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Evaluation of *Eichhornia crassipes, Pistia stratiotes* and *Vetiver zizanoides* in phytoremediation of a Hospital Wastewater Effluent

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Abstract
Water contamination poses negative impacts on humans and the ecosystems leading to issues of water scarcity, water stress, drought and mortality related to water diseases and hence the need for wastewater recycling for future reuse. The study assessed the physicochemical parameters from a hospital wastewater treatment plant in Zaria, Kaduna using standard method for water and wastewater examination and its phytoremediation by hydroponic treatment method using *Eichhornia crassipes* (waterhyacinth), *Pistia stratiotes* (Waterlettuce) and *Vetiveria zizanoides* (Vetiver grass). Results recorded were analyzed by one way analysis of variance at 95% confidence, multiple comparison tests and quantitative linear relationship. The study showed higher concentrations of Electrical Conductivity (EC) (951.83 µs/cm), Phosphate (PO_4) (64.46 mg/l) , Biological Oxygen Demand (BOD) (244.90 mg/l), Chemical Oxygen Demand (COD) (625.50 mg/l), Total Suspended Solid (TSS) (27.30 mg/l), Potassium (K) (40.67mg/l) above the permissible limit standard by WHO and FAO, Phytoremediation showed there was significant difference at $P \leq 0.05$ in reduction capacity across treatment between waterhyacinth, waterlettuce and vetiver grass for EC (49.8, 41.6 and 51.4%), TDS (51.3, 47.0 and 63.0%), PO_4 (49.0, 45.3 and 53.0%), COD (47.3, 48.4 and 57.1%), NO_3 (51.6, 43.1 and 64.6%), TSS (49.5, 42.7 and 60.3%), NH_3 (44.7, 40.8 and 53.2%), SO4 (53.9, 47.2 and 62.8%). Plant analysis result showed higher concentration of contaminant in the roots of waterhyacinth than in the shoot, higher concentration in the shoot of vetiver grass than its roots and there was even distribution in roots and shoots of water lettuce. Therefore, the three plants can be used effectively in phytoremediation of wastewater contaminants due to cost effectiveness, ecofriendliness as emerging cheaper technology for a lasting solution to the problems of water contamination to both humans and the environment at large.
Key words: Wastewater, Contamination, Phytoremediation, Vetiver grass, Waterhyacinth, Waterlettuce

1.0 Introduction
Water is essential and important to human life. Fresh water is a vulnerable resource subjected to various contaminants, while in use for human development and the environment. One of the fundamentals of life for human survival, health and productivity on our planet, is to have access to clean water, but due to increasing demand by the rising population, the resource is under threat (Arimieari *et al*., 2014). Pollution is regarded as an environmental problem in the world due to its adverse effect on living organism and the past few decades, uncontrolled urbanization has caused serious pollution problem due to sewage disposal, industrial and hospital effluents discharged to water bodies (Tamil *et al*., 2012). Pollution of water is measured by assessing the physicochemical parameters of the water (WHO, 2017). Therefore, it is important to assess the quality of water before utilizing for irrigation, drinking, fisheries, industrial purposes and also in understanding the processes of complex interaction between climatic and biological activities in the water body [Ramachandra, *et al*., 2014].
Wastewater is “utilized water from any mix of residential, mechanical, Health, business or farming exercises, surface overflow or storm water, and any sewer inflow or sewer penetration” [Suhad *et al*., 2018].

