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Editorial

Kenya Aquatica Vol. 5 continues to feature additional papers presented during Kenya Coastal Development Project (KCDP) Conference held at the Voi Lodge, Taita Taveta County in March, 2016. This is the final series of papers submitted by the key authors. The Editorial Board of Aquatica has compiled and published these papers with support from KMFRI, Pwani University and the Technical University of Mombasa.

This final Volume on the Conference contains papers on themes covering indigenous coastal knowledge, innovative application of environmental biotechnology, assessment of reverine harzadous waste, ecological modelling, sanitation and water quality.

The Editorial Board acknowledges the following reviewers: Ms. Damaris Mutia, Dr. Cosmas Munga, Mr. Stephen Mwangi, Ms Mary Ontomwa, Mr. Boaz Ohowa and Dr. Majambo Jarumani.

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Contents

Potential of Vetiver Grass (*Chrysopogon zizanioides*) in Phytoremediation of Kipevu Wastewaters

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ABSTRACT

Wastewater treatment worldwide has mainly been done using physical, chemical and biological treatment systems such as activated sludge and biological nutrient removal technologies. Mombasa produces large amounts of pollutant-laden effluent water that is collected and primarily treated and discharged into the Indian Ocean. Various pollutants such as nitrates, phosphates, zinc and cadmium in water bodies pose a threat to the aquatic species. The aim of this study was to explore the potential of vetiver grass as a phytoremediation technology to minimise pollutants in water bodies. Field experiments were carried out using plastic containers of five litres which were set-up simultaneously with planted grass hydroponically. Wastewater was characterized for physicochemical parameters before and after treatment with the grass. The parameters studied were: effects of vetiver on reduction of pollutants with time and effect of biomass (number of Vetiver plants) on treatment efficiency. Wastewater had high levels of Chemical Oxygen Demand (1440 mg/L), Biological Oxygen Demand (75 mg/L) nitrates (775 mg/L), phosphates (25 mg/L) and Total Dissolved Solids (1432 mg/L) , which were beyond the maximum contaminable levels. A drastic increase in the pollutants uptake with time was observed. The levels of nitrates, phosphates, chemical oxygen demand, biological oxygen demand and total dissolved oxygen reduced by 89.76%, 85.6%, 84.51%, 69.33% and 16.76% respectively over 28 days. During the same period, a direct correlation in the reduction of Chemical Oxygen Demand and nitrates with increased number of plants was observed. The potential of vetiver grass after four weeks hydroponic treatment was found to be efficient in the reduction of pH, nitrates, phosphates, total nitrogen, total phosphorous, total dissolved solids, chemical oxygen demand and biological oxygen demand. The study recommends that vetiver grass be introduced in the aeration tanks to improve the efficiency of Kipevu waste water treatment plant.

***Keywords**: Wastewater, Vetiver, hydroponic, phytoremediation.*

INTRODUCTION

Humans are inducing environmental changes in the planet as a whole in fact, the human fingerprint is extensively visible on the global atmosphere, the oceans and land (Miller, 2006). Clean water is increasingly becoming one of the most scarce and valuable resources in the twenty-first century. This is because its supply is finite and its traditional source is easily polluted by industries and population growth. The production and discharge of untreated wastewater is rapidly increasing in Kenya, most especially in Mombasa city, due to population growth, urbanization, and economic development. However, lack of investment capacity for construction and operation of adequate treatment facilities (Van Lier & Lettinga, 1999). Consequently, it threatens the quality of surface water, soils and groundwater to which wastewater is discharged. The treatment of these wastewater effluents has not been given due attention to date. One of the reasons for the lack of attention is the capacity and the cost associated with the construction and operation of wastewater treatment plants (Bedewi, 2010). Wastewater has serious negative impact not only on underground, surface water bodies and land in the surrounding area but also on the aquatic ecological system (Gebre-Mariam & Beshah, 2002). Treatment of wastewater has mainly been done using physical, chemical and biological wastewater treatment systems such as activated sludge and biological nutrient removal technologies. However, these technologies are expensive and depend on power source and skill hence conventional treatment technologies are either ineffective or wasteful and costly (Nhapi, 2004; Oron, 1994). Many studies on phyto-remediation technologies are on-going, of the many plant species that have been widely studied, Vetiver grass (*Vetiveria zizanioides*) has been identified to be of significant potential (Njau & Mlay, 2003; Liao et al., 2003).

Vetiver (*Chrysopogon zizanioides*) belongs to Order Poales and Family Poaceae (Mcmahon, 2014).The Vetiver system depends on the use of this unique tropical plant which can be grown over a very wide range of climatic and soil conditions. If planted correctly, it can be used virtually anywhere under tropical, semi-tropical, and Mediterranean climates. It has characteristics that in totality are unique to a single species. When Vetiver grass is grown in the form of a narrow self-sustaining hedgerow it exhibits special characteristics that are essential to many of the different applications that comprise the Vetiver system. It has unique morphological and physiological characteristics. Vetiver has been successfully used in the field of environmental protection. It is excellent for the removal of heavy metals from contaminated soil (Roongtanakiat & Chairoj, 2001) and rehabilitating landfills (Roongtanakiat *et al.*, 2003). Even though it is not an aquatic plant, Vetiver can be established and survive under hydroponic conditions. It can purify eutrophic water, garbage leachates and wastewater from pig farms (Kong et al., 2003). Therefore, Vetiver has high potential to be used for wastewater treatment.

MATERIALS AND METHODS

The main experimental materials used were Vetiver plants, and municipal wastewater collected from the aeration tanks of Kipevu Waste Water Treatment Plant (WWTP). The vetiver plant tillers were sourced from Voi in Taita-Taveta County.

The research was conducted at Kipevu WWTP between August 2015 and January 2016. The plant is located at Changamwe Sub County in Mombasa County. The study area is described by latitude 4°03'16''S, longitude 39°39'48''E.

The sampling of wastewater in the treatment plant was done just before the water enters the aeration tanks. 90 litres and 200 litres of waste water were collected for the first and second set of the experiment respectively. Five litres of effluent waste water were poured into each of the 12 plastic containers of five litre capacity. Three sampling bottles (500 mL each) were also filled with untreated wastewater for characterization. The effect of treatment time on water quality was studied by planting hydroponically two plants in a 5 L volume of wastewater per treatment. The three treatments were replicated thrice and 500 mL volume sampled for chemical analysis at 7, 14, 21 and 28

Fig. 1: Treatment time experiment

days (figure 1). The experiments were carried out between August and September 2015.

Variation of biomass was studied by planting hydroponically four (4), eight (8) and twelve (12) plants in a 5 L volume of wastewater (figure 2). The three treatments were replicated thrice and sampled for physico-chemical analysis. The experiment was carried out between December and January 2016.For each treatment of biomass there were twelve observations making a total of thirty six observations 7, 14, 21 and 28 days.

Fig.2: Biomass experiment

The influent and effluent were sampled at seven-day intervals and analyzed for Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Nitrates, Phosphates Total Nitrogen (TN), Total Phosphorus (TP) and Total Dissolved Solids (TDS) according to standard methods for water and wastewater analysis (Eaton, 2005). The collected data was analysed using one way analysis of variance (ANOVA) to test for the significant difference between effluent wastewater pollutant levels of the different treatments.

RESULTS AND DISCUSSIONS

The results of physicochemical analysis of Kipevu raw wastewater are shown in Table 1. The values were compared to the maximum permissible limits of effluent waste water set by NEMA (EMCA, 2015).

The pH, Zinc, Cadmium and EC values of the wastewater met NEMA effluent standards. Other heavy metals were below detectable levels. The low concentration of heavy metals could be as a result of the source of the wastewater at that period of sampling. The concentration of pollutants varies from time to time because the plant receives waste water from different sources thus close monitoring of these pollutants is required. However, the average concentration levels of BOD_5 and COD were above NEMA Maximum Contamination Level (MCL) limits by 60% and 2880% respectively.

Table 1: Physico-chemical parameters of Kipevu Wastewater

Parameter	Units	Value	NEMA MCL
pH	pH units	7.81	6.5-9.0
EC	µS/cm	1573	2000
BOD_5	mg/L	75	30
COD	mg/L	1440	50
TDS	mg/L	1432	1200
TN	mg/L	138	-
TP	mg/L	13.45	-
Nitrates	mg/L	775	45
Phosphates	mg/L	25	5
Ammonia	mg/L	1.22	100
Cadmium	mg/L	0.007	0.1
Zinc	mg/L	0.0216	5

The high BOD_5 and COD may be due high concentration of organic matter present in the wastewater released from different sources of waste water in the treatment plant. The high level of BOD_5 indicates the pollution strength of the wastewaters and low oxygen availability for living organisms in the wastewater when utilizing the organic matter present in the wastewater. High COD level implied that there was high oxidizable wastes that require very high dissolved oxygen. The COD: BOD_5 ratio is an important indicator of the biodegradability of the pollutants in wastewater. The results showed a ratio of 19.2 (i.e. 1440/75=19.2). If the ratio is less than two (<2), the load is considered easily biodegradable ((Rehm et al., 1999). Therefore, in this study the ratio of COD to BOD_5 is »2 hence the pollutant load was not easily biodegradable.

The study found out that the wastewater at the Kipevu plant contained 775 mg/L nitrates and 25 mg/L phosphates. These were above the minimum NEMA contaminable limits of 45 mg/L and 25 mg/L. The high concentration of nitrates may be due to the discharge of animal and human wastes with decaying organic matter. High phosphates levels in the wastewater could possibly be the result of soaps being used in washing places, restaurants, industries and showers which drains into the treatment plant.

In general, the results of this analysis indicated that high level of contamination of the wastewater with both organic and inorganic pollutants. The concentration of BOD_5, COD, Nitrates, Phosphates and EC were found to be beyond the maximum level of their respective permissible values set by NEMA. Thus, this wastewater requires treatment before discharging into the environment.

The study found that pH reduced by 0.77% in the first week, 1.42% in the second week, 2.09% in three weeks and 3.44% in four weeks. A 72% reduction was realised in the entire four weeks. The pH values of Vetiver sets were slightly lower than the control set in all treatment time (figure 3).

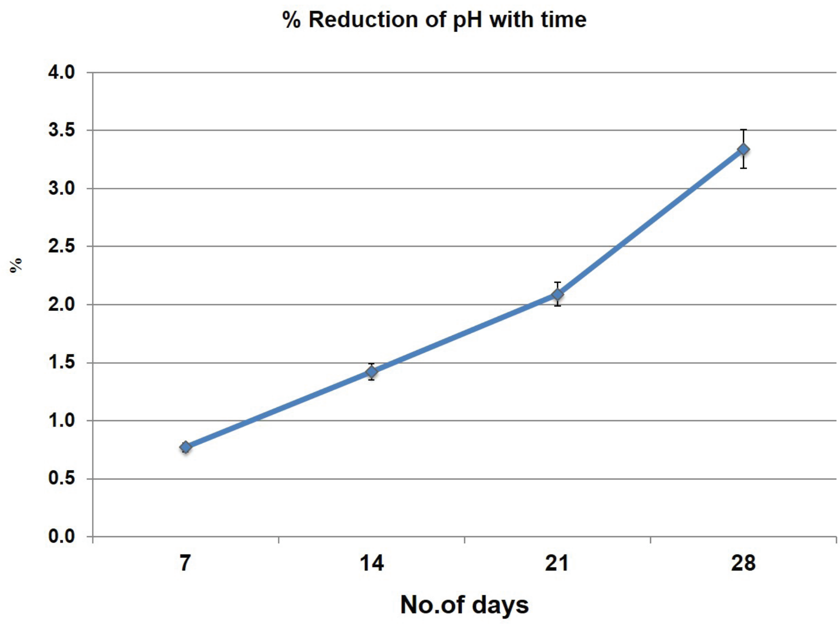

Fig.3: Percentage reduction of pH with time

The analysis of variance on the net reduction amounts coming from Vetiver and control treatment during the entire experiment period on pH was statistically significant (p = 0.027).

Vetiver treatment resulted in lower pH value (7.23) compared to that obtained under the control treatment (7.81). From these finding, it is clearly evident that higher organic matter decomposition rate resulting in CO_2 and acid production lowered the pH values of the wastewater under Vetiver treatment (Bedewi, 2010).

COD concentrations were also reduced from 1440±1 mg/L at the establishment of experiment to 38.19% in one week, 27.53% in two weeks, 28.22% in three weeks and 51.84% in four weeks. Cumulatively percentage removal was 84.51% in four weeks treatment. BOD_5 reduced from 75±1 to 14.76% in one week, 25.56% in two weeks, 34.04% in three weeks and 38.71%. 74.67% was the cumulative percentage reduction. Moreover, during the entire time series analysis it could be depicted that the concentration of BOD_5 and COD of experimental sets planted with Vetiver were lower than the control set (Figure 4).

Vetiver plants support the BOD_5 and COD level reduction processes by availing atmospheric oxygen in their submerged stems, roots and tubers, which is then utilized by the microbial decomposers attached to them below the level of the water to digest the organic matter in wastewater. The analysis of variance showed that the effect of Vetiver on reduction of BOD_5 concentration was statistically significant (p=0.007) over control (without vetiver) treatment. The effect of Vetiver treatment on the reduction in BOD_5 concentration (19 mg/L) was statistically significant over control (75 mg/L) treatment. The analysis of variance also showed effectiveness of Vetiver in COD reduction was statistically significant (p=0.006).

Treating wastewater with Vetiver resulted in a statistically significant reduction in COD concentration (1440mg/L-54mg/L). From this result, it is distinctly clear that the effectiveness of wastewater treating potential of Vetiver in terms of organic

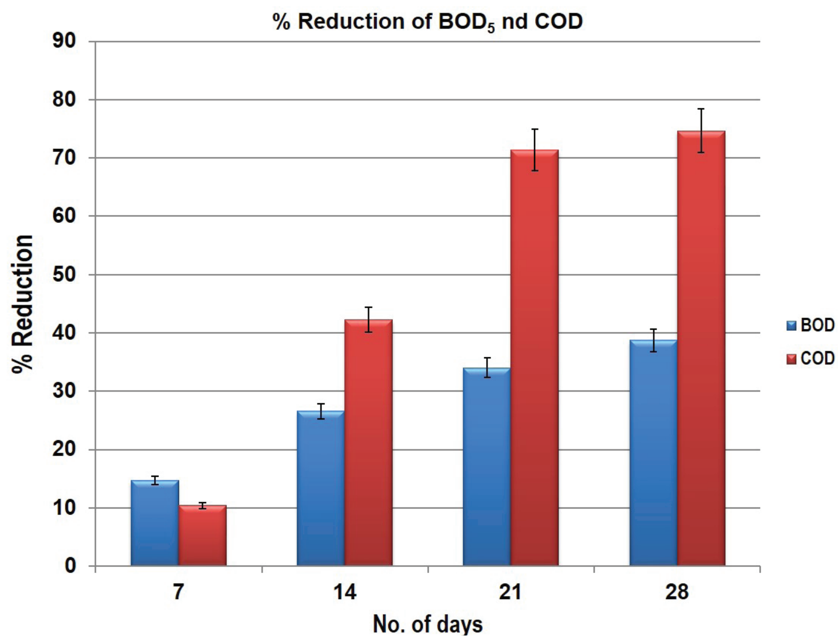

Fig.4: Percentage reduction of COD and BOD_5 with time

matter removal (BOD_5 and COD) as compared to control. BOD_5 and COD results obtained in the present study was higher than the removal results obtained in the field from piggery wastewater had reported 35.8% BOD_5 as well as 64% COD removal after four weeks treatment (Liao et al, 2003). The efficiency of organic matter removal depends on the strength of wastewater and the treatment time (Ronnachai et al., 2007). Therefore, the pragmatic incongruity in the organic matter removal efficiency with different literatures might be due to difference in concentration of wastewater, treatment time and method of Vetiver application.

On average, the effectiveness of Vetiver for pollutant treatment in the present experiment was not so distinct in the first few weeks'. This was probably due to the relatively smaller biomass and the relatively poorer adaptation to the wastewater environment. From these results, it is seen that Vetiver has great potential in treating waste water. After four weeks hydroponic treatment, the BOD5, and COD met the maximum discharge limit set by NEMA.

The results clearly show that the uptake of concentrations of TDS and EC increased with increase in quantity of biomass. TDS percentage reduction was for 76%, 83.1% and 89.3%, 4 plants, 8 plants and 12 plants respectively for duration of four weeks while that of EC 60.8%, 72.7% and 86.4% for 4 plants, 8 plants and 12 plants respectively for twenty eight days (figure 5). The electrical conductivity values of Vetiver sets were lower than the

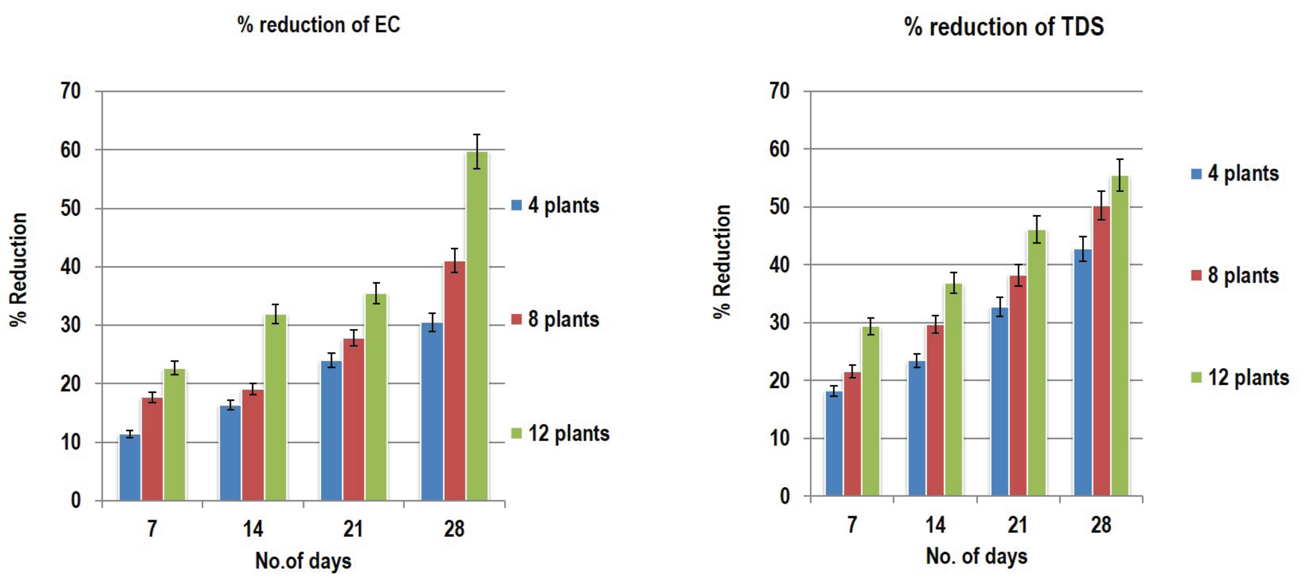

Fig.5: Percentage reductions of EC and TDS with biomass variation

control sets in all hydroponic treatment because Vetiver absorbed most of the ions responsible for conductivity. The analysis of variance shows that the effectiveness of Vetiver in removal of TDS were statistically significant for 4 plants treatment ($p=0.018$), 8 plants treatment ($p=0.002$) and 12 plants treatment ($p=0.005$) hence indicating the potential use of Vetiver grass in the treatment of effluent wastewater. Reduction of total dissolved solids in this study might be due to the variation of wastewater concentration, configuration of hydroponic vetiver set-up (container as growing medium), temperature, treatment time, quantity of vetiver applied and hydraulic retention time (HRT) used in this study could be another factor (Bedewi, 2010).

