The Effects of Native Plant Species Vegetated in Constructed Wetland on Removal of Selected Pollutants in Water of Bomachoge Sub County in Kisii County-Kenya

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Abstract

The relationship between water quality and health should be appreciated by engineers and researchers so that water resources users can be sensitive to water contamination and associated health impacts. It's against this background that this study was carried out to establish the quality of water before and after passing through vegetated constructed wetlands. This is presumably a less costly method of naturally reducing pollutant load in water sources. This study evaluated the levels of microbial and chemical parameters of water in surface flow through constructed wetlands vegetated with *Colocasia esculenta*, *Cyperus esculentus* and their combination to determine the efficacy of plant species in removal of these parameters. The study was conducted from August 2015 to February 2016 at Bomachoge Sub County of Kisii County, Kenya. Measurements and laboratory examinations of water samples was carefully done and results revealed that there was significant ($p<0.005$) removal of all selected parameters, whereby *Colocasia esculenta* was more effective than *Cyperus esculentus* and their mixture. *C. esculenta* (A), *C.esculentus* (B) and their combination (AB) removed selected bacteriological parameters such as faecal coliforms by (A 98%, B 16%, AB 36%), faecal *Streptococcus* (A 100%, B 13%, AB 15%), total coliforms (A 98%, B 18%, AB 26%) and *Escherechia coli* (A 97%, B 11%, AB 34%) and physico-chemical parameters such as, dissolved oxygen increased by (A-244%, B-318%, AB -107%) conductivity removed by (A 65%, B 41%, AB -28%), phosphates (A 95%, B -20%, AB 37%), nitrates (A 86%, B -38%, AB 40%) and turbidity (A 65%, B 67% AB -19%) from the result DO increased significantly. These results show that *C. esculenta* performance was higher compared to *C.esculentus* and the mixture of both plants, however, the combination of two plant species performed better than *C. esculentus*. Statistica statistical program was used to compare the overall, among and within samples. From the findings it shows that both plant species significantly removed pollutants from water. It was recommended that *C.esculenta* and *C.esculentus* plant species to be conserved and more studies to be done on other native species in other places of kisii county to ascertain their efficacy in water purification.

Keywords: Faecal, Contamination, Anova, Parameters, Coliforms, Wetland, Bacteriological.

1. Introduction

Constructed wetlands are used around the world to treat domestic water, agricultural, and industrial wastewater, storm water runoff, and other water contaminants. They are designed either as free water surface wetlands with standing water or as subsurface flow wetlands with water below the soil or on the surface (Kadlec and Knight, 1996).

Polluted water flows with regulated velocity either horizontally or vertically through the established plants in wetland. Pollution level of water in constructed wetlands is determined by examination of physico-chemical and bacteriological parameters (Kadlec and Knight 1996). Constructed wetlands are designed to increase the predictability and efficiency of treatment, enabling use of wetlands that are less land intensive (Engelhardt and Ritchie, 2001).

Use of native plant species for water purification in artificial wetlands is generally favoured since native plants require less maintenance and pose few environmental and human risks than genetically modified species or exotic species. Well selected native plant species are tolerant to local climatic conditions, soils and seasonal changes (Shutes, 2001). The plant species *Colocasia esculenta* is commonly known as 'Cocoyam',it is a wetland herbaceous perennial, found in the tropics and much of sub tropics (Bindu *et al.,* 2008). *C. esculenta* have a good growth rate and they spread very fast over water masses and colonize marshy land areas (Kurien and Ramasany, 2006). *C. esculenta* is a rooted emergent type of weed and spreads all over the marshy places, on the banks of main streams, canals, ponds and any other area with favourable conditions (Kurien and Ramasany, 2006). Their thick vegetation causes no harm to water bodies in which they colonize.

When *C. esculenta* spreads on surrounding land areas where soil moisture is high especially during rain seasons they develop into thick bushes harboring undesirable organisms or become breeding ground for vectors (Bindu and Ramasany, 2005). *C. esculenta* like other weeds have excellent growth potential and high productivity which can be utilized meaningfully (Kurien and Ramasany, 2006). Researchers have been trying to find out ways of utilizing *C. esculenta,* so that the cost eliminating it mechanically or uprooting it as a weed can be fully recovered from its benefits, the efforts include use as a source of compost and energy (Bindu and Ramasany, 2005). As part of these more research has been done on other macrophytes ranging from duckweeds, water hyacinth, cattails, reeds and sedges like *C.esculentus* as wetland plants to check pollution and ecosystem dynamics (Vaillant *et al*., 2003).

