Science of the Total Environment 955 (2024) 177118

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Surface-derived groundwater contamination in Gulu District, Uganda: Chemical and microbial tracers

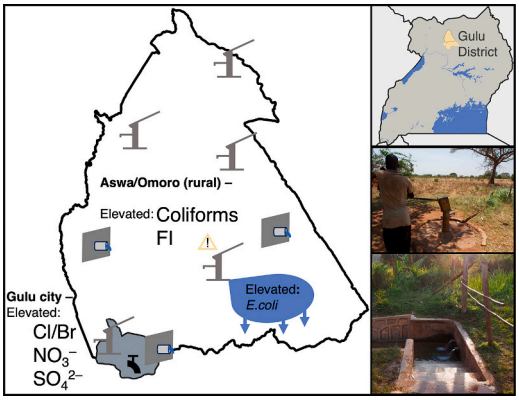
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HIGHLIGHTS

- Paucity of drinking water quality data in groundwater-reliant Sub-Saharan Africa
- Wastewater tracers quantified in groundwater and taps of Gulu District, Uganda
- NO_3^-, SO_4^{2-} and Cl/Br were elevated in urban groundwater compared to rural levels.
- Spot checks demonstrated condition of borehole may influence *E. coli* contamination.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: JV Cruz

Keywords:
Groundwater
Dissolved organic matter (DOM)
Water, sanitation and hygiene (WASH)

ABSTRACT

Groundwater is consumed by over 2 billion people globally, though it can be impacted by microbial and chemical contamination in both rural and (peri-)urban areas. This issue is particularly pertinent in regions like East Africa, where rapid urbanisation has strained local infrastructure, including water and sanitation systems. We use selected tracers of human and animal waste to assess the quality of community drinking sources with regards to surface-derived groundwater inputs and to compare urban *versus* rural water quality, under the rapidly

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https://doi.org/10.1016/j.scitotenv.2024.177118
Received 9 July 2024; Received in revised form 23 September 2024; Accepted 19 October 2024
Available online 22 October 2024
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Sanitary risk assessment
Urban *versus* rural water quality
Sub-Saharan Africa

developing urban area of Gulu, Northern Uganda. Specifically, we examine bulk and fluorescent dissolved organic matter (DOM), microorganisms (total coliforms, *E. coli*) and inorganic tracers of anthropogenic waste (NO_3^-, SO_4^{2-}, Cl/Br) from various sources: boreholes (12–76 m depth; $n = 90$), protected springs ($n = 11$) and municipal taps ($n = 4$). Our results show that NO_3^- and SO_4^{2-} were elevated in groundwater sources in the Gulu city urban area and the Cl/Br ratio was elevated in springs, compared to concentrations in the more rural Aswa and Omoro County area ($p < 0.05$). Interestingly, human and animal waste indicators *E. coli* and Tryp:FA (the ratio of tryptophan-like to fulvic-like fluorescence) displayed no significant difference between rural and urban settings ($p > 0.05$), though total coliforms were significantly higher in rural boreholes ($p < 0.05$). The presence of a pollution source, pollution carrier and a breakdown of a sanitary barrier at the borehole, as spot-checked by a visual sanitary risk assessment, was significantly associated with groundwater *E. coli* abundances. Evidence suggests monitoring and mitigation should be improved for all water types in Gulu District to meet WHO and Uganda Standard guidelines for potable water. This study offers valuable insights for water management planning and risk assessment of community water sources particularly in the context of East Africa and similar settings.

1. Introduction

Groundwater is consumed by over 2 billion people and is generally considered one of the safer available drinking water sources (Alley et al., 2002; Parker et al., 2010). However, groundwater chemical and microbial quality can deteriorate from causes such as surrounding land use (Abanyie et al., 2023; Khan et al., 2017; Lerner and Harris, 2009; Salman et al., 2018; Wilson et al., 2023), (hydro)geological processes (Addison et al., 2020; Howard et al., 2003; Nayebare et al., 2020; Nsubuga et al., 2004; Podgorski and Berg, 2020; Saunders et al., 2005; Sorensen et al., 2021; WHO, 2022) and unsuitable borehole construction (Banks et al., 2021; Ercumen et al., 2017; Takavada et al., 2022). Contamination of drinking water may cause acute water-borne infectious diseases such as cholera, dysentery and typhoid (Bwire et al., 2017; Eurien et al., 2021; Mpenyana-Monyatsi et al., 2012; Murphy et al., 2017); and chronic illness including cardiovascular disease (Moon et al., 2012; Sagheer et al., 2024) and cancer (Baines et al., 2021), depending on part on the type(s) of pollutants present.

In Uganda, many rely primarily on shallow groundwater and protected springs for drinking water and the provision of treated municipal-supply piped water is generally concentrated in dense urban zones (Okot-Okumu and Otim, 2015; Silva-Novoa Sanchez et al., 2020). The majority of groundwater quality issues here are thought to be related to microbial quality, rather than chemical pollutants (Abaasa et al., 2024; Lapworth et al., 2020). In Gulu District, latrines are often built on unstable sandy soils, despite 80 % having no reinforcement to their structure (Mubatsi et al., 2021). As a consequence, latrines may be susceptible to collapse and could result in the dilapidation of water, hygiene and sanitation (WASH) facilities and groundwater pollution (Nyenje et al., 2013). Improper management of waste has been speculated as a major cause of surface-derived contamination of groundwater in urban areas of Uganda (Baguma et al., 2023; Nsubuga et al., 2004; Nyenje et al., 2013).

The issue of groundwater quality in Gulu District remains largely underreported (Arwenyo et al., 2017; Richards et al., 2023). Therefore, our aim was to assess the characteristics of drinking water sources in Gulu and evaluate the influence of surface-derived contamination on groundwater quality. Our methodology integrates fluorescent dissolved organic matter (fDOM), a fast and informative method of DOM characterisation (Baker, 2002; Cory and McKnight, 2005; Sorensen et al., 2021; Wünsch et al., 2019), with inorganic tracers of anthropogenic waste (nitrate – NO_3^-, sulfate – SO_4^{2-} and the chloride to bromide molar ratio – Cl/Br; Cronin et al., 2007; McArthur et al., 2012; Nyenje et al., 2013; Shalev et al., 2015), to investigate the nature of surface-derived contamination. We also use World Health Organization (WHO) sanitary risk assessments (Kelly et al., 2020; WHO, 1997), to investigate potential reasons for the drinking water quality observed in the District.

In this study we considered the following research questions: (i) What is the quality of groundwater, springs and taps in Gulu District, according to tracers of human and animal waste?; (ii) How does human settlement affect wastewater tracers in groundwater in Gulu District? and (iii) Are there associations between WHO sanitary assessments and measured chemical and microbial parameters in Gulu District?

2. Methods

2.1. Site description and selection

Gulu is a rapidly developing city in the Northern Region of Uganda with approximately 97,000 residents in 2024, increasing 2.7 % *per annum* (UBOS, 2019). Recent insurgencies (1986–2008) have resulted in mass displacement from rural communities into municipal areas, exacerbating stress on urban infrastructure and services (Atkinson, 2009; UNCDF, 2018). The city is split into four administrative divisions: Pece, Layibi, Bar Dege and Laroo. Gulu District is comprised of Gulu city (~3500 people/km^2), Aswa County (~90 people/km^2) and Omoro County (~150 people/km^2; Fig. 1; UBOS, 2019).

Gulu District is underlain by predominantly high grade metamorphic rocks which constitute the Gneissic-Granulitic Complex (Schlüter, 1997). Crystalline bedrock is typically overlain by 27–35 m weathered material (*e.g.* saprolite or laterite; Owor et al., 2021; Taylor and Howard, 1994). Groundwater is abstracted from both the fractured bedrock (0.8–1.9 m^2/d transmissivity) and the shallow saprolite-saprock aquifer (3.2 m^2/d transmissivity; Owor et al., 2022). The mean depth to the static groundwater level varies from 8 to 21 m (Owor et al., 2021). Gulu experiences 1390 mm rainfall/year with a distinct rainy season between April–October (Harris et al., 2020), noting there was near-normal monthly precipitation in Gulu District during February 2023 (~25 mm; Diem et al., 2014; UNMA, 2024).

