multi-Risk sciEnce for resilienT commUnities undeR a changiNg climate

Codice progetto MUR: **PE00000005** – F83C22001660002

Deliverable title: Methodology for ecological risk assessment of combined exposure to multiple chemicals.

Deliverable ID: DV 4.4.2

Due date: 30/11/2024

Submission date: 30/11/2024

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1. Technical references

Project Acronym	RETURN
Project Title	multi-Risk sciEnce for resilienT commUnities undeR a changiNg climate
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Project Duration	December 2022 – November 2025 (36 months)

Deliverable No.	DV 4.4.2
Dissemination level*	CO
Work Package	WP4 - Multi risk assessment, and proof of concepts
Task	T4.2 - Screening and advanced (probabilistic and uncertainty based) methodologies for risk assessment, chemical and chemical/particle mixture, individual based effect models, in silico tools and QSAR approaches
Lead beneficiary	Stefano Albanese (UNINA)
Contributing beneficiary/ies	

* PU = Public

PP = Restricted to other programme participants (including the Commission Services)

RE = Restricted to a group specified by the consortium (including the Commission Services)

CO = Confidential, only for members of the consortium (including the Commission Services)

2. Document history

Version	Date	Lead contributor	Description
0.1	22.04.2025	Stefano Albanese (UNINA)	First draft
0.2	25.04.2025		Critical review and proofreading
0.3	27.04.2025		Edits for approval
1.0	28.04.2025		Final version

3. Abstract

The deliverable presents the methodology and proof-of-concept development for an Ecological Risk Assessment (ERA) framework addressing combined exposures to multiple chemicals in complex environmental systems. The work is motivated by the need for reliable tools to evaluate ecological impacts in riverine-to-marine transitional environments, where multiple stressors interact under anthropogenic and climatic pressures. The proposed framework is designed as a tiered, decision-support system (DSS) aligned with OECD and EU guidelines, and it integrates traditional, probabilistic, and multi-stressor approaches.

The document begins with an overview of the state of the art, highlighting the evolution of ERA methodologies from simplified regulatory screening tools to advanced, site-specific assessments incorporating mixture toxicity models, biomarkers, and ecotoxicological endpoints. Key guiding principles include tiered assessment, weight-of-evidence approaches, and the incorporation of climate-related stressors. Particular attention is given to the challenges posed by chemical mixtures, for which both whole-mixture and component-based approaches are considered, as well as to the need for probabilistic models that account for variability and uncertainty.

Problem formulation is developed through a systematic review of methods for assessing soil and sediment contamination. Potentially toxic elements (PTEs) are evaluated using individual and aggregate pollution indices, including Igeo, EF, CF, PLI, and PERI. Their strengths, limitations, and requirements for analytical rigor are critically discussed, with emphasis on the role of bioavailability, geochemical baselines, and speciation analyses. Parallel to this, bioplastics are addressed as emerging contaminants, with a review of biodegradation standards across compost, soil, digestate, and marine environments. Experimental designs were defined to test the fate of selected commercial bioplastics under both controlled and uncontrolled conditions, simulating diverse environmental compartments.

Data gathering combined existing geochemical datasets from the Sarno River basin with new surveys carried out in 2024. Sediments and waters were analysed for PTEs, organic pollutants, and ecotoxicological responses. Complementary experimental studies assessed bioplastic degradation under composting, soil, and marine conditions, as well as the influence of enzymatic pre-treatment on anaerobic digestion performance. Results indicate differential degradation rates across products and environments, with implications for persistence and risk.

The final section outlines the proof-of-concept ERA framework. A novel methodological proposal integrates local geochemical baselines with traditional indices to better capture spatial variability and reduce bias in contamination assessment. Applied to Sarno River sediments, the approach improved detection of anthropogenic signals and enabled the identification of contamination sources through principal component analysis, distinguishing industrial and agricultural-urban pressures. The framework demonstrates the feasibility of coupling geochemical, ecotoxicological, and experimental data within a unified risk assessment procedure.

Overall, Chapter 5 provides an integrated methodology for ERA under multiple chemical exposures, combining regulatory guidance, innovative analytical tools, and experimental validation. The proposed DSS,

though at an early stage of technological readiness, offers a structured basis for future development of multi-stressor ecological risk assessment at regional scale, supporting adaptive management in the context of climate change and evolving environmental challenges.

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5.1 Introduction

5.1.1 State of the art

Several ERA procedures have been developed during the last two decades, and, in general, the assessment process can follow three main paths, following the kind of data used as input:

- Comparison of chemical concentration values found in environmental media with predetermined environmental quality standards (EQS)
- Analysis of biomarkers for the assessment of the contaminant impact at the sub-organism level of biological organisation and different phases of stress in model organisms
- Use ecotoxicological endpoints (e.g. survival and reproduction) to assess the direct effect at the population level.

Different disciplines usually work on the definition of ERA procedures; the complexity of natural systems affected by anthropogenic pressure, together with the concomitance of multiple stressors, makes the assessment of risk challenging at any level due to the sensitive degree of uncertainty accompanying the results.

One of the most complex environments to deal with in the framework of an ERA procedure is the one that connects a riverine system with the marine environment. In this system, we can at least delineate four environmental sectors, including:

- The headwater area (usually characterized by a steep morphology with a poorly anthropized environment), where erosion is very active.
- The transfer zone area (usually characterized by a hilly morphology with an anthropized environment) where the amount of deposited and transported materials is balanced.
- The “terrestrial” depositional zone (usually characterized by a flat or quasi-morphology, often with a broad and diffuse anthropized environment) where the energy of the stream progressively decays and a huge amount of transported materials are deposited even at a distance from the river course when floods occur.
- The “marine” depositional zone, including the transition zone between the riverine system and the sea, where peculiar geological, sedimentary, and biological features occur.

