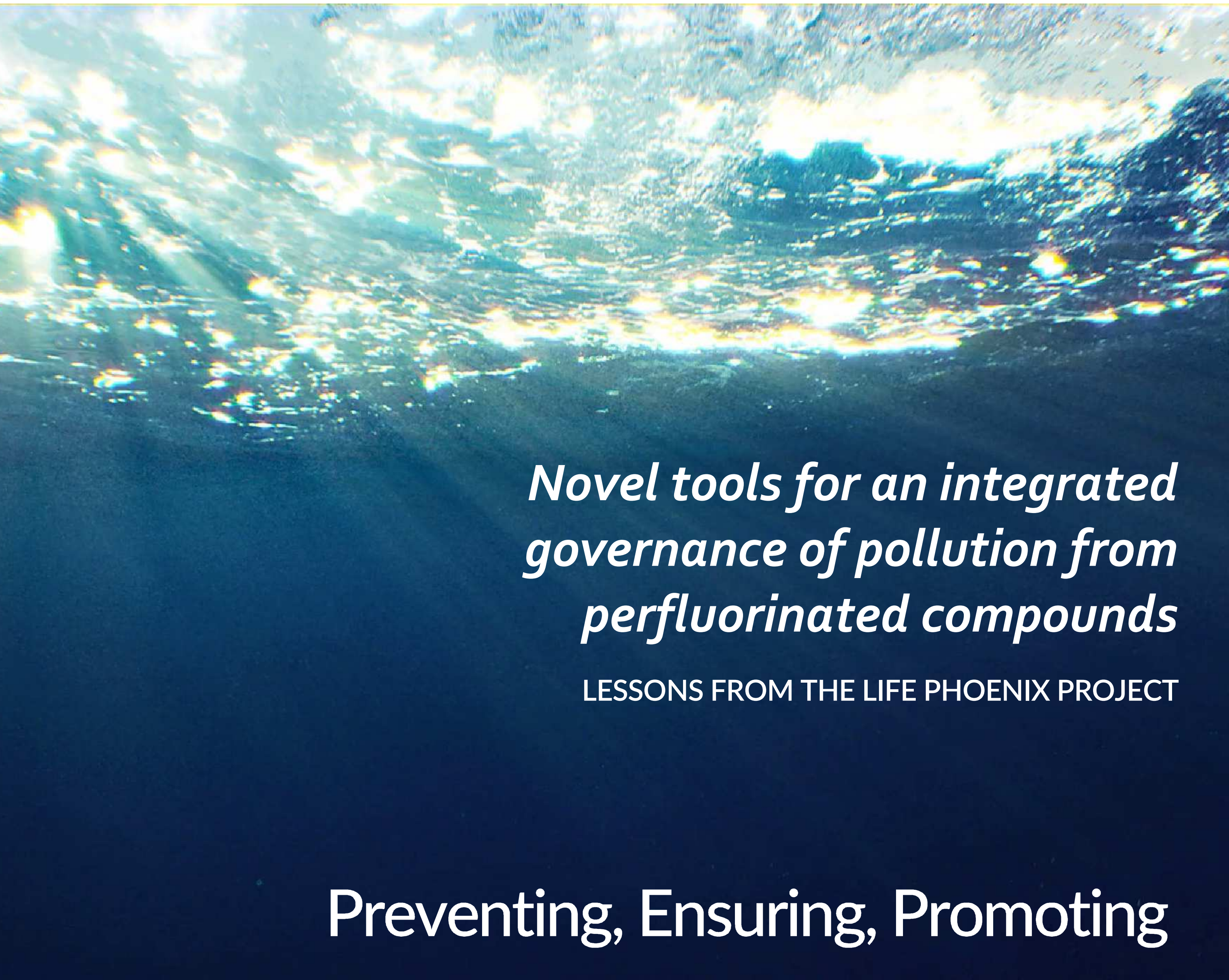
lifephoenix.eu

Life Phoenix

Perfluorinated compounds HOlistic ENvironmental Interinstitutional eXperience



Novel tools for an integrated governance of pollution from perfluorinated compounds

LESSONS FROM THE LIFE PHOENIX PROJECT

Preventing, Ensuring, Promoting

LIFE PHOENIX Project

An integrated approach for the effective management of water pollution risks from emerging contaminants

Perfluorinated compounds
HOlistic ENvironmental
Interinstitutional eXperience

Novel tools for an integrated governance of pollution from perfluorinated compounds

LESSONS FROM THE LIFE PHOENIX PROJECT

Scientific curator: Gisella Pitter (Azienda Zero)

Editing & publishing: Roberto Di Gennaro (e2B Consulting)

Those who contributed towards the making of this document include the following:

Marco Bonato (UNIPD), Matteo Chinellato (Azienda Zero), Francesca Corrà (UNIPD), Franco Corti (UNIPD-CRIEP), Francesca Daprà (ARPAV), Paola Favaretto (Regione Veneto), Claudia Ferrario (IRSA-CNR), Vanessa Groppi (Regione Veneto), Laura Guidolin (UNIPD), Roberto Lava (ARPAV), Massimo Mazzola (ARPAV), Francesco Pintus (UNIPD-CRIEP), Gisella Pitter (Azienda Zero), Stefano Polesello (IRSA-CNR), Nadia Raccanello (Azienda Zero), Francesca Russo (Regione Veneto), Gianfranco Santovito (UNIPD), Laura Tagliapietra (Regione Veneto), Laura Tallandini (UNIPD), Sara Valsecchi (IRSA-CNR), Francesca Zanon (ARPAV)

COORDINATOR

REGIONE DEL VENETO

WITH THE CONTRIBUTION OF THE LIFE FINANCIAL INSTRUMENT OF THE EUROPEAN UNION
LIFE16ENV/IT/000488 - LIFE PHOENIX

PARTNERS

REGIONE DEL VENETO AZIENDA ZERO

arpav Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto

IRSA CNR

Università degli Studi di Padova

Contents

SECTION 1
The Scenario: PFAS pollution in the Veneto Region

The direct or indirect discharge of chemicals into the aquatic environment may lead to hazards to human health, harm to living resources and to aquatic ecosystems and interferences with all the legitimate uses of water. Recently, the emission of the so-called "emerging" organic contaminants has become a priority environmental issue. Emerging contaminants are widely used in everyday life, as human and veterinary pharmaceuticals, personal care products, pesticides, surfactants and water- and grease-repelling products. Contaminants of emerging concern are not necessarily new chemicals, but generally, they are neither regulated by the legislation nor newly detected in the environment.

Among emerging contaminants a particular interest is currently focused on Persistent Mobile Organic Contaminants (PMOC), which are highly polar compounds capable to move and persist in the water cycle and in raw waters used for drinking water production and irrigation. Because they degrade very slowly, are very mobile in aqueous medium and the preferable accumulation matrix is water (including water contained in the biological tissues), the release of PMOC into the environment can lead to a long-term exposure of the population with consequent adverse health effects, which in many cases cannot be adequately assessed because monitoring data and knowledge on eco-toxicological properties are lacking.

Among the PMOCs, perfluoroalkyl and polyfluoroalkyl substances (PFAS) have been causing major concern in recent years due to their peculiar properties of persistence and mobility in the environment, their widespread use and their suspected health effects. PFAS comprise more than 5,000 anthropogenic organic chemicals that have been used since the late 1940s in a wide variety of commercial products and industrial applications due to their unique surfactant and repellant properties. PFAS are persistent environmental contaminants, resistant to biodegradation, photo-oxidation, direct photolysis, and hydrolysis (OECD, 2018). PFAS are also capable of bioaccumulation and biomagnification and some congeners have a very long half-life in the human serum ranging from 2.7 to 5.3 years (Li et al., 2018). Sources of exposure to PFAS for humans may include food, drinking water, house dust, air, and breast milk for infants (EFSA, 2020). PFAS exposure has been associated to a number of adverse health effects, including immunotoxicity, hepatotoxicity, metabolic and reproductive alterations, and testicular and kidney cancer (EFSA, 2020; IARC, 2016).

The first European project that identified the presence of PFAS in waters and sediments of European rivers was PERFORCE (Perfluorinated organic compounds in the European environment, EU Research Program FP6-POLICIES, 2006). The outcomes of PERFORCE raised the environmental problem created by PFAS and were the starting point for more detailed investigations conducted by the Italian Institute for Water Research of the National Research Centre (IRSA-CNR) that ultimately led to the discovery of the PFAS contamination in the Veneto Region.

In 2011, the Italian Ministry for the Environment, Land and Sea commissioned the IRSA-CNR to study PFAS contamination in major Italian river basins (Valsecchi et al., 2015). Results of this study revealed surface water and groundwater in a large area of the Veneto Region in North-East Italy was contaminated with PFAS that were also found in drinking water samples. The results of the study were communicated to the Regional Government in late spring 2013. In July 2013, the Agency for Environmental Prevention and Protection of the Veneto Region (ARPAV) set up an environmental monitoring plan that is still ongoing to determine the extent and level of groundwater and drinking water contamination, to identify its source, and to track its development over space and time. A manufacturing plant located in the town of Trissino that produced PFAS since the late 1960s was identified as the source of water contamination. Specific studies demonstrated the vast propagation of PFAS through the phreatic table and the surface water network, due to the close interconnection between surface and ground water bodies. Overall, the contamination extended over an area of 930 km^2 located inside the Brenta-Bacchiglione river basin and involving three different Provinces (Vicenza, Padova and Verona). The groundwater contamination plume propagated across decades from Trissino up to 18 km in the east direction and up to 35 km in the south-southeast direction reaching a total estimated extension of 190 km^2. Thirty municipalities were totally or partially served by contaminated waterworks with roughly 140,000 inhabitants potentially exposed to high doses of PFAS through drinking water (Pitter et al., 2020).

During the emergency phase several PFAS were detected with range of concentration from 10 up to 25000 ng/L along the plume axis. Between July and August 2013, water treatment plants were equipped with granular activated carbon (GAC) filters that led to an abrupt reduction in PFAS concentrations in drinking water distributed by public waterworks, and the effectiveness of water treatment continued to improve so that by 2018 PFAS congeners were undetectable in the majority of samples. A comprehensive description of the management of this environmental disaster from a public health perspective can be found elsewhere (WHO, 2017).

The management of the pollution posed a number of challenges to Public Authorities. First of all, in 2013, when the pollution was discovered, PFAS were unregulated substances and no legal thresholds had been established for their levels in the different matrices, including drinking water. Moreover, knowledge on the environmental fate of PFAS and their migration through food was very limited. Other difficulties came from the absence of analytical standards and validated methods for the determination on PFAS in the various matrices. Last but not least, the complex and multifaceted nature of the contamination and its associated risks imposed the need for an interdisciplinary evaluation and decision-making process based on shared data and information, that had never been experimented before in the Veneto Region and required a great effort to be put in place.

The LIFE PHOENIX project was conceived in the abovementioned context to help overcome the several difficulties that the Veneto Region's Authorities were facing. The main aim of LIFE PHOENIX was to demonstrate how a model of governance based on a permanent inter-institutional network supported by innovative information and forecast tools can work for the prevention and mitigation of damages caused by PMOCs, with the ultimate goal to improve population health, preserve the environment, avoid negative socio-economic consequences and save public money.

SECTION 2
Specific objectives and partnership of the LIFE PHOENIX project

The LIFE PHOENIX project pursued the following specific objectives:

- To establish an inter-institutional structure in charge of assessing and managing environmental risks with possible impacts on human health
- To develop instruments and tools to aid in risk assessment, such as guidelines, information systems, forecast tools
- To raise awareness of risks related to contamination of the water resources and on prevention measures, both among stakeholders and among citizens
- To experiment innovative technologies for depuration of drinking water and irrigation water
- To promote transferability of the model of governance and its tools to other contexts.

LIFE PHOENIX was coordinated by the Veneto Region's Directorate for Prevention, Food Safety, Veterinary. Veneto Region, according to the Italian law, is a public administration with institutional commitments concerning the definition and implementation of policies in many fields, including the competence about the organisation of healthcare services. Among the regional offices, the Directorate for Prevention, Food Safety, Veterinary is responsible for the governance of public health policies, including those concerning the Environment & Health sector, through planning, direction, control and coordination of the Regional Health Service.

The project involved the following partners as associated beneficiaries:

- Azienda Zero: a regional public company that exerts a role of coordination and governance of the regional healthcare service and guarantees a centralised technical and administrative support to the Veneto Region and to the Local Health Units;
- the regional Agency for Environmental Prevention and Protection (ARPAV): a public agency established with the regional law n. 32/1996 that guarantees environmental monitoring, environmental modelling and control activities on pressure sources, with the goal to help identify and eliminate risks to humans and the environment;
- the Italian Institute for Water Research of the National Research Centre (IRSA–CNR): a public institute established in 1968 with the task of carrying out research in the areas of water resource management and protection and in the development of methodologies and technologies for water purification and treatment of waste water (urban and industrial);
- the University of Padua (UNIPD) – Biology and Engineering Departments: a public university founded in 1222, among the oldest in the world; its role in civil society and in the international context is proved by its many activities, such as the uncountable research cooperation projects promoted by professors and researchers.

SECTION 3
Actions and results of the project

3.1 GOVERNANCE MODEL FOR THE ASSESSMENT AND MANAGEMENT OF ENVIRONMENTAL ISSUES WITH A POSSIBLE IMPACT ON HUMAN HEALTH

As already stated, the main aim of LIFE PHOENIX was to build up and test a novel organisation for the integrated governance of Environment & Health issues. One of the pillars of this model was the establishment of a structured inter-institutional system based on a permanent Regional Commission for Environment & Health supported by inter-disciplinary expert groups.

The Commission for Environment & Health of Veneto Region has been established with Deliberation of the Regional Government in 2017 and has the task to assess risks for human health associated with environmental contamination and to identify the most appropriate actions to be carried out to control those risks and/or mitigate impacts. The Commission is an inter-sectorial and inter-institutional decision-making body composed of representatives of all the regional offices and Entities with competencies in the fields of Public Health, Environment, and Agriculture:

- the Director General of the Health and Social Area
- the Director General of the Area for Land Protection and Safety
- the Attorney Coordinator of the Veneto Region's Legal office
- the Director of the Directorate for Environment
- the Director of the Directorate for Soil Protection
- the Director of the Directorate for Agriculture, Hunting and Fishing
- the Director of the Directorate for Prevention, Food Safety, Veterinary
- the Director of the Unit for Integrated Water System and Water Protection
- the Director of the Unit for Protection of the atmosphere
- the Director of the Unit for Reclamation and irrigation
- the Director of the Unit for Environmental remediation and the Venice project
- the Director of the Regional Epidemiological Service
- the Director General of ARPAV
- experts from ARPAV for specific issues.

To provide the Commission with all the evaluations and analyses needed to take appropriate decisions, a permanent Technical Scientific Committee has been established with Deliberation of the Regional Government in 2018. The Technical Scientific Committee is composed by high profile technicians from public agencies and research institutions (e.g., National Health Institute, ARPAV, CNR, Universities) with role of supporting the Commission for Environment and Health on the following themes:

- identification of dedicated present and future water resources;
- identification of hazards that are potential sources of contamination for water resources intended for human use;
- identification of protective measures;
- identification of guidelines and the best technical measures available;
- identification of technologies for the mitigation of contaminants.

During the course of the project, the Commission for Environment & Health, supported by the Technical Scientific Committee and with the aid of the forecast tools developed under the operative actions, took some collegial decisions concerning the management of PFAS contamination:

- redefinition of the contaminated area: using the outputs of the action "Implementing innovative and integrated forecast tools", the groundwater contamination plume was reassessed and the area served by contaminate drinking water resources updated
- extension of the monitoring activities conducted by ARPAV: starting from 2019 the PFAS congener cC6O4 was added to the monitoring of surface and groundwater bodies, including the withdrawal points upstream of the area of collection areas for drinking purposes ("POT" points) of contamination, in order to give completeness to the level of attention on these substances.

Different tools have been developed to support the work of the Commission for Environment & Health and of the Technical Scientific Committee and will be described thereafter:

- Management guidelines and Self-analysis Check-list
- Integrated information system
- Forecast hydro-geological models.

Management guidelines and Self-analysis Check-list

The document entitled "*Guidelines to control and manage issues related to contamination from emerging pollutants and risk prevention*" summarises the innovative approach that has been successfully test within the LIFE PHOENIX project providing a structured track as well as methodological instruments to replicate this approach in different situations or contexts. The document has been published on the project's website. The guidelines are complemented with a "*Self-analysis Check-list*", a tool conceived to help institutions assess their ability to implement the proposed governance model and the resources needed to fill some gaps. The self-analysis check-list summarizes, for each specific operational, monitoring and dissemination action, what are the minimum conditions to be able to act following the model proposed by LIFE PHOENIX and what are the fundamental steps to ensure the successful outcome of the implementation at local/regional level.

Integrated information system

One of the key outputs of the LIFE PHOENIX project is an innovative information system for Environment & Health, designed to facilitate and support the risk analysis process regarding environmental risks with a possible impact on human health. This information system is composed of two components:

- a Data warehouse (DWH) that collects data extracted from multiple institutional databanks owned by different Entities
- an online geo-portal with multi-layer structure organised in several thematic areas that allows integration of the different datasets and production of various types of reports, either tables, graphs, or maps with geo-referenced objects.

The process that led from the first conceptualisation of the information system to its detailed design and eventually development was very complex and inter-disciplinary, involving experts and representatives of many different entities also outside the project's partnership.

As a first step, the project's Management Board identified the key questions the information system should be able to answer, that can be summarised as follows:

- Geographical distribution of a chemical variable
- Distribution of chemical variables related to the territorial pressures (e.g. disposal sites, wastewater treatment plants, industrial discharges, etc.)
- Distribution of chemical variables related to the territorial uses (agriculture, breeding farms, pints of water abstraction, etc.)
- Seasonal variations of a chemical variable
- Temporal trend of a chemical variable.

Starting from those key questions, the Management Board identified the existing databanks that should be interrogated to provide answers and contacted the owning Entities to assess the actual availability of information of interest and the willingness of the Entities to provide those information on a regular basis. Then an accurate analysis was carried out in strict cooperation with owning Entities to define in detail the attributes of different datasets, the variables to be extracted and uploaded in the information system, the periodicity of data transfer and the procedures to check for data completeness and accuracy.

At present, the DWH includes the following:

- a basal geographical layer
- a polygonal shapefile of municipalities
- a polygonal shapefile of river basins
- a polygonal shapefile of irrigation districts
- a geo-coded dataset of pressure sources (productive plants, incinerators, landfills, wastewater treatment plants, farms, etc), including data on type of activities, environmental authorisations, points of wastewater discharge
- a geo-coded dataset of catchment points of irrigation water, including information on the related irrigation district
- data on population size by municipality and on drinking water companies (population and municipalities served)
- a geo-coded dataset of drinking water catchment points and treatment plants
- datasets of results of official analyses carried out on several environmental matrices (soil, sludge, surface water, groundwater, wastewater, leachate, drinking water) including geo-coded sampling points
- aggregated biomonitoring data on serum PFAS concentrations in the population residing in the contaminated area, by municipality of residency.

In parallel, the Management Board and computer scientists worked together to design the statistical reporting system. It was deemed essential that the system was able to produce various types of reports, both ready-to-use and customizable upon free interrogation of the system by users. Figure 1 shows an example from the pre-established set of reports already available on the web portal.

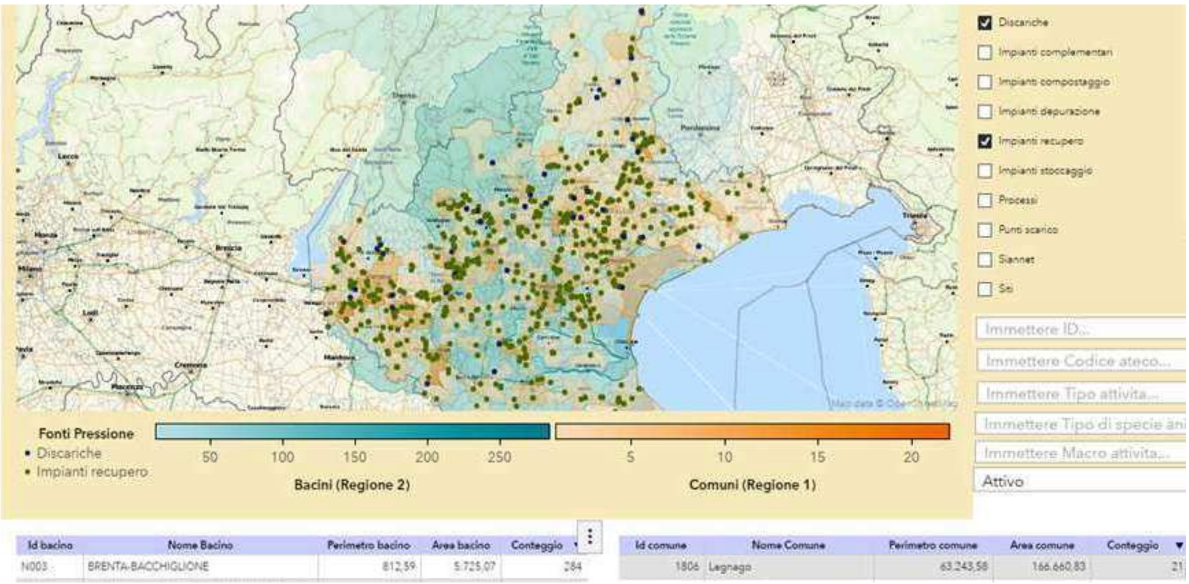


Figure 1. Example of user interface of the information and statistical system: some types of pressure sources (landfills and waste recovery plants) are geo-localized on the map of the Veneto Region in relation to river basins and municipalities.

The information and statistical system is an advanced business intelligence tool intended for use by experts belonging to Entities with competencies in the fields of environment and health. In order to ensure appropriate use and adequate protection of the data contained, access will be allowed only to users formally identified by the Entities, on the basis of a written protocol defining roles and responsibilities that will be approved by the Commission for Environment & Health.

A web-based platform based on a unified, open and flexible high-performance architecture that supports cloud data processing has been identified for the creation of the information and statistical system. The platform integrates a Data Warehouse and advanced multi-layer reporting and predictive tools.

Forecast hydro-geological models

Another key action of the project has led to the development and validation of a predictive mathematical model fed with environmental monitoring data and capable of predicting the spread of the pollutants' contamination plume in space and time. This tool will be crucial to guide risk analysis and decision-making as it allows to forecast the fate of contaminants in the environment also estimating the time the contaminants will take to reach critical targets such as drinking water catchments. The forecast model is described in detail in Section 3.2.

References

- EFSA Panel on Contaminants in the Food Chain (EFSA CONTAM Panel), Schrenk D, Bignami M, Bodin L, Chipman JK, Del Mazo J, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, Leblanc JC, Nebbia CS, Nielsen E, Ntzani E, Petersen A, Sand S, Vleminckx C, Wallace H, Barregård L, Ceccatelli S, Cravedi JP, Halldorsson TI, Haug LS, Johansson N, Knutsen HK, Rose M, Roudot AC, Van Loveren H, Vollmer G, Mackay K, Riolo F, Schwerdtle T. Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA J. 2020 Sep 17;18(9):e06223
- International Agency for Research on Cancer (IARC). 2016. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 110. monographs.iarc.fr/ENG/Monographs/vol110/index.php
- Li Y, Fletcher T, Mucs D, Scott K, Lindh CH, Tallving P, Jakobsson K. Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. Occup Environ Med. 2018 Jan;75(1):46-51
- Organisation for Economic Co-operation and Development (OECD), 2018. Toward a new comprehensive global database of per- and polyfluoroalkyl substances (PFASs): Summary Report on updating the OECD 2007 List of Per- and Polyfluoroalkyl Substances (PFASs)
- Pitter G, Da Re F, Canova C, Barbieri G, Zare Jeddi M, Daprà F, Manea F, Zolin R, Bettega AM, Stopazzolo G, Vittorii S, Zambelli L, Martuzzi M, Mantoan D, Russo F. Serum Levels of Perfluoroalkyl Substances (PFAS) in Adolescents and Young Adults Exposed to Contaminated Drinking Water in the Veneto Region, Italy: A Cross-Sectional Study Based on a Health Surveillance Program. Environ Health Perspect. 2020 Feb;128(2):27007
- Valsecchi S, Rusconi M, Mazzoni M, Viviano G, Pagnotta R, Zaghi C, Serrini G, Polesello S. Occurrence and sources of perfluoroalkyl acids in Italian river basins. Chemosphere. 2015 Jun;129:126-34
- World Health Organization (WHO) Regional Office for Europe. Keeping our water clean: the case of water contamination in the Veneto Region, Italy. World Health Organization 2017. ISBN 978 92 890 5246 7

3.2 Production and validation of the flow and transport numerical model

Introduction

The purpose of action B4.1 of the LIFE PHOENIX project was to build up, calibrate and validate a three-dimensions hydrogeologic flow and transport model for forecasting. To this end several fate and transport run simulations were performed for old (PFOA) and new generation PFAS (cC6O4 e HPFO-DA).

