Int.J.Curr.Microbiol.App.Sci (2026) *15*(6): 176-187

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 15 Number 6 (2026)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2026.1506.018

Comprehensive Analysis and Characterisation of Graywater in a College Campus, Mumbai, Maharashtra, India

Sohini Dasgupta[1*] and Zaine P. Bhathena[2]

[1]Department of Microbiology, Bhavan's College, Munshi Nagar, Andheri (West), India
[2]Bhavans Research Center, Palanji Sadan, Bhavan's College Campus, Munshi Nagar, Andheri (West), Mumbai, 400058, India

**Corresponding author*

Keywords

Graywater, Physicochemical parameters, Pathogens, *Cryptosporidium*, *Giardia*

Article Info

Received:
18 April 2026
Accepted:
29 May 2026
Available Online:
10 June 2026

A B S T R A C T

Graywater reuse helps to address global water scarcity, but its safe application requires careful quality assessment to reduce potential health risks. An intervention work was set up to quantify and analyse the amount of graywater generated within a college campus and to provide an overview of its quality. This study quantified graywater generation and characterised its quality across two residential buildings on a college campus, analysing 120 samples collected over 02 years. Physicochemical parameters (TOC, COD, BOD, TSS, nitrate, surfactants, chlorine, and turbidity) and microbial indicators were evaluated. Microbial assessments targeted total heterotrophs, total coliforms, *Escherichia coli*, *Staphylococcus* spp., and pathogens including *Pseudomonas* spp., *Salmonella* spp., and *Enterococcus* spp. Total coliforms ranged from 1.48 ±1.60 x10^3 to 5.42 ±1.28 x10^4CFU/mL ($p<0.05$), and *E. coli* from 1.01±0.03×10^2 to 3.81±1.65×10^4 CFU/ mL ($p<0.05$), indicating consistent high-level bacterial contamination. The present study also highlights the occurrence of protozoan, specifically *Cryptosporidium* oocysts and *Giardia* cysts, when evaluated using formalin–ether concentration and calcium carbonate flocculation techniques, both of which detected low levels in the samples. Overall, the results confirm elevated pathogenic contamination, underscoring the need for treatment prior to reuse.

Introduction

Water scarcity is one of the major challenges faced in arid and semi-arid regions of the world, which has slowly but steadily reached the mega-cities. IWMI estimated that one in three people will face water scarcity by 2026 in India due to exponential population growth and the associated increase in water demand (Edwin *et al.,* 2014). Thus, government bodies dealing with water management have initiated measures aimed to reduce water usage through increased awareness, installation of rainwater harvesting technologies and graywater treatment systems, especially in areas of developing countries that are more vulnerable to water scarcity (Abedin *et al.,* 2013).

Graywater is the wastewater or wash water discharged from bathtubs, showers, sinks, washing machines, and

dishwashers that typically contains significantly lower nitrogen levels than the black waters obtained from toilet discharges (Benami *et al.,* 2013), making it more suitable for gentler treatment and reuse (CDPH, 2001), as Graywater makes up the largest proportion of the total wastewater flow from households in terms of volume.

Graywater, contains diverse physico-chemical and microbiological constituents due to the diverse activities that generate it, varying its key physico-chemical parameters like pH, electrical conductivity, total dissolved solids, turbidity, suspended solids, and organic load (Birks *et al.,* 2007). In addition to these chemical characteristics, graywater may also harbour levels of bacteria, which pose potential health risks if discharged untreated or reused. Furthermore, protozoan such as *Giardia* spp and *Cryptosporidium* spp may also be present, often as resistant cysts or oocysts, as they are capable of surviving environmental exposure, posing a threat to human health in rural as well as in urban environments if these water supplies are untreated (Chaudhary *et al.,* 2023). Exposure to these protozoans through ingestion or contact can result in gastrointestinal illnesses, particularly affecting children, immunocompromised individuals, and the elderly.

Thus, untreated discharge of graywater enhances environmental and public health risks. Effective treatment is essential not only to mitigate these risks but also to promote sustainable water reuse and reduce the burden on centralised wastewater treatment systems (Rangan *et al.,* 2019). Thus, the primary objective of this study was to evaluate the influent domestic graywater samples from communities like college campus by assessing its physico-chemical and microbiological characteristics with an aim to develop effective treatment and reuse strategies for creating water sustainability.

Materials and Methods

Study site

To identify the sampling locations (Figure 1), assistance was taken from Hostel authorities and BMC floor plans were used to locate different graywater sources, such as the washbasin, kitchen sink and bathroom. Outlet pipes that were clearly separated from the central wastewater collection system were shortlisted to collect the graywater.

Sample Collection

Initially, 30 graywater samples were collected to determine suitable sampling time intervals based on diurnal variation in flow and quality. Subsequently, a total of 120 graywater samples were collected over a period of 2 years and subjected to detailed physicochemical analysis from two residential buildings on a college campus. 03 litre samples were collected in sterile HDPE bottles by using the grab technique and analysed immediately or kept sealed and stored under cold and dry conditions (<4ºC) for not more than a day and processed as per the standard protocols of APHA, 2005. Graywater samples were collected from 3 different point sources:

a) B28 building-wastewater obtained from the Kitchen sink outlet labelled as B28K and a combined outlet of the washbasin and bathrooms labelled as B28B.
b) B29 building-wastewater obtained from a combined outlet of washbasin and bathrooms labelled as B29B.

A multiple-response survey study was first administered to all residents across both buildings. The survey was designed to evaluate residents water consumption patterns at different times of the day. The outcome obtained was crucial for identifying the optimal periods for graywater collection and subsequent analysis. The survey findings were systematically summarized.

Analysis of Sampled graywater

Physicochemical analysis of graywater

A 100 mL aliquot of each sample was analysed to assess its physicochemical parameters such as pH, temperature, conductivity, turbidity, TSS, COD, BOD, chlorine content, and water hardness as per USEPA (600), 1983 standards. pH of the samples was measured using a pH meter (Universal Enterprises), while turbidity was determined using a nephelometer (Sistronic Digital). Total suspended solids (TSS), chemical oxygen demand (COD), and biochemical oxygen demand (BOD) were analysed using a UV chemical analyser, while electrical conductivity was measured with a conductometer (Sistronic Digital). Chlorine test was performed by DPD test kit (WT006D) (AQUA Check), and Total Hardness of water was analysed by using AQUASOL kit (Rakiro Biotech system Pvt ltd).

