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PRIORITY CHEMICAL SUBSTANCES IN ENVIRONMENTAL WATERS UNDER THE IMPLEMENTATION OF THE WATER FRAMEWORK DIRECTIVE

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ABSTRACT

The thesis work, financially supported by the Environmental Protection Agency of Region Veneto (ARPAV) through the I.S.PER.I.A. Project, has addressed issues related to the implementation of the Water Framework Directive (2000/60/EC) by the institutional technical organisms in relation to the control of dangerous substances in the aquatic environment of Veneto Region. Attention was firstly focused on the definition of *good chemical status* of the aquatic environment, described by the national legislation that transpose and implement the EU legislation, and the concepts of "*pollutant*" and "*hazardous* and/or *priority substance*". These concepts are related to toxicity, persistence and bioaccumulation properties of the substances and connected with the interactions if discharged into the aquatic environment. Criteria for the adjustment of monitoring plans and of analytical control laboratories and for a characterization of the territory were considered with the target of a better knowledge of effective environmental problems. Consequently, a deep awareness of necessary requirements for a better control of pressure sources of pollution in the territory becomes the primary goal of environmental protection.

Into specific, the issues addressed by the thesis focused in a first moment purely on chemical-analytical aspects, following by a territorial survey of the Region.

First of all a simple and efficient multiresidue method for the simultaneous determination of aniline, nitrobenzene and their chlorinated derivatives (chloroaniline and chloronitrobenzenes), considered hazardous substances by Italian legislation, was developed. The method is based on SPE in basic conditions and GC-MS analysis without any derivatization. Afterwards the method was validated in terms of linearity, repeatability, recovery and ruggedness including a first assessment of uncertainty estimation, with the aim to adopt the procedure in a routine/control environmental laboratory. The same procedure was tested on environmental water samples to check the feasibility and to investigate the real presence of these substances as pollutants of the aquatic system in the Region.

The requirement given by the Water Framework Directive to reach a *good chemical status*, both for surface waters and groundwaters, prompted the development of further investigations related to the new emerging organic pollutants that influence the aquatic ecosystem. The attention was focused on a class of substances very similar, for chemical properties, to the other already considered: nitrogen derivatives

of benzotriflurides. The knowledge of a past pollution episode of groundwater due to some of these substances in the Vicenza Province area and dated back to the Seventies, allowed to reconsider the event and to related it with the current situation in the same area. The modern technology survey, both analytical and territorial, allowed investigating the study case in more details starting from the few and not homogeneous past data held. Hence the elaboration and assessment of historical available data was performed following by a development of suitable analytical methods for the determination of BTFs, based on the previous developed method for chloroanilines and chloronitrobenzene. The result obtained helped to compare the current situation with the past one, to detect new pollutants never described on literature and to identify their traceability at groundwater level. The environmental persistence of these substances, detected in some cases in considerable amount even after decades, and their propagation in the groundwater of Valleagno area, raise the question of how important it is to reassess and not neglect past pollution events, as the territory can be considered a sort of chemical contamination "archive".

Finally the thesis work addressed the issues to the intrinsic environmental toxicity of some of these substances with a preliminary assessment of ecotoxicological test based on three different trophic levels.

SOMMARIO

Il lavoro di tesi, finanziato dall'Agenzia per la Protezione e Prevenzione Ambientale della Regione Veneto (ARPAV) attraverso il Progetto I.S.PER.I.A., ha affrontato problematiche inerenti l'implementazione della Direttiva Quadro sulle Acque (2000/60/CE) da parte di organismi istituzionali tecnici in relazione al controllo delle sostanze pericolose immesse nell'ambiente acquatico della Regione Veneto. L'attenzione è stata posta innanzitutto sulla definizione di *buono stato chimico* dall'ambiente acquatico, descritto dalla legislazione nazionale che recepisce ed implementa quella europea, e ai conseguenti concetti di "*inquinante*" e "*sostanza pericolosa* e/o *prioritaria*". Questi concetti sono legati alle proprietà di tossicità, persistenza e bioaccumulo delle sostanze in questione con le relative conseguenze dovute alle interazioni una volta immesse nell'ambiente idrico. Sono stati presi in considerazione criteri per l'aggiustamento dei piani di monitoraggio, per l'adeguamento dei laboratori analitici di controllo e per una caratterizzazione del territorio con lo scopo di aver una maggior conoscenza degli effettivi problemi ambientali. Di conseguenza un'approfondita consapevolezza delle esigenze necessarie, per un maggiore controllo delle fonti di pressione d'inquinamento, diventa obiettivo primario di protezione ambientale.

Nello specifico, le tematiche affrontate dal lavoro di tesi hanno riguardato in un primo momento l'aspetto prettamente chimico-analitico, seguito da una indagine territoriale calata nella realtà regionale.

Innanzitutto è stato sviluppato un metodo multiresiduale rapido ed efficiente per la determinazione simultanea di anilina, nitrobenzene, e loro derivati clorurati (cloroaniline e cloronitrobenzeni), considerati dalla legislazione nazionale sostanze pericolose per l'ambiente idrico. Il metodo adotta l'estrazione SPE in ambiente basico con conseguente analisi in GC-MS senza alcun tipo di derivatizzazione. Una validazione della procedura in termini di linearità, recupero, ripetibilità e robustezza, nonché una preliminare valutazione di stima dell'incertezza, si sono rese necessarie per l'applicazione del metodo a livello di agenzia di controllo e laboratorio di analisi di routine su matrici ambientali. Lo stesso metodo è stato testato su matrici ambientali reali per verificarne la fattibilità e per indagare sulla presenza di queste sostanze come inquinanti dell'ambiente acquatico della regione.

L'esigenza data dalla Direttiva Quadro di ottenere un *buono stato chimico*, sia per acque superficiali che per acque sotterranee, ha spinto allo sviluppo di ulteriori indagini legate alla problematica di nuovi inquinanti organici che in qualche modo influenzano la qualità dell'ecosistema acquatico. L'attenzione si è quindi concentrata su una classe di sostanze affine, per caratteristiche chimiche, a quelle già considerate: derivati azotati dei benzotrifluoruri (BTFs). La conoscenza in letteratura di un episodio d'inquinamento della falda idrica dovuta ad alcune di queste di sostanze nella zona del Vicentino e risalente alla fine degli anni settanta, ha permesso di riconsiderare l'evento per verificare la situazione attuale nelle zone interessate dalla passata contaminazione. Le tecnologie d'indagine odierne, sia chimico-analitiche nonchè territoriali, hanno permesso di riprendere il caso e studiarlo in modo maggiormente approfondito partendo dai pochi e disomogenei dati di cui si era in possesso. E' stato quindi possibile elaborare in modo maggiormente dettagliato i dati storici disponibili e quindi successivamente è stato possibile sviluppare specifici metodi di determinazione analitica partendo da alcune modifiche della procedura già considerata per l'analisi di cloroaniline e cloronitrobenzeni. I risultati hanno permesso di confrontare la situazione passata con quella attuale, di individuare la presenza di ulteriori contaminanti non descritti dalla letteratura come inquinanti ambientali e di identificarne una loro tracciabilità nel territorio. La persistenza ambientale di tali sostanze, riscontrabili in alcuni casi in considerevole quantità anche a distanza di decenni, e la propagazione di queste sostanze a livello di falda acquifera nella zona della Valleagno, pone la questione di quanto sia importante rivalutare e non trascurare eventi passati poiché il territorio può fungere da "archivio" per contaminazioni chimiche dell'ambiente.

Infine il lavoro di tesi ha affrontato problematiche connesse alla tossicità ambientale di alcune di queste sostanze indagando, seppur in modo preliminare attraverso tests ecotossicologici effettuati su tre livelli trofici diversi, sulla reale pericolosità intrinseca delle sostanze pure.

Technical-Scientific production

Technical production:

- A.A.V.V. "Indagine sulle sostanze pericolose nelle acque superficiali. I risultati del progetto I.S.PER.I.A." - Regione Veneto Pubblication May 2009;
- Polesello S., Carere M., Lava R. "Specific potentially present and on identified and localised main pressure sources in four Bulgarian pilot areas" - Report for the EU twinning Project BG 06 IB EN 01, July 2009

Scientific production related to the thesis topic:

- Lava R., Menegus L., Pojana G., Marcomini A., Simultaneous determination of chloroaniline and chloronitrobenzene derivatives on environmental waters – manuscript submitted at the *International Journal of Environmental Analytical Chemistry* on January 2009

Oral presentation:

- Tallinn - Estonia, 14 May 2008: "Dangerous substances: the effects assessment and the chemical tests under the REACH Regulation" EE 05-IB-TWP-ESC-09 Workshop on "Rising awareness on exposure based risk assessment of chemicals";
- Simitli - Bulgaria, 18 February 2009: "Methods for determination of priority substances of WFD list" BG 06-IB-EN-01 Workshop on "Strengthening of surface water monitoring network operations";
- Verona - Italy, 25 March 2009: "A future approach of the Environmental Protection Agency of Region Veneto to the ecopharmacovigilance" Workshop on Ecopharmacovigilance at the International Society of Pharmacovigilance (ISoP) Annual Meeting;
- Bucarest - Romania, 7 September 2009: "Current situation about REACH Regulation enforcement and the Authorities Inspection System" RO 06-IB-EN-10 Workshop on "Support to the NEG to strengthening capacity on inspection and control".

Abbreviations and Acronyms

AA-EQS – Annual Average Environmental Quality Standard
AN - Aniline
ANOVA – Analysis Of Variance
APAT – Agenzia Protezione Ambientale nazionale
APPI – Atmospheric Pressure Photoionization
ARICA – Aziende Riunite Collettore Acque Provincia Vicenza
ARPAV – Agenzia Regionale di Protezione e Prevenzione Ambientale del Veneto
BTA – Best Technologies Available
BTF – Benzotrifluoride
CAs – Chloroanilines
CBs - Chlorobenzenes
CNBs – Chloronitrobenzenes
CNR – Centro Nazionale di Ricerca
CRM – Certified Reference Material
DAD – Diode Array Detector
DSD – Dangerous Substances Directive
EPA – US Environmental Protection Agency
E-PRTR – European Pollutant Release and Transfer Register
EQS – Environmental Qualità Standard
ESI – Electrospray Ionization
EU – European Union
EU-RAR – European Union Risk Assessment procedure
FID – Flame Ionization Detector
FS – Full Scan
GC – Gas Chromatography
GHS – Globally Harmonized System
GPC – Gel Permeation Chromatography
HPLC – High Performance Liquid Chromatography
HR – High Resolution
HS – Head Space
IPPD – N-isopropyl-N’-phenyl-1,4-phenylenediamine
IRSA – Istituto di Ricerca sulle Acque
ISPERIA – Identificazione di Sostanze PERicolose Immesse nell’Ambiente Acquatico

ISPRA – Istituto Superiore di Protezione Ambientale
ISS – istituto Superiore di Sanità
IT – Information Technologies
LC – Liquid Chromatography
LLE – Liquid-Liquid Extraction
LLLME – Liquid-Liquid-Liquid Micro Extraction
LPME – Liquid-Phase Micro Extraction
LOD – Limit of Detection
LOEC – Low Observed Effect Concetration
LOEL – Low Observed Effect Level
LOL – Limit of Linearity
LOQ – Limit of Quantification
LV-OC – Large Volume on Column
MAC-EQS – Maximum Allowable Environmental Quality Standard
MAE – Microwave-assisted Extraction
MBOCA – 4,4’-Methylenebis(2-chloroaniline)
MMLLE – Microporous Membrane Liquid-Liquid Extraction
MS – Mass Sprectroscopy
NB – Nitrobenzene
NOEC – Non-Observed Effect Concentration
NOEL – Non-Observed Effect Level
NPD – Nitrogen Phosphorous Detector
PA - Polyacrylate
PAH – Polycyclic Aromatic Hydrocarbon
PDMS - Polidimethylsiloxane
PLE – Pressurized Liquid Extraction
PTV – Programmed Temperature Vaporizing injector
P&T – Purge and Trap system
QA/QC – Quality Assurrance / Quality Control
REACH – Registration, Evaluation, Authorization and Restriction of Chemicals
RP – Reverse Phase
RSD – Relative Standard Deviation
SD – Standard Deviation
SFE – Supercritical Fluid Extraction
SIM – Selected Ion Monitoring

SIR – Selected Ion Recording
SME – Small Medium Enterprise
S/N – Signal to Noise ratio
SPE – Solid Phase Extraction
SPME – Solid Phase Micro Extraction
TeCE – Tetrachloroethylene
TGD – Technical Guideline Document
TIC – Total Ion Current
TOC – Total Organic Carbon
TOF - Time of Flight mass detector
TrCE - Trichloroethylene
UV – Ultraviolet
VOC – Volatile Organic Compound
WFD – Water Framework Directive
WWTP – Wastewater Treatment Plant

1. INTRODUCTION

1.1 Thesis objectives

1.1.1 Aim of the work

The research activity has been developed at ARPAV (Environmental Protection Agency of Region Veneto)[1] laboratories in Venice Mestre. ARPAV, established by Regional Law 18/10/1996 n.32[2], is delegated by Veneto Region as scientific/technical control agency to carry out monitoring and controls on different environmental sectors, including water protection.

With the adoption at the EU level of Directive 2000/60/EC Water Framework Directive (WFD), it was necessary to investigate from the scientific point of view, before the applicative and technical ones, on criteria for the adjustment of environmental monitoring plans, competence of analytical laboratories and for an anthropic impact assessment through better knowledge of environmental problems, characterization of the territory and control of pressure sources.

During the last years ARPAV was involved, in collaboration with the National Environmental Agency (APAT, now ISPRA) and the Health Ministry (i.e. ISS), in many activities regarding WFD implementation, such as the drawing up of the guidelines for the monitoring (*Guidelines of monitoring for the Water Framework Directive*, European Commission 2003) and the project of the Regional Water Protection Plan as provided by national legislation.

In 2003, through Environmental Ministry Decree n.367 of November 2003, the I.S.PER.I.A. (Identificazione di Sostanze PERicolose Immesse in Ambiente aquatico) Project was set up in Veneto, to address the lack at Regional level on control and monitoring of dangerous and priority substances on environmental waters. The work of this thesis is financially supported by the I.S.PER.I.A Project, with the aim of providing a scientific contribution to the technical and applicative activities that the Agency carries out every day.

1.1.2 Questions to be answered

Since the first draft of the I.S.PER.I.A. Project, ARPAV realized the need to allocate more resources to fulfil the legislative requirements. The requests of the Agency in specific were:

1) a development of suitable methods for the determination of some classes of 160 pollutants regulated by Decree 367/2003, not considered in the previous regional monitoring plan of environmental waters;

2) a strong validation work in support of the internal methods due to the lack of standardized methods in scientific literature and especially at regulated official method levels;

3) an application of the methods developed to obtain environmental samples in support of the monitoring activities planned with I.S.PER.I.A monitoring plan and a testing of the validity of the analytical determination on real samples;

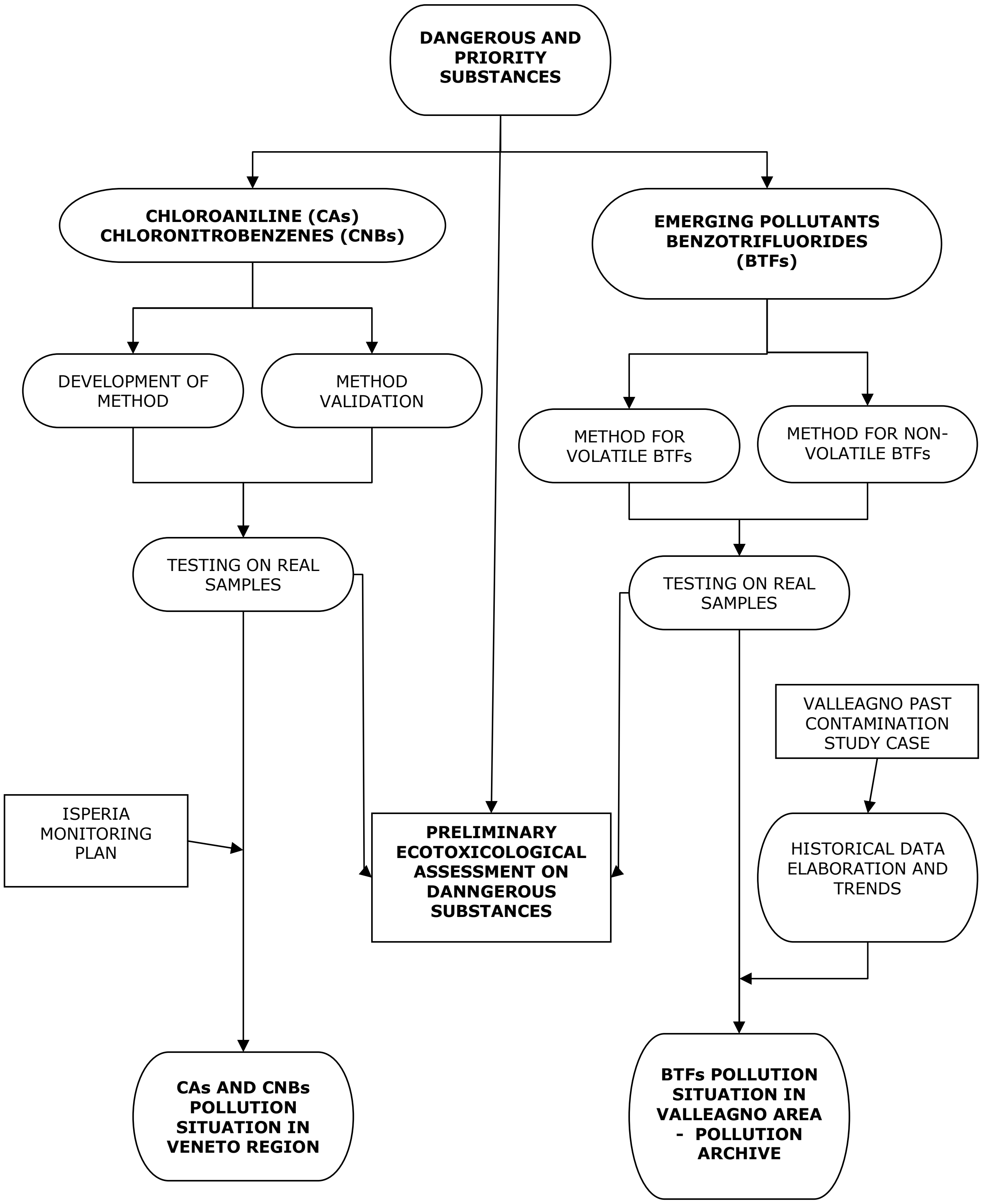
4) a first survey, in collaboration with other Departments of the Agency, of the use in human activities and thus the presence of these contaminants in the Veneto Region area.