Numerical groundwater contaminated modelling is a powerful methodology to investigate the pollutants space-temporal evolution. In addition, it supports the decision makers with scientific and technical information.

The implementation of the model was carried out in the territory contaminated by PFAS in the Veneto region (Figure 2). The reconstruction of the pollution carried out by ARPAV, allowed the identification of the origin of the pollution – a fluorochemical plant - and the first delimitation of the contaminated territory. The extensive environmental surveys highlighted that the fluorochemical plant had an important impact on the water of large areas of the Veneto region, more than 200 square kilometers between Vicenza, Padua and Verona district. The maximum concentration value in the groundwater is over 70.000 nanogram per liter (out of source contaminated site).

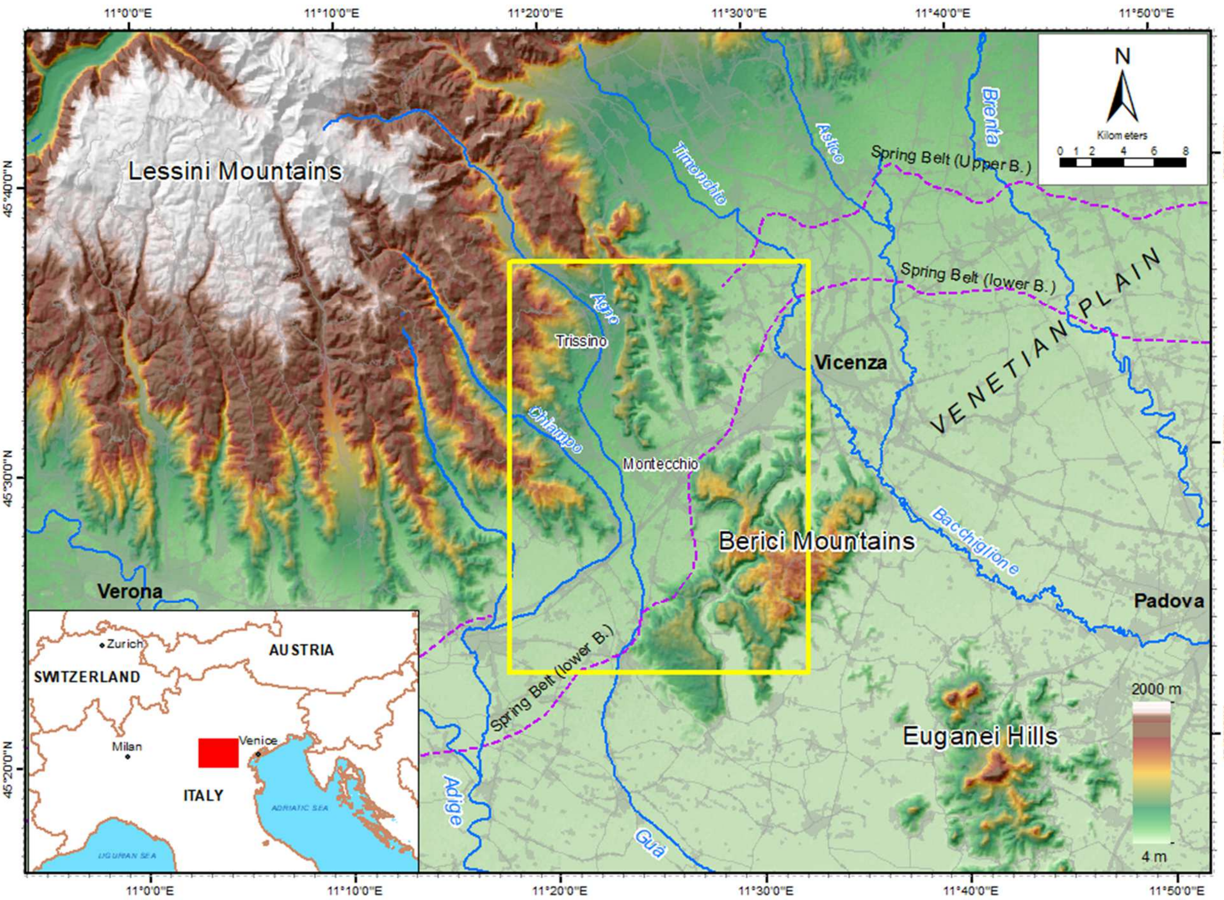


Figure 2. Geographic frame with study area.

Model implementation

A preliminary geological and geomorphological analysis is necessary to properly define the three-dimension conceptual model.

Many geological, structural, geomorphological and geophysical data supported the development of a three dimensions geologic conceptual model. Moreover, the gravel distribution provided the definition of zone with different hydraulic conductivity. In order to provide a basin-scale geological model, the alluvial aquifer has been simplified into two distinct layers. The first one represents the surface covering layer of clay that becomes thicker in the middle plain; the second one represents the porous aquifer layer composed of gravel, sand and silt.

The effective infiltration, evaluated according to the Kennesey's methodology, has been developed and integrated with the water irrigation contributes. In addition, river discharges have been implemented according to bibliography studies. Water wells, withdrawals have been divided in three main groups: withdrawals for public pipeline purposes; local withdrawals which have a flow rate >10 l/s; widespread withdrawals with a flow rate lower than <10 l/s.

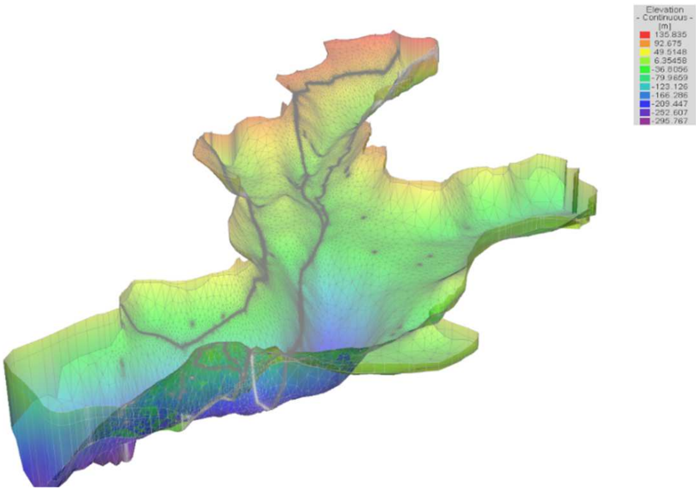


Figure 3. Bedrock geology model.

Moreover, geophysical and hydrogeological surveys supported and improved these conceptual models, which have led to a numerical model for groundwater flow and contaminant transport simulation and fate analysis. In order to improve the knowledge of sorption processes in the specific study area, experimental laboratory determination of the partition coefficients has been performed.

Calibration and validation

Flux calibration and validation processes are important steps for the modelling implementation. The calibration and the validation ensure that a model is a "true" simulation of reality. In other words, these phases are decisive to define the quality and the accuracy of the model. The main target of the flow calibration and validation processes is to limit, as much as possible, differences between measured groundwater level and simulation outcomes levels. In particular, in the final validation phase, has been used the groundwater dataset levels collected by ARPAV in the 2017s drought. The comparison process between the outputs of simulated flux model and the measured values in different hydrologic conditions ended positively. The plots of the observed and simulated water levels indicate a good correlation between the simulated and observed water levels in the area of interest. These important phases allowed to improve calibration model; besides, the input parameters (hydraulic conductivity values and areas, boundary conditions) have been refined improving the flux model and thus minimizing the statistical output error. The iterative and feedback process of refinement of the input parameters allowed to further decrease the RMS from 2.01 to 1.07 m. (Figure 4).

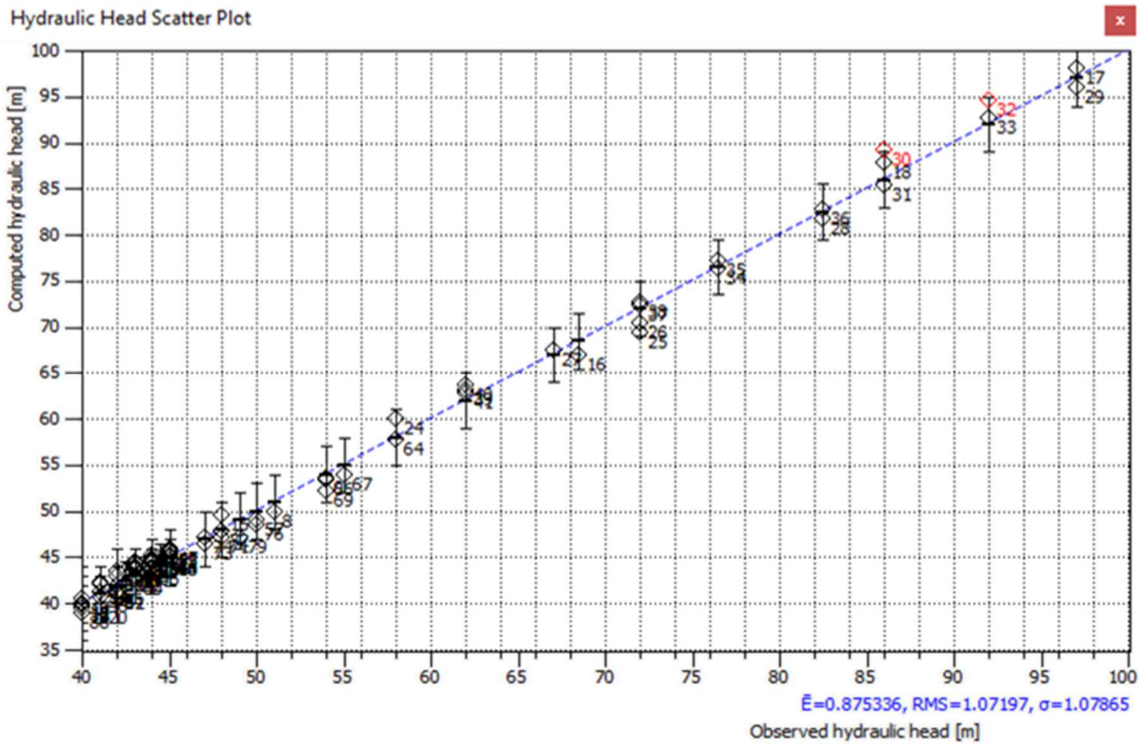


Figure 4. The deviation between observed data and computed results in validation process.

The groundwater flow field

The numerical model simulates the real trend of groundwater flow with a progressive decrease of average hydraulic heads from upstream (about 105 m a.s.l.) to downstream (29 m a.s.l.) as highlighted in figure 5.

In the upper part of the domain, in the middle valley, the groundwater contours are fairly regular, with little local variations that could indicate lateral anisotropies in the hydraulic conductivity. In the centre of the domain, close to Montecchio/Alte, there is an important flow field variation. In fact, in this area there is a significant reduction of velocity, a strong decrease of hydraulic gradients and an important divergence of the flow lines. The streamlines take two directions: southbound and eastward. The numerical model provides a possible hydrodynamics explanation about this hydrogeological condition: the significant increase of aquifer section flow area causes a decrease in flow rate and so a lower hydraulic gradient. Further at south, the concave contour lines indicate a drainage axis. In this area there is the first springs system of the Brendola stream. These results are in accordance with other freatimetric reconstructions performed in the area.

The hydraulic gradient varies between 0.03% in the lower valley and 0.8 % in the upper valley with an average value of 0.2 %.

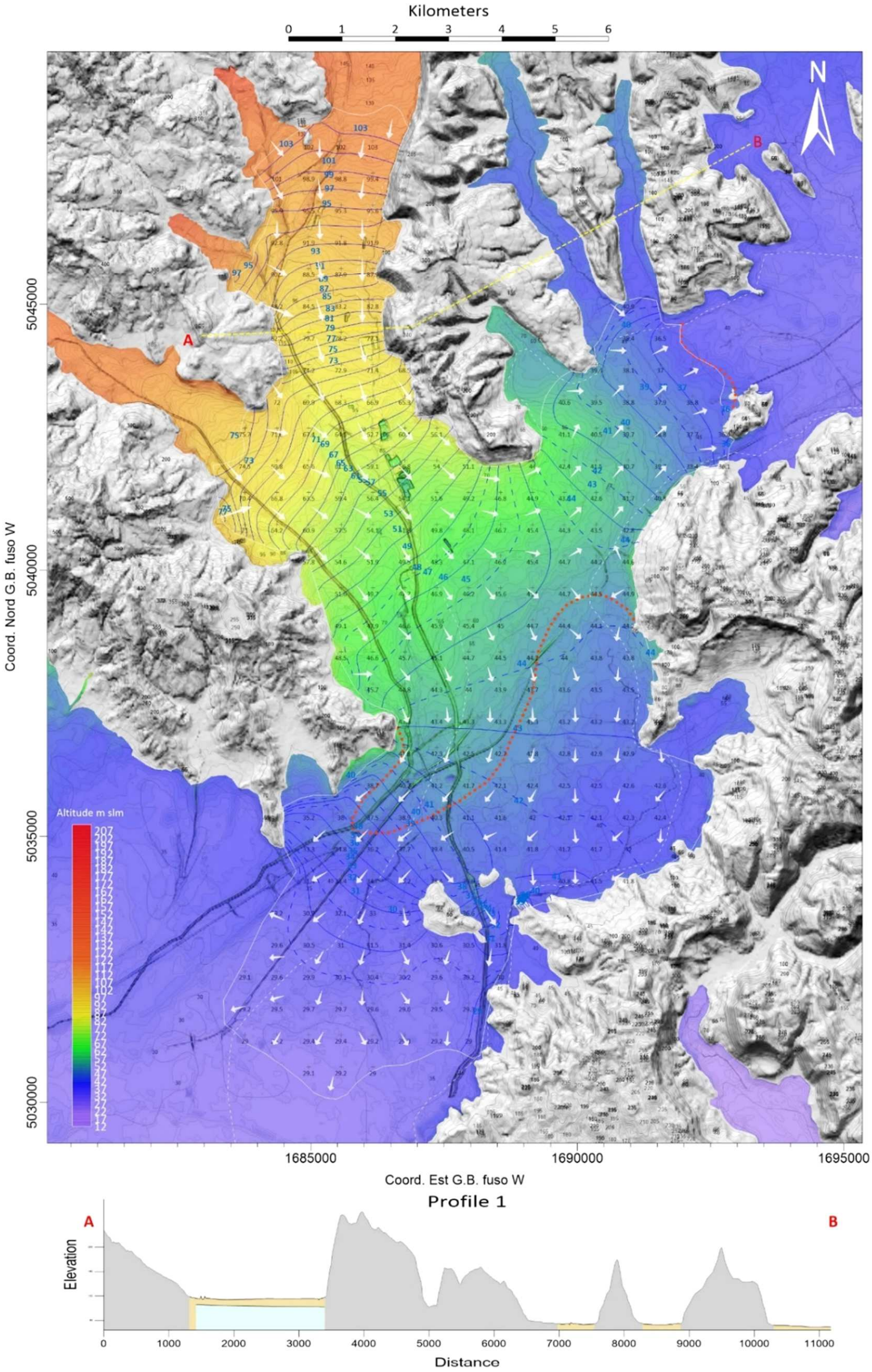


Figure 5. Contour lines and vector map of simulate groundwater flow with a hydrogeological cross section of the Agno valley.

Hydrogeological water balance

One of the most important checks performed moreover during simulation was the analysis of the overall or partial water mass balances of the model. The steady-state flow model, through the quantification of the inflows and outflows, provides a hydrogeological water balance of the alluvial aquifer system under average conditions. Water balance also provides important information on recharge and drainage factors of aquifer. The terms of the hydrological balance as average input and output flow rate are described below. The groundwater inflows from the northern sectors aquifer, equal to 3.26 m^3/s, are the main recharge factor of the hydrogeological system. The second recharge term of the hydrogeological mass balance is the rivers dispersion with a flow rate over 2.1 m^3/s. The recharge operated by effective infiltration (rainfall and irrigation) in the north of the model domain is less relevant since does not exceed 0.46 m^3/s.

The output mass flow of the model is higher than 3.9 mc/s of which 1.08 m^3/s on the east side and 2.14 m^3/s from the southern sector towards south. Finally, the outflow rate from plain springs is 0.65 m^3/s. This value indicates that the springs system plays an important role in groundwater and for the possible migration of contaminants into surface water. The withdrawal of greater wells is 1.48 m^3/s while the diffuse withdrawal is equal to 0.68 m^3/s. Wells are the most important artificial outflow of the mass balance.

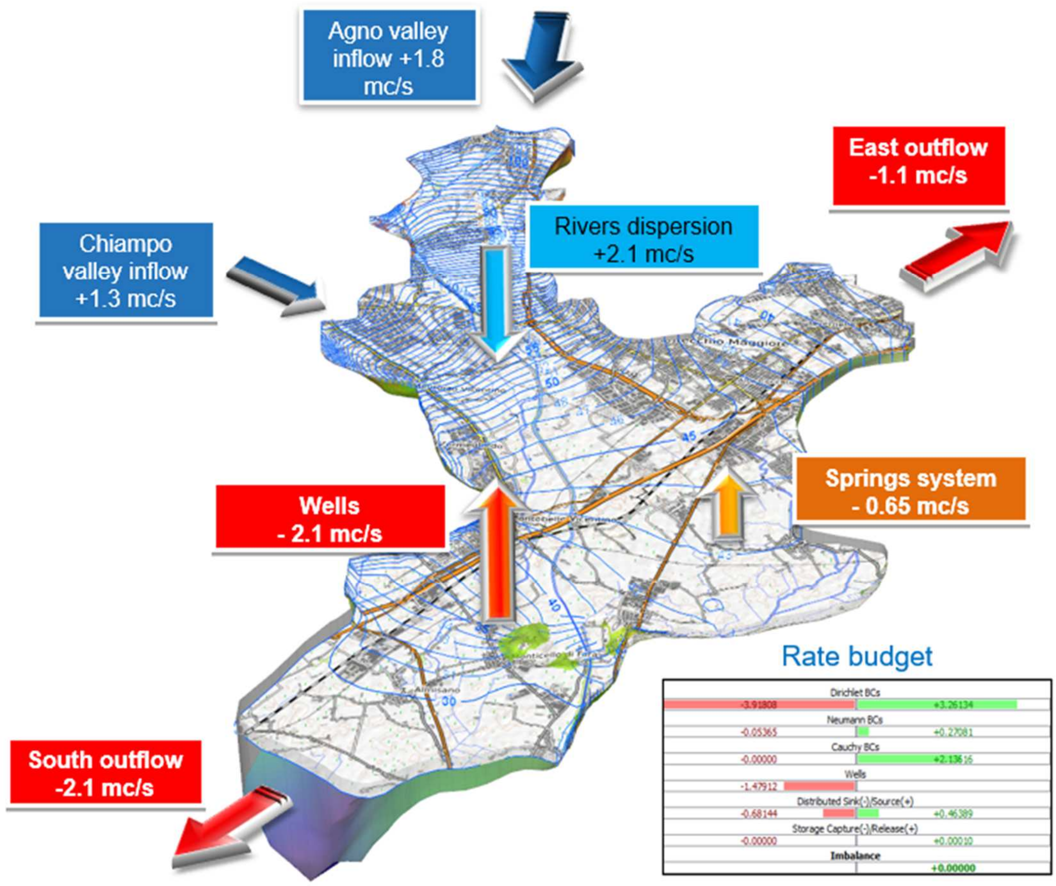


Figure 6. Terms of the hydrogeological flow balance.

Fate and transport model simulations

The last and most important phase was the implementation of the numerical transport model and the developed of transport and fate model simulations able to predict the spatial and temporal evolution of a pollution through the development of forecasting scenarios. This potential, implicit in numerical modelling, was used in the last phase of the activities by introducing various transport scenarios concerning in particular cC6O4, HFPO-DA and, finally, PFOA (used as tracer of historical pollution by PFAS).

All simulations produced are based on the reconstruction of the conceptual model of the source term and the currently available groundwater pollutant concentration data. This activity covered both predictive transport simulations from observed concentrations in groundwater (Figure 7) and development, for example, scenarios reconstructed to simulate the transport of some of the representative substances belonging to the PFAS family such as PFOA (long chain), cC6O4 and HFPO-DA (short chain). This "scenario-based" approach was necessary for two reasons:

1. The quantification of the term source of pollution (Trissino site) is not yet defined;
2. The data necessary for a complete reconstruction of the term source are not available for the past, considering that the emissions from the source site varied in time and space.

All scenarios reconstructed for the project were therefore based on the currently available data and, when the data were insufficient or missing, used assumptions which, if not confirmed, they may partially invalidate the results of the simulations. It is therefore clear that the results of these simulations present all the limits of reliability of an approach "by hypothesis" and then to be confirmed through further analysis.

The contamination plumes have form, size, and speed all determined by type of contaminant (solubility, sorption, etc...) and by the velocity of groundwater. Advection is the dominant transport process in the pollution cases studied here and, consequently, the speed of the groundwater flow is the main factor to define the speed of the pollution plumes.