In this kind of system, humans' interaction with the natural environment can generate several “contamination” processes that can overlap, generating an overall relevant pressure whose individual parts are not easily recognizable due to their mixing.

The Framework for Risk Assessment of Combined Exposures to Multiple Chemicals (OECD, 2018) 's main objective is to develop a “fit for purpose” assessment strategy that uses only the resources necessary to support a decision. The framework outlines a tiered approach that can be applied based on available data, considering the possible methods and level of refinement for conducting the hazard and exposure assessments and subsequent risk characterization.

5.1.2 Objectives

From a historical perspective, some contaminants are of greater environmental concern than others, and this has been assumed as a reference for developing a Decision Support System (DSS) to apply an ERA at the regional scale.

The DSS is characterized by a medium-low degree of maturity (at a Technology Readiness Level [TRL] set at 3/4] aiming at the development of an experimental POC, according with the project requirements and in line with the European Commission definitions1

Potentially toxic elements and bioplastics in sediments have been used for the DSS development.

The activities carried out were distributed across three main phases:

- Problem definition
- Data gathering, production, preparation and analysis
- ERA DSS procedure development

5.2 Problem definition

5.2.1 Summary of main principles/methodologies for Ecological Risk Assessment Risk Assessment (ERA)

5.2.1.1 Introduction to the Ecological Risk Assessment

An extensive review of the main principles and methodologies for Ecological Risk Assessment (ERA) was produced (**Annex 1**) as a sound base for the development of research activities within the framework of the planned activities

ERA is defined as an iterative process that evaluates the likelihood that adverse ecological effects may occur as a result of exposure to one or more stressors (USEPA, 1992). Risk assessment approaches, originally developed in the mid-20th century for human health toxicology, have been progressively expanded to cover environmental and ecosystem-level evaluations (Tarazona and Ramos-Peralonso, 2023). More recently, ERA has also been applied on large spatial scales, addressing complex issues such as regional and urban ecological risks.

ERA vs Environmental Risk Assessment

In Europe, the terms Environmental Risk Assessment (ERA) and Ecological Risk Assessment are often used interchangeably, but subtle differences exist (Simon, 2019). Environmental risk assessments encompass both ecological and human impacts, while ERA focuses specifically on ecosystems. Unlike human health risk assessments, which aim to protect the individual, ERA aims to safeguard populations, communities, ecosystem integrity, and ecosystem services (OECD, 2018; EFSA, 2019). Typical endpoints include reproductive impairment, population growth, mortality, and disruption of ecosystem services.

ERA methodologies can be divided into prospective (predictive, pre-market assessments) and retrospective (post-market, site-specific evaluations). According to Tarazona and Ramos-Peralonso (2023), three main levels are commonly recognized:

1. **Level 1 ERA:** Simplified, scenario-based approaches primarily for regulatory purposes, assessing whether acceptable risk thresholds may be exceeded.
2. **Level 2 ERA:** Probabilistic methods that account for temporal and spatial variability, increasing complexity and often requiring expert risk communication.
3. **Level 3 ERA:** Retrospective, site-specific assessments that link monitoring data with observed ecological effects, often used for contaminated sites (USEPA, 1998; Environment Agency UK, 2008a; FCSAP, 2012).

The Water Framework Directive (WFD), together with the Environmental Quality Standards (EQSD) and Groundwater Directives (GWD), establishes the EU framework for the protection of aquatic environments. These are complemented by directives such as the Marine Strategy Framework Directive (MSFD), the Urban Wastewater Treatment Directive (UWWTD), and the Nitrates Directive. Risk-based principles underpin all these regulations (EC, 2018). The technical guidance (EC Guidance Document No. 27) defines how to derive EQS, generally using conservative assumptions to protect ecosystems and human health.

However, despite the ambition of the EU legislation, there is no common framework specifically guiding ERA in the management of contaminated sites. In Italy, for example, ERA applications have been sporadic, often driven by proactive actions of local authorities or academic initiatives (Bizzotto et al., 2023).

ERA is structured around three main phases (USEPA, 1998):

1. **Problem Formulation:** Identification of stressors, receptors, conceptual models, assessment and measurement endpoints, and protection goals (ECHA, 2014).
2. **Analysis:** Assessment of exposure and effects, including measured and modelled data, to establish relationships between contaminants and ecological impacts.
3. **Risk Characterization:** Integration of exposure and effect data, uncertainty analysis, and communication of results to stakeholders (OECD, 2019).

5.2.1.2 Ecological Risk Assessment: principles and methodologies

Key guiding principles include the use of tiered approaches (progressively refining the assessment as new data become available), multiple Lines of Evidence (LoE), and the Weight of Evidence (WoE) approach, which integrates heterogeneous data for transparent decision-making (Linkov et al., 2009; USEPA, 2016).

One of the main challenges in ERA is assessing risks from chemical mixtures. Since testing every possible mixture is unfeasible, predictive models are used (OECD, 2018; EFSA, 2019). Two major approaches are applied:

Whole Mixture Approach (WMA): Evaluates the mixture as a single entity, capturing synergistic effects but lacking detail on individual components.

Component-Based Approaches (CBA): Estimate combined risks from the toxicological profiles of individual components, typically using concentration addition (CA) or response addition (RA) models.

Tiered strategies are recommended, starting with conservative screening (hazard quotients, PEC/PNEC ratios), and advancing to probabilistic and biologically based models such as Toxicokinetic-Toxicodynamic (TK–TD) models and Species Sensitivity Distributions (SSDs).

Climate change introduces additional stressors such as temperature shifts, extreme weather events, and altered habitats, which interact with chemical contaminants (Rattner et al., 2023). Adaptive management and probabilistic tools like Bayesian networks are recommended to capture complex dynamics and uncertainty. ERA must increasingly move toward a multistressor framework, integrating ecological, chemical, and climatic drivers of risk.

