Consequently, the uptake of this heavy metal is through carriers used for uptake of essential metals for plant metabolism. The toxic effects of Cr are primarily dependent on the metal speciation, which determines its uptake, translocation and accumulation. Further details on these aspects are well documented (Cervantes et al. 2001; Wallace et al. 1976; Skeffington *et al*.1976; Ramachandran *et al.* 1980; Golovatyj *et al*. 1999; Huffman & Allaway 1973a; Shanker *et al*. 2004a).

2.5.2 Effects of Chromium on Plant growth and development

As reported by Shanker *et al*. (2005), the toxic effects of Cr to plant growth and development include alterations in the germination process as well in the growth of roots, stem and leaves which may affect total dry matter production and yield.

Most notably, seed germination is the first physiological process affected by chromium and the ability of seed to germinate in a medium containing Cr(VI) would be the indicator of its level of tolerance to this metal (Rout *et al*. 2000; Zeid 2001; Amin *et al.* 2013).

2.5.3 Effects of Chromium on plant physiology

Cr also causes deleterious effects on plant physiological processes such as photosynthesis, water relations and mineral nutrition (Shanker *et al*. 2005).

It is one of the important factors that affect photosynthesis in terms of CO_2 fixation, electron transport, photophosphorylation and enzyme activities (Clijsters & Van Assche 1985). As based on several studies on plants, such as wheat, peas, rice, maize, beans and sunflower, its effects include inhibition of electron transport, inactivation of Calvin cycle enzyme, reduction of Carbon fixation, disorganization of chloroplast (Davies *et al*. 2002; Bishnoi *et al*. 1993a, b; Zeid 2001; Shanker 2003).

Cr effects on water relations has also been investigated, using plants such as bush beans, sunflower and mug bean. It is reported to cause decrease in water potential, increase respiration rate, and reduce diffusive resistance, wilting, reduction in tracheary vessel diameter (Vasques *et al*. 1987; Barcelo *et al.*1993; Davies *et al*. 2002).

Shanker *et al*. (2005), further reported on its effects on the processes of mineral nutrition, enzymes and other compounds, based on studies on Soybean, tomato, bush bean, sunflower, maize, *Nymphaea alba* and various cereals and legumes.

2.5.3.1 Effects of chromium on the process of photosynthesis through chlorophyll content reduction

Plants commonly respond to metal stress by a decrease in the chlorophyll content in the leaves of the plant, then subsequent the reduction in photosynthesis that finally leads to lower biomass production (Monteiro *et al*. 2009). Chromium stress is one of the important factors that affect photosynthesis (Shanker *et al*. 2005). According to Mishra & Bharagava (2016), several earlier researchers have reported five effects of Cr(VI) toxicity on the process of photosynthesis as; electron transport inhibition, reduced CO_2 fixation, chloroplast disorganization, Calvin cycle inactivation and photo-phosphorylation.

Shanker *at al.* (2005), indicates that in higher plants, the effect of Cr on photosynthesis is well documented. However, it is not well understood to what extent Cr induced inhibition of photosynthesis is due to disorganization of chloroplast ultrastructure, inhibition of electron transport or the influence of Cr on the enzymes of the Calvin cycle (Vasques *et al*. 1987). Clijsters & Van Assche (1985), reported that the disorganization of the chloroplast ultrastructure and inhibition of electron transport processes due to Cr and a diversion of electrons from the electron donating side of PS 1 to Cr(VI) is a possible explanation for Cr- induced decrease in photosynthetic rate. It is possible that electrons produced by the photochemical process were not necessarily used for carbon fixation as evidenced by low photosynthetic rate of the Cr stressed plants. The overall effect of Cr ions on photosynthesis and excitation energy transfer could also be due to Cr(VI) induced abnormalities in the chloroplast ultrastructure like poorly developed lamellar system with widely spaced thylakoid and fewer grana. Cr can cause ultra-structural changes in the chloroplast leading to inhibition of photosynthesis, and such alterations in chloroplast have been observed in many plants such as *Lemna minor*, *Pistia* specie, *Taxithelium nepalanse* (Choudhary & Panda, 2004).

Several studies on Cr toxicity effect on the photosynthetic pigment of various crops and trees are well documented (Barcelo *et al*. 1993; Sharma & Sharma 1996; Vajpayee *et al*. 1999). Furthermore, Cr stress in plants causes decrease in chlorophyll a, chlorophyll b, total chlorophyll and carotenoids (Panda & Patra 1997, 2000; Tripati & Smith 2000; Panda & Khan 2003; Choudhury & Panda 2004; Panda & Choudhury 2005). Panda & Choudhury (2005) showed that Cr can impact the photosynthetic pigments in three ways as follows:

(i) Cr has the ability to degrade the δ-aminolevulinic acid dehydratase, an important enzyme involved in chlorophyll biosynthesis, thereby affecting the δ-aminolevulinic

acid (ALA) utilization (Vajpayee *et al*. 2000). Heavy metals inhibit chlorophyll biosynthesis (Xiong 1997).

(ii) Mostly as Cr(VI), it can replace Mg ions from the active site of many enzymes thus depleting the chlorophyll content (Vajpayee *et al*. 2000).

(iii) Like other heavy metals, Cr can induce degradation of carotenoids in some plants, such as in *Vallisneria spiralis* and other aquatic plants (Baszynski *et al.* 1981; Rai *et al*. 1992), However, an increase in carotenoids content was observed under Cr treatment (Tripatti & Smith 2000; Vajpayee *et al*. 2001). The increased carotenoids content may act as antioxidant to scavenge reactive oxygen species (ROS) such as H_2O_2, O_2^-, OH^-, generated as a consequence of a wide range of abiotic stress including heavy metals (Panda & Choudhury 2005).

Both Cr(III) and Cr(VI) reduce chlorophyll content (Panda & Patra 2000; Pati *et al*. 2014). The chlorophyll concentration decreased significantly with the increase in Cr concentration. Pati *et al*. 2014, in their study on the physiological responses induced by Cr(VI) toxicity to *Cucumis sativus L.* and *Macrotyloma unifloroum* Lam, reported that the total chlorophyll were reduced gradually with the rise in concentration (5-20 ppm) and also time duration (7 - 21 days). They attributed the observed reduction in chlorophyll content at higher concentrations of Cr(VI) to the breakdown of the thylakoid and chloroplast envelope, as was previously reported by Dodge & Lawes (1974). Bera *et al*. (1999), in a study on 6-day-old mung bean seedlings, exposed to a tannery effluent, reported that the amount of chlorophyll a, chlorophyll b and total chlorophyll decreased irrespective of the concentration of Cr(VI) present. Similarly, Sharma & Sharma (1996) reported that chlorophyll content decreased as a marked effect of various concentrations of different Cr compounds of Cr(III) and Cr(VI), in *Tritium aestivum.*

Based on a study on *Salvinia minima*, Nicholis *et al*. (2000), reported that a concentration increase of 1 and 2 mg/l Cr(VI), caused a significant reduction in the chlorophyll a, chlorophyll b and carotenoid concentrations. Similar findings were also reported by Panda & Khan (2003) and Tripathi & Smith (2000). Shanker *et al*. (2003), postulated that a decrease in the ratio of chlorophyll a/b ratio (meaning an increase in chlorophyll b than a), could indicate that Cr toxicity possibly reduces the size of the peripheral part of the antenna complex. Whereas, the alternative could be due to the destabilization and degradation of the proteins of the peripheral part.

The general reduction in chlorophyll content in a plant under Cr stress, could also be attributed to the inactivation of the enzymes in chlorophyll biosynthetic pathway by the Cr (Shanker *et al*. 2005). Moreover, heavy metals are known to interfere with chlorophyll synthesis, either through direct inhibition of an enzymatic step or by inducing deficiency of an essential nutrients and as reported in earlier studies chlorophyll content decreases significantly in plants kept under Cr(VI) stress at higher concentrations (10 – 40 ppm) and also negatively affects plants growth and development (Oliveira 2012). On the contrary, a study on Cr and Ni tolerance on *Echinochloa colona* by Rout *et al*. (2000) revealed higher chlorophyll content in plants under high Cr concentration.

2.6 Phytoremediation of heavy metals contaminated soil and water

According to Raskin *et al*. (1994), the term phytoremediation was used for the first time in 1991 and was defined as plant-based action (phyto - plant, remediation – to recover). Subsequently, USEPA (1998), defined phytoremediation as the direct use of living plants for in-situ, or on-site remediation of contaminated soils, sludge, sediments and groundwater through contaminant removal, degradation or containment. Phytoremediation is considered an effective, low cost and

environmental friendly technology to cleanup heavy metal-polluted sites. It is based on the capacity of some plants called hyper-accumulators, for taking these metals from the soil and accumulating them above a threshold value in their harvestable tissues (Fernandez *et al.* 2011).

As described by Orcutt & Nelsen (2000), whenever plants are exposed to heavy metals in their environment, they are ultimately forced to develop relevant mechanisms for adaptation to the metal stress, and this could either be metal resistance or metal sensitivity. Resistance to metal stress means that in spite of the metal toxicity, plants react in a way that allows them to survive high concentrations of metals and to produce the next generation of plants, whereas, in cases of sensitivity to metals would result in injury or death of the plants. Plants resist metal stress through two mechanisms; avoidance, as a mechanism for external protection of the plant from metal stress and secondly, tolerance, as a mechanism in which the plant is able to survive internal stress imposed by high internal metal concentrations (Shahid *et al*. 2013a).

According to Schnoor (1997), plants ideal for phytoremediation should fulfil four main requirements;

1. They must be fast growing and have high biomass
2. Have deep roots
3. Have easily harvestable aboveground portion,
4. Accumulate large amounts of metals (~1000 mg/kg) in aboveground biomass.

According to Schat *et al*. (2000), the possibility of effective phytoremediation of heavy metal-contaminated soil depends on the availability of plants varieties with high rates of accumulation and tolerance of the metal(s) to be extracted. Moreover, plants that exhibit extremely high rates of metal accumulation in their tissues are called hyper-accumulators. This term was first used by Brook *et al.* (1998), to describe plants that accumulated high concentration of Nickel in their

tissues and it has then since generally been applied to the accumulation of high concentrations of other metals. Hyper-accumulators can concentrate metals in their aboveground tissues far exceeding those present in the soil or in the non-accumulating species growing nearby. Yang *et al*. (2005) further refined the definition of hyper accumulator that the plant must accumulate metal in the amount exceeding its amount in the soil. To date, there is a long list of hyper-accumulators (Kadukova & Kavulicova 2011). There are also plants called metallophytes, with specific biological mechanisms that enable them to tolerate high metal concentrations and therefore grow on soils that are contaminated with high level of metals and these can be classified as accumulator, indicator or excluder, according to the concentrations of metals found in their tissues (Kadukova & Kavulicova 2011).

The hyper - accumulating plants have the following characteristics (Chaney *et al*. 1997

1. Have the ability to tolerate high levels of the element in root and shoots cells (hyper-tolerance)
2. Have ability to translocate an element from roots to the shoots at high rates
3. There must be a rapid uptake rate for the element at level that occur in soil solution

However, there are significant differences in the degree of tolerance, uptake and accumulation of Cr among plant species (Shahadeh & Hossner 2000).

In a review by Mukhopadhyay & Maiti (2010), many typical plants used in cleaning up metal contaminated sites were listed. Some of these metals accumulator plants (genus only or species names) are classified according to the accumulatable metal as follows; As accumulators *(Paspalum, Eriochloa, Holcus, Pinnestum juncus, Scirpus and Thymus*), Pb accumulators *(Brassica juncea, Vetiveria, Sesbanaia, Minuartia, Juncus, Scirpus* and *Thymus*), Cu accumulators (*Ammania baccifera, Scleranthus*), Zn and Cd accumulators (*Vetiveria, Sesbania, Viola, Sedum,*

Rumex). These plants were used in studies and have been recommended for further research to develop fast growing and high biomass plants with improved metals uptake abilities, increased translocation and tolerance of metals through genetic engineering for effective phytoremediation of metal contaminated sites.

In a study by Takahashi *et al. (*2005), on phytoremediation from abandoned rice field, *Rumex crispus L.* subsp. *Japonicus*, a kind of curly dock, a member of the Polygonaceae was found to be one of the best in Remzol Brillinant Blue R (RBBR) decolorization assay. Moreover, Rumex species are perennial and form thick taproots, which might be suited for cleanup pollutants. This supports an earlier study by Cha (1992), which reported that *Rumex crispus* removed cadmium ions from wastewater. As such *Rumex lanceolatus* was selected in this study to investigate its phytoremedial properties.

Hall (2002) described a range of potential cellular mechanisms possessed by plants and applied in the detoxification of heavy metals and tolerance to metal stress. These includes the roles; for mycorrhiza and for binding to cell wall and extracellular exudates, for reduced uptake or efflux pumping of metals at the plasma membrane for chelation of metals in the cytosol by peptides such as phyto-chelatins, for the repair of stress damaged proteins and for the compartmentation of metals in the vacuoles by tonoplast-located transporters.

2.7 The significance of solids, organics and nutrients in water quality assessment

As stated by Zaghloul *et al*. (2019), pollution is objectionable changes in any given ecosystem trailing to potential health hazards. The effluent from UWWTP plays a significant role in the

quality and amount of water resources available for the Central region. It is therefore imperative that a wholistic view on the potential pollution threat is taken. Moreover, assessment of pollution level is continually recognized by several pollution indicators, biological, chemical and physical. Chemical indicators always validate fair information about the evenness between ecosystem components (Zaghoul *et al*. 2019). With this holistic view in mind, the following key water quality variables were also considered (Chapman, 1996 and Sawyer *et al.* 2003); pH, Temperature, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Chemical Oxygen Demand (COD), Total Kjeldal Nitrogen (TKN) and Ortho-Phosphate (ortho - PO_4). The concentrations limit as set out by the NWQSE for these parameters are as indicated in table 1 below this section.

2.7.1 Temperature

As described by Chapman (1996), temperature affects physical, chemical and biological processes in water bodies and therefore the concentration of many variables. Increase in temperature generally increases the rate of chemical reactions, evaporation and volatilization of substances, it also decreases the solubility of gases such as O_2, CO_2, N_2, CH_4 and others in water. It affects the metabolic, respiration and decomposition rates in aquatic environment. Temperature plays a major role in the population growth of bacteria and phytoplankton, macrophyte and algal blooms and leads to eutrophication of water bodies in water with high nutrient loads. Surface water has a normal temperature ranging between 0 and 30 ^{o}C. It is therefore an essential parameter to understand biological and chemical processes in water bodies. It should be measured in situ, with the use of a thermometer or temperature probe.

2.7.2 pH

The pH is also an important parameter in water quality assessment as it also influences many biological and chemical processes within a water body and all processes associated with water

supply and treatment. It is useful in determining the extend of the effects of an effluent discharge plume in the water body. It is a measure of the acid balance of a solution and is defined as the negative of the logarithm to the base 10 of the hydrogen ion concentration. It has scale runs from acidic to alkaline (0 – 14), with neutral (pH 7). In unpolluted waters, pH is principally controlled by the balance between the carbon dioxide, carbonate and bicarbonate ions as well as other natural compounds such as humic and fulvic acids. The pH of most natural waters is between 6.0 and 8.5, although lower values can occur in dilute waters high in organic content, and higher values in eutrophic waters, groundwater brines and salt lakes (Chapman 1996).

2.7.3 Solids

To assess the water quality of any effluent discharge, Total Suspended Solids (TSS) and Total Dissolved Solids (TDS) are the two analysis of significance. The term "solids" is widely used for the majority of compounds which are present in natural waters and remain in a solid state after evaporation. TSS and TDS correspond to non-filterable and filterable residue, respectively (Chapman 1996)

2.7.3.1 TSS

TSS (mg/l) are the solids retained on a standard filter (usually a glass fibre "GF/C" grade) and dried to a constant weight at 105° C (Bartram and Ballance, 1996). Suspended solids can lead to the development of sludge deposits and anaerobic conditions when untreated wastewater is discharged in the aquatic environment (Tchobanoglous *et al*. 2003). The settleable and suspended solids determination are of great value in assessing the strength of domestic, industrial waste water and lightly polluted waters. Moreover, suspended solids determination is important in the analysis of polluted waters. It is one of the major parameters used to evaluate the strength of domestic waste waters and to determine the efficiency of treatment units (Sawyer *et al*. 2003).

2.7.3.2 TDS

According to Chapman (1996), TDS (mg/l) is also determined gravimetrically and can also be obtained by multiplying the conductance by a predetermined factor for each water body and is commonly between 0.55 and 0.75. This multiplication factor remains approximately constant provided the ionic proportions of the water body remain stable. It is close to 0.67 for waters in which sodium and chloride dominate, and higher for waters containing high concentrations of sulphates. The conductivity, or specific conductance, is a measure of the ability of water to conduct an electric current. It is sensitive to variations in dissolved solids, mostly mineral salts. The degree to which these dissociate into ions, the amount of electrical charge on each ion, ion mobility and the temperature of the solution all have an influence on conductivity. Conductivity of most freshwaters ranges from 10 to 1,000 µS cm-1 but may exceed 1,000 µS cm-1, especially in polluted waters, or those receiving large quantities of land run-off. In addition to being a rough indicator of mineral content when other methods cannot easily be used, conductivity can be measured to establish a pollution zone, for example around an effluent discharge, or the extent of influence of run-off waters. It is usually measured in situ with a conductivity meter, and may be continuously measured and recorded. Such continuous measurements are particularly useful in rivers for the management of temporal variations in TDS and major ions.

2.7.4 Organics

As described by Tchobanoglous *et al.* (2003), biodegradable organics, composed principally of proteins, carbohydrates and fats. They are measured most commonly in terms of Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). Most importantly, if discharged untreated to the environment, their biological stabilization can lead the depletion of natural oxygen

resources and to the development of septic conditions. COD is the most widely test used to measure the organic strength of domestic and industrial wastes (Sawyer *et al*. 2003). It is a measure of the oxygen equivalent of the organic matter in a water sample that is susceptible to oxidation by a strong chemical oxidant, such as dichromate. The COD is widely used as a measure of the susceptibility to oxidation of the organic and inorganic materials present in water bodies and in the effluents from sewage and industrial plants. The test for COD is non-specific, in that it does not identify the oxidizable material or differentiate between the organic and inorganic material present. Similarly, it does not indicate the total organic carbon present since some organic compounds are not oxidized by the dichromate method whereas some inorganic compounds are oxidized. Nevertheless, COD is a useful, rapidly measured, variable for many industrial wastes and has been in use for several decades. The concentrations of COD observed in surface waters range from 20 mg l-1 O2 or less in unpolluted waters to greater than 200 mg/l O_2 in waters receiving effluents. Industrial wastewaters may have COD values ranging from 100 to 60,000 mg/l O_2 (Chapman 1996)

2.7.5 Nutrients

Both nitrogen and phosphorus, along with carbon are essential nutrients for growth. Consequently, when discharged to the aquatic environment, these nutrients can lead to the growth of undesirable aquatic life, equally, when discharged in excessive amounts on land, they can also lead to the pollution of groundwater (Tchobanoglous *et al*. 2003).

2.7.5.1 Nitrogen

Nitrogen is an important constituent of proteins including genetic material and is therefore of great importance in water resources, in the atmosphere and in the life processes of plants and animals It

has a complex chemistry, as it can assume several oxidation states and that the changes are brought about by living organisms (Sawyer *et al*. 2003).

It is converted from inorganic to organic forms by plants and micro-organisms. In the environment, inorganic nitrogen occurs in a range of oxidation states as nitrate (NO_3^-) and nitrite (NO_2^-), the ammonium ion (NH_4^+) and molecular nitrogen (N_2). It undergoes biological and non-biological transformations in the environment as part of the nitrogen cycle (Chapman 1996).

Nitrogen species are also of atmospheric concern such as; photochemical smog, global warming and stratospheric ozone depletion. In aquatic environments, it is an indicator of sanitary quality. Studies has established that freshly polluted waters mostly contain organic (protein) nitrogen and ammonia. As time progresses, the organic nitrogen is gradually converted to ammonia nitrogen and later on if the aerobic conditions prevail, oxidation of ammonia to nitrite and nitrate. Consequently, freshly polluted waters are of great danger to public health than the long time previously polluted water, in a time period of 0 – 50 days (Sawyer *et al*. 2003). Unpolluted waters contain small amounts of ammonia and ammonia compounds, usually < 0.1 mg/l as N. Total ammonia concentrations measured in surface waters are typically less than 0.2 mg/l N but may reach 2-3 mg/l N. Higher concentrations of total ammonia could be an indication of organic pollution such as from domestic sewage, industrial waste and fertilizer run-off. Ammonia is, therefore, a useful indicator of organic pollution. Natural seasonal fluctuations also occur as a result of the death and decay of aquatic organisms, particularly phytoplankton and bacteria in nutritionally rich waters. High ammonia concentrations may also be found in the bottom waters of lakes which have become anoxic. Organic nitrogen consists mainly of protein substances (e.g. amino acids, nucleic acids and urine) and the products of their biochemical transformations (e.g. humic acids and fulvic acids). Organic nitrogen is also naturally subject to the seasonal fluctuations

of the biological community because it is mainly formed in water by phytoplankton and bacteria, and cycled within the food chain. Increased concentrations of organic nitrogen could indicate pollution of a water body (Chapman 1996).

The four nitrogen forms; ammonia, nitrite, nitrate and organic nitrogen are of interest in water resources. Although they all have different methods of determination, they are all customary reported as nitrogen, for a straightforward interpretation without the use of a factor (Sawyer *et al*. 2003).

Organic nitrogen is usually determined using the Kjeldahl method which gives total ammonia nitrogen plus total organic nitrogen (Kjeldahl N). The difference between the total nitrogen and the inorganic forms gives the total organic nitrogen content (Chapman 1996). The Kjeldahl method is a standard procedure and uses Sulfuric acid as the oxidizing agent (Sawyer *et al*. 2003). To assess the quality of the effluent discharged at UWWTP, in terms of its nitrogen content, the Total Kjeldahl Nitrogen (TKN) results are therefore considered.

2.7.5.2 Phosphorus

As described by Chapman (1996), Phosphorus is an essential nutrient for living organisms and exists in water bodies as both dissolved and particulate species. It is generally the limiting nutrient for algal growth and, therefore, controls the primary productivity of a water body. Artificial increases in concentrations due to human activities are the principal cause of eutrophication. In natural waters and in wastewaters, phosphorus occurs mostly as dissolved orthophosphates and polyphosphates, and organically bound phosphates. Changes between these forms occur

continuously due to decomposition and synthesis of organically bound forms and oxidized inorganic forms. The equilibrium of the different forms of phosphate that occur at different pH values in pure water. The recommended expression of phosphate concentrations is mg/l PO_4 – P. Natural sources of phosphorus are mainly the weathering of phosphorus-bearing rocks and the decomposition of organic matter. Domestic waste-waters (particularly those containing detergents), industrial effluents and fertilizer run-off contribute to elevated levels in surface waters. In most natural surface waters, phosphorus ranges from 0.005 to 0.020 mg/l PO_4 - P.

Phosphorus concentrations are usually determined as orthophosphates, total inorganic phosphate or total phosphorus (organically combined phosphorus and all phosphates). The dissolved forms of phosphorus are measured after filtering the sample through a pre-washed 0.45 µm pore diameter membrane filter. Particulate concentrations can be deduced by the difference between total and dissolved concentrations (Chapman 1996).

All the polyphosphates (molecularly dehydrated phosphates) gradually hydrolyze in aqueous solution and revert to the ortho from which they were derived. Therefore, determination of phosphate involves conversion to orthophosphate which is then measured colorimetrically (Sawyer *et al*. 2003 and Chapman 1996).

2.8 Guidelines and regulatory limits for chromium, solids, organics and nutrients

According to Mishra & Bharagava (2016), environmental and occupational exposure to Cr(VI) is still considered a "major human health issue". They further argued that, the need for greater understanding of chromium toxicity continues to spur research and in turn, new regulations. Due to toxicity concerns in the United States, concentrations of total Cr are regulated at 0.1 mg/l Cr in

drinking water and 5 mg/l leached from solids. The Cr(VI) is relatively soluble and can move more readily through soil to groundwater. The typical ratio of chromium in plants to chromium in soil is estimated to be 0.0045 or 0.45%.

The USEPA (1998) has set an equal limit of 0.10 mg/l for both Cr(III) and Cr(VI) in drinking water and regulates the different chromium emission under the Clean Air Act of 1990. The WHO has set the provisional guideline value for total Cr content in drinking water at 0.050 mg/l Cr, pending additional data and review, because the effects are determined largely by the oxidation state. In Namibia, as according to the Water Resources Management Act, Number 11 of 2013 (Namibian Government 2013) there are two guidelines in relation to Cr, as annexures as follows:

1. The Namibian Water Quality Standards for Effluents (NWQSE); for effluents to be discharged or disposed of in areas with potential for drinking water source contamination; international rivers and dams and in water management and other areas (Table 1).
2. The Namibian Water Quality Standard for Potable Water (NWQSPW); specific for water quality intended for human consumption and piped water supply.

Total chromium: ideal guideline; < 0.050 mg/l Cr and Acceptable standard; < 0.10 mg/l Cr Cr(VI); Not specified.

The NWQSE is the applicable standard for compliance of the effluent from UWWTP.

