

Biomarkers for monitoring antibiotic resistance in aquatic environments

A. Margarida Teixeira^a (s-anmariteixeira@ucp.pt), Ivone Vaz-Moreira^a, David Calderón Franco^b, David Weissbrodt^{b,g}, Sabina Purkrtova^c, Stanislav Gajdos^d, Giulia Dottorini^e, Per Halkjær Nielsen^e, Leron Khalifa^f, Eddie Cytryn^f, Jan Bartacek^d, Célia M. Manaia^a



CATOLICA

CBQF · CENTRO DE BIOTECNOLOGIA E QUÍMICA FINA LABORATÓRIO ASSOCIADO

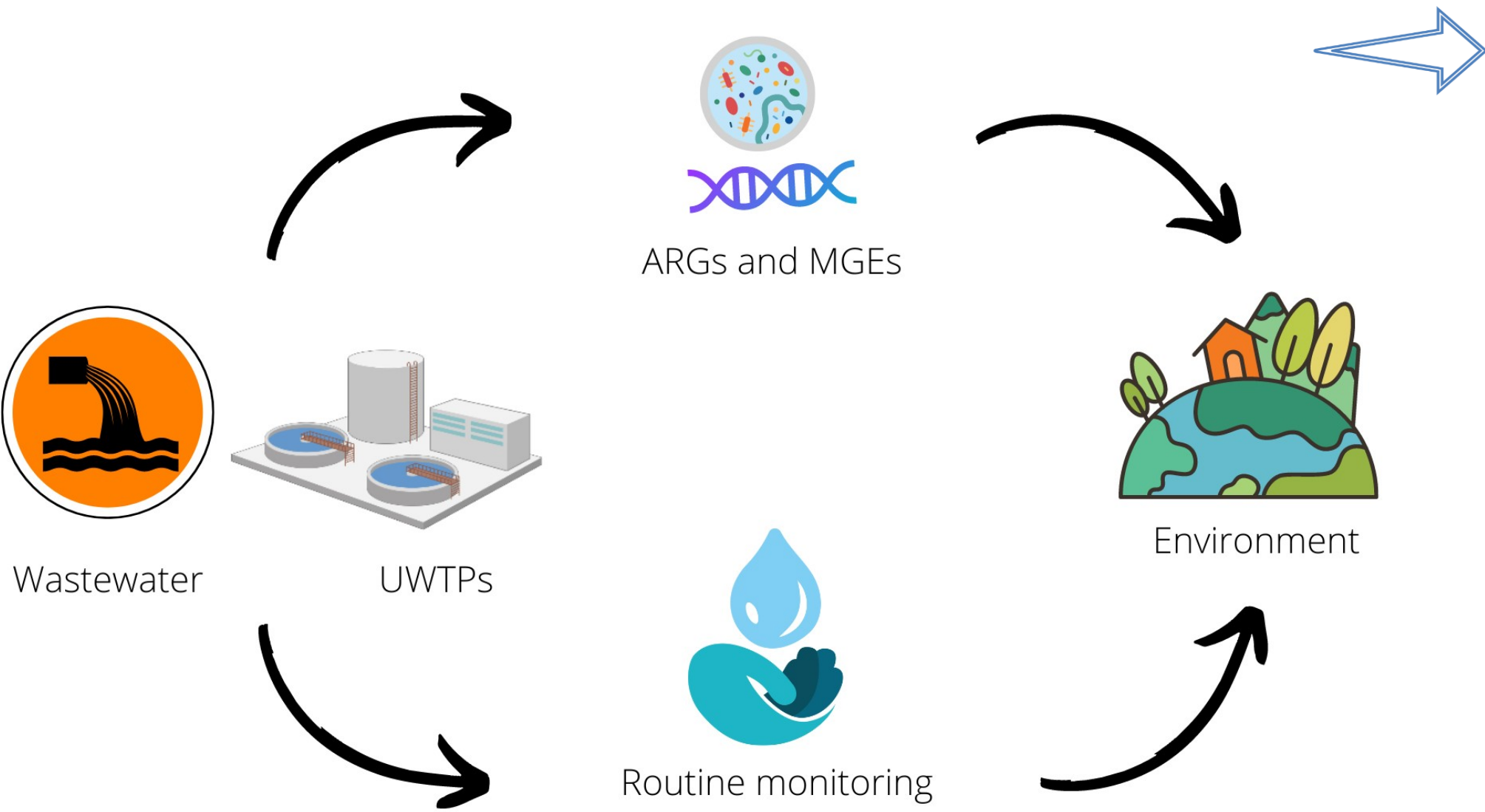
CBQF

PORTO



^a Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, Rua de Diogo Botelho 1327, 4169-005 Porto, Portugal
^b Department of Biotechnology, Environmental Biotechnology Section, Delft University of Technology, van der Maasweg 9, Delft 2629 HZ, the Netherlands
^c Department of Biochemistry and Microbiology, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, 5 Technická, 166 28 Prague, Czech Republic
^d Department of Water Technology and Environmental Engineering, Faculty of Environmental Technology, University of Chemistry and Technology Prague, 5 Technická, 166 28 Prague, Czech Republic
^e Department of Chemistry and Bioscience, Section of Biotechnology, Center for Microbial Communities, Aalborg University, 9220 Aalborg, Denmark
^f Institute of Soil, Water and Environmental Sciences, The Volcani Institute, Agricultural Research Organization, P.O. Box 15159, Rishon LeZion 7528809, Israel
^g Department of Biotechnology and Food Science, Norwegian University of Science and Technology, 7034 Trondheim, Norway

