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Original article

Isolation of microorganisms and screening of heavy metals from Municipal Council's treated sewage from Nakuru sewage treatment plant in Nakuru County, Kenya

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A B S T R A C T

Diarrhoeal cases due to taking of contaminated water are on the increase Nakuru County. They are a leading cause of human mortality and morbidity. The current study was aimed at isolating microorganisms from Municipal's council treated sewage and screening it for heavy metals followed by testing the efficacy of Moringa stenopetala in removing the heavy metals and treating the sewage against the microbial isolates. In addition, susceptibility test of the microbial isolates to antibiotics and Moringa stenopetala was also carried out. A total of 492 samples were collected out of which 46 % (226/492) were positive for all the microorganisms isolated and heavy metals. Standard methods were used in isolating microorganisms and screening of the sewage for heavy metals. Antimicrobial susceptibility test was carried out using Karby Bauer's disk diffusion test. The percentage reduction of heavy metals was Cadmium (97 %), nickel (97 %), lead (83.17 %) and Chromium (88 %) after treatment of the Municipal council's sewage with Moringa stenopetala. In addition, the microbial isolates reduced by 95.3 % after treatment with Moringa stenopetala. There was significant difference in both heavy metals (p=0.036) and microbial isolates (p=0.02) before and after treatment of Municipals council treated sewage with Moringa stenopetala. There was also a significant difference (p=0.01) in susceptibility patterns of the microorganisms against antibiotics and Moringa stenopetala. Treated municipal council's sewage is highly contaminated with microorganisms that can cause diseases in addition to having highly harmful heavy metals if ingested in food. There is need for proper sewage treatment in Nakuru town in addition to in cooperating Moringa stenopetala in sewage treatment in the town.

1. Introduction

Safe disposal of sewage is a big menace not only in Nakuru County but also in the entire world. The problem has been greatly increased by the rising human populations being witnessed especially in developing countries such as Kenya. Nakuru County is located in the Great Rift Valley, 160 km Northwest of Nairobi at an altitude of 1, 859 m above sea level. The county has a population of 1, 603,325 and an area of 2,325.8 km2. The unique features of the area are volcanic soils, calderas and hot springs (ROSA, 2007).

Sewage treatment is the process of removing contaminants from waste water and household sewage, both runoff and domestic (Korrapati et al., 2010). Sewage-treatment plants attempt to clean up the water that has gone down the drain in homes, businesses and hospitals. Yet even after treatment, this water may still carry microbes and antibiotics. If sprayed into the environment, that water now can spread potentially harmful disease causing microorganisms and traces of the antibiotics (Idzelis et al., 2010).The presence of antibiotics in sewage can be attributed to incomplete metabolism in humans and animals (Pueyo et al., 2001). The more time that bacteria encounter antibiotics, the more likely they are to evolve DNA changes that allow them to survive these germ killers. What's more, even harmless microorganisms that acquire these changes can be problematic (Dacela, 2007).

In addition, sewage has high organic and inorganic substances together with living organisms which include pathogens. As a result sewage becomes a suitable habitat for spread of antibiotics resistance (Guarbassi and Dalsgaard, 2002). However, antibiotic resistance is not always attributed to overuse of misuse of drugs. Sometimes it occurs due to other substances that occur in sewage such as heavy metals (Wireman et al., 2003).

The presence of heavy metals in sewage is a rising concern because the water may be used in irrigation especially of vegetables. Such vegetables can be a health risk when consumed either raw or after processing (Mapanda et al., 2004). Further studies carried out elsewhere indicate that these metals influence the activities of beneficial soil microorganisms (McBride, 2003). Moreover, the behaviour of heavy metals in soils and their plant uptake are dependent on the nature of the metal, the physico-chemical properties of the soil and the plant species (McBride, 2003).