Generally, there was reduction of both nitrates and TN in all treatments. Nitrates reduced by 91.2%, 94.9% and 97.2% for 4 plants, 8 plants and 12 plants respectively in four weeks treatment while TN concentrations reduced by 70%, 79.2% and 90.1% for 4 plants, 8 plants and 12 plants respectively in four weeks of treatment (figure 6). The highest reduction of nitrates and TN was evident in the 12 plants treatment.

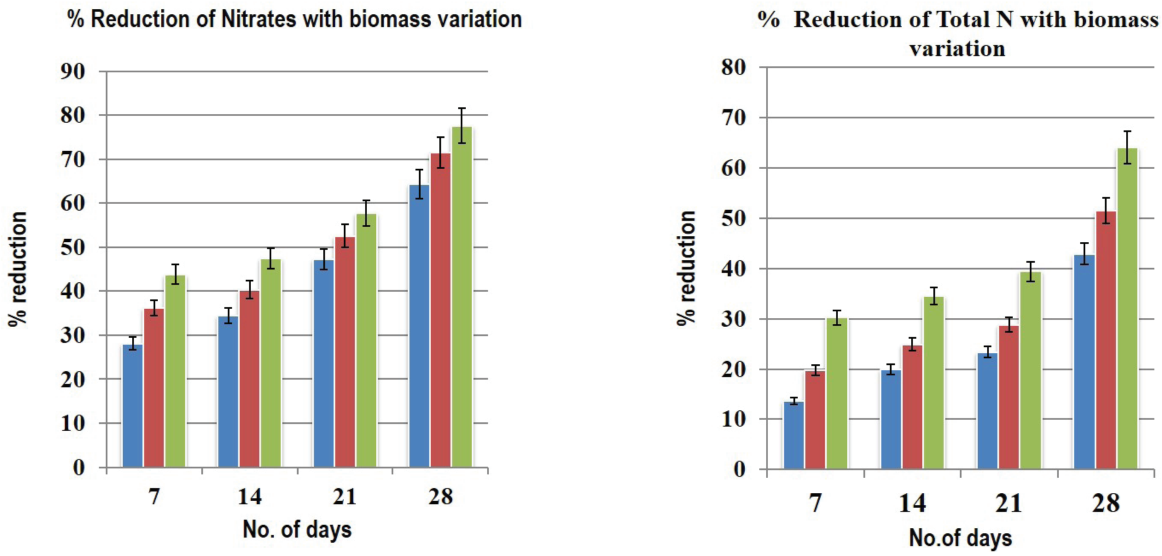

Fig.6: Percentage reductions of TN and nitrates with biomass variation

The optimum pH and temperature condition for nitrification process is in the range of 6.5 to 8.6 and 20 °C to 30 °C respectively (Grunditz & Dalhammar, 2001).The reduction of nitrogen and nitrates in the wastewater might have occurred due to microbial activities; nitrification and denitrification, evaporation and plant and bacterial uptake phenomenon. In addition, the accumulation of oxygen around the roots leads to more nitrification making the nitrogen and nitrate removal more probable (Dhanya & Jaya, 2013).

The result of the analysis of variance revealed that the potential of Vetiver in reduction of TN was significant at p=0.018 for 4 plants, p=0.002 for 8 plants and 0.012 for 12 plants. Results obtained for TN and nitrates were higher than those reported earlier, 43.85% and 76.2% reduction of TN and nitrates were reported at a HRT of twenty eight days and 3 plants of Vetiver planted hydroponically (Danh et al., 2006). Generally, the results strongly indicate that there was a linear correlation between quantity of Vetiver plants used and uptake of pollutants i.e. 12 plants >8 plants >4 plants.

CONCLUSION AND RECOMMENDATIONS

The study shows that vetiver was effective in wastewater treatment since all the units that contained vetiver plants performed better than the plant controls. Vetiver grass growing under hydroponic with no supporting medium, can effectively remove organic matter and nutrients from municipal wastewater. The potential of Vetiver grass after four weeks hydroponic treatment was found to be efficient for the reduction of pH, chemical oxygen demand, biological oxygen demand, total nitrogen, nitrates and electrical conductivity from wastewater. There was a linear correlation of biomass with reduction of pollutants. From the results obtained and the challenges faced during the experiment period, the following recommendations are made:-

1. Vetiver is effective in reduction of BOD, COD and nutrients to the required levels for disposal of wastewater to the environment, hence can be used at Kipevu WWTP.
2. Vetiver can be introduced in the two aeration tanks to reduce electricity costs at Kipevu WWTP.
3. Further research should be conducted to monitor pollutant removal in the aeration tank with a given amount of Vetiver biomass per the volume of wastewater at the retention time currently in practice.
4. Further research regarding amounts of nutrient and heavy metals accumulation in roots and shoots of Vetiver should be done. And how the toxic contaminants can be converted into less harmful sub-

stances to avoid transfer of pollution from one source to another.

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Influence of Nutrients on Seagrass Distribution in Malindi, Kenya

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ABSTRACT

Seagrass are marine flowering plants that occupy both tidal and subtidal marine environments. They are highly productive ecosystems that are useful indicators of environmental health due to their sensitivity to changes in the quality of the marine environment. Nutrients enrichment is known to be a leading cause in the declining of seagrass meadows worldwide. The changes of seagrass and the effects of nutrients fluctuations on their distribution pattern in Malindi have not been documented widely. The aim of the study was to determine the influence of nutrients on the distribution of seagrass in Malindi. Malindi Marine Park (MMP) sites were selected; one at the, the Malindi Marine Reserve (MMR), and the Malindi Marine Buffer (MMB) zone. At each site 30m transects were laid perpendicularly to the shore and samples collected in triplicate at intervals of 10m. Water and sediment samples were collected using plastic bottles and corers, respectively. Nutrients (nitrates, nitrites, ammonia and phosphates) in both water and sediments were analysed colorimetrically. Five species, *viz Thalassia hemprichii, Enhalus acoroides, Syringodium isoetifolium, Cymodocea serrulata and Thalassodendron ciliatum* were identified throughout the sites. Nutrients in sediment were generally higher than in water. Total biomass was predominantly influenced by nitrates (r=-0.141) and nitrites (r=+0.488) in sediments and ammonia (r=-0.364) and nitrites (r=+0.50) in water. The Malindi marine environment could still be oligotrophic but further studies across greater timescales and space are required to unravel seagrass variations due to tourism, urbanization and watershed changes, it was recommended that such be integrated in the monthly monitoring effort of the Kenya Wildlife Service (KWS) and Environmental Impact Assessment (EIA) efforts of the National Environment Management Authority (NEMA).

***Keywords**: Seagrass, Biomass, Nutrients-enrichment, Marine environment.*

INTRODUCTION

Seagrass are marine flowering plants that occupy tidal and sub tidal environments of the oceans. They are highly productive ecosystems and are important to the lifecycles of aquatic plants and animals (Thangaradjou & Kannan 2005). The ecosystem goods and services of seagrass beds are also critical to the economic and cultural lives of hundreds of coastal communities. Some of these services, such as controlling shoreline erosion, nutrient recycling and carbon capture serve mankind and are estimated at over USD 100 billion each year. They also serve as nurseries and habitats for other invertebrates, fish and mammals, source of food to animals such as dugong and sea turtles, stabilize the ocean bottom and improve water quality by trapping fine sediments in water (Cullen & Unsworth, 2016). Seagrass communities could also be useful indicators of environmental health as they are sensitive to changes in the quality of the marine environment. Ecological parameters such as light, temperatures, salinity, epiphytes, pollution, nutrients, and turbidity correlate with above and below-ground biomass and diversity of species (Thangaradjou & Kannan, 2005). These parameters also correlate with human activities around these ecosystems and could therefore, provide a basis for evaluating the impact of human development on the marine ecosystems. Globally, about 50% of seagrass species have gone extinct over the past 50 years. It is estimated that 7% of seagrass cover is lost every year (Kneer *et al*., 2014). These losses are profound noting the interdependencies among marine creatures with seagrass beds. For instance, highly endangered megafauna such as the green sea turtle or the dugon feed exclusively on seagrass and face imminent extinction owing to the seagrass losses. The significance of seagrass to biodiversity is demonstrated further in their subtle functions of securing coral reefs from sediments and excess nutrients and the mangroves from strong tidal action. Seagrass beds are also crucial reserves of oceanic carbon and their continued decline present a great obstacle to the global campaign against climate change. In the tropics, seagrass account for more than 25% of the biomass of fish consumed locally, demonstrating its importance to not only food security but livelihoods in sectors such as fisheries. Seagrass declines have been attributed to reduction of light, nutrient enrichment, changes in sediment conditions, strong grazing, wave motions and climate change (McKenzie *et al*., 2016). These changes are increasingly driven by human activities such as illegal fishing practices, coastal developments, mining and pollution. Once degraded, seagrass rehabilitation and recovery is normally slow and costly, sometimes taking centuries depending on the severity of damage and species involved (Holon *et al*., 2015).

In response, several government agencies are adopting measures to conserve seagrass; such as adopting measures to safeguard water quality through the creation of marine protected areas, legislation, environmental impact assessments and restriction to destructive fishing (de la Torre-Castro 2006) and have appealed greatly to various government agencies (Short *et al* 2007). Although Kenya has ratified some of these actions, such as the marine protected areas (MPAs), limitations in funding and monitoring capacity have hindered the comprehensive evaluation of these measures (Maina *et al*., 2011).

Nutrients have a profound effect on water quality and productivity of marine and estuarine environments and could significantly influence the diversity, spatial and temporal variations of seagrass. Nutrient concentrations and bioavailability are increasingly being influenced by human activities such as poor sewage disposal. The nutrients regime of coastal and estuarine waters could therefore offer considerable lessons to evaluate the impact of specific industries such as tourism or fisheries or development policies that promote urbanization on critical coastal ecosystems such as seagrass (Phillips & Menez, 1988).

Nutrients in the ocean are not uniformly distributed. Areas of upwelling provide supplies of nutrients from euphotic zones. Coastal zones provide nutrients from runoff and upwelling. In as much as seagrass growth is influenced by the availability of sunlight, temperature and water, nutrients play a significant role and previous studies have developed an equation to demonstrate this relevance (Hemming & Duarte 2000):

Sunlight + $106CO_2$+ $16NO_3^-$ + HPO_4^{2-} + $122H_2O$ +$18H^+$ + trace elements→

$[(CH_2O)_{106}(NH_3)_{16}(H_3PO_4)]$

The availability of nutrients is thus critical for seagrass growth. For instance, Duarte (2002) observed that low nutrient concentrations in shallower marine waters became the major factor limiting the growth of marine seagrass. Seagrass absorb nutrients through their leaves or from sediment pore water through their root system (Touchette & Burkholder, 2000a; 2000b). The nutrient uptake kinetics in seagrass tissues reflect its adaptations to life in water columns with low nutrients and relative high nutrients in sediment-pore water. Nutrients increase plant productivity through physiological changes such as increase in rate of photosynthesis, photosynthetic efficiency and increases Chlorophyll concentration. The respective contributions of leaf and root nutrient uptake vary depending on the nutrient concentration. Seagrass allocate most of their biomass in their below ground tissue under low sediment nutrient available and more above ground biomass in areas with higher nutrient concentration. The leaves of seagrass increase in high sediment nutrient condition due to increase in leaf nutrient demand (Lee & Dunton, 1999).

Malindi Marine Park is one of the best parks in coastal Kenya hosting large seagrass meadows. However, little has been done on seagrass studies and the impacts of nutrients fluctuations on seagrass. This study was aimed at evaluating monthly variation of nutrients influence on seagrass distribution in Malindi.

MATERIALS AND METHODS

The study sites lies at latitude -3.22^{0}S and longitude 40.1^{0}E, with temperatures ranging between 20 - 30^{o}C.

The sites are endowed with magnificent resources such as fringing reefs, coral gardens in the lagoons, seagrass beds, mangroves, mudflats, marine mammals and high fish diversity.

Samples were collected across two seasons, *viz*, short rains (October- December) and dry season (January- March). Water samples were collected in clean plastic bottles just below the water surface. Sediment samples were collected using a corer. Samples were immediately transferred to a cooler box cooled and brought to the lab for refrigeration and analysis. Water samples were filtered using a Whatman GF/C filter paper while sediment samples were sieved to remove shells and corals and afterwards treated with concentrated sulphuric acid centrifuged and homogenized. The water samples and filtrate were then analysed in accordance with the method described by Strickland and Parsons (1972).

In this study, seagrass diversity was conceived as the physical counts of seagrass species in a randomly cast quadrant along the nutrient sampling transects. Similarly, seagrass distribution was defined both in terms of species diversity and above or below ground biomass of all seagrass species encountered within a randomly cast quadrant. To determine the biomass, seagrass harvested from the quadrant were oven dried at 50°C for 1hour and weighed on a calibrated weighing balance in grams.

Data on nutrient levels and biomass was recorded in micrograms per litre (mg/L) and grams (g), respectively. The data was entered on separate sheets on Microsoft Excel for cleaning and for computing basic statistical tests such as arithmetic means, drawing tables and pie charts for presentations. Once cleaned, the sheets were copied onto Minitab statistical package for spearman correlation and regression analyses. Correlation dialogue boxes have been included for further output on the tests of association between nutrient levels in water and sediment against seagrass biomass and diversity.

RESULTS AND DISCUSSION

Five species of seagrass were recorded in the study area (Table 1). These were *Thalassodendron ciliatum; Syringodium isoetifolium, Enhalus acoroides, Thalassia hemprichii* and *Cymodocea serrulata.*

Table 1: seagrass species and their distribution

Species	MMR	MMB zone	MMP
Thalassia hemprichii	+	+	+
Enhalus acoroides	+	-	-
Syringodium isoetifolium	+	-	+
Cymodocea serrulata	+	-	-
Thalassodendron ciliatum,	+	+	+

Where: (+) shows presence and (-) shows absence

Of the five species *Thalassodendron ciliatum* was dominant forming pure strands mostly in the sublittoral zone on sandy and rocky substrate. *Enhalus acoroides* occurred in the reserve only. It occurred mostly in the sub-tidal zone on muddy sand substrates and was exposed during low tides. *Thalassia hemprichii* was recorded in all the sampling sites in the sub-tidal zones. It inhabited muddy and coarse substrates and had short leaves that appeared curved and sickle shaped. *Syringodium isoetifolium* inhabited fine sediments in the upper intertidal zones. It exhibited narrow fleshy leaves some of which were covered by dead leaves of other seagrasses and was found in the reserve and the park. *Cymodocea serrulata* grew on fine sandy sediments. It occurred in the reserve only. It had flattened and reddish colored leaf sheaths Seagrass percentage cover varied from 45% to 98% with the highest value observed in May and the lowest value recorded in the month of February (Figure 1).

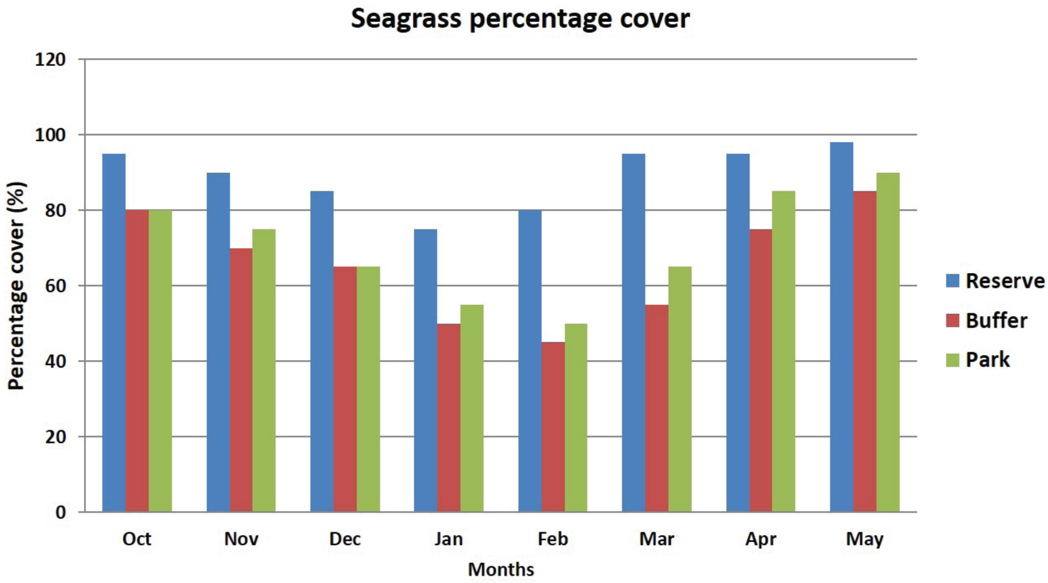

Fig. 1: Monthly variation of seagrass percentage cover

The declines occurred due to heavy rains which raised the volume of turbid freshwater runoff. It is possible, therefore, that seagrass beds were devastated by uprooting, low light conditions due to elevated turbidity, deposition of suspended waste on the leaves and seagrass burial by sediment from runoff from River Sabaki (Ahmad-Kamil *et al.*, 2013).

The highest and lowest total biomass was recorded in the months of May and December, with means of 256.790±91.2g dry wt/m^2 and 136.390±91.2g S.D dry wt/m^2, respectively (Figure 2). Seasonally, maximum biomass was observed during the long rains followed by the dry season and minimum biomass recorded during the short rains.

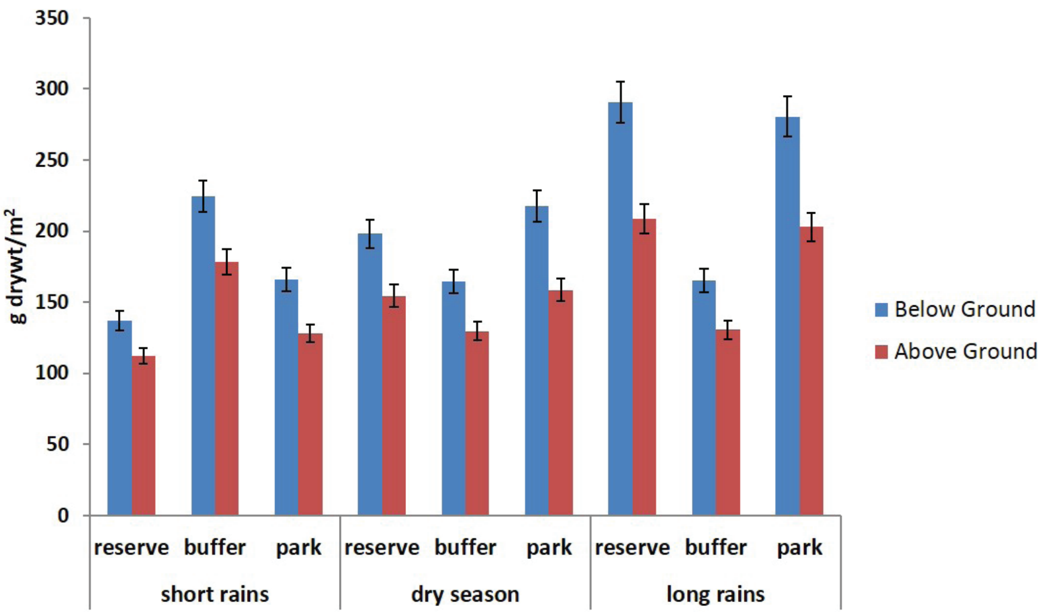

Fig. 2: Seasonal distribution of seagrass biomass

Below ground biomass recordings were greater than above ground biomass. This may be attributed to: the hard sediment that hinders rhizomes from obtaining nutrients from the substrate and demands for more energy for penetration of roots, the low nutrient levels generally observed in these areas due to the presence of coarser sediment, and low light due to reflection by the white sand sediments in the park and buffer. These findings confirm those of Paynter, Cortés and Engels (2001) and Dawes (1998).

