Session C: Microbials diversity

Can microbes be used as early warning systems for quality assessment of anthropogenically disturbed coastal zones

Ines Petrić*

* Ruđer Bošković Institute, Bijenic street 54, 10000 Zagreb, Croatia, *ipetric@irb.hr*

IRB | HRZZ Croatian Science Foundation | Project MicroLink

Can microbes be used as early warning systems for quality assessment of anthropogenically disturbed coastal zones (sciforum-051474)

Abstract With anthropogenically-driven pressures drastically changing marine coastal ecosystems much international efforts have been focused on its protection. For that reason, Mediterranean states have accepted numerous regional and international contracts, including Water (WFD) and Marine Strategy Framework (MSFD) Directives, to protect and preserve its rich and unique natural heritage. Following guidelines defined in these directives impact of human activities on the ecological status of marine ecosystems is defined through measurement of an array of different parameters, however, these tools are still facing many challenges. Within the frame of the **MicroLink project**, funded by the Croatian Science Foundation, we will focus on the effect of anthropogenic pressures onto benthic microbial assemblages, including ecological network of bacteria, archaea, fungi and viruses. We believed that microbial assemblages could offer potential answer to some of the challenges facing marine protection programs: (i) challenge of determining effects of multiple pollution pressures on the integrity of the coastal marine ecosystem, (ii) possible improvement of the existing biological indicators pool (iii) potential of applying fast and reliable state-of-the-art approaches to determine structural and functional integrity of marine ecosystems; and (iv) problems associated with assessment of the ecological status of marine sediments. Even though microbes dominate marine ecosystems and have pivotal role in biogeochemical cycling and pollutant elimination they are neglected in the EU quality legislation protocols. Changes exerted on the level of microbial communities could alter the trophic structure of the whole food web, eventually impairing marine ecosystem services. We will tackle this issues by combining a multi-trophic/ multilayer approach and study microbial communities (in anthropogenically-impacted vs reference environments) by using 4 different state-of-the-art methodologies (next generation sequencing, real-time PCR, multi-trophic network analysis, shotgun metagenomics) and on 4 different trophic levels (bacteria, archaea, fungi and viruses). Finally, there is an urgent need to define possible contribution and importance of monitoring microbial assemblages in achieving of Good Environmental Status which could offer us first step toward potential integration of microbial assemblages as indicators of marine environment quality.

Keywords: marine pollution, microbial diversity, benthic microbial community, health status indicators

IECD 2022

Marine ecosystems are facing tremendous pressures driven by human activities:

- *urban pollution*
- *pollution from industry*
- *agriculture apollution*
- *aquaculture activities*
- *habitat degradation*
- *introduction of invasive species*
- *climate change*

→ affecting all components of the marine ecosystems

Adriatic Sea

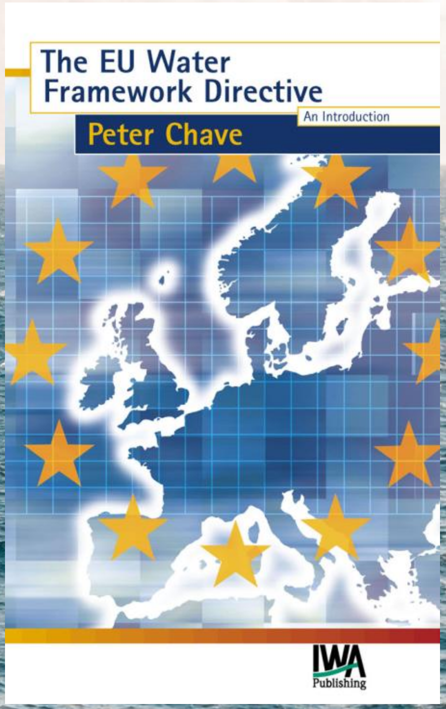
Tools for protection of Mediterranean basin, including its sub-region Adriatic Sea:

- *United Nations Environmental Programme Mediterranean Action Plan (UNEP-MAP)*
- *Barcelona Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean*
- *Euro-Mediterranean Partnership (EUROMED)*
- *Directive for Maritime Spatial Planning (MSP)*
- *Integrated Coastal Zone Management (ICZM)*
- *Water and Marine Strategy Framework Directive (WFD, 2000/60/EC, MSFD, 2008/56/EC)*