The bioactive potential of Moringa spp. has been reported as back as 1981 (Choubey et al., 2012). Amagloh and Benang (2009) reported that its concentration of 12.0 g/1000 ml loading dose as a coagulant gives similar effect on turbidity compared with alum of loading dose of 10.00 g/1000 mm. According to Pritchard reported that Moringa oleifera removed 84 % turbidity of water initially at 146 NTU (Pritchard, 2010). Apart from the turbidity removal properties, Moringa spp. has been reported to have antimicrobial properties in water (Pritchard, 2010). It has also been reported to have the ability to remove heavy metals from water (Subramanium et al., 2011; Vikashni et al., 2012). However, the potential Moringa stenopetala in treatment of sewage remains unexplored (Yongabi, 2010).

The most common heavy metals are Cadmium (Cd), Lead (Pb), Nickel (Ni) and Arsenic (As) (Tang et al., 2006). Many countries have imposed rules and regulations for controlling the levels of these metals in treated waste water because of their harmful effects more so in body poisoning (Idzelis et al., 12). However, their presence in treated sewage continues to be witnessed in many parts of the world where their harmful effects continue being felt (Korrapati et al., 2010). This study was therefore conceived in order to isolate microorganisms that are resistant to both heavy metals and antibiotics and to determine the best treatment method that can be effective in treating the water.

2. Materials and methods

2.1. Sample size and sample collection

The sample size was calculated by the formula of (Lwanga and Lememshows, 1991) using prevalence rate of 20 % diarrhoeal cases associated with taking contaminated water. The high sample size (492) was used in order to increase the power and reliability of the study and its findings. The following formula was used in sample collection;

$$n = \frac{Z^2 pqD}{d^2}$$

where; n = sample size, p = anticipated prevalence which was 20 % (0.2) in this study, q = failure which was calculated as 100-20 giving 80 % (0.8), Z = is the appropriate value from the normal distribution for the desired confidence level which was 1.96 in this study, d = allowable error (0.05) and D = design effect which was given a value of 2 because replication was carried out. Based on 20 % prevalence and Z value of 1.96 the sample size was;

$$n = \frac{1.96^2 (0.2 \times 0.8) 2}{0.05^2} = 491.724 = 492$$

2.2. Isolation of microorganisms

Municipal council's treated sewage was collected from the treatment plant in a sterile 250 ml beaker and filtered through Whatman No. 40 filter paper. One milliliter of the filtrate was inoculated into 9 ml lactose broth and incubated with at 37 oC for 24 hrs. From this, 0.1 ml was inoculated into nutrient agar (NA) plate and incubated for 24 hrs at 37 oC. Distinct and representative colonies following sub culturing in NA plates were further purified and stored on NA slants in a refrigerator. The isolates were subjected to standard morphological and biochemical tests for identification based on the criteria of Krieg and Holt (1984) and Cowan and Steel (1965). The procedure was repeated after treating the samples with Moringa stenopetala powder. Treated sewage samples from the treatment plant were collected from three different points every four days from January to December 2013.

2.3. Screening for heavy metals

The treated sewage from the treatment plant was analyzed in terms of its heavy metals content using the Standard Methods for the Examination of Water and Wastewater (WHO, 2000). Heavy metals were analyzed using Inductively Coupled Plasma with mass Spectroscopy (ICP-MS) Perkin-Elmer model ELAN 9000 after microwave digestion.

2.4. Antimicrobial susceptibility tests

The antimicrobial susceptibility testing was carried out by use of Kirby Bauer disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI) (Bauer et al., 1996). Sterile wire loop was used to separately pick 3 colonies of each microorganism followed by emulsification in 3 ml of sterile physiological saline. Standardization of the suspended colonies was performed by diluting the normal saline suspension until the turbidity matched the 0.5 McFarland Standards. A sterile cotton swab was dipped into the standardized suspension, drained, and used for inoculating 20 ml of Mueller-Hinton agar in a 150 mm disposable plate (STERLIN, UK).

