PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

Report Form for PhD student annual evaluation (XXXVII and XXXVIII cycles)

Name of PhD student: Matteo Fanelli

Title of PhD research: Biogeochemical cycle of contaminants in marine waters

Name of PhD supervisor: Silvia Illuminati
Research lab name: Analytical Chemistry for the Environment and Food

Cycle:
[ ] XXXVI
[X] XXXVII

PhD Curriculum::
[ ] Marine biology and ecology
[ ] Biomolecular Sciences
[X] Civil and environmental protection

DISVA instrumentation labs/infrastructure eventually involved in the project:
[X] Actea Mobile Laboratory
[ ] Advanced Instrumentation lab
[ ] Aquarium
[ ] MassSpec lab
[ ] MaSBiC
[ ] Simulation/informatics lab
[X] Other. Please, indicate: Clean Room

ABSTRACT:
Potentially Toxic Element, PTEs are naturally occurring elements presents in the environment in very low concentrations. PTEs evaluation is a key parameter to reach the Good Environmental State (GES) according to the MSFD (Directive 2008/56/EC). The PhD Project aims to evaluate (1) the characterization of PTEs in seawater, surface sediments and atmospheric depositions; (2) the possible influence of atmospheric pollution on the marine biogeochemical cycle of PTEs; (3) the partitioning and the interaction between the different matrices, and (4) the seasonal evolution of the pollutant contents in the different matrices. Here, the main results of the first sampling campaign in the coastal area of Ancona are presented. Although a great variability related to seasonality and site features, seawater and marine sediments showed a good environmental quality status. On the contrary, the air quality is strongly affected by the anthropogenic activities characterizing the Ancona port area.

Part 1. Scientific case of the PhD **Research**

- BACKGROUND: The city of Ancona lies on the mid-Adriatic coast and its port is an important junction for the distribution of goods and the transfer of passengers at European level. It is also dotted by several industries, mainly located along the Esino Valley and characterized by the presence of a big oil refinery in Falconara. Potentially Toxic Elements (PTEs) are persistent and naturally occurring elements, capable of generating toxicity for marine organisms by tissues bioaccumulation and - for Hg - even biomagnify. Anthropogenic pollution can release such contaminants in the environment, increasing natural concentrations [1]. Moreover, nutrients (N, P, Si) are essential for organisms' growth, but whenever high concentrations occur, may generate eutrophication, especially for a susceptible region such the Adriatic. The main sources for nutrients are riverine runoff and sewage discharge outflows, particularly if untreated [2].

- SCIENTIFIC AIMS: The overall goal of the project is to biogeochemically characterize the coastal area of Ancona subjected to several anthropogenic pressures. In particular, the second year of my PhD Project focuses on (a) the evaluation of the seasonal evolution of PTEs in the different matrices (seawater, sediments, atmospheric depositions); (b) the assessment of the possible sources and the relationships or fluxes between the different matrices; (c) the microbiome characterization of the atmospheric deposition over the Ancona port area.

- WORKPLAN AND RESEARCH ACTIVITIES

From July 2021 samples of seawater, marine sediments and atmospheric depositions have been collected each month along the coastal area of Ancona. Three sites with different anthropogenic impacts and environmental features were individuated along the coast of Ancona, in three different area: 1) Palombina (PAL), characterized by low hydrodynamics, very shallow waters (<1 m), the proximity of API refinery and next to untreated sewage discharge points, Esino River and the presence of bathers in the summer season; 2) Marina Dorica (MD), close to the Ancona port, characterized by intense maritime traffic and close to industrialized areas and 3) Portonovo (PN), two miles far from the coastline, characterized by the absence of direct anthropogenic impact or industries proximity, except for the maritime traffic, particularly intense during the summer season.

WP 1. Seawater

Methods. Seawater samples were monthly collected from February 2022. Surface seawater samples were collected manually by pre-cleaned HDPE bottles. Bottom waters were sampled only in the Portonovo site through a Niskin bottle and then preserved in HDPE bottles. Once collected, seawater samples were vacuum filtrated through pre-weighed 0.45-µm MCE filters to determine the PTEs dissolved (M_{diss}) and particulate (M_{part}) fractions. The total Suspended Particulate Matter (SPM) was obtained after the differential weighing procedure of the 0.45-µm MCE filters and related to the filtration volume. The filtrated solution is acidified with ultrapure HCl at 2-10% v/v, while MCE filters were microwave digested in a acid mixture of HNO_3:H_2O_2:HF, 5:1:1) Dissolved As, Cd, Cu, Hg and Pb are determined by Atomic Spectroscopy Fluorescence (AFS), while particulate PTEs are determined by GF-AAS and DMA. Dissolved inorganic nutrients (N-NO_2, N-NO_3, N-NH_4, Si-SiO_2, P-PO_4) are determined colorimetrically following the APHA or EPA certified standard procedures.

