







Understanding Impact of the Informal Use of Untreated Wastewater and Faecal Sludge in Agriculture on Health and the Environment

Interdisciplinary Research in Urban Sanitation 2021 Programme

Research Report December 2022



National Institute of Urban Affairs



With





By







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Abbreviations and Glossary

AMRUT	Atal Mission for Rejuvenation and Urban Transformation		
BORDA	Bremen Overseas Research & Development Association		
BWSSB	Bengaluru Water Supply and Sewerage Board		
CFU/ml	Colony forming Unit per ml		
СМС	City Municipal Corporation		
COPD	Chronic obstructive pulmonary disease		
CPHEEO	Central Public Health & Environmental Engineering Organisation		
DNA	Deoxyribonucleic acid		
EP ACT	Environment (Protection) Act		
FAO	Food and Agriculture Organization of the United Nations		
FGD	Focussed Group Discussions		
FS	Faecal Sludge		
FS&S	Faecal Sludge and Septage		
FSSM	Faecal Sludge and Septage Management		
FSTP	Faecal Sludge Treatment Plant		
GoK	GoK - Government of Karnataka		
НН	House Hold		
HS	Honey Sucker		
IHHL	Individual Household Latrine		
KLD	Kilo Litres per Day		
KUWSDB	Karnataka Urban Water Supply and Drainage Board		
LAHDC	Ladakh Autonomous Hill Development Council, Leh		
LEDeG	Ladakh Ecological Development Group		
LPCD	Litres per capita per day		
МСК	Municipal Committee, Kargil		
MCL	Municipal Committee, Leh		
MCSCH	Molecular Solutions Care Health, LLP		
MLD	Million Litres per Day		
MODI	Mission Organic Developing Initiative		
MoHUA	Ministry of Housing and Urban Affairs		
MSL	Mean Sea Level		
N.I.S.R	National Institute of Sowa Rigpa		
NCBS	National Center for Biological Sciences		
NIUA	National Institute of Urban Affairs		
OIE-WAHIS	World Organisation for Animal Health		
PHC	Primary Health Centre		
RNA	Ribonucleic Acid		
SBM	Swachh Bharat Mission		
SDG	Sustainable Development Goals		
SFD	Shit Flow Diagram		
SS	Salmonella Shigella		
SSP	Sanitation Safety Plan		
STP	Sewage Treatment Plant		
TCBS	Thiosulfate-Citrate-Bile Salts-Sucrose		
TDS	Total dissolved solids		
TIGS	Tata Institute for Genetics and Society		
TMC	Tumakuru Muncipal Corporation		
ULB	Urban Local Body		
USEPA	U.S. Environmental Protection Agency		
UT	Union Territory		
UTI	Urinary tract infection		
WASH	Water, Sanitation and Hygiene		
WHO	World Health Organization		
WWTP	WasteWater Treatment Plants		

1. Structure of the Report

The structure of the report follows the objectives of the study. The introductory sections are in Chapters 1-3. This is followed by Chapter 4 which outlines the overall research methodology; Chapter 5 which documents the informal use of untreated wastewater and faecal sludge in agriculture in the five study locations in this order: (i)Vijayapura, Karnataka (ii)Tumakuru, Karnataka (iii)Leh (iv)Kargil and (v)Vikas Nagar, Uttarakhand. Chapter 6 which captures the qualitative data on the health and environmental impact due to the use of untreated wastewater and faecal sludge; captures quantitative data on the health and environmental impact due to the use of untreated wastewater, wastewater, faecal sludge and soil samples. And finally Chapter 7 provides recommendations on de-risking and mitigation measures based on the findings.

2. Introduction to Wastewater Reuse and Rationale of the Study

This document comprises the report on a study commissioned by the National Institute of Urban Affairs (NIUA) on "Impact of the Informal Use of Untreated Wastewater and Faecal Sludge in Agriculture on Health and the Environment". The study was undertaken under the 'Interdisciplinary Urban Sanitation Research 2020' Programme of the NIUA by the BIOME Environmental Solutions Private Limited (Biome) with its consortium partners Ladakh Ecological Development Group (LEDeG) and Bremen Overseas Research & Development Association (BORDA) with the support of the Tata Institute for Genetics and Society (TIGS), National Center for Biological Sciences (NCBS) and Molecular Solutions Care Health, LLP (MSCH).

Wastewater has been reused for agriculture in India and many other parts of the world for decades. This has been the consequence of freshwater scarcity in many parts of the country. With the increasing adverse effects of climate change on water availability, currently, 10% of the global population consumes food that is produced using wastewater (treated, partially treated or diluted) (Ungureanu N et al. 2020). The usage of wastewater for agriculture, while common, is also controversial, with concerns of safety. On the one hand, the benefits of wastewater are many: for instance, an estimate suggests that treated wastewater can annually irrigate about 1 to 1.5 Million Hectares of land area (Sengupta, 2008). It also has the potential to contribute about one million tons of nutrients and 130 million person-days of employment (Minhas and Samra, 2004). (Ungureanu, N., et al. 2019) describes the multiple benefits of using wastewater in agriculture: year-round availability of large amounts of wastewater from daily domestic discharge, unaffected by climatic conditions, high nutrient content that allows reduced use of chemical fertilizers thus saving costs, increased productivity on less fertile soils, reduced damage to freshwater ecosystems associated with eutrophication and algal blooms, and many more . Additionally, reusing wastewater in agriculture reduces the burden on freshwater systems, since 70% of all freshwater sources are utilized for agricultural purposes.

Studies have shown high yields of various crops which have been irrigated with wastewater: Jang et al. 2013 observed a 15% increase in rice productivity, Emongor et al. observed a 114.9% increase in tomato productivity and in the productivity of crops like potato, onion, cabbage, avocado, cotton, sorghum, wheat, apples, grapes (Winpenny, J. et al 2010).

On the other hand, there could also be multiple drawbacks of wastewater. Using wastewater without proper safety gear could expose farmers to various diseases. It could also end up in the accumulation of heavy metals, salts, antibiotics, growth hormones, and other hazardous substances in the soil; low hydraulic conductivity due to clogging of soil pores with suspended solids from wastewater; decreased quality of agricultural crops due to clogging of soil pores with suspended solids from wastewater, and decreased quality of agricultural crops due to clogging of soil pores.

3. Objectives of the Study

The importance of wastewater reuse is well established. However, the unregulated reuse of wastewater and faecal sludge (FS) particularly in agriculture is a cause of concern. The adverse impact on health and the environment has not been adequately studied /understood.

The overall aim of the research is to help address knowledge gaps and inform practice in the urban sanitation sector with a focus on faecal sludge and wastewater management. Specific objectives are to:

- 1. Document the informal use of untreated wastewater and faecal sludge in agriculture
- 2. Capture qualitative data on the health and environmental impact due to the use of untreated wastewater and faecal sludge
- 3. Capture quantitative data on the health and environmental impact due to the use of untreated wastewater and faecal sludge through tests of groundwater, wastewater, faecal sludge and soil samples
- 4. Provide recommendations on de-risking and mitigation measures based on the findings

4. Overall Research Methodology

The methodology was three-pronged and comprised



Review of literature:

Key national and international resources comprising regulations, standards, guidelines, research, policy, documentation of practices, and project interventions from national and global institutes of eminence were assessed. The regulatory framework in India including key acts and policies/advisories was reviewed. A detailed study of , the 'Guidelines for the Safe use of Wastewater, Excreta and Greywater' and the 'Sanitation Safety Plan (SSP) manual' developed by WHO on the health and environmental risk mitigation from the application of untreated wastewater and faecal sludge use in agriculture, and the use of Reclaimed Water and Sludge in Food Crop Production, National Research Council was conducted. Academic and research papers on the health and environmental impacts of the reuse of wastewater (WW) and faecal sludge (FS) in agriculture were also considered. Noteworthy research and documentation of practices in the reuse of wastewater and faecal sludge were also reviewed.

Qualitative Data Collection through interviews:

The study locations were selected to represent diverse agro-climatic regions comprising five towns/cities – two from Karnataka, two from the Union Territory of Leh, and one from Uttarakhand. Each city was also selected to bring out specific unique elements of faecal sludge and wastewater reuse. It was envisaged that documenting prevalent practices in each city study would contribute to an integrated perspective.



Prevalent practices concerning reuse of WW/FS in the cities were documented, in consultation with public health experts a checklist was drawn up to elicit qualitative information from farmers, residents and local leaders on the experience and perceived health/ environmental impacts if any, from the use of untreated wastewater and faecal sludge. Detailed interviews/ FGDs with key informants i.e., five farmers/users from each of the selected towns were conducted. Doctors at government health centers were interviewed to ascertain the prevalence of dermatological conditions, water-borne and vector-borne diseases.

Quantitative Data Collection Through Testing of Water and Soil Samples from Wastewater Treated and Control Sites:

The selection of samples was done based on the physical observations by the researchers in consultation with experienced epidemiologists and local population/farmers.

In the selected site, tests were conducted on a combination of samples from the wastewater/ faecal sludge used for agriculture and corresponding soil treated with this source. The bacterial load was examined for samples from Vijaypura and Tumakuru. The sampling protocol was decided in consultation with experts, based on the prevalent practices of the town/city. Further details on the exact methods have been given in the relevant section of this document. Our original aim in this section was to conduct an overall metagenomic analysis of all the bacterial species found in the samples collected through a next-generation sequencing methodology, in partnership with Tata Institute for Genetics and Society (TIGS), the National Center for Biological Sciences (NCBS). Towards the end, samples were collected, shipped and frozen for RNA and DNA analyses. However, due to the pandemic second wave, the required additional resources, including the test kit, were not available immediately due to disruptions in the supply chain. This has been subsequently planned for.

5. Reuse Practices

This section captures city-wise details of the reuse practices of faecal sludge and wastewater in the cities. This diagram provides an overview of FS and WW reuse in the study cities.

City	WW Treatment	FS Treatment	Formal WW Reuse	Formal FS Reuse	Informal WW Reuse*	Informal FS Reuse**
Vijayapura					\checkmark	\checkmark
Tumakuru	~				~	\checkmark
Leh	~	\checkmark		\checkmark	~	\checkmark
Kargil		~			~	\checkmark
Vikas Nagar	\checkmark				\checkmark	

Figure 1: City-Wise Status of Treatment and Reuse of wastewater and Faecal Sludge/ Septage

Note: Formal is classified as practices under the state supervision with the legal and institutional framework in place. Informal is understood as small private sector players with no legal or institutional guidance.

*There may be more instances of informal reuse of wastewater

** There may be more instances of informal reuse of faecal sludge/septage management

***In Tumakuru, treated WW is used to fill lakes and there is a proposal to sell this to industries.

**** In Vikas Nagar Sewage Farm, wastewater is partially treated before land application.

5.1 Vijayapura, Karnataka

5.1.1 Physiography

Table 1: Basic information, Vijayapura

Area	16 sq km.
Population	34,866
Geography & Climate	Falls under Eastern-Dry agro-climatic zone. The town receives 750 mm of annual average rainfall. The average minimum temperature is 16°C and the maximum temperature is 31°C. Most areas have red loamy soil
Population density	2,179 per sq. km.
Blocks	23 wards

5.1.2 Water Supply

Vijayapura is currently entirely driven by groundwater. Historically, a major source of water used to be Badanekere, a downstream tank filled a few decades ago, and open wells were also used for domestic water. Borewells are drilled in and around this lake to source water. A total of 1.89 MLD of water is supplied at 57 LPCD. The drinking water is piped to communities for two days in the week, for a limited period¹. The region is extremely water-scarce and groundwater is only available source and has high levels of TDS. Vijayapura lies in a belt where the likelihood of fluoride presence in groundwater is very high (Urban wastewater management in Karnataka, NIUA, 2018).

5.1.3 Wastewater Reuse

Containment: The 2011 Census report says that Vijayapura has a total of 7,377 toilets of which 1,451 are pit toilets; 5,675 are flush types and 251 of miscellaneous categories. The town has a combination of open and closed drainage systems. According to this data, 91.2% of the households had toilets in 2011. Most on-site sanitation systems are in the "extension" areas that have emerged as the city grew. They are mostly single leach pits rather than septic tanks. Only black water goes into these systems, greywater typically flows into open stormwater drains.

Conveyance: Vijayapura is the only Urban Local Body in Bangalore Rural District with a sewer network. Vijayapura got the underground drainage (UGD) connection more than two decades ago. According to the Municipal records, nearly 63% of households have a sewer connection and 34% of the households have an on-site sanitation system and the remaining 3% of the household do not have a toilet facility. Households with onsite sanitation are likely to discharge greywater into open stormwater drains.

Treatment: Vijayapura does not have a sewage treatment plant (STP). Hence, black water from the underground drainage gets mixed with greywater and flows out into the drains that are connected to two main stormwater drains, each discharging raw sewerage into Badanekere lake. However, the wastewater reaches this tank only during the rainy season. In other seasons, farmers capture this wastewater for agricultural purposes.

Informal reuse in agriculture: The wastewater flowing in stormwater drains and in UGD lines is tapped by the farmers for irrigation. This wastewater is collected in structures similar to that of farm ponds, approximately 5-10ft wide and 2-3ft deep, and is left untouched with exposure to the sun for a few days. The sludge settles at the bottom of the pond, while the wastewater is pumped out and used in irrigation. After drying, the sludge is used as plant fertilizer. Farmers in Vijayapura grow non-edible crops like Mulberry extensively followed by fodder crops like maize and grass for livestock, using this wastewater.