The inoculated plates were air dried, and antibiotic discs (ABTEK BIOLOGICAL LTD., UK) were placed on the agar using sterile forceps and were gently pressed down to ensure contact. The following antibiotic discs were used: gentamicin (10µg), lincocin (30 µg), rifampin (10 µg), erythromycin (30 µg), chloramphenicol (20 µg), streptomycin (30 µg), ampiclox (30 µg) co-trimoxazole (30 µg), ampicillin (30 µg) and ofloxacin (10 µg). After incubation for 24 h at 37 oC and measurement of inhibition zone diameters, susceptibility ranges were decided according to Prescott et al., (1999). Control plates were incubated without antibiotic discs. Multiple antibiotic resistance (MAR) was also determined. The MAR Index of an isolate is defined as a/b, where a represents the number of antibiotics to which the isolate was resistant and b represents the number of antibiotics to which the isolate was subjected (Bauer et al., 1996).

2.5. Sourcing and preparation of *Moringa Stenopetala* seeds

Seeds were identified by the Kenya Forestry Research Institute. Moringa stenopetala seeds were obtained from Whizpop Products Ltd., Nairobi. Seeds were deshelled by hand and ground in a coffee mill (National MX-J210PN), to obtain a fine powder. Defatted cakes were prepared by cold solvent extraction of the powdered seed with hexane fraction followed by removal of traces of fat by washing with diethyl ether. Drying in a vacuum oven (Gallenkamp OVL 570 010 J) at 40 ÚC and 600 mbars for 24-48 h was then carried out followed by crushing of the cakes to obtain powder (Mangale et al., 2012).

2.6. Antibacterial activity of *Moringa Stenopetala* against the isolates

Dilutions were made up to10-4 using 3 g of Moringa stenopetala powder in 20 ml of distilled water as original sample. Microbial cell cultures isolated from treated sewage from the treatment plant were separately prepared up to a dilution of 10 -4. Nutrient agar (NA) plates in petri dishes were separately inoculated with 0.1 ml of these cultures and spread using a sterile glass spreader. Watman No 1 sterile paper disks were saturated with dilutions of Moringa stenopetala powder and asceptically placed on the petri dishes having NA and the inoculums of microbial isolates. These plates were incubated at 37 °C for 24 h Prescott et al., 1999). Antibacterial activity against the microbial isolates was recorded by measuring the diameter of the inhibition zone. In addition, Multiple antibiotic resistance (MAR) was also determined.

3. Results

3.1. Heavy metal and microbial isolates

The amount of nickel isolated from Municipal council's treated sewage ranged between 99-34 mg/kg higher than that in M. stenopetala treated sewage (2.8-1.0) while that of Pb was 193.7-107.7 mg/kg in Municipal council's treated sewage and in M. stenopetala treated sewage range was 31.6-6.0. Cr varied between 153-50 mg/kg in Municipal council's treated sewage which reduced to 31.6-6.0 after treatment with M. stenopetala. The level of Cd was between 6.3-4.8 in Municipal council's treated sewage which reduced to 0.6-0.1 after treatment with M. stenopetala. After the Municipal council's treated sewage was treated with M. stenopetala Cd and Ni reduced by 97 %, Pb (83.17 %) and Cr (88 %) (Table 1). There was significant reduction in the amount of heavy metal upon treatment of the Municipals council treated sewage with M. stenopetala ($p=0.036$).

Table 1
Heavy metal and microbial isolates from treated sewage obtained from Nakuru sewage treatment plant from January to December 2013.