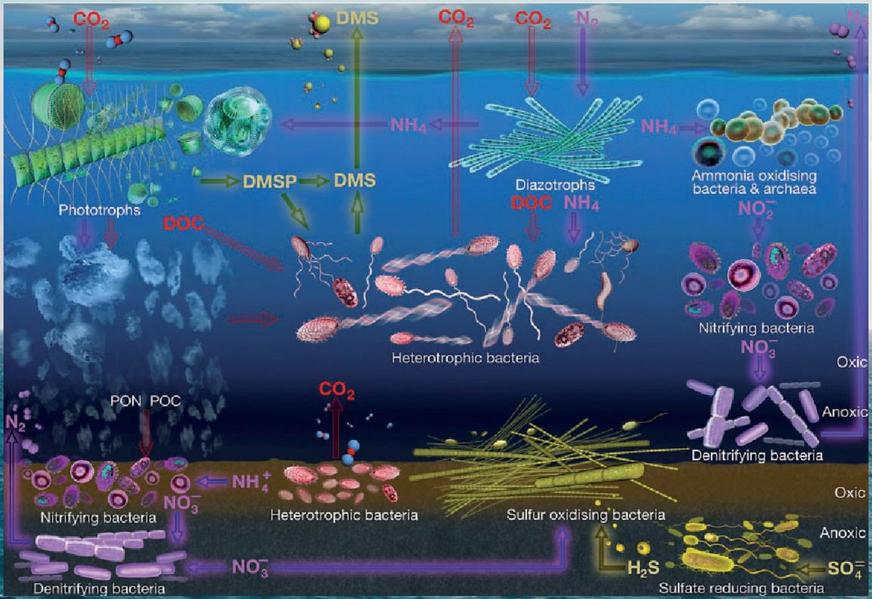
Water Framework Directive (WFD) and Marine Strategy Framework Directive (MSFD)

...”impact of human activities on the **ecological status** of marine ecosystems is defined through measurement of an array of different parameters...”

- WFD uses chemical, physicochemical, hydromorphological and biological elements
- MSFD define ecological status by monitoring changes in 11 qualitative Descriptors



Microbial component within the WFD and MSFD ?



In marine quality assessment **MICROORGANISMS (MO)** are considered only based on their pathogenicity – determining presence of intestinal enterococci (UNEP IMAP, Bathing Water Quality Directive (EU, 2006/7EC))

- ✓ **neglecting MO crucial role in marine ecosystem functioning !!**

1st

PROKARYOTES (**bacteria, archaea, viruses and fungi, protists**) numerically dominate all marine ecosystems, play pivotal role in mediating biogeochemical cycling of all major elements, C sequestration, elimination of pollutants and are vital for sustainability of the marine ecosystem

2nd

Existance of strong link between anthropogenic pressures and changes in microbial assemblages (alternations on the level of diversity, structure, abundance, activity and/or biogeochemical cycle rate)

MicroLink project funded by *Croatian Science Foundation* is intended to find missing link between changes in microbial assemblages and ecosystem quality, paving the way toward integration of microbes among qualitative Descriptors of the MSFD Good Environmental Status (GES)

2 main scientific questions:

(1) Can we find a general relation between microbial communities dynamics and anthropogenic-associated pollution?

(2) At which level (different trophic and/or investigation level) within microbial assemblages changes are best to be recognized?

Methodological approach:

(i) multi-domain approach – study changes in microbial assemblages on 4 different trophic levels: bacteria/archaea, viruses, fungi, protists
(ii) multi-layer approach - investigations on 4 different resolution levels: changes in structure, networks, function and biodegradation potential
(iii) state-of the-art methodologies: NGS, qPCR, shotgun metagenomics, data-mining and modelling
(iv) investigations under multiple pollution pressures conditions, in both field and laboratory conditions
(v) focusing on the sediment ecosystem compartment i.e. benthic microbial assemblages.