1 Bengaluru Rural Zilla Panchayat & Government of Karnataka (2014). Bengaluru Rural District Human Development Report, 2014.

Figure 2: Mulberry farm applied with wastewater



5.1.4 Faecal Sludge Management

Containment: As per 2011 census, 34% of the households are connected with an onsite sanitation system. The system is dominated by single leach pits. These pit toilets hold black water from the households and the greywater is discharged into open drains. These pit toilets are usually around 10 ft deep and 3-4 ft wide and made up of concrete rings. The old households' leach pits are made up of bricks. These pit toilets need to be emptied by the desludging operators once full, on an average of twice a year.

Emptying and Transport: There are four desludging operators in the town - two owned by the Municipality (one jetting and one sucking) and two owned by the private operators. Municipal vehicles engage in cleaning the manholes and private vehicles provide pit emptying services for the households. Each desludging vehicle is of 3000 litres capacity. Both the municipal and private desludging operators charge ₹ 1,000 for cleaning a single pit toilet. Municipal vehicles empty the FS in the nearest manholes. Private vehicles empty the FS in the farm fields. Mostly mulberry and fodder crops are grown using the FS.



Figure 3: Desludging vehicle unloading Faecal sludge in a manhole

Treatment & Reuse: There is no FS treatment plant in Vijayapura. Farmers around Vijayapura play an important role in resource recovery and the reuse of nutrients in two different ways. If the client owns a farm, this faecal sludge is deposited on the client's farm. If not, the faecal sludge is deposited on the land of any farmer willing to use it. Thus, several farmers have an informal arrangement with the desludging vehicle operators who deposit the faecal sludge on the lands, with no cost to the farmer. This sludge is allowed to further decompose in large pits and later applied to the field. Some farmers also apply faecal sludge directly on the land and allow it to dry before ploughing.

5.2 Tumakuru, Karnataka

5.2.1 Physiography

Table 2: Basic information, Tumakuru

Area	48.6 sq. km
Population	302,143 (2011 census)
Administration	City Municipal Council with 35 wards
Geography & Climate	Elevation: 822 metres. Arid to semi-arid zone and is a drought prone region
Rainfall	The average annual rainfall of 780 mm with 45 rainy days

5.2.2 Water supply

Tumakuru city has no perennial water resources. The city is dependent on Hemavathi reservoir located 170 km away. Around 50 MLD of total water is supplied at 107 LPCD with a frequency of once in 3 days. Under the Smart City Mission and funded by AMRUT at an estimated cost of ₹ 258.73 crores, Tumakuru city is moving towards a major milestone in water supply, by implementing a 24/7 water supply, to all households including low-income houses.

5.2.3 Wastewater management

The below diagram captures the sanitation chain of Tumakuru city:



Figure 4: Sanitation chain, Tumakuru

Containment: According to KUWSDB and SLB 2016 data, 90% of the HH have toilets. Tumakuru city has a total of 88,525 households, of which 65% of households are connected to the UGD, while 35% have onsite sanitation systems. These are pit toilets, wherein only black water is contained and the greywater is openly led into the stormwater drains.

Conveyance: Tumakuru city has 65% of households connected to the UGD, according to the SLB data. The sewer network exists only in the core areas of the city. Under Tumakuru Smart City Mission in 2017, KUWSDB is aiming for a 100% UGD network, to connect every single household in the city to the sewerage system.

Treatment: The wastewater from the households that are connected to the UGD network is taken into the Sewage Treatment plant which has been operational since 2004. The STP is 24.75 MLD capacity following the technology of Aerated Oxidation Pond. The treated wastewater is let into Bheemasandra lake (50 acres) which overflows into an adjacent lake called Melekote lake (90 acres).

Figure 5: Left - Sewage Treatment Plant; Right - Map of the lakes receiving treated wastewater after treatment at the STP



Informal reuse of treated wastewater in Baje cultivation: Farmers from the command area (area around the lake, which gets irrigated from the lake water, via a canal) of both Bheemasandra and Melekote lakes are using treated wastewater in irrigation informally, to cultivate a wetland crop locally called Baje (Sweet Flag), (Scientifically referred to as Acorus Calamus or also known as Vacha and Vasambu in local Indian languages). It is a rhizome that is used widely in pharmaceutical industries and is a part of indigenous and Ayurveda as well, for treating various health problems such as digestive disorders, stimulating brain functioning, asthma, speech impediments, hair care, sedative, cold, etc.

Baje follows flood irrigation. The Lake water is allowed to flow continuously through sluice gates and is channelled until the last plot of agricultural land using bunds. Baje is dried under the sun for about a week, rhizomes are removed using machines and cut into small pieces of three to four inches.

Baje has a high market value and farmers are making profits. Farmers and wage labourers who are in contact with wastewater say they have not faced any health-related issues.



Figure 7: Baje cultivation in the command area of Bheemasandra lake (left) and Baje root (right)



5.3.4 Faecal sludge management

Containment: In 35% of the households in the city, black water is contained in pit toilets. The greywater is openly led into stormwater drains.

Emptying and Transport: Desludging operators are engaged in pit emptying, transporting and disposal of FS, who operate both within city limits and also in neighboring villages. There is only one government-owned desludging vehicle for Tumakuru city and 20 private-owned vehicles. As the city expanded the number of operators in this sector also witnessed a rise. The government-owned vehicle charges ₹ 500 per trip, and dispose the FS in the nearest manhole or in the STP inlet, whereas private vehicles can charge between ₹ 1000-1800, based on the distance traveled, and dispose it at arecanut and coconut fields.

Figure 8: Government-owned (left) and one of the private-owned desludging vehicles



Treatment & Reuse: There is no Faecal Sludge Treatment Plant (FSTP) in Tumakuru city. The FS is applied on fields, as a manure after some preliminary onfarm treatment. Farmers compost the FS for 5-6 months before application on field, by composting them in shallow ponds. Alternatively, some spread it across the field directly during sowing time, and give it adequate time to dry. FS is applied rowwise (furrows) to Arecanut and Coconut farms.

Figure 9: Use of faecal sludge in areca nut plantation



5.3 Leh, UT of Ladakh

Leh is located in the trans-Himalayan region and also a district located in Union Territory (UT) of Ladakh. Leh is one of the highest permanently inhabited regions of the world with a low population density.

5.3.1 Physiography

Table 3: Basic information, Leh (LAHDC, Leh)

Area	45,110 sq. km.
Population	1,33,487
Geography & Climate	Altitude varies between 8,000 and 21,000 ft above MSL. Cold desert, with very less annual precipitation.
Population density	3 heads per sq. km.
Administration	Ladakh Autonomous Hill Development Council, Leh with 8 Tehsils
Blocks	16

5.3.2 Water supply

Leh is part of Indus River Basin. The main source of water is from springs, melting glaciers along with groundwater drawn using borewells and tubewells; ie 90% groundwater and 10% surfacewater. A total of 5 MLD of water is supplied during summers at 70 LPCD and 1.6 MLD in winters at 30 LPCD. During winters the water bodies and the water in distribution networks get frozen which leads to a deficit of 83%. There are 5200 households with a functional household tap connection as opposed to 1600 households without one. (BORDA & LEDeG, 2022).

Central Government is working on Jal Jeevan Mission in Ladakh which aims to provide safe drinking water to every household by the end of 2022². In this mission, a total of 125 villages are said to be covered with piped water supply in the UT of Ladakh with a budget of ₹362 crores.

5.3.3 Wastewater management

Leh generates around 10.9 MLD of wastewater during summers, but this decreases to 3.23 MLD in winters due to the less floating population (BORDA & LEDeG, 2017). Only 40% sewerage network is completed, and is yet to be fully functional as the construction of the Sewage Treatment Plant (3MLD) is still in progress. For the remaining 60% an decentralised system is being developed which is in DPR stage. The sewage collected through the sewers is currently being conveyed to STP.

The below diagram captures the sanitation chain of Leh:

Value Chain	Onsite (% based on population)		Off-site (% based on population)		
	Condition	Issues	Condition	Issues	
Containment system	Fully lined or lined Sometimes it overflows and groundwater gets contaminated		UGD system is under construction	The construction is still in progress and only 40% of the work is completed. It is still	
Emptying	1 service provider; 4 trips per day and 350 KL collected monthly.	Frequency of emptying is irregular, done once it gets full		not functional.	
Conveyance	Trucks carry it to FSTP for treatment.				
Treatment	12 KLD FSTP plant.				
Reuse	Soil conditioner				

Table 4: Sanitation chain of Leh

Greywater disposal: In Leh district, there are two primary areas that dispose of the greywater into land streams that flow into the Indus. They are the residential areas of Ibex Colony and Housing Colony. The primary form of disposal of greywater in this region is direct disposal onto the ground. In many places, greywater and wastewater is treated as same and hence disposed in the same manner. There are places where they drain greywater into the streams. The natural regenerative ability of the streams causes them to auto-purify within a certain distance of contamination. Certain households are implementing soak pits that store the faecal sludge, where the greywater is drained into. There are two pilot projects which are present in managing the greywater in Gangles village wherein the treated water is either fed to the greenhouse or disposed of in the fields.



Figure 10: The greywater treatment unit

5.3.4 Faecal Sludge Management

Containment: The most prevalent on-site sanitation system in Leh is a lined pit with semipermeable walls and open bottom, with no outlet and overflow, and the team observed that this has posed a significant risk of groundwater pollution in Leh. A study that LEDeG had commissioned had shown that there was a steady increase in faecal coliform in borewells especially in the lower Leh region. The study showed that the areas with higher concentration of these pits tend to have contaminated groundwater especially in the upper grounwater strata. The contamination also tends to move southwards with the slope. After an MCL mandate, fully lined tanks (sealed) were introduced which are mostly the containment unit for hotels and guest houses.

The toilets on the east side of the Leh namely Ibex Colony, Housing Colony, Leh Main Market, Leh Old Town and Skampari are connected to the sewerage system. This wastewater from the remaining households is managed onsite, in pits with open bottoms and hence leaks in to the groundwater/environment.

People in Leh use the traditional dry toilets. The excreta collected in the chamber is converted into compost. The compost is a stable product that is used as a soil conditioner in agricultural fields (Dry toilets have not been considered while making the SFD).



Figure 11: SFD of Leh town (including dry toilet data)

The SFD Promotion Initiative recommends preparation of a report on the city context, the analysis carried out and data sources used to produce this graphic Full details on how to create an SFD Report are available at:std.susana.org

Conveyance: The city is dependent on Municipal owned desludging tankers for services for emptying of faecal sludge (FS). The Municipal Committee of Leh (MCL) has four desludging trucks which are operational. A fee of ₹ 3,000-3,500 per trip is charged for tankers of different capacities. The variation in fees depends upon the size of the containment system. Emptying of sludge in Leh is done on demand and most of the demand is from hotels and guest houses which are using fully lined tanks (sealed). The frequency of emptying varies from 3 to 15 years. Blue Water Company (BWC) is a private entity that is in charge of running the FSTP in Leh. They also handle the vacuum trucks and employ people for the upkeep of the plant. The plant is however owned by the MCL. Hence, the percentage of containment emptied for fully lined tanks is assumed to be 100%.

The waste from dry toilets are collected by informal sanitation workers. There are about 45-50 informal sanitation workers who work in groups of 4 or 5. They are usually employed for three months - December, January, and February The sanitation workers are usually employed only in the winter months because that is the time when the floating population reduces considerably and work is scarce this allows for such activities plus these are the months when the dry toilet waste is easier to empty with less odour. These sanitation workers are employed to manually empty the existing dry pits in Leh city. The ones in rural areas usually do not employ sanitation workers. Emptying pits is usually seen as a community activity, and if the need be, friends and neighbors of the household emptying the pit join them.

Treatment: An FSTP was installed in the year 2017 to tackle the issue of the growing amount of faecal sludge produced by the hotels. The FSTP is of 12 KLD capacity and there is a plan for another 20 KLD FSTP. The FSTP uses an up-flow anaerobic sludge blanket and gravitational process. The treated solids are used as a soil conditioner and treated liquid is used in irrigation. The FSTP is not functional in winter (November to March) due to the sub-zero temperature.

Figure 12: Steps of faecal sludge treated in the existing FSTP plant



5.3.5 Reuse of manure from dry toilets in agriculture

Each and every household has a dry toilet. This is a native and unique architecture that is found all over the Union Territory of Ladakh. The practice of using manure from dry toilets has been followed for decades. The usual behaviour is to empty the pit under the dry toilet once every year (between end Feb - March, during summer). No special equipment is used to empty. The emptied matter is covered with soil and stored either in drums or on the side of the field. These stay there for 10 days up to 1 month depending on weather, requirements, etc.

Figure 13: The traditional dry toilet (left); the manure stored on the side of a field in Leh (right)



During the field study, Shey, Chuchot, Gangles villages were primarily covered for the survey. Crops like wheat, some local pulses, tomatoes, cauliflowers, and turnips are the mainstay of agriculture in these regions. Only one cropping cycle from April to August is observed in these areas. Irrigation canals have been dug from streams, which provide the primary source of irrigation water for about 6 months in a year.

Most of the crops harvested are for self-consumption, of the villagers. If ever there is a surplus, it is sold in markets. Organic compost increases soil fertility whereas urea boosts growth. The use of chemical fertilizers like urea, Muriate of Potash (MOP) and Di-ammonium Phosphate (DAP) are prevalent in some parts, but the percentage of usage with respect to organic compost is low (approx. 10%). The use of chemicals in the fields has been on the decline for the last 4-5 years. Flush toilets have been set up alongside dry toilets in the last 5-6 years due to the impact of urbanization.