Detection and Enumeration of total heterotrophic bacteria

Samples for microbiological analysis were processed according to USEPA and APHA methods (Table 1). Indicator organisms such as *Escherichia coli* (CFU/mL), total coliforms (CFU/mL), and *Enterococcus* spp. (CFU/mL) were detected by membrane filtration technique using selective media such as Rapid HiColiform Agar and Bile Esculin agar respectively, while the load of heterotrophic organisms were determined by the standard plate count method using Standard Plate Count (SPC) Agar, with colonies being enumerated following incubation for 24 to 48 hrs at 28±0.5°C. The presence of suspected pathogenic organisms such as *Vibrio cholerae*, *Salmonella* sps, *Pseudomonas* spp., *Enterococcus* spp. and *Staphylococcus aureus* (CFU/mL) from the graywater samples was determined by membrane filtration using selective media like Thiosulfate–citrate–bile salts–sucrose (TCBS) agar, Xylose-Lysine Deoxycholate (XLD) agar, Cetrimide Agar and Mannitol salt agar, respectively. 100ml of each sample was filtered through a 0.45 µm filter and placed on the respective selective media and incubated for 24hrs at 37 ± 0.5°C. Isolates showing typical colonies were further purified and subjected to identification based on biochemical reactions and those showing positive biochemical reactions were identified as per Bergey's Manual of Determinative Bacteriology and counted as CFU/mL. All assays were performed in triplicate. Confirmation of few isolates was undertaken through an Analytical Profile Index (API) based identification performed on a VITEK 2 system.

Detection and enumeration of protozoan oocysts and cysts

Approximately 60 graywater samples were processed for analysis in accordance with USEPA Method 1623 and IS15553:2006 by membrane filtration method, followed by formalin–ether concentration process and calcium carbonate flocculation method with slight modification.

Sample collection, filtration and concentration

a) Membrane filtration method-

Graywater samples were collected from both buildings B28 and B29 for the detection of *Cryptosporidium* spp oocysts and *Giardia* spp cysts. For samples with turbidity <30 NTU, direct filtration was performed using a 1.2 µm pore size, mixed cellulose ester membrane filter [Merck, India], followed by an elution process. For high-turbidity samples (≥30 NTU), a double filtration process was carried out. Samples were first passed through a cellulose watmann filter paper of 11 µm pore size pre-filter (Himedia Labs Pvt Ltd) and then again by a 1.2 µm pore size, mixed cellulose ester membrane filter [Merck, India]. The filter membrane pad was then eluted with 20-30ml of eluting fluid (phosphate-buffered saline pH 7.3±0.2, 1% Tween 20, 0.5% SDS) using a mechanical stomacher bag, and the eluate was centrifuged at 2,000 g for 10 -15min in 50 ml centrifuge tubes at 4 °C, with the process being repeated. The supernatant was discarded, and pellets from all centrifuge tubes were pooled into a single sterile centrifuge tube.

The pellet was resuspended in 50 mL sterile saline, after which 10 mL of 10% formalin (Sigma, USA) and 4 mL of 99% diethyl ether (Sigma, USA) were added. The tube was sealed and vigorously shaken to ensure contact of the solvent with the sediment, followed by centrifugation at 1,000 ×g for 5 min. After centrifugation, the supernatant was carefully discarded, and the final pellet was resuspended in 100 µL of 0.85% sodium chloride solution and subsequently subjected to the staining procedure.

b) Modified Calcium Carbonate flocculation method-

1L Graywater samples were mixed with 40-50ml of 1M Calcium chloride ($CaCl_2$) solution and 40-50ml of 1M Sodium hydrogen carbonate ($NaHCO_3$) solution. The contents were mixed after which 50ml of 1M Sodium hydroxide (NaOH) solution was added and mixed thoroughly. The contents were allowed to stand at room temperature (25°C) for 4-5 hours, such that the flocs settled.

The supernatant was aspirated into another sterile bottle without disturbing the flocs. A 1.5%v/v 1M HCl was added to dissolve the flocs and combined with the supernatant. The mixture was transferred to sterile 50 mL centrifuge tubes, 0.05% Tween-20 was added, and samples were again centrifuged at 2,500 × g for 15 minutes. Supernatants were discarded, and pellets were pooled into a single tube with 5 mL of distilled water and then stained.

Staining and identification process

Each concentrated sample was stained using the modified Ziehl Neelson Carbol Fuchsin (ZNCF) [Himedia Labs Pvt Ltd] staining method, wherein 3% sulphuric acid instead of 1% was used to ensure appropriate decolourisation for the identification under 100 X. The stained slides were screened for the presence of *Cryptosporidium spp* oocysts. Structures that appeared pink colored, round to oval of size 4–6 µm, were counted as positive oocysts by the ZNCF method. Staining of each sample were performed in triplicate.

Each concentrated sample was resuspended on a slide using the Lugol's iodine wet-mount procedure. 20 µl of sample were mixed with 10–15 µL of Lugol's iodine (Himedia Labs Pvt. Ltd.), a coverslip was placed gently, and the preparation was allowed to stand for approximately 30–60 seconds to permit stain penetration into cysts. Slides were examined at 40× to inspect for *Giardia* spp. cysts. Oval structures of the 8–12 µm size showing internal nuclei and median bodies in the iodine mount were recorded as positive. Staining was performed in triplicate for each sample.

Statistical Data Analysis

Data are presented as mean ± standard deviation (SD). Statistical analyses were conducted using SPSS version 23.