The work initially focuses on two classes of compounds relatively new for EU legislation and not considered at Italian level before Decree 367/2003 came into force: chloroanilines and chloronitroaromatics derivatives. ARPAV laboratories had no methods for determining these classes of contaminants. A method for the simultaneous determination of these two classes of compounds was developed to obtain an easy and rapid multiresidue procedure to apply in a routine/control environmental laboratory. At the same time a work of validation was carried out to demonstrate the ruggedness of the procedure. The method was applied on specific real samples to examine the feasibility and to check the presence of these substances on territory as water pollutants. Consequently, a set of samples from different environmental waters collected on Region Veneto was analysed after a planned study of possible sources drawn according the information already available from the territorial-industrial survey of I.S.PER.I.A Project. Afterwards the procedure will be use by the Agency for the control of these classes of substances on the future campaigns provided by the forthcoming monitoring plans.

On the other hand, from 2003 to 2009, the environmental legislation at the national level has undergone many important and relevant changes with a more awareness of the investigation of new potential chemical pollutants, arising from new knowledge of the territory and from assessments of toxicological and ecotoxicological factors on the aquatic systems. Particular considerations came up during the work of thesis focusing attention on groundwater resource and its *good chemical status* as detailed by WFD requirements. A study case of a past chemical contamination of groundwater from benzotrifluoride derivatives during the Seventies, in the area of Vicenza Province (Valleagno), was considered. The similarity from the chemical point of view between the benzotrifuoride compounds, detected in a first moment only at qualitative level, addressed part of the thesis objectives to apply the same method used for the analysis of choroaniline and chloronitroaromatics pollutants with the proper modifications. A quantitative determination of volatile and non-volatile benzotrifluorides then was carried out together with an evaluation of the few past data available about the specific contamination episode. Moreover the development of modern analytical techniques, related to a better knowledge of the territory, helped to aim the work on a comparison between the past and the nowadays situations of the pollution still detected on the area studied. The attention was focused on the real persistence of benzotrifluoride compounds on the territory and on the importance of a sort of "pollution archive" obtained studying the trends of data.

Finally a preliminary assessment of ecotoxicological effects on the environment of some of the dangerous substances considered at analytical level was carried out because of a lack of toxicological information in the scientific literature. The preliminary work compared the substances examined at three trophic levels including a first investigation on the toxicological effects due to the different substituents of the molecules and a preliminary evaluation of effects as suggest on the indication expressed by the WFD.

The following flow chart summarizes the work carried out during the thesis period:



1.2 Regulatory aspects involved in the implementation of the WFD

This chapter considers the water protection and management from the point of view of the Directive 2000/60/EC Water Framework Directive (WFD) and the following "daughters" Directives, focusing the problems connected to dangerous and harmful substances. Surface and inland water protection, as well groundwater protection, are considered because these are the topics that the experimental part of the work investigated In a similar way, transposition to the Italian legislation and to the national environmental regulation were referred.

1.2.1 The Water Framework Directive and the concept of RBDs

On 23 October 2000, EU sets out Directive 2000/60/EC Water Framework Directive[3] (WFD) that established a framework for Community action in the water field supporting the principle for a sustainable policy of water management. The Water Framework Directive is the most substantial piece of water legislation ever produced by the European Commission, and will provide the major drivers for achieving sustainable management of water in all the Member States. It requires that all inland and coastal waters, within defined river basin districts (RBD), must reach at least a *good status* by 2015 and defines how this should be achieved through the establishment of environmental objectives and ecological targets for surface waters. The result will be a healthy water environment achieved by taking due consideration of environmental, economic and social factors.

Over the past 30 years, a series of EU Directives had a strong influence on Member States' water laws and regulations. They addressed priority issues, such as water quality objectives for waters used for drinkable purposes, the control of dangerous substances, the protection of the sea, the preservation of fundamental biological and ecological balances and the adoption of industry specific measures to reduce pollution. WFD's fundamental principals are:

1) preventive protection of the asset "water";
2) integrated protection in all the matrixes involved;
3) protection of water with the scope of a sustainable use.

The WFD addressed its aims to protect and enhance the status of aquatic ecosystems and of terrestrial ecosystems and wet areas directly dependant on aquatic ecosystems, with an approach that considers the global safeguard of these ecosystems. Details of the key features of the WFD directive can be summarized as follows.

- The concept of river basin management is introduced to all Member States through the establishment of *river basin districts* (RBDs) as basic management units. For international rivers these river basin districts will transcend national boundaries (Article 3).
- For each RBD a river basin management plan must be developed, including a programme of measures, and these will form the basis for the achievement of water quality protection and improvement (Articles 11 and 13).
- Although its most important aim is the environment, the Directive embraces all three principles of sustainable development. Environmental, economic and social needs must all be taken into account when river basin management plans are being developed (Article 9).
- The river basin management plans will not allow further deterioration to existing water quality. With certain defined exceptions, the aim is to achieve at least *good status* for all water bodies in each RBD. Definitions of good status for surface and groundwater are given below. (Article 4).
- The two previously competing concepts of water quality management, the use of Environmental Quality Standards and the use of emission limit values are brought together by the Directive in a new dual approach (Article 10).
- The previous water environment regulations and a number of existing directives will be replaced when new local standards are developed to meet the Directive requirements. These local standards must be at least as stringent as those being replaced. Daughter Directives are introduced to deal with groundwater quality and for priority substances (formerly known as dangerous substances) (Article 16).
- Measures to conserve water quantity are introduced as an essential component of environmental protection. Unless minimal, all abstractions must be authorised and, for groundwater, a balance struck between abstraction and the recharge of aquifers (Article 11).
- The “polluter pays" principle is incorporated through a review of measures for charging for water use, including full environmental cost recovery (Article 9).
- Public participation and the involvement of stakeholders is a key requirement of the RBD planning process. (Article 14).

The Directive will impact on every aspect of water use: domestic, industrial, agricultural, leisure and environmental conservation. Besides restrictions on point source discharges, the achievement of *good status* will mean tackling the problem of diffuse pollution from agriculture and contaminated land. In some instances, it may require river re-grading work or the reversal of land drainage schemes to restore lost habitats. The sustainable objectives on the use of water resource and the needs of its integrated implementation were focused on the VI° Environmental Action Program (VI° EAP- Decision 1600/2002/CE)[4]. The program provided that:

- ✓ an high level of quality of all surface and groundwater hydric bodies can be ensured;
- ✓ a complete implementation of the WFD with a prevention of pollution and a promotion of sustainable use of hydric resources can be reached;
- ✓ satisfactory conditions from the ecological, chemical and quantitative points of view can be created.

In particular for the last point a coherent and sustainable management is strictly connected with the elaboration of actions to stop the discharges, the emissions and the leaking of dangerous and harmful substances into water bodies. The Water Framework Directive sets out clear deadlines for each of the requirements which add up to an ambitious overall timetable. Table 1.1 listed the key milestones and deadlines involved with WFD. There are a number of objectives in respect of which the quality of water is protected. The key ones at European level are: general protection of the aquatic ecology, specific protection of unique and valuable habitats, protection of drinking water resources and protection of bathing water. All these objectives must be integrated into each RBD. It is clear that the last three, special habitats, drinking water areas and bathing water, are applied only to specific bodies of water and their supporting special wetlands, i.e. those identified for drinking water abstraction and those generally used as bathing areas. On the other hand, ecological protection should apply to all waters. The central requirement of the treaty is that the environment will be protected to a high level in its entirety. Under the Directive for river basin management planning, the RBD may comprise one or more river basins. These may encompass lakes, streams, rivers, groundwater and transitional waters (estuaries) together with the coastal waters into which they flow. In the natural state, their ecology will depend on such factors as their hydromorphology and their physicochemical state. Coastal inlets and bays also influence the ecology of river basins.

Table 1.1: key deadlines on the application of the WFD.

Year	Issue	Reference
2000	Directive entered into force	Art. 25
2003	Transposition in national legislations Identification of RBDs and Authorities	Art. 23 Art. 3
2004	Characterisation of RBDs: pressures, impacts and economic analysis	Art. 5
2006	Establishment of monitoring network Start public consultation	Art. 8 Art. 14
2008	Present draft river basin management plan	Art. 13
2009	Finalise river basin management plan including programme of measures	Art. 13 & 11
2010	Introduce pricing policies	Art. 9
2012	Make operational programmes of measures	Art. 11
2015	Meet environmental objectives (*good status*) First management cycle ends Second river basin management plan & first flood risk management plan	Art. 4
2021	Second management cycle ends	Art. 4 & 13
2027	Third management cycle ends, final deadline for meeting objectives	Art. 4 & 13

1.2.1.1 The Ecological Status of surface waters

Each Member State had to harmonize its legislation with the Directive 2000/60/EC at a national level. The application must follow the “subsidiarity principle” to guarantee the *good status* of an aquatic environment at the EU level. Art.4 of WFD establishes that this *good status* must be reached within 15 years of the adoption of the Directive.

"*Good status* of surface waters" means the status reached by a surface hydric body when its ecological and chemical profile can be defined "good" according art.2. Art.8 prescribes the obligation for Member States to establish a monitoring plan to estimate the Ecological Status and the Chemical Status of water bodies according the provision of quality classes explained on Annex V.

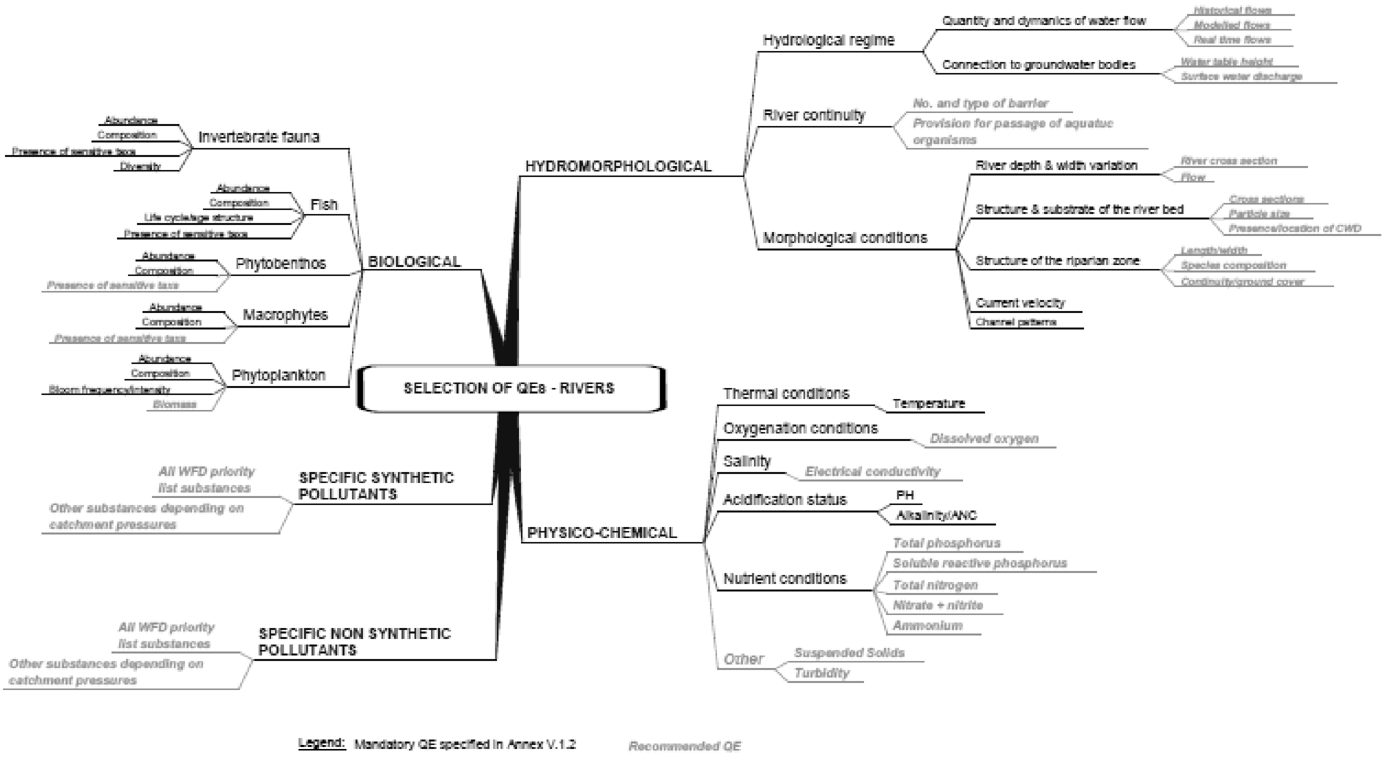
"Ecological status" is defined in terms of the quality of the biological community, the hydrological characteristics and the chemical characteristics. As no absolute standards for biological quality can be set which apply across the Community because of ecological variability, an identification of reference conditions specific for each hydric surface body is necessary. The controls are specified as allowing only a slight departure from the biological community which would be expected in conditions of minimal or no anthropogenic impact. For each surface water body, type-specific hydro-morphological and physicochemical conditions just shall be established representing the values of quality elements specified in point 1.1 of Annex V for that surface water body type at high ecological status as defined in the relevant table in point 1.2 of Annex V.

A set of procedures for identifying that point for a given body of water, and establishing particular chemical or hydromorphological standards to achieve it, is provided, together with a system for ensuring that each MS interprets the procedure in a consistent way to ensure comparability. The system is complicated, but this is inevitable due to the extent of ecological variability and the large number of parameters which must be dealt with.

Figure 1.1 represents the frame of elements for the monitoring of surface water bodies following the WFD approach that is also explained on the "Guidance on monitoring for the Water Framework Directive"[5] edited in 2003. The strategy provides for each water body:

1) a classification of the typology of water body (river, lake, lagoon, artificial water body,…);
2) a selection of the reference site with a good ecological status for each typology as reference condition;
3) a comparison between the ecological status of the investigated area and the reference site;
4) a definition of the quality classes as explained on Annex V of WFD.

Figure 1.1: selection of Quality Elements for river classification. Legend: mandatory QE specified in Annex V (dark grey), recommended QE (light grey) [from European Commission, 2003 *Guidance on monitoring*].



Since the purpose of the Directive is to maintain water quality where this is already at least of *good status*, and to restore waters failing to achieve this, it is essential to have an effective means of classifying the status of the various types of water bodies. The classifications for surface water bodies are as follows:

1) *High status:* very little, or no, anthropogenic alteration to the physicochemical and hydro-morphological quality elements and biological quality elements expected for the type of water body in pristine condition.

2) *Good status:* reflecting undisturbed conditions or minimal disturbance. Values of the biological quality elements for the water body type show low levels of distortion resulting from anthropogenic activity but deviate

only slightly from those normally associated with the water body under undisturbed conditions.

3) *Moderate status:* the values of the biological quality elements for the water body deviate moderately from those normally associated with that body under undisturbed conditions and show moderate signs of distortion resulting from anthropogenic activity and are significantly more disturbed than under conditions of *good status*.

Member States may further classify waters as *Poor* or *Bad* to reflect stages of deterioration beyond *Moderate status*.

Whilst the Directive indicates in qualitative terms the nature of the hydro-morphological, physicochemical and biological elements, the quantitative values for these elements are ensured by some elements:

- MS use comparable ecological quality assessment systems and harmonised ecological quality criteria.
- Ecological criteria are agreed for the designation of sites of *good ecological status.*
- Agreed numerical *Ecological Quality Ratio* values are established for the class boundaries between high-good and good-moderate classes.
- Use of intercalibration exercise.

