

Commentary

Open Access



# Environmental proteomics: a potential tool in wastewater-based epidemiology

Antoni Ginebreda<sup>1\*</sup> , Damià Barceló<sup>1,2</sup>, Yolanda Picó<sup>3</sup>, Montserrat Carrascal<sup>4</sup>, Joaquin Abián<sup>4</sup>

<sup>1</sup>Department of Environmental Chemistry, Institute of Environmental Assessment and Water Studies, Spanish National Research Council (IDAEA-CSIC), Barcelona 08034, Spain.

<sup>2</sup>Water Quality Area, Catalan Institute for Water Research (ICRA), Girona 17003, Spain.

<sup>3</sup>Food and Environmental Research Group (SAMA-UV), Research Desertification Centre (CIDE) (CSIC-University of Valencia-GV), Moncada 46113, Spain.

<sup>4</sup>Proteomics Laboratory CSIC/UAB, Institute of Biomedical Research of Barcelona, Spanish National Research Council (IIBBSIC/IDIBAPS), Barcelona 08036, Spain.

\***Correspondence to:** Prof. Antoni Ginebreda. Department of Environmental Chemistry, Institute of Environmental Assessment and Water Studies, Spanish National Research Council (IDAEA-CSIC), Jordi Girona 18-26, Barcelona 08034, Spain. E-mail: agmqam@cid.csic.es

**How to cite this article:** Ginebreda A, Barceló D, Picó Y, Carrascal M, Abián J. Environmental proteomics: a potential tool in wastewater-based epidemiology. *Water Emerg Contam Nanoplastics* 2023;2:6. <https://dx.doi.org/10.20517/wecn.2023.19>

**Received:** 22 Mar 2023 **Revised:** 31 Mar 2023 **Accepted:** 3 Apr 2023 **Published:** 6 Apr 2023

**Academic Editors:** Wei Chen, Guangming Jiang **Copy Editor:** Ke-Cui Yang **Production Editor:** Ke-Cui Yang

## Abstract

Wastewater-based epidemiology (WBE) is a new complementary alternative to traditional epidemiological surveillance approaches for community health surveys. It provides objective, flexible, and comprehensive data on diseases, exposure to toxicants and pathogens, social habits, and lifestyle. Wastewater is regarded as an integrated pooled sample of the entire population served by a certain wastewater system, thus providing an average picture of its health status. WBE relies on the extraction, analysis, and subsequent interpretation of appropriate health status indicators, including both chemical and biological components, either exogenous or endogenous, altogether constituting the exposome. Instrumental development, especially mass spectrometry (MS) for the chemical analysis of small and large molecules, has been critical to the success achieved through WBE. Whereas all previous studies have been restricted to small molecules, the use of proteomics in WBE is an obvious extension of the intensive ongoing pursuit of biomarkers for community surveillance of diseases and health status. The relevance of human proteins present in wastewater as potential WBE indicators of population-prevalent diseases has been previously postulated, although no real examples to demonstrate their feasibility have been available until recently. These findings provide new insights into the characterization of wastewater proteomics useful for practical



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



purposes related not only to WBE applications but also to environmental monitoring and wastewater treatment plant management.

**Keywords:** Environmental proteomics, wastewater-based epidemiology, sewage epidemiology, biomarkers, water fingerprinting, mass spectrometry

## WASTEWATER-BASED EPIDEMIOLOGY: AN OVERVIEW

The term “Epidemiology” is a broad concept that has undergone significant changes in its definition over time, with the most widely accepted definition nowadays being “the study of the distribution and causes of health-related states or events in specified populations, and the application of this study to control health problems”<sup>[1,2]</sup>. The recent worldwide outbreak of COVID-19 has highlighted the need for new epidemiological tools to assess population health. Factors like globalization - which is stimulated by trade and tourism- together with climate change, population growth, uncontrolled urbanization, and changes in land use contribute to the emergence or recurrence of infectious disease outbreaks, as well as to their rapid spread. Current approaches to epidemiological surveillance based on individual patient clinical monitoring data or statistical changes in rates (mortality/morbidity and drug prescribing rates), although very valuable, are insufficient as rapid alert systems. In this context, wastewater-based epidemiology (WBE)<sup>[3]</sup>, also known as sewage epidemiology<sup>[4]</sup>, has appeared as a new complementary alternative capable of providing comprehensive health and environmental information about communities. Under this approach, wastewater is considered an integrated aggregate sample of the entire population served by a certain sewage system; thus, its monitoring provides an average picture of its health status<sup>[5,6]</sup>. Some of WBE’s well-established advantages are<sup>[4]</sup>: (a) it provides objective, flexible, and comprehensive information (for example, surveillance of multiple aspects of health, such as disease, exposure to toxic substances and pathogens, social habits and lifestyle); (b) samples are available continuously and easily, allowing results to be obtained in (near) real-time; (c) it is scalable across the whole wastewater system and catchment area; (d) the samples are anonymous and, consequently, are not subject to specific personal and ethical consent requirements; and (e) it is cost-effective and thus economically feasible.