Expected/Obtained Results. In the second year of my PhD project, nutrients and PTEs measurements were extended until March 2023. Nutrients showed the following concentrations (values reported as mean ± SD): 5.8 ± 5.8 µmol L^{-1}, N-NO_3; 0.52 ± 0.60 µmol L^{-1}, N-NO_2; 0.51 ± 0.42 µmol L^{-1}, N-NH3; 0.14 ± 0.12 µmol L^{-1}, P-PO_4; 4.0 ± 3.5 µmol L^{-1} Si-$Si(OH)_4$. Overall, nutrient concentrations are similar among the sampling sites (no statistically significant differences). Seasonal trends can be observed for some nutrients, with high concentrations measured during winter and low during summer mainly because of the increase in precipitations and, consequently, of riverine outflows carrying nutrients from land. While in MD and PAL this effect is more visible because of their proximity with the Esino River, in PN nutrient concentrations show higher variability

throughout the year and seem to be more affected by resuspension phenomena than by freshwater inputs. Redfield Ratio calculated from our data confirmed the P-limitation typical of the Adriatic Sea.

The analysis of both dissolved and particulate fractions of As, Cd, and Hg showed that the dissolved phase of all elements accounted for up to 90% of the total. The dissolved fractions showed the following mean (±SD) values: 0.81 ± 0.29 μg L^{-1}, As_{diss}; 26 ± 16 ng L^{-1}, Cd_{diss}; 18 ± 13 ng L^{-1}, Hg_{diss}. The particulate fractions of TEs show the following concentrations: 2.1 ± 1 .2 μg g^{-1}, As_{part}; 36 ± 32 ng g^{-1} Cd_{part}; 38 ± 23 ng g^{-1}, Hg_{part}. Overall, concentrations varied as follows: As > Cd > Hg. Statistically significant differences were observed between the sampling sites, highlighting the geographic different position of each study site. As_{diss} values were generally lower in summer and higher in autumn, probably because of the combined effects of increasing precipitations and remobilization from sediments. Cd_{diss} showed the opposite trend with higher concentrations in spring/summer and lower in autumn. Hg_{diss} did not show a pronounced seasonal trend. Higher concentrations for both As_{part} and Cd_{part} were measured in Portonovo bottom waters (PNb) with respect to Portonovo surface waters (PNs), probably because of the decomposition of dead algal cells at the bottom that had previously uptaken these elements in surface waters during their growth. Concentrations of As, Cd, Hg resulting from our analysis are in the same order of magnitude as those reported by other studies carried out in the Northern Adriatic Sea. For both nutrients and TMs, concentrations were within the legal limits established by the European and Italian law.

WP 2. Marine sediments

Methods. Samples of superficial sediment have been collected once a month from March 2022 until now, by manual core in PAL and by grab sampler in PN and MD. Sediment samples were subjected to freeze drying lyophilization and subsequently homogenization processes. About 0.15 g was used for the mercury quantitative determination through Directly Mercury Analyzer (DMA). Another aliquot (~0.1 g) was subjected to microwave-assisted acid digestion (3 mL HNO_3 + 1 mL HCl + 1 mL HF). Metal concentrations (Al, As, Cd, Co, Cr, Fe, Mn, Ni, Pb) were determined in the digested solutions by GF-AAs. Organic Matter (OM) was measured by Loss on Ignition in oven at 450°C for 4h and by differential weighing procedure.

Expected/Obtained Results. In the second year of my PhD project, the collection and analysis of marine sediments were extended until March 2023. Results obtained are reported in Table 1. The main difference between the sediments is given by the grain size and Organic Matter content. MD and PAL are coarse and sandy, while PN grain size is fine and rich in OM. This difference, as expected, greatly influences the PTEs adsorption processes. Although a great variability between sampling sites, Fe, Al, Mn showed an increasing trend passing from PAL to PN. Cd, Cu, Cr and Hg follow the same distribution of the OM, increasing from PAL to PN. Pb, As and Co show the higher concentrations in MD. Mn median concentration also follow this trend. This could be related to the combustion of fossil fuel (maritime and vehicular traffic).
Ni and V showed comparable concentrations between PN e MD. V and Ni are usually used as marker of maritime traffic, which is the main anthropogenic pressure of MD and PN in summer.
Sediments PTEs content was well-below the Environmental Quality standard for Cd, Hg and Pb, meanwhile Cr showed values next or above the EQS only for Portonovo site (Table 1).