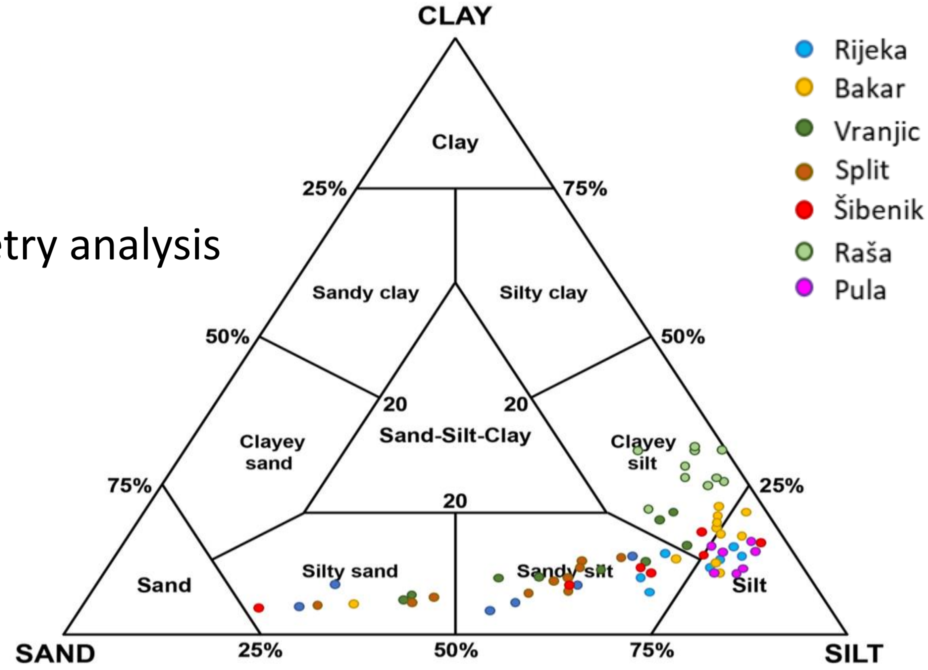
Sampling campaign and comprehensive sediment characterization

1. Sampling of surface sediments within 7 selected „hot spot” harbors in the Adriatic sea (Croatia) (and reference sites)

Sampling campaign and comprehensive sediment characterization

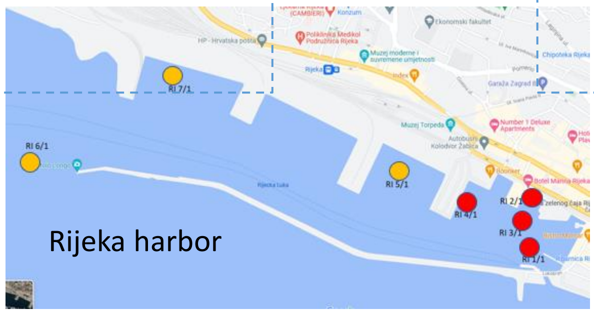
2. Comprehensive physico-chemical characterization of the sediment: granulometry analysis, sediment toxicity testing (Microtox), sediment redox potential, T, O_2, pH, salinity, TC, TN, TP, measuring concentrations of selected organic pollutants in sediments (TBT, Hg, metals...)

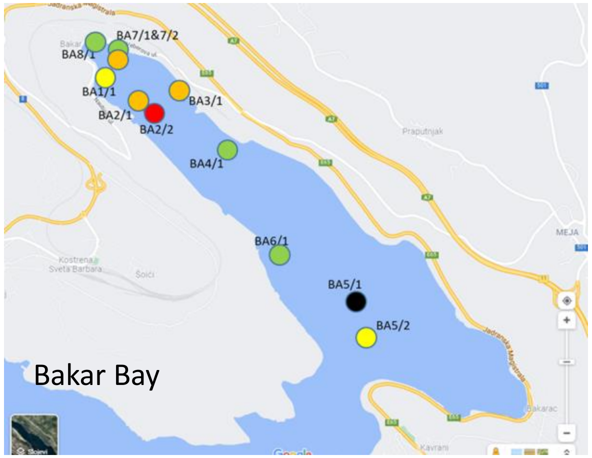
granulometry analysis



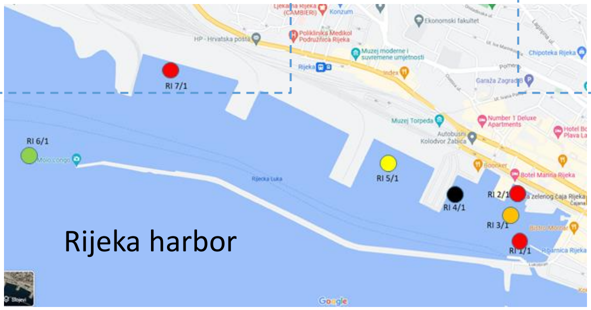
Sampling campaign and comprehensive sediment characterization

