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Evaluation of quality and toxicological aspects of river water treated with slaked lime

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ABSTRACT

Objectives: This study was carried out to investigate the quality and toxicological effect of slaked lime treated river water on the liver and kidney of white albino rats (*Rattus novergicus*) at contact times varying from 0 to 504h.

Methodology and results: Agba River was treated with lime at concentration of 0.5g dissolved in 100ml of river water sample. After treatment, the total bacteria count ranged between 8.6 x 10^3 to 0.5 x10^3cfu/ml, total coliform count ranged between 7.7 x 10^3 to 0.2 x10^3cfu/ml, pH ranges from 6.6 to 4.4, water temperature was between 23^{o}C and 29^{o}C, turbidity also ranges from 0.301(NTU) to 0.018(NTU), and total dissolve solid ranges from 0.1mg/l to 0.0mg/l. Six bacterial species were isolated viz; *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaricus, Bacillus subtilis* and *Staphylococcus epidermidis.* Histopathology of the liver and kidney after force feeding the rat with lime treated water at varying contact times revealed no destruction to the tissues from 0 to 504 hours. The centrolobular (central vein) and portal tract that forms the liver plates with portal space of the liver and urinary space and glomerular capillary of the kidney appeared normal as compared to the control.

Conclusion and application of findings: The efficacy of slaked lime in treating river water has been demonstrated with its non- toxic effect on the kidney and liver of rats at a concentration of 0.5g per 100ml of river water sample. Slake lime can therefore be recommended as an alternative coagulants and antimicrobial agent in current approaches for water treatment.

Key words: Slaked lime, river water, histopathology, rat.

INTRODUCTION

Oceans, streams, lakes and rivers constitute surface water. The quality of surface water depend on its contact with living organisms and by the amount of mineral and organic matter which may have picked from the surface that may have seeped into the ground and accumulated above impervious strata (Morgan, 1990). Water and heath are intricately linked to surface water since water acts as a conveyance medium for pathogens as also it provides the habitat for vectors and intermediate hosts of pathogens (Olayemi, 2007).

Diseases associated with lack of access to clean water are measured principally using Disability-Adjusted Life Years (DALY). Data are

organized by age and include information on sex and geographical region for diarrhea, malaria, schistosomiasis, lymphatic filariasis, onchocerciasis, dengue, Japanese encephalitis, trachoma, intestinal nematode infections, protein energy malnutrition and drowning (Lechevallier *et al., 1983)*. In 2002, diarrhea diseases and malaria accounted for 1.8 and 1.3 million deaths, respectively, which were almost entirely children under 5 years of age. Diarrhea remains the leading cause of death from water-related diseases in children. In developing countries it accounts for 21% of all deaths in children aged below 5 years. Although children mortality rate is decreasing, the proportion of deaths due to persistent diarrhea and dysentery is increasing (WHO and UNICEF, 2006). Water purification generally means freeing water from any kind of impurity such as contaminants or microorganisms through a process of many steps that vary depending on the kind of impurities that are present (Lenntech, 2008).

Chemical treatment entails addition of certain chemicals that cause changes in the structure of the contaminants so that they can be removed more easily (Lenntech, 2008). Calcium hydroxide [Ca $(OH)_2$] traditionally called slaked lime, hydrated lime, or pickling lime, is a colourless crystal or white powder, that is obtained when calcium oxide (*lime* or *quicklime*) is mixed, or "slaked" with water (Halstead & Moore, 1957). In terms of annual tonnage, lime ranks first among chemicals used in the treatment of potable and industrial water supplies. Lime is used by many municipalities to improve water quality, especially for water softening and removal of arsenic. The American Water Works Association has issued standards that provide for the use of lime in drinking water treatment (Graymont, 2008).

The liver is the largest internal organ in the human body, and it plays a major role in metabolism and has a number of other functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis and detoxification (Sanjiv, 2002).The kidneys are complex organs that have numerous biological roles, primarily in maintaining the homeostatic balance of bodily fluids by filtering metabolites (such as urea) and minerals from the blood and excreting them, along with water, as urine (Bruce, 2004). Today, the availability of portable water to each country is unique and usually constant; yet the demand for water continues be on the increased daily. The problem now is how to balance demand and supply. In the near future, the availability of water rather than land will be the main constraints to the development of countries. This present study intended to review the process of sedimentation, clarification and disinfection as important steps in improving water treatment using effective, easily and locally available and highly efficient coagulants and disinfectants.

This study investigated the efficacy of slaked lime in treating surface water and the possible toxicological effects of the treated water on the kidney and liver of white albino rats.

MATERIALS AND METHODS

A total of 64 albino rats (*Rattus novergicus*) weighing between 120-150g were obtained from the small animal unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. Calcium hydroxide (slake lime) was procured from Sigma chemical company, London. All other reagents used were of analytical grade and were prepared using distilled water.