Importantly, treated wastewater plays a major role in meeting the growing water demand in the society, supporting sustainable agriculture, enhancing energy production and industrial development. Hospitals are an essential asset of population and waste production is usual outcome from its service delivery. Wastewater from hospitals contains harmful pollutants generated from all activities from medical and non-medical facilities [Tsegahun, 2018]. Phytoremediation is a process of using plants to extract and reduce or detoxify waste products and pollutants from both soil and water bodies. About 300 years ago, plants were proposed for use in the treatment of wastewater and have gained increasing attention since last two decades, as an emerging cheaper technology (Carolin *et al*., 2017). The remediation technique for wastewater involves specific planting arrangements in a hydroponic system, employing floating-plants and numerous other configurations [Abebe, *et al*., 2018]. The aquatic plants have been reported for long to detoxify environmental pollutants. The notable environmental contaminants reduced are inorganic and organic pollutants which can be Phytoremediated in various ways (Isiuku and Enyoh, 2019).
Waterhyacinth (*Eichhornia crassipes*. Mart solms.) is a perennial aquatic macrophyte of the *Pontederiaceae* family that is invasive in the amazons. Its impact and potentials on the physicochemical parameters of water, are the ability to decline Biological Oxygen Demand (organic load), and nutrient levels. This makes them suitable for the treatment of wastewater. [Gupta, *et al*., 2012].
Water lettuce (*Pistia stratiotes* L.) is a floating perennial macrophyte of the family *Araneae*. It is capable of removing nutrients and heavy metals from the sewage sludges and drainage ditches. The physicochemical parameters reduce progressively from the effluent ponds in tropical areas [Dipu, *et al*., 2011].
Vetiver Grass (*Vetiveria zizanoides)* is a perennial grass that can grow up 2m high and 3m deep in the ground. It has a strong dense and vertical root system. It is capable of growing in different environmental conditions and can be effectively use for bioremediation application [Paz-Alberto, *et al*., 2013].

Plate I: ***Eicchornia crassipes***
Source: Stuart xchange.org.

Plate II: ***Pistia stratiotes***
Source:Semanticscholar.org

Plate III : ***Vetiveria zizanoides***
source: Research gate

Due to usable water scarcity, which is a global issue, wastewater has to be treated for re-use for certain purposes such as irrigation. More and more countries are experiencing water stress, and increasing drought and desertification is already worsening these trends therefore, Protecting and restoring water-related ecosystems is essential. However, cost of conventional treatment of wastewater is high, not suitable for all contaminant removal and in the current economic recession, there is the need for an environmentally friendly and cost-effective treatment method, as bioremediation.
In the present study, the main objective is to evaluate the absorbed contaminant in roots and shoots of the three plants and determine the phytoremediation efficiency of the three plants.

2.0 Methodology

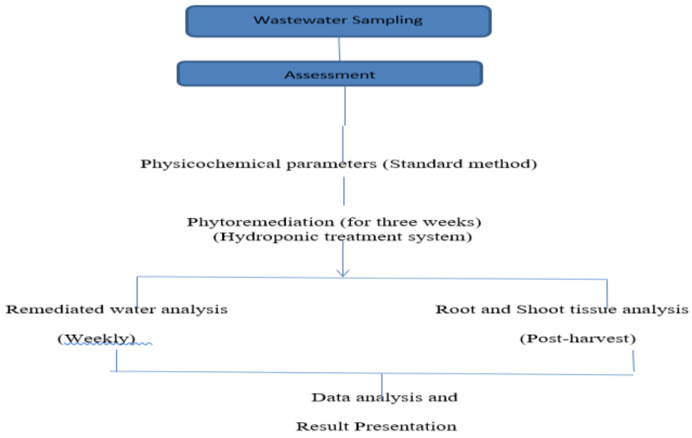

Figure 2.1 The Experimental Scheme of the study.

2.1 Determination of Physicochemical Parameters

Water Samples were taken from the effluent of the hospital wastewater plant. The parameters were determined as described in the standard methods for the examination of water and wastewater 23rd Edition, APHA. (2017). One-liter polyethene bottles were properly washed with mild detergent and then leached with 1:1 HCl overnight. At the Sampling sites, the containers were rinsed several times with deionized water and rinsed three times with the wastewater before the samples was collected. The pH meter (Jenway model 3310), thermometer and conductivity meter (Hach model C0150) where used on-site by immersion of the calibrated instruments. The wastewater sample was taking to the laboratory for further analysis.

2.2 Hydroponic Phytoremediation using *Chrysopogon zizanoides, Eichhornia crassipes and Pistia stratiotes*.

Six plastic containers 0.35m width x 0.55m length x 0.25m / 0.35m depth each was set up with 3 controls. The containers were filled with wastewater to an effective depth of 0.35m by considering the root potential growth of Vetiver grass while a shallow depth of 0.25m for water hyacinth and water lettuce were used. Control unit were filled with wastewater to an effective depth of 0.15m. Sets of floating polystyrene rafts for each replicate hydroponic treatment unit were set up for supporting vetiver tillers on wastewater surface which allowed the vetiver roots to be fully immersed. In each floating platform, 6 holes of 10 x10cm intervals were made. The roots were washed carefully with tap water to remove adhering soil and sediments prior to use. The plants were acclimatized in distilled water for a week. Then manually, 150gram vetiver grass was splitted carefully to (avoid damage to the roots) into tillers. Similar size healthy vetiver tillers were selected, pruned to 20cm for the shoots and 10cm for the roots (stem and leaves) to reduce transpiration. Each tiller was planted into the holes in the platform foam and approximately 10cm of the roots was submerged under wastewater during the experimental period. 150g to 50g Water hyacinth and Water lettuce plant stems respectively were remained above the water level, while their roots grow down through the buoyant structure and into the water column.