The plant species *Cyperus esculentus* belongs to a family of monocotyledonous graminoid flowering plants known as sedges. *C. esculentus* provide edible tubers commonly called tigernut, nut grass or earth almond. *C. esculentus* is a perennial crop cultivated particularly in tropics and subtropical areas worldwide and extensively in Africa, Asia and other European countries for their sweet tuber. An ecosystem with greater plant richness would be expected to display a wider range of functional traits, with increasing opportunities for more efficient resource use due to the variation in survival characteristics (Bouchard *et al.,* 2007). Effective resource use enhances productivity, resulting to effective performance in reducing pollutant load in wetlands.

According to Engelhardt and Ritchie, (2001) environmental heterogeneity promotes complementary effects between species as evidenced by effects of species richness on pollutant removal that come from surrounding terrestrial area (Bouchard *et al.,* 2007). In nutrient rich ecosystem, strong competition for space and other scarce resources synergetic effects are likely to occur (Engelhardt and Ritchie, 2001). The performance of combined species in constructed wetlands and the link between species richness and ecosystem functioning is a major question in ecology (Bouchard *et al*., 2007). Plant *C. esculentus* effectively support food webs by recycling nutrients, as they manufacture and absorb some from the environment (Bouchard *et al*., 2007). Generally, wetlands are currently degraded by both natural and anthropogenic activities, which deteriorate their quality, and push them to extinction in the process of poorly planned development, giving rise to the need for suitable conservation strategies. The objectives of carrying out physico-chemical and bacteriological analysis of water bodies is achieve the natural ecological balance and safe aquatic life. This is the integral part in wetland evaluation (Taylor *et al.,* 2002)

It is therefore important that the relationship between water quality and health be fully appreciated by engineers and researchers so that water resources users can be sensitive to water contamination and associated health impacts. It's against this background that this study was carried out to ascertain the quality of water in wells and springs, and also asses the cost effective methods of naturally reducing pollutant load in water at the source using local resources like plant species and sand.

2. Materials and methods

2.1 Study area.

The study was set up at Magenche area in Bomachoge Sub-county of Kisii County. The sub-county has a population of about 107, 200. In terms of topography, the Sub County is mainly hilly with several ridges, with excellent drainage system. The sub-county has a highland equatorial climate with annual average rainfall of 1,500mm which is reliable (KCDDP, 2008)

2.2 The design of the reservoir, two constructed vegetated wetlands and control

The wetlands were established in an identified location which was adjacent to the existing natural spring and communal reservoir. These are the main water sources for watering animals and domestic uses. The vegetated constructed wetlands were located at Maroba in Magenche area. This was an experimental site and the set up was designed in such a way that it did not affect the existing natural spring and native plants in the surrounding ecosystem. The samples were collected and analyzed following procedure described by APHA (1998). The experimental reservoir was constructed for the purpose of storing and supplying water to constructed wetlands in experimental area; the flow rate of water to constructed wetlands was reduced by the backward slant delivery pipes. Young shoots of native plants *Colocasia esculenta* and *Cyperus esculentus* were collected from the surrounding natural wetland. And the shoots were planted in two constructed wetlands at a density of 20shoots/m^2, the third wetland was vegetated with a combination of *Colocasia esculenta* and *Cyperus esculentu*s plant species with a density of 20 shoots/m^2. The contaminated water from communal reservoir was put in the experimental reservoir which feed the constructed wetlands through the polyvinylchloride pipes, with loading rate of 30Lm^{-2}/day^{-1}. Plant shoots were allowed to establish in the constructed wetlands for 2months to make the vegetation have a proper biological transport system, prior to the start of experiment. Water was allowed to flow constantly into each wetland for 7 consecutive months. The wetlands were constructed in such a way that their floors slanted backwards to reduce velocity of influent water so that plant are able to maximally absorb the contents.

2.3 Laboratory examination of water samples

Test for faecal coliforms

Testing for faecal coliforms in water samples was done using modified membrane filtration method as described in (APHA, 1998). Apparatus to be used are dilution bottles, pipettes, graduated measuring cylinder, Petri dishes(60 x 15,,) membrane filters (0.45mm), filtration unit, absorbent pads, forceps, spirit lamps and incubators. Laboratory testing of faecal coliforms, sample water will be measured into a measuring cylinder. A small amount of dilution water was added to the funnel before filtration is done to aid in uniform dispersion of bacteria suspension over entire effective filtration surface. Sterile membrane filter paper was placed over porous plate using sterile forceps to be sterilized by flaming. The grid side of the filter membrane was placed to face-up. The funnel unit was carefully matched over the receptacle and locked in place.