Water sample was collected from Agba River between 9.00 am and 11.00amaccording to the procedures of APHA (1985), and immediately transported to the laboratory for analysis.

Slaked lime water: To prepare slaked lime water 0.5g of calcium hydroxide was dissolved in 100ml of water sample collected. The chemical reaction is CaO(s) + H_2O (l) ↔ Ca $(OH)_2$(s). Water sample was treated with slake lime at the concentration of 0.5%w/v.The treated water was then analyzed for total bacteria count, total coliform count, and physio-chemical properties before administering it to white albino rats at contact time interval of 0, 24, 48, 72, 96, 168, 336 and 504 hours.

Animal studies: The rats were fed *ad libitum* for 24 to 48 hours to stabilize them before being subjected to the experimental study. The rats were placed randomly into two groups, each having 32 albino rats. After having been fasted overnight, the rats were allocated into treatment groups as indicated below;

Group I: Rats fed with 5ml of treated water daily;
Group II: Rats fed with 5ml of sterile distilled water daily (control).

Four rats in each group were sacrificed 24 hours after daily doses (Akanji & Nlumanze, 1987). All groups were fed with double distilled water with batex feed (commercially formulated feed) after daily doses of treatment administration. The experimental set–up was observed over a period of 504 hours (21 days). Histopathological studies on the liver and the kidney were carried out according to the method described by Krause (2001).

Isolation and identification of bacteria from treated water: Serial dilution was done for the treated water sample (experimental sample) at the fold of 1/1000 or 10^{-3} dilution after treatment was carried out and inoculated into sterile Petri-dishes using pour plate method. The media used for isolation of bacteria and coliforms from the river water were Nutrient agar (oxoid), Eosine Methylene Blue (EMB) and Methyl Red-Voges-proskauer broth. Media were prepared according to the manufacturer's instructions. The plates were inoculated in duplicates and then incubated at 35°C for 24h. Total bacteria and total coliform counts were carried out and isolates were subjected to biochemical tests and tentative identification done using the Bergey's Manual of Determinative Bacteriology (Buchanan & Gibbons, 1974).

Physico-chemical analysis: The hydrogen ion concentration (pH) of the treated water sample was measured using a pH meter with glass electrode (Philip Model 9418). Water temperature was determined using a mercury bulb thermometer dipped up to about 15cm below the surface of the container and left for about 3 minutes (when the mercury fluid remain steady).

To determine the total dissolved solids**,** the weight of dried filter paper (membrane filter paper) was measured, the water sample was filtered about 10ml through the filter paper, the filter paper was dried in an oven at 80°C until a constant weight was obtained, the filter paper was then weighed. The final weight of the filter paper was then taken and subtracted from the initial weight.

The turbidity was determined by the use of spectrophotometer (Cam Spec N105). Five milliliter of water sample was put into a cuvette and the turbidity values read off the indicator at 690nm.

RESULTS

The pH of the treated water ranged from 6.6 to 4.4 (Table 1), being highest at 0 hour contact time and gradually decreasing to 4.4 after 504h. The temperature ranged between 23 and 29°C. Turbidity of the water sample after treatment was 0.0301(NTU) immediately after treatment (0h) and thereafter it gradually decreased to 0.018(NTU) after 504h. The total dissolved solids ranged from 0.10 to 0.00mg/l at 0 and 24h, respectively, and thereafter remained constant to the end of the experiment. The highest bacterial count was 8.6 $x10^3$ at 0h, and there was a significant reduction in the colony counts to 0.5 $x10^3$ after 504h. The coliform counts at 0h were 7.7 x 10^3 with a gradual reduction to 0.2 x 10^3 at 504h contact time (Table 2). Six bacterial isolates were obtained from the slake lime treated water which include *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus epidermidis, Staphylococcus aureus,* and *Proteus vulgaricus* (Table 3).

The histopathologic examination of the liver and kidney after feeding rats on treated water for varying periods of time did not show any damage to the tissues of the liver and kidney as they resembled those of the control (Figures 1 to 4). Result revealed that at the varying contact times of the experiment the histology features of the liver (Figures 1 and 2) revealed that there was normal centrolobular (a central vein), the portal tract forming the liver plates that anastomose freely, limiting the space occupied by the sinusoids PT stain. The sinusoids contain the kupffer cells (i.e. liver macrophages). A portal space with its characteristic small artery, vein lymph vessels, and bile duct surrounded by connective tissue are shown, indicating that no damage was done to the tissues as compared with the control. Also,the histological features of the kidneys at varying contact times of the experiment (Figures 3 and 4) revealed no damage, but present distinctively cell bodies that are normal. The urinary space and the glomerular capillary are as well indicated. The renal cortex is shown with the convoluted tubules and the juxtaglomerular rennin secreting cells, which appear stained. The brush borders formed by the microvilli of the cuboidal cells of the proximal convoluted tubules are seen on PT stain. All these cells and their arrangement showed no damage or inflammation as compared with the control.

