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**Effiom E. Oku  
Catherine V. Nnamani  
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**UNITED NATIONS UNIVERSITY  
INSTITUTE FOR NATURAL RESOURCES IN AFRICA  
(UNU-INRA)**

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## **About UNU-INRA**

The United Nations University Institute for Natural Resources in Africa (UNU-INRA) is the second Research and Training Centre / Programme established by the UN University. The mandate of the Institute is to promote the sustainable management of Africa's natural resources through research, capacity development, policy advice, knowledge sharing and transfer. The Institute is headquartered in Accra, Ghana, and also has five Operating Units (OUs) in Cameroon, Ivory Coast, Namibia, Senegal and Zambia.

## **About the Project**

This project was jointly implemented by UNU-INRA and Ebonyi State University, Nigeria. The aim was to evaluate the effectiveness of the African species of vetiver grass in treating wastewater from industrial and domestic sources.

## **About the Authors**

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## **Abstract**

The discharge of untreated domestic and industrial wastewater into the environment is common in Africa and other developing nations of the world. The conventional treatment systems available in developed economies are very expensive for many African countries. The quest to find a unique and greener way of treating the growing wastewater volume, both at the micro and macro scale in Africa, led to this study. The study evaluated biological and mechanical properties of an unknown African endemic species of Vetiver grass (*Chrysopogon nigritanus*) and the South Indian species (*Chrysopogon zizanioides*). It further analysed their effectiveness in cleaning industrial effluent and compared the potential of the African species with the South Indian species, which potential in treating wastewater is well-established by research. Untreated effluents were collected from an abattoir, a quarry site and fertilizer and cassava processing companies. Untreated leachate from a public refuse dump, wastewater from urban drains and crude oil polluted water were also collected and treated with these bio-resources (*Chrysopogon nigritanus* and *Chrysopogon zizanioides*). Pre and post treatment properties of effluents assessed were BOD, COD, pH, N, P, Cd, Pb, Zn, Ar, Ni, Fe, Mg, among others. All pre-treatment properties exceeded the WHO/FAO and USEPA safe levels for wastewater before discharge or re-use. Treatment using the African bio-resource for 2, 4 and 6 days reduced the pollutants significantly to acceptable safe levels. In some cases, contaminants were completely removed bringing the wastewater quality to acceptable safe levels for urban agriculture. The African species, just like the South Indian species, has a high potential in cleaning wastewater. However, while the African species was more effective in removing phosphate, the South Indian species was more effective on nitrate. Both species fit into the global call for promoting a Green Economy, more specifically, in the area of managing wastewater with bio-resources before discharge or for re-use.

*Keywords: Vetiver grass, wastewater, effluents, bio-resource*

## Table of Contents

Acknowledgements.....	iv
Abstract.....	v
List of Tables.....	vii
List of Figures.....	vii
List of Plates.....	viii
Acronyms and Abbreviations.....	ix
1.0 Introduction.....	1
1.1 Overview of Wastewater Management Challenges.....	1
1.2 Drivers of Wastewater Use.....	3
1.3 Challenges of Wastewater.....	4
1.4 Impact of Wastewater on Environmental Health.....	5
1.5 Toxicity of Heavy Metals and Concerns for Human Health.....	6
1.6 Benefits/Opportunities in Wastewater.....	7
1.7 Wastewater Use in Agriculture in Developing Countries.....	9
2.0 Materials and Methods.....	10
2.1 Study Area and Establishment of Vetiver through Hydroponic Structure.....	10
2.2 Wastewater Sources, Collection and Treatment.....	12
2.3 Physicochemical Analysis of Wastewater.....	12
2.4 Anatomical Studies.....	12
3.0 Results and Discussions.....	14
3.1 Wastewater Reaction (pH).....	14
3.3 Biological Dissolved Oxygen (BOD) and Chemical Dissolved Oxygen (COD) reduction.....	18

3.4 Heavy Metals Removal.....	20
3.5 Anatomy of African and South Indian Species of Vetiver and their Phytoremediative Potentials .....	25
4.0 Conclusion and Recommendations.....	30
4.1 Conclusions .....	30
4.2 Recommendations .....	30
References .....	31

## List of Tables

Table 1. Associated diseases of some hazardous heavy metals and maximum acceptable level. ....	7
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## List of Figures

Figure 1. Pre and post-treatment pH levels of quarry industry effluents .....	15
Figure 2. Pre and post treatment levels of pH of cassava processing industry effluent.....	15
Figure 3. Pre and post treatment pH levels of crude oil polluted water .....	16
Figure 4. Pre and post-treatment levels of nitrate contaminant in a public dumpsite leachate.....	17
Figure 5. Pre and post-treatment phosphate contaminant level in a public dumpsite leachate .....	18
Figure 6. Pre and post treatment levels of Biological Dissolved Oxygen (BOD) in cassava industry effluent .....	19
Figure 7. Pre and post-treatment level of Chemical Dissolved Oxygen in an abattoir effluent.....	19

Figure 8. Pre and post-treatment levels of Lead (Pb) in quarry industry effluent...	21
Figure 9. Pre and post-treatment Cadmium contaminant levels in fertilizer company effluent.....	22
Figure 10. Pre and post-treatment cyanide contaminant level in cassava processing industry .....	22
Figure 11. Pre and post treatment zinc contaminant levels in cassava processing industry .....	23
Figure 12. Pre and post-treatment Arsenic contaminant level in fertilizer company effluent.....	23
Figure 13. Pre and post-treatment Arsenic contaminant levels in quarry industry effluent.....	24
Figure 14. Pre and post-treatment Manganese contaminant level in abattoir effluent.....	24

## List of Plates

Plate 1. Vetiver dug from the soil with roots and shoots pruned washed clean of soil using water. ....	10
Plate 2. Vetiver planted through holes in a floating material.....	11
Plate 3. Vetiver roots spout as observed 15 days after the set-up.....	11
Plate 4. Transverse Sections of Roots of both Species .....	26
Plate 5 Transverse Sections of stems of both Species. ....	27
Plate 6. Transverse sections of leaves of both species .....	28
Plate 7. Stomatal features in <i>C. nigrifolius</i> x 400 (Abaxial).....	28
Plate 8. Stomatal features in <i>C. zizanioides</i> X 400 (Abaxial) .....	29

## **Acronyms and Abbreviations**

As	Arsenic
BOD	Biochemical Oxygen Demand
Co	Cobalt
COD	Chemical Oxygen Demand
CN	Cyanide
Cu	Copper
FAO	Food and Agriculture Organization
Fe	Iron
Hg	Mercury
Mn	Manganese
Ni	Nickel
Pb	Lead
UNEP	United Nations Environment Programme
UN-HABITAT	United Nations Human Settlements Programme
USEPA	United States Environmental Protection Agency
WHO	World Health Organisation
Zn	Zinc

## 1.0 Introduction

### 1.1 Overview of Wastewater Management Challenges

Water bio-resource-engineering are techniques that harness plants' biological and mechanical properties to provide remedies for contaminated water. In developing countries including Sub-Saharan Africa (SSA), almost all industrial units discharge their untreated wastewater into waterbodies, wetlands and the soil. It is reported that an estimate of 90 percent of the developing world wastewater does not pass through any treatment before it is discharged into the environment (UNEP and UN-Habitat, 2010). Aside contamination of waterbodies, land, and food chain, wastewater related emissions of methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) are more harmful than carbon dioxide (CO<sub>2</sub>) (UNEP and UN-Habitat, 2010). In developing countries where there is limited financial and physical resources to treat wastewater, the socioeconomic situation and the context of urbanization create the conditions for unplanned and uncontrolled wastewater use (Drechsel, *et al.*, 2010).

The high cost of installing conventional engineering treatments, cost of maintenance, and energy insecurity are some of the reasons for the practice of discharging untreated wastewater into the environment particularly in Africa and Asia. According to UNU-INRA (2013), this practice will continue to produce sick soil and water, sick food and sick people as a result of crops irrigation with untreated wastewater. Therefore a smart low-cost effective and efficient solution is needed to revolutionize wastewater management in Africa and other parts of the world.

According to UNEP and UN-Habitat (2010), any smart solution for wastewater treatment must be economically and environmentally viable for the future and also be socially and culturally inclusive. The water bio-resource engineering employed in this study fits into the above criteria. The utilization of Vetiver species from South Indian (*Chrysopogon zizaniodes*) as a bio-resource has been shown to be smart and effective in wastewater treatment (Truong *et al.*, 2008). The potentials of the African species (*Chrysopogon nigritanus*) is not known and literatures not readily available. Moreover, earlier studies and existing literature on the use of the three known species of Vetiver including the North Asian species (*Chrysopogon nemoralis*) in erosion control showed that all the three species differ in their potentials to control erosion (Truong *et al.*, 2008). The effectiveness of the

African species in domestic and industrial wastewater treatment is not well known or documented.