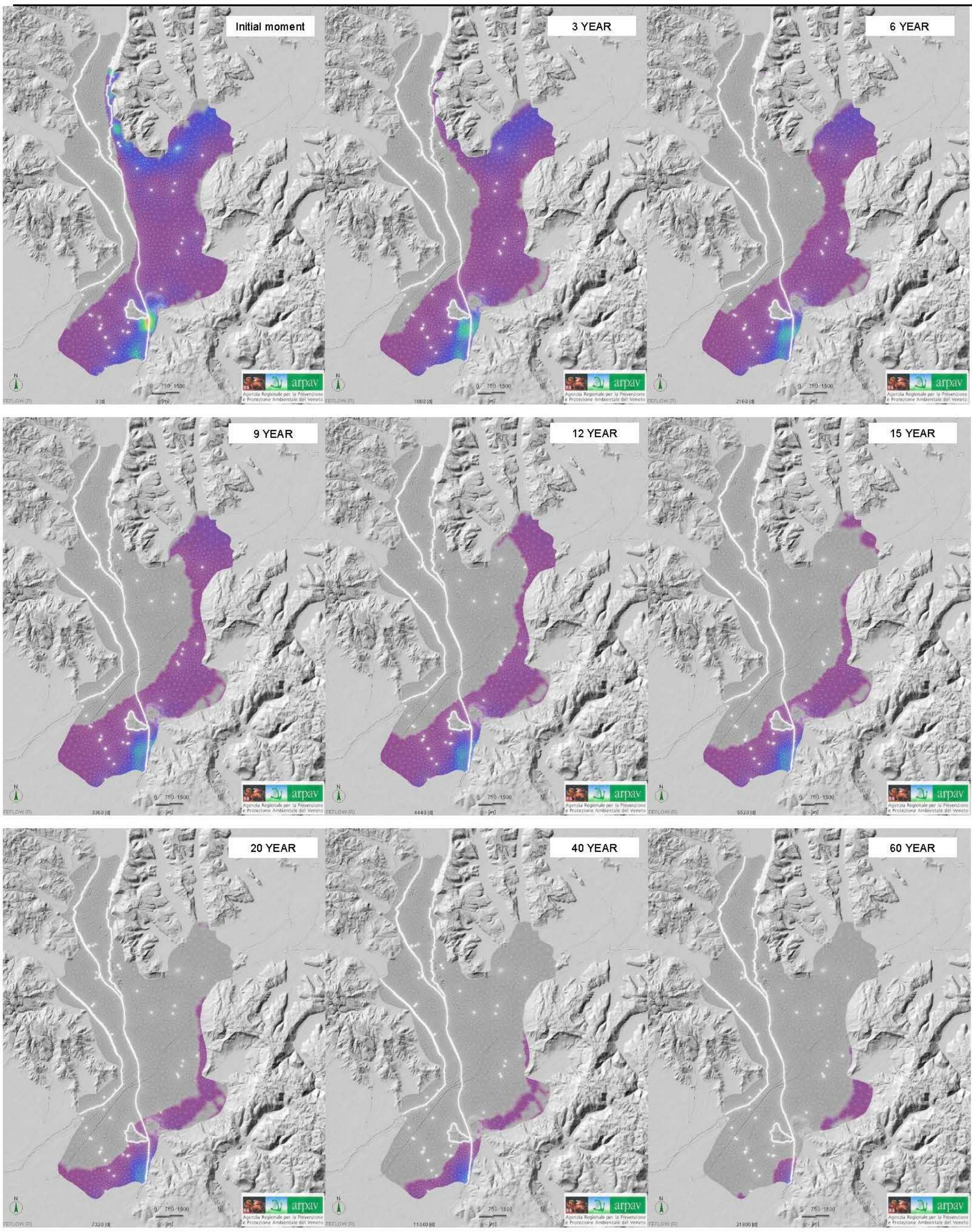
A further element to consider in the following simulations is the limit of quantification (LOQ) that is typical for each substance. By reducing this limit the ability to detect substances increases and therefore the distributions detected may be different.

The salient features and the findings of all these simulations are summarized in the final conclusions paragraph.

PFOA – forecasting scenario

This scenario simulates the progressive attenuation of the pollutant phenomenon following the complete removal of the source of contamination and the natural replacement of groundwater.

B.4.1 - PRODUCTION AND VALIDATION OF THE FLOW AND TRANSPORT NUMERICAL MODE



PFOA forecasting scenario

Figure 7. Predictive simulation of the evolution of PFOA contamination in the scenario following a complete removal of the source of pollution.

cC6O4

In this paragraph the propagation scenario of the cC6O4 in groundwater is represented.

This substance, as with the GenX, had not yet been detected when the LIFE PHOENIX project started.

This demonstrates the full potential and flexibility of the numerical modelling approach or the possibility of simulating any type of substance.

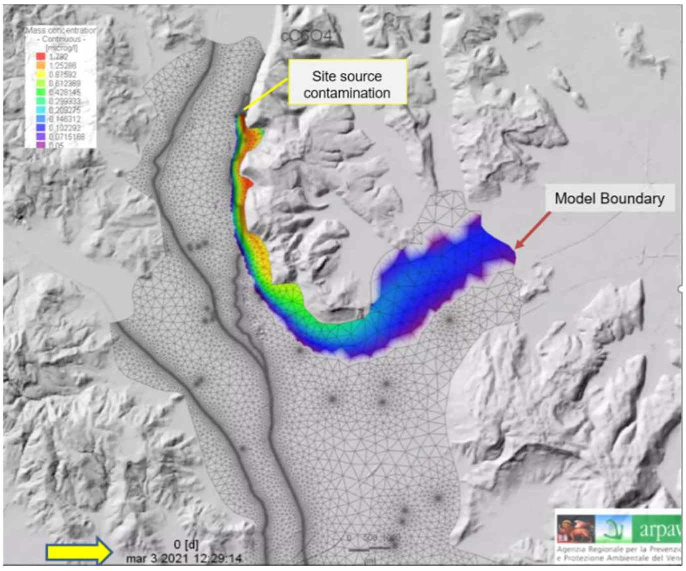


Figure 8. Simulation run of the transport of cC6O4 in groundwater. The model domain is highlighted by the mesh (the dark grey lines). Simulation time 3360 days. LOQ 50 ng/l.

The release pathway of this substance was through the contaminated subsoil. The concentration values used must be comparable to those measured. This scenario uses LOQ 50 ng/L, with different LOQ the simulation evolution plume will be different. The distribution of concentrations can be seen in the figures below with a comparison between the simulation results and the observed values.

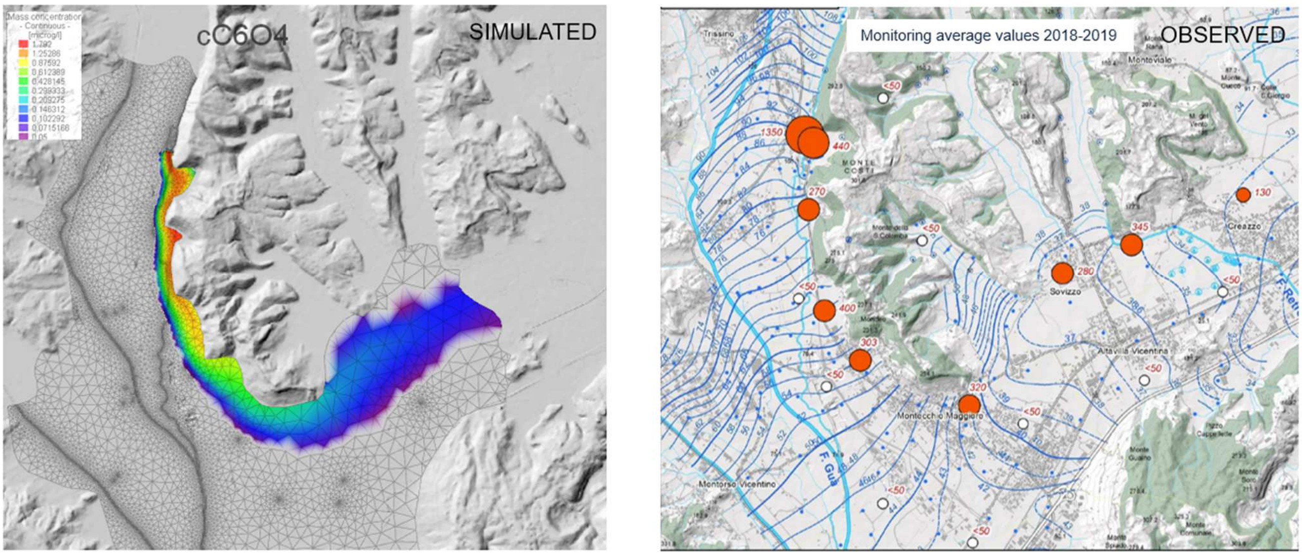


Figure 9. Comparison of simulation results with monitoring data. In the left one we can see the result of simulation (ten years run), in the right side the real measured values.

HFPO-DA

This section shows the simulation of another new generation short chain's PFAS: HFPO-DA or Genx. As in the previous case, the reconstructed conceptual model of the site source is composed only by polluted subsoil and the concentration values entered shall be coherent with the values measured by monitoring in groundwater. This scenario uses current LOQ 5 ng L. Even in this case with different LOQ the evolution of plume simulation will be different.

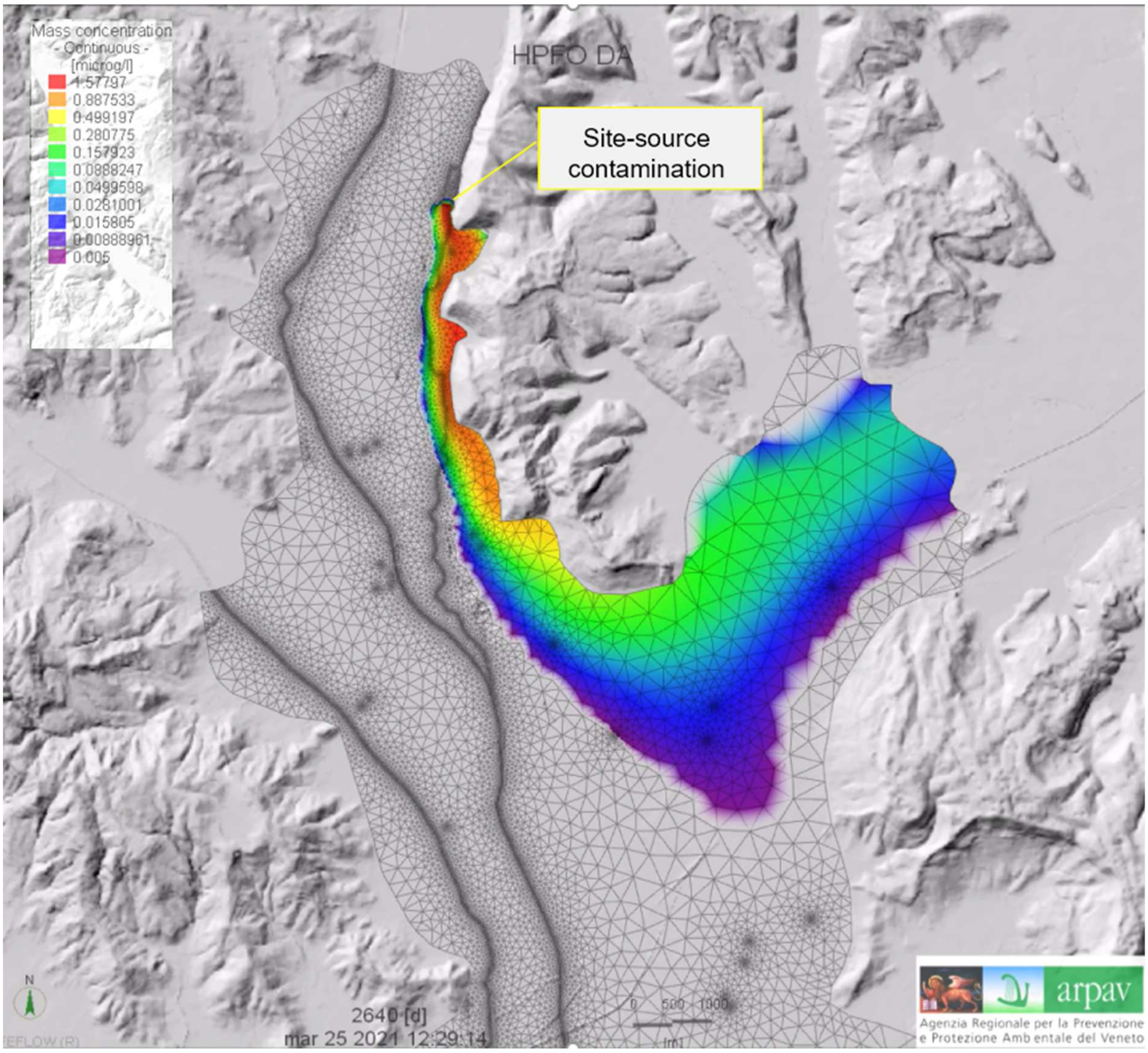


Figure 10. Transport simulation of HFPO-DA in groundwater. Simulation time 2640 days. LOQ 5 ng/l.

PFAS (PFOA)

In this reconstructed scenario, PFOA has been used as a PFAS pollutant tracer due to its greater concentration and extension in the different environmental matrices.

The implementation of the term source suffers from the limited information available also in consideration of the industrial production in the chemical plant changed significantly over time (see figure 11) and therefore, probably, also the quantities and types of emissions.

Many other factors, some of which are not well known, have had to be estimated. All these reasons make this simulation affected by a wide margin of uncertainty and therefore to be treated with caution. This uncertainty is greatest when approaching the southern end of the model. The reconstructed conceptual model of the term source is composed of two components: the polluted subsoil and the stream Poscola used as receptor of industrial discharges (Figure 12).

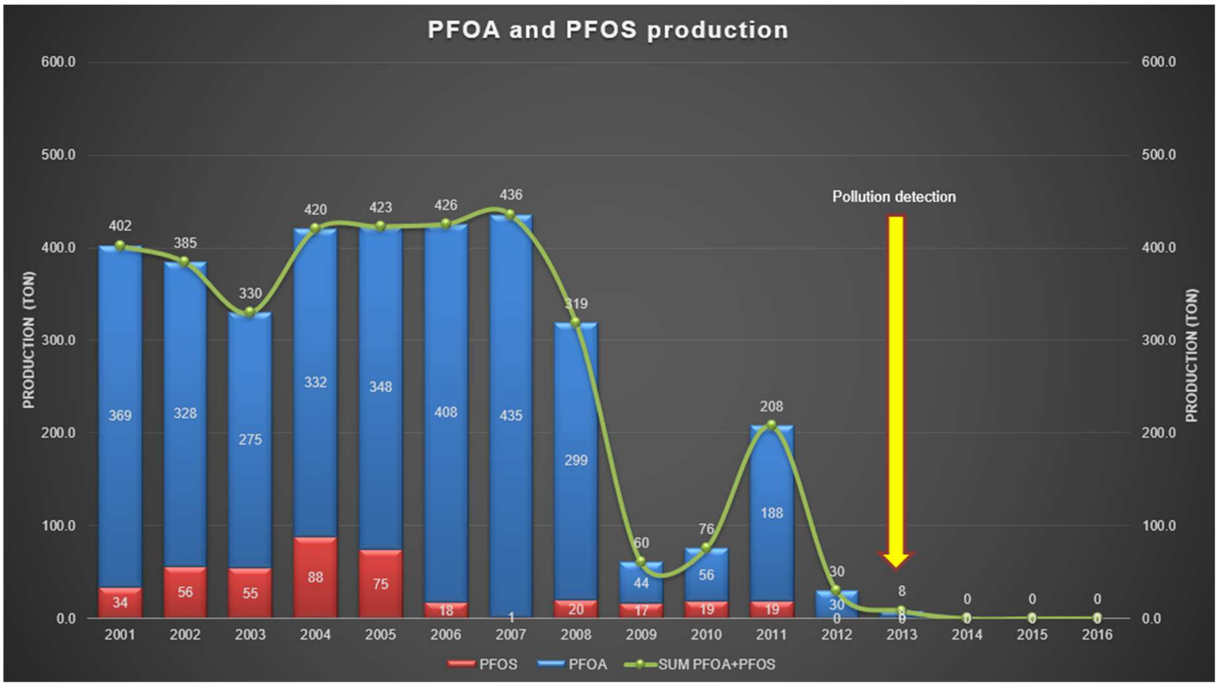


Figure 11. PFOA and PFOS production since 2001 in the fluorochemical plant of Trissino (Company data)

.

Both of these terms have varied over time and, as regards discharges on the Poscola, also in space. The simulation begins in 1967 and ends in 2013 year.

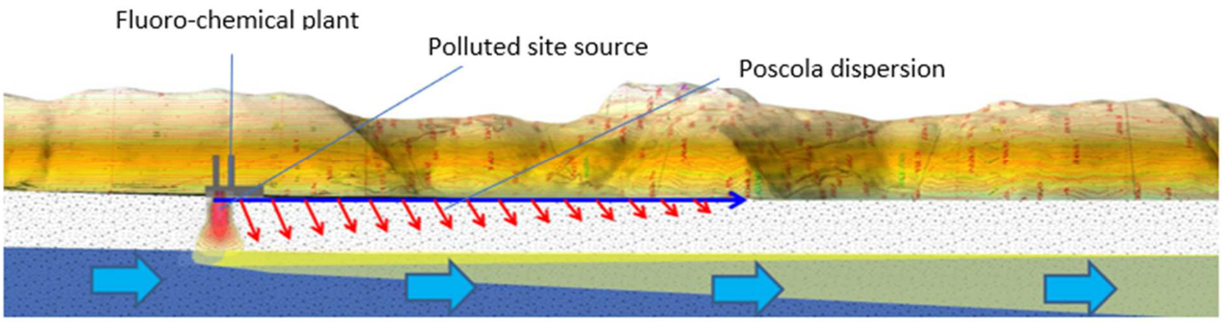


Figure 12. Conceptual model of the term source.

The following figure (Figure 13) shows the final moment of the transport simulation with the pollutant plume extended both to the east and to the south, otherwise to the new generation PFAS are present in this time only in the flow direction to the east. This is in accordance with the monitoring data detected by ARPAV in recent years.

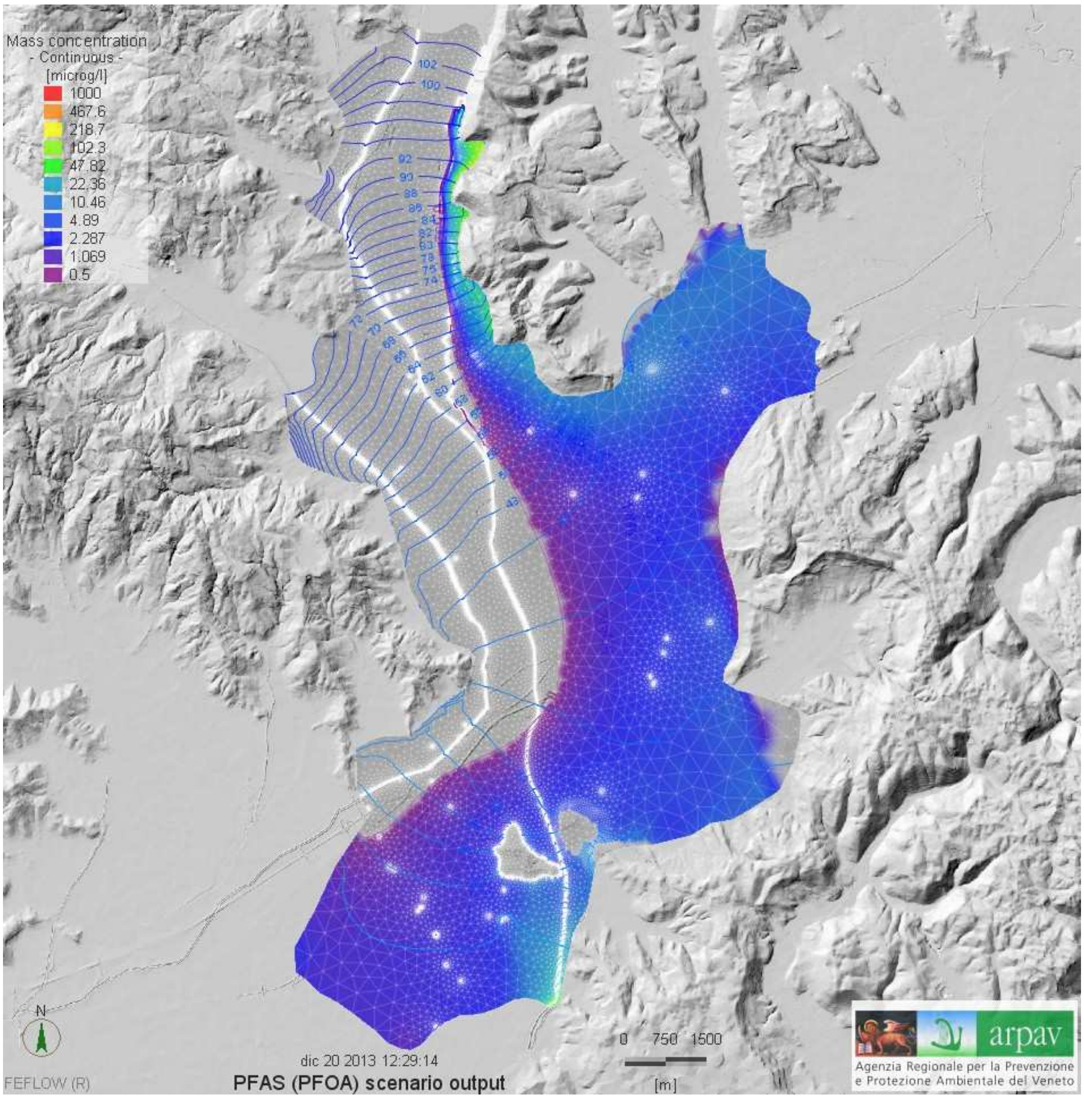


Figure 13. PFAS (PFOA) plume contamination by reconstructed emission scenario. Simulation time 16790 days. LOQ 500 ng/l.

Conclusions

A 3D finite elements numerical model (FEM) of the aquifer sedimentary basin was developed and tested for predictive purposes. Advection, diffusion, hydrodynamic dispersion and adsorption have been simulated.

Several transport run simulations were performed for old and new generation PFAS (PFOA, cC6O4 e HFPO DA). For older generation PFAS (PFOA) emission scenarios have been reconstructed on the basis of the limited information available before 2013, in order to overcome uncertainty from the source of pollution. All these reconstructed scenarios were then made with the few data currently available and resorted, when the data were insufficient or missing, to assumptions and/or simplifications that, if not confirmed, they may partially invalidate the results of the same simulations. For this reason, the results of these simulations present all the limits of reliability of an approach of this type and therefore to be confirmed through further studies.

All the run simulations allow to get important information about the PFAS contamination event such as:

1. The contamination plumes have form, size, and speed determined by source terms (concentration values and pathways emissions) and by contaminant properties (solubility, sorption, etc…). In particular, the different distribution observed appears in close relation to the different length of the molecular chain.
2. The transport simulations identify a polluting axis with greater concentration along the Trissino - Montecchio Maggiore – Sovizzo - Creazzo junction towards the east, as detected of monitoring survey by ARPAV. This finding reflects a preferential direction of pollution due to hydrodynamic field status confirmed by ARPAV groundwater monitoring.
3. PFAS pollution is plausible with an event occurring over a decades-long time scale, likely to have started more than 50 years ago. This indication is consistent with the start of industrial production in the former chemical plant and with the monitoring of pollution by ARPAV in the last 8 years.
4. The reconstructed scenarios are consistent in tracing the origin of the contamination plumes from PFAS, cC6O4 and HFPO-DA to the chemical plant of Trissino.
5. The groundwater flow and the adsorption process are the main factors to define the transport and so the fate of pollution of PFAS. As with most groundwater systems, advection is the dominant transport process in this case as well.
6. The stream near the chemical plant (used for industrial discharge), probably in the past has played an important role in the dispersion of pollution both in terms of propagation speed and extent of contamination;
7. The reconstructed simulation scenario for older generation PFAS (PFOA used as tracer) indicates that the concentration values are compatible with the vestiges of massive pollution likely continued at least until 2007. This indication is consistent with the pollution concentrations detected on the contamination front in 2013 by ARPAV.
8. The propagation kinetics analysis finds that the contamination of water bodies is occurred progressively as a distance from the pollution source. The most likely scenario, with river Poscola dispersions source, estimated (with LOQ 0.5 microg/l) the arrival of the polluted front in less of two years for the Montecchio Maggiore town, and in about ten / fifteen years later for Almisano (Lonigo).
9. The run simulation of HFPO-DA estimates the possible beginning of the contamination in 2014.
10. The total amount of contamination about cC6O4 and HFPO-DA released in the environmental from site source cannot be estimate due to the uncertainty of the source terms. The magnitude estimated is in the order of 10 kg/year.
11. The forecasting scenarios, on equal terms and considering the detection limit, indicate that natural depletion times are different for each single pollutant (please note that the pollution is a mixture of several PFAS substances). In the best-case scenario as plume of cC6O4 (low concentration, small area) with LOQ 50 ng/l, the natural depletion time, in the modelled area, is about 10 years. In the worst-case scenario as PFOA (high concentration and wide extension) it is greater than 60 years.
12. The estimated amount of PFAS released in the environmental from site source is possible only as an order of magnitude for lack of data about source term. The scenario reconstructed assumes an emission in the environment of several hundred kg per year with maximum values in the period when the chemical plant was in full production with the production discharges directly on the Poscola river.