The *Ecological Quality Ratio* for a water body is simply the ratio of the observed biological value to the reference biological value. It is close to one for a water body exhibiting high status and close to zero for a water body exhibiting bad status. The intercalibration exercise, instead, will cover all surface water body types and may include heavily modified and artificial water bodies to facilitate the identification of maximum, good and moderate ecological potential for an alternative classification system for such water body types. The intercalibration exercises were completed by 2006. The classification process provides the basis for the following outputs:

- Identification of the characteristics of *good status* for all groups of water bodies.
- Establishment of the current status of all water bodies in a RBD using existing data.
- Determination of where actions are needed.

1.2.1.2 The Chemical Status of surface waters

The WFD also requires MS to establish environmental monitoring of waters and a list of priority substances. According to Art.16, a “strategy against pollution of waters” from chemicals with a procedure for identification of priority substances and priority hazardous substances needs to set out by MS, as well the adoption of specific measures against pollution. The chemical monitoring programs should be set out for the substances of the priority list of Annex X of the WFD and for the river basin pollutants. The European list defines the chemical status of a surface water body while the river basin specific pollutants are part of the ecological status assessment. Monitoring both priority list (Annex X of WFD) and other river basin specific pollutants of WFD substances for the purpose of determination of the chemical and ecological status shall be performed according to Article 8 and Annex V of WFD. The monitoring plan should include column waters, sediment and biota.

A *good chemical status* is defined in terms of compliance with all the Environmental Quality Standards (EQS) established for chemical substances at European level. The Directive provides a mechanism for renewing these standards and establishing new ones by means of a prioritisation mechanism for hazardous chemicals. This will ensure at least a minimum chemical quality, particularly in relation to very toxic substances, everywhere in the Community. The river-basin specific pollutants should be selected taking into account the family or groups of substances described on Annex VIII of WFD; from the same list the priority substances at EU level of Annex X were considered. This list is shown on Table 1.2.

The highest chemical status is reached when the single synthetic pollutants concentrations are close to zero or, at least, under the LOD of the best analytical techniques generally available. For single no-synthetic pollutants, the best status is reached when concentrations are close to the background level.

Table 1.2: indicative list of the main pollutants (Annex VIII of WFD).

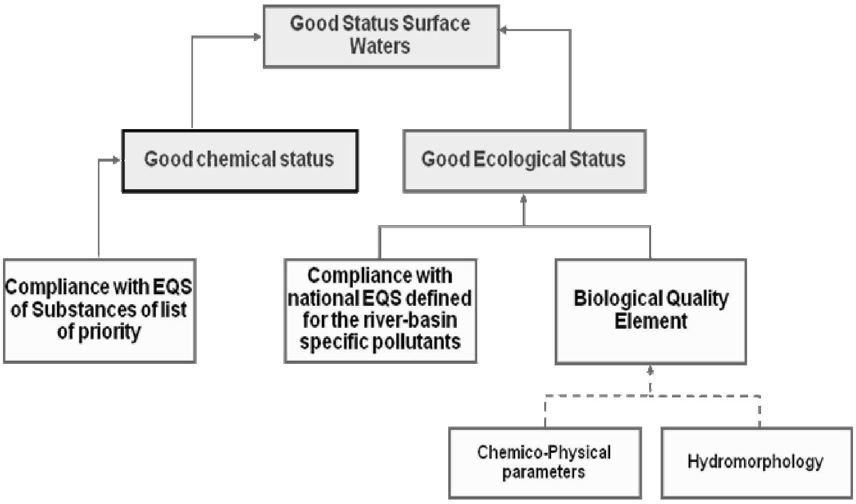
1. Organohalogenated compounds and substances which may form such compounds in the aquatic environment
2. Organophosphorous compounds
3. Organotin compounds
4. Substances and preparations, or the breakdown products of such, which have been proved to possess carcinogenic or mutagenic properties or properties which may affect steroidogenic, thyroid, reproduction or other endocrine-related functions in or via the aquatic environment
5. Persistent hydrocarbons and persistent and bioaccumulable organic toxic substances
6. Cyanides
7. Metals and their compounds
8. Arsenic and its compounds
9. Biocides and plant protection products
10. Suspended matter
11. Substances that may increase eutrophication (in particular nitrates and phosphates
12. Substances with negative effects on oxygen balance

Figure 1.2 shows how compliance with EQS of priority substances and the determination of river basin specific pollutants can be considered for the final status of surface water.

The EU policy concerning the control of dangerous and hazardous substances on environmental waters was introduced by "Council Directive on pollution caused by discharge of certain dangerous substances" (Directive 76/464/EEC)[6], also called Dangerous Substances Directive (DSD). Several substances have been regulated in specific "daughter" Directives[7,8,9,10,11] during the 1980s by defining Community-wide emission limit values and quality objectives for surface waters.

Directive 76/464/EEC was based on a "one-way" approach with the primary aim of human health protection. It identified two substances lists based on toxicity, persistence and bioaccumulation properties. List I detailed the substance to phase out for the aquatic environments while List II proposed a list of substances to be reduced on disharge and emission. Only with the WFD was the approach integrated with both human health and aquatic ecosystem safeguards. As part of the ongoing restructuring of the Community water policy, the DSD is now integrated in the WFD, while Directive 76/464/EEC will be fully repealed in 2013. In particular Article 7, related to the list II substances, remains a key element for the control of dangerous substances in the aquatic environment. For this purpose, MS may apply the principles of the identification of pollution problems and the substances causing them, the establishment of quality standards and the adoption of measures laid down in the WFD. The List II of the DSD includes indeed many groups of substances that should be part of Annex VIII of the WFD.

Fig 1.2: block diagram explaining the compliance with WFD.



The first list of priority substances was adopted in 2001 with Decision N.2455/2001/EC[12]. The aim of this decision was to identify a list of substances of concern (priority substances) that presented a significant risk to or via the aquatic environment and so to complete Annex X of WFD. The preparation of the first list of priority substances included a procedure called COMMPS (Combined Monitoring-based and Modelling-based Priority Setting) which was developed to identify the substances of highest concern at Community level[13]. In the application of COMMPS procedure about 820000 monitoring data from waters and sediments from all MS were evaluated and data for more than 310 substances on production, use and distribution in the environment were used for modelling if available monitoring information was not sufficient. The result was the identification of a list of 33 substances, or group of substances, which have been shown to be of major concern for EU waters. Within this list, 11 substances have been identified as priority hazardous substances which are of particular concern for the inland, transitional, coastal and territorial waters (Table 1.3). These substances will be subject to cessation or phasing out of discharges, emissions and losses within an appropriate timetable that shall not exceed 20 years. A further 14 substances were identified as being subject to review for identification as possible "priority hazardous substances". This issue has been addressed in the Commission proposal on EQS that then became the Directive on Priority Substances (Directive 2008/105/EC)[14].

The definition of hazardous substances, priority hazardous substances and pollutant are:

- ✓ "Hazardous Substances" means substances or groups of substances that are toxic, persistent and liable to bioaccumulate, and other substances or groups of substances which give rise to equivalent levels of concern.
- ✓ "Priority Substances" (PS) means substances identified in accordance with Art. 16(2) and listed in Annex X that present a significant risk to or via the aquatic environment. Among these substances there are "Priority Hazardous Substances" (PHS) meanings substances identified according with Art 16(3) and (6) for which measures have to be taken according to Art. 16(1) and (8).
- ✓ "Pollutant" means any substances liable to cause pollution, in particular those listed in Annex VIII.

For PS, a progressive reduction of discharges, emissions and losses is planned while for PHS, cessation of discharge, emissions and losses or phasing-out is compulsory by 2020.

The *good chemical status* is reached when the concentration of priority substances of Annex X are under the fixed EQS of Annex IX ("Emission values" and Environmental Quality Standards). Art.10 of WFD provide that all emission sources (point or diffuse) were choose with the combined approach of emission controls based on the Best Available Technologies (BAT), of emission limit values and on the application of best environmental procedure. The characterization of chemical contamination from point and diffuse sources is described by Annex II, point 1.4 of WFD. Annex II of WFD clearly requires the identification of all significant pressures, from point to diffuse, and speaking about "discharges, emissions and losses". It is evident that a broad interpretation of the term "discharge", which covers all sources and pathways into the aquatic environment, must be considered throughout the WFD. Actually in the CIS Technical Guidance on Surface Water Chemical Monitoring[15] a substance is considered being discharged into a river basin when it is being introduced via point or diffuse sources or through accidental releases.

MS should monitor the specific river basin pollutants that are discharged in significant quantities in river basins or sub-river basins. "Significant quantities" means quantities that could compromise the achievement of one of the Directive's objectives. As said the specific river-basin pollutants are part of the ecological status while the analysis of pressures and impacts is the first important step towards the identification of those pollutants. The starting point in the WFD is consequently the list of 'main pollutants' mentioned in annex VIII (Table 1.2). This list can be considered equivalent to the "universe of chemicals", since no chemical substance or pollutant can be excluded from the beginning. The challenge is to develop an iterative approach which narrows the endless list of substances down to a manageable number of pollutants in a pragmatic and targeted step-by-step approach. The final aim is to target the measures and the monitoring to those substances first which are most affecting the aquatic environment on the different levels mentioned-above. In that respect, the national EQS set in accordance to Annex V, 1.2.6 is the most important benchmark since it represents the boundary between "good" and "moderate" status.

The list of relevant pollutants may change during the different steps in the implementation of the WFD mainly due to a refinement of the analysis and assessments. But during this process, it is important that the evolution of the list of relevant pollutants remains transparent and clearly linked to the objectives and the requirements of the WFD.

1.2.2 The "daughter" Directives of WFD

The two most important "daughter" Directive necessary for the implementation of the WFD regarded the priority substances and the groundwater protection. The "daughter" Directive 2008/105/EC defined the chemical status of aquatic environments through the setting of EQS of Annex IX of WFD and given quantitative emission values for priority substances. The "daughter" Directive 2006/118/EC was necessary for a specific groundwater protection policy. Groundwater quality is essential for the several human use (i.e. in-take for drinkable purpose) and as environmental agent sensitive to contamination, especially the chemical one.

1.2.2.1 The Priority Substances Directive (2008/105/EC)

The “daughter” Directive 2008/105/EC on environmental quality standards (EQSs) for surface water priority substances has been published on December 2008 in compliance with the requirements of the article 16 of WFD[14]. It was base on proposal COM(2006) 398 proposed by EU Commission on July 2006[16]. The key components of the Directive are:

1) the definition of the chemical status through the setting of EQSs for the water phase for the 33 priority substances and for 8 dangerous substances (so-called other pollutants) derived from List I of the DSD and the setting of biota EQSs for hexachlorobenzene, mercury and hexachlorobutadiene;
2) the identification of the priority hazardous substances that have to be eliminated from all discharges, emissions and losses within 20 years from the publication of the directive;
3) a list of new substances, mostly emerging pollutants (table 1.4), for which the European Commission will do a review to evaluate their inclusion in the list of priority substances or priority hazardous substances;
4) the obligation for MS to elaborate an inventory of emission, discharges and losses of all the priority substances.

Annex II of this new directive replaces Annex X of WFD referring to the list of priority substances. Member States shall take actions to meet those quality standards by 2015 as part of chemical status (Article 4 and Annex V point 1.4.3). EQSs have been established on the basis of:

- EU Risk Assessment (EU-RAR)
- Technical Guidance Documents (TGD) according to Regulation 793/93[17] (i.e. TGD on Risk Assessment of Chemicals[18])
- Application of “safety factors” to NOEC (Not Observed Effect Concentration) measured in ecotoxicological and/or human toxicological tests.

Water EQSs are listed on Annex I (Part A and B) and for each substance 2 EQSs have been stated for surface internal water (rivers, lakes, streams, artificial river bodies) and for other surface waters (coastal, transitional, lagoon and territorial waters). The EQS are expressed in μg/L as (table 1.3):

✓ AA-EQS: annual average EQS based on monthly measurements;

✓ MAC-EQS: maximum allowable concentration, applicable only for some substances characterized by seasonal discharges.

Table 1.3: EQS (in μg/L) for priority substances and certain other pollutants established on Annex I and II of Directive 2008/105/EC and that replaced Annex X of WFD.

No	Name of substances	CAS number	AA-EQS inland surface waters	AA-EQS other surface waters	MAC-EQS inland surface waters	MAC-EQS other surface waters
1	Alachlor	15972-60-8	0.3	0.3	0.7	0.7
2	Antrachene	120-12-7	0.1	0.1	0.4	0.4
3	Atrazine	1912-24-9	0.6	0.6	2.0	2.0
4	Benzene	71-43-2	10	8	50	50
5	Brominated diphenylether	32534-81-9	0.0005	0.0002	na	na
6	Cadmium and its compounds	7440-43-9	≤ 0.08 0.08 0.09 0.15 0.25	0.2	≤ 0.45 0.45 0.6 0.9 1.5	≤ 0.45 0.45 0.6 0.9 1.5
6a	Carbon tetracloride	56-23-5	12	12	na	na
7	C10-C13-chloroalkanes	85535-84-8	0.4	0.4	1.4	1.4
8	Chlorfenvinphos	470-90-6	0.1	0.1	0.3	0.3
9	Chlorpyriphos-ethyl	2921-88-2	0.03	0.03	0.1	0.1
9a	Cyclodiene pesticides: Aldrin Dieldrin Endrin Isodrin	 309-00-2 60-57-1 72-20-8 465-73-6	Σ = 0.01	Σ = 0.005	na	na
9b	Total DDT/p,p-DDT	50-29-3	0.025/0.01	0.025/0.01	na	na
10	1,2-dichloroetane	107-06-2	10	10	na	na
11	Dichloromethane	75-09-2	20	20	na	na
12	DEHP	117-81-7	1.3	1.3	na	na
13	Diuron	330-54-1	0.2	0.2	1.8	1.8
14	Endosulfan	115-29-7	0.005	0.0005	0.01	0.004
15	Fluoranthene	206-44-0	0.1	0.1	1	1
16	Hexachlorobenzene	118-74-1	0.01	0.01	0.05	0.05
17	Hexachlorobutadiene	87-68-3	0.1	0.1	0.6	0.6
18	Hexachlorocyclohexane	608-73-1	0.02	0.002	0.04	0.02
19	Isoproturon	34123-59-6	0.3	0.3	1.0	1.0
20	Lead and its compounds	7439-97-6	7.2	7.2	na	na
21	Mercury and its compounds	7439-97-6	0.05	0.05	0.07	0.07
22	Naphtalene	91-20-3	2.4	1.2	na	na

23	Nickel and its compounds	7440-02-0	20	20	na	na
24	4-Nonylphenol	104-40-5	0.3	0.3	2.0	2.0
25	Octylphenol	140-66-9	0.1	0.01	na	na
26	Pentachlorobenzene	608-93-5	0.007	0.0007	na	na
27	Pentachlorophenol	87-86-5	0.4	0.4	1	1
28	PAH:					
	Benzo(a)pyrene	50-32-8	0.05	0.05	0.1	0.1
	Benzo(b)fluorantene	205-99-2	Σ = 0.03	Σ = 0.03	na	na
	Benzo(k)fluorantene	207-08-9			na	na
	Benzo(g,h,i)perylene	191-24-2	Σ = 0.002	Σ = 0.002	na	na
	Indeno(1,2,3-cd)pyrene	193-39-5			na	an
29	Simazine	122-34-9	1	1	4	4
29a	Tetrachloroethylene	127-18-4	10	10	na	na
29b	Trichloroethylene	79-01-6	10	10	na	na
30	Tributhyl cations	36643-28-4	0.0002	0.0002	0.0015	0.0015
31	Trichlorobenzenes	12002-48-1	0.4	0.4	na	na
32	Trichloromethane	67-66-3	2.5	2.5	na	na
33	Trifluralin	1582-09-8	0.03	0.03	na	na

na: not applicable

For organic pollutants the total concentration in the whole sample is considered (dissolved and particulate fractions). For metals (Cd, Pb, Hg, Ni) only dissolved fraction, after filtration at 0.45 μm, is considered. MS may also, when assessing the monitoring results against the EQS for metals and their compounds, take into account the natural background if they prevent compliance with EQS value as well other parameters that can affect bioavailability of metals (e.g. hardness, pH). For metals, the Directive set different provisions: for example for cadmium the EQS changes in comparison to the concentration of $CaCO_3$.

The provision of chemical data by MS shall be ensured by analytical methods conform to relevant international or national standards or, alternatively, by analytical methods that are not standardized but provide data of equivalent or better scientific quality and comparability than standard analytical methods. It is established a minimum performance criteria for methods of analysis to be applied by MS when monitoring water, sediment and biota status. In particular, according to standard ISO 6107-2:2006[19]:

- U% (Relative Uncertainty, k=2) ≤ 50 % at EQS level
- LOQ ≤ 1/3 EQS.

If there are no methods that satisfied the minimum criteria, the BAT have to be considered[20]. Annex III of the "daughter" Directive, finally, listed substances subject to review for possible identification as PS or PHS. For other pollutants relevant at river basin level, but not compliance with EQS at EU level, national EQS are defined.