The *E. crassipes, P. stratiotes* and *V. zizanoides* planted in the floating form were left to grow for three (3 weeks) and the wastewater were analyzed weekly. Dead shoots were replaced after monitoring for survival conditions [Calheiros *et al*., 2009].

2.3 Laboratory Plant Analysis after Remediation

At the end of the experiments, biomass of the three plants samples from each treatment unit of the wastewater, was harvested from the water platform and transported to the laboratory for analysis. Adhering material such as soil particles on the plants were cleaned by hands. The roots and shoots were separated, rinsed using tap water for 5mins then shaken off. The roots and shoots were then submerged in distilled water for 2mins, dried at 60^oC for 72 hrs and milled to a fine powder (0.5 to 1.0mm) in a grinder. The grinded sample was analyzed for physicochemical factors. Contaminants in the roots and the shoots of the plants where assessed for the percentage removal efficiency.

3.0 Result and Discussion

3.1 Percentage Reduction of Electrical Conductivity, Total Dissolves Solid, Chlorine and Phosphate

Figure 1A shows the percentage reduction in EC using the three plants for phytoremediation. There is significant difference between *V. zizanoides* and *P. stratiotes* while no significant difference was observed at $P \leq 0.05$ between *E. crassipes* and *V. zizanoides*. Control shows a slight reduction in EC.

Figure 1B shows there is significant reduction in TDS after phytoremediation with three plants. *V. zizanoides* has the highest percentage of reduction and significantly different with *P. stratiotes* while *E. crassipes* is insignificantly different with both.

Figure 1C and 1D shows percentage reduction in chlorine and phosphate with *P.stratiotes* having lower potential, significantly different with *V. zizanoides* while insignificantly different with *E. crassipes*. *V. zizanoides* was insignificantly different with *E. crassipes*. The phosphate reduction in control was higher than in chlorine, EC and TDS.