Table 1: Concentration limits for the water quality parameters of interest as set by the NWQSE (Source: Namibian Government, Water Resources Management Act 11 of 2013)

Parameter	**NWQSE**	
	Special standard	**General standard**

	(95 Percentile)	(95 percentile)
Temperature (^oC)	Not more than 10 ^oC higher than the recipient water body	
pH	6.5 – 9.5	6.5 – 9.5
TSS (mg/l)	< 25	< 100
TDS (mg/l)	< 500	< 500
Conductivity (mS/m)	< 75	< 75
COD (mg/l)	< 45	< 100
TKN (mg/l - N)	< 18	< 33
Soluble ortho-PO_4 (mg/l PO_4-P)	< 0.2	3.0
Cr, Total (mg/l Cr)	< 0.050	< 1.0
Cr (VI) (mg/l Cr)	<0.010	0.050

3 CHAPTER 3: MATERIALS AND METHODS

3.1 Study Area

3.1.1 Location of the study area

The UWWTP is located in the north eastern part of the city of Windhoek, at about 3 km north east of the Nampower's Von Eck coal power station, along the B1 western bypass high way and before the newly settlement of Elisenheim Village (Figure 1). The KWR flows in a north westerly direction, joining the Otjiseru ephemeral river which is part of the Upper Swakop ephemeral river

catchment and discharge into the Swakoppoort Dam. This dam forms part of the Central Water Supply Scheme (CWSS), which is the source of over 60% of potable water used in Windhoek supplied through the Namibian National Water Corporation (NAMWATER). The study therefore compared plants in two sites along the KWR; (1) the control site located at a distance of about 2000 m south of the UWWTP effluent discharge point and (2) the experimental site located at about 500 m north of the UWWTP effluent discharge point (Figure 2). The control site was placed at such a further distance from the discharge point to avoid proximity to the decommissioned UOPS, which was filled with sludge from its operation days. Both the control and the experimental site were about 600 m by 10 m in sizes and lies between the following GPS coordinates: Control site (-22 492128, 17.085389 and -22 498 135, 17.085314) Experimental site (-22 470517, 17.083254 and -22 465559, 17.080765).

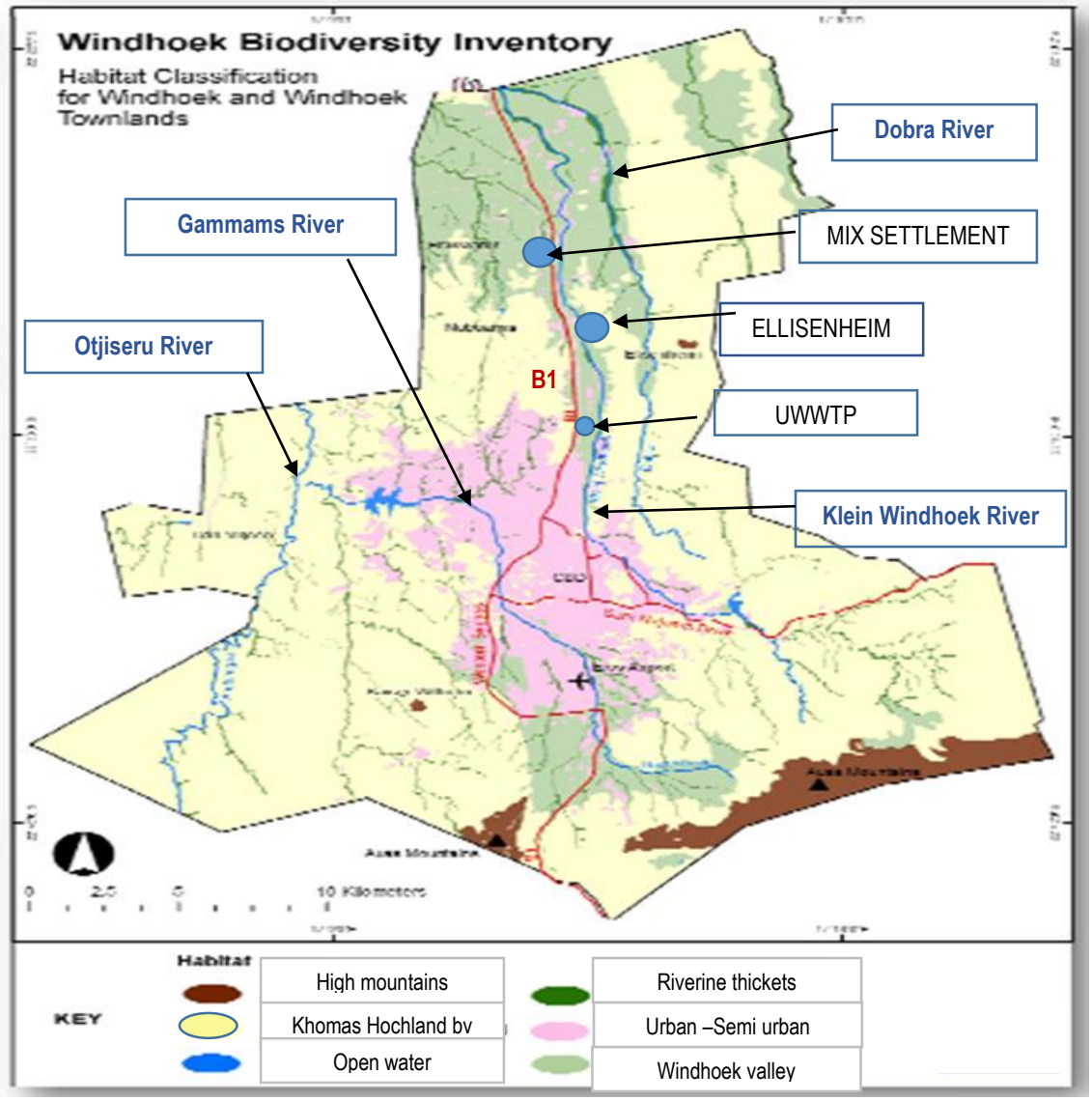

Figure 1: The map of Windhoek townland showing the location of the study area (Source: Enviro-dynamics 2009)

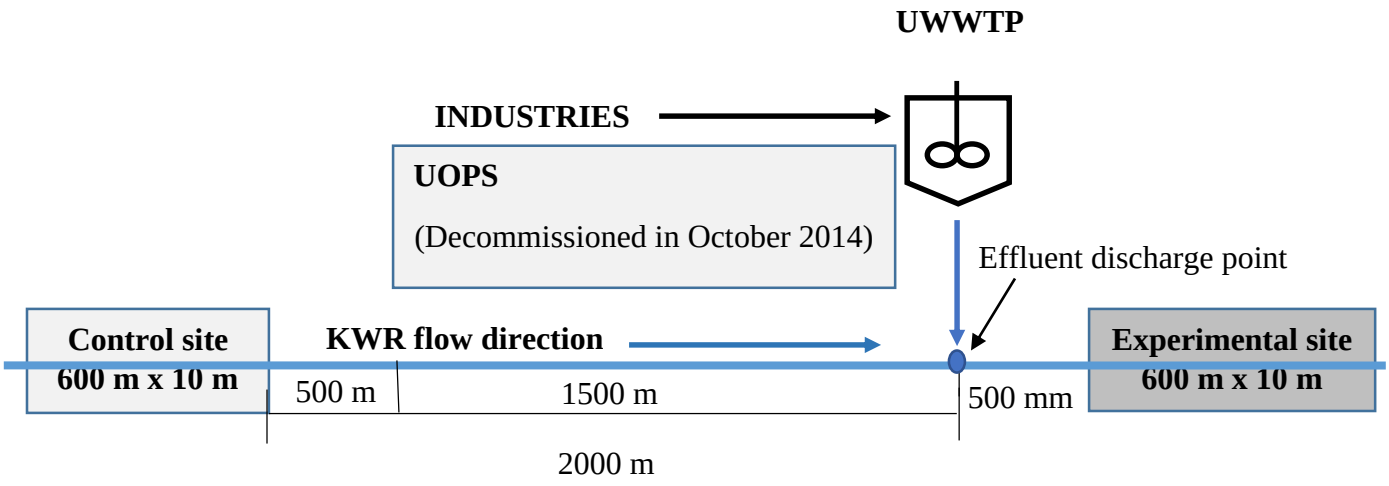

Figure 2: The sketch diagram of the study site, showing the control and the experimental site in relation to the UWWTP along the KWR. Not to scale

3.1.2 Climate

According to Barnard (1998) and Mendelson *et al*. (2002), the climate in Windhoek can be described as semi-arid. The summer or the wet season ranges from November to April, with a dry season mostly between May to October. The maximum temperatures range from 30 and 32 ^{0}C and the minimum temperatures lie between 5 and 18 ^{o}C (Government of the Republic of Namibia, Ministry of Works and Transport: Meteorological Services 2012). Windhoek mostly experiences summer rains, with a mean annual rainfall ranging between 300 and 360 mm (Barnard 1998).

3.1.3 Flora

The UWWTP lies in the Windhoek valley habitat, divided into alluvial and lowlands plains (Strohbach & Seely 2003). The vegetation in this valley includes three of the protected species such as *Vachellia erioloba, Boscia albitrunca* and *Searsia lancea,* as well as others such as *Vahlia capensis* subsp, *capensis, Selago dinteri* subsp. d*interi*, including some alien species such as *Prosopis glandulosa, Nicotiana glauca, Argemone ochroleuca, Datura inoxia, Arundo donax, Datura ferox* and *Pennisetum clandestinum (*Mannheiner *et al.* 2009). Other species also include *Senegalia mellifera, Leucosphaera baines*ii (Strohbach & Seely 2003). The KWR runs through

this valley from Avis dam along the western side of Windhoek. In this river there are species such as *Rumex lanceolatus, Cullen obtusifolia* and *Plantago major.*

3.1.4 Fauna

According to Strohbach & Seely (2003), the Windhoek valley habitat is naturally home to plain and sand burrowing animals (Strohbach & Seely, 2003; Envro-dynamics 2009). These could be classified as follows:

1 Mammals**:** include large herbivores such as the Red Hartebeest (*Alcelaphus buselaphus*), Duiker *(Sylvicapra grimmia*), Springbok (*Antidorcas marsupialis*), Steenbok (*Raphicerus campestris*), Oryx gazelle and Kudu (*Tragelaphus strepsiceros)*. Small mammals**: Endemic**; Hairy footed gerbil (*Gerbillurus paeba*),

Near threatened; South African hedgehog (*Atelerix frontalis*), Kaokoveld ground squirrer (*Xerus princeps*),

Vulnerable; Pangolin (*Mannis temminckii*), **Allien invasive**; House mouse (*Mus musculus*).

2 Birds species include; Dam (Red billed hornbill) *Tockus damarensis*, Monteiro's hornbill (*Tockus monteiri*), Carps (Black) Parus carp, **Endagered**: Booted eagle (*Aquila pennat*us).

3 Amphibians: **Endemic**; Dombe toad (*Bufo dombensis*), Hoesch's toad (*Bufo hoeschi*), Marbled rubber frog (*Phrynomntis annectens*).

4 Arthropods: includes; *Mantophasma zephyra*

5 Reptiles: Several **endemic species** of snakes such as; Boyle's beaked blind snake (*Rhinotyplops boylei*), Cape cobra (*Naja nivea*), Black necked spitting cobra (*Naya*

nigricollis nigricintha), Dwarf gecko (*Lydodactylus Bradfield*), Dwarf python (*Pytho anchietae*) and Namibian Rock Agama (*Agama planiceps*).

3.1.5 Geology, soil and the physical environment

According to Africon & EnviroNomics (2004) and Brink (1981), the geology riverine area of the KWR is characterized by biotite schist formation intersected by north-south running band of sand calcrete gravel and alluvium. The sand calcrete gravel can best described as an unconsolidated surficial deposit consisting of sand and calcrete, which acts as an infiltration medium for surface water. The presence of calcrete indicates perched water as it is highly permeable. In this area, as in most parts of Windhoek, there is generally poorly developed thin top soil, which is a product of the alluvium deposits of mainly fine sand and silts, intermixed with residual quartz pebbles. These river alluviums along ephemeral rivers courses and valleys, such as the KWR, comprises of sand gravel and stones to form the thickest soils.

3.2 Experimental design

A quantitative research was used in the experiment. As tabulated in Table 2 below, the components of the experimental design as considered for the three main aims of the study (i) to assess the potential uptake of Cr(VI) by *Rumex lanceolatus* and *Cullen obtusifolia*, and its potential toxicological effects on the total chlorophyll content in the leaves of these two species exposed to the treated industrial effluent from UWWTP. (ii) To assess the concentration of Cr(VI) discharged from UWWTP into the KWR, its compliance to NWQSE and its relationship to the increasing distance away from the discharge point. (iii) To assess the performance of the UWWTP and its compliance to the NWQSE with regard to the concentrations of solids, organics and nutrients in the effluent discharged into the KWR.

Table 2: The components of the experimental design for the three main aims of the study.

Main	**Groups**		**Repeated**	**Variables**		
Objective	**Control**	**Experimental**	**Trials**	**Independent**	**Dependent**	**Controlled (Significant)**
(i)	Plants not exposed to UWWTP effluent	Plants exposed to UWWTP effluent	60	Leaves Cr(VI) Concentrations	Total chlorophyll	Plant species, moisture, sunlight, soil type, etc.
(ii)	NWQSE: 0.050 mg/l Cr (VI)	UWWTP Effluent Cr(VI) concentrations	10	Distance Away from UWWTP	Effluent Cr(VI) Concentration	Flowrate, evaporation rate, rainfall, sediment/soil type, etc.
(iii)	1. UOPS removal efficiency for solids, nutrients and organics	UWWTP removal efficiency for nutrients, organics and nutrients	28	UWWTP treatment process	UWWTP removal efficiency for nutrients, organics and nutrients	Incoming concentrations of Solids, Organics and Nutrients, Flowrate
	2. NWQSE for Solids, Organics and Nutrients	UWWTP effluent concentrations for Solids, Organics and Nutrients	125	UWWTP treatment process	UWWTP effluent concentrations for Solids, Organics and Nutrients	Incoming concentrations of Solids, Organics and Nutrients, Flowrate

3.3 Selection of study plant species

In the month of April 2018, soon after the rainy season, a site familiarization tour was carried out on both the control and experimental sites, to observe the herbaceous plants that grow along the KWR and to identify herbaceous plants. Five herbaceous plant species were observed, which appeared to be well adapted to the environment, widely dispersed along the river and could be considered as natural occurring vegetation of the KWR.

A second visit to the study area was conducted for further observation and specimen collection in July of 2018. It was noted that two of the five herbaceous species initially identified were no longer available on the control site. This could be attributed to the dry weather, although some limited plants of the same specie could be observed on the experimental site where moisture content is high. Specimens of the three available plants species were collected and taken to the National Botanical Research Institute (NBRI) for identification. These three specimens were identified (Appendix 19) by the National Herbarium of Namibia, as *Rumex lanceolatus* from the Polygonaceae family, *Cullen obtusifolia* from the Fabaceae family and *Plantago major* from the Plantaginaceae family. *Plantago major* could not be found on the control site at the time of field study and it was therefore excluded from the study. The study finally focused on two species; *Rumex lanceolatus* and *Cullen obtusifolia.*

Figure 1: Photo of Rumex lanceolatus (Photo: Iiputa, G. 2019)

Figure 2: Photo of Cullen *obtusifolia* (Photo: Iiputa, G. 2019)

Species 1: *Rumex lanceolatus* (Figure 3), is an herbaceous plant with large green, smooth leaves that looks very similar to spinach leaves and browsed by porcupines. Its leaves are slightly wavy on the edges, borne on stalks and can grow up to 30 or 40 cm. It produces clusters of pale yellow flowers, which eventually turn into pale brown little fruits with dark brown seeds. It is one of the

two species commonly known as the common dock, smaller dock or smooth dock. Its distribution includes rivers, dams and various wet places and it is a naturalized exotic weed and has become common in many parts of the world. Traditionally the roots are used for the treatment of internal parasites (tapeworm and roundworm), and the whole plant is widely used for vascular diseases and internal bleeding. It is also applied externally to treat abscesses, boils and tumors. It contains oxalic acids, which in high concentrations causes oxalate poisoning in stocks, but medically used as a hemostatic agent. It also contains chrysophanol and glycosides which imparts its laxative effects. Its nitrates sometimes accumulate in toxic concentrations and contains other potential toxins such as rumicin, anthraquinone, chrysarobin, malic acid, tannic acid and tartaric acid (Hyde *et al*. 2019; Hutchings *et al.* 1996).

Species 2: *Cullen obtusifolia* also known as *Psoralea obtusifolia or Trigonella tomentosa*, (Figure 4) It is a herb which grows up to 60 cm tall, often forming a mat or cushion. It contains white strigose or grey hair but eventually glabrous, with many pale branches up to 75 cm long and widely spreading prostate to ascending from much branched rootstock. Its leaves are pinnately 3-foliolate, leaflets are 3 -30 by 2 -20 mm obovate or elliptic, obtuse at the apex (Hyde *et al*. 2019; Grimes 1997). Grimes (1997) further describes its habitat as grassy areas, edges of pans and rivers, hot and dry wooded grassland and scrub, usually on sandy soils, and an altitude range of up to 1000 m. Its worldwide distribution covers; Angola, Botswana, Namibia, Mozambique, South Africa and Zimbabwe. No recorded uses.

3.4 Data Collection

3.4.1 Determination of the total chlorophyll content in the leaves of the study plants

Two methods for determining chlorophyll content in the plant leaves were considered; (i) Laboratory based method (Spectrophotometric method) and (ii) field method (SPAD-502Plus

method). The Laboratory based methods such as atomic spectroscopy (Porra *et al*. 1989) and spectrophotometric method (Arnon 1949). These are the conventional methods used to estimate photosynthetic pigments in plant tissues in a laboratory and requires extraction of the pigments from the plant tissues, with a solvent such as N-N-dimethylformamide (DMF), ethanol, methanol, acetone or Diethyl ether (DEE), then followed by estimation of the photosynthetic pigment using the spectrophotometer (Sumanta *et al.* 2014). These methods are described as destructive in nature, time consuming, requiring specific equipment and solvents which are toxic to human health and environments (Kumar & Sharma 2019).

In contrast, the Soil-Plant-Analysis-Development (SPAD) or SPAD 502Plus instrument, is a handy, lightweight, self-calibrating, portable diagnostic devise to estimate the quantity of chlorophyll in the field (Minolta 2009). It has the advantage over the spectrophotometric method that it is non-destructive, convenient, accurate and time efficient (Schaper & Chacko 1991; Britol *et al.* 2011; Neto *et al*. 2005)

Although the SPAD-502Plus method saves time, space and resources in comparison to the conventional methods of determining the photosynthetic pigments, there is a need to validate the results obtained with it. In a study on comparison of four different non-destructive chlorophyll meters; CL-01, SPAD, Dualex and CCM-200, in estimating the chlorophyll content of plants under nutrient deficiency conditions, Kalaji *et al.* (2017) concluded that these devices provided reasonably accurate results under optimal nutrient conditions, the same devices gave different values for the same plants under the same nutrient deficiency. This therefore pointed out that these devices need to be validated against destructive methods under nutrient deficiency conditions. Furthermore, Samsone *et al*. 2007 concluded that the accuracy of the SPAD chlorophyll meter is specie specific and therefore its use must be preceded with accurate calibration of the chlorophyll

readings against a destructive method determined chlorophyll content. Against this background, the SPAD-502 Plus was chosen as the study method and validated against the spectrophotometric chlorophyll readings, for both *R. Lanceolatus* and *C. obtusfolia.*

3.4.1.1 The Spectrophotometry method

3.4.1.2 Plant sample collection

Approximately 1.0 to 2 kg of plant tissues consisting of shoots with leaves were collected and labeled with the following details: plant species, plant number and study site (1 or 2 for control or affected site respectively). A total of 20 plants tissues were collected from different plants of each species, from both the control (Not affected by UWWTP effluent) and the experimental site (affected by the UWWTP effluent), placed in black plastic bags and transported to the Laboratory. The plant tissues were then stored in a refrigerator at 4 ^{0}C.

3.4.1.3 Apparatus and materials

UV visible spectrophotometer (UV-1600PC UV-VIS) with 1cm glass cuvettes.

3.4.1.3.1 Plant sample analysis

From each of the 20 plants tissue samples collected as stated above, three leaves were taken from each plant and a 1cm^2 peace was caught from each fresh leave. Each 1cm^2 was immersed in 5ml of acetone (80%) in a properly labelled snap cap specimen tube and left in the fridge overnight. In this way the chlorophyll was extracted from the plant leaf.

Thereafter, the green extract was transferred to a glass cuvette, special care was taken not use plastic cuvettes as they will be attacked by the acetone. Then the Optical Density of these solutions were read taken with a UV visible spectrophotometer, at the wavelength of 645 and 663 nm, for

Chlorophyll *a* and *b* respectively. The total chlorophyll could then be determined in accordance with the following equation (Arnon 1949);

$$\text{Total chlorophyll (mg/l)}, C = C_a + C_b = 0.0202 \times OD_{645} + 0.00802 \times OD_{663} \quad (1)$$

For each plant, the average OP at both wavelengths for all the three leaves was taken and substituted in the above equation to give the Total chlorophyll, C, for each plant. A total of 20 chlorophyll concentration readings were recorded for both *R. lanceolatus* and *C. obtusifolia* plant species per study site (control and experimental), this gave a total of 40 chlorophyll concentration readings for each species. These chlorophyll concentration readings were then plotted on a scatter plot against the SPAD-502Plus values to establish a linear relationship between the two methods for each plant species. The resultant calibration equations were used to convert the SPAD value to chlorophyll concentration (mg/l) for each plant species.

3.4.1.4 The SPAD-502Plus method

The Chlorophyll meter SPAD-502Plus (Konica Minolta Inc.: 91001873) (Konica Minolta 2009) was used to determine the relative amount of chlorophyll present in the plant leaf. The relative chlorophyll amount is calculated based on the amount of light transmitted by the leaf in two wavelength regions in which the absorbance of chlorophyll is different. The SPAD-502Plus has two Light Emitting Diodes (LED) built in its measuring head, which emits lights in sequence when the head is closed. These LEDs are red and infrared with peak wavelengths of approximately 650 nm and 940 nm respectively.

The amount of chlorophyll present in the plant leaves is an indicator of the overall condition of the plant itself. Generally, healthier plants contain more chlorophyll than less healthy ones (Neto et al.

2017). This supports an earlier conclusion by Netto *et al*. 2005, based on their study on photosynthetic pigments, nitrogen, chlorophyll *a* fluorescence and SPAD-502 readings in coffee leaves, that SPAD values lower than 40 showed impairment in the photosynthetic process. The SPAD value determined by this instrument provided an indication of the relative amount of chlorophyll present in plant leaves.

The SPAD-502Plus instrument was firstly calibrated in accordance with the manufacturer's operational manual, before any measurement was taken. All measurements were taken in the field, with the leaves still attached to the plants. The instrument requires a measuring area of 2 mm x 3 mm and leaf thickness of up to 1.2 mm. The measurements were carried out as follows:

4. The individual 2x3 mm leaf sample to be measured were timeously inserted into the sample slot of the instrument measuring head

5. Care was taken to ensure that the leaf was clean and free of dust or water, by wiping it with a paper towel or cloth.

6. The leaf sample was placed between the emitting and receiving windows. Only the thin part of the leaf was used for these measurements (Figure 5); extremely thick parts of the leaf were avoided. Only one reading was taken from each leaf because the leaves of both study plants species had fine leaves, without many veins.

7. After inserting the leaf between the windows, the measuring head was closed by pressing the finger rest and holding it firmly until a beep sound was heard and the measured values appeared in the display. The step was repeated, whenever a series of beep sound were heard and an error message appeared. This indicated that the measurement was not performed correctly or the measuring head was not closed completely.

8. The SPAD value, which corresponds to the total chlorophyll content present in the sample leaf was depicted in the little instrument screen.

Figure 3: An example of how to take a chlorophyll reading (SPAD value) of a plant leaf in the field. (Photo: Iiputa, G. 2019)

For each of the two study plant species, 20 individual plants were randomly selected for both the control and affected site. Readings were repeated three times for each of the 20 plants, which then

resulted in a total of 60 SPAD value readings per plant species per site (Control and Experimental site).

3.4.1.5 Validation of the SPAD-502Plus chlorophyll meter method

A scatter plot was prepared for both *R. lanceolatus* (n = 40) and *C. obtusifolia* (n =40) to determine correlation between chlorophyll estimates given by the SPAD-502Plus chlorophyll meter method and the spectrophotometric method (Figure 7a and 7b) A regression analysis were also carried out (Appendix 1 and 2)

3.4.2 Determination of the concentration of Cr(VI) in the plant leaves samples by Direct Spectroscopic (DS) Method

3.4.2.1 Plant tissue sample preparation

Using the plant tissue samples collected in 3.4.1.1.1, the plant leaves were separated from the shoots then rinsed with tap water, followed by washing with deionized water twice before drying them in an oven (Scientific series 2000, Economy oven, model: 220) at 60 ^{o}C for 24 hours and in accordance with EPA Method 3050B (USEPA 1996). Thereafter the dried plant leaves were ground into fine powder using a pestle and mortar and placed in labeled 50 ml plastic snap cap vials. They were then stored at room temperature in cupboards in the laboratory.

3.4.2.2 Plant tissue samples digestion

The samples were digested in accordance with the EPA Method 3050B (USEPA 1996). From each 50 ml plastic snap cap vial, three replicates of 250 mg of the powdered plant leaves samples were weighed, using a weighing balance (Make: Adam, Model: AFP800L) and each transferred to 15 ml glass vial. A total of 60 vials were prepared for each plant species per site (Control and affected

site). The plant leaves samples and a blank (deionized water) were then digested in one step digestion procedure, by adding a di-acidic mixture of 4 ml nitric acid (HNO_3) and 1 ml hydrochloric acid (HCL) (4:1 v/v) in a fume hood. These vials were closed and immersed in a water bath and heated to about 90 ^oC for two hours. After 2 hours of heating, the plant leaves were completely digested, with a golden yellow fume build up in the vials above the mixture. The samples were left to cool down for at least another one hour, after which the vials were opened and filled up with deionized water. The cooled samples were filtered through a filter paper (Whatman No.1, Whatman International Ltd) into 100 ml volumetric flasks, and later filled up to 100 ml with deionized water.

3.4.2.3 Preparation of the calibration curve for the DS method

A volume 250 ml containing 50 mg/l Cr as a Potassium dichromate ($K_2Cr_2O_2$) stock solution was prepared by dissolving 35.35 mg of $K_2Cr_2O_2$, pre-dried in an oven at 110 ^0C for 1 hour, followed by 30 min in a desiccator, in deionized water in a 250 ml volumetric flask then further filled to the mark with deionized water. This stock solution was then covered with aluminum foil and stored in a closed cupboard.