After the second agreement with the European Parliament there is a provision approved by Council and Parliament that concerns the sediment and biota: there is the possibility for MS to set EQS for sediment or biota for specified priority substances and for some specific types of water bodies. The EQS for sediment have to guarantee the same level of protection of the EQS derived for water. Sediment and biota chemical analysis should also be fundamental for the assessment of long-term trend and to evaluate the no-deterioration objective required by WFD.

Table 1.4: list of substances subject to review for possible identification as priority substances or priority hazardous substances (Annex III, Directive 2008/105/EC).

CAS number	Substance
1066-51-9	AMPA
25057-89-0	Bentazon
80-05-7	Bisphenol-A
115-32-2	Dicofol
60-00-4	EDTA
57-12-5	Free cyanide
1071-83-6	Glyphosate
7085-19-0	Mecoprop (MCPP)
81-15-2	Musk xylene
1763-23-1	Perfluorooctane sulphonic acid (PFOS)
124495-18-7	Quinoxyfen (5,7-dichloro-4-(p-fluorophenoxy)quinoline)
-	Dioxins
-	PCB

It is clear, from Annex III and from the five-years system of revision of the lists, the approach at EU level to investigate in new potential pollutants and a continuous updating work of the same list and of method to assess potential candidates to the list based on the best scientific and technological knowledge.

1.2.2.2 The Groundwater Directive (2006/118/EC)

One of the innovations of the WFD is that provides a framework for integrated management of groundwater and surface water for the first time at European level. While the quantitative status objectives were clear in the WFD, aiming to ensuring a balance between abstraction and recharge of groundwater, chemical status criteria were more complex to be defined at the time of the adoption of the WFD so it was decided to request the Commission to come forward with a proposal for a "daughter" Directive clarifying good chemical status criteria and specifications related to the identification and reversal of pollution trends of groundwater. Therefore the Groundwater Directive (2006/118/EC)[21] has been adopted on 12 December 2006 according to Art.17(1) and (2) of WFD. The presumption in relation to groundwater should broadly be that it should not be polluted at all. It is the most important reservoir for drinkable water and about 75% of EU inhabitants depend on it for their water supply[22]. For general protection, another approach was necessary. It was essentially a precautionary approach that comprises a prohibition on direct discharges to groundwater, and, to cover indirect discharges, a requirement to monitor groundwater bodies so as to detect changes in chemical composition and to reverse any anthropogenic induced upward pollution trend. Taken together, these should ensure the protection of groundwater from all contamination, according to the principle of minimum anthropogenic impact. For this reason, no EQS values were set out for groundwater but only guidelines, on Annex II, to each MS for determination of EQS at national level. Few standards have been established at EU level in accordance with previous Directives[23,24,25] only for particular issues: nitrates, pesticides and biocides with their metabolites (Annex I). The classification of groundwater bodies is not as complicated as that for surface waters, although the monitoring of change to groundwater status can be very complex. Groundwater bodies are classed with *good status* when both there are a *good quantitative status* and a *good chemical status*. This is reachable when direct and indirect abstractions have minimal effect on the water body’s ability to support dependant ecosystems and when it complies with Directive requirements for maximum levels of defined pollutants. A *good status* need to be achieved by 2015.

Before the end of 2008 MS were obligated to set out the EQS at national level or at RBD level for priority substances and pollutants and to start the monitoring with the scope of assessment water quality trends. Furthermore each MS assessed procedure for testing compliance to *good chemical status* and the identification and reversal of significant and sustained upwards trend. Annex II (Part B) gives a minimum list of substances for which MS are required to establish threshold values according to Art. 4(2). This list is divided in:

1) substances or ions which may both occur naturally and as result of human activities;
2) man-made synthetic substances;
3) conductivity as important parameter to understand salinity intrusion.

Finally the groundwater management plan had to establish that for all indirect discharge or emission on groundwater an authorization is necessary at condition that the *good chemical status* was not altered.

1.2.3 Implementation of WFD at Italian level

1.2.3.1 From Decree 367/03 to Ministry Decree 56/2009

The innovative approach of WFD on dangerous substances establishes the duty of set out and of apply EQS for water bodies directly to MS. From the old approach based on List I and II of Directive 76/464/EEC, with the objectives to phase out substances of List I and reduce emissions of substances of List II, the new approach was introduced with an indicative list of main pollutants to search (Annex VIII) and a more specific list of mandatory priority substances to monitor (Annex X) as already explained in the previous paragraphs. The Environmental Ministry Decree n. 367 of 6 November 2003[26] was the transposition at national level of Directive 76/464/EEC on pollution caused by discharge of certain dangerous substances into water bodies. Decree n.367/2003 introduced EQSs for inland surface waters, for coastal-transitional waters and for sediments of coastal-transitional waters. 160 substances, divided into 10 classes, were chosen as pollutants, PS and PHS substances with different values to achieve before 31 December 2008 (Column A) and before 13 December 2015 (Column B). As well EQSs for 27 substances in the sediments of marine-coastal waters, lagoons and coastal ponds were identified. The first deadline was established to reach a general chemical status for human health protection, the second deadline, instead, was introduced to have a *good chemical status* for the entire aquatic ecosystem. The necessary actions for protection of water bodies have been related to analytical result obtained on a health and environmental risk assessment if the application of BTA is not able to reach EQS pre-established. For each situation the aims were to phase out all 160 PS below LOD, or at a background level for natural substances, for 1 January 2021. Each Region had to search all available information on pollution sources, impact of pollutions, production and usage of chemicals or derivatives before 1 January 2006 with a following monitoring plan to update the anthropogenic pressures. The classes established by Decree n.367/2003 are listed on table 1.5.

Table 1.5: priority substances classes considered by Italian Environmental Ministry Decree n.367/2003

Classes	Number of pollutants	Number of PS
Metals	6	4
Organometals	6	2
Polycyclic Aromatic Hydrocarbons (PAH)	9	9
Volatile Organic Compounds (VOC)	37	5
Nitroaromatics	7	0
Halogenated phenols	9	1
Aniline and derivatives	5	0
Pesticides	54	14
Semivolatile Organic Compounds	8	1
Other compounds	19	8

The Legislative Decree n.152 of 6 April 2006[27], introduced later and considered actually the most important piece of environmental legislation at Italian level, set out new requirements for PS and PHS with more monitoring of biological and hydromorphological parameters. With Decree 152/2006 general issues of WFD approach were introduced at national level because Decree 367/2003 was based on the old water legislation in force in Italy until 2005[28]. In particular, general requirements for a monitoring and classification of environmental waters based on generic EQSs were considered repurposing the WFD design with a first draft of technical criteria. On Annex I of Part III of Decree 152/2006, 36 chemical parameters divided into 6 classes are specified for surface water monitoring with limit values in μg/L and relative standard methods for analytical determination based on National

Research Council Water Institute (CNR-IRSA) studies. A second list of additional parameters to monitor was introduced with no specific EQS that will be introducing by Environmental Ministry only in case of real detection on the territory. These two lists included all the 160 substances of Decree 367/2003 even if there was no clear evidence of repealing of Decree 367/2003. Recently, the last amendment of Decree 152/2006, the Environmental Ministry Decree n.56 of 14 April 2009[29] clarifies the criteria of classification of water bodies and adopts new AA-EQS as specified in the "daughter" Directive 2008/105/EC for all priority substances plus other chemicals considered dangerous for aquatic system in the RBDs of Italian territory.

"Quantitative and chemical status" of groundwater of WFD was generally introduced by Decree 152/2006 while the Groundwater Daughter Directive 2006/118/EC were fully transposed at national level with the Legislative Decree n.30 of 16 March 2009[30]. This Decree takes into account also Directive 2008/105/EC giving EQS at national level to achieve the *good chemical status* of groundwater in Italy. For these reasons Annex II, in application of Art. 7(2), of Decree 30/2009 reported a minimum list of dangerous substances considered as PS or PHS by Directive 2008/105/EC. Annex III, on the other hand, considers the chemical status giving definition of *good chemical status*, adopting the EQS at EU level for groundwater (nitrates, pesticides and biocides with their metabolites), proposing national threshold values in μg/L for groundwater and for groundwater with close interaction with surface waters (e.g. resurgences). The compliance with threshold values and EQS is verified on average results of data based on monitoring plan. The provision of chemical data by shall be ensured by analytical methods conform to relevant international or national standards. For some pollutants where no standards method according EN ISO 17025[31] are yet available (e.g. C_{10}-C_{13} chloroalkanes), the analytical results will be obtained by the application of BTA from the scientific and economic points of view. Decree 30/2009 gives also details for the determination of the quantitative status as well technical guidelines for groundwater monitoring plan.

1.2.3.2 The application at Regional level: ISPERIA Project

The objective of I.S.PER.I.A. Project[32] (Identificazione delle Sostanze PERicolose Immesse nell'Ambiente acquatico) was to implement the Decree n.367/2003 at regional level. In the Decree quality standards for 160 hazardous substances in inland surface waters, transitional and coastal and marine quality standards for 27 substances in the sediments of marine-coastal waters, lagoons and coastal ponds were identified and a subsequent work of impact analysis at Regional level was necessary simultaneously the adoption of a Regional Water Protection Plan.

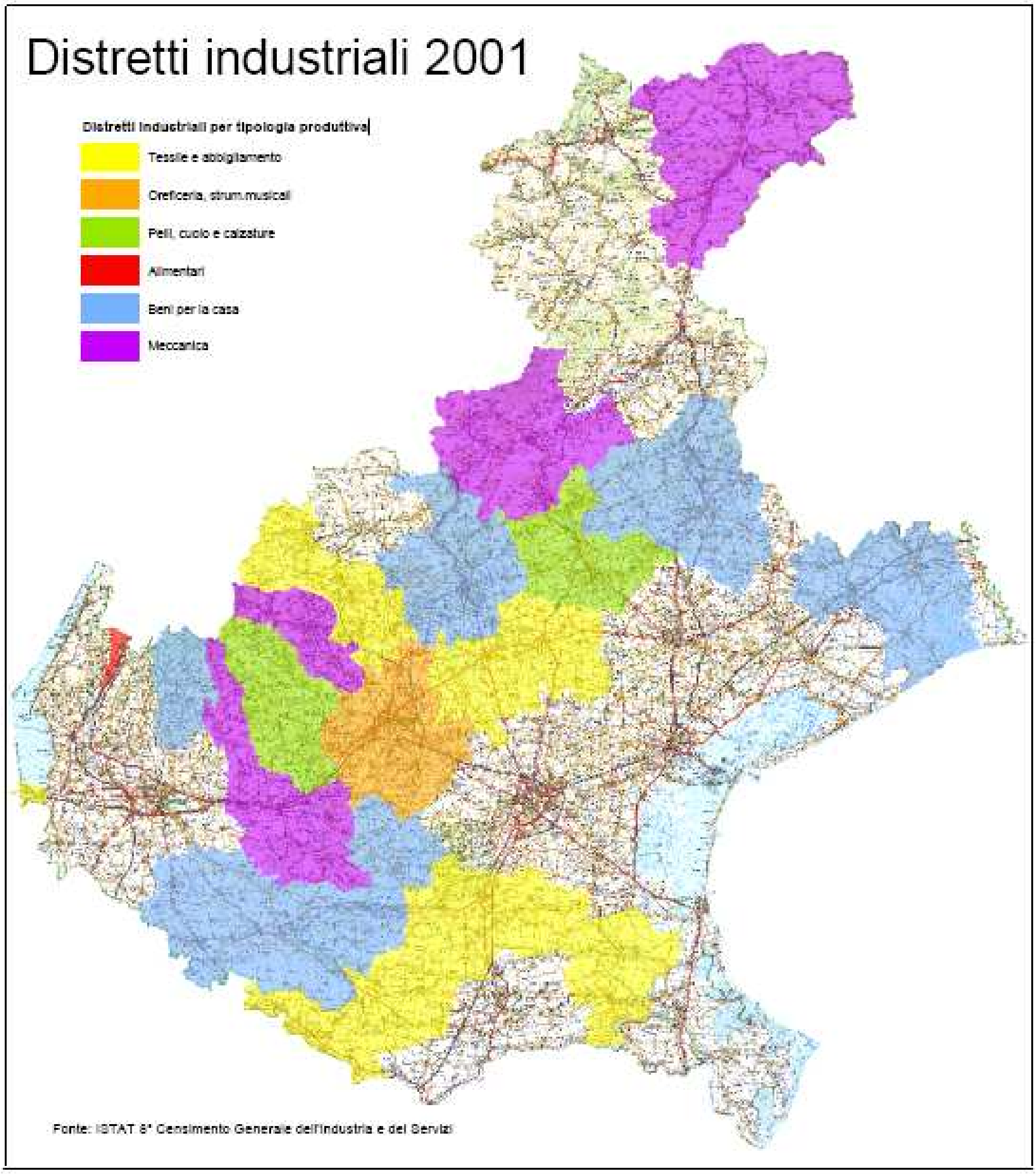
The project was divided into a number of specific objectives:

a) to establish a list of dangerous and harmful substances used in Veneto area;
b) to draw up the list of sources of releases of hazardous substances identified (exhaust and industrial emissions, sewage treatment, agriculture...);
c) to locate the sources of origin;
d) to update the current monitoring ARPAV network of inland surface waters, coastal and marine transition waters enhancing the number and the location of monitoring stations also with the introduction of new searched parameters;
e) to adequate the network of regional laboratories and the analytical instrumentation to be able to analyse the new pollutants arriving at LOD significant for the EQS established by Decree 367/03;
f) to identify the programs of actions for the reduction or elimination of hazardous substances achieving the objectives expected to be observed by Decree 367/03

All these actions, especially the latter, were in support of operational decisions of the Veneto Region. Among the expected results there was the establishment of a list of potentially dangerous substances in the region and their sources of origin, the adaptation of analytical methods and Regional Laboratories for analysis of dangerous substances, an initial knowledge of environmental status of surface waters in the Veneto and to the presence of hazardous substances. The ISPERIA Project was concluded and presented to Veneto Region at the end of 2007 and all the work was useful to help the Regional Environmental Agency to understand better the vulnerable areas and the potential chemical contamination situation. The activities were divided into two planned actions: 1) the monitoring plan on field with a final elaboration of data of dangerous substances determined in waters samples collected during the period 2004-2007 from strategic monitoring stations and 2) a territorial analysis.

The territorial analysis consisted in a deep investigation of industrial districts analysing industrial process activities and their potential impacts on the environment and a study of use and distribution of pesticide at Regional level. Figure 1.3 shows the macro-districts considered for the initial pressure analysis.

Figure 1.3: industrial districts in Veneto Region in 2001 based on ISTAT census data (taken from ISPERIA Project final report)



Fonte: ISTAT 8° Censimento Generale dell'Industria e dei Servizi

1.3 Selected priority substances of thesis concern

This chapter considers in specific some classes of substances harmful and dangerous for the environment. These substances are synthetic and are used in several industrial processes. They can be released into the environment as pollutants during their production, transport or use. Besides, can be incurring in the environment as degradation products or metabolites of more complex substances, especially if these are used for agrochemical purpose. Annex VIII of WFD shows and indicative list of chemicals that can be considered as pollutant for the aquatic system. The list is not specific and, excluding the selected priority substances for which the control is compulsory, a procedure for identification of priority substances and priority hazardous substances needs to set out by each MS. Indeed river basin specific pollutants are part of the ecological status assessment as well the adoption of the specific measures against pollution at regional/local level.

1.3.1 Chloronitrobenzenes and chloroaniline

Chlorinated nitrobenzenes (CNBs) and chlorinated aromatic amines (CAs) are two classes of substances generally dangerous because of their toxicity. They are considered by Italian environmental legislation as "pollutants of main concern" according the EU Commission first drafts of the Water Framework Directive 2000/60[33]. WFD requires Member State to establish environmental monitoring of waters and a list of priority substances according specifically to Art.16. Therefore a "strategy against pollution of waters" from chemicals, including a procedure for the identification of PS and PHS, was set out by Italy as well the adoption of specific measures against pollution of water bodies. Annex VIII of the WFD explains the indicative list of main pollutants from which the Environmental Ministry Decree n.367 of 6 November 2003 was resulted. Decree n.367/2003 introduced EQSs for inland surface waters, for coastal-transitional waters and for sediments of coastal-transitional waters and 160 substances were selected divided into 10 classes. CNBs and CAs were considered into those lists.

1.3.1.1 Chloronitrobenzenes and chloroaniline properties

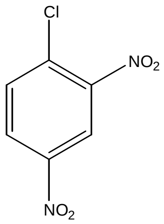
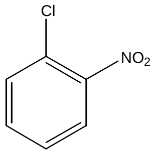
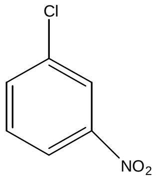
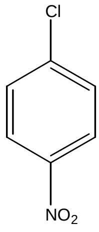
The compounds considered are aromatics characterized by, at least, one atom of chlorine and a nitrogen-group, as nitro (-NO_2) or amino (-NH_2), substituted to the benzene ring. Organochlorine compounds derive exclusively from anthropic synthetic processes since they are not found as natural product. The presence of a C-Cl bond induces particular stability properties at the molecule at the degradation; the chlorine atoms presence reduces reactivity of the other functional groups of the molecules for its deactivating properties. The lack of natural removal system at organism level associated at the hydrophobic properties, gives to chlorine-substituted substances, and to halogenated-substituted substances in general, the problem of bioaccumulation in organisms[34].