Sampling was conducted according to two selection criteria: i) the Gulu urban area was sampled systematically ($n = 32$; Fig. 1c) using circular search zones (500 m radius) which were spatially distributed within a 7 × 7 km grid; ii) Aswa County sites ($n = 72$; Fig. 1c) were selected randomly from a Ministry of Water and Environment spreadsheet of community drinking water source locations ($n = ~600$), to reflect the spatial distribution of boreholes in Aswa County. For instances where a sampling location was not found under the search criteria, the closest suitable alternative was sampled. Depth variability was assessed post-sampling.

2.2. Drinking water sampling and in-situ measurements

Water was sampled in February 2023 from handpumps (12–76 m reported depth; $n = 88$), protected springs ($n = 11$), municipal supply taps ($n = 4$), a public stand post ($n = 1$) and a solar pump ($n = 1$). The screened depths of the boreholes were documented in borehole completion logs or reported by Local Council (LC) leaders and committee members found at the site, noting that the exact physical characteristics of each borehole was largely unknown. In instances where only the number of pipes was known, a pipe depth of 3 m was assumed.

All boreholes and taps were in regular use and were purged for ~120 s to obtain a representative sample from the aquifer. Water samples were filtered in the field (Minisart® 0.45 μm regenerated cellulose membrane) into glass bottles and sealed with Parafilm. Prior to sampling, bottles were acid-washed (10 % HNO_3 for >8 h), rinsed copiously with MilliQ®-grade deionized water and then baked at 450 °C. All samples were preservative-free (due to transport restrictions on nitric acid) and kept at 4 °C until analysis to prevent changes to analytes, except where this was not feasible (*e.g.* during transit from Gulu to Manchester). Several repeats ($n = 5$) and procedural blanks ($n = 3$) were taken to check the consistency of the sampling method and to test for possible sources of procedure-induced contamination. Measurement of pH and Oxidation-Reduction Potential (ORP) were made (Myron L Ultrameter™ II 6PFC) with regular 3-point calibration at pH 4, 7 and 10 and HI 7021 ORP solution, respectively.

An estimation of the microbial contamination of the water was achieved *in-situ* using the Aquagenx® Compartment Bag Test (CBT EC + TC) Most Probable Number (MPN) kits (Stauber et al., 2014). Estimations of total coliforms (TC) and *Escherichia coli* (*E. coli*) were measured from a thoroughly rinsed beaker of sample. As instructed by the manufacturer, the growth medium and a sodium thiosulfate tablet was dissolved in 100 mL sample in a sterile Whirl-Pak® Thio-Bag and immediately transferred into a Compartment Bag. To estimate the prevalence of microorganisms, the color sequence of the Compartment Bag was matched to one of the 32 color-coded sequences provided by Aquagenx®, after incubation between 25 and 27 °C for ~48 h. Some samples ($n = 3$) were repeated to estimate the variation between subsamples.

2.3. Sanitary risk score (SRS)

Sanitary inspections were undertaken visually at handpumps to identify possible sources and pathways of microbial contamination (Kelly et al., 2020; Sorensen et al., 2016; WHO, 2024). The inspection consisted of ten yes-no questions relating to potential sources of contamination, carriers of contamination and breakdown of preventative barriers (Table 1; full methods previously published in WHO, 1997). The sum of the positive responses was recorded as the total sanitary risk score (SRS). A contamination pathway was identified where three conditions coincided: i) a potential contamination source ii) a carrier and

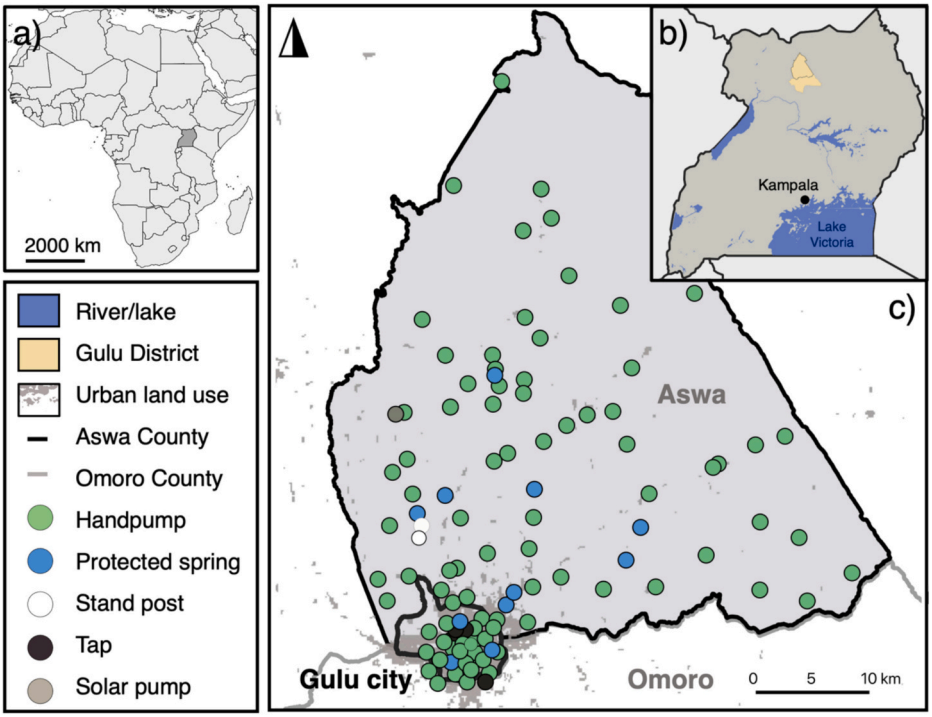


Fig. 1. The location of the sample points within a) the African continent; b) Uganda – the yellow area delineates c) Gulu District – comprised of Aswa County, Omoro County and Gulu city. Samples were taken from handpumps (from 12 to 76 m depth; $n = 88$), protected springs ($n = 11$), municipal supply taps ($n = 4$), a public stand post ($n = 1$), a solar pump ($n = 1$) in February 2023.

Table 1
Ten sanitary risk questions (adapted from WHO, 1997) were spot-assessed at boreholes and categorised into contamination sources, carriers and barrier breakdowns according to Kelly (2021).

	Sanitary Risk Question	Category
1	Is there a latrine < 10 m from the well?	Source
2	Is the nearest latrine on higher ground than the well?	Source
3	Is there any other pollution (e.g. animal excrement, rubbish) < 10 m of the well?	Source
4	Is the drainage poor, causing stagnant water < 2 m of the well?	Carrier
5	Is the drainage channel well missing/cracked/broken?	Barrier breakdown
6	Is the fence around the well missing or inadequate (e.g. allowing animals in)?	Barrier breakdown
7	Is the concrete base < 1 m around the well?	Barrier breakdown
8	Does water collect on the concrete base around the well?	Carrier
9	Are there any cracks/damage in the concrete base around the well?	Barrier breakdown
10	Is the handpump loose at the point of attachment to the base, such that water could enter the casing?	Barrier breakdown

IF (Q1 || Q2 || Q3 = Yes) && (Q4 || Q8 = Yes) && (Q5 || Q6 || Q7 || Q9 || Q10 = Yes) –> **YES**, IF ELSE –> **NO** [Eq. (1)].

iii) a barrier breakdown (Kelly et al., 2021; Eq. 1).

2.4. Quantification of dissolved organic matter (DOM)

Bulk dissolved organic matter (DOM) was quantified at the Manchester Analytical Geochemical Unit (MAGU) laboratory (University of Manchester) using a Shimadzu® Total Organic Carbon Analyser (TOC-VCPN) alongside an ASI-V autosampler with 24-mL glass vials. The determination of DOM, specifically non-purgeable carbon (NPOC), was achieved with the 680 °C combustion catalytic oxidation method (Shetty and Goyal, 2022). Several samples ($n = 9$) were repeated to estimate the quality of the data.