5.2.2 Systematic review of available methods to assess the degree and severity of chemical contamination of soil and sediments.

5.2.2.1 Potentially Toxic Elements

A critical review of a selection of pollution indices, quantitative tools designed to evaluate contamination levels and ecological risks posed by chemical elements in soils and sediments, was completed. These indices are classified into individual indices (e.g., Igeo, EF, CF, PI*) and aggregate multi-element indices (e.g., PLI, Cdeg, mCdeg, PERI). Their reliability hinges on rigorous analytical methodologies, including proper sampling, geochemical background/baseline selection, and speciation analysis.

An overview of pollution indices (individual and complex) was generated, fixing the specific features for each approach.

Specifically,

a) for Individual Indices:

- Geoaccumulation Index (Igeo) assesses contamination by comparing element concentration to geochemical background, adjusted by a lithogenic factor (1.5). The method does not account for bioavailability.
- Enrichment Factor (EF) identifies anthropogenic influence by normalising element concentration (C_n) against a reference metal (C_m, e.g., Fe, Al). The choice of the reference element can affect the reliability of results if not done critically.
- Contamination Factor (CF) evaluates contamination relative to preindustrial levels.
- Pollution Index (PI) compares element concentration to the regional background. The use of the geometric mean may mask extreme values.

b) for Aggregate Indices:

- Pollution Load Index (PLI) aggregates contamination factors (CF) of multiple elements into a geometric mean. Ranges from $PLI < 1$ (unpolluted) to $PLI > 1$ (polluted).
- Degree of Contamination (Cdeg or CD) and Cumulative Contamination index (CCI) sums CF and EF values, respectively, to quantify cumulative contamination.
- Modified Contamination Factor (mCdeg) averages CF values for a normalized assessment. Ranges from $mCdeg \leq 1.5$ (very low) to $mCdeg \geq 32$ (ultra-high).
- Potential Ecological Risk Index (PERI) integrates toxicity and contamination for ecological risk. Toxicity factors may lack regional relevance.

The critical analysis led to some key conclusions useful for the for the task objectives:

- Analytical rigor is paramount:
    - Reliable indices depend on validated analytical methods, certified reference materials, and consideration of grain size (e.g., fine fractions <0.062 mm adsorb more metals).
    - Speciation analysis is essential for elements like Cr(VI) and Hg(II), where toxicity varies by oxidation state.
- Index selection must align with objectives:
    - For source identification, the use of EF or CF is a suitable option
    - For ecological risk is better to select PERI or E_r (toxicity-weighted).
    - For a holistic assessment, a combination of PLI (aggregate pollution) with Igeo (individual metals) is a potential solution
- Challenges and limitations:
    - Individual indices typically use geochemical background/baseline values as a reference to assess the degree of contamination using a ratio. Typically, a unique reference value is selected for each element within a study area. This may be a critical point in the process, particularly when applied on a large scale to classify the entire basin due to its high geological variability.
    - Most of the aggregate indices are based on a linear aggregation function as the sum of individual indices; they implicit that the individual indicator's influence on the aggregate indicator's value should be constant, independently of the indicator's value.
- Practical recommendations and potential research targets:
    - The use of metal bioavailability in place of total concentration and dynamic modelling could refine the risk predictions.
    - The development of methods for assessing regional or sub-regional-specific geochemical background could improve indices accuracy.

5.2.2.2 Bioplastics

The assessment of bioplastics degradation across diverse environments, such as soil, compost, digestate, and marine water, is critical to understanding their real-world performance and potential environmental impact. This is a crucial step for the evaluation of the risk that incomplete or slow degradation may pose to ecosystems, especially if bioplastics are expected to accumulate in sensitive environments. Current testing methods vary significantly in scope, duration, and realism, often lacking standardization across conditions. While industrial composting and anaerobic digestion tests are relatively well-established (e.g., EN 13432 and ISO 14853, respectively), methods for soil and marine environments are less mature, with limited reproducibility and relevance to natural settings. Biodegradation testing in soil relies on standards such as ISO 17556 and ASTM D5988. In these tests, bioplastic samples are buried in soil and tested at controlled temperature, moisture, and oxygen levels. There are several standards to simulate different marine environments, depending on the specific marine zone, such as the eulittoral (ISO 22404), pelagic (ISO 23977-1), or sublittoral (ISO 19679) zones. In all cases, test results must be carefully interpreted as they can be useful to define the potential degree and severity of contamination associated to bioplastic residues. Some of the most used standards for assessing the biodegradability of bioplastic products at the laboratory scale are reported in **Table** for comparison.

Table 5.2.1. Overview of main standards used to assess the biodegradation of bioplastics in different environments.

Standard	Environment	Test matrix	Duration	T [°C]	Bioplastics concentration	Key Metric
ISO 14855	Compost	mature compost	up to 180 days	58	160 gTS/kgTS(compost)	CO_2 evolution
ISO 14853	Digestate	digestate	up to 90 days	35 – 52	20 mgTOC/L(digestate)	Biogas evolution
ISO 17556	Soil	natural soil	6 – 24 months	20 – 28	1 gTS/kgTS(soil)	CO_2 evolution
ISO 19679	Marine (sublittoral)	seawater+sediment	1 – 2 years	15 – 25	150 mg/L(water+sediment)	CO_2 evolution

Based on the standards reported above, the experimental design shown in Errore. L'origine riferimento non è stata trovata. was defined for testing during the project. Dedicated experiments were set up to explore the biodegradation features under the indicated bioplastic characteristics and environmental conditions. These were aimed at exploring the fate of bioplastic residues in different environments, estimating their lifetime in various contexts and inferring their presence in the environmental compartments under both controlled and uncontrolled management scenarios.

