

Research Application Summary

Use of multistage phytoremediation technique in wastewater treatment

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Abstract

Efficient treatment of wastewater before discharge into aquatic ecosystems is a requirement to prevent the detrimental effect of polluting the environment and to safeguard public health. Adoption of green technology in wastewater treatment is paramount. This study was carried out to investigate the efficiency of multistage technique in phytoremediation of wastewater. The experimental set up was a four-stage treatment system. It comprised of four columns with different arrangements of macrophytes which included *Ceratophyllum demersum*, *Typha latifolia*, *Nymphaea sp.* and *Azolla pinnata*. Wastewater was sampled and put in four, twenty litres pre-sterilized plastic containers and allowed to flow to plastic troughs each containing the experimental plants from stage 1 to stage 4 with a retention time of five days. Water indicator parameters, nutrients and heavy metals in the wastewater were analysed at the beginning of the treatment process and from each trough in each column at the end of the retention time of five days for a period of 20 days to determine the changes in the levels of the parameters investigated. Means of mentioned parameters were calculated and analyzed using ANOVA and significant means separated using Tukey's test at 5% level. Removal efficiency of the investigated parameters was calculated and the range was as follows; TDS 79.13-82.27%, pH 14.12-16.67%, conductivity 66.92-71.48%, turbidity 67.97-80.54%, faecal coliforms 100%, faecal streptococcus 100%, phosphates 93.72-100%, nitrates 89.79-100% Cd 83.40-100%, Cu 83.39-88.60%, Ni 100%, Co 100%, Pb 100%, Mn 100%, Zn 100% and Fe 88.60-95.77%. Multistage technique was found to be efficient in wastewater treatment. This technique is a green technology which is efficient and economical hence recommended for remediation of wastewater.

Key words: Macrophytes, multistage technique, phytoremediation, wastewater management

Résumé

Le traitement efficace des eaux usées avant leur rejet dans les écosystèmes aquatiques est une exigence visant à prévenir les effets néfastes de la pollution de l'environnement et à préserver la santé publique. L'adoption de technologies vertes dans le traitement des eaux usées est primordiale. Cette étude a été réalisée pour évaluer l'efficacité de la technique à plusieurs étages dans la phytoremédiation des eaux usées. Le dispositif expérimental était un système de traitement à quatre étages. Il comprenait quatre colonnes avec différents agencements de macrophytes, notamment *Ceratophyllum demersum*, *Typha latifolia*, *Nymphaea sp.* et *Azolla pinnata*. Les eaux usées ont été échantillonnées et placées dans quatre contenants en plastique pré-stérilisés de vingt litres, puis laissées s'écouler dans des rigoles en plastique contenant chacune les plantes expérimentales de l'étape 1 à l'étape 4, avec un temps de rétention de cinq jours. Les paramètres indicateurs de l'eau, les éléments nutritifs et les métaux lourds dans les eaux usées ont été analysés au début du processus de traitement et à partir de chaque rigole de chaque colonne à la fin du temps de rétention de cinq jours, sur une période de 20 jours, afin de déterminer les changements dans les niveaux des paramètres étudiés. Les moyennes des paramètres mentionnés ont été calculées et analysées.

à l'aide de l'ANOVA, et les moyennes significatives ont été séparées à l'aide du test de Tukey au niveau de 5%. L'efficacité d'élimination des paramètres étudiés a été calculée et la plage était la suivante : DCO 79,13-82,27%, pH 14,12-16,67%, conductivité 66,92-71,48%, turbidité 67,97-80,54%, coliformes fécaux 100%, streptocoques fécaux 100%, phosphates 93,72-100%, nitrates 89,79-100%, Cd 83,40-100%, Cu 83,39-88,60%, Ni 100%, Co 100%, Pb 100%, Mn 100%, Zn 100% et Fe 88,60-95,77%. La technique à plusieurs étages s'est révélée efficace dans le traitement des eaux usées. Cette technique, qui est une technologie verte efficace et économique, est donc recommandée pour la remédiation des eaux usées.

