DISCOVERY

58(316), April, 2022

Use of multistage phytoremediation technique in wastewater treatment

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To Cite:
Wanjohi L, Mwamburi L, Mwasi S, Meso D, Isaboke J. Use of multistage phytoremediation technique in wastewater treatment. *Discovery*, 2022, 58(316), 252-263

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Peer-Review History
Received: 29 January 2022
Reviewed & Revised: 31/January/2022 to 03/March/2022
Accepted: 05 March 2022
Published: April 2022

Peer-Review Model
External peer-review was done through double-blind method.

DISCOVERY
SCIENTIFIC SOCIETY

ABSTRACT

Efficient treatment of wastewater before discharge into aquatic ecosystems is a requirement to prevent the detrimental effect of polluting the environment and safeguard public health. Adoption of green technology in wastewater treatment is paramount. This study was carried out to investigate the efficiency of the multistage technique in the phytoremediation of wastewater. The experimental setup was a four-stage treatment system. It comprised of four columns with different arrangements of macrophytes that included *Ceratophyllum demersum*, *Typha latifolia*, *Nymphaea* spp., and *Azolla pinnata*. Wastewater was sampled and put in four twenty-liter 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 5 days. Water indicator parameters, nutrients, and heavy metals in the wastewater were analyzed at the beginning of the treatment process and from each trough in each column at the end of the retention time of 5 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; total dissolved solids 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%, cadmium 83.40-100%, copper 83.39-88.60%, nickel 100%, cobalt 100%, lead 100%, manganese 100%, zinc 100% and iron 88.60-95.77%. The multistage technique was found to be efficient in wastewater treatment. This technique is a green technology that is efficient and economical hence recommended for remediation of wastewater.

Keywords: Multistage technique, phytoremediation, wastewater, macrophytes, heavy metals.

1. INTRODUCTION

Water is one of the most precious natural resources on earth, without which life would be non-existent. It is an essential component of life as it plays a vital role in sustaining all life on earth (Rolston *et al*., 2017; Rajan & Nisha, 2020). It is the backbone of growth and prosperity for humankind and a key determining aspect for economic growth in a country (Balamurugan, 2020); hence its scarcity limits economic and social development.

The world is currently facing severe water pollution and treatment challenges. There is a dire need to increase the levels of wastewater treatment to achieve Target 6.3, which is paramount for achieving the entire Agenda 6 that is concerned with uncontaminated water and hygiene. Thus, the Sustainable Development Goals Target 6.3 states that by the year 2030, there should be improved water quality by decreasing water pollution, eradicating discarding, and reducing disposal of hazardous chemicals and materials, halving the proportion of unprocessed wastewater, and impressing recycling and reusing (United Nations, 2017). The consequences of water pollution are poor public health, a contaminated environment, and poverty.

Sources of water in Kenya are being polluted from municipal and industrial wastes, agricultural chemicals such as fertilizers and pesticides, and the 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 significant constraints on a scarce resource. Poor urban planning and weak implementation of environmental policies have escalated this problem. There is also a lack of technical know-how, inadequate assessment and monitoring of water quality, and limited capital, which has engendered severe 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 normally higher than 5 g/cm^3 (Anjuli *et al.*, 2012). Pollution of heavy metals in soils and freshwater environments is an issue in developing countries generating and disposing untreated waste. Currently, eutrophication is another major challenge in 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 they can lead to eutrophication (Lu *et al.*, 2019).

Phytoremediation has been applied successfully in treating contaminated environments. It is a concern research category in environmental studies because of the advantages of being environmentally friendly and the possibility of harvesting the plants to extract accumulated pollutants such as heavy metals that cannot be easily biodegraded for recycling. It has little installation and maintenance costs compared to other remediation methods. To clean up wastewater, selecting an appropriate and efficient plant system is highly essential. Also, the arrangement of plants in a constructed wetland is essential as it influences its efficiency. This has prompted a search for the best plants to be used for cleaning wastewater and the best technique to be employed to achieve maximum efficiency. Such methods include multistage phytoremediation with good purification effect, low cost, convenient operation and management, and good landscape and ecological benefits. Despite the abundance of literature on phytoremediation, there is a shortage of information on multistage phytoremediation techniques. This type of research is less attempted, hence requires in-depth investigation.

2. MATERIALS AND METHODS

Study area

The study was carried out at the University of Eldoret, located in Uasin Gishu County, about 9 km northeast of Eldoret town (Figure 1). Uasin Gishu County is situated 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 setup

Young and healthy macrophytes samples from Marura wetland were sampled randomly by hand. The plants were put in plastic containers and transported to the laboratory at the University of Eldoret within a few hours of collection. The plants were cleaned sensibly using running tap water to eliminate any dirt.