The sample of the test water was passed through filter membrane under partial vacuum, 30-50ml sterile buffered water was used to rinse the filter between the samples. The funnel was unlocked after all the water was filtered and the forceps were used to remove the filter membrane which was placed on sterile agar with a rotating motion to avoid entrapment of air. The liquid medium was used and the culture dish will be saturated with 1.8-2.0ml of prepared M-ENDO medium.The agar was placed directly on the Petri-dish then incubated for 22 to 24 hours at 37^{0}C ± 0.5 in incubator, after incubation, the number of bacteria colonies were counted and expressed as colonies in 100ml of sample water.

Test for faecal streptococcus.

This was done by using Bile Esculine Azide Agar, a selective media for isolating faecal streptococcus from water samples. One militer of each tube containing different sample dilution was drawn into the Petri-dish using a sterile pipette and molten agar was poured into mixture and incubated at 37.0 ±0.5 ^{o}c for 48 hrs. Blackening colonies in the media denote the presence of fecal *Streptococcus* and fail of the media to form black colonies indicates negative results (APHA, 1998).

Enumeration of Total coliforms and *E.coli*.

Colilert -18 test procedure.

Colilert-18 detects total coliforms and E.coli in water; it is based on patented defined substrate technology (DST). When total coliforms metabolizes colilert-18 nutrient indicator ONPG, The sample turns yellow. When *E.coli* metabolize colilert-18 nutrient indicator MUG, the sample fluoresces. Colilert-18 simultaneously detects total coliforms and *E.coli* within 18 hours.

Contents of 100mls of water sample was added in sterile water container, container was tightly sealed and shaken until it dissolved, reagent mixture was poured into quanti tray/2000 and tray sealed, the sealed tray was placed in 37±0.5 ^{O}C for total coliforms. Yellow equal to or greater than the comparator when incubated at 37±0.5 ^{o}C indicates the presence of total coli forms and absence of yellow color is negative result. Yellow and fluorescence equal to or greater than the comparator when incubated at 37±0.5 ^{O}C indicates positive *E.coli* and absence of yellow and fluorescence indicate negative results. Fluorescence is checked with a 6-watt, 365-nm Uv light within 5inches of the sample in a dark environment, light should face a way from eyes and towards the sample.

Turbidity

Instrument used to determine nitrate levels in water samples was HACH colorimeter (DR/820).Turbidity was measured by following absorptometric method, where a stored program number for turbidity was entered, and when a program number was pressed the FAU unity for turbidity was displayed and zero icon. A sample of 10ml de-ionized water or blank was placed into cell holder and instrument cap tightly fixed. Zero FAU was displayed in the screen after reading it was removed. 10mls of sample water was fixed tightly into cell holder and read button key was pressed, reading was displayed in Formazin Attenuation Units (FAU) and the values were recorded.

Phosphorous (PO_4)

Amino acid method was used in measurement of phosphorous in water samples, HACH calorimeter (DR/820) was used in the measurements. Phosphorous is measured in mg/L. The stored program number for reactive phosphorous was pressed and mg/L, PO_4 and zero appeared in the screen, in a 25 ml sample one mls of Molybdate reagent was added using 1ml calibrated dropper and then mixed well and the sample was placed in cell holder and tightly covered with the instrument cap, after 10 minutes the read button was pressed, the result in mg/LPO_4 was displayed and reading recorded.

Nitrate (NO_3^--N).

Cadmium Reduction method was used in measurement of nitrate levels in collected water samples using the HACH colorimeter (DR/820). Nitrates in water were measured in mg/L. The stored program number was entered, for high range nitrate nitrogen (NO_3^--N), program mg/L, NO_3—N was entered and zero icon was displayed. Contents of one Nitra Ver 5 Nitrate reagent powder pillow were added to prepared sample of 10 mls, mixture was mixed well and the sample was sealed with the cap of the cell and one minute reaction time was allowed. Read button pressed and the result in mg/LNO^3-N was displayed

Measurement of dissolved Oxygen in samples

Dissolved Oxygen its normally expressed in concentration, that is the amount in weight of Oxygen in a given volume of water sample, in this study DO was measured using a HANNA DO meter (H1 9143).To reduce errors which may affect Oxygen levels during transportation from the field, the initial measurement of dissolved oxygen was done in the area where samples were collected. The machine calibrations were adjusted to read or display 100% active air concentration and the tip of the probe was immersed into the sample in a container and the machine allowed to stabilize before obtaining the actual level of Oxygen in parts per million (ppm) which is the same as mg/l.

