MICROCOKIT: MICROBIAL COMMUNITY-BASED SEQUENCE ANALYSIS LINKED TO ANTROPOGENIC PRESSURES TO ADDRESS THE WATER QUALITY

A. Barra Caracciolo[1*], P. Grenni[1], J. Hugg[2], G. Mengs[3], L. Medlin[4], V. Ferrero[5], N. Ademollo[1], L. Patrolecco[1], M.L. Saccà[1], J. Pinto Grande[5], T. Lettieri[5]

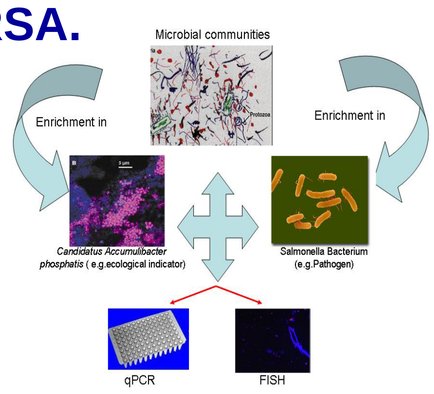
[1]National Research Council, Water Research Institute (Rome) Italy, [2]LGC (London) UK [3]Natural Biotec SL (Madrid) Spain, [4]The Marine Biological Association of the United Kingdom (Plymouth) UK, [5]European Commission, Joint Research Centre, Institute for Environment and Sustainability (Ispra) Italy - *e-mail: barracaracciolo@irsa.cnr.it

Project financed by European Union through FP7 (PEOPLE) Industry - Academia Partnerships & Pathways - Marie Curie Actions. (FP7-PEOPLE-2012-IAPP)

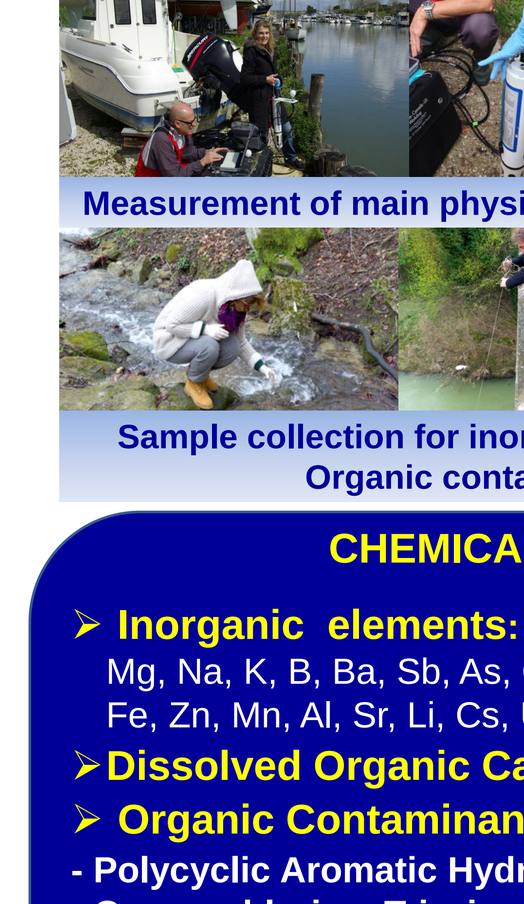
MicroCokit is a Marie Curie Industry-Academia Partnerships and Pathways (MC-IAPP) project entitled "Microbial Community-based sequencing analysis linked to anthropogenic pressures: MicroCoKit to address the water quality". It involves a close cooperation between academic groups with pan-European academic laboratories and leading private enterprises, and is coordinated by CNR-IRSA.

Its main aims are to:

- **investigate and identify aquatic complex stressor indicators based on microbial communities;**
- **foster the transfer of knowledge among the partners with the final goal to bring to market faster, more sensitive and robust tools as bioindicators of water quality.**

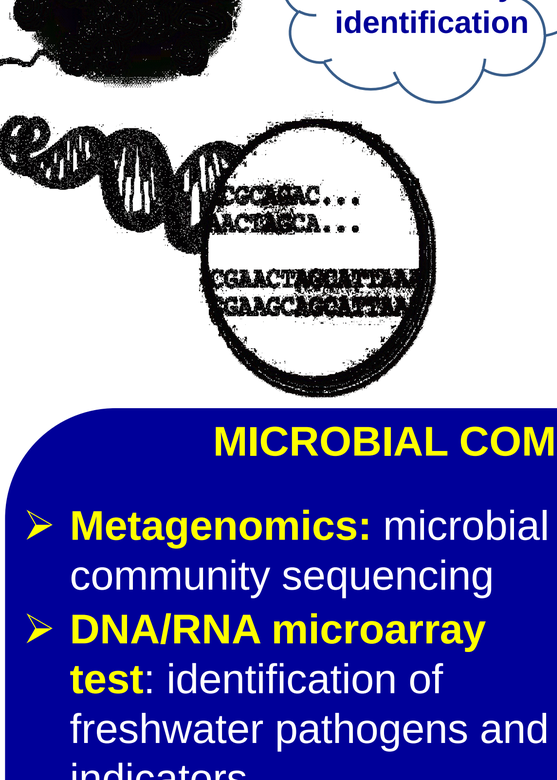


The figure shows the final goal of MicroCoKit project which will be to generate two tools for water quality assessment, one based on quantitative real time (qPCR) and the other one on Fluorescence in situ Hybridization (FISH)