Result and Discussion

Sample collection and survey reports

A household water-use survey was administered to assess daily per-household consumption in B28 and B29, with 52 responses being received from 60 flats. Respondents (primarily aged 25–45 years) reported that 80–85% of water usage occurred in the morning (08:00–10:00), 10–15% during midday (12:00–14:00), and 0–5% in the evening (16:00–18:00) (Figure 2). Survey results indicated that overall household water use in B28 exceeded that in B29, due to various routine domestic activities; this suggested spatial variability in graywater volumes and characteristics. To substantiate these findings, preliminary monitoring of raw graywater was performed using 30 samples collected over three months. Morning samples consistently exhibited higher pH, turbidity, TSS, COD, BOD, TOC, and hardness compared with afternoon and evening samples, supporting the observed temporal variation in both graywater quantity and quality (Figure 3) as per CPCB 2008 and USEPA 2012 prescribed limits.

Based on the survey and sampling data, household water consumption was estimated at approximately 150–200 L per family per day, assuming an average per-capita use of 50–100 Lpcd; this is comparable to Birks *et al.,* (2007), who reported 185 Lpcd with 60–65% of household discharge classified as graywater. Conversely, Abedin *et al.,* (2013) reported higher urban middle-class consumption (200–300 L per household per day), with around 67% of building discharge being graywater and higher usage on weekends than weekdays, a pattern consistent with observations in the present study (Figure 2). These findings confirm clear daily variation in graywater generation and underscore its substantial contribution to overall household water demand. Since most of the activities within the household occurred in the morning, as per the data collected by preliminary studies, leading to the production of graywater that is rich in additives, it was thought prudent to restrict our collection time to morning sampling only.

Analysis of morning graywater samples

Physicochemical analysis

Analysis of 120 samples for pH, turbidity, TSS, BOD, and COD from building B28, compared with those from building B29, is summarised in Table 2. Though the temperature ranged between 25-28ºC for samples collected from B28 building, it was found to be comparatively warmer than that of B29 building. Alhough both the building samples showed a basic pH value, the average pH value of B28 building (7.30 to 7.95) was slightly higher than samples of B29 building (7.1 to 7.3), this may be due to the higher usage of soap, detergents, floor cleaner etc as B28 was more of a residential building having a kitchen as compared to B29 which was of a hostel type building. There was no significant difference in mean pH between building B28 and B29 ($F_{1,118} = 0.73, p = 0.39$), nor in mean temperature ($F_{1,118} = 1.12, p = 0.29$). This contrasts with that reported by Abedin *et al.,* (2013) who reported Dhaka city graywater to be slightly acidic. This data was also similar to that reported by Eze *et al.,* (2015) who observed a pH value of 5.9-6.3 in graywater studied for hostels in Nigeria. However, Kotut *et al.,* (2011) reported

the pH value obtained from Homa Bay town to be more basic due to the presence of alkaline compounds and organic loads within its primary water reservoirs.

Electrical conductivity is used as an indicator for the concentration of dissolved salts and minerals in water. Conductivity of samples obtained from the B28 building was in the range of 126 to 134 µS/cm while that of the B29 building waters was determined in the range of 99 to 105 µS/cm again indicating the presence of additives by the members residing in it. Kotut *et al.,* (2011) reported the value of conductivity in the range of 240 to 588µS/cm while that observed by Birks *et al.,* (2007) was 327µS/cm in domestic graywater analysed. From the previous studies, it has also been observed that the conductivity was higher in most of the cases, due to the dissolved ions in detergents used for household activities. B28 building samples also showed higher turbidity (30 to 55 NTU) than B29 building (21 to 41 NTU). Turbidity values as high as 241 NTU were reported by Abedin *et al.,* (2013), although Birks *et al.,* (2007) found that the impurity concentration was significantly lower, at 25 NTU, because of the impurity settling in the settlement tank. The total suspended solids (TSS) were measured in the range of 86 to 120 mg/l for the water of the B28 building and 30 to 40 mg/l for the B29 building. The higher TSS observed in B28 may be due to the kitchen wastewater, which typically contains much higher suspended solids than bathroom wastewater, which generally has a much lower solids content, resulting in lower TSS values in B29. Similar data was reported by Pachkor *et al.,* (2017) also due to the presence of organic material.

The BOD/COD ratios of the water samples obtained from B28 and B29 buildings were 0.82 and 0.67, respectively, which is much lower than that observed by Edwin *et al.,* (2014). The observed BOD/COD ratios indicate a moderate degree of biodegradability of graywater. Eze *et al.,* (2015) reported that the graywater samples from the Nigerian off-campus dormitories had similar lower BOD and COD values. Chlorine content of B28 building was in the range of 2 to 3 ppm whereas the water of B29 building were within the range of 1 to 2 ppm as measured by DPD kit method, and remained in a much lower range as compared to that found by Patil *et al.,* (2022). According to the CPCB 2008 and USEPA 2012 guidelines pH, turbidity, TSS, COD and BOD are important factors in assessing the quality of water and the kind of treatment needed for reuse. Some similar trends were observed in this study, with increases in TSS, COD, and BOD concentrations accompanied by corresponding increases in electrical conductivity (Kotut, K.; 2011) (Tilve, M. M.; 2014). The results showed that there was significant difference in the mean values of TSS ($F_{1,118}$=15.2, p<0.001); COD, ($F_{1,118} = 9.4,\ p = 0.003$); BOD, ($F_{1,118} = 6.7,\ p = 0.011$) among the two buildings.

Building B28, a residential block with kitchens, showed higher values than the hostel-type building B29, with samples exhibiting greater pH, turbidity, TSS, BOD, and COD in summer compared with the monsoon and winter seasons. Previous studies have also shown that parameters like turbidity, TSS, BOD, COD, and surfactants often peak in dry seasons (summer) when graywater is more concentrated and organic load is higher, while some dilution and slight improvement in quality can occur during rainy seasons (Tilve, M. M.; 2014)

Detection of indicator and pathogenic organisms predominant in raw graywater

A total of 251 bacterial isolates were obtained from graywater samples collected from both buildings. Total heterotrophic count of the two buildings ranged from 1.26 x10^5 CFU/mL to 4.78 x10^7 CFU/mL (Mean ±SD). It is observed that higher viable count of both buildings was observed during the summer season than those collected during the monsoon and winter seasons (Figure 5). Total coliform count of both the building ranged from (1.48 ±1.60) x10^3 to (5.42 ±1.28) x10^4CFU/mL (Mean ±SD) and *E. coli* count ranged from (1.01±0.26) ×10^2 to (6.43±1.65) ×10^3CFU/mL (Mean ±SD), (Mean±SD) Faecal contamination in graywater pipelines may have contributed to high counts in graywater samples collected from the bathroom area.