5.4 Kargil, UT of Ladakh

Kargil is the district in the Union Territory of Ladakh. It lies along the Suru river and experiences wide diurnal and seasonal fluctuations of temperature. Kargil is an arid cold desert. The lack of rainfall, dry overall weather and extreme climatic conditions that prevail in Kargil causes it to rely on the very few resources that it has at its disposal.

5.4.1 Physiography

Table	5:	Basic	informa	ation.	Kargil
	- · ·				

Area	14,036 sq km	
Population	1,40,802 (Union Territory of Ladakh, 2021)	
Population density	10 heads per sq.km (Union Territory of Ladakh, 2021)	
Geography	High altitude area between 8000 to 23000 ft. above the sea level	
Administration	Ladakh Autonomous Hill Development Council, Kargil	
Blocks	9	

5.4.2 Water Supply

There is no perennial source of water in Kargil. Except for the villages along the rivers (R. Wakha, R. Suru etc.), Kargil is a water-scarce region. Springs are the primary source of drinking water. In the recent past, water from the Suru River was also being treated and piped to households by Municipal Committee, Kargil (MCK). A total of 250 KLD water is supplied at 30 LPCD. Much of the drinking water is piped to communities on alternate days for a maximum of 3 days a week, for a limited period. Only 28% have tap connections, whereas the rest depend on tankers and other private sources. This causes the population to rely on other water sources like springs and rivers. However, as observed on ground, water sourced from the Suru River may be a potential cause of diseases, as much of the sewer network drains into the river. So, when the residents draw water directly from the river without any pre-treatment, it poses significant risks. The groundwater table is uneven, and the use of handpumps is rare. River water via canals is used in irrigation. These canals run on both sides of the main road, and the water is available from March until October.

5.4.3 Wastewater Management

While on the ground, no reuse of wastewater was identified. Much of the greywater generated was disposed off outside the home onto barren lands or valley sides. No separate blackwater is generated outside of the faecal sludge which is discussed broadly in the next section.

5.4.4 Faecal Sludge Management

The primary toilet in use is the dry toilets, but for 10-15 years, flush toilets have also been in use. The western style-flush toilets have been growing in preference in and around Kargil town over the last two decades and most of the flush toilets in the Kargil district exist in Kargil town. Flush toilets have been installed recently in some peri-urban regions but are limited in their use. Containment chambers are built underneath the flush toilets. These are unlined chambers where the liquid component seeps through the gravelly soil layer at the bottom, leaving behind the sludge. This sludge needs to be emptied regularly.

However, this unplanned growth in the number of bottomless pits had an externality in the form of groundwater contamination. During the survey, we observed that although the groundwater table is quite irregular and relatively unexplored, there were some pockets of groundwater exploration along the Suru Valley (Faroona, Sankoo, etc.). In discussion with respondents, we were told that the effluent from the holding tanks reaches the groundwater, which attributes an offensive odour to the water that was pumped out. Over the last 10-12 years, many hand pumps had to be decommissioned, for that matter. The number of hand pumps currently in use is a few.





The SFD Promotion Initiative recommends preparation of a report on the city context, the analysis carried out and data sources used to produc Full details on how to create an SFD Report are available at: sfd.susana.org

Containment: The most prevalent on-site sanitation system in Kargil is lined with semipermeable walls and open bottom, with no outlet and overflow, with a significant risk of groundwater pollution (T2A5C10, 50%). Residents usually construct pits with stone masonry for the sidewalls and the bottom is left open. The top is usually sealed with reinforced concrete. The faecal sludge (FS) from these containment systems is not emptied as, in some areas, the desludging trucks are not accessible or the residents don't have any knowledge about the Requirement of MCK. Due to these reasons, only 10% of these containment systems demand emptying.

30% of the population have traditional dry toilets, wherein the FS, bulking material, and cleansing water is contained in a chamber where it decomposes. After a certain period, the chamber is emptied where the decomposed material is collected and used in agricultural lands. These containment systems are accounted as lined tanks (sealed), with no outlets and overflow in the SFD (T1A3C10 30%).

Around 5% of the toilets in Kargil are connected to the open drains/natural drains or end up in open drains/natural drains. These are mostly from the slum areas of Kargil (Chanchik, Poyen and Baltibazar).

Around 15% of the population use pits which are never emptied but abandoned when full and covered with soil with no outlet and overflow, where there is a 'significant risk' of groundwater pollution (T2B7C10, 15%).

Emptying: The city is dependent on Municipal owned desludging vehicles for emptying of FS. Municipal Committee of Kargil has four desludging tankers which are operational. Emptying of sludge in Kargil is done on demand and most of the demand is households with pits with semi-permeable walls and open bottom. The city has narrow and congested roads and some households are inaccessible. Fees depend upon the size of the containment system and accessibility.

Transportation: The emptied faecal sludge is transported using a desludging tanker, owned by MCK. Vacuum tankers have a capacity of 2000 liter capacity. These vehicles cover 7-12 km per trip on average from the FSTP to the household and back to the FSTP. There are on average 20 trips in a month during the peak season and charges about Rs.2000 per trip, for emptying and transporting.

Treatment & reuse: Kargil has an FSTP of 10 KLD, located just outside the city that caters to the sludge emptied from the holding tanks. Septage from the septic tanks is transported to the FSTP using 4 suction Trucks (run by MCK) with a capacity of 2000 Liter each. The FSTP is operated by a private company on a turnkey basis. The FSTP is not functional during the winter months (November – April) due to lack of inflow. However, these desludging operations are not a regular occurrence, which often causes the FSTP to be out of commission due to a lack of input. The FSTP had a cost of ₹ 3.04 Cr and an additional cost of ₹ 70 Lakhs was proposed for the transmission of electricity and other services (BORDA Baseline study, Kargil). The treated water is proposed to be used in agriculture fields and the treated sludge is composted and used as manure.



Figure 15: Septage Treatment Plant, Kargil

5.4.5 Reuse of manure from dry toilets in agriculture

The dry toilet consists of a slab with a hole cut out, placed directly on top of the pit, which acts as a containment chamber. No water is used. The pit built under the dry toilet holds the faecal sludge for about 6-7 months, depending on the emptying cycle practiced in the local area. The dry toilet pit is emptied twice a year, once in August-September and again in February-March before the two cropping seasons begin. Once it gets composted, it is used as a manure and soil amender in agricultural fields.

Kargil has two cropping seasons in most of its places except in a few regions having one cropping season. The primary crops grown in the first cropping season include wheat, barley, bajra, cauliflower, carrot, turnip, cabbage, etc. and fodder for cattle, turnips, and vegetables are grown in the second season. Chemical fertilizers (Urea, MOP, DAP) are used for vegetables, while composted manure from the dry toilet is used mainly for seed crops like wheat.



Figure 16: Typical dry toilet, Kargil

5.5 Vikas Nagar, Uttarakhand

5.5.1 Physiography

Table 6: Basic information, Vikas Nagar

Area	2.40 sq. km		
Population	24,019 (Post expansion of municipal boundaries in 2018)		
Population density	5,378 (Post expansion of municipal boundaries in 2018)		
Geography	 Soil: Deep to moderately deep fine and coarse loamy soils and silty Soil with slight to moderate erosion Average Elevation: 452 metres Average water table across the city is between 15 ft bgl to 150 ft bgl Warm temperate climate which drops to an average of 6°C in the winters. The average temperature is 6°C to 37°C 		
Administration	Municipal Council with 11 wards		

5.5.2 Water Supply

Vikas Nagar Municipal Council of Dehradun district in Uttarakhand has no river flowing within its municipal boundary. However, the river Yamuna flows at a distance of 5 km, and the river Asan flows at a distance of 3 km from the city centre.

Vikas Nagar Municipal Council has more than 95% coverage of piped water connections. Groundwater is the main source of water supply. The average water supply in the city is 125 lpcd. Water is supplied for 6 hours a day to the HHs by Jal Sansthan after primary treatment (chlorination), in the overhead tanks itself. There are 5 hand pumps across the city to provide people access to potable water. Currently, there are no Water Treatment Plants (WTPs) in the city.

5.5.2 Wastewater management

Vikas Nagar is a net producer of approximately 2.4 MLD wastewater. The below diagram captures the sanitation chain of Vikas Nagar city:



Figure 17: Sanitation chain, Vikas Nagar, Uttarakhand

Containment: 100% of the households have access to individual household latrines (IHHL). Out of the 11 wards, four wards ie. ward no. 4, 5, 10 and 11 are completely dependent on on-site sanitation systems. Total population of these four wards is 8,741 (36.3% of total ULB's population) with 2,124 households (39.4% of total households in ULB). In the remaining 7

wards, some households are connected to the sewer network and some are dependent on on-site sanitation systems. Total population of these wards is 15,278 (63.7%) with 3,254 households (60.6%).

Conveyance: Sewer lines in Vikas Nagar were laid in 1978 by Pey Jal Nigam and were handed over to Jal Sansthan in 1980. Initially, 30% of the city was connected to sewer lines but 10% became non-functional over the years. Thus, at present, almost 20% of the city is connected to sewer lines which are functional.

Treatment: Presently, there is no formal treatment facility in Vikas Nagar city. The sewage from the 20% HHs in the city connected with sewer lines is reused at the Sewage Farm for irrigation and fertilization of land.

5.5.3 Sewage farm

Since there is no STP present in the city, the municipal council is using the sewage generated by 20% of the population of the ULB connected to sewer lines for irrigation and fertilisation on the 32 acres of agricultural land primarily owned by Jal Sansthan, considering it an immediate interim measure for safe disposal of sewage. The sewage reaching the farm is passed through a screening chamber followed by a settling chamber, and after the settlement of solids at the bottom, comparatively cleaner water is used for farming activities at the sewage farm. The process of this is discussed in detail in the coming sections. The land is located just outside the city limits in a village called Bhimawala. This Sewage farm was set up in the city back in 1978 by Pey Jal Nigam with the idea to treat and reuse sewage water. It was handed over to Jal Sansthan in 1980.



Figure 19: Picture of Sewage farm showing ready crops at left and land being prepared for cropping at right.

Sewage farm flow: The sewage enters the grill chamber (10mx10mx5m), where the primary screening is done. Then it goes to the settling tank (10mx10mx5m) in the crematory adjacent to the Sewage farm. The walls of the collection tank are made of brick masonry, and the bottom of the tank is made of cement concrete. The solids settle at the bottom of the settling tank, and the liquid goes to the Sewage farm to be used for irrigation purposes through channels. Both tanks are cleaned as and when required (mostly once a month). The sludge that settles at the bottom of the tanks is used as fertilizer on the farm itself.

Figure 20: Sewage farm - Process Flow







Sewage at the inlet

Grill chamber and settling tank

Water at the outlet

As per key informant interviews (KII), households in ward no. 10 and ward no. 11 have agriculture fields, and they most commonly dispose of their FS in their fields only.

Business model

The agriculture practice on the Sewage farmland is being carried out by a private party, for which a contract is awarded by Jal Sansthan to an eligible candidate through a tendering process. The contract is usually for 3 years, but at present it was only for one year due to Covid-19 restrictions. The private party makes a one-time payment (amount decided at the time of tender) to the Jal Sansthan, after which the private party is responsible for the O&M of the Sewage farm. The private party sells the produce from the farm in the local mandis.

The contract gives clear instructions to the private party that sewage water must not be used for any vegetable/ crop which is consumed raw (without cooking) or crops of a creeper nature.



As informed by the current contract holder, they use the sewage water only to irrigate and fertilize the land before sowing crops. In current practice, they are growing crops like corn, coriander, radish, spinach, wheat, sugarcane, seasonal vegetables, etc., which are also consumed raw, and this is the reason why sewage water is used only to prepare the land and never used directly on the crops. Water demand for such crops is quite high, thus they have also installed a tube well to meet the clean water demand of the farm. Also during the same KII, it was informed that many sewer lines are damaged and experience leakage, because of which very little sewage reaches the farm.

Economics of Sewage Farm:

- Land and basic infrastructure like grill chambers, settling tanks, and water channels were provided by Jal Sansthan
- A third-party contractor makes the capital investment to set up/ procure an on-field shelter, tube wells, farming equipment, tractors, trolleys, agriculture supplies, fertilizers, electricity generator, livestock, transportation cost, and manpower
- Generates full-time employment for approximately 20 persons and marginally employs 40 to 50 persons
- Wages vary between ₹ 250 and ₹ 350 per day based on the nature of work
- Generates profit by selling farm produce in local mandis
- Farmers, daily wage labourers, and their families are directly dependant on the Sewage Farm for their living
- The households connected to sewer lines are indirectly dependant on the Sewage Farm for disposal and reuse of WW

Figure 23: Sewage farm & agricultural fields



Sewage outlet channel running across the farm



Corn produced at the Sewage farm



Tube well installed at the Sewage farm by private party to maintain clean water supply for crops



Channels running across the farm land

Challenges

- Insufficient wastewater reaching the farm
- Increased dependency on fresh water
- Increased expenses for fertilizers
- Poor maintenance of basic infrastructure

Current Status

- Due to the choking and leakage of existing sewer lines covering 20% of the city, not enough WW is reaching the farm
- The third-party contractor is taking water from a nearby canal and paying tax for the same to the irrigation department
- Third-party contractor has also set up a tube well to meet the additional water demand

- Previously, FS generated from the OSS was also getting disposed of in one of the manholes and was going to the sewage farm through sewer lines, but ULB has put a restriction on this practice, and the third-party contractor also raises objection to such events
- Artificial fertilizers are being used to increase the yield

Benefits

- Reuse of sewage during irrigation and fertilization of farm land
- Reduction in demand for fresh water for irrigation and fertilisation of farm land reduces the load on ground water as it is the main source of water supply in the area
- The probability of illegal and unsafe disposal of FS is reduced
- The probability of groundwater and surface water contamination due to unsafe disposal of FS and sewage is reduced
- Increase in yield without using artificial and chemical-based fertilisers
- The cost of farming is reduced as artificial/chemical-based fertilisers are not needed

Concerns

- No testing of the soil has ever been done to make sure there are no negative health impacts due to the use of sewage for agricultural purpose
- As seen in the excreta flow diagram below, 45% of the total wastewater and FS generated in the city is not being disposed of/treated safely
- Sewage farm alone is not sufficient to manage the FS of the entire city
- No testing of the water has ever been done at the outlet of the settling tank
- Testing of surrounding water sources has never been done as well
- The treatment technology being used at the sewage farm is obsolete considering the character of the sewage has changed over the years as per KIIs and field observation
- The concentration of the sewage has also changed with population growth
- The sewer lines covering 20% of the city are also in a dilapidated condition and pollute the environment due to leakage

5.5.4 Faecal Sludge Management

Containment: Overall, almost 80% of the city is dependent on on-site sanitation systems. Unlined honeycomb tanks are the predominant type of containment across the city. The average size of containments is: length-12ft, breadth- 10ft and depth- 10ft. The type of construction used is mainly brick masonry. The average desludging frequency in the city is 5-10 years.