A $K_2Cr_2O_2$ standard solution was then prepared daily from the stock solution, by transferring a volume of 10 ml stock solution with the use of a pipette into a 100 ml volumetric flask, filled up to the mark with deionized water to make a concentration of 5 mg/l Cr solution.

A freshly made standard solution was used to make serial dilutions of $K_2Cr_2O_2$ standard solutions in 100 ml volumetric flasks: The required volumes of the standard solution were determined using

the serial dilution equation (Equation 2), for the Cr(VI) concentrations of; 0.0 (blank), 0.02, 0.04, 0.06, 0.08 and 0.10 mg/l Cr(VI)

$$C_1V_1 = C_2V_2, \quad (2)$$

Where C_1 equals to the Cr (VI) concentration in mg/l Cr(VI) of the standard solution and V_1 the required volume (liters, l) of the standard solution, while C_2 and V_2 are Cr(VI) concentrations (known) and Volume of the solution to be made respectively. The volumetric flasks were filled to the mark with deionized water.

The absorbances readings of the solutions were then taken with the **UV** visible spectrophotometer (UV-1600PC UV-VIS), measured directly at 363 nm with a path length of 1 cm using quartz cuvettes. The solutions with their corresponding volumes and readings of absorbance taken were tabulated and a further used to construct a calibration curve (Table 3).

Table 3: The prepared Cr(VI) solutions and their respective absorbance readings.

Solution No.	C, (C_1), mg/l Cr(VI)	Vol. (V_1), ml	Absorbance			Average Absorbance
			1	2	3	
1	0.00	0.00	0	0	0	0.00
2	0.02	0.20	0.422	0.422	0.421	0.42
3	0.04	0.40	0.982	0.979	0.982	0.98
4	0.06	0.60	1.523	1.52	1.52	1.52
5	0.08	0.08	1.93	1.898	1.9	1.91
6	0.10	1.00	2.303	2.304	2.301	2.30

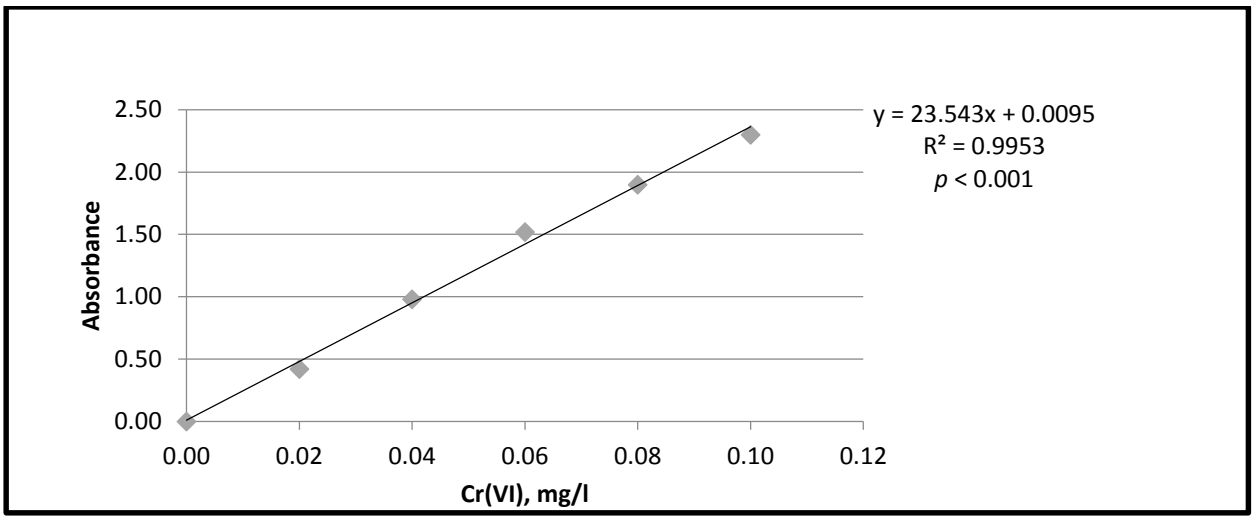

Figure 4: The calibration curve for the Cr(VI) prepared standard solutions using the UV-Vis spectrophotometry at 363 nm (n = 6)

As shown in Figure 6, the Coefficient of determination for the measured Cr(VI) concentration R^2= 0.9953. This shows that about 99.5% of the absorbance readings taken can be attributed to the Cr(VI) concentration. The regression analysis (Appendix 4) further revealed that there was a very strong relationship between the Cr(VI) concentration and the absorbance readings, with r(10) = 0.998, p = 0.001. Therefore, all points accurately fit the statistical model with minimal variability.

This calibration curve gave equation, $y = 23.543x + 0.0095$ (3)

Where, **y** = absorbance readings for the samples given by the **UV** visible spectrophotometer (UV-1600PC UV-VIS), at 363 nm wavelength,

x = the corresponding Cr(VI) concentration in mg/l Cr(VI), which can be calculated as the subject of the equation.

The regression analysis equation is therefore used to predict all the concentrations of Cr(VI).

3.4.2.4 Validation of the DS method

The validity of the direct spectrometric method was evaluated by comparing it to the commonly accepted 1.5-diphenylcarbazide (DPC) colorimetric assay kit, supplied by Merck & Co, carried out by the Scientific Services Laboratory of the City of Windhoek. In this exercise, three samples namely, Raw, Taxidermy and Paints were organized by the Chemistry section of the Scientific Services Laboratory, City of Windhoek, as part of their routine waste water monitoring program. These samples were shared with the Ecological studies laboratory at UNAM, in volumes of 250 ml, contained in glass bottles and then analyzed independently between these two laboratories. The Scientific Services Laboratory used the DPC kit method whereas UNAM Ecological studies laboratory used the Direct Spectroscopy method. The results obtained are as shown in the results section (Figure 11)

3.4.2.5 Sample analysis by DS method

The concentration of Cr(VI) in the digested plant leaves solutions was determined by direct spectroscopic method, using the **UV** visible spectrophotometer (UV-1600PC UV-VIS), measured directly at 363 nm with a path length of 1 cm using quartz cuvettes (Kim & Om 2013). All samples were analyzed within 24 hours after digestion, in order to minimize any possible change in the concentrations of Cr(VI). All absorbance for the samples in each 100 ml volumetric flask were measured in triplicates. The plant leaves sample digestion and analysis procedures were repeated three more times, making it two times for each plant species.

The Cr(VI) concentration data collected in this section was used in combination with Chlorophyll concentration data from section 3.3.1 as inputs for the objective of determining the effect effects of Cr(VI) concentration on the chlorophyll content of the plants.

3.4.3 Effect of distance from discharge point on Cr(VI) concentration.

In order to determine changes in concentrations of Cr(VI) in the water discharged from the UWWTP, water samples were collected at six points along the KWR; at 0, 2, 4, 6, 8 and 10 km (Table 4).

Table 4: Water sampling points from the water discharged from UWWTP along the KWR

Sample point number	Site Description	Approximate distance from the UWWTP effluent discharge point (km)	GPS Coordinates:	
			South	East
1	UWWTP discharge	0	-22,473895	17,084036
2	Elisenheim bridge	2	-22,470596	17,083265
3	Before Elisenheim WWTP discharge	4	-22,440800	17,074639
4	West of Dobra turn off	6	-22,420252	17,075025
5	IJ Smith Trans Co. bridge	8	-22,405017	17,073652
6	Mix informal settlement crossing	10	-22,386527	17,070905

Water samples were collected in 250 ml glass bottles at the above sampling points, one bottle per sample point. The samples were collected in the morning between 10h00 – 12h00, over one month

between 15 August – 19 September 2019. A total of 10 samples were collected from each sampling point and gave an overall total of 60 samples.

The samples were transported to the Laboratory and analyzed within 5 hours at room temperature. These samples were analyzed for Cr(VI), ug/l, using the same direct spectroscopy method described above. The following parameters were recorded from each water sample collected using a handheld multi-parameter meter- Conductivity (mS/m), pH and Temperature (^{0}C).

The Cr(VI) concentration results from the effluent samples from the discharge point and further along the river were compared to the Namibian Water Quality Standards for Effluents (NWQSE) of < 0.05 mg/l Cr(VI) as part of the last study objective.

3.4.4 Comparisons of the performance of the UOPS and the UWWTP in terms of their removal efficiencies for solids, organics and nutrients

The data for this objective were provided by the City of Windhoek from its extensive database of weekly analysis part of their regular monitoring program. The initial data supplied included; pH, Temperature (^{o}C), NH_4 (mg/l as N) NO_2 (mg/l as N), NO_3 (mg/l as N), Conductivity (mS/m), TSS (mg/l), COD (mg/l), TKN (mg/l as N), ortho-PO_4 (mg/l as P). They were taken from samples collected from the raw effluent into UOPS and UWWTP as well as the final effluent released.

The data compared were for the year 2012 (UOPS) and 2017 (UWWTP), the data set of 28 (n = 28). The parameters considered were TDS (mg/l) calculated from Conductivity (mS/m) with a multiplication factor determined for UWWTP of 6.7, TSS (mg/l), COD (mg/l), TKN (mg/l as N), ortho-PO_4 (mg/l as P). The removal for each parameter were calculated as differences between the concentration in the influent raw water and the final effluent from each plant. Subsequently, the

Removal Efficiencies (RE, %) were determined for each parameter for each plant, using Equation 4 (Tchobanoglous *et al.* 2003)

$$RE = \frac{(C_o - C_e)}{C_o} \times 100 \quad (4)$$

Where,

C_0 = Concentration in the influent raw (mg/l)

C_e = Concentration in the final effluent (mg/l)

3.4.5 Comparison of the concentrations of solids, organics and nutrients in the effluent discharged from the UWWTP into the KWR with the allowable concentration limits set by the NWQSE general standard.

The data for this objective were supplied by the City of Windhoek. The data considered were taken from the UWWTP, final effluent sample point, sampled over a period of 2.5 years, as from January 2017 to May 2019. These gave a total data set of 125 (n = 125). The parameters considered were TDS calculated (mg/l), TSS (mg/l), COD (mg/l), TKN (mg/l as N), ortho-PO_4 (mg/l - P). The concentrations of these parameters were compared with the corresponding NWQSE general standards values as shown in Table 1.

3.5 Data analysis

The data analysis was carried out using Microsoft Excel and IBM-SPSS-STATISTICS, Version 26, according to the study objectives.

3.5.1 Comparison of the total chlorophyll content in the leaves of *Rumex lanceolatus* and *Cullen obtusifolia,* exposed to the effluent from UWWTP and in those not exposed to the effluent from UWWTP

The chlorophyll data were firstly tested for normality using Shapiro Wilk Test and then followed the applicable test for significance difference. The percentage change in the Chlorophyll concentration between the two groups were also determined for each species using Equation 5:

$$\text{Percentage Change in Concentration} = \frac{(C_2 - C_1)}{C_1} \times 100\% \quad (5)$$

Where,

C_1 = Average or Median Concentration of Chlorophyll or Cr(VI) for the plants Not exposed to UWWTP effluent. C_2 = Average or Median Concentration of Chlorophyll or Cr(VI) for the plants Exposed to UWWTP effluent.

3.5.2 Comparisons of the concentration of Cr(VI) in the leaves of *Rumex lanceolatus* and *Cullen obtusifolia,* exposed to the effluent from UWWTP and those not exposed to the effluent from UWWTP.

The Cr(VI) data were firstly tested for normality using Shapiro Wilk Test and then followed the applicable test for significance difference. The percentage change in the Cr(VI) concentration between the two groups were also determined for each species using Equation 5:

3.5.3 Relationship between the concentration of Cr(VI) in the plant leaves and the total chlorophyll content in the leaves of *Rumex lanceolatus* and *Cullen obtusifolia* exposed to the effluent from UWWTP.

A simple linear regression analysis was used to determine the relationship between the concentration of Cr(VI) in the plant leaves and the total chlorophyll content in the leaves of *Rumex lanceolatus* and *Cullen obtusifolia* exposed to the effluent from UWWTP.

3.5.4 Effect of distance from the discharge point on the concentration of Cr(VI)

A simple linear regression analysis was used to test the relationship between distance from the discharge point and the concentration of Cr(VI).

3.5.5 Comparison of the concentration of Cr(VI) in the effluent discharged from the UWWTP into the KWR with the allowable general standards set by the NWQSE

A one sample T-test (df = 59) at 95% confidence interval was used to compare the mean concentration of Cr(VI) measured in the effluent discharged from the UWWTP into the KWR and flowed over a distance of 10 km, to the allowable general standard of 0.05 mg/l Cr(VI) as set by the NWQSE.

3.5.6 Comparisons of the performance of the UOPS and the UWWTP in terms of their removal efficiencies for solids, organics and nutrients.

The data for the removal of TSS, TDS, COD, TKN and ortho-PO4 where tested for normality with Shapiro-Wilk test and further with the relevant test for significant difference between the removal

of these parameters by UOPS and UWWTP, at 95% confidence interval (df = 54). The Median Removal efficiencies were also calculated and compared.

3.5.7 Comparison of the concentrations of solids, organics and nutrients in the effluent discharged from the UWWTP into the KWR with the allowable standards set by the NWQSE.

One sample T-test were carried out as follows; TSS (df = 124), TDS (df = 124), COD (df =125), TKN (df =122) and ortho-PO_4 (df = 124), at 95% confidence interval, to compare the mean concentrations of TSS, TDS, COD, TKN and ortho-PO_4 measured in the effluent discharged from the UWWTP into the KWR to the allowable general standards (Table 1) as set by the NWQSE.

4 CHAPTER 4: RESULTS

4.1 Validation of the SPAD-502Plus chlorophyll meter method

Figure 7 and 8 respectively shows the linear relationship between the SPAD value and the spectrophotometric method determined chlorophyll concentration taken from the leaves of *R. lanceolatus* and *C. obtusifolia* plants from both the affected and not affected by UWWTP effluent sites.

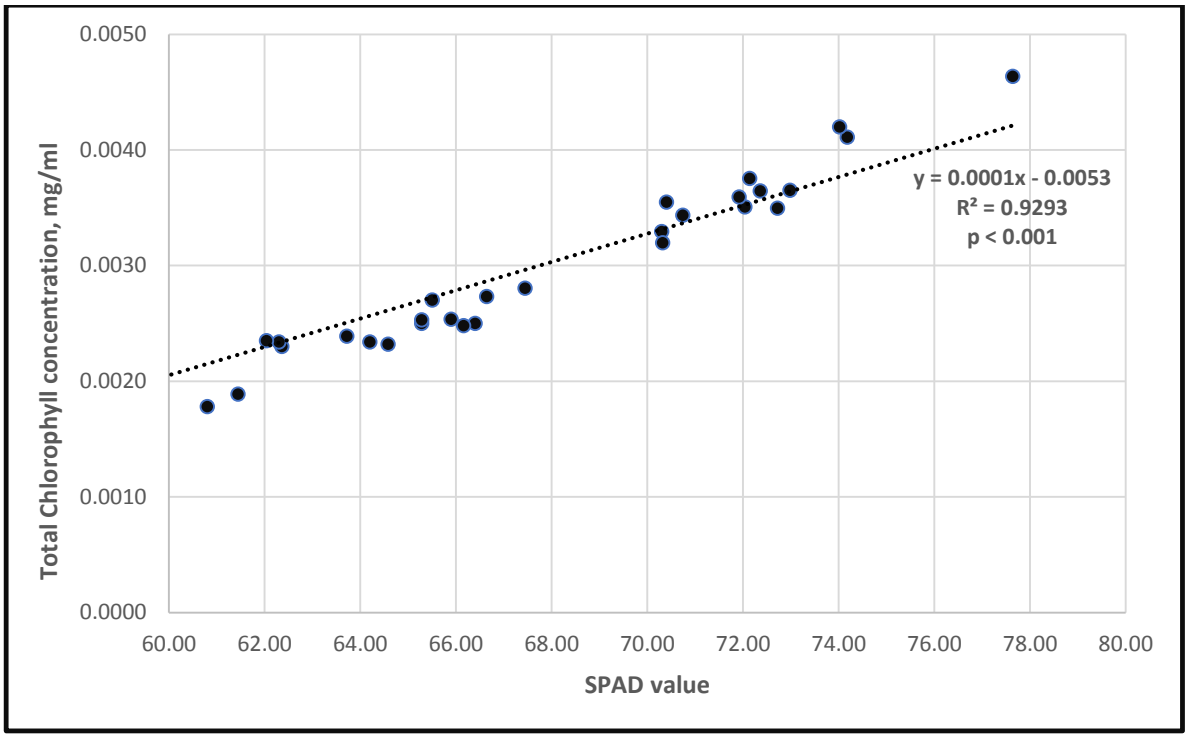

Figure 5: The linear relationship between the SPAD value and Spectrophotometric chlorophyll (mg/ml), for R. lanceolatus, both exposed and not exposed to UWWTP, (n = 40)

As shown in Figure 7, the Coefficient of Determination for the measured SPAD value; R^2= 0.929. This shows that about 92.9% of the SPAD value can be attributed to the concentration of chlorophyll in the leaves of *R. lanceolatus* as determined by the spectrophotometric method. The regression analysis further revealed that there was a very strong relationship between the SPAD values and the concentration of chlorophyll of the *R. lanceolatus* as determined by the spectrophotometric method $r(38) = 0.96$, $p < 0.001$ (Appendix 1)

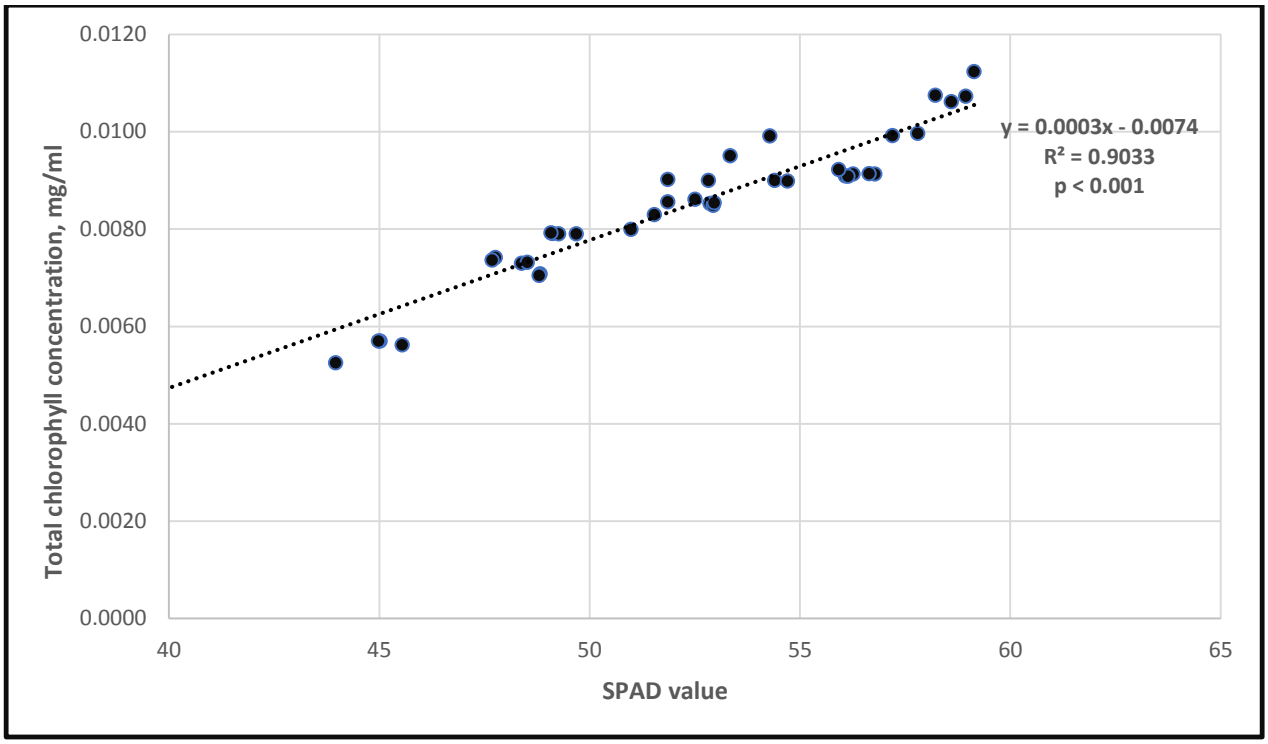

Figure 6: The linear relationship between the SPAD value and Spectrophotometric chlorophyll (mg/ml), for *C. obtusifolia*, both exposed and not exposed to UWWTP effluent, (n = 40)

Figure 8 also shows that the Coefficient of Determination for the measured SPAD value; R^2= 0.903. This shows that about 90.3% of the SPAD value can be attributed to the concentration of chlorophyll in the leaves of *C. obtusifolia* as determined by the spectrophotometric method. The regression analysis further showed that there was a very strong relationship between the SPAD values and the concentration of chlorophyll of *C. obtusifolia* as determined by the spectrophotometric method $r(38) = 0.95$, $p < 0.001$ (Appendix 2)

These results (Figure 7 and 8) shows that the SPAD values accurately fit the statistical model with minimal variability. This therefore assures that the SPAD 502Plus is 90% reliable as a method to estimate the chlorophyll content in *R. lanceolatus* and *C. obtusifolia.*

4.2 Total chlorophyll content in the leaves of *Rumex lanceolatus* and *Cullen obtusifolia,* exposed to the effluent from UWWTP and in those not exposed to the effluent from UWWTP

The Shapiro -Wilk test (Appendix 5) showed that the total chlorophyll content data for *Rumex lanceolatus* from the site not exposed to the effluent from UWWTP were not normally distributed ($df = 60$, $p < 0.015$). This data set had an outlier of -2.39 mg/l, it was adjusted to a data average of 1.7 mg/l, but it still did not make any significant change in the distribution of the data. The data for the plants exposed to the effluent from UWWTP were normally distributed ($df = 60$ and $p = 0.298$). Consequently, a Mann-Whitney U test for non-parametric data (Appendix 5) was used to test for significant differences in the chlorophyll content data between the two sites at 95% confidence interval ($df = 120$).

For *Cullen obtusifolia*, the chlorophyll data were normally distributed from the plants on both the site exposed and not exposed to the effluent from UWWTP ($df =60$, $p = 0.519$) and ($df = 60$, $p = 0.731$) respectively (Appendix 6). Then followed by an independent t-test, at 95% confidence interval, was used to tested for significant differences in the chlorophyll content data between the plants from the two sites.

The results for the tests for significant difference (Appendix 7) showed significantly lower total chlorophyll content of both plants species, *R. lanceolatus (*Mann-Whitney U test, $U = 396$, $df = 120$, $p < 0.001$) and the Independent samples t-test; *C. obtusifolia* ($t(118) = 4.496$, $p < 0.001$) (Appendix 8) naturally growing in the soil containing the treated effluent from the UWWTP than the plants growing in the control sites free from the UWWTP effluent pollution. Figure 9 and 10 depicts the graphical representations of the effect of Cr(VI) in the effluent from UWWTP on the chlorophyll concentrations of both plant species.

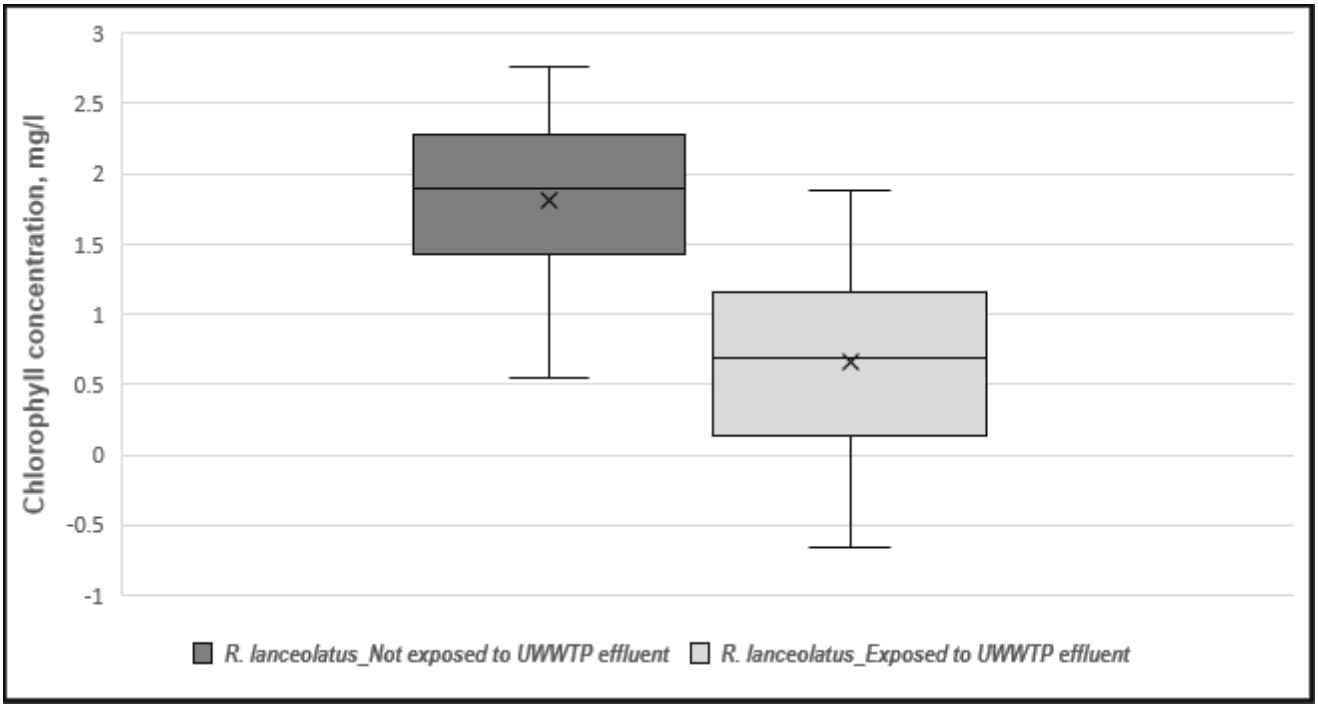

Figure 7: Effect of Cr(VI) in effluent from UWWTP discharge on chlorophyll content in the leaves of *R. lanceolatus* (n = 60).