The experimental plants were initially subjected 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. *Azolla pinnata*, *Nymphaea* spp., *Typha latifolia*, and *Ceratophyllum demersum*. The experimental setup was a four-stage treatment system. It is comprised of four columns with different arrangements of macrophytes. The first column of plants was selected randomly, followed by a systematic arrangement.

Approximately 120 litres of wastewater were sampled from the university of Eldoret wastewater treatment plant using sterile plastic containers. 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.

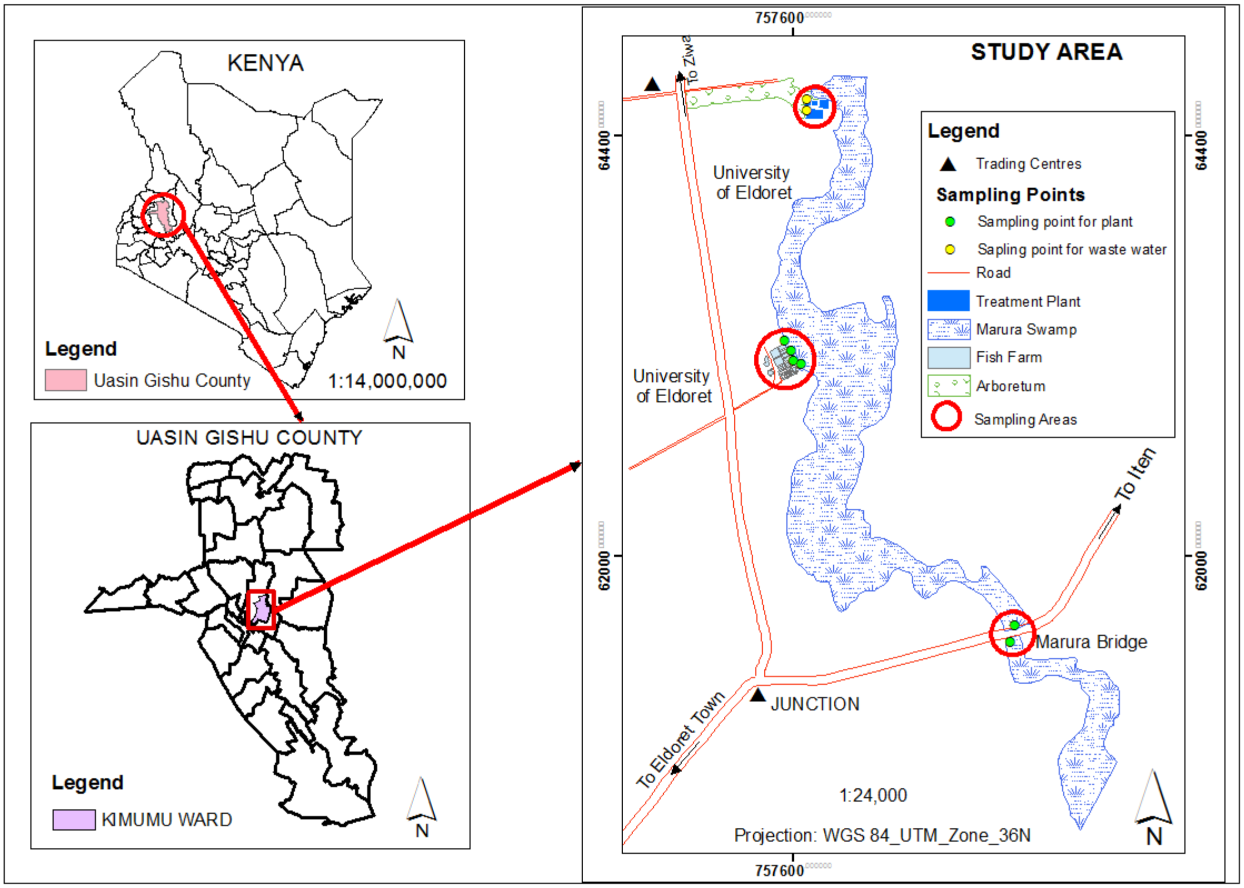

Figure 1. The study area map showing the location of Marura wetland and University of Eldoret wastewater treatment plant, Uasin Gishu County, Kenya