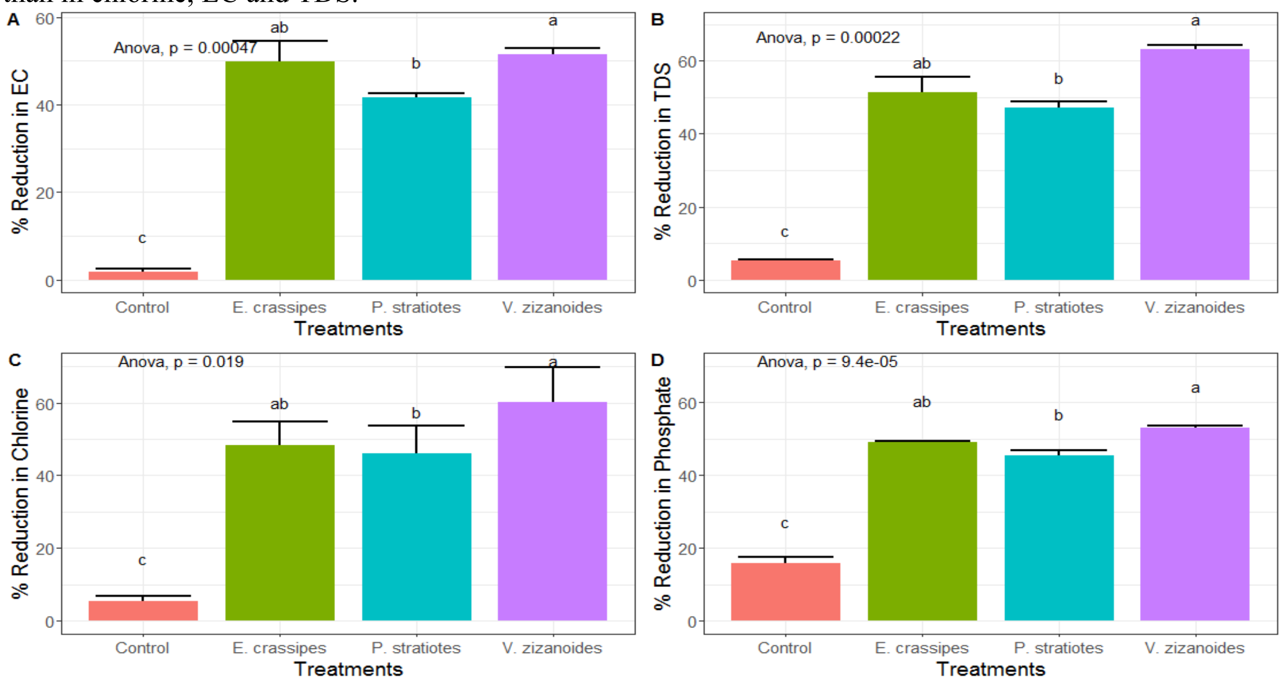

Fig 3.1 Percentage Reduction of Electrical Conductivity, Total Dissolves Solid, Chlorine and Phosphate by phytoremediation.

3.2 Percentage Reduction in HCO_3, BOD, COD and Sodium.

Figure 2E, 2F, 2G and 2H showed the percentage reduction in HCO3, BOD, COD and Na using the three plants respectively. No significant difference at $P \leq 0.05$ between reduction potentials of *V.zizanoides, E.crassipes* and *P.stratiotes* for the parameters.