Table 5.2.2. Experimental design adopted

Test	T [°C]	Tested bioplastics	Bioplastic size
discontinuous biodegradation in compost	58 °C	cutlery, shopper, dental floss, sticker, coffee capsule	<0.1 cm
coupling of enzymatic (pre-)treatment and anaerobic digestion	55 °C	cup, plate, shopper	2 × 2 cm^2
controlled degradation in natural soil	22 °C	coffee capsule, chips, plate, shopper, sticker	1 × 1 cm^2
uncontrolled degradation in natural soil	environmental conditions	coffee capsule, cup, plate, shopper	2 × 2 cm^2
controlled degradation in marine water and sediment	18 °C	shopper	1 × 1 cm^2
uncontrolled degradation in marine water	environmental conditions	bottle, box	⌀ 4 mm

5.3 Data gathering, production, and preparation.

5.3.1 Potentially toxic elements chemical data (in sediment and soil) acquisition and preparation for inclusion in the ERA procedure

5.3.1.1 Former datasets

Data gathering from previous geochemical environmental projects at UNINA was completed. The data refer to different sampling media, including 283 topsoils (Cicchella et al., 2016) and 89 stream sediments (Albanese et al., 2013), collected within the Sarno River catchment basin (Southern Italy) and analysed to determine PTE contents and, on a limited number of samples, the concentration of some organic pollutants (OCPs, PAHs).

5.3.1.2 New data

During May and June 2024, a comprehensive geochemical survey was carried out in the framework of the RETURN project to explore the environmental impact of multiple anthropic stressors on a changing environment. The Sarno River was chosen for the purpose, considering the pre-existing database and the need to evaluate whether climatic changes (and related hydrological regime variations) can significantly influence contaminant distribution along a water course and its river banks. Twenty-four sampling points were selected along the main stream of the Sarno River (including the Solofrana and Cavaiola tributaries), and at each location, stream sediments and waters (when available) were collected (**Figure. 5.3.1**).

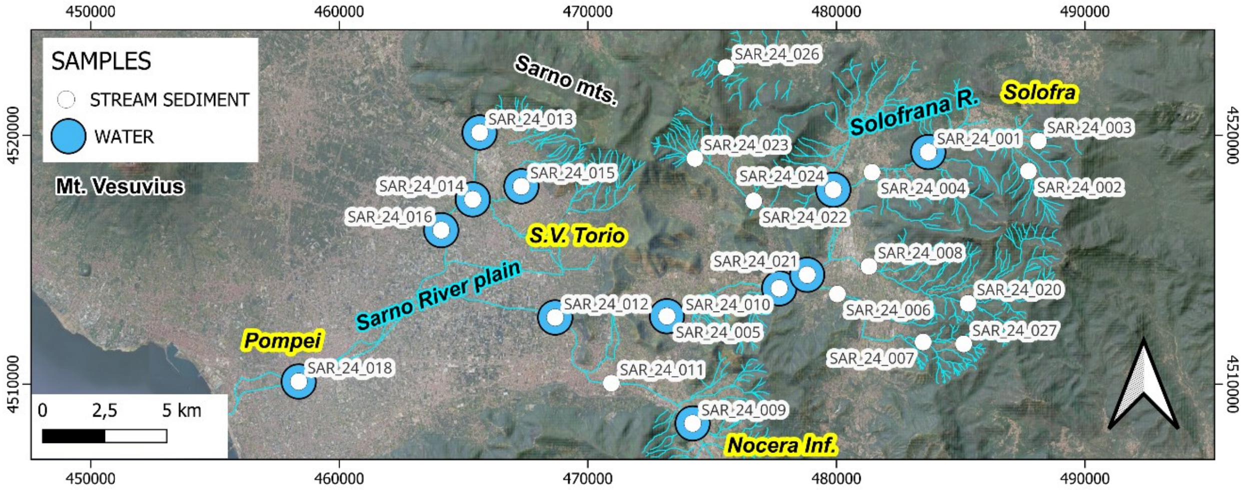

Figure 5.3.1. Stream water and sediment sampling locations (May/June 2024) in the Sarno River basin.

Waters were analysed at the Environmental Geochemistry lab of the Department of Earth, Environment and Resource Sciences (University of Naples Federico II) using the Palintest 7500 photometer. ENEA (IMPACT Division) analysed sediments, assessing the concentration of a selection of potentially toxic elements and some additional metals and organic compounds (including PAHs, PCBs, OCPs, organotin compounds, and others). Ecotoxicological tests were also carried out at ENEA on both sediments and waters. The total content of PTES (Be, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Pb, U, V, Hg) in the sediments was obtained by solubilising the samples using the microwave-assisted acid digestion procedure according to the EPA 3052 method. Data were quality-controlled and assessed, and georeferenced for subsequent digital mapping

5.3.2 Development of experimental testing methods to assess the transformation and physical/chemical/biological degradation of bioplastics

5.3.2.1 Aerobic composting of commercial bioplastics before and after mechanical reprocessing

Aerobic composting degradation tests were conducted on bioplastic cutlery (knives and forks) both neat and after undergoing varying numbers of extrusion cycles, specifically after 1 and 8 cycles (Ext1 and Ext8, respectively), to assess the impact of mechanical recycling on their biodegradability. The tests were carried out in duplicate under controlled composting conditions at 58 °C for a duration of 180 days. **Figure 5.3.2**Figure presents the monitoring of CO_2 evolution over time. The results show no significant differences in CO_2 production or overall biodegradation performance among the three samples, indicating that repeated extrusion did not adversely affect the biodegradability of the materials. Thermogravimetric (TG) analyses were conducted at various stages of the degradation process to monitor alterations in the material composition. The results revealed a progressive loss of polylactic acid (PLA) as the initial and more readily degradable component. Only at later stages of degradation was a noticeable reduction in polybutylene adipate terephthalate (PBAT) observed, which is the more recalcitrant fraction. Aerobic degradation appears to be more favorable under the tested conditions; however, it is important to note that the test setup (180 days at 58 °C) does not fully reflect real-world industrial composting environments. In actual facilities, elevated temperatures are typically sustained only for a few weeks during the thermophilic phase. This discrepancy suggests that while laboratory results indicate good biodegradability, the performance of the materials in operational composting plants may differ, and the extent of degradation could be lower due to shorter exposure to optimal conditions.