References

- M. Mazzola, I. Saccardo e R. Cappellin, Stato dell'inquinamento da sostanze perfluoroalchiliche (PFAS) in provincia di Vicenza, Padova e Verona, ARPAV, Padova 2013.
- R. Antonelli, R. Dazzi, G.M. Mari, G. Mozzi e G. Zambon, Correlazioni litostratigrafiche e idrostrutturali nel complesso alluvionale della bassa valle del Fiume Agno-Guà e nella antistante pianura vicentina., in «Boll. Serv. Idrogeol. It., Roma», (1993).
- R. Antonelli e G.M. Mari, Carta della vulnerabilità naturale: ricerca sulla vulnerabilità naturale e sul rischio di inquinamento delle acque sotterranee nelle valli dell'Agno-Guà e del Chiampo e dell'antistante pianura., in «National Research Centre Publication», CMXLVII (1993).
- E. Pilotto, G. Ghezzi, M. Marchetti, G. Perin, G. Sandri, S. Stevanato. Studio geologico e chimico dell'inquinamento della falda acquifera nei comuni di Montecchio M., Creazzo, Sovizzo e Altavilla V., IRSEV - Istituto Regionale di Studi e Ricerche Economico-Sociali del Veneto, Venezia 1979.
- A. Sottani, L. Altissimo, S. Bertoldo, A. Schiavo, Progetto Giada. Rapporto conclusivo, UE Financial support LIFE 00 ENV/IT/00184., 2014.
- I. Saccardo, S. Cremonese, M. Sangati, G. Boso, M. Dal Col, M. Zasso, C.G. Moretto, S. Benacchio, Studio e analisi della ricarica delle falde sotterranee nei progetti di difesa del suolo - Relazione tecnica conclusiva. ARPAV, 2019
- G. Bartolomei, Il deflusso delle falde acquifere nella conoide Agno-Chiampo in base a due traccianti chimici, in «Mem. Acc. Olimpica di Vicenza», (1984).
- M. Mazzola, G. Boso, R. Gnech e M. SangatiI, Carta dei deflussi freatici nella media e bassa valle dell'Agno. Inedita, ARPAV, Padova 2017.
- M. P. Anderson, W. W. Woessner, R.J. Hunt, Applied groundwater modeling - 2nd Edition: Simulation of Flow and Advective Transport, Academic Press
- C.A.J. Appelo e D. Postma, Geochemistry, Groundwater and Pollution, A.A. Balkema 2005
- DHI-Wasy Feflow 7.3, Finite element Subsurface, Flow & Transport Simulation System, User Manual, 2020
- H.J. Diersch, Feflow - Finite Element Modeling of Flow, Mass and Heat Transport in Porous and Fractured Media, Springer, 2014

Acronym	Description
ARPAV	Veneto Region Environmental Agency
CAS	Chemical Abstracts Service
cC6O4	Difluoro{[2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl]oxy}acetic acid
HPFO-DA	Hexafluoropropylene oxide-dimer acid
LOQ	Limit of quantification
microg/L	microgram per liter, typical reporting units for analyses of micropollutants in water, equivalent to ppb Parts per billion for water samples with a density of 1 gram per centiliter (i.e., low total dissolved solids)
ng/L	nanogram per liter, typical reporting units for analyses of PFAS in water, equivalent to ppt for water samples with a density of 1 gram per centiliter (i.e., low total dissolved solids)
PFAS	Per- and polyfluoroalkyl substances.
PFOA	Perfluorooctanoic acid (C8)
PFOS	Perfluorooctanesulfonic acid

3.3 Study of PFAS fate in different environmental compartments, including edible plants

The LIFE PHOENIX project envisaged an extensive monitoring program to assess the distribution of PFAS in different environmental matrices (irrigation water, soil, plants) in agriculture areas in Veneto, with a specific focus on more mobile but shorter chain PFAS.

Collected samples were analysed by methods which were redefined and optimized at the beginning of the project. In particular, the analytical procedure to determine PFAS in vegetable samples was developed, validated and submitted for publication on a scientific journal.

Development of method for PFAS extraction from vegetal samples

A method for the determination of 12 PFAS in vegetal samples was developed to optimize the detention of short-chain PFAS (C<8) due to their higher potential to be translocated and bioaccumulated in plants than long-chain congeners. The analytical procedure, based on ultrasonic extraction, clean-up and HPLC-MS/MS analysis, determined PFAS in the different plant tissues allowing to study the PFAS distribution and partition in vegetal compartments. The proposed approach is particularly relevant in edible plant investigation because PFAS levels recorded in the comestible fractions provide information for human risk assessment due to vegetable consumption. Furthermore, data on the remaining not edible parts, intended for breeding forage, are also useful for the assessment of the PFAS transfer in the breeding trophic chain. The performance of this method was validated by analysing samples (root, stem and leaf) of reed grass. The validated procedure was then applied to vegetal samples collected in agricultural areas impacted by a fluorochemical plant discharge in Veneto.

Sample preparation

At harvest, each plant was split in their different parts: root, stem, leaf and, in case of maize plant, corn cob and corn kernel. Root was washed using tap water and deionized water to remove soil residues and dried with cleaned paper. Each part of plant was considered as a single sample, which was divided in two portions. A few grams of sample were dried in oven at 105°C for 24h to determine the percentage of humidity. The remaining sample was transferred into a food storage bag and placed in freezer at -20 °C. Shortly before extraction phase, a sufficient portion of frozen sample was thawed in oven at 60°C until the complete drying (constant weight), then crushed by grinder and wrapped in aluminium foil until analysis.

Sample extraction

The extraction was carried out according to Mazzoni et al. (2016) with minor modifications. For PFAS quantification, about 1 g (2 g for corn kernel) of dry crushed sample was placed into a PP tube and spiked with 100 µL of 40 µg L-1 SIL-IS. The extraction was then performed with the addition of 10 mL of a mixture of water and ACN (10:90 v/v) and 140 µL of formic acid followed by vortex agitation for 30 sec, sonication for 15 min and then centrifugation for 12 min (8000 rpm, 10°C). The same steps were repeated twice more by adding 5 mL of ACN and 70 µL of formic acid. After each centrifugation, supernatant was transferred within a single PP tube where 0.5 g of NaCl and 2 g of anhydrous $MgSO_4$ were added later. The PP tube was immediately shaken to prevent coagulation of $MgSO_4$, centrifuged and stored at -4°C for one night. After that, the extract was concentrated to 1 mL under a gentle stream of nitrogen, purified using a prewashed phospholipid removal SPE cartridge (Phree™) and transferred into a glass vial. 0.2 mL of extract were then transferred into an Eppendorf tube and evaporated to dryness under a gentle nitrogen stream. The residue was dissolved in 0.2 mL of a mixture of a buffer solution (2 mM ammonium acetate/5% MeOH) and MeOH (95:5 v/v). The extract was then agitated by vortex for 30 s, sonicated for 15 min and centrifuged for 2 min (3200 rpm, 10°C). After that, 100 µL of sample were transferred into a micro-vial and acidified by adding 5 µL of formic acid before the injection. Procedural blanks were included during analyses and handled in the same manner of samples.

Instrumental analysis

All samples were analysed by UHPLC-MS/MS equipped with Water Acquity UPLC BEH C18 column. Injection volume was 20 µL. Mobile phases A and B were 2 mM ammonium acetate/5% MeOH and MeOH, respectively. The chromatographic separation was achieved in 12 min with a constant flow rate of 0.3 ml min-1. The mobile phase composition varied according to the following gradient program: starting composition of 97.5% of A and 2.5% of B for 2 min, then 30% of A and 70% of B for 3.5 min and lastly 100% of B for 6.1 min.

A triple quadrupole mass spectrometer equipped with a heated-electrospray ionization (HESI-II) probe operating in negative mode was used. The mass spectrometer operated at a resolution of 0.7 Da in negative multiple reaction monitoring (MRM) mode. The Xcalibur 4.0 (Thermo Scientific) was used for instrument control, data acquisition and processing.

Confirmation and Quantification

Compound identification was performed by comparing their retention times (RT) with those of the SIL-IS (deviation ≤ 0.25%) or with the RT of the reference standards if SIL-IS was unavailable. Quantification was performed by using the isotopic dilution method. Calibration curves were prepared using ACN standard solutions with concentration from 0 to 20 µg L-1. 100 µL of each standard solution were transferred in micro-vial and spiked with 10 µL of 40 µg L-1 SIL-IS and evaporated to dryness under a gentle nitrogen stream following the same procedure adopted for the sample extracts. The residue was redissolved in 100 µL of a mixture of the buffer solution and MeOH (95:5 v/v) and finally acidified with 5 µL of formic acid before injection. Standard calibration curves were acquired before and at the end of each analytical sequence. Solvent blank samples were injected every five samples. Procedural blanks were injected at the beginning and at the end of each analytical sequence.

Method development

According to Yamazaki and co-workers (2019), samples were dried before extraction. The introduction of this step brought considerable advantages. First of all, dry samples are easier to crush than the wet one. Moreover, percentage of humidity in the analysed sample was very variable, ranging from 2% to 72%. The water content in specimen affects some extraction aspects such as the final volume of the supernatant obtained after centrifugation and the amount of salts to be added. In particular, drying of samples allowed to standardized these quantities, to obtain a smaller volume of supernatant, speeding up then its concentration, lower the interference of water in the pretreatment procedures (Xiang et al., 2017) and reduce the amount of salts to add.

The extraction procedure of Mazzoni et al. (2016) was developed for the detection of PFAS in animal tissues and sediments and its application on dry plant samples required some changes. Compared to Mazzoni et al. (2016) procedure, the extraction was repeated once more adding a higher total amount of ACN (19 mL instead of 9.5 mL) because vegetal sample tends to absorb extraction solvents. Moreover, a higher amount of salts (0.5 g of NaCl and 2 g of $MgSO_4$ instead of 0.2 g and 0.6 g, respectively) were required to prevent coagulation of $MgSO_4$ in the supernatant. In both methods, phospholipids were removed passing the concentrated extract through Phree™ cartridge. Indeed, phospholipids are essential components of biological membranes and signal transduction cascades in plants but can interfere with the analysis of PFAS (Honda et al., 2018).

In-line clean up procedure of the extracts by turbulence flow chromatography (TFC) (Mazzoni et al., 2016) was avoided and the plant extracts were analysed by direct injection. The latter injection method was adopted to improve the recovery of early-eluting short-chain PFAS, which are more bioaccumulable in edible plants (Scher et al., 2018). To achieve optimal separation efficiency of short-chain PFAS, the mobile phase gradient was optimized. In particular, at the beginning of the chromatographic run, the mobile phase was composed by a 97.5% buffer solution to improve the shapes of early-eluting peaks.

Method validation

The method was subjected to the validation procedure by analysing samples (root, stem and leaf) of reed grass (*Phragmites australis*). The procedure was verified to be satisfactory in term of matrix effect, recovery, repeatability, linearity and sensitivity. The obtained results highlighted also the matrix effects of vegetal components on the PFAS extraction and determination, mainly due to the complex composition of the vegetal tissues (e.g., cuticular waxes wrapped on the aerial parts). The obtained results are showed in detail and discussed in Ferrario et al. (2021).

Environmental monitoring results

Matching the results of the biomonitoring study to those of chemical analysis, Veneto local authority divided the contaminated area in three zonas with different health impacts: zone of max exposure (red area), of precaution (yellow area) and not directly impacted (green area). Nine sampling stations representative of the framed areas with different levels of PFAS pressures were selected (Figure 14).

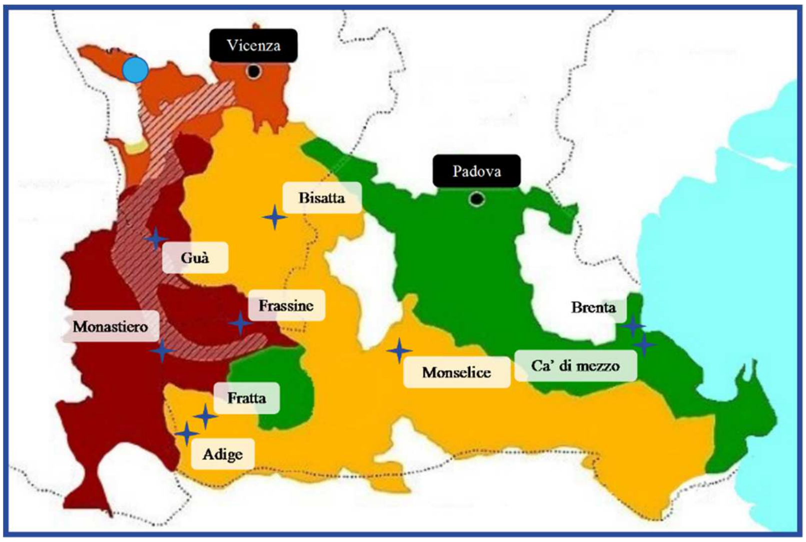


Figure 14. Sampling area. Sampling sites, emission source (blue point) and contamination plume (hatched area) are reported.

A "reference" site (located north of the impacted area) was also included. All sampling stations are characterized by the presence of a water body used for irrigation, agricultural soil, a ubiquitous aquatic vegetal species (*Phragmites australis*) and vegetable crops (*Zea mais*, *Lactuca sativa*, *Cichorium intybus*, *Allium cepa*). From May 2018 to October 2020, irrigation waters were monthly collected (n = 225), while soil (n = 119) and plant samples (n = 83) were collected twice a year during harvest season.

This monitoring plan allowed to study the variability of PFAS concentrations in irrigation waters, to verify the pollution from these substances in agricultural soils subject to irrigation and to evaluate the accumulation capacity of PFAS by spontaneous and cultivated plants.

Figure 15 shows the concentrations of PFAS recorded in the considered environmental matrices. The levels of these compounds in irrigation waters are characterized by a high variability due to the different water supply throughout the year. Events of higher contamination were occasionally recorded. On the other hand, soil pollution reflects the area classification based on different levels of PFAS pressures: the highest concentrations were indeed found in samples from the red area while the lowest levels were detected in those from the green area. This matching was not observed in plants whose contamination level was similar in the three areas, regardless of sampling site. It is also noteworthy that PFAS concentrations in edible plant samples (onion, lettuce, corn kernels) were lower than those measured in spontaneous species and never exceeded 2 ng g-1 per wet weight.

PFAS is a group of heterogeneous substances with different chemical-physical properties. The behaviour of these compound is mainly driven by the length of their fluorinated alkyl chain. In accordance with this, regardless of sampling site, irrigation water contamination was dominated by more water-soluble short-chain compounds ($C<8$), while soil was mainly polluted by more lipophilic long-chain PFAS ($C\geq8$). On the contrary, the composition of vegetal contamination was affected by the sampling site (Figure 16). In particular, plants collected in the green area were mainly polluted by long-chain compounds ($C\geq8$), released in the past, while short-chain chemicals ($C<8$), of more recent origin, were dominant in vegetable samples taken in the red area, which is directly impacted by the fluorochemical plant discharge in surface waters.

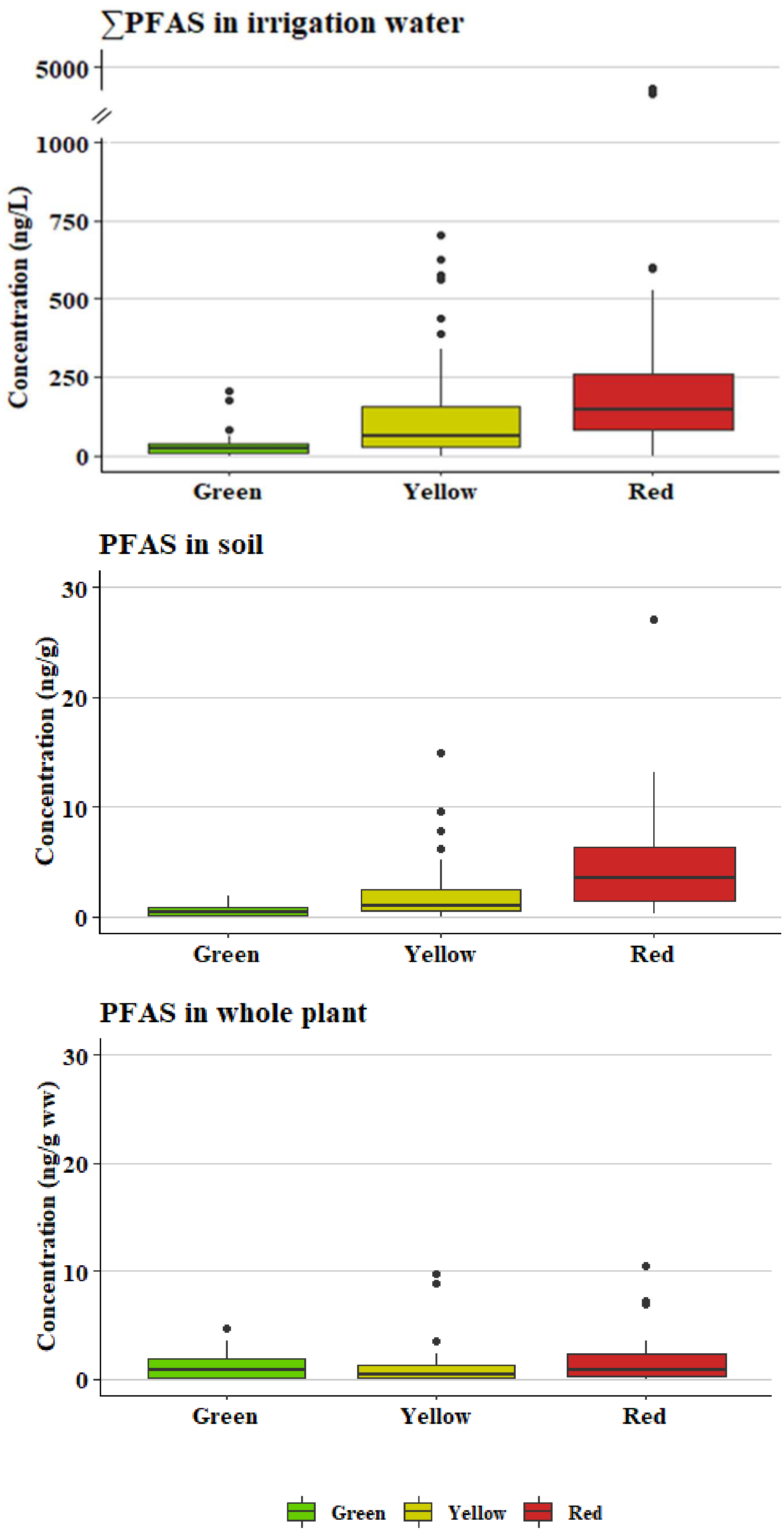


Figure 15. PFAS concentration in samples from area of max exposure (red area), of precaution (yellow area) and not directly impacted (green area).

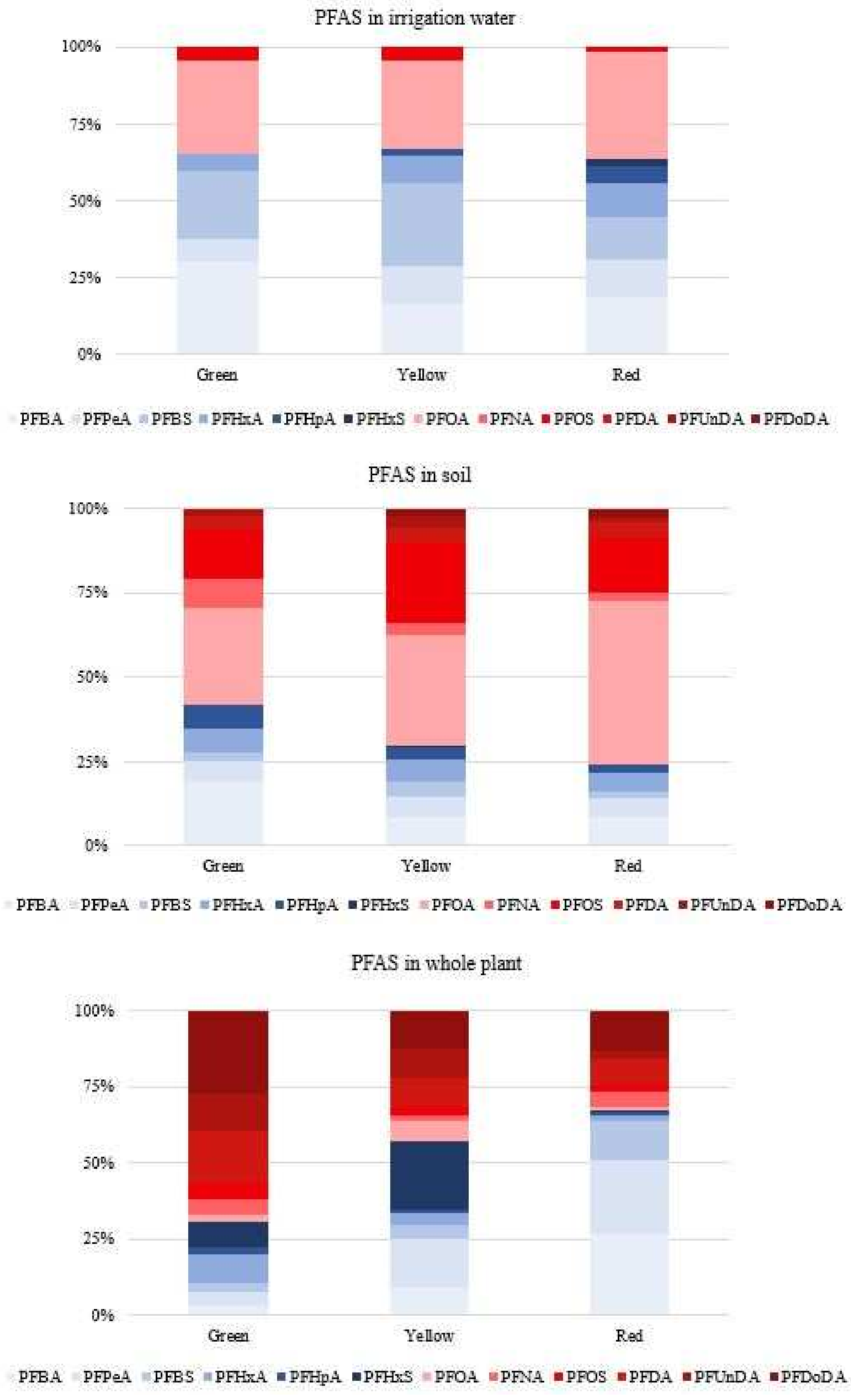


Figure 16. PFAS contamination in samples. Short- (C<8) and long-chain (C≥8) compounds are represented in shade of blue and red, respectively.

In conclusion, the monitoring activity carried out within the LIFE PHOENIX project made it possible to quantify the level of irrigation water and agricultural soil contamination in one of the areas most impacted by PFAS release. Moreover, the study was focused on PFAS determination in plants, in which these compounds were occasionally found. In particular the attention was paid on PFAS uptake in edible crops in order to provide information for human risk assessment due to vegetable consumption.