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 (a_1) *Azolla pinnata*, trough (b_1) *Typha latifolia*, trough (c_1) *Ceratophyllum demersum*, and trough (d_1) *Nymphaea* spp. After five days, the partially treated wastewater was allowed to flow into stage 2. This stage contained troughs with the following plants: trough (a_2) *Typha latifolia*, trough (b_2) *Nymphaea* spp., trough (c_2) *Azolla pinnata*, and trough (d_2) *Ceratophyllum demersum*. This was achieved by opening the control valve connecting trough 1 to trough two at the termination of the five days. This procedure continued until the partially treated wastewater finally flowed into stage 4. The plants in stage 3 were: trough (a_3) *Ceratophyllum demersum*, trough (b_3) *Azolla pinnata*, trough (c_3) *Nymphaea* spp., and trough (d_3) *Typha latifolia*. The plants in stage four were: trough (a_4) *Nymphaea* spp., trough (b_4) *Ceratophyllum demersum* trough (c_4) *Typha latifolia*, and trough (d_4) *Azolla pinnata*. A control was set up without the plants to assess the role of macrophytes in the elimination of pollutants.

Approximately 200 ml of the wastewater was sampled from the four plastic containers at the start of the treatment process and from each trough in each column at the finishing of the retention time of 5 days. They were analyzed in triplicate for the physicochemical and bacteriological parameters, nitrates, phosphates, and heavy metals.

Analysis of wastewater

According to (APHA 2005), water indicator parameters were analyzed using standard methods. The analyzed physicochemical parameters included temperature, pH, TDS, and conductivity, which were determined using a multi-tester digital pH meter. DO meter was used to measure Dissolved oxygen, and a colorimeter measured turbidity.

Bacteriological analysis

Aseptic techniques were employed in all bacterial analyses. Serial dilution and 1ml of the diluted sample were inoculated using the pour plate method on sterile media. MacConkey agar was used to culture fecal coliforms, and Bile esculine azide agar was used for Feacal streptococci. The number of colonies per 1ml as calculate in the equation below

$$\frac{\text{Cfu}}{\text{ml}} = \frac{\text{No. of colonies x dilution factor}}{\text{Volume plated (ml)}} \qquad (1)$$

