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Town scale response of water viral communities to town source surface water contamination with hydrochemical parameters.

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Abstract. This study aims to explore the relationship between human enteric viruses found in town-scale surface water sources and certain chemical contaminants present in the water. From October 2010 to April 2012, water samples were collected and analyzed using a combination of biophysical and molecular techniques to detect the presence of human adenoviruses (HAdV) and human enteroviruses (HEV) as well as chemical parameters as predictors for virus survival. The concentrations of 12 chemical contaminants were found to be within WHO-recommended limits. The study found positive and negative associations between viral genome detection and four out of the 12 metal and nonmetal analytes. Specifically, there was a correlation between Cd and HAdV genome detection (rho = 0.146, p = 0.032) and between Pb and Fe with HEV (rho = 0.156, p = 0.022) and (rho = 0.148, p = 0.029), respectively. For nonmetals, phosphates were slightly negatively correlated to HEV (rho = 0.174, p = 0.010). The results of the study did not provide support for the hypothesis of an association between the presence of human enteric viruses and the levels of twelve chemical contaminants.

Keywords. Enteric viruses, WHO, virus stability, hydrochemicals.

1 Introduction

Urban water sources chemical contaminants and microbes' interactions are all important terms related to urban water quality issues caused by increased population growth and socio-economic activities. In Kenya, towns, and cities along massive ecosystems such as Lake Victoria (LV) experience chemical and biological contamination issues in their recreational and drinking water supplies to a greater degree, due to changes in land use and agricultural and industrial development. The chemical contamination pathways to the city's water sources may include surface runoff, leaching, and water seepage through tailing piles from mines and other anthropogenic sites as well as from natural mineralized deposit zones. These chemically contaminated water supplies can directly impact the health of cities' populations as well as act as reservoirs for the incidence and survival of potentially pathogenic microorganisms such as enteric viruses [1, 2]. For the human population, studies have shown that exposure to elevated concentrations of heavy metals and non-metals can have adverse effects on the contact population, including diseases, disorders, and complications of major organs and organ systems [3]. Heavy metals such as mercury, lead, cadmium, zinc, nickel, copper, and arsenic can desaturate the protein, inhibit cell division, enhance transcription and enzymatic activity, and induce nucleic acid damage [3].

The different chemical contaminants can also affect the growth and survival dynamics of bacterial, fungal, archaeal, and protozoan communities in various aquatic ecosystems over time, thus potentially exacerbating their pathogenicity [4]. Studies have shown that most heavy metals, for example, may be essential for various metabolic activities and the maintenance of ionic concentrations in various microbes [5]. However, exposure to higher concentrations can result in negative effects such as osmotic imbalance and alterations in the microbe's structure, which may lead to a decrease in diversity, prevalence, biomass, and distribution of the microbes. Interference with the microbial community profile may negatively or positively affect the ecosystem balance [6].

Most research activities, however, have focused on the interaction between chemical environmental pollution and interaction with bacterial communities, however, few studies have explored the interaction of viruses with the concentration of these pollutants in the water environment, which may present challenges in risk modeling. Some studies found that the concentrations of the anions studied had not significantly affected the occurrence of any of the enteric virus groups [7]. This study highlights the importance of different chemical components in the occurrence of enteric viruses in an open freshwater water source. Heavy metals such as cadmium, iron, and chromium have been found to influence viruses' survival on different fomites, while copper (Cu) can have negative effects on certain viruses such as noroviruses [7]. These effects can be attributed to different mechanisms of action of the chemicals on the virus's biochemical processes, ranging from binding to viral proteins to the activation of the reverse transcription process. Some ions typically form an integral part of viral proteins, while others are involved in different activities such as genomic material maturation, activation and catalytic activity, reverse

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transcription, initial integration, and defense of newly synthesized DNA materials [7].

