Since 1959

# Wastewater-Based Epidemiology


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Israel-IITM Capacity building on wastewater treatment and reuse, Nov. 18-20, 2024

# Epidemiology

"The study of what is upon the people"
Derived from Greek.
***epi*** 'upon, among',
***demos*** 'people, district', and
***logos*** 'study, word, discourse'

Epidemiology is the study and analysis of the distribution (who, when, and where), patterns and determinants of health and disease conditions in defined populations.

# WBE

WBE is a technique for determining the **consumption of, or exposure to chemicals or pathogens in a population.** This is achieved by **measuring chemical or biological entities in wastewater** generated by the people contributing to a wastewater treatment plant catchment.




wikipedia.org/wiki/Wastewater-based epidemiology.
Sims, Natalie, and Barbara Kasprzyk-Hordern. *Future Perspectives of Wastewater-Based Epidemiology: Monitoring Infectious Disease Spread and Resistance to the Community Level*. *Environment International*, vol. 139, p. 105689, 2020.

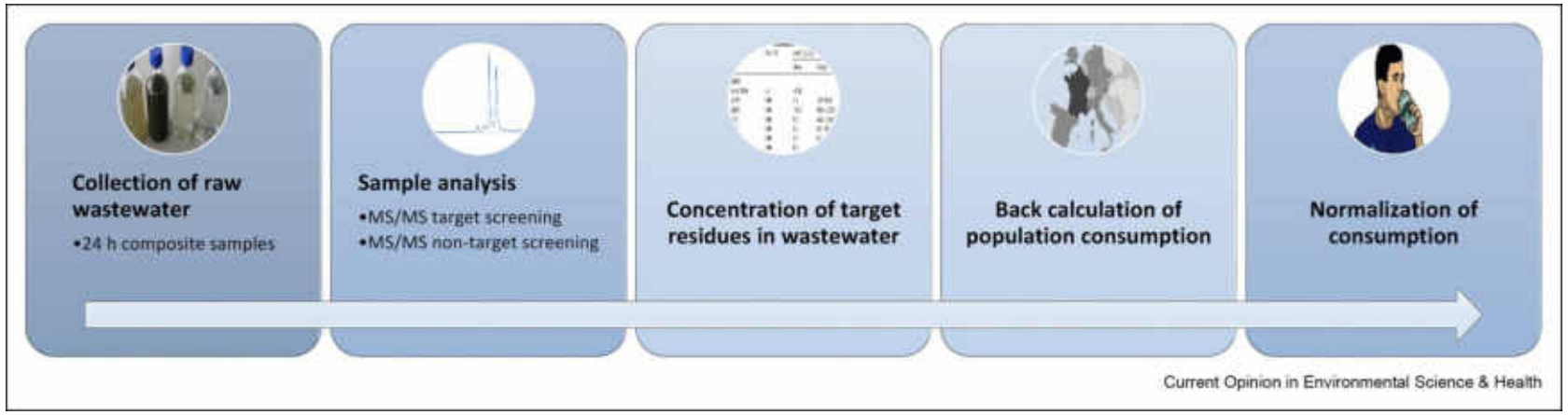
# Wastewater-based epidemiology

Predicting public health through wastewater

Wastewater-based epidemiology (WBE) has been consolidated as a tool to provide real-time information on consumption of legal and illegal drugs of abuse by the population.

However, WBE is expected to achieve more ambitious objectives such as:

1. Establishing exposure to certain agents such as pesticides, personal care products.
2. Abuse of medicated and psychoactive drugs.
3. Incidence of specific diseases (diabetes, allergies).
4. Determination of some lifestyle consequences or environmental factors (increase in temperature) in populations.

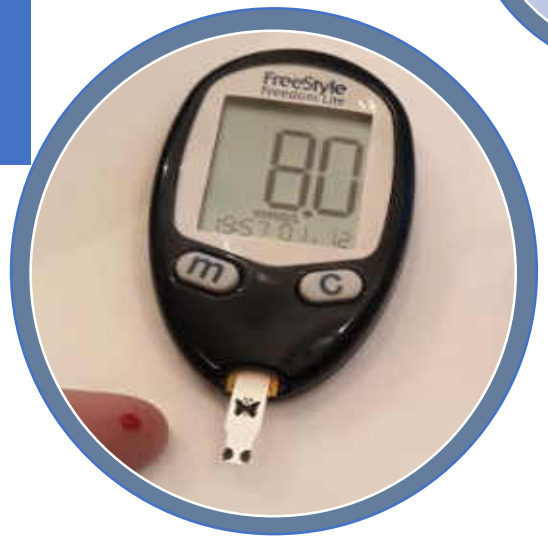


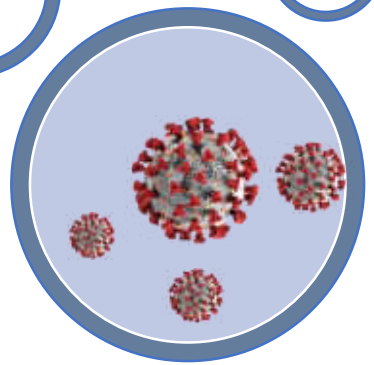

Lorenzo, M. & Picó, Y. Wastewater-based epidemiology: current status and future prospects. *Current Opinion in Environmental Science & Health* **9**, 77–84 (2019).

# Why WBE is essential?

Estimating the percentage of diabetic people in a society, non-invasively through disease biomarkers

**WBE is faster and does not require clinical data**



Evaluating the dynamics of viruses in a community without

Door-to-door testing

**WBE provides early warning & is economical as samples are very few**

Daily illicit drugs consumption monitoring

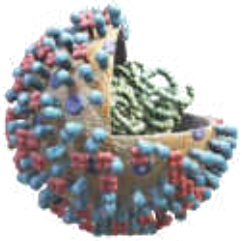
**WBE overcomes need for reporting**

Estimating economics through wastewater

**WBE is time conserving by eliminating need for surveys**

# Historical context of infectious diseases

Major epidemics and monitoring evolution



**Major epidemics since 2000**

Overview of significant epidemics including SARS, H1N1, Ebola, Zika, MERS, Nipah, and COVID-19, highlighting their global impact on public health.


**Evolution of disease monitoring**

The shift from traditional monitoring methods to innovative approaches like Wastewater-Based Epidemiology (WBE), emphasizing the importance of timely responses to emerging infectious diseases.

# Risk of infectious diseases in metropolitan cities

1. International and national tourists visiting places
2. Higher movement of the population during festivals
3. Drastic seasonal changes – attract diseases such as flu and dengue

THE ECONOMIC TIMES | News

English Edition ▾ | 27 September, 2022, 08:16 PM IST | Today's Paper

## Delhi reports 8th monkeypox case as 30-yr-old Nigerian woman tests positive; India's tally rises to 13

**Synopsis**

According to the sources, another person suspected to be suffering from monkeypox has also been admitted to the Delhi government-run hospital.



Representative Image

Delhi reported its eighth monkeypox case on Friday. A 30-year-old Nigerian woman has tested positive for monkeypox in the national capital, said PTI sources.

This is the country's 13th case of the viral disease.

The woman has been admitted to LNJP Hospital, they said.

https://commons.wikimedia.org/wiki/File:New_Delhi_montage.png

# Global concerns of drug abuse

Understanding the public health and socioeconomic impact of drug abuse

**275,000,000**

## High prevalence of drug use

In 2016, approximately 275 million people aged 15-64 used illicit drugs at least once.