Table 1. PTEs concentration in Marine Sediments

		Metal concentration											
	%	mg/g			μg/g								ng/g
Site	OM	Al	Fe	Mn	As	Cd	Co	Cr	Cu	Ni	Pb	V	Hg
PAL	0.9 ± 0.3	0.8 ± 0.5	3.5 ± 2.0	0.67 ± 0.06	7.7 ± 0.8	0.06 ± 0.01	5 ± 0.7	15.6 ± 5.7	2.2 ± 0.2	7.4 ± 1.6	8.2 ± 0.9	17.9 ± 3.5	4.4 ± 0.5
MD	2.2 ± 0.5	1.1 ± 0.7	18.0 ± 19.9	0.92 ± 0.23	12.7 ± 3.1	0.07 ± 0.01	13.4 ± 4.6	34.7 ± 10.1	8.8 ± 1	32 ± 8.3	16 ± 2.1	86.1 ± 34	16 ± 3
PN	4.2 ± 0.7	1.4 ± 0.3	17.9 ± 4.4	1.09 ± 0.34	11.4 ± 1.5	0.09 ± 0.02	13.3 ± 4.7	64.6 ± 10.4	20.5 ± 7.7	37.3 ± 11.1	14.2 ± 3.5	102.5 ± 21.4	35 ± 9
EQS*					12	0.3		50			30		300

*Environmental Quality Standard for coastal marine sediments included in D.Lgs 13/10/2015 n.172.

To assess the possible sources of PTEs in the marine sediments, Enrichment Factors (EF) evaluations was performed using Fe as indicator, as described in [3]. None of the determined elements was above the EF>40 (extreme enrichment). However, some metals showed an enrichment from moderate (V, Cr, Cu) to very high (As). As results to be the element most affected by anthropogenic sources and tends to be as much enriched as it is closer to the riverine and sewage discharge outflows. Other elements, such as Ni, Al, Hg, and in PN, also Pb, Cd and Co showed a natural, crustal origin.

WP 3. Atmospheric Depositions:

Methods. From July 2021, atmospheric depositions were collected by means of passive samplers (sampling bulks) in only one site of the study area (Marina Dorica). Once collected, samples were vacuum-filtered through pre-weighed 0.45-µm membrane filters to separate the soluble and insoluble fractions. The total particle load from atmospheric deposition were measured according to the gravimetric method, following a procedure previously set-up and optimized [4]. Soluble and insoluble metal fractions were determined by analysing filtered and residual fractions. A quarter of each bulk-set filters (¼ filter with residue + ¼ filter with particulate from the funnel walls) was microwave (MW) digested in Teflon bottles, after addition of ultrapure HNO_3+H_2O_2+HF, 5+2+1 mL. Digested solutions were analysed by GF-AAS for metal determination. The total mercury content in residual fractions was quantified by Direct Mercury Analyzer (DMA). Soluble concentrations of metals were determined by AFS (Hg, As, Cd) and AAS. For each metal and each metal fractions the daily depositional flux, expressed in ug m^{-2} d^{-1}, was computed.

Expected/Obtained Results. In the second year of my PhD Project, results for the first samples, from July 2021 to November 2022 are reported. During the sampling period, the Ancona port area was characterized by a total flux of 8.0±0.4 mg m^{-2} d^{-1}. The total average daily fluxes (given as the sum of each metal fraction depositional flux) of elements follow the following decreasing order: Fe > Al > Cu > Mn > Ni > Cr > Pb > V > As ≅ Cd > Hg. Daily depositional fluxes showed a high seasonal variability with high summer values that decreased in winter. This variability could be highly influenced by the port area and the increasing maritime traffic in summer.