References

- Ferrario, C., Valsecchi, S., Lava, R., Bonato, M., Polesello, S., 2021. Determination of perfluoroalkyl acids in different tissues of graminaceous plants. Royal Society of Chemistry, Anal. Methods, 2021, 13, 1643 - 1650 DOI: https://doi.org/10.1039/D0AY02226H
- Honda, M., Robinson, M., Kannan, K., 2018. A rapid method for the analysis of perfluorinated alkyl substances in serum by hybrid solid-phase extraction. Environ. Chem. 15, 92–99. https://doi.org/10.1071/EN17192
- Ingelido AM, Abballe A, Gemma S, Dellatte E, Iacovella N, De Angelis G, Zampaglioni F, Marra V, Miniero R, Valentini S, Russo F, Vazzoler M, Testai E, De Felip E. Biomonitoring of perfluorinated compounds in adults exposed to contaminated drinking water in the Veneto Region, Italy. Environ Int. 2018 Jan;110:149-159. doi: 10.1016/j.envint.2017.10.026. Epub 2017 Nov 3. PMID: 29108835.
- Mazzoni, M., Polesello, S., Rusconi, M., Valsecchi, S., 2016. Liquid chromatography mass spectrometry determination of perfluoroalkyl acids in environmental solid extracts after phospholipid removal and on-line turbulent flow chromatography purification. J. Chromatogr. A 1453, 62–70. https://doi.org/10.1016/j.chroma.2016.05.047
- Scher, D.P., Kelly, J.E., Huset, C.A., Barry, K.M., Hoffbeck, R.W., Yingling, V.L., Messing, R.B., 2018. Occurrence of perfluoroalkyl substances (PFAS) in garden produce at homes with a history of PFAS-contaminated drinking water. Chemosphere 196, 548–555.
- Xiang, L., Sun, T.F., Chen, L., Xiao, T., Cai, Q.Y., Li, H., He, D.C., Wong, M.H., Li, Y.W., Mo, C.H., 2017b. A Robust Method for Routine Analysis of Perfluorooctane Sulfonate (PFOS) and Perfluorohexane Sulfonate (PFHxS) in Various Edible Crop Matrices. Food Anal. Methods 10, 2518–2528. https://doi.org/10.1007/s12161-017-0807-4
- Yamazaki, E., Taniyasu, S., Noborio, K., Eun, H., Thaker, P., Kumar, N.J.I., Wang, X., Yamashita, N., 2019. Accumulation of perfluoroalkyl substances in lysimeter-grown rice in Japan using tap water and simulated contaminated water. Chemosphere 231, 502–509. https://doi.org/10.1016/j.chemosphere.2019.05.022

3.4 BIOLOGICAL AND ECOTOXICOLOGICAL EARLY WARNING SYSTEMS: CELL BIOMARKERS OF STRESS IN A SOIL BIOINDICATOR IN PFAS-POLLUTED AREAS

This action of the LIFE PHOENIX project had the following objectives:

- the optimization of a "smart methodology" based on some "early warning" biomarkers of environmental stress, and earthworms as bioindicator organisms,
- the use of this analytical tool for the ecotoxicological assessment of some sites in the Veneto region affected by PFAS pollution.

Therefore, it was to develop an action system that can be successfully applied in cases of pollution comparable to that under study in the Veneto Region. We therefore worked to improve the effectiveness of the use of biomarkers, by implementing an adaptation of the protocols that would allow a faster evaluation of the classic procedures, in order to increase the number of processed samples and increase the significance of the data.

Terrestrial oligochetes are detritivorous organisms considered an index of good soil quality and, indicated by the OECD since 1984 among the 5 best terrestrial bioindicators (OECD 1984, 2009). These animals are bioaccumulators of PFAS, and in particular the intakes of PFOS, PFOA and PFBS have been much studied.

During the project, earthworms were sampled from the soils of the three areas identified by the Veneto Region with different degrees of pollution (Fig. 17).

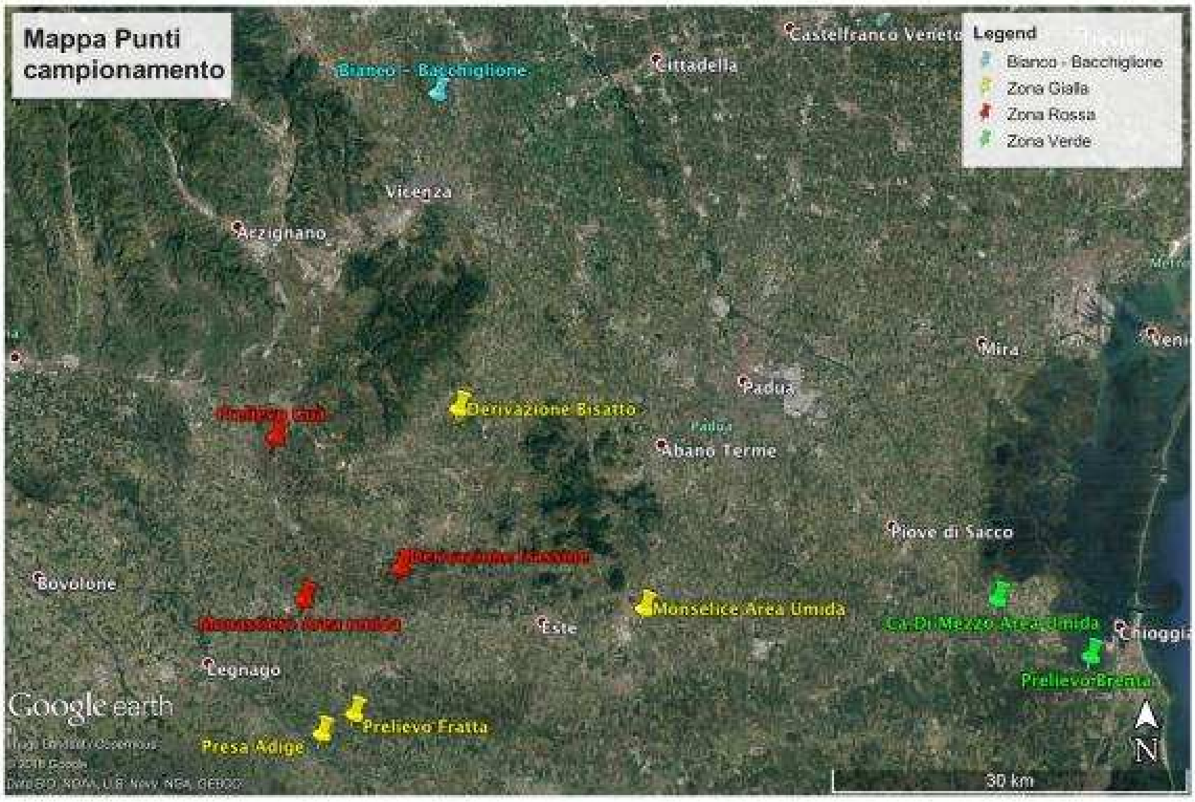


Figure 17. Map of Veneto with the ten sampling sites.

In the initial stages of the project, a series of laboratory tests were carried out in order to validate the reliability of the proposed tests in these organisms. For these tests, which preceded the activities in the field, earthworms of a species very common in European soils, *Eisenia hortensis* also known as *Dendrobaena veneta* (Fig. 18), purchased in specialized shops and coming from uncontaminated farms, were used. The earthworms found in the sampling sites were instead transported in the laboratory with their soil and kept alive.

For each specimen we proceeded:

a) to the recovery, using physiological methods (Brousseau et al., 1994), of samples of the coelomic liquid from which the coelomocytes used to test five biomarkers were isolated,
b) to dissect each organism into three portions.
c) to the use of tissues of these three portions for the determination of PFAS levels, the establishment and implementation of the biobank, and the maintenance in the freezer at -80 °C of sample aliquots for any subsequent analysis.

The analyses performed on coelomocytes concerned the five biomarkers indicated in the project: cell mortality, stability of lysosomal membrane, production of reactive oxygen species (ROS), total antioxidant capacity (TAC) and DNA fragmentation.

The cell mortality test is based on the integrity of the plasma membrane, the cellular component that allows the maintenance of cellular homeostasis, separating the intracellular environment from the extracellular one and ensuring the selective transport of the molecules that have to enter or leave the cell itself. By the cell mortality test, the loss of this function is highlighted, indicating that the cell is dead. The analysis was conducted by optical microscopy, testing the Cell Drop tool (De Novix Inc., Wilmington, DE, USA) in order to increase speed and efficiency of the test. In figure 19, the dead cells are those coloured in blue due to the entry into the cytoplasm of the Trypan blue dye.

Figure 18. Specimen of *Eisenia hortensis* also known as *Dendrobaena veneta*.

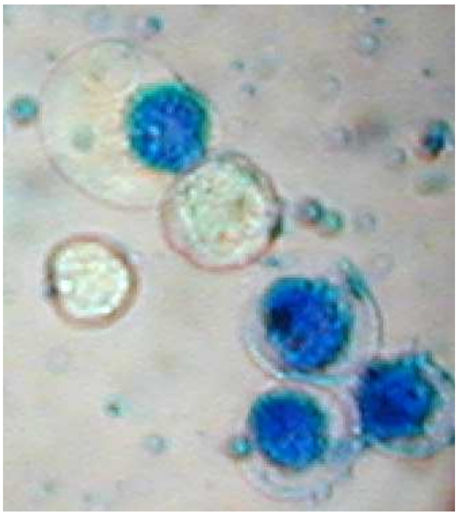


Figure 19. Cell mortality test with Trypan Blue dye: blue cells are dead, white cells are viable.

The second biomarker evaluates the stability of lysosomal membrane, which is believed to be a generalist measure of cellular stress (Martinez-Gomez *et al.*, 2015). In lysosomes with an unstable membrane, enzymes such as hydrolases leave this intracellular structure and react with the substrates present in the cytosol, damaging cellular homeostasis. Optical microscopy analysis was performed by optimizing the original method (Weeks and Svendsen, 1996) to reduce execution timing. In figure 20 the cells that develop the red colour are those in which lysosomes are damaged, and therefore no longer able to retain the Neutral Red dye that can thus diffuse into the cytoplasm.

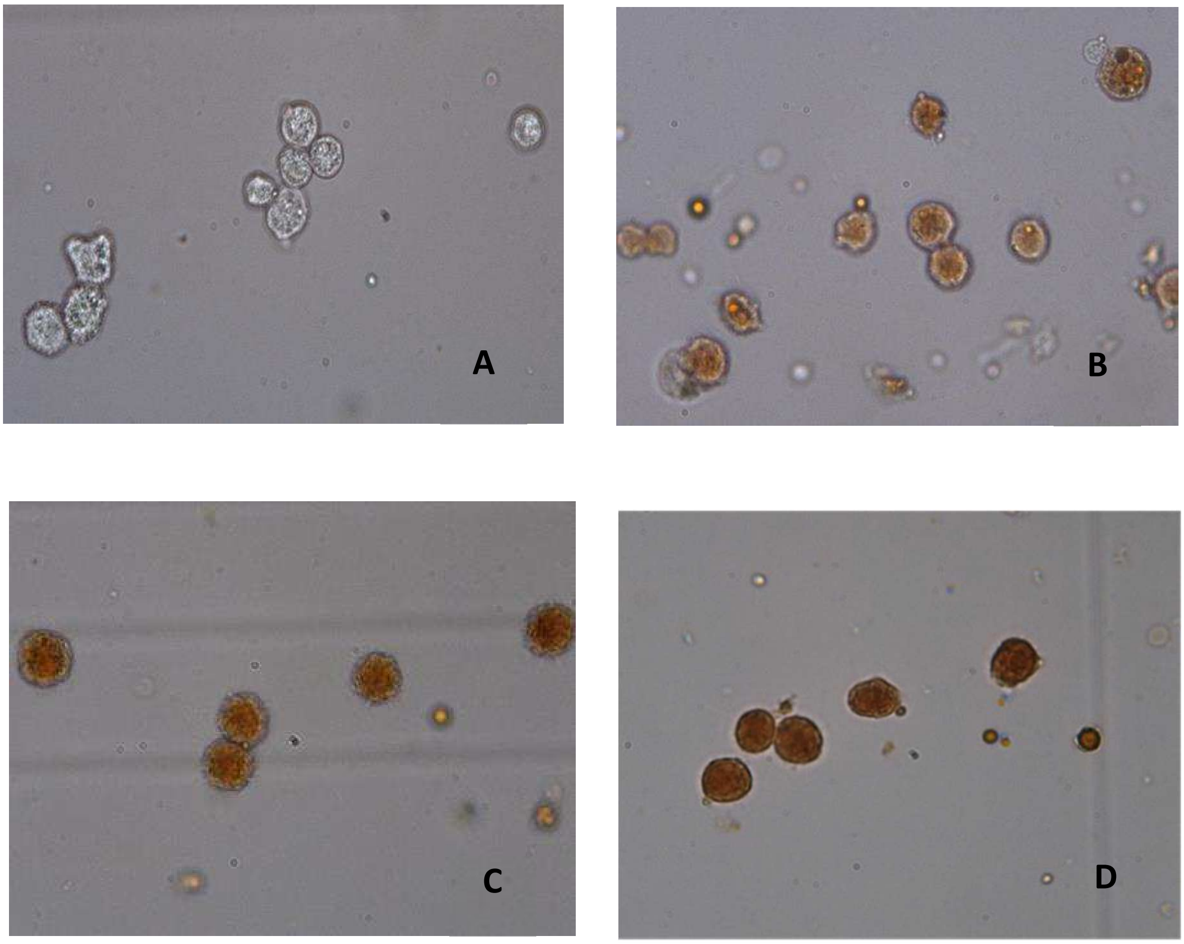


Figure 20. Lysosomal membrane stability test with the neutral Red dye. In the four images, from A ($T_{0\ minutes}$) to D ($T_{60\ minutes}$), we can observe the increase in the red coloration of the cells over the time. Magnification 400× in white light microscopy.

ROS, such as superoxide and hydroxyl radicals, hydrogen peroxide, and various organic peroxides, are normally produced during major cellular metabolic reactions, but their rate of formation can easily increase when an organism is exposed to various exogenous factors, including environmental pollutants. If ROS are produced in excess, an imbalance is generated between their production and elimination, increasing the risk of oxidative stress that can lead to damages to biological macromolecules and finally to cell death (Bonato et al., 2020). ROS production was evaluated by optical fluorescence microscopy (Fig. 21) and the use of specific probes for different cellular districts (dihydroethidium for cytosol and nucleus, dihydrorodamine for mitochondria), implementing the quality of the analysis and the number of processed samples thanks to the use of 96-well plates (instead of single sample reading) and the Operetta CLS automatic image reader, equipped with Harmony software (PerkinElmer Inc., Waltham, MA, USA).

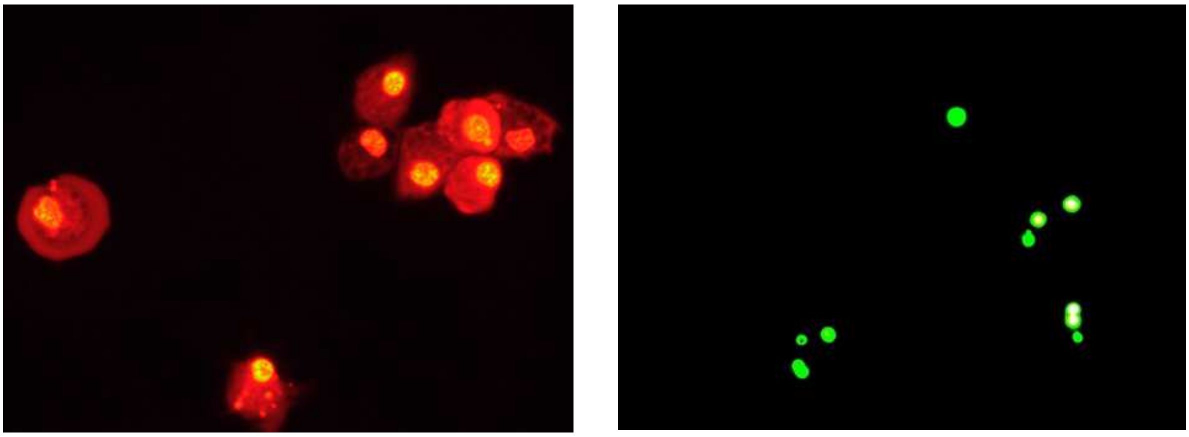


Figure 21. ROS production in coelomocytes. On the left, the dihydroethidium probe marks with a red fluorescence the ROS that are forming in the cytoplasm and nucleus; on the right, the dihydrorodamine probe with a green fluorescence the ROS that are forming in mitochondria. 400× magnification in fluorescence.

The TAC evaluates the ROS elimination activity of the complex of antioxidant molecules present in the cell. Antioxidants can be small molecules such as glutathione and vitamins, or enzymes such as superoxide dismutase, catalase and glutathione peroxidase. The antioxidant activity of individual molecules is very often evaluated, but the method we used allows to have a more general picture of the possibilities that cells can put into play to defend themselves against the negative effects produced by ROS. Moreover, the development of this method on the specific biological model allowed to increase the number of analyses (in UV/VIS spectrophotometry), thanks to the use of 96-well plates (instead of reading on a single sample).

The last considered biomarker is the DNA fragmentation, an index of genotoxicity and cytotoxicity produced by chemical and physical agents, analysed by gel electrophoresis and optical fluorescence microscopy. The method, known as Comet Assay as the damaged DNA appears with a comet shape after electrophoresis, is very long and laborious, and therefore we aimed to increase the number of replicates simultaneously analysed using 96-well plates and same instrumental support used for the ROS evaluation (Operetta CLS automatic image reader equipped with Harmony software).

Thanks to the activities under controlled laboratory conditions, it was possible to work on the finalization, on *Eisenia hortensis*, of the methods related to the five chosen biomarkers. In particular, for four of them (cell mortality, lysosomal membrane stability, ROS levels and total antioxidant capacity) we were able to optimize the methods in relation to the specific model organism (earthworm). The optimization of the DNA damage method took much longer than expected, in addition to providing results that were lower than expected, and therefore was not used for the subsequent ecotoxicological assessment of the various environmental situations affected by PFAS pollution.

The experimental laboratory activity also served to evaluate the possibility of using the biomarkers selected in the project as a smart methodology of environmental stress, exposing earthworms for 30 days to mixtures of multiple PFAS (PFBA, PFBS, PFPeA, PFHxA, PFHpA , PFNA, PFDA) at the concentrations detected following environmental monitoring carried out (in the order of ng/L). This treatment did not produce any detectable biological effect using the considered biomarkers. While this datum is positive from an ecotoxicological point of view, because it indicates that exposure to these pollutants for a short time (acute exposure), at the environmental concentrations currently present in the Veneto region, does not produce immediate cell damages in animals experiencing this adverse condition, on the other hand it does not give full-scale information, in which earthworms are chronically exposed to PFASs. In fact, following this treatment, the accumulation of the individual PFAS in earthworm tissues is extremely low, unlike earthworms sampled in nature (which were chronically exposed to these pollutants) that have significantly higher accumulation levels.

To have confirmation of our hypothesis, we carried out laboratory tests by exposing specimens of the same species to much higher concentrations of PFASs (in the order of μg/L and mg/L), obtaining both an accumulation of PFAS and a significant variation of the different biomarkers compared to the controls (specimens not exposed to the pollutant) only in earthworms exposed to the highest concentrations. For example, earthworms exposed to concentrations of PFOA and PFBS of the order of mg/L (i.e. 10^6 times higher than those found in polluted environments) showed a significant accumulation of these two PFASs in the coelomocytes already after 14 days of exposure (8.5 μg PFOA/g of dry weight and 400 μg PFBS/g of dry weight), whereas specimens exposed for 30 days to concentrations of PFOA and PFBS of the order of μg/L (i.e. 10^3 times higher than those found in polluted environments) did not show any accumulation of PFASs. Simultaneously with the accumulation of PFAS there was a variation of biomarkers in accordance with evidence of coelomocyte damage in earthworms exposed to PFBS, such as a stability of the lysosomal membrane three times lower (compared to controls), a halving of the TAC and an increase in the formation of ROS in the cytoplasm (+ 100%), in the nucleus (+ 300%) and in the mitochondria (+ 700%).

Therefore, experimentation under controlled laboratory conditions has confirmed that PFASs can cause significant damage to the body, but only after acute exposure to very high concentrations of these pollutants (which most likely will never be found in the environment) or following of prolonged exposure over time, which favours a slow but progressive accumulation of these substances within the animal's body. It also confirms the validity of the selected biomarkers, as their variations are consistent with a toxicological picture in which the entry of PFAS into the cell causes an increase in ROS production in all districts, and this condition causes damages not only to cell structures, such as the plasmatic and lysosome membranes, but also to the proteins of the antioxidant system, which is therefore not adequately able to counteract the production of these cytotoxic elements. In conclusion, although the selected biomarkers have not proved useful for the evaluation of acute stress from short-term exposures at environmental concentrations, they can certainly represent a smart methodology in contexts of chronic environmental stress.

This hypothesis was further confirmed by the results obtained in the ecotoxicological assessment carried out on the ten sampling sites, using earthworms as bioindicator organisms and four biomarkers. The most relevant results were obtained from three of these ten stations, where high levels of PFAS were not always present in the soil or water, but the resident earthworms showed a conspicuous accumulation of PFAS in their body.

In particular, in the earthworms of station 4, located in an area that the Veneto Region indicated as focus area (yellow), the highest levels of PFHxA, PFHpA, PFDA and PFUnA were detected during the sampling of May 2019 (Fig. 22). This corresponds to high levels of cell mortality, ROS production in all cellular compartments (cytoplasm, mitochondria, and nucleus) and total antioxidant capacity, with increases ranging from 250% to 850%, with respect to normal values. These four PFAS therefore exert a generalized response of the various biomarkers in the earthworm, but their coexistence at high levels does not allow us to identify specific early warnings for each of them.

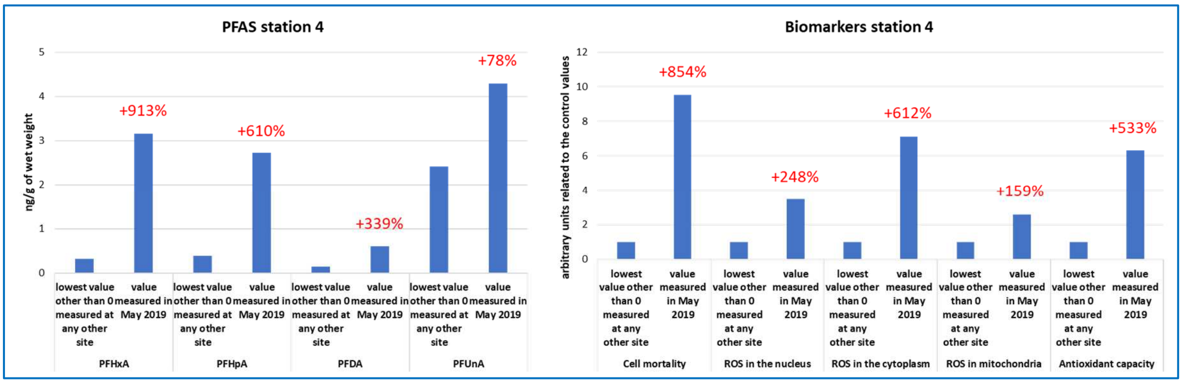


Figure 22. Levels of PFAS and biomarkers in earthworms of station 4 sampled in May 2019. The highest levels of PFHxA, PFHpA, PFDA and PFUnA correspond to high levels of cell mortality, ROS production (in all cellular compartments) and total antioxidant capacity.

In the earthworms of station 8, located in an area that the Veneto Region had indicated as the area of maximum exposure (red), the highest levels of PFBS were detected during the sampling of June 2020 (Fig. 23). This corresponds to high levels of ROS production at mitochondrial level (+250%). This biomarker can therefore be proposed as specific early warning for PFBS in the specific bioindicator organism.

In the earthworms of station 9 (also in the red area) the highest levels of PFOA, PFPFDoDA and PFOS were detected during the sampling of May 2019 (Fig. 24). This corresponds to high levels of cell mortality, with an increase of 800%. Therefore, cell mortality can be proposed as a specific early warning for these PFAS in the specific bioindicator organism, and in particular of PFOA which is the one present in greater quantities.

The highest levels of PFNA were detected in station 9 earthworms during the October 2019 sampling (Fig.25). This corresponds to high levels of ROS production in the nucleus (+250%). This biomarker can therefore be proposed as specific early warning for PFNA, using the earthworm as a bioindicator organism.