**11**

## Significant drug use disorders

11% of drug users suffer from drug use disorders, highlighting serious heath concerns.

**54**

## Surge in overdose deaths

The United States saw a 54% increase in drug overdose deaths between 2011 and 2016.

# Analyzing wastewater to analyze community health and substance use

1

## Community-wide estimation

Explores consumption or exposure across the entire population effectively

2

## Innovative methodology

WBE analyses target chemicals in wastewater for community health insights

3

## Cost-effective approach

Offers a more economical alternative to traditional survey methods.

4

## Early warning System

Serves as a timely alert mechanism for identifying drug use trends

5

## Public health biomarkers

Identifies emerging psychoactive substances impacting public health

# Conventional methods v/s WBE

Analyzing biases in substance use estimation techniques

## Limitations of traditional methods

Conventional methods like surveys and hospital reports are prone to biases leading to underreporting

## Drug seizures as indicators

May not represent actual usage since they depend on law enforcement, not actual consumption

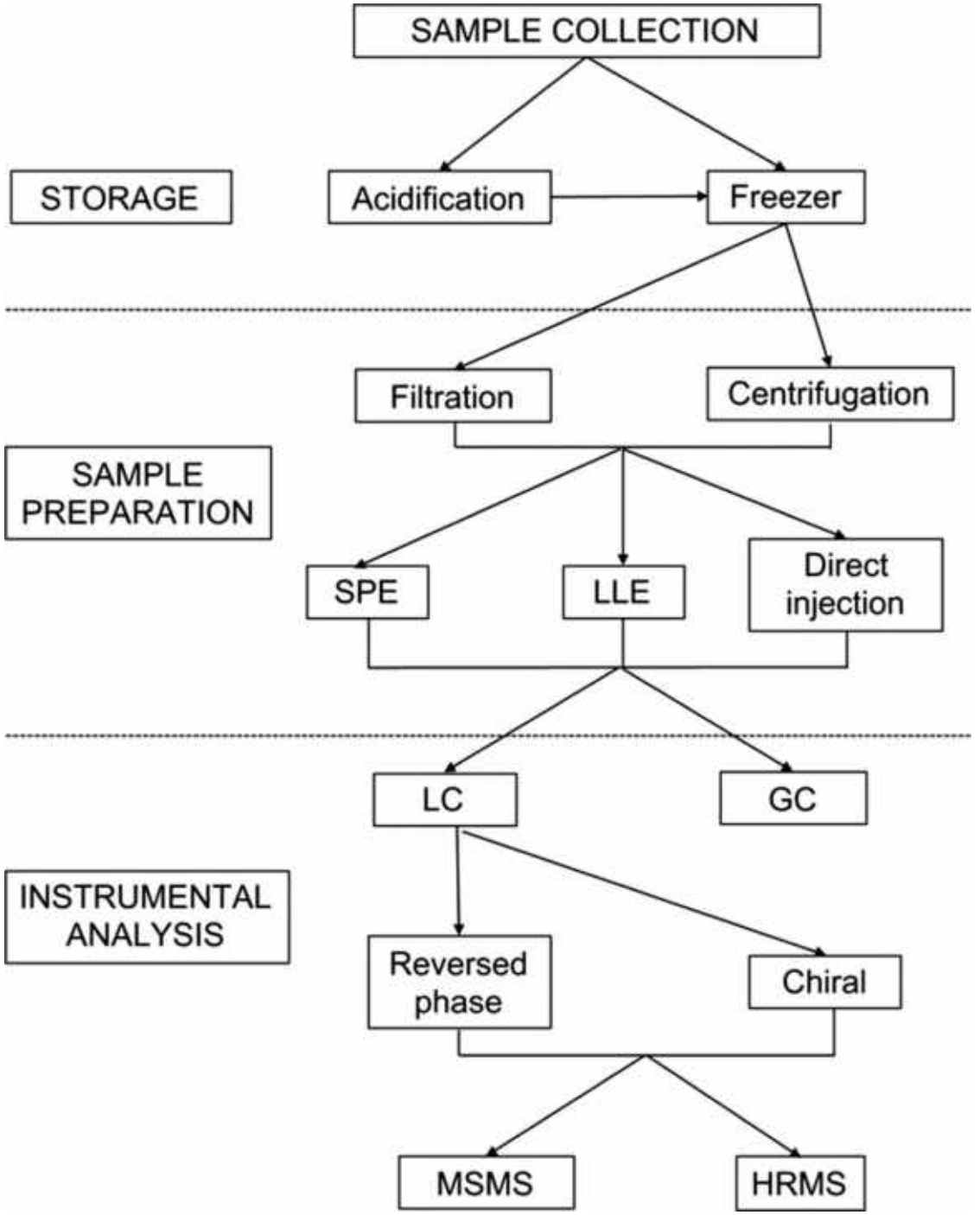
## Introduction to WBE

WBE analyses drug residues in wastewater providing a novel and unbiased perspective

## Real-time data collection

WBE provides timely data on community drug consumption, which can inform public health responses effectively

# Methodology




**SPE**: Solid phase extraction
**LLE**: Liquid-liquid extraction
**LC**: Liquid chromatography
**GC**: Gas chromatography
**MS/MS**: 2nd order Mass Spectroscopy
**HRMS**: High-resolution mass spectroscopy

# Sample preparation

Pre-treatment of the sample before analysis

Sample collection

Sample transportation

Sample centrifugation

Solid phase extraction

Sample concentrator

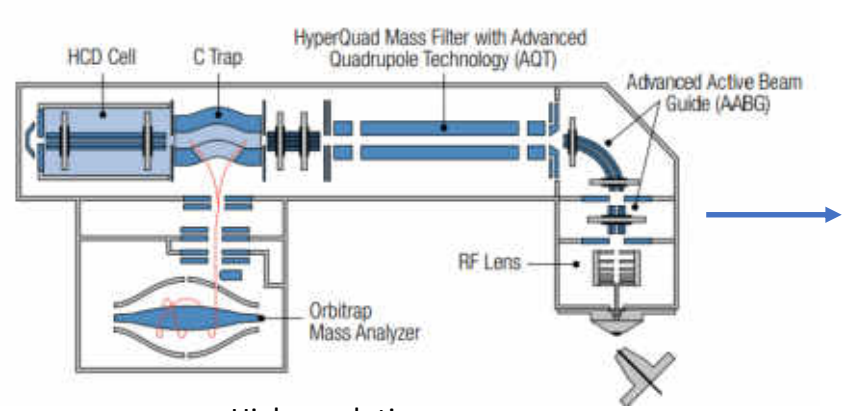
Liquid chromatography coupled to High-resolution mass spectrometry

Gas chromatography-mass spectrometry

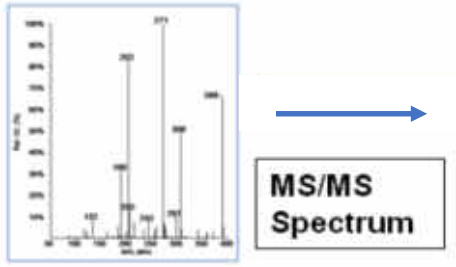
Inductively coupled plasma-mass spectrometry

# Infrastructure – 1. Advanced mass spectrometry in WBE

Preconcentrated wastewater sample




High resolution mass spectrometer



MS/MS Spectrum

Data analysis

Total mass of metabolite

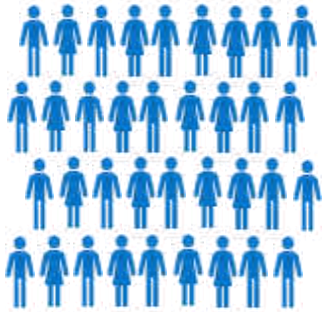
Excretion rate, metabolite and drug mass difference, degradation factor

Total mass of metabolite excreted/1000 people

Flow rate of water, population dynamics, correction factors

Total mass of target drug consumed /1000 people

Control of drug abuse



Wastewater

WWTP

https://en.wikipedia.org/wiki/File:Tandem_ms.png
https://www.thermofisher.com/in

# Population consumption

How do we analyze the amount?