The water and sediment samples collected were analysed for nutrient content. Results indicated that there was a higher variability in nutrient levels in sediment samples compared to seawater and that ammonia and phosphates exhibited the highest variations in both seawater and sediment (Table 2). The low concentration of nutrients in water samples compared to those observed in sediment samples could probably be the result of tidal dilution and phytoplankton and microalgae and vascular plants uptake (Mallin *et al*., 2007) as well as the losses into the sediments since sediments have a higher affinity for nutrients as compared to water. Nutrients in the water column as well as those that are adsorbed on the suspended solids could also have been trapped at the bottom sediment during the process of sedimentation resulting to higher nutrients concentration in sediments than in water (Wetzel, 2001).

Table 2: Comparison of nutrients in water and sediments

Variable	N	Mean	StDev	Minimum	Maximum
NO_3_water	72	0.0294	0.0448	0.000238	0.167
NO_2_water	72	0.0101	0.0196	0.001	0.158
NH_3_water	72	0.031	0.1825	0.001	1.554
PO_4_water	72	0.0289	0.0366	0.002	0.1759
NO_3_sediment	72	7.662	7.442	0.26	25.824
NO_2_sediment	72	4.067	6.822	0.05	35.014
NH_3_sediment	72	7.899	8.465	0.083	36.382
PO_4_sediment	72	11.96	19.78	0.055	82.21

A Spearman's Correlation test was used to determine the relationship between sea grass biomass and nutrients in water, nutrients in sediment and sediment texture. Below ground biomass showed a moderate linear relationship with nitrates and nitrites in sediment, and nitrites and ammonia in water with r values of -0.388, -0.514, +0.490 and -0.380 respectively (Figures 3 and 4). Other parameters (ammonia(r=+0.065) and phosphates (r=-0.196) in sediment, phosphates in water(r=-0.105) sand (r=+0.151) and clay (r=-0.13) showed a weak linear relationship with below ground biomass. Nitrates in water did not show any significant relationship with below ground biomass.

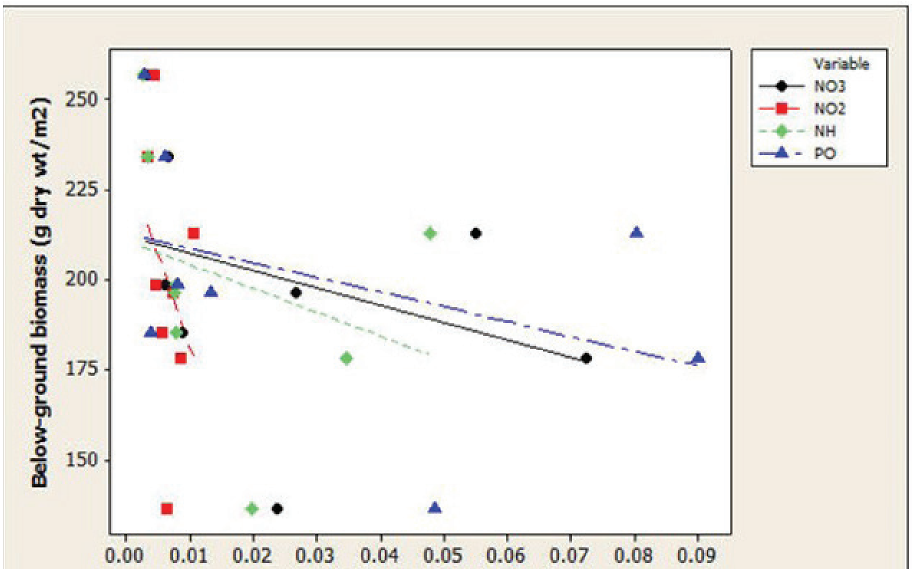

Fig 3: Correlation between nutrients in water and below ground biomass

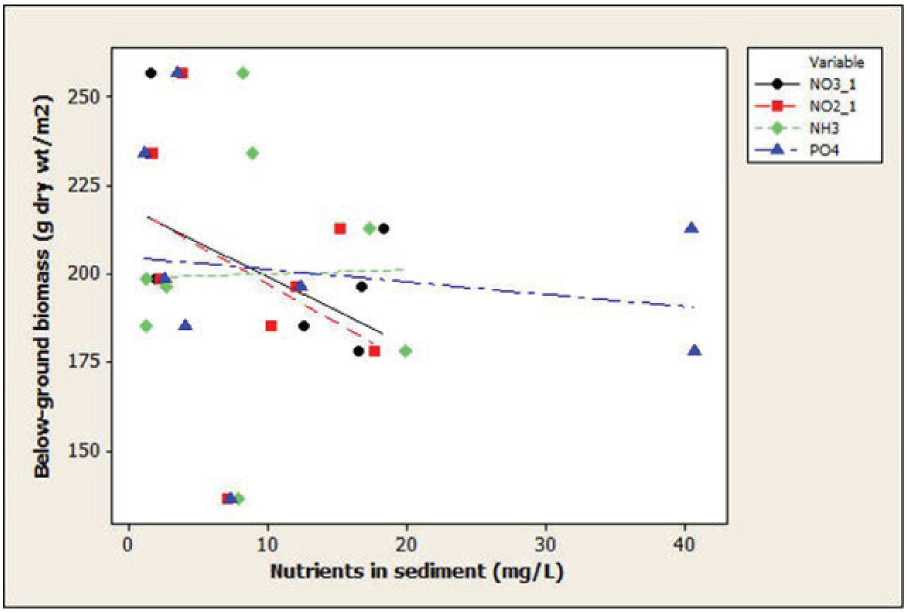

Fig 4: Correlation between nutrients in sediment and below ground biomass

This implies that seagrass below ground biomass were predominantly sensitive to variations in nitrites level in water and nitrates levels in sediment. Other nutrients had very close to zero correlation coefficient with above ground biomass suggesting limited influence on above ground biomass of seagrass.

Above ground biomass showed a linear relationship between nitrates and nitrites in sediment with r values of -0.431 and -0.427 respectively, and between nitrites and ammonia in water with r values of 0.489 and -0.321 respectively (Figure 5 and 6). Clay, Silt and sand showed a weak relationship with above ground biomass (r=0.99, 0.083 and 0.84 respectively).

Nitrate and nitrites in sediments and nitrites and am-

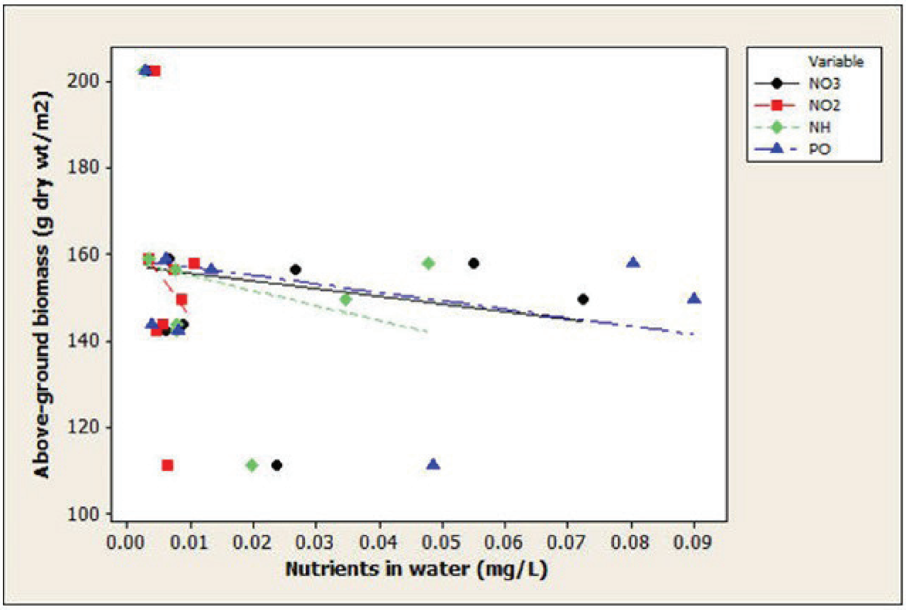

Fig 5: Correlation between nutrients in water and above ground biomass

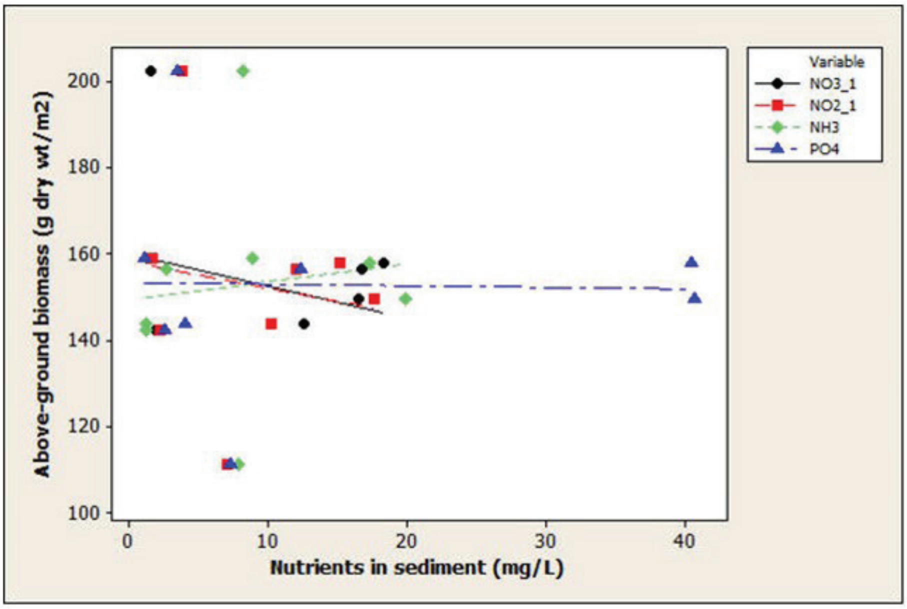

Fig 6: Correlation between nutrients in sediment and above ground biomass

monia in water showed a higher strength of correlation with above ground biomass. This shows that these nutrients influence the growth of seagrass but their decrease results to increase of above ground biomass of seagrass.

Total biomass showed a moderate relationship between nitrates and nitrites in sediments with r values of -0.141 and +0.488 respectively. In water, total biomass showed a strong relationship between nitrites and ammonia with r values of +0.500 and -0.364 respectively, while with sediment texture, total biomass showed a weak relationship with r values of 0.126, 0.002 and 0.166 for sand, silt and clay, respectively (Figure 7 and 8).

Total biomass was predominantly influenced by ni-

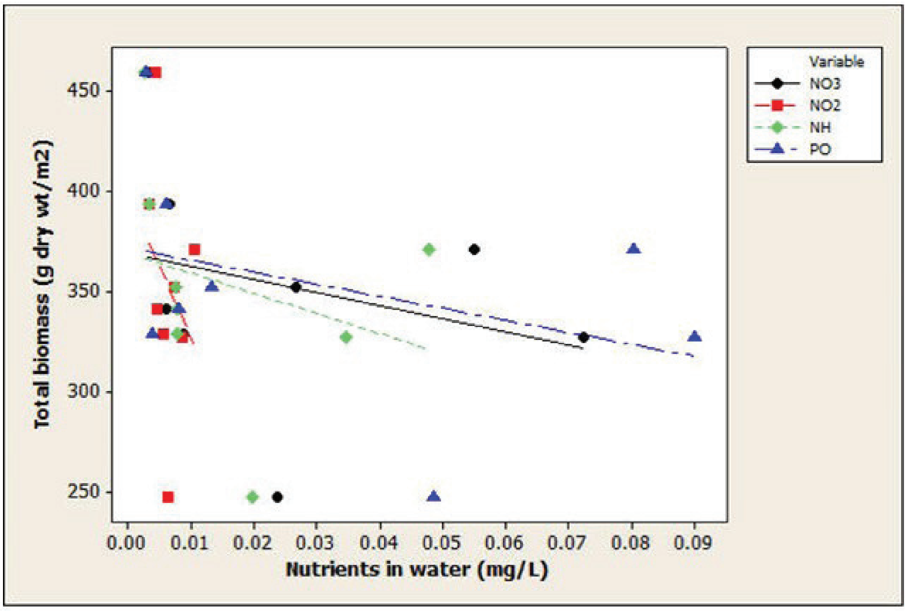

Fig. 7: Correlation values for nutrients in water and total biomass

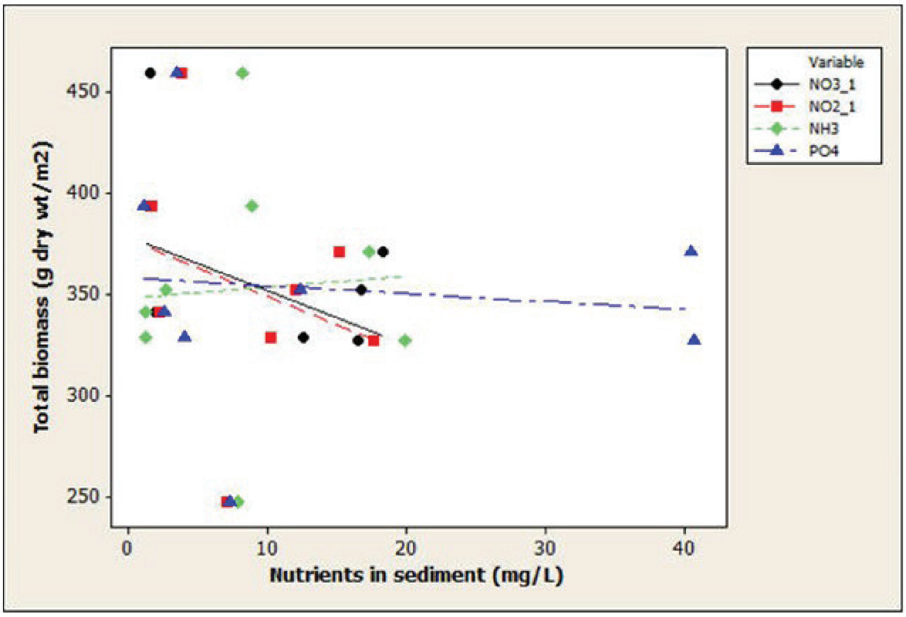

Fig. 8: Correlation values for nutrients in sediments and total biomass

trates and nitrites in sediments and ammonia and nitrites in water. Other nutrients showed a correlation close to zero reflecting minimal influence of these nutrients to the total biomass of seagrass. Except for ammonia levels in sediment, all other nutrients analysed in this study had a limited influence on seagrass percentage cover in all stations. Ammonia concentrations in sediment explained as much as 60% of the variation in seagrass percentage cover in the Park (r=58.5 P=0.027) but could not predict cover in the Reserve and Buffer.

CONCLUSION AND RECOMMENDATIONS

This study brought out the significance of nutrient concentrations and sediment texture on seagrass distributions in Malindi Marine Park and Reserve. Since seagrass have the ability to absorb nutrients from both water and sediments it is the conclusion of this study that nutrients constitute an important factor in influencing the biomass of seagrass.

Biomass of seagrass was higher in the park than in the other sampling sites this showed that the marine parks serves its purpose of protecting marine ecosystems. Low biomass in the reserve could be attributed to human disturbances around the area such as including inputs such as nutrients run-off and sediment loading. Sediment, nutrients and freshwater from River Sabaki reaching the sampling area during heavy rain season could explain the seasonal mortalities of seagrass and corals in this fragile marine ecosystems.

Nutrients play a major role in seagrass distribution. The nutrient distribution pattern indicates a very strong influence of river discharge during the short and long rainy season. During the short and long rains nutrients levels were higher and this caused death to seagrass and low mobility organisms. During the dry seasons nutrients levels were low and this resulted to recovery and growth of seagrass. The high nutrient values observed can be attributed to rich nutrient waters from runoff, river discharge, agricultural activities and use of fertilizer in the area surrounding the river bank. It can also be attributed to the local sources within the area surrounding the study area such as the sewage effect, use of detergents and decomposition of organic materials. However due to mixing and phytoplankton intake nutrients concentration tend to drop. Nutrients may be interlinked with and influenced by human activities and natural processes and are individually or jointly governing the distribution, diversity and biomass of seagrass depending up on seasonal changes.

It is vital, therefore, that measures seeking the long-term conservation of seagrass in Malindi should contemplate much broader strategies such as integrated watershed management of R. Sabaki and sustainable urbanization of Malindi town and her environs. Long-term monitoring is essential and could be achieved by integrating nutrients, sediment and biomass data in the monthly monitoring exercises of the Kenya Wildlife Services. Similar data could be integrated into the Environmental Impact Assessment (EIA) and management plans of major land-use change projects and programs along Malindi area and R. Sabaki.

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Land-Based Activities as Pollution Sources on Fresh Water Resources: A Case Study of Selected Heavy Metal Contamination in River Mukurumudzi, Kwale County

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ABSTRACT

Pollution of water-body ecosystems by heavy metals through uncontrolled anthropogenic activities lead to serious ecological problems in many parts of the world. These metals continue to accumulate to high toxic levels as they are discharged into water-bodies from agricultural activities, industrial and domestic wastes, and mining operations. This study focused on assessing the concentration of selected heavy metals (Fe, Pb, Cu, Cd and As) in the waters and sediments of River Mukurumudzi in Kwale County, Kenya. Four sampling points along the course of the river were purposely chosen owing to their proximity to identified land based pollution sources, these were titanium mining sites, large scale sugarcane plantations, human settlements and a control site. Samples were collected in three different seasons (long rain, short rain, and dry season). The heavy metals were analyzed using Atomic Absorption Spectrophotometer (AAS). The results showed that the level of Fe and Cu in water was the highest during the rainy season while all the other metals were found to be below detectable limits. In sediments, Fe (0.64 mg/L, 1.97 mg/L, 0.33 mg/L and 0.21 mg/L), Cu (0.1 mg/L, 0.14 mg/L, 0.14 mg/L and 0.1 mg/L), and Pb (0.14 mg/L, 0.21 mg/L, 0.12 mg/L and 0.33 mg/L) were detected at Shimba Hills, Nguluku, Bomani and Gazi consecutively. The concentrations of metals in water were found to be within the NEMA and WHO safe limits. Though River Mukurumudzi is not contaminated with the selected heavy metals it is established that the anthropogenic activities along the river are contributing some of the heavy metals. As such, this study recommends that conservation measures be put in place to avoid contamination of the river.

Key Words: *Anthropogenic activities, Heavy metals, Land-based sources, Water pollution.*

INTRODUCTION

Availability of freshwater is one of the most essential factors in development. Freshwater constitutes only 3% or less of the earth's surface water, most of which is in form of ice and snow in polar region or in underground aquifers. However, despite its importance, mismanagement of water as a resource has been observed in many parts of the world (Fakayode, 2005). Human activities have been the major causes of water pollution and this has been documented in many parts of the world (Adekunle, 2009). Many rivers, lakes, wetlands, ground waters and oceans suffer a great loss of degradation from various human activities. These activities have effects on water quality, changing both its physiochemical and biological parameters making it unsuitable not only for domestic use, but also for other purposes. Water pollution also has effects on habitats, causing species migration while exterminating others due to impacts that affect their reproductivity.

Freshwater ecosystems are among the most critical ecosystems in Kenya. Human activities have put such ecosystems under a lot of pressure. According to the National Environmental Policy Kenya, 2014, activities such as unsustainable land use practices, poor soil and water management behaviors, and pollution lead to the degradation of natural resources in Kenya. Natural resources such as land, freshwater, marine water and biodiversity are scarce and their degradation threatens livelihoods of a many people. Furthermore, environmental degradation in Kenya contributes to climate change impacts like the rising cost of water treatment.