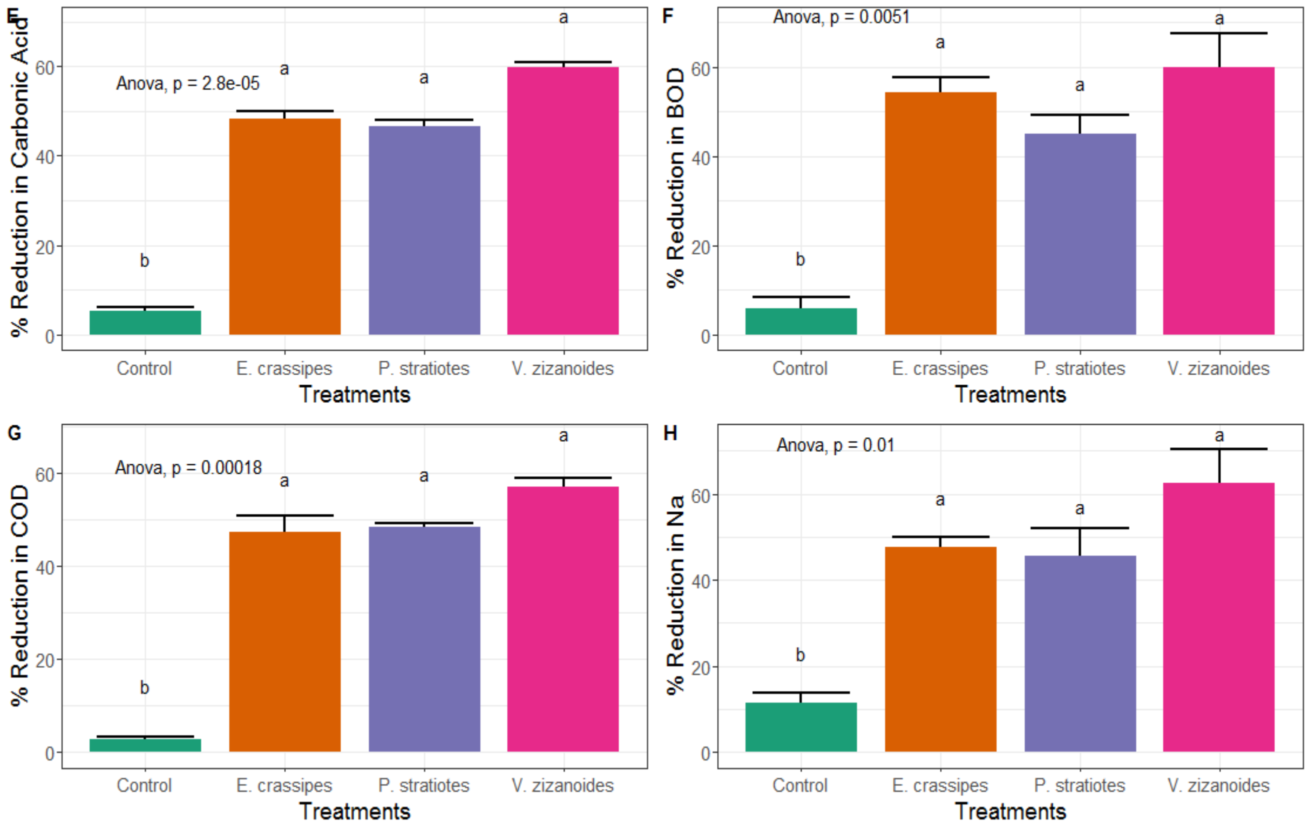

Fig 3.2 Percentage reduction in HCO_3, BOD, COD and Sodium by phytoremediation

3.3 Percentage Reductions in Potassium (K), Nitrate (NO_3), Nitrogen (N) and Total suspended Solid (TSS)

Figures 3I, 3J, 3K and 3L shows the percentage reduction in K, NO_3, N and TSS respectively using the three plants for phytoremediation. There was significant difference at $P \leq 0.05$ between *V.zizanoides* and *P.stratiotes* in reduction of the above parameters while insignificantly different with *E.crassipes*. *V.zizanoides* having the highest percentage potential.

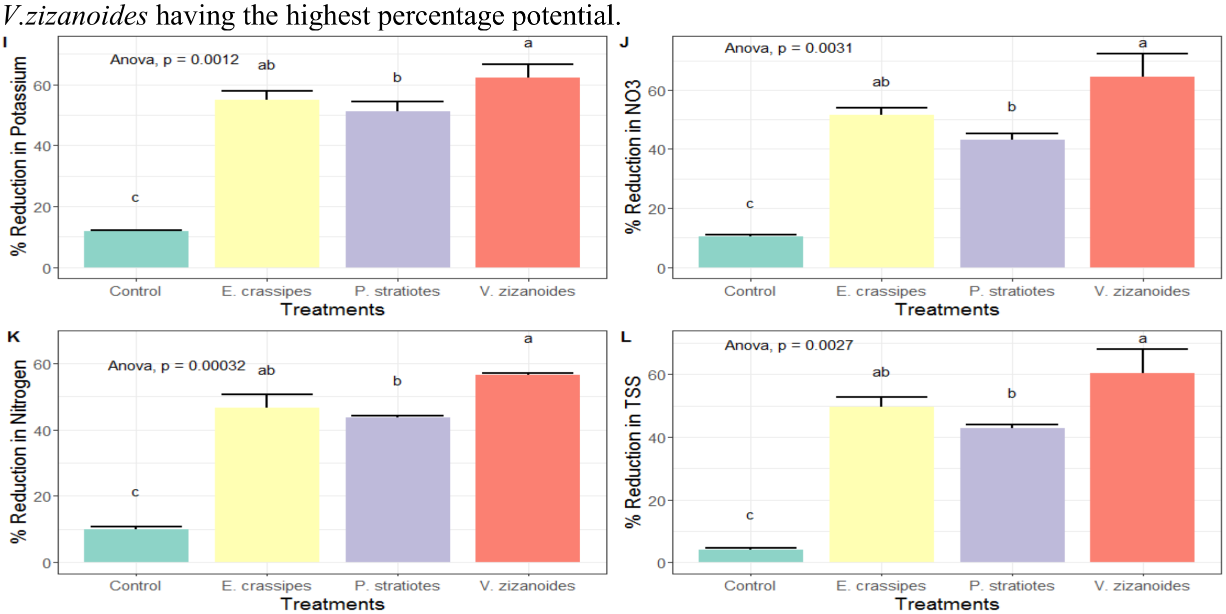

Fig 3.3 Percentage Reductions in Potassium (K), Nitrate (NO_3), Nitrogen (N) and Total Suspended Solid (TSS).