Mass load is the amount of individual drugs introduced into the WWTP (mg/L)

$$\text{Mass load} = C \times F \times \left(\frac{100}{100 + \text{Stability}}\right) \times \frac{1}{1.0 \times 10^6}$$

Conversion factor nanograms to milligrams

'C' is the total nanograms of analyte in 1L of wastewater influent and SPM (ng/L)
'F' is the daily average flow rate of influent wastewater (L/day)
Stability is a measure of the stability change (%) of the analyte in wastewater (up to 12h)

Consumption is in mg/day/1000 people

$$\text{Consumption/1000 people} = \text{Mass Load} \times \left(\frac{100}{\text{Excretion}}\right) \times \left(\frac{MW_{par}}{MW_{met}}\right) \times \left(\frac{1000}{\text{Population}}\right)$$

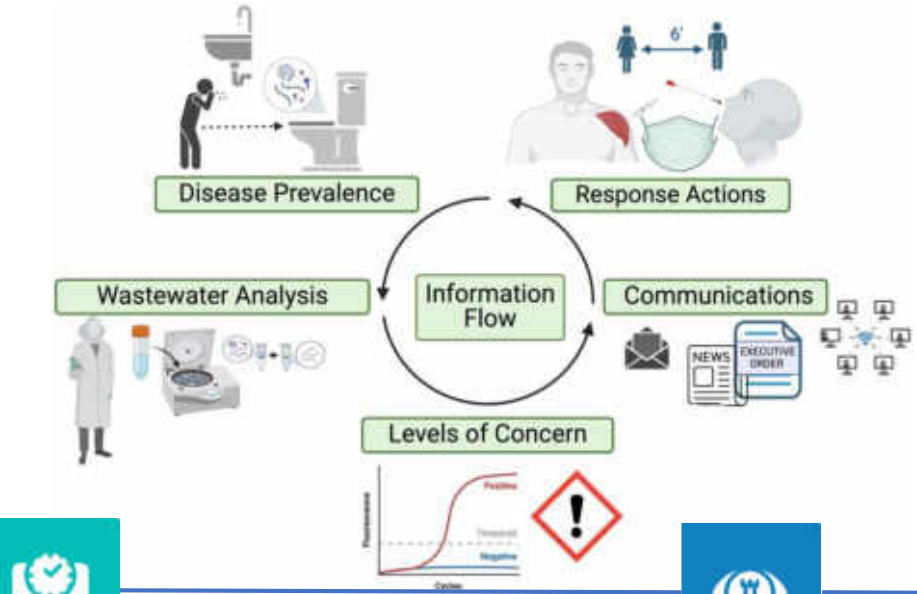
Percentage conversion

Correction factor for metabolite

Excretion rate (%) of the parent drug or metabolite excreted from the human body after administration
$MW_{par}$ is the molar mass of the parent compound
$MW_{Met}$ is the molar mass of the metabolite
Population is the number of people served by the WWTP

# Importance of efficient disease monitoring

Addressing challenges in public health surveillance




**Effective Monitoring**
Effective monitoring is crucial for preventing and managing infectious disease outbreaks.

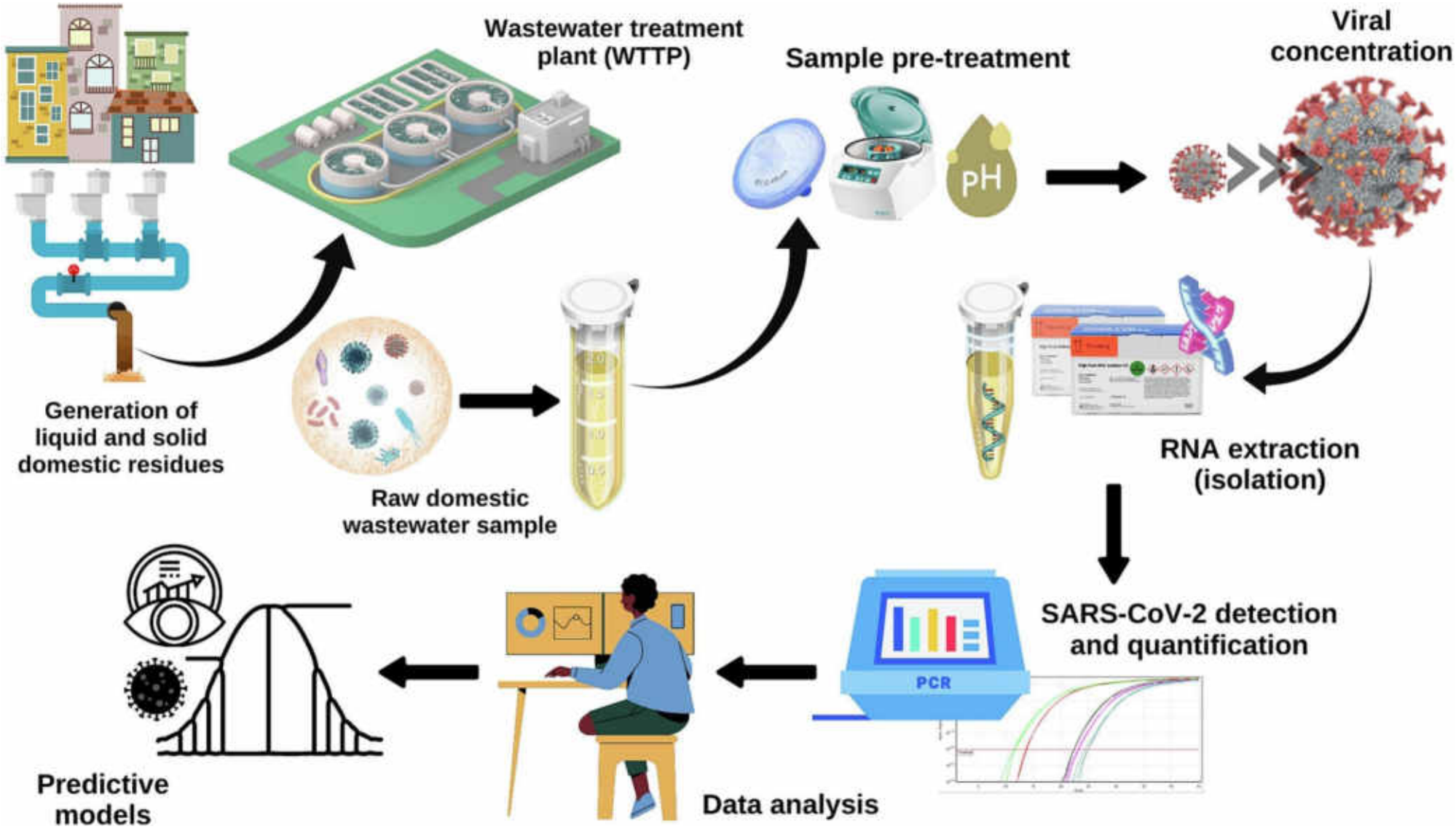
**Limitations of Traditional Monitoring**
Traditional monitoring has limitations such as resource insensitivity and bias.