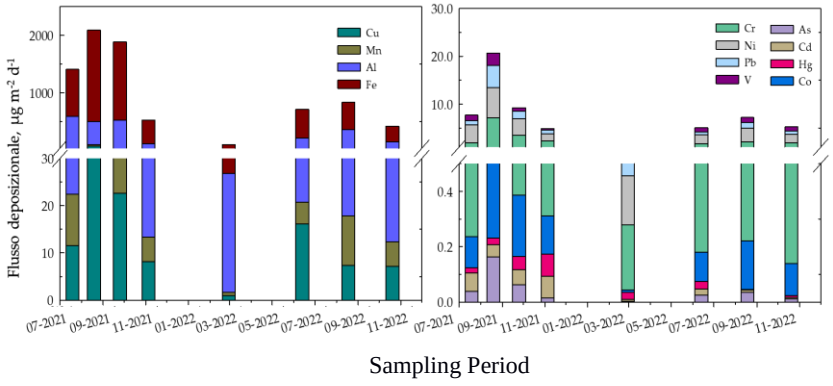


Fig. 1: Seasonal trend of depositional fluxes. a) Al, Cu, Fe, Mn; b) Cd, Co, Cr, Hg, Ni, Pb, V, expressed in µg m^{-2} d^{-1}.

The EFs analysis highlighted a high enrichment for Cu, a moderate enrichment for Cd, Cr and Pb, and a low enrichment for Hg and Ni. All other elements showed a lithogenic origin. In general, August and October were the months with the air quality mainly affected by anthropogenic activities. To deepen in the characterization of the air quality over Ancona, the analysis of microbiome was carried out in collaboration with the CNR-IRBIM of Ancona. The DNA extraction and the following High Throughput Sequencing of the gene 16S rRNA and procedures were carried out according to [5]. The low presence of Actinobacteria, which are commonly associated with crustal sources, suggested that atmospheric transport of soil-related microorganisms had a minimum influence on the airborne microbic communities here identified. The multivariate analysis (CCA) identified Hg and Cd as statistically significative variables having an influencing role on the formation of the atmospheric microbiome community analysed.

Future Perspectives

From March 2023, the collection of seawater, marine sediments and atmospheric depositions continue to have a more complete scenario on the anthropogenic effects on nutrients and PTEs distribution in the coastal area of Ancona.

The third year of the PhD project will focus on:

- Set up of an analytical procedure for the determination of dissolved fractions of PTEs (Al, Cr, Fe, Mn, Ni, Se, Pb) by Atomic Absorption Spectroscopy (GF-AAS)
- Analysis of inorganic contaminants in all the samples collected in the second sampling campaign
- Nutrients determination in the atmospheric depositions
- Source apportionment of the airborne elements and microbiome in atmospheric aerosol over Ancona
- Chemometric treatment of data

REFERENCES

[1] Vareda, J. P., Valente, A. J., & Durães, L. (2019). Journal of environmental management, 246, 101-118.

[2] Volf, G., Atanasova, N., Kompare, B., & Ožanić, N. (2013). Journal of hydrology, 504, 182-193.

[3] Aprile, F. M., & Bouvy, M. (2008). Brazilian Journal of Aquatic Science and Technology, 12(1), 1-8.

[4] Illuminati, S., Bau, S., Annibaldi, A., Mantini, C., Libani, G., Truzzi, C., Scarponi, G., (2016). Gazi University Journal of Science, 24(1), 29-34.

[5] G.M. Quero, L. Perini, G. Pesole, C. Manzari, C. Lionetti, M. Bastianini, M. Marini, G.M. Luna (2017) Mol. Ecol. 26 (21) 5961–5973

Part 2. PhD student information on the overall year activity (courses/seminars/schools, mobility periods, participation to conferences)

List of attended courses/seminars/schools

1. Formazione specifica salute e sicurezza sul lavoro Rischio Medio (24/01/2023)

2. Seminari public speaking: comunicazione efficace per la scienza_introduzione alla comunicazione al public speaking (11/04/2022)

4. A shot of science: Toxicological effects of cigarette butts for marine organisms (20/12/2022)

5. Tips and tricks, CASS-Scifinder (07/02/2023)

6. Lezioni di informatica, Corso getting started with R: environmental computing (curricular)

7. A shot of science: Evaluation of phytoplankton communities in two Long-Term Ecological Research stations through statistical approaches

8. Progetto scienziato per un giorno: centri estivi sportivi JUMP (03/09/2023)

9. Computer science: Theory and application of complex networks (Maria Grazia Ortore), curricular

List of periods spent abroad

1.None

List of conferences/workshops attended and of contributions eventually presented

1. 5th MS Envy Day, Milan (ITA) **Fatty acids as potential biomarkers in marine suspended particulate matter across the Southern Ocean: extraction and quantification by gas chromatography-mass spectrometry** C. Truzzi, F. Girolametti, A. Annibaldi, M. Fanelli, B. Ajdini, S. Illuminati.