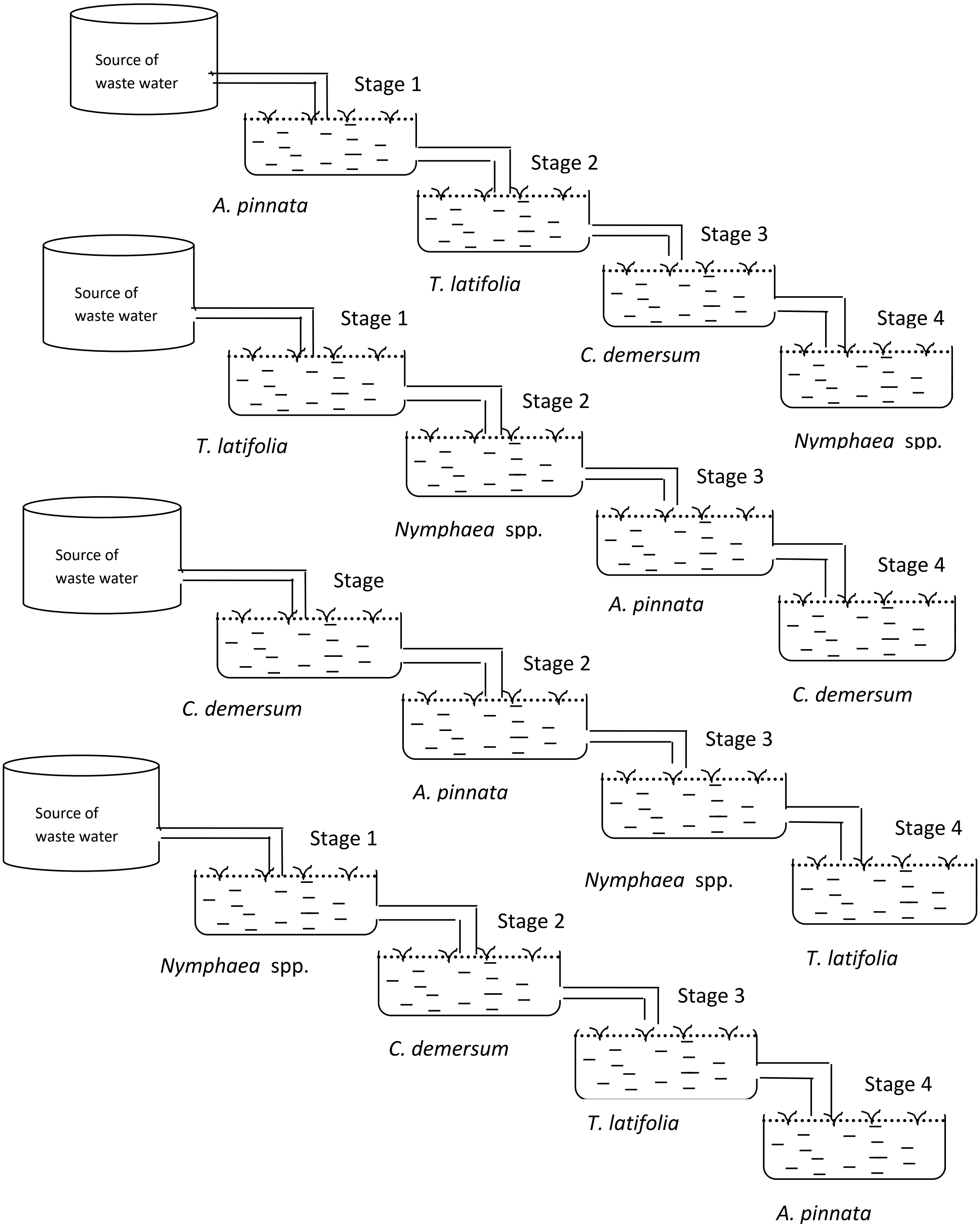

Figure 2. The experimental setup for the phytoremediation of University of Eldoret wastewater using four macrophytes in multistage technique

Nutrient analysis

Phosphates in wastewater were determined by the ammonium molybdate method. Absorbance of the samples were measured using UV-Vis spectrophotometer at a wavelength set at 650nm. Nitrates in wastewater samples were determined by the brucine method. The UV-Vis spectrophotometer measured the absorbance of the samples at a wavelength set at 420nm.

Heavy metal analysis

The wastewater samples were digested using the nitric acid digestion method (APHA, 2005). Used reagents were of analytical grade, and all the vessels were prepared according to procedures outlined in APHA (2005) to avoid the external contributions of heavy metals. Wastewater samples were analyzed in the AAS using an air/acetylene flame.

Data analysis

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

$$\text{Removal efficiency } (E_r) = \frac{\text{Initial concentration} - \text{final concentration}}{\text{Initial concentration}} * 100 \qquad (2)$$

3. RESULTS AND DISCUSSION

The results indicates that there were significant reductions in physicochemical parameters, nutrients, bacterial loads, and potential toxic elements in the University of Eldoret wastewater by the four macrophytes in the multistage technique.

Dissolved Oxygen (DO)

All macrophyte columns showed an increase in dissolved oxygen (Table 1, Figure 3e). 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%. There was a significant difference in the mean DO obtain for the different macrophytes columns (P = 0.00). The increase in DO may be attributed to the addition of oxygen during photosynthesis (De Godos *et al.,* 2010). This is due to the fact that oxygen transmission by aquatic plants into the root zone is important in promoting the growth of aerobic bacteria in the root zone and, as a result, enhanced organic matter breakdown in wastewater. (Reddy *et al.,* 1987; Wießner *et al.*, 2005). Therefore, the reduction of degradable matter leads to increased DO (Lee *et al.*, 2008).

pH

There was a pH decrease in all macrophyte columns (Table 1, Figure 3d). 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%. There was a significant difference in pH reduction, with columns 2 and 4 performing better than the other columns. The results were in agreement 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 abbaitor effluents. The pH reduction may be ascribed to the absorption of pollutants by macrophytes (Mahmood *et al.*, 2005).

Total dissolved solids (TDS)

There was a significant reduction of TDS by all macrophyte columns (Table 1, Figure 3a). Column four had the highest TDS deduction with a removal efficiency of 84.18%. It was followed closely by column 2, which had a removal efficiency of 82.27%. Column 1 reduced TDS by 79.13%, while column 3 reduced TDS by 79.90 %. There was no significant difference in TDS reduction among the different macrophytes columns. Reduction of TDS may be attributed to the reduction of organic matter and other dissolved salts due to the utilization of these substances by macrophytes during their growth and development. Previous studies by Krems *et al.* (2013), some aquatic macrophytes' roots were shown to be capable of storing both coarse and fine particle organic elements contained in wastewater to sustain their development. This can be accomplished through the electrical charges associated with root hairs, which react with the opposing charges on colloidal particles, resulting in lower TDS levels in the effluent. (Krems *et al.*, 2013).