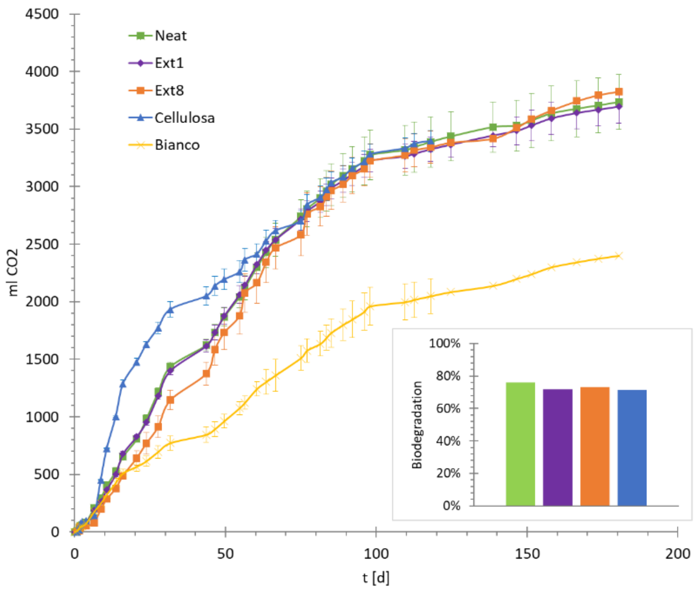

Figure 5.3.2. CO_2 evolution and biodegradation degree for the bioplastic products under aerobic degradation in mature compost.

5.3.2.2 Enzymatic (pre-)treatment of bioplastic products

In this study, lipase was selected and applied in both free and immobilized forms as an enzymatic treatment for bioplastic materials. Initial tests were conducted using powdered PLA-based bioplastic cups treated with the immobilized enzyme, but no observable effects were detected. Similarly, sonication proved ineffective under the conditions tested. Subsequently, free lipase was used as a pre-treatment on PLA-based cups prior to anaerobic digestion. The cups were incubated with the enzyme in a buffer solution at 37 °C for 96 hours in a thermostatic shaker. Three different enzyme concentrations were tested (0.5, 2 and 10 mg/ml). This was followed by thermophilic anaerobic digestion for 50 days (biogas evolution shown in Figure 5.3.a), which led to the complete degradation of the cups. However, no significant impact from the enzymatic pre-treatment was observed. Further anaerobic degradation tests were carried out on three different bioplastic products (a cup, a plate, and a shopping bag) in the presence of lipase. Biogas evolution is shown in Figure 5.3.b-d. Again, three concentrations of the enzyme (0.5, 2 and 10 mg/ml) were used, but this time the lipase was added directly into the digestate. While the enzyme did not seem to significantly affect the overall degradation, an increased degradation rate was observed during the initial 1–2 days of the process with higher enzyme concentrations. Conversely, higher enzyme concentrations appeared to negatively affect the degradation rate in the later stages of the process. Final degradation levels were similar across all tests: complete for cups and plates, and approximately 30% for the shopping bags, regardless of enzyme concentration.