Microbial enumeration was performed by membrane filtration on selective and differential media, followed by biochemical tests, to detect the presence of *Escherichia coli, Enterococcus* species, *Pseudomonas* species, *Salmonella* species, *Staphylococcus* species and *Vibrio* species. Among all the samples that were evaluated, the frequency % of *Escherichia coli* of B28 building was 51% while that of B29 building was 42% whereas the coliforms count of B28 building 75% and B29 building 62%. The presence of total coliforms and *E. coli* shows faecal contamination, indicating the possible presence of pathogens.

Table.1 Standard methods used to determine bacterial load within raw graywater.

Parameters	Reference method
Heterotrophic organisms	APHA 9215A (2005)
E.coli	USEPA 1603 (2014)
Coliforms	USEPA 1604 (2002)
***Pseudomonas* spp**	ISO 16266
S.aureus	APHA 9213 (2005)
***Salmonella* spp**	USEPA 1682 (2006)
***Enterococcus* spp**	USEPA 1600 (2009)
Vibrio cholerae	USEPA139/600 (2010)

Table.2 Physicochemical analysis of morning raw graywater samples from B28 and B29 buildings. (n=120 samples.

Parameters (Units)	Standard Limits*	B28 Mean values ±SD	B29 Mean values ± SD
pH	6.0-9.0	7.65±0.32	7.2±0.12
Temperature (°C)	NA	25±0.2°C	26±0.1°C
Odour (Non-offensive)	NA	-	-
Colour	NA	-	-
Conductivity (µS/cm)	NA	130.6±4.16 $*10^{-6}$	102±3.15 $*10^{-6}$
Turbidity (NTU)	-	35.1±13.2	21.2±11
TSS (mg/L)	≤ 10–30 mg/L	83.5±17.1	40±10.2
COD ((mg/L)	≤ 50 mg/L	135±17.78	91±13.1
BOD (mg/L)	≤ 10 mg/L	80.5±6.8	61±9.3
TOC (mg/L)	NA	41±10.2	36±9.3
Nitrate (mg/L)	NA	3.5±1.5	2±0.5
Surfactant (mg/L)	NA	2±1.4	1.5±0.2
Chlorine (>1ppm)	>1ppm b	1.9±0.83	1.2±0.5
Water hardness (ppm)	NA	13±1.6	5.2±0.8

*As per USEPA 2012 & CPCB 2008

Table.3 Summary of the positive samples by Formalin ether concentration and Calcium carbonate flocculation process.

Protozoans prevalence	B28 building (no of positive samples)		B29 building (positive samples%)	
	Formalin ether concentration process	Calcium carbonate flocculation process	Formalin ether concentration process	Calcium carbonate flocculation process
***Cryptosporidium* oocysts**	04	01	01	-
***Giardia* cysts**	01	-	-	-

Figure.1 Sampling site location (Source -Google Earth).

Figure.2 Survey responses from residents of buildings B28 and B29 (n = 52).

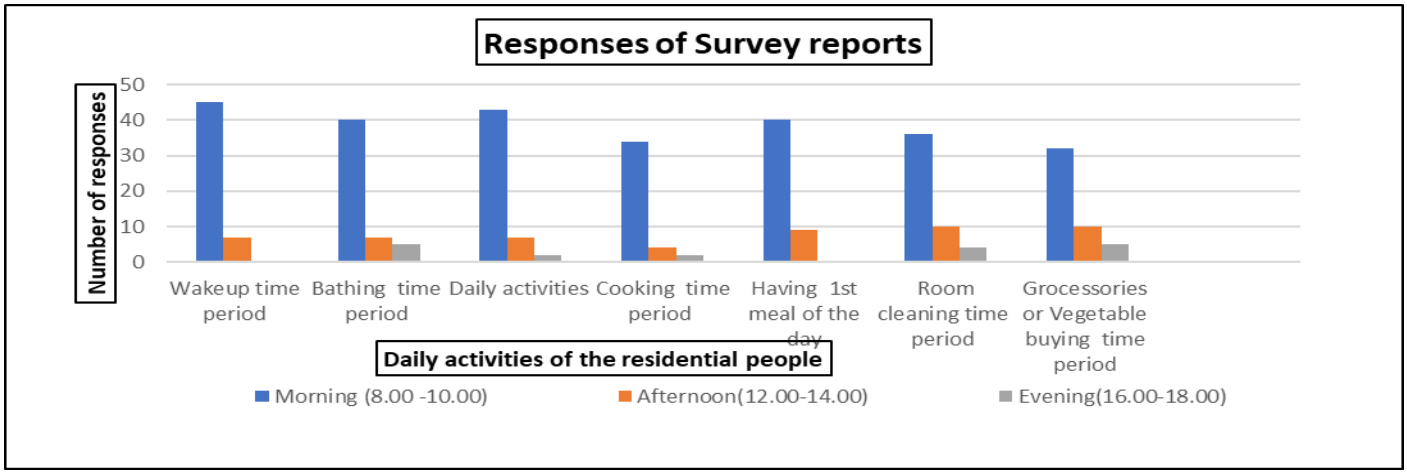


Figure.3 Preliminary assessment of graywater quality (n = 30 samples).