Anthropogenic activities are a major cause of concern for water pollution in the world (Adekunle, 2009). Additionally, research has proven that high levels of toxic heavy metals are discharged through human activities (Gao *et al*., 2010; Nduka & Orisakwe 2011; Kassim *et al.* 2011). Pollution of ecosystems such as rivers, oceans, lakes and wetlands by heavy metals resulting from anthropogenic activities causes serious ecological problems in many parts of the world. These metals continue to accumulate to higher levels of concentration as they are discharged into water-bodies from agricultural, industrial and domestic wastes, pesticides or mining operations resulting into severe toxicological effects on humans and the aquatic ecosystem (Jung, 2001; Ezeh & Chukwu, 2011). Anthropogenic activities like mining release huge amounts of tailing waste containing heavy metals. Agrochemicals introduced as soil nutrients to improve fertility contain metals, which in most cases exceed the limits set for land application and their continuous use can exacerbate their accumulation in agricultural soils (Lim *et al*. 2008; Hesterberg, 1998).

The increase in the cost of water treatment as a result of pollution has led to the rise in the price of useable water. As such, protection of water sources from anthropogenic activities that are likely to compromise water quality should be encouraged. There is therefore need to evaluate the impact of land based activities on the quality of water-bodies and provide mitigating measures.

MATERIALS AND METHODS

The sampling was conducted in River Mukurumudzi, Kwale County, Kenya. Four sampling points (S1, S2, S3, and S4; Figure 1) were purposely chosen along the course of the river. Three sampling points were chosen owing to their proximity to anthropogenic land based activities that were suspected to be sources of heavy metals. The fourth sampling point was chosen upstream of the river to act as a pristine site free from metal contamination.

The first sampling point at Shimba hills is close to the source of the river and not much of human activities takes place at this area except that since the water is much cleaner people would come to fetch water at this site. The second site is at Nguluku area where titanium mining activities are undertaken. The third site was at Bomani area where largescale sugarcane plantations are carried out. The fourth sampling was more downstream of the river where different human activities such as small-scale agricultural activities, laundry, bathing and motorcycle washing are done.

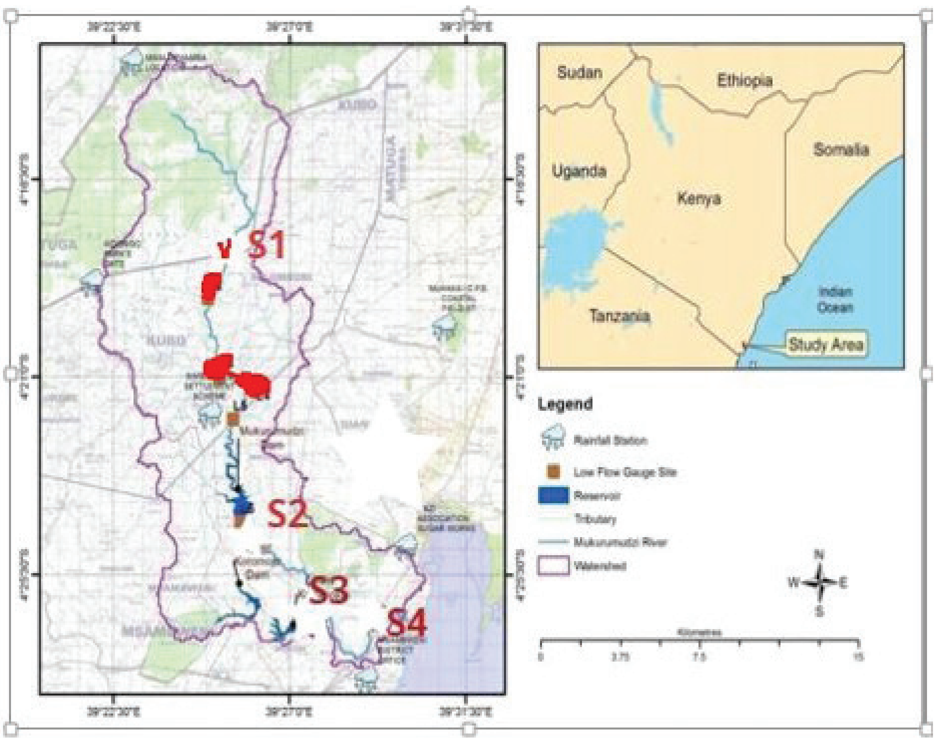


Fig. 1: A map of the study area showing sampling points.

Water and sediment samples were collected in duplicate from the four sampling points. Sampling was conducted in three different seasons; short rain season (August-October 2015), dry season (January-March 2016) and during the long rains (April-July 2016).

A total of 72 samples were collected (36 water samples and 36 sediment samples). After collection the water and sediments were acidified with nitric acid of a pH of 2 and then transported to the laboratory.

Heavy metals were analysed using AAS. The samples were handled carefully. The glassware was properly cleaned using chromic acid solution and distilled water. Chemicals and reagents of analytical grade were used all through the process. Instrument readings were established using blank samples.

Sediment sample of 10 to 20 g was weighed accurately in a tarred silica dish. The samples were then oven dried at 180ºC. The dishes were then placed in a muffle furnace at an ambient temperature and the temperature was gradually raised to 450ºC at a rate of 50ºC/h. After the attainment of 450ºC the samples were left there for a minimum of 8 hours. Care was taken to avoid losses by volatilization of elements. After cooling the dishes of the samples were removed from the furnace. After this, the samples were digested in 50% nitric acid on a hot plate. The samples were then filtrated using Whatman filter paper No. 44 into a 100 mL volumetric flask. The sampled was then topped to the mark using distilled water.

Water of 100 mL was taken from each sample and put in a beaker. The samples were then digested on a hot plate by 5 mL nitric acid. The samples were then filtrated to a 100 mL volumetric flask using Whatman filter paper No. 44 and then made to mark with distilled water.

The standard solution for each metal was prepared for the calibration of the AAS before analyses was performed. All samples were prepared by chemicals of analytical grade. 1 g of Cd, Cu, Pb was dissolved in HNO_3 solution; 1 g of Fe, and As were dissolved in HCL solution. All the samples were made up to 1 L in a volumetric flask using distilled water. From which the stock solution of 1000 mg/L of all elements were prepared (Cantle *et al*., 1983). Then 100 mL of 0.1, 0.25, 0.5, 0.75, 1.0 and 2.0 mg/L of working standards of each metal was prepared from the stocks using micropipettes in 5 mL 2N HNO_3. Reagent blank was also prepared to avoid contamination.

Atomic Absorption Spectrophotometer was set up with the flame condition and observance was optimized for the analyses. The blanks, standards, sample blanks and samples were then aspirated into the flame. Calibration curves were obtained for concentration against absorbance. The concentration of each sample was then established.

One way Analysis of Variance (ANOVA) was used to show the variations of concentration of heavy metals between different seasons and sites. The variations were graphically represented. Pearson's product moment correlation matrix was done to identify the relation among metals to make the results obtained from multivariate analysis.

RESULTS AND DISCUSSION

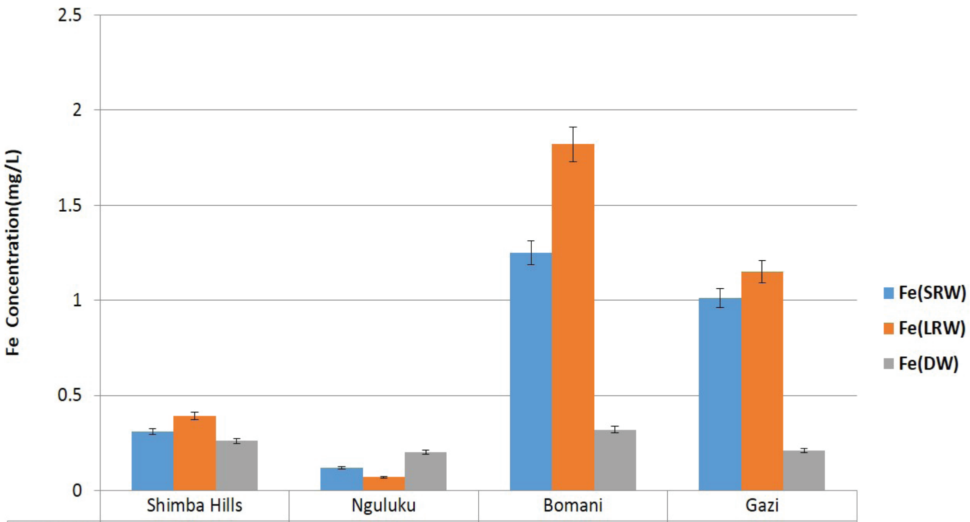
The study established that the concentration of the selected heavy metals in water were within WHO and NEMA permissible limits with the exception of Fe. In sediments, all metals were found to be below detection limit (BDL). There was no significance differences between the concentration of heavy metals in sediment and water ($p > 0.05$).

The highest concentration of Fe in water (1.82 mg/L) was recorded during the long rainy season at Bomani sampling point while the lowest (0.07 mg/L) was recorded during the long rainy season at Nguluku sampling point. Similar observations were found by Maina (2008). He stated that the level of Fe in Mrima Hills was high and they could be influenced by the geology of the area. Okuku et al (2011) similarly found a high concentration of Fe in sea water sampled along Gazi Creek. Further analysis revealed that heavy metal content in this area was contributed by natural sources. In general, the level of Fe was higher during the long rain season across all points with the exception of the Nguluku sampling point. The sampling points of Gazi and Bomani human settlements showed an increased concentration of Fe in water. Since these points were downstream of the titanium mining zone, it could be interpreted that there is an enrichment of Fe from the mining activities. Another attribute to the high Fe concentration along Bomani human settlements could be the use of cosmetic products. Mascara and oils are known to be rich in Fe (Kayumba, 2014). Ladies and women using the river to bathe and launder clothes might be enriching Fe from the use of these products.

The highest concentration of Cu (0.11 mg/L) was found during long rains season at Bomani sampling point. The lowest (0.08 mg/L) was detected in other sampling points during the long rain season. However, during all the other seasons the concentration of Cu was found to be below detection limit (<0.01 mg/L) at all four sampling points.

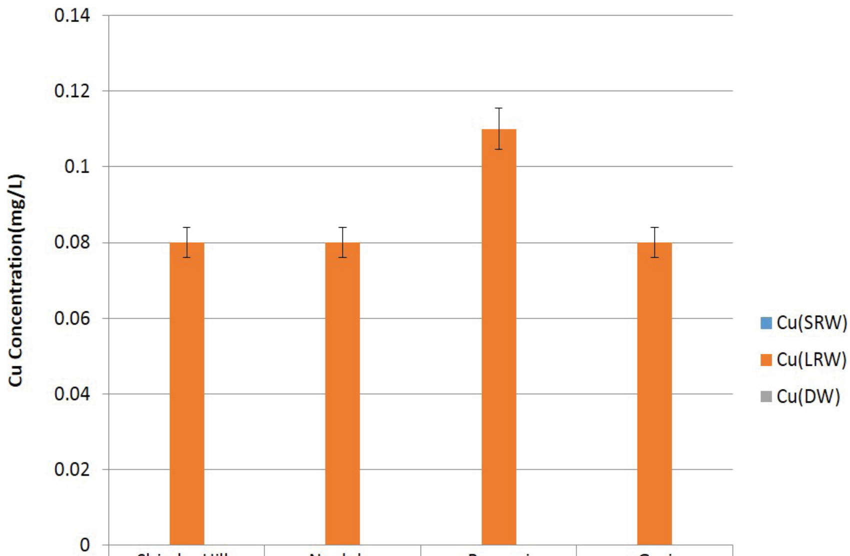
The levels of Pb in water were below the detection limit (<0.001 mg/L) across all the points during short rains (SRW), long rains (LRW) and dry season (DW). This suggests that there is minimal if any Pb contamination from the anthropogenic sources to the waters of River Mukurumudzi. The levels of Pb in water were lower than the WHO (1993) and WASREB (2008) standards for domestic waters. A study done by Chege, Hashim and Merenga (2013) documented that the levels of Pb in groundwater from wells sampled within the area, ranged between BDL-1.397mg/L. The study also found out that 62.3% of the samples had a concentration above the WHO reference level of 0.01 mg/L.

In this study, the concentration of Cd and As in water was found to be (<0.01mg/l) along all the four points during all the seasons. Therefore, the results of Cd and As are not displayed in graphs. Chege *et al.*, (2013) found the level of Cd in 42% of the sampled groundwater sources to be above 0.005mg/l. This study shows there is no Cd enrinchment in surface waters as it is in groundwater.



	Shimba Hills	Nguluku	Bomani	Gazi
Fe(SRW)	0.31	0.12	1.25	1.01
Fe(LRW)	0.39	0.07	1.82	1.15
Fe(DW)	0.26	0.2	0.32	0.21

Fig. 2: Fe concentration in water for the three different seasons



	Shimba Hills	Nguluku	Bomani	Gazi
Cu(SRW)	0	0	0	0
Cu(LRW)	0.08	0.08	0.11	0.08
Cu(DW)				

Fig. 3: Cu concentration in water during the long rain season

The level of metals in sediments was higher than in water. In general, the level of Fe in sediments was high during the long rain season (LRS) across all the four points of sampling and low during the dry season. The level of Fe in sediments along Nguluku was higher than all the other points, this could be attributed to the mining of the titanium by Base Titanium Company. Titanium is mined in ores like ilmenite ($FeTiO_3$) which contains iron. Mangala (1987) also showed a high concentration of Fe in the area. Another study by Maina (2008) showed a low concentration of Fe in the soils of the area, this differed with the findings of this study. It could therefore be linked to the mining activities that are taking place in the area. The Shimba Hills area also had the second highest levels of Fe in its sediments. This could also be as a result of the mineral composition of the area (Chege *et al.*, 2013). The sediments along Gazi and Bomani settlements had the lowest levels of Fe.

Sediments along Gazi had the highest concentration of Pb during the dry (DS) and short rain seasons (SRS) 0.54±0.0067 mg/L and 0.27±0.0033 mg/L respectively, this could be attributed to the use of agrochemicals and fertilizers that contain Pb especially in the Kwale International Sugar Company Limited (KISCOL) plantations. Sediments along the Shimba Hills area had the lowest level of Pb during the dry season (0.08±0.012) mg/L, since this is an area upstream the river where not much of land based activities are happening, these levels could be attributed to the soil mineral contents in the area. Along Nguluku, the level of Pb was 0.12±0.0088 mg/L during the dry season while along the Bomani settlements it was 0.16±0.001 mg/L. Sediments along Nguluku had the highest concentration of Pb 0.34±0.0057 mg/L during the Long rain season, this shows that there could be a possibility of pollution from anthropogenic sources through run-off and leaching of water and sediments from the surrounding

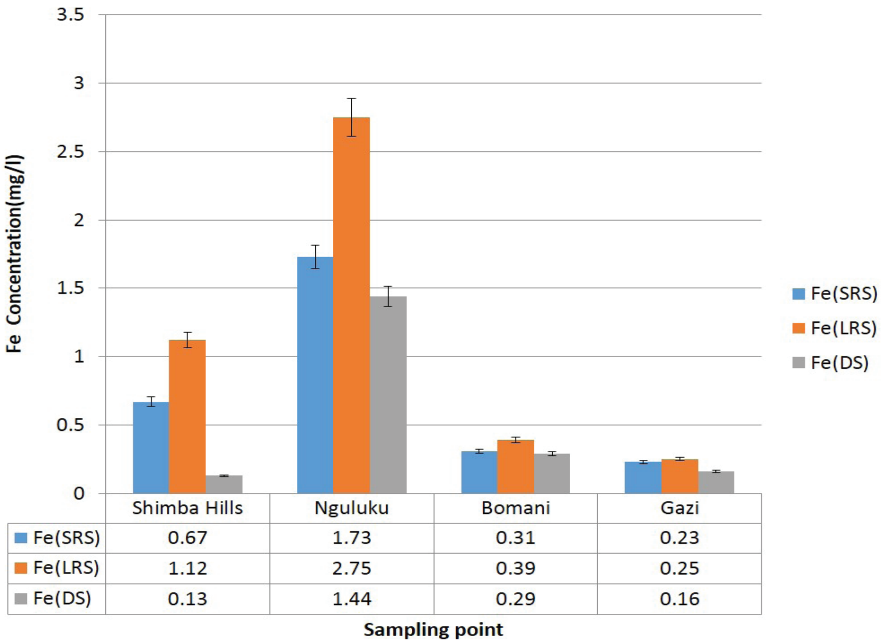


Fig. 4: Fe concentration in sediments in all the seasons

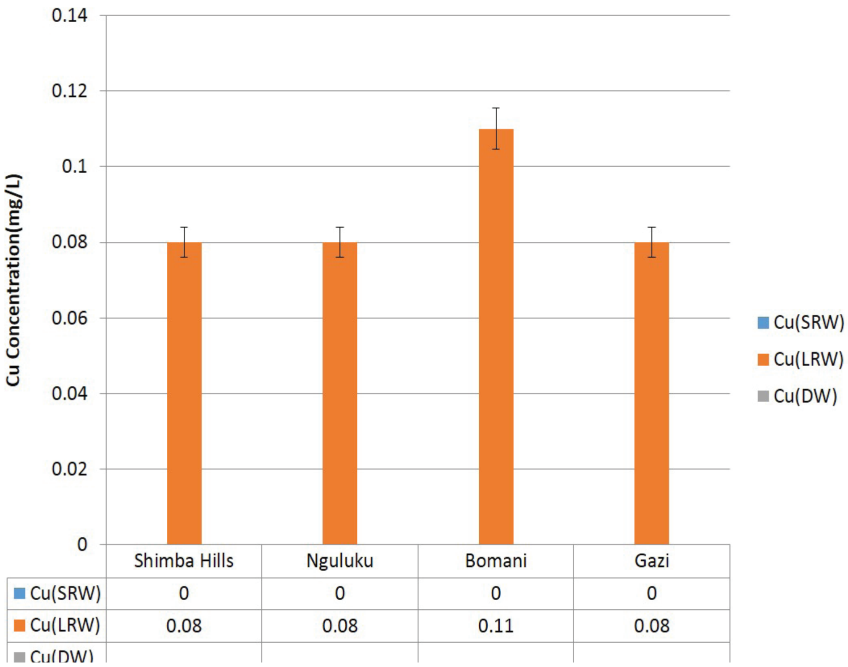


Fig.5: Cu concentration in water during the long rain season

mines. The detection of Pb in sediments and not in water strongly suggests that sediments are sinks of pollutants in aquatic environments as recorded by Horowitz, Meybeck, Idlafkih and Biger (1990). There was no correlation in the levels of Pb in water and those in sediments. This could be interpreted to mean that the sources of Pb in River Mukurumudzi are anthropogenic.

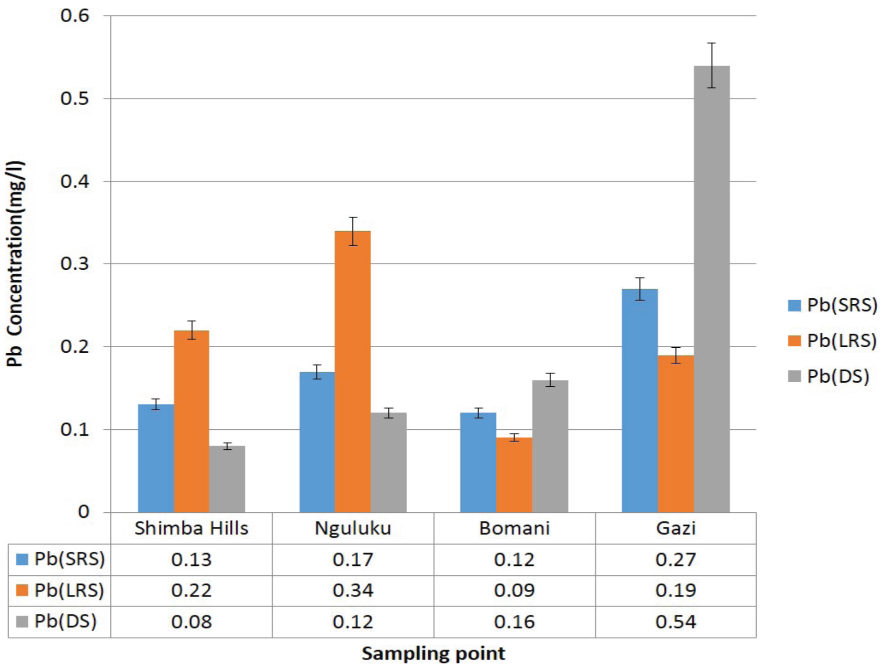


Fig. 6: Pb concentration in sediments in all the seasons

The concentration of Cu in water and sediments across the different sampling points had shown no significant difference.