The most important issue is that enteric viruses are of public health importance due to their link to infections such as gastroenteritis in communities from poor sanitation settings [8]. This study investigated the association between town-scale surface water chemical attributes and enteric virus contamination from LV waters in Homa Bay town (HB), Kenya. Six heavy metals, iron (Fe), cadmium (Cd), zinc (Zn), lead (Pb), copper (Cu), and mercury (Hg), and six anions, chlorides (Cl^{-}), carbonates (CO_3^{2-}), sulphates (SO_4^{2-}), fluorides (F^{-}), nitrates (NO_3^{-}) and phosphates (PO_4^{3-}) were analyzed from the water samples. The average concentrations of the chemical pollutants were also compared with the standards prescribed by the WHO [9] for environmental waters to determine the quality and suitability of the waters for human use by the town population. Despite the study area being characterized by multiple anthropogenic activities that could be linked to sources of surface water pollution with different chemical and viral contaminants, no relevant research has been reported on the same. The data generated from the present study may be useful in informing the early design and development of initiatives to counter cities' scale water chemical pollution and mitigate incidences of potentially pathogenic viral contamination for society's benefit.

2 Materials and methods

2.1 Study area

The study was conducted in Homa Bay, Kenya, a town characterized by anthropogenic activities such as horticulture, water transportation, car washing, and industrialization. The regional geological structure favors deposits of minerals that may contain the mineralized forms of the chemical contaminants, thus raising the chances of pollution of the lake waters through erosion and underground reservoirs. Artisanal mining of some of the mineral deposits has been ongoing along the lakeshore for more than a decade, and other mineral deposits in the region include limestone, gold, soda ash, niobium, phosphate, zinc, and copper. The town has a size of approximately 29 km2 and an estimated population of 45,000 [10]. Gold mining activity at Macalder in bordering Migori County, has been reported to lead to contamination with Hg through the receiving waters. Factors such as inadequate sewage maintenance and treatment systems, poor urban drainage systems, and the rolling topography of the area have been identified as contributing to the contamination [10].

2.2 Samples collection

The Central Business District was surveyed from a sampling strip carefully selected from an area of 9 km2, with six sampling points designated as P1-P6 (Fig. 1). Water samples were collected into sterilized 10-liter polyethylene containers and transported to the Institute of Primate Research in Nairobi for viral analysis, with an additional 1 liter of the samples collected from the same spots for chemical analysis for six months from October 2011 to April 2012. The total number of samples collected for both analyses was 216.

2.3 Hydrochemistry analyses

The chemical contaminants were analyzed following the methods adopted from the standard methods for the examination of water and wastewater [11]. The heavy metals were analyzed using the Atomic Absorption Spectrometer (AAS) and Buck Scientific (210 VPG) while other spectrophotometry techniques were used to analyze the anions. The Standard Solutions were prepared using specific analar grade reagents for each of the chemical elements to be analyzed, which were dissolved in distilled water and calibrated curves before analyzing the water samples.

2.4 Molecular detection of viruses

Viral genome recovery was carried out using the glass wool adsorption-elution technique and the polyethene glycol (PEG) precipitation technique [12]. Briefly, the whole water sample was passed through a column of oiled sodocalcic glass wool filters, and negative pressure was applied using a vacuum pump. Glycine beef extract buffer (GBEB), pH 9.5, was used to elute the virus particles. Secondary concentrates were subjected to an overnight incubation at 4 °C followed by centrifugation for 45 min at 4,200 rpm. A phosphate-buffered saline (PBS) solution was then used to resuspend the resultant pellets for another round of centrifugation, from which the resultant supernatant was used for nucleic acid extraction.

DNA extraction was done using an automated commercial MagNA Pure total DNA extraction kit (large volume) and the RNeasy mini kit (QIAGEN). Uracil DNA glycosylase was used to treat the nucleic acid extracts to reduce the probability of amplifying contaminant DNA. The PCR process was adopted from methods originally described by Puig et al. [13], using nested primer pairs previously described [14]. cDNA was synthesized by reverse transcription (RT) from the RNA extracts using Reverse Transcriptase-Superscript II (Invitrogen, Carlsbad, CA), 20 U of RNasin (Promega).