Emptying and Transport: Currently, Jal Sansthan owns one suction vehicle of 3,000 L capacity dedicated to the cleaning of the sewer line. The urban local body (ULB) also owns one vehicle with a capacity of 3000 L to provide desludging services to the households. The ULB charges ₹1000/trip to provide desludging services within the city limits and ₹1,500/trip outside city limits. House owners also call private desludging operators from Selakui and Daakpathar, which are neighbouring ULBs at a distance of 22 km and 7 km, respectively. Private operators charge between ₹800 and ₹1600 per trip. The ULB vehicle makes 1-2 trips every month, depending on demand, and private operators make 8-10 trips per month on average.



Figure 18: Suction Vehicle owned by Jal Santhan, Vikas Nagar

Treatment and Reuse: Desludging operators empty and discharge faecal sludge at the private tanks set up in local farmlands under an informal contract. Farmers, after drying the faecal sludge, use it for fertilization of farmlands. The desludging operator and farmer do not pay anything for discharging or using the faecal sludge. This is practiced with a mutual understanding between the two.

VikasNagar Shit Flow Diagram



Assumptions taken while preparing the SFD:

- 1. Out of 80% of the population dependent on the lined pits with semi-permeable walls and open bottom, about half the population is considered at significant risk and the remaining half at low risk. As the groundwater table in the city ranges between 15ft bgl to 150 ft across seasons, and there is no data available on groundwater contamination, hence this assumption is made based on the water supply which is provided by Jal Sansthan through borewells with disinfection treatment
- 2. The average floating population on a peak day in the city is 400
- 3. The rock types in unsaturated zones is fine sand, silt, and clay
- 4. The sanitation facilities that are located less than 10 m from groundwater sources are less than 25%
- 5. The sanitation facilities that are located uphill of the groundwater source are less than 25%
- 6. More than 25% of drinking water is sourced from groundwater
- 7. Protected boreholes are the main water source used in the city
- 8. Overall, groundwater pollution is at low risk

6. Analyses and Findings

This section is organized as follows:

I. Qualitative data

- a. Interviews with farmers, desludging operators, and contractors
- b. Interviews with health professionals and others, such as environmental engineers

II. Quantitative data

- a. Sample collection & testing processes
- b. Results

III. Overall observations

6.1 Interviews/Discussions (farmers, desludging operators, health professionals, etc.)

We had FGDs and individual semi-structured interviews in each of the towns/cities. These are the key points that emerged from our discussion with farmers, faecal sludge operators, and health professionals from each city. The detailed questionnaire followed can be found in **Annexure 1**.

6.1.1 Interviews with farmers

Vijayapura: In Vijayapura, what is seen is a conscious choice of lower-risk crops by farmers, hygiene practices by farmers to ensure their own safety, and in some cases conscious irrigation practices to ensure the safe use of wastewater. What is also observed in Vijayapura is the washing of produce with freshwater before it is sent to the market thus reducing the health risks for consumers greatly.

Faecal sludge is used as fertilizer for agriculture by farmers in Vijayapura. Here again, farmers have developed practices of the use of faecal sludge in a way that significant hazards are reduced when actually applied to crops. faecal sludge is usually composted and used or spread across fields during sowing time and given adequate time to dry.

Mr. M is the owner of 9 acres of farmland where mulberry plants are grown. For the past 20 years, he has been using wastewater for irrigation. The wastewater is collected in a pit and left untouched and exposed to the sun for a few days. The sludge settles at the bottom of the pit, while the wastewater is pumped out and used for irrigation. After drying, the sludge is used as plant fertilizer.



Figure 24: Farm pond used to collect wastewater (left) Dried up wastewater, leaving dark residues (right) Mr. M has been using wastewater for irrigation without any protective gear, such as gloves or gumboots, for nearly twenty-five years, but has reported no illness or infection. As observed by the medical team from MSCH, there were no visible signs of any skin conditions. M's wife and daughter also mentioned that they don't feel sick. However, the young generation has been wearing protective gear whenever they are in the field because they are aware of the health risks of using wastewater. In the words of Mr. M, livestock consuming the fodder grown using wastewater are in good health and they hadn't observed any diseases or infections in them. Mr. M credits the availability of wastewater for the irrigation of his farms and the increase in his income. He has been able to build a house for his family and purchase more pieces of land.

Another farmer told us that he grows non-edible crops and flowers using wastewater. He too credits the use of wastewater for irrigation of his lands for his family's upward mobility: his sons are completing undergraduate degrees in engineering.

"I have been using the wastewater for more than two decades. I have not faced any health-related issues with wastewater. Often there is no contact with wastewater as I follow furrow irrigation. The misconceptions about the use of wastewater like crop failure due to diseases and the health of cattle are also seen in farms where there is no use of wastewater. Thus, one shouldn't fall to the conclusion that wastewater is harmful. My produce receives higher prices in the market and I don't use any artificial fertilizers. There is a tendency to ignore the harmful effects of using artificial manure and stress only on the use of wastewater. More stories like mine should come to the light so that the stigma and the yuck factor associated with wastewater would change."

M, Mulberry farmer, Vijayapura

"Crops grown using borewell water require artificial manure which is very expensive. It costs Rs.50,000 annually. Whereas the crops grown using wastewater don't require artificial manure as the water is rich in nutrients. The cost of growing crops in borewell water is much more expensive than that of wastewater. The quality of Mulberry grown using wastewater is better when compared to Mulberry grown using borewell water. Thus, in the market, we get a better price for mulberry grown using wastewater."

K, Mulberry farmer, Vijayapura

Tumakuru: Lake water from Bheemasandra and Melekote lakes has provided a perennial source of water for irrigation. Cultivation of Baje has contributed to the increase in income of the farmers and is seen as a most reliable source of livelihood for wage laborers during harvest season. Treated wastewater has also provided a livelihood for grass cutters like Typha cutters who travel from more than 50kms distance.

Fishing in the lakes tendered by the government is generating huge revenues to the contractor whereas informal fishing (folks fishing in small quantities without the coracle) is generating livelihood for the fishermen.

"I own no land and depend completely on agriculture for my livelihood. Baje is non-seasonal and the harvest begins in December and goes on till February-March. Just after the harvest the new planting begins. Baje gives livelihood to thousands of wage labourers like me when we don't get any work in the season. I travel for 10 kms to work in the Baje field. I have been working in the Baje fields for a decade now. Skin allergies are mostly seen for the newcomers and they gradually get used to it. The tenants complain of smell and mosquitos are nothing in front of our livelihoods. One should arrive at a conclusion where all of us can co-exist. Increasing the quality of treatment might be of help in reducing the smell and mosquito breeding".

M, Wage labourer, Baje farm, Tumakuru

With the use of faecal sludge manure, Areca nut and Coconut farmers save costs upto ₹ 50,000, on buying commercial manure. In the partnership of desludging operators and farmers, due to the need for a safe space to dispose of the faecal sludge, they provide it to the farmers for free.

Mr. K is a farmer from Gulur village of Tumakuru taluk which is around 10 kilometers from the city. Krishnegowda owns eight acres of Areca nut and Coconut farm. He has been using faecal sludge for the past five years. Every day the desludging operators dump a minimum of two to five truckloads in his field. "My farm was depleting in quality. The yield was less and trees were lost. Once the desludging operator approached me and asked if they could empty the faecal sludge in my land. I saw faecal sludge as rich manure and asked them to dump it directly. In just two years, I found my farm thriving. There is an increase in quantity and quality of the yield and trees are growing very well".

K, Farmer, Tumakuru

Leh: Organic manure is being used by each and every farmer in Leh. This practice has been carried out for ages. Farmers reported that there were no health issues so far due to the use of organic manure in the fields. The government was providing chemical fertilizers but there is a decrease in usage over the past 4-5 years. Chemical Fertilizers were resulting in higher growth but the farmers also believed that the cause of cancer, and some GI tract infections are due to the use of chemical fertilizers. On further prodding about use of organic compost only, everyone mentioned that no cases of disease outbreaks were ever recorded.

Figure 25: Process of reuse of manure from dry toilets



Dry toilets

faced any health issues so far."

 Greenhouse • Agricultural Lands

 Fruits Vegetables

"I have been practicing farming for the past 40 years and I have been using organic manure in my fields which I was observing from my past generations. Our pits are emptied by our family members. We use gloves and masks as protection while emptying our pits. There is also an NGO that was promoting the use of only organic manure and to reduce the use of chemical fertilizers in Gangles village. The fertility of the soil depends on the nutrients in the soil and the chemical fertilizers will reduce the fertility. I have not

Farmer P

"The difference between clean and dirty waste was ascertained through "Smell". If the waste is clean with no foul odor (enough soil has been put on the faeces) and decomposed properly, there will be farmers willing to buy them. If they are dirty with foul odor (not used enough soil after defecating), there will be no demand and they will be disposed of in the area "Bombgard" as solid waste. Sometimes, they are given away free of cost and sometimes they are sold."

Pit Emptier

"Use of organic manure, either from dry toilet pits or sheds of farm animals is an age-old practice. We have been seeing this since we were little kids," said a 65-year-old farmer. There was no use of chemical fertilizers in the past. But now from the past 10-20 years there is a use of chemical fertilizers along with organic manure". Organic manure imparts better taste to the food but the growth is less. We have not faced any health problems so far."

Farmer N

Kargil: The preparation procedure of organic manure follows varied methods; Manures usually consist of only human faecal matter decomposed with soil from the field. The decomposition process takes place in the pit and on the field when the pit is emptied in September-October. Sometimes, layers are made with cow dung and goat faeces, which are covered with what is emptied from the pit. They are left in the field for co-composting.





There exists a wide gap in the perception, acceptance, and usage of chemical fertilizers in the agricultural field. Places like Bhibat, Ningoor and Tambis use government-supplied chemical fertilizers as much as the native homegrown organic manure. On the other hand, places like Hunderman, Saliskote and Trespone do not use chemical fertilizers.

"Chemical fertilizer causes more growth, but it also causes many diseases. Therefore, we restrict our use of chemicals in the field."

Farmer H, Trespone village

Vikas Nagar: Currently, a third-party contractor has been working at the sewage farm for the past 15 years. According to him, very little amount of wastewater is reaching the farm due to leakage and choking of sewer lines, which is insufficient to irrigate even a small part of the land. He uses canal water and pays taxes to the irrigation department for the same. He has also installed a tube well to meet his additional water needs. He suffered significant losses during the COVID period and is still trying to recover from it. He is completely in favour of using wastewater for better yield. He stated that wastewater is used directly only on empty land to fertilize it, so there can be no negative health or environmental impacts.

Third-party contractor's interview

"We directly apply wastewater to empty land in order to make it fertile. The farm receives very little wastewater, which is insufficient to irrigate even 15-20 bighas of land. Here we have 165 bighas of land. We use canal water for irrigation. I pay taxes to the irrigation department for the use of canal water. Because sewer lines are small and choked, not enough wastewater reaches the farm. In order to meet water needs, I also installed a tube-well. We have been at a loss for the past two years. Laborers were unable to come to the farm during covid times, so we had to destroy the ready crops. I have requested the department to extend my contract for an additional period of time to allow me to recover from the loses.

> -Irshad, Third Party Contractor for 15 years



Farmers at Vikas Nagar Sewage Farm consider wastewater to be a resource and facility. According to them, it reduces the effort required for farmland fertilization while increasing productivity and, thus, overall profit. The sewage farm provides a steady source of income and a better quality of life for them and many other daily wage labourers. Farmers now require wastewater from sewage farms, and a lack of supply due to breaks and leakage in sewer lines is a major concern. They want an uninterrupted wastewater supply for better yields without the use of artificial fertilizers and better revenues. Farmers see no environmental or health risks from applying wastewater to farmland. Farmers and their families, who are the direct beneficiaries, are completely in favour of using wastewater for farming purposes.

Farmer's interview

"Work is good here, farming is good, the employer is good; everything is good for us. Wastewater should continue to flow here. Farming was not very good when I was in the mountains, but it is now. While freshwater yield is poor, wastewater yield is good. Additional fertilizers are not required. The wastewater supply should be maintained as before."