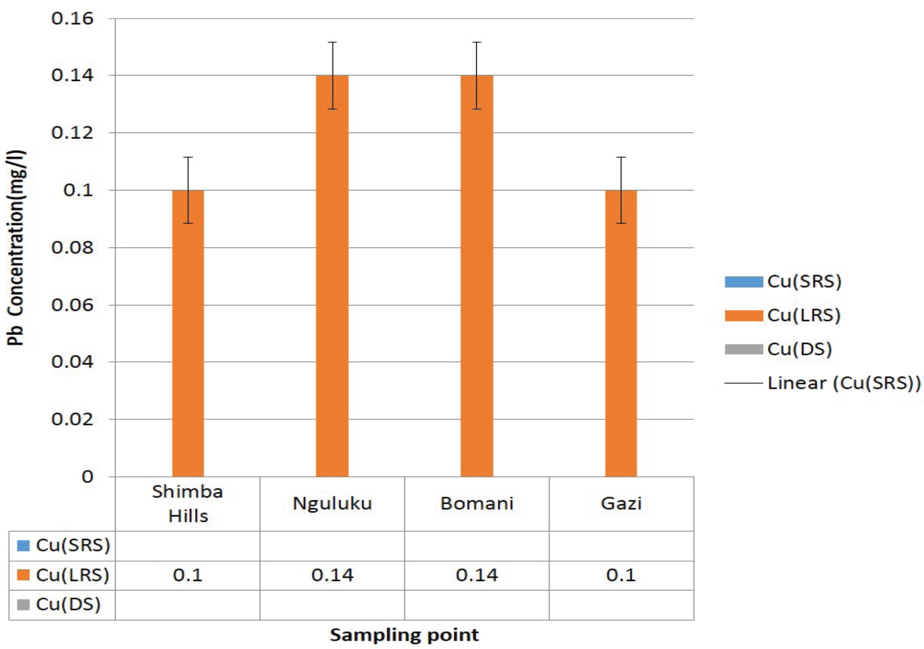


Fig. 7: Cu concentration in sediments during long rain season

Interrelations between metals in water and sediments provide important information on aquatic environment regarding their sources and pathways. Very strong, strong and moderate correlation indicates that, their sources of origin are similar, especially from industrial effluents, anthropogenic wastes and agricultural inputs (Schober, Boer, & Schwarte, 2018).

There was a strong positive correlation of the concetration of Fe in water across all the sampling areas (Table 1). This can be interpreted to mean that the contamination sources of Fe are the same across the different sampling points. Bomani and Nguluku exhibited the strongest (r=0.800) correlation of (r=0.999). The lowest correlation was between Gazi and Bomani.

Table 1: Correlation of Fe in water

	Fe-Shimba Hills	Fe-Nguluku	Fe-Bomani	Fe-Gazi
Fe-Shimba Hills	1	-	-	-
Fe-Nguluku	0.934584	1	-	-
Fe-Bomani	0.926456	0.999754	1	-
Fe-Gazi	0.966916	0.812916	0.799787	1

There was a negative correlation of Fe in sediments between Nguluku and other stations (Table 2). The correlation (-0.965) between Nguluku and Shimba Hills, (-1.00) between Nguluku and Bomani and (-0.96825) between Nguluku and Gazi. This is interpreted to mean that the source of Fe in Nguluku sediments is different from the sources at the other locations. All the other stations exhibited a positive correlation of Fe in sediments.

Table 2: Correlation of Fein sediments

	Fe-Shimba Hills	Fe-Nguluku	Fe-Bomani	Fe-Gazi
Fe-Shimba Hills	1	-	-	-
Fe-Nguluku	-0.96512	1	-	-
Fe-Bomani	0.963733	-0.99999	1	-
Fe-Gazi	0.869017	-0.96825	0.969542	1

There was a positive correlation (0.990) of Pb in sediments between Shimba Hills and Nguluku (Table 3). There was also a positive correlation (0.976) of Pb in sediments between Bomani and Gazi. A negative correlation (-0.970) of Pb in sediments was observed between Shimba Hills and Bomani and (-0.893) between Shimba Hills and Gazi.

Table 3: Correlation of Pb in sediments

	Pb-Shimba Hills	Pb-Nguluku	Pb-Bomani	Pb-Gazi
Pb-Shimba Hills	1			
Pb-Nguluku	0.989991	1		
Pb-Bomani	-0.96995	-0.9259	1	
Pb-Gazi	-0.89283	-0.82033	0.975585	1

Cu in water and sediments was strongly correlated across all the sampling points (Table 4).

Table 4: Correlation of Cu in sediments

	Cu-Shimba Hills	Cu-Nguluku	Cu-Bomani	Cu-Gazi
Shimba Hills	1	-	-	-
Nguluku	1	1	-	-
Bomani	1	1	1	-
Gazi	1	1	1	1

One way ANOVA showed a significant difference (p=0.024) between the concentration of Fe in water in Nguluku and the control site along the Shimba Hills area. There was also a significant difference (p=0.050) in the concentration of Fe in sediments along Nguluku with those along the Shimba Hills area. There was no significant difference (p=0.139) in the level of Fe in water along the Bomani settlements and Shimba Hills area, there was also no significant difference (p=0.342) in the concentration of Fe in sediments along these two areas. There was no significant difference (p=0.212) in the level of Fe in sediments along Gazi and Shimba Hills area, the level of Fe in water in these two areas was also not significant (p=0.187). The difference in level of Pb in sediments along Shimba Hills and Nguluku was not significant (p=0.442). There was also no significant difference (p=0.684) in the level of Pb in sediments along Shimba Hills and Bomani settlement. There was also no significant difference (p=0.170) in the level of Pb in sediments along Shimba Hills and Gazi. This suggests that there are different sources of Pb input to the River Mukurumudzi.

CONCLUSION AND RECOMMENDATIONS

River Mukurumudzi is an important source of fresh water in Kwale County. Most of the populace use the river to get water for their domestic activities. The probable sources of contamination in the river are anthropogenic activities such as agriculture, mining and washing of clothes, motorcycles and bathing in the river. Based on this study it is concluded that the river is not contaminated by the selected heavy metals since they are all below the WHO and NEMA permissible limits. It is also concluded that there is a higher concentration of metals in sediments than waters. Conservation measures should be put in place in order to protect River Mukurumudzi from contamination. The extensive construction of dams on the river should also be controlled because it's affecting the levels of water downstream hence, contributing to the accumulation of contaminants.

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Mathematical Models For Animal Population Dynamics

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ABSTRACT

Researchers have always been interested in systems consisting of multiple autonomous agents. Many systems are inspired from the observation of animals that move from one area to another or a system where the species living in a certain ecosystem have some form of relationship, either the prey-predator, cooperation or competition relationship. In this article, a literature survey of some of the methodologies employed by researchers to population dynamics modeling is discussed. Suitable application with respect to the mathematical models is also reported. This work seeks to contribute to a better understanding of the ecological dynamics of ecosystems using mathematical models. It demonstrates the need to investigate the nature of the animal populations for one to precisely model the population dynamics. This study shows that different models could be used in different situations employing difference equations, ordinary differential equations and Markov Chains. Movements and population dynamics of some species are used for illustrations. Aggregation methods are used to reduce the complexity of the model, which uses actual parameters. These suggested models make the population dynamic modeling more realistic by capturing essential features of the growth process.

Keywords: *Population dynamics, Ecological dynamics, Aggregation methods, Multiple autonomous agents*

INTRODUCTION

Researchers have been interested in systems consisting of multiple autonomous agents. Bonabeau *et al.* (1999) ; 2000 explains that many systems are inspired from the observation of animals that move from one area to the other or a system where the species living in a certain ecosystem have some form of relationship, either the prey-predator, cooperation or competition relationship. Such observations have been used to extract ideas, models and philosophies underlying natural systems and apply them to artificial problems. When applying rules extracted from natural systems to artificial problems, we need specific mathematical models to understand the internal mechanism of the systems with respect to real and simulation environments. Natural systems have flexibility and reliability but artificial systems are not. This is the reason why the mathematical model plays a vital role in modelling population dynamic systems. Principally, mathematical modeling has been used in a wide range of applications with various domains.

This article illustrates how differential equations and markov chains can be used to describe a wide range of processes in the physical, biological, and social sciences, and devote this section to a number of ways to model and analyze the long term behavior of animal populations.

This work starts by considering the simplest form where we have a single population for example, grazers only, then improving to a two populations for instance predators and prey modeling. These ideas are then adapted to the population dynamics of virtually all living things. In each model, the variables that describe what is happening are identified then we establish how these variables change overtime. After examining the predictions of our simple model and checking how well they correspond to reality, we make modifications. Models can provide successive approximations to reality, or we might describe the same features in different ways. Gradually, through a succession of refinements of our original simple model, we hope for descriptions that come closer and closer to the real situation we are studying.

Bayindir and Sahin (2007) while modelling swarm robot classified the taxonomy in five modelling axes namely, modelling, behaviour design, communication, analytical studies and problems. Dudek, Jenkin, Milios and Wilkes (1993) also noted that, individual robot behaviour is simple but they exhibit the complex behaviours for the desired tasks. The interaction among the robots and their environment is very important to achieve the overall group performance. Dudek *et al.* (1993) says that specific parameters to understand the behavior of the whole system are needed. Real and Sensor-based experiments are the well known aspects to observe the behaviors of the system with different parameters. On the other hand, real and simulated experiments are very expensive and time-consuming, and also a problem in size, noise and other environmental issues for the overall system performance. Barrera, Flores and Fuerte-Esquivel (2008) noted that mathematical models can rapidly and efficiently be used to study the swarm systems in order to understand the behaviour of the system with real and simulated environments.

Martinoli, Ijspeert and Gambardella (1999) presented a modeling technique based on rate equations which were successfully applied to several scenarios. Many works which followed this approach can be found not only by Martinoli himself but also by Lerman, Martinoli and Galstyan (2004). A methodologically very sophisticated approach is reported by Lerman and Galstyan (2002) where parameters of the rate equations are determined by the use of the system identification techniques. Galstyan, Hogg and Lerman (2005) presents a generalization of Master equation for densities and continuous space systems. They used microscopic approach to study collective behavior of animals engaged in cluster aggregation and collaborative stick-pulling in which the interactions with environment are modelled as a series of stochastic events, with probabilities determined by simple geometric considerations and systematic experiments.

Apart from these studies population dynamics of systems using the probabilistic models also exist. These consider only the average number of species in a certain state at a point in time but do not take into account the spatial distribution of the animals. Nevertheless, such models have shown strong quantitative agreement with a series of real world case studies where the performance metric is non spatial. Object clustering, collaborative manipulation, inspection have been obtained for model prediction and realistic simulation as presented by Lee, Kim and Kazadi (2005).

Differential and difference equations play a vital role in population dynamics modelling, in order to understand the analytical and control behaviour of the system. Sumpter and Pratt (2003) showed that the Markov chains can be transcribed in to a system of difference or differential equations (one for each state) that summarize the average state transitions and thus track the average number of robots in each state. In many cases, interactions among the species lead to the transition probabilities that are a function of the number of species in other states, and thus yield a system of differential/difference equations that are nonlinear. Lerman, Xu and Tsatsaroni (2002) present a mathematical model of foraging in a homogeneous group using the behaviour based control theory suggested by Lerman and Galstyan (2002). In their work they demonstrated the interference technique by using the series of coupled differential equations.

PROBABILITY MODELS, DIFFERENTIAL EQUATIONS AND MARKOV CHAINS

In this section different models are introduced starting with models for single populations to prey-predator interaction models. It is illustrated that differential equations easily capture real life situations when modelling population dynamics. The theory is then extended to models using markov chains, difference equations and use of partial differential equations.

Single Species Population

Consider a population with N_o on a large, unpopulated area that has plenty of food for survival. Our interest is how this population might vary over time. If we let $N(t)$ be the population at time t (measured in months, say), we would like to make some predictions about the function $N(t)$ as well as find its distribution. It would be ideal to have a formula for $N(t)$ but this is not usually possible. Nevertheless, there may still be a great deal we can say about the behavior of $N(t)$. The first stage is constructing a single species model. After the predictions are made, we look at various ways to adjust the model so that it approximates reality more closely. For the first model, we assume that at any time t, the rate at which the Constant per capita growth of the population changes is simply proportional to the size of the population present at that time. For instance, if there were twice as many animals, then the rate at which the population would be increasing would also double. In mathematical terms, our assumption takes the rential equation

$$\frac{dN(t)}{dt} = kN(t) \quad \text{...1}$$

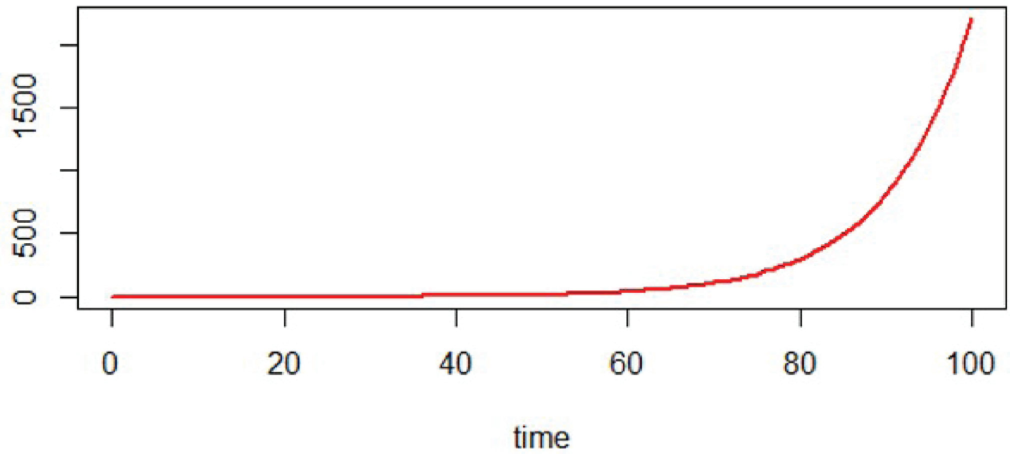

Fig 1: Single population growth curve.

The multiplier k is called the per capita growth rate (or the reproductive rate) One way out of the problem of unlimited growth is to modify equation (1) to take into account the fact that any given ecological system can support only some finite number of creatures over the long term. This number is called the carrying capacity of the system. We expect that of the environment when a population has reached the carrying capacity of the system, the population should neither grow nor shrink (Figure 1). At carrying capacity, a population should hold steady and its rate of change should be zero

$$\frac{dN(t)}{dt} = kN(t)\left(1 - \frac{N(t)}{C}\right) \text{..(2)}$$

$$\frac{dN}{dt} = kN\left(1 - \frac{N}{C}\right) \Rightarrow \frac{dN}{N\left(1-\frac{N}{C}\right)} = kdt \Rightarrow \int \frac{dN}{N\left(1-\frac{N}{C}\right)} = \int kdt$$

Rewrite $\frac{1}{N\left(1-\frac{N}{C}\right)}$ as $\frac{1}{(C-N)} + \frac{1}{N}$ and integrate each term separately. The equation is now $\int \frac{1}{(C-N)} dN + \int \frac{1}{N} dN = \int kdt$

$$-ln(C-N) + ln(N) = kt + D \Rightarrow ln(C-N) - ln(N) = -kt - D$$

$$ln\left(\frac{C-N}{N}\right) = -rt - D \Rightarrow \frac{C-N}{N} = e^{-kt-D} \Rightarrow \frac{C-N}{N} = e^{-kt}e^{-D}$$

Let e^{-C} be A and rearranging become

$$N = \frac{C}{1+A\,e^{-kt}}$$

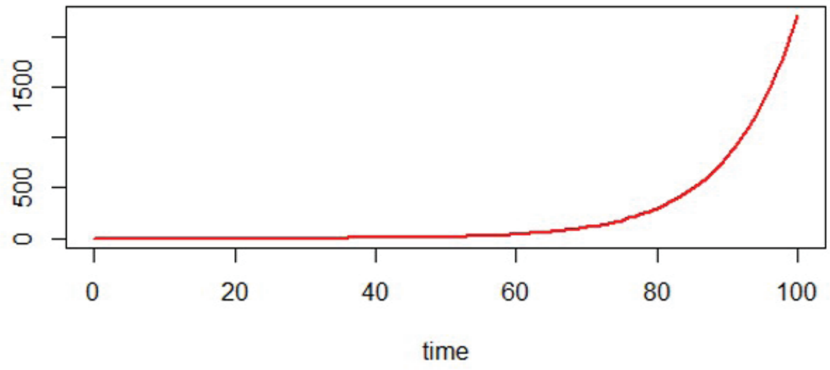

Fig 2: Logistic growth in a single population.

The coefficient k in equation 2 is called the natural growth rate and it plays the same role as the per capita growth rate in equation (1) while the number C is the carrying capacity. As the C approaches the carrying capacity, the population reaches a stable state (Figure 2).

Two-species Population

No species lives alone in an environment. In any habitat there are predators and preys (Figure 3). One species of the population will probably have to deal with predators of various sorts. The model in equation (1) is enriched by adding a predator that will prey on the first species .We will continue to suppose that the prey lives on abundant food, and we will now assume that it's the sole food supply for the predators. Will the number of predators and the preys level off and reach a "steady state" where their numbers don't vary? Or will one species perhaps become extinct? Let $N_1(t)$ denote the number of the prey, and $N_2(t)$ the number of the predator at time t. As before, measure the time t in months. We seek differential equations that describe how the growth rates $\frac{dN_1(t)}{dt}$ and $\frac{dN_2(t)}{dt}$ are related to the population sizes $N_1(t)$ and $N_2(t)$. We make the following assumptions.

i. The prey population increases exponentially in the absence of the predator
ii. The predator population decreases exponentially in the absence of the prey
iii. The two species encounter each other at a rate proportional to their populations
iv. The prey population decreases as a result of the predator species.

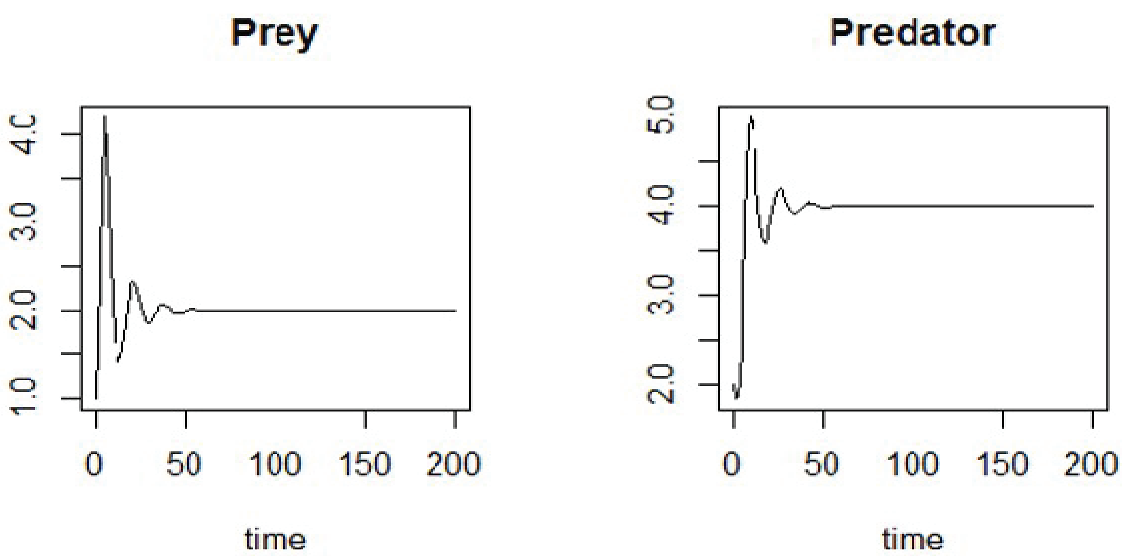

Fig 3: Rosenzweig-MacArthur Predatory-prey Model

Our assumptions are about birth and death rates, so we can convert them quite naturally into differential equations. The assumptions translate into these differential equations:

$$\frac{dN_2(t)}{dt} = aN_2(t)\left(1 - \frac{N_2(t)}{C}\right) - cN_1(t)N_2(t)$$, which simplifies to

$$\frac{dN_2(t)}{dt} = aN_2(t) - \frac{a}{C}[N_2(t)]^2 - cN_1(t)N_2(t)$$

$$\frac{dN_1(t)}{dt} = dN_1(t)N_2(t) - eN_1(t) \quad \text{.......} \quad (3)$$

The differential equations (3) can be solved to obtain the population of the prey and the predator variations as shown in figure 4 and 5. The Lotka–Volterra predator-prey model equations with bounded growth which were originally proposed by A. J. Lotka and V. Volterra in the 1920's. The coefficients a, b, c, d, and e are parameters constants that have to be determined through field observations in particular circumstances.