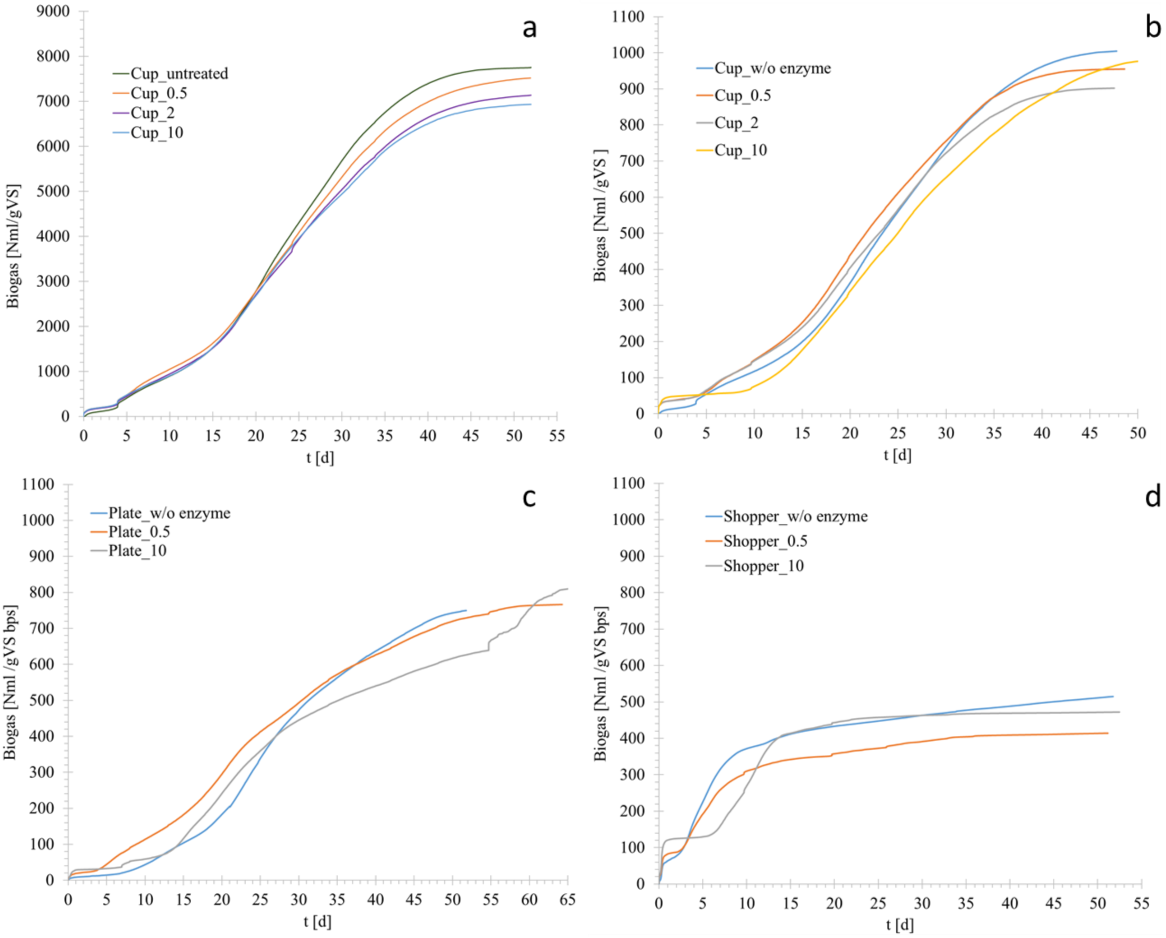

Figure 5.3.3. Biogas evolution during the anaerobic digestion of bioplastic cups after the enzymatic pre-treatment with lipase (a) and anaerobic digestion of bioplastic cup, plate and shopper with digestate enriched with lipase (b-d).

5.4 Risk assessment general framework development and concept proofing on specific topics

5.4.1 Development of the ERA general framework based on phases 1 and 2 outputs, including methodological proposals for weighting and scoring of available data. Concept proofing on specific topics

5.4.1.1 Focus on Potentially Toxic Elements (PTEs) in stream sediment.

Stream sediment data from the former dataset related to the Sarno River (Campania region) provided by UNINA were used to develop a new method to assess the degree of contamination (related to PTES). The key target of the method was to consider the variability of the background/baseline values along a catchment, the geological variations of the substrate, and the dynamic nature of the transported sediment. The method aimed at evaluating whether all the considered variables have the same weight in the aggregate function and, when not, explore how equilibrating the contribution of each variable involved could improve the used indices.

For this purpose, the traditional Upper Regional Baseline Limit (URBL) and a new method using Local Baselines (LBs) derived from Sample Catchment Basins (SCBs) were applied. The goal was to see if using local baselines improves contamination assessments.

The total concentration of potentially toxic elements in 97 stream sediment samples was used. URBLs were calculated for single elements using ProUCL software to get a regional value, while LBs were determined by weighting element concentrations based on lithological units within each SCB, considering the morphology of terrain to account for slope.

The results showed that LBs were significantly lower than URBL for all elements (Wilcoxon test $p<0.001$), indicating URBL might overestimate contamination. Using LBs in contamination indices (CF, EF) and aggregate indices (CD, CCI) provided more detailed spatial patterns, highlighting contamination in urban/industrial areas (**Figure 5.4.1**)

In addition, sensitivity analysis revealed that the use of LBs balanced element contributions better than URBL (**Figure 5.4.2**).

Results obtained by applying the new method showed that local baselines account for geological and land-use variability, leading to more accurate contamination detection. They reduce false negatives by adjusting for natural variations, especially in heterogeneous regions. The SCB approach further improves baseline accuracy when considering terrain slope. This method better identifies anthropogenic impacts in specific areas, like downstream industrial zones, which URBL might overlook.

An attempt was made to identify the primary historical sources of contamination in the Sarno River, based on applying a multivariate statistical analysis technique using PTE concentrations determined on stream sediment samples collected during May/June 2024.