-Mrs. Seema, Farmer at Sewage Farm, Vikas Nagar

Farmer's interview

"We were previously living on the mountains and were unable to attend school. We've been going to school and studying since we arrived here. My father works here, and we manage decently. I am in the 12th grade and will continue my education after that. Working with wastewater has no negative effects on my father's health, but if the yield is low and we suffer losses, we become weak. Wastewater should continue to flow here because productivity has decreased as supply has decreased."

-Raveena, 17 years, Farmer's daughter

Perception of desludging vehicle owner: Mr. Rajesh has been a desludging operator in the city for over 17 years now. He has one desludging vehicle that he drives himself and hires daily wage labourers to empty. He believes that the business is more profitable during the monsoon season because the frequency of emptying increases significantly when compared to the summer season.

Private Operator's Interview

"I work in partnership with one more person. We only have one truck. We don't get jobs every day. In the summer, we hardly get any work. During the monsoon season, we work more frequently, about once every 2-3 days. Other private service providers are also there, coming from different cities like Herbertpur, Ponta, and Selakui. People call the person with whom they have terms. I charge based on the distance travelled and the amount of work completed. My tanker has a capacity of 5.5K litres. We leased land from private farmers in Jalali and disposed of the septage in tanks we built. Once the tank is full, farmers of nearby land use the dry sludge for farming purposes."

Rajesh, Private Desludging Operator, Working in Vikas Nagar for 17 years



6.1.2 Interviews with health professionals

Vijayapura: We visited the Community Health Centre Premises Vijayapura, the local Primary Health Centre (PHC), and spoke with Dr. Swaroop. We gathered information on the patient load of the PHC as well as the age group, gender, and frequency of patients. He informed us that a number of women had presented with urinary tract infections (UTIs). When we asked about the causes of these diseases, we were told that poor sanitary conditions were to blame. We found no link between wastewater and women's diseases during our brief discussion. Patients do not follow up on their health and do not finish their doctor-prescribed medicine dosage, according to our sources. We were also told that rickettsial fever was common among the male patients during the season changes.

Leh: Interviews with the medical superintendent of Sonam Nurboo Memorial (SNM) Hospital and Director of National Institute of Sowa Rigpa (N.I.S.R) revealed a critical gap in studies revolving around the environmental cause of diseases. The growing trend in diseases, however, align with those seen elsewhere i.e., lifestyle-oriented diseases like high blood sugar, high blood pressure, cancer etc. The Medical Superintendent of SNM hospital mentioned some diarrhoeal diseases were reported seasonally which can be co-related to food consumption changes like consumption of ripe Apricots in the month of August and green leafy vegetables on the onset of summer.

The Medical Superintendent pointed out the rise in cases of Hep-B across Ladakh but could not pinpoint a definite cause for the same. Construction work along Khardungla by Border Road Organization (BRO) every summer causes contamination of streams with human excreta which causes an annual outbreak of diarrhoeal diseases.

Kargil: Mr. M, the sub-divisional officer, gave us an insight into the history of agriculture in Kargil. The use of night soil as a soil amender has been a longstanding practice. In the last couple of decades, the use of government-supplied chemical fertilizers has gained traction. However, under Mission Organic Developing Initiative (MODI), many blocks of UT of Ladakh have been converted into organic farming regions. The mission objective is to convert all the agricultural areas of Ladakh into organic farming zones. So, in some selected blocks, chemical fertilizer supply has been stopped, and focus is being put on upscaling faecal manure reuse.

However, as Mr. H pointed out, there have been no major disease outbreaks that can be linked to the use of faecal manure in agriculture.

The emergence of unplanned, unlined containment chambers for flush toilets has potentially caused the groundwater to contaminate. But the low temperature coupled with very low relative humidity creates unfavorable conditions for the disease environment; the result is lesser diseases in crops or humans.

To find data points, the data regarding health conditions prevailing in the district was collected to find the correlation between existing practices to environmental and health impacts. Interviews of Medical Officers, Block Medical Officers, and Epidemiologists in Kargil were undertaken for the same. The officers shared that in communicable diseases, Hepatitis B and Tuberculosis are the most reported whereas among the U18 population ascariasis is common. Among non-communicable diseases, Chronic obstructive pulmonary disease (COPD) is ubiquitous, along with asthma, hypertension, and diabetes, especially among people of age or those with a predisposition to illness. The medical officers are aware of faecal sludge disposal hotspots, contamination due to overflowing dry toilets, use of contaminated water for drinking and cooking and advocate remedial measures for the same.

6.2 Testing of wastewater and soil samples for various pathogens that can constitute health risks

Wastewater can contain a number of pathogens, many of which have already been studied in detail in other published work. Our original aim in this section was to conduct an overall metagenomic analysis of all the bacterial species found in the samples collected through a next generation sequencing methodology. Towards this end, we collected the samples and froze them down at -80C for RNA and DNA analyses (A detailed process of sample collection, shipping and sample preservation method has been shared in Annexure 2). However, this aspect required funds and additional resources, including the test kit, that were unavailable immediately due to disruptions in supply chain because of the pandemic. These took time to source and hence could not be completed during this study period. They are currently planned.

Some of these samples were thawed overnight at room temperature and tested for bacterial growth and resistance. These results have been included in Annexure 2.

To complete the mandated requirements of this study, we collected fresh samples from Vijayapura and Tumkuru, which are locally adjacent. We tested for the following:

a) Bacterial pathogens: Salmonella species, Shigella species and Vibrio species were specifically selected for and quantified. In addition, we also tested the resistance of some of the isolated colonies for a sentinel antibiotic called Ertapenem (shared in Annexure 3). Gastrointestinal pathogens were focused upon, as they would be the obvious parameters to monitor health risks in this particular context. To a smaller extent, we also studied common bacteria that cause urinary tract infections (such as *Klebsiella sp.*)

Vijayapura:

from:

A. Water samples from:

irrigated field

B. Paired Soil samples

Wastewater

Same day of

irrigation • 4/5 days post

irrigation

irrigated field on

Wastewater

The results of these samples have been given below.

Sample collection & testing protocol

Sample description

Sample collection (March 2022)

Vijayapura:

A. Water samples from:

- Borewell irrigated field
- Wastewater irrigated field

B. Paired Soil samples from:

- Borewell irrigated field
- Wastewater irrigated field on
- Same day of irrigation
- 4/5 days post irrigation
- 1 month post irrigation

Tests done:

Bacterial load:

- Total Gram negative load by bacterial counts on MeConkey agar
- Total Salmonella/Shigella load by bacterial counts on SS plates
- Total Vibrio load by bacterial counts on TCBS agar
- Ertapenem resistance study

Sample collection (September 2022)

Tumkur:

- A. Water samples from: • Lake 1 receiving
 - wastewater • Lake 2 receiving wastewater and
 - water from lake 1
 - fields

- Wastewater irrigated

Tests done:

Bacterial load:

- Total Gram negative load by bacterial counts on Salmonella/Shigella media plates
- Identification of Salonella and Shigella from samples collected

• • • 25

• Wastewater irrigated

B. Paired Soil samples from:

- field on
- Same day of irrigation

Paired wastewater and soil samples were collected for the experiment in March 2022 and in September 2022, from Vijayapura, for a bacterial study as detailed below. Water samples were grabbed - collected from the furrows into sterile 5mL cups. Soil from the field fertilized with this water was collected on the day of irrigation and either 4 or 5 days after irrigation. The March collection also included a soil sample 1 month after wastewater application. This was absent in the September collection. Soil samples were collected at a depth of 1 inch from the top using spatula and placing them into sterile 5mL cups.

In Tumkuru, local treated and untreated wastewater flows into the Bheemasandra lake and thence to the Melukote lake. Water from both these lakes was collected. This wastewater is also used in farms. Paired water and soil samples were collected as above. All collections were done in Sept 2022. Collection of samples was done as described above. Table 7 summarizes the types of samples collected from each place:

Location	Type of Sample	Details
Vijayapura Town, Bangalore (samples collected September 2022)	Water 1	Wastewater sample used for irrigation of soil in a farm
	Soil 1	Soil sample from the same farm irrigated with the wastewater containing fecal sludge, collected on the same day of irrigation
	Soil 2	Soil from the same farm irrigated with the wastewater containing fecal sludge, collected on 5th day after irrigation
Vijayapura Town, Bangalore (samples collected March 2022)	Water 2	Wastewater sample used for irrigation of soil in a farm
	Soil 3	Soil sample from the same farm irrigated with the wastewater containing fecal sludge, collected on the same day of irrigation
	Soil 4	Soil from the same farm irrigated with the wastewater containing fecal sludge, collected on 5th day after irrigation
	Soil 5	Soil sample from the same farm irrigated with wastewater 1 month prior to collection
	Water 3	Bheemasandra lake wastewater
Tumkuru, Bangalore	Water 4	Melekote lake wastewater
(September 2022)	Water 5	Wastewater used in baje farm
	Soil 6	Soil sample from same farm

Table 7: Types of samples collected

Microbiological procedures:

1. Sample preparation

- Water sample: 1ml of water was measured and added into a fresh sterile Tarson tube and diluted 1:1000 with 0.9% saline and was plated on McConkey, SS and TCBS plates (March 2022) and just SS plates (Sept 2022).
- Soil sample: 1 gram of soil was measured and added into a fresh sterile Tarson tube and diluted 1:1000 with 0.9% saline and was plated on McConkey, SS and TCBS plates (March 2022) and just SS plates (Sept 2022).

2. Sample plating

Plates used were McConkey, SS and TCBS pre-made plates.

MacConkey agar is a selective and differential media that facilitates the growth of gramnegative bacteria only. The media is pink in color due to the presence of neutral red, a pH indicator. It can help differentiate between lactose fermenting and lactose non-fermenting bacteria. Lactose fermenting bacteria appear red or pink in the agar. Non-lactose fermenting bacteria appear yellow in color. SS Agar facilitates the growth of *Salmonella* and *Shigella* and other Enterobacteriaceae. Salmonella ferments xylose to form black colonies, whereas Shigella doesn't ferment xylose and thus is denoted as colorless colonies.

Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) Agar is a selective and differential medium that facilitates the growth of *Vibrio sp.* Various species of vibrio produce either yellow or green colonies based on their sucrose fermenting capabilities.

50 μ I of each diluted sample was taken using a sterile 200 μ I microtip and plated onto the corresponding SS agar plates and spread evenly using a sterile spreader till the sample was absorbed onto the media. The plates were dried completely and were kept in an inverted position. Each sample was plated in triplicates. Plates were incubated in an incubator overnight at 37°C.

3. Results

The results were observed the next day and colonies were counted manually.

Identification of Salmonella/ Shigella: A colony of *Salmonella* species appears to either be completely black in color, or have a black center with white border on SS agar plate. *Shigella* colonies on SS agar plates are colorless and opaque in appearance. These colonies matching the above-mentioned morphological criteria were taken for further biochemical analysis. 200 ml of LB broth (Himedia M1245) was prepared following the manual provided. 15 ml tarson tubes were taken and 5ml of LB broth was added in each tube and labeled accordingly. The selected colonies were incubated in an incubator overnight at 37°C.

Himedia biochemical kits KB002 was used for biochemical testing. These kits contain a set of 12 standardized colorimetric tests, such as beta-galactosidase presence, arginine dihydrolase activity, lysine decarboxylase activity and others, which help in identification of the Gram Negative bacteria. After 24 hours of incubation, each biochemical test strip was inoculated with culture broth following the manual and incubated overnight at 37°C. Results were observed and interpreted by following the manual.

Bacterial load calculations and Effect of sunlight on bacterial load

Sample details: Vijayapura collectic (September 2022)	on	Dilution factor	Average number of colonies on	Colony forming units on		
Sample Information	DI elpmaS		Salmonella Shigella agar (SS)	Salmonella Shigella agar (SS) CFU/ml		
Wastewater sample used for irrigation of soil in a farm	Water 1	10 ³	39	780000		
Soil sample from the same farm irrigated with wastewater on the same day	Soil 1	10 ³	60	1200000		
Soil sample from the same farm irrigated with wastewater 5 days prior to collection	Soil 2	10 ³	11	220000		

Table 8: CFU/ml data of wastewater and wastewater irrigated soil Vijayapura, Karnataka (September 2022)

*Test samples were repeated in triplicates.

Figure 27: Graphical representation of CFU/ml data of wastewater and wastewater irrigated soil Vijayapura, Karnataka (September 2022)



Table 9: CFU/ml data of wastewater and wastewater irrigated soil Vijayapura, Karnataka (March 2022)

Sample details: Vijayapura collecti 2022)	on (March	Dilution factor	Average number of colonies on	Colony forming units on
Sample Information	Sample ID		Salmonella Shigella agar (SS)	Salmonella Shigella agar (SS)
				CFU/ml
Wastewater sample used for irrigation of soil in a farm	Water 2	10 ³	55	1100000
Soil sample from the same farm irrigated with wastewater on the same day	Soil 3	10 ³	45	900000
Soil sample from the same farm irrigated with wastewater 5 days prior to collection	Soil 4	10 ³	0	0
Soil sample from the same farm irrigated with wastewater 1 month prior to collection	Soil 5	10 ³	34	680000

*Test samples were repeated in triplicates.

Figure 28: Graphical representation of CFU/ml data of wastewater and wastewater irrigated soil Vijayapura, Karnataka (March 2022)



Colony counts of bacteria found in environmental samples from Vijaypura, Karnataka (March 2022).