Determination of conductivity

This is the measure of how well water can pass an electric current. It is an indirect measure of the presence of inorganic dissolved solids such as nitrates, phosphates, sodium, magnesium, calcium, iron and many others. The presences of these substances increase conductivity in water body. In the current study conductivity of water samples were measured in the field, where samples were drawn using JENWAY 3405 Electrochemical analyzer. Conductivity was measured in μs/cm

Water temperature is a controlling factor for aquatic life. In this study temperature was measured using a HACH thermometer. Temperature measurements were done in the study area and readings recorded in Celsius (^{O}C). All readings were recorded.

Data analysis

Since the data was in counts, scores transformation was necessary. Logarithmic transformation was made to allow use of completely randomized design. The design was used to compare the overall, among and within studied parameters. Data from coded, scored and analyzed using SPSS, statistical programs, one way ANOVA after appropriate transformations was used. Relationships from measurements were analyzed using simple and multiple regressions.

3. Results

Effects of *Colocasia esculenta* and *Cyperus esculentus* plant species in constructed wetlands on water quality improvement**.**

Experiment started after establishment of selected plant shoots.

Transformation of the dependent variables was done using Box-Cox transformation methods. Comparison was done using one-way ANOVA. Negative percentage implies an increase while a positive percentage implies a decrease

Table 7: Average percentage reduction in the levels of biophysical pollutant load in water going through monoculture and polyculture plant species

Variable	Plant	Unfiltered Mean ± SD	Filtered Mean ± SD	% Reduction	F - value	P - value
TC	A	378.4 ± 38.9	7.6 ± 4.3	98%		
	B	371.6 ± 44.8	294.6 ± 75.6	18%	9.92	0.0003
	AB	319.0 ± 43.1	232.5 ± 45.1	26%		
FC	A	326.3 ± 37.0	6.3 ± 4.2	98%		
	B	296.1 ± 48.8	289.9 ± 60.7	16%	12.69	<0.0001
	AB	290.7 ± 35.7	184.1 ± 39.5	36%		
EC	A	251.9 ± 73.9	5.7 ± 3.7	97%		
	B	292.1 ± 46.1	315.1 ± 85.9	11%	58.77	<0.0001
	AB	230.5 ± 50.0	153.7 ± 57.1	34%		
FS	A	359.5 ± 37.7	1.7 ± 2.1	100%		
	B	342.3 ± 54.6	288.9 ± 63.0	13%	7.88	0.001
	AB	58.0 ± 23.3	49.3 ± 19.1	15%		

A – *Colocasia esculenta*; B – *Cyperus esculentus*. AB-Mixture of *Colocasia esculenta* and *Cyperus esculentus*.

Table 8: Average percentage reduction in the levels of chemical pollutant load in water going through monoculture and polyculture plant species

Variable	Plant	Unfiltered Mean ± SD	Filtered Mean ± SD	% Reduction	F - value	P - value
NO3	A	13.8 ± 3.1	1.8 ± 0.3	86%		
	B	43.7 ± 10.7	55.3 ± 15.4	-38%	1.87	0.166
	AB	50.4 ± 9.1	29.5 ± 11.7	40%		
PO4	A	19.7 ± 2.7	0.9 ± 0.5	95%		
	B	144.8 ± 29.2	168.3 ± 28.7	-20%	8.14	0.001
	AB	19.2 ± 4.4	11.6 ± 3.8	37%		
DO	A	4.1 ± 0.7	13.6 ± 3.2	-244%		
	B	3.7 ± 1.0	14.3 ± 3.4	-318%	7.85	0.001
	AB	32.7 ± 11.9	56.6 ± 12.6	-107%		
TURBIDITY	A	318.5 ± 59.6	107.3 ± 16.8	65%		
	B	321.7 ± 59.2	102.6 ± 25.8	67%	49.74	<0.0001
	AB	203.6 ± 54.0	157.9 ± 28.3	-19%		
CONDUCTIVITY	A	322.3 ± 29.7	111.9 ± 23.8	65%		
	B	317.0 ± 36.4	183.0 ± 57.3	41%	10.60	0.0002
	AB	211.7 ± 81.0	136.4 ± 17.4	-28%		

A – *Colocasia esculenta*; B – *Cyperus esculentus*. AB- A mixture *Colocasia esculenta* and *Cyperus esculentus*.