Positive and negative controls were introduced alongside the samples during the PCR process. Positive control for the adenoviral genome was derived from the HAdV-C2 human adenovirus strain (ATCC VR-1079 AS/Rab), while that for the enterovirus was drawn from poliovirus type 1. Nuclease-free water was used as a negative control. Samples were analyzed for PCR inhibition by

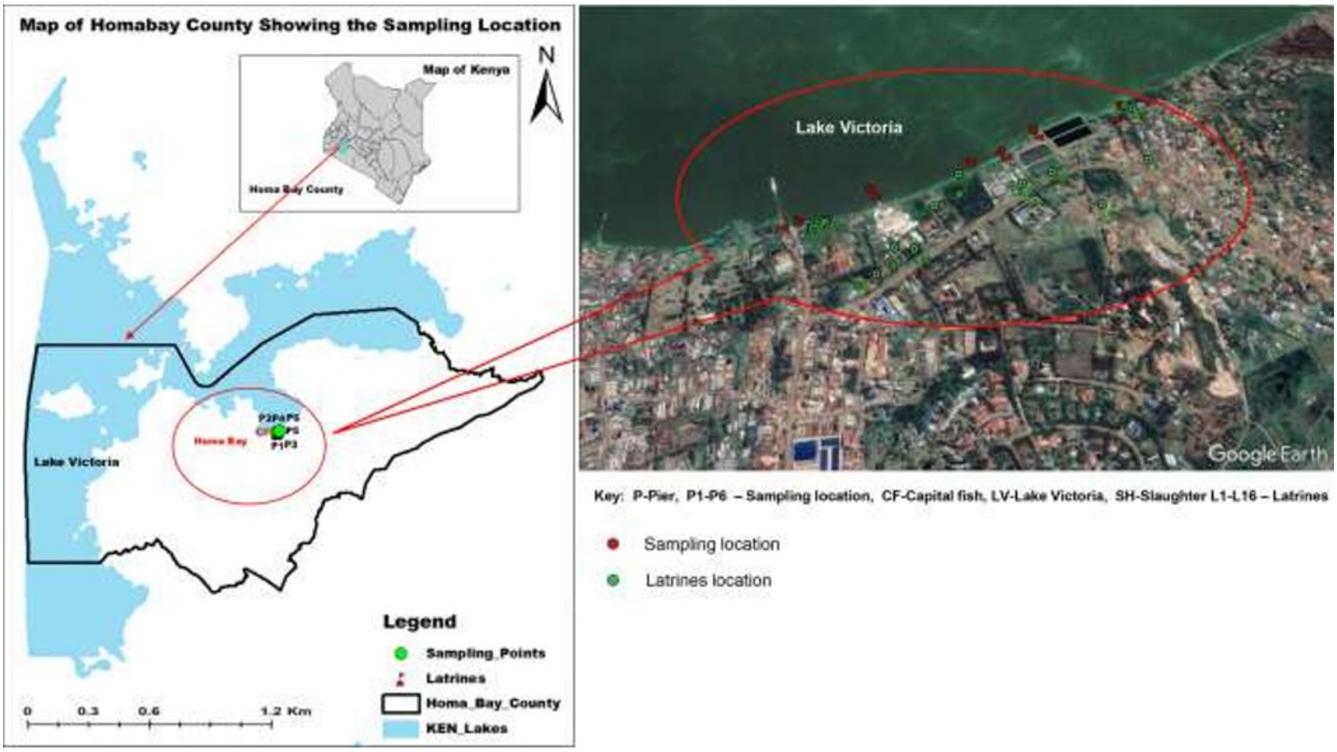

Fig. 1. Map of Homa Bay town showing the sampling sites, water infrastructure and the orientation to the possible sources of contaminants.

spiking some of the aliquots that had returned positive and negative results with each of the specific virus-positive controls for another round of PCR processes.

2.5 Data analysis

We used descriptive statistics, Spearman's correlation coefficient, and logistic regression analysis to examine the link between chemical pollutants and enteric viruses. To determine the likelihood of detection, we utilized SPSS 20.0.