WBE is based on extracting, analyzing, and subsequently interpreting appropriate indicators of health status. These must be considered in their widest sense, encompassing both exogenous and endogenous chemical and biological components, making up, on the whole, the so-called exposome<sup>[7,8]</sup>. The success of WBE can be linked to the development of analytical instrumental technologies, particularly mass spectrometry (MS) for the analysis of small and large molecules and, more recently, polymerase chain reaction (PCR) for genetic material analysis<sup>[9]</sup>. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) with triple quadrupole (QqQ) or linear quadrupole ion trap (QTRAP) has risen as an effective analytical method for simultaneously detecting multiple biomarkers in wastewater. High-resolution mass spectrometry (HRMS), utilizing quadrupole time-of-flight (QqTOF) or Orbitrap, can often be a preferable choice for the direct identification of human biomarkers in sewage water, thus offering novel possibilities in WBE. Most studies utilizing LC-MS/MS do target analysis, where *a priori* selected analytes are monitored, thereby ensuring maximum sensitivity in the analytical determination. However, this approach may overlook the detection of other compounds present in the sample. Due to the complexity of wastewater samples potentially containing a large number of compounds, it is unlikely that the biomarkers of interest are the primary constituents. To address this analytical challenge, the so-called “suspected screening” approach has been developed. This requires constructing a database of reported compounds in the literature or other databases to assist in identifying relevant biomarkers<sup>[10-12]</sup>. *In silico* tools, including the prediction of MS fragmentation, retention times, metabolism, biodegradation pathways, or transformation products, have proven effective in biomarker identification from raw MS data<sup>[11,13]</sup>. Processing of wastewater

MS data profiles (fingerprinting) has been used as well for such purposes. However, this methodology demands the intensive application of bioinformatics and chemometric tools to support filtering and extraction of acquired MS data, cluster classification, which organizes in different groups ionic signals sharing common characteristics, estimates ionic signal intensity in space, and applies advanced multivariate statistical methods to develop predictive models.

Some successful applications of WBE deserving mention include the consumption of illegal drugs<sup>[14,15]</sup>, pharmaceuticals and personal care products<sup>[16,17]</sup>, tobacco<sup>[18]</sup> and alcohol use<sup>[19]</sup>, the exposure to toxicants like pesticides<sup>[20]</sup>, and Bisphenol A<sup>[21]</sup>, and concerning biological response, oxidative stress<sup>[22]</sup> or the monitoring of coronavirus prevalence during the recent COVID-19 outbreak using reverse transcription-PCR (RT-PCR) and other related methods<sup>[23,24]</sup>.

## ENVIRONMENTAL PROTEOMICS IN WBE

While current WBE studies primarily focus on small molecules such as xenobiotics and their metabolites and transformation products, the potential for using proteomics in WBE is gaining interest as a means of identifying biomarkers for community biomonitoring of diseases and overall health status. Rice and Kasprzyk-Hordén<sup>[6]</sup> previously suggested that human proteins found in sewage water could serve as indicators of prevailing diseases affecting a population. This approach emerged as a new concept in the assessment of community health, even though no experimental evidence was yet available to support its feasibility as a practical WBE tool, hence showing that the road from theory to reality is not straightforward. Although the use of large molecules in WBE has so far been scarce, recent studies<sup>[25,26]</sup> have shown that analysis of influent and effluent from wastewater treatment plants (WWTPs) can provide valuable information about the large molecules present. As far as we know, these are the earliest non-target proteomics studies dealing with the analysis and identification of a range of peptides and proteins in the various steps of the WWTP process. Wastewater proteomics is thus a complex field that is still in its early stages of development, as it deals with the analysis of samples containing diverse molecules from various sources. Therefore, the amount of available information in this area is nowadays fairly limited, though more work is ongoing.