UNU-INRA (2013) opined that the use of the African species is still at its infancy when compared to the extensive use of the South Indian species in the continent of Asia and beyond. In this regard, the United Nations University Institute for Natural Resources in Africa (UNU-INRA) collaborated with Ebonyi State University in Nigeria to carry out a detailed study of the histology of the African indigenous species and compared it to the South Indian species while also assessing the potentials of the anatomical features to clean domestic and industrial wastewater. Generally, Vetiver offers a cost effective, efficient and environmentally friendly solution, particularly, in the face of the global need for green industrial effluent management and reduced industrial carbon footprint.

Wastewater is water that has been used by households, industries, and commercial units that contain contaminants and therefore do not serve a useful purpose unless treated (Raschid-Sally, *et al.*, 2008). Non-treatment of the water before discharge into the environment raises major human, crop and environmental health concerns. Effluents from hospitals, urban-runoff, agro and aquaculture effluents, dissolved and suspended matter were further added by Corcoran *et al.*, (2010) to the list of sources of wastewater. Though it is seen as water that is not useful unless treated, Hamilton, *et al.*, (2007), reported that around 20 million hectares of land are irrigated globally with untreated wastewater. This is not only likely to increase, but will also expand in developing nations as water stress intensifies with population, urbanization and industrialization increasing within the few decades.

Inorganic and organic contaminants of wastewater have the potential to cause diseases such as bacteria, viruses and parasitic infections. Heavy metal contents in wastewater can also be dangerous to human health. Therefore untreated wastewater use in agriculture creates risks for both the population and economy. The main concern of wastewater treatment is to kill or remove contaminants to internationally acceptable safe levels before discharge into the environment or water bodies where it poses no threat to the environment and public health. In the course of achieving this, metals, energy and nutrient loads of wastewater can be recovered, allowing for gainful re-use of water and recovered resources. Untreated wastewater is widely and commonly used for irrigation by urban and peri-urban farmers in developing countries. This practice is expanding as it provides food (fresh vegetables) to the cities, and a source of livelihood for women, youths, unemployed persons and low-income city workers who take to urban agriculture as an additional source of

income. Importantly, climate change impact, promotes water stress and scarcity, converting once productive rural land to infertile land, thus, contributing to more vulnerable people migrating from rural areas to cities. According to Dr Elias Ayuk, Director of United Nations University Institute for Natural Resources in Africa (UNU-INRA) quoted by Nutakor (2014), it may be impossible to meet future demand for water without revolutionising wastewater management.

Wastewater, on one hand, promotes food production and food security, particularly in urban centres. This is because it is readily available even during normal dry seasons and droughts to support crop production (Barry, 2011). An effective alternative to conventional engineering wastewater treatment system is the use of innovative, cost-effective and efficient climate smart wastewater bioengineering technique. The technique presented in this monograph utilizes mechanical and biological properties of two Vetiver species from Africa and South India to absorb contaminants from wastewater to acceptable internationally safe disposal levels. The objective of this monograph is therefore to present a study on the assessment of the effectiveness of this bio-resource engineering technique using an African species of Vetiver grass (*Chrysopogon nigritanus*). In addition, histological studies were conducted to compare the features and potentials of *Chrysopogon nigritanus* and *Chrysopogon zizanioides*.

## **1.2 Drivers of Wastewater Use**

Agriculture is currently accounting for over 70 percent of global fresh water resources withdrawals and 86 percent of the world's total fresh water consumption (Mateo-Sagasta, *et al.*, 2013). Fresh water is also becoming scarce with increase in population, urbanization, industrialization and climate change intensification. In Africa and Asia, according to UNEP (2008), an estimated 85–90 percent of all fresh water resources are used for agriculture while water is an increasingly scarce resource. Agricultural water withdrawals have already exceeded 90 percent of total water withdrawals according to the same report. In Africa, agriculture is the major occupation and over 70 percent of the population is into agriculture. . The predominant rain-fed system of Africa's agriculture is unable to meet crop demand for water, therefore putting increasing high demand on the use of wastewater as can be seen particularly in cities.

Wastewater therefore, becomes readily available for irrigation and food production. With increasing demand for water by municipal and industrial sectors, competition for water will continue to increase. Freshwater now used

for agriculture will be diverted to the urban domestic and industrial sector uses. Wastewater use ranges from the formal use of ultrapure recycled water for advanced industrial purposes to the informal use of untreated and raw wastewater in vegetable production in a peri-urban area (Mateo-Sagasta, *et al.*, 2015).

Meanwhile, in their contribution, Raschid-Sally, *et al.*, (2008), stated that the main drivers of wastewater use in irrigated agriculture are a combination of three factors in most cases:

- 1) Increasing urban water demand and related return flow of used but seldom treated wastewater into the environment and its water bodies, causing pollution of traditional irrigation water sources;
- 2) Urban food demand and market incentives favouring food production in city proximity where water sources are usually polluted;
- 3) Lack of alternative (cheaper, reliable or safer) water sources. In contrast, Drechsel, *et al.*, (2010), reported that in more developed countries, water reuse and recycling are driven by physical water scarcity (including climate change and drought management), water reallocations from agriculture to other uses and also as an economic response to costly inter-basin transfers. An additional factor influencing recycling is the stringent environmental standards, which make the land application of wastewater and sludge both unavoidable and economically feasible (Drechsel, *et al.*, 2010).

### **1.3 Challenges of Wastewater**

There are currently no globally comparable data on the percentage of wastewater treatment at the national scale to aid in the assessments of this effort (Burrian, *et al.*, 2000). Centralized wastewater treatment systems in developing countries are not always affordable, and when they are in place, there is usually lack of the needed skills, institutional and financial capacities to handle these systems. (Raschid-Sally, *et al.*, 2008). Lack of capacity to manage wastewater does not only compromise the natural capacity of marine and aquatic ecosystems to assimilate pollutants, but also causes the loss of a whole array of benefits provided by waterways and coasts that are taken for granted. (UNEP & UN-Habitat, 2010). The UN report further speculates that the financial, environmental and societal costs in terms of human health, mortality and morbidity and decreased environmental health are projected to increase dramatically unless wastewater management is given very high priority and dealt with urgently.

However, many countries do not have national guidelines for the acceptable use of wastewater for irrigation. In many other countries, the capacity to apply these guidelines and best practice recommendations are insufficient and need substantial strengthening (UNEP & UN-Habitat, 2010). Information on the quantity of wastewater generated, treated, and used at the national scale is often “unavailable, limited, or outdated” (Sato, *et al.*, 2013). Evers, *et al.* (2008) also observed that farmers often lack knowledge of water quality, including nutrient content, so they combine nutrient-rich irrigation water with chemical fertilizers making agriculture a source of pollution.

#### **1.4 Impact of Wastewater on Environmental Health**

Nitrate and phosphate are major pollutants that cause algal blooms and eutrophication in water bodies. The nitrate and phosphate are excess nutrients that allow aquatic micro-organisms to rapidly multiply and consume all available oxygen in the water, thus suffocating marine animals. Eutrophication, in turn, can lead to fish die-offs from lack of oxygen conditions. In humans, other effects such as the emerging issue of endocrine disruption can occur in part due to the presence of pharmaceutical products or chemicals in waterways (UNEP & UN-Habitat, 2010). According to Hamilton, *et al.*, (2007), ecological impacts include; (1) accumulation of bio-available forms of heavy metals and fate of organics in soil, (2) impact from extensive use on catchment hydrology and salt transport, (3) microbiological contamination risks for surface water and groundwater, and (4) transfer of chemical and biological contaminants to crops.

The primary health risk is diarrheal disease for consumers and farmers as well as skin and worm infections for all those in contact with wastewater (Raschid-Sally, *et al.*, 2008) but the impact on health varies depending on location and type of contaminant. However, bacteria and intestinal worm infestations have been shown to pose the greatest risk (Drechsel, *et al.*, 2010). Mateo-Sagasta, *et al.*, 2013 also characterised the effect of wastewater use in terms of agriculture on public health, ecology and crop and soil resources in general as follows; a high concentration of chemical pollutants in wastewater may be toxic to plants. Accumulation of nitrogen, phosphorus, dissolved solids and other constituents such as heavy metals in the soil affect its productivity and the sustainability of land use for agriculture. Salt accumulation in the root zone may have harmful impacts on crop yields.

Ecological impacts: drainage of wastewater from irrigation schemes into water bodies may indirectly affect aquatic life and negatively influence

overall biodiversity, e.g. the presence of water birds. Groundwater resources are affected through leaching of nutrients and salts included in wastewater which potentially affects the quality of groundwater. The degree of impact depends on several factors, including the quality of groundwater, the depth of the water table, soil drainage and the amount of wastewater applied for irrigation. On the other hand, excessive concentrations of nitrogen in wastewater can lead to over-fertilization and cause excessive vegetative growth, delayed or uneven crop maturity and reduced quality (Jiménez, 2006 and Qadir, *et al.*, 2007). Excessive concentrations of some trace elements may also cause plant toxicity and sometimes become a health risk for crop consumers.