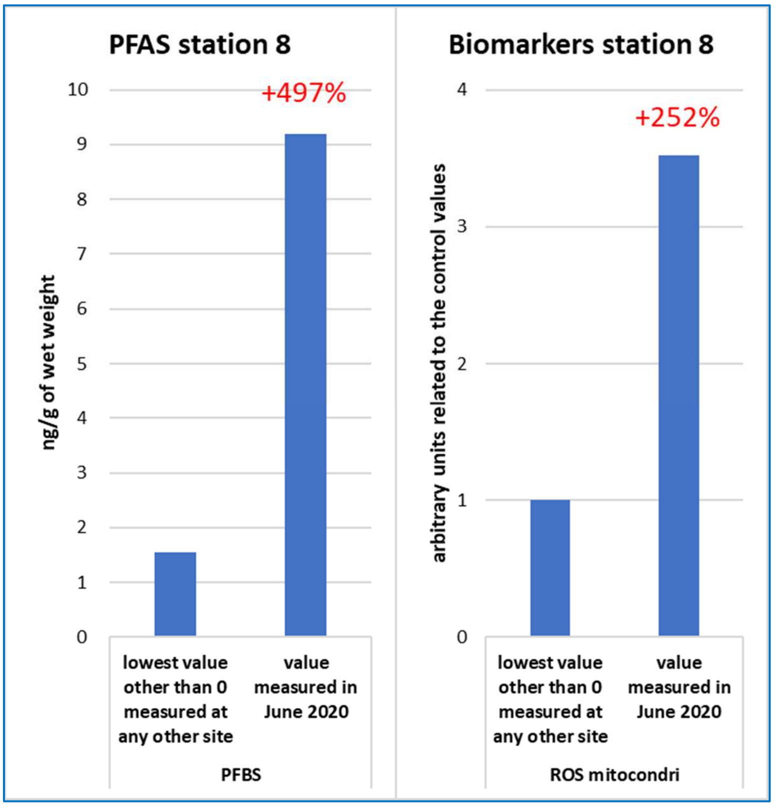


Figure 23. Levels of PFAS and biomarkers in earthworms of station 8 sampled in June 2020. The highest levels of PFBS correspond to high levels of lysosomal fragility and ROS production in mitochondria.

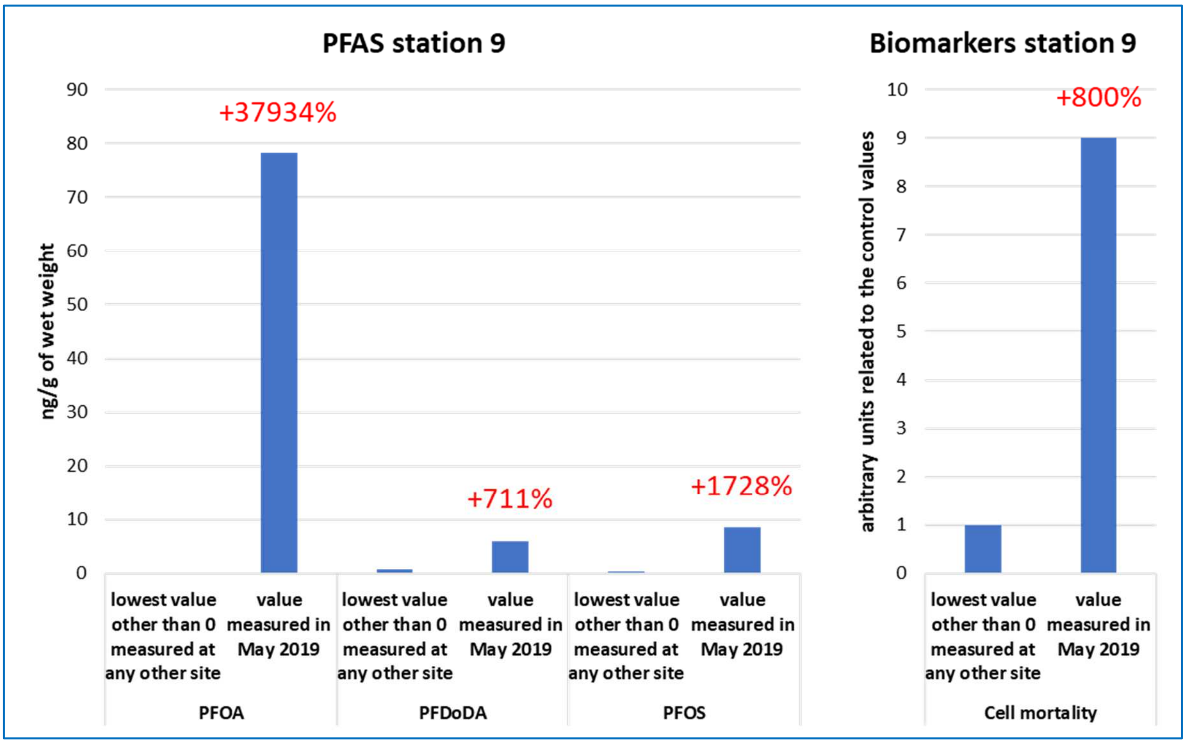


Figure 24. Levels of PFAS and biomarkers in earthworms of station 9 sampled in May 2019. The highest levels of PFOA, PFDoDA and PFOS correspond to high levels of cell mortality.

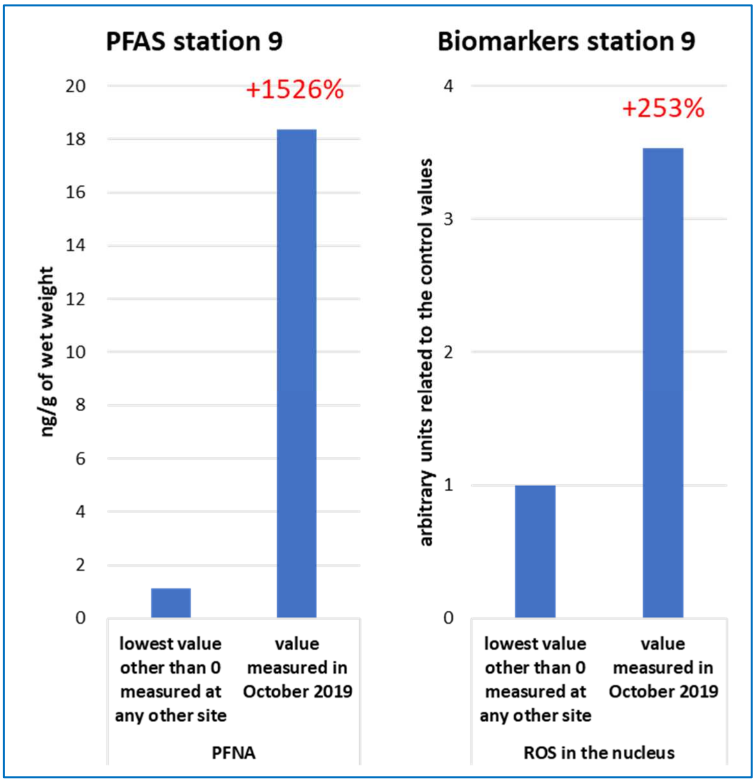


Figure 25. Levels of PFAS and biomarkers in earthworms of station 9 sampled in October 2019. The highest levels of PFNA correspond to high levels of lysosomal fragility and ROS production in the nucleus.

In conclusion, we can confirm the effectiveness and usefulness of using this set of biomarkers as a smart methodology for assessing the environmental impact by PFAS. Although it has been used in the specific reality of the Veneto region, we believe that it can also be applicable to larger areas, thus constituting a methodological approach that can be replicated in other European and world contexts. The tool that we developed is obviously implementable, for example by improving the part relative to the Comet analysis method which.

References

- Bonato, M., Corra, F., Bellio, M., Guidolin, L., Tallandini, L., Irato, P., Santovito, G., (2020) PFAS Environmental Pollution and Antioxidant Responses: An Overview of the Impact on Human Field. Int. J. Environ. Res. Public Health 17(21), 8020; https://doi.org/10.3390/ijerph17218020
- Brousseau, P., Fugère, N., Bernier, J., Coderre, D., Nadeau, D., Poirier, G., M. Fournier (1997) Evaluation of Earthworm Exposure to Contaminated Soil by cytometric assay of coelomocytes phagocytosis in Lumbricus terrestris (Oligochaeta), Soil Biology and Biochemistry 29, 681-684. https://doi.org/10.1016/S0038-0717(96)00029-6
- Martínez-Gómez, C., Bignell, J., Lowe, D. (2015) Lysosomal membrane stability in mussels. ICES Techniques in Marine Environmental Sciences vol 56, 41pp. http://dx.doi.org/10.25607/OBP-240
- OECD (Organisation for Economic Co-operation and Development) (1984). Earthworm, acute toxicity test. OECD Guidelines for testing of chemicals No. 207, OECD Paris.
- OECD (Organisation for Economic Co-operation and Development) (2009). Bioaccumulation in terrestrial oligochaetes. Proposal for a new guideline for OECD Guidelines for the testing of chemicals. OECD Paris.
- Weeks, J.M., Svendsen, C. (1996) Neutral red retention by lysosomes from earthworm (Lumbricus rubellus) coelomocytes: A simple biomarker of exposure to soil copper. Environmental Toxicology and Chemistry 15, 1801-1805. https://doi.org/10.1002/etc.5620151022

3.5 TEST OF INNOVATIVE METHODS FOR WATER PURIFICATION: ION EXCHANGE RESINS FOR DRINKING WATER AND CONSTRUCTED WETLANDS FOR IRRIGATION WATER

In the LIFE PHOENIX project, two innovative methodologies have been tested and developed to mitigate the spread and concentration of PerFluorinated Alkyl Substances (PFAS) in drinking water and surface water used for irrigation. For drinking water ion exchange resins have been employed, while for irrigation water, constructed wetlands were tested in two different conditions: in a small pilot plant and on a larger scale in a wet area of real size.

Ion exchange resins for drinking water

The pilot plant

The technology of ion exchange resins (Zaggia et al., 2016)has been identified and tested because, unlike actived Carbon Beds, can also be adopted for specific types of short-chain PFAS such as PFBS (perfluorobutanesulfonic acid) and PFBA (pentafluorobenzoic acid). Furthermore, the resins have the possibility of being regenerated and reused several times directly in the plant. In this way there could be an environmental advantage, as well as an economic advantage compared to the technology of the activated carbon filters.

The experimentation took place at the drinking water distribution plant of Acque Veronesi (s.c.a.r.l.) (Fig. 26), near Lonigo (Vicenza), which has both a polluted pit, no longer used, because it's contamination of PFAS, and a technical room available for setting up the pilot plant and for all logistical needs (electricity, water supply, etc.).

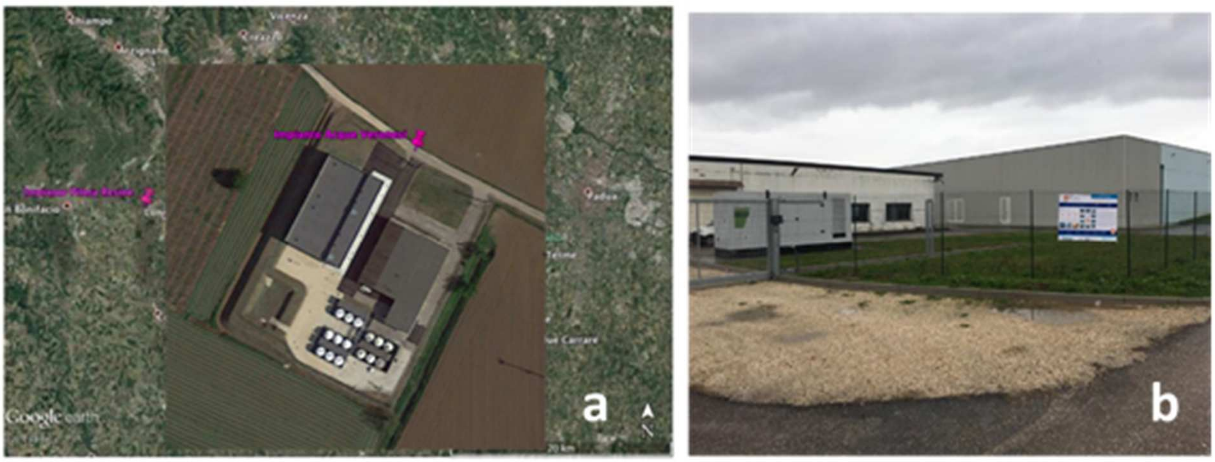


Figure 26. Acque Veronesi (s.c.a.r.l.) Plant near Lonigo (Vicenza): a) aerial view; b) Site of the pilot plant with ionic exchange resins

The pilot plant uses water with a minimum flow rate, allows measurement and regulation using field instruments and reduces the effects of interference with walls or by-pass. The size of the implant allows for transport and the resin is easily replaceable or regenerable.

As we can see from figure 27, the system is completely automated, consisting of a tank (filter) filled with 50 liters of ion exchange resin and equipped with valves that allow the management of the service and regeneration phases. A 200/L tank for the regenerating solution was installed next to the resin tank. The raw water is filtered before it passes through the resin bed exiting the treated water connection. Once the resin saturation is reached, the valves switch to the regeneration position: the inlet of raw water is stopped and the regenerating solution is pumped into the spent resin bed. Once the resin regeneration is complete, the implant returns to its service position.

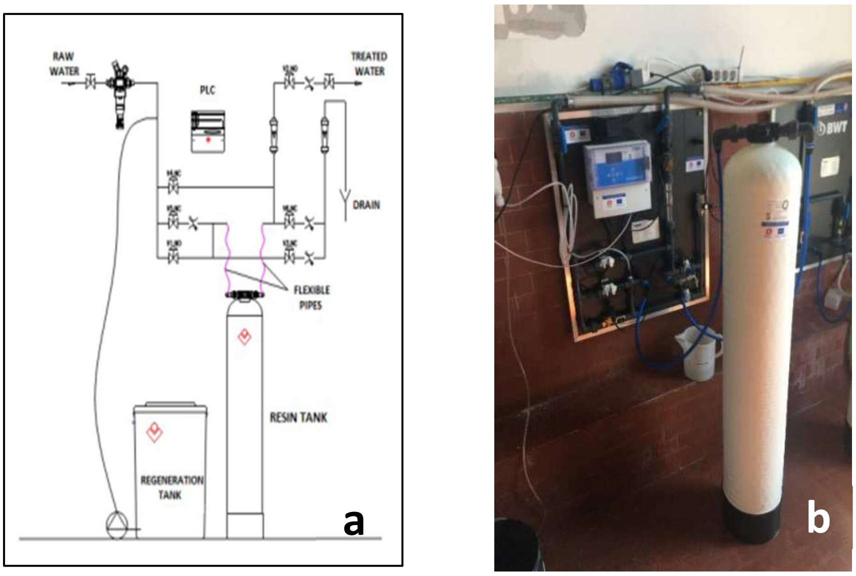


Figure 27. Pilot plant: a) control panel; b) regenerable ion exchange resins

A second plant was added to the system, however, equipped with a filter with non-regenerable ion exchange resins in order to have more data to compare (Fig. 28).

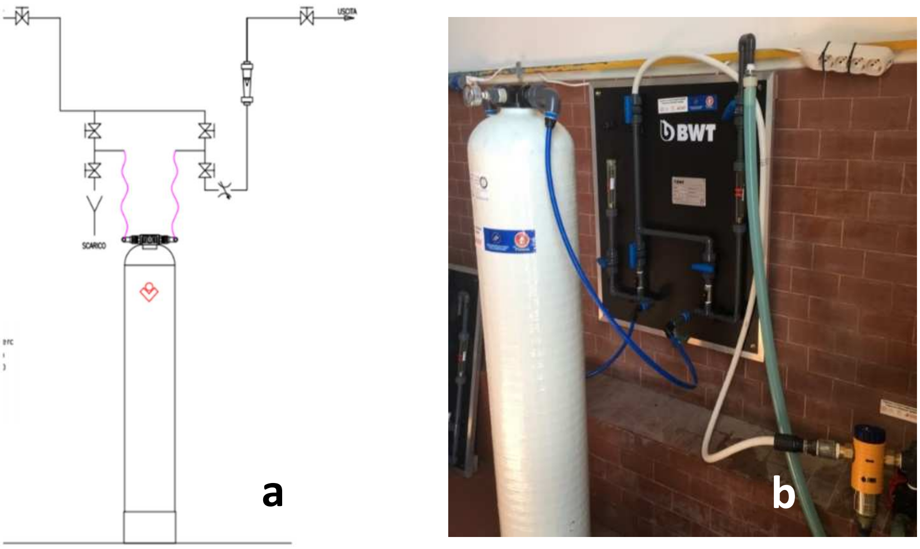


Figure 28. Pilot plant: a) control panel; b) non-regenerable ion exchange resins

Efficiency test

During the various tests carried out, the water was sampled at the inlet before the filter, and separately at the outlet from the filters of the two different types of resins and from the activated carbon system filter. Thus, three different types of samples were collected. In all the samples the concentration of PFAS was analysed, the efficiency rate of the resins and of the activated carbon calculated with two parameters:

- the *Time of use* of each method before the breakthrough (the moment in which the pollutant comes out from the filters),
- the *Adsorption Index* (AI - Adsorption Index) which allows to determine the amount of contaminant removed from a unit mass of filter from the beginning of the test to the "breakthrough", that is, before the contaminant escapes. The formula used is the following: AI = $M_{c,b}/M$, where $M_{c,b}$ is the mass of contaminants removed in the "passage" and M is the mass of the filters (Acque Veronesi s.c.a.r.l.).

Results of the tests carried out

The tests were carried out at intervals of 7-10 days from October 2019 to February 2020 for a total of 110 days. For each test, 12 samples of water matrices both for incoming and outgoing water were used, on which the analyses of the PFAS species within the samples were carried out (Tables 1 and 2):

- *Resin Usage Time*: Our ion exchange resin system has been shown to work for 34 days prior to "breakthrough".
- *Adsorption Index* (AI): During the 34 days of operation our system treated 378 m^3 of water with an average inflow concentration of 382.6 ng / L and removed 144.62 mg of PFAS with an AI of 3.024. The activated carbon system had a use time of 91 days before breakthrough, where it treated 933 m^3 of water, removing 345.3 mg of PFAS with an AI of 3.74. The daily adsorption of PFAS by the ion exchange column is 4.25 mg, that of the activated carbon column is 3.79 mg.

Conclusion

Both technologies have proved to be valid for the purification of drinking water, with a similar adsorption index. Although the data are comparable to those of activated carbon, the positive use of resins allows us to broaden the spectrum of methodological approaches related to the PFAS pollution problems of drinking water. In particular, ion exchange resins could solve some adsorption problems, by activated carbons, of molecules with a low number of carbon atoms, such as PFBA and PFBS and more, which have largely replaced the 8-atom PFAS (PFOA and PFOS), now banned.

In conclusion, from the data obtained from our experimentation we could state that the use of ion exchange resins could be considered a promising technology with interesting and more advanced results than the use of activated carbons. However, this technology requires at the moment further fine-tuning to extend the duration of their time of use (e.g. optimization of the saturation matrix) and to reduce costs for regeneration activities. In the future, thanks to these results, plants could be experimented in which these two technologies will be combined.

Table 1. Ion exchange resin pilot plan: concentration of PFAS (ng/L) in the inlet water during the experimentation from 24 October 2019 to 11 February 2020

Sampling date	PFBA	PFPeA	PFBS	PFHxA	PFHpA	PFHpS	PFHxS	PFOA Linear*	PFNA	PFDA	PFOS Linear*	TOTALE
24/10/2019	63	40	53	35	7	<5	12	131	<5	<5	18	359
31/10/2019	72	35	66	30	<5	<5	9	124	<5	<5	22	358
07/11/2019	70	46	67	43	8	<5	13	154	<5	<5	24	425
15/11/2019	45	40	62	40	7	<5	16	160	<5	<5	28	398
21/11/2019	57	36	64	32	8	<5	11	146	<5	<5	19	373
29/11/2019	46	35	65	40	7	<5	11	144	<5	<5	19	367
06/12/2019	58	41	61	47	7	<5	14	130	<5	<5	20	378
20/12/2019	46	35	65	40	7	<5	11	144	<5	<5	19	367
30/12/2019	42	33	52	35	6	<5	11	152	<5	<5	17	316
16/01/2020	47	28	67	30	8	<5	11	141	<5	<5	28	360
28/01/2020	42	27	55	32	9	<5	7	129	<5	<5	15	316
11/02/2020	58	31	57	32	9	<5	13	133	<5	<5	17	350

* *Perfluoroalkyl substances with linear chains of carbon atoms greater than 70%.*

Table 2. Ion exchange resin pilot plan: concentration of PFAS (ng/L) in the outlet water during the experimentation from 24 October 2019 to 11 February 2020

Sampling date	PFBA	PFPeA	PFBS	PFHxA	PFHpA	PFHpS	PFHxS	PFOA Linear*	PFNA	PFDA	PFOS Linear*	TOTALE
24/10/2019	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	0
31/10/2019	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	0
07/11/2019	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	0
15/11/2019	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	0
21/11/2019	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	0
29/11/2019	10	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	10
06/12/2019	29	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	29
20/12/2019	46	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	46
30/12/2019	56	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	56
16/01/2020	56	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	56
28/01/2020	65	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	65
11/02/2020	37	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	37

* *Perfluoroalkyl substances with linear chains of carbon atoms greater than 70%.*

Phytoremediation of irrigation water

The pilot plant

The LIFE PHOENIX project was one of the first projects at a European level and the first at an Italian level to address the problem of perfluorinated compounds (PFASs) in water for irrigation purposes, testing low-impact and low-cost methods, such as phytodepuration, to mitigate naturally the concentration of these pollutants in the irrigation waters.

A first step of the study involved the setting up of a phytoremediation pilot plant to test the adsorption and storage capacity of PFAS by an aquatic plant, which was ubiquitous in Veneto, such as *Phragmites australis*, commonly called straw of swamp, a plant that grows where water is available in abundance.

The plant was located in Lonigo (Vicenza) at the garden center "Vivai Dall'Ava", which provided us technical and logistical support in order to carry out our tests. The main function of our pilot plant was to capture the PFAS from a contaminated well, which already existed within the nursery. The plant (Fig. 29) consists of two plastic tanks: the first, smaller (Fig. 29a) is used as a water storage tank (approximately 1 m^3) and has the function of feeding the main tank (volume of water about 3 m^3, Fig. 29b), containing *P. australis* in the central section.