Sample	Before treatment with *M. stenopetala*								After treatment with *M. stenopetala*							
	Heavy metal (mg/kg D.W.)				Microorganism				Heavy metal (mg / kg D.W.)				Microorganisms			
	Ni	Pb	Cr	Cd	Vib	Sal.	Shi.	E. coli	Ni	Pb	Cr	Cd	Vib	Sal.	Shi.	E. coli
1	44.0	145.0	153.0	4.8	1.7	2.3	4.3	5.5	1.3	24.0	18.4	0.1	7.9	1.1	2.0	2.6
2	66.0	107.7	83.0	6.3	1.2	3.1	3.7	3.0	2.0	18.1	10.0	0.2	5.6	1.5	1.7	1.4
3	92.0	115.0	50.0	4.9	1.9	3.4	2.9	5.3	2.8	19.4	6.0	0.6	8.9	1.6	1.4	2.4
4	34.0	190.0	263.0	5.2	1.5	2.8	4.1	4.7	1.0	32.0	31.6	0.2	7.1	1.3	1.9	2.2
5	99.0	193.7	123.0	5.7	1.1	4.6	2.4	2.5	3.0	32.6	14.8	0.2	1.2	2.2	1.1	1.2
Mean	67.2	150.2	134.4	5.4	1.5	3.2	3.5	4.2	2.0	25.3	16.2	0.3	6.1	1.5	1.6	2.0
WHO std	20 µgNi/l	10 µgPb/l	50 µgCr/l	5.0 µgCd/l	Zero	Zero	Zero	Zero	20 µgNi/l	10 µgPb/l	50 µgCr/l	5.0 µgCd/l	Zero	Zero	Zero	Zero

Samp, Sample; D.W., Dry weight; Ni, Nickel; Pb, lead; Cr, Chromium; Cd, Cadmium; Vib, vibro; Sal., Salmonella; Shi., Shigella; std, standard.

On the other hand, Vibrio spp. Ranged between 1.9 x104-1.1 x104 in municipal council's treated sewage which was reduced to 8.9 x102-1.2 x102 after treatment with M. stenopetala. Before treatment with M. stenopetala, the Salmonella ranged between 4.6 x104-2.3 x104 in Municipal council's treated sewage while in M. stenopetala treated sewage the variation was 2.2 x102-1.1 x102. Shigella varied between 4.3 x104 -2.4 x104 in Municipal council's treated sewage which reduced to 2.0 x102-1.1 x102 after treatment with M. stenopetala. In addition, E. coli ranged between 5.5 x104 -2.5 x104 in Municipal' council's treated water which went down to 2.6 x104-1.2 x102 in M. stenopetala treated sewage (Table 1). The general reduction of microorganisms after treating Municipal council's treated sewage with M. stenopetala was 95.3 %. There was also a significant reduction in the level of microorganisms upon treatment of the Municipals council treated sewage with M. stenopetala (p=0.02).

3.2. Antimicrobial susceptibility

In municipal council's treated sewage, Vibrio spp. had high resistance to Lincocin (90 %), Ampicillin (70 %), rifampin (60 %) and ofloxacin (60 %). On the other hand Vibri spp had high sensitivity to chloramphenicol (80 %), Gentamicin (70 %) and erythromycin (60 %). Salmonella spp. was highly resistant to Lincocin (90%), ampicillin (80 %), rifampin (70), erythromycin (70 %), ofloxacin (70 %) and ampiclox (60 %). However, bacteria showed high sensitivity to chloramphenicol (90 %), gentamicin (80 %) and co-trimoxazole (80 %). In Shigella spp, high resistance was witnessed in lincocin (80 %), rifampin (70 %), ampiclox (70 %) and ampicillin (60 %) while it was highly sensitive to Gentamicin (90%), chloramphenicol (60 %) and ofloxacin (60 %). All E coli were resistant to lincocin in addition to being highly resistant to ampicillin (70 %) and rifampin (60 %). However it was highly sensitive to ampiclox (80 %), co-trimoxazole (70 %), cloramphenicol (60 %), and ofloxacin (60 %) (Table 2).