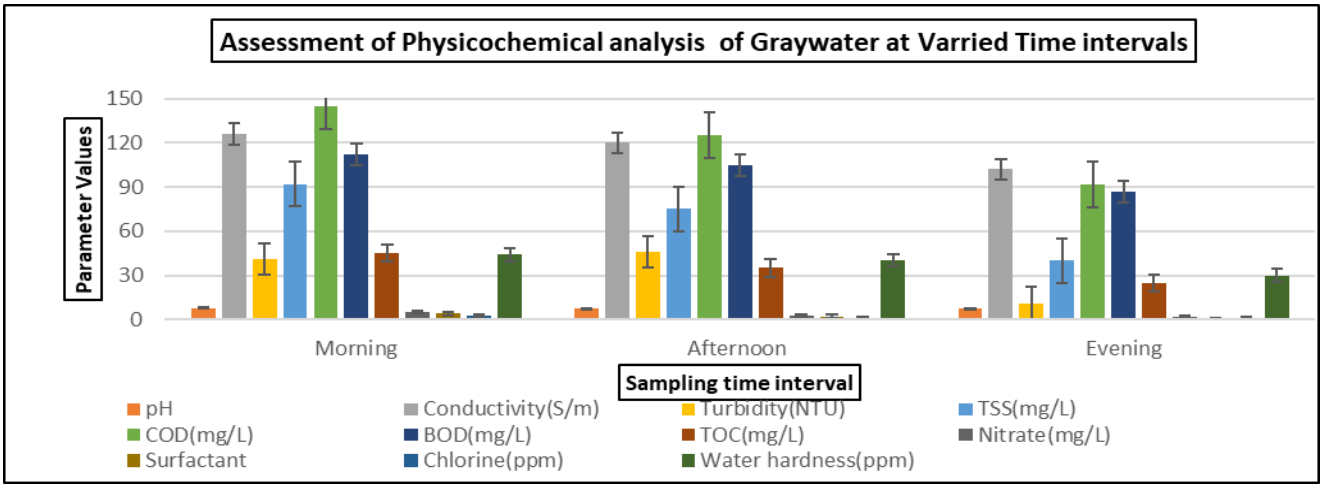


Figure.4 Season-wise variation in physico-chemical characteristics of graywater from B28 and B29 buildings (n=120 samples)

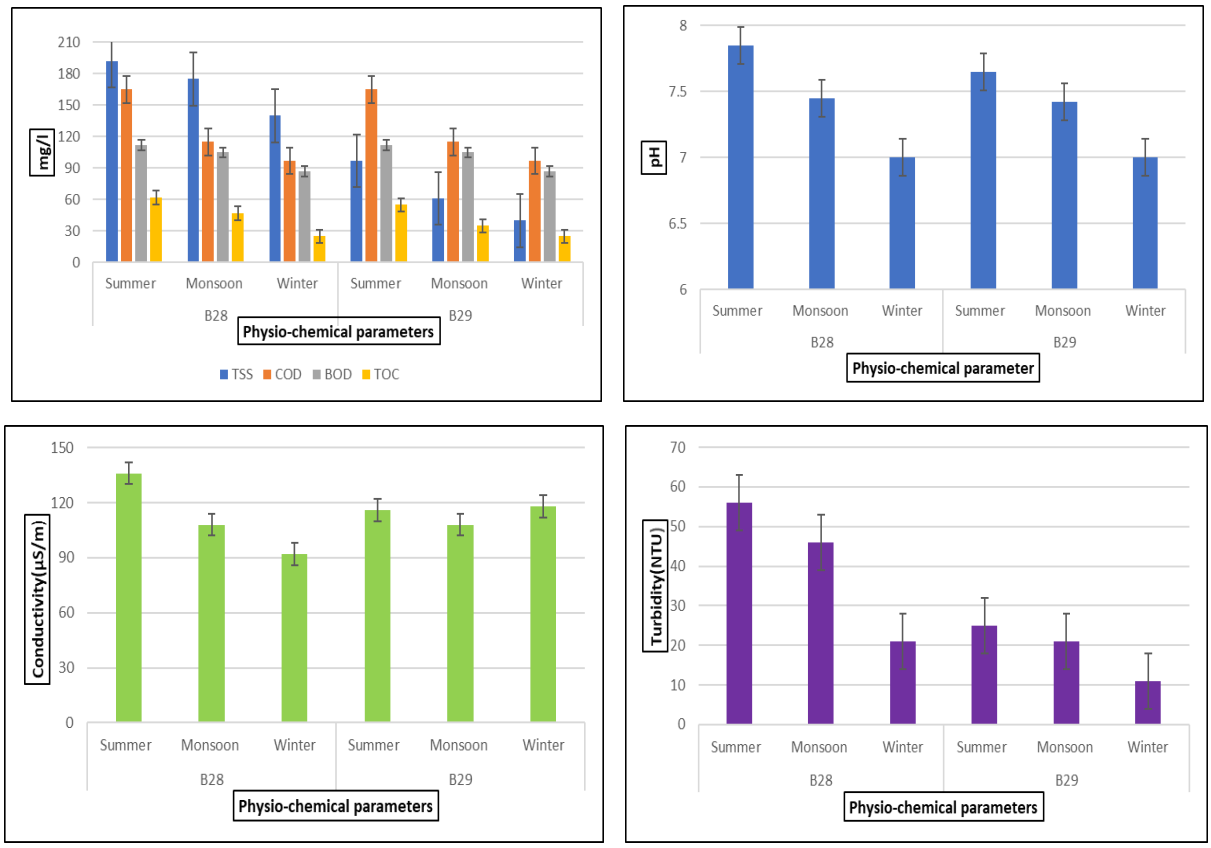


Figure.5 Total Heterotrophic count within graywater samples collected from B28 and B29 building (Mean ±SD)