### **1.5 Toxicity of Heavy Metals and Concerns for Human Health**

Lead (Pb) is one of the most abundant toxic metals that pose a serious threat to human beings, animals and phytoplanktons (Singh, Tiwari, & Gupta, 2012). In humans, it is absorbed directly into the blood stream and is stored in soft tissues, bones and teeth (David, Than, & Tun, 2003). It can also affect the kidney and most importantly the nervous system and brain accumulation over a long-time can cause diseases such as anaemia, encephalopathy, hepatitis and nephritic syndrome (Singh, Tiwari, & Gupta, 2012). Lead also influences the aquatic system as certain communities of aquatic invertebrate populations are more sensitive than others and adaptation to low oxygen conditions can be hindered by high Pb concentration (Singh, Tiwari, & Gupta, 2012). Zinc supports normal growth and development during pregnancy, childhood and adolescence and is required for proper sense of taste and smell (National Institutes of Health, 2013). According to Lenntech (2015), excessive zinc concentration in the human body can cause problems, such as stomach cramps, skin irritations, vomiting, nausea and anaemia. Additionally, it can cause damage to the pancreas, disturb the protein metabolism, and cause arteriosclerosis. Extensive exposure to zinc chloride can cause respiratory disorders (Lenntech, 2015).

The body stores cadmium (Cd) in the liver and kidneys, which is slowly excreted in the urine, but high concentrations of Cd can cause abdominal pain, nausea, vomiting and intestinal bleeding. Furthermore, exposure at lower concentrations over many years can damage the kidneys, bones, lungs, liver and nervous system and may cause several types of cancer (Northern Territory Government, 2013). Cyanide (CHN) is extremely toxic to humans and long term exposure negatively affects the central nervous system, cardiovascular and respiratory systems, as well as an enlarged thyroid gland, and irritation to the eyes and skin (USEPA, 2000).

Heavy metals contained in wastewater may lead to signs associated with some specific illness, as presented in Table 1. Through the consumption of crops and animals that grazed on contaminated soil and plants, heavy metals enter the human system and food chain, further leading to bioaccumulation and subsequent illness (Abudullahi, 2013 and Akpor, *et al.*, 2014).

**Table 1. Associated diseases of some hazardous heavy metals and maximum acceptable level.**

Heavy metals	Toxicities	MCL (mg/l)
Arsenic	Skin diseases, visceral cancers, vascular diseases	0.05
Cadmium	Kidney damage, renal disorder, human carcinogen	0.01
Chromium	Headache, diarrhoea, nausea, insomnia	0.05
Copper	Liver damage, Wilson disease, insomnia	0.25
Nickel	Dermatitis, nausea, chronic asthma, coughing human carcinogen	0.20
Zinc	Depression, lethargy neurological signs and increased thirst	0.80
Lead	Damage to fetal brain, kidney disease, circulatory and nervous system	0.006
Mercury	Rheumatoid arthritis, kidney disease, circulatory and nervous system	0.00003

Sources: Babel and Kurniawan, 2003, and Barakat, 2011

## 1.6 Benefits/Opportunities in Wastewater

Across major cities in West Africa, between 50 and 90 percent of vegetables consumed by urban dwellers are produced within or close to the cities where much of the water used for irrigation is wastewater (Drechsel, *et al.*, 2010). From the microbiological perspective, wastewater is perceived more as a biophysical hazard. Its chemical content presents a more complex situation with both positive and negative impacts on soils, crops and water bodies,

which are important considerations, not only for the farmer but also for managing wastewater treatment and discharge (Qadir, *et al.*, undated).

An important factor which makes wastewater valuable is that it is a reliable source of water, available all year round, unlike rainfall and freshwater from streams that are seasonally available (Raschid-Sally, *et al.*, 2005). Wastewater does not only permit cropping throughout the year and higher crop yields but expands cropping areas into the drier areas of the nation (Drechsel, *et al.*, 2010). The increased productivity and related income/food supply gains allow farmers a more reliable livelihoods. The use of income from sales of produce produced with wastewater for education and obtaining health care is some of the indirect benefits. The more direct benefit to cities and communities are in terms of food and nutritional security (Drechsel, *et al.*, 2010). For example, in Accra, Ghana, more than 200,000 people eat vegetables cultivated with wastewater every day (Amoah, *et al.*, 2007)

Considering that in Sahelian countries such as Burkina Faso, Mali, and Senegal, forage biodiversity has decreased over time (FAO, 2006), the demand for dairy in cities is increasing with urbanization and changing diets. Therefore reusing wastewater for fodder production appears an important and comparatively low-risk avenue for these countries which can contribute to enhancing the resilience to climate changes and food insecurity in these developing countries. Another advantage of wastewater reuse is the nutrient content. Even when treated, wastewater recycles organic matter and a larger diversity of nutrients than any commercial fertilizer can provide. Bio-solids, sludge and excreta when contained in wastewater provide numerous micronutrients such as cobalt, copper, iron, manganese, molybdenum and zinc, which are essential for optimal plant growth (Drechsel, *et al.*, 2010). These elements need to be present for proper crop growth, but when available in levels above the safe limit, it constitutes health challenges to humanity, crops and the environment. It is estimated that 1000 cubic metres of municipal wastewater used to irrigate one hectare can contribute 16–62 kg total nitrogen, 4–24 kg phosphorus, 2–69 kg potassium, 18–208 kg calcium, 9–110 kg magnesium, and 27–182 kg sodium (Qadir, *et al.*, 2007) and therefore can reduce the demand for chemical fertilizers.

Drechsel, *et al.*, (2010) accounted for a few studies that have quantified the economic gains from nutrients in wastewater under actual field conditions. According to them Keraita *et al.*, (2008) estimated in Guanajuato, Mexico, saving arising from using wastewater to supply the required nitrogen and phosphorus for crops to be US\$135 per hectare. A similar study comparing vegetable production using freshwater and untreated wastewater in

Haroonabad, Pakistan, found that the gross margins were significantly higher for wastewater (US\$150 per hectare) because farmers spent less on chemical fertilizers.

### **1.7 Wastewater Use in Agriculture in Developing Countries**

Substitution of freshwater by treating wastewater is already seen as an important water conservation and environmental protection strategy, which is simultaneously contributing to the maintenance of agricultural production (Raschid-Sally, *et al.*, 2008). However, in Africa, the lack and insufficient treatment of water and wastewater with rapid population growth and urbanization is a great challenge. At present, however, the efforts to improve drinking water quality and wastewater treatment are not keeping pace with population growth and urbanization (Wang, *et al.*, 2013). A number of case studies of city and country assessments of varying detail conducted in middle and low-income countries of Africa, Asia and Latin America have recognized that the use of untreated wastewater for the irrigation of high-value cash crops in and close to urban centers is a widespread practice (Raschid-Sally, *et al.*, 2008).

Raschid-Sally and Jayakody (2008) suggests from a survey across the developing world that wastewater without any significant treatment is used for irrigation purposes in four out of five cities. Few studies have quantified the aggregate contribution of wastewater to food supply. In Pakistan, about 26 per cent of national vegetable production is irrigated with wastewater (Ensink *al.*, 2004), while in Hanoi, Vietnam, which is much wetter than Pakistan, about 80 per cent of vegetable production is from urban and peri-urban areas irrigated with diluted, but untreated wastewater (Lai, 2002), as cited in (Drechsel, *et al.*, 2010) (Raschid-Sally, *et al.*, 2008).

On-site sanitation systems such as pit latrines and septic tanks are widely used in rural and semi-urban areas in Africa. However, the maintenance and management of these pit latrines are very poor and hence deteriorates groundwater quality. With many pit latrines full and the wastewater flowing, natural wetlands are used for wastewater treatment or disposal in some countries such as Uganda; however, due to increasing pollutant loads, more and more natural wetlands are weakened or diminished (Wang, *et al.*, 2013).

## 2.0 Materials and Methods

### 2.1 Study Area and Establishment of Vetiver through Hydroponic Structure

The study was carried out in Nigeria. A Vetiver nursery was raised in the field for *Chyosopogon nigritanus* (African species) and *Chyosopogon zizaniodes* (South Indian species) as recommended by Troung et al., 2008 and Oku, et al., 2011. In the screen house, Vetiver plant was raised hydroponically, as described by Truong and Hart (2001) in Queensland Australia, where the system was first developed. The hydroponic screen house set-up in this study was a 40 diameter and 60 cm high bucket. Vetiver plant was first dug out from the soil as in Plate 7 with the roots and shoots pruned. The root was washed in a pool of water to remove all soil attached to the roots as in Plate 1.



**Plate 1. Vetiver dug from the soil with roots and shoots pruned washed clean of soil using water.**

The Vetiver was split and planted into a hole of a floating material as in Plate 2. This was to allow the plant float in the effluent when fully established and to be used for the effluent treatment. The raised plant was then planted using nutrient water as the medium (instead of soil). The nutrient medium was a pool of water in a bucket container to which NPK fertilizer was added. This was to provide nutrient in the water to enable

Vetiver roots and shoots established and grow as in Plate 3 before using for wastewater treatment



**Plate 2. Vetiver planted through holes in a floating material**



**Plate 3. Vetiver roots spout as observed 15 days after the set-up.**

The set-up was allowed in the open for 8 weeks for the roots and shoots to establish. The established roots and shoots aid in removing contaminants from the effluents and in the shoots.