Treating the municipal council's treated sewage with Moringa stenopetala greatly reduced antibiotic resistance. In Vibrio spp., 70 % of the bacteria were sensitive; intermediate were 10 % while the proportion that was resistant was 20 %. The Salmonella spp. that were senstitive were 80 %, intermediate 10 %, and those that were resistant (10 %). The sensitivity of Shigella spp. M. stenopetala was 70 %, intermediate (15 %) while the resistant ones were 15 %. Lastly sensitivity of E. coli to M. stenopetala was 70 %, intermediate 10 % and those resistant were 20 % (Table 3).

In Municipal council's treated sewage, highest MAR index was shown by E. coli (0.41), followed by Salmonella spp. (0.35), Vibrio spp. (0.31) while the least was in Shigella spp. However, the MAR index reduced after treatment with Moringa stenopetala. In this case, the highest MAR was exhibited by E. coli (0.70), followed by Shigella spp. (0.16), Vibrio spp. (0.14) and the least was in Salmonella spp. (0.10). The highest increase in susceptibility was observed in Vibrio spp. (80 %) followed by Salmonella spp. (70 %), Shigella spp. (70 %) and lastly E. coli (53.30).

Table 2

Antimicrobial susceptibility test (%) results for ten antimicrobials in Nakuru sewage treatment plant from January to December 2013 before sewage treatment with M. stenopetala.

Antib	Vibrio spp.			Salmonella spp.			Shigella spp.			E.coli		
	S	IR	R	S	IR	R	S	IR	R	S	IR	R
Gen (10μg)	70.0	0.0	30.0	80.0	0.0	20.0	90.0	0.0	10.0	50.0	0.0	50.0
Lin (30 μg)	0.0	10.0	90.0	10.0	0.0	90.0	10.0	10.0	80.0	0.0	0.0	100.0
Rifa (10μg)	30.0	10.0	60.0	10.0	20.0	70.0	20.0	10.0	70.0	30.0	10.0	60.0
Ery (30μg),	60.0	10.0	30.0	30.0	0.0	70.0	40.0	10.0	50.0	50.0	0.0	50.0
Chlo (20μg)	80.0	0.0	20.0	90.0	0.0	10.0	60.0	20.0	20.0	60.0	10.0	30.0
Strep (30μg)	50.0	40.0	10.0	60.0	10.0	30.0	30.0	30.0	40.0	50.0	10.0	40.0
Ampi (30μg)	20.0	30.0	50.0	20.0	0.0	80.0	20.0	20.0	60.0	80.0	0.0	20.0
Cot (30 μg),	50.0	10.0	40.0	80.0	0.0	20.0	40.0	10.0	50.0	70.0	0.0	30.0
Amp (30 μg)	20.0	10.0	70.0	30.0	10.0	60.0	20.0	10.0	70.0	30.0	0.0	70.0
Oflo (10μg).	30.0	10.0	60.0	10.0	20.0	70.0	60.0	10.0	30.0	60.0	10.0	30.0
Mean	41.0	13.0	46.0	42.0	6.0	52.0	39.0	13.0	48.0	48.0	4.0	48.0

Antib, Antibiotic; S, sensitive; IR, intermediate resistant; R resistant; Gen, gentamicin; Lin, lincocin; Rifa, rifampin; Ery, Erythromycin; Chlo, chloramphenicol; Strep, streptomycin; Ampi, ampiclox, Cot, co- trimoxazole, Amp, ampicillin, Oflo, ofloxacin (10 μg).

Table 3
Susceptibility test (%) results for the isolates against M. stenopetala in Nakuru sewage treatment plant from January to December 2013.

Microorganism	Sensitive	Intermediate	Resistant
Vibrio spp	70.0	10.0	20.0
Salmonella spp.	80.0	10.0	10.0
Shigella spp.	70.0	15.0	15.0
E.coli	70.0	10.0	20.0
Mean	72.5	11.25	16.25

Table 4
Multiple Antibiotic Resistance (MAR) and % increase in susceptibility values of microbial isolates in Nakuru sewage treatment plant from January to December 2013.