2. Bioanalitica 2023, Florence (ITA)**Chemical characterization of a deep Ionian Sea sediment core** M. Fanelli, S. Zampatti, F. Girolametti, B. Ajdini, A.Annibaldi, C. Truzzi, S. Illuminati, F. Spagnoli. Poster presentation by M.Fanelli

3. Bioanalitica 2023, Florence (ITA)**Chemical composition of European tea leaves: elemental content and health risk assessment for consumers** F.Girolametti, A. Annibaldi, S. Illuminati, B. Ajdini, M. Fanelli, E. Damiani, E. Giorgini, P. Carloni, C. Truzzi. Poster presentation by F.Girolametti

4. TUMA 2023, Francavilla al Mare (ITA) **Mercury (Hg) and other potentially toxic elements (Cd, Pb, As) in honey from the Marche Region (Central Italy):risk assessment for human health** F. Girolametti, B. Ajdini, A. Mezzaluna, A. Annibaldi, S. Illuminati, M. Fanelli, C. Truzzi. Poster presentation

5. TUMA 2023, Francavilla al Mare (ITA) **Up-take of potentially toxic elements in the novel food Acheta domesticus grown of seaweed-enriched media: risk assessment for human health** B. Ajdini, I. Biancarosa, S. Illuminati, A. Annibaldi, F. Girolametti, M. Fanelli, C. Truzzi. Oral presentation by B.Ajdini

6. Science for the Planet, Campobasso (ITA) **Studio delle deposizioni atmosferiche nell'area portuale di Ancona** M. Fanelli, F. Girolametti, B. Ajdini, M. Lorenzo, A.Falgiani, G.M. Quero, M. Basili, P. Penna, E. Frapiccini, A. Annibaldi, G.M. Luna, C. Truzzi, S. Illuminati

7. IV International Ross Sea Conference 2023, Naples (ITA) **From New Zealand to Antarctica (Ross Sea): the fatty acid composition of marine suspended particulate matter** F. Girolametti, A. Annibaldi. S. Illuminati, M. Fanelli, B. Ajdini, A. Ardini, C. Truzzi. Oral presentation by F.Girolametti

8. Analitica 2023, Vasto (ITA) **Nutritional value of house cricket (Acheta domesticus) fed diets supplemented with different levels of the seaweed Palmaria palmata in the feeding media** B. Ajdini, I. Biancarosa, G. Cardinaletti, S. Illuminati, A. Annibaldi, F. Girolametti, M. Fanelli, G. Pascon, M. Martinoli, C. Truzzi XX National Congress of the Division of Environmental Chemistry and Cultural. Oral presentation by B.Ajdini

9. XX National Congress of the Division of Environmental Chemistry and Cultural Heritage, Ischia (ITA) **Chemical characterisation in arctic marine sediments in Kongsfjorden** M. Fanelli, L. Massi, F. Girolametti, B. Ajidini, A. Annibaldi, C. Truzzi, Ø. Mikkelsen, S. Illuminati. Poster presentation

10. XX National Congress of the Division of Environmental Chemistry and Cultural Heritage, Ischia (ITA) **Trace elements and nutrients evaluation in the caostal area of Ancona subjected to different anthropogenic pressures** M. Fanelli, A. Pelella, F. Girolametti, B. Ajdini, C. Truzzi, A. Annibaldi, S. Illuminati. Poster presentation

Part 3. PhD student information on publications

In preparation: Vertical distribution of Hg in sediment cores of the western-central and southern-Adriatic Sea.

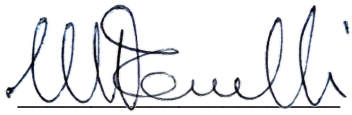
If not yet published, please indicate the publication status (submitted, accepted, in preparation...)

List of publications on international journals

J1. . Girolametti, F., Illuminati, S., Annibaldi, A., Ajdini, B., Fanelli, M., & Truzzi, C. "Mercury in honey from the Marche region (central Italy). Risk assessment from human consumption and its use as bioindicator of environmental pollution." ***Heliyon***, 2023, 9(10), e20502, DOI:https://doi.org/10.1016/j.heliyon.2023.e20502

13/10/2023

Student signature



Supervisor signature