Quantifying sub-fractions of DOM provides an indication of the source and nature of organic compounds (Cory and McKnight, 2005; McKnight et al., 2001; Murphy et al., 2013; Stedmon and Markager, 2005; Wünsch et al., 2019). Fluorescence and absorbance measurements were undertaken at the British Geological Survey (Wallingford, UK) in August 2023. Fluorescence analysis was conducted using a Varian Cary Eclipse fluorescence spectrophotometer with an excitation (Ex.) scanning range of 250 to 400 nm (5-nm bandwidth) and an emission (Em.) range of 250 to 500 nm (2-nm bandwidth); the photomultiplier detector operated at a voltage of 725 V. Fluorescence data, normalised to the Raman peak value of ultrapure water blanks at Ex. 350 nm, Em. 397 nm (Murphy, 2011), were reported in Raman Units (RU) after blank subtraction. Fluorescence analysis was performed in quartz cuvettes with a 1-cm path length. Absorbance at 254 nm was determined using a Varian UV–Vis spectrophotometer and the same cuvette of water used to determine the fluorescence of the sample. Absorbance correction of the data was undertaken using a method from Lakowicz (1994). Reagent-grade Ultrapure water (ASTM type 1, including a UV cracker) served as blanks and to clean the cuvette between samples. Further details were previously published elsewhere (Richards et al., 2019). A subset ($n = 9$) underwent repeated measurements to gauge the quality of the data.

Following data collection and processing of fluorescence DOM (fDOM) data, reference wavelength regions of the excitation-emission matrix (EEM) were quantified: i) fulvic acid-like (FA-like), ii) tryptophan-like (Tryp-like), iii) Tryp:FA – the ratio of Tryp-like to FA-like fluorescence, used to differentiate labile to recalcitrant DOM (Baker, 2002; Sorensen et al., 2018) and iv) the fluorescence index (FI) – used to differentiate microbial and terrestrial DOM (McKnight et al., 2001). Data was processed and indices were calculated in R according to Lapworth and Kinniburgh (2009; R Core Team, 2023).

2.5. Quantification of anions/cations

Anions (SO_4^{2-}, NO_3^-, NO_2^-, Cl^-, Br^-) and selected cations (Ca^{2+}) reported here were quantified by ion chromatography (IC; Dionex ICS5000 Ion Chromatograph with Dionex IonPac AS18 columns) and inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin-Elmer Optima 5300 dual view), respectively, both through MAGU. The Cl^- to Br^- ratio was reported as a molar (M) ratio. Multi-anion Certified Reference Materials (CRMs; LGC6020, LGC6026 and VHG-ICM1-500, LGC Standards, UK and QC1364, Sigma Aldrich, USA) were used to validate the analysis.

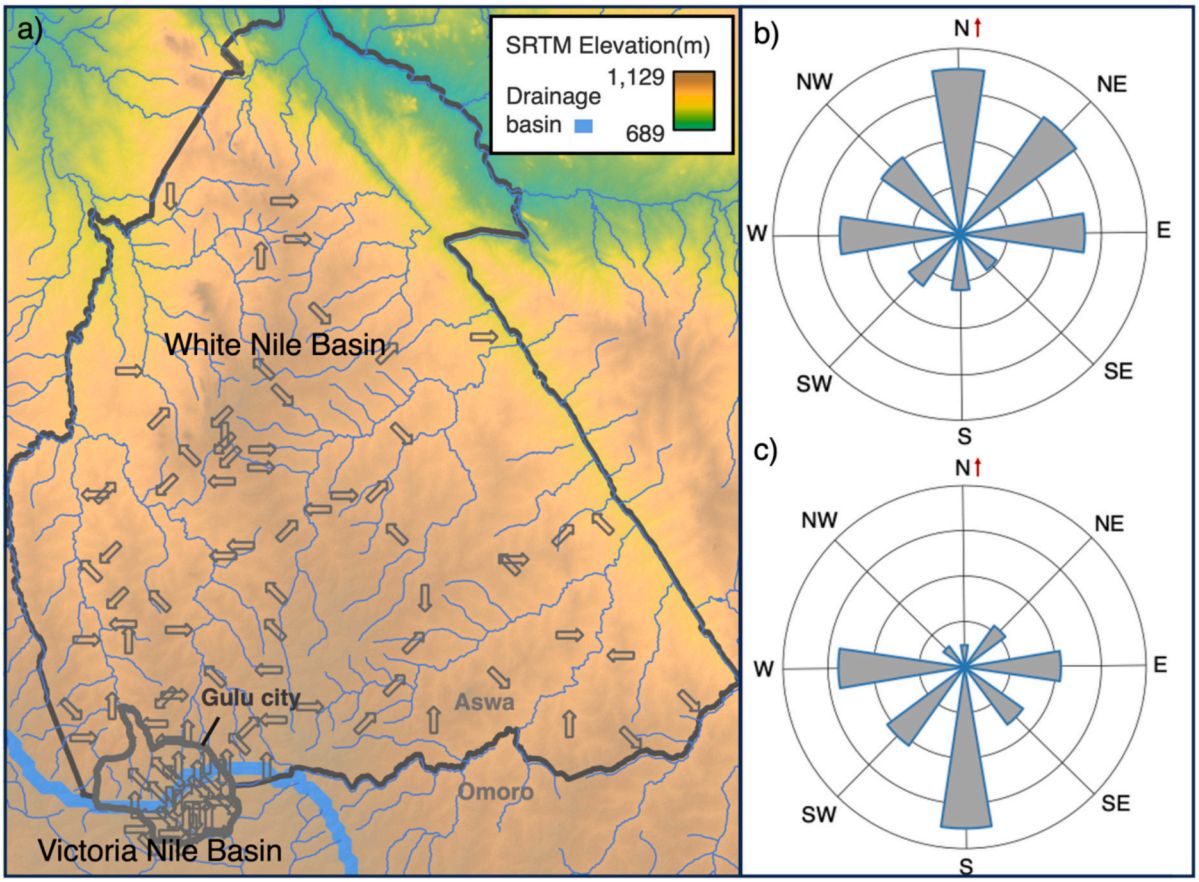


Fig. 2. Modelled groundwater flow directions in the Victoria Nile and White Nile 6 HydroSHEDS basins calculated from a digital elevation model (DEM; Lehner and Grill, 2013). The delineation between Victoria Nile and White Nile HydroSHEDS catchments is shown by a thick blue line. A sink fill algorithm based on Wang and Liu (2006) was used to identify and remove surface depressions in the SRTM DEM (Digital Elevation Model of the Shuttle Radar Tomography Mission) and to model a) groundwater flow direction from Victoria Nile and White Nile basins in the extent shown as an 8-wind compass direction at each sampled point (black arrows) and the predominant flow direction rose plots for b) White Nile and c) Victoria Nile basins for the spatial extent displayed in **a**, plotted in ggplot2 (R Core Team, 2023).

2.6. Groundwater flow direction

In order to understand the hydrochemistry of Gulu District, the groundwater flow directions in the Victoria Nile and White Nile basins were modelled, assuming that they were broadly determined by topographic gradients, using a digital elevation model (DEM; Lehner and Grill, 2013) in QGIS (3.22.6-Białowieża). The cell size of the DEM was downsampled from 30 to 500 m resolution to smooth out topography and better simulate a general groundwater flow direction from the elevation data. A sink fill algorithm based on Wang and Liu (2006) was used to identify and remove surface depressions in the DEM and to determine inferred groundwater flow directions (Fig. 2a).

Gulu city is situated on a groundwater divide – springs and groundwater in the north of the city and in Aswa County predominantly flow north–northeast (Fig. 2b) towards the Achwa River and White Nile, whilst water in the south of the city and Omoro County flows southward towards the Victoria Nile (Fig. 2c).

2.7. Statistical tests

All statistics were performed using R (4.3.2) in the RStudio (2023.12.1+402) environment (R Core Team, 2023). The relationship between nonparametric continuous variables was assessed using a Spearman rank-correlation test and reported in the format "*r(degrees of freedom) = r value; p = p value*". A Mann-Whitney *U* Test was carried out to compare the means of unpaired non-parametric populations; the results were reported in the format "*U = Mann-Whitney U, p = p value*", with U representing the difference betweeen the two rank totals. All statistical tests were conducted at a 95 % confidence level ($\alpha = 0.05$) and to help mitigate the risk of Type I errors, a Bonferroni correction ($\frac{\alpha}{n}$) was applied for increased conservatism for instances where multiple tests were performed concurrently, where n = the number of hypotheses tested.