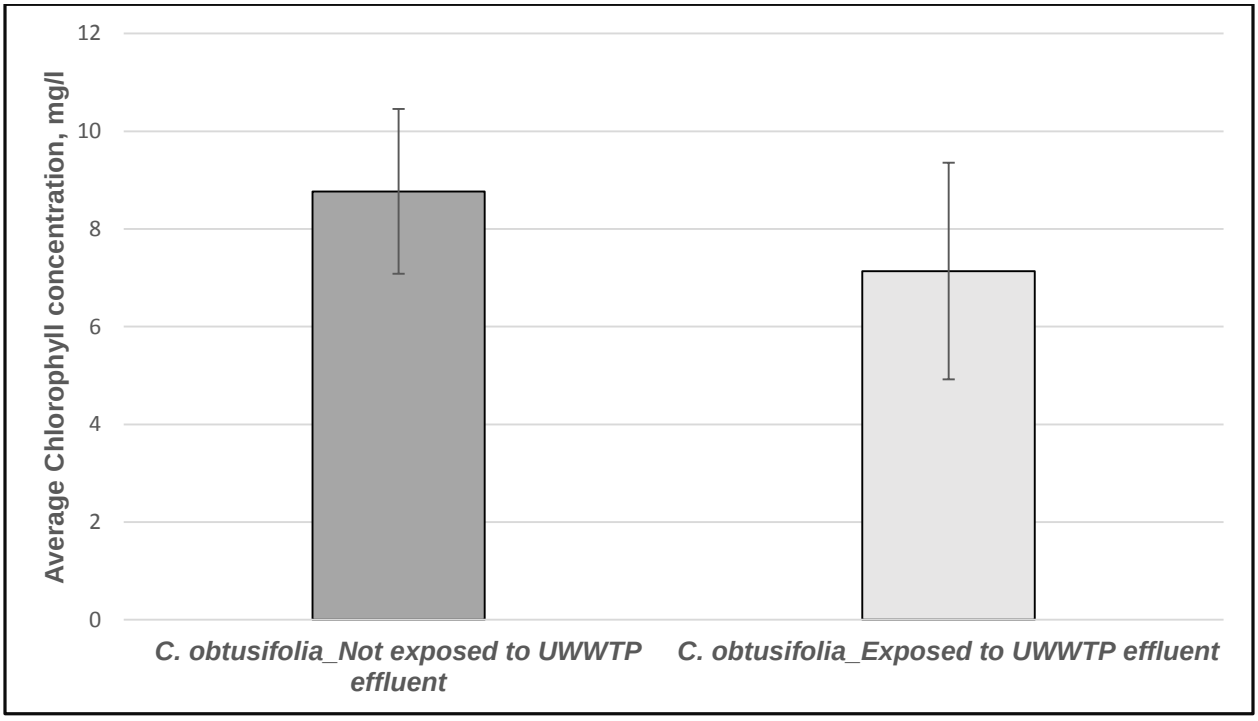

Figure 8: Effect of Cr(VI) in effluent from UWWTP discharge on chlorophyll content in the leaves of *C. obtusifolia*. Bars represent Standard Deviation (n = 60).

As shown in Figure 9, the results for chlorophyll concentration in the leaves of *R. lanceolatus* has a median of 1.89 mg/l for plants not exposed to the effluent from UWWTP and 0.856 mg/l plants exposed to the effluent from UWWTP. The box plot further confirmed that the data between the two sites were significantly different. Furthermore, Figure 10 shows that the average chlorophyll concentration in the leaves of *C. obtusifolia* plants not exposed to effluent from UWWTP exhibited 8.77 mg/l of chlorophyll and 7.14 mg/l of chlorophyll for the plants exposed to the effluent from UWWTP. The results (Table 5) further showed that the reduction in the chlorophyll content in plants exposed to Cr(VI) containing effluent was greater in *R. lanceolatus* (63.8%) than in *C. obtusifolia* (18.6%).

Table 5: The percentage reduction in Chlorophyll content of the plants as a result of Cr(VI) exposure.

Plant species	**Chlorophyll Concentration, mg/l**		**% Reduction**
	Not exposed UWWTP effluent	**Exposed to UWWTP effluent**	
R. lanceolatus (Median)	1.890	0.685	63.8
C. obtusifolia (Average)	8.77	7.14	18.6

4.3 Concentration of Cr(VI) in the leaves of *Rumex lanceolatus* and *Cullen obtusifolia,* exposed to the effluent from UWWTP and those not exposed to the effluent from UWWTP

4.3.1 Validation of the DS Method

Figure 11 shows the results obtained from the analysis of three samples using the DS method and the 1,5-dipenylcarbazide (DPC) Kit method.

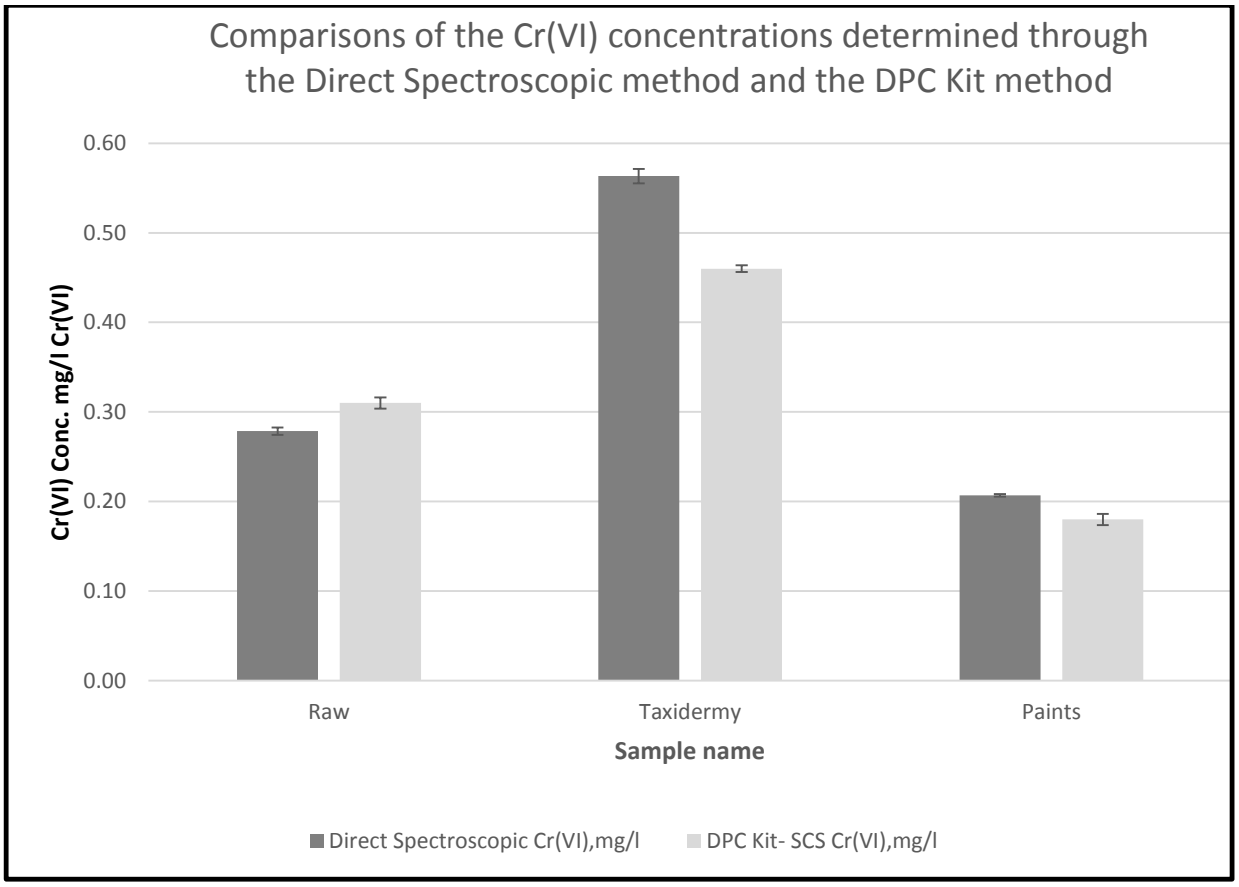

Figure 9: Comparison of the Cr(VI) concentrations determination by the Direct Spectroscopy method and by the 1,5-dipenylcarbazide (DPC) Kit method at the Scientific Services Laboratory (City of Windhoek). Bars represent Standard deviation (n = 3).

As shown in Figure 11, the two Cr(VI) determination methods of Direct Spectroscopy and DPC kit compares statistically well with minimal variability. The data were tested for normality with Shapiro Wilk Test, which showed that the two methods are 88% correlated, the data vs z-score plot showed a Linear and therefore the data was approximately normally distributed. The data was further tested for significance with the independent sample t-test, which showed that, the analytical results given by the DPC Kit method (M = 0.35, SD = 0.19, n = 3) were hypothesized to be equal to the analytical results given by the DS method (M = 0.32, SD = 0.14, n = 3) and there is no significant difference between them, t(3) = 2.78, p = 0.41 (one tail) (Appendix 4)

4.3.2 Concentration of Cr(VI) in the plant leaves

The Shapiro Wilk test showed that the data for concentration of Cr(VI) in the leaves of *Rumex lanceolatus* exposed to the effluent from UWWTP and those not exposed to the effluent from UWWTP were normally distributed, with df = 60 for both sites and (p = 0. 498 and 0.336) for control and experimental site respectively. Therefore, an independent t-test, at 95% confidence interval, was used to tested for significant differences in the concentration of Cr(VI) in the leaves of this plant species between the two sites (df = 118).

This test however, showed that the data for the concentration of Cr(VI) in the tissues of *C. obtusifolia* not exposed to the effluent from UWWTP were normally distributed (df = 60 and p = 0.582), while the Cr(VI) data for *C. obtusifolia* from the site exposed to the effluent from UWWTP were not normally distributed (df = 60 and $p < 0.001$). There were high values considerably outliers; 1.313 and 2.963 mg/l Cr(VI) in this data set from the plants exposed to the effluent from UWWTP and were changed to the data average of 0.41, but still did not make any change in the distribution of the data. Consequently, a Mann-Whitney U test for non-parametric data was carried

out to test for significant difference in the concentration of Cr(VI) between the two sites at 95% confidence interval (df = 120).

The results for the tests for significance showed a significantly higher concentration of Cr(VI) in the plant leaves of *R. lanceolatus;* independent samples t-test ($t(118) = 5.692$, $p < 0.001$) naturally growing in the soil containing the treated effluent from the UWWTP as compared to their controls growing in an environment free from the UWWTP effluent pollution. They however showed no significant difference between the two groups for *C. obtusifolia;* (Mann-Whitney U test, $U = 1512.50$, df= 120, $p = 0.131$), Figure 12 and 13 below depicts the graphical representation of the comparisons of the Cr(VI) concentrations for each plant between the two study sites.

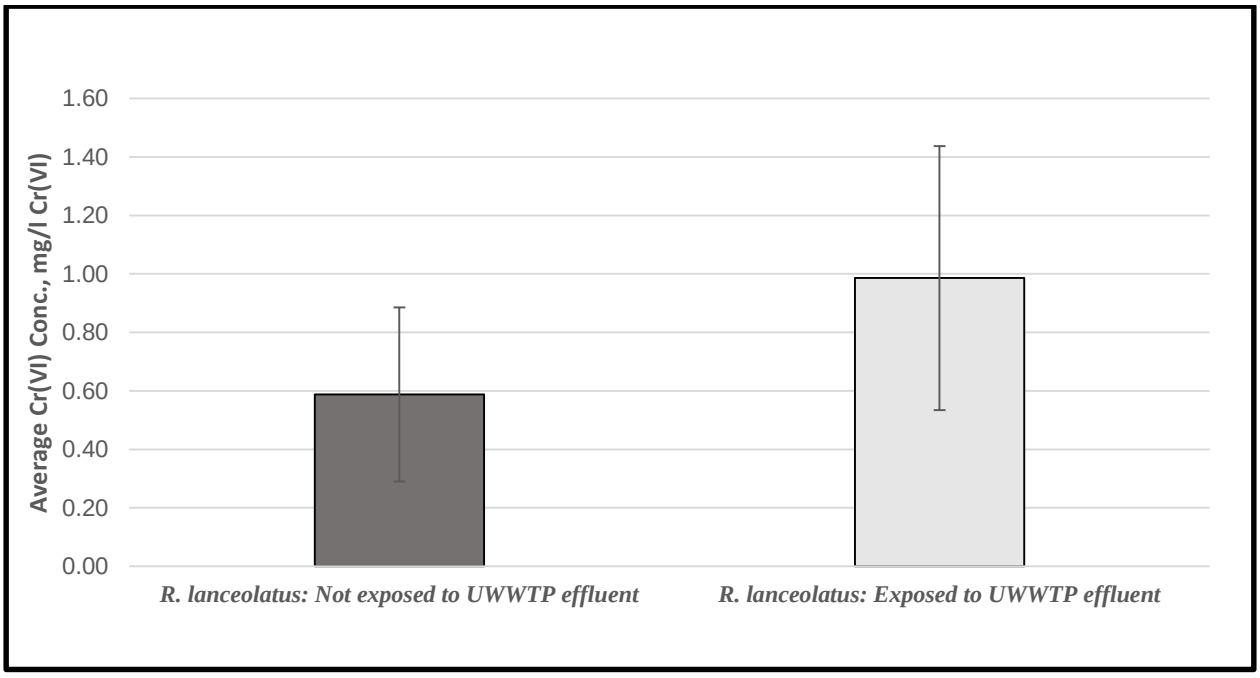

Figure 10: Effect of the effluent discharged from UWWTP on the Cr(VI) concentration in the leaves of *R. lanceolatus*. Bars represent Standard Deviation (n = 60).

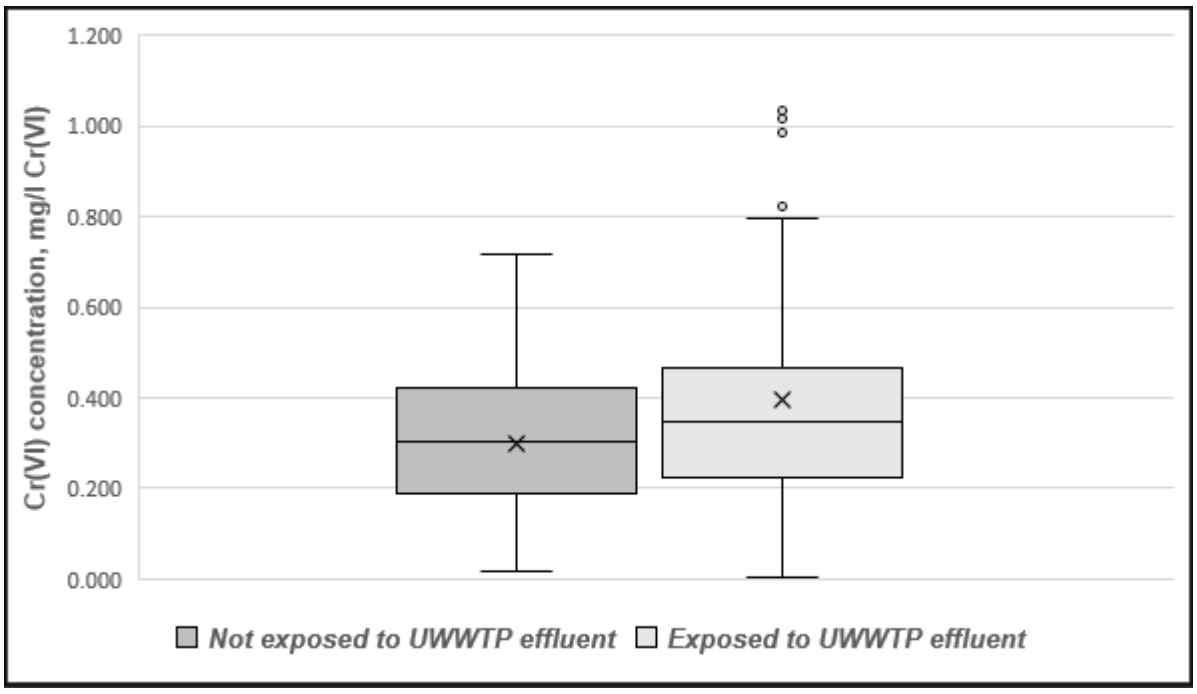

Figure 11: Effect of the effluent discharged from UWWTP on the Cr(VI) concentration in the leaves of *C. obtusifolia* (n = 60).

As shown in Figure 12, the concentrations of Cr(VI) in the leaves of *R. lanceolatus* displayed an average of 0.59 *m*g/l for the plants not exposed to the effluent from UWWTP and a higher average of 0.99 mg/l. Figure 13 present the not normally distributed data for Cr(VI) concentration in the leaves of *C. obtusifolia*, with a median of 0.301 mg/l for the plants not exposed to UWWTP effluent and higher median of 0.346 mg/l for the plants exposed to UWWTP effluent. These results (Table 6) indicated increases in the Cr(VI) concentrations in the leaves of these plants exposed to the effluent from UWWTP, at 67.8% for *R. lanceolatus* and 15.0% for *C. obtusifolia*. The results also showed that *R. lanceolatus* accumulates nearly 4 times as much Cr(VI) in its leaves than *C. obtusifolia* growing in the same environment.

Table 6: The percentage increase in the Cr(VI) concentration in the leaves of the study plants exposed to UWWTP effluent.

Plant species	Cr(VI) concentration in the leaves, mg/l Cr(VI)		% Increase
	Not exposed to UWWTP effluent	Exposed to UWWTP effluent	
R. lanceolatus (Average)	0.59	0.99	67.8
C. obtusifolia (Median)	0.301	0.346	15.0

4.4 Relationship between the concentration of Cr(VI) in the plant leaves and the total chlorophyll content in the leaves of *Rumex lanceolatus* and *Cullen obtusifolia* exposed to the effluent from UWWTP.

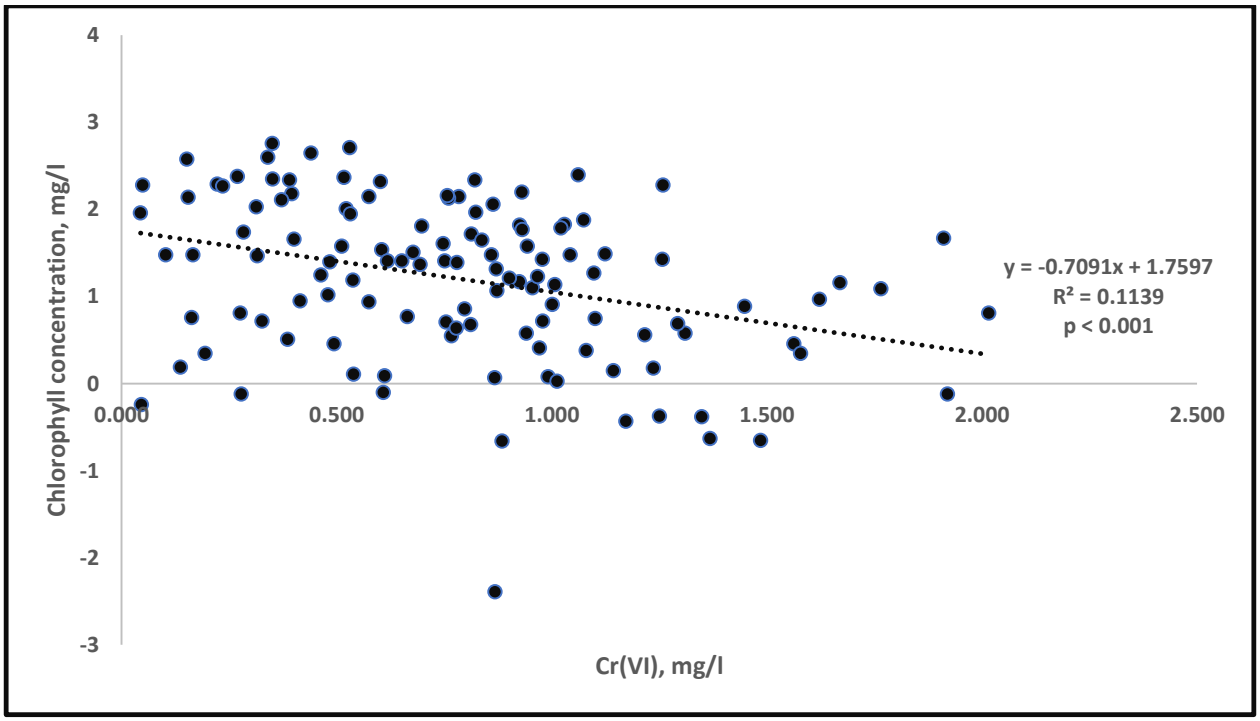

Figure 12: The effects of Cr(VI) concentration on the Chlorophyll concentration in the leaves of *R. lanceolatus* (n = 120)

Figure 14 shows that the Cr(VI) concentration accounted for only about 11.4% (R^2 = 0.1139) of the observed variations in the chlorophyll concentration data. Regression analysis (Appendix 13) revealed a highly significantly negative relationship between the Cr(VI) concentration and the total chlorophyll concentration in the leaves of the of *R. lanceolatus* exposed to the effluent from UWWTP (r(118) = 0.34, p < 0.001).

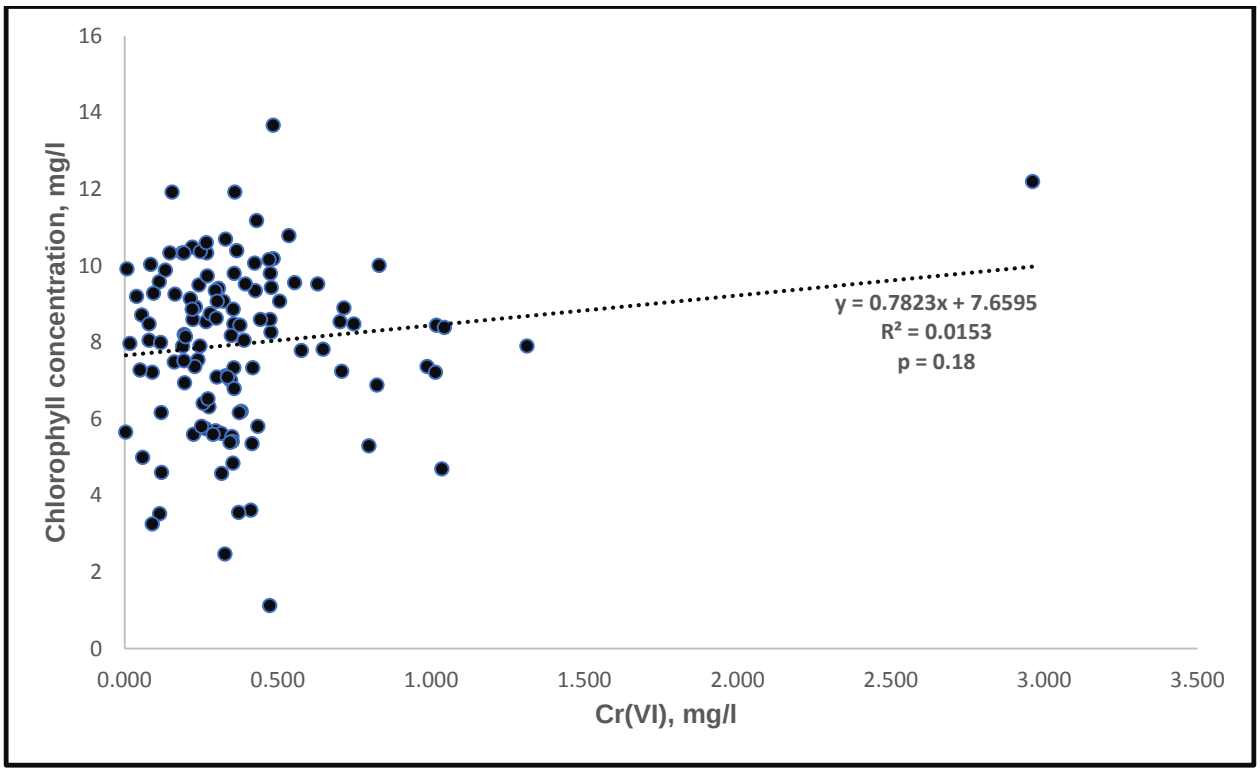

Figure 13: The effects of Cr(VI) concentration on the Chlorophyll concentration in the leaves of *C. obtusifolia (n = 120)*

Regression analysis (Figure 15) showed that there was no significantly relationship between the Cr(VI) concentration and the total chlorophyll concentration in the leaves of the of *C. obtusifolia*

exposed to the effluent from UWWTP ($r(118) = 0.12$, $p = 0.18$) (Appendix 14). It also shows that the Cr(VI) concentration in the leaves accounted for only about 1.5% ($R^2 = 0.0153$) of the observed variations in the chlorophyll concentration data for *C. obtusifolia*.