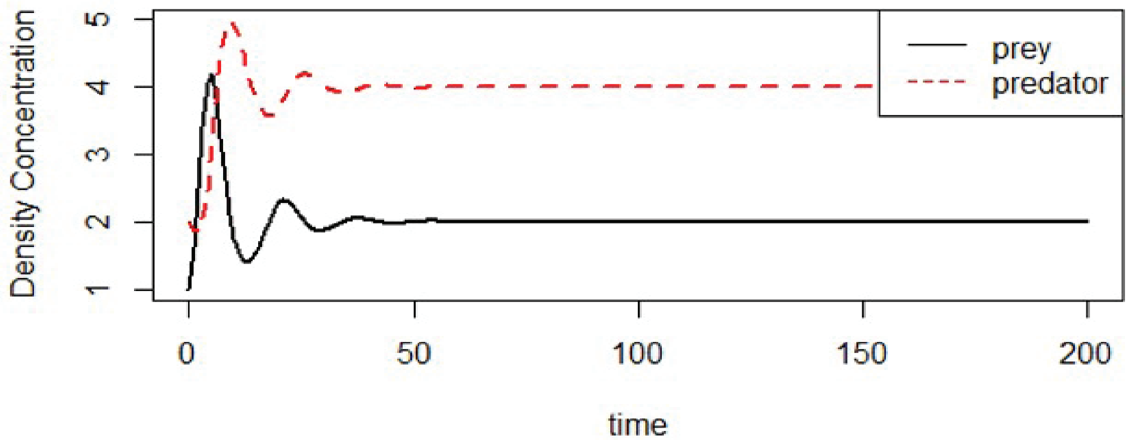

Fig 4: Lotka-Volterra Prey-Predator

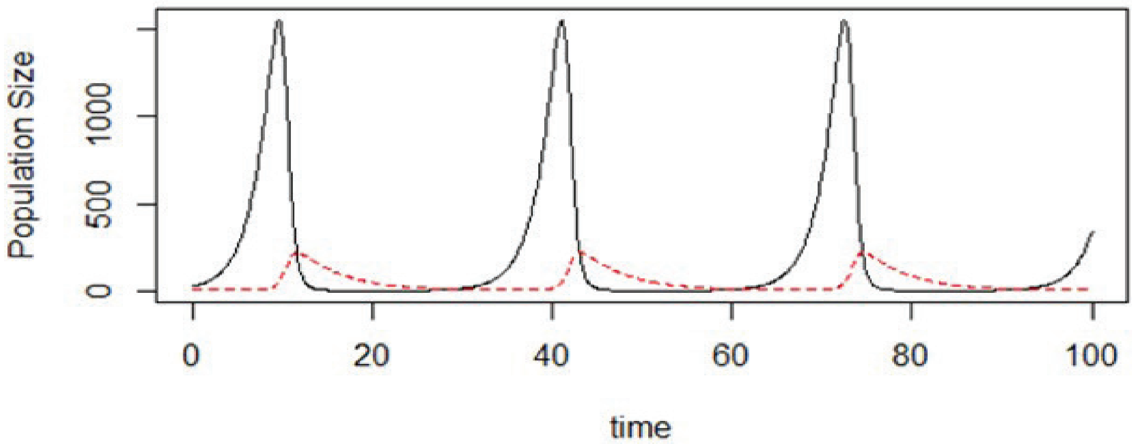

Fig 5: The Prey-Predator interaction

In this section a three species Lotka-Volterra model is considered where we add a second predator species to the model in equation (3). The system of equation therefore becomes

$$\frac{dN_1(t)}{dt} = aN_1(t) - bN_1(t)N_2(t)$$

$$\frac{dN_2(t)}{dt} = -cN_2(t) + dN_1(t)N_2(t) - eN_2(t)N_3(t)$$

$$\frac{dN_3(t)}{dt} = -fN_3(t) + gN_2(t)N_3(t)$$

The three species Lotka-Volterra Model has the following additional assumptions

i. The second species decreases exponentially in the absence of the first predator species

ii. The population of the first predator species decrease with the number of encounters with the second predator species

iii. The second predator species increases with the number of encounters with the first predator species

The additional two constants govern the behavior of the second predator species. Here e is the rate of decrease of first predator species as a result of predation by the second predator species, f is the rate of death of the second predator species and g is the rate of growth of the second predator species as a result of capturing first predator species.

The above systems of equations are solved and the results of the relationship of the three species is shown in figure 6.

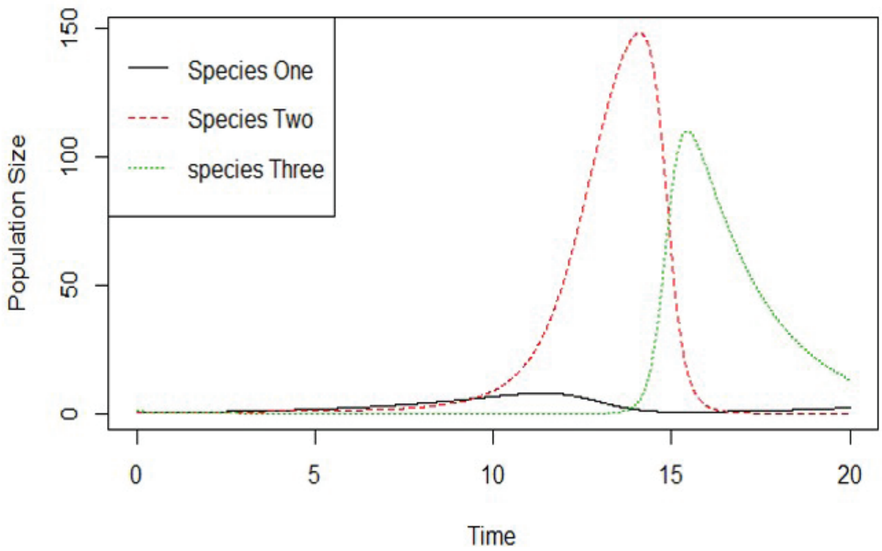

Fig 6: The three species Lotka-Voltera model

Modelling of Joint Probabilities using Markov chains

In environments where we have two species we are usually interested in the population of each of the species so that you would create a balance between the prey and the predators. In this case you would consider estimating the joint probability of having a population of size n_1 for the predator and n_2 for the prey at $time\ t$. A simple birth-death process is studied, which provides a useful introduction to this stochastic modeling. These processes are natural stochastic generalizations of the deterministic population model for population growth of a single species inhabiting an environment in which the amount of resources never changed and the number of other species also remained fixed. On this note, we will consider a natural stochastic generalization of the classical predator-prey model. The classical model is formulated by considering two interacting populations at a time t, a predator species, denoted $N_1(t)$ and a prey species, $N_2(t)$. We formulate this as follows. Let $N_1(t)$ be the size of the predator population and $N_2(t)$ be the size of the prey population at time t. In the model to be formulated, it is now assumed that instead of a (deterministic) rate of predator and prey births and deaths, there is a probability of a predator and prey birth or death. Thus $N_1(t)$ and $N_2(t)$ are time dependent random variables

Let the probability of there being n_2 preys and n_1 predators at time t be denoted by

$$P_{n_1,n_2}(t)=P[N_1(t)=n_1,N_2(t)=n_2]\text{, for } n_1=0,1,2,\ldots,\ n_2=0,1,2,\ldots \qquad \ldots\ldots\ldots\ldots.(4)$$

As in the simple birth-death process for a single species, births and deaths in this process occur proportional to the population size. We assume that the infinitesimal probability of an individual prey birth during the small time interval Δt is $\alpha n_2 \Delta t + o(\Delta t)$, where δ > 0 is the prey birth rate. Similarly, we assume that the infinitesimal probability of an individual predator death during the small time interval Δt is $\delta n_1 \Delta t + o(\Delta t)$, where $\delta > 0$ the predator death rate is. To mimic the deterministic model interaction term, we assume that the infinitesimal probability of a prey death occurring during Δt is $\beta n_1 n_2 \Delta t + o(\Delta t)$ where β > 0 is the prey death rate. Similarly, a predator birth occurs during Δt with infinitesimal probability $\beta\ n_1 n_2 \Delta t + o(\Delta t)$ with γ > 0. Thus, this predator-prey process can be described as having the following transitions and rates:

Transition rate

$$
\begin{array}{lll}
n_2 \to n_2 + 1 & n_1 \to n_1 & \alpha n_1 \\
n_2 \to n_2 - 1 & n_1 \to n_1 & \beta n_2 \\
n_2 \to n_2 & n_1 \to n_1 + 1 & \gamma n_1 n_2 \\
n_2 \to n_2 & n_1 \to n_1 - 1 & \delta n_2
\end{array}
\quad \text{...(5)}
$$

The standard argument using the forward Kolmogorov equation is used to obtain $P_{n_1,n_2}(t)$ by considering the probability $P_{n_1,n_2}(t+\Delta t)$ .This probability is obtained as the sum of the probabilities of the following mutually exclusive events:

a) There are n_2 prey and n_1 predators by time t and no birth or deaths of either species occur in (t, t + Δt).

b) There is $n_2 - 1$ prey and f predators by time t and a prey birth occurs in $(t, t + \Delta t)$.

c) There are n_2 prey and $f - 1$ predators by time t and a predator birth occurs in $(t, t + \Delta t)$.

d) There are $n_2 + 1$ prey and f predators by time t and one prey death occurs in $(t, t + \Delta t)$.

e) There are n_2 prey and $f + 1$ predators and one predator death occurs in $(t, t + \Delta t)$.

It is assumed that Δt is sufficiently small to guarantee that only one such event can occur in $(t, t + \Delta t)$. These probabilities yield

$$
\begin{aligned}
P(t+\Delta t) = {} & (1 - \beta n_2 n_1 + \delta n_1 + n_2 \alpha + n_2 n_1 \gamma + o(\Delta t))\Delta t P_{n_1,n_2}(t) \\
& + (\alpha(n_2 - 1) + o(\Delta t))\Delta t P_{(n_1-1,n_2)}(t) + (\gamma(n_1 - 1)r + o(\Delta t))\Delta t P_{(n_2,n_1-1)}(t) \\
& + (\beta(n_2+1)n_1 + o(\Delta t))\Delta t P_{(n_2+1,n_1)}(t) + (\delta(n_1+1) + o(\Delta t))\Delta t P_{(n_2,n_1+1)}(t) \qquad \text{.........(6)}
\end{aligned}
$$

Rearranging (6) and letting Δt → 0, we obtain for r = 0, 1, 2 . . .
and f = 0, 1, 2 . . ., the state equations for this process as

$$
P'_{n_1,n_2}(t) = \lim_{\Delta t \to 0} \frac{P_{n_1,n_2}(t+\Delta t) - P_{n_1,n_2}(t)}{\Delta t}
$$

$$
= -(\beta n_1 n_2 + \delta n_1 + \alpha n_2 + n_1 n_2 \gamma) P_{n_1,n_2}(t) + \alpha(n_2 - 1) P_{(n_2-1,n_1)}(t) + \gamma(n_1 - 1) n_2 P_{(n_2,n_1-1)}(t) + \beta(n_2+1) f P_{(n_2+1,n_1)}(t) \qquad \text{.........(7)}
$$

The doubly infinite system (7) of differential equations is not easily solved and in fact, it appears to be an open problem to obtain its closed form solution. It should be noted that a numerical solution for birth-death type systems is possible through the method of randomization. The system can however, be studied by letting

$$
\phi(z_1, z_2, t) = \sum_{n_1-0}^{\infty} \sum_{n_2=0}^{\infty} P_{n_1,n_2}(t) z_1^{n_1} z_2^{n_2} \qquad \text{.........(8)}
$$

be the probability generating function (p.g.f.) for this system. Then, φ can be expressed as a partial differential equation. In particular, using (7) in (8) gives

$$\sum_{r=0}^{\infty}\sum_{f=0}^{\infty}P'_{n_1,n_{21}}(t)z_1^{n_1}z_2^{n_2}=\sum_{n_1=0}^{\infty}\sum_{n_2=0}^{\infty}(-(\beta n_1 n_2+\delta n_1+n_2\alpha+n_1 n_2\gamma))P_{n_1,n_2}(t)z_1^{n_1}z_2^{n_2}$$
$$+\sum_{n_1=0}^{\infty}\sum_{n_2=0}^{\infty}(\alpha(n_2-1)P_{(n_2-1),n_1}(t)z_1^{n_1}z_2^{n_2}+\sum_{n_1=0}^{\infty}\sum_{n_2=0}^{\infty}(\gamma(n_1-1)n_2P_{n_2,(n_1-1)}(t)z_1^{n_1}z_2^{n_2}$$
$$+\sum_{n_1=0}^{\infty}\sum_{n_2=0}^{\infty}\beta(n_2+1)n_1P_{(n_2+1),n_1}(t)z_1^{n_1}z_2^{n_2}+\sum_{n_1=0}^{\infty}\sum_{n_2=0}^{\infty}\delta(n_1+1)n_1P_{n_2,(n_1+1)}(t)z_1^{n_1}z_2^{n_2} \quad ....(9)$$

Expanding by distributing the summation through the first term in (5), we obtain

$$\frac{\partial\phi(z_1,z_2,t)}{\partial t}=-\sum_{n_1=0}^{\infty}\sum_{n_2=0}^{\infty}n_1n_2\gamma P_{n_1,n_2}(t)z_1^{n_1}z_2^{n_2}-\sum_{n_1=0}^{\infty}\sum_{n_2=0}^{\infty}n_1\alpha P_{n_1,n_2}(t)z_1^{n_1}z_2^{n_2}-\sum_{n_1=0}^{\infty}\sum_{n_2=0}^{\infty}\delta n_1n_2P_{r,f}(t)z_1^{n_1}z_2^{n_2}$$
$$=\sum_{n_1=0}^{\infty}\sum_{n_2=0}^{\infty}\beta(n_2+1)n_1P_{n_2+1,n_1}(t)z_1^{n_1}z_2^{n_2}+\sum_{n_1=0}^{\infty}\sum_{n_2=0}^{\infty}\delta(n_1+1)P_{n_2,(n_1+1)}(t)z_1^{n_1}z_2^{n_2}$$

Recognizing partial derivative of the probability generating function in (8) we have

$$\frac{\partial\phi(z_1,z_2,t)}{\partial t}=\beta[z_2(1-z_1)-\lambda z_1z_2(1-z_2)\frac{\partial^2\phi(z_1,z_2,t)}{\partial z_2\partial z_1}+\delta(1-z_2)\frac{\partial\phi(z_1,z_2,t)}{\partial z_2}-\alpha z_1(1-z_1)\frac{\partial\phi(z_1,z_2,t)}{\partial z_1} \quad ....(10)$$

as the partial differential equation for the probability generating function of the system (7). From this expression, the (possible) intractability of the system (7) is apparent. The probability generating function (8) can be used to find the expected population sizes. In particular, differentiating (10) with respect to z_1 gives an expression for the expected prey population size as

$$\frac{\partial}{\partial z_1}\frac{\partial\phi(z_1,z_2,t)}{\partial t}=(2z_1-1)\alpha\frac{\partial\phi(z_1,z_2,t)}{\partial z_1}+z_1(z_1-1)\alpha\frac{\partial^2\phi(z_1,z_2,t)}{\partial z_1^2}+(1-z_2)\delta\frac{\partial^2\phi(z_1,z_2,t)}{\partial z_2\partial z_1}$$
$$=(\text{-}z_2\beta+(z_2^2-z_2)\gamma\frac{\partial\phi(z_1,z_2,t)}{\partial z_2\partial z_1}+[z_2(1-z_1)\beta+z_1z_2(z_2-1)\gamma]\frac{\partial^2\phi(z_1,z_2,t)}{\partial z_2^2}$$

Substituting $z_1=z_2=1$ and recognizing expectations gives

$$\frac{\partial}{\partial t}E(N_1(t))=\alpha E[N_1(t)]-\beta E[N_1(t)N_2(t)] \quad ....(11)$$

where $E(\cdot)$ is the expectation. Similarly, the expectation for the predators is found by differentiating (10) with respect to z2. In particular

$$\frac{\partial}{\partial z_2}\frac{\partial\phi(z_1,z_2,t)}{\partial t}=z_1(z_1-1)\alpha\frac{\partial^2\phi(z_1,z_2,t)}{\partial z_1\partial z_2}-\delta\frac{\partial\phi(z_1,z_2,t)}{\partial z_2}+(1-z_2)\delta\frac{\partial^2\phi(z_1,z_2,t)}{\partial z_2^2}$$
$$=+[(1-z_1)\beta+(2z_1z_2-z_1)\gamma]\frac{\partial^2\phi(z_1,z_2,t)}{\partial z_1\partial z_2}+[z_2(1-z_1)\beta)+z_1z_2(z_2-1)\gamma]\frac{\partial^3\phi(z_1,z_2,t)}{\partial z_1\partial z_2^2}$$

Evaluating this expression with $z_1=z_2=1$ and we obtain

$$\frac{\partial}{\partial t}E(N_2(t))=\gamma E[N_1(t)N_2(t)]-\delta E[N_2(t)] \quad ....(12)$$

where $E(\cdot)$ is the expectation. Similarly, the expectation for the predators is found by differentiating (10) with respect to z2. In particular

CONCLUSION

The aim of the article was to show some examples of mathematical models that could illustrate the dynamics in animal populations. Lotka-Volterra population dynamic models were used to illustrate the dynamic nature of the predator-prey relationship. The paper also showed how such problems of the ordinary differential equations could easily be solved using standard statistical software such as R. Finally it was shown that joint probabilities of the proportion of species could be modelled as Markov processes.

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Evaluation of Water Quality in Shallow Wells at Mokowe Village in Lamu County

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ABSTRACT

Inadequate supply of fresh piped water at Mokowe Village in Lamu County has forced the residents to depend on alternative water sources such as shallow wells for their daily needs such as shallow wells. However, most of the wells are located near residential houses less than 30 m from the pit latrines. This closeness contaminates the aquifer that supplies water to the wells. Gastrointestinal infections are among the challenges experienced in Mokowe Village. This study investigated the quality of water in shallow wells at different times of the day. The objective of the study was to evaluate the physico-chemical parameters and microbial contamination of water from two main shallow wells, namely Salim Hassan and Baoni, with respect to their distance from the pit latrines. Water samples from Himwa tap was collected as a control. Total coliforms and *Escherichia coli* (*E.coli*) were analyzed by the most probable number (MPN) method. Physicochemical parameters analyzed were fluorides, nitrates, iron, sodium, potassium, pH, total dissolved solids (TDS), chloride, magnesium, calcium and total hardness, These were determined using DR 6000 Spectrophotometer, flame photometer, pH meter gravimetric analysis and wet methods (titration) respectively. Odour and taste were determined using organoleptic methods while colour was analyzed using a colour comparator. Total coliforms and *Escherichia coli* levels in the two wells were very high in the rainy season compared to dry season while Himwa tap water had no *E.coli*. However, total coliforms counts in Himwa tap water was beyond the permissible levels given by National Environment Management Authority (NEMA). Chloride, nitrate, sodium, potassium and calcium were beyond the allowable Maximum Contamination Level (MCL) provided by NEMA. Levels of pH for all the water samples were within the NEMA standards. The results indicate that water from the two main shallow wells were highly contaminated hence prompt chlorination and water quality monitoring should be undertaken. It was recommended that the public health office should ensure compliance on distance from the buildings when a new well is being constructed.