3. Results

3.1. Data quality control/quality assurance

Repeated measurements were taken throughout sampling and analysis to quantify heterogeneities between sub-samples and to assess the quality of measurements made. For total coliforms and *E. coli*, the mean sampling error and 95 % confidence interval (C.I.) of CBT repeats ($n = 3$) was 33 % and 33 % (± 64 and 65 %), respectively. For FI, DOM and Tryp:FA, the mean analytical repeat ($n = 9$) error and 95 % C.I. was 228 %; 96 % and 53 % (± 441; 47 and 56 %) whilst the sampling repeat ($n = 5$) error was 35 %; 5 % and 23 % (± 117; 44 and 80 %), respectively. It is plausible that the high analytical and sampling biases on the organic parameters is a result of very low DOM (<1 mg/L) in most samples. For NO_3^-, SO_4^{2-} and Cl/Br [M], the mean analytical repeat ($n = 9$) error and 95 % C.I. was 11 %; 2 % and 10 % (± 15; 1 and 12 %) whilst the sampling repeat ($n = 5$) error was 10 %; 2 % and 5 % (± 34; 6 and 10 %), respectively. The average CRM bias for NO_3^-, SO_4^{2-}, Cl^-, Br^-, and Ca^{2+} was ±6.4 %, ±3.8 %, ±1.6 %, ±0.7 % and ± 2.4 %, respectively. The Hubaux-Vos limit of detection (LoD) of SO_4^{2-}, NO_3^-, NO_2^-, Cl^-, Br^- was 0.06 mg/L, 0.13 mg/L, 0.07 mg/L 0.04 mg/L and 0.09 mg/L. respectively (Hubaux and Vos, 1970). The LoD for Ca^{2+} was 0.23 µg/L, which was determined from the calibration curve according to ICH, (2005).

Furthermore, MilliQ®-grade deionized water (DIW) analytical blanks ($n \geq 3$) and procedural blanks ($n = 7$; 50–350 mL DIW sequentially filtered in integers of 50 mL through Minisart® 0.45 µm RC membranes) were analysed to quantify potential filtration-induced contamination and assess instrument performance. Relative to the Gulu sample dataset, Tryp-like, FA-like, SO_4^{2-} and NO_3^- were low in both analytical blanks (0.0 ± 0.0 RU and 0.0 ± 0.0 mg/L; $n = 3$) and procedural blanks (>0.1 RU, >0.1 RU, >0.5 mg/L and > 0.1 mg/L; $n = 7$), respectively. Whilst TOC DIW analytical blanks were very small (0.1 ± 0.0 mg/L; $n = 16$), DOM up to 0.4 mg/L was detected in procedural blanks ($n = 7$), noting that this varied as a function of flushed volume (50–250 mL) and thus the DOM was likely arising from the filter materials used (noting that these were unused single-use syringe filters). As DOM was of a similar concentration (0.1–1.8 mg/L) in groundwater in Gulu, DOM has been presented with caution to reflect potential filtration-induced DOM contamination of up to a maximum of ~0.4 mg/L (Figs. 3; 5; 7 and 8).

3.2. Depth distribution of wastewater indicators in groundwater in Gulu District

In order to examine evidence of wastewater contamination in Gulu District, geochemical tracers were plotted as a function of reported depth in groundwater in Gulu District (Fig. 3). The upper detection limit for *E. coli* and total coliforms (100 MPN/100 mL) was exceeded in 8 % and 57 % respectively in all groundwater sources, such that regression analysis with reported depth would not be a meaningful test (Fig. 3a). Importantly, both *E. coli* and total coliforms had high concentrations (> 100 MPN/100 mL) in samples across the entire depth range sampled. NO_3^-, SO_4^{2-}, DOM and FI exhibited a peak in groundwater at approximately 20 m reported depth. None of the tracers investigated (NO_3^-, SO_4^{2-}, Tryp:FA, Cl/Br [M], FI) except DOM were correlated with reported depth ($p < 0.05$; Fig. 4).

3.3. Comparison between protected springs and handpump-derived groundwater

Geochemical tracers were used to quantify potential anthropogenic contamination of groundwater and protected springs in Gulu District. NO_3^-, SO_4^{2-} and Cl/Br [M] were not significantly different between the two water sources ($U = 1397$; 1394; 915 $p = 0.36$; 0.37; 0.05 respectively). Whilst the point of supply may differ between the borehole and protected spring, the groundwater feeding a spring could share similar characteristics to groundwater drawn from a shallow borehole.

Though for all sites, DOM in protected springs (0.2–1.8 mg/L) was significantly higher than handpump-derived groundwater (0.1–1.8 mg/L; $U = 1658$, $p = 0.01$). Both *E. coli* and total coliforms were detected (> 0 MPN/100 mL) in 100 % of springs in Gulu District, compared to 71 % and 93 % detection rate of total coliforms and 25 % and 41 % detection of *E. coli* in boreholes in Gulu city and Aswa/Omoro County, respectively. *E. coli* and total coliforms were significantly higher in protected springs than groundwater from handpumps in Gulu District ($U = 1157$; 1053, $p < 0.01$ for both). Poor microbial quality of protected springs elsewhere in Uganda has also been reported (Nsubuga et al., 2004; Okot-Okumu and Otim, 2015; Wamyil et al., 2023). It is likely that deeper sources of groundwater have undergone a larger degree of filtration through saprolite and/or laterite soil horizons and thus coliforms are less prevalent (Owor et al., 2021; Worthington and Smart, 2017). However, FI, an indicator of microbial fDOM, was not significantly different between water sources ($U = 1289$; $p = 0.79$). The Tryp:FA ratio was significantly higher in handpump-derived groundwater than protected springs ($U = 518$; $p < 0.01$), which suggests deeper groundwater sources may contain relatively more protein-like DOM and could point towards the construction and/or pumping of boreholes facilitating the ingression of labile DOM into groundwater (Banks et al., 2021; Ercumen et al., 2017; Viban et al., 2021; Wilson et al., 2023). In order to further investigate surface-derived ingression, a comparison between rural and urban areas was made.

3.4. Comparison between urban and rural areas in springs and handpumps in Gulu District

A comparison between the geochemical indicators of Gulu city and more rural surrounding areas of Aswa and Omoro County revealed

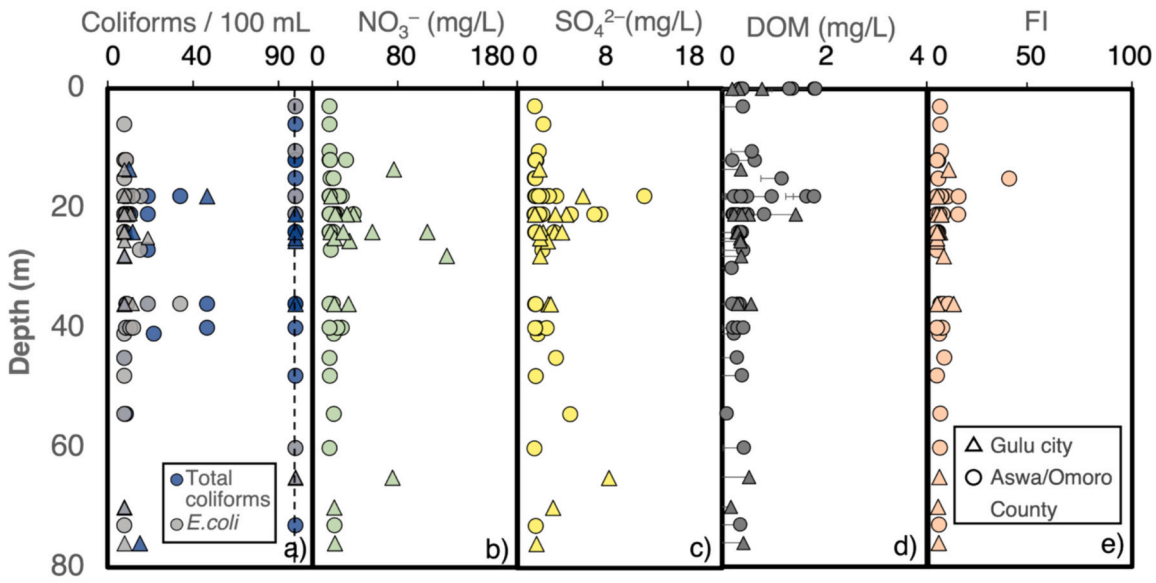


Fig. 3. Characterisation of wastewater indicators a) total coliforms and *E.coli*, b) NO_3^-, c) SO_4^{2-}, d) dissolved organic matter (DOM) and e) Fluorescence Index (FI; McKnight et al., 2001) in groundwater sites in Gulu District as a function of reported depth. The detection limit of coliforms was 100 MPN/100 mL (most probable number; the upper limit is indicated by a dashed line). The error bars in **d** indicate a concentration of DOM (0–0.4 mg/L) that could have resulted from procedural-induced contamination due to filtration (Section 3.1).