Table 1: The pH, temperature, turbidity and total dissolved solid of treated water over time.

Contact time interval (hours)	pH ±0.10	Temperature (°C) ±0.12	Turbidity (NTU) ±0.0003	Total Dissolved Solid (mg/l) ±0.001
0	6.6	23	0.301	0.10
24	6.3	29	0.193	0.10
48	5.4	27	0.049	0.00
72	5.2	26	0.025	0.00
96	5.2	28	0.022	0.00
168	5.0	26	0.021	0.00
336	5.1	25	0.020	0.00
504	4.4	27	0.018	0.00

Values are mean of three replicates determination (± SEM)

Table 2: Total bacteria and coliform count in the - river water sample treated with slaked lime.

Contact time interval (hours)	Total Bacteria count ($x10^3$) cfu/ml	Coliform count ($x10^3$) cfu/ml
0	8.6	7.7
24	8.0	5.8
48	7.5	4.4
72	6.6	3.9
96	4.0	3.5
168	3.5	1.5
336	1.5	0.5
504	0.5	0.2

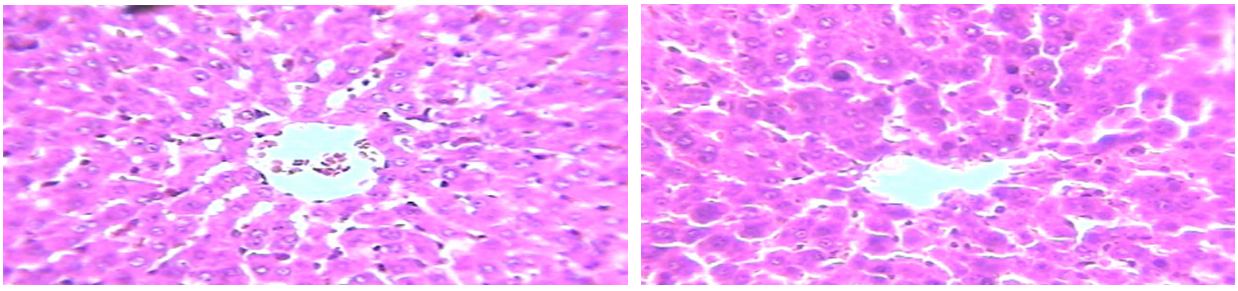

Figure 1 (left): Histopathology of liver of the control rats (fed on water without lime) (stained with Haematoxylene and Eosin) (x400). The field shows normal centrolobular (central space) and portal tract. **Figure 2 (right)**: Histopathology of liver of rat fed on water with slaked lime at varying contact time interval of 504hours. All others appeared the same (stained with Haematoxylene and Eosin) (x 400). Field shows normal centrolobular (central space) and portal tract.

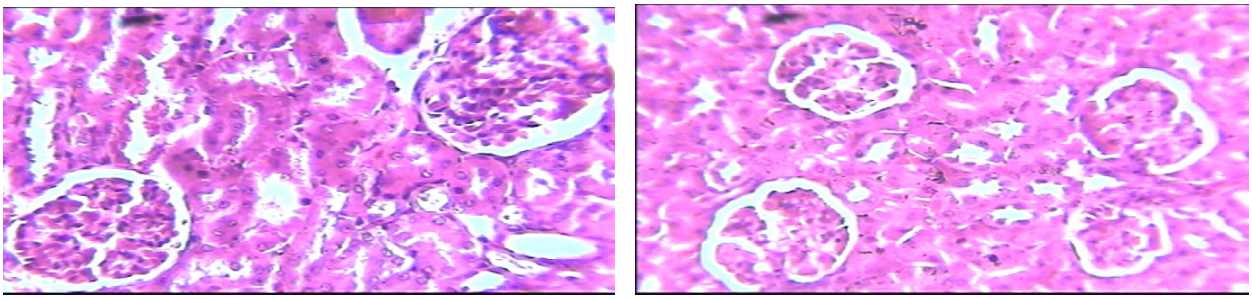

Figure 3 (left): Histopathology of kidney of the rats fed on water without slaked lime (stained with Haematoxylene and Eosin) (x400). The field shows normal glomerular and urinary space. **Figure 4 (right):** Histopathology of kidney of rat fed on water with slaked lime at varying contact time interval of 504 hours. All others appeared the same (stained with Haematoxylene and Eosin) (x400). Field shows normal glomerular and urinary space.