Microtox

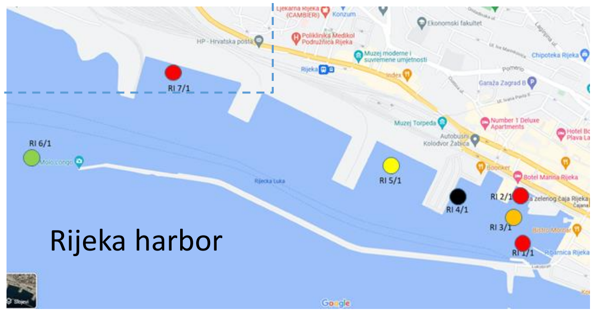


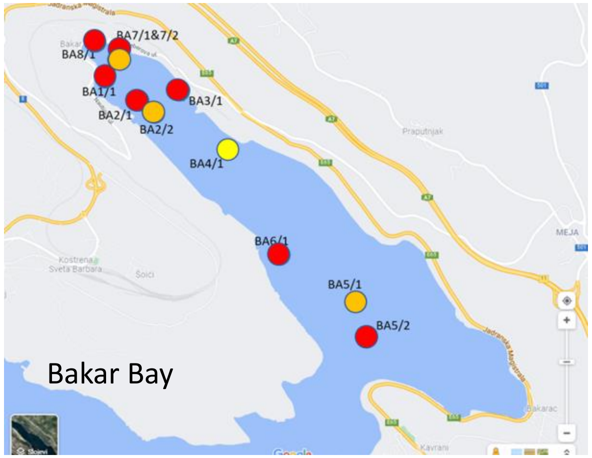


Hg [mg/kg s.t.]



TBT [ng/g]





TOX	
>250	Black
(125-250)	Red
(32-125)	Orange
(8-32)	Yellow
<8	Green

I - nonpolluted	<0,15
II - no toxic effects / good	0,15-0,63
III - toxic effects at chronical exposures /moderate	0,63-0,86
IV - toxic effects at acute exposures /bad	0,86-2
V - significant toxic effects at acute exposures / very bad	>1,6

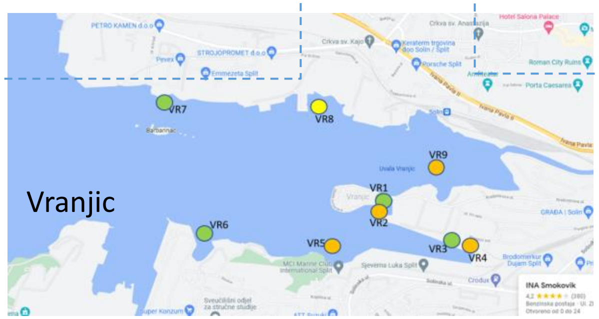
zeleno	Background	background levels
žuto	Good	no toxic effects
naranč	Moderate	toxic effects following chronic exposure
crveno	Bad	toxic effects following short term exposure
crno	Very bad	severe acute toxic effects

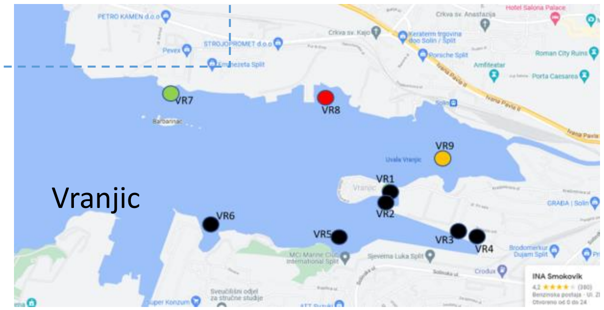
Sampling campaign and comprehensive sediment characterization