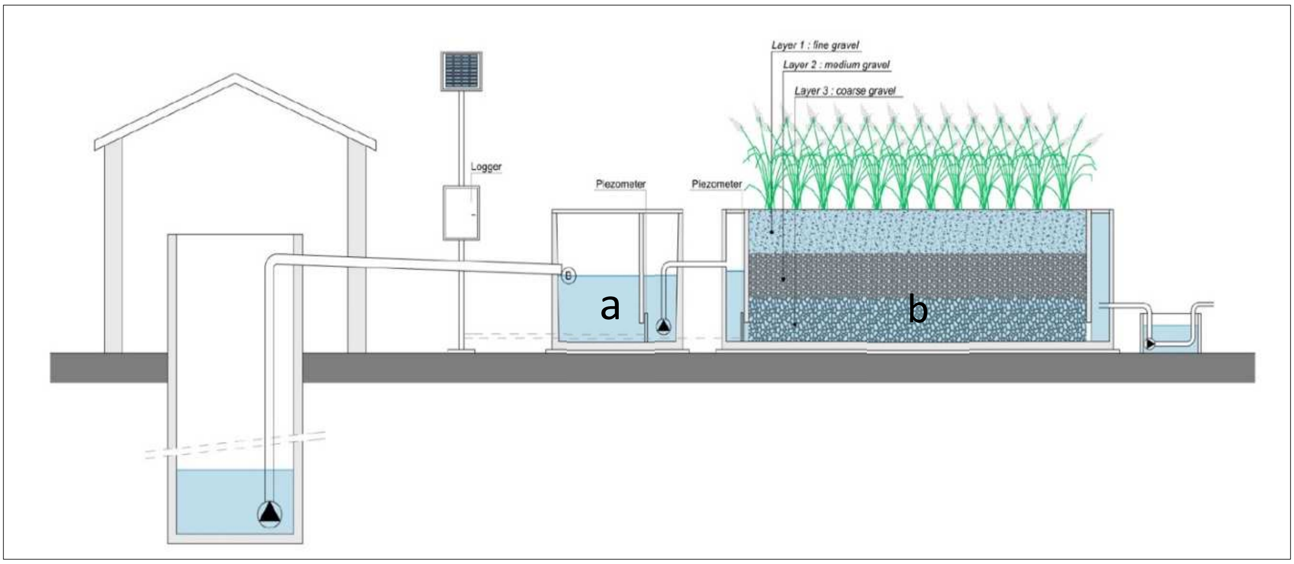


Figure 29. Operation scheme of the phytoremediation pilot plant: a) storage tank; b) main tank

The main tank is filled with a substrate consisting of three different layers of stones of different granulometry: on the bottom, and almost 50 cm in height, we find coarse gravel formed by pebbles of 8-10 cm in diameter (Fig. 30a); the intermediate layer, 25 cm high, is made up of medium gravel (20-35 mm in diameter); the last layer, 3-5 cm, is made up of fine gravel. In March 2018, 200 seedlings of *Phragmites australis* were planted in this thin layer (Fig. 30b).

The two tanks are connected to the pit and to each other by a series of pipes and taps. To optimize the operation of the pilot plant, two probes were also installed (for measuring the water level in each tank) and a timer (to automatically activate the pumps to fill the two tanks).

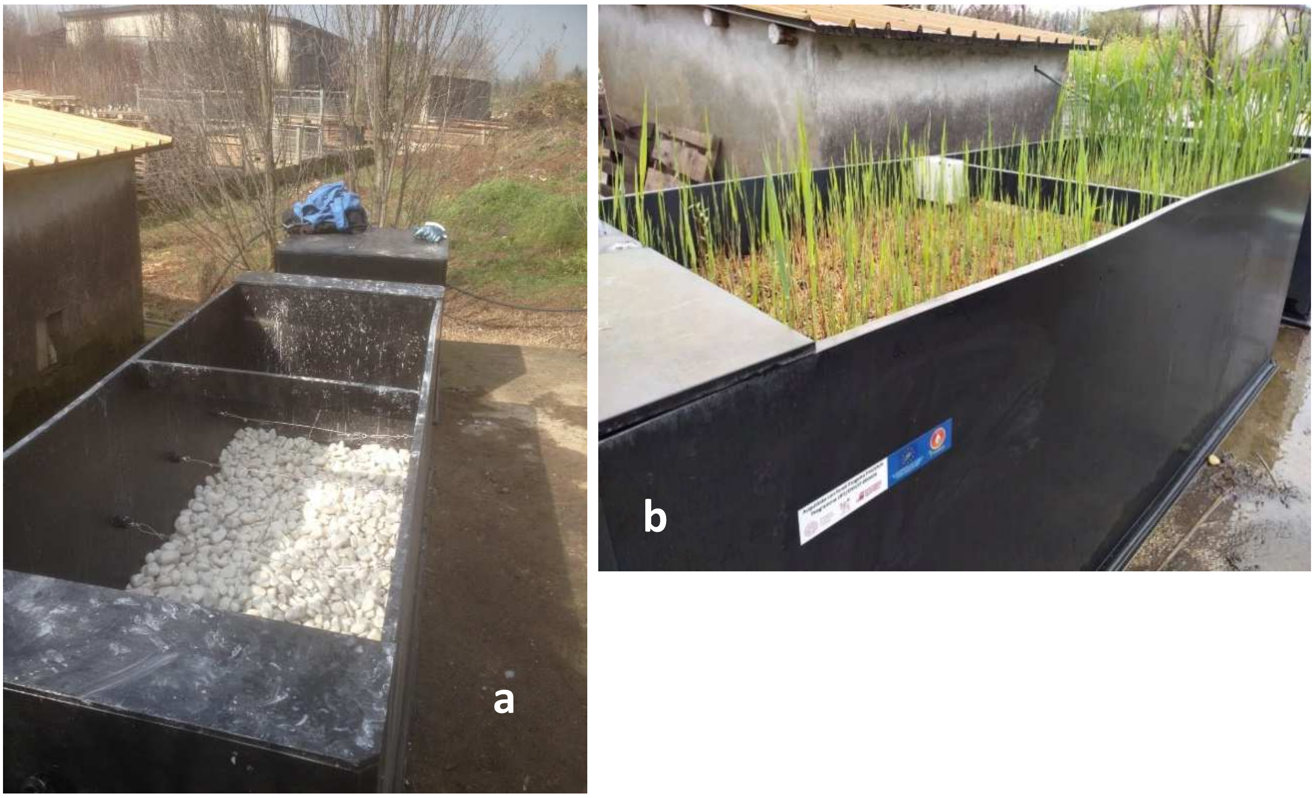


Figure 30. Phytoremediation pilot plant Main tank: a) during the preparation with layers of stone; b) with young plants of *Phragmites australis* planted for a few weeks

Functioning

In a phytoremediation plant, the water is pumped into the storage tank directly from the polluted well. Then, through the use of a small pump, the water fills the main tank containing the plants and flows slowly until it drains, taking a few days (from 5 to 7), that is the time necessary to allow to the *Phragmites* to sequester the pollutants present in the water and carry out its phytodepuration processes.

The water level inside the two tanks is regulated by two piezometers and is displayed in real time on a web portal, while the water speed is regulated by the influx of the pump.

Efficiency test

To prove the adsorption capacity of the PFAS, by *Phragmites australis*, several tests were carried out during the project, to confirm or not the filtering capacity of the system. The concentrations of PFAS in the water entering and leaving the pilot plant were recorded for 7 days.

The time interval between the inlet and outlet sampling allowed to compare the values between each other, given the residence time of the water previously evaluated within the system.

System settings

Furthermore, during the research activities, in three different periods in 2020, other kind of data were collected in order to evaluate all the variables (e.g. rainfall of incoming rainwater, environmental temperature, concentration of PFAS, etc.) useful for calculating the evapotranspiration and the water balance in the system, as well as the concentration of PFASs in the incoming and outgoing water: the first period was carried out in June, the second in early September and the third, more intense, in late September. In figure 31 it is possible to see the calculated water balance.

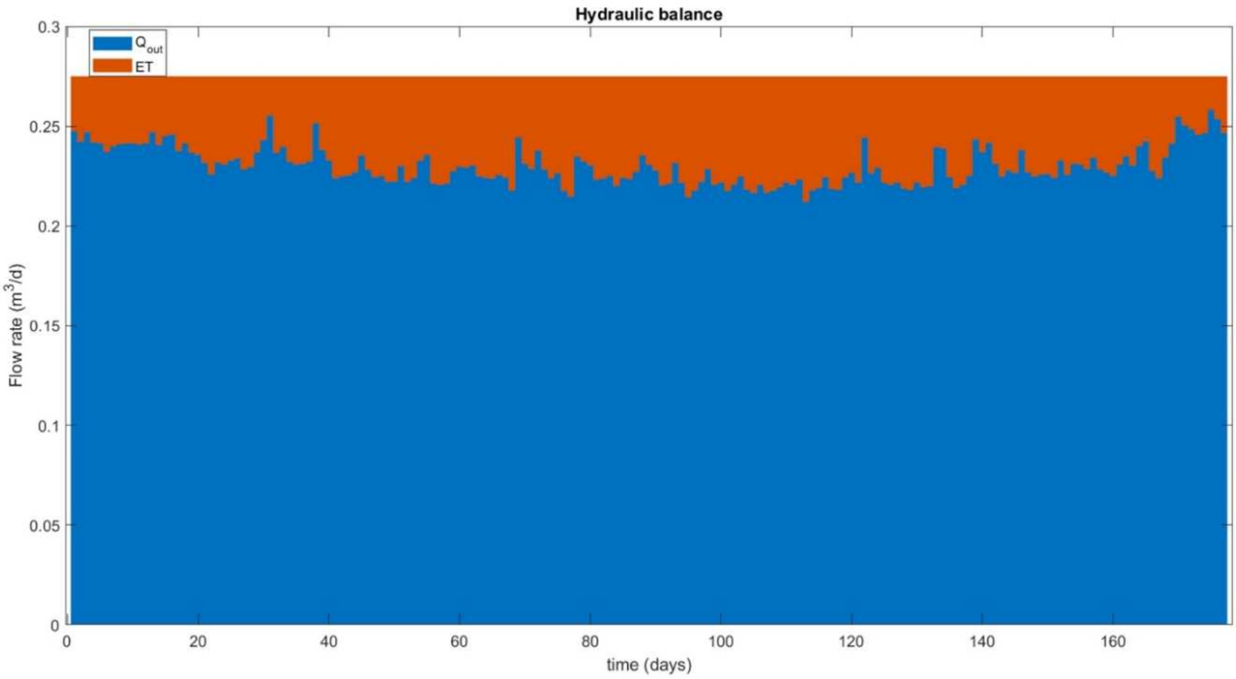


Figure 31. Phytoremediation pilot plant - Calculated Mass balance: Q_{out}, outflow total water volume; ET, total water volume absorbed by plants by the evapo-transpiration process

In our pilot plant system, the water inflow value was fixed (275 L / d) and the outflow volume could vary approximately between 210 and 250 L / d, with an evapotranspiration volume that was at least 10% less. compared to that of the influx. The concentrations of PFAS were recorded both in and out of the pilot plant system and the results are shown in Table 3. The tests were performed several times during the growing season of the plants (from April to September).

The data obtained allowed us to set up our mathematical model.

Results

If we analyze the data in a superficial way, considering only the PFAS concentrations of the inlet and outlet water, the results may seem not very appreciable, because the water entering and leaving the pilot plant contain approximately the same concentration of PFAS. In fact, to understand the abatement capacity of a wet area, mass flows must be evaluated. In fact, the primary objective of a treatment plant is to reduce the mass flow of an outgoing pollutant compared to the incoming one. Therefore, both PFAS concentrations and water flows must be considered.

Table 3. Phytoremediation pilot plant: mean PFAS concentrations in ng/L detected in inlet and outlet water in June and September 2020

PFAS	June Test (20 - 26)		September Test (6 - 12)		September Test (24 - 30)	
	Mean Concentration Inlet (ng/l)	Mean Concentration Outlet (ng/l)	Mean Concentration Inlet (ng/l)	Mean Concentration Outlet (ng/l)	Mean Concentration Inlet (ng/l)	Mean Concentration Outlet (ng/l)
PFBA	806	736	1.306	1.029	1.001	1.005
PFPeA	472	455	1.004	881	642	614
PFHxA	464	416	757	642	590	566
PFHpA	102	96	86	96	123	123
PFOA	1.868	1.714	1.837	2.037	2.681	2.720
PFNA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
PFDA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
PFUnA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
PFDoDA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
PFBS	444	467	512	546	517	542
PFHxS	34	38	<LOD	<LOD	36	39
PFOS	68	70	<LOD	60	70	67
TOTALE	4.258	3.992	5.502	5.291	5.660	5.676

The mass flow corresponds to the water flow multiplied by the PFAS concentration and, while the concentration is almost the same in both the inflow and outflow, the volume of the water runoff is clearly less than the inflow, due to the not negligible effect of evapotranspiration during a whole "seasonal sampling".

According to the hydraulic balance we calculated, the evapotranspiration volume was at least 10% of the inlet flow (Bettio D., 2018). In this way the plant has a positive abatement capacity for PFAS.

Several mathematical models were tested to find the one that best suited our conditions in order to obtain the best estimate of the water runoff as a function of the evapotranspiration of the plants.

With an inflow volume of 275 L / d, a quantity of PFAS equal to approximately 1,413,500 ng / d was pumped into the pilot plant system every day. If we consider the average abatement capacity of the plant of 12% (which corresponds to the reduction in the volume of outgoing water), the quantity of PFAS absorbed by the pilot plant is equal to 169,620 ng/d. We have to remember, that the system worked for 180 days for a total of 49.5 m^3 of water pumped in the pilot plant, with a

PFAS abatement of 30.53 mg. This result seems far better than hypothesized in the LIFE PHOENIX project (30 mg of PFAS abatement for 250 m^3 of water pumped in the pilot plant).

In conclusion, we can say that the phytoremediation pilot plant is able to absorb an appreciable amount of PFAS from polluted waters. However, it is a mitigation action that does not immediately reduce the concentration of PFAS in the water, as can be seen in Table 3, but which, in the long term, could be used as a mitigation strategy for the whole irrigation system.

Large-scale phytoremediation in three wetlands of the Veneto region

A wetland is a so-called transition zone, an area partially covered by water or saturated with water. Within the project area, three different wetlands with similar features have been identified (Fig. 32):

- Ca' di Mezzo in the green area (low risk from PFAS),
- Monselice in the yellow zone (medium risk from PFAS),
- Monastiero in the red zone (high risk from PFAS).

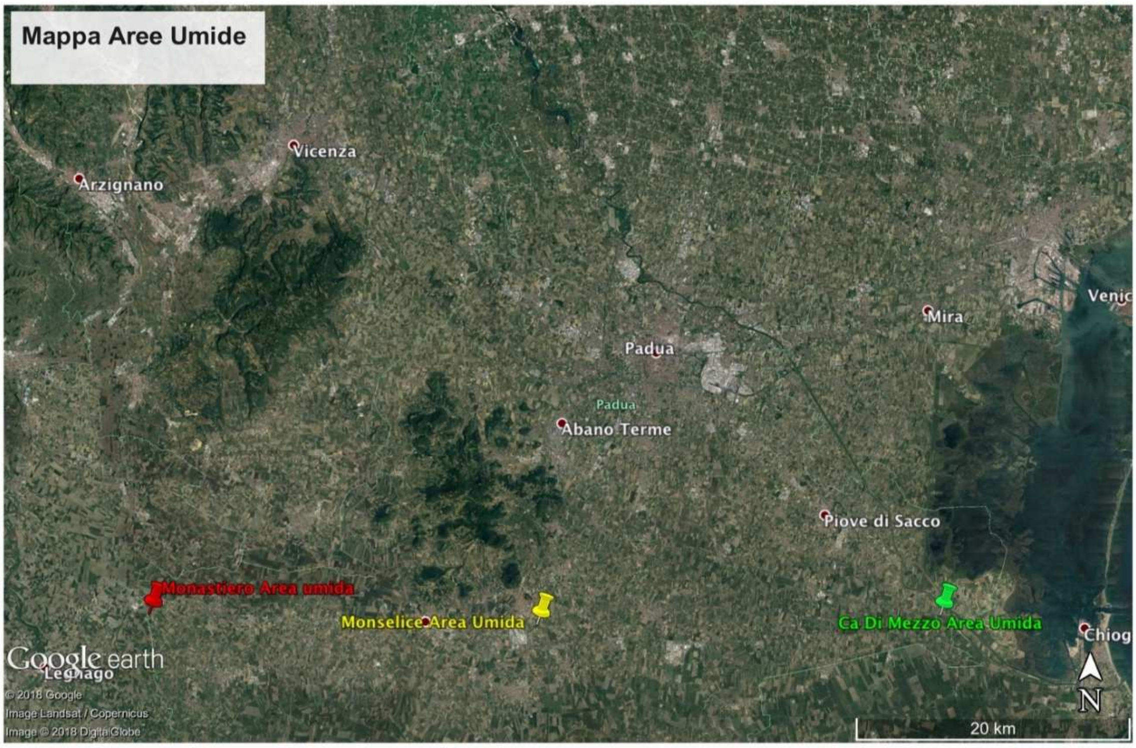


Figure 32. Map of the three wetlands system: Ca' di Mezzo - Padova (green zone), Monselice - Padova (yellow zone) and Monastiero - Verona (red zone)

The three wetlands are managed by the Adige Euganeo Reclamation Consortium. All three wetlands have an entry point, where irrigation water enters in the system, a series of small channels, or ponds, to decrease the speed of the water flow, and a runoff point that could allow the possibility to manage the water flow speed in order to maximize the phytodepuration and adsorption capacity of the plants. The concentrations of PFASs were recorded both at the entrance and at the exit, and the results are reported in tables divided for each wetland (see Tables 4 - 7).

Table 4. Wetlands: mean PFAS concentration (ng/L) measured in the inlet and outlet water

WETLANDS	Ca' di Mezzo		Monselice		Monastiero	
PFAS	Mean Concentration		Mean Concentration		Mean Concentration	
	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
PFBA	38	9	14	13	59	9
PFPeA	20	<LOD	7	8	7	5
PFHxA	40	3	14	13	7	6
PFHpA	20	<LOD	4	4	3	2
PFOA	31	10	17	17	116	10
PFNA	9	<LOD	<LOD	<LOD	<LOD	<LOD
PFDA	3	<LOD	<LOD	<LOD	<LOD	<LOD
PFUnA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
PFDoDA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
PFBS	13	6	7	7	74	50
PFHxS	<LOD	<LOD	3	3	<LOD	<LOD
PFOS	5	0	0	0	58	0
TOTALE	179	28	66	65	324	82

Table 5. Daily water flow in the three wetlands investigated

	Ca' di Mezzo		Monselice		Monastiero	
Flow	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
m^3/day	21.600	17.280	12.960	8.640	8.640	7.776

Table 6. Mass flow of PFAS in and out of the three wetlands investigated

	Ca' di Mezzo		Monselice		Monastiero	
PFAS	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
g/day	3,87	0,48	0,86	0,56	2,8	0,64

Table 7. Percentage of abatement of PFAS in the three wetlands investigated

Wetlands	V_{in} (m^3)	V_{out} (m^3)	PFAS $_{in}$ (ng/l)	PFAS $_{out}$ (ng/l)	PFAS (g) inlet	PFAS (g) outlet	Abatement %
Ca' di Mezzo	3.888.000	3.110.400	179,0	28,0	696,0	87,1	87,5
Monselice	1.000.944	846.834	66,0	65,0	66,1	55,0	16,7
Monastiero	194.400	174.960	324,0	82,0	63,0	14,3	77,3

Results

Wetland test results look very encouraging. In particular, in the two areas, Ca 'di Mezzo and Monastiero, the PFAS abatement efficiency is 87.5% and 77.3% respectively. As regards, however, the wet area of Monselice, the PFAS abatement efficiency is 16.7% much lower than the other two. This data, not comparable with the efficiencies observed for the other two wetlands, is affected by an important management by the Consorzio di Bonifica Adige Euganeo, which uses the area as an expansion tank, or storage basin, depending on the different climatic and seasonal conditions. This means that the area undergoes, in an irregular way, significant reductions in the flow rates treated.

The heterogeneity of these systems makes it very delicate to evaluate the efficiency of these plants (Kadlec R. and Wallace S., 2008): the wet areas Ca 'di Mezzo and Monastiero are fed continuously through an in-line channel, while the humid area of Monselice is fed by a lifting system activated according to management needs.

Conclusion

The phytodepuration pilot plant gave good results, confirmed and indeed surpassed by the tests in the wet areas on a full scale. Phytodepuration has proved to be a very important strategy to mitigate the PFAS present in irrigation waters. At the moment, other water mitigation systems are not being studied or applied. In Italy, it is the first attempt in the environmental field to use constructed wetlands for PFAS, a low-impact and low-cost methodology.

The appreciable results of the experimentation with the pilot plant were a strong stimulus to the application of tests on a full scale, in particular in three wetlands managed by the Adige Euganeo Reclamation Consortium, with the concrete possibility of being able to evaluate the mass flows in natural environments and, therefore, to follow the mitigation of PFAS in the water.

Currently, the Veneto Region hosts over 300 wet areas of various types and, at least half, would be usable in order to immediately apply phytoremediation.

On the basis, of the observed results, we can state that the constructed wetland system is a methodology applicable to the mitigation of the concentration of PFAS in irrigation waters and replicable in situations similar to the one considered in the LIFE PHOENIX project.

For the system to work on a large scale, however, it is essential to implement synergistic actions between the different "actors" of the territory to create a more advanced governance of the agri-environment.

The points to consider, in any case, could be:

- Review and reprogram the management of the pruning and mowing of ditches and wetlands in general, adopting targeted rules in order to recover the material produced and, if PFASs were present, treat it as a possible waste, thus avoiding their release into the environment.
- Update and expand the regulations currently in force on waste so as to be able to manage the mowing of wet areas for possible transfer to specific disposal plants.
- Think of an innovative management of irrigation water, in which the wetlands are the central hubs of the "phytoremediation" system of most of the irrigation waters present in the Veneto Region. Furthermore, the management of ditches and second-level canals should be rethought, treating them as widespread wetlands. This would increase the usability of the territory also from the naturalistic point of view.

Finally, the possibility of a wide-ranging use of phytoremediation to remove PFAS from irrigation water could be an "added value" in the perception of the quality of the agri-food chain of the Veneto Region.

References

- Bettio D. (2018). Analisi e modellazione del funzionamento di un impianto pilota di "fitorisanamento" per l'estrazione di composti PFAS dall'acqua. Tesi di Laurea Magistrale in Ingegneria Ambientale. Gruppo di ricerca LASA del Dipartimento di Ingegneria Industriale dell'Università di Padova Università di Padova.
- Kadlec R. and Wallace S. (2008). Treatment Wetlands. Ed. CRC Press: pp 1046.
- Zaggia A., Conte L., Falletti L., Fant M., Chiorboli A. (2016) Use of strong anion exchange resins for the removal of perfluoroalkylated substances from contaminated drinking water in batch and continuous pilot plants. Water Res.91:137-46. doi: 10.1016/j.watres.2015.12.039.

3.6 Assessment of the socio-economic impact of the PFAS contamination

The monetization of social benefits: a meta-analysis application

The estimation of the economic benefits of a specific policy action (or regulation) requires a good knowledge concerning the different methodologies adoptable for this purpose. In the context of environmental protection interventions, as the one object of the LIFE-PHOENIX project and developed in the action B.3 and B.4, the main problem in the benefit analysis is related to the valuation of the so-called *non-market commodities.* The latter are environmental resources whose price is not clearly defined since they are not exchanged in a real market (because they are not excludable or separable) and therefore a variation in their quantity or quality is not intuitive to monetize.

In the framework of the LIFE-PHOENIX project, the commodity whose level would change thanks to the mitigation strategies introduced is represented by drinking and irrigation water quality. Indeed, from an economic perspective, a reduction in PFAS contamination may be considered as equivalent to an increase in water quality, whose monetary value represents a social benefit for the households living in the contaminated area. Since it does not exist any real market for water quality, the valuation procedure aimed at monetizing a positive variation in its level relies on the classical economic theory on individual preferences and utility. Despite ecosystem services are not regularly traded, in fact, individuals always preserve a personal valuation about the utility they get from them with respect to other general goods.

The best strategy that may be implemented to estimate the monetary value of an environmental resource, therefore, involves surveys in which individuals are directly asked how much they value a specific change in the quality/quantity level of a natural service. In particular, the economic valuation of a certain environmental improvement is generally measured as the amount of money – in a fixed unit of count (e.g., €/2018) – an individual would be willing to trade to obtain that improvement. The latter quantity is technically called Willingness to Pay (WTP) and is the outcome of our meta- analysis for the PFAS area.

Hence, it is clear that the assessment of the WTP for an environmental quality change would require data gathered from a well-designed survey distributed in the contaminated area. Indeed, the first best to estimate social benefits of the LIFE-PHOENIX project would be to ask[1] people living in the Red, Orange and Yellow Area how much they would be willing to pay to restore the quality level in drinking/irrigation water.