Keywords: *Shallow wells, Escherichia coli, Pit latrines, Physicochemical parameters, Microbial contamination*

INTRODUCTION

Mokowe Village is located in Hindi Ward, Lamu County. Lamu, is in the north coast of Kenya, borders Kilifi County in the southwest, Garissa County to the north, Republic of Somalia to the northeast and the Indian Ocean to the South (County Government of Lamu [CGL], 2018). In Mokowe Village, the residents have been experiencing difficulties in accessing clean water for drinking and other household needs. Water is one of the most vital basic needs that mankind has to be supplied with. In addition, water is important in many ways, such as, drinking, washing, transportation, chemical uses, heat exchange, fire extinction, recreation and in industrial applications such as; in the production of energy (e.g. hydroelectricity) and food processing among others (Department of Water Affairs and Forestry [DWAF], 1996). Water makes up 2/3 of human body composition; the human brain is made of 95% of water, blood 82% and lungs 90%. Therefore, without water human being can only survive for a few days (Sharma & Sanghi, 2012).

The inhabitants of Mokowe have been experiencing difficulties in accessing adequate clean water for drinking and other domestic needs, as a result the people of Mokowe Village have for a long time relied on the slightly salty water from shallow wells for their daily needs. According to the Lamu water and sewerage company (LAWASCO), inhabitants of Mokowe Village used to receive clean piped water from Amu Island. The project was initiated and managed by the Ministry of Water; however, following revenue losses incurred, the supply of water to Mokowe Village was terminated. It was at this time that the residents of the village reverted to shallow Wells as the alternative source of water to meet their needs.

In the study area shallow wells are surrounded by households with inbuilt sanitation which are not so well designed; with poor and inadequate groundwater protection. The distance between the wells and the pit latrines is less than what is required in the Public Health regulations; this could lead to draining of latrine waste-water into the aquifer supplying water in shallow wells. Majority of disease causing organisms lack the property to propel themselves through the environment in which they live and those that can propel themselves are not capable of traveling very long distances. Instead, pathogens are carried from one point to another within the medium in which they live and in the case of pit latrines water transports the contamination into the aquifer and nearby wells.

The inhabitants complained of gastrointestinal infections since the situation exacerbated in 1997 when cholera struck the area and this compelled the Ministry of Public Health to treat the wells as many lives were lost. In 1998 the Ministry of water discovered a worthy water resource in Chomo, Hindi Ward, Lamu County to supply fresh clean water to the villages around, including Mokowe Village. This forestalled the demand by natives of these villages on shallow wells and other treated water sources. Since the discovery of the new source of water, the ministry relegated the priority of shallow wells and therefore they were unattended to by the Public Health officials.

The new water supply service didn't last beyond the year 2000 as a result of deterioration in the quality and quantity of water. This compelled the community in Mokowe to revert back to harvesting water from the shallow wells, which have hitherto become their only source of water. Shallow wells are therefore a very essential source of water in the study area despite the health risks that may emanate from them due to their close proximity to pit latrines. This study therefore, investigated the quality of water in shallow wells in Mokowe Village in Lamu County to determine its suitability for domestic use.

MATERIALS AND METHODS

The study was conducted at Mokowe Village in Lamu County during rainy and dry seasons; between August and September of 2015 and January and February of 2016 respectively. Mokowe Village is located at 2° 13' 52" South, 40° 51' 31" East and is 242 km from Mombasa. The predominant form of sanitation in the study area is the pit latrine. The area has a total of six (6) shallow wells which are surrounded by households and the study focused on two most used shallow wells which are the main source of water for the residents.

The study focused on two areas within Mokowe village namely Tumbo la Kati and Majengo where the two main shallow wells are located. One well is situated in Majengo area namely Kisima cha Salim Hassan, coordinates 2˚14'19.6"S, 40˚50'46.2′ E, and the other in Tumbo la Kati area namely Kisima cha Baoni, coordinates 2˚14'17.4"S, 40˚50'50.5"E. The choice of the sites was based on the high population that obtains water from these wells for domestic use.

Sampling bottles and tools were sterilized using an autoclave. The study employed purposive sampling, to select the shallow wells for sampling. Two wells were selected namely Salim Hassan and Baoni, these are the only wells used by the community living in Mokowe Village owing to less salinity compared to the rest, which are highly saline and unused.

The study employed both qualitative and quantitative methods. For quantitative method samples were obtained from the shallow wells using a conventional method by the community (a rope tied to a rope). Sampling was carried out three times during the rainy season in August to September 2015, and three times during the dry season in January-February 2016. A total of 50 samples were collected during rainy and dry seasons. Two samples (one for physicochemical and another for microbial tests) were collected from the two most used shallow wells. Samples were collected in the morning at 4 am before the community commenced their daily chores of drawing water from the wells. Two samples were collected after the day activities at 8 pm hence making up a total of eight samples per visit. Water from Himwa tap was collected for comparison (control). The samples were collected in sterilized, sealed and labeled 1L polyethylene bottles for analysis.

The average distance of pit latrines to shallow wells was also measured. Questionnaires were employed as the main data collection instruments for qualitative method. A conventional formula of 10% was used to obtain the size of the sample (sub group). The study applied simple random sampling procedures to obtain the respondents for questionnaires without bias. A total of 225 (10% of households) questionnaires were administered to the residents of Mokowe in October 2015. Three enumerators were hired and trained on how to collect data via questionnaires over 45 day duration.

The physicochemical parameters and microbial levels in the sampled water were tested and analyzed using standard methods and procedures at the Government Chemist Laboratories in Mombasa. Total coliform and *E. coli* were analyzed by most probable number (MPN) method while physicochemical parameters such as fluoride and iron were analyzed using the DR 6000 Spectrophotometer, sodium and potassium were analyzed using flame photometer, pH was measured using pH meter, nitrates amount was determined by DR 6000 Spectrophotometer and chloride, magnesium, calcium and total hardness were analyzed by wet methods (titration). Two-Sample T-Test and Mann-Whitney Test were used to compare the physicochemical and microbial loads between the short rains and dry seasons respectively. Wilcoxon Signed Rank Test and Paired T-test were also used to show disparity between the sampling times (8 pm and 4 am) per visit during short rain and dry season.

RESULTS AND DISCUSSION

The microbial analysis indicates the water in the two wells was highly contaminated with Total Coliforms and *E. coli* (Table 1). The levels of total coliforms were above the NEMA recommended values. However, Himwa sample had *E. coli* within the recommended levels while total coliforms were beyond the allowable limits.

The rainy season did not have a significant change in the Total Coliforms in the two wells at the different sampling times of the day. However, in the dry season, *E. coli* levels were relatively lower than in the wet season. This could be attributed to surface run-off during wet spells that increases pollution into the existing wells as well as transport of faecal materials form the pit latrines.

Table 1: Microbial analysis of water samples

			RAINY SEASON		DRY SEASON	
Site	**Visit**	**Time**	**T. coliform**	***E. coli***	**T. coliform MPN/100 mL**	***E. coli.* MPN/100 mL**
Salim Hassan well	1	8:00pm	>2400	>2400	>2300	220
Salim Hassan well		4:00am	>2400	>2400	1700	18
Salim Hassan well	2	8:00pm	>2400	>2400	1700	36
Salim Hassan well		4:00am	>2400	1100	1700	18
Salim Hassan well	3	8:00pm	>2400	93	2300	220
Salim Hassan well		4:00am	240	240	1700	42
Baoni well	1	8:00pm	>2400	>2400	>1300	130
Baoni well		4:00am	>2400	>2400	>2400	14
Baoni well	2	8:00pm	>2400	>2400	1700	9
Baoni well		4:00am	>2400	1100	2300	0
Baoni well	3	8:00pm	>2400	>2400	>2400	130
Baoni well		4:00am	>2400	>2400	>2400	18
Himwa Tap			1100	0	1100	0
NEMA maximum contamination level (MCL)			10	0	10	0

In the rainy season, total coliforms ranged from highest value of >2400 MPN/100 mL to lowest value of 240 MPN/100 mL recorded in the third sampling in September at Salim Hassan Well. These levels were clearly beyond the maximum contamination levels of 10 MPN/100 mL recommended (NEMA, 2006). Whilst, the total Coliforms for dry season ranged from 1300 to > 2300; these were beyond the maximum contamination levels recommended by NEMA of 10 MPN/100 mL.

E. coli levels in the two wells during rainy season ranged from 93 to > 2400 MPN/100 mL, while for dry season they ranged from 0 to 220 MPN/100 mL. Nevertheless, Baoni Well visit 2 at 4:00 am indicated no *E. coli*, whereas the rest were beyond the maximum contamination level (MCL). Total coliforms in Himwa tap water were 1100 MPN/100 mL which is beyond the recommended levels while *E. coli* level was nil. In the rainy season, both Total coliforms and *E. coli* levels in the two wells were very high compared to the dry season. The smaller the amount of water in the pit latrines, therefore, the lower the risk of water point contamination (Sugden, 2006). The larger the number of users, the higher the amount of water drawn from a well and the higher the hydraulic gradient between the well and the latrine, consequently the higher rate of flow to the well and hence more contamination (Kiptum & Ndambuki, 2012).

Total coliforms and *E. coli* are used as indicators of possible sewage contamination because they are commonly found in human feaces. Therefore, their presence in the sampled water from the selected wells suggests that pathogenic micro-organisms might also be present and consumption of water from these sources might pose a severe health risk (Abdulkadir *et al*, 2015).

The outcome of the physicochemical analysis for both rainy and dry seasons (Table 2) was compared to the acceptable levels designed by NEMA to indicate the suitability of water from Baoni and Salim wells. Odour of samples from both wells in both rainy and dry seasons were earthy-musty. This was attributed to natural biological process compared to Himwa tap water which was odourless. Water from the two wells had a sour taste compared to Himwa tap water which was sweet. The water samples were colourless during rainy season and turbid during dry season. Turbidity in the dry season was attributed to low water levels in the two wells as bucket use to draw the water from the well unsettled water leading to turbidity while Himwa water was colourless. The pH values of all the water samples ranged from 7.20 to 7.82 which were within the NEMA standards. Sodium levels ranged from 714 to 220 mg/L that exceeded the (NEMA, 2006) standard guideline of 200 mg/L.

Fluoride values for both wells during rainy and dry seasons ranged from 0 to1.27 mg/L these were below the standard limit of 1.5 mg/L while Himwa water had 1.2 mg/L. Nitrate levels during both rainy and dry seasons ranged from 0 to 32 mg/L. However in Baoni and Salim Hassan visit 1 at 8 pm and visit 3 at both 8 pm and 4 am respectively during the rainy season, Nitrate levels were beyond the MCL. Similarly nitrate levels of the water sample from Salim Hassan Well visit 1 at 4 am, visit 2 and 3 at 8 pm during the dry season were beyond the MCL. Baoni Well visit 1, 2 and 3 at both 8 pm and 4 am for the dry season had nitrate levels beyond the MCL (Max limit of 10 mg/L). Himwa water had nitrate levels beyond the maximum allowable limits. Mg values were below the permissible level of 6.72-36.2 mg/L (Max limit of 100 mg/L). Chloride and potassium values 450 to 660 mg/L and 280 to 480 mg/L respectively of the two shallow wells were beyond the limits of 200 mg/L Cl and of 100 mg/L K compared to Himwa tap water with potassium and chloride levels fell within the maximum allowable limits.

The total hardness values ranged from 330 to 544 mg/L which exceeded the limit of 300 mg/L compared to Himwa tap with 280 mg/L which was within permissible levels. The total dissolved solids of all the samples ranged from 1800-3800 mg/L and exceeded the MCL of 1200 mg/L. However, Fe levels from all the samples ranged from 0-0.1 mg/L which is below the NEMA limits of 0.3 mg/L (NEMA, 2006). Calcium level of Baoni Well visit 2 at 8 pm, Salim Hassan Well visit 1 and 3 at 8 pm, visit 2 at 8 pm and 4 am during the rainy season were beyond the MCL. Similar trend was seen in Baoni visit 1, 2 and 3 and Salim Hassan visit 1, 2 and 3 at 4 am in the dry season, respectively. Calcium values in both rainy and dry seasons for Himwa water were below the limits (150 mg/L). Two-sample t-test and Mann-Whitney test were employed to show comparison between rainy and dry seasons. However, the

Table 2: - Physiochemical parameters of samples collected from Salim Hassan and Baoni Wells for both rainy and dry seasons.

Parameters	Maximum Limit by NEMA	Time	WELLS PER VISIT DURING RAINY SEASON						WELLS PER VISIT DURING DRY SEASON						Him-wa Tap
			Baoni			Salim Hassan			Baoni			Salim Hassan			
			1	2	3	1	2	3	1	2	3	1	2	3	
Sodium	200(mg/L)	8 pm	872	860	720	1120	1220	1160	722	720	720	1200	1200	1210	350
		4 am	740	740	714	1220	1160	1100	840	842	840	1180	1182	1180	
Fluoride	1.5(mg/L)	8 pm	1.22	1.27	0.8	0.7	1.21	0.8	0.72	0.68	0.70	0.84	0.88	0.82	1.2
		4 am	0.78	0	0.66	0.83	0.66	0.82	1.26	1.24	1.26	0.7	0.8	0.7	
Nitrates	10(mg/L)	8 pm	14	0	20	12	1.2	12	16	14	18	10	12	12	22
		4 am	0	1.4	32	0	1.1	11	22	20	22	12	10	10	
Chloride	240(mg/L)	8 pm	660	540	450	620	640	580	460	462	462	650	652	653	200
		4 am	480	452	540	656	596	652	550	550	550	590	590	590	
Potassium	100(mg/L)	8 pm	472	360	280	452	480	456	280	280	280	398	398	398	55
		4 am	300	300	340	480	460	430	350	352	352	462	460	460	
Magnesium	100(mg/L)	8 pm	36.2	29.76	8.5	12.4	8.64	16.2	6.42	6.42	6.42	34.2	34.2	34.2	7.5
		4 am	6.72	8.64	8.22	35.52	17.28	32.4	30.2	30.0	30.0	16.4	16.2	16.2	
Calcium	150(mg/L)	8 pm	134.2	168	120.2	174.4	174.4	162.2	120	122	122	130.8	130	130	82
		4 am	120	118.4	118.2	132.8	163.2	142.4	170	170	170	160.0	160.2	160	
pH	6.5-8.5	8 pm	7.62	7.57	7.62	7.56	7.52	7.58	7.4	7.4	7.4	7.6	7.2	7.4	7.2
		4 am	7.72	7.53	7.82	7.61	7.59	7.4	7.48	7.46	7.4	7.4	7.4	7.2	
Total Hard-ness	300(mg/L)	8 pm	482	544	330	462	472	482	330	332	332	448	450	452	280
		4 am	328	332	340	480	480	402	550	550	550	462	460	462	
TDS	1200(mg/L)	8 pm	3200	2700	2200	3300	3300	3120	2450	2450	3100	3120	3120	3120	1800
		4 am	2300	2200	2600	3180	3200	3200	2800	2800	3820	3300	3400	3300	
Iron	0.3(mg/L)	8 pm	0.01	0.01	0.02	0	0	0	0.01	0.01	0.01	0.1	0.1	0.1	0.01
		4 am	0.05	0.01	0.05	0.01	0	0.02	0.01	0.01	0.01	0.01	0.01	0.01	

outcomes were not responding since P-values (significant levels) were beyond 0.05. Average levels were used to show comparison and it was apparent that the rainy season had the highest concentration of Physicochemical parameters compared to the dry season since mean and median levels, revealed by both Two sample t-test and Mann Whitney, respectively, showed that the rainy season was high compared to the dry season.

Results obtained from the administered questionnaire on main source of water are represented in figure 1 below. A total of 91% of the respondents used well water as their major source of domestic water, since tap water was not reliable. Majority of the villagers live below the poverty lines and cannot afford to buy water from the nearby village, thus inclining on shallow wells some of which are uncovered at the top. However, 9% said they used tap water to alleviate their needs; they are middle class and said to have been buying water from the nearby Village (Hindi). None were using boreholes or reservoir (Figure 1).

Figure 2 shows the results collected from the respondents in terms of their awareness of the safety of the water. Most respondents (67%) reported to having used water from the wells. These respondents considered the water to be safe for consumption due to numerous efforts made by public health officers to lessen the contamination. Among the efforts they recalled were taking care of the wells in case of an outbreak in the neighboring village and by engaging the villagers through community health workers, distribution of chlorine tablets in households and training households on how to use the tablets. However, 32% reported that the quality of the water was not safe and 1% did not respond. The distance between the pit latrines and the two wells was 11m; which is less than half, the recommended distance.

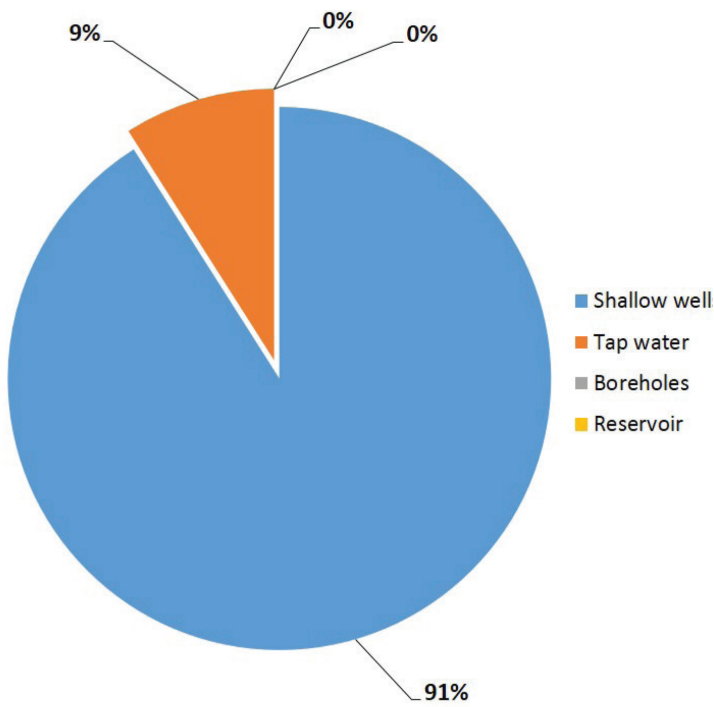

Fig. 1: Sources of water

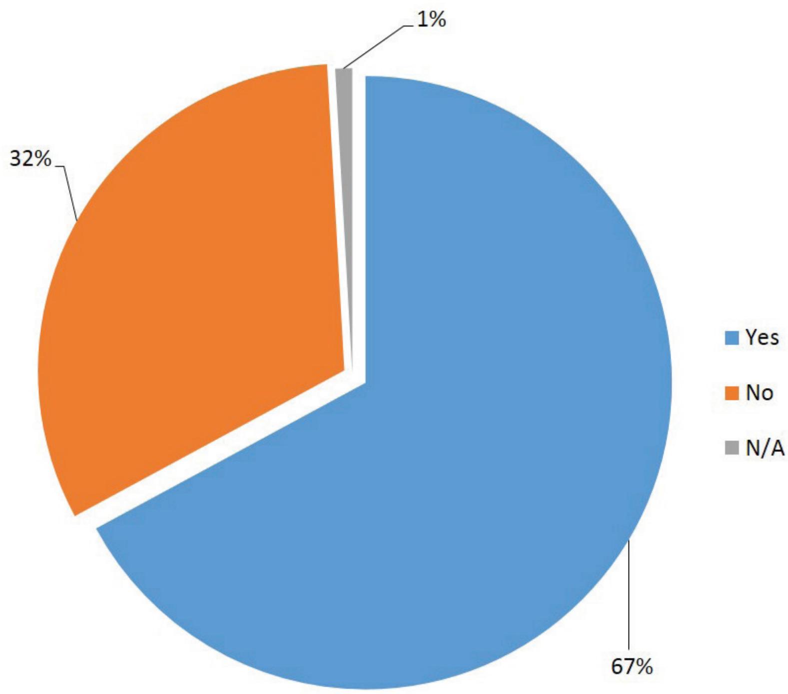

Fig. 2: Safety of water in the shallow wells.