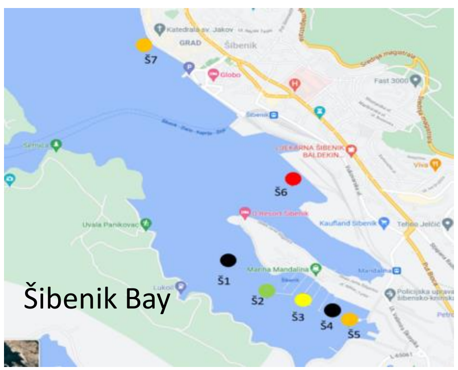
Microtox

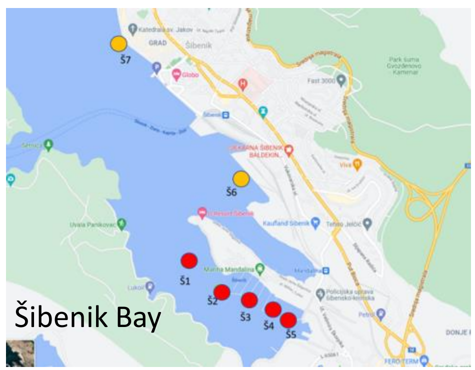
Hg [mg/kg s.t.]

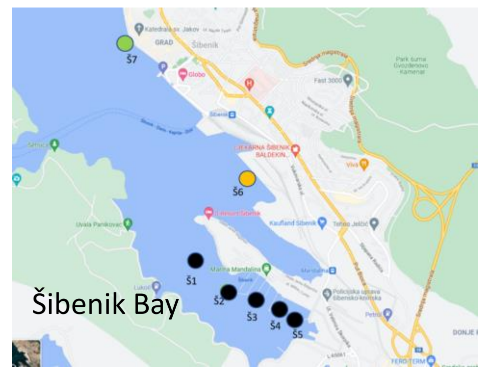
TBT [ng/g]











TOX	
>250	Black
(125-250)	Red
(32-125)	Orange
(8-32)	Yellow
<8	Green

I - nonpolluted	<0,15
II - no toxic effects / good	0,15-0,63
III - toxic effects at chronical exposures /moderate	0,63-0,86
IV - toxic effects at acute exposures /bad	0,86-2
V - significant toxic effects at acute exposures / very bad	>1,6

zeleno	Background	background levels
žuto	Good	no toxic effects
naranč	Moderate	toxic effects following chronic exposure
crveno	Bad	toxic effects following short term exposure
crno	Very bad	severe acute toxic effects