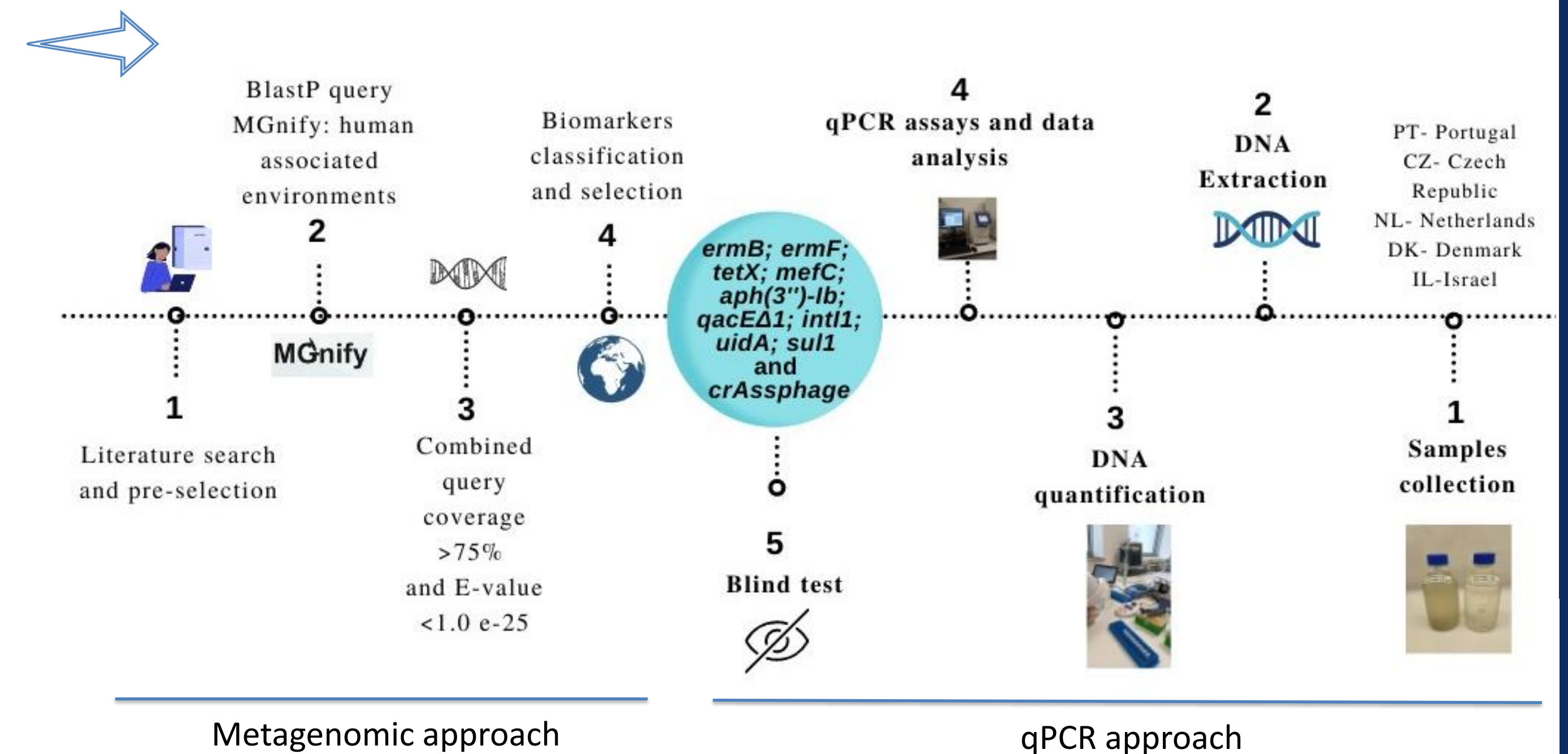
Introduction



Objectives

- ✓ Identify appropriate biomarkers, whose detection and quantification could indicate anthropogenic sources of contamination;
- ✓ Track the spread of antibiotic resistance in (waste)water;
- ✓ Facilitate regular antibiotic resistance monitoring for regulatory purposes.