3.4 Percentage Reduction in Ammonia (NH_3) and Sulphate (SO_4)

Figures 4M and 4N, shows percentage reduction in NH_3 and SO_4 respectively. *V.zizanoides* having the highest reduction potential and significantly different at $P \leq 0.05$ with *E.crassipes, P.stratiotes* and control while no significant different between *E.crassipes* with both *V.zizanoides* and *P.stratiotes.*

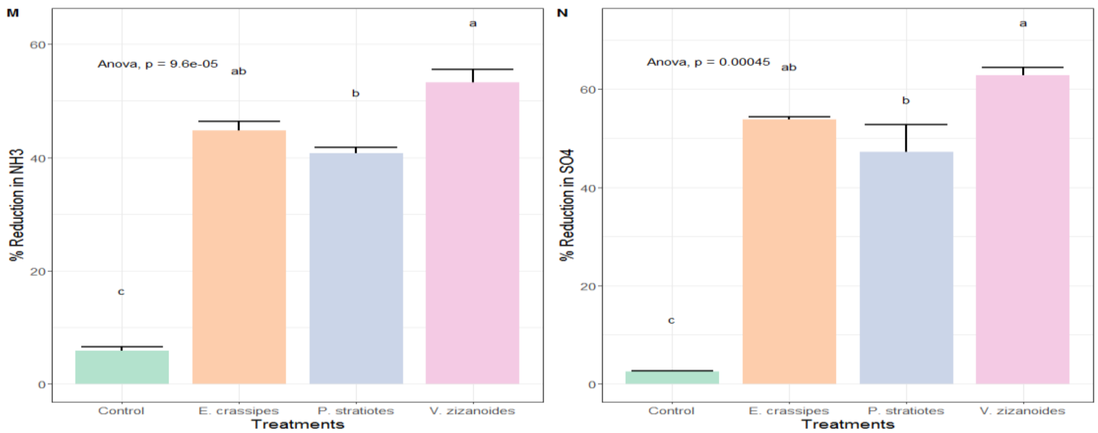

Fig 3.4 Percentage Reductions in Potassium (K), Nitrate (NO_3), Nitrogen (N) and Total suspended Solid (TSS)

3.5 Mean Variation in Percentage Reduction Potential of Phytoremediation of Physicochemical Parameters Across Treatment.

Table 3.5 showed the mean variation in percentage reduction of physicochemical parameters across treatment. There was significant difference at $P \leq 0.05$ (95% confidence) in reduction capacity between *E. crassipes, P. stratiotes* and *V. zizanoides* for EC (49.8, 41.6 and 51.4%), TDS (51.3, 47.0 and 63.0%), PO_4 (49.0, 45.3 and 53.0%), HCO_3 (48.4 46.5 and 59.8%), COD (47.3, 48.4 and 57.1%), NO_3 (51.6, 43.1 and 64.6%), N(46.6, 43.5 and56.4%), TSS (49.5, 42.7 and 60.3%), NH_3 (44.7, 40.8 and 53.2%) and SO_4 (53.9, 47.2 and 62.8%). Highest removal percentage was recorded with *V. zizanoides* (50% and above).

Table 3.1 Mean variation in the percentage reduction of physicochemical parameters across the Treatment conditions.

Physicochemical Parameters(mg/l)	Control	*E. crassipes* %	*P. stratiotes* %	*V. zizanoides* %	P-value
EC(µs/cm)	1.90 ± 0.70^{b}	49.80 ± 6.78^{a}	41.60 ± 1.55^{b}	51.45 ± 1.90^{a}	0.000
TDS	5.30 ± 0.28^{c}	51.35 ± 5.72^{b}	47.05 ± 2.33^{b}	63.00 ± 1.83^{a}	0.000
Cl	5.45 ± 2.05^{b}	48.35 ± 8.98^{a}	46.05 ± 10.96^{a}	60.10 ± 13.71^{a}	0.0194
PO_4	15.80 ± 2.54^{c}	49.05 ± 0.49^{ab}	45.35 ± 2.19^{b}	53.02 ± 0.87^{a}	0.000
HCO_3	5.25 ± 1.20^{c}	48.40 ± 2.26^{b}	46.50 ± 2.12^{b}	59.85 ± 1.48^{a}	0.000
BOD	5.80 ± 3.81^{b}	54.30 ± 4.94^{a}	44.90 ± 6.22^{a}	59.85 ± 10.96^{a}	0.005
COD	2.64 ± 0.79^{c}	47.30 ± 5.09^{b}	48.40 ± 1.27^{b}	57.15 ± 2.75^{a}	0.000
Na	11.35 ± 3.46^{b}	47.60 ± 3.39^{a}	45.60 ± 9.05^{a}	62.50 ± 11.17^{a}	0.0105
K	11.80 ± 0.56^{b}	55.05 ± 4.17^{a}	51.10 ± 4.80^{a}	62.35 ± 6.15^{a}	0.0011
NO_3	10.35 ± 1.06^{c}	51.60 ± 3.53^{ab}	43.10 ± 2.96^{b}	64.60 ± 10.74^{a}	0.003
N	10.05 ± 0.91^{c}	46.65 ± 5.44^{b}	43.50 ± 0.98^{b}	56.40 ± 0.98^{a}	0.003
TSS	4.05 ± 0.63^{c}	49.50 ± 4.52^{ab}	42.75 ± 1.76^{b}	60.35 ± 10.96^{a}	0.022
NH_3	5.85 ± 0.91^{c}	44.75 ± 2.33^{b}	40.80 ± 1.41^{b}	53.20 ± 3.25^{a}	0.000
SO_4	2.50 ± 0.14^{c}	53.90 ± 0.70^{ab}	47.20 ± 7.91^{b}	62.85 ± 2.19^{a}	0.000