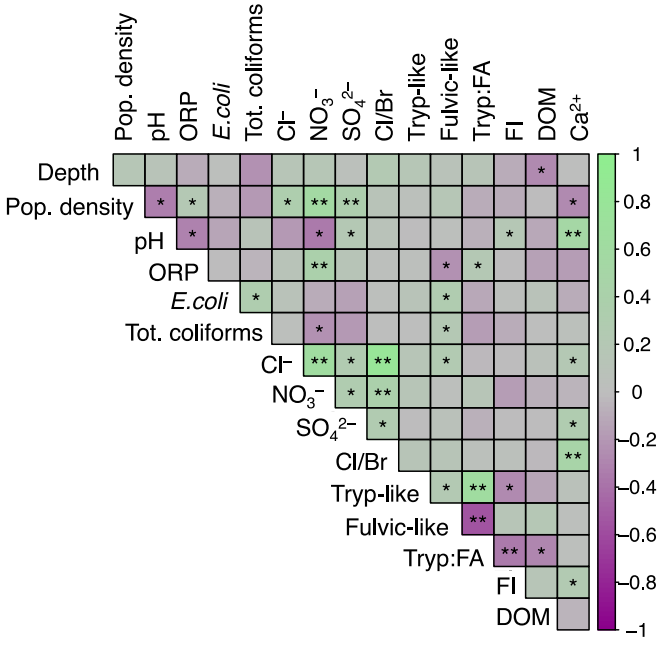


Fig. 4. Spearman rank correlation matrix for the Gulu District dataset (all groundwater; $n = 101$) revealed the statistical relationship between independent variables. *Result significant to $p < 0.05$, **result significant to Bonferroni-adjusted p-value ($\frac{\alpha}{n}$), to mitigate from type I error.

spatial differences in water quality. Interestingly, total coliforms were significantly higher in the rural handpumps than the urban handpumps ($U = 545$, $p = 0.04$; Table 2), whilst higher FI in rural boreholes ($U = 542$, $p = 0.02$) also suggested that DOM was of predominantly microbial nature. Total coliforms were above detection limits (>100 MPN/100 mL) in all protected springs in Gulu District. Abundance of *E. coli* did not differ between the urban and rural areas in handpumps ($U = 632$, $p = 0.21$) or protected springs ($U = 15$, $p = 0.6$). Anions NO_3^- and SO_4^{2-} were significantly elevated in boreholes ($U = 1347$; 1140, $p < 0.01$, respectively) and protected springs ($U = 23$; 24, $p = 0.02$; 0.01, respectively) in Gulu city compared to rural sites. In fact, 28 % ($n = 7$) of handpumps in Gulu city exceeded the guideline value of NO_3^- for Uganda Standard guidelines (45 mg/L; UNBS, 2014), compared to 0 % exceedance in Aswa/Omoro County boreholes and 0 % in protected springs in Gulu District. Despite the measured NO_3^- values in spring-derived groundwater (10.3–42.7 mg/L), the apparent absence of NO_3^- in very shallow urban handpumps (0–15 m; Fig. 3b) likely reflects the lack of groundwater wells present at this reported depth in Gulu city. The Cl/Br molar ratio was significantly different with respect to springs ($U = 22$; $p = 0.05$), but not for handpumps ($U = 765$; 0.84), between the urban and rural sites. This suggests a hydrological connection between protected springs and Cl-rich waste (Nsubuga et al., 2004). Bulk DOM and Tryp:FA did not differ between urban and rural areas in handpumps ($U = 746$; 752 $p = 0.70$; 0.74, respectively) or springs ($U = 4$; 19, $p = 0.13$; 0.19, respectively; Table 2).

To investigate a potential link between urban land use and groundwater contamination, inorganic (NO_3^- and SO_4^{2-}) and organic (DOM and FI) tracers of anthropogenic waste were compared to an estimated population density (Tatem, 2017; Fig. 5) and settlement data (Fig. 6b). The population density yielded a modest positive correlation with NO_3^- (Fig. 5a; $r(99) = 0.58$, $p < 0.01$) and SO_4^{2-} (Fig. 5b; $r(99) = 0.35$, $p < 0.01$) in water sources in Gulu District. A significant correlation between SO_4^{2-} and Ca^{2+}, $r(99) = 0.29$, $p < 0.01$, in groundwater in Gulu District (Fig. 4) could plausibly be increased by calcium-sulphur evaporites (*e.g.* gypsum or anhydrite), though considering the largely homogenous granitoid hydrogeology of Gulu District (GTK, 2014; Owor et al., 2021), it is perhaps a result of gypsum fertilisers (Okello et al., 2010) and/or animal and human excreta and other urban-derived waste (Nyenje et al., 2013). Elevated NO_3^- was associated with densely populated areas that have been inhabited for long periods of time (Fig. 6a and b). Despite FI being significantly higher in Aswa County than Gulu city (Table 2), neither FI nor DOM was significantly correlated to population density, $r(99) = -0.10$, < 0.01, $p = 0.34$, 0.95 (Fig. 5d and c; respectively) in Gulu District, noting that low DOM (typically <1 mg/L) likely made the organics data susceptible to large analytical and sampling error (Section 3.1). Since DOM production rate is controlled by primary productivity and the decomposition of plant material, bacteria and algae (Søndergaard and Thomas, 2004) rather than human activity (McDonough et al., 2020), this finding is perhaps unsurprising.

Table 2
Geochemical indicators reveal significant differences in wastewater indicators in handpumps and protected springs between Gulu city and Aswa and Omoro Counties. Results are reported in the format 'median' ('range'). MPN = most probable number. FI = Fluorescence Index (McKnight et al., 2001). Tryp:FA = tryptophan-like to fulvic-like fluorescence. A Mann-Whitney *U* test compared the distribution of the two sample groups; a significantly different distribution ($p < 0.05$) is indicated in bold (R Core Team, 2023).

	Gulu city (Urban)	Aswa and Omoro Counties (Rural)	Mann-Whitney U test *p*-value
Handpumps	($n = 25$)	($n = 63$)	
Total coliforms (MPN/100 mL)	**29 (0.0–100+)**	**100+ (0.0–100+)**	**0.04**
E. coli (MPN/100 mL)	0.0 (0.0–100+)	0.0 (0.0–100+)	0.21
NO_3^- (mg/L)	**20.0 (0.10–137)**	**0.90 (0.00–33.0)**	**0.00**
SO_4^{2-} (mg/L)	**1.6 (0.1–8.7)**	**0.4 (0.0–13)**	**0.00**
DOM (mg/L)	0.4 (0.2–1.4)	0.4 (0.1–1.8)	0.70
FI	**0.6 (0.0–8.1)**	**1.4 (0.0–54)**	**0.02**
Cl/Br (M)	696 (68–2170)	802 (136–5350)	0.84
Tryp:FA	4.9 (1.1–55)	5.5 (0.4–170)	0.65
Protected springs	($n = 3$)	($n = 8$)	
Total coliforms (MPN/100 mL)	100+ (100+)	100+ (100+)	–
E. coli (MPN/100 mL)	4.7 (4.7–14)	3.1 (1.0–100)	0.60
NO_3^- (mg/L)	**14.6 (10.3–42.7)**	**0.10 (0.00–12.3)**	**0.02**
SO_4^{2-} (mg/L)	**1.5 (1.0–1.7)**	**0.1 (0.0–0.9)**	**0.01**
DOM (mg/L)	0.3 (0.2–0.8)	0.9 (0.4–1.8)	0.13
FI	0.57 (0.5–1.0)	1.3 (0.0–1.8)	0.08
Cl/Br (M)	**956 (922–1610)**	**342 (218–1320)**	**0.05**
Tryp:FA	4.7 (2.7–16)	1.5 (0.4–20)	0.19