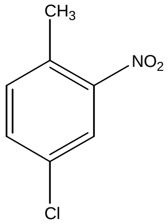
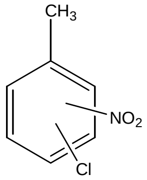
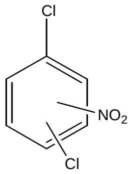
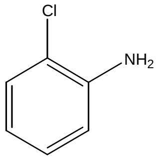
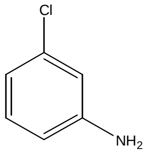
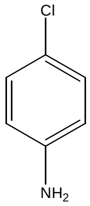
CAs are organic compounds with relative polar and alkaline properties, with a reasonable solubility on water and a moderate volatility[35]. The aromatic ring decreases the alkalinity of the amine, but the amino group strongly increases the reactivity of the aromatic ring because of an electron-donating effect on nitrogen. Strong electron-attractor groups, normally deactivation groups, decreases the alkalinity of aniline nitrogen as effect of the attraction of electronic density from the basic site. Substituent as -NO_2 and –Cl are two examples respectively as a strong and a weak deactivating groups. If the substituent group is on *p*- or *o*- position compared the amino group, inductive and resonance effects are possible. Electron-donor groups instead, as -NH_2 or –CH_3, yield the site more active than aniline. The value of pK_a of CAs clearly shows the not negligible alkalinity of the molecules.

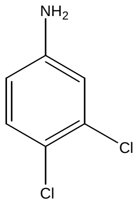
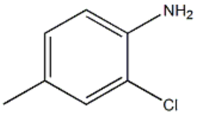
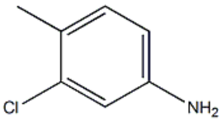
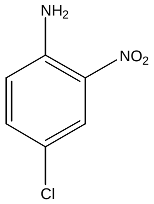
CNBs, on the other hand, are semivolatile compounds, less water-soluble and are the oxidized form of correspondent CAs. The presence on the molecule of two deactivating substituent, -NO_2 and –Cl, give the molecule a sort of stability and not a lot reactivity in the environment.

Since environmental distribution and persistence are controlled by the physicochemical properties, they are important to determine the transport pathway and the redistribution and partitioning in the environment of CAs and CNBs. Properties of importance are vapour pressure (V_p), water solubility (S), Henry's Law constant (H_{LC}), octanol/water partition coefficient (K_{OW}), organic carbon/water partition coefficient (K_{OC}) and ionization constant (pK_a) for ionisable compounds. Some of these properties are related or derived through estimation of experimental values as for example H_{LC} of K_{OC} that is correlated to K_{OW}. The environmental distribution and partitioning among air, water, soil, sediment and organic phase so can be estimated from these properties taking into account that ionisable compounds phase distribution is strongly controlled by S, pK_a and pH of water or soil[36]. The most important physicochemical properties of the considered CNBs and CAs are shown on table 1.6 the effect of adding chorine to aromatic molecules can be seen by an increase of K_{OW}, resulting in greater partitioning into organic carbon phases of soil and sediments. An increase of K_{OW} is generally connected to a great bioaccumulation property as compounds with log $K_{OW} > 3$ tends to bioaccumulate in aqueous environments[37].

Table 1.6: list of pollutants considered with main properties and EQS expressed on Decree n.367/2003 and on the new EQS establish by Decree n.56/2009.

COMPOUND	PHYSICO-CHEMICAL PROPERTIES*	EQS** (μg/L)
1-CHLORO-2,4-DINITROBENZENE  **(C24DNB)** 97-00-7	MW: 202.56 mp: 53.4°C bp (atm): 315°C V_p: $8.5\cdot10^{-5}$ mmHg ρ: 1.70 g/cm^3 Log K_{ow}: 2.17 H_{LC}: $2.8\cdot10^{-6}$ atm·m^3/mol S: 8 mg/L	2008: ND 2015: -
2-CHLORONITROBENZENE  **(2CNB)** 88-73-3	MW: 157.56 mp: 33°C bp (atm): 245.5 °C V_p: 0.027 mmHg ρ: 1.37 g/cm^3 Log K_{ow}: 2.52 H_{LC}: $4.45\cdot10^{-5}$ atm·m^3/mol S: 307 mg/L	2008: 1 D 0.1 M-L (provv.) 2015: - AA-EQS: 1 D 0.2 M-L
3-CHLORONITROBENZENE  **(3CNB)** 121-73-3	MW: 157.56 mp: 46°C bp (atm): 235.5 °C V_p: 0.097 mmHg ρ: 1.53 g/cm^3 Log K_{ow}: 2.41 H_{LC}: $7.37\cdot10^{-5}$ atm·m^3/mol S: 256 mg/L	2008: 1 D 0.1 M-L (provv.) 2015: - AA-EQS: 1 D 0.2 M-L
4-CHLORONITROBENZENE  **(4CNB)** 100-00-5	MW: 157.56 mp: 83°C bp (atm): 242 °C V_p: 0.094 mmHg ρ: 1.52 g/cm^3 H_{LC}: 2.39 Henry const: $5.44\cdot10^{-5}$ atm·m^3/mol S: 154 mg/L	2008: 1 D 0.1 M-L (provv.) 2015: - AA-EQS: 1 D 0.2 M-L

4-CHLORO-2-NITROTOLUENE  **(4C2NT)** 89-59-8	MW: 171.58 mp: 38.5°C bp (atm): 240 °C ρ: 1.26 g/cm^3	2008: 1 D 0.1 M-L (provv.) 2015: - AA-EQS: 1 D 0.2 M-L
CHLORONITROTOLUENEs (isomers sum)  **(CNT)**	MW: 171.58	2008: 1 D 0.1 M-L (provv.) 2015: - AA-EQS: 1 D 0.2 M-L
DICHLORONITROBENZENEs (isomers sum)  **(DCNB)**	MW: 192.01	2008: 1 D 0.1 M-L (provv.) 2015: -
2-CHLOROANILINE  **(2CA)** 95-51-2	MW: 127.57 mp: -2.3°C bp (atm): 208 °C V_p: 0.204 mmHg ρ: 1.21 g/cm3 Log K_{ow}: 1.90 H_{LC}: $4.2\cdot10^{-6}$ atm·m^3/mol S: 8.165 g/L pKa: 2.66	2008: 1 D 0.1 M-L 2015: 0.1 D 0.001 M-L AA-EQS: 1 D 0.3 M-L
3-CHLOROANILINE  **(3CA)** 108-42-9	MW: 127.57 mp: -10.4°C bp (atm): 230.5 °C V_p: 0.054 mmHg ρ: 1.21 g/cm3 Log K_{ow}: 1.88 H_{LC}: $1.68\cdot10^{-6}$ atm·m^3/mol S: 5.4 g/L pKa:3.52	2008: 2 D 0.2 M-L 2015: 0.2 D 0.02 M-L AA-EQS: 2 D 0.6 M-L
4-CHLOROANILINE  **(4CA)** 106-47-8	MW: 127.57 mp: 72°C bp (atm): 232 °C V_p: 0.027 mmHg ρ: 1.70 g/cm3 Log K_{ow}: 1.83 H_{LC}:$1.16\cdot10^{-5}$ atm·m^3/mol S: 3.9 g/L pKa: 3.98	2008: 1 D 0.1 M-L 2015: 0.1 D 0.01 M-L AA-EQS: 1 D 0.3 M-L

3,4-DICHLOROANILINE  **(3,4DCA)** 95-76-1	MW: 162.02 mp: 71°C bp (atm): 272 °C V_p: $6.32 \cdot 10^{-3}$ mmHg ρ: 1.33 g/cm3 Log K_{ow}: 2.69 H_{LC}: $1.46 \cdot 10^{-5}$ atm·m^3/mol S: 92 mg/L	2008: 0.1 D 0.01 M-L 2015: 0.05 D 0.005 M-L AA-EQS: 0.5 D 0.2 M-L
2-CHLORO-4-METHYLANILINE **(2C4MA)** 615-65-6 	MW: 141.60 mp:7°C bp (atm): 219°C ρ: 1.15 g/cm3	2008: ND 2015: -
3-CHLORO-4-METHYLANILINE **(3C4MA)** 95-74-9 	MW: 141.60 mp: 26°C bp (atm): 242°C Log K_{ow}: 2.58 H_{LC}: $1.6 \cdot 10^{-6}$ atm·m^3/mol S: 4.5 g/L	2008: ND 2015: -
4-CHLORO-2-NITROANILINE  **(4C2NA)** 89-63-4	MW: 172.57 mp: 116°C bp (atm): 245.5 °C V_p: $3.8 \cdot 10^{-4}$ mmHg Log K_{ow}: 2.64 H_{LC}: $3.62 \cdot 10^{-12}$ atm·m^3/mol	2008: ND 2015: -

[*] MW: molecular weight, mp: melting point, bp: boiling point, ρ: density, V_p calculated at 25°C, solubility on water.

[**] 2008 and 2015 are the old EQS values related to Decree 367/2003, while AA_EQS referred to the new EQS vales established with Decree 56/2009. D: EQSs for inland surface waters, M-L: EQSs for coastal-lagoon waters, ND: not determined.

1.3.1.2 Chloronitrobenzenes and chloroaniline uses

In general chlorinated-organic compounds are made from reaction between chlorine with hydrocarbons and have wide uses in different technological and chemical sectors because of their high stability and relative reduced reactivity. Both CNBs and CAs are used as intermediate and as final products on chemical industry. CNBs have a great industrial versatility as primary intermediates due to the wide range of products obtained by nucleophylic substitution reaction of the chlorine substituent. The three isomers of monochloronitrobenzene and the 1-chloro-2,4-dinitrobenzene are synthesized by nitration of chlorobenzene, obtaining a mixture of isomers mostly of *o*- and *p*- substituted products as result of the –Cl substituent. The reaction products yields are: 63-65% of 4CNB, 34-36% of 2CNB and 1-3% of 3CNB. C24DNB is obtained by second nitration of 2CNB with an *m*-oriented addition. It is estimated a worldwide production of around 100000 tonnes/y for the main CBNs. CNBs have a great versatility as primary intermediate for a wide selection of derivatives through nucleophilic substitution reaction of chlorine atom, electrophilic substitution of the aromatic ring with the insertion of other functional groups in *m*-position at the nitro-group or reduction of the nitro group to give the CAs. The reduction reaction is carried out by an hydrogenation at low pressure in presence of a catalyst (i.e. Fe-HCl)[38] In particular CNBs are used as intermediate in the synthesis and production of polymers and resins, dyes, pesticides and drugs[39]. 2CNB is an intermediate for the pesticide *carbofuran*, for 2-nitrophenols and 2-amminophenols, used as developer agent in the photographic industry. Moreover, its alkaline reduction, was a fundamental step for the production of 3,3′-dichlorobenzidine, a pigment used on the production of inks and dyes. 3CNB is a reagent used for pentachloronitrobenzene synthesis through an exhaustive chlorination. 4CNB is an important intermediate for the *dapsone* production, a medicine used in the treatment against leprosy and malaria, for *parathion* and *nitrofen* (herbicides), for other additives for technical oils and antioxidant for rubbers and polymers as IPPD. The dinitrochlorobenzenes are particularly involved in nucleophilic reaction in aqueous solution to give pesticides, herbicides, algaecides and dyes productis. Between the ten chloronitrotoluenes available on the market, only five have a potential technological concern for dyes and other azo-compound products[39,40].

The reduction reaction of the nitro group is the way to obtain correspondent CAs. Nevertheless CAs are technologically used as intermediate for the production of several dyes and pigments, pesticides, herbicides, cosmetics and pharmaceuticals, rubbers and polymers[41,42]. 2CA is an intermediate for MBOCA (4,4′-methylbis(2-chloroaniline)), considered a carcinogenic for animals and a probably carcinogenic substances for humans. MBOCA was widely used as vulcanizing agent in the production of polyurethane pre-polymers[43]. *Chloropropan* has 3CA as intermediate for its synthesis while 4CA is used for production of green pigments and herbicides (i.e. *monuron*, *monolinuron*, *diflubenzuron*) as well cosmetic products (i.e. 4-chlorophenols and chloroexidrine). It was estimated that in 1988, globally, 3500 tonnes of 4CA were producted for: 60% pesticides and herbicides, 20% cosmetic industry, 7.5% pigments and dyes industry[44]. 4C2NA is used for the production of yellow colour dyes and 34DCA (12000 tonnes/y in 1991) is used exclusively for the 3,4-dichlorophenylisocianate synthesis, an intermediate for the herbicide production (i.e. *diuron*, *linuron, propanil*) and cosmetic/deodorant industries (trichlorocarbanilide). *Diuron* is used not only as herbicide, but also as algaecide in substitution of the forbidden organotins compounds[45]. These compounds may be released in the environment as chemicals residues of degradation or metabolites of some pesticides as phenylcarbamate, phenylurea herbicides[46,47,48].

This study considers five CAs (2-chloroaniline, 3-chloroaniline, 4-chloroaniline, 3,4-dichloroaniline, 4-chloro-2-nitroaniline), five CNBs (2-chloronitrobenzene, 3-chloronitrobene, 4-chloronitrobenzene, 4-chloro-2-nitrotoluene, 1-chloro-2,4-dinitrobenzene) and two other compounds (2-chloro-4-methylaniline, 3-chloro-4-methylaniline) listed by Decree 367/03 as dangerous in its tables 1.7, 1.5 and 1.10 respectively. Limits of these compounds considered by decree 367/2003 as environmental quality standard (EQSs) are shown on table 1.6 where was established to have a good chemical status that preserve human health to reach before 2008 and in some cases with lower values for a complete protection of aquatic ecosystem before 2015. Nowadays the decree is repealed by the new national legislation in force, Legislative Decree 152/2006, which requires the monitoring of possible presence in the environment of these pollutants. The last amendment of Decree 152/2006 is the Legislative Decree 56/2009 that replaces old EQS with AA-EQS (annual average EQS) based on monthly measurements based on "daughter" Directive 2008/105/EC. Also new AA-EQS are represented on table 1.6 for substances which are established. For the other substances no EQS values are at the moment available. Only in case of relevant chemical contamination due to these chemicals, specific local EQS will be determined after a specific environmental risk assessment investigation of the pollution episode.

1.3.2 Benzotrifluoride compounds

Benzotrifluoride derivatives compounds are characterized by a -CF_3 bonded to the benzene ring. This functional group gives to molecules strong stability and hydrophobic characteristics. The insertion of a trifluoride group on a ring decrease normally the solubility in water compared to the same molecules not substituted. The compounds considered are not listed as priority by EU legislation, but consideration on WFD on procedures for identification of priority substances and priority hazardous substances cannot exclude there compounds from the indicative list of main pollutants of Annex VIII of WFD. Besides no information about their impact on the environment are available on literature. Table 1.7 shows some physicochemical properties of benzotrifluoride derivatives considered on this study.

1.3.2.1 Benzotrifluoride compounds: uses and properties.

Over 50% of benzotrifluoride derivatives production is used in the agrochemicals field. The ability of fluorine atom to be target more effectively than other halogens and than hydrogen means that a much smaller quantity of product is required to achieve effective results and a less amount used with obvious benefit for the environment. For this reason a wide range of biocide products and agriculture crop protection agents were developed with –CF_3 group. BTF is a colorless liquid used for dyes and pharmaceuticals, as solvent and vulcanizing agent and for insecticides. Other 40% are used as processing aids, paint additives, dyes and in chroming plating and about 10% goes to the pharmaceutical industry as intermediate in a series of new generation drugs (e.g. antidepressant, diuretocis, antibiotics, analgesics)[49]. Benzotrifluoride (BTF) is used in several chemical productions as in dye chemistry, high polymer chemistry, as solvent, dielectric fluid, vulcanizing agent, insecticide, and as an intermediate for pharmaceuticals[50]. It may result its release to the environment through various waste streams. If released to soil, BTF will have low mobility while volatilization may be important from moist and dry soil surfaces from an estimation of K_{OC} based on K_{OW} experimental value[51]. If released to water, it may adsorb to suspended solids and sediment. Experimental BCF values of 26-54 and 31-58 suggest that BTF bioconcetration in aquatic organisms is an important fate process[52]. If released to the atmosphere, BTF will exist solely in the vapour phase.

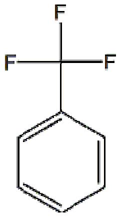
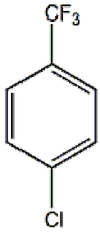
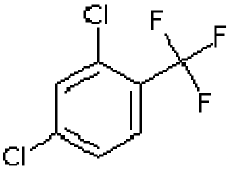
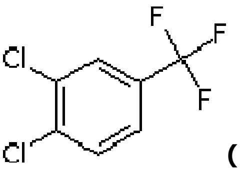
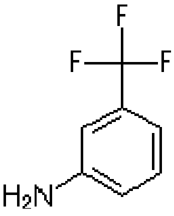
4-chlorobenzotrifluoride (4CBTF) is a key intermediate for the synthesis of dyes, pharmaceuticals and pesticides, and is used as solvent and dielectric fluid. In specific it is an intermediate for herbicides with a diphenyl ether structure as *fluorodifen* and *acifluorfen* and for the insecticide *fluvalinate*[38]. If released to air, a V_p of 7.63 mmHg at 25 °C indicates 4CBTF will exist mainly as a vapour in the ambient atmosphere. An estimated BCF of 320 was calculated for 4CBTF using an estimated log Kow of 3.6[53] and from the regression-derived equation[54]. According to a classification scheme[55], this BCF suggests the potential for bioconcentration in aquatic organisms is high.