3 Results and discussion

The detection rate for enteric viruses was 8.33% (18/216), with HAdV at 5.09% (11 of 216 samples) and HEV at 3.24%. A comparatively higher percentage of viral genome detection was reported from the sampling points to the east of the stretch (P5 and P6), with a total contamination percentage of 6.02%. HAdV detection was at 0.93% (2/216) from the other middle region sampling point (P3), while HEV detection was not detected. Adenovirus contamination was detected only at Al at a lower rate of 0.46% (1/216) (Table 1).

Table 1. Detection rates of the viral genomes by site (n=216).

	P1	P2	P3	P4	P5	P6
HAdV	1 (0.46)	0 (0.00)	2 (0.93)	0 (0.00)	5 (2.31)	3 (1.39)
HEV	0 (0.00)	1 (0.46)	0 (0.00)	1 (0.46)	3 (1.39)	2 (0.93)
Total	1 (0.46)	1 (0.46)	2 (0.93)	1 (0.46)	8 (3.70)	5 (2.31)

All the recorded average concentrations for the 12 chemical contaminants were lower than levels recommended by the WHO, with the concentrations of Pb being higher at an average of 0.0039 mg/L and Hg at 0.000023 mg/l, both significantly below the WHO permissible limits of 0.01 mg/L and 0.006 mg/L, respectively. However, the concentration of a few chemicals in some samples was higher than the WHO standards, as shown by the maximum values that were recorded. For example, the maximum concentrations of iron and phosphate reported were higher than the WHO recommendations of 0.3 mg/L and 0.5 mg/L, respectively. Certain samples returned values below the contaminants' detection limits, even though the average values of the contaminants per site were computed. For instance, some samples fell below the 0.1 mg/L phosphate detection limit (Table 2).

The results showed that all the heavy metals (Fe, Pb, Zn, Cu and Hg) had no major association with an adenoviral gnome except for Cd (Table 3). There was a small, negative, insignificant correlation between Fe and HAdV, while Cd had a weak significant positive relationship. All the other 5 heavy metals did not record any significant relationship with H EV. There were no significant anions correlation to the HEV except for phosphate ion ($PO4^{3-}$) in which a negative association existed. Binary logistic regression analysis was conducted to determine the probability of using 12 chemical pollutants as predictors for the of the town waters' viral pollution (Table 4 and 5). The model's fitting regression equation showed that HAdV and the heavy metals had positively related to the concentration of Cd ($p < 0.05$) and that higher concentrations of Pb

Table 2. Concentration of the chemical contaminants by site.

Analyte (mg/L)	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**Combined descriptive statistics**				**WHO Limits**
							Mean	**SD**	**Min**	**Max**	
Fe	0.1278	0.1181	0.1361	0.1244	0.1231	0.1286	0.1263	0.07802	0.03	0.80	0.3
Cd	0.0002	0.0002	0.0001	0.0001	0.0007	0.0006	0.000308	0.0002514	0.0001	0.0009	0.003
Zn	0.0082	0.0084	0.0074	0.0072	0.0087	0.0079	0.007968	0.0014694	0.0050	0.0150	-
Cu	0.0817	0.0892	0.0750	0.0836	0.1019	0.0922	0.0873	0.05031	0.03	0.60	2
Hg	0.00002	0.00002	0.00003	0.00003	0.00002	0.00002	0.000023	0.0000072	0.0000	0.0000	0.006
Pb	0.0034	0.0029	0.0030	0.0027	0.0039	0.0036	0.003257	0.0008633	0.0015	0.0066	0.01
Cl^{-}	14.7222	15.8611	12.3056	14.1389	20.8611	17.8056	14.972	4.7012	5.0	26.0	250
F^{-}	0.0211	0.0339	0.0297	0.0264	0.0203	0.0242	0.0259	0.01519	0.01	0.09	1.5
CO_3^{2-}	33.3333	43.6944	57.4167	69.5833	65.2778	60.8056	55.019	28.4252	25.0	230.0	-
NO_3^{-}	9.0000	11.1111	10.3611	12.7778	15.2222	13.7222	12.032	4.0063	5.0	28.0	45
PO_4^{3-}	0.1578	0.1439	0.1653	0.1811	0.1400	0.1381	0.1544	0.09020	0.05	0.90	0.5
SO_4^{2-}	21.1111	20.0556	11.7778	13.0278	11.1111	19.8681	16.157	7.2416	6.0	48.0	400