Table 10: CFU/ml data of samples collected from lakes rejuvenated wastewater irrigated soil, water and soil samples collected from farms using wastewater Tumkur, Karnataka.

Sample details: Tumkuru (Septem	ıber 2022)	Dilution	Average number	Colony forming units on	
Sample Information	Sample ID		Salmonella Shigella agar (SS)	Salmonella Shigella agar (SS) CFU/ml	
Bheemasandra lake wastewater	Water 3	10 ³	59	1180000	
Melekote lake wastewater	Water 4	10 ³	48	960000	
Wastewater used in baje farm	Water 5	10 ³	27	540000	
Soil sample from same farm	Soil 6	10 ³	25	500000	

*Test samples were repeated in triplicates.

Figure 29: Graphical representation of CFU/ml data of samples collected from lakes rejuvenated wastewater irrigated soil, water and soil samples collected from farms using wastewater Tumkur, Karnataka



*Test samples were repeated in triplicates.

Identification of Salmonella and Shigella:

Salmonella sp and Shigella sp were identified morphologically upon plating water and soil samples on SS plates (Table 3). 75% of colonies were confirmed by biochemical tests to be Salmonella sp (specifically Salmonella enterica), 12.5% of colonies were confirmed to be Shigella sp (specifically Shigella boydii), and 12.5% couldn't be identified.

6.3 Observations and Discussions:

It emerged during the interviews with stakeholders and through field observations that the following practices/safeguards are in place while using untreated WW and FS for agriculture, which may be considered while preparing a sanitation safety plan.

- Drying/desiccation of the faecal matter before use
- Mixing of faecal sludge with other organic matter before application
- Wearing personal protective gear (such as wearing non-absorbable shoes, mask, gloves etc. while handling the faecal matter)
- Minimizing the number of people who handle it, thus reducing exposure to the faecal matter
- Ensuring FS is covered thoroughly with leaves and soil after application
- Using a shallow settling tank to allow exposure of wastewater (with or without faecal sludge) to sunlight for a few days before use

Stakeholders further observed that additional safe practices like ensuring direct application of FS is confined to non-edible crops, ensuring that edible crops do not come in direct contact with the applied sludge and suspending the application of WW/FS before a few days of harvest and thorough disinfection of vegetables and fruits at the consumer end etc., may be adopted.

Our study indicates that these safeguards and that of holding wastewater (with or without faecal sludge), merit further study in the potential transport of pathogenic organisms from one region to another.

Exposure to sunlight of soil treated with wastewater reduces the colony count: CFU/ml data of soil irrigated with wastewater on the day of collection was high when compared with its corresponding wastewater. But there was a reduction in CFU/ml counts in the soil sample which was collected 5 days after wastewater application (Samples from Vijayapura collected

in September 2022). Table 8 shows a stark difference in the bacterial load between samples irrigated with wastewater on the day of collection vs samples irrigated with wastewater 5 days prior to collection. The weather condition was reported as sunny and hot during the span of 5 days. As there is a decrease in the bacterial load of samples collected 5 days after irrigation, we can say that the effect of sunlight plays a major role in it. There are multiple studies stating that sunlight can lead to reduction of bacterial load in water samples^{3 4} and in soil samples^{5 6} and indeed has been the scientific foundation of a new type of disinfection of soil, called soil solarization⁷.

There were no significant differences in CFU between wastewater samples and the wastewater irrigated soil tested, collected from Baje field, Tumkur, Karnataka.

Risk Classification of wastewater irrigated soil:

Our study shows that wastewater irrigated soil and the wastewater carry high loads of bacteria belonging to the Salmonella and Shigella genera. However, sunlight exposure reduces this bacterial load significantly. Hence the risk classification of wastewater irrigated soil reduces from a medium to a low category.

This is likely similar to what happens in Leh, Kargil and other farms in high altitude-temperate regions, which are exposed to sub-zero temperatures for many months of the year.

Learnings for future studies in this field:

- Trans-disciplinary coalitions are needed for studying such questions of cross-cutting significance: this study combined fields of water, agriculture, urban practices and planning, rural livelihoods, public health, microbiology, clinical medicine and environmental ecology. Our core learning was in the creation of trans-disciplinary teams
- Technical parameters to consider for future studies: This is a preliminary pilot. As such, a broad scoping study was attempted over multiple agro-climatic zones. Future studies to clearly delineate and reduce the risk of wastewater usage in agriculture would require deeper dives into each type of agro-climatic zone as well as obtaining primary clinical information from local primary health care centers, understanding local practices and determining local knowledge in different farming communities.
- Multiple valuable insights were obtained, many of which require deeper study. These include cost-efficacy studies of using wastewater, the actual safety practices observed, the range of medical conditions experienced, the prevalence of antimicrobial resistance, locally relevant risk mitigation methods, and so on. These not only require deeper study, but also a mindset that is less disease-focused and more open to systemic problem-solving.

Highlights of study

- Treated and untreated wastewater have been used for agricultural irrigation in many farms in India for decades in the face of severe droughts and rainfall scarcity.
- Preliminary interviews with farmers and their families indicate no adverse health impacts despite using untreated wastewater for agricultural irrigation for over two decades. This was corroborated by the local primary health care center physician. All the farmers interviewed reported that 90-100% of irrigation requirements are met through untreated wastewater and attributed their economic growth and stability to it.
- We found that bacterial loads of Gram-negative bacteria were similar in wastewater and corresponding soil samples.
- A finding of significance was that 5 days after the application of wastewater, the soil contained a significantly lower count of bacteria when exposed to sunlight during two different periods in the year.
- Thus, a risk reduction step to make wastewater safer and enable its wider use in irrigation could likely be something as simple as exposing the wastewater to sunlight (in tropical regions) or to subzero temperatures (in high altitude, temperate regions like Leh or Kargil). Environmental exposure to sunlight or freezing temperatures will significantly reduce the gastro-pathogenic bacterial load in the soil and wastewater.

³ https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7064263/

⁴ https://www.int-res.com/articles/ame/11/a011p135.pdf

⁵ https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2435.13061

⁶ https://digitalcommons.hope.edu/cgi/viewcontent.cgi?article=1098&context=curcp_16

 $^{^7\,}https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/soil-solarization$

7. Recommendations

7.1 Areas for further study

This study sets the stage for further work needed to understand the comprehensive health impact of using untreated wastewater. Future work will entail the following measurements in the various sources of water used for irrigation: a) Heavy metal load b) Pesticide and/or antibiotics c) Measurement of helminth levels

Currently, there is very little organized data from India on the health and livelihood impacts on the environment, animals, and humans due to the use of untreated wastewater and faecal sludge. On the basis of the literature review, interviews as well as sample results the following gaps were identified and need to be studied further:

- How does the use of wastewater affect the ecosystem in terms of soil health, epidemiology of pathogen populations (such as helminths, viruses, bacteria, drug-resistant bacteria, and fungi?)
- Is there a movement of heavy metals, antibiotics, pesticides and other chemicals from urban areas which are the sources of wastewater to farms surrounding them?
- How does this movement affect the ecosystem and the communities that depend on the water and the land?
- What are the longer-term effects on the soil, and the surrounding ecology of animal and bird life?
- What are the short, intermediate, and longer-term impacts on reproductive health, longevity, and wellness of farming communities exposed to untreated wastewater?
- What are the short and long-term impacts of edible farm produce that is generated on farms using wastewater for irrigation if any?
- What are the economic benefits or risks of using wastewater for agricultural irrigation in the farming and consumer communities?
- What are other resources, such as bacteriophages, from wastewater that can be identified, isolated and exploited for improving health?
- What are the specific pathogens that need to be looked at to evaluate health risks and ensure livelihoods?

Multi-disciplinary partnerships must be put in place to carry out studies and formulate a protocol for wastewater quality surveillance system and its application for irrigation.

7.2 Quantifying the use of untreated wastewater and faecal sludge in agriculture

The extent of treated wastewater is captured in official data. However, despite the widespread use of untreated WW/FS in agriculture, it has not yet been quantified with utmost accuracy. Such efforts should also be considered while documenting interventions in the use of WW/FS in agriculture.

7.3 Formulation of 'fit-for-purpose' standards for untreated wastewater/faecal sludge application

Multiple safeguards while using untreated faecal sludge or wastewater are in place.

Intermediate standards may be specified for wastewater and faecal sludge application in agriculture for edible and non-edible crops along with mandating risk management measures.



A small wetland in the stilling and storage basin. The wetland cleans and polishes the wastewater. This wastewater is then pumped through a drip irrigation system and flowers such as roses, marigolds being grown.



Farmers have developed a simple filtration and ponding of tapped wastewater (2-3 hrs to 24 hrs retention). The storage pond with a mesh filter separates solid waste, before use via furrow irrigation.



Devanahalli Centre for vegetable washing: Farmers specifically set up infrastructure for vegetable washing, wherein vegetables are washed with freshwater before packing.



In the use of untreated wastewater from Dakshina Pinakini river, outskirts of Bengaluru, farmers pump up wastewater upto 20 km to meet their irrigation needs. Specially designed pipes draw the wastewater into a storage structure, either a pond or a dried-up well, where the wastewater is allowed to sediment. Additionally, filters fitted to the pump help filter it before sending through the drip irrigation and sprinkler system.



Faecal sludge is emptied into a storage pit. This is covered in a layer of soil and cow dung/sheep dung, and allowed to dry and compost for 3-6 months, before application on land.

7.4 Issue Guidelines on Sanitation Safety Planning in agriculture

A comprehensive sanitation safety plan should be prepared and implemented. The following steps may be followed:

- Formation of an 'SSP in Agriculture' Task Force Mayors, representatives from city, water board, irrigation, health department, pollution control board, civil society, farmers, desludging operators, consumer representatives. The Task Force will deploy experts and oversee the SSP
- Document the process flow of wastewater and faecal sludge including spatial mapping
- Mapping of exposure groups and risk rating
- Preparation of a risk management action plan comprising a hazard identification and mitigation matrix with the following components
 - 1. exposure route
 - 2. prevalent control mechanism
 - 3. validation of control
 - 4. risk assessment by exposure type to map likelihood, severity, score and risk level
 - 5. proposed mitigation action and resource requirement including a wastewater quality surveillance system^{*}

An advisory on the application of SSP may be provided to city governments and appropriate capacity development and training programmes.

An illustrative example of mapping risk and exposure for a town has been shared below:

SSP Planning for Wastewater management

Figure 30: Process flow diagram for Liquid Waste Management System in Town X



Table 6: Exposure groups identified in the wastewater collection system

Exposure group: WORKERS (W)							
No.	Exposure sub-group	Number of individuals					
W1	Open drain workers from	60*					
W2	Solid waste collectors – Tractors (Drivers and Helpers)	10					
Exposure group: FARMERS (F)							
No.	Exposure sub-group	Number of individuals					
F1	Farmers using open drain water	25-30					
Exposure group: LOCAL COMMUNITY (L)							
No.	Exposure sub-group	Number of individuals					
No. L1	Exposure sub-group Community/Citizens	Number of individuals 28,000					
No. L1 L2	Exposure sub-group Community/Citizens Households in proximity to open drains	Number of individuals 28,000 150					
No. L1 L2 L3	Exposure sub-group Community/Citizens Households in proximity to open drains Households in proximity to WW irrigated fields	Number of individuals28,00015030-50					
No. L1 L2 L3 L4	Exposure sub-group Community/Citizens Households in proximity to open drains Households in proximity to WW irrigated fields Household near the outflow of the drain	Number of individuals 28,000 150 30-50					
No. L1 L2 L3 L4 Exposure	Exposure sub-group Community/Citizens Households in proximity to open drains Households in proximity to WW irrigated fields Household near the outflow of the drain group: CONSUMERS (C)	Number of individuals 28,000 150 30-50					
No. L1 L2 L3 L4 Exposure No.	Exposure sub-group Community/Citizens Households in proximity to open drains Households in proximity to WW irrigated fields Household near the outflow of the drain group: CONSUMERS (C) Exposure sub-group	Number of individuals 28,000 150 30-50 Number of individuals					

Table 11: Hazard identification Exposure assessment – Wastewater management of town xx

Hazards – Microbiologi Hazardous Events – Op	cal (bacteria/virus/p erations & maintena	orotozoa), Helminth, Ch ance, infrastructure bre	າemical and Physica eakdown, climatic eາ	l vents (floods),	L=Likelihood: Se=	Severity:	Sc=Score	e: R=Risk	level	
indirect & cumulative h Exposure Groups – Sani	azards tary workers, Comn	munity, Farmers and Co	nsumers							
	Hazard/	L	Description of	Validation of	Risk assessment					Comment (ex. General
Element of KKK chain	Hazardous event	Exposure route	existing control	control	Exp. Gr.	_	Se	Sc	X	observations, etc.)
P7: Cleaning the solid	Injuries through sharp items	Injury through contact – Cut	PPE gloves, boots are	PPE not used	W1	4	4	16	high	Awareness should be created among
waste from the drains : Tractors	mixed in solid waste	injuries from sharps	provided		W2	c	4	12	medium	community not to throw solid waste in to the
			Tools are provided	Tools are appropriate						drains
P8: Clogged drains	Clogged drains lead to water	Mosquito bites (Houseflies (in	None	N.A	L2	4	4	16	high risk	vector- borne diseases are heavily
	stagnating sites	presence lot of organic content is								underreported in India
	hazard for	there)			W2	e	2	9	low risk	