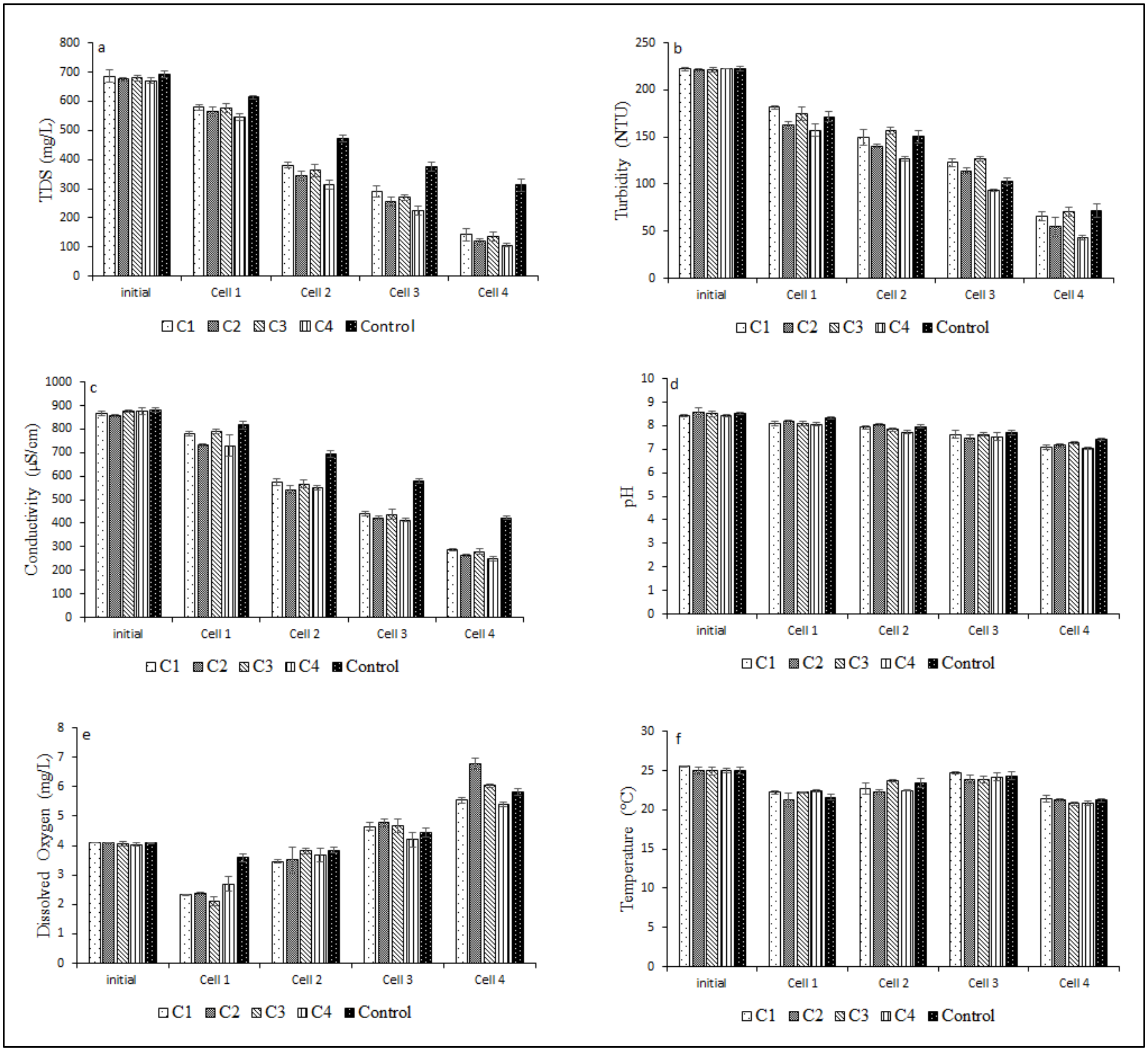

Figure 3. Remediation of water quality indicator parameters by four macrophytes in multistage technique
(a = Total Dissolved Solids (TDS), b = turbidity, c = conductivity, d = pH, e = Dissolved oxygen and f = temperature).

Conductivity

Column 4 had the highest removal efficiency of 71.48%, while column 1 had the lowest removal efficiency of 66.92% (Table 1, Figure 3c). Column 2 reduced conductivity by 69.26%, while column 3 had a removal efficiency of 68.06%. There was a significant difference in conductivity reduction 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 might be related to removing salts from the effluents through plants uptake and root absorption (Ran *et al.*, 2012).