4.5 Effect of distance from discharge point on Cr(VI) concentration

Regression analysis plot (Figure 16) showed that there was a significant negative relationship between the distance from the UWWTP discharge point and the Cr(VI) concentration in the flowing effluent ($r(5) = 0.88$, $p = 0.02$) (Appendix 15)

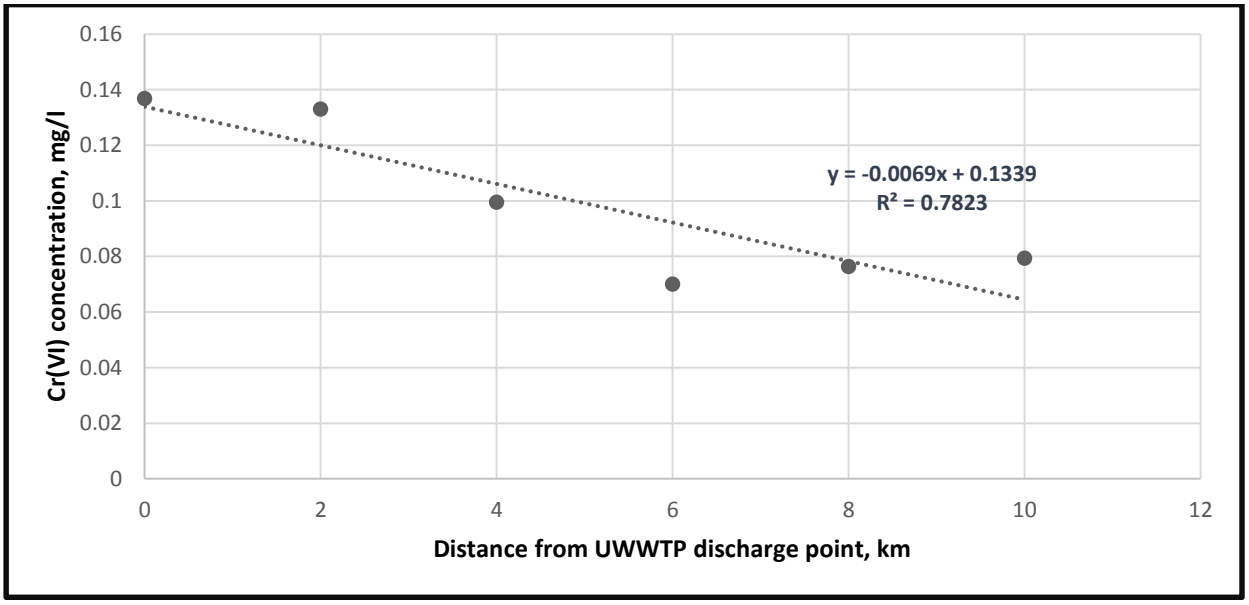

Figure 14: The effect of increase in distance away from the discharge point on the concentration of Cr(VI) in the effluent discharged from UWWTP into the KWR (n = 6)

The study revealed that increase in the distance from the UWWTP discharge point accounted for about 78.2% ($R^2 = 0.7823$) of the observed variations in the Cr(VI) concentrations (Figure 16).

4.6 Comparison of the concentration of Cr(VI) in the effluent discharged from the UWWTP into the KWR with the allowable standards set by the NWQSE

The results of the one sample t-test (Appendix 16) showed that the concentrations of Cr(VI) discharged from the UWWTP are significantly higher than the allowable concentration limit of 0.050 mg/l Cr(VI) as set by the NWQSE ($t(59) = 12.475$, $p < 0.001$).

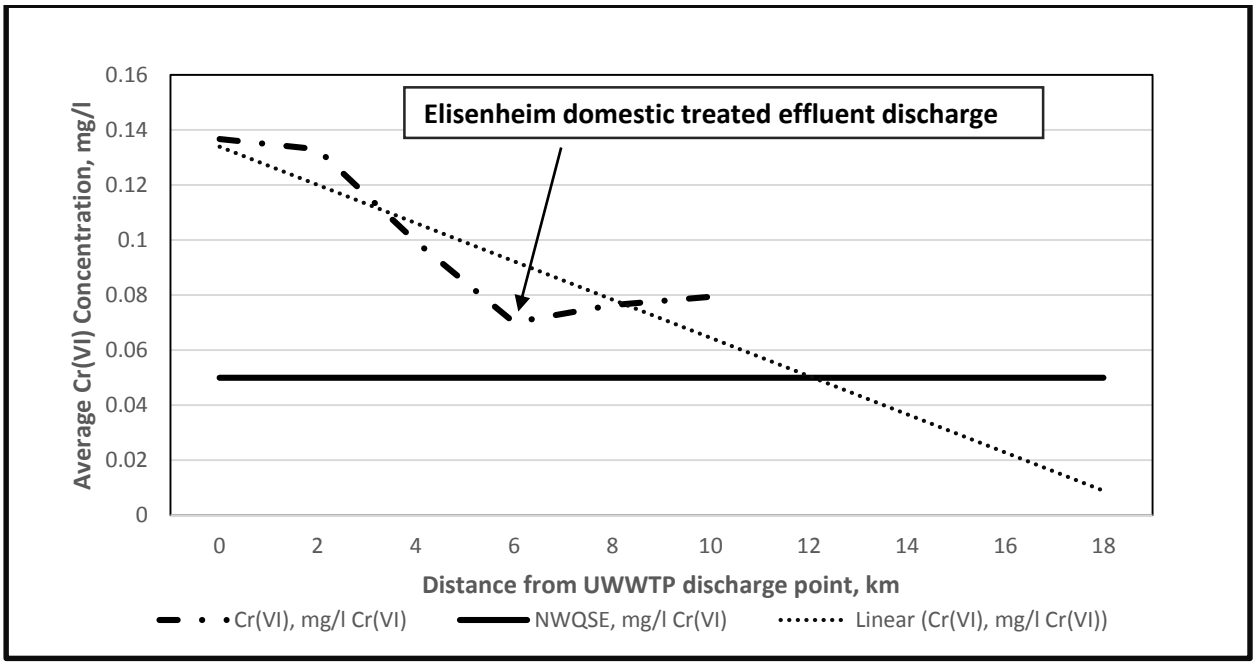

Figure 15: Compliance of the Cr(VI) concentration in the effluent discharged from UWWTP to the Cr(VI) concentration limits set by the NWQSE and the effects of increase in the distance from the UWWTP discharge point (n = 60)

As shown in Figure 17, the study revealed that the concentration of Cr(VI) in the effluent discharged from the UWWTP had an average of 0.136 mg/l Cr(VI) and reduced to 0.079 mg/l Cr(VI), after flowing for at least 10 km in the KWR.

The results also showed that there is an increase in the concentration of Cr(VI) in the area between the Elisenheim settlement domestic treated effluent discharge point and the Dobra crossing at Nampower premises.

Lastly, in the absence of this unknown source of Cr(VI), the concentration of Cr(VI) in the effluent would hypothetically reach a level below 0.050 mg/l Cr(VI) after an approximate flowing distance of 12 km.

4.7 Comparisons of the performance of the UOPS and the UWWTP in terms of their removal efficiencies for solids, organics and nutrients.

4.7.1 TSS

The Shapiro-Wilk test showed that the data for the removal of TSS concentrations by both UOPS and UWWTP were not normally distributed ($df = 54$, $p < 0.001$). Consequently, a Mann-Whitney U test for non-parametric data was used to test for significant difference between the removal of TSS by UOPS and UWWTP at 95% confidence interval. The results showed a significant difference in the removal of TSS by UOPS and UWWTP; *(*Mann-Whitney U Test, $U = 230$, $n = 54$, $p = 0.021$). The median removal efficiency was 71.12% for UOPS and 99.2% for UWWTP. The data distribution for both UOPS and UWWTP are shown in a box plot (Figure 18).

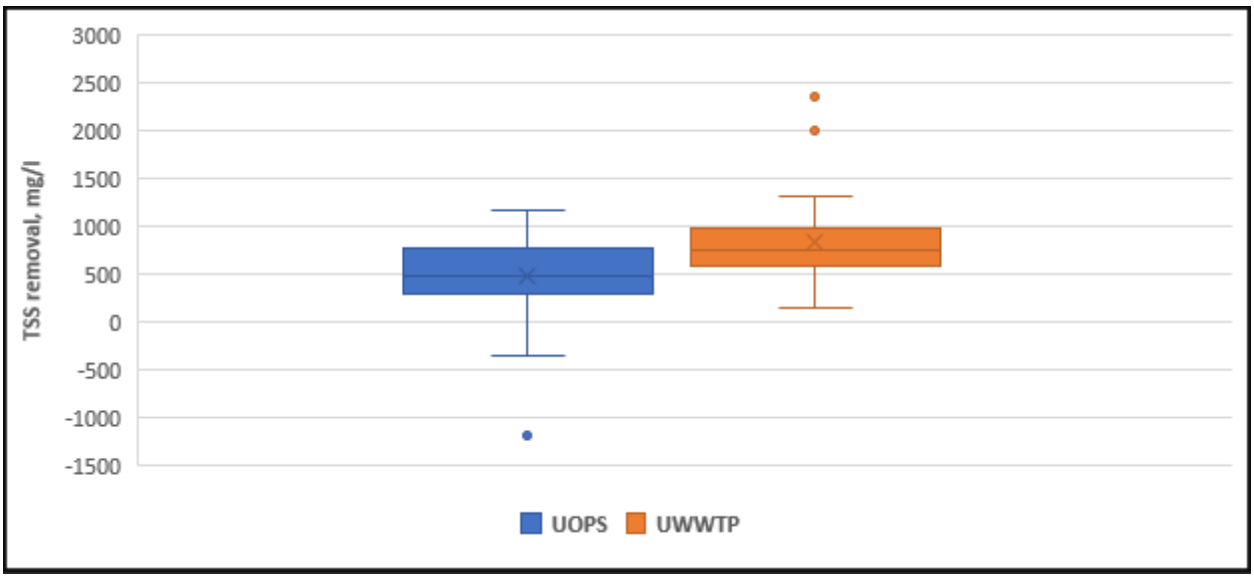

Figure 16: The comparison of the UOPS and UWWTP in removing TSS from the industrial effluent received (n = 54)

As depicted in the box plot (Figure 18) above, the data for the median value for the removal of TSS by the UOPS was 480 mg/l and lower than 746 mg/l for UWWTP. The outliers were disregarded as isolated incidences therefore not part of these data set. The UWWTP has a higher removal efficiency for TSS than the former UOPS.

4.7.2 TDS

The Shapiro -Wilk test showed that the data for the removal of TDS concentrations by both UOPS and UWWTP were normally distributed (df = 54, p = 0.163). Consequently, an independent samples t-test was used to test for the significant difference between the removal of TDS by UOPS and UWWTP at 95% confidence interval (df = 52). The results (Appendix 17.8) showed a significant difference between the amount of TDS removed by UOPS and by UWWTP*;* independent samples t-test (t(52) = -3.089, p = 0.003). The average TDS concentration removed

with standard deviation error were as depicted in Figure 19. The mean removal efficiency was -11.26% for UOPS and 19.55% for UWWTP.

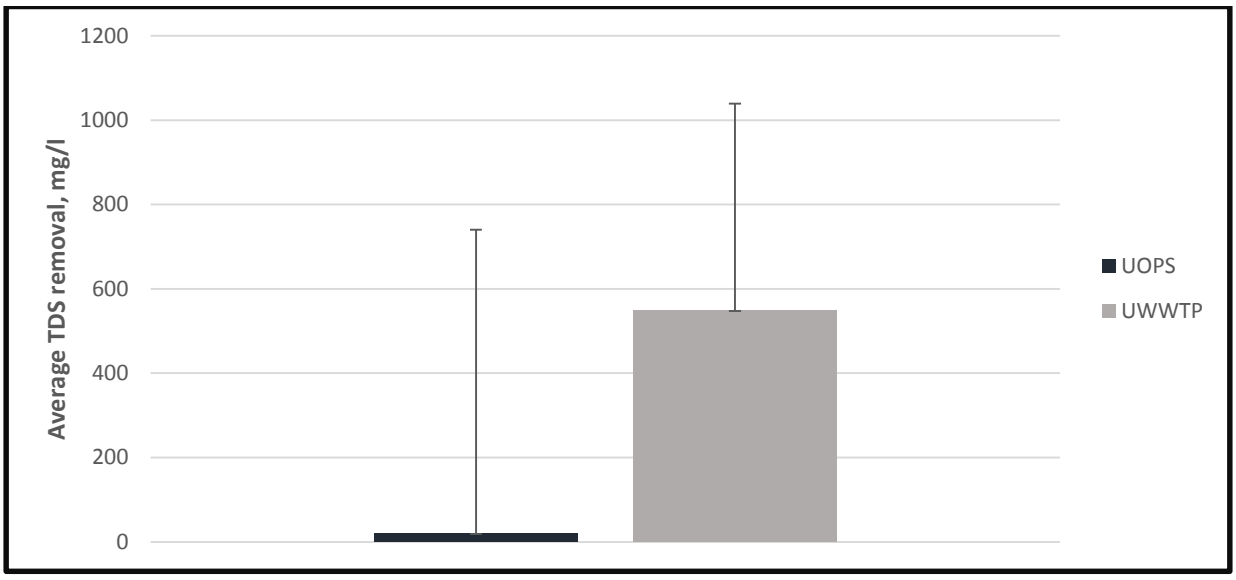

Figure 17: The comparison of the UOPS and UWWTP in removing TDS from the industrial effluent received. Bars represent Standard Deviation (n = 54).

As depicted in the bar chart Figure 19, the mean value for the removal of TDS by the UOPS was 19.33 mg/l and lower than 548.37 mg/l for UWWTP. The UWWTP has a higher removal efficiency for TDS than the former UOPS.

4.7.3 COD

The Shapiro-Wilk test showed that the data for the removal of COD concentrations by both UOPS and UWWTP were not normally distributed ($df = 54$, $p < 0.001$). Consequently, a Mann-Whitney U test for non-parametric data was used to test for significant difference between the removal of

COD by UOPS and UWWTP at 95% confidence interval. The results (Appendix 17.4) showed that there is no significant difference in the removal of COD by UOPS and UWWTP *(*Mann-Whitney U Test, U = 280, n = 54, p = 0.146). The data distribution for both UOPS and UWWTP are shown in a box plot (Figure 20). The median removal efficiency was 75.86% for UOPS and 98.0% for UWWTP.

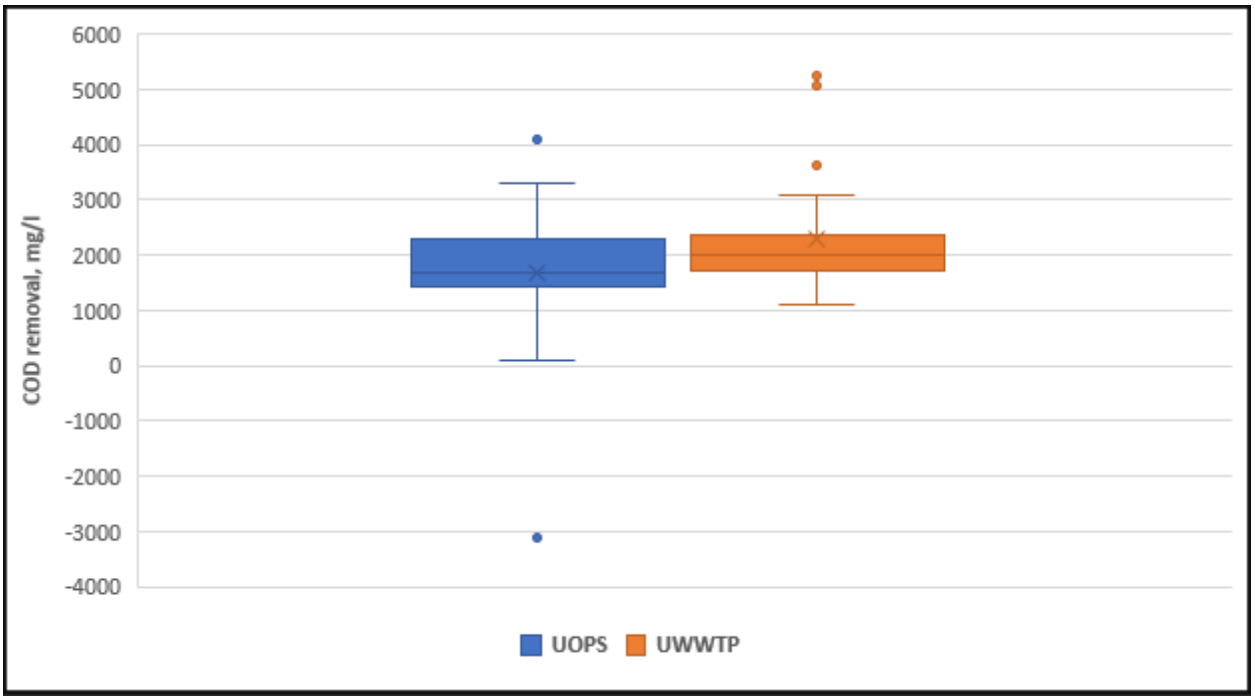

Figure 18: The comparison of the UOPS and UWWTP in removing COD from the industrial effluent received (n = 54).

As depicted in the box plot Figure 20, the data for the median concentration of COD removed by the UOPS was 1670 mg/l and was close to 2002 mg/l for UWWTP. As also confirmed by the statistical test above, the box plot also shows that the upper quartile value for the concentration of COD removed are nearly similar for both UOPS and UWWTP, the difference the concentration of

COD removed by UOPS and UWWTP was negligible. The UWWTP has not made a significant difference in the removal of COD from the industrial effluent received.

4.7.4 TKN

The Shapiro-Wilk test showed that the data for the removal of TKN concentrations by both UOPS and UWWTP were normally distributed (df = 54, p = 0.083). Consequently, an independent samples t-test was used to test for the significant difference between the removal of TKN by UOPS and UWWTP at 95% confidence interval. The results (Appendix 17.2) showed a significant difference between the amount of TKN removed by UOPS and by UWWTP*;* independent samples t-test (t(52) = -10.447, p < 0.001). The average TKN concentration removed with standard deviation error were as depicted in Figure 21 The mean removal efficiency was -13.06% for UOPS and 97.50% for UWWTP.

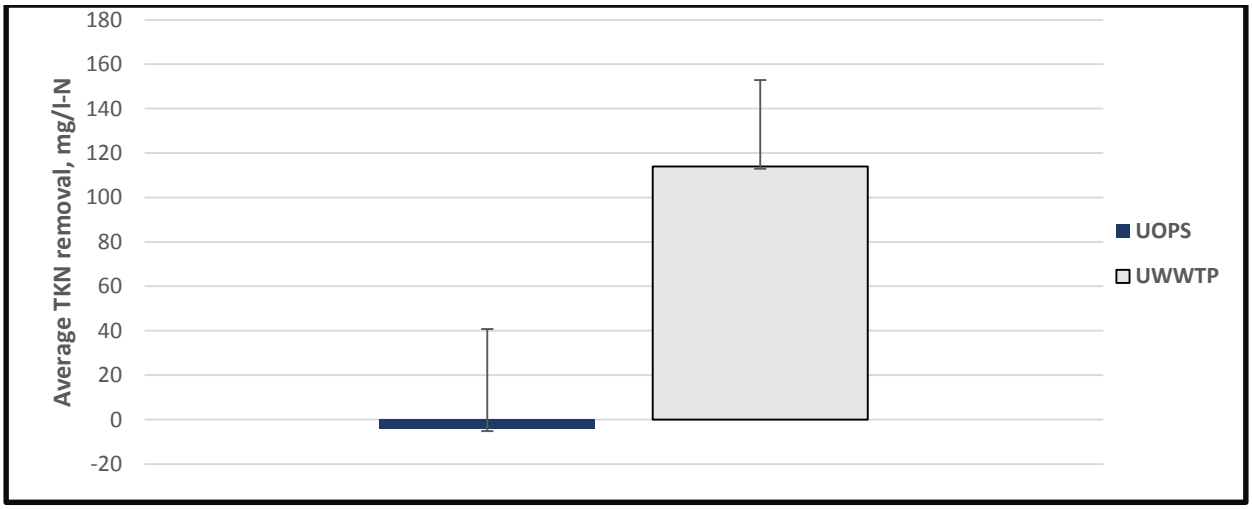

Figure 19: The comparison of the UOPS and UWWTP in removing TKN from the industrial effluent received. Bars represent Standard Deviation (n = 54).

The comparison of the means for the TKN removal data as shown in the bar chart (Figure 21), showed a low mean value of -4.301 mg/l N for UOPS and higher value for UWWTP at 113.90 mg/l N. Consequently, the UWWTP has a higher removal efficiency for TKN than the former UOPS.

4.7.5 Ortho PO_4

The Shapiro-Wilk test showed that the data for the removal of ortho-PO_4 concentrations by both UOPS and UWWTP were normally distributed (df = 54, p = 0.086). Consequently, an independent samples t-test was used to test for the significant difference between the removal of ortho-PO_4 by UOPS and UWWTP at 95% confidence interval. The results (Appendix 17.10) showed a significant difference between the amount of ortho-PO_4 removed by UOPS and by UWWTP*;* independent samples t-test ($t(52) = -6.860$, $p < 0.001$). The average ortho-PO_4 concentration removed with standard deviation error were as depicted in Figure 22.

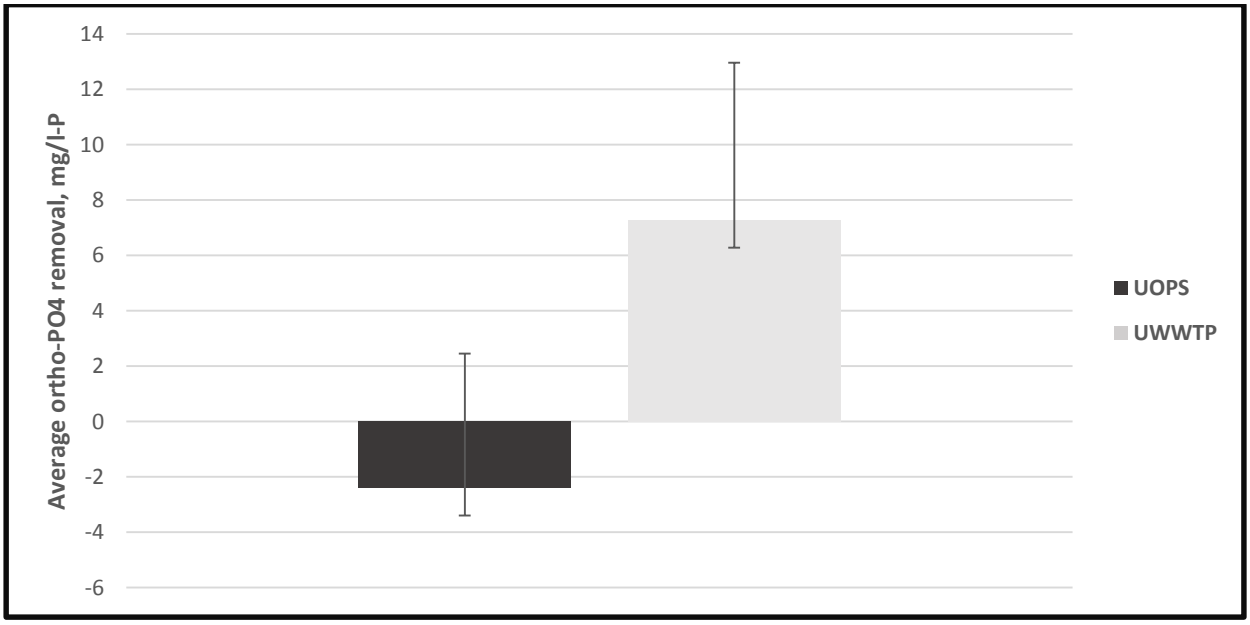

Figure 20: The comparison of the UOPS and UWWTP in removing ortho-PO_4 from the industrial effluent received. Bars represent Standard Deviation (n = 54).

As depicted in the bar chart (Figure 22), the average concentration of ortho-PO_4 removed by the UOPS was -2.39 mg/l and lower than 7.28 mg/l for UWWTP. The mean removal efficiency was -124.20% for UOPS and 43.76% for UWWTP. The UWWTP has a higher removal efficiency for ortho-PO_4 than the former UOPS.

4.7.6 Comparative summary of the Removal efficiency

The calculated removal efficiency for all parameters were compiled and plotted as shown in Figure 23.

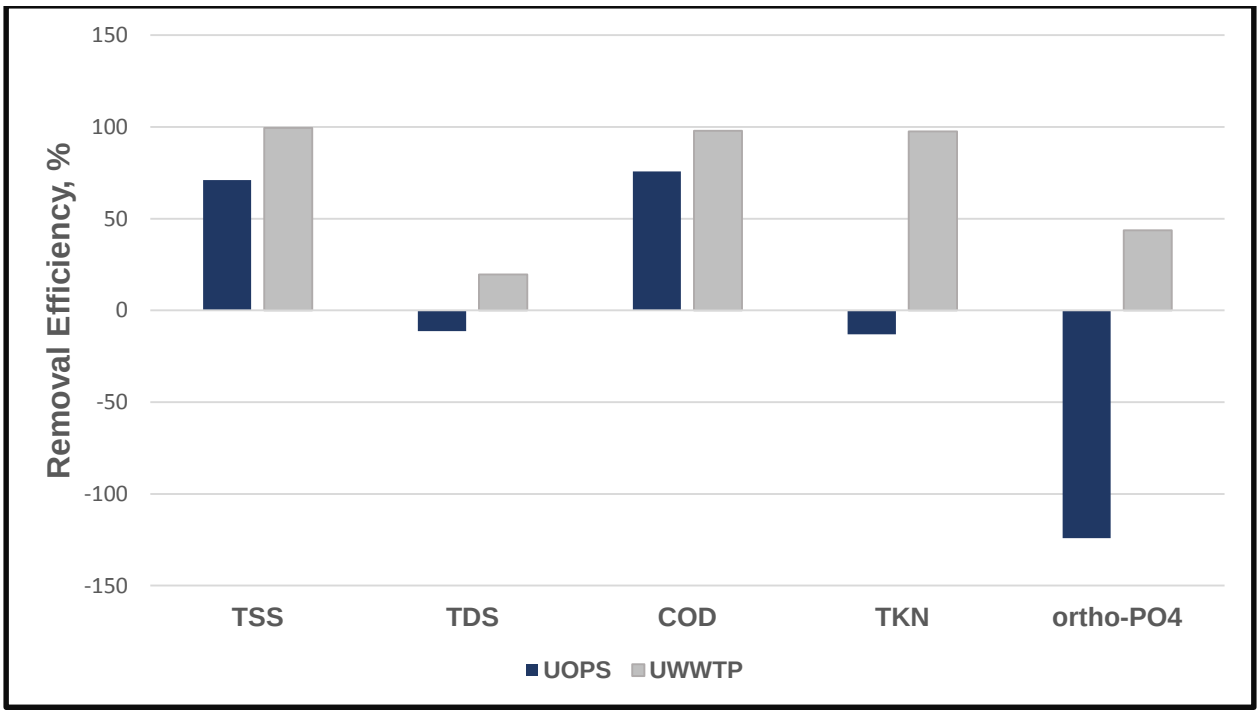

Figure 21: Comparative summary of the Removal Efficiency for UOPS and UWWTP with regard to the removal of TSS, TDS, COD, TKN and ortho-PO_4 from the incoming raw industrial effluent received in 2012 (UOPS) and 2017 (UWWTP) (n = 54)

As depicted in Figure 23, the UOPS could only remove TSS and COD and increased the concentrations of other parameters such as TDS, TKN and Ortho PO_4 into the discharged effluent. In contrast, the UWWTP removes about 90% of TSS, COD and TKN. The results however showed that it had challenges with the removal of TDS and ortho-PO_4, with average removal efficiencies of about 19.5% and 43% respectively.