**Demand for Novel Approaches**
There is a demand for flexible, cost-effective, and scalable monitoring systems.

**WBE as a Solution**
Wastewater-Based Epidemiology (WBE) serves as a solution to address the gaps in traditional surveillance methods.

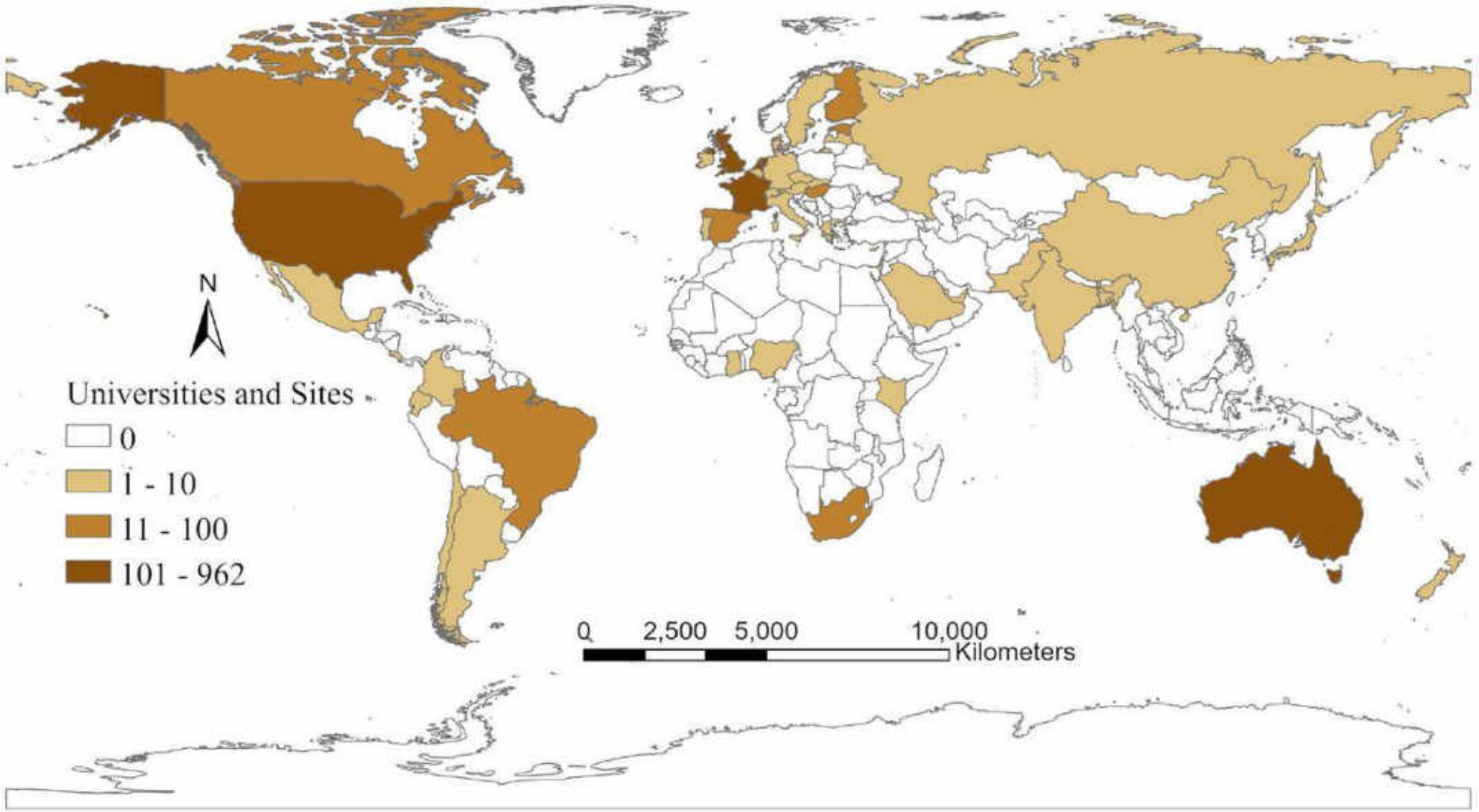
Brosky, H.; Prasek, S. M.; Innes, G. K.; Pepper, I. L.; Miranda, J.; Brierley, P. E.; Slinski, S. L.; Polashenski, L.; Betancourt, W. Q.; Gronbach, K.; Gomez, D.; Neupane, R.; Johnson, J.; Weiss, J.; Yaglom, H. D.; Engelthaler, D. M.; Hepp, C. M.; Crank, K.; Gerrity, D.; Stewart, J. R.; Schmitz, B. W. A Framework for Integrating Wastewater-Based Epidemiology and Public Health. Front. Public Health 2024, 12.

# Infrastructure – 2. Polymerase chain reaction in WBE




Ciannella, S.; González-Fernández, C.; Gomez-Pastora, J. Recent Progress on Wastewater-Based Epidemiology for COVID-19 Surveillance: A Systematic Review of Analytical Procedures and Epidemiological Modeling. Sci. Total Environ. 2023, 878, 162953. https://doi.org/10.1016/j.scitotenv.2023.162953.

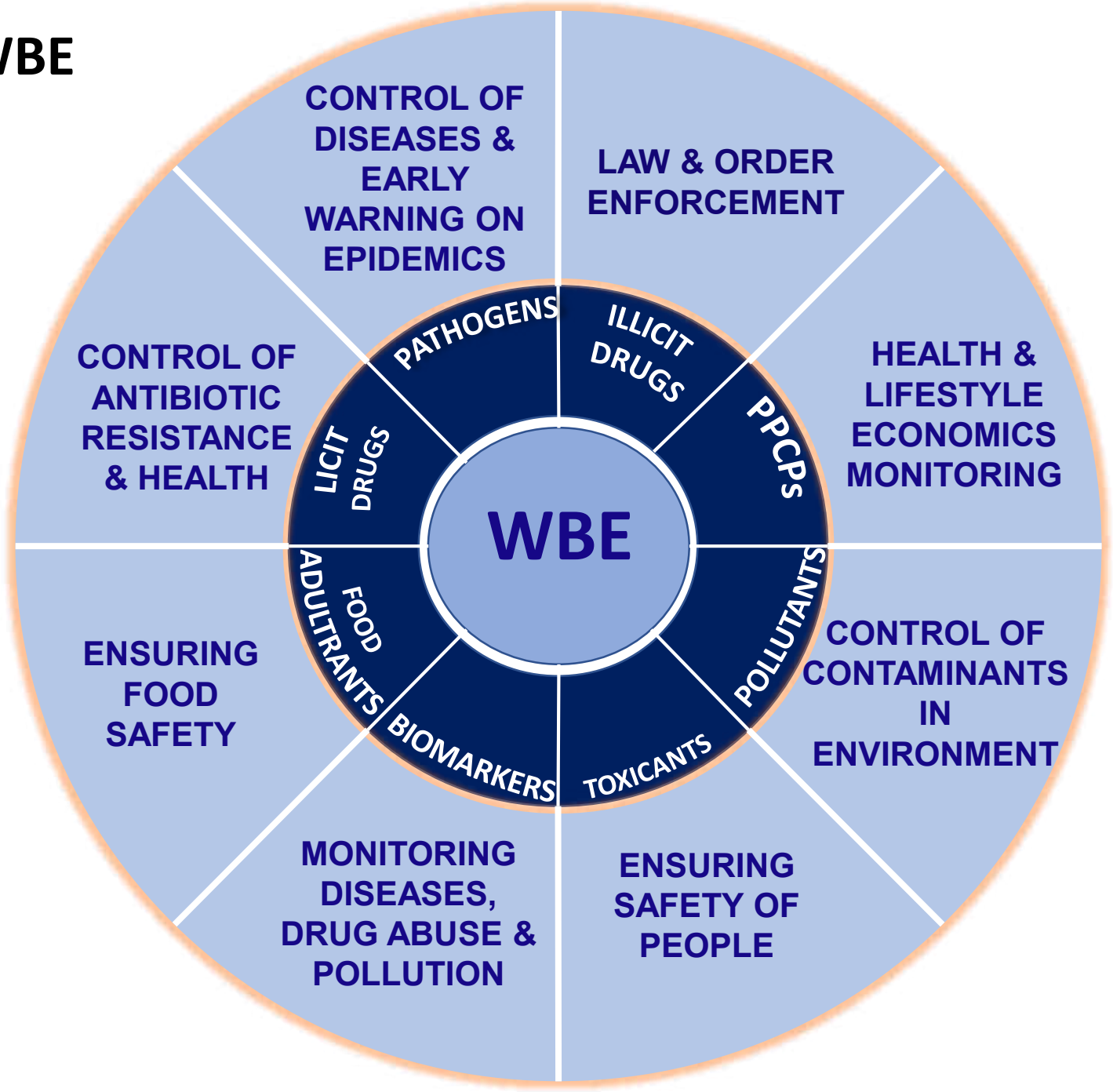
# WBE worldwide - SARS-CoV-2 monitoring countries