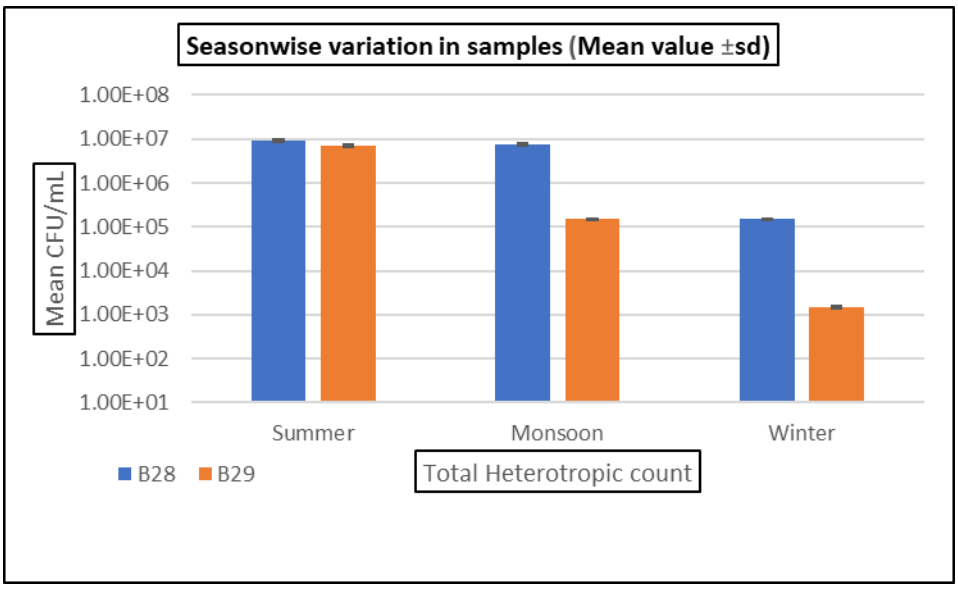


Figure.6 Seasonwise variation of environmental isolates from B28 and B29 building

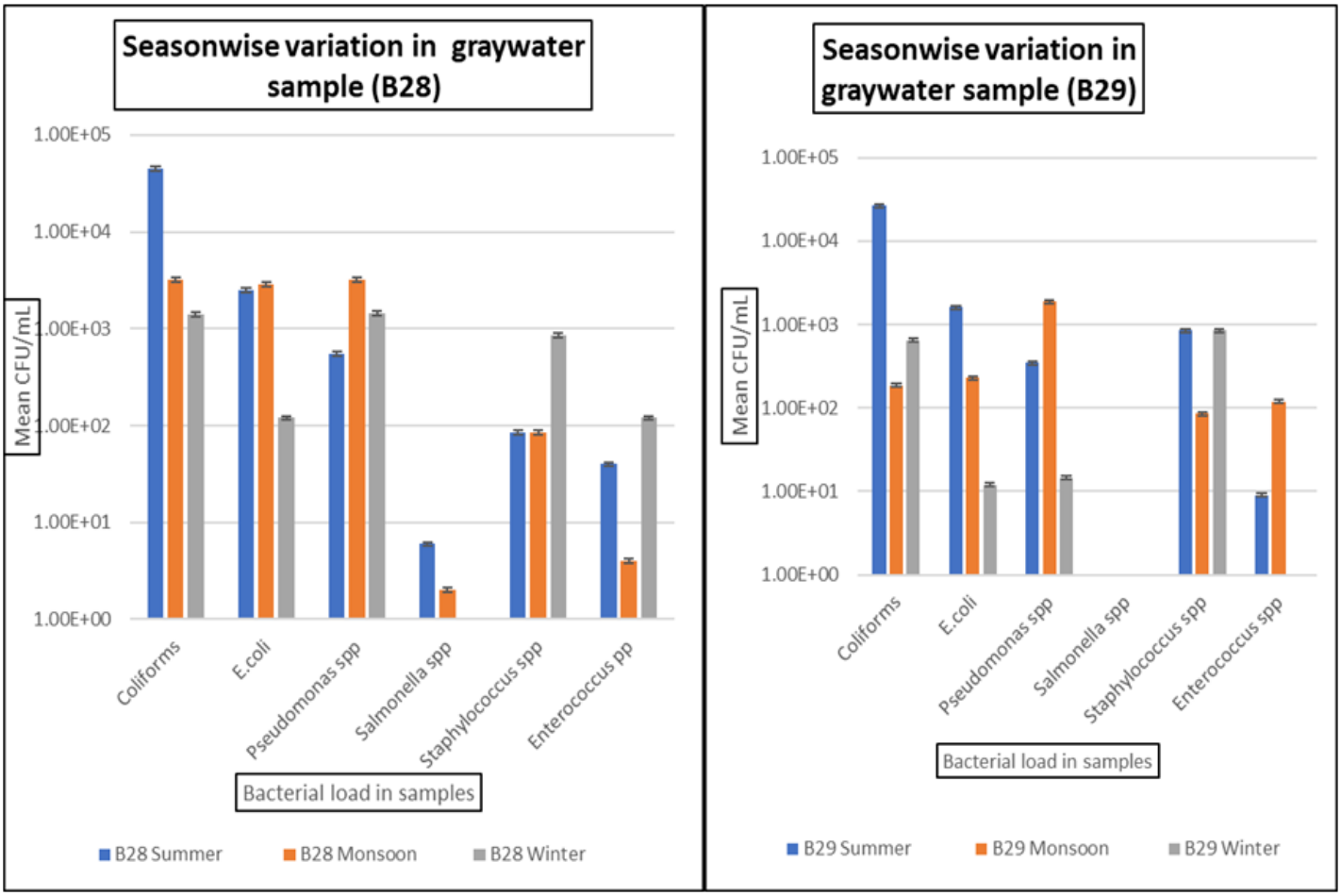
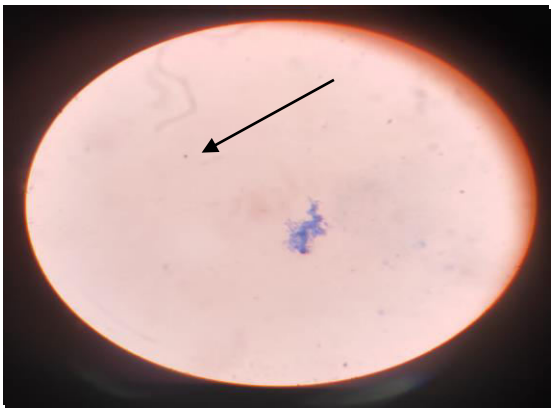
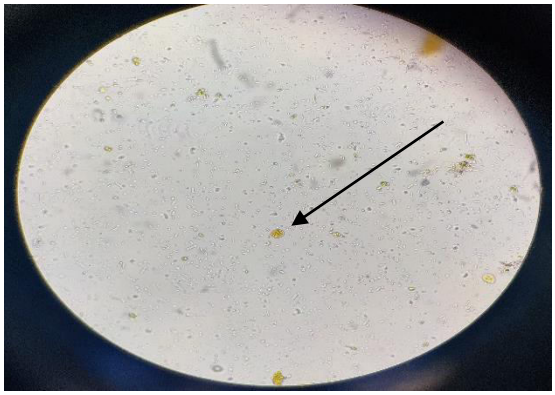


Figure.7 Microscopic examination of oocysts and cysts -A) ZNCF stained oocysts (100X) B) Iodine (Lugol's) wet mount (40x).