Methods



Results

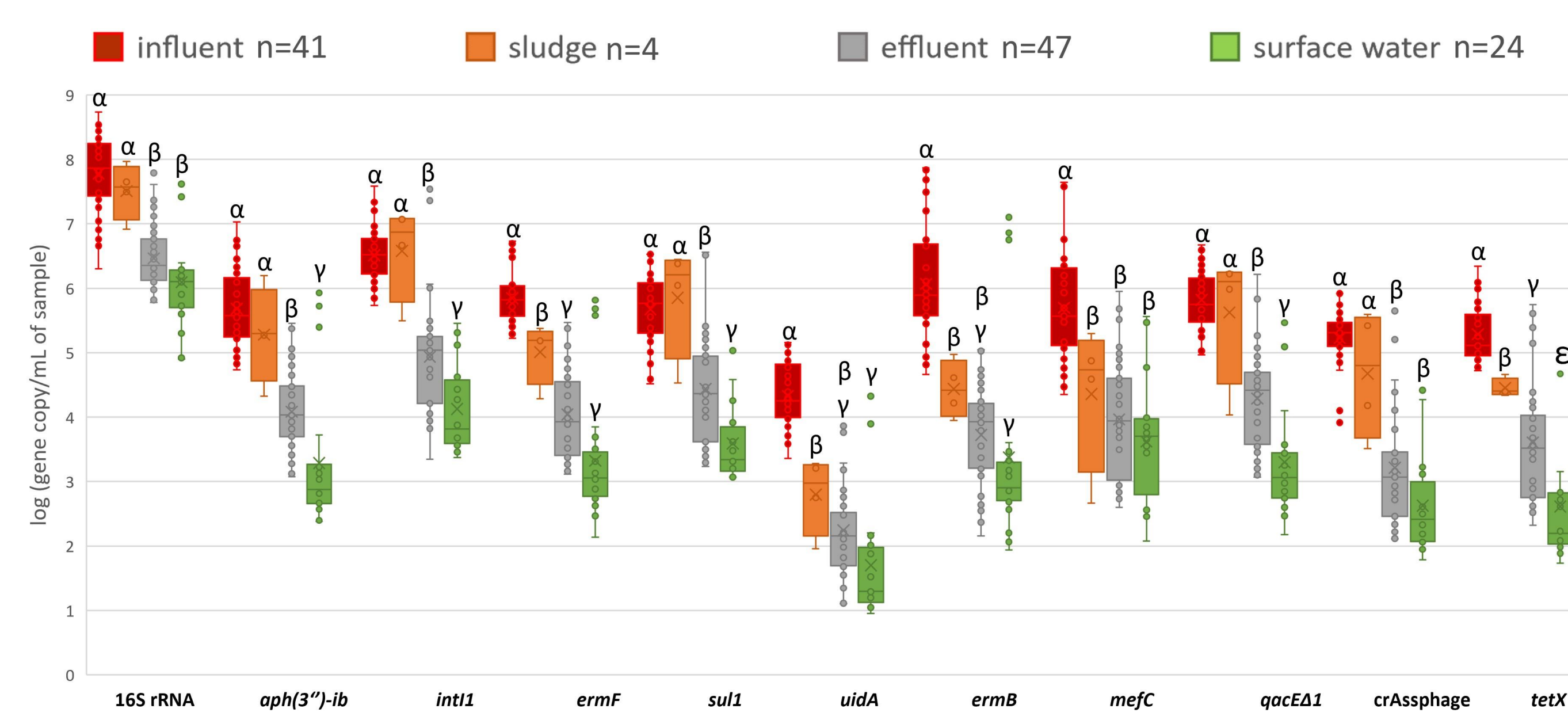


Figure 1: Gene abundance (log (gene copy/mL of sample)) in wastewater and surface water. α , β , γ and ϵ indicate significantly ($p < 0.05$) different Tukey's groups comparing the genotype of samples: influent, sludge, effluent, and surface water.