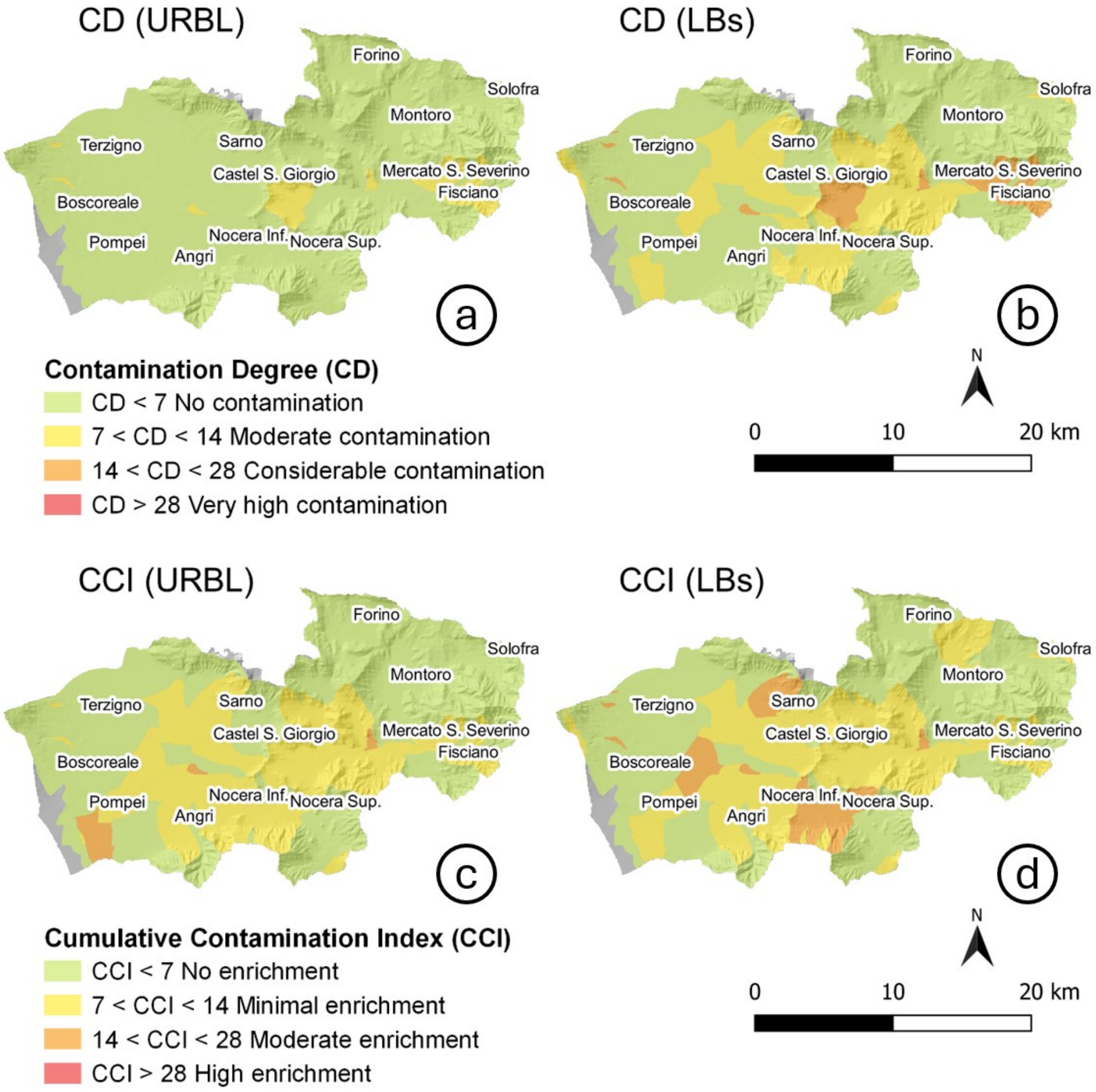

Figure 5.4.1. Aggregated function distribution maps for a) Contamination Degree (CD) derived from URBL, b) Contamination Degree (CD) derived from LBs, c) Cumulative Contamination Index (CCI) derived from URBL, d) Cumulative Contamination Index (CCI) derived from LBs.

Figure 5.4.2 . Sensitivity analysis using raw data and SCB-corrected data for a) CF vs CD, b) EF vs CCI.

The concentration of the single PTEs was mapped to evaluate their spatial variations along the water course. Further, geochemical associations were determined to associate specific multi-elemental patterns with known contamination sources. To do so, a Principal Component Analysis (PCA) was performed on a selection of highly correlated elements. The PCA results allowed to classify samples into two main groups, one predominantly affected by the industrial pressure exerted by the tannery pole (Upper course of the Solofrana) (featuring Cr, Ni, Co, Vn, Mn) and one mainly influenced by agricultural activities and urbanisation (Lower course) (featuring featuring Hg, Cu, Pb, Zn) (**Figure 5.4.3**). We also considered the potential influence of the sediment grain size on metal accumulation, but found no firm evidence of correlation.

A comparison of the statistical distribution of concentrations of PTEs in the Sarno River sediments was also carried out, comparing former data (cfr. §5.3.1.1) with new data (cfr. §5.3.1.2)

Results showed that the application of multivariate analysis can help determine the primary sources of contamination in an environmental context and guide policymakers in identifying environmental priorities. Previous data can also be beneficial in determining the evolution trends of contamination in a basin whose environmental balance is affected by different anthropogenic sources.

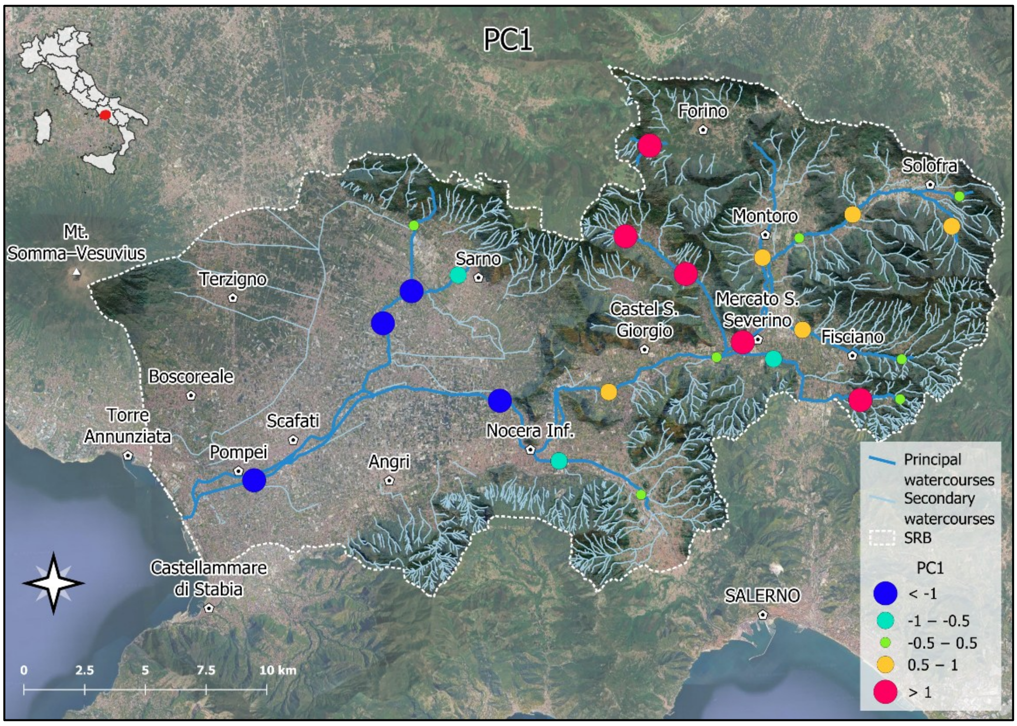

Figure 5.4.3. Distribution map of the scores of the first component generated by PCA. Positive values are associated with Cr, Ni, Co, Vn, Mn and negative values are related to the Hg, Cu, Pb, Zn.