4.8 Comparison of the concentrations of solids, organics and nutrients in the effluent discharged from the UWWTP into the KWR with the allowable standards set by the NWQSE.

4.8.1 TSS

The graphical comparison of the effluent TSS concentration with allowable NWQSE standard is depicted in Figure 24. The results of the one sample t-test (Appendix 18.3) showed that the concentrations of TSS in the final effluent discharged from the UWWTP are significantly lower than the allowable concentration limit of less than 100 mg/l TSS as set by the NWQSE general standard ($t(124) = -98.42$, $p < 0.001$).

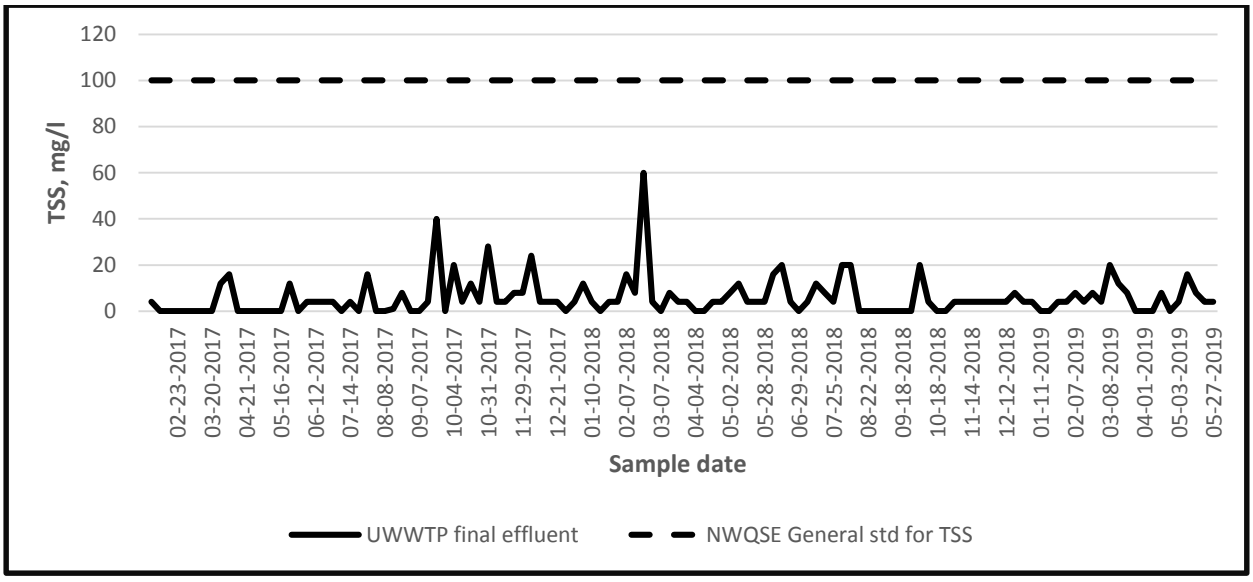

Figure 22: The compliance of the TSS concentration in the effluent discharged from UWWTP to the limits for the TSS concentration set by the NWQSE general standard (< 100 mg/l TSS) (n = 124).

In Figure 24, the statistical test confirmed that the concentration of TSS in the effluent from UWWTP is significantly lower than the NWQSE standard of 100 mg/l. The TSS concentration in the effluent has a maximum of 60 mg/l and an average of 6.59 mg/l.

4.8.2 TDS

The graphical comparison of the effluent TDS concentration with allowable NWQSE standard is depicted in Figure 25. The results of the one sample t-test (Appendix 18.4) showed that the concentrations of TDS in the final effluent discharged from the UWWTP are significantly higher than the allowable concentration limit of less than 500 mg/l TDS as set by the NWQSE general standard ($t(124) = 37.778$, $p < 0.001$).

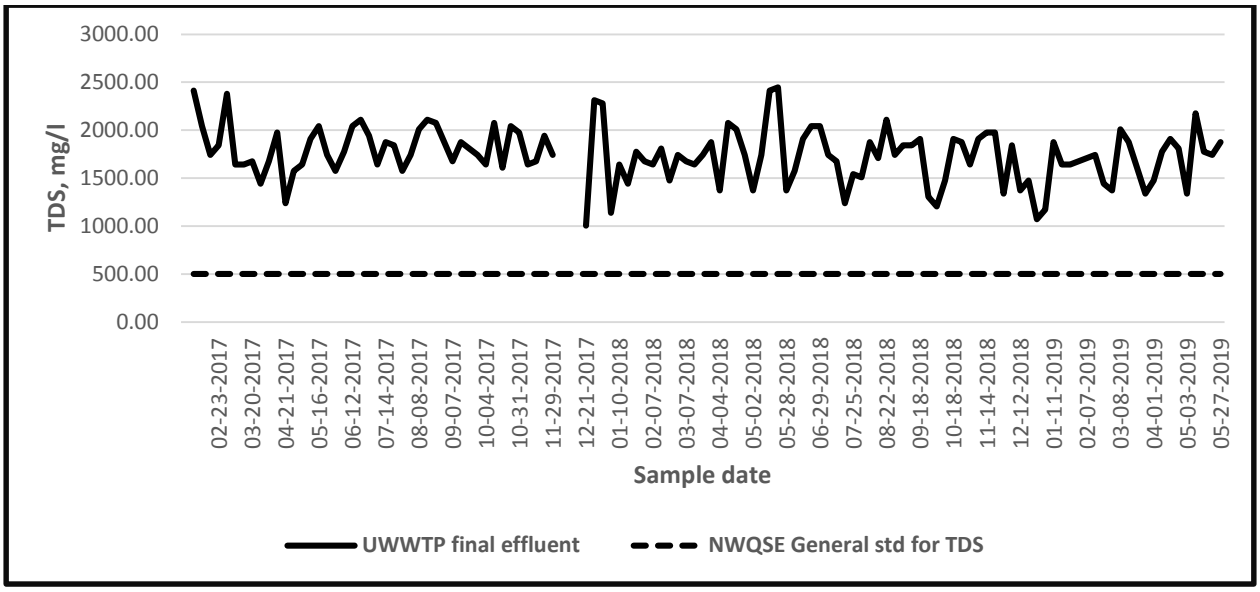

Figure 23: The compliance of the TDS concentration the effluent discharged from UWWTP to the limits for the TDS concentration set by the NWQSE general standard (< 500 mg/l TDS) (n = 124).

Figure 25 also shows that the TDS concentration in the effluent from UWWTP was consistently higher than the allowable level as set by the NWQSE standards of < 500 mg/l TDS. It had an average of 1714.17 mg/l and a minimum of 1005 mg/l. No analysis recorded on the 07 and 19th of December 2017.

4.8.3 COD

The graphical comparison of the effluent COD concentration with allowable NWQSE standard is depicted in Figure 26. The results of the one sample t-test (Appendix 18.2) showed that the concentrations of COD in the final effluent discharged from the UWWTP are significantly lower than the allowable concentration limit of less than 100 mg/l COD as set by the NWQSE general standard ($t(125) = -36.127$, $p < 0.001$).

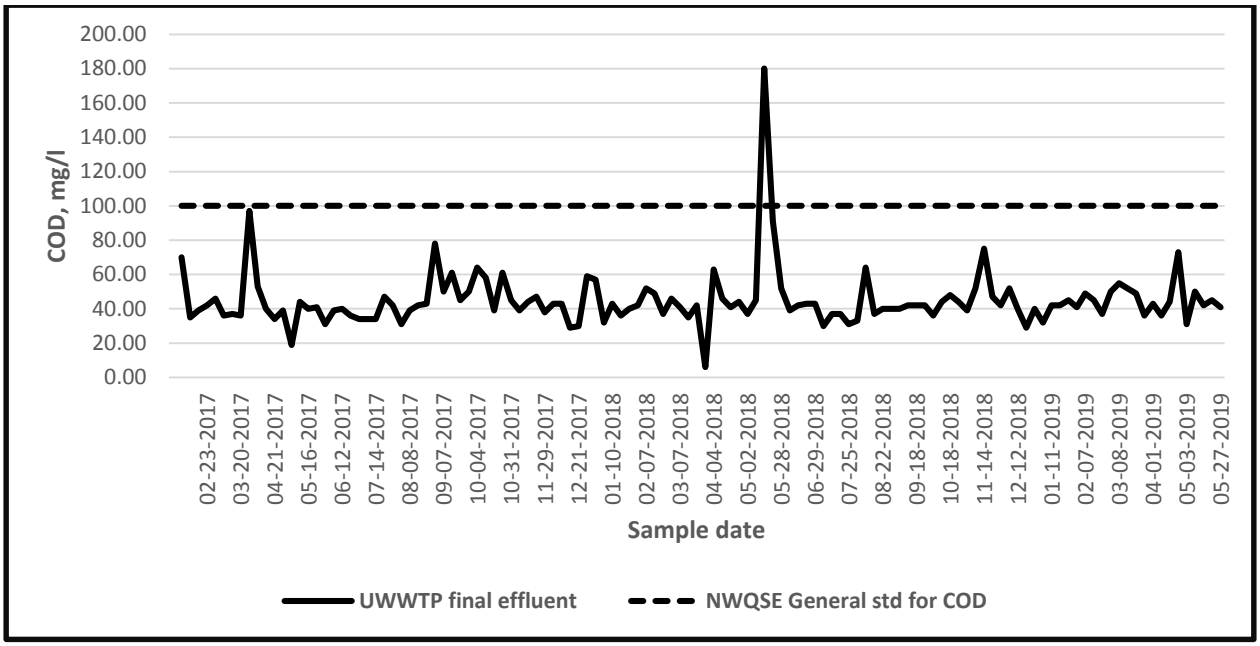

Figure 24: The compliance of the COD concentration in the effluent discharged from UWWTP to the limits for the COD concentration set by the NWQSE general standard (< 100 mg/l COD) (n = 125).

The COD concentration in the effluent from UWWTP (Figure 26) had an average of 44.92 mg/l and a maximum of 180 mg/l, which was an isolated case.

4.8.4 TKN

The comparison of the effluent TKN concentration with allowable NWQSE standard is shown in Figure 27. The results of the one sample t-test (Appendix 18.1) showed that the concentrations of TKN in the final effluent discharged from the UWWTP are significantly lower than the allowable concentration limit of less than 33 mg/l as N as set by the NWQSE general standard ($t(122) = -60.837$, $p < 0.001$).

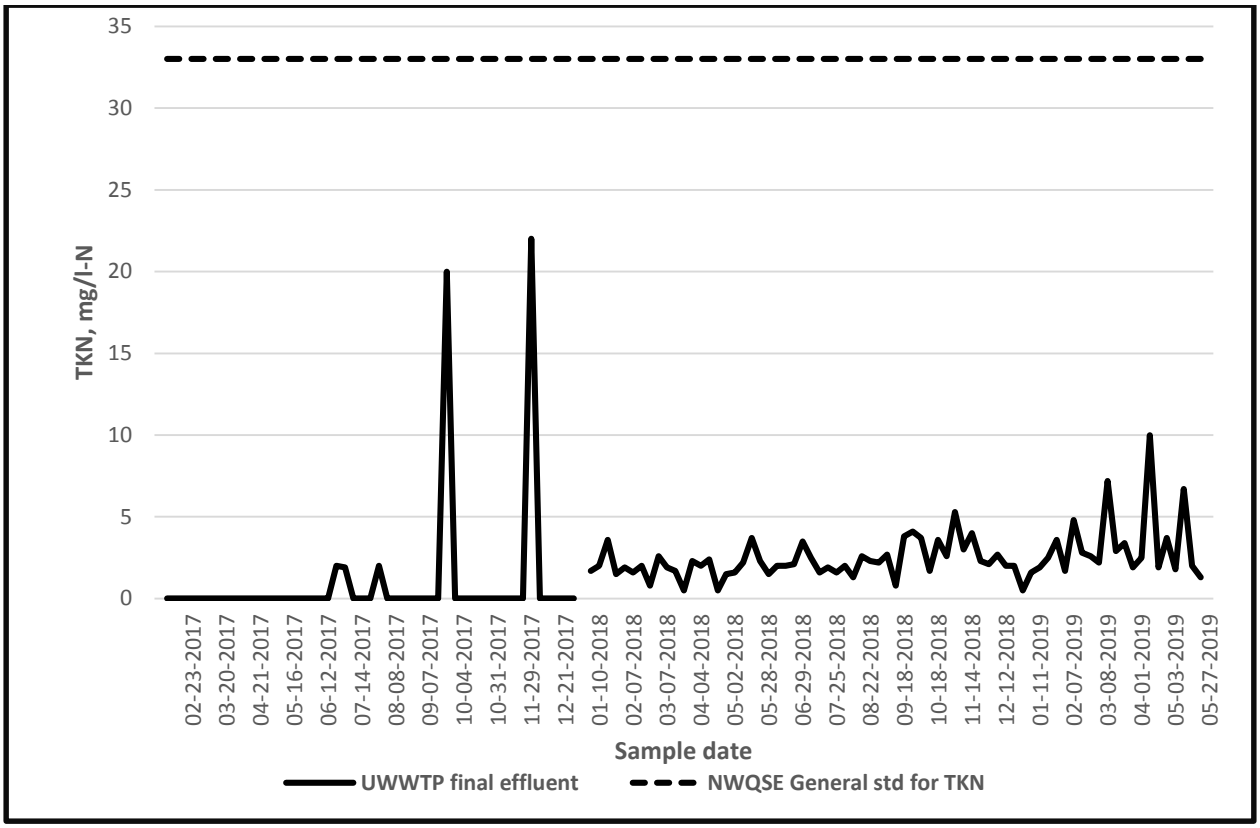

Figure 25: The compliance of the TKN concentration in the effluent discharged from UWWTP to the limits for the TKN concentration set by the NWQSE general standard (< 33 mg/l - N) (n = 122)

As shown in Figure 27, the TKN concentration in the effluent from UWWTP had an average of 2.99 mg/l - N and a maximum of 22 mg/l – N. It is also consistently below the allowable limit as set by the NWQSE. No analysis was recorded on 29/12/2017.

4.8.5 Ortho PO_4

The comparison of the effluent ortho-PO_4 concentration with allowable NWQSE standard is depicted in Figure 28. The results of the one sample t-test (Appendix 18.5) showed that the concentrations of ortho-PO_4 in the final effluent discharged from the UWWTP are significantly

lower than the allowable concentration limit of less than 3.0 mg/l as P as set by the NWQSE general standard ($t(124) = -44.435$, $p < 0.001$).

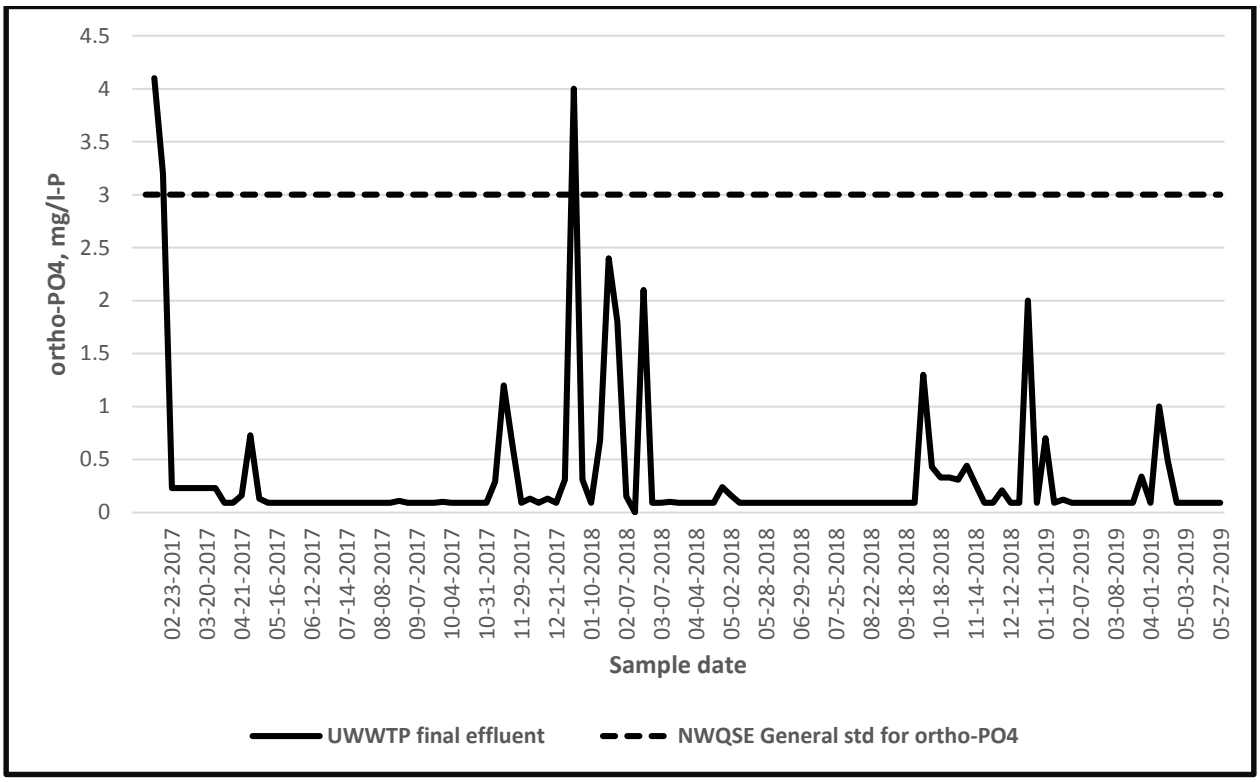

Figure 26: The compliance of the ortho-PO_4 concentration in the effluent discharged from UWWTP to the limits for the concentration of ortho-PO_4 set by the NWQSE general standard (< 3.0 mg/l-P) (n = 124).

As shown in Figure 28 the ortho-PO_4 concentration in the effluent from UWWTP had an average of 0.3 mg/l - P and a maximum of 4.1 mg/l – P.

5 CHAPTER 5: DISCUSSION

5.1 Total chlorophyll content in the leaves of *Rumex lanceolatus* and *Cullen obtusifolia,* exposed to the effluent from UWWTP and in those not exposed to the effluent from UWWTP

The results indicated a significantly ($p < 0.001$) lower total chlorophyll content (Figure 9) of both plant species, *R. lanceolatus* and *C. obtusifolia*, naturally growing in the soil containing the treated effluent from the UWWTP as compared to those growing in an environment free from the UWWTP effluent pollution. The results further showed that the reduction in the chlorophyll content with the exposure to Cr(VI) containing effluent was greater in *R. lanceolatus* (63.8%) than in *C. obtusifolia* (18.6%) (Table 5). This results are in agreement with those obtained by Bera *et al.* (1999), who reported a significant reduction in the amount of total chlorophyll irrespective of the concentration of Cr(VI) present, based on a study on 6 days mug bean seedlings exposed to a tannery effluent. These results are further consistent with findings by Pati *et al*. 2014, that the total chlorophyll in *Macrotyloma uniflorum* and *Cucumis sativus* were reduced gradually with the rise in Cr(VI) concentration (5-20 mg/l) and also time duration (7 - 21 days).

Considering that the subject plants of the study, *Rumex lanceolatus* and *Cullen obtusifolia* have been exposed to the high concentrations 0.136 mg/l at an effluent flowrate of 5175 m^3/d giving an estimate Cr(VI) loading rate of 0.703 kg/d, over an indefinite time. It is inevitable that their chlorophyll content will be significantly affected. This indicate that Cr(VI) is toxic to plants even at low concentrations and therefore given the high Cr(VI) in the UWWTP effluent, the reduction in the chlorophyll in both *R. lanceolatus* and *C. obtusifolia* can be attributed to its presence.

Although no biochemical analysis was carried out in this study to understand and identify the possible mechanism that led to the reduction in the chlorophyll content of *R. lanceolatus and C.*

obtusifolia, factors based on some previous studies by Pati *et al.* (2014) could be used to explain this reduction. As the chlorophyll pigments are present in thylakoid within chloroplast, any damage brought to these structures could lead to denaturation of these pigments. It may thus be suggested that the observed decrease in chlorophyll content (Table 5) at higher concentration of chromium might be due to the breakdown of thylakoid membranes and chloroplast envelope as was previously reported (Dodge & Lawes 1974).

Furthermore, Vajipayee *et al.* 1999, showed that Cr affects pigment biosynthesis by, for instance, degrading δ-aminolaevulinic acid dehydratase (AIAD), an essential enzyme in chlorophyll biosynthesis. It is likely that low chlorophyll content in chromium exposed *R. lanceolatus and C. obtusifolia* might be due to the inhibition of chlorophyll biosynthesis by altered AlAD activity. Cr (VI) also causes Fe and Zn deficiency, leading to interrupted chlorophyll biosynthesis, as previously reported by Barcelo *et al.* 1985. Vernay *et al*. (2007) also presented evidence that Cr competes with Mg and Fe for assimilation and transport to leaves, affecting therefore pigment biosynthesis.

Low reduction in the chlorophyll content (Figure 10) of *C. obtusifolia* could be an indication of high Cr tolerance in this species. Rout *et al*. (2000) also reported high concentrations of Cr were associated with higher chlorophyll content in tolerant calluses in *Echinochloa colona.* They concluded that *Echinochloa colona* is tolerant to Cr. This suggests that some plants have mechanisms to cope with the Cr(VI) induced stress. The reduction in chlorophyll content is a plant's common response to metal stress (Monteiro *et al.* 2009). The lower reduction of chlorophyll content (Table 5) in the leaves of *C. obtusifolia* could therefore be an indication of the level of this plant species' response to the Cr(VI) induced stress.

5.2 Concentration of Cr(VI) in the leaves of *Rumex lanceolatus* and *Cullen obtusifolia,* exposed to the effluent from UWWTP and those not exposed to the effluent from UWWTP.

The result in Figure 12, showed that the concentration of Cr(VI) were significantly higher ($p < 0.001$) in the leaves of *R. lanceolatus* exposed to the Cr(VI) containing effluent from UWWTP, at 67.8% (Table 6) more Cr(VI) concentration than in the leaves of the same plants specie not exposed to this effluent. However, for *C. obtusifolia* (Figure 13), although the plants exposed to the UWWTP effluent had 15% more Cr(VI) concentration in the leaves than those not exposed to the same effluent, the difference was statistically insignificant. Therefore, *R. lanceolatus* accumulated nearly over four times more Cr(VI) concentration in its leaves than the *C. obtusifolia* growing in the same environment.

The disposal of large quantity of Cr(VI) have been reported to overcome the reducing capacity of the environment and thus persist for long period as a pollutant (Mishra & Bharagava 2016). Cr(VI) eventually accumulating in crops from contaminated soils (Broadway *et al*. 2010). Cr is toxic, non-essential element to plants, hence they do not possess specific mechanisms for its uptake. Consequently, the uptake of this heavy metal is through carriers used for uptake of essential metals for plant metabolism (Shanker *et al*. 2005).

Interestingly, although Cr(VI) is a non-essential element, it easily gains entry into the plant cells and accumulates, even at lower concentrations of Cr(VI) released into the environment, a little percentage will most likely enter up in the cells, accumulates over time to higher levels, such that toxicological effects may become more pronounced. The difference in the amount of Cr(VI) in the leaves of *R. lanceolatus* and *C. obtusifolia* shows an important fact that the entry of Cr(VI) into the plant cells is dependent on the plant species, as some species has developed adaptation

mechanisms to withstand its toxic effects. As reported by Shahid *et al.* (2013a), this could be through either one or a combination of the following mechanisms; (i) avoidance, which offers external protection of the plant from metal stress and (ii) tolerance, whereby the plant is able to survive internal stress imposed by high internal metal concentrations.

5.3 The relationship between the concentration of Cr(VI) in the plant leaves and the total chlorophyll content in the leaves of *Rumex lanceolatus* and *Cullen obtusifolia,* not exposed and those exposed to the effluent from UWWTP

There is a significant negative linear relationship ($p < 0.001$) between Cr(VI) concentration and the total chlorophyll concentration in the leaves of *R. lanceolatus* exposed to the effluent from UWWTP (Figure 14). However, *C. obtusifolia* (Figure 15) showed no significant relationship ($p = 0.18$) between the Cr(VI) concentration and the total chlorophyll in the leaves.

In the case of *R. lanceolatus,* the observed negative relationship could indicate that *R. lanceolatus* is sensitive to increases in the concentrations of Cr(VI) and therefore will be markedly affected by the continuous introduction of Cr(VI) into its immediate environment. Furthermore, the high Cr(VI) concentration in the leaves of this plant species exposed to UWWTP effluent (67.8%) (Table 6), and corresponding to a reduction in chlorophyll content of (63.8%) (Table 5) observed in this plant species shows that *R. lanceolatus* has neither avoidance nor tolerance as the mechanism to handle high Cr(VI) stress. Moreover, plants exposed to heavy metals are ultimately forced to develop mechanisms for adaptation to the metal stress, these could either be; 1. Metal resistance (avoidance), 2. Metal sensitivity (tolerance) (Orcutt & Nelson 2000; Shahid *et al.* 2013a). In the absence of these mechanisms, continuous exposure to high metal concentration

would result in injury or death of the plant. As Reported by Rout *et al*. (2000), tolerant plants would have high chlorophyll content under high Cr(VI).