Note: Means with the same superscript are not ($P \leq 0.05$) significantly different across the rows

KEY: EC= Electrical conductivity, TDS = Total Dissolved Solids, DO = Dissolved oxygen, BOD = Biological oxygen demand, COD = Chemical oxygen demand, NO_3 = Nitrate, TSS = Total suspended solids, NH_3 = Ammonia, SO_4 = Sulphate.

3.5 Concentration of Parameters in Roots and Shoots of *E.crassipes, V. zizanoides* and *Pistia stratiotes*

Figure 5A and B below showed the concentration of parameters in *E.crassipes* and *V. zizanoides* plant. There was higher concentration of parameters in the roots than in the shoot of *E.crassipes* and shoot of *V. zizanoides* than the roots of the plants.

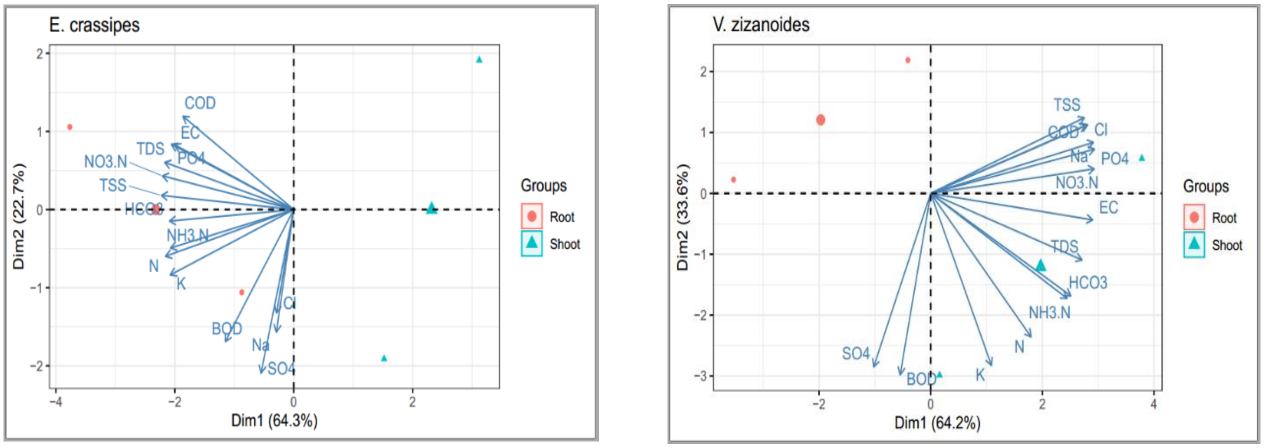

Fig 5A and B Concentration of parameters in Roots and Shoots of *E.crassipes* and *V. zizanoides.*

3.6 Concentration of parameters in Roots and Shoots of *P.stratiotes* plant.

Figure 6 below shows the concentration of parameters in *P.stratiotes* plant. There was even distribution with of parameters in the shoots and roots of the plants.