## 2.2 Wastewater Sources, Collection and Treatment

Untreated wastewater was collected from city open drain, public un-engineered dumpsite, fertilizer blending company, quarry site, cassava processing industry, crude oil polluted water and abattoir. Wastewater from the different sources were put under treatment and each treatment was replicated three times, using a total of 21 containers with effluents for each Vetiver species. The study had a total of 42 containers. Wastewater from the different sources mentioned above was collected in plastic buckets and the 8 weeks old hydroponically raised Vetiver was allowed to sit on the effluents with the roots immersed in the effluents. The immersed roots removed contaminants by absorbing the contaminants from the roots and storing in the leaves above.

## 2.3 Physicochemical Analysis of Wastewater

The physicochemical properties were determined for both the pre and post treatment effluents. Heavy metals and other properties determined were; pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrate, phosphate, cyanide. Others were; lead, zinc, iron, cobalt, cadmium, mercury, manganese, arsenic, nickel and copper. All elements were analysed using standard methods (APHA, 2005 and Udo *et al.*, 2009).

Contaminant removal rate was calculated using the pre and post-treatment effluents results (Boonsong and Chanirp, 2008; Bedewi, 2010) as described below:

$$\text{Percentage removal} = \frac{\text{Pre-treatment contaminant level} - \text{Post-treatment contaminant level}}{\text{Pre-treatment level}} \times 100$$

Both the pre and post-treatment physicochemical properties were compared with the WHO/FAO (2006) and USEPA safe and allowable standards.

## 2.4 Anatomical Studies

### Young Stems and Leaves

The young stems, leaves and roots of the two plants were collected and sectioned with the aid of a Sliding Sledge Microtome into transverse sections (TS) using Fixatives of Formalin, Acetic acid and Alcohol (F A A) in a Botanical Laboratory at the University of Nigeria, Nsukka. Sections were mounted on glycerol and examined under a light microscope at both x 100

and x 400 magnifications. Parameters assessed include: vessel lumen diameter, vessel density, height and diameter of vessels in stems (culms) Photomicrographs of some important features were made with Olympus CH Bi-nocular microscope fitted with Cannon Digital Camera at x100 and x 400.

### **Leaf Epidermal Structures**

Epidermal peels were obtained by standard Impression Technique as modified by Onyeike and Osuji, 2003, Esau, 1977). Peels were made from both adaxial and abaxial surfaces. The epidermal features studied include nature of the epidermal cells, distribution and types of stomata, stomatal frequency, length and breadth of stomata, breadth of guard cells, pore length and breadth, nature of subsidiary cell and Stomatal index (SI) which was

given as:  $SI = \frac{S}{S + E} \times 100$  Where: S = the number of stomata per field of view, E = the corresponding number of epidermal cells Metcalfe and Chalk (1957).

### **2.5 Data Analysis**

Data derived from these were further subjected to statistical analysis. The percentage reduction of pollutants and their mean standard error were determined. Characters of wood and terminologies for all anatomical assessments were selected according to Nnamani and Nwosu (2012), Metcalfe and Chalk (1957), Esua (1977). Photomicrographs of some of the important features were made using 650 IS Cannon Digital Camera at x 100 and x 400 magnifications

## 3.0 Results and Discussions

### 3.1 Wastewater Reaction (pH)

The hydrogen ion activity (pH) in the wastewater is measured by acidic and basic nature of effluents. Figures 1, 2 and 3 reveal the pre and post-treatment pH of industrial effluents from quarry industry, cassava processing industry and water polluted with crude oil. On the pH scale, the pre-treatment value of cassava processing effluent was 3.8 indicating strongly acidic, the value of 12.8 for the quarry industry effluent indicates strongly alkaline and pH of water polluted by crude oil was 9.2 indicating weak alkaline. However, these values are higher or lower than the pH value of the 7.25 recommended by WHO (2006) as the acceptable maximum level of pH for treated wastewater before discharge into the environment. Intervention or treatment with two different species of Vetiver grass brought quarry industry effluent from 12.8 to 8.32 and 7.16 with African (*Chyosopogon nigritanus*) and South Indian (*Chyosopogon zizanioides*) species, respectively (Fig. 1).

The pH of cassava processing factory effluent was positively affected by Vetiver treatment, moving from the pre-treatment value of 3.8 to 5.77 and 6.23 with African and South Indian species, respectively. The corresponding pH of crude oil polluted water changed from 9.2 to 7.9 and 8.03 respectively. The pH was brought from either acidic or basic condition to near neutral. Thus Vetiver grass gave the industrial effluent favourable pH suited for wastewater before discharging into water bodies. The pH of industrial effluents and its adjustment during conventional treatment methods, allows dissolved contaminants to be separated from the wastewater during treatment processes (Brand, 2014).

The conventional engineering treatment system requires that the solution is agitated or catalyzed to maintain a certain pH for removal of particular heavy metal. This is not the case with treatment using Vetiver in the water bio-engineering system, all heavy metals regardless of the pH of the solution were positively influenced by Vetiver intervention. This is proved with the survival of Vetiver in both the highly acidic and basic solution (wastewater). A similar report had earlier been reported for the South Indian species by Truong (2008) and Oku (2010). The survival of the African species of Vetiver grass shows its comparative potential to grow under both acidic and alkaline conditions.

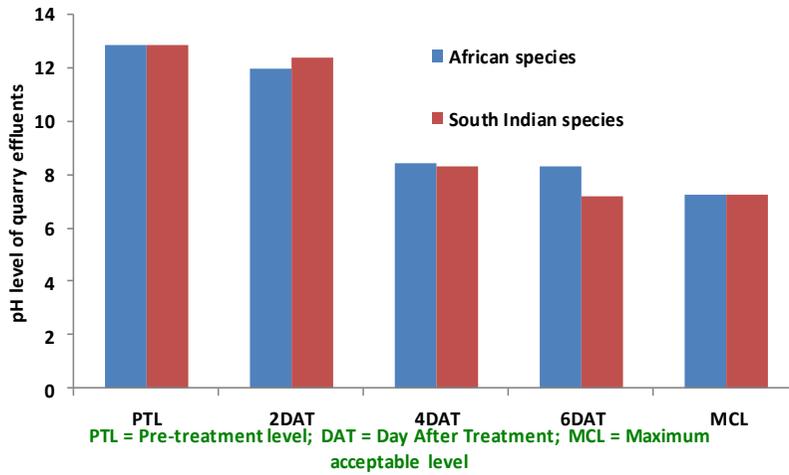


Figure 1. Pre and post-treatment pH levels of quarry industry effluents

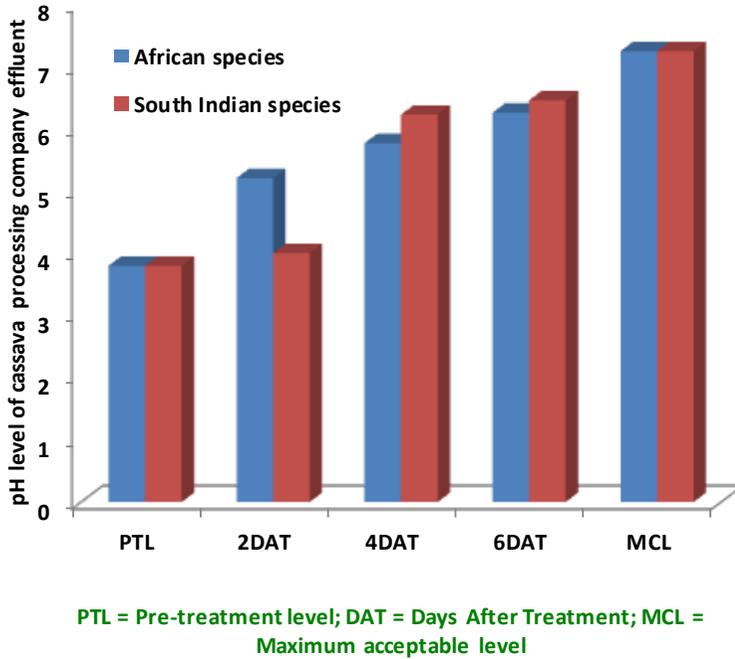


Figure 2. Pre and post treatment levels of pH of cassava processing industry effluent