Turbidity

All macrophytes columns reduced turbidity levels (Table 1, Figure 3b). Column 4 had the greatest removal effectiveness of 80.54%, while column 3 had the lowest removal efficiency of 67.97%. There was a notable disparity in turbidity reduction (P = 0.001).

Reduction in turbidity may be connected to the uptake and degradation of organic and inorganic substances by plants and the adsorption of some suspended substances to the macrophyte tissues (Aisien *et al.,* 2015).

Table 1. Reduction of water quality indicator parameters by four macrophytes in multistage technique

	Water quality indicator parameters						
	Dissolved oxygen	pH	Total dissolved solids	Conductivity	Turbidity	Phosphates	Nitrates
Columns	% Reduction						
Column 1 *ATCN*	-35.20^{ab}	15.48^{ab}	79.13^{a}	66.92^{b}	70.31^{b}	93.72^{a}	100^{a}
Column 2 *TNAC*	-65.77^{d}	16.28^{a}	82.27^{a}	69.26^{ab}	75.19^{ab}	95.55^{a}	89.79^{b}
Column 3 *CANT*	-48.77^{c}	14.12^{ab}	79.90^{a}	68.06^{ab}	67.97^{b}	100^{a}	100^{a}
Column 4 *NCTA*	-33.66^{a}	16.67^{a}	84.18^{a}	71.48^{a}	80.54^{a}	100^{a}	100^{a}
Control	-42.65^{bc}	12.94^{b}	54.80^{b}	51.93^{c}	67.81^{b}	55.35^{b}	73.96^{c}

Means followed by the same letter within the same column are not significantly different at $P \leq 0.05$.
(-) = increase. A = *Azolla* sp., T = *Typha* sp., C = *Ceratophyllum* sp., N = *Nymphaea* spp.

Temperature

There was no significant difference in the levels of temperature obtained in various sampling among different columns (P = 0.079). The temperature ranged from 20.5°C to 25.5°C (Figure 3f). The temperature recorded was dependent on ambient temperature.

Phosphates

All macrophytes columns reduced the levels of phosphates in the wastewater effluent (Table 1). Columns 3 and 4 had a removal efficiency of 100%. Column 1 condensed phosphates by 93.72%, while column 2 had a removal efficiency of 95.55%. The reduction of phosphates may be ascribed to the uptake by plants for their growth and development. Most aquatic macrophytes can assimilate phosphates directly through their thalli, shoots, and leaves. Also, periphyton and microorganisms associated with macrophytes can remove nutrients instantly from the wastewater (Reddy *et al.,* 1987). In control, phosphates removal may be as a result of algae uptake (Basilico *et al.,* 2015).

Nitrates

There was a general reduction of the levels of nitrates by all macrophytes columns (Table 1). Columns 1, 3, and 4 had a removal efficiency of 100%. Column 2 reduced the nitrates by 89.79%. There was a significant difference in the reduction of nitrates among the macrophytes columns. The removal efficiency of column two was significantly different from the others (P = 0.00). Nitrate and nitrite in the constructed wetland system were removed by plant uptake or denitrification (Basilico *et al.,* 2015). Denitrification may also have accounted for the nitrates removal in control (Vymazal, 2013).

Total coliforms, Feacal coliforms, and Feacal streptococcus

There was no significant difference in reducing feacal coliforms and fecal streptococcus among the macrophyte columns (P = 0.948 and p = 0.973), respectively (Table 2). The reduction efficiency of the total coliforms varied from to 68.41% to 73.74% (Table 2). Generally, bacteria decreased in number at each subsequent sampling. There was a 100 % removal efficiency of fecal coliforms and streptococcus in all macrophyte columns and the control. The results were in harmony with the findings of Dar *et al.* (2011) and Aisien *et al.* (2015), 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, physico-chemical processes that all reduce bacteria populations. The removal efficiency depends on wastewater and plant species (Ottová *et al*., 1997).

Table 2. Reduction efficiency of coliforms by four macrophytes in multistage technique

Columns	Total coliforms	Feacal coliforms	Feacal streptococcus
	% Reduction		
Column 1 *ATCN*	68.41^a	100^a	100^a
Column 2 *TNAC*	77.82^a	100^a	100^a
Column 3 *CANT*	71.33^a	100^a	100^a
Column 4 *NCTA*	73.74^a	100^a	100^a
Control	72.15^a	100^a	100^a

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.