could increase HEV activity ($p < 0.05$). However, the Hosmer and Lemeshow test showed that there was no statistically relevant contribution to the model. For anions, the expectation of water contamination with HEV was negative with phosphate ($PO4^{3-}$) concentration but statistically insignificant for the other 5 predictors (Data not shown). This suggests that the higher the $PO4^{3-}$ concentration, the less probable the HEV could contaminate the waters.

The study found that only Cd among the heavy metals (Fe, Pb, Zn, Cu, and Hg) had a significant correlation with an adenoviral genome, while the other five did not show any significant relationship with HEV. Additionally, the phosphate ion ($PO4^{3-}$) had a negative association with HEV. A binary logistic regression analysis was conducted to predict viral pollution in town waters using 12 chemical pollutants as predictors. The model showed that higher concentrations of Cd were positively related to HAdV and heavy metals, while Pb was positively related to HEV activity. However, the Hosmer and Lemeshow test did not show any significant contribution to the model.

HAdV and HEV detection rates were highest at the upper-east side of the sampling stretch (P5 and P6). The two sampling points were situated in a region associated with possible sources of virus contamination via fecal pollution, including a wastewater treatment plant and an informal settlement estate. The results were consistent with previous studies that have reported an association between enteric viruses' contamination of surface waters and pollution from sewage [15]. On the contrary, points P1 and P2 situated in the lower section of the sampling stretch, respectively, had a viral contamination rate of 0.46%, which could be accounted for by the decrease in chances of effluent contamination as well as transportation of the virus particles from distant reservoirs because of waves and constant water flow [15].

The research aimed to link the occurrence of HAdV and HEV in water samples with the concentration dynamics of the chemical parameters. The chemical parameters were evaluated in comparison to the levels agreed by the WHO for safe water use, as exposure to higher proportions of certain chemicals, such as heavy metals, in water supplies can compromise the dependent population's health [16]. This was important as LV waters serve a population that is solely dependent on it for domestic, agricultural, and drinking purposes. The findings showed a notable pattern of contamination levels, with the average concentration levels of chemical pollutants being minimal and below the WHO-approved levels. However, certain notable variations such as higher levels of phosphates at point P4, were likely due to the proximity of the site to agricultural activities and runoff from polluted industrial sections to the lakefront. The mean Cd concentration was highest at points P6 and P5, respectively. The possible explanation for the higher concentration at these sites may be related to the use of galvanized steel pipes for piping water, as well as the sewage effluent and infiltration from the nearby sewerage treatment plant [17]. The average

Table 3. Spearman correlation of hydrochemicals and the viruses.

S/No	Chemical contaminants	*n*	HAdV	HEV
1	Fe	216	−0.120	0.148*
			0.077	0.029
2	Pb	216	0.079	0.156*
			0.246	0.022
3	Cd	216	0.146*	0.090
			0.032	0.186
4	Zn	216	−0.027	0.085
			0.697	0.212
5	Cu	216	0.029	0.093
			0.669	0.174
6	Hg	216	−0.106	0.028
			0.119	0.687
7	Cl^-	216	−0.059	0.132
			0.385	0.053
8	F^-	216	0.016	−0.109
			0.814	0.110
9	CO_3^{2-}	216	0.045	0.025
			0.509	0.713
10	NO_3^-	216	0.007	−0.025
			0.915	0.716
11	PO_4^{3-}	216	0.012	−0.174*
			0.856	0.010
12	SO_4^{2-}	216	−0.081	−0.005
			0.233	0.941

Correlation coefficient (rho), probability (P), and *Correlated characteristics with some level of statistical significance (p 0.05)

concentration of Fe was generally higher, with a maximum value above the standard WHO concentration of 0.3 mg/L. This could be attributed to pollution from the runoff from the contaminated sites. Homa Bay County had been home to an iron ore mining operation, on the slopes of Homa Hills overlooking the town and located about 33 kilometers away across the lake. Leaching effects from residual deposits may still be possible at the site [10]. Nitrate levels were within the WHO recommended limits, but NO_3^- concentrations are normally exacerbated by human and animal wastes as well as fertilizer usage and thus high concentration values could still be reported in the future.