Colleen C. Naughton *et al*. ***Show us the Data: Global COVID-19 Wastewater Monitoring Efforts, Equity, and Gaps.*** medRxiv, .03.14.21253564, 2021.

# Possibilities in WBE



# WBE for special events and seasonal trends

Monitoring drug consumption during public events

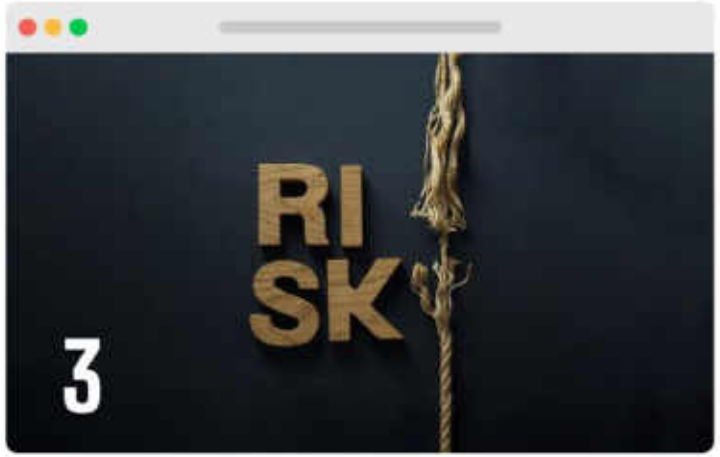

## Monitoring during special events

WBE effectively tracks drug consumption spikes during New Year and Music festivals


## Increased THC-COOH

A study in Kentucky revealed a significant increase in THC-COOH levels during these festivals

Tetrahydrocannabinolic acid A

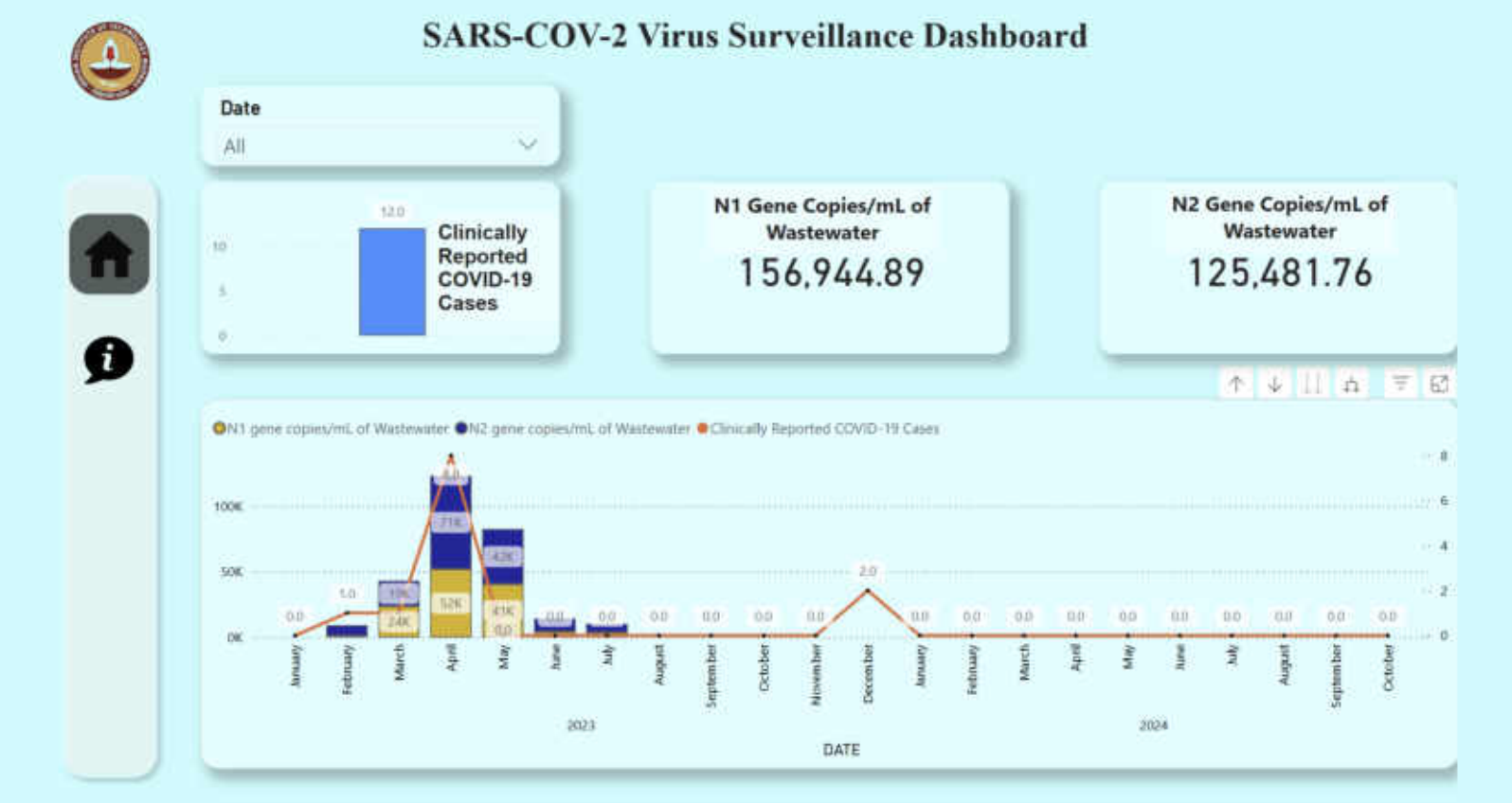



## Public health awareness

This monitoring capability aids authorities in preparing for and responding to potential public health