4. Discussion

The wetlands vegetated with *Colocasia esculenta* was more efficient in removal of selected water parameters than *Cyperus esculentus* and a combination of *Colocasia esculenta* and *Cyperus esculentus.* A combination of *Colocasia esculenta* and *Cyperus esculentus* were significantly (p<0.05) more efficient than *Cyperus esculentus* in removal of physico-chemical and bacteriological parameters from contaminated water. All parameters measured such as total coliforms, faecal coliforms, *E.coli,* faecal *Streptococcus*, nitrates, phosphates, turbidity, conductivity were significantly reduced except dissolved oxygen which increased significantly.

These results were also found to be within the range of removals reported on a constructed wetlands for wastewater treatment (Reinoso *et al.,* 2008). Both *Colocasia esculenta* and *Cyperus esculentus* plants recorded significant removal of parameter which was measured. However*, C.esculenta* plant species was more efficient with percentage removals of individual bacteria of about 98% (Table1). Whereas *C.esculentus* had ability to remove the bacteria strains of about 15% and their combination significantly removed bacteriological parameters of about 28% better than *Cyperus esculentus* alone (Table 1).

Generally, the vegetated constructed wetland was more efficient in the reduction of parameters. This can be associated with their additional aeration at the roots to enhance the degradation process by aerobic bacteria (Kadlec and Knight *et al*., 1996).

In this study the experimental plants reduced nitrates and phosphates significantly from water (Table 2). This may be due to the functions of plants in constructed wetlands which include absorption of nitrates, supplying organic carbon for denitrification and providing shelter for denitrifying bacteria. In addition, nitrate removal can be attributed to any or mechanisms of uptake by plants, volatilization of ammonia or bacterial nitrification/denitrification. This explains why bacterial processes have the most effect on the overall nitrogen removal (Weisner *et al*., 1994). Nitrosomonas and nitrobacter nitrify ammonia into nitrates which is made available for plant and microbial uptake. Nitrate is also converted into gaseous state by denitrifying bacteria (Weisner *et al*., 1994).

The mechanisms of phosphates adsorption, plant uptake and biotic assimilation might have reduced the phosphate level in the effluent (Watson *et al.*, 1998). The test for significant change in the pollutant load in the current study showed that both plant species as well as the combination of the two plant species resulted in a significant reduction in the pollutant load (P<0.05). The results also show that there was a significant increase in the level of dissolved oxygen for each of the plant species as well as the combined plant species. This confirms findings by Kadlec and Knight, (1996) that wetlands with vegetation rely on biological, chemical and physical processes in a natural environment to remove pollutants in water, they further found out that constructed wetlands differ from natural wetlands in that operators have greater control over natural processes in the constructed wetlands because flows are more stable. Whereas, natural wetlands are subject to the variability of precipitation probably this could be the reason why plants in constructed wetlands in this study reduced pollutant load significantly. Biological removal of pollutants is perhaps the most important pathway that reduced contaminants in this study, Literature also reveals that the most widely recognized biological process for contamination removal in wetlands is plant uptake (Reinoso *et al.,* 2008).

Comparison of the average change in the pollutant load between the plant species as well as the combination of the two plant species showed that both plant species as well as the combination of the two plant species performed differently. The test for difference in the mean pollutant levels using one-way ANOVA was statistically significant ($P<0.01$). These differences in reductions of pollutants observed in individual species (monoculture) and combined species (polyculture) are similar to those observed by Torrens et al. (2009) in both monocultural and polycultural systems.

5. Conclusion

Management and conservation of wetlands systems require a serious attention from all stalk holders so as to sustainably utilize wetlands today and in future without depletion. The long-term efficiency and sustainability of these wetland systems is critically dependent on integrated understanding of their biological, chemical and hydrological processes. Reduction of pollutants in contaminated water by native plants in wetlands is a great achievement in water purification systems and therefore the current study concludes that *Colocasia esculenta* and *Cyperus esculentus* plant species be conserved and be utilized for pollution assessments in water systems.

6. Recommendations

It was recommended that more research be done on other native plant species of Kisii County arrea to discover their potential in reducing pollution of water and other roles in the ecosystem.

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