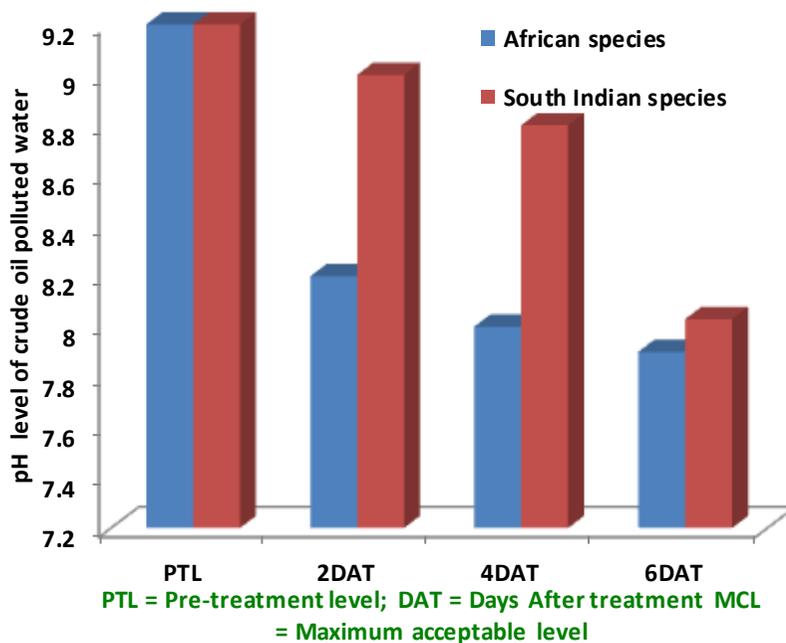


Figure 3. Pre and post treatment pH levels of crude oil polluted water

### 3. 2 Nitrate and Phosphate removal

Nitrate and phosphate are the major contaminants of water bodies. The high removal rate of both nutrients by the South Indian species had been reported (Zheng *et al.*, 1997 and Danh *et al.*, 2009). The pre-treatment and post-treatment results show a decrease levels of nitrate in the refuse dumpsite leachate. The removal rate of the African species was 54, 58 and 58 percent, as measured on two, four and six days after treatment respectively (Fig. 4). Whereas the South Indian species removed nitrate contaminants by 55, 58 and 62 percent as measured by two, four and six days after treatment respectively. The rate of phosphate contaminant removal by both species is presented in Fig. 5. Treatment of leachate from public unengineered refuse dumpsite using African species of the Vetiver grass removed phosphate from  $92.9 \text{ mg l}^{-1}$  by 49 percent, 72 percent and 78 percent following two, four and six of treatment respectively. The corresponding phosphate removal rate with the South Indian species was 43 percent, 55 percent and 56 percent. Similar results had been obtained with the South Indian species (Danh *et al.*, 2015),

where N and P were reduced to significant levels in 48 to 72 hours of treatment.

The fast and very high contaminant absorbing capacity of South Indian species (Truong and Hart, 2001; Truong 2003) can also be reported true for the African species. It was observed, while the African species was more effective in removing phosphate, the South Indian species was more effective in removing nitrate in industrial effluent. However, use of both the African and South Indian species of Vetiver grass presents a great opportunity to remove both contaminants from wastewater. Both species removed contaminants to safe level of  $50 \text{ mg l}^{-1}$  and  $30 \text{ mg l}^{-1}$  for nitrate and phosphate respectively.

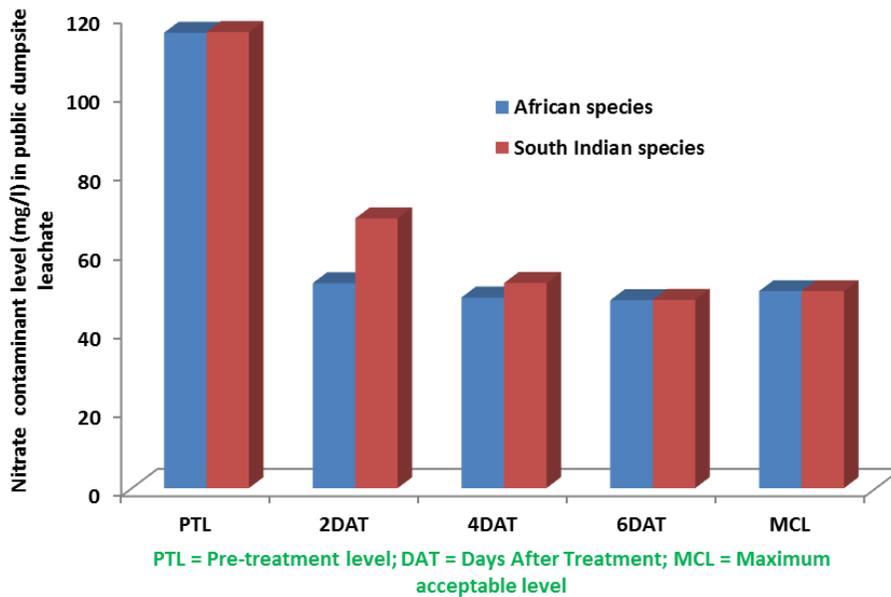
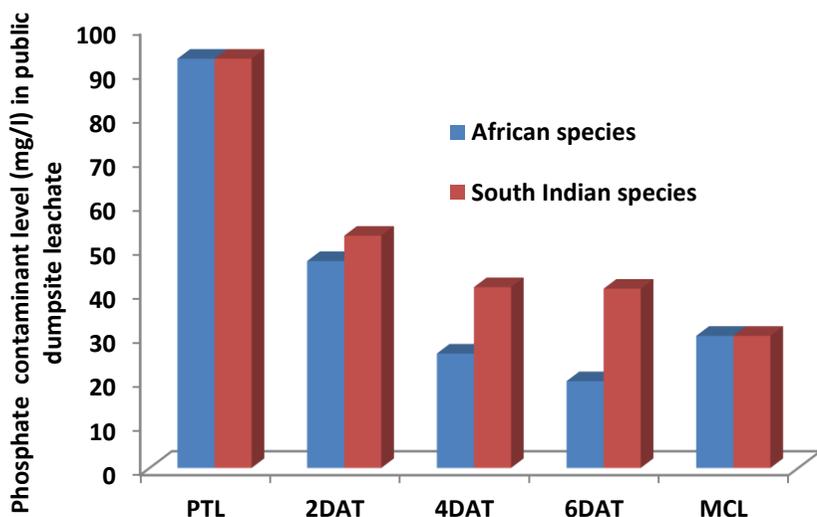


Figure 4. Pre and post-treatment levels of nitrate contaminant in a public dumpsite leachate



PTL = Pre-treatment level; DAT = Days After Treatment; MCL = Maximum acceptable level

Figure 5. Pre and post-treatment phosphate contaminant level in a public dumpsite leachate

### 3.3 Biological Dissolved Oxygen (COD) and Chemical Dissolved Oxygen (COD) reduction

Whereas COD gives an idea of biodegradable and non-biodegradable substances in the wastewater, BOD gives an indication of only biodegradable substances. Figure 6, presents the pre and post- treatment levels of Biological Oxygen Demand (BOD) of effluent obtained from a cassava industry. The BOD value favourably decreased by 59 percent, 95 percent and 96 percent, two, four and six days after treatment respectively, with the African vetiver species, whereas the corresponding values for the South Indian species were 47 percent, 71 percent and 96 percent. After treatment of abattoir effluent with the African species, Chemical Oxygen Demand (COD) decreased from a pre-treatment level by 67 percent, 82 percent and 86 percent, after two, four and six days of treatment respectively (Fig. 7). Corresponding values for the South Indian species were 52 percent, 85 percent and 88 percent. Both species of Vetiver grass effectively reduced BOD and COD to internationally acceptable limits of  $80 \text{ mg l}^{-1}$  and  $150 \text{ mg l}^{-1}$  respectively.

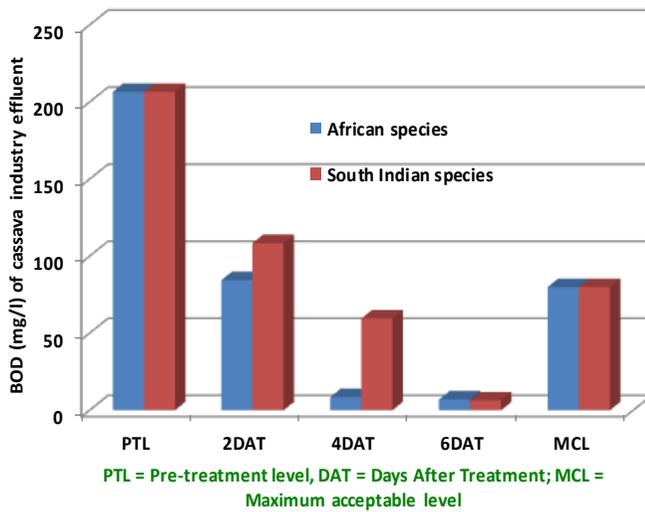


Figure 6. Pre and post treatment levels of Biological Dissolved Oxygen (BOD) in cassava industry effluent

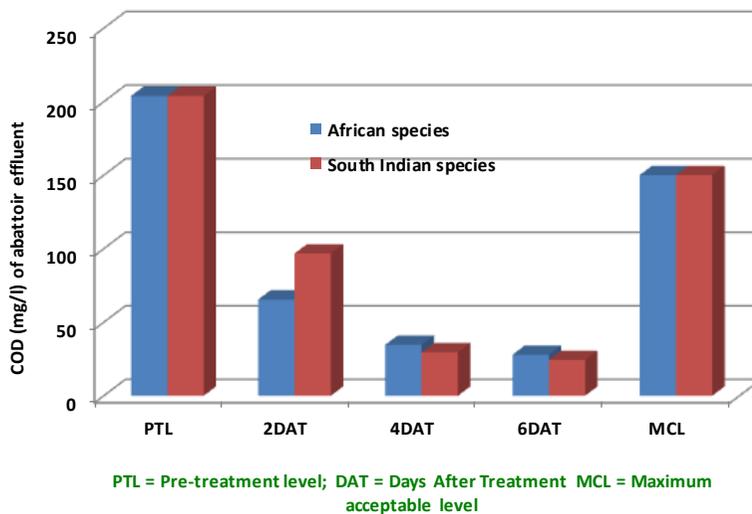


Figure 7. Pre and post-treatment level of Chemical Dissolved Oxygen in an abattoir effluent

### 3.4 Heavy Metals Removal

Vetiver has a high tolerance for heavy metals as reported for the south Indian species (Truong, 2008). This explains why rather than heavy metals killing Vetiver, the Vetiver rather “killed” the heavy metals (As, Cd, Mn, Pb, Zn). Lead (Pb) contaminant from the quarry site effluent was absorbed by the African species of Vetiver grass, by 80 percent, 83 percent and 83 percent (Fig 8) after two, four and six days of treatment respectively. The South Indian species removed Pb contaminants by 13 percent, 76 percent and 96 percent in two, four and six days of treatment respectively. Cadmium (Cd) contaminants in the fertilizer industry effluent and removal rate by the two species of Vetiver grass is presented in Fig. 9. The African and South Indian species removed 50 percent and 63 percent of the contaminants in two days after treatment.