Heavy metals

Cadmium

All macrophytes columns reduced the levels of cadmium in the wastewater effluents (Table 3). Columns 3 and 4 had the most effective elimination efficiency 100%, while column 1 had the lowest removal efficiency of 83.40%. Column 2 had a removal efficiency of 91.44%. The slower removal of Cadmium compared to the other metals present in the wastewater effluent may result to its toxicity (Verbruggen *et al.*, 2009). Most plants do not have mechanisms to absorb non-essential elements such as cadmium, and uptake is through Ca^{2+}, Fe^{2+}, $Mn^{2+,}$ and Zn^{2+} transporters when plants absorb essential trace elements (Marchard *et al.*, 2010). Hence, Cadmium being non-essential to plants, maybe excluded during absorption, especially in other important metals (Verbruggen *et al.*, 2009).

Copper

Column 4 reduced to Copper by 81.98%, while column 1 had a removal efficiency of 88.60% (Table 3). Column 2 reduced copper by 86.40%, and column 3 had a removal efficiency of 83.39%. The slower removal of Copper compared to the other metals may be ascribed to the high initial concentrations of Copper compared to the rest of the heavy metals (Keith *et al.*, 2006).

Iron

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

Table 3. Reduction efficiency of toxic elements by four macrophytes in multistage technique

	Heavy metals					
	Cadmium		**Copper**		**Iron**	
Columns	Mean ± SE	% E_r	Mean ± SE	% E_r	Mean ± SE	% E_r
Column 1	0.040 ± 0.00^a	83.40	0.588 ± 0.00^a	88.60	2.837 ± 0.00^a	100
Column 2	0.048 ± 0.00^a	91.44	0.563 ± 0.00^a	86.40	2.789 ± 0.00^a	98.24
Column 3	0.053 ± 0.00^a	100	0.543 ± 0.00^a	83.39	2.716 ± 0.00^a	95.77
Column 4	0.053 ± 0.00^a	100	0.534 ± 0.00^a	81.98	2.837 ± 0.00^a	100
Control	0.008 ± 0.00^b	14.37	0.127 ± 0.00^b	19.52	0.504 ± 0.00^b	17.61

% Er = % reduction efficiency

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

Lead, Manganese, Zinc, Nickel, and Cobalt

All macrophyte columns were efficient in removing cobalt, nickel, lead, manganese, and zinc as they all attained a removal efficiency of 100% (Table 4 & 5). There was no discernible change in removing these metals among the macrophyte columns, but there was a considerable difference between the macrophytes and the control. Some of these heavy metals such as nickel, zinc, iron, manganese, cobalt, and Copper are required for plant growth in physiological quantities as they perform essential functions in living organisms. They are essential in maintaining the appropriate structure and operation of enzymes and making up metal-organic compounds such as metalloproteins (Nyquist *et al*., 2007). However, they are toxic in greater concentrations, limiting root growth and damaging root cells. In addition, they change the permeability of cell membranes and slow down the transport of electrons in the photosynthesis process (Krems *et al*., 2013). A moderate reduction in all heavy metals in control is due to microorganisms and their biological activities.

Table 4. Reduction efficiency of potential toxic elements by four macrophytes in multistage technique

	Heavy metals					
	Lead		**Manganese**		**Zinc**	
Columns	Mean ± SE	% E_r	Mean ± SE	% E_r	Mean ± SE	% E_r
Column 1	0.139 ± 0.00^a	100	1.128 ± 0.00^a	100	0.421 ± 0.00^a	100
Column 2	0.139 ± 0.00^a	100	1.128 ± 0.00^a	100	0.421 ± 0.00^a	100
Column 3	0.139 ± 0.00^a	100	1.128 ± 0.00^a	100	0.421 ± 0.00^a	100
Column 4	0.139 ± 0.00^a	100	1.128 ± 0.00^a	100	0.421 ± 0.00^a	100
Control	0.029 ± 0.00^b	26.69	0.239 ± 0.01^b	21.21	0.070 ± 0.00^b	16.69

% Er = % reduction efficiency

Means in the same column that are preceded by the same letter are not statistically different P ≤ 0.05.

Table 5. Reduction efficiency of toxic elements by four macrophytes in multistage technique

	Heavy metals			
	Nickel		**Cobalt**	
Columns	Mean ± SE	% E_r	Mean ± SE	% E_r
Column 1	0.039 ± 0.00^a	100	0.272 ± 0.00^a	100
Column 2	0.038 ± 0.00^a	100	0.272 ± 0.00^a	100
Column 3	0.039 ± 0.00^a	100	0.272 ± 0.00^a	100
Column 4	0.038 ± 0.00^a	100	0.272 ± 0.00^a	100
Control	0.012 ± 0.00^b	30.73	0.088 ± 0.00^b	32.61

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 (Ingole and Bhole 2003; Keith *et al.*, 2006). The initial concentration of heavy metals in the wastewater was low, especially for cobalt, nickel, manganese, zinc, and lead. This could have contributed to their high removal efficiency of 100%. The initial concentration of Copper was higher compared to the other metals. This could have contributed to its lower removal efficiency. Heavy metal buildup is also affected by the kind of heavy metal (Vahdatiraad and Khara 2012; Ikpesu & Ariyo, 2021). The accumulation of the eight heavy metals was different among the plants. The essential heavy metals were removed at a higher percentage than the non-essential and toxic heavy metals.