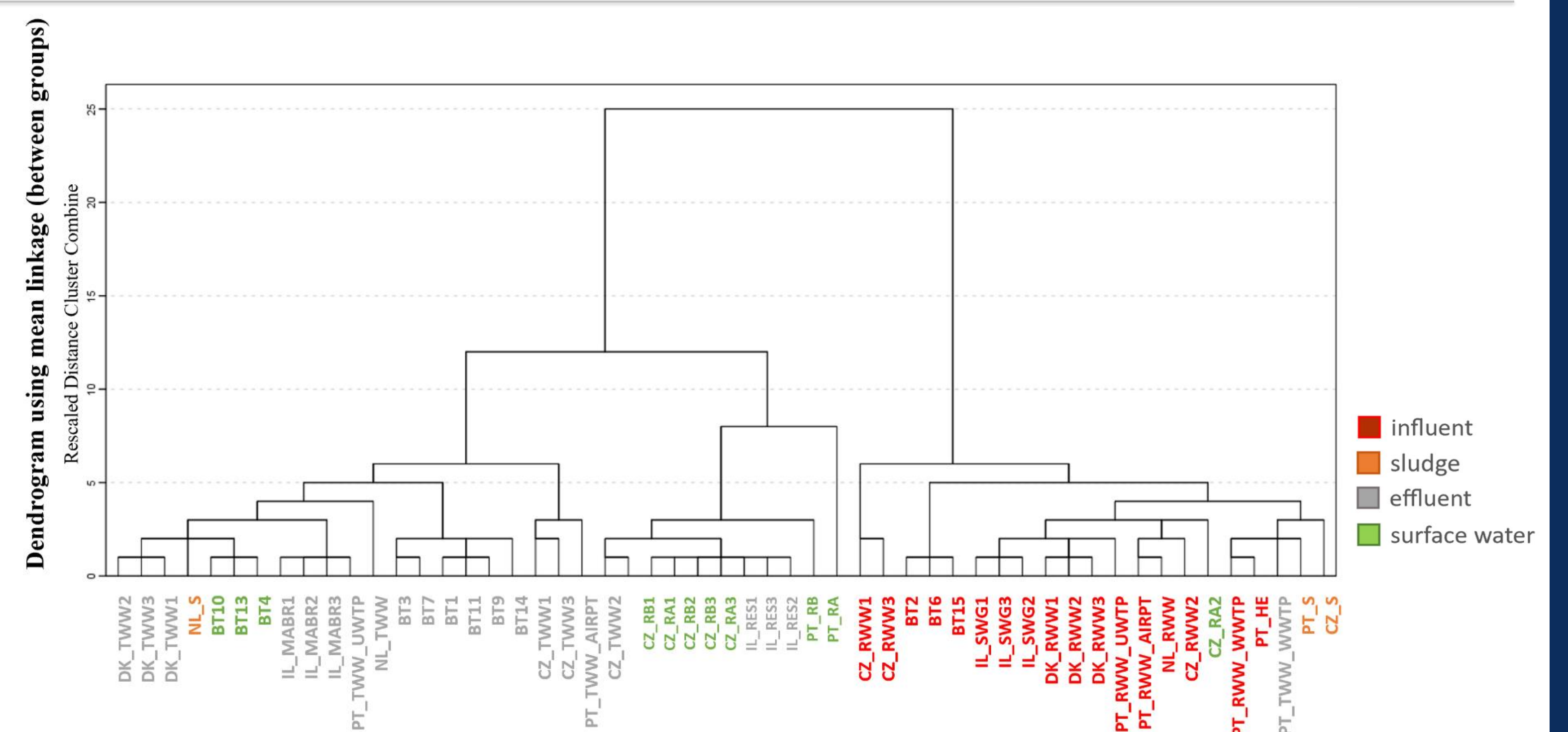


Figure 3: Biomarkers abundance hierarchical cluster analysis. Euclidean distance using mean linkage between average samples from influent, sludge, effluent and surface water and blind test samples (BT1-15) from influent, effluent (secondary and UV treatment), surface water (river) for all biomarkers (*int1*, *sul1*, *ermB*, *ermF*, *aph(3)-Ib*, *uidA*, *qacEΔ1*, *tetX*, *mefC* and *crAssphage*).