However, in our context funding limitations and time constraints are significant, as often happens in environmental valuation studies. If this is the case, an optimal alternative developed in the literature to estimate benefits from ecosystem services quality change is the benefit transfer methodology. Benefits transfer refers to the procedure of "adapting values estimated from past researches to assess the value of a similar, but separate, change in a different resource". Applied to

[1] By applying valuation methods widely used in environmental studies: Averting Expenditure (revealed preferences); Contingent Valuation (stated preferences); Travel costs (revealed preferences); Hedonic pricing (revealed preferences).

the environmental valuation context, willingness to pay values for a certain quality change, estimated by previous empirical papers in different sites (*study sites*), are combined and applied for the estimation of the same value in a new, but similar, site (*policy site*).

In particular, one of the procedures aimed at exploiting benefit transfer is the Meta-Analysis, defined as a systematic and quantitative summary of the results reported in existing scientific contributions about the valuation of the same environmental resource. After the collection of several primary studies analysing a common empirical outcome, researchers investigate the relationship of the estimated values (in our case WTP for higher water quality) with the socio-economic characteristics of each site (*core-economic variables* - e.g., income, population density, sex, age) and/or the methodological characteristics of the specific paper (*study design variables* - e.g., how data are gathered, how the question about WTP is formulated).

Initially, we selected all studies aimed at valuing positive groundwater quality changes in general, collecting a total of more than 90 studies. Afterwards, among them we chose only the ones which respect the study selection criteria defined as follow:

1. Papers must estimate the same economic outcome: annual willingness to pay for better drinking water quality.
2. Results on willingness to pay must be available in the study in the form of mean or median of the distribution;
3. A contamination event prior to the survey was not considered to be a necessary condition to judge a valuation study suitable for our purpose, since we decided to control for contamination directly in the regression function;
4. We exclude studies valuing groundwater quality that does not specify if the groundwater object of the analysis is used as source for drinkable and home use;
5. We exclude studies which clearly claim the bad quality of their results due to asymmetry between the characteristics of the sample interviewed and the target population.

Lastly, it is key to precise that we even drop all studies which investigate quality changes in drinking water in developing countries, because in that case we would make a big mistake due to commodity inconsistency.

Filtering by these criteria, we ended up with a total of 40 studies and 72 observations. A full list of the researches selected is provided inside Table 8.

Table 8. List of papers estimating WTP in analogous study sites selected for the MA.

Study	Year	Study site	Contaminant
Harrington et al.	1986	Luzerne County, Pennsylvania, US	Giardia
Edwards	1988	Cape Cod, Massachussets (Barnstable and Plymouth Counties)	Nitrate
Shulz & Linsday	1990	Dover, New Hampshire	Generic contaminants
Abdalla et al.	1992	Perkasie, southestern Pennsylvania	TCE
Musser et al.	1992	Milesburg, Pennsylvenia	Giardia
Sun et al.	1992	Dougherty County, Georgia	Agricultural chemicals
Collins & Stainback	1993	West Virginia	Bacteria contaminatin
Du Vair & Loomis	1993	California, US	Heavy metals
Hauser & Van Kooten	1993	Abbotsford region of british columbia, Canada	Nitrate
Jordan & Elnagheeb	1993	Georgia	Nitrate
Wattage	1993	Iowa, US	Acgricultural chemicals
Bergstrom, J.C. and J.F. Dorfman	1994	Dougherty County, Georgia	Agricultural chemicals
Powell et al.	1994	Massachussetts, Pannysilvania, New York	TCE, Dissel fuel
Tervonen et al.	1994	Oulu, Finland	N.C.
Laughland et al.	1996	Milesburg, Pennsylvenia	Giardia
Cho	1996	Southwestern Minnesota	Copper and sulfates
Crutchfield et al.	1997	Mid-columbia basin	Nitrate
Luzar & Cosse	1998	Lousiana, US	N.C.
Piper	1998	Montana, US	N.C.
Poe & Bishop	1999	Portage County, Wisconsin	Nitrate
Abrahams et al.	2000	Georgia, US	Generic contaminants
Bergstrom et al.	2001	Aroostock County, Maine	Nitrate
Epp & Delavand	2001	Lebanon and Lancaster counties, Pennsylvania	Nitrate
Koss, P. and M.S. Khawaja	2001	California, US	Water shortage
Eisen-Hect & Kramer	2002	Catawba basin, US	N.C.
Hurley et al.	1999	Iowa, US	
Um et al.	2002	Pusan, South Korea	E. Coli and nitrate
Kim & Cho	2002	Southwester Minnesota	Copper
Brox et al.	2003	Grand River, Souther Ontario	Chemical spills
Nielsen et al.	2003	Denmark	Toxic substances
Dupont 2005	2005	Canada	E. coli
Genius & Tsagarakis	2006	Haraklion, Greece	Water shortage
Aulang et al.	2006	Alsace Region, France	Clorinant solvents
Genius et al.	2008	Rethymno, Grecce	Soil infiltration
Beaumais et al.	2010	10 OECD countries (Italy, Korea and Brazil)	N.C.
Kanishi & Adachi	2010	Minnesota, US	Arsenic
Polyzou et al.	2011	Mitylene, Greece	Water shortage
Kwak et al.	2013	Pusan, South Korea	N.C.
Alvarez & Asci	2014	Florida	
Tanellari et al.	2015	Northern Virginia and Maryland	Generic contaminants
Nielsen-Pincus et al.	2017	The McKenzie River watershed, Oregon	N.C.
Chatterjee et al.	2017	Jacksonville, Florida	Chemical components
Guerrini et al.	2018	Italy, Province of Verona	N.C.

The meta-analysis is implemented by estimating a regression model which explains how estimates of WTP, of all the studies selected, vary according to: a set of core economic variables of the country/region considered; some study design variables which control for papers heterogeneity concerning the methodology applied in the data gathering process and analysis.

The estimated log-log model for the study s is the following:

$$ln(WTP)_S = \beta_1\, mean_s + \beta_2\, elicitation_s + \beta_3\, payment_s + \beta_4 contamination_s + \beta_5\, CVM_s + \beta_6\, phone_s + \gamma_1 income_s + \gamma_2\, density_s + \gamma_3\, sex_s + \gamma_4\, age_s + \gamma_5\, education_s + \epsilon_s$$

Where the dependent variable is $ln(WTP)_S$ = Natural log of annual willingness to pay per household estimated in the study expressed in 2018 $1.

The explanatory variables, according to the WSTU approach, are divided in two groups. The study design variables are the following:

- $mean_s$: 1 if the estimated WTP is the mean; 0 if the estimated WTP is the median;
- $elicitation_s$: 1 if dichotomous choice in the WTP question; 0 otherwise;
- $payment_s$: 1 if the payment vehicle used in the survey is extra taxes on water bills; 0 otherwise;
- $contamination_s$: 1 if the valuation is related to a contamination event; 0 if it is not;
- CVM_s: 1 if the study employs the contingent valuation methods; 0 if the study employs averting behavior method or others;
- $phone_s$: 1 if the survey is conducted by phone interviews; 0 otherwise;

While core economic-demographic variables are the following:

- $income_s$: Natural log of the average annual personal income in the study site in $;
- $density_s$: Natural log of population density in the study site in h/$km2$;
- age_s: 1 if the average age in the site is bigger or equal than 45; 0 otherwise;
- sex_s: 1 if the percentage of female in the population is bigger or equal than 50%; 0 otherwise;
- $education_s$: Percentage of people in the population who holds a bachelor's degree or higher.

The valuation function behaves statistically well, with a percentage of variance explained by the model (R^2) equal to 98 % and nine out of eleven parameters estimated significantly different from zero (eight of them at 1% significance level and one at 5 %).

In particular, from a methodology point of view, the WTP estimate is significantly higher when is considered the mean of the collected values w.r.t. the median ($\beta_1 = 0.7785$), if dichotomous choice method is applied in the WTP question ($\beta_2 = 0.6437$) and if WTP is aked after a previous contamination event ($\beta_4 = 0.7785$). Looking at the socio-economic features, instead, WTP estimate is significantly higher for an higher-income ($\gamma_1 = 0.3821$), older ($\gamma_4 = 0.8448$) and female ($\gamma_3 = 1.2096$) individuals. Eventually WTP estimate is significantly lower the higher is the population density of the policy site (($\gamma_2 = -0.1749$).

Using the parameters estimated in Table 9, and plugging in the value function the explanatory variables for the PFAS area we obtain a annual WTP which stands in the interval [267,48 €; 496,13 €] per household, given a measurement errors of 30% applied to the point estimate.

Given the number of households living in each area, annual social benefits from a reduction in PFAS drinking water pollution stands therefore in the interval [33.432.466,94 €; 62.088.867,17 €][2].

[2] Even if this interval appears to be quite big, literature suggests that measurement error is the main defect of benefit transfer procedure. As a consequence, 30% of range around the point estimate is necessary to overcome this source of inaccuracy.

Table 9. Estimated parameters from the meta-analysis regression (value function).

Source	SS	df	MS
Model	1965.3862	11	178.671473
Residual	34.3778178	61	.563570783
Total	1999.76402	72	27.7745003

Number of obs	=	72
F(11, 61)	=	317.03
Prob > F	=	0.0000
R-squared	=	0.9828
Adj R-squared	=	0.9797
Root MSE	=	.75071

ln_WTP	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
mean	.7785108	.2374209	3.28	0.002	.3037584	1.253263
elicitation	.6437578	.2078202	3.10	0.003	.2281957	1.05932
payment	-.4500623	.2087158	-2.16	0.035	-.8674154	-.0327092
contamination	.6880508	.2462852	2.79	0.007	.195573	1.180529
CVM	-.4419198	.3739158	-1.18	0.242	-1.189611	.3057711
phone	.2293906	.2821408	0.81	0.419	-.3347849	.793566
income	.3821446	.0539489	7.08	0.000	.2742671	.4900221
density	-.1749186	.0601461	-2.91	0.005	-.2951883	-.054649
sex	1.209659	.2053935	5.89	0.000	.7989494	1.620369
age	.8448168	.2159875	3.91	0.000	.4129231	1.276711
education	.1158186	.0363882	3.18	0.002	.0430558	.1885813

Analysis of health costs in emergency cases of contamination: the experience of the Veneto Region

The analysis of the socio-economic impact of the project has been implemented with a focus on the Health Surveillance Program organized by the Regione Veneto for the target population. The organizations involved are: Veneto Region, for planning and coordination of the Health Surveillance Plan, Aulss 8 Berica, Aulss 9 Scaligera and the Arpav agency for laboratory analysis.

We have proceeded with the identification of the phases of the Health Surveillance Plan, finding three main phases: preliminary phase, with studies and evaluations for the adoption of the health surveillance plan and convocation of the interested population, first level phase of analysis, with interview on socio-demographic characteristics, personal health history and tests on non-fasting blood and urine, and finally a second level phase, with specialistic examinations and medical services.

It has been delivered a questionnaire to all the institutions involved in order to collect the necessary information and data, related to the expenses incurred in this program (already pre-centralized by the Veneto Region), and then the information collected have been reclassified according to common and homogeneous criteria.

The first cycle of monitoring programme, which started at the end of 2016, was meant to finish by 2020, but due to the Covid19 pandemic there were several interruptions of the activities and the expected end has been postponed to 2021. Therefore, by the end of the LIFE PHOENIX project the monitoring activities are still ongoing and as a consequence some of the evaluations must be considered not definitive and limited to the existing data. The aim of this analysis is to elaborate a recognition of the Healthcare costs, direct and indirect, necessary to develop further economic evaluations, intended as a comparison of alternative courses of action in terms of their costs and consequences (Drummond et al., 2015). Moreover these partial evaluations could provide useful information for whole-disease models in comparing expected costs with real-world cost data (Buja et al., 2021).

Data collection of the costs related to the emergency - Health surveillance plan on the population exposed to PFAS

Direct Costs amounts to 3.853.333 € related to the different phases of monitoring activity, planning and coordination, distribution of the questionnaire, first phase of examinations and sample collection, second phase of control and specialist examinations:

- The cost of preparing and managing the health surveillance plan (hours of work of the Medical Directors, Health sector personnel, Administrative Section employees) and coordination between the institutions from 2016 to following years amounts to 856.643,52 €. This cost has been estimated multiplying the hours of the personnel involved with the Standard Cost Table of the Veneto Region available for the LIFE PHOENIX project in 2017.
- The unit cost of letters: Cost of letters, processing, printing, enveloping and postage (including the cost of administrative staff) amounts to 143.583,00 € has been estimated multiplying the number of letters sent with the unit cost of necessary to produce.
- The external postal service, including administrative activities for the text elaboration, preparation of agendas, creating invitations, file extraction, postal software data entry, amounts to 72.559,46 € has been estimated multiplying the number of letters sent with the unit cost of necessary to and delivery
- The number of individuals involved in First Level in the screening program as of DGRV 2133/2016 and DGRV 691/2018 and the related invitation: 76.025 are the people invited at 18/12/20, and 94.331 is the number of letters sent (included further progress and solicitations).
- First Level activity began in December 2016 with interviews and blood/urine sample collection and the estimated cost amounts to 2.324.027,72 € including the tools and the additional analysis provided by Arpav. We have collected the costs which correspond to serological analysis, and investment in equipment for serological screening analysis (high-performance liquid-phase chromatography and automated robotic system for preparation of biological samples).
- Second Level activity began in December 2017 with specialistic examinations and the estimated cost amounts to 456.519,63 €. We looked for the number of individuals subjected to the first and second level of (ad hoc follow-up examinations) considering ad hoc in-depth examinations in internal, cardiological or antidiabetic centers.

Indirect costs are considered as costs related to patients and family members not directly attributable to the NHS and require purely economic (≠ financial) evaluations. We have looked for the number of individuals subject to the first level of analysis considering different methods of convocation (data extracted from the Regional Information System). This information is relevant to estimate the amount of time dedicated by the population to the program. Other information relevant for the estimation were the average patient permanence within Ulss clinics, the average time spent delivering and counseling to a patient, the call center activities.

In order to provide an economic assessment of the time dedicated we first proceeded to obtain an estimate for the monetary value of an hour of free time and working time, following the methodology of added value to GDP (De Luca, 2000). For the estimation of the value of the time we have adopted the "social" value of time compared to the marginal contribution to the local (Veneto) GDP, reaching a value of 1.205.544,21 € as evaluation of the time spent.

Conclusion

The meta-analysis developed within the LIFE PHOENIX Project has been able to produce an exportable econometric model which relates the WTP (Willingness to Pay) estimates for better drinking water quality - collected from more than 90 past papers - to core economic variables of the study sites and study-design variables. The best model specification is a log-log linear regression and it has been estimated with OLS. Parameters estimated allow us to compute the WTP for potentially curbing PFAS pollution in the Red Area (our policy site) and therefore the related social benefits.

Further research may be dedicated at computing WTP for better irrigation water quality, which is actually an open issue since really few past studies developed such a specific analysis. As a consequence, there are not the optimal condition to apply benefit transfer to compute WTP for a reduction in PFAS pollution in irrigation water and an original survey should be conducted on the exposed population to obtain a complete configuration of social benefits.

We also developed an economic and financial valuation of the cost sustained by the Veneto Region to implement the surveillance plan on the exposed population. The study adopts the perspective of the Veneto Regional Healthcare Service, considering only the direct costs sustained by the public health authorities, and using cost data gathered with questionnaires and from official reimbursement tariffs in 2020.

All the most known complete economic evaluations techniques in Healthcare (CMA, CBA, CEA and CUA) adopt the analysis of the costs expressed in monetary values and are different for the kind of comparisons of interventions or the evaluations of benefits. The data gathered could be useful to formulate the analysis of costs and comparing expected costs with real-world costs (Buja et al. 2021), and to study the side of benefits intended as alternative use of resources and spared costs (Drummond et al. 2015).

References

- Allen & Loomis (2007). The decision to use benefit transfer or conduct original valuation research for benefit-cost and policy analysis. Western Economic Association International.
- Bergstrom & Taylor (2006). Using meta-analysis for benefit transfer: theory and practice. Ecological Economics.
- Boutwell & Westra (2013). Benefit Transfer: a Review of Methodologies and Challenges. Resources.
- Buja, A., Rivera, M., Polo, A. D., Brino, E. di, Marchetti, M., Scioni, M., Pasello, G., Bortolami, A., Rebba, V., Schiavon, M., Calabrese, F., Mandoliti, G., Baldo, V., & Conte, P. (2021). Estimated direct costs of non-small cell lung cancer by stage at diagnosis and disease management phase: A whole-disease model. Thoracic Cancer, 12(1), 13–20.
- De Luca, M. (2000). Manuale di pianificazione dei trasporti—FrancoAngeli. FrancoAngeli. https://www.francoangeli.it/Ricerca/scheda_Libro.aspx?codiceISBN=9788846417398
- Drummond, M. F., Sculpher, M. J., Claxton, K., Stoddart, G. L., & Torrance, G. W. (2015). Methods for the economic evaluation of health care programmes. Oxford university press.
- Nielsen et al. (2003). Impact of socio-demographic factors on Willingness to pay for a reduction of future health risk. Journal of Environmental Planning and Management.

SECTION 4
Benefits brought by the project to the regional system and development prospects

The LIFE PHOENIX project has represented an important test for the integration between Environment and Health, demonstrating that a shared approach to issues of environmental origin with an impact on the health of the population is not only possible but guarantees better results than working by sectors. During the project, an intersectoral working method was tested and common tools were developed that constitute a solid basis for continuing along the intersectoral path, as also indicated by the National Prevention Plan 2020-2025 recently approved with an agreement between the Italian State and the Regions.

In the next few years, the working method and operational tools developed in LIFE PHOENIX will find concrete application in the context of the safety of the drinking water supply chain, with the aim of supporting the adoption throughout the Region of a new holistic approach based on the model of the Water Safety Plans proposed by the World Health Organization. This approach, which shifts the focus from retrospective control on distributed water to risk prevention and management in the drinking water supply chain, extended from collection to taps, is actively promoted in Italy by the Ministry of Health and the Istituto Superiore di Sanità with a view to strengthen water quality control strategies, remodelling them on the basis of knowledge deriving from risk analysis. The regional Directorate for Prevention, Food Safety, Veterinary will make the information and statistical system developed within LIFE PHOENIX available to the competent Bodies to support the risk analysis process that each Integrated Water Service Manager will have to conduct on its own supply systems, with the collaboration of the Local Health Unit and of ARPAV. This will also make it possible to test the functionality of the information system in the field and highlight the need for improvement and development.

The numerical model of flow and transport of pollutants, successfully implemented and tested in the area of the upper and middle Veneto plain affected by PFAS pollution, has proved to be an important and effective tool for interpreting, quantifying and predicting the propagation of pollutants in groundwater, providing estimates of the masses of contaminants released and reliable predictions on the future evolution of contamination. Numerical modelling therefore represents an advanced and powerful analysis tool to support decisions in order to respond to the most diverse types of real scenarios (e.g. accident with the release of a new contaminant) proving to be a valuable tool in the management of environmental emergencies or, if applied for preventive purposes, in the implementation of the Water Safety Plans. This tool needs to be further developed and perfected for a better understanding of the extensive PFAS contamination event that occurred in Veneto: a greater knowledge of the geological structure, hydrological and hydrodispersive parameters would allow to expand the model area for a more complete and exhaustive analysis of the polluted area. In particular, the extension of the model in the plain served by irrigation, through a full integration of surface water, groundwater and soil waters, could provide important information regarding the impact of contamination on the food chain and therefore on population exposure (also retrospectivelly).

It is now evident, and this project has brought numerous supporting data, that the high mobility and persistence of PFAS in the aqueous matrix favors their diffusion in all environmental compartments, with a contribution not yet sufficiently studied by atmospheric transport. From this it follows that a significant point source can impact a very large area, exposing ecosystems and population to the risk caused by this class of substances. Soil is one of the pollution receptor compartments of both water and the atmosphere. As demonstrated during the project, soil constitutes an effective buffer system for long-chain PFAS but not for short-chain ones that are captured by plants. However, this does not mean that soil is a sequestration compartment for these substances, because they can be reintroduced into ecosystems through the terrestrial trophic network, from invertebrates such as earthworms to birds and mammals. Further studies are needed on the role of such trophic network in the dispersion of PFAS. Another aspect to be explored with regard to soils is the determination of background concentrations in soil and agricultural products in order to determine the background exposure of organisms through the different exposure routes. In the absence of an European legislation for soils, it is necessary to promote extensive international cooperation to establish background values of PFAS contamination in the various sectors and of exposure for the population.

On the environmental analysis and monitoring front, the LIFE PHOENIX project has made it possible to develop a package of methods and procedures that can be used and further refined in the near future, also through application on a larger scale, with the aim of early detection of the contamination of the various environmental matrices and monitoring its progress over time, also providing indications on the effectiveness of control and mitigation measures. The determination of a set of cellular biomarkers of stress in the earthworm, a bioindicator organism of soil quality, tested in the specific reality of the Veneto Region, has proved to be a promising methodological approach for the assessment of the environmental impact by PFAS and to be potentially applicable to larger areas. Extensive biomonitoring campaigns based on this approach would make it possible to analyze a large number of samples and obtain significant results in an adequate time frame to provide precise indications on the ecotoxicological situation.

Alongside the tools for forecasting, analysis and monitoring, the LIFE PHOENIX project also intended to test innovative tools for the mitigation of PFAS contamination. On this front, promising results have been obtained by applying a method based on natural processes, phytodepuration, to irrigation water. In Italy, it is the first attempt in the environmental field to use constructed wetlands as a low-impact and low-cost depuration methodology for PFAS. The good results obtained are particularly interesting for the territorial context of the Veneto Region which is very rich in humid areas of various types potentially usable for phytodepuration. The large-scale use of this natural method of water purification requires the adoption of synergistic actions between the various "actors" involved in the management of the territory, in order to transform the wetlands (including those of ditches and second-level canals) in central junctions of the phytodepuration system of most of the irrigation waters present in the Veneto Region. Such an approach would also bring benefits from a naturalistic point of view and the usability of the territory.

Finally, the project activities allowed an initial quantification of the direct and indirect costs that the contamination from PFAS entailed for the regional system. In fact, through a meta-analysis procedure, an exportable and replicable econometric model has been developed which allows to estimate the Willingness To Pay (WTP) for a better quality of drinking water starting from a series of socio-economic and methodological variables. The application of this econometric model to the PFAS-contaminated area of the Veneto Region returns an estimate of the WTP in the range between € 267.48/year and € 496.13/year per household. In addition, an economic and financial survey was carried out of the direct costs incurred by the Veneto Regional Health Service for health surveillance activities in favor of the exposed population, resulting in € 3,853,333.

The data and information collected so far may be refined in the future with further research, for example by evaluating the WTP through surveys carried out in the specific context and taking into account, in addition to drinking water, also other vulnerable environmental matrices such as irrigation water. The cost recognition carried out represents a fundamental prerequisite for any type of economic analysis aimed at evaluating and comparing possible alternative uses of resources.

“Strategic project aimed at timely, effective and efficient action in case of pollution of drinking water and irrigation water”

Life Phoenix

COORDINATOR

REGIONE DEL VENETO

Contacts

Regione del Veneto - Area Sanità e Sociale
Direzione Prevenzione, Sicurezza Alimentare, Veterinaria
Rio Novo – Dorsoduro 3493
30123 Venezia ITALY
info@lifephoenix.eu
lifephoenix.eu

PARTNERS

REGIONE DEL VENETO AZIENDA ZERO

arpav Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto

IRSA CNR

Università degli Studi di Padova

Budget

2.176.493 €
EU co-financing: 1.264.369 €

Duration

from 01/09/2017
to 31/03/2021

WITH THE CONTRIBUTION OF THE LIFE FINANCIAL INSTRUMENT OF THE EUROPEAN UNION
LIFE16ENV/IT/000488 - LIFE PHOENIX

Elaborazione e sviluppo grafico e2Bi CONSULTING moving Energy into your Business