A



B

This is similar to earlier studies conducted by Birks *et al.,* (2007) and Kotut *et al.,* (2011), which found a higher number of total coliform and *Escherichia coli*. Frequency % of isolates such as *Pseudomonas* species and *Staphylococcus aureus* of B28 building were 12% and 15% whereas of B29 building were 11% and 18% respectively. The % isolates of *Salmonella* species, *Enterococcus* species and of B28 building were 9%, 15% and of B29 building were 2%, 15%. Few other studies conducted have reported a similar kind of distribution of bacterial isolates [Eze *et al.,* (2015); Jahne *et al.,* (2007) and Kotut *et al.,* (2011)]. None of the graywater samples showed the presence of *Vibrio* species. Thus, the data obtained from microbial analysis showed a high load of faecal coliform, *E. coli* and *Pseudomonas* species, *Enterococcus* species, and *Staphylococcus aureus* in comparison to *Salmonella* species, similar data have been reported by Birks *et al.,* (2007). Rare pathogenic organisms such as *Enterococcus casseliflavus* and *Paenibacillus lautus* prevalence were confirmed by Vitek analysis of identification, and were also detected in lower concentrations, such as 3% and 9% in B28 building, with prevalence of *Enterococcus casseliflavus* being observed (5%) in B29 building samples.

Building B28 consistently had higher bacterial counts than B29, possibly due to greater household activity or higher organic matter in its graywater. In summer, the warm temperature, higher organic content, and greater household activity is likely to promote bacterial growth, whereas in monsoon rainfall, the dilution effect contributes to reduced counts as compared to summer. Additionally, cooler temperatures in winter suppress microbial multiplication and lower their metabolic activity, thereby reducing bacterial load as compared to other seasons (Figure 6). These results demonstrate that monitoring of pathogenic organisms in raw graywater, along with seasonal monitoring in influent graywater, is recommended to identify environmental sources of contamination and obtain an informed decision about the design and operational timing of treatment systems for safe campus-scale graywater reuse.

Detection of Protozoan cysts and oocysts in raw graywater

A total of 60 raw graywater samples from buildings B28 and B29 were analysed for protozoa using both the formalin–ether concentration (FEC) and calcium carbonate flocculation (CCF) methods; a comparative summary is presented in Table 3. Overall, the FEC method yielded higher and more efficient recovery of cysts/oocysts in comparison to CCF method under the study conditions. Seasonal variation in Cryptosporidium oocysts and Giardia cysts were not statistically evident, because their levels in raw graywater stayed very low in the collected graywater samples,

Cryptosporidium oocysts were detected at low concentrations (3-4 oocysts/L) with building B28 samples showing 05 positive samples, while building B29 had only 01 positive sample. These counts are below commonly reported infective doses (10^1–10^3 oocysts /L) for untreated graywater, and are therefore of limited immediate public-health concern. This is similar to the earlier investigations of Birks *et al.,* (2007) and Jahne *et al.,* (2017) who reported no detectable oocysts in various water sources. In contrast Eze *et al.,* (2015) reported that the occurrence of *Cryptosporidium* oocysts in untreated wastewater generally falls within the range of 10–10^3 oocysts/L, although this may vary according to geographic location and seasonal factors.

Giardia cyst counts in the raw graywater were very low (1–3 cysts /L). Only building B28 yielded detectable cysts in 01 positive samples, whereas no cysts were observed in samples from building B29. These concentrations are below the commonly reported infective range of 10^2–10^4 cysts/L in untreated graywater and are therefore unlikely to pose a significant health risk. Similar observations were reported by Birks *et al.,* (2007), who detected Giardia in 63% of samples but considered the risk low because concentrations remained below the infective dose of approximately 10–25 cysts/L.

In conclusion, amidst global challenges like climate change and rapid urbanisation, ensuring sustainable water management is of prime importance. To reduce the use of potable water for non- consumptive purposes and to enable graywater reuse, it is important to assess the quality of graywater generated by residential buildings on a college campus.

The present study highlights variation in graywater quality from both buildings, with higher pH, turbidity, TSS, COD and BOD during summer than in the monsoon and winter. Microbiological results followed the similar trends, with increased total heterotrophs, coliforms, *E. coli, Pseudomonas* spp., *Staphylococcus* spp., *Salmonella* spp. and *Enterococcus* spp. in summer

compared to rainy and winter season Pathogenic organisms such as *Enterococcus casseliflavus* (3%) and *Paenibacillus lautus* (5%) prevalence were also detected, identified, and confirmed by Vitek analysis. Protozoan (oo) cysts were detected at low levels by both formalin–ether and calcium carbonate flocculation methods. While the staining techniques used here may serve as preliminary screening tools, confirmatory approaches such as molecular assays or FITC staining are recommended for more accurate detection of protozoa.

This research also highlights that based on the observed physicochemical parameters, *E. coli* and faecal coliforms counts, the graywater would require minimal additional treatment for reuse in irrigation and other non-potable applications, in accordance with CPCB (2008) guidelines.

Author Contributions

Sohini Dasgupta: Investigation, formal analysis, writing—original draft. Zarine Bhathena: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Abedin, S. B., & Rakib, Z. bin. (2013). Generation and Quality Analysis of Graywater at Dhaka City. Environmental Research, Engineering and Management, 64 (2). https://doi.org/10.5755/j01.erem.64.2.3992.