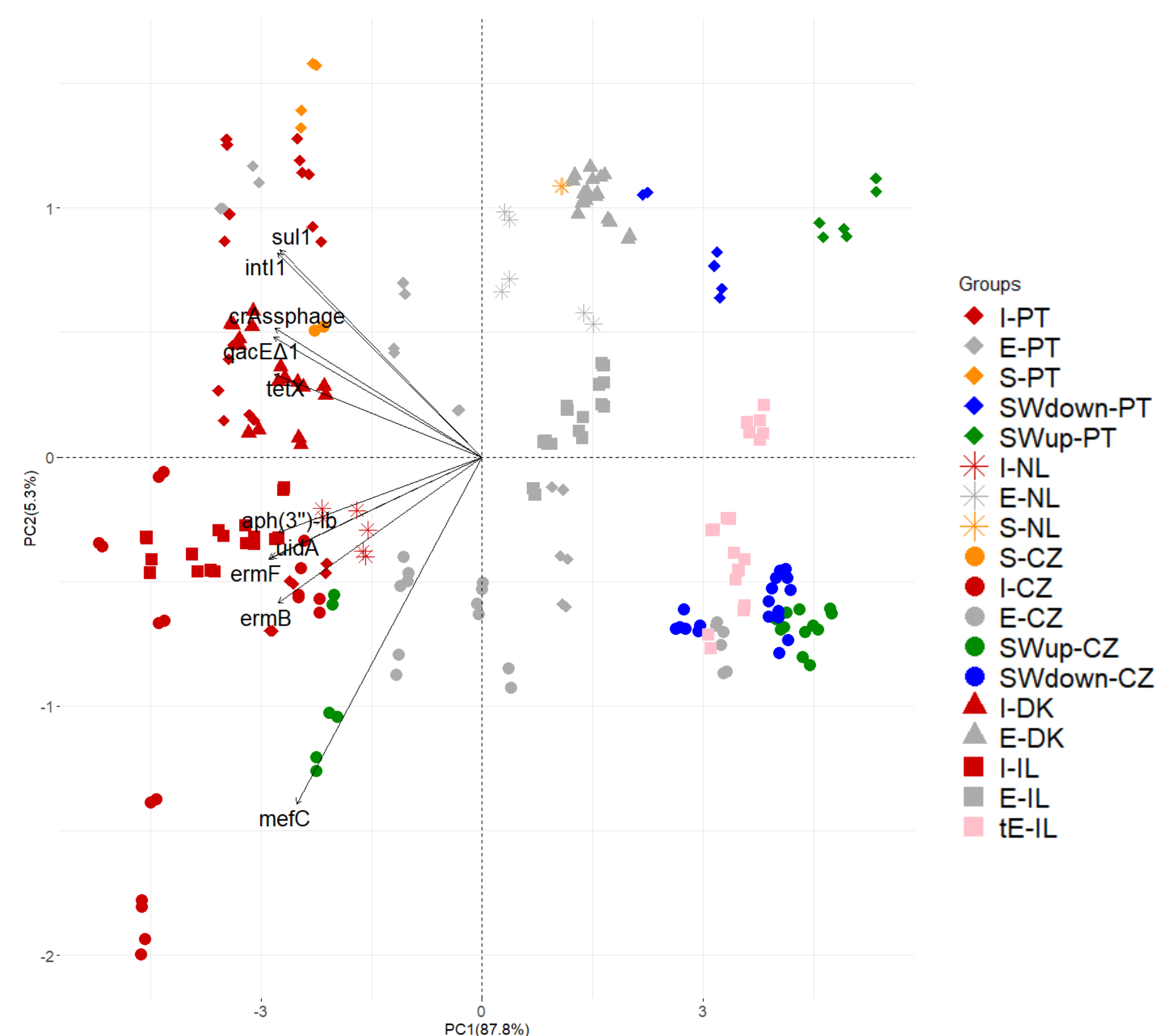


Figure 2: Principal Component Analysis (PCA) based on biomarker distribution in the different types of sample: influent (I), effluent (E), sludge (S) and surface water (SW up and down) samples, based on the quantification by qPCR of the ten biomarkers (*int1*, *sul1*, *ermB*, *ermF*, *aph(3)-Ib*, *uidA*, *qacEΔ1*, *tetX*, *crAssphage* and *mefC*), for the five countries in the study (NL = The Netherlands, CZ = Czech Republic, DK = Denmark, IL=Israel and PT= Portugal).