Table 12: Characteristics of the various categories of workers in Town xx Wastewater Management

high risk

16

4

4

C1 root crops

- Washing after harvesting in safe water

product 2. Skin contact with

contamination of agricultural

Microbial

P15 Agricultural produce

soil 3.Accidental

fungi, viruses, helminths)

product (bacteria,

1. Ingestion of contaminated

hazard for vector breeding

low risk

 \sim

က

W2

medium

12

4

က

C1 surface crops

low risk

4

2

2

C1 high growing crops

ingestion worm infestations/ E. coli

Other control Measures other than PPEs suggested	
Any symptoms from last 2 weeks	
Reasons for not using PPEs	
Whether Appropriate for the task	
Use of PPEs	
PPEs recommended	
Specific Training for the job	
Perception of Health Risks associated with daily work	
Category of worker	

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SSP Planning for Faecal sludge management



Figure 31: Process flow diagram for faecal sludge management system in Town X

Table 13: List of identified exposure groups involved in Faecal Sludge Management system in xx Town

xposure sub-group Desludging vehicle - Driver	Number of individuals
Desludging vehicle - Driver	1
	±
Desludging vehicle - Helper	2
group: FARMERS (F)	Number of individuals
xposure sub-group	
armers using faecal sludge	5-8
group: LOCAL COMMUNITY (L)	Number of individuals
xposure sub-group	
Devanahalli community	28'000
louseholds in proximity to faecal sludge dumping sites	
group: CONSUMERS (C)	Number of individuals
xposure sub-group	
Consumers of vegetables grown with faecal sludge	More than 28'000
	esludging vehicle - Helper group: FARMERS (F) kposure sub-group armers using faecal sludge group: LOCAL COMMUNITY (L) kposure sub-group evanahalli community ouseholds in proximity to faecal sludge dumping sites group: CONSUMERS (C) kposure sub-group onsumers of vegetables grown with faecal sludge

	Comment (ex. General	observations, etc.)	Mechanised process reduces the exposure of faecal sludge to hands. The workers get exposed while handling the pipes that are used to connect the pit to machine						
Risk level			high risk	medium risk	high risk	high risk	high risk	low risk	medium risk
Score; R=I			16	12	16	20	16	5	ω
erity; Sc=9			4	4	4	4	4	-	7
e=Seve	Ļ		4	м	4	5	4	2	4
L=Likelihood; S	Risk assessmen		W1 (skin contact)	W1 (accidental ingestion)	F1	C1 Root crops	C1 Surface crops	C1 High growing crops	F1
	Validation of control		Inconsistent use of mask is observed		Not Appropriate face mask would be better				
	Description of existing control		The workers wear mask at the collection point Mechanized process (use of suction and jetting pump in the vehicle)	Wash hands with soap/antiseptic solution and water after the activity	None -cover mouth/nose with cotton cloth	Washing of the vegetables after	harvesting in safe water.	Roots and tubers are washed thoroughly,	whereas green leafy vegetables and mint, coriander etc are not washed thoroughly as the shelf life will decrease.
	Exposure route		 Skin contact Accidental ingestion Inhalation of aerosols (rare) 		Inhalation	1. Ingestion of contaminated product	2. Skin contact with	3.Accidental ingestion worm infestations/	E.coli
	Hazard/ Hazardous event		Microbiological hazards: Pathogens (Bacteria, Fungi, Viruses, Helminths)		Exposure to malodour	Microbiological contamination of	Agriculture Produce: Pathogens (Bacteria, Fungi Viruses,	Helminths)	
	Element of RRR chain		T1 Desludging operator		P7 Faecal sludge application	P8 Agricultural	produce		

Table 9: Hazard identification Exposure assessment - Faecal sludge management of town xx

Other control Measures suggested	None
Any symptoms from last 2 weeks	1. 2. Headache 3. Malodour 3.
Reasons for not using PPEs	Not available
Whether Appropriate for the task	Yes
Use of PPEs	Yes, but not consistent
PPEs recommended	Mask
Specific Training for the job	Driving license for driving Heavy Motor Vehicle
Perception of Health Risks associated with daily work	 Malodour Road Traffic Accident Nausea Vomiting
Category of worker	Desludging vehicle- Driver
SI. No.	1

Table 14: Characteristics of the various categories of workers in Town X Faecal Sludge Management

Annexure 1:

Questionnaire for interviews

General information

- 1. Name of the farmer & age
- 2. Contact details
- 3. Land details (total area owned)

Identification of stakeholders

- 1. Institutional: ULB's- Water supply and sewerage board and Municipality (Health inspector, Environmental Engineer), CPCB, CPHEEO, CGWB,
- 2. Non-institutional: Farmers, Desludging Operators,....

Part 1: Informal use of wastewater in agriculture

A. Water/source of water

- 1. What is the source of wastewater in irrigation?
 - Grey water
 - Treated Wastewater
 - Untreated wastewater
- 2. What is the quantity of wastewater used in irrigation?
- 3. What is the area being irrigated with wastewater?
- 4. Is there a possibility of industrial effluents getting mixed with wastewater?
- 5. If treated wastewater is used informally, does it meet the treatment standards of CPCB?
- 6. What are the levels of parameters tested in wastewater? (N, Iron, Phosphates, BOD, COD, DO etc)
- 7. Before the availability of wastewater, what was the source of water for irrigation?

B. Methods of wastewater/FS use

- 1. Are famers pumping the wastewater or using it through gravity?
- 2. If pumping, are they using diesel pumps or electric motors?
- 3. How much is the money spent on pumping?
- 4. What is the distance being pumped?
- 5. What is the method of irrigation?
 - Furrow
 - Flood
 - Drip
- 6. Are farmers using wastewater directly in irrigation (without any pre-treatment)?
- 7. What are the methods of pre-treatment before using wastewater on the field? (retention ponds, wells ...)
- 8. If farmers are passing the wastewater through retention ponds, what is the retention time? (hours/days)

C. Cropping pattern

- 1. What all crops are grown using wastewater?
- 2. How many crops do they grow in a year?
- 3. Are farmers growing root crops using wastewater? If yes, what are the root crops grown? If there is an issue to grow root crops, what are those?
- 4. After the use of wastewater, did farmers change their cropping patterns?
- 5. If yes, what were they growing before the availability of wastewater?
- 6. Which crop is said to thrive on wastewater?
- 7. How many crop cycles do you grow in a year? What is the quality of produce?

D. Marketing

- 1. Where are farmers selling their produce?
- 2. Is there market acceptance of crops grown in wastewater?
- 3. Overall, has there been any change in market prices because of the availability of water and increase in quantity of produce?

E. Impact on Health

- 1. Do farmers report any issues in using wastewater?
- 2. If yes, what are issues faced?
 - Skin allergies
 - Mosquito breeding
 - Gastrointestinal disorders
 - Water Waterborne
- 3. When are the health hazards seen? Or How long does it take to witness health hazards?
- 4. How are crops produced using we impacting the consumers? (if possible)
- 5. What are the safety measures taken?
 - Hand gloves
 - Furrow irrigation
 - Non-edibles

F. Impact of Environment

- 1. What is the impact of wastewater on groundwater? Or Do people report groundwater salinity?
- 2. What is the impact of wastewater on groundwater table? (Farmers not drilling borewells and thus groundwater is preserved)
- 3. Do farmers report a decrease in soil fertility due to the use of WW?
- 4. Are farmers using a decreased quantity of artificial fertilizers?
- 5. Did wastewater result in the change of species of fish? Or is there a threat to native varieties of fishes after the entry of WW? (If possible- What is the impact on consumers of fisheries?)

Part 2: Informal use of fecal sludge in agriculture

Introduction:

- 1. Type of containment
 - Pit toilet
 - Septic tank
 - Dry toilet
- 2. How many honeysuckers are there in the city/town?
- 3. Is there a formal FS emptying site?
- 4. If there is no formal FS emptying site, where are HS emptying the FS?
- 5. If FS is used in irrigation, how many farmers are using FS?

A. Cropping pattern

1. What are the crops grown using fecal sludge?

B. Methods of usage

- 1. Do farmers use fecal sludge directly on the land?
- 2. What are different methods of pretreatment involved?
 - Retention ponds
 - Composting
 - Drying

C. Impact on Health

- 1. Identify the exposure groups with risks?
 - Farmers
 - Consumers
- 2. What are the safety measures taken?
 - Hand gloves
 - Pre-treatment measures
- 3. What are the health hazards reported?

Part 3: SSP risk rating

- 1. What is the risk rating for hazardous events, hazards, exposure groups?
 - Low risk
 - Medium risk
 - High risk
 - Very high risk
- 2. What are the health risks?
 - Helminth infections
 - Parasitic worms
 - Viruses
 - Protozoa
 - Bacteria

Annexure 2

For bacterial counts and antibiotic sensitivity assays

Items needed:

- 1. Sterile urine collection tubes: should be available with most medical suppliers
- 2. A wooden/ plastic spoon that can be cleaned easily. This is for collecting soil samples. A spatula would be ideal, but these are much cheaper and more accessible alternatives
- 3. Ziplock bags
- 4. Permanent marker
- 5. Tissue paper
- 6. A backpack with multiple compartments that can be opened horizontally (like a suitcase. Many options online)
- 7. Gel packs that can be thrown in a fridge or freezer before use (need to check if truly necessary)
- 8. A styrofoam box (need to check if truly necessary... in a pinch, the cold gel packs can be tossed into a plastic cover and kept in the same backpack compartment as the collected samples)



Type of collection tubes

- Gloves
- GPS/app on mobile with lat-long for recording

Samples to be collected on sites:

- at the inlet,
- in the middle of the farm,
- at the far end of the farm away from the inlet.
- Also, water source, if possible.

Samples to be collected at each site:

- Water
- Soil (at the surface. Ideally would be good to also check soil at different depths, but for this
 initial sweep, let's stick to whatever is easier)
- Some of the produce (leaves/ root)

Amount of sample to be collected: About 3/4th of the collection tube should be filled with water or soil. Tightly close the lid.

Labeling of samples: Immediately label the tube after closing the lid. Labeling should follow a standard procedure. Ideally this is the information it should contain:

Date

Site

Type (water, soil, produce... this would be obvious from a quick glance.) We can add this only if there are two types of water/ soil/ produce samples being collected from the same site (for instance, if one wants to collect the newer leaves and older leaves from the same site, then each type of leaf goes into a different tube and they are appropriately labeled)

All samples from a single site need to be placed into one ziplock which is then labeled in detail:

Date

District/Town/Village

Location: GPS coordinates

Farm (first farm visited, second farm visited etc)

Site description:

Thus, each farm will have a ziplock containing all the samples labeled by site and type (if not obvious), and the ziplock itself will be labeled with all the pertinent details regarding the farm.

- If samples are going to be reaching the lab within 6 hours, can continue to keep them at room temperature.
- If the ambient temperature is very hot (say > 35°C), then best to keep samples in a styrofoam box with ice packs in it. You will need to freeze the ice packs a day before and keep them in the styrofoam box. The box itself should be firmly closed (may need to use cello-tape to keep it closed).
- If shipping the samples to a different city, then definitely seal the styrofoam box before shipping.

Annexure 3

Examination of the farms irrigated with treated and untreated wastewater to evaluate the risk of antimicrobial resistance (AMR)

Introduction:

Antimicrobial resistance (AMR) has been increasingly seen to be a major public health concern. Multiple lines of study show that AMR is not just due to overexposure of antibiotics in human healthcare settings, but also due to antibiotic use in veterinary and environmental settings and the transport of the causative genes through environmental routes to humans who may have never been exposed to antibiotics in the first place. Thus, the transport of wastewater from urban to rural areas could be a crucial pathway of AMR dissemination. We focused on a particular antibiotic called ertapenem. This is a last line antibiotic in the carbapenem class which is used exclusively through the intravenous route in hospitals for severe cases of sepsis or bacterial infections. Thus, resistance to ertapenem is a sentinel metric for widespread antibiotic resistance. Hence, we included this parameter in our testing matrix.

Specifically, in the context of AMR, wastewater is considered a unique source and might well provide an integrated view of the AMR patterns from human and non-human sources. In this preliminary study, we sought to examine farms irrigated with treated and untreated wastewater to better evaluate the risk of antimicrobial resistance (AMR) and to generate a map of the AMR patterns of Gram-negative enteric pathogens against multiple antibiotics depending on the type of wastewater being used in the farms.

In this sampling, we specifically focused on the antibiotic Ertapenem. Ertapenem is a broadspectrum antibiotic typically reserved for serious infections for both hospital inpatients and outpatients. Ertapenem is used to treat Urinary Tract Infections (UTIs), abdominal infections, and skin diseases and is exclusively given through the intravenous (IV) route. It belongs to a group of antibiotics known as Carbapenems, which are considered the last line of antibiotics and can be considered sentinel antibiotics for surveillance of resistance. Earlier studies of wastewater in Bengaluru have shown resistance to ertapenem is present and in some areas is as high as 33% in Gram-negative bacterial isolates. These antibiotic-resistant bacteria can spread through wastewater from urban areas, where the usage is likely to be high, especially from hospitals, to sub-urban or peri-urban areas. Wastewater can act as a conduit for transport of ertapenem resistance from urban to suburban/ peri-urban/rural areas when it is used in agricultural irrigation.

Methods:

Types of samples collected:

In Vijayapura, wastewater from open drains or sewage pipes flowing from town is collected in a pit (Fig 32 A & B). The water is allowed to flow through the farm through furrows in the ground (Fig 32 C). The specific farm we visited had been utilizing locally sourced wastewater for irrigation for the past 20 years. Paired locally sourced wastewater and soil samples, that is, wastewater samples from Vijayapura town, used for agricultural irrigation and the soil samples from fields irrigated with the same wastewater, were collected for the experiment.