# Work done

SARS-Cov 2 surveillance in IIT Madras

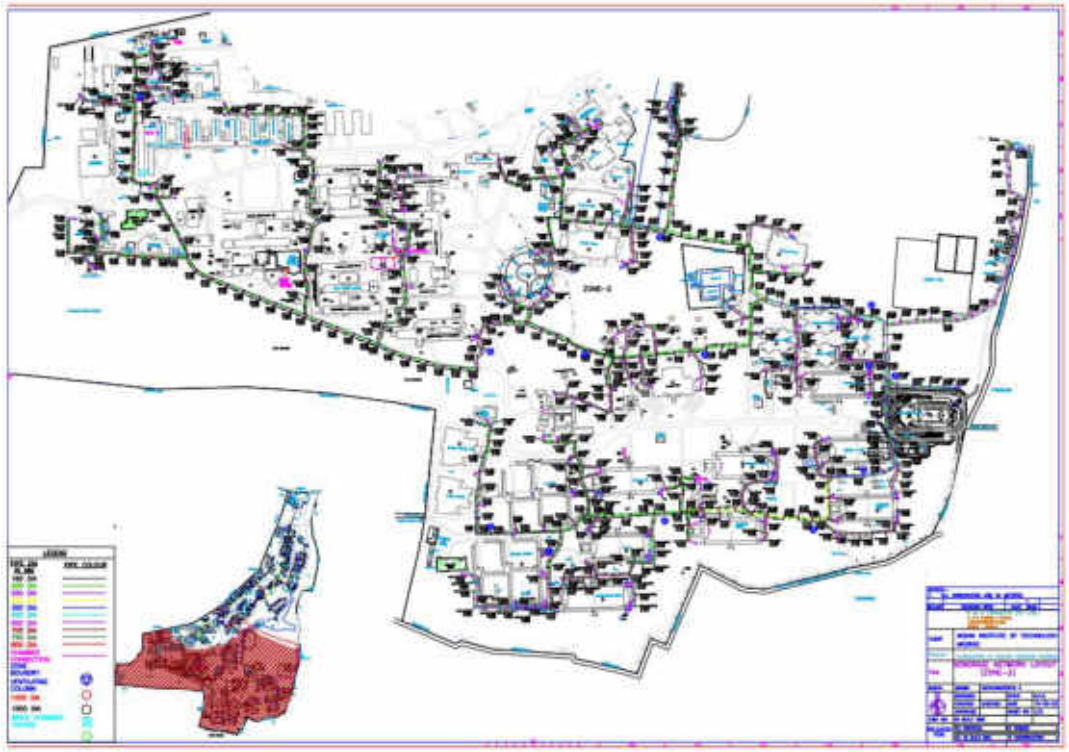



**Conceptual link:**

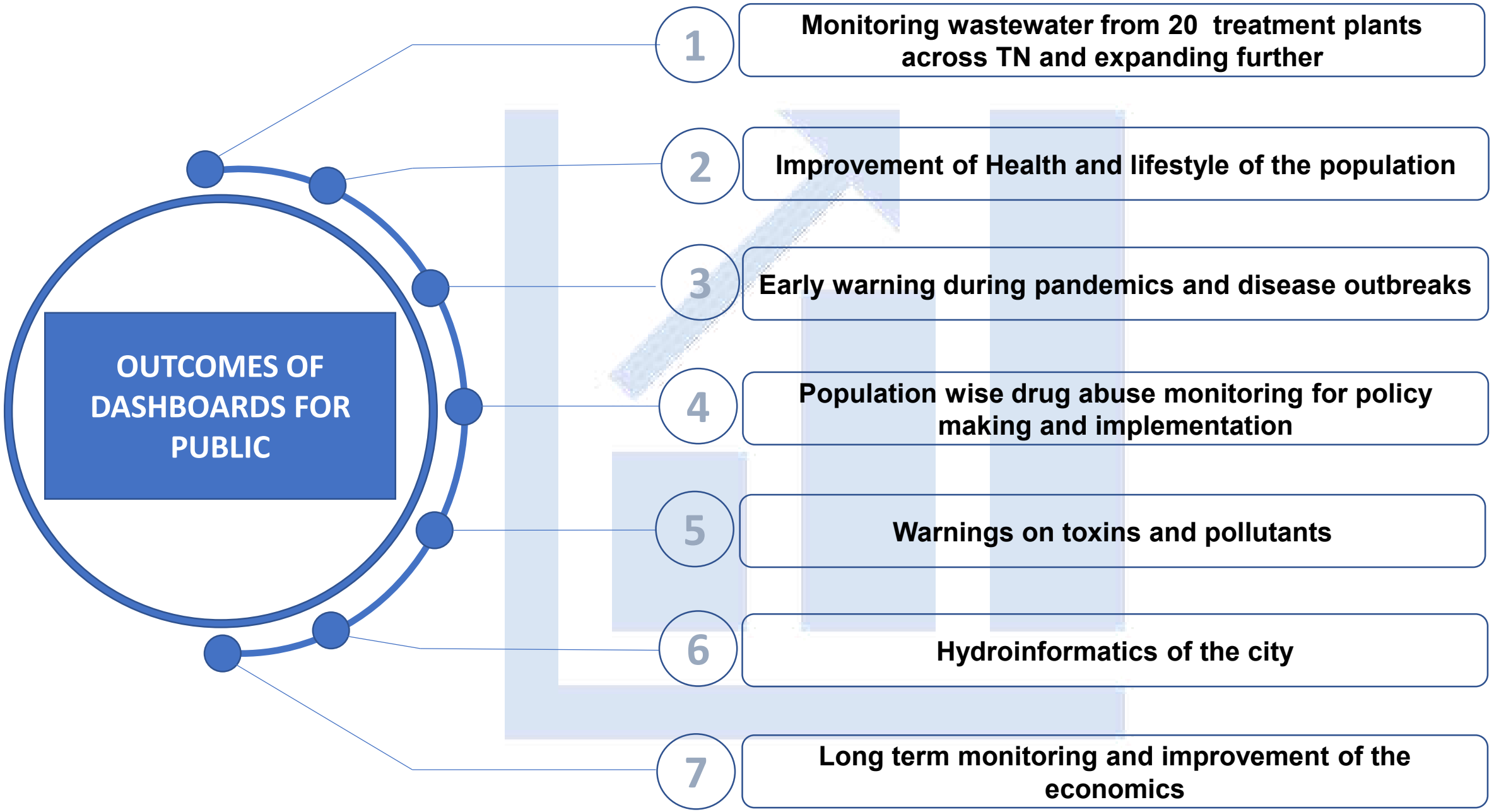
- **N1 and N2 gene copies**: These represent fragments of SARS-CoV-2 RNA detected in wastewater. Higher concentrations indicate higher viral shedding in the community, often correlating with more COVID-19 cases.
- **Clinically reported cases**: These are confirmed cases from testing. However, not everyone gets tested, so clinical data may underreport actual infections.