Sampling campaign and comprehensive sediment characterization

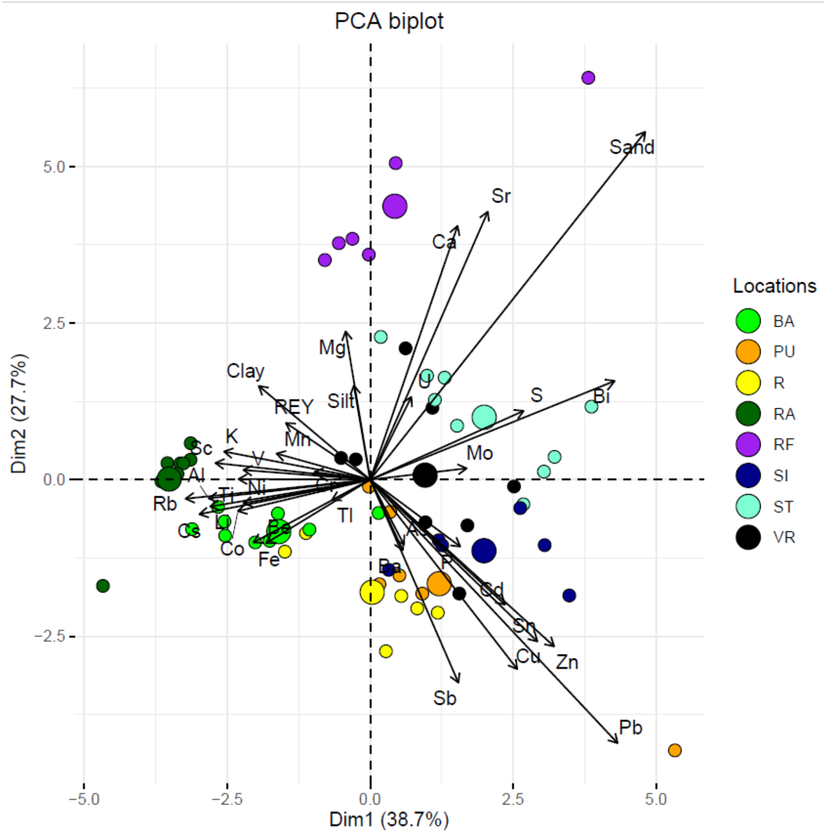
Site	Sample	Mo	Cd	Sn	Pb	Bi	Cr	Co	Ni	Cu	Zn	Sb	As
RIJEKA	RI1_1	2,23	2,69	6,51	5,59	3,26	1,89	1,19	1,32	7,67	3,71	2,94	1,22
	RI2_1	5,34	8,60	8,29	11,37	14,02	1,87	1,15	1,42	10,03	6,85	3,85	1,62
	RI3_1	2,85	5,71	7,38	10,51	6,19	1,56	1,09	1,33	10,80	6,60	4,07	1,73
	RI4_1	4,57	8,56	5,82	10,10	9,29	1,92	1,13	1,43	9,60	6,62	5,34	1,91
	RI5_1	6,51	10,14	4,27	6,60	6,39	1,42	1,06	1,19	5,91	4,93	2,82	1,61
	RI6_1	2,36	2,36	2,49	3,58	3,25	1,28	1,04	1,16	3,41	1,96	2,01	0,86
	RI7_1	1,69	1,55	4,72	3,06	8,34	1,26	1,07	0,90	3,63	2,14	1,32	1,27
BAKAR	BA1_1	2,18	1,74	3,55	2,07	2,62	1,32	1,04	1,12	2,84	1,63	2,17	1,09
	BA2_1	1,94	2,10	1,82	1,85	1,52	1,07	0,96	1,07	2,33	1,37	1,42	1,10
	BA2_2	2,48	1,80	2,07	1,90	27,16	1,18	1,00	1,07	2,32	1,38	1,73	1,21
	BA3_1	4,94	2,62	5,17	2,40	4,69	1,27	1,68	1,68	5,19	1,79	3,80	1,54
	BA4_1	1,81	2,04	1,49	1,66	2,11	1,00	0,93	1,00	1,87	1,24	1,42	1,20
	BA5_1	1,62	1,43	1,68	1,52	1,80	1,12	0,99	1,01	1,69	1,10	1,24	0,91
	BA5_2	1,56	1,54	1,80	1,38	1,56	1,01	0,90	0,94	4,55	1,20	1,13	0,79
	BA6_1	1,82	1,39	1,69	1,55	2,02	1,11	0,94	1,02	1,82	1,13	1,24	0,95
	BA7_1	2,18	2,00	2,87	2,37	2,27	1,23	0,99	1,09	2,88	1,49	1,59	1,13
	BA7_2	2,00	1,86	5,37	2,33	1,91	1,36	1,05	1,15	5,47	1,48	1,79	0,67
	BA8_1	7,92	8,81	6,37	8,47	25,77	1,41	1,21	1,15	8,73	7,48	4,11	1,77
RAŠA	RA1	1,55	1,90	0,95	0,70	1,47	1,22	1,18	1,22	2,08	1,04	0,92	0,49
	RA2	1,34	2,02	0,85	0,60	1,09	1,17	1,08	1,18	1,89	0,92	0,79	0,55
	RA3	1,29	2,03	0,81	0,63	1,08	1,14	1,08	1,21	1,95	0,97	0,76	0,60
	RA4	1,28	1,95	0,93	0,56	0,97	1,13	1,08	1,19	1,77	0,88	0,75	0,62
	RA5	1,45	1,43	0,97	0,79	1,32	1,19	1,05	1,18	1,79	0,98	0,85	0,88
	RA6	1,27	1,66	0,91	0,68	1,01	1,07	0,98	1,15	1,67	0,91	0,71	0,82
	RA7	1,40	1,65	0,86	0,76	1,19	1,05	0,96	1,12	1,56	0,93	0,81	1,12
	RA8	1,42	1,60	0,86	0,77	1,07	1,02	0,92	1,04	1,46	0,82	0,77	0,97
	RA9	1,45	1,57	1,49	0,74	1,83	1,19	1,11	1,21	1,79	0,92	0,85	0,77
	RA10	1,24	1,82	0,88	0,67	2,15	1,12	1,05	1,18	1,88	0,95	0,79	0,55
PULA	PU1	3,30	2,76	7,36	8,66	6,75	1,02	0,62	0,72	7,30	4,48	2,43	2,09
	PU1_1	4,95	3,92	10,10	11,03	9,74	1,17	0,63	0,77	8,68	5,90	2,55	2,09
	PU2_1	21,12	12,01	149,58	880,03	22,25	1,12	1,13	1,33	67,46	53,99	19,30	2,64
	PU3_1	2,92	1,73	8,98	8,85	6,44	1,19	0,74	0,77	6,34	3,88	2,45	2,75
	PU3_2	2,59	2,07	9,90	11,08	7,44	1,12	0,68	0,73	6,73	4,13	2,00	2,33
	PU4_1	3,07	1,37	11,19	7,83	5,09	1,16	0,84	0,83	5,75	3,84	2,54	2,53
	PU5_1	2,53	2,26	4,56	5,43	3,55	1,03	0,54	0,67	4,17	2,94	1,94	1,73