It is worth mentioning that the field of wastewater proteomics is in many cases capable of distinguishing human proteins from those of animal, plant, or microbial origin that are also present in these waters. Instead of being seen as a source of interference, these non-human proteomes provide valuable insights into the human environment<sup>[25]</sup>. This initial discovery paved the way for the identification of human protein biomarkers in wastewater, which in conjunction with other biomarkers found in sewage water, may serve as indicators of the different anthropogenic activities occurring in the surroundings (i.e., domestic, agricultural, and industrial), pest control, as well as the population health status as assessed through WBE.

From the experimental point of view, protein analysis in wastewater involves certain preparative steps such as cleaning, concentration, and digestion. This is usually done by gel electrophoresis. Prior to the analysis by LC-HRMS, the bands with the concentrated proteins are excised and digested with trypsin<sup>[27]</sup> to liberate the constituent peptides. MS methods based on LC-Orbitrap-MS and matrix-assisted laser desorption/ionization (MALDI)-TOF may allow for fast analysis and regular peptide monitoring, and related protein identification<sup>[25,26]</sup>. Owing to the large amount of MS data acquired, as in the case of small molecules, the use of bioinformatics tools to process the complex omics pattern becomes crucial<sup>[26]</sup>.

Up to now, studies on sewage proteomics have predominantly focused on characterizing the bacterial microbiome in either sludge<sup>[28]</sup> or wastewater<sup>[29]</sup>, which includes extracellular proteins<sup>[30]</sup>. However, the

aforementioned recent studies<sup>[25,26]</sup> have expanded upon this work. By utilizing passive sampling polymeric devices and LC-HRMS shotgun proteomic methods, these researchers have been able to profile the wastewater proteome beyond prokaryotes and include eukaryotic higher organisms, such as plants, animals, and human proteomes. In terms of higher organisms, these studies have identified not only major constituents, such as albumins and keratins, but also less abundant biomarkers of disease, including S100A8, uromodulin, and defensins, proposed as health markers associated with renal function and stress-related changes<sup>[31]</sup>. This groundbreaking work represents the first attempt to identify the entire wastewater proteome, yet it also highlights the experimental and analytical challenges involved in its characterization, such as the need to overtake the predominance of bacterial proteins in comparison with those of other organisms.

Finally, it is worth noting that the feasibility of detecting disease-related viral structural proteins in wastewater, particularly in the case of SARS-CoV-2, has been discussed in the literature<sup>[9]</sup>. However, it is important to point out that although MS has been utilized to identify viral proteins and peptides in clinical studies, its usage on WBE is not yet accomplished. In the case of SARS-CoV-2, the virion particle contains four structural proteins: spike, membrane, envelope, and nucleocapsid (referred to as S, M, E, and N, respectively). Some of these proteins have already been identified by MS in clinical samples and may also be present in wastewater. Therefore, the possibility of detecting them in wastewater should not be dismissed, so that they can be used as WBE biomarkers<sup>[9]</sup>. At present, the foremost limitations to its practical application are the complexity of the sewage samples to be analyzed, together with the insufficient sensitivity of the currently available MS instruments.

## FINAL REMARKS

In this commentary, we have outlined the recent progress of WBE application to large molecules. Notably, there are few studies currently available that refer to proteins and peptides as exposure biomarkers<sup>[25,26]</sup>. However, their recent findings provide new insights into the characterization of wastewater proteomics that allow proposing specific bioindicators useful for practical purposes related not only to WBE surveys, but also to environmental monitoring, pest control, and wastewater treatment plant management.

Although significant progress has been made in studying the wastewater proteome, it should be noted that there is still much to discover and understand about the dynamics, fate, and emission rates of proteins in wastewater. Altogether, this highlights the need for continued research to fully grasp the potential of these biological molecules as WBE and environmental biomarkers.