Microbial isolate	MAR*	MAR**	% increase in susceptibility
Vibrio spp.	0.31	0.14	80.00
Salmonella spp.	0.35	0.10	70.00
Shigella spp.	0.30	0.16	70.00
E. coli	0.41	0.70	53.30

MAR*, MAR before treatment with M. stenopetala; MAR**, MAR after treatment with M. stenopetala.

4. Discussion

Nakuru town is located in the Great Rift Valley which has many unique features. The population of the town has been increasing at a rate of 5.6 % per annum. The current population estimate of the town is 760 000 (ROSA, 2007). In addition, the town is home to many industries which could further be aggravating the problem.

The high level of heavy metal isolates in this study attest to the fact that Nakuru has many industries which release their wastes into the sewers ending up in the sewage treatment plant. These results agree with previous studies by Arafat and Mohamed (Arafat and Mohamed, 2013). In addition, it may be an indicator of poor methods of heavy metal removal from Municipal sewage treatment in the current study area. The high success in reduction of heavy metals in this study contradicts with a study by Madrona (Madrona, 2012). The differences in the amount of M. stenopetala could be a contributing factor.

There was high level of microbial isolation from treated sewage in this study than in a previous by Onyunka et al. (2013). The increase in population of Nakuru town especially after the 2007 tribal crashes has strained the resources so much leading to allot indiscriminate wastes disposal. This has increased the rates of infections which have contributed to antimicrobial abuse and misuse. As a result the town has witnessed rising levels of antimicrobial resistance (ROSA, 2007). Failure of the Municipal council of Nakuru to embrace modern methods of water treatment may have also heightened the problem.

However, the potency of M. stenopetala in reducing the microbial level in Municipal council's treated water is a great stride in solving this problem. But this method does not make the water completely safe. This suggests further treatment of the sewage before it is released into the environment (Amogloh and Benang, 2009). The reasons for these results could be vested in sub-optimal use of M. stenopetala coupled with antimicrobial resistance of the isolates.

Microbial resistance of antimicrobials is a growing concern to medics and public health officers. The microbial isolates in the current study were most resistant to lincocin and rifampin and most sensitive to gentamicin and chloramphenicol in Municipal council's treated sewage. This could be probably because lincocin and rifampin have been used for a long time. In addition, the emergence of sensitivity to old generation drugs such as gentamicin and chloramphenicol suggest reduction of resistance to these drugs due to reduced usage (Aminov and Mackie, 2007).

Despite the high resistance to antibiotics observed with Municipal council's treated sewage, all microorganisms indicated high sensitivity to M. stenopetala. These results are in agreement with a previous study by (Subramanium et al., 2011). This could be attributed to low usage of M. stenopetala therapy as is the case with the tested antibiotics (Mangale et al., 2012). The MAR indexes in microorganisms isolated from Municipal council's treated sewage were beyond 0.02 indicating that there has been high risk of exposure to antibiotics. This is in concurrence with a study carried out by (Atieno et al., 2014). The possible reasons could be indiscriminate use of antibiotics in both human and animal therapy. However, the MAR index of the microorganisms when using M. stenopetala was lower than 2.0 suggesting low risk of exposure. There was also high percentage increase in microbial sensitivity to antibiotics indicating high capability of M. stenopetala in solving problems of antibiotic resistance. The results agree with a study carried out elsewhere by (Amogloh and Benang, 2009). This could be have resulted from low mutation of the microorganism to the active ingredients present in M. stenopetala (Alo et al., 2012).

4. Conclusion

The Municipal council's treated sewage in Nakuru is highly contaminates with potentially harmful microorganisms. It also has high levels heavy metals which posses a big risk if taken for example when feeding on vegetables grown using the treated sewage. However, M. stenopetala has a high potential of rectifying the problem. We recommend thorough treatment of sewage and use of M. stenopetala in removing the heavy metals and reducing the microorganisms if not eradicating them all together.

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