Furthermore, the absence of any significant relationship between Cr(VI) and chlorophyll shown by *C. obtusifolia* (Figure 15), could be viewed as a sign of some form of biological adjustments by the plant to protect itself, such that any further increase in the Cr(VI) content absorbed in the tissue, will have no further effect on the chlorophyll content. This plant species appeared to use avoidance as a stress handling mechanisms, that it absorbed as little Cr(VI) amount into its leaves (15%) (Table 6) and corresponding to a 18.6% reduction in chlorophyll content (Table 5). *C. obtusifolia* is therefore considered a non-hyper-accumulator of Cr(VI) in its above ground tissues.

These results confirm that Cr(VI) is toxic to plants and that the toxicity of Cr(VI) and Cr(VI) content in plants is species-specific (Cervantes *et al.* 2001). Most plants commonly respond to metal stress by a decrease in the chlorophyll content in their leaves and this subsequently leads to the reduction in photosynthetic capacity of the plants and finally to lower biomass production (Monteiro *et al.* 2009). Cr(VI) affect the chlorophyll by depleting the chlorophyll content and interrupting the chlorophyll biosynthesis processes through three mechanisms as reported by Choudhury & Panda (2004) and Vajpayee *et al.* (2000).

5.4 The effects of distance from the discharge point on the concentration of Cr(VI)

There was a significant ($p = 0.02$) negative relationship between the flowing distance (km) away from the UWWTP effluent discharge point and the concentration of Cr(VI) in the effluent flowing in the KWR from the UWWTP (Figure 16).

The reduction in the concentration of Cr(VI) in the effluent can be attributed to its metal characteristics. According to Chapman (1996), metals in natural waters can exist in dissolved, colloidal and suspended forms. The proportions of these forms vary for different metals and for different water bodies. Consequently, the toxicity and sedimentation potential of metals change depending on their forms. For instance, Cr(VI) in its ionic form is the most toxic form of this metal, but under certain conditions, metallo-organic, low -molecular compounds formed in natural water exhibit toxicities greater than the uncombined form (Chapman 1996). Given these characteristics, Cr(VI) would therefore most likely binds itself to suspended or solid organic and inorganic materials as well as to the sediments at the bottom of the KWR as the water flows, depending on other factors including the pH and temperature of the water as well as the level of organic matters.

Lehmann (2010) and Moss (1996) also state that high temperature, low pH and the presence of other metals generally heightens heavy metals toxicity. The presence of heavy metals harms most bacteria and can therefore interfere with the self-healing processes of surface waters. Heavy metals have a high bioaccumulation potential in plants and animals (Moss 1996). According to Merriam-Webster (n.d.), bioaccumulation is defined as the accumulation over time of a substance and especially a contaminant, such as a pesticide or heavy metal in a living organism.

In view of this, the informal usage of this effluent particularly at the Mix informal settlement and the constructions industry could present a health hazard and need to be addressed. Alternatively, there is a greater need to study and understand the factors that affect substrate binding for the Cr(VI) in this water and establish the safest point of extraction for all these possible usages as a

valuable resource particularly to the building industry which is currently happening without any safety concerns.

5.5 The comparison of the concentration of Cr(VI) in the effluent discharged from the UWWTP into the KWR with the allowable standards set by the NWQSE

The concentrations of Cr(VI) in the effluent discharged into the KWR is significantly higher than the limits set by the NQWSE of 0.05 mg/l Cr(VI) (Figure 17). The chart further shows that the concentration of Cr(VI) in the effluent increases after the discharge point of the Elisenheim settlement domestic treatment effluent discharge point.

Cr(VI) is the most toxic form of Cr metal and like most other heavy metals, pollution does not only result in the death of specific organisms, but there may be also reduced growth rate of those that survive and accumulation of the metals in their bodies by factors of many thousands over the concentration found in the environment (Moss 1996). Furthermore, the KWR has been known in the past as one of the leading pollution sources into the Swakoppoort dam, which suffers from severe level of anthropogenic pollution emanating from the catchment area (Lehmann 2010). This high concentrations of Cr(VI) as well as other pollutants in this effluent present a new industrial waste cycle and would highly endanger the innovative and unique waste water reclamation of Windhoek (Lehmann 2010).

The increase in the concentrations of Cr(VI) after the Elisenheim domestic waste water treatment plant discharge point (Figure 17) could be attributed to two possible explanations; there is an unidentified industrial source and need to be investigated or it is due to other natural factors. The later could be true as according to Moss (1996) and Chapman (1996), the concentrations of heavy

metals in water and therefore the concentrations at which they may cause physiological or behavioral changes or death varies not only with species but with life stages, concentrations of other heavy metals in the water, pH, temperature, Oxygen, bicarbonates, organic matter. Therefore, the introduction of the Elisenheim domestic effluent may affect the Cr(VI) concentration in the KWR depending on its constituents and thereby enhancing its toxicity level downstream. In fact, the addition of the Elisenheim domestic effluent or any other effluent to the UWWTP effluent in the KWR may pose a significant level of Cr(VI) toxicological danger to the people of Mix informal settlement who utilize this water, as it dislodges the substrate bound Cr(VI), accumulated over many years, into the water solution. This is of outmost importance and need to be addressed with urgency to safeguard human lives.

The discharged effluent met the NWQSE limit of less than 0.050 mg/l Cr(VI) after a flowing distance of about 12 km, in the absence of the Elisenheim domestic effluent (Figure 17). According to Moss (1996), the nature of Cr(VI) as stated earlier presents a challenge in deciding what concentration can safely be released to a natural environment. However, the set level of 0.050 mg/l Cr(VI) might be exceeded if more Cr(VI) is added from the accumulated substrate in the water depending on other factors, such as the rate of introduction of other effluents with different physico-chemical compositions.

Finally, it is worth noting that there has been few analysis of Cr(VI) carried out in the past five years, in the period between August 2015 to February 2018 and no other samples until April 2019. The WHO guideline on drinking water safety strongly recommends a preventative integrated management approach, with collaboration from all relevant agencies, as the most applicable approach to ensuring drinking water safety for humans. Lastly, given its extreme toxicity and further consideration as a “human carcinogen” by the IARC, USEPA and WHO (Yadav, 2009), it

is therefore imperative that any Cr(VI) related operation in any land is given rigorous monitoring and scientific evaluation, especially in the soil-plant system, as a first step towards ensuring environmental safety and human health.

5.6 Comparisons of the performance of the UOPS and the UWWTP in terms of their removal efficiencies for solids, organics and nutrients.

The results showed that UOPS could only remove TSS (71.12%) and COD (75.86%) (Figure 18 & 20). The median COD removal was found to be the same as that of the UWWTP (Figure 20). The UOPS served as a recharging reservoir at it increased the concentrations of other key parameters such as TDS (-11.26%), TKN (-13.06%) and Ortho-PO_4 (-124.2%) (Figure 19, 21 & 22) into the discharged effluent. This fully confirmed why it was replaced, as it was a serious source of pollution to the downstream environment including the Swakoppoort dam for several years. As reported by Lehmann (2010), that the Swakoppoort dam suffers from severe level of anthropogenic pollution emanating from the catchment area, particularly from KWR and Otjiseru River, with high levels of heavy metals such as Chromium, Cadmium, Nickel as well as high loads of Phenol, Formaldehyde, ammonia and nutrients like phosphates and Dissolved Organic Carbon (DOC).

In contrast, the UWWTP lived up to its expectation as its replacement. Its establishment was a great necessity to reduce the pollution load onto the KWR and downstream environment. The results showed that it had a high removal efficiency for TSS (99.2%), COD (98%) and TKN

(97.5%) (Figure 23). It also showed a low removal efficiency for ortho-PO_4, with an average removal efficiency of about 43%, but this could be an operational objective as it still meets the general limits of the NWQSE of less than 3.0 mg/l-P in the effluent discharged. The results however revealed problems with TDS, with the lowest removal efficiency of about 19.55%. The removal of TDS is however not highly anticipated due to the absence of the right technology, its removal could only be accomplished through the use principal unit operations and processes such as; 1. ion exchange, 2. Reverse osmosis, 3. Electrodialysis and 4. Distillation (Tchobanoglous *et al.* 2003). The high TDS in the effluent discharged into the KWR is still a threat to the environment and need an urgent solution.

5.7 Comparison of the concentrations of solids, organics and nutrients in the effluent discharged from the UWWTP into the KWR with the allowable standards set by the NWQSE.

As shown in Figure 24 - 28, the effluent discharged at UWWTP met the required NWQSE general standard limits in terms of TSS, COD, TKN and ortho-PO_4. It however did not meet the NWQSE general limits with regard to TDS.

The UWWTP is performing in accordance with its design a purpose which entails the removal of suspended solids, organics and nutrients. As stated by Tchobanoglous *et al.* (2003), both nitrogen and phosphorus, along with carbon, are essential nutrients for growth. When discharged to the aquatic life environment, they can lead to growth of undesirable aquatic life. When discharged in excessive amounts on land, they can also lead to the pollution of groundwater. This step is

commendable, considering the important role of the receiving Swakoppoort dam, as a source of potable water to the central region of Namibia.

Regarding the removal of TDS (Figure 25), it requires principal unit operations and processes as stated in the preceding subsection (5.6). The use of these operations and processes comes at high cost and given the small treatment capacity required, it may not be economical. As Moss (1996), puts it, too stringent standards may cost the industry too much in special extracting plant to remove the pollutant that it becomes unprofitable and an appeal procedure discourages the pollution offices from allowing too great a safety margin.

The continuous discharge of high TDS concentrations in the KWR will impact the water quality of the Swakoppoort dam part of the sources of potable water to the central regions. As there are no effective processes and operations currently in Namibia to remove these dissolved ions, it will have a greater impact on the quality of water supplied particularly in Windhoek, which already have high TDS because of the reclaimed water portion. Given the Windhoek's closed potable water circulation system, the TDS is constantly being circulated, increases with time and may impact the health of the people among the general population.

6 CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

From this study the following conclusions were drawn.

1. Both *R. lanceolatus* and *C. obtusifolia* exposed to effluent containing Cr(VI) showed lower total chlorophyll content than their controls (not exposed to effluent containing Cr(VI). *R. lanceolatus* showed a higher reduction in chlorophyll content than *C. obtusifolia*.

2. Plants exposed to an effluent containing Cr(VI) showed an accumulation of Cr(VI) in their leaves. The amounts accumulated differed significantly between plant species with both species having more Cr(VI) in their leaves than their controls.

3. The effects of Cr(VI) on the total chlorophyll content differed with plant species, for instance, Cr(VI) concentration in the leaves negatively affects the chlorophyll content in R. lanceolatus. In fact, there was a 63.8% reduction in the total chlorophyll content, however only 11.4% of this variation could be attributed to the concentration of Cr(VI) in its leaves. Whereas, there was no relationship between the Cr(VI) concentration and the chlorophyll content in the leaves of *C. obtusifolia.*

4. The concentration of Cr(VI) discharged in the KWR from the UWWTP reduced with the increase in the distance away from the UWWTP discharge point. In fact, the increase in the distance accounted for 78.2% of the reduction in the concentration of Cr(VI) in the UWWTP effluent flowing in the KWR.

5. The concentration of Cr(VI) in the effluent from UWWTP was higher than the limit set by the NWQSE general standard of 0.05 mg/l Cr(VI) and therefore the effluent did not comply with NWQSE requirement. The concentration of Cr(VI) in the effluent from UWWTP discharged into the KWR increased after the Elisenheim domestic wastewater treatment discharge point.

6. The UWWTP displayed a higher removal efficiency for TSS, TKN and ortho-PO_4 than the former UOPS. The former UOPS only removed TSS and COD and the rest were mostly enhanced. The UWWTP therefore has played a significant role in reducing the pollution load onto the KWR and the downstream environment.

7. The effluent from UWWTP complied with the NWQSE general standard in terms of TSS, COD, TKN, ortho-PO_4 concentrations. It however did not comply in terms of TDS concentrations, which could be attributed to the lack of effective treatment processes.

6.2 Recommendations

The following would be recommended:

1. Further studies on the avoidance and tolerance of both *R. lanceolatus* and *C. obtusifolia* as response mechanisms to Cr(VI) induced stress at different concentrations in a controlled environment should be conducted to get a better understanding of the relationship in a relatively shorter time.
2. Laboratory based observations and biochemical analyses should be carried out to understand the likely mechanisms by which the Cr(VI) contained in the plant tissues impacts the chlorophyll content of the plant leaves.
3. Laboratory studies should be done on these plants, analyzing their hyper-accumulation potential, phyto-extraction efficiency, metal accumulation index, translocation index, bioaccumulation factors, extraction coefficient among others, as tools to evaluate these plants as ideal for phytoremediation.
4. A regular water quality monitoring program, including; Cr(VI), Cadmium, Nickel and Conductivity/TDS for the UWWTP discharged effluent. Considering that this effluent reaches into the Swakoppoort Dam, which forms part of the sources of potable water supply to the central regions of Namibia, including Windhoek, and the suspected human carcinogenicity of this heavy metal, Cr(VI), it would be of outmost importance that regular monitoring of these parameters is established. The WHO guideline on drinking water safety strongly recommends a preventative integrated management approach, with collaboration

from all relevant agencies, as the most applicable approach to ensuring drinking water safety for humans.

5. Many warning signs should be placed along the KWR, to warn people of the dangers associated with the utilization of this effluent for any domestic or agricultural uses.
6. A follow up study is proposed on the impact of high TDS discharged into the Swakoppoort dam on the potable water distributed in Windhoek.

7 CHAPTER 7: REFERENCES

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8 APPENDICES

8.1 APPENDIX 1: Regression analysis; The relationship between SPAD-502Plus value and spectrophotometric chlorophyll determined concentrations: *R. lanceolatus*

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.96398
R Square	0.92926
Adj. R Square	0.92740
Std Error	0.00023
Observations	40.00000

ANOVA

	df	*SS*	*MS*	*F*	*Sig. F*
Regression	1.00000	0.00003	0.00003	499.17132	0.00000
Residual	38.00000	0.00000	0.00000		
Total	39.00000	0.00003			

	Coefficients	*Std Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*
Intercept	-0.00529	0.00036	-14.82462	0.00000	-0.00602	-0.00457	-0.00602
SPAD value	0.00012	0.00001	22.34214	0.00000	0.00011	0.00013	0.00011

Conclusion:

There is a significant positive relationship between SPAD values and the spectrophotometric chlorophyll concentration for *R. lanceolatus* , r(38) = 0.96, p < 0.001, thus less than 0.05.

8.2 APPENDIX 2: Regression analysis; The relationship between SPAD-502Plus value and spectrophotometric chlorophyll determined concentrations: *C. obtusifolia*

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.9504
R Square	0.9033
Adj. R Square	0.9008
Std Error	0.0005
Observations	40.0000

ANOVA

	df	*SS*	*MS*	*F*	*Sig. F*
Regression	1.0000	0.0001	0.0001	354.9535	0.0000
Residual	38.0000	0.0000	0.0000		
Total	39.0000	0.0001			

	Coefficients	*Std Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-0.0074	0.0008	-8.8087	0.0000	-0.0091	-0.0057	-0.0091	-0.0057
SPAD	0.0003	0.0000	18.8402	0.0000	0.0003	0.0003	0.0003	0.0003

Conclusion:
There is a significant positive relationship between SPAD values and the spectrophotometric chlorophyll concentration for *C. obtusifolia*, r(38) = 0.95, p < 0.001, thus less than 0.05.

8.3 APPENDIX 3: Regression analysis; The relationship between Cr(VI) concentration and the absorbance

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.997654
R Square	0.995313
Adjusted R Square	0.994141
Standard Error	0.067584
Observations	6

ANOVA

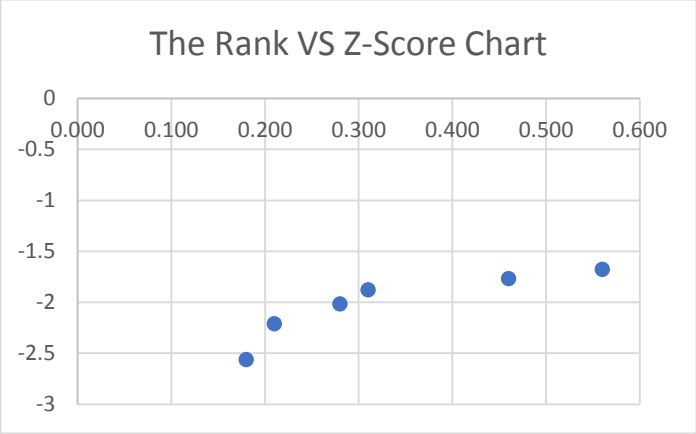
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	3.879863	3.879863	849.4279	8.25E-06
Residual	4	0.018268	0.004568		
Total	5	3.898131			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.009524	0.048914	0.194706	0.855113	-0.12628	0.14533	-0.12628	0.14533
X Variable 1	23.54286	0.807785	29.14495	8.25E-06	21.30009	25.78563	21.30009	25.78563

8.4 APPENDIX 4: Comparisons: DPC and DS Methods

Shapiro Wilk test: for normlity

Method	Cr(VI), mg/l	Rank	Index	z-score	correl
1	0.280	3	0.021920668	-2.015604622	0.882309
1	0.560	6	0.04697286	-1.674941448	
1	0.210	2	0.013569937	-2.20950011	
2	0.310	4	0.030271399	-1.876819787	
2	0.460	5	0.038622129	-1.766904288	
2	0.180	1	0.005219207	-2.560957471	



Comments:
The two methods are 88% correlated.
The plot of Rank vs z-score is also linear
Therefore, the data is approximately normally distributed.

Sample	Direct Spectroscopic Cr(VI),mg/l	DPC Kit-SCS Cr(VI),mg/l
Raw	0.28	0.31
Taxidermy	0.56	0.46
Paints	0.21	0.18

Descriptive statistics:

Column1	*DS Method*	*Column2*	*DPC Method*
Mean	0.349596483	Mean	0.316666667
Standard Error	0.108818061	Standard Error	0.080897741
Median	0.278596483	Median	0.31
Mode	#N/A	Mode	#N/A
Standard Deviation	0.18847841	Standard Deviation	0.140118997
Sample Variance	0.035524111	Sample Variance	0.019633333
Kurtosis	#DIV/0!	Kurtosis	#DIV/0!
Skewness	1.454605824	Skewness	0.213619059
Range	0.356333333	Range	0.28
Minimum	0.206929816	Minimum	0.18
Maximum	0.56326315	Maximum	0.46
Sum	1.048789449	Sum	0.95
Count	3	Count	3

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	0.349596483	0.316666667
Variance	0.035524111	0.019633333
Observations	3	3
Pooled Variance	0.027578722	
Hypothesized Mean Difference	0	
Df	4	
t Stat	0.242855458	
P(T<=t) one-tail	0.410031157	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.820062315	
t Critical two-tail	2.776445105	

Conclusion:
The analytical results given by the DPC method (M = 0.35, SD = 0.19, n = 3) were hypothisised to be

equal to the analytical results given by the DS method (M = 0.32, SD = 0.14, n = 3) and there is no significant
difference between them , t(3) = 2.78, p = 0.41 (one tail).

8.5 APPENDIX 5 Shapiro Wilk test for normality*: R. lanceolatus*: Chlorophyll

Descriptives				
			Statistic	Std. Error
Chlorophyll Site 1	Mean		70.0370	.64135
	95% Confidence Interval for Mean	Lower Bound	68.7644	
		Upper Bound	71.3096	
	5% Trimmed Mean		70.3078	
	Median		70.7500	
	Variance		41.132	
	Std. Deviation		6.41346	
	Minimum		50.00	
	Maximum		80.90	
	Range		30.90	
	Interquartile Range		8.20	
	Skewness		-.642	.241
	Kurtosis		.239	.478
Site 2	Mean		60.0520	.69974
	95% Confidence Interval for Mean	Lower Bound	58.6636	
		Upper Bound	61.4404	
	5% Trimmed Mean		60.0778	
	Median		60.0000	
	Variance		48.964	
	Std. Deviation		6.99742	
	Minimum		46.10	
	Maximum		74.50	
	Range		28.40	
	Interquartile Range		10.43	
	Skewness		-.046	.241
	Kurtosis		-.685	.478

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Site 1	.070	100	.200*	.968	100	.015
Site 2	.047	100	.200*	.985	100	.298
*. This is a lower bound of the true significance.						
a. Lilliefors Significance Correction						

Chlorophyll for *R.lanceolatus* at Site 1 is not approximately normally distribution (p-values for Shapiro is less than 0.05); whereas for Site 2, the chlorophyll is approximately normally distributed (both p-values are greater than 0.05).

8.6 APPENDIX 6: Shapiro Wilk Test: *C. obtusifolia*: Chloropyll

Descriptives

	Site			Statistic	Std. Error
Chlorophyll	Site 1	Mean		53.900	.7323
		95% Confidence Interval for Mean	Lower Bound	52.435	
			Upper Bound	55.365	
		5% Trimmed Mean		53.848	
		Median		54.650	
		Variance		32.177	
		Std. Deviation		5.6725	
		Minimum		41.3	
		Maximum		70.2	
		Range		28.9	
		Interquartile Range		6.6	
		Skewness		-.017	.309
		Kurtosis		.432	.608
	Site 2	Mean		48.465	.9617

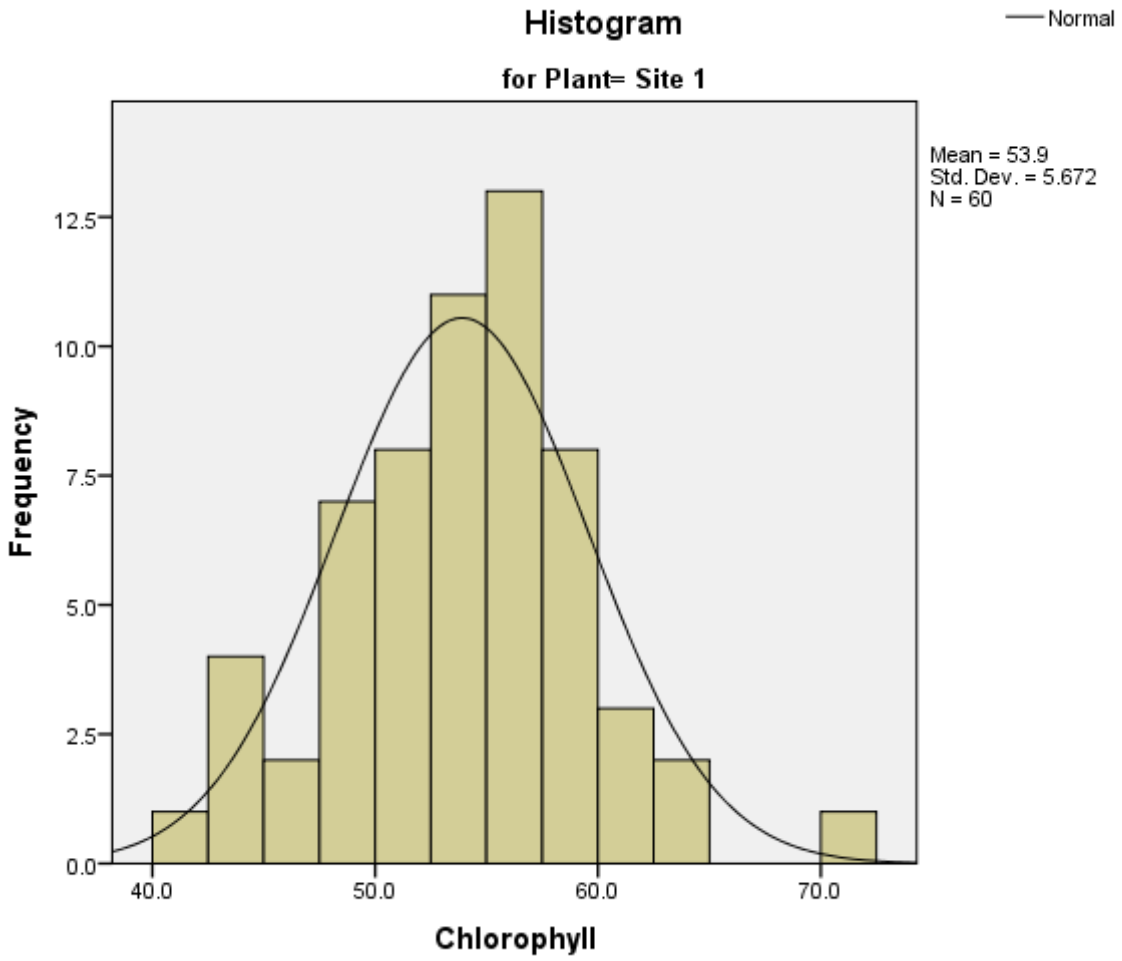
	95% Confidence Interval for Mean	Lower Bound	46.541	
		Upper Bound	50.389	
	5% Trimmed Mean		48.639	
	Median		49.200	
	Variance		55.497	
	Std. Deviation		7.4496	
	Minimum		28.4	
	Maximum		65.3	
	Range		36.9	
	Interquartile Range		10.0	
	Skewness		-.329	.309
	Kurtosis		-.026	.608