Suggestions were made in the literature for future surveillance of viral proteins such as SARS-CoV-2 in WBE<sup>[9]</sup>, integrating previous experience gained in the use of LC-MS in biomedical studies to identify viral proteins and peptides. While PCR methodologies currently dominate in this area, MS detection may provide more detailed data concerning virus-specific peptides. In that sense, we believe that WBE MS could be in the future a convenient analytical methodology to complement PCR. However, for now, further improvements in MS sensitivity are needed to make it competitive with PCR tools.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to the conception, writing, review, and editing: Ginebreda A, Barceló D, Picó Y, Carrascal M, Abián J

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

This work was supported by the Spanish Ministry of Science and Innovation (MICINN, Spain) (Project nos. PID2020-114065RB-C22 and PID2020-114065RB-C21).

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2023.

## REFERENCES

1. Winkelstein W. From the editor. *Am J Epidemiol* 2001;154:93. [DOI](#)
2. Vitale D, Morales Suárez-varela M, Picó Y. Wastewater-based epidemiology, a tool to bridge biomarkers of exposure, contaminants, and human health. *Curr Opin Environ Sci Health* 2021;20:100229. [DOI](#)
3. Daughton CG. Illicit drugs: contaminants in the environment and utility in forensic epidemiology. In: Whitacre DM, editor. *Reviews of Environmental Contamination and Toxicology Volume 210*. New York: Springer; 2011. pp. 59-110. [DOI](#) [PubMed](#)
4. Sims N, Kasprzyk-Hordern B. Future perspectives of wastewater-based epidemiology: monitoring infectious disease spread and resistance to the community level. *Environ Int* 2020;139:105689. [DOI](#) [PubMed](#) [PMC](#)
5. Daughton CG. Monitoring wastewater for assessing community health: Sewage Chemical-Information Mining (SCIM). *Sci Total Environ* 2018;619-620:748-64. [DOI](#) [PubMed](#) [PMC](#)
6. Rice J, Kasprzyk-hordern B. A new paradigm in public health assessment: Water fingerprinting for protein markers of public health using mass spectrometry. *TRAC-Trend Anal Chem* 2019;119:115621. [DOI](#)
7. Vermeulen R, Schymanski EL, Barabási AL, Miller GW. The exposome and health: where chemistry meets biology. *Science* 2020;367:392-6. [DOI](#) [PubMed](#) [PMC](#)
8. Wild CP. Complementing the genome with an “exposome”: the outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiol Biomarkers Prev* 2005;14:1847-50. [DOI](#) [PubMed](#)
9. Picó Y, Barceló D. Mass spectrometry in wastewater-based epidemiology for the determination of small and large molecules as biomarkers of exposure: toward a global view of environment and human health under the COVID-19 outbreak. *ACS Omega* 2021;6:30865-72. [DOI](#) [PubMed](#) [PMC](#)
10. Andrés-costa MJ, Andreu V, Picó Y. Liquid chromatography-mass spectrometry as a tool for wastewater-based epidemiology: assessing new psychoactive substances and other human biomarkers. *TrAC Trends Anal Chem* 2017;94:21-38. [DOI](#)
11. Boogaerts T, Quireyns M, Covaci A, De Loof H, van Nuijs ALN. Analytical method for the simultaneous determination of a broad range of opioids in influent wastewater: optimization, validation and applicability to monitor consumption patterns. *Talanta* 2021;232:122443. [DOI](#) [PubMed](#)
12. Kinyua J, Psoma AK, Rousis NI, et al. Investigation of biotransformation products of p-methoxymethylamphetamine and dihydromephedrone in wastewater by high-resolution mass spectrometry. *Metabolites* 2021;11:66. [DOI](#) [PubMed](#) [PMC](#)
13. Andrés-Costa MJ, Andreu V, Picó Y. Analysis of psychoactive substances in water by information dependent acquisition on a hybrid quadrupole time-of-flight mass spectrometer. *J Chromatogr A* 2016;1461:98-106. [DOI](#) [PubMed](#)
14. Mastroianni N, López-García E, Postigo C, Barceló D, López de Alda M. Five-year monitoring of 19 illicit and legal substances of abuse at the inlet of a wastewater treatment plant in Barcelona (NE Spain) and estimation of drug consumption patterns and trends. *Sci Total Environ* 2017;609:916-26. [DOI](#) [PubMed](#)
15. Thomas KV, Bijlsma L, Castiglioni S, et al. Comparing illicit drug use in 19 European cities through sewage analysis. *Sci Total Environ* 2012;432:432-9. [DOI](#) [PubMed](#)
16. Burgard DA, Banta-Green C, Field JA. Working upstream: how far can you go with sewage-based drug epidemiology? *Environ Sci Technol* 2014;48:1362-8. [DOI](#) [PubMed](#)
17. Gao J, O'Brien J, Du P, et al. Measuring selected PPCPs in wastewater to estimate the population in different cities in China. *Sci Total*