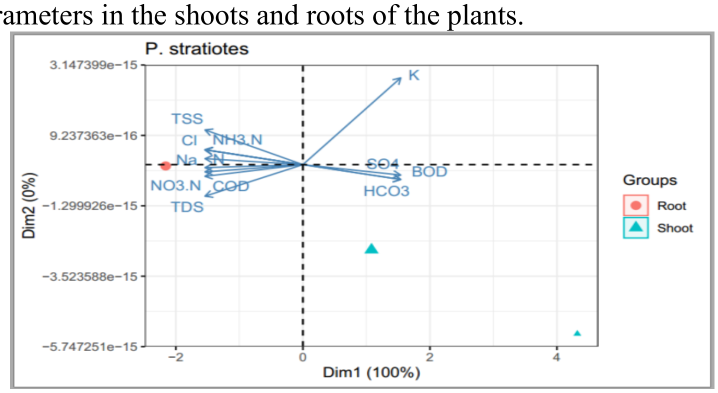

Fig 6: Concentration of parameters in Roots and Shoots of *P.stratiotes* plant.

Discussion

The potentials of water hyacinth (*Eicchornia crassipes),* waterlettuce (*Pistia stratiotes*) and Vetiver grass(*Chrysopogon zizanoides*) in treatment of wastewater assessed in this study was also reviewed by Gupta *et al*, (2012) for assessing phytoremediation techniques in the Treatment of water using water hyacinth, waterlettuce and Vetiver grass, various contaminants such as TDS, TSS, EC, BOD, COD, Nitrogen, phosphorous, heavy metals and other contaminants have been minimized using the three plants and therefore reported to be cost effective compared to other methods. High potential of vetiver in this study was also reviewed by [Darajeh, *et al*., 2019], in effectiveness of vetiver grass versus other plants such as *Cyperus* species, *Phragmites* species, and *Typha* species for phytoremediation of contaminated water and wide range of industrial and domestic wastewater due to its extraordinary and unique morphological and physiological characteristics. But oppose the findings of [Gupta, *et al*., 2015], in the study of the treatment of ground water using phytoremediation technique at Kolar Gold Fields, India, where conclusively it revealed that water hyacinth had higher contaminant reduction capacity than water lettuce and vetiver grass. This could be due to less contaminants concentration in groundwater than wastewater which are toxic and hinder the extraction ability of water lettuce.

The highest accumulation capacity for nutrients of water hyacinth (*E.crassipes*) more than water lettuce *(P.stratiotes*) in this study was also observed by Nayanathara and Bindu, (2017) in a review of the effectiveness of water hyacinth and water lettuce for the treatment of Grey water were water hyacinth was shown to be more efficient than water lettuce .These results may be attributed to the larger total root surface, active absorption area and leaf area and higher root activity, root biomass and net photosynthetic rate of water hyacinth than those of water lettuce. Even distribution of contaminants in *pistia stratiotes* living tissues was also reported by Daniel *et al*. (2019) in the study of *Pistia stratiotes* for the phytoremediation and post treatment of domestic sewage, where the bioaccumulation of contaminants was detected in the living tissues of *P. stratiotes*.

Concentration of elements more in the shoots of *Vetiver* in this study collaborated with the findings of [Keshtar *et al*., 2016], in application of a *Vetiver* system for unconventional water treatment, were parameters were distributed more in the shoots than the roots of the vetiver due to over saturation of components in the roots from the initial. But oppose the findings of [Ashton *et al*., 2017], in the study of phytoremediation potential of vetiver grass (*Vetiveria zizanoides*) for treatment of metal-contaminated water, where there were higher uptake of heavy metals in the roots than the shoots due to roots length and higher density of plants.

4.0 Conclusion

The assessed physicochemical parameters EC (951.83 µs/m), PO_4 (64.46 mg/l), BOD (244.90 mg/l), COD (625.50 mg/l), TSS (27.30mg/l) and K (40.67mg/l) of ABUTH has concentrations above acceptable threshold of WHO and FAO indicating contamination, and not appropriate for direct use or discharge into water bodies and use for irrigation purposes. Vetiver grass *(V. zizanoides)* had the highest potential with percentage removal efficiency of (NO3 and Co=64.60%, TDS=63.0%, K, Cr and Zn=62%, BOD=59.85% and Cu=60.60%) followed by *E. crassipes* and then *P. stratiotes* and hence significant reduction of contaminants was recorded after phytoremediation and highest concentration of contaminants was in the roots of *V. zizanoides*, shoots of *E.crassipes* and heterogeneous distribution in roots and shoots of *P. stratiotes.*

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