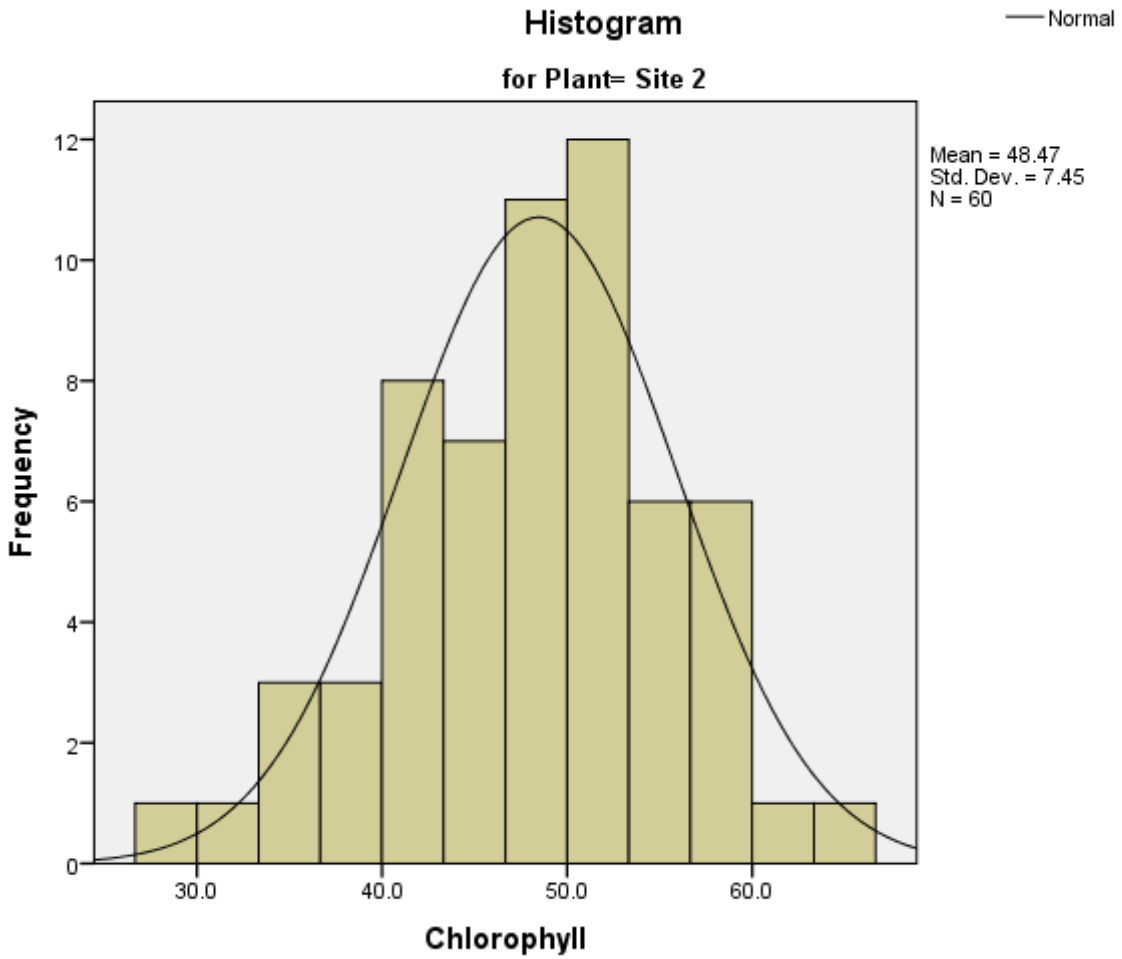
Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Site	Statistic	df	Sig.	Statistic	df	Sig.
Chlorophyll	Site 1	.071	60	.200*	.982	60	.519
	Site 2	.079	60	.200*	.986	60	.731

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction


Histogram
for Plant= Site 1
Normal
Mean = 53.9
Std. Dev. = 5.672
N = 60
Frequency
12.5
10.0
7.5
5.0
2.5
0.0
40.0
50.0
60.0
70.0
Chlorophyll


Histogram
for Plant= Site 2
Normal
Mean = 48.47
Std. Dev. = 7.45
N = 60
Frequency
0
2
4
6
8
10
12
30.0
40.0
50.0
60.0
Chlorophyll

8.7 APPENDIX 7: Non Parametric Test: *R. lanceolatus* Chlorophyll

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
Chlorophyll	120	65.018	9.0361	29.1	80.6
Plant type	120	1.50	.502	1	2

Mann-Whitney Test

Ranks

	Plant type	N	Mean Rank	Sum of Ranks
Chlorophyll	Site 1	60	83.90	5034.00
	Site 2	60	37.10	2226.00
	Total	120		

Test Statistics[a]

	Chlorophyll
Mann-Whitney U	396.000
Wilcoxon W	2226.000
Z	-7.369
Asymp. Sig. (2-tailed)	.000

a. Grouping Variable: Plant type

8.8 APPENDIX 8: T-Test: *C. obtusifolia* Chlorophyll

Group Statistics

	Site	N	Mean	Std. Deviation	Std. Error Mean
Chlorophyll	Site 1	60	53.900	5.6725	.7323
	Site 2	60	48.465	7.4496	.9617

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Chlorophyll	Equal variances assumed	4.595	.034	4.496	118	.000	5.4350	1.2088	3.0412	7.8288
	Equal variances not assumed			4.496	110.203	.000	5.4350	1.2088	3.0395	7.8305

95% CI: (3.0412, 7.8288)

Std. Error: 1.2088

8.9 APPENDIX 9: Shapiro Wilk Test: *R. lanceolatus*: Cr(VI) in the leaves

Descriptives

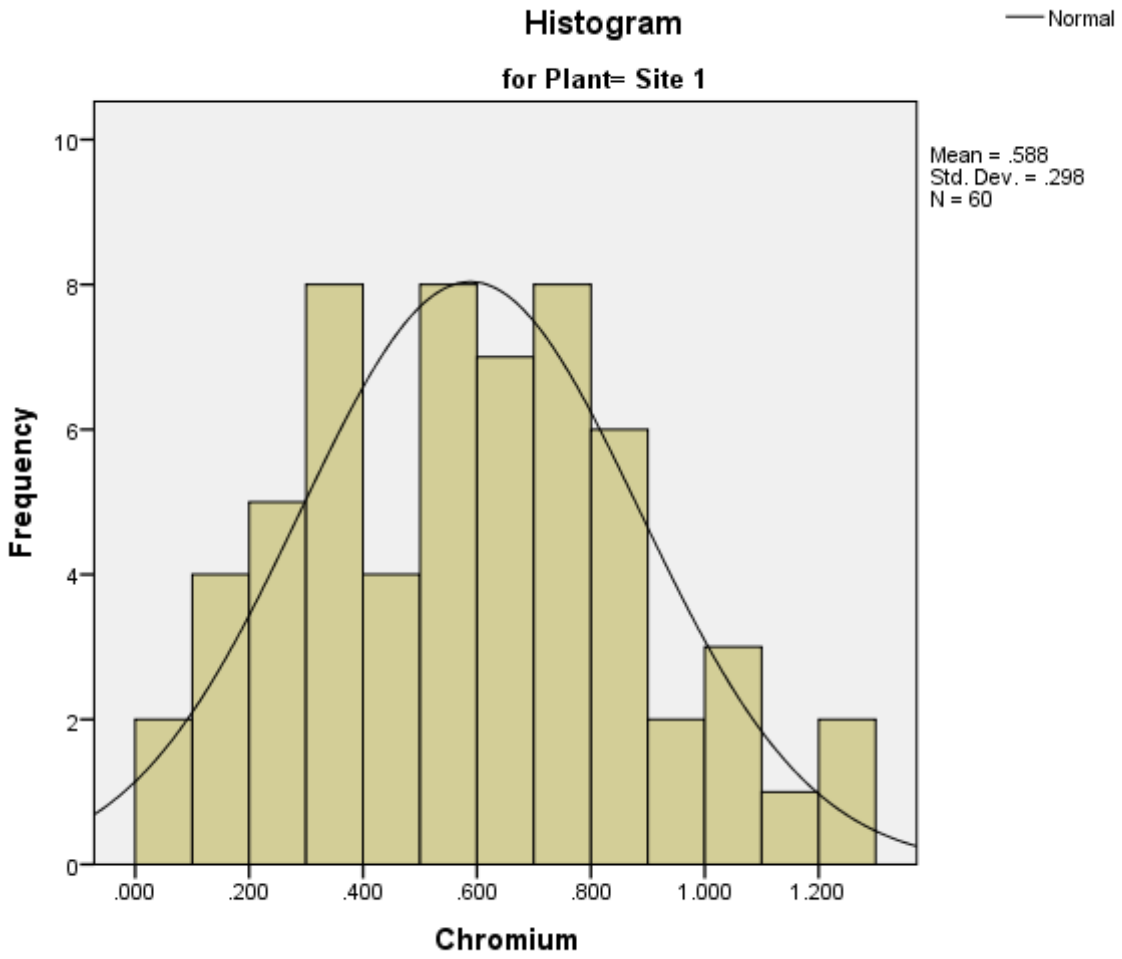
	Site			Statistic	Std. Error
Chromium	Site 1	Mean		.58810	.038438
		95% Confidence Interval for Mean	Lower Bound	.51119	
			Upper Bound	.66501	
		5% Trimmed Mean		.58285	
		Median		.57500	
		Variance		.089	
		Std. Deviation		.297739	
		Minimum		.044	
		Maximum		1.258	
		Range		1.214	
		Interquartile Range		.444	
		Skewness		.208	.309
		Kurtosis		-.508	.608
	Site 2	Mean		.98572	.058325
		95% Confidence Interval for Mean	Lower Bound	.86901	
			Upper Bound	1.10243	
		5% Trimmed Mean		.98061	
		Median		.97450	
		Variance		.204	
		Std. Deviation		.451785	
		Minimum		.046	
		Maximum		2.015	
		Range		1.969	
		Interquartile Range		.486	
		Skewness		.098	.309
		Kurtosis		-.072	.608

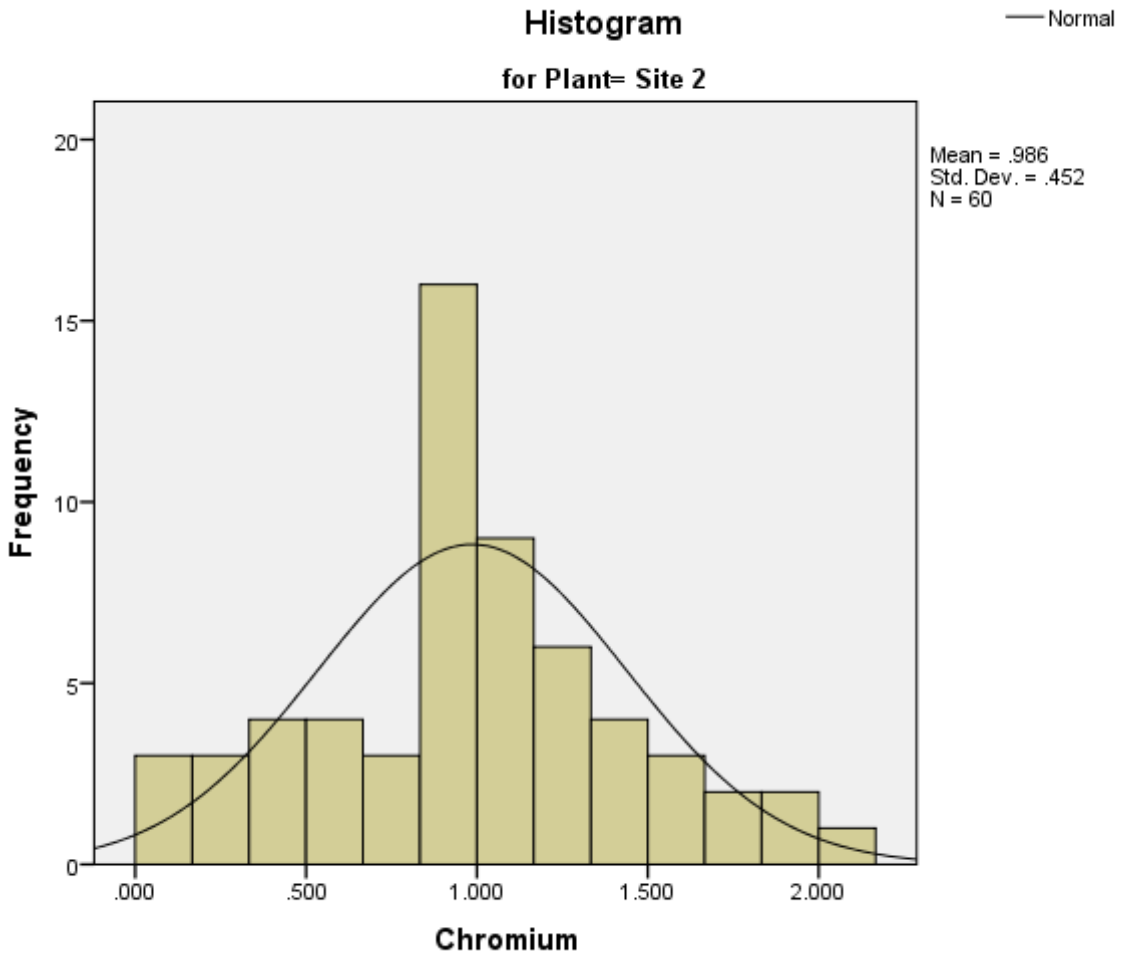
Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Site	Statistic	df	Sig.	Statistic	df	Sig.
Chromium	Site 1	.070	60	.200*	.982	60	.498
	Site 2	.107	60	.084	.978	60	.336

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction




Histogram
for Plant= Site 2
Normal
Mean = .986
Std. Dev. = .452
N = 60
Frequency
20
15
10
5
0
.000
.500
1.000
1.500
2.000
Chromium

8.10 APPENDIX 10: Shapiro Wilk Test: *C. obtusifolia*_ Cr(VI) in the leaves

Descriptives				
			Statistic	Std. Error
Cr(VI) Site 1	Mean		.3009	.01975
	95% Confidence Interval for Mean	Lower Bound	.2614	
		Upper Bound	.3404	
	5% Trimmed Mean		.2978	
	Median		.3015	
	Variance		.023	
	Std. Deviation		.15297	
	Minimum		.02	
	Maximum		.72	
	Range		.70	
	Interquartile Range		.23	
	Skewness		.196	.309
	Kurtosis		-.328	.608
Cr(VI) Site 2	Mean		.3964	.03489
	95% Confidence Interval for Mean	Lower Bound	.3266	
		Upper Bound	.4662	
	5% Trimmed Mean		.3822	
	Median		.3460	
	Variance		.073	
	Std. Deviation		.27023	
	Minimum		.00	
	Maximum		1.04	
	Range		1.04	
	Interquartile Range		.24	
	Skewness		1.052	.309
	Kurtosis		.392	.608

Tests of Normality						
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Site 1	.054	60	.200*	.983	60	.582
Site 2	.202	60	.000	.884	60	.000
*. This is a lower bound of the true significance.						
a. Lilliefors Significance Correction						

8.11 APPENDIX 11: T TEST: *R. lanceolatus*: Cr(VI) in the leaves

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Chromium	Equal variances assumes	4.231	.042	-5.692	118	.000	-.397617	.069852	-.535943	-.259291
	Equal variances not assumes			-5.692	102.116	.000	-.397617	.069852	-.536166	-.259067

Group Statistics

	Site	N	Mean	Std. Deviation	Std. Error Mean
Chromium	Site 1	60	.58810	.297739	.038438
	Site 2	60	.98572	.451785	.058325

95% CI: (-0.535943; -0.259291)

Standard error Difference: 0.069852

8.12 APPENDIX 12: Non Parametric Test: *C. obtusifolia* Cr(VI) in the leaves

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
Chromius	120	.37745	.338316	.004	2.963
Site	120	1.50	.502	1	2

Mann-Whitney Test

Ranks

	Site	N	Mean Rank	Sum of Ranks
Chromius	Site 1	60	55.71	3342.50
	Site 2	60	65.29	3917.50
	Total	120		

Test Statistics[a]

	Chromius
Mann-Whitney U	1512.500
Wilcoxon W	3342.500
Z	-1.509
Asymp. Sig. (2-tailed)	.131

a. Grouping Variable: Site

8.13 APPENDIX 13: Regression analysis: *Rumex lanceolatus*; The relationship between Cr(VI) and chlorophyll.

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.33755382
R Square	0.11394258
Adjusted R Square	0.10643362
Standard Error	0.00854171
Observations	120

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.00110712	0.00110712	15.17421	0.000163054
Residual	118	0.008609371	7.2961E-05		
Total	119	0.009716493			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.07059702	0.001630822	43.2892337	2.89E-74	0.067367545	0.07382649	0.06737	0.07382
Cr(VI), mg/l	-0.00709103	0.001820355	-3.89540926	0.000163	-0.010695827	-0.00348062	-0.0107	-0.00348

8.14 APPENDIX 14: Regression analysis*: C. obtusifolia*: The relationship between Cr(VI) and chlorophyll.

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.123633
R Square	0.015285
Adjusted R Square	0.00694
Standard Error	0.007111
Observations	120

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	9.3E-05	9.26E-05	1.831637	0.178521
Residual	118	0.00597	5.06E-05		
Total	119	0.00606			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.050198	0.00097	51.49889	1.02E-82	0.048268	0.052129	0.048268	0.052129
Cr(VI), mg/l	0.002608	0.00193	1.35338	0.178521	-0.00121	0.006423	-0.00121	0.006423

8.15 APPENDIX 15: Regression analysis; The relationship between the distance and Cr(VI) in the effluent discharged from UWWTP.

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.884474
R Square	0.782294
Adjusted R Square	0.727868
Standard Error	15.32897
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	3377.423	3377.423	14.37341	0.019249
Residual	4	939.9088	234.9772		
Total	5	4317.332			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	133.9224	11.09429	12.07129	0.00027	103.1197	164.7251	103.1197	164.7251
Distance, km	-6.94614	1.832162	-3.79123	0.019249	-12.033	-1.85925	-12.033	-1.85925

8.16 APPENDIX 16: One Sample t-test: Cr(VI) Concentration vs NWQSE (50 mg/l Cr)

t-Test: One sample: Cr(VI) vs NWQSE < 0.05 mg/l Cr(VI)

	Cr (VI), ug/l
Mean	99.19365582
Variance	932.8847671
Observations	60
Hypothesized Mean	50
df	59
t Stat	12.47586661
P(T<=t) one-tail	1.71796E-18
t Critical one-tail	1.671093032
P(T<=t) two-tail	3.43592E-18
t Critical two-tail	2.000995378

Ho: U less/equal 50
H1: u greater than 50

test statistics

t Stat	12.47586661	greater than t one tail
t Critical one tail	1.671093032	
p =	1.71796E-18	

Since t Stat > t Critical, we reject the Null Hypothesis and also
p is less than 0.05, therefore reject Null Hypothesis
Conclusion: There is enough evidence to infer that the Cr(VI) conc. In the UWWTP effluent is greater than 0.05 mg/l Cr(VI) as stipulated by NWQSE.
$(t(59) = 12.48, p = 0.001)$

8.17 APPENDIX 17: Comparisons: UOPS and UWWTP

8.17.1 Shapiro Wilk test – TKN

Descriptives

			Statistic	Std. Error
TKN	Mean		52.9165	9.80884
	95% Confidence Interval for Mean	Lower Bound	33.2424	
		Upper Bound	72.5905	
	5% Trimmed Mean		52.1636	
	Median		61.1000	
	Variance		5195.524	
	Std. Deviation		72.07998	
	Minimum		-148.00	
	Maximum		231.80	
	Range		379.80	
	Interquartile Range		109.88	
	Skewness		-.024	.325
	Kurtosis		.334	.639

8.17.2 Independent T test - TKN

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
TKN	1	28	-3.7143	43.38788	8.19954
	2	26	113.9035	39.00416	7.64934

Independent Samples Test

Levene's Test for Equality of Variances	t-test for Equality of Means

								95% Confidence Interval of the Difference		
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper	
Equal variances assumed		.100	.753	-10.447	52	.000	-117.61775	11.25861	-140.20980	-95.02570
Equal variances not assumed				-10.489	51.950	.000	-117.61775	11.21360	-140.12000	-95.11549

8.17.3 Shapiro Wilk test - COD

Descriptives

			Statistic	Std. Error
COD	Mean		1994.0741	158.69799
	95% Confidence Interval for Mean	Lower Bound	1675.7662	
		Upper Bound	2312.3819	
	5% Trimmed Mean		1994.6399	
	Median		1892.0000	
	Variance		1359992.787	
	Std. Deviation		1166.18729	
	Minimum		-3120.00	
	Maximum		5250.00	
	Range		8370.00	
	Interquartile Range		871.25	
	Skewness		-.757	.325
	Kurtosis		7.545	.639

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
COD	.168	54	.001	.833	54	.000

a. Lilliefors Significance Correction

8.17.4 Mann Whitney test - COD

Ranks

	Group	N	Mean Rank	Sum of Ranks
COD	1	28	24.50	686.00
	2	26	30.73	799.00
	Total	54		

Test Statistics[a]

	COD
Mann-Whitney U	280.000
Wilcoxon W	686.000
Z	-1.454
Asymp. Sig. (2-tailed)	.146

a. Grouping Variable: Group

8.17.5 Shapiro Wilk test - TSS

Descriptives

			Statistic	Std. Error
TSS	Mean		810.9074	178.67308
	95% Confidence Interval for Mean	Lower Bound	452.5346	
		Upper Bound	1169.2802	
	5% Trimmed Mean		657.6461	
	Median		671.0000	
	Variance		1723899.671	
	Std. Deviation		1312.97360	
	Minimum		-1190.00	
	Maximum		9540.00	
	Range		10730.00	
	Interquartile Range		536.00	
	Skewness		5.701	.325
	Kurtosis		38.297	.639

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.

TSS	.311	54	.000	.434	54	.000

a. Lilliefors Significance Correction

8.17.6 Mann Whitney test – TSS

Ranks

	Group	N	Mean Rank	Sum of Ranks
TSS	1	28	22.73	636.50
	2	26	32.63	848.50
	Total	54		

Test Statistics[a]

	TSS
Mann-Whitney U	230.500
Wilcoxon W	636.500
Z	-2.311
Asymp. Sig. (2-tailed)	.021

a. Grouping Variable: Group

8.17.7 Shapiro Wilk test - TDS

Descriptives

			Statistic	Std. Error
TDS	Mean		280.7796	89.96254
	95% Confidence Interval for Mean	Lower Bound	100.3376	
		Upper Bound	461.2216	
	5% Trimmed Mean		258.9702	
	Median		251.2500	
	Variance		437035.943	
	Std. Deviation		661.08694	
	Minimum		-1139.00	
	Maximum		2010.00	
	Range		3149.00	
	Interquartile Range		795.63	
	Skewness		.554	.325
	Kurtosis		.480	.639

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
TDS	.092	54	.200*	.968	54	.163

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

8.17.8 Independent T-test – TDS

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
TDS	1	28	32.3036	708.26850	133.85016
	2	26	548.3692	491.04260	96.30138

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means						
								95% Confidence Interval of the Difference	
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Equal variances assumed	2.079	.155	-3.089	52	.003	-516.06566	167.09071	-851.35769	-180.77363
Equal variances not assumed			-3.130	48.230	.003	-516.06566	164.89337	-847.56514	-184.56618

8.17.9 Shapiro Wilk test – PO4

Descriptives

			Statistic	Std. Error
PO4	Mean		2.1343	.98874
	95% Confidence Interval for Mean	Lower Bound	.1511	
		Upper Bound	4.1174	
	5% Trimmed Mean		1.9471	
	Median		2.1500	
	Variance		52.791	

	Std. Deviation	7.26576	
	Minimum	-18.10	
	Maximum	25.91	
	Range	44.01	
	Interquartile Range	8.47	
	Skewness	.440	.325
	Kurtosis	2.065	.639

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
PO4	.081	54	.200*	.962	54	.086

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

8.17.10Independent T test - PO4

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
PO4	1	28	-2.6464	4.94851	.93518
	2	26	7.2827	5.68389	1.11470

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
PO4	Equal variances assumed	.184	.669	-6.860	52	.000	-9.92912	1.44749	-12.83372	-7.02452
	Equal variances not assumed			-6.824	49.755	.000	-9.92912	1.45503	-12.85200	-7.00624

8.18 APPENDIX 18: Comparisons: UWWTP and NWQSE

8.18.1 One sample T-test – TKN

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
TKN	123	3.4733	5.38271	.48534

One-Sample Test

	Test Value = 33					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
TKN	-60.837	122	.000	-29.52675	-30.4875	-28.5660

8.18.2 One sample t-test – COD

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
COD	125	44.8168	17.07786	1.52749

One-Sample Test

	Test Value = 100					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
COD	-36.127	124	.000	-55.18320	-58.2065	-52.1599

8.18.3 One sample t-test – TSS

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
TSS	125	7.3040	10.52970	.94180

One-Sample Test

	Test Value = 100					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
TSS	-98.424	124	.000	-92.69600	-94.5601	-90.8319

8.18.4 One sample t-test – TDS

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
TDS	125	1711.7160	358.60257	32.07439

One-Sample Test

	Test Value = 500					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
TDS	37.778	124	.000	1211.71600	1148.2318	1275.2002

8.18.5 One sample t-test – PO4

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
PO4	125	.3274	.67244	.06014

One-Sample Test

	Test Value = 3					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
PO4	-44.435	124	.000	-2.67256	-2.7916	-2.5535

8.19 APPENDIX 19: **NBRI: PLANT SPECIES IDENTIFICATION REPORT**

Ministry of Agriculture, Water and Forestry

National Herbarium of Namibia (WIND)

Identification Report

Report No.: 2019/404

16 May 2019

Collector/s: Mr G. Iiputa
Address: UNAM
Main Campus
WHK

Number	ID cat.	Identification
sp. 1	1	Rumex lanceolatus Thunb.
sp. 2	1	Cullen obtusifolia (DC.) C.H.Stirt.
sp. 3	1	Plantago major L.

Comment:

Curator
National Herbarium of Namibia (WIND)

CURATOR
NATIONAL HERBARIUM
OF NAMIBIA
NATIONAL BOTANICAL
RESEARCH INSTITUTE
Private Bag 13184
WINDHOEK
NAMIBIA

Identification categories: 1. Certain identification 2. Closest to 3. Certain to genus only 4. Unable to identify

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