However, Cd could not be detected in effluent when it was analyzed on the fourth and sixth day after treatment. This implies Cd was completely removed from the effluent after two days of treatment with both species of Vetiver grass. Cassava industry effluent contained a high level of cyanide ( $4.53 \text{ mg l}^{-1}$ ) as against the international acceptable level ( $0.2 \text{ mg l}^{-1}$ ) of cyanide as presented in Fig. 10. Treatment with the African species of Vetiver grass removed 76 percent, 97 percent and 97 percent of cyanide contaminants when measured at two, four and six days after treatment respectively. The South Indian species removed cyanide, by 31 percent, 97 percent and 97 percent with two, four and six days of treatment respectively. There was no significant difference between the fourth and sixth day treatment results.

Figure 11 shows pre and post treatment of zinc contaminant and removal levels by the two species of Vetiver grass. After the treatment of cassava effluent with the African species, zinc contaminants were removed and reduction levels were 67 percent, 77 percent and 99 percent, after two, four and six days of treatment respectively. Contaminant removal rate using the South Indian species was 53 percent and 86 percent in two and four days of treatment respectively. Zinc contaminant could not be detected in effluent when the treated effluent was analysed on the sixth day of treatment. Effluent from the fertilizer company was found to contain Arsenic (As) to the level of  $0.2 \text{ mg l}^{-1}$  far above the acceptable level of  $0.05 \text{ mg l}^{-1}$  of wastewater as presented in Fig. 12. Treatment of the effluent for two days with African and South Indian species of Vetiver grass removed 50 percent and 65 percent of arsenic contaminants, respectively. Quarry effluent treated using the African species of Vetiver grass as in Fig. 13 showed that arsenic contaminant was removed by 55 percent compared to 45 percent using the

South Indian species. However, the arsenic contaminant was not seen in the effluent after two days of treatment under both species.

Manganese pre and post-treatment removal levels in an abattoir effluent is presented in Fig. 14. After treatment of abattoir effluent with the African species, manganese contaminants were removed by 24 percent, 45 percent and 87 percent, under two, four and six days of treatment respectively, whereas from the same pre-treatment level, the South Indian species removed the contaminants by 23 percent, 90 percent and 93 percent with two, four and six days of treatment respectively.

Vetiver has been used to accumulate heavy metals as it retains a significant amount of absorbed heavy metals as Pb, Cd, As, and Zn in the roots (Srisatit *et al.*, 2003), however, nutrients are transferred to the shoots and this makes Vetiver suitable for grazing hence poses no health danger to grazing animals (Truong, 2008). The ability of Vetiver to store the toxic metals in its roots without transferring to the shoots is an advantage in its use against other plants (Jadia and Fulekar, 2009).

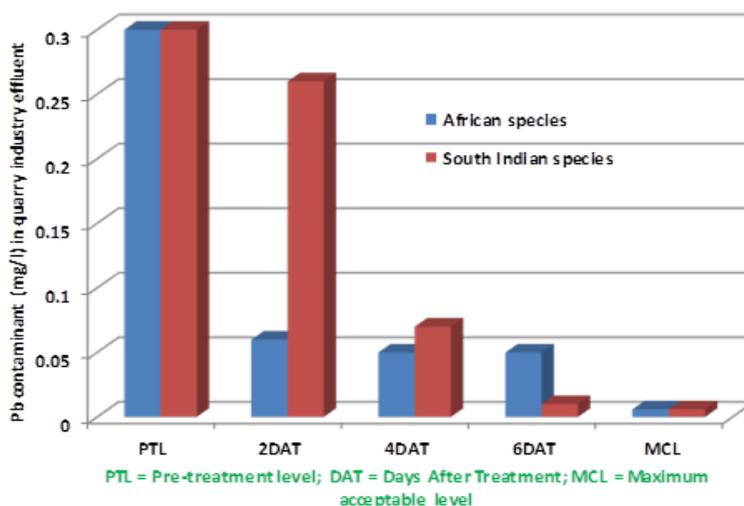


Figure 8. Pre and post-treatment levels of Lead (Pb) in quarry industry effluent

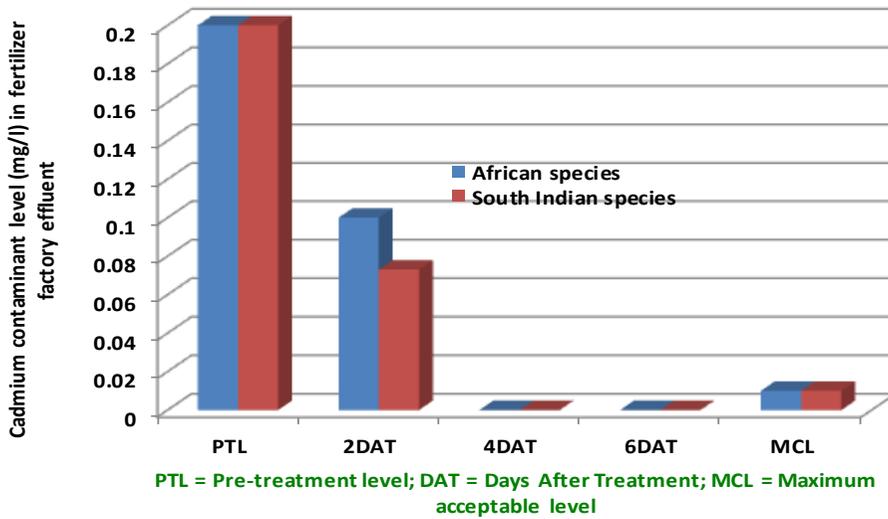


Figure 9. Pre and post-treatment Cadmium contaminant levels in fertilizer company effluent

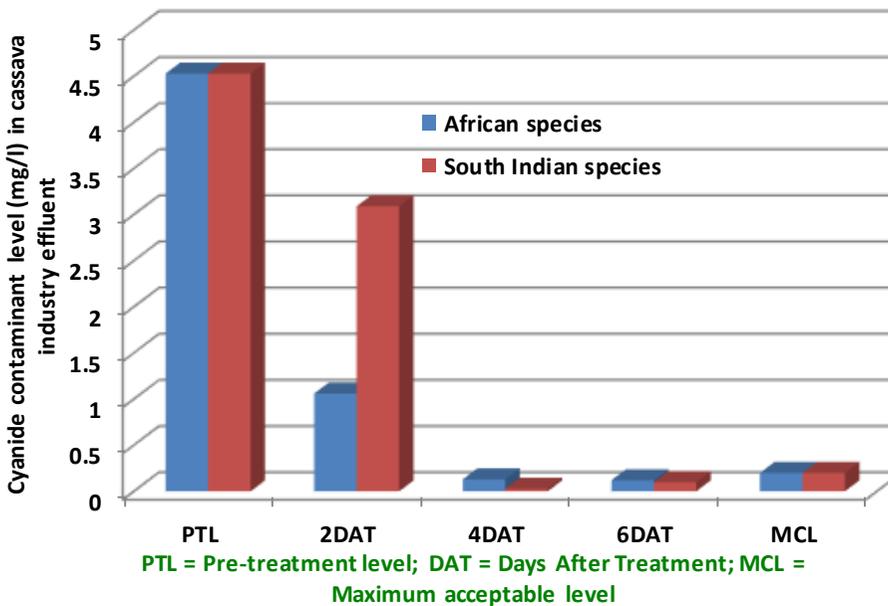


Figure 10. Pre and post-treatment cyanide contaminant level in cassava processing industry