Mots-clés : Macrophytes, technique à plusieurs étages, phytoremédiation, gestion des eaux usées

Introduction

Water is one of the most precious natural resource that exists on earth, without which life would be non-existent. It is an essential component of life as it plays a vital role in the sustenance of all life on earth (Rolston *et al.*, 2017). It is the backbone of growth and prosperity for mankind and a key determining aspect for economic growth in a country hence its scarcity limits economic and social development.

The world is currently facing serious water pollution and treatment challenges. Sustainable Development Goals Target 6.3 states: "by 2030, improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally" (United Nations, 2017). There is a dire need to increase the levels of wastewater treatment in order to achieve Target 6.3, which is paramount for achieving the entire agenda 6 that is concerned with clean water and sanitation. The consequences of water pollution is poor public health, contaminated environment as well as increase in poverty.

Sources of water in Kenya are under pressure from urban and industrial wastes, agricultural chemicals such as fertilizers and pesticides in addition to production of hydroelectric power. There is increased demand for water resources as the population tries to acquire water to meet its diversified needs. This has caused major constraints on the scarce resource. Poor urban planning and weak implementation of environmental policies has escalated this problem. There is also lack of technical knowhow, poor assessment and monitoring of water quality and limited capital which has engendered serious water pollution and treatment challenges. The rapid growth of industrialization has given rise to the problem of heavy metal contamination. Heavy metals are metals of specific gravity that is normally higher than 5 g/cm³ (Anjuli *et al.*, 2012). Pollution of heavy metals in soils and freshwater environments is a potential environmental problem in developing countries due to the disposal of untreated or partially treated industrial wastewaters. Currently, eutrophication is another major challenge in most of the developing countries. Eutrophication of water has been increased by anthropogenic activities that accelerate the rate of nutrient input in aquatic ecosystems. These nutrients are generally nontoxic at the concentrations typically found in nature but when in excess, they can lead to eutrophication (Xiaotian *et al.*, 2019).

Phytoremediation has been applied successfully in the remediation of contaminated environments. It is a growing field of research in environmental studies because of the advantages in being environmental friendly and the possibility of harvesting the plants for the extraction of accumulated pollutants such as heavy metals that cannot be easily biodegraded for recycling. Despite the abundance of literature on phytoremediation, there is a dearth of information on multistage phytoremediation. This type of research is less attempted, hence require in-depth investigation.

Methods

Study area. The study was carried out at the University of Eldoret located in Uasin Gishu County, about 9 km north east of Eldoret town (Figure 1). Uasin Gishu County is located in mid-western Kenya, between 34°55'33" and 36°38'58"E and between 0°2'44"S and 0°55'56"N.

Sample collection and experimental set up. Young and healthy macrophytes samples were sampled randomly by hand from Marura wetland. The plants were put in plastic containers and transported to the laboratory within few hours of collection where they were cleaned carefully using tap water to remove dirt and dust. The experimental plants were initially subject to acclimatization in stock tanks containing tap water for one week, after which they were thoroughly washed with sterile distilled water before being introduced in the experimental troughs. These plants were *Azolla pinnata*, *Nymphaea sp.*, *Typha latifolia* and *Ceratophyllum demersum*. The experimental set up was a four-stage treatment system. It comprised of four columns with different arrangements of macrophyte. The first column of plants was selected randomly followed by systematic arrangement. Approximately, 120 litres of wastewater were sampled from the university of Eldoret wastewater treatment plant using clean sterilized plastic container. They were transported to the University of Eldoret. The wastewater was put in four, twenty litres pre-sterilized plastic containers and allowed to flow to plastic troughs each containing the experimental plants from one stage to the other as shown in Figure 2. They were stacked at different heights next to each other. The retention time for each stage (cell) was 5 days. A 2.5 cm diameter plastic pipe with a control valve was used to facilitate the flow from one stage to another after the 5 days retention time.