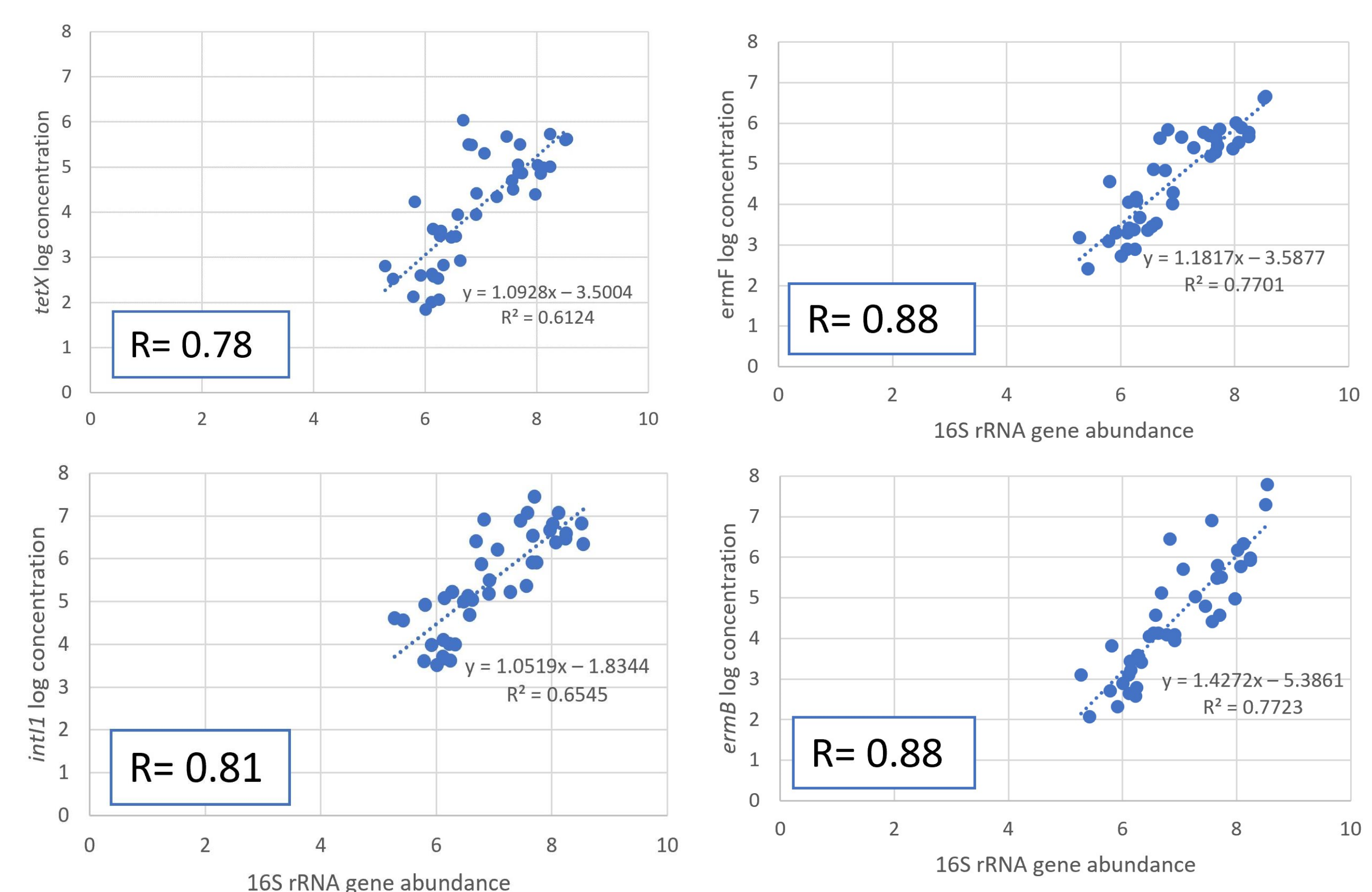


Figure 4: Pearson's correlation coefficient (R) between the concentration (log (gene copy/mL of sample)) of the biomarkers *int1*, *ermB*, *ermF*, *tetX* and the 16S rRNA gene abundance (log (gene copy/ 16S rRNA copy number)).

Conclusions

- ✓ The tested biomarkers were found to discriminate between different types of sample, allowing the assessment of the efficacy of wastewater treatment or the impact of discharges from WWTPs or other sources on the aquatic environment.
- ✓ The selection of suitable biomarkers that can typify different water sources and levels of contamination with ARGs and MGEs, together with harmonised qPCR procedures, may facilitate regular and integrated regulatory requirements for antibiotic resistance monitoring in wastewater and related aquatic environments.

Acknowledgements: This work has received funding from European Research Council under European Union's Horizon 2020 Research and Innovation through the projects "REPARES" Programme Grant Agreement 857552, and "DSWAP" under PRIMA grant 1822. National Funds from FCT - Fundação para a Ciência e a Tecnologia (UID/Multi/50016/2019). AMRT was supported by the FCT Ph.D grant UI/BD/151388/2021 and FSE (Fundo Social Europeu).