For example, zinc, Copper, cobalt, and manganese are used to yield the enzyme required to synthesize other physiologically active particles. Apart from copper, these metals had a removal effectiveness of 100 percent. Even at low concentrations, some heavy metals, like as cadmium, can produce oxidative stress, which inhibits chlorophyll production and promotes lipid pre-oxidation, resulting in membrane damage. (Harguinteguy *et al.*, 2013). According to Aravind *et al.* (2005), the existence of metal-metal interactions can influence the accumulation of one metal in the occurrence of another. For example, supplementation of PTE zinc in growth media comprising cadmium resulted in a decrease in cadmium accumulation in *Ceratophyllum demersum.*

There was a difference in removal potential for different PTEs within a particular macrophyte column. Marbaniangand (2014) The additional metal concentrations in the medium have been found to be one of the most important factors on individual heavy metal absorption.

The variance might also be related to environmental variables such as chemical speciation, initial heavy metal concentrations, redox potential, and the interaction of distinct heavy metals with one another, all of which generally control phytoremediation capability. According to Rofkar *et al.,* (2014), The inclusion of silicon and copper reduced arsenic buildup by Azolla caroliniana.

The buildup of heavy metals varies according to the kind of plant species and the capability of either their roots or shoots to concentrate the contaminants (Hazra *et al.,* 2015). The presence of microbial symbionts such as rhizosphere bacteria and fungi can affect the gathering of metals in plants (Lin *et al.,* 2010). The root-colonizing bacteria and mycorrhizal fungi present in the root area increase metals' bioavailability (Khan *et al.,* 2016). Plants support transformations of pollutants mediated by microbes by supplying fixed-carbon as a foundation of energy for bacteria and by changing the chemical environment in their root zone (Lin *et al.,* 2010).

Contaminants can be reversed in plants by varying biophysico-chemical processes such as sorption, transportation and transformation, hyperaccumulation, and mineralization. The biosorption processes of metals by plant cells can be either through surface sorption or extra and intracellular accumulation. Sedimentation processes, induced changes in biogeochemical cycles by plants and bacteria combined with oxidation and precipitation of metals as insoluble salts effectively remove metals from the medium (Bednar *et al.,* 2013).

The composition of macrophytes in column two performed better in removing pollutants from wastewater than in the other columns. This column had *T. latifolia* in the first stage. This is an emergent plant, and hence its efficiency could not be affected by turbidity. It had *C. dermersum,* a submerged plant in the last stage, mitigated the outcome of turbidity on this plant which could have compromised its performance by hindering light penetration.

Combining different species of plants in multistage techniques may improve tolerance to altering 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. The multistage technique can be employed in developing high-efficient nutrient phytoremediation systems. An ecosystem rich and diverse with plant species would be expected to display a broader range of functional traits with increasing opportunities for more efficient resource use due to variation in survival characteristics. Effective resource usage boosts productivity, which leads to improved performance in decreasing pollutants in artificial 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).

Multistage technology has a great potential in becoming the root principle of sustainable development, which promotes activities that do not adversely affect the environment. The wastewater treatment with this method also allows for the creation or restoration of wetlands. Wetlands for environmental enhancement and serve other ancillary functions such as sequestration of CO_2 hence mitigating climate change. The multistage phytoremediation technique offers a considerable opportunity to reduce wastewater treatment costs.

4. CONCLUSION

The multistage phytoremediation technique was noted to be effective in phytoremediation. This technique was noted to be fast in removing pollutants and achieving higher removal efficiencies. Column two comprised of Typha latifolia in stage 1, Nymphaea sp. in stage 2, Azolla pinnata in stage 3 and Ceratophyllum demersum in stage 4 were more efficient in phytoremediation of wastewater compared to the other columns. The order of reduction efficiency was column 2 > column 4 > column 3 > column 1. This technique is a green expertise that is efficient and economical hence recommended for wastewater treatment.

Acknowledgement

My appreciation goes to the University of Eldoret for providing laboratory space and facilities.

Funding

This study has not received any external funding.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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