- Environ* 2016;568:164-70. [DOI](#) [PubMed](#)
18. Castiglioni S, Senta I, Borsotti A, Davoli E, Zuccato E. A novel approach for monitoring tobacco use in local communities by wastewater analysis. *Tob Control* 2015;24:38-42. [DOI](#) [PubMed](#)
  19. Ryu Y, Barceló D, Barron LP, et al. Comparative measurement and quantitative risk assessment of alcohol consumption through wastewater-based epidemiology: An international study in 20 cities. *Sci Total Environ* 2016;565:977-83. [DOI](#) [PubMed](#)
  20. Rousis NI, Gracia-Lor E, Zuccato E, et al. Wastewater-based epidemiology to assess pan-European pesticide exposure. *Water Res* 2017;121:270-9. [DOI](#) [PubMed](#)
  21. Lopardo L, Petrie B, Proctor K, Youdan J, Barden R, Kasprzyk-Hordern B. Estimation of community-wide exposure to bisphenol A via water fingerprinting. *Environ Int* 2019;125:1-8. [DOI](#) [PubMed](#)
  22. Ryu Y, Gracia-Lor E, Bade R, et al. Increased levels of the oxidative stress biomarker 8-iso-prostaglandin F(2 $\alpha$ ) in wastewater associated with tobacco use. *Sci Rep* 2016;6:39055. [DOI](#) [PubMed](#) [PMC](#)
  23. Alygizakis N, Markou AN, Rousis NI, et al. Analytical methodologies for the detection of SARS-CoV-2 in wastewater: protocols and future perspectives. *Trends Analyt Chem* 2021;134:116125. [DOI](#) [PubMed](#) [PMC](#)
  24. Barceló D. Wastewater-Based Epidemiology to monitor COVID-19 outbreak: present and future diagnostic methods to be in your radar. *Case Stud Therm Eng* 2020;2:100042. [DOI](#)
  25. Carrascal M, Abian J, Ginebreda A, Barceló D. Discovery of large molecules as new biomarkers in wastewater using environmental proteomics and suitable polymer probes. *Sci Total Environ* 2020;747:141145. [DOI](#) [PubMed](#)
  26. Perez-lopez C, Ginebreda A, Carrascal M, Barceló D, Abian J, Tauler R. Non-target protein analysis of samples from wastewater treatment plants using the regions of interest-multivariate curve resolution (ROIMCR) chemometrics method. *J Environ Chem Eng* 2021;9:105752. [DOI](#)
  27. Casanovas A, Carrascal M, Abián J, López-Tejero MD, Llobera M. Discovery of lipoprotein lipase pI isoforms and contributions to their characterization. *J Proteomics* 2009;72:1031-9. [DOI](#) [PubMed](#)
  28. Kuhn R, Benndorf D, Rapp E, Reichl U, Palese LL, Pollice A. Metaproteome analysis of sewage sludge from membrane bioreactors. *Proteomics* 2011;11:2738-44. [DOI](#) [PubMed](#)
  29. Westgate PJ, Park C. Evaluation of proteins and organic nitrogen in wastewater treatment effluents. *Environ Sci Technol* 2010;44:5352-7. [DOI](#) [PubMed](#)
  30. Zhang P, Zhu J, Xu XY, Qing TP, Dai YZ, Feng B. Identification and function of extracellular protein in wastewater treatment using proteomic approaches: a minireview. *J Environ Manage* 2019;233:24-9. [DOI](#) [PubMed](#)
  31. Mattes W, Yang X, Orr MS, Richter P, Mendrick DL. Biomarkers of tobacco smoke exposure. Elsevier; 2014. pp. 1-45. [DOI](#)