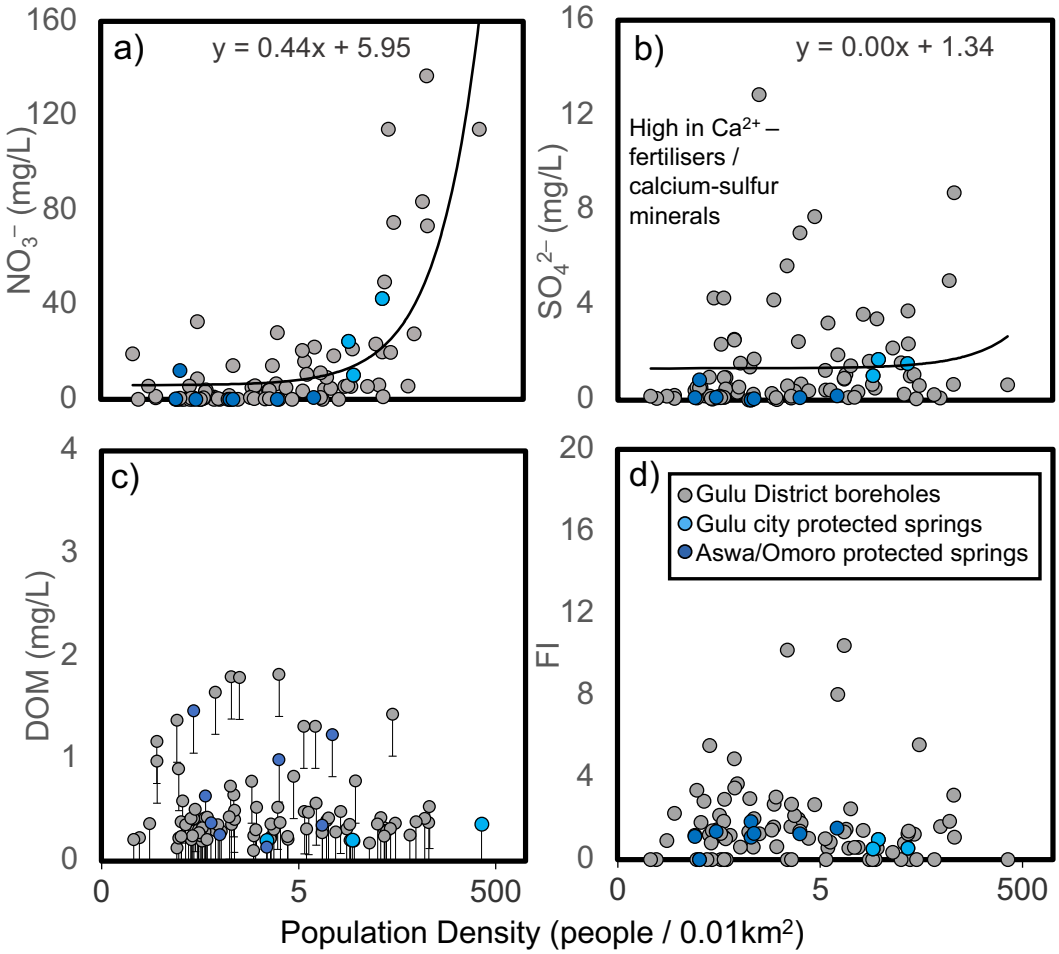


Fig. 5. The population density estimate (Tatem, 2017) was correlated ($p < 0.05$) to a) NO_3^- (solid black line) and b) SO_4^{2-}, but not to c) DOM and d) Fluorescence Index (FI; $p > 0.05$; McKnight et al., 2001) in groundwater and springs in Gulu District, Uganda. The population density (x-axis) is displayed as a logarithmic scale. The error bars in **c** indicate a concentration of DOM (0–0.4 mg/L) that could have resulted from procedural-induced contamination (Section 3.1).

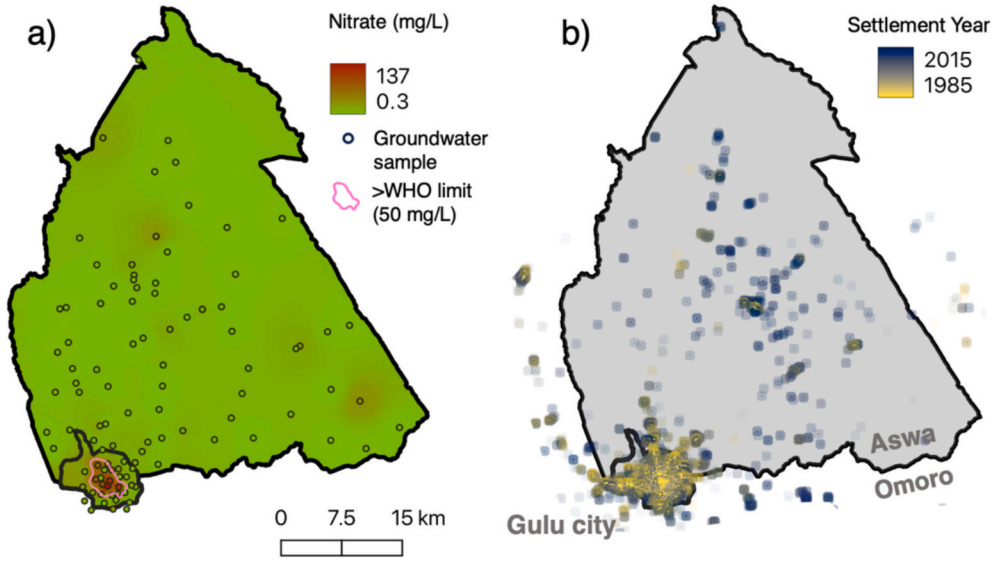


Fig. 6. a) Interpolated groundwater nitrate (NO_3^-) concentration and b) World Settlement Footprint-Evolution (WSF-Evolution) land use data (Marconcini et al., 2021) show a spatial association between high NO_3^- (>50 mg/L; pink area) and settlement in Gulu District. Samples were taken from boreholes (n = 90; 12–76 m depth) in February 2023 and analysed using ion chromatography. Interpolation was conducted using the inverse distance weighting (IDW) method. Non-interpolated data is displayed as circles. The areas of settlement in **b** were highlighted as squares for clearer visualization.