At the start of the experiment, wastewater was allowed to flow into the first stage of treatment. This stage contained troughs with the following plants; trough (a1) *Azolla pinnata*, trough (b1) *Typha latifolia*, trough (c1) *Ceratophyllum demersum* and trough (d1) *Nymphaea sp.* After 5 days in these troughs, the partially treated wastewater was allowed to flow into stage 2. This stage contained troughs with the following plants: trough (a2) *Typha latifolia*, trough (b2) *Nymphaea sp.*, trough (c2) *Azolla pinnata* and trough (d2) *Ceratophyllum demersum*. This was achieved by opening the control valve connecting trough 1 to trough 2 at the end of the 5 days. This procedure continued until the partially treated wastewater finally flowed into stage 4. The plants in stage 3 were: trough (a3) *Ceratophyllum demersum*, trough (b3) *Azolla pinnata*, trough (c3) *Nymphaea sp.* and trough (d3) *Typha latifolia*. The plants in stage four were: trough (a4) *Nymphaea sp.*, trough (b4) *Ceratophyllum demersum* trough (c4) *Typha latifolia* and trough (d4) *Azolla pinnata*. A control was set up without the plants to assess the role of macrophytes in the removal of pollutants. 200ml of the wastewater were sampled from the four plastic containers at the beginning of the treatment process and from each trough in each column at the end of the retention time of 5 days. They were analysed in triplicate for the physicochemical and bacteriological parameters, nitrates and phosphates and heavy metals.

Analysis of wastewater. Water indicator parameters were analysed using standard methods according to (APHA, 2005). Physicochemical parameters that were analysed included, pH, TDS and conductivity, which were determined using a multi tester digital pH meter. DO was measured using a DO meter and turbidity was measured using colorimeter. BOD₅ was determined as the difference between the oxygen concentrations of an appropriately diluted sample before and after incubation for 5 days at 20 ± 1°C while COD was determined using micro digestion method.

Bacteriological analysis. MacConkey agar was used to culture faecal coliforms and Bile esculine azide agar was used for faecal streptococci. The number of colonies per 1ml was calculated as follows

$$\frac{\text{Cfu}}{\text{ml}} = \frac{\text{No.of colonies x dilution factor}}{\text{Volume plated (ml)}}$$

Nutrient analysis. Phosphates in wastewater was determined by ammonium molybdate method. Nitrates in wastewater samples were determined by brucine method.

Heavy metal analysis. The wastewater samples were digested using nitric acid digestion method and analyzed in the AAS using an air/acetylene flame (APHA, 2005).

Data analysis. The removal efficiency for the investigated parameters was calculated as follows:

$$\text{Removal efficiency (E}_r\text{)} = \frac{\text{Initial concentration} - \text{final concentration}}{\text{Initial concentration}} * 100$$

Results and Discussion

Total dissolved solids (TDS). There was a significant reduction of TDS by all macrophyte columns. Column four had the highest removal efficiency of 84.18%, column 2 had 82.27%, column 1 had 79.13% while column 3 reduced TDS by 79.90 % (Figure 1a). There was no significance difference in reduction of TDS among the different macrophytes column. Reduction of TDS may be attributed to the reduction of organic matter and other dissolved salts as a result of utilization of these substances by macrophytes during their growth and development. The roots of some aquatic macrophytes are capable of retaining both coarse and fine particulate organic materials present in wastewater to support their growth. This is mainly achieved through the electrical charges associated with the root hairs, which reacts with the opposite charges on colloidal particles. This reduces the levels of TDS in the wastewater. Generally, salinity of a solution influence the sorption of heavy metals, where reduced salinity leads to increased sorption efficiency by macrophytes (Krem, 2013).