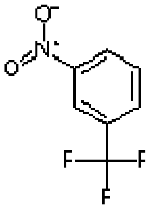
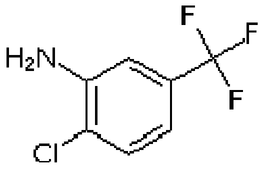
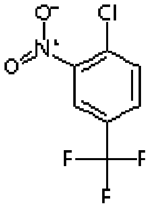
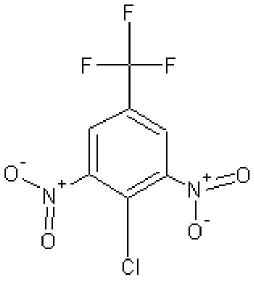
Between the dichlorine-substituted BTF, 3,4-dichlorobenzotrifluoride (34DCBTF) has the most important industrial use. 34DCBTF's production is for the manufacture of herbicides and may result in its release to the environment through various waste streams. If released to air, a V_p of 2.36 mmHg at 25 °C indicates 34DCBTF also will exist generally as a vapour in the ambient atmosphere. If released to soil, 34DCBTF is expected to have slight mobility based upon an estimated Koc of 4800. Volatilization from moist soil surfaces may be an important fate process based upon an estimated Henry's Law constant of $2.6 \cdot 10^{-2}$ atm·m^3/mol. 34DCBTF may potentially volatilize from dry soil surfaces based upon its V_p. However, adsorption to soil is expected to attenuate volatilization. A mean BCF of 1500 in rainbow trout suggests bioconcentration in aquatic organisms is very high[56]. Biodegradation is expected to be slow because of the high number of halogens. No biodegradation data for 3,4-dichlorobenzotrifluoride were found; biodegradation is expected to be slow as reported for other compounds containing a large number of halogens[57].

3-aminobenzotrifluoride (3ABTF) is industrially obtained by nitration of BTF to 3-nitrobenzotrifloride (3NBTF) followed by hydrogenation. 3ABTF and 3NBTF are used as intermediate for phenothiazine derivatives, intermediate for herbicides (e.g. *fluometron* and *norflurazon*), antihypertensive and diuretics drugs. In literature there are no information about their environmental fate and interaction with the environment as well there are no reported cases of chemical contamination from these pollutants. 3N4CBTF is synthesized by reaction of 4-chlorobenzotrichloride with anhydrous hydrogen fluoride under atmospheric of high pressure conditions, followed by nitration of the resulting 4CBTF, while 3A4CBTF is subsequent reduction of the –NO_2 functional group.

3-amino-4-chlorobenzotrifluoride (3A4CBTF) and 3-nitro-4-chloro-benzotrifluoride (3N4CBTF) are also used as intermediate for herbicides and drugs (e.g. *fluorodifen*)[38].

Table 1.7: list of benzotrifluoride derivatives considered with main physico-chemical properties

COMPOUND	PHYSICO-CHEMICAL PROPERTIES
BENZOTRIFLUORIDE  **(BTF)** 98-08-8	MW: 146.11 mp: -29.05 °C bp (atm): 103.46°C V_p :38.83 mmHg ρ: 1.19 g/cm^3 Log K_{ow}: 3.01 H_{LC}: 0.017 atm·m^3/mol S: 451 mg/L
4-CHLOROBENZOTRIFLUORIDE  **(4CBTF)** 98-56-6	MW: 180.6 mp: -33.0 °C bp (atm): 139.3 °C V_p: 7.63 mmHg ρ: 1.33 g/cm^3 Log K_{ow}: 3.60 H_{LC}: 3.5·10^{-2} atm·m^3/mol
2,4-DICHLOROBENZOTRIFLUORIDE  **(24DCBTF)** 320-60-5	MW: 215.00 mp: 10.0 °C bp (atm): 117.5 °C ρ: 1.48g/cm^3
3,4-DICHLOROBENZOTRIFLUORIDE  **(34DCBTF)** 328-84-7	MW: 215.00 mp: -12.5 °C bp (atm): 173.5 °C V_p: 2.36 mmHg ρ: 1.48 g/cm^3 Log K_{ow}: 2.39 H_{LC}: 2.6·10^{-2} atm·m^3/mol S: 15 mg/L
3-AMINOBENZOTRIFLUORIDE  **(3ABTF)** 98-16-8	MW: 161.12 mp: 5.5 °C bp (atm): 187.5 °C V_p:2.00 mmHg ρ: 1.30 g/cm^3 Log K_{ow}: 2.15 S: 5 g/L

3-NITROBENZOTRIFLUORIDE  **(3NBTF)** 98-46-4	MW: 191.12 mp: -5.0 °C bp (atm): 203.0 °C V_p: 0.204 mmHg ρ: 1.437 g/cm3 Log K_{ow}: 2.81 S:
3-AMINO-4-CHLOROBENZOTRIFLUORIDE  **(3A4CBTF)** 121-50-6	MW: 195.57 mp: 10°C bp (atm): 82 °C V_p: 0.11 mmHg ρ: 1.42 g/cm3 Log K_{ow}: 2.71 H_{LC}: atm·m^3/mol S: 11.0 g/L
4-CHLORO-3-NITROBENZOTRIFLUORIDE  **(3N4CBTF)** 121-17-5	MW: 225.55 mp: -2.0 °C bp (atm): 222.0 °C V_p: 0.04 mmHg ρ: 1.51 g/cm3 Log K_{ow}: 3.43 S: insoluble
4-CHLORO-3,5-DINITROBENZOTRIFLUORIDE  **(35DN4CBTF)** 393-75-9	MW: 270.55 mp: 57°C bp (atm): °C V_p: mmHg ρ: g/cm3 Log K_{ow}: H_{LC}: atm·m^3/mol S: mg/L

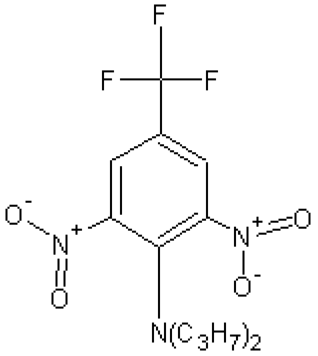
MW: molecular weight, mp: melting point, bp: boiling point, ρ: density, vapour pressure calculated at 25°C, solubility on water

4-chloro-3,5-dinitrobenzotrifluoride (35DN4CBTF) is obtained by reaction of p-chlorotoluene with hydrogen fluoride to produce 4CBTF, which then reacted with nitric and sulphuric acids for nitration[38]. 4C35DNBTF is mostly used for the production of the class of dinitro herbicide. The most important, and one of the most used herbicide worldwide, is *trifluralin* but a long list of herbicide derivatives of new generation are continuously developed to obtain more efficient, but at the same time, more "environmentally friendly" products. Figure 1.4 shows *trifluralin* chemical formula and the most important dinitro herbicides derivatives, all characterized by $-NO_3$ groups in position 3 and 5 to $-CF_3$ and with a different functional group that substitutes the chlorine atoms of 4-chloro-3,5-dinitrobenzotrifluoride giving the specificity of the molecule.

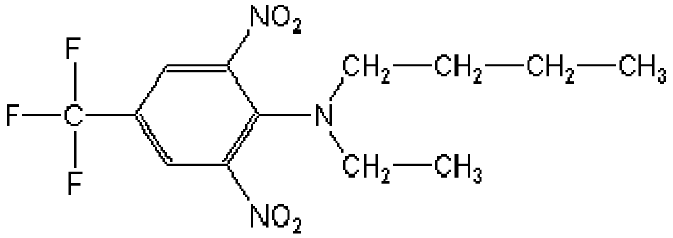
1.3.2.2 Trifluralin and dinitro herbicides

Trifluralin ($C_{13}H_{16}F_3N_3O_4$) has the IUPAC name α,α,α-trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine (CAS: 1582-09-8) and is an orange crystalline solid compound with a molecular weight of 335.5. It was introduced in 1960s as a soil-acting herbicide. Most important commercial names of the active principle are: Treflan, Trigard, Digermin, Tristar. It is used to control a wide range of annual grasses and broadleaf weeds in vegetable crops, root crops, flowers, sunflowers and shelterbelts plants[58]. *Trifluralin* appears to act as a mitotic poison affecting root growth and may also affect other metabolic reaction as lipid synthesis[59]. The main release route into the environment is from its application to agriculture crops, but could also be released from accidental discharges and spillage during manufacture, transport and storage. Volatilization is the major dissipation pathway and the occurrence of it in air generally follows the seasonal use of the herbicide. Another transportation pathway is surface runoff from treated fields although a minimal movement from soils it is expected because of its low solubility and strong adsorption to soil[60].The dinitro functional group in *trifluralin*, as well in other dinitro herbicides, extensively decreases the molecule's water solubility as it makes hydrogen bonds with alkyl groups of surrounding molecules (Table 1.8). For these reasons *trifluralin* concentration can generally be two to three orders of magnitude higher in sediments than in the surrounding environmental waters[61]. Its concentrations in streams from areas where it is used, have range from 0 to 1.8 μg/L and often are below LOD. Concentration in surface waters increase during spring runoff too[62].

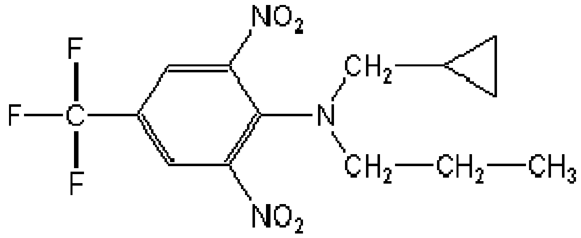
Figure 1.4: chemical formulas of most used herbicides of the dinitro class.



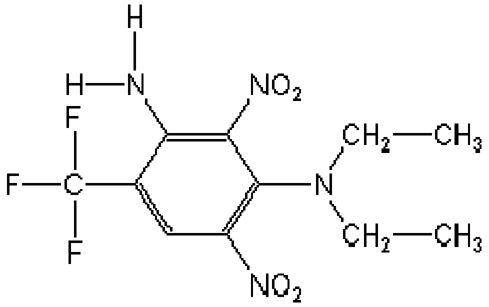
TRIFLURALIN



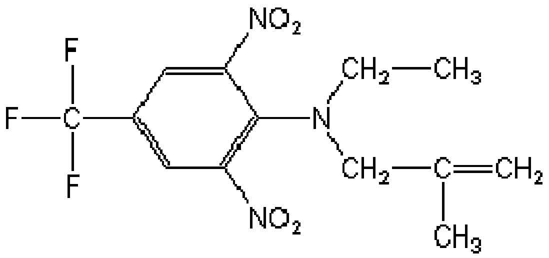
BENFLURALIN



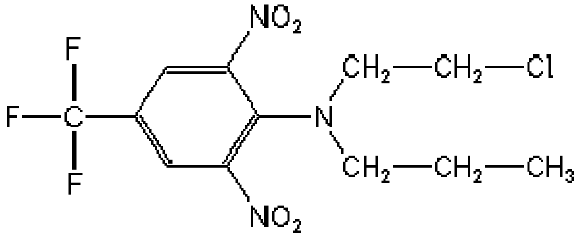
PROFLURALIN



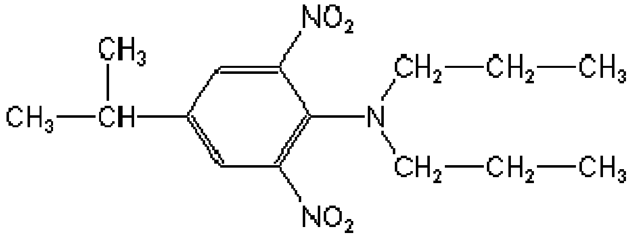
DINITRAMINE



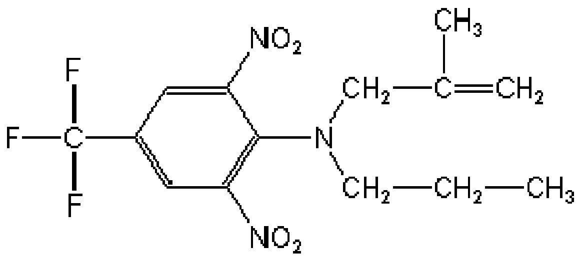
ETHALFLURALIN



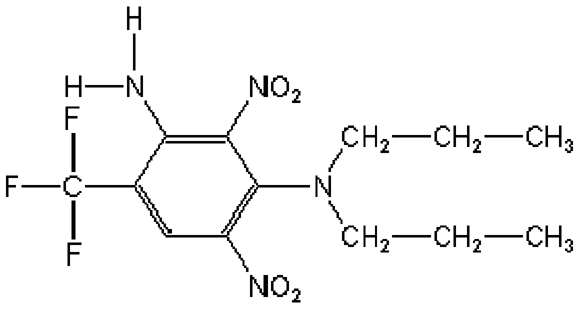
FLUCHLORALIN



ISOPROPALIN



METHALPROPALIN



PRODIAMINE

Trifluralin appears to have low toxicity for mammals and birds and so may not be as acutely harmful to terrestrial wildlife as some other pesticides (LD_{50} birds >5000 mg/Kg, Oral LD_{50} rat > 5000 mg/Kg)[63]. However aquatic organisms are highly susceptible to *trifluralin*. Other toxicological parameters are explained on table 1.8.

Table 1.8: Main physicochemical and toxicological properties of *trifluralin*.

Trifluralin physicochemical properties		
Molecular wheight	335.60	
Water solubility (25°C)	0.3 mg/L	Hornsby *et al*,. 1996
Vapour pressure (25°C)	$9.5 \cdot 10^{-3}$ Pa	Tomlin, 1994
log K_{OW}	5.34	Muller *et al*, 1994
log K_{OC}	3.87	Grover, 1991
Henry constant	4.02 $Pa \cdot m^3/mol$	Muller *et al*, 1994
$t_{1/2}$ into soil	60 days	Wauchope *et al*, 1992
Trifluralin toxicological parameters		
EC_{50} (96h, *Selenastrum capricornutum*)	0.7 mg/L	Fairchild *et al*, 1996
NOEC (*Selenastrum capricornutum*)	$1.5 \cdot 10^{-2}$ mg/L	Fairchild *et al*, 1996
	$5.6 \cdot 10^{-2}$ mg/L	RIVM, 1994
EC_{50} (24h, *Daphna magna*)	0.27 mg/L	RIVM, 1994
	0.56 mg/L	Tomlin, 1994
LC_{50} fishes	0.42-1.60 mg/L	RIVM, 1994

For its relevant toxicity of the aquatic organisms, *trifluralin* was listed for the first time in Annex II of Directive 2008/105/EC as priority substance in the field of water policy and today its use as active principle for pesticides and for agricultural crop protection is forbidden. Also Italian legislation with the new Environmental Ministry Decree n.56 of 14 April 2009 adopted restrictions and monitoring of *trifluralin* with an AA-EQS value of 0.03 µg/L, even for internal surface water and coastal/lagoon/transitional waters.

Table 1.9 shows the sales data[64] of *trifluralin* and some other dinitro herbicides in Italy and Veneto area in the last years.

Table 1.9: sales market data in Kg of trifluralin and most used dinitro herbicide at national (Italy) and regional (Veneto) level.

(Kg)	TRIFLURALIN		DINITRAMINE		BENFLURALIN		ETHALFLURALIN	
YEAR	**Italy**	**Veneto**	**Italy**	**Veneto**	**Italy**	**Veneto**	**Italy**	**Veneto**
1996	196751	12165	2325	825	2212	105	867	596
1997	191535	11123	3050	1375	1653	114	510	349
1998	113107	16378	1775	1375	2923	140	119	33
1999	91631	10592	1197	1100	2605	79	607	0
2000	217367	8132	2468	850	1460	20	530	0
2001	656244	10688	1325	625	1677	20	64	0
2002	*no data*	*available*						
2003	*no data*	*available*						
2004	59757	11973	227	209	14328	49	0	0
2005	25433	7996	45	44	861	66	4	0
2006	*no data*	*available*						
2007	*no data*	*available*						

Sales data from AAAF working group Report (2007)

1.4 Past and present sources of environmental contaminants of groundwater

1.4.1 Contamination of groundwater from organic pollutants

Groundwater is the most critical natural resource of water for use as water supply for human consumption. It is an expensive source of safe water that generally doesn't require treatments and a reservoir to buffer against extended periods of drought and because it has limited lost through evaporation or runoff. For these reasons it represents 98% of world liquid fresh water supply[65] and provides:

- 50% of world drinkable water supply;
- 40% of industrial demand;
- 20% of water used for irrigation in agriculture.