DISCUSSION

The results showed that the pH of the water after treatment was between 6.6 and 4.4. The hydrogen ion concentration of water is important because it affects the chemical reactions and many biological systems which function only in relatively low pH ranges (Prescott *et al*., 2008). In this study, the decline in pH as the contact time intervals increases could possibly be due to the decreased in total dissolved solids and organic matter present in the river water. pH condition increases due to the presence of varying amounts and types of organic matter and minerals in water (Dunne & Leopold, 1978).The reduced pH in this study is similar to that reported by Dalsgaard *et al*. (1997) and Mata *et al*. (1994) and this is desirable as it is effective against some pathogens like *Vibrio cholerae* and coliforms. Epidemiological studies during cholera outbreaks in Guinea-Bissau showed that lime juice in rice foods was strongly protective against the disease, and this observation was corroborated by laboratory studies showing that the presence of lime juice inhibited *V. cholera* growth. Our finding was contrary to that of Graymont (2008) who reported that by raising the pH of water to 10.5-11 through the addition of lime and retaining the water in contact with lime for 24-72 hours, an adverse environment is created that suppresses the growth of bacteria and certain viruses. This application of lime is utilized where "phenolic water" exists, because chlorine treatment tends to produce unpalatable water due to the phenol present.

The result from this study showed that temperature ranges from 23 to 29^{o}C. Temperature is important in controlling the microbial growth in drinking water and affecting directly or indirectly a wide array of physical and chemical parameters (Lechevallier *et al*., 1983). This finding was in line with Fransolet *et al.,* (1985) who revealed that most of the bacteria isolated from the water were mesophiles. The observed reduction in water turbidity is due to the settlement of the heavier flocs that are present and this may be attributed to effectiveness of lime as a coagulant. This result is in agreement with the WHO Guideline for drinking water quality, which recommends that the median value of turbidity before terminal disinfection must not exceed 1(NTU) and must not exceed 5(NTU) in single samples (WHO, 2008).

After 72hours, there were no detectable dissolved solids, probably due to settlement or complete dissolving or decomposition of the organic and inorganic matter in water. TDS is generally considered not as a primary pollutant but it is rather used as an indication of aesthetic characteristics of drinking water and as an aggregate indicator of the presence of a broad array of chemical contaminants with high TDS levels generally indicating hard water (John, 1997). Research has shown that high level of TDS in water is compounded in toxicity when other stressors are present, such as abnormal pH, high turbidity or reduced dissolved oxygen with the latter stressor acting only in the case of animalia (Claude, 1999). A number of studies have been conducted that indicate various species' reactions range from intolerance to outright toxicity due to elevated TDS. The threshold of acceptable aesthetic criteria for human drinking water is 500 mg/l (USEPA, 1991) which is in line with the findings in this study.

The gradual reduction in total bacterial and coliform count was probably due to lowering of pH towards acidic from slightly neutral, as reported by Ademoroti (1980). Six bacterial isolates were isolated from the water sample after treatment with lime which included *Proteus vulgaricus, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermis, Staphylococcus aureus,* and *Bacillus subtilis.* The presence of *E.coli* in the water indicated that the water was faecally contaminated possibly through run-off, animals or human excretion near the river (Joan, 1988).

The presence of *Staphylococcus aureus, Staphylococcus epidermidis* could be attributed to human swimming or cattle grazing around the surface river water. *Proteus vulgaricus* is mostly found in the soil (Mathess, 1982). *Pseudomonas aeruginosa* presence indicates pollution from the environment since surface water is constantly exposed to pollution. *Bacillus subtilis* undergo sporulation, an attribute which helps them to survive adverse conditions in the environment (Thimann, 1963).

The histopathological result for the liver indicating that no damage was done to the tissues as compared to the control were in agreement with the work of Luiz and Jose, (2003) who observed similar anatomical features. IPCS (2004) reported that slaked lime did not produce tumours in male or female mice when given in drinking water or in diet. Also the cell arrangements in the histological features for the kidney did not show any damage or inflammation as compared with the control. This anatomical finding was in line with the report of IPCS (2004) However, this finding is in disagreement with the report of IPCS (1985) which indicated that betel-lime quid with or without tobacco is carcinogenic to experimental animals.

Conclusively, this study showed that treatment of surface water with slake lime water at concentration of 0.5w/v was an effective means of reducing bacterial load by lowering of the pH, acting as a coagulating agent and induces no toxicological effect to albino rats (*Rattus novergicus*) as demonstrated through liver and kidney histopathology . The treatment of surface water using slaked lime is therefore recommended as an alternative measure to currently available methods. It is more economical and easily available to the rural dwellers thereby making portable water more assessable for their consumption. Further research into the toxicological effects of the treated water on the liver and kidney enzymes function indices would be desirable.

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