Turbidity. All macrophytes column reduced the levels of turbidity. Column 4 had the highest removal efficiency of 80.54% while column 3 had the lowest removal efficiency of 67.97% (Figure 1b). There was a significance difference in turbidity reduction ($P = 0.001$). Reduction in turbidity may be attributed to the uptake and degradation of organic and inorganic substances by plants and also the adsorption of some suspended substances to the macrophyte tissues. During the process of phytoremediation, both microorganisms and plants participate in the degradation process, either independently or through synergistic effects. Turbidity reduction may be also ascribed to the reduction of microbes in the wastewater effluents.

Conductivity. Column 4 had the highest removal efficiency of 71.48% while column 1 had the lowest removal efficiency of 66.92%. Column 2 reduced conductivity by 69.26% while column 3 had a removal efficiency of 68.06% (Figure 1c). There was a significance difference in reduction of conductivity among the macrophyte columns ($P = 0.00$). The results were in line with the findings of Aisien *et al.*, (2015) who reported a reduction efficiency ranging from 63.4 to 89.3 %. The reduction in conductivity may be attributed to the removal of salts from the effluents through plants uptake and root absorption (Ran *et al.*, 2012).

pH. There was reduction of pH in all macrophyte columns. Column 4 had the highest removal efficiency of 16.67% while column three had the lowest removal efficiency of 14.12%. Column 1 reduced the pH by 15.48% while column 2 reduced the pH by 16.28% (Figure 1d). There was a significance difference in the reduction of pH ($P = 0.03$). The results were in harmony with the findings of Aisien *et al.*, (2015) who reported a reduction efficiency of pH ranging from 13.3 to 20%

while working with macrophytes to improve the abattoir effluents. pH reduction may be ascribed to the absorption of pollutants by macrophytes. pH is one of the most essential parameter in the bio sorption process. It affects the chemistry of the metals in solution, the activity of the functional groups in the biomass and the competition of metallic ions.

Dissolved Oxygen (DO). All macrophyte columns showed an increase in dissolved oxygen. Column 2 had the highest DO addition of 65.77 %, column 4 added DO by 33.66%, macrophytes in column one increased the DO by 35.20 %, and those in column 3 increased the DO by 33.66% (Figure 1e). There was a significance difference in the mean DO obtained for the different macrophytes columns ($P = 0.00$). The increase in DO may be attributed to the addition of oxygen during the process of photosynthesis. Oxygen transfer by aquatic plants in to the root zone plays a significant role in supporting the growth of aerobic bacteria in the root zone and subsequent increased degradation of organic matter in wastewater. Reduction of degradable matter leads to increased DO.

Temperature. There were no significance differences in the levels of temperature among different columns ($P = 0.079$). The temperature ranged from 20.5 to 25.5°C (Figure 1f).

Feecal coliforms and Feecal streptococcus. There was 100 % removal efficiency of fecal coliforms and fecal streptococcus in all macrophyte columns and the control. There was no significance difference in the reduction of fecal coliforms and fecal streptococcus among the macrophyte columns ($P = 0.948$ and $p = 0.973$) respectively (Table 1). The results were in harmony with the findings of Aisien *et al.*, (2015) and Dar *et al.*, (2011) who reported a removal efficiency of 100%. Aquatic plants significantly reduce microbial contaminants in wastewater by playing a role in biofiltration via a combination of biological, physical and chemical processes which all take part in the reduction of bacteria populations. The removal efficiency depends on associated characteristics of wastewater and plant species.

Phosphates. All macrophyte columns reduced the levels of phosphates in the waste water effluents. Column 3 and 4 had removal efficiency of 100%. Column 1 reduced phosphates by 93.72% while column 2 had a removal efficiency of 95.55% (Table 1). The reduction of phosphates is ascribed to the uptake by plants for their growth and development. Most aquatic macrophytes species can assimilate phosphates directly through their thalli, shoots and leaves. Also periphyton and microorganisms associated with macrophytes can removes nutrients directly from the wastewater. In the control, phosphates removal may be due algae uptake.