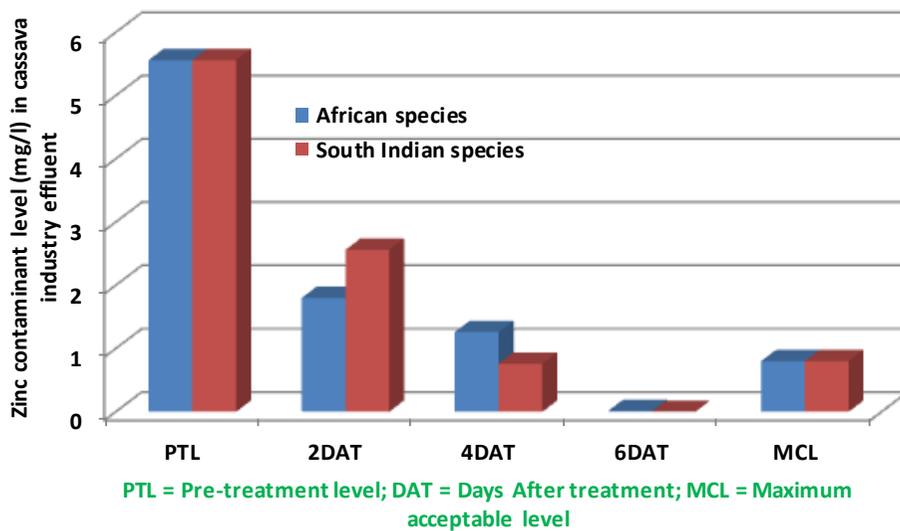


Figure 11. Pre and post treatment zinc contaminant levels in cassava processing industry

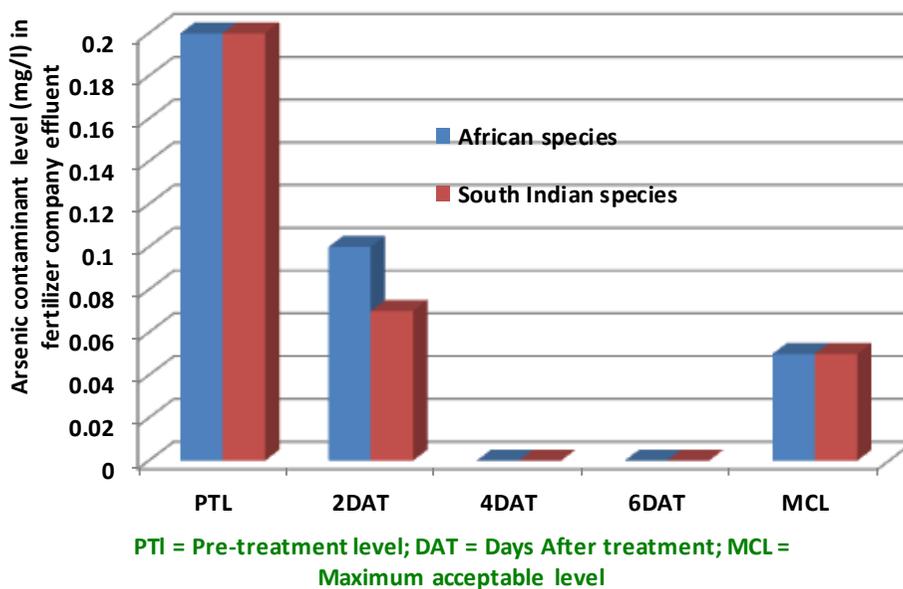


Figure 12. Pre and post-treatment Arsenic contaminant level in fertilizer company effluent

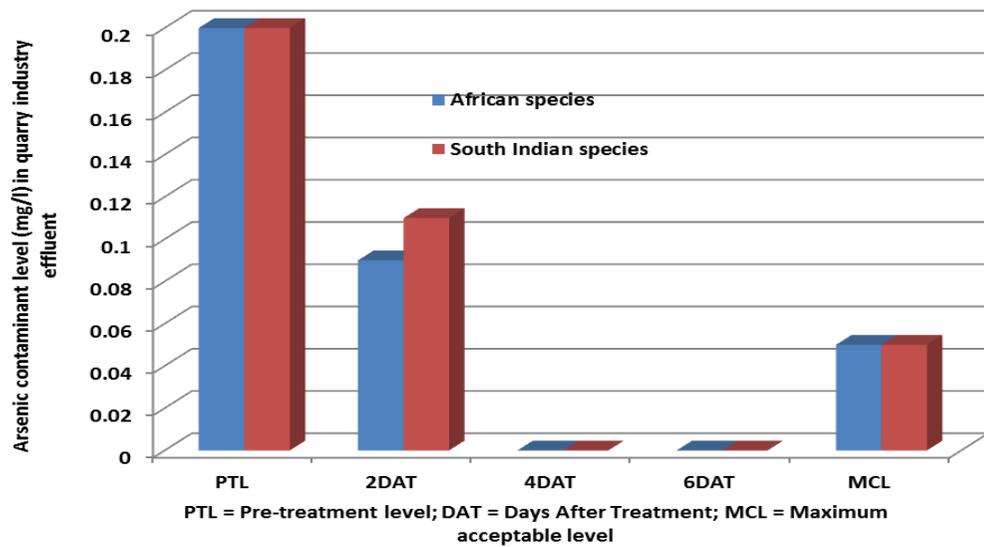


Figure 13. Pre and post-treatment Arsenic contaminant levels in quarry industry effluent

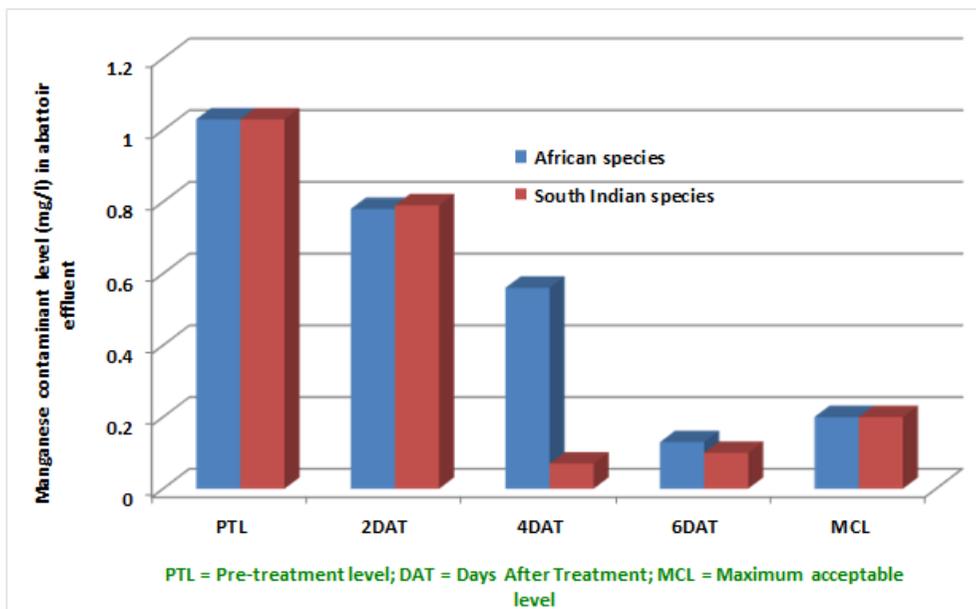


Figure 14. Pre and post-treatment Manganese contaminant level in abattoir effluent

### **3.5 Anatomy of African and South Indian Species of Vetiver and their Phytoremediative Potentials**

#### **Vetiver Roots**

Root hairs were observed in *C. zizanioides* species whereas, it was absent in the African species *C. nigrifolius*. The presence of root hairs in the South Indian species tend to give it comparatively additional contaminant absorbing capacity, though the difference was not statistically significant in most cases. An average of fourteen (14) metaxylemlacunae was observed to occur in pairs forming a ring within the endodermis of *C. zizanioides* as in Plate 4. A comparative count in *C. nigrifolius* gave ten (10) single metaxylemlacunae also found to be in the ring. Transverse section of roots of Vetiver as shown in Plate 4 also show the presence of aerenchyma in root cortex and air cavities in pits of both the *Chrysopogon nigrifolius* (African species) and *Chrysopogon. zizanioides* (South Indian species). The presence or possession of these natural facilities enable Vetiver plants to survive in aquatic environments. This is in line with the description of Khnema and Thamathaworn (2011) who reported the presence of large airspaces in the roots of *C. zizanioides* in Thailand. The anatomy of Vetiver is a possible indicator of its ability in cleaning up wastewater.

Aerenchyma which enables dry land plants to grow deep into the soil and resist drought also gives buoyancy to plants growing in waterlogged conditions. This structure, according to USDA (1997) is a relief towards the effects of drought and flooding which have posed major challenges in environmental management. As an adaptation to waterlogged conditions, aerenchyma in Vetiver has been reported to increase in size and number when transplanted from land to water (Liao *et al.*, 2003). Aerenchyma also plays a leading role in the remediation of polluted soil and water. Foy (1983) reported that aerenchyma releases oxygen into polluted media, and this may oxidize toxic minerals resulting in compounds which are less harmful. Plant roots may respond to physical and chemical changes in the environment by developing certain physical structures such as aerenchyma (Jin and Zheng 2000).