Metals pollution level – „enrichment factor"

Sutherland, 2000.

EF Value	Category	Enrichment level
<2	1	Deficiency to minimal enrichment
2-5	2	Moderate enrichment
5-20	3	**Significant enrichment**
20-40	4	**Very high enrichment**
>40	5	**Extremely high enrichment**

Sampling campaign and comprehensive sediment characterization



Next step:
CORRELATE SEDIMENT GROUPS WITH MICROBIAL ASSEMBLAGES CHARACTERISTICS (currently working on...):

1. Structure of benthic microbial communities (bacteria 16SrRNA, fungi ITS, protist 18SrRNA)
2. Abundance of key functional genes involved in biogeochemical cycling (qPCR analysis)
3. Degradation potential of benthic microbial assemblages (shotgun metagenome sequencing)

MAIN DELIVERABLES

Proposing list of “microbial endpoints/attributes” that could be used as potential indicators/Descriptors of ecological quality status of benthic environment:

D1. Proposing monitoring structural changes within microbial assemblages and networks as criteria for achieving of GES

D2. Proposing monitoring functional changes within microbial assemblages as criteria for achieving of GES

D3. Proposing monitoring functional catabolic gene/network within microbial assemblages as criteria for achieving of GES

Proposed “new” criteria can be potentially introduced within MSFD Descriptor 4 (4.3.1 Abundance trends of functionally important selected groups/species), Descriptor 1 (1.7.1 Composition and relative proportions of ecosystem components) and/or Descriptor 6 (6.2.1 Presence of particularly sensitive and/or tolerant species; 6.2.2 Multi-metric indices)

Thank you for your attention

MicroLink
http://hrzz-microlink.unaux.com/

HRZZ
Croatian Science Foundation

MicroLink TEAM MEMBERS:

Ines Sviličić Petrić, PhD, Ruđer Bošković Institute, Croatia, project Leader
Ana Ramljak, PhD student, Ruđer Bošković Institute, Croatia
Nikolina Udiković-Kolić, PhD, Ruđer Bošković Institute, Croatia
Ivana Babić, PhD, Ruđer Bošković Institute, Croatia
Martina Furdek Turk, PhD, Ruđer Bošković Institute, Croatia
Slavica Matijević, PhD, Institute of Oceanography and Fisheries, Croatia
Maja Fafanđel, PhD, Ruđer Bošković Institute, Croatia
Dimitrios Karpouzas, PhD, University of Thessaly, Greece
Antonis Chatzinotas, PhD, Helmholtz Centre for Environmental Research GmbH, Germany
Zlatan Morić, Bsc, University College Algebra, Croatia
Vladimir Kuzmanovski, PhD, Jožef Stefan Institute / Aalto University, Slovenia / Finland