Nitrates. There was a general reduction of the levels of nitrates by all macrophyte columns. Column 1, 3 and 4 had a removal efficiency of 100%. Column 2 reduced the nitrates by 89.79% (Table 1). There was a significance difference in the reduction of nitrates among the macrophyte columns. The removal efficiency of column two was significantly different from the others ($P = 0.00$). Nitrate and nitrite in the constructed wetland system are removed by plant uptake or denitrification. Once nitrogen has been denitrified, it is released in the atmosphere as nitrous oxide or dinitrogen gas. Denitrification could account for the nitrates removal in the control.

Heavy metals

Cadmium. All the macrophyte columns reduced the levels of Cd in the wastewater effluents. Column 3 and 4 had the highest removal efficiency of 100% while column 1 had the lowest removal efficiency of 83.40%. Column 2 had a removal efficiency of 91.44% (Table 1). The slower removal of Cd in comparison to the rest of the metals present in the wastewater effluent may be due to its toxicity. Cd is non-essential to plants and hence may be excluded during absorption especially in presence of other essential metals. Most plants do not have mechanisms to absorb non-essential elements such as Cd and uptake is through Ca^{2+} , Fe^{2+} , Mn^{2+} and Zn^{2+} transporters when plants absorb essential trace elements (Marchard *et al.*, 2010).