**Plate 4. Transverse Sections of Roots of both Species**

A: Root of *C. nigritanus* x 40

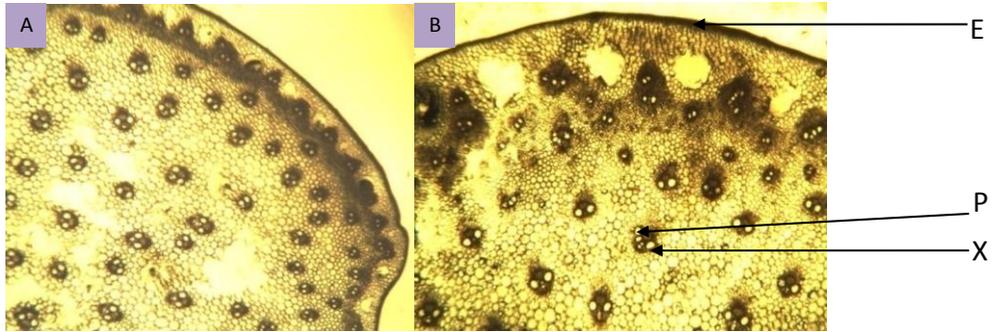
B: Root of *C. zizanioides* x 40

**Legend:**

E = Epidermis, C = Cortex with Aerenchyma, En = Endodermis, Rh = Root Hair, P = Pith and M = Metaxylem Lacunae.

**Stems**

Transverse section of stems of both species of Vetiver showed scattered vascular bundles with pitted tracheids as seen in Plate 5. The present study further supports earlier reports by Zarinkamar (2006) in Iran and Arcana and Rodriguez (2009) in Vietnam and Khnema and Thammathaworn (2011) in Thailand. All the above mentioned researchers had independently studied the anatomy of *Chrysopogon zizanioides*. The length, diameter and density of vessels in both species were significantly different ( $P < 0.05$ ). Curved end-walls were observed in the tracheids of *C. nigritanus*, while, *C. zizanioides* has straight end-walls. However, the vessel lumen diameter in both species was not significantly different ( $P > 0.05$ ). This is a possible reason for their similar abilities in wastewater cleaning and the close generic properties.



**Plate 5 Transverse Sections of stems of both Species.**

A: Culm of *C. nigritanus* X 40

B: Culm of *C. zizanioides* X 40

**Legend:**

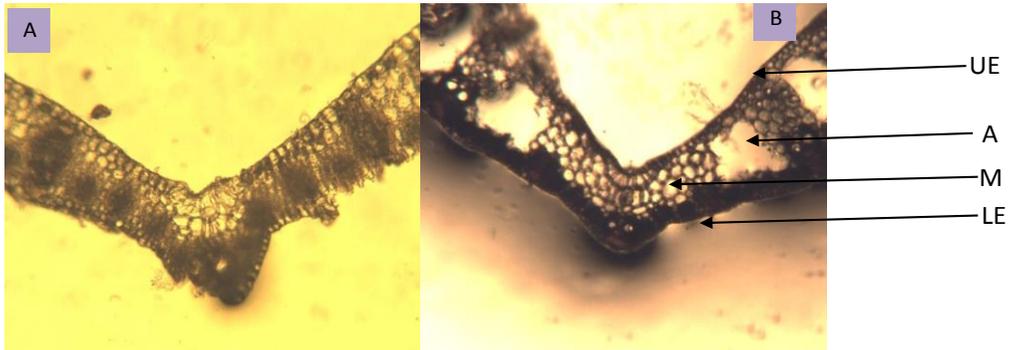
E = Epidermis, P = Phloem, X = Xylem

**Leaves:**

The leaves of Vetiver are tough and slender (1 – 3 meters) and 0.01meter wide at maturity. The edges are rough due to the presence of tiny barbs (NRC, 1993). Transverse sections of leaves of both species of Vetiver revealed large intercellular spaces and “V” shaped contours (Plate 6). Stomata were abaxially placed (at the back side of leaves) in *C. zizanioides*, but found on both sides of leaves in *C. nigritanus*. This Stomatal positioning suggests the South Indian species will be conservative in the release of water to the atmosphere under water scare condition. Whereas, the African species will be generous in water release even under water scare conditions. This further suggests that the African species could be more suitable in environments with an abundance of water including hydroponic systems, while *C. zizanioides* may be more suitable in arid conditions. This is in line with the report by Truong *et al.* (2008) who noted that the South Indian species (*C. Zizanioides*) is the most suitable for drought prone areas or related conditions.

The stomata of Vetiver may also play a role in wastewater cleaning. Liao *et al.* (2003), in an experiment to compare the anatomical features between Vetiver grown in dry land and in constructed wetland in China, reported that air chamber density and stomatal apparatus increased in the wetland Vetiver. Similarly, Fei *et al.* (1999) reported that the area of stomatal apparatus in Vetiver reduces when there is a reduction in water supply. Paracytic stomata arranged in parallel rows were observed in both species. In the stomata of *C. nigritanus*, the points of attachment of subsidiary cells form two prominent

arcs (like wings) on both sides as shown in Plate 7. This was not observed in *C. zizanioides* as shown in Plate 8.



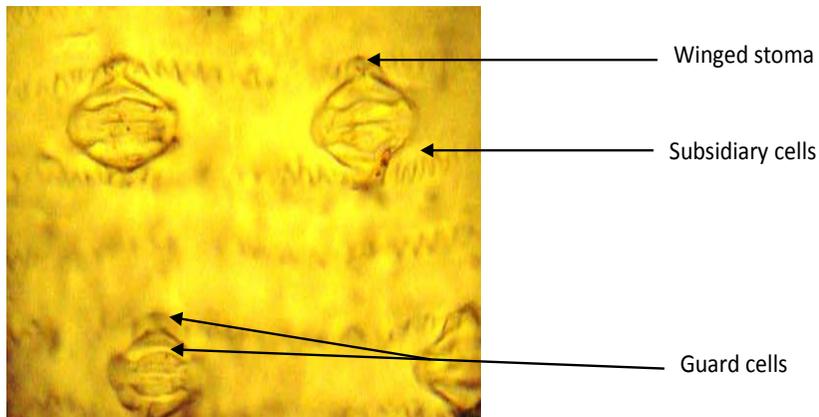
**Plate 6. Transverse sections of leaves of both species**

A: Leaf of *C. nigritanus* X 40

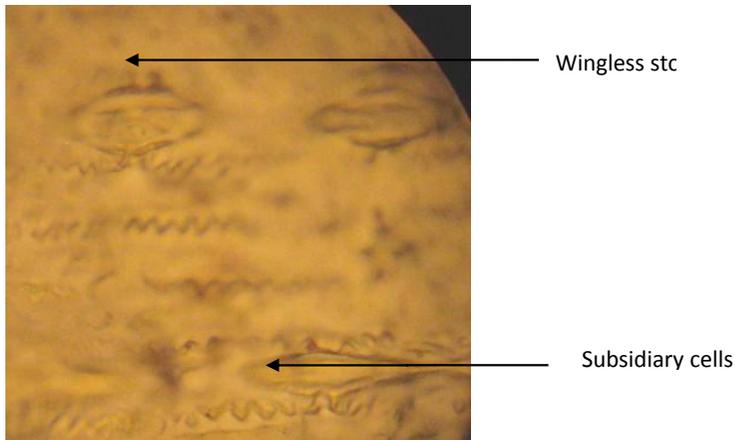
B: Leaf of *C. zizanioides* X 40

**Legend:**

UE = Upper Epidermis, A = Air Cavities, M = Mesophyll and LE = Lower Epidermis



**Plate 7. Stomatal features in *C. nigritanus* x 400 (Abaxial)**



**Plate 8. Stomatal features in *C. zizanioides* X 400 (Abaxial)**

The inflorescence of the plants shows slight differences in whorl arrangement; incomplete whorls were observed in the inflorescence of *C. zizanioides*, while complete whorls were found in *C. nigritanus*. The overall anatomical differences found in the plants have not significantly affected their relatedness in their wastewater cleaning abilities. These similarities suggest that the African species (*C. nigritanus*) is closely related to the South Indian species (*C. zizanioides*) and has similar potentials in phytoremediation.

## **4.0 Conclusion and Recommendations**

### **4.1 Conclusions**

Vetiver technology comes in handy as one of the smart wastewater management solutions industries should sort to reduce carbon footprints in their wastewater management chain. Though some plants have been proven to have phytoremediation potentials, the advantage of Vetiver over these plants is its ability to store toxic materials in the roots without transmitting to the shoot. The use of Vetiver in industrial wastewater cleaning also fits into the present global call for the greening of industries.

Furthermore, it is both financially cost effective and environmentally efficient, mitigating green houses and eliminating the use of conventional energy. The study confirms that African species of Vetiver grass (*Chrysopogon nigritanus*) has comparative potentials as the South Indian species in cleaning industrial wastewater. The use of exotic species of plant in Africa has not been greeted with enthusiasm by users. This makes this finding critical for the continent.

### **4.2 Recommendations**

Government intervention through policy is required to change the “Business-as-Usual” and drive this Green Technology down the industrial units. This technology is easy to instruct and well suited for treating wastewater for urban and peri-urban agriculture crop irrigation. Knowing the importance of urban agriculture practice to food security, for human nutrition, employment and livelihoods sustenance, the government could work with researchers to develop farmers’ capacities to use this technology.

Further studies on the anatomical adaption of these species should be investigated.

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