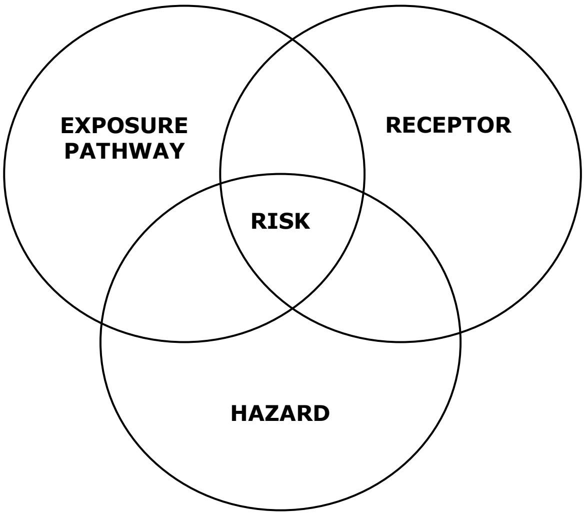
Considering groundwater a valuable natural resource, it should be protected from deterioration and chemical pollutions. If Directive 2000/60/EC sets out general provisions for the protection and conservation of groundwater, measure to prevent and control groundwater pollution should be adopted, including criteria for assessing the good chemical status and criteria for identification of significant and sustained upward trends and for the definition of starting points for trend reversals. Therefore at EU level was decided to come forward with a specific "daughter" directive for groundwater clarifying good chemical status criteria and specifications related to the identification and reversal of pollution trends. Consequently Groundwater Directive (2006/118/EC) has been adopted according to WFD requirements. In the directive it is also clearly expressed that researches should be conducted in order to provide better criteria for ensuring groundwater ecosystem quality and, first of all, to check groundwater quality.

Virtually all aquifers are subjected to qualitative degradation and to qualitative decline which could render substantial portions of groundwater unusable for the future. Often the extension and the severity of groundwater contamination of unsatured zones and aquifers have long been underestimated owing to the relative inaccessibility of aquifers. Its protection is a focal point for all water resource management.

Assessment procedures for determining the chemical status of a groundwater body needs to be carried out in relation to estimate its quality, specific if related at a pollution episode. Risk assessment examines the three basic elements which constitute risk: the chemical hazard, the receptor and the exposure pathway by which the receptor is exposed to the hazard. A correct risk management strategy can be used to reduce risks by reducing the degree of exposure or the severity of the hazard[66].

The hazard identification is the starting point with the individuation of possible specific sources of chemical contamination that may pose an human health or environment risk. An identification of all receptors that may be exposed to the hazard, integrated with estimation or a survey of its characteristics, is then necessary. Finally a qualitative and quantitative evaluation of the degree to which the receptors should be exposed to the hazard is at the base of the exposure assessment. All these elements connected give the quantitative assessment of the risk of each hazard to each receptor and the potential consequence on it. (Figure 1.5).

Figure 1.5: risk assessment bases.



The hazard identification includes the individuation of all chemical species that may concern. A subsequently survey of all significant pressures, point or diffuse sources or accidental releases, is necessary to understand the discharges, emissions or losses of the contaminant. Table 1.10 shows some examples of point and diffuse sources of contaminants that can be considered on a case of groundwater contamination.

Table 1.10: example of main point and diffuse sources of pollutants necessary to consider in the process inquiry for determination of river basin pollutants.

Point sources

- Industry
- SME
- IPPC industry
- Mine / ore extractions
- Waste deposit / landfill
- Underground storage of chemicals
- Households
- Historical contaminated land

Diffuse sources

- Atmospheric depositions
- Transports
- Indirect drainage of deep groundwater reservoirs
- Agriculture
- Traffic and non urban infrastructures
- Accidental spills
- Release from materials
- Natural sources

The sources considered in table 1.10 are defined as primary because of there is a direct release of the contaminant into the environment. From the source of contamination and through a specific pathway, the receptor is exposed to the chemicals. To clarify relevant factors for distribution of contaminants, on the other hand, it is necessary to understand the relation between the point source and the plume behaviour. The aim of a first investigation is to asses the uses and degree to which a groundwater body is at risk. Analysis may employ existing hydrological, geological, pedological, land use, discharge and abstraction data as provide by WFD. All data are necessary to identify:

- boundaries of the groundwater body
- pressure of contamination
- interaction with linked surface water
- abstraction/ artificial recharge
- a general characterization of the overlying strata in the catchments area.

Further deep investigations following the initial one, if the groundwater body has been identified as being at risk, in order to establish a more precise assessment and identification of measure are required. This characterization shall include relevant information on:

- geological characteristic of the groundwater body including the type of geological units;
- hydrogeological characteristics of the groundwater (i.e. hydraulic conductivity, porosity and confinement;
- characteristics of the superficial deposits and soil in the catchment from which the groundwater body receives its recharge (i.e. thickness, porosity, adsorption properties) ;
- stratification characteristic of the groundwater into the aquifer;
- associated surface systems, as terrestrial ecosystems and surface waters, with which the groundwater body is dynamically linked;
- estimation of direction, rate and exchanges of water between the groundwater body and the associated surface systems;
- characterization of chemical composition of groundwater and the hydrochemical behaviour to establish the background levels of the groundwater body.

As clear, considerations typical for surface waters cannot be valid also at groundwater level. A characterization of the hydrochemical behaviour is of strategic importance to understand also possible secondary sources of contaminants. A pollutant can reacts under particular groundwater conditions acting as secondary source of contamination with result of products of reaction/degradation or metabolites. Often the products of these reactions can be substances that are more toxic or dangerous than reagents.

Lots of processes can control the fate of several groups of organic contaminants with different hydrochemical properties and several mechanisms tend to prevent or retain the migration of organic substances from land surface or soil zone into the deeper parts of subsurface environment[67]. These mechanisms include chemical precipitation, volatilization, chemical degradation, biological degradation and adsorption. Most organic substances have extremely low solubility in water and this is an important property that limits possibility of appreciable migration of large loads into groundwater. However, because many organics are very toxic also at low concentrations, solubility is not the only property to consider for prevention of migration at significant concentration levels. Volatilization is another pathway and the result is a loss of contaminants by diffusion from soil to atmosphere. This process can greatly reduce concentration available for transportation in subsurface waters, however the process become irrelevant below the water table where species occur only in dissolved form and are not more in contact with a gas phase. A fundamental process is due to soil bacteria activity that can transform organic substances under biochemical degradation. The soil contains a multitude of bacteria that can convert a great numbers of chemicals. Also particular physicochemical conditions of the soil can address the degradation or the reaction according with the hydrochemical behaviour (i.e. pH, reduction/oxidation conditions and presence of metals). The main mechanism that prevents the migration into the aquifer system, however, is the adsorption to soil. At soil level, under particular and limited geological and hydrochemicals conditions, an interaction between sediments and organic contaminants can prevent the leakage until the groundwater.

Conseguently organic contaminants that pose the greatest threat to the quality of groundwater are those relatively soluble, not volatile and particularly stable to chemical/biological degradation. Stability of the molecule and its "refractory" properties can store and load contaminants into the subsurface levels for years and also decades. Contaminations occur for long temporary period hence subsurface soil and groundwater can represent a sort of "pollution archive". This archive is exposed to a range of alterations during the time as transport factors (velocity, dispersivity), property of the behaviour (geology, hydrochemicals properties) and the chemical partitioning of the organic pollutants.

During the assessment of a groundwater pollution risk, the aquifer pollution hazard will be addressed by two possible approaches: through direct methods or indirect methods. Direct methods are appropriate for a chemical quality monitoring with on-site sampling and analytical determination of the concentration of chemical contaminants. They give indication of the real degradation of pollutants. Indirect methods, instead, involve survey of substance contaminant load and estimation of pollution vulnerability of the contaminated aquifer often using modelling techniques and simulations.

The complexity of some hydrogeological conditions, the groundwater recharge and the variable flow regimes bring often to a dependency upon indirect methods as result of inadequacy of groundwater quality monitoring networks in term of spatial design as well analytical investigation[68].

Despite the use of both direct and indirect methods, a continuous development of strategies and technologies involved is necessary[77]. For a better characterization and determination, new technologies can be adopted even from the analytical point of view (new analytical techniques, more efficient chemical determination)[20] as well IT tools (i.e. Geographical Information System, simulation and modelling software)[70]. Attention be focused on new emerging pollutants that can be discharge as well contaminants already discharged during the past that more scientific knowledge assess their toxic or dangerous properties. In this contest it is clear the importance of a traceability of past pollution events that may occur after long period and the condition of the "archive" already considered. It is important to understand, first of all, the level of the past event, then the relation with the actual situation. The better scientific and technological knowledge nowadays, as well a more awareness of environmental problems and protection, can reconsider past events of chemical contamination and help with a better management of groundwater resource.

2. MATERIALS AND METHODS

This section is dedicated to the experimental part of the work, especially with considerations on the materials used and the methods developed during the laboratory activities. The experimental work was mostly carried out at the Regional Laboratory Service of Mestre-Venice ARPAV using technologies and instrumentation for the routine control and monitoring of environmental samples. The Operative Units involved were: the Chemical Unit for the analysis of macrodescriptor of water samples, the Organic Micropollutant Unit for the main activities of chromatographic and analytical parts and the Biology Unit for the ecotoxicological testing. The water samples extractions and the HPLC-HRTOF-MS determinations were performed at the Environmental Chemistry Laboratory at the Venice University. Under ARPAV requests and I.S.PER.I.A. Project needs, the first experimental part investigated on a suitable and useful method for an environmental control laboratory, allowing the simultaneous determination of chlorinated anilines and chlorinated nitrobenzenes. The method was developed after a deep study of the proceures available in literature for the two singlw classes and was successfully tested on real environmental samples as required by the legislation in force. A second part of the work investigated the benzotrifluoride derivatives compounds. The study resumed data of an historical contamination of groundwater of these compounds in the seventies in Vicenza province. No updated data of environmental contamination from benzotrifluoride is described in literature. This part of the work focussed the attention on the actual situation compared with the data available at the period of the pollution episode and during the last three decades, including a development of methods for the quantitative determination of benzotrifluorides compounds. A preliminary assessment of the ecotoxicological effects of these compounds was finally investigated in support of the awareness of new European legislation in the protection of human health and environment from chemicals.

2.1 Chloroaniline and chloronitrobenzenes determination

2.1.1 Brief review of analytical methods for the analysis of chloroanilines and chloronitrobenzenes

Several methods for the determination of chloroanilines (CAs) and chloronitrobenzenes (CNBs) in aqueous samples have been proposed, but none considered the simultaneous analysis of the two classes regulated by Italian Decree 367/2003. In the literature only the EPA[71,72] methods took into account the determination of a wide range of each class of these compounds[35]. Some of the reviewed methods are focussed on the pre-treatment phases of the samples (extraction/pre-concentration/clean-up) while others gave more attention on the chromatographic separation of the analytes.

It is important to evaluate the type of matrices to analyse and how it influences the performance of the methods, such as the LOD. Since the decree considered Environmental Quality Standards in two different aqueous matrices, river-inland water and lagoon-coastal water, it has been necessary to evaluate a wide range of literature methods to develop a suitable method. Indeed complex matrices, contaminated by many pollutants as a wastewater treatment plant (WWTP) effluent or a contaminated soil, are very difficult to consider and treat for an analytical control. The LOD of contaminants in complex matrices can be sometimes more than 10000 fold greater than, e.g., in a groundwater sample.

The most widespread considered extraction methods are the liquid-liquid extraction (LLE) and the solid-phase extraction (SPE) although recently there was an increase in the use of the solid-phase micro extraction technique (SPME). Other techniques explained are the liquid-phase micro extraction (LPME), the supercritical fluid extraction (SFE), the microwave-assisted extraction (MAE), the pressurized liquid extraction (PLE) and the micro dialysis, but most of these techniques are quite used at the research levels and not applicable for a routine analytical monitoring. The LLE is efficient, but it often shows the disadvantage of requiring a large amount of organic extraction solvent with the consequent problem of waste disposal. Furthermore, the formation of emulsions and the accurate clean-up phase make the technique laborious and time-consuming[47]. The SPE exceeds these disadvantages and nowadays it is the most used technique, due to the wide range of stationary commercial phases to fill the cartridges[73,74]. This technique is applied off-line as well as on-line systems. The first solid-phases used for this purpose were silica-based adsorbent materials (C_8, C_{18}, C_{18}-OH) and polymeric-based materials as styrene-divinylbenzene (PRP-1, PLRP-S). These phases may obtain a good extraction performance if coupled with chromatographic systems like HPLC, but show some lacks on recoveries due to breakthrough phenomena of more polar compounds. Nowadays new generations phases exist and are applicable to different environmental matrices. They are specially designed for a wide range of pollutants and have the characteristic of a high surface area (LiChrolut™ EN, Isolute™ END, ENVI-Chrom™ P, BondElut™ ENV). These phases give a high recovery of the majority of the polar analytes, but suffer the difficulty of desorption, due to the high adsorption capacity and, as result, the broadening of chromatographic peaks when used in an on-line system[75]. With SPE remains the problem that high amounts of samples, particularly of complex matrices, can overload the cartridge until blocking it. The SPME technique is recently considered for the fast extraction/pre-concentration combined steps. Moreover the use of a minimum amount of sample and the no-solvent use dramatically simplify the analytical steps[47]. Otherwise, in the literature discordance is shown about this technique due to reproducibility data problems, especially if not used as head-space, which are minimized only in a small part by on-line techniques and the new generation of autosamplers[76]. The main uses of SPME are qualitative and semi-quantitative analysis and rapid screening technique.

The most used instrumental separation methods for the analysis of CA and CNB pollutants are gas-chromatography (GC) and liquid-chromatography (LC). Generally gas-chromatography is coupled with a flame-ionization detector (FID) or an electron-capture detector (ECD) more specific for the determination of halogenated organic compounds; the nitrogen-phosphorus detector (NPD) is more selective because of the presence of the nitrogen atoms in the contaminants[41,76,78,79]. The gas-chromatography coupled with a mass-spectroscopy detector (GC-MS) requires injecting bigger quantities of purified extract for the low sensitivity in comparison with more specific detectors, but has the advantage of a qualitative confirmation. For the not negligible polarity of CAs, some different separation columns were tested. In literature, however, some articles explain derivatization methods of the aromatic amines to remedy the polarity and low volatility properties and to enhance the sensitivity and the selectivity of the GC method[80,81,82]. Less extraction steps and problems can be obtained if the CAs analysis is carried out with the liquid-chromatography with UV detector, photo-diode array detector (PDA) or with more efficient results with mass-spectroscopy detection[48,75,83,84,85] (LC-MS).

The official EPA method (EPA 8091, 1996)[72] can be used for the detection of some CNBs, such as 2-chloronitrobenzene (2CNB), 4-chlorobenzene (4CNB), chloro-2,4-dinitrobenzene (C24DNB), three chloronitrotoluene isomers, five dichloronitrobenzenes, two trichlorobenzenes and two tetrachlorobenzenes. The EPA method for the CAs analysis (EPA 8131, 1996), instead, includes a broad range of CAs. Both the methods described a preliminary liquid-liquid extraction followed by a clean-up step for more complex matrices with a gel-permeation chromatography system (GPC). The separation method used the GC-NPD with a fused-silica column crosslinked with 95% dimethylsiloxane and 5% diphenylpolisiloxane (DB-5, RT_x-5, SPB-5 type or equivalent) or 14% cianopropylphenylsiloxane and 86% dimethylsiloxane (DB-1701, RT_x-1701 type or equivalent) for CNBs. The column types as SE-54 and SE-30 (J&W Scientific, Folsom CA US) in fused-silica are suitable for CA. Gatermann showed an analytical approach to quantify the presence and distribution of CNB as organic micropollutants in the North Sea[39]. In this study, 2CNB, 3CNB, 4CNB, 1,2-dichloronitrobenzene (12DCNB) and 14-dichloronitrobenzene (14DCNB) were considered applying LLE with *n*-hexane, followed by clean-up on an alumina column. The quantitative analysis was carried out with GC-AFID and GC-ECD (column NB-54 Nordibond) and the confirmation with a GC-MS triple quadrupole detector (column DB5-MS) with LOD about 0.03 and 1 ng/L.

Di Corcia[86] (1990) presented one of the first works for the analysis of CA as trace contaminants of environmental water, where selective liquid-solid extraction of 14 CAs was achieved by using a two-trap tandem system cartridges, one containing a non-specific adsorbing material, such as graphitized carbon black (Carbopack B), and the other one filled with a resin-based strong cation exchanger. The quantitative determination was obtained with a LC-UV detector system (LC-18-DB-RP Supelco column). The limits of detection of the analytes considered were below 0.1 μg/L. The monochoroanilines (2CA, 3CA and 4CA) had greater mobility on the graphitized carbon black sorbent than the di-substituted chloroanilines. Schmidt[82] and Less[81] (1998) developed a method to extract 56 aromatics amine (2CA, 3CA, 4CA, 34DCA) in river and wastewater with SPE. Seven different types of sorbents were compared on the basis of different recoveries; the analyses were performed by a GC-ECD (DB5 J&W

column) and HPLC-PDA (RP-C_{18} column) after derivatization in iodobenzenes compounds obtaining LOD of 0.5-20 μg/L.