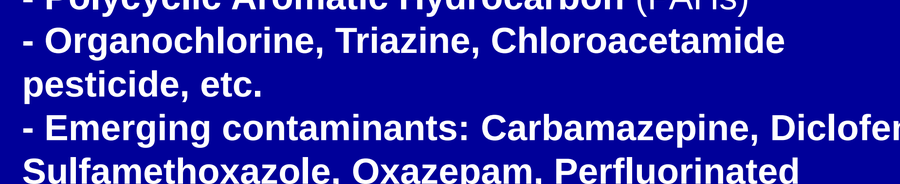
The river Tiber has been chosen as a pilot case study and four areas, including Emilia Romagna, Umbria and Lazio region, have been selected on the basis of various kinds of anthropogenic pressures.

Water sampling points:

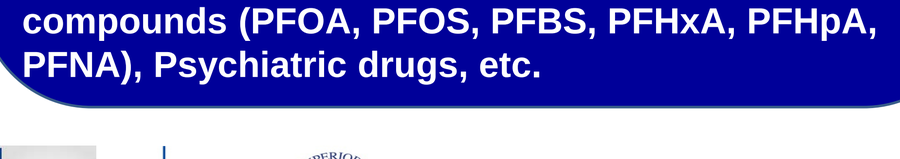


1. Monte Fumaiolo, the river source which is in the pristine area;
2. Attigliano, an agricultural area affected by widespread pesticide use;
3. River Aniene (Tiber tributary), which undergoes industrial contamination at the confluence with the river Tiber in the city of Rome;
4. Scafa, which is an anthropogenic area downstream from the Magliana WWTP of southern Rome, very close to the sea.

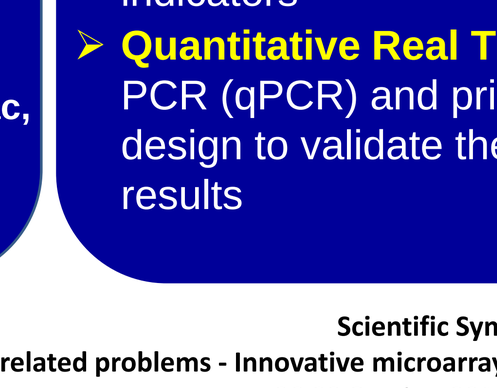
2 samplings per year (1 in Autumn and 1 in Spring) performed in the 4 sampling points



Measurement of main physico-chemical parameters



Sample collection for inorganic elements, DOC, Organic contamininants



Water collection for community analysis by FISH

Water filtration for community analysis (DNA/RNA extraction)

CHEMICAL ANALYSIS

- **Inorganic elements:** NO_2, NO_3, Cl, SO_4, F, Ca, Mg, Na, K, B, Ba, Sb, As, Cd, Cr, Cu, Pb, Hg, Ni, Se, V, Fe, Zn, Mn, Al, Sr, Li, Cs, U, Co
- **Dissolved Organic Carbon**
- **Organic Contaminants:**

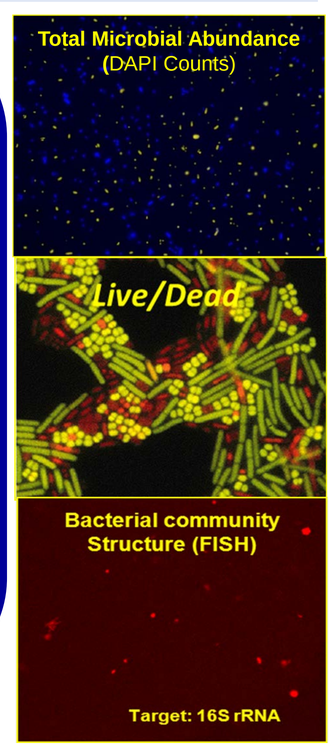
- Polycyclic Aromatic Hydrocarbon (PAHs)
- Organochlorine, Triazine, Chloroacetamide pesticide, etc.
- Emerging contaminants: Carbamazepine, Diclofenac, Sulfamethoxazole, Oxazepam, Perfluorinated compounds (PFOA, PFOS, PFBS, PFHxA, PFHpA, PFNA), Psychiatric drugs, etc.

MICROBIAL COMMUNITY ANALYSIS

- **Metagenomics:** microbial community sequencing
- **DNA/RNA microarray test**: identification of freshwater pathogens and indicators
- **Quantitative Real Time** PCR (qPCR) and primer design to validate the results

Epifluorescence Microscopy

- **Total Microbial Abundance:** DAPI Counts
- **Cell Viability:** Live/dead method
- **Microbial Community Characterization: Fluorescent In Situ Hybridization (FISH)**



Scientific Symposium
Small solutions for big water-related problems - Innovative microarrays and small sensors to cope with water quality and food security
26-28 October 2014, Rome – Italy