6. Conclusions

Chapter 5 has presented the development and preliminary application of a methodological framework for Ecological Risk Assessment (ERA) under combined exposures to multiple chemicals. The approach integrates state-of-the-art principles, systematic reviews, experimental testing, and case study data into a coherent decision-support system (DSS). Several key conclusions can be drawn:

Methodological advances – The ERA framework moves beyond conventional single-contaminant assessments by adopting tiered, weight-of-evidence and probabilistic approaches. The inclusion of both potentially toxic elements (PTEs) and bioplastics highlights the capacity of the methodology to address heterogeneous stressors of emerging concern.

Soil and sediment contamination indices – The critical review of pollution indices revealed both strengths and limitations in their application. The adoption of local geochemical baselines, instead of regional thresholds, proved effective in improving accuracy, reducing bias, and enhancing the detection of anthropogenic contamination in heterogeneous catchments such as the Sarno River basin.

Bioplastics as emerging contaminants – Standardized methods for testing biodegradation exist mainly for composting and anaerobic digestion, but are less mature for soil and marine environments. Experimental results showed that degradation strongly depends on environmental conditions and material type. Mechanical reprocessing did not affect composting performance, while enzymatic pre-treatment only marginally influenced anaerobic digestion, confirming that real-world persistence of bioplastics may differ from laboratory expectations.

Integration of new and existing data – The combination of legacy geochemical datasets with newly collected samples allowed for both temporal comparison and spatial refinement of contamination patterns. Multivariate statistical analyses (e.g., PCA) successfully identified major contamination sources and linked them to industrial and agricultural-urban activities.

Proof-of-concept risk framework – The integration of chemical, ecotoxicological, and experimental evidence into a unified ERA framework demonstrates the feasibility of applying a structured DSS at the basin scale. Although at an early stage of technological readiness, the framework already highlights its potential to inform environmental management and policy decisions.

In summary, the document provided consolidates methodological innovations, experimental insights, and field applications into a coherent framework for assessing ecological risks in complex and multi-stressor contexts. The approach, while requiring further refinement and upscaling, provides a strong foundation for advancing multi-risk ecological assessments in line with European and international guidelines, particularly under the additional challenges imposed by climate change.

7. References

Albanese, S., Iavazzo, P., Adamo, P., Lima, A., & De Vivo, B. (2013). Assessment of the environmental conditions of the Sarno river basin (south Italy): A stream sediment approach. Environmental Geochemistry and Health, 35, 283–297. https://doi.org/10.1007/s10653-012-9483-x

Allard, P., Fairbrother, A., Hope, B. K., Hull, R. N., Johnson, M. S., Kapustka, L., Mann, G., McDonald, B., & Sample, B. E. (2010). Recommendations for the development and application of wildlife toxicity reference values. Integrated Environmental Assessment and Management, 6(1), 28–37. https://doi.org/10.1897/IEAM_2009-054.1

Bizzotto, E. C., et al. (2023). ERA guidelines for contaminated sites in Italy. [Journal/Publisher details needed].

Cicchella, D., Hoogewerff, J., Albanese, S., Adamo, P., Lima, A., Taiani, M. V. E., & De Vivo, B. (2016). Distribution of toxic elements and transfer from the environment to humans traced by using lead isotopes: A case-study in the Sarno River basin, southern Italy. Environmental Geochemistry and Health, 38(2), 619–637. https://doi.org/10.1007/s10653-015-9748-2

European Chemicals Agency (ECHA). (2014). Guidance on information requirements and chemical safety assessment. Publications Office of the European Union.

European Commission (EC). (2018). Technical guidance for deriving environmental quality standards: Guidance document No. 27. Publications Office of the European Union.

European Food Safety Authority (EFSA). (2019). Guidance on harmonised methodologies for risk assessment of combined exposure to multiple chemicals. EFSA Journal, 17(3), e05634. https://doi.org/10.2903/j.efsa.2019.5634

Environment Agency UK. (2008a). Guidelines for ecological risk assessment of contaminated sites. Environment Agency.

Federal Contaminated Sites Action Plan (FCSAP). (2012). Ecological risk assessment framework. Government of Canada.

Linkov, I., Satterstrom, F., Kiker, G., et al. (2009). From comparative risk assessment to multi-criteria decision analysis and adaptive management: Recent developments and applications. Environment International, 35(2), 263–277. https://doi.org/10.1016/j.envint.2008.07.009

Organisation for Economic Co-operation and Development (OECD). (2018). Considerations for assessing risks of combined exposure to multiple chemicals (OECD Series on Testing and Assessment No. 296). OECD Publishing.

Organisation for Economic Co-operation and Development (OECD). (2019). Guidance on weight of evidence in environmental risk assessment. OECD Publishing.

Rattner, B. A., Melancon, M. J., & Meyer, J. S. (2023). Climate change as a stressor in ecological risk assessment. Environmental Toxicology and Chemistry, 42(5), 1123–1138. https://doi.org/10.1002/etc.5610

Simon, J. (2019). Distinctions between environmental and ecological risk assessment in Europe. Ecotoxicology and Environmental Safety, 182, 109418. https://doi.org/10.1016/j.ecoenv.2019.109418

Tarazona, J. V., & Ramos-Peralonso, C. (2023). Advances in ecological risk assessment methodologies. Science of the Total Environment, 857, 159211. https://doi.org/10.1016/j.scitotenv.2022.159211

United States Environmental Protection Agency (USEPA). (1992). Framework for ecological risk assessment (EPA/630/R-92/001). U.S. Environmental Protection Agency.

United States Environmental Protection Agency (USEPA). (1998). Guidelines for ecological risk assessment (EPA/630/R-95/002F). U.S. Environmental Protection Agency.

United States Environmental Protection Agency (USEPA). (2016). Weight of evidence in ecological risk assessment (EPA/100/R-16/001). U.S. Environmental Protection Agency.