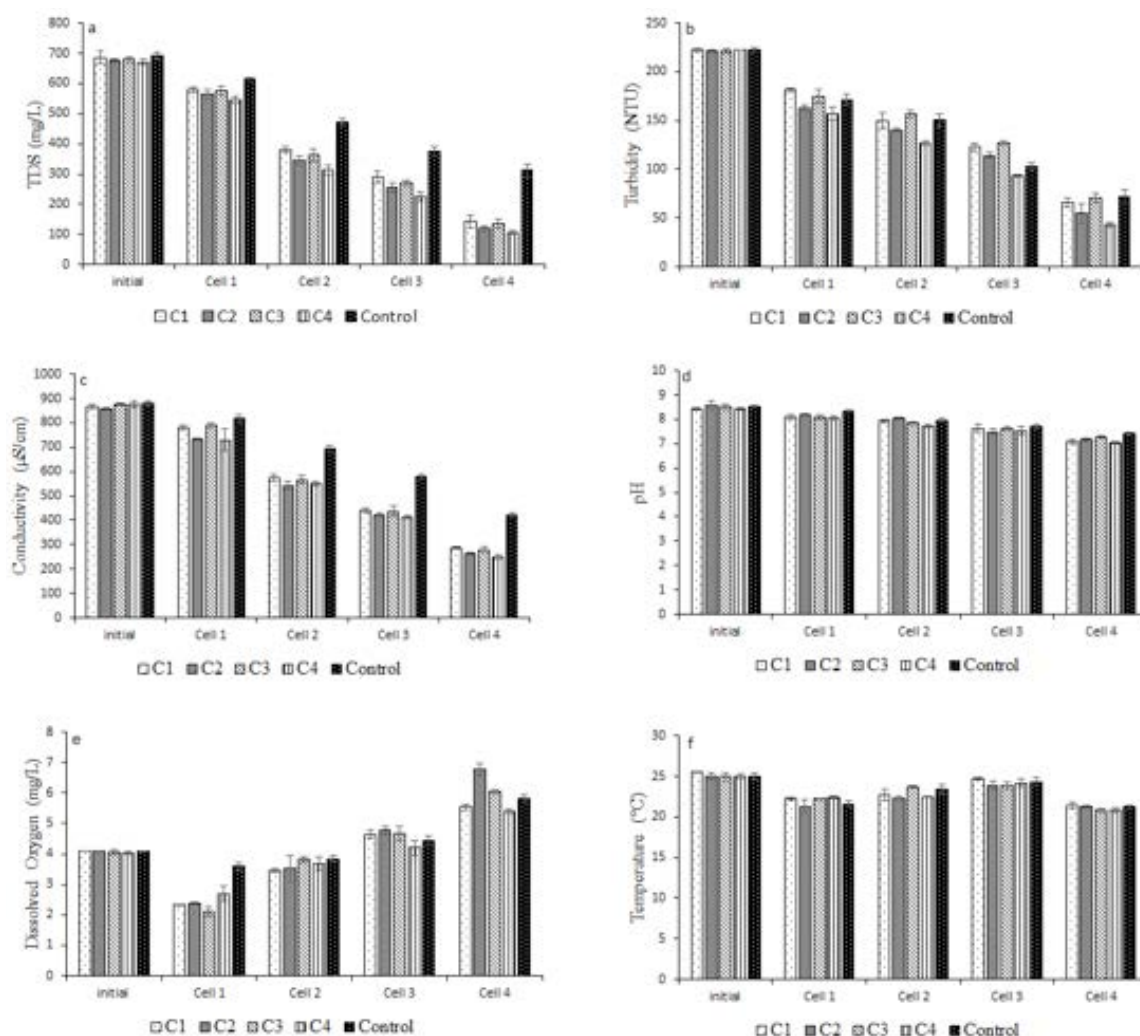


Figure 1. Phytoremediation of water quality indicator parameters in multistage technique (a = TDS, b = turbidity, c = conductivity, d = pH, e = DO and f = temperature).

Table 1. Reduction efficiency of nutrients, coliforms and Heavy metals in multistage technique

Parameter	Column 1 ATCN	Column 2 TNAC	Column 3 CANT	Column 4 NCTA	Control No plants
Phosphates	93.72 ^a	95.55 ^a	100 ^a	100 ^a	55.35 ^b
Nitrates	100 ^a	89.79 ^b	100 ^a	100 ^a	73.96 ^c
Total coliforms	68.41 ^a	77.82 ^a	71.33 ^a	73.74 ^a	72.15 ^a
Feecal coliforms	100 ^a	100 ^a	100 ^a	100 ^a	100 ^a
Feecal streptococcus	100 ^a	100 ^a	100 ^a	100 ^a	100 ^a
Cadmium (Cd)	83.40 ^a	91.44 ^a	100 ^a	100 ^a	14.37 ^b
Copper (Cu)	88.60 ^a	86.40 ^a	83.39 ^a	81.98 ^a	19.52 ^b
Iron (Fe)	100 ^a	98.24 ^a	95.77 ^a	100 ^a	17.61 ^b
Lead (Pb)	100 ^a	100 ^a	100 ^a	100 ^a	26.69 ^b
Manganese (Mn)	100 ^a	100 ^a	100 ^a	100 ^a	21.21 ^b
Zinc (Zn)	100 ^a	100 ^a	100 ^a	100 ^a	16.69 ^b
Nickel (Ni)	100 ^a	100 ^a	100 ^a	100 ^a	30.73 ^b
Cobalt (Co)	100 ^a	100 ^a	100 ^a	100 ^a	32.61 ^b

Means followed by the same letter within the same column are not significantly different at $P \leq 0.05$.

A = *Azolla sp.* T = *Typha sp.* C = *Ceratophyllum sp.* N = *Nymphaea spp.*

Copper. Column 4 reduced copper by 81.98% while column 1 had a removal efficiency of 88.60%. Column 2 reduced copper by 86.40% and column 3 had a removal efficiency of 83.39% (Table 1). The slower removal of Cu in comparison with the other metals may be ascribed to the high initial concentrations of copper compared to the rest of the heavy metals.

Iron (Fe). Column 1 and 4 reduced iron by 100% while column 2 had a removal efficiency of 98.24%. Column 3 reduced Fe by 95.77% (Table 1). Iron is essential to plants and hence was utilized in their growth and development.