# Wastewater of IITM

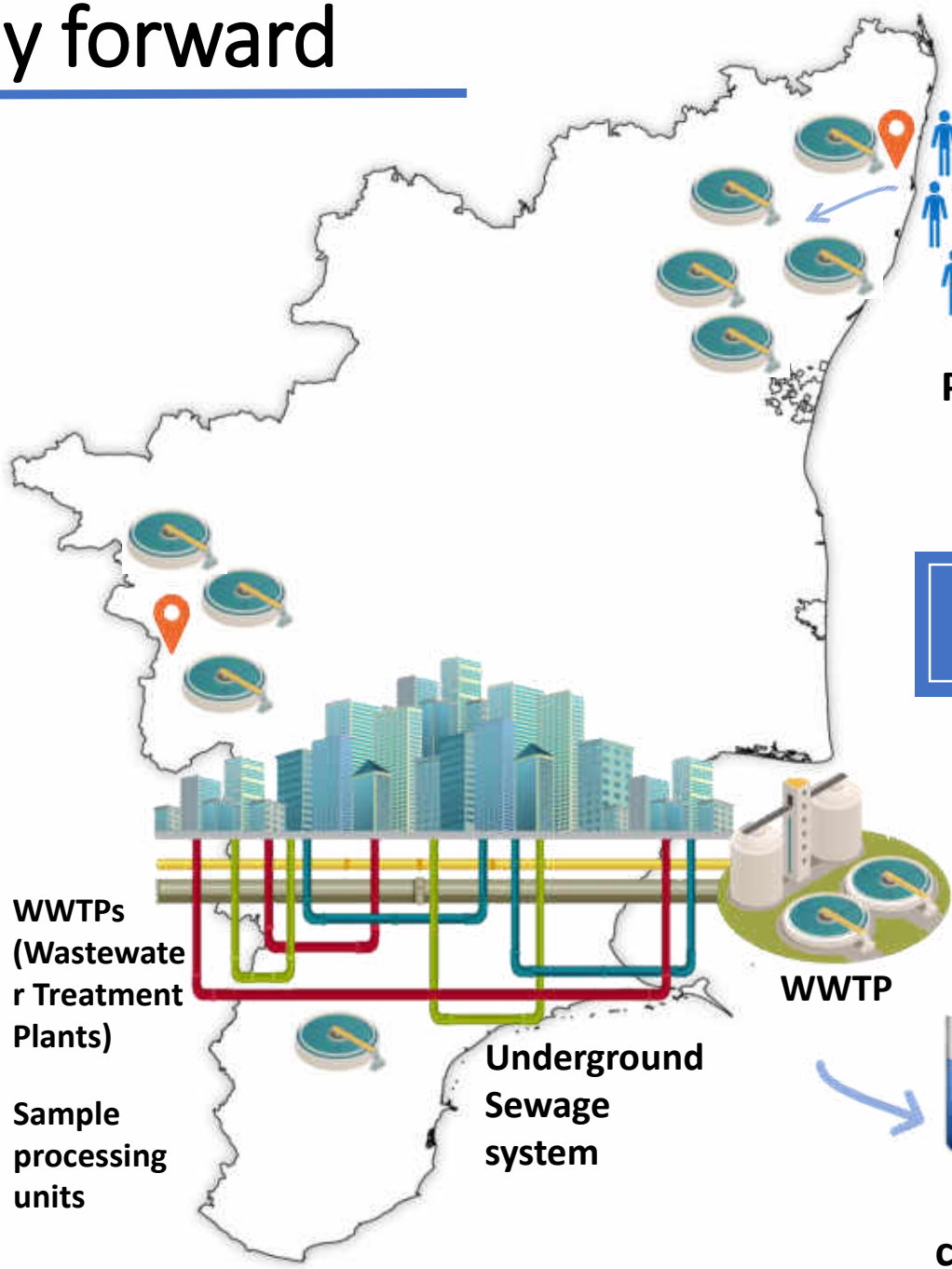
Already established the protocols



**17,000 residents, in IIT Madras**



OUTCOMES OF DASHBOARDS FOR PUBLIC
1
Monitoring wastewater from 20 treatment plants across TN and expanding further
2
Improvement of Health and lifestyle of the population
3
Early warning during pandemics and disease outbreaks
4
Population wise drug abuse monitoring for policy making and implementation
5
Warnings on toxins and pollutants
6
Hydroinformatics of the city
7
Long term monitoring and improvement of the economics

# Way forward



WWTPs (Wastewater Treatment Plants)

Sample processing units

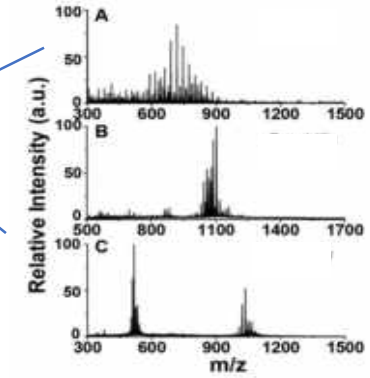
Underground Sewage system

WWTP

Sample collection

Sample Storage

Analysis




Public Health Monitoring

Epidemiology Modelling

Data Storage

**WBE for TN**

# Infectious Disease Monitoring

| Origin of infection | Monitored through WW | Disease caused | Benefits in WBE |
|---|---|---|---|
| Virus | SARS-CoV-2 | Respiratory infection | Establishing continuous monitoring platforms for disease outbreaks and tracking mutations, eliminates need for door to door testing |
| | Hepatitis A | Liver infection | Safety of effluent water from WWTPs, Early warning compared to clinical data |
| | Zika | Mild infection, microcephaly | Early warning system |
| | Dengue | Severe flu-like illness | Control on diseases induced by seasonal changes |
| | Influenza A | Respiratory infection | Spatiotemporal mapping of disease hotspots to curb further spread |
| | Norovirus (GI) | Gastroenteritis | Controlling spread through contact before clinical tests data is available |
| | Norovirus (GII) | Gastroenteritis | Monitoring WW from nursing homes and hospitals |
| Bacteria | *Klebsiella pneumoniae* | Pneumonia, UTI, bacteraemia and endophthalmitis | • Safety of effluent water from WWTPs<br>• Early warning system<br>• Control on diseases induced by seasonal changes<br>• Imposing food safety regulations on disease instances |
| | *Pseudomonas aeruginosa* | Pneumonia, UTI, gastrointestinal infections | |
| | *Enterococcus faecalis* | UTIs, bacteraemia, septicaemia | |
| Antimicrobial resistance (AMR) strains | Methicillin resistance | Severe infections which are difficult to treat<br>Diseases causing prolonged illness and mortality | • Monitoring AMR strains in the wastewater<br>• Understanding the spread and evolution of AMR among communities<br>• Investigating the cause behind the spread of AMR<br>• Implementing policies to monitor Antimicrobial Drug consumption<br>• Review of policies implemented on long term without the need for clinical data |
| | Erythromycin resistance | | |
| | Sulphonamide resistance | | |
| | Beta-lactam resistance | | |
| | Tetracycline resistance | | |
| Fungus | Candida species | Candidiasis | Evaluation of the basic health and hygiene of a population |
| | Aspergillus (*Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus flavus*) | Chronic pulmonary aspergillosis, pulmonary and nasal allergies, asthma, pneumonitis | |
| Parasites | *Giardia lambli* | Small intestine infections | Population wise evaluation of water and food contamination |
| | *Cryptosporidium* | Gastrointestinal illness | |



Maria Lorenzo and Yolanda Picó, *Wastewater-based epidemiology: current status and future prospects, Current Opinion in Environmental Science & Health*, Vol. 9, 77-84, 2019.