Samples were also collected from wastewater containing faecal sludge collected from nearby homes. Collection of this watery mixture occurred as soon as the truck carrying it emptied its contents into a pit. This mixture is collected in a pit and left untouched and exposed to the sun for a few days. The sludge settles at the bottom of the pit, while the wastewater is pumped out and used for irrigation. After drying, the sludge is used as plant fertilizer. Soil from the field fertilized with this dried sludge was also collected for testing (32 D). This collection occurred 3 days after the soil had recently been treated with dried faecal sludge.



Figure 32 : Pictures of wastewater being stored and used in the farm



Figure 33: Sample collection from from different fields

In Tumkuru, local treated and untreated wastewater that flows into the Bheemasandra lake and thence to the Melukote lake was collected. The standing untreated wastewater from the farms, paired with soil samples was collected.

In Vikas Nagar, wastewater from sewage pipes or drains flows to the sewage and sludge farm. Wastewater is allowed to flow through trenches on the ground throughout the farm. Paired water and soil samples were collected from the point of wastewater flow initiation into the field (inlet) and the point of wastewater flow to exit from the field (outlet). Groundwater borewell outlet samples were also collected. Soil from a field that had previously been fertilized with wastewater (3 months ago) was collected for testing.

From Leh and Kargil, only soil samples treated with composted manure and the manures from dry toilets were collected.







Figure 34: Sample collection sites in Leh

All samples from Vikas Nagar, Leh and Kargil were meant for microbiome analysis initially and had been frozen at -80C for 6 months. They were thawed at room temperature overnight before use in these experiments.

Location	Type of Sample	Details	Zone	Sampling strategies	
Vijayapura Town, Bangalore	Water	Wastewater sample used for irrigation of soil		Grab collection using sterile 50ml sample collection	
	Water	Fresh faecal sludge- containing wastewater transported from town	_	cup using syringe (water samples) and Spatula (soil	
	Soil	Soil sample from farm irrigated with the wastewater	Dry climate Agro- climatic zone: Southern Plateau	samples)	
	Soil	Soil from a farm typically enhanced with faecal sludge containing wastewater	and Hills		
Tumkuru,	Water	Melekote lake wastewater			
Bangalore	Water	Bheemasandra lake wastewater			
	Water	Wastewater used in Baje farm			
	Soil	Soil sample from Baje farm			
Vikas Nagar, Uttarakhand	Water	Wastewater samples from Inlet pipe			
	Water	Wastewater samples from outlet pipe			
	Water	Groundwater- Borewell outlet	Humid subtropical climate. Agroclimatic zone:		
	Soil	Soil sample irrigated with wastewater start of the field	Upper Gangetic Plains		
	Soil	Soil sample irrigated with wastewater end of the field	-		
	Soil	Soil sample for ready crop field (wastewater applied 3 months ago)	_		
Leh, Union Territory of Leh	Soil	Topsoil taken from field with manure			
	Soil	Bottom soil taken from field with manure	ken from field Agroclimatic zone: Western Himalayan		
	Co- compost	Manure			
Kargil, Union Territory of Leh	Soil	Topsoil taken from field with manure			
	Soil	Bottom soil taken from field with manure	-		
	Co- compost	Manure	-		

Table 1: Sample data

All samples were sent on ice to Tata Institute of Genomics and Society (TIGS), Bengaluru for metagenomic sequencing to identify all bacterial species in the samples. At TIGS, they were frozen down at -80 $^{\circ}$ C for eventual DNA extraction.

These results will be shared as updates when ready.

One aliquot of these frozen samples was thawed and plated. This data has been shown below:

Media preparation

Luria Bertani (LB) is a medium for E. coli and other related Gram-negative enteric bacteria (MacWilliam M et al. 2006). LB agar plates for 12 samples {12 Controls and 46 (3 sets of tests)} were prepared following the below-mentioned protocol.

	Control media plates	Test media plates (LB agar + Antibiotic)	
	(LD agar)	For 1 set	For 3 sets *
Quantity of media (ml)	200	200	600
Luria Bertani broth (gms)	5	5	15
Agar (gms)	3	3	9
Distilled water (ml)	200	200	600
Antibiotic (µg/ml)	-	400	1200

*Test samples were repeated in triplicates.

Table 2: Media preparation data

The Himedia manuals M1245 and RM026 were used to create the LB broth and agar, respectively. The measured amount of the media was added to separate flasks containing distilled water, cotton plugs, and labels that complied with the requirements (control and Test media). The medium was autoclaved at 121°C and 15 pressure for 15 minutes and the media was allowed to cool to a temperature of 55°C before handling. It was essential to keep the area clean. LAF was cleaned using cleaning agents. A sterile lamp was lit. Tarson media plates were labeled accordingly:

- For Control plates: [Date]–[Sample name]
- For Test plates: [Date]–[Sample name] [Set 1]
- Same was repeated with set 2 and set 3.

The media was poured into a respective petri dish and was allowed to solidify. The antibiotic Ertapenem (2 μ g/ml) [Standard followed by CLSI performance standards for antimicrobial susceptibility testing M100-S26] was added to the conical flask containing the test sample's LB medium. The media was poured onto labeled Petri dishes and was allowed to cool. Media plates were ready to use.

Sample plating

LAF was cleaned with cleaning agents. The control set of media plates was taken and arranged accordingly. 100 μ l of each diluted sample was taken using a sterile 100 μ l microtip and plated onto the corresponding LB agar plates and spread evenly using a sterile spreader till the sample was absorbed onto the media. The plates were dried completely and were kept in an inverted position. The same procedure was repeated for the control set and test set samples. Plates were incubated in an incubator overnight at 37°C.

Samples from Vijayapura mentioned in Table 1 are taken and using a sterile inoculation loop, 10 µl of the sample was streaked onto the media plates and labeled accordingly.

The results were observed the next day and colonies were counted manually.

Test results

Bacterial load and Ertapenem resistance in paired water and soil samples:

Bacterial load in water and soil samples were evaluated in:

- Either wastewater alone or fresh liquid containing wastewater and faecal sludge in Vijayapura, Karnataka.
- Wastewater samples from lakes restored with treated wastewater (Melukote and Bheemasandra), water and soil samples from a farm irrigated with untreated wastewater from Tumkur, Karnataka.
- Water and soil samples from a farm irrigated with untreated wastewater in Vikas Nagar, Uttarakhand. Samples were collected near the inlet and the outlet of the wastewater pipe.
- Manure used from dry toilets and soil samples (from different depths) from a farm in Leh.
- Manure used from dry toilets and soil samples (from different depths) from a farm in Kargil.

Vijayapura							
Type of sample/ colony count	Wastewater sample used for irrigation of soil	Soil sample from farm irrigated with the wastewater	Fresh faecal sludge containing wastewater from the city	Soil from a farm typically enhanced with faecal sludge containing wastewater			
Total Bacterial load	70	100	170	164			
Ertapenem Resistant bacterial load	0	0	42	0			
Tumkur							
Type of sample/ colony count	Water sample from Lake 1 (Melukote)	Water sample from Lake 2 (Bheemasandra)	Wastewater used in farm irrigation	Soil from wastewater irrigated farm			
Total Bacterial load	141	153	190	185			
Ertapenem Resistant bacterial load	0	5	0	0			
Vikas Nagar							
Type of sample/ colony count	Inlet wastewater sample	Soil sample irrigated with inlet water	Outlet wastewater sample	Soil sample irrigated with outlet water			
Total Bacterial load	141	160	199	165			
Ertapenem Resistant bacterial load	0	0	0	0			
Leh							
Type of sample/ colony count		Topsoil mixed with manure	Bottom soil mixed with manure	Manure			
Total Bacterial load		3	1	2			
Ertapenem Resistant b	oacterial load	0	0	0			
Kargil							
Type of sample/ colony	/ count	Topsoil mixed with manure	Bottom soil mixed with manure	Manure			
Total Bacterial load		2	1	2			
Ertapenem Resistant b	oacterial load	0	0	0			

*Each sample was done in triplicates, with a dilution factor of 100

Table 3: Bacterial load of environmental samples and its corresponding Ertapenem resistant colony

Out of a total of 18 samples, 2 samples showed resistant colonies: one is a wastewater sample containing fresh faecal sludge and the second is water from a lake supplied with treated wastewater.



Discussion

In this sampling, we focused on the problem of antimicrobial resistance and sought patterns in the prevalence of resistant bacteria from the wastewater (that is flowing into this region from a neighboring city) and the soil. We obtained samples from three different zones: the cold-arid climatic zone (Leh and Kargil) the Upper Gangetic agroclimatic zone (Vikas Nagar, Uttarakhand) and the Southern Plateau/Hills agroclimatic zone (Karnataka). The average rainfall received at Vikas Nagar is about 81 inches and in the Vijayapura/Tumkura regions is about 35-40 inches. The climate in Vikas Nagar is considered to be temperate-tropical and in Vijayapura/Tumkur to be tropical. In both regions, wastewater is commonly used to augment the soil for irrigation and compost use.

We examined the degree of resistance to ertapenem among bacteria that grow that LB agar, that is, E.coli and other related enteric species of bacteria. Our results show that ertapenem resistance is relatively low in wastewater transported from cities to agricultural lands for irrigation. The only sample showing significant levels of ertapenem resistance was wastewater that contained fresh faecal sludge being pumped into a manhole before it was used for manure and irrigation. The faecal sludge in the wastewater is presumably from multiple different parts of the city where it was collected, including from hospitals. However, the soil that had been treated with this faecal sludge earlier showed very little resistance. This begs the question: what happens to the AMR bacteria in the soil over time? Do the antibiotics that are probably present in the same sample (not tested) degrade over time and do the bacteria lose the AMR due to an absence of continued evolutionary pressure?

The half-life of ertapenem in the human body is estimated to be around 20h (Pletz, Mathias WR, et al 2004). Left in a plastic syringe at 25°C, it is estimated to degrade completely within 1-5 hours (Kuti et. al. 2016). If the antibiotic degrades significantly over the course of a single day, and if farmers are only applying the faecal sludge containing wastewater to their farms irregularly (once in a month or once in a quarter), then perhaps the soil microbiome is not being exposed to ertapenem consistently and therefore the levels of ertapenem-resistant bacteria are significantly lower.

This could have very significant implications: even if antibiotics are disposed of incorrectly by people and medical institutes, holding the wastewater (with or without faecal sludge) for 1-2 days before applying it on the farms might remove or reduce the possibility of transporting AMR (to ertapenem, as per this pilot) from urban areas to rural farms. As the antibiotic degrades, the evolutionary pressure on the bacteria to hold on to the AMR plasmids reduces, and the chance of introducing resistant bacteria to the soil and thereby to the produce should reduce.

Annexure 4:

Integrated disease surveillance from wastewater in Bengaluru

Biome Environmental Trust is a part of the Precision Pandemic Health program. The <u>Precision</u> <u>Health program</u>, after successful completion of its pilot Wastewater Based Epidemiology (WBE) initiative on SARS-CoV-2 surveillance in Bangalore from May 2021 to April 2022, is looking to explore the potential of environmental surveillance for the detection and testing wastewater samples for new pathogens which are an existing and emerging concern for the city. With rising cases of Monkeypox in India and high disease burden of Influenza H1N1, the platform has launched <u>Integrated Disease Surveillance initiative</u> in Bengaluru city as a pilot study on testing wastewater samples for the presence of these pathogens, along with SARS-CoV-2, with a goal of:

- Using WBE, where it can be (a) scaled beyond the current pandemic and
- can be integrated into the existing public surveillance systems and response mechanisms.
 To read more about the prevalence of above diseases, please visit <u>here</u>.

The team works closely with the Bangalore municipal corporation, Bruhat Bengaluru Mahanagara Palike (BBMP) and Bangalore Water Supply & Sewerage Board (BWSSB), with an aim to support local governance by integrating the insights in the public health framework and thus, help in preventing and controlling spread of these diseases in the city. There may be other stakeholders, such as the Government of Karnataka or the Pollution Control Board, who may also have interest in these findings.

Details of Methodology:

Each wastewater sample is collected from the existing sewer systems in the city and tested for each of the targeted pathogens.

- Sample collection sites 28 functional sewershed sites spread across Bangalore city
- Sample collection frequency Once a week from each sample collection site
- Total wastewater samples collected in a week: 28 (Sampling for the integrated surveillance began on the 17th of August. Initially 5 sites were sampled Mallathahalli, Cubbon Park, Lalbagh, V Valley and Mailasandra. The first batch of 9 samples were collected from these 5 sites between 17th Aug to 23rd of Aug. At the end of one month, as of 17th September 2022, 34 sewershed sites were sampled once a week. A single sample is collected which is then tested for the presence of multiple pathogens namely SARS-CoV-2, Influenza panel (H1N1, Influenza A and Influenza B) and MonkeyPox (MP).
- Sampling methodology Grab sampling



Site positivity for SARS CoV2 RNA:









We also tested these samples for Influenza B and Monkeypox, both of which were negative.

Presence of RNA does not indicate presence of live virus. In fact, multiple lines of evidence suggests that live viruses do not survive for long in the wastewater (perhaps due to the multiple other chemicals and biological materials that exist in it)8 (refs). However, much of this evidence is recent, especially in the case of SARS CoV2 and Monkeypox. In the early days of the Covid pandemic, wastewater was thought to have transmission potential (refs), thereby putting millions of people in urban and rural areas at risk had SARS CoV2 indeed been able to be transmitted through sewage networks. Thus, testing for these viruses had deep public health relevance.



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