CONCLUSIONS AND RECOMMENDATIONS

The results showed that 91% of the respondents used shallow wells as their main source of domestic water. The findings show that the water from the two main shallow wells was not safe for consumption due to the high levels of microbial contamination and physicochemical parameters. Fluoride, magnesium and iron levels were below the acceptable limits by NEMA while the remaining parameters were beyond the limits for portable water standards.

Himwa water was also not safe for drinking owing to high levels of total coliform which was beyond the permissible levels. Most of the physicochemical parameters were within the acceptable limits except for sodium and nitrate, which were beyond the recommended limit, this can be amended by addition of coagulant such as alum.

The Public Health Officers should enforce set procedures for sinking wells and enhance awareness through enlightenment campaign on the danger of digging shallow wells close to pit latrines. The County Government of Lamu should ensure adequate and efficient public water supply through the provision of piped water.

Treatment of water should be recommended before use and more water sources should be created to avoid enormous pressure on the two main shallow wells which has led to drying of the wells.

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Exploitation of Scented Volatiles and Essential Oil of *Mkilua fragrans* Verdc. for Livelihood Enhancement Among the Coastal Communities of Kenya

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ABSTRACT

Essential oils and volatiles are complex mixtures of biologically active substances used since time immemorial as flavoring agents and constituents of a number of commercial products. The fragrant plant, *Mkilua fragrans* Verdc. forms part of a biodiversity of Keny's coastal forests that has remained under-utilized in a heavily impoverished region. Fragrance from the flowers of the plant have been used for decades alongside *Jasminum L. bignoniaceum* G. Don to enhance the quality of the scent commonly applied as perfume in weddings and other important ceremonies by Swahili, Digo and Arab women. In this study, essential oils of *M. fragrans* were extracted by different procedures and quality of fragrance determined by sensory evaluation. The essential oils were extracted from flowers of *Mkilua fragrans* obtained from Kwale, Kilifi and Mombasa counties by hydro distillation, solvent extraction and effleurage. GC-MS analysis of the extracted oils and volatile compounds from effleurage of fresh *Mkilua fragrans* flowers revealed the presence of esters (16.70%), ethers (14.11%), alcohols (12.87%) and hydrocarbons (11.62%). Esters were in highest proportions in headspace trapping for the three stages with (36.00%), (40.48%) and (47.47%), respectively and also in cold effleurage (16.8%). These results suggest that sesquiterpenes and esters, contribute greatly to the strong and original scent of *Mkilua fragrans*. The stability of the extracts varied from 0 to 52% after 30 days exposure to light under room temperature. These findings provide a basis for a chemical formulation that mimics the *Mkilua* fragrance, which may be exploited in the perfume industry thereby enhancing revenue generation and preservation of the plant.

Keywords: *Mkilua fragrans Verdc., Essential oils, Livelihood enhancement, Coastal communities*

INTRODUCTION

Mkilua fragrans Verdc. *(M. fragrans)* belongs to the Family Annonaceae and is part of a rich forest biodiversity along the coast of Kenya and Tanzania (Muhammed, Pakia & Wainaina, 2014). The plant has been used for decades as a source of perfume by the Swahili and Arab women. According to Verdcourt (1971), *M. fragrans* is identified variably between two Kenya coastal communities as 'Mkilua', 'Mlua' and 'Muua' (Swahili), and 'Mlua', 'Mchilua', 'Mrua' and 'Chingade' (Digo). Apart from the name Chingande from the Digo community, all the other names maintained the prefix M, to denote the recognized growth habit of *M. fragrans* as being a tree ('Mti' and 'Muhi', respectively) and the suffix -ua (flower) (Pakia, Cooke & Van Staden, 2003). *M. fragrans* is sold together with other flowers in a vase as part of decoration during celebrations and ceremonies, including weddings. It is also used in an artistic floral design known as *Kikuba, Kishada, Koja* and *Joho* with a collection of flowers including Ylang ylang, Jasmine, Rose, and Nargis (Muhammed *et al.*, 2014). Previous studies indicate that ethanolic extracts of the root and stem bark of *M. fragrans* were found to be very potent against *E.Coli.* Lyantagaye, (2014) scented extracts from fruits and stem barks of *M. fragrans* gave *mkiluynoic* acid 1 and *mkiluynoic* acid 2, which exhibit antifungal activity against *Candida albicans*. (Baraza, Nkunya, Jonker, Juma & Waibel, 2006). Essential oils extracted from the leaves of *M. fragrans* by hydrodistillation gave limonene, β-elemene and caryophylleneoxide as terpene components (Odalo, 2004). These essential oils were repellent to the mosquito, *Anopheles gambiae* (Odalo *et al*., 2005).

This study as carried out in recognition of the rich biodiversity of the Kenya coastal forest and the increasing threat to these forests, which are partly associated with increased poverty among the local communities. The coastal forests of Kenya are a part of the ''ancient coastal vegetation mosaic'' of eastern Africa, rich in biodiversity, and part of the most important biological systems of the world (Robertson & Luke, 1993). It is also evident from previous studies that there exists untapped biodiversity in this region. There is, therefore, a need to evaluate more plant species as sources of perfume. *M. fragrans* has been in use as a source of perfume for decades. This builds on an already existing local practice, hence would be easily accepted at local level. This in turn is expected to enhance economic empowerment of the local community and especially women who have been at the forefront in the use of *M. fragrans* (Muhammed *et al.*, 2014). The application of *M. fragrans* in perfume has not been fully exploited while alternative potential uses of the scent from the flower such as fragrance for soaps and other toiletries, candles, soft drinks and medicines have not been exploited Muhammed *et al.* (2014) in previous studies, reported that the volatile components of *M. fragrans* were isolated by hydro distillation and solvent extraction. The less pleasant essential oil obtained from the leaves by hydro distillation revealed the presence of camphene, caryophylleneoxide, (-)-dehydroaromadendrene, 4-isopropylbenzene-methanol, limonene and a-ylangene as the major constituents (Odalo, 2004). The methanolic extracts of the flowers revealed the presence of *mkiluaynoic* acid 1 and *mkiluaynoic* acid 2 as the major components (Baraza *et al.*, 2006). The reported data so far has not revealed phytochemical components of the plant's fragrant molecules from effleurage.

MATERIALS AND METHODS

The plant was identified by a botanist and voucher specimen no. HM.NOV./2012/01 is logged at the University of Nairobi Herbarium for future verification. Flowers for extraction by effleurage were collected in two locations namely Mtaani area in Kilifi Town, Kilifi County and Bakarani in Mtopanga, Mombasa County, Kenya. In this study four development stages of the *M. fragrans* flower were identified based on the aroma, floral and petals shape. Extraction by effleurage using virgin coconut oil was done using stage III of the *Mkilua* flower.

Virgin coconut oil was used as a solvent to extract scented volatiles from *M. fragrans* flowers. Both cold and hot effleurage methods were investigated using flower petals of *M .fragrans* Stage III.

Cold effleurage was conducted in two ways either by preserving the oil as well as the flowers according to Putthita *et al*., (2009) with modification where palm stearin was melted at 80^0 C and spread over two frame glass sheets. In this study Virgin coconut oil was used instead of palm stearin and *M. fragrans* flowers soaked in glass bottles. The amount of flowers used ranged from 1000g to 3500g/200mL of palm stearin, while 50g/200 mL of virgin coconut oil were used for *M. fragrans* flowers.

Virgin coconut oil of 200mL was placed in a glass bottle and 50g of *M. fragrans* flowers were added and the mixture left at room temperature (approximately 27^0C) for 48 hours. The flowers were then removed and a clear sample of the virgin coconut oil and Volatile Organic Compounds (VOCs) was withdrawn and set aside for Gas Chromatography Mass Spectroscopy (GC-MS) analysis. Another 50g of fresh flowers were added to the remaining oil, left for 48 hours and liquated again. The procedure was repeated for the third time and another aliquot similarly withdrawn. The three aliquots were analyzed by GC-MS to establish the oil content.

Hot effleurage was done by heating 50 g of flowers/100mL of coconut oil at 60^o C for 30 minutes. The mixture was then cooled and kept in a refrigerator overnight at 4^0C. The oil was then agitated for 3 to 4 days, filtered and finally a clear sample was taken for GC-MS analysis Putthita *et al.* (2009).

Characterization, identification and determination of relative amounts of the components of extracts from effleurage, was done by gas chromatography-linked with mass spectrometry (GC-MS). The GC-MS machine used was of the order Finnigan GC 8000 series, interfaced with a voyager EI-MS detector (CE Instruments, Milan, Italy) used for separation, detection and quantification of the samples. Column Rtx- 5MS dimensions 20m x 0.25mm x 0.25 µm. Injector temp: 220^{0}C Tempprog-initial temperature of 60^{0}C held for 2 minutes then the temperature ramped at a rate of 18^{0}C/min to 240^{0}C and maintained for 10 minutes. Scanning range: 50-450mz 99.99% pure and the gas flow rate were at 25 Kpa. The compositions of essential oils and volatile compounds were identified using standard procedures adapted from (Odalo *et al.*, 2005). The volatile compounds were identified by comparison of their retention index (RI) relative to (C_6-C_{40}) n-alkanes with those of literature and/or those of authentic standards as adopted from mass spectral libraries and comparison with other published spectral data (Robertson & Luke, 1993).

RESULTS AND DISCUSSION

GC-MS analysis of scented volatile compounds extracted from *M. fragrans* with coconut oil yielded a total of 8 compounds. These included hydrocarbons (54.41%), ethers (16.97%), heterocompounds (12.44%) and esters (7.97%) (Table 1).

Table 1: *Mkilua fragrans* flower from cold effleurage with preserved oil sample (first to third) Flower sample

SN.	Compound	% Peak Area		
		Sample 1	Sample 2	Sample 3
1.	tetrahydro-2-(1-methylethoxy)-2H-pyran	5.89	7.78	-
2.	1-(ethenylthio)-octane	12.44	15.53	14.11
3.	3-methyltridecane	1.16	0.89	11.60
4.	n-propylheptylether	1.00	-	-
3.	Trans-2,3-bis(1-methylethyl)oxirane	10.08	11.51	0.82
6.	Isopropyl palmitate	9.25	4.13	10.62
7.	2-(2-hydroxyethoxy)-octadecanoate	-	5.93	6.08
8.	1,1-Dodecanediol	0.84	-	-
9.	11-tridecen-1-ol	7.97	11.16	12.87
10	Bis(2-ethylhexyl)phthalate	-	30.94	-
11.	1-(pentyloxy)-2-hexene	-	7.78	-
12.	2,3,7-trimethyldecane	50.26	-	-
13.	3-methyltetradecane	2.95	-	-
14.	O-(2-methylpropyl)-Hydroxylamine	-	-	2.35
15.	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	-	-	1.47
16.	3-methylpentadecane	-	-	0.65
17.	2,6,10,14-tetramethylheptadecane	-	-	13.12
18.	2-methylpentadecane	-	-	1.79

Preserving the extraction oil lead to a general increase in concentration of the components as depicted by enhanced peaks 1,2,3,4 and 5 (Figure 1 and Table1) as compared to the first sample. The GC-MS analysis (Figure 2 and Table 1) of second flower sample gave esters (40.0%), ethers (23.13%), heterocompounds (15.56%), alcohols (12.10%) and hydrocarbons (0.89%). The main compounds were Bis(2-ethylhexyl)phthalate (30.94%), 1-(ethenylthio)-octane (15.56%), trans-2,3-bis(1-methylethyl) oxirane (11.51%), 11-tridecen-1-ol (11.16%), tetrahydro-2-(1-methylethoxy) 2H-Pyran (7.78%) and isopropylpalmitate (4.14%). The presence of additional compounds in the second sample may have been due to incomplete extraction in sample. Some compounds were missing in this sample and it is thought that they were exhaustively extracted and their concentrations below detectable levels.

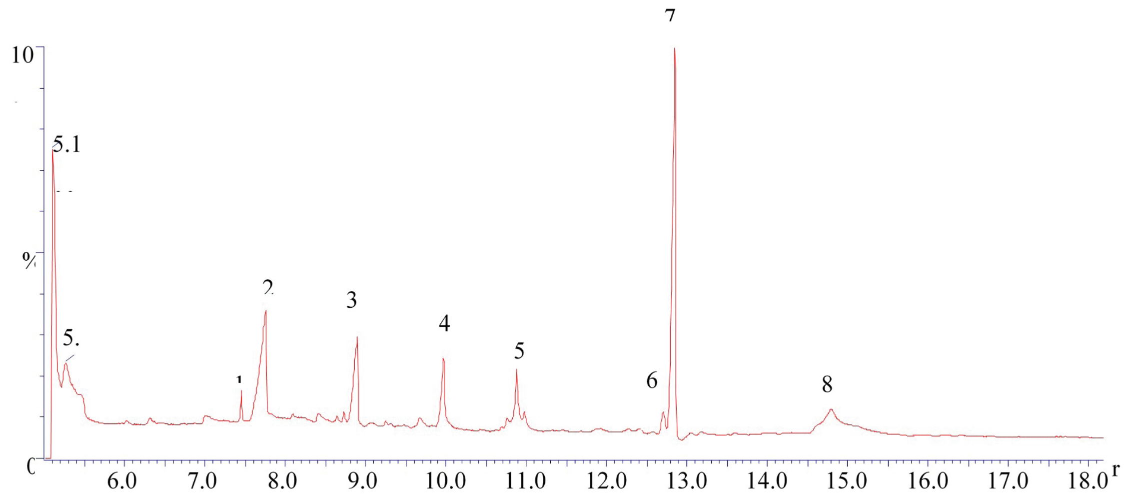

Figure 1: GC-profile of scented compounds from *Mkilua* flowers with preserved coconut oil first flower sample

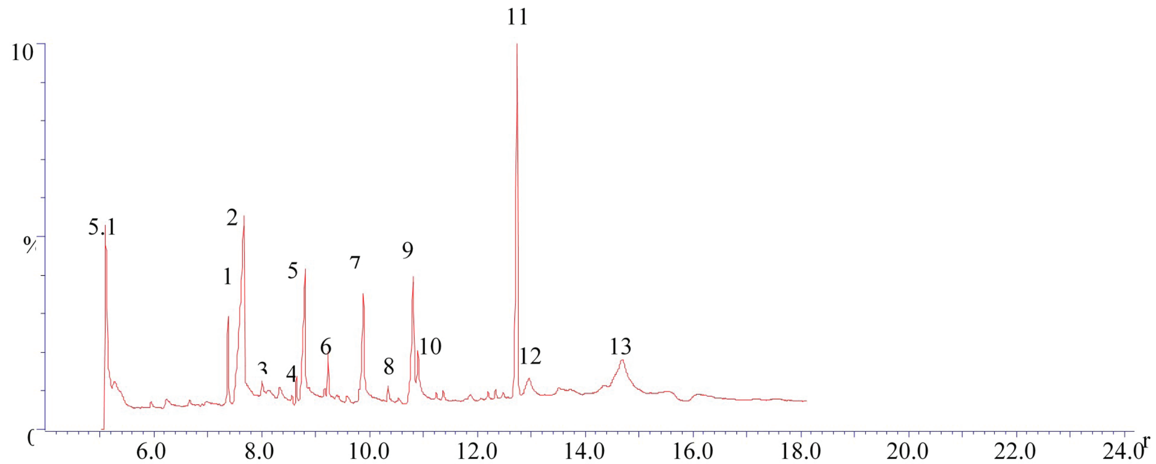

Figure 2: GC-profile of scented compounds from *Mkilua* flowers with preserved coconut oil second flower sample

The extraction of fresh flower samples with the oil used in the second extraction revealed esters (16.70%), ethers (14.11%), alcohols (12.87%) and hydrocarbons (11.62%) by GC-MS analysis. Tetrahydro-2-(1-methylethoxy)- 2H-Pyran was missing in sample 3 while new compounds were extracted such as 3-hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion and O-(2-methylpropyl)-Hydroxylamine. The main compounds were 1-(ethenylthio)-octane (14.11%) 2, 6, 10, 14-tetramethylheptadecane (13.12%), 3-methyltridecane (11.60%) and Isopropyl palmitate (10.62%). (Figure 3 and Table 1).

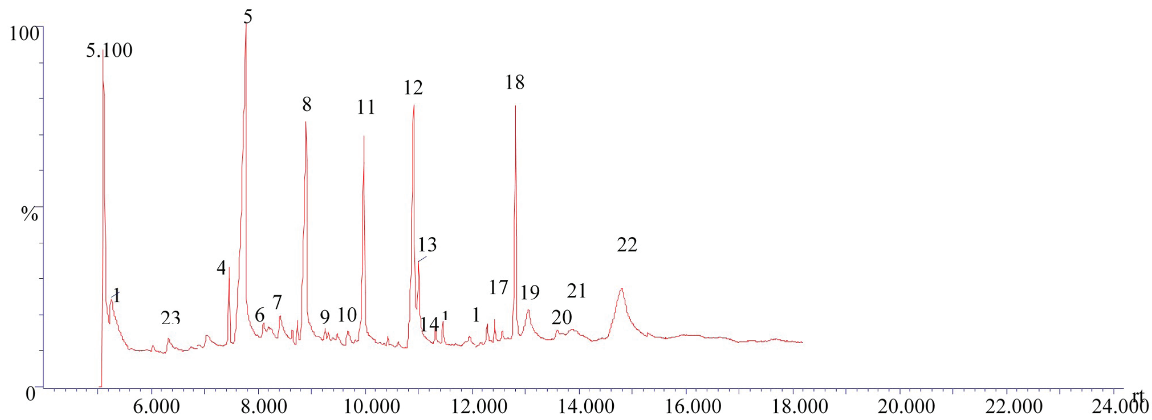

Figure 3: GC-profile of scented compounds from *Mkilua* flowers with preserved coconut oil third flower sample

There was a cumulative extraction of scented volatile compounds by coconut oil making it a suitable solvent for extracting the stated compounds thus qualifying its use for decades by the local Swahili and Arab women in extracting scented volatile molecules from *M. fragrans* flowers. It is evident that sample 3 was more saturated and contained a high proportion of the scented volatile compounds than in sample 2 and 1. This extraction by preserving oil helps to obtain a more scented and concentrated oil extract. Several additional compounds were also obtained from sample 1 to 3. The oil was also more scented than the one extracted from same flower and oil sample. The presence of additional compounds in sample 2 and 3 suggests that the oil/flower ratio was limiting and may not have been optimal for complete extraction of all the scented VOCs.

CONCLUSION AND RECOMMENDATONS

This study established that scent from the *Mkilua fragrans* flower can now be associated with both qualitative and quantitative aspects of the VOCs extracted by effleurage. The fragrance is attributed to the high proportions of esters in the Mkilua oil. The plant should be preserved and artificial fragrance be developed based on established chemical components.

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