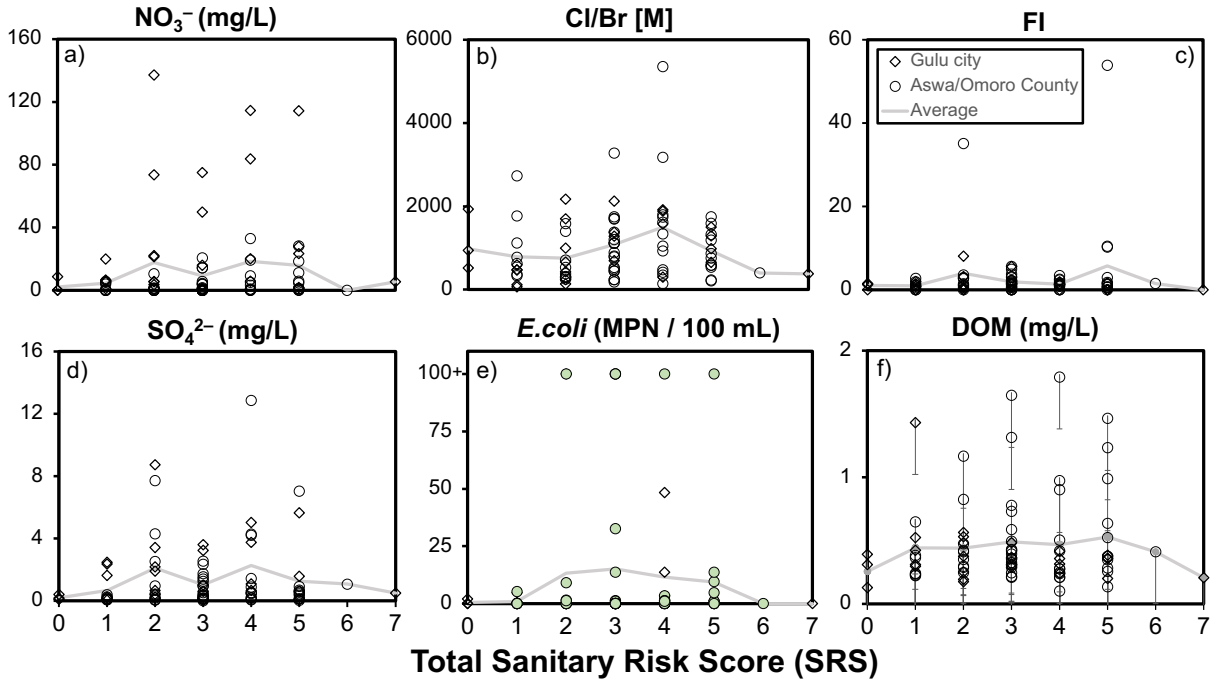


Fig. 7. The total Sanitary Risk Score (SRS; WHO, 1997) compared to tracers of human and animal waste in boreholes in Gulu District (n = 89) FI = Fluorescence Index (McKnight et al., 2001). The public stand post site was not included in the analysis because the groundwater source was at a different location to the pump. The mean (grey line) represents all sites. The error bars in **f** indicate a concentration of DOM (0–0.4 mg/L) that could have resulted from procedural-induced contamination (Section 3.1).

3.5. Sanitary risk score assessment of boreholes

A simple sanitary inspection of boreholes, according to WHO (1997), was used to assess the possibility that groundwater was contaminated at the point of supply based on visual factors evaluated with a spot-check assessment. A series of ten yes-no sanitary risk questions addressed potential sources, carriers and breakdowns of sanitary barriers with respect to the boreholes in Gulu District. As a total Sanitary Risk Score (SRS; /10), there was no direct correlation with any of the wastewater indicators measured (NO_3^-, Cl/Br, FI, SO_4^{2-}, *E. coli*, DOM; Fig. 7a–f; to p = 0.05). Interestingly, an SRS within the range of 3 to 5 was associated with maximum values of the wastewater tracers (Fig. 7a–f), suggesting that groundwater contamination through human and animal waste may be directly impacted or facilitated by factors assessed in the risk assessment, in some instances. However, an apparent decrease in NO_3^-, Cl/Br, FI, SO_4^{2-}, *E. coli* and DOM at <1 and > 5 SRS (Fig. 7a–f) would undoubtedly be influenced by the distribution of the data – there were few observations at both low and high total SRS (mean SRS = 3.0;

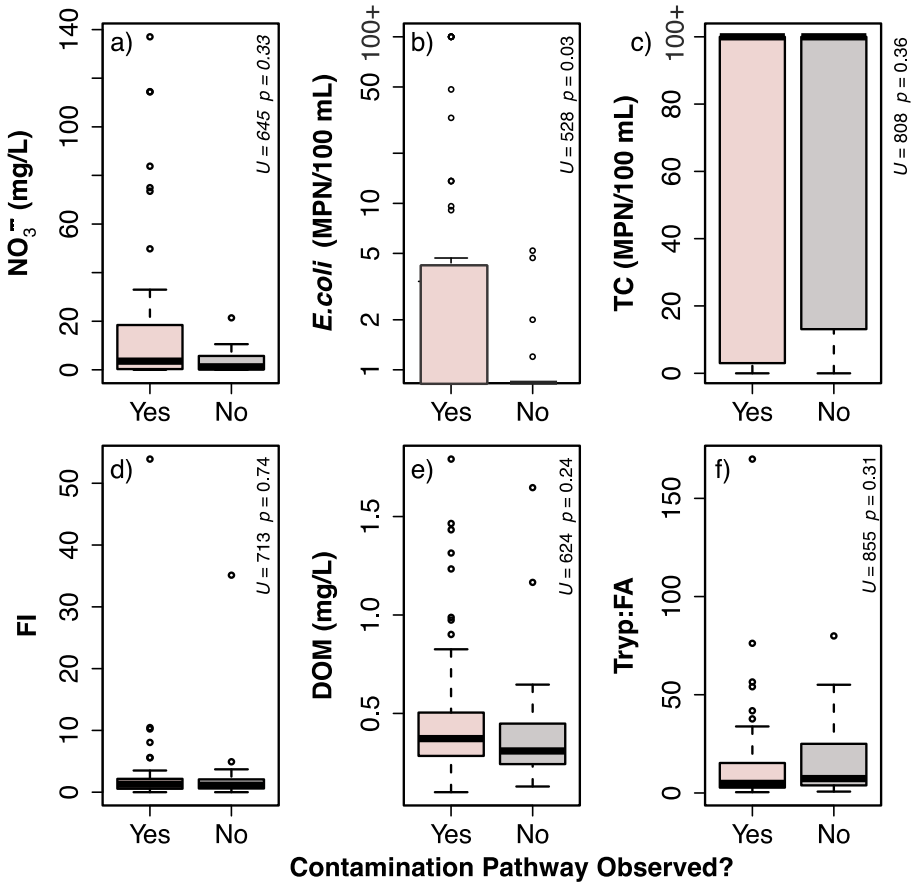


Fig. 8. Sites with a complete contamination pathway, as assessed by WHO sanitary risk scores, were significantly higher in *E. coli* ($p < 0.05$) than sites that did not have a complete contamination pathway consisting of a source, carrier and a barrier breakdown (Kelly et al., 2021). A non-linear y-axis was used in plot **b**.

standard deviation = 1.5 and $n = 89$).

Additional investigation between the SRS of the boreholes and tracers of human and animal waste focused on instances where three conditions coincided: i) a potential contamination source ii) a carrier and iii) a barrier breakdown (Kelly et al., 2021; Fig. 8a–f; Eq. (1)). Out of all sampled handpumps and solar pumps, 74 % ($n = 66$) met all three conditions and 26 % ($n = 23$) did not. Tracers of anthropogenic waste NO_3^-, total coliforms, FI, DOM and Tryp:FA were not significantly different in the presence of an observed contamination pathway compared to sites where this was not observed ($U = 645, 808, 713, 624, 855$; $p = 0.33, 0.36. 0.74, 0.24, 0.31$; Fig. 8a, c–f; respectively). On the other hand, the probable abundance of *E. coli* was significantly different between the two populations ($U = 528$; $p = 0.03$; Fig. 8b), which could indicate that a combination of risk factors, consisting of a contamination source, carrier and a barrier breakdown may significantly affect presence of faecal contamination in boreholes in Gulu District (WHO, 2022). Other research has also identified links between sanitary risk assessment of boreholes and *E. coli* (Olalemi et al., 2023; Viban et al., 2021) in sub-Saharan Africa.

3.6. Gulu city tap water

To understand the quality of community drinking water sources supplied *via* distribution system taps in the city, we characterised several treated water taps ($n = 4$; Fig. 1c) and compared these to the groundwater in Gulu city. Interestingly, NO_3^-, *E. coli*, total coliforms and FI were not significantly different between the water sources ($U = 76; 35; 49; 29$, $p = 0.11; 28; 0.97; 0.18$; respectively). The range of NO_3^- concentrations for taps was 0.2–48.1 mg/L. Out of 4 tap sources tested, 50 % tested positive for *E. coli* (3.4–100+ MPN/100 mL) and 50 % tested negative (0 MPN/100 mL; UNBS, 2014). Although a small sample size, these results are consistent with more comprehensive studies on distributed tap water in Uganda (Walekhwa et al., 2022). The average total coliform count across measured water sources was protected springs > groundwater > taps, whilst for *E. coli* it was taps > springs > groundwater. Cl/Br, DOM and SO_4^{2-} were significantly higher in tap water than in borehole-derived water in Gulu city ($U = 4; 1; 10$, $p < 0.01; 0.00; 0.01$; respectively) perhaps because the tap water consisted of a mixture of groundwater and organic-rich surface water that has been treated with chlorine (NWSC, 2022). The Tryp:FA was significantly lower in taps than boreholes in Gulu city ($U = 93$; $p = 0.01$).