Benami, M., Gross, A., Herzberg, M., Orlofsky, E., Vonshak, A., & Gillor, O. (2013). Assessment of pathogenic bacteria in treated graywater and irrigated soils. *Science of the Total Environment*, *458–460* (May 2011), 298–302. https://doi.org/10.1016/j.scitotenv.2013.04.023

Birks, R., & Hills, S. (2007). Characterisation of indicator organisms and pathogens in domestic graywater for Recycling. Environmental Monitoring and Assessment, 129 (1–3), 61–69. https://doi.org/10.1007/s10661-006-9427-y

Chaudhary, A., Rana, S., Singh, R., Gurian, P. L., Betancourt, W., Kumar, A., & Kumar, A. (2023). Non-potable water reuse and the public health risks from protozoa and helminths: a case study from a city with a semi-arid climate. *Journal of Water and Health*, *21* (8), 981–994. https://doi.org/10.2166/wh.2023.283

Dasgupta, S., & Bhathena, Z. (2026).Pilot-Scale Efficacy of Bacteriophage Cocktail For Reducing Enteric Bacteria In Domestic Graywater. *Journal of Emerging Technologies and Innovative Research* (Vol. 13). http://www.jetir.org/papers/JETIR2603233.pdf

Edwin, G. A., Gopalsamy, P., & Muthu, N. (2014). Characterization of domestic gray water from point source to determine the potential for urban residential reuse: a short review. Applied Water Science, 4 (1), 39–49 https://doi.org/10.1007/s13201-013-0128-8

Eze, V. C., Onwuakor, C. E., & Mgbeokwere, E. U. (2015). Comparative Analysis of the Microbiological and Physicochemical Characteristics of Graywater Sources in Off-Campus Hostels at Michael. In Int.J. Curr.Microbiol. App.Sci (Vol. 4, Issue 8). http://www.ijcmas.com

Jahne, M. A., Schoen, M. E., Garland, J. L., & Ashbolt, N. J. (2017). Simulation of enteric pathogen concentrations in locally-collected graywater and wastewater for microbial risk assessments. *Microbial Risk Analysis*, *5*, 44–52. https://doi.org/10.1016/j.mran.2016.11.001

Keely, S. P., Brinkman, N. E., Zimmerman, B. D., Wendell, D., Ekeren, K. M., de Long, S. K., Sharvelle, S., & Garland, J. L. (2015). Characterization of the relative importance of human- and infrastructure-associated bacteria in grey water: A case study. *Journal of Applied Microbiology*, *119* (1), 289–301. https://doi.org/10.1111/jam.12835

Khurana, S., Sharma, P., Sharma, A., & Malla, N. (2012). Evaluation of Ziehl-Neelsen staining,

auramine phenol staining, antigen detection enzyme linked immunosorbent assay and polymerase chain reaction, for the diagnosis of intestinal cryptosporidiosis. *Tropical Parasitology, 2* (1), 20–23. https://doi.org/10.4103/2229-5070.9723

Kotut, K. (2011). Physico-Chemical and Microbial Quality of Graywater from Various Households in Homa Bay Town. The Open Environmental Engineering Journal, 4 (1), 162–169. https://doi.org/10.2174/1874829501104010162

Laaffat, J., Aziz, F., Ouazzani, N., & Mandi, L. (2019). Biotechnological approach of graywater treatment and reuse for landscape irrigation in small communities. Saudi Journal of Biological Sciences, 26 (1), 83–90. https://doi.org/10.1016/j.sjbs.2017.01.006

Mandal, D., Labhasetwar, P., Dhone, S., Dubey, A. S., Shinde, G., & Wate, S. (2011). Water conservation due to graywater treatment and reuse in urban setting with specific context to developing countries. Resources, Conservation and Recycling, 55 (3), 356–361. https://doi.org/10.1016/j.resconrec.2010.11.001

Motie, I., Burns, K., Thompson, R., Friar, E., Bermingham, I., Ranasinghe, U., & Wiese-Rometsch, W. (2022). Acinetobacter radioresistens and *Enterococcus casseliflavus* co-infection with endocarditis, bacteremia, and pneumonia. IDCases, 30.

Ottoson, J., & Axel Stenstr om, T. (2003). Faecal contamination of graywater and associated microbial risks. In *Water Research* (Vol. 37).

Oteng-Peprah, M., Acheampong, M. A., & deVries, N. K. (2018). Graywater Characteristics, Treatment Systems, Reuse Strategies and User Perception—a Review. *Water, Air, and Soil Pollution*, *229* (8). https://doi.org/10.1007/s11270-018-3909-8

Pachkor, R. T., & Parbat, D. K. (2017). Quantification and Characterization of Grey Water-A Case Study of Shraddha Nagar, Pusad. www.ijraset.com

Patil, P. D., Bhange, V. P., Shende, S. S., & Ghorpade, P. S. (2022). Graywater characterization of an Indian household and potential treatment for reuse. Water-Energy Nexus, 5, 1–7. https://doi.org/10.1016/j.wen.2021.12.001

Platzer, M., Cáceres, V., & Fong, N. (2004). The reuse of treated wastewater for agricultural purposes in Nicaragua; Central America

Rangan, R. P., & Heenalisha, K. (2019). Grey-Water Treatment and Reuse: A Review. In Asian Journal of Science and Applied Technology (Vol. 8, Issue 1). www.trp.org.in

Sáez-Nieto, J. A., Medina-Pascual, M. J., Carrasco, G., Garrido, N., Fernandez-Torres, M. A., Villalón, P., & Valdezate, S. (2017). *Paenibacillus* spp. isolated from human and environmental samples in Spain: detection of 11 new species. New Microbes and New Infections, 19, 19–27.

Shamabadi, N., Bakhtiari, H., Kochakian, N., & Farahani, M. (2015). The Investigation and Designing of an Onsite Grey Water Treatment Systems at Hazrat-e-Masoumeh University, Qom, IRAN. Energy Procedia, 74, 1337–1346. https://doi.org/10.1016/j.egypro.2015.07.780

Tilve, M. M. (2014). Seasonal Variation In Greywater Quality For A Real Life System. In *IJRET: International Journal of Research in Engineering and Technology*.

How to cite this article:

Sohini Dasgupta and Zaine P. Bhathena. 2026. Comprehensive Analysis and Characterisation of Graywater in a College Campus, Mumbai, Maharashtra, India. *Int.J.Curr.Microbiol.App.Sci.* 15(6): 176-187.
doi: https://doi.org/10.20546/ijcmas.2026.1506.018