Table 4. Logistic regression analysis of metals and HAdV

Predictor	*B*	*S.E.*	Wald's $\chi 2$	*df*	*Sig.*	Exp (*B*)
Fe	−12.473	7.736	2.599	1	0.107	0.000
Pb	399.520	533.455	0.561	1	0.454	3.232E3
Cd	2510.514	1225.125	4.199	1	0.040	.
Zn	−294.130	298.688	0.970	1	0.325	0.000
Cu	7.095	8.855	0.642	1	0.423	1205.413
Hg	−56777.929	39676.018	2.048	1	0.152	0.000
Constant	−0.949	2.685	0.125	1	0.724	0.387

Cox and Snell R2 = 0.054. Nagelkerke R2 = 0.164.

Table 5. Logistic regression analysis of the metals and HEV

Predictor	B	S.E.	Wald	Df	Sig.	Exp (B)
Fe	21.849	11.333	3.717	1	0.054	3081513717.143
Pb	1365.281	643.649	4.499	1	0.034	.
Cd	392.394	1591.470	0.061	1	0.805	2.599E + 170
Zn	91.499	250.891	0.133	1	0.715	5.462E + 39
Cu	15.202	11.990	1.608	1	0.205	4002075.783
Hg	47732.080	59260.752	0.649	1	0.421	.
Constant	−14.546	4.751	9.373	1	0.002	0.000

Test	$\chi 2$	*Df*	*p*
Goodness of fit test			
Hosmer and Lemeshow	7.538	8	0.480

The presence of viruses in an environment can be affected by different chemicals, and the concentration of these chemicals can be used to signal the probability of the occurrence of the viruses [9]. Most chemical parameters did not correlate with virus detection, but some notable interactions between some of the chemical parameters and the viruses were observed. Pearson correlation and logistic regression analyses between the heavy metals and the two sets of viruses indicated a positive relationship between the concentrations of HAdV and Cd, as well as between HEV, Pb, and Fe. Heavy metals such as iron, copper, and zinc play an important role in maintaining the virus' structure and functions, which may affect the virus' stability and survival [7]. They interact with the viruses by binding to the protein and altering biochemical processes such as reverse transcription, translation regulation, RNA cleavage, and catalytic activity [18]. Some studies have shown no relationship between the concentration of HEV and phosphate, although sulphate, nitrate, phosphate, and fluoride were not correlated [19]. The present study did not show any correlation except for phosphates. Phosphates are also reported to be involved in the interaction of proteins and nucleic acids, thus affecting viral stability [20].

4 Conclusion

The average concentrations of 12 chemical parameters were found to be within the standards given by the WHO, thus qualifying safety with regards to these pollutants. Heavy metals like Cd at some of the sampling points at slightly higher concentrations could be an indication of a possibility of a potential undesirable level due to continued accumulation. There was no clear correlation to draw a fair inference or state categorically whether the chemicals studied reliably affect the stability of enteric viruses in the waters. Long-term multisampling approaches and concentrations of other potentially pathogenic viruses, such as rotavirus are recommended to determine the true position of the use of chemical parameters as possible indicators for the occurrence of enteric viruses in the town source waters. In sub-Saharan Africa, data on environmental monitoring is limited, so this study aims to inform contact populations about the need to reduce exposure to contaminants from an environmental pollution control perspective. It could also inform remediation strategies to reduce chemical and viral exposure and monitor pollution from multiple sources. Data on the association of the chemical parameters with viruses' detection could contribute to understanding the impact of interactions of viruses with different chemicals in the environment, helping to inform knowledge and understanding of strategies for reducing potential pathogenic viral elements and bioremediation.

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