4. Discussion

4.1. Drinking water quality in Gulu District, Uganda

In terms of human and animal waste indicators, our results revealed the quality of urban and rural springs and boreholes. In relation to the Uganda guidelines for unpiped water (*E. coli* – 0 CFU/100 mL; total coliforms – 10 CFU/100 mL; CFU = colony forming unit), the measured springs exceeded the regulatory guidelines in all sites in Gulu District for both parameters (UNBS, 2014). For handpumps in Gulu District, 36 % were above the guideline *E. coli* value and 71 % above the suggested limit for coliforms. The WHO guidelines for drinking water are also 0 CFU/100 mL for *E. coli*, though total coliforms are not used as an indicator of sanitary quality by WHO (2022).

Whilst SO_4^{2-} did not exceed the Uganda Standard (US EAS 12: 2014) guideline value (400 mg/L) in any water sample in this study, groundwater NO_3^- exceeded the Uganda Standard and WHO guideline values (45 and 50 mg/L; UNBS, 2014; WHO, 2022; respectively) in 28 % and

24 % of samples from Gulu city, respectively. In fact, NO_3^- concentrations from Gulu were found to be around 10 times higher than in a previous study from a decade ago during the same season (Arwenyo et al., 2017), though this is plausibly due to an unsuitable detection range used in their spectrophotometric cadmium-based method (Hach, 2023), rather than a profound change over a relatively small timeframe. Whilst Arwenyo et al. (2017) observed a surprisingly high R^2 between NO_3^- and *E. coli* in Gulu city, we found no correlation between NO_3^- and *E. coli* ($p > 0.05$), suggesting that the dominant source of these contaminants in groundwater in our study may be independent of one another. Whilst our study reports on human and animal waste tracers, the quantification of health-threatening inorganic chemical contaminants (*e.g.* arsenic, uranium and fluoride) was not documented here and is the topic of ongoing investigation by co-authors.

4.2. Land use influences water quality in Gulu District

Elevated concentrations of groundwater NO_3^- and SO_4^{2-} around Gulu is plausibly a result of polluting activities from human inhabitancy (Arwenyo et al., 2017; Taylor and Howard, 1994; Fig. 6). Previous studies have suggested that NO_3^- was associated with latrines in settlements such as Gulu and Ibadan, Nigeria (Adetunji and Odetokun, 2011; Arwenyo et al., 2017). Poor management of domestic, market, hospital and commercial/industrial solid waste, is also a known source of NO_3^- and SO_4^{2-} to groundwater in Uganda (Matagi, 2002; Nyenje et al., 2013). Existence of latrines at very shallow depths (typically 0.6–2.5 m), in combination with sandy soils exhibiting a high infiltration rate (Jacob et al., 2008), likely contributes to an elevated concentration of NO_3^- in shallow groundwater of the urban centre of Gulu (Mubatsi et al., 2021). Both prolonged and dense human settlement were associated with elevated NO_3^- in Gulu District (Figs. 5 and 6). Though beyond the scope of this current work, further study may systematically compare the effects of population density and settlement duration on groundwater quality. Nevertheless, our results are concurrent with other studies that suggest urban centres in Uganda contribute to inorganic pollution of groundwater (Arwenyo et al., 2017; Kulabako et al., 2007; Matagi, 2002; Nyenje et al., 2013).

Our study suggested rural land seemed to have little influence on elevated NO_3^- in groundwater in Gulu District. This is perhaps unsurprising, since Ugandan farmers use very little fertiliser (2.4 kg/ha/year, compared to 22.6 kg/ha/year average in sub-Saharan Africa; FAO, 2021) and even less so in Gulu (Benson et al., 2013). In Gulu District groundwater, a significant correlation between SO_4^{2-} and Ca^{2+} ($p < 0.05$) suggested that fluctuations in SO_4^{2-} in rural areas could be a product of calcium-sulphate evaporites or gypsum-based fertiliser. Microbial indicators FI and total coliforms were elevated in Aswa and Omoro County, compared to Gulu city. Since groundwater predominantly flows S—N from Gulu to Aswa County (Fig. 2), this suggests the source of microbial contamination is in the agricultural area, plausibly from livestock or the practice of open defecation (Ntaro et al., 2022). We also investigated borehole-induced short circuits to groundwater flow using sanitary risk score.

4.3. Usefulness of sanitary risk scores

A spot assessment of boreholes was carried out to identify pollution sources, carriers and barrier breakdown (WHO, 1997). Unsurprisingly, our results indicated a poor association between total SRS and measured groundwater contamination. Kelly et al. (2020, 2021) highlight that correlation between borehole quality and risk scores should not be expected and that the value of a sanitary inspection is derived from its utility to visually and simply assess potential contributors which may negatively impact water quality, rather than to provide an accurate assessment or prediction of risks.

Findings here indicate SRS assessors should ideally be considered in groupings that identify contamination in context of the source-pathway-receptor model (Holdgate, 1979; Kelly et al., 2021; Fig. 8), which describes the flow of environmental pollutants from a source, through a pathways and to a receptor – groundwater, in this context. In cases of an evident contamination flowpath, significantly elevated *E. coli* may be a result of orders of magnitude difference between microorganism abundance at the surface than in groundwater (WHO, 2022; Fig. 8b), rather than a smaller concentration difference for chemical contaminants (Fig. 3). Indeed, Ercumen et al. (2017) and Banks et al. (2021) conclude that a dominant contamination pathway in shallow groundwater boreholes can be through bypass of the wellhead, rather than subsurface transport, although a range of factors (*e.g.* recharge rate, pumping-induced surface-derived ingress and other processes impacting surface-groundwater interactions) may impact subsurface microbial water quality (Graham et al., 2015).

5. Conclusions

The assessment of water quality revealed that microbial quality was generally poor in rural Gulu District whilst inorganic tracers indicated contamination below Gulu city urban area was consistent with wastewater ingress. Prolonged and/or dense human settlement has likely increased NO_3^-, SO_4^{2-} and Cl/Br in groundwater in Gulu District, plausibly through poor management of urban-derived waste and latrines. We suggest that the significantly higher coliforms in rural Gulu District ($p < 0.05$) could arise from a combination of factors, including livestock presence, open defecation practices as well as less sanitary boreholes in rural areas than Gulu city. The average total coliform abundance for measured water sources was protected springs > groundwater > taps, whilst for *E. coli* it was taps > springs > groundwater.

This research highlights the usefulness of sanitary risk questions in the assessment of boreholes for microbiological quality (*e.g. E. coli*) to identify potential causes of contamination, when applied in consideration of pollution pathways. We therefore recommend the use of similar spot checks in risk management plans of water sources in similar settings, noting however that these should not be used as a replacement of direct water quality testing of relevant chemical and microbial parameters.

Evidence here suggests monitoring and mitigation should be improved for all water types to meet the relevant guidelines for potable water. This study highlights the need to further identify the likely source (s) of microbial and chemical pollution and calls for improvement to drinking water sources in Gulu District.

CRediT authorship contribution statement

George J.L. Wilson: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Derick Muloogi:** Writing – review & editing, Investigation. **Rajabu Hamisi:** Writing – review & editing, Investigation, Funding acquisition. **Timna Denwood:** Writing – review & editing, Investigation, Conceptualization. **Prosun Bhattacharya:** Writing – review & editing, Funding acquisition. **Expedito Nuwategeka:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Daren C. Gooddy:** Writing – review & editing, Supervision, Methodology, Conceptualization. **David A. Polya:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Jonathan J. Huck:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Laura A. Richards:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Acknowledgements

A Dame Kathleen Ollerenshaw Fellowship is acknowledged for LAR and for GJLW's PhD studentship. A UKRI-GCRF-Newton-ODA 2022–2023 award to LAR et al. *via* an institutional allocation to The University of Manchester supported this project. Loy Asiimwe and her team at the Ministry of Water and Environment are thanked for sharing regional well information from the 2017 Uganda Water Supply Atlas, which informed sampling. We thank Nancy Aromo and Monica Adokorach (both formerly Gulu University) and Yasin Koire for field support. Jordan Gaskell, Abby-Ragazzon Smith and Rosie Byrne (all The University of Manchester) are acknowledged for analytical support through MAGU. DCG publishes with the permission of the Executive Director of the British Geological Survey (UKRI). The reviewers are thanked for their suggestions which improved the manuscript. The views expressed here do not necessarily represent those of any of the institutions, funders or individuals whose support is acknowledged.

Data availability

Data will be made available on request.

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