# Licit and Illicit drugs

| Licit drug | Biomarker | Significance in WBE |
|---|---|---|
| Alcohol | Ethyl sulfate<br>Cocaethylene | Associated risks in terms of exposure at the community level, Tracking the pattern of consumption |
| Caffeine | 1,7-dimethyluric acid | Tracking the population level consumption and its variations |
| Tobacco | Cotinine, nicotine, trans-3-hydroxycotinine | Understanding the consumption for localized control measures |
| Nicotine | 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone | Population-wide exposure to toxicants and carcinogens through third-hand smoke |

| Illicit drug | Biomarker | Significance in WBE |
|---|---|---|
| Cannabis (Marijuana) | 11-nor-9-carboxy-$\Delta^9$-tetrahydrocannabinol (THC-COOH) | Control of drug abuse with community wise estimation of drug consumption |
| Cocaine, amphetamine, opiates | Benzoylecgonine (BEG), cocaine, cocaethylene (for cocaine), Amphetamine (AMP), Methamphetamine (METH) 3,4-methylenedioxymethamphetamine (MDMA) | |
| Ketamine | Norketamine | |
| New psychoactive substances (NPS) | meta-Chlorophenylpiperazine (mCPP), 2,5-dimethoxy-4-bromophenethylamine, and 4-fluoroamphetamine, mephedrone, benzylpiperazine, trifluoromethylphenylpiperazine, 1-(3-chlorophenyl)piperazine (mCPP), synthetic opioid AH-7921, arylcyclohexylamine, methoxetamine | Tracking the entry of NPS into the city and evaluating the consumption on a daily basis with data shared over dashboards to the public |
| Weight loss stimulants | Ephedrine, norephedrine, methylhexanamine, and 2,4-dinitrophenol | Use of doping agents by the general population and during sports events |



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# PPCPs and Lifestyle

| Drug | Biomarker | Significance in WBE |
|---|---|---|
| Metformin | Metformin | Public health care and diabetes control |
| NSAIDs | Ibuprofen carboxylic acid/carboxyibuprofen, salicylic acid, diclofenac, parecoxib, valdecoxib, celecoxib, etoricoxib, indomethacin, ketoprofen, piroxicam, paracetamol, pregabalin, amitriptyline, gabapentin, naproxen | Monitoring self medication during epidemics |
| Carbamazepine | Carbamazepine | Efficiency of wastewater treatment |
| Histamine | 1,4-methylimidazoleacetic acid | Temporal mapping of drugs consumption for asthma and allergies – tracking the effect of pollution |
| Antivirals | Acyclovir, Carboxy-abacavir | Understanding self medication during epidemics |
| Antifungals | Ketoconazole, Miconazole, Clotrimazole | Understanding the prevalence and dynamics of fungal infections in the population |
| Antibiotics | n-Demethyl azithromycin, Ciprofloxacin, Erythromycin | Respiratory tract infections, Understanding self medication |
| Pain killers | Acetaminophen, Ibuprofen | Estimating the overall consumption |
| Antidepressants and psychoactive pharmaceuticals | Aripiprazole, quetiapine, lorazepam, alprazolam, diazepam, oxazepam, nordiazepam, carbamazepine, venlafaxine, bupropion, sertraline, and citalopram, hydroxyalprazolam, lormetazepam | Monitoring the population well being |
| Atenolol, acesulfame, naproxen | Atenolol, acesulfame, and naproxen | Effect of temperature on the population through drugs consumed |
| Sildenafil | Desmethyl sildenafil and desethyl sildenafil | Counterfeit medication |

| Personal Care Products | Biomarker | Significance in WBE |
|---|---|---|
| UV filters | Benzophenone-1, 4,40-dihydroxybenzophenone, 4-benzylphenol, homosalate; octocrylene, 3-benzylidene camphor | Control of endocrine disruptors present in the products |
| Parabens | Methyl, ethyl, n-propyl, butyl (n- and iso-), and benzyl parabens | Curbing the potential risk due to human exposure to parabens, through direct quantification of WW |
| Synthetic musks | Musk xylene and musk ketone | Contamination of sewage through industrial effluents |
| Disinfectants/ antiseptics | Triclosan, 4-Chloroxylenol, Chlorophene, 3,4,5,6-Tetrabromo-o-cresol, p-Benzylphenol | Exposure to chemical agents which indicate the impact on environment and economy |



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# Toxicants and Pollutants

| Toxicant | Category | Biomarker | Significance in WBE |
|---|---|---|---|
| Bisphenol A (BPA) | Plasticizer | BPA sulfate | Human exposure including occupational exposure |
| Phthalates | Plasticizer | Phthalate monoesters | Human exposure (children's exposure), health risk exposure, early warning system of exposure |
| organochlorines (chlordecone), triazines, organophosphates, and pyrethroids | Pesticides | bis(Chlorophenyl)acetic acid, Glufosinate, Monohydroxyl penconazole | Controlling pesticide usage and their exposure |
| 1,4-methylimidazoleacetic acid | Asthma medicine and antihistamines | Acesulfame, saccharin, sucralose, aspartame and cyclamate | Air quality monitoring and pollution remediation through drugs consumed |



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# Food safety, stress & Population markers

| Molecule/ Targets | Category | Biomarker | Significance in WBE |
|---|---|---|---|
| Antischizophrenics, sedative–hypnotic–anxiolytics, and antidepressants | Psychoactive pharmaceuticals | Aripiprazole, quetiapine, lorazepam, alprazolam, diazepam, oxazepam, nordiazepam, carbamazepine, venlafaxine, bupropion, sertraline, and citalopram, hydroxyalprazolam, lormetazepam | Understanding the overall lifestyle and health of the population |
| Triazines, organopho-sphates, pyrethroids | Pesticides | Triazines, organopho-sphates, pyrethroids | Exposure to harmful substances such as pesticides through food |
| Preservatives, Food colors, Ripeners, etc | Food adulterants | Formalin, DDT, Calcium carbide | Helps in improving food safety through continuous monitoring without rapid tests and clinical inputs |
| Parameters which indicate the population dynamics | Population marker | Biological oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen and total phosphorous, Artificial sweeteners | Indicates the changes in the population such that the data analysis can be precise |



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# Strategic challenges to WBE implementation

Addressing key challenges for large-scale implementation of WBE


## Access to wastewater samples

Securing reliable access to wastewater sample is crucial for accurate WBE implementation

## Securing funding

Funding is essential for sustaining WBE projects; challenges exist in obtaining adequate financial support,

## Privacy concerns

Addressing privacy concerns is vital to ensure community trust and participation in WBE

## Community stigmatization

Potential Stigmatization of communities participating in WBE needs to be carefully managed

## Collaboration among stakeholders

Effective collaborations with public health authorities, policymakers and researchers are essential to overcome these

# Conclusion and future directions

WBE as a complementary tool towards public health monitoring

- **Real-time data collection**
  - WBE allows for an immediate gathering of accurate data enhancing public health monitoring
- **Complementary to traditional methods**
  - WBE serves as a powerful tool alongside conventional survey methods for better monitoring
- **Methodological challenges**
  - There are no specific methodological issues that need to be addressed to improve WBE effectiveness
- **Privacy concerns**
  - Addressing privacy issues is essential to gain public confidence in WBE
- **Interdisciplinary collaborations**
  - Future research should promote collaboration across disciplines to enhance WBE
- **Refinement of analytical methods**
  - Current analytical methods can be further improved to increase the capability of WBE.

# Additional reading materials

## Papers

1. Bowes, D. A.; Driver, E. M.; Choi, P. M.; Barcelo, D.; Beamer, P. I. Wastewater-Based Epidemiology to Assess Environmentally Influenced Disease. *J. Expo. Sci. Environ. Epidemiol.* **2024**, *34* (3), 387–388. https://doi.org/10.1038/s41370-024-00683-w.

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