Cobalt, Lead, Manganese, Zinc and Nickel. All macrophyte column were efficient in removal of Co, Ni, Pb, Mn and Zn as they all attained a removal efficiency of 100% (Table 1). There was no significance difference in removal of these metals among the macrophyte columns but there was significant difference between the macrophytes and the control. Some of these heavy metals such as Ni, Zn, Fe, Mn, Co and Cu are required for plant growth in physiological quantities as they perform important functions in living organisms. They are essential in maintaining the appropriate structure and operation of enzymes and also make up metal-organic compounds such as metalloproteins. However, they are toxic in greater concentrations as they limit root growth and damage root cells. In addition, they change permeability of cell membranes and slow down the transport of electrons in photosynthesis process (Krems *et al.*, 2013). A moderate reduction in all heavy metals in the control might be due to the presence of microorganisms and their biological activities.

Phytoremediation efficiency of metals greatly depends on the concentration of these metals in solution, the lower the concentration of the metals in the solution the higher the removal efficiency. The initial concentrations of heavy metals in the wastewater were low especially for Co, Ni, Mn, Zn, and Pb. This could have contributed to their high removal efficiency of 100%. Initial concentration of Cu was higher compared to the other metals. This could have contributed to its lower removal efficiency. The accumulation of heavy metals also depends on the type of heavy metals (Vahdatirad and Khara 2012). The accumulation of the eight heavy metals was different among the plants. The essential heavy metals were removed at a higher percentage compared to the non-essential and toxic heavy metals. For example, Zn, Cu, Co and Mn are used to produce the enzyme required in synthesis of other, physiologically active particles. These metals attained a removal efficiency of 100% apart from Cu. Some heavy metals such as Cd can cause oxidative stress, even at low concentrations which stops chlorophyll biosynthesis and accelerates lipids pre-oxidation, which leads to damage of cellular membranes (Harguinteguy *et al.*, 2013).

The accumulation of heavy metals depends on the type of plant species and the capability of either their roots or shoots to concentrate the contaminants (Moushumi *et al.*, 2015). Presence of microbial symbionts such as rhizosphere bacteria and fungi can affect the accumulation of metals in plants. Contaminants can be remediated in plants by varying biophysical and biochemical processes such as sorption, transportation and transformation, hyper accumulation and mineralization.

Combining different species of plants in multi stage technique may improve tolerance to changing environmental conditions and function in stabilizing the biogeochemical processes. It may contribute to optimal environmental conditions through improved physicochemical parameters and increased productivity through more efficient use of available resources such as nutrients hence reducing their load in wastewater. Multistage technique can be employed in developing high-efficient nutrients phytoremediation systems. An ecosystem which is rich and diverse with plant species would be expected to display a wider range of functional traits with increasing opportunities for more efficient resource use due to variation in survival characteristics. Effective resource use enhances productivity resulting to effective performance in reducing pollutants in constructed wetlands.

It can be conceded that roots of all the three species do show high retention of heavy metals as well

as trace elements in their root structure thus they can prevent bioaccumulation in higher trophic level (Ahmad *et al.* 2014). Ancillary functions include primary production of organic carbon by plants; oxygen production through photosynthesis; production of wetland herbivores, as well as predator species that range beyond the wetland boundaries; reduction of export of organic matter and nutrients to downstream ecosystems; and creation of cultural values in terms of educational and recreational resources. One or more of these ancillary functions may be an important goal in some constructed wetland projects. For detailed descriptions of ancillary functions, the reader is referred to information presented elsewhere (Feierabend, 1989; Sather, 1989; Knight, 1992).

The multistage technology has a great potential in becoming the basis of the principle of sustainable development which promotes activities that do not have adverse effect on the environment. The treatment of wastewater using this technology also provides an opportunity to create or restore wetlands for environmental enhancement and also serve other ancillary functions such as sequestration of CO₂ hence mitigating climate change. The use of multistage phytoremediation technique offers a considerable opportunity for realizing sizeable savings in wastewater treatment.

Conclusion

Multistage phytoremediation technique was fast and efficient in phytoremediation. The order of reduction efficiency was column 2 > column 4 > column 3 > column 1. This technique is a green technology which is efficient and economical hence recommended for wastewater treatment.

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