Lacorte[46] (1999) showed a performance comparison between five SPE cartridges for the analysis of CAs, among which 2CA, 3CA, 4CA and 34DCA, in industrial waste water with high content of organic matter and high values of TOC (1-70 mg/L). The sample volume analysed was 200 mL and the results were quantified by LC-ED with an Interchim Nucleosil C_{18} column. The LOD of the method varied between 10 and 40 ppb and depended from the analysed compound. The comparison study provided a good performance at μg/L levels with no great differences in the recoveries between different cartridges, but with high RSDs values on the analysis of spiked industrial effluent water, which made quantification difficult. On the contrary, Di Corcia[48] (1999) used graphitized carbon black of new generation cartridges (Carbograph 4) also to estimate phenylurea herbicides and their metabolites in environmental water. With a LC-EC-MA (column C_{18} RP Alltech), limits of detection of 3-4 ng/L in drinkable water, 6-21 ng/L in groundwater and 36-75 ng/L for river water were reached. In this paper, a procedure of pre-treatment of the samples with $NaBH_4$ was explained to avoid the making of reversible bonds between aromatic amines and the humic and fulvic acids of the environmental matrix. It was explained that this type of bonding is possible only at pollutants concentration of ng/L. Patsias[75] (2000) focussed the attention on the development of a method to simultaneously determinate anilines, phenols and caffeine in aqueous samples. The 4CA and 34DCA were studied in this paper. A comparison between two different SPE sorbents, 2 commercial trade mark cartridges (Hysphere-GP™ and Hysphere-SH™) with a styrene-divinylbenzene based solid phase was performed. Hysphere-GP™ was more sensitive and better selective than Hysphere-SH™. The instrumental determination was based on HPLC-ED with a Nucleosil 100-S C18 Macherey-Nagel column through the extraction of 10-150 mL of sample volume, reaching limits of detection between 50 and 200 ng/L. Weigel[87] (2001) developed a method to extract organic micropollutants with solid phase from large amounts (10 L) of marine water samples. The multi layer filtration-extraction system worked in large scale on-site with different types of sorbents. Bacaloni[88] (2007) developed a SPE-HPLC with a tandem mass spectroscopy analytical method, suitable for the simultaneous analysis of benzidines, dichlorobenzidines and chloroanilines and based on a normal phase LC separation using a cyano propyl methyl silica column coupled with an APPI interface that permits to overcome the limitations in ionizing the less polar compounds. A shortcoming of this method was the lack in robustness because small changes in the ionization environment may provoke in substantial changes in the ionization yield and ion type formation, so working with standard addition was necessary.

Some papers that explained SPME techniques are presented below.
Muller[41] (1997) developed a method based on SPME with a CW-DVB (CarboWax-DiVinylBenzene) fiber, normally used for polar substances, to determine 4CA and 34DCA in river water and groundwater collected in a polluted industrial area near Milan (Italy). With a GC-MS system working on Single Ion Monitoring (PTA base-deactivated Supelco column) it was possible to treat 10 mL of sample obtaining calibration curves on the range of 0.05-5 µg/L.
Other methods that employed SPME as extraction technique were described by Huang, Lambropoulou, Yang and Berrada. Huang[78] (1997) analysed several aromatic amines, although not chlorinated, using PA (PolyAcrylate) and PDMS (PoliDiMethylSiloxane) SPME fibers with a GC-FID. With a CBP-10 Shimadzu column and 3 mL of real sample, it was possible to reach LODs of 5-10 µg/L. Lambropoulou[47] (2000) considered some fungicides as *dicloran* and its metabolites. Four different SPME fibers coupled with a GC-ECD (DB-1 J&W column) and a GC-MS for qualitative confirmation (DB-5-MS J&W column) were studied. 3 mL of environmental sample (groundwater, lake, river and sea water) were extracted with a final LOD range between 1 and 60 mg/L. Yang[89] (2001) considered a series of aromatics amines (two monochloroanilines and four dichloroanilines) in aqueous solution with an IR spectrometer as detector to reach limit of detection of 5-50 mg/L. Berrada[79] (2004) determined phenylurea herbicides and their metabolites, like 4CA and 34DCA, into vegetable matrices by using a PA fiber and GC with NPD detector (BP10 SGE column) with variable limits of detection, ranging between 10 and 20 µg/Kg.

Two papers in the literature focussed the attention on the analysis of CAs and CNBs in soil and sediment matrices. Fromberg[76] (1996) used the HS-SPME with PA fiber to analyse 20 chloro- and nitro-benzenes. The HS technique is simpler than the immersion one, but presents the limit that only the volatile fraction of pollutants is detectable. The compounds considered are: 2CNB, 3CNB, 4CNB, 4C2NA, 34DCNB and 25DCNB. A GC/ECD was used following confirmation with a MS detector (HT-8 SGE column) and obtaining a calibration range of 0.5-250 µg/L equipped with a ECD after extraction of 5g of soil. The curves were not linear whereby the technique resulted inadequate for the quantitative determination of trace pollutants. Anyway the method was rapid and effective if used as a screening method for soils and solid environmental matrices.

Kristenson[35] (2004) evaluated the complex matrix of soil and developed a miniaturized pressurized liquid extraction system (PLE) for CA. This method reduced a lot the amount of sample to extract (50-100 mg) and the quantity of organic solvent used (100 μL). In this paper only 2CA and 3CA were searched for with a GC-MS analysis and two different injection systems were compared: PTV and LV-OC. Using column DB-XLB (Agilent) for LV-OC injector and column ZB-5 (Phenomenex) for PTV injector, it was possible to reach limits of detection on soil of 0.2-25 ng/g (PTV) and 1-35 ng/G (LV-OC).

Zhu[90] (2002), on the base of the significant polarity of aromatic amines, had developed a technique based on ion chromatography (IC) working with an Ion Pack CS12 column (Supelco) and an amperometric detector. Several aromatic amines, as 4CA, were analysed in industrial waste water with LOD between 2 and 25 μg/L (16.6 μg/L with 4CA).

Jen, Zhao, Peng and Zhou, finally, had proposed some different methods for CAs analysis based on innovative and new extraction/pre-concentration methods. Jen[84] (2000) had proposed a method to detect aniline and 2CA, testing it on polluted wastewater in an area of production of polymers. Within the microdialysis and HPLC-UV (column Supelcosil RP C_{18}, Supelco) and the extraction of 50 mL of highly contaminated samples, LOD of 100-500 μg/L were reached. Zhao[91] (2002) developed a liquid-liquid-liquid microextraction (LLLME) technique coupled with HPLC-UV (Inertsil ODS-2 Chrompack column) to investigate anilines such as 3CA, 4CA and 34DCA with LOD of 80-100 ng/L, extracting 4 mL of aqueous sample. Peng[85] (2005) tested the technique of HS-LPME at high temperature using a non-volatile ionic liquid (1-butyl-3-methylimidazoliumhexafluorophospate) to absorb the analytes from 20 mL of tap, drinkable and river water. 2CA, 4CA, 24DCA and 34DCA were determined with a LOD range of 0.5-1 μg/L, injecting directly the ionic liquid on a HPLC-DA (C18 column ODS-P Inertsil, GL Science). Zhou[92] (2004) used a microporous membrane liquid-liquid extraction (MMLLE) coupled with a capillary electrophoresis to detect aromatic amines in general water with LOD levels between 0.16-7.5 ug/L.

From the reviewed literature, the following considerations are important to produce and to explain. Firstly, many studies were proposed for the analysis of CAs because they are more common environmental pollutants compared to CNB due to their spread use, especially during the past, and the use of compounds that can give CAs as metabolites or products of degradation. CAs are substances with not negligible polar properties (i.e. $pK_a = 2.6 \div 3.9$ for monochloroanilines, see table 1.6) so an accurate and well selected extraction method from different environmental aqueous matrices is important. Even if the sample derives from very complex environmental matrices as for example WWTP effluent, water with high TOC or from polluted industrial areas, problems with cleanup, low recoveries and high relative standard deviations with replicate extractions were common. A delicate point appears to be the state and the maintenance of the conditions of the separation column as the presence of high boiling residues may dirty the column or give co-elution of analytes and interferents. For this reason a mass spectrometer detector is often necessary for confirmation after the chromatographic separation. Otherwise for a mass spectroscopy confirmation, a good purification step and a detectable amount of analytes are important. CAs are substances less stable than other semivolatile organic micropollutants, hence it is necessary to work with fresh standard and working solutions followed by everyday calibration curves. A derivatization step enhances the performance of the method especially for the determination of CAs using a gaschromatographic system, but it is not always possible especially in analytical laboratories working everyday with a huge number of samples.

Annex I of this work summarises the most important methods considered in this brief review about the analysis of CAs and CNBs on environmental samples. Both are explained together with the most important characteristics and performance features.

2.1.2 Reagents and standards

The tested CAs and CNBs were purchased from Dr Ehrenstorfer (Augsburg, Germany) with purity between 99.0 and 100.0 % (Table 2.1); aniline, pure nitrobenzene, aniline-d_5 used as internal standard and nitrobenzene-d_5 as surrogate, in concentration of 2000 μg/mL in dichloromethane, were from Supelco (Sigma-Aldrich, St. Louis, MO, US). Stock primary solutions in a range of 700-2400 mg/L of each compound were prepared in 2-propanol. Working solutions (6 levels in the range 5-1000 μg/L) were prepared by dilution of stock solutions with a mixture of methanol/ethylacetate 1:1. Stock and working solutions were stored at 4°C, except aniline-d_5 solutions stored at -20°C. Aqueous solutions were prepared by spiking water with appropriate amount of working solution. The water used was purified with a Milli-Q system (Millipore®, Billerica, MS USA) and the organic solvents, i.e. methanol, ethylacetate, *n*-hexane, dichloromethane, acetonitrile and 2-propanol were all pesticide grade (VWR Milan, Italy). The pH 9 buffer solution (sodium tetraborate/hydrochloric acid) was Fluka Fixanal grade (Sigma-Aldrich, St. Louis, MO, US) and NaOH used to prepare a solution of 10% w/w was purchased from Merck (Readingtong, NJ US). Formaldehyde 37% in methanol was purchased from Fluka (Sigma-Aldrich, St. Louis, MO, US) and used for the storage of some water samples.

Table 2.1: the tested anilines and nitrobenzene compounds.

Compounds	CAS number	Fabricant	Purity %	Stock primary solution concentration* [mg/L]
aniline-d_5 (int standard)	4165-61-1	Supelco	-	2000 μg/mL in CH_2Cl_2
aniline	62-53-3	Supelco	99.5	1158.18
nitrobenzene-d_5 (surrogate)	4165-60-0	Supelco	-	2000 μg/mL in CH_2Cl_2
nitrobenzene	98-95-3	Supelco	100	1378.00
2-chloroaniline	95-51-2	Dr Ehrenstorfer	99.0	1346.40
3-chloroaniline	108-42-9	Dr Ehrenstorfer	99.0	1679.04
4-chloroaniline	106-47-8	Dr Ehrenstorfer	99.5	794.01
2-chloro-4-methylaniline	615-65-6	Dr Ehrenstorfer	99.0	1304.82
3-chloronitrobenzene	121-73-3	Dr Ehrenstorfer	99.5	1086.54
2-chloronitrobenzene	88-73-3	Dr Ehrenstorfer	99.5	905.45
4-chloronitrobenzene	100-00-5	Dr Ehrenstorfer	99.0	937.64
3-chloro-4-methylaniline	95-74-9	Dr Ehrenstorfer	99.5	2258.65
4-chloro-2-nitrotoluene	89-59-8	Dr Ehrenstorfer	99.0	1059.30
3,4-dichloroaniline	95-76-1	Dr Ehrenstorfer	99.0	908.82
1-chloro-2,4-dinitrobenzene	97-00-7	Dr Ehrenstorfer	99.0	2356.20
4-chloro-2-nitroaniline	89-63-4	Dr Ehrenstorfer	99.5	776.10

* stock primary solution prepared in 2-propanol.

2.1.3 Sample collection

A litre of each water sample was collected in borosilicate amber glass bottles following the EN ISO 5667-3: 2003(E) procedure[93] for water quality sampling. Samples were stored at 4°C and analysed in the days after the sampling. Before the storage, the pH of each sample was tested with a Crison pHmeter GLP 21 (Barcelona, Spain). The real samples analysed to check the method validity were from: surface rivers, groundwater, piezometers of polluted area, leakages from landfills and wastewater from input and output of treatment plants. For waste water, leakages of landfills and water collected from WWTPs, an amount of formaldehyde 37% in methanol, in the quantity of 1% for output plant samples and 4% for the input plant samples, was added before the storage as recommended by the ISO procedure. This procedure was necessary both to destroy the microbic and bacterial load, dangerous for the biological degradation of the sample, and especially as a safety precaution for the operators. A quantity of 200 mL of each sample was analysed and the rest of samples was stored in fridge for further extractions or confirmations.

2.1.4 Sample extraction and SPE procedure

The procedure described below was adopted after the development of the method described on paragraph 3.1.

Each 200 mL sample at room temperature, spiked or real, was prepared by adding 10% of buffer and 2.5% of methanol. The real samples were filtered with 0.45 µm filters (Sartorius, Goettingen Germany). The pH was eventually adjusted to 9 adding NaOH 10% drop wise and controlled with the pHmeter. Then 50 µL of surrogate nitrobenzene-d_5 (1.6 mg/L) were added to each sample. The extraction technique was carried out using a Gilson Aspec XL Automatic Solid Phase Extraction Station equipped with a Gilson Valvemate^{TM} (Middleton, MA US). For the final developed method Oasis HLB^{TM} cartridges (Waters, Milford MS, US) filled with a copolymeric poli(divinylbenzene-*N*-vinylpyrrolidone) resin (PDB+VP) were used. According with the manufacturer the polymeric sorbent is balanced for lipophylic substances as well as for hydrophylic compounds[94]. Each cartridge was activated with 5 mL of ethylacetate and equilibrated with 5 mL of methanol followed by 5 mL drop wise at 3 mL/min of Milli-Q water buffered at pH 9. Avoiding the cartridge to dry, the amount of sample was passed thorough the conditioned cartridge at a flow-rate of 5 mL/min. After the extraction, the cartridge was rinsed with 3 mL of Milli-Q water at flow-rate of 3mL/min to remove possible impurities and co-retained interferences from the matrix.

Afterwards, each cartridge was dried under vacuum for about 1 h. It is important to be sure that all the cartridges are without any residue of water. The elution was performed in two steps both with 3 mL of ethylacetate at 1mL/min, waiting 2 minutes between them, and the elute was collected in a 10 mL vial. To concentrate, a gentle stream of nitrogen was applied until the final volume of 0.5 mL using a Turbovap II (Zymark, Basel Switzerland) concentration evaporator station. Before the analysis, a further amount of 50 μL of internal standard aniline-d_5 was added to each sample to obtain a final volume of 0.55 mL into the vial. After the preparation step, the vials were stored in a fridge until the GC-MS analysis. During the development of the method, and before the adoption of the final protocol, several cartridges with different stationary phases were tested. They are described in detail in the *Results and Discussion* chapter.

2.1.5 Instrumental analysis

GC-MS analysis was carried out using a Thermo Finnigan Trace GC Ultra equipped with a DSQ single quadrupole mass detector (Walthman, MA US). A Varian (Palo Alto, CA US) VF-5MS column (30 m, 0.25mm ID, 0.25 μm film thicknesses) with 5% phenyl - 95% dimethylpolysiloxane was installed and a PTV injector in CT splitless modality was used for the investigations. The injector was maintained at 270°C with a split flow of 40 mL/min and a splitless time of 0.8 min; 1 μL of sample was injected. The following oven conditions were used:

- initial temperature of 75°C for 1 min,
- ramp of 6 °C/min from 75°C to 190°C,
- hold time of 4 min,
- ramp of 15°C/min until 300°C,
- final hold time of 5 min at 300°C.

Helium was used as carrier gas at constant flow of 1 mL/min for a total run time of about 26 minutes. The ion source and the transfer line were kept at 250°C and 300°C respectively. From the spectrum of each compound, one ion (normally the parent ion) and three ions for the qualitative determination were selected to evaluate the response in SIM (Selected Ion Monitoring) mode. These ions were chosen after acquisitions both in Full Scan (FS) modality, in a range of mass between 29 and 300 amu, and Total Ion Current (TIC) as shown in Table 2.1. The instrumental and processing methods were both managed by the Xcalibur® Thermo software.

Table 2.2: the ions used for the qualitative (qual) and quantitative (quan) determination of the analytes.

COMPOUND	Retention time [min]	SIM (quan) [amu]	SIM* (qual) [amu]
aniline-d_5 (int standard)	5.35	98	98(100) 71(42) 70(34)
aniline	5.37	93	93(100) 66(47) 65(20)
nitrobenzene-d_5 (surrogate)	7.25	82	82(100) 54(76) 128(35)
nitrobenzene	7.27	77	77(100) 123(53) 51(43)
2-chloroaniline	8.10	127	127(