

## How much spatial and temporal variation in groundwater microbiology can occur following open dumping of municipal solid waste?

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### ABSTRACT

The effects of open dumping of municipal solid waste on local groundwater microbiology were assessed in Ranchi, India. Both the spatial and temporal variations of groundwater microbiology were studied. Groundwater was analyzed to determine heterotrophic plate count (HPC), total coliforms (TC) and faecal coliforms (FC). Highest HPC was  $4.5 \times 10^4$  CFU/mL and the highest total coliform count was  $3.7 \times 10^4$  CFU/mL. Faecal coliforms were detected in most of the groundwater samples. The highest faecal coliform count of  $2.1 \times 10^4$  CFU/mL was from a hand pump located adjacent to the dumping site. Spatial variations revealed dispersion of bacterial contamination up to 5 km from the periphery of the municipal solid waste dump. The level of bacterial contamination decreased with an increase in distance from the municipal solid waste dump. The maximum bacterial count was detected in the monsoon whereas the lowest count was found in summer. Molecular characterization of morphologically similar faecal coliform colonies indicated the presence of *Klebsiella pneumoniae* (*Klebsiella ssnkb1*, KU647674) in one of the groundwater samples that showed maximum faecal coliform count. Results of this study indicated that groundwater in the area is highly contaminated with a bacterial load which can be a major health risk.

**Key words:** faecal coliforms, *Klebsiella pneumoniae*, MSW dumpsite, pathogens, water

### HIGHLIGHTS

- Spatial and temporal variations in groundwater microbiology were assessed due to municipal solid waste dumping.
- Faecal coliforms were found in most of the samples.
- Bacterial contamination dispersed up to 5 km from the periphery of municipal solid waste dump.
- Molecular characterization indicated the presence of *Klebsiella pneumoniae* in groundwater.

### INTRODUCTION

Worldwide proper disposal of municipal solid waste (MSW) is a major challenge, and this is more so in poor and developing countries due to un-scientific waste dumping in unlined landfills (Mukherjee *et al.* 2015). Open dumping of MSW is the most-followed practice in India mainly due to its simplicity and cost-effectiveness (Mohan & Joseph 2021). MSW disposal in unlined landfills is known to cause several environmental and health hazards and often groundwater is the most significantly affected system (Chakraborty & Kumar 2016; Samadder *et al.* 2017; Abiriga *et al.* 2020).

Open dumping exposes the waste to several environmental variables such as precipitation and moisture, which leads to the generation of complex landfill leachate (Abiriga *et al.* 2021a). Landfill leachate is complex effluent, which is known to contain dissolved organic substances, inorganic substances (ammonia, calcium, magnesium, sodium, potassium, iron, sulphate, chloride etc.), metals (cadmium, chromium, copper, lead, zinc, nickel etc.) and xenobiotic organic compounds (Thitame *et al.* 2010; Abiriga *et al.* 2020; Lee *et al.* 2020). Leachate has been reported to migrate from unsaturated zone to groundwater where it gets mixed and forms plume which spreads with groundwater movement (Shu *et al.* 2018; Abiriga *et al.*, 2021b).

Pathogenic microbes in groundwater due to pollution can pose a severe risk to the human population. Microbial contamination of groundwater may lead to several water-borne diseases such as cholera, typhoid

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etc. (Giglio *et al.* 2016; Kapembo *et al.* 2019). Presence of microorganisms such as *Escherichia coli*, *Enterobacteriaceae*, *Campylobacteriaceae*, *Cryptosporidium sp*, *Giardia sp*, *Citrobacter*, *Streptococcus sp*, *Staphylococcus sp*, *Salmonella sp*, *Klebsiella sp*, *Shigella sp* etc. in groundwater is highly undesirable for human consumption (Aydin 2007; Hrudehy & Hrudehy 2007). Several water-borne diseases due to contaminated groundwater are constantly being reported across the globe irrespective of the economic status of the region (Zhang *et al.* 2014; Andrade *et al.* 2018). *E. coli* is regularly used as an indicator organism to check faecal discharges and ensure biological safety of water sources (Navab-Daneshmand *et al.* 2018; Gizaw *et al.* 2022).

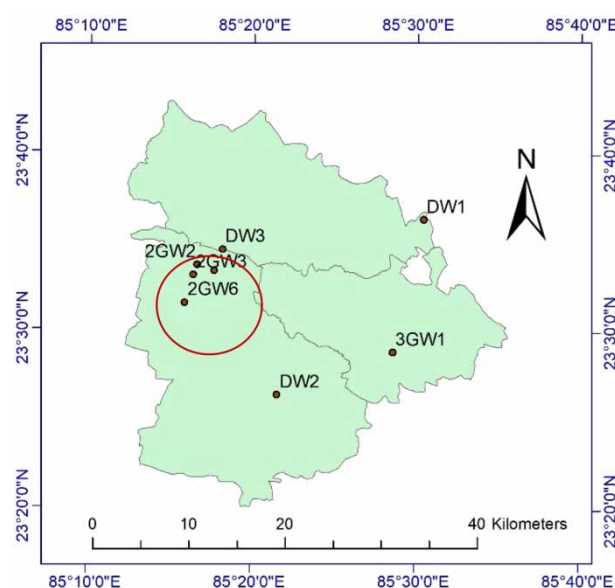
Getting a scientific outlook on this important issue of groundwater quality in times of high rate of industrialization and urbanization will help make management decisions to protect the water resources and human health. Most of the studies on open MSW dumping and its impact on regional water resources have generally focused on the changes in physicochemical characteristics, comparatively fewer studies have focused on the presence of pathogenic bacteria and that too mostly as a one-off sampling event. In the current work, the impacts of open dumping of MSW were assessed on the groundwater bacterial density around the dumping site in Ranchi, Jharkhand, India. The major aim of this study was to gain firsthand information on the spatio-temporal variations in the abundance of heterotrophs, total coliforms and faecal coliforms in groundwater around the MSW dumpsite.

## MATERIALS AND METHODS

### Study area description

MSW dumpsite at Jhiri locality is situated 20 km away from Ranchi (23°35'N and 85°33'E), the capital of Jharkhand State in East India. MSW dumpsite covers an area of 8.9 ha and has been in operation since 2000. Ranchi is situated at an average elevation of 652 m from the mean sea level with an annual rainfall of 1,400 mm and the average number of rainy days is 75. The climate of Ranchi is subtropical with substantial rainfall occurring in the monsoon months (June to September). Summer season is from March to June and November to February is winter. The maximum mean annual temperature is 29.6°C whereas the minimum is 18°C.

The dumpsite (23° 22'N and 85° 20' E) in Ranchi is divided into two parts, a larger area presents the old dumping yard which is also near a village, and another part is the new dumpsite where fresh waste is deposited daily which is close to agricultural land (Figure 1). Adjacent to the landfill area, there is a village with a population of 3,486 spread over an area of 258.6 ha (Census of India 2011). The landfill does not have any type of liner material at the base to contain the migration of leachate. Waste segregation and compaction at the landfill is not carried out and there is no practice of covering the deposited waste with soil cover, thereby exposing the waste to the



**Figure 1** | Location of groundwater sampling points at the dumping site in Ranchi, Jharkhand, India (circle indicates dumpsite location).

environmental conditions. Approximately 700 tons/day of co-mingled waste are collected from the various sites of Ranchi, and it is deposited without any kind of processing/treatment. The dumpsite gets waste from residential sector, commercial sector, local vegetable and fruit markets, slaughterhouses and fish markets etc. Often, biomedical waste is co-disposed along with MSW and further, the waste collected from drains in the form of wet silts is also disposed of in a landfill.

### Groundwater sampling

Groundwater for bacteriological analyses was sampled in 50 mL sterilized high-density polyethylene bottles. Samples were collected to ensure homogeneity and representativeness by flushing the source water till the point temperature was constant. Following collection, the groundwater samples for microbial analysis were kept in an ice box and immediately taken to the laboratory and stored at 4 °C for further analyses as per the standard methods (APHA 2017). Sampling locations were chosen around the MSW dumping site in a radius of 0.5 to 5 km from the periphery of the landfill to get representative data. Sampling points were selected based on the availability of groundwater samples throughout the year and the ones being used by the residents of the village near the dumpsite.

A total of eight groundwater sampling locations (dug wells and bore wells) already available were identified during the initial site survey with major selection criteria as year around water availability and site accessibility. Groundwater sampling locations were named as 2GW1, 2GW2, 3GW1, DW1, DW2 etc. GW indicates hand pump samples and DW indicates dug well samples. A control site (DW1) was also included in the sampling set that was far away from the MSW dumpsite. Sampling sites 2GW1, 2GW2, 2GW3, 2GW6 and 3GW1 are located around the old part of the dumpsite and sampling locations DW2 and DW3 are located around the new part of the dumpsite (Figure 1). Different zone of the sampling locations was identified by using the prefix number 2 or 3 based on their distance from the dumpsite. Sampling could not be carried out in the 1st zone due to the unavailability of groundwater sources at the periphery of the MSW dumpsite. Monthly sampling was carried out for one year to determine the seasonal variations (summer, monsoon, and post-monsoon) in groundwater microbiology. In this study, March to June is referred as summer, July to September as monsoon and October to February as the post-monsoon season.

### Microbiological analysis

Heterotrophic bacteria, coliforms and faecal coliforms were enumerated to assess the microbiological status of groundwater in the study area (APHA 2017). All the analyses were undertaken in <24 h of sampling. Groundwater samples were first serially diluted up to  $10^{-1}$  using sterile distilled water. 100  $\mu$ L of each sample was plated on nutrient agar, MacConkey agar and Eosin methylene blue agar (EMB) using the spread plate technique to determine HPC, TC and FC, respectively. The inoculated plates were incubated for 24 h at 37 °C. A digital colony counter was used to count the number of bacterial colonies present in the groundwater samples as colony-forming units per millilitre (CFU/mL).

### Molecular characterization

Molecular characterization was performed for faecal coliform colonies found on EMB agar plates, which were found to be morphologically identical during all the sampling events. A sample for molecular characterization was taken from 2GW2 (within 500 m from dumpsite), which presented the highest faecal coliforms count. 16S rDNA sequencing method was used to identify bacteria from the identical colonies. For this purpose, DNA was first isolated from the cultured sample by using a DNA isolation kit. The isolated DNA was amplified by PCR using 8F and 1492R primer and ran on 0.8% agarose gel to check the quality of the DNA. Amplicon from PCR were purified and processed for sequencing of nucleotide. Forward and reverse sequencing reactions of PCR amplicon was performed using 704F and 907R primers (BDT v3.1 sequencing kit – ABI 3730xl Genetic Analyzer). A consensus sequence of 1,486 bp 16S rDNA was produced from forward and reverse sequencing data by using the software. The 16S rDNA sequence was utilized to perform BLAST alignment search tool (NCBI gene-bank database). Maximum identity score of ten sequences were chosen and aligned with the multiple alignment software (Clustal W). Finally, the phylogenetic tree was constructed following the protocol detailed in the literature (Kumar *et al.* 2016).

## Data analysis

Data generated from the study were analyzed using MS Office-Excel. Groundwater microbiology data were also analyzed to obtain correlation coefficients to determine the relationship between distance from the dumpsite and bacterial contamination. Single-factor ANOVA and two-factor ANOVA were applied to study the variance in the data. All the statistical tests were performed using the MS Office-Excel with Analysis Toolpak. Data on spatial variations in the microbial count was interpolated by applying the IDW method (inverse distance weighting) using ArcGIS (Reed *et al.* 2000; Adhikary & Dash 2017). GIS maps to show the spatial variations were also produced using the IDW process in ArcGIS.

## RESULTS AND DISCUSSION

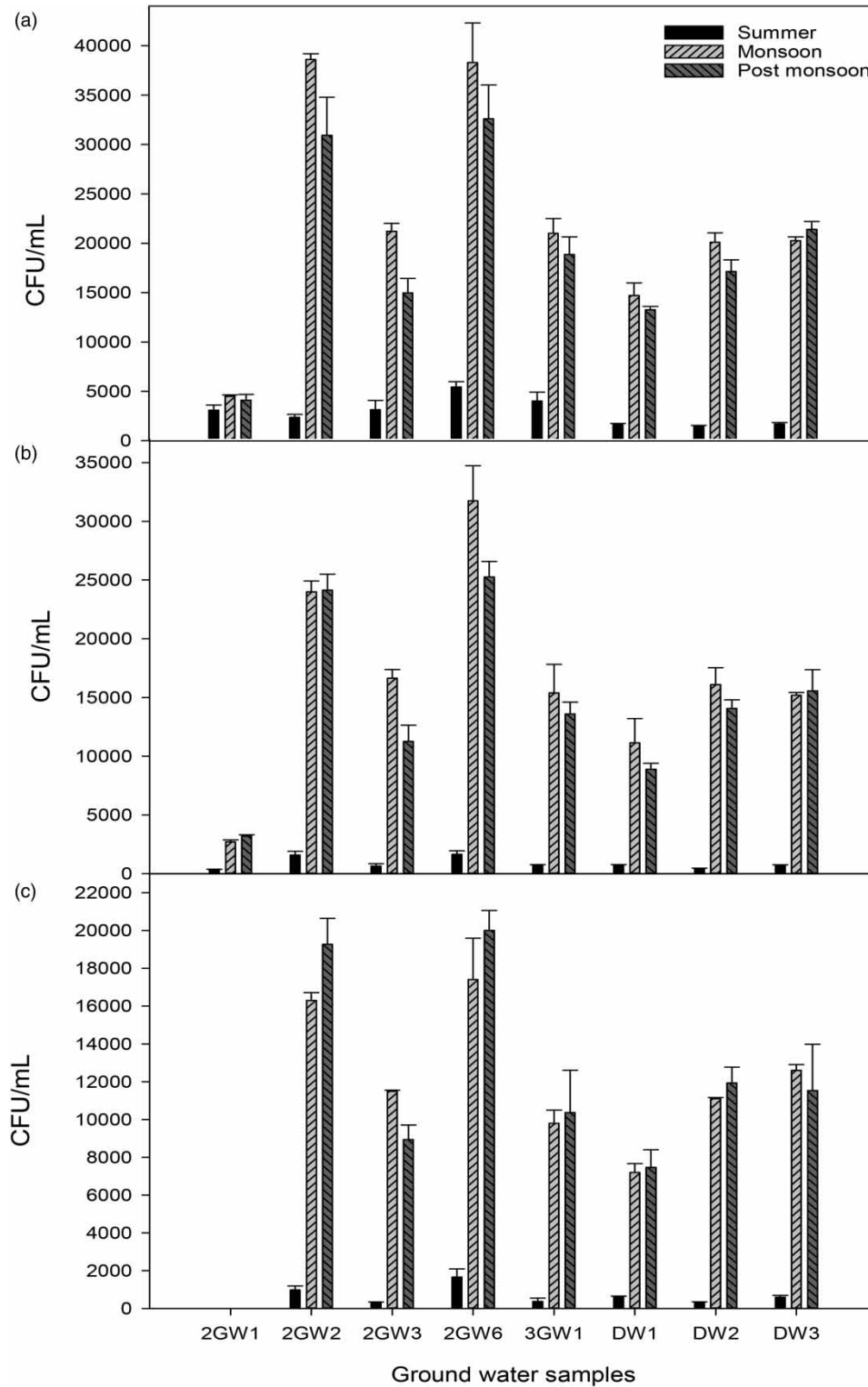
### Groundwater microbiology

Coliforms were present in most of the samples collected from various sites around the dumpsite. Heterotrophic bacterial density was from  $4.2 \times 10^2$  to  $4.5 \times 10^4$  CFU/mL and total coliforms ranged from  $2.0 \times 10^2$  to  $3.7 \times 10^4$  CFU/mL (Figure 2). Major reasons for such high bacterial contamination of groundwater could be due to poor sanitary conditions of human settlements, livestock faecal matter, in addition to open dumping of MSW that contains a variety of waste such as slaughterhouse waste biomedical waste, and kitchen waste. Faecal coliforms were also found in most of the samples, which is of major concern as the water is used for different domestic uses in the area. The highest faecal coliform count ( $2.1 \times 10^4$  CFU/mL) was found from the hand pump (2GW2) located very close to a solid waste dumping site, coliform bacteria were detected at a higher range during monsoon and post-monsoon. During the monsoon, there is a higher discharge of leachate and surface runoff compared to the dry season, which could be one of the factors responsible for higher numbers of coliforms in groundwater in the vicinity of the dumpsite. Further, the water sources in the area are not protected by any measures to minimize pollution.

Landfill leachate often offers a conducive environment for bacterial growth and thereby may contain high bacterial density (Grisey *et al.* 2010; Abiriga *et al.* 2021b). Grisey *et al.* (2010) reported total coliform density as 20,000 CFU/100 mL, *E. coli* and *Enterococci* density as 15,199 CFU/100 mL and 3,290 CFU/100 mL, respectively in groundwater collected near an abandoned sanitary landfill. Their study demonstrated that the landfill was not a significant source of bacterial load in this case and the major reason for the high coliform count was faecal inputs from septic tanks. The faecal coliform count can be in the range of  $10^8$  CFU/100 mL in regions significantly influenced by septic tank influents (Sinton 1982). Like the higher bacterial load found in this study, Gwimbi *et al.* (2019) also reported that the *E. coli* count was substantially lower (maximum 4,800 CFU/100 mL) in protected water sources (springs, open wells, streams) compared to water sources that were unprotected ( $4.35 \times 10^7$  CFU/100 ml). The main reasons attributed to such high counts of enteric pathogens were due to faecal contamination due to open defecation, faecal matter from livestock, latrines near water sources and unhygienic routines.

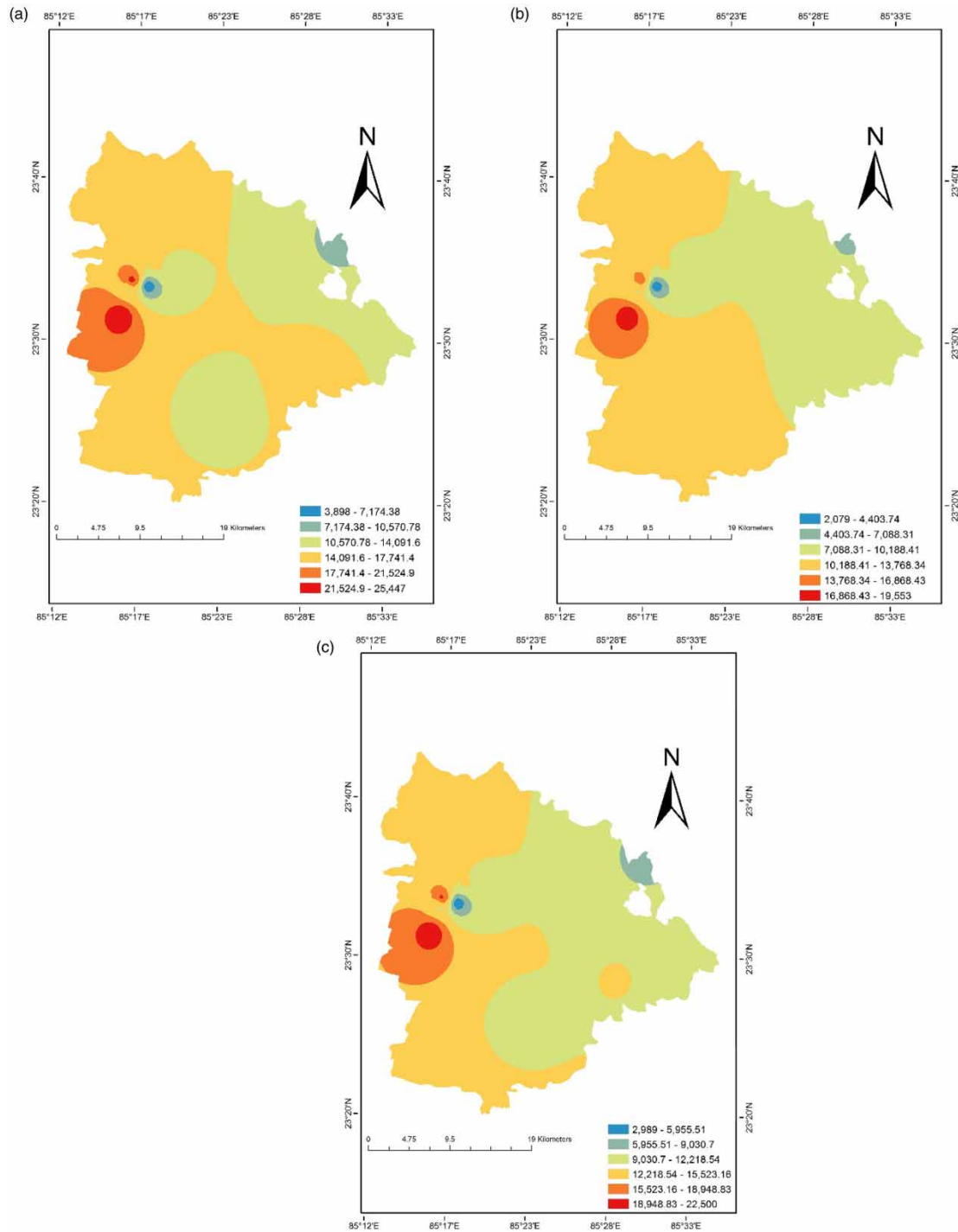
Bacterial contamination in groundwater was found to be low during summer but the bacterial density increased considerably during the monsoon season. HPC in the summer season was in the range of  $4.2 \times 10^2$  to  $5.1 \times 10^3$  CFU/mL and it increased to  $3.9 \times 10^4$  CFU/mL during the monsoon season. Similar results were also recorded for faecal coliforms ( $1.7 \times 10^4$  CFU/mL) in the monsoon, and it was comparatively lower in summer ( $1.6 \times 10^5$  CFU/mL). Contrary to the present study, Grisey *et al.* (2010) noted that the coliform count in groundwater influenced by MSW landfill and faecal inputs was higher in summer than in the monsoon season. However, the present results agree with the work of Pujari *et al.* (2007) who recorded that the bacterial population can be higher during the monsoon than in summer as the survival rate of pathogens is better due to higher water flux. They also concluded that during the monsoon the pathogens can move faster in the unsaturated zone.

Spatial variations in microbial populations were also assessed (Figure 3). The degree of bacterial load in various zones declined as the distance increased from the dumpsite. The zone-wise data showed that the nearest point to the dump site (within 0.5 km) where three samples (2GW1, 2GW2 and 2GW3) were taken, exhibited HPC of  $1.3 \times 10^4$  CFU/mL and faecal coliform of  $6.8 \times 10^3$  CFU/mL. The control site DW1 showed fewer numbers of faecal coliforms ( $3.9 \times 10^5$  CFU/mL) when compared to other sites. Faecal contamination detection at the control site can be attributed to the poor sanitation conditions maintained by the residents resulting in easy contamination of the open dug well (Gwimbi *et al.* 2019). Low microbial contamination was recorded in DW2 and DW3 as they are far (~5 km) from the dumpsite. 2GW6 and 3GW1 were identified as moderately



**Figure 2** | Mean ( $n = 3 \pm S.D.$ ) seasonal variations in (a) heterotrophic plate count, (b) total coliform count and (c) a faecal coliform count in groundwater samples.

affected areas as the level of microbial pollution was relatively lower than that in sites close to the dumpsite. HPC, TC and FC of these sites were  $1.2 \times 10^4$  CFU/mL,  $8.2 \times 10^3$  CFU/mL and  $5.8 \times 10^3$  CFU/mL, respectively. Suthar *et al.* (2009) also suggested that an increased bacterial population can be found more around nearby solid waste dumpsites. Bacterial load based on the distance from the dump site confirmed that the area is getting affected by the open MSW dumpsite.



**Figure 3** | Spatial variations in (a) heterotrophic plate count (CFU/mL), (b) total coliform count (CFU/mL) and (c) fecal coliform count (CFU/mL) in groundwater around the MSW dumpsite.

The correlation coefficient was determined between the distance of sampling points with HPC, TC and FC which showed a negative correlation between distance and HPC (-0.998), distance and TC (-0.969) and distance with FC (-0.906) (Table 1). These data signify that groundwater samples around the MSW dumping site were impacted due to unscientific disposal of solid waste and landfill leachate generation where the degree of microbial contamination gradually lowered with increasing distance from the dump site. On the other hand, HPC showed a high degree of correlation with TC (0.976) and FC (0.921) while TC was significantly correlated with FC (0.981). Data were subjected to one-way ANOVA to test the differences in the mean of different types of bacteria zones wise. For instance, there was no significant difference between HPC, TC and FC at the nearest

**Table 1** | Correlation coefficients of relationship between microbial contamination and distance of groundwater source from the MSW dumpsite

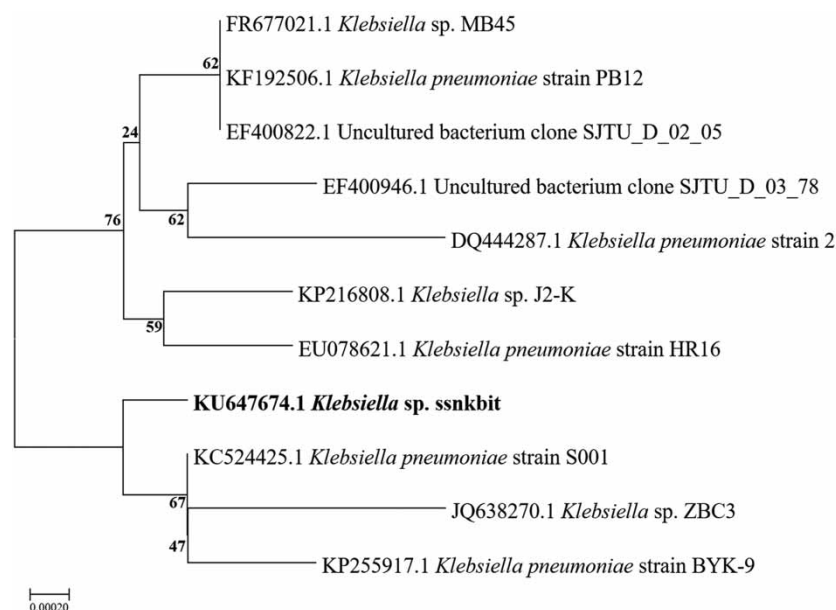
	Distance	HPC	TC	FC
Distance	1			
HPC	-0.998*	1		
TC	-0.969*	0.976*	1	
FC	-0.906*	0.921*	0.981*	1

\*Denotes significant correlation.

zone (0.5 km) and farthest zone (5 km) of the dumpsite. There was a significant difference between different bacterial groups ( $F_{5,14} = 5.82$ ,  $P < 0.05$ ) at 1.5 km from the dumpsite. Data was also tested with two-factor ANOVA to find the statistical significance of means between the bacterial groups and the distance of the site from landfill. There was a significant difference between the types of bacteria detected ( $F_{3,40} = 21.45$ ,  $P < 0.05$ ) and the distance from landfill was statistically significant in terms of influencing the existence of bacteria ( $F_{3,00} = 5.29$ ,  $P < 0.05$ ) (see supplementary data for ANOVA tables). Another study at same site has shown that the groundwater quality in this region was poor and there was lateral movement of leachate up to 5 km from the boundaries of MSW dumpsite (Chakraborty & Kumar 2016).

### Molecular characterization

The presence of faecal coliforms in groundwater is a major concern as it affects the potability of water, whereas the local population in developing countries mainly depends only on these water sources for drinking often without any type of treatment or minimal treatment. To know the major types of bacteria present, morphologically identical colonies were primarily screened visually which were members of the *Enterobacteriaceae* family as indicated by typical colony characteristics such as mucoid, smaller, pinkish colonies when cultured on EMB agar. EMB agar medium helps to differentiate *E. coli* and *Enterobacter*, where the presence of *E. coli* can be easily identified by greenish metallic sheen colonies. The secondary screening was carried out by using the 16S rDNA genome sequencing method to identify the species and establish the taxonomic position of the isolates. Figure 4 indicates that the isolate (*Klebsiella ssnkbit*, KU647674) was almost 99% similar (through NCBI BLAST) with *K. pneumoniae*, strain S001 (Accession No. KC524425).



**Figure 4** | Maximum composite likelihood-based neighbour-joining (NJ) phylogenetic reconstruction of 16S rRNA gene sequence of *Klebsiella* sp. ssnkbit. (The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches).

*K. pneumoniae* is known to cause pneumonia, infant bronchitis, several pulmonary diseases etc. It must be noted that the main pathway for the spread of *K. pneumoniae* is person-to-person contact and it is typically not linked with waterborne diseases. NCBI BLAST tree view software was used to construct the phylogenetic tree (Query\_64527) based on a neighbour-joining analysis of 1,000 replicates (Figure 4). Suthar *et al.* (2009) also reported the presence of *K. pneumoniae* in 90% of the groundwater samples of rural areas in northern Rajasthan, India. The presence of faecal coliforms and subsequent detection of *K. pneumoniae* highlighted that the groundwater in the study area is seriously impacted due to open dumping of MSW. Open dumping appears to be the major source of bacterial contamination and it is not to be discounted on the fact that human settlements near dumpsites (with very poor sanitary conditions), livestock faecal matter and surface runoff may also contribute to bacterial density in groundwater that is completely unprotected. Further studies on identifying the contribution of landfill leachate and other sources of bacterial load will be interesting to gain detailed insights for site remediation and management.

## CONCLUSIONS

Groundwater sources around the MSW dumpsite were evaluated to find the temporal and spatial variations in the bacterial population. Open MSW disposal is strongly affecting the groundwater in terms of bacterial contamination. Most of the groundwater sources in the study area exhibited a wide range of the bacterial population. The heterotrophic plate count of groundwater samples showed the highest bacterial population of  $4.5 \times 10^4$  CFU/mL and the maximum faecal coliform of  $2.1 \times 10^4$  CFU/mL was at a hand pump source near the MSW dumping area. Bacterial contamination was found to be very high during monsoon and the contamination gradually declined with increasing atmospheric temperature. In terms of spatial contamination, a nearby area of the MSW dumping site was most affected, whereas the impact was found to be dispersed up to 5 km from the dumping site. Molecular characterization of the faecal coliform colonies revealed the presence of *K. pneumoniae* in groundwater samples of the area, a causative agent for pneumoniosis, infant bronchitis, several pulmonary diseases etc. The groundwater microbiology indicates a high level of contamination, which requires disinfection before consumption and other domestic use. It will be better to avoid using untreated groundwater at least 5 km from the periphery of the dumpsite and groundwater from such areas must not be consumed directly without effective disinfection during any season. Further research on source identification to know the contribution of landfill leachate and other sources of bacterial load will be useful for site remediation and management.

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## CREDIT ROLES

**Shubhrasekhar Chakraborty:** Conceptualization, Data curation, Formal analysis, Methodology, Field survey, Writing – original draft. **Mohini Verma:** Methodology, Data curation, Field survey. **Jawed Iqbal:** Software, Validation, Supervision, Writing – review and editing. **R. Naresh Kumar:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review and editing.

## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

## DECLARATION OF COMPETING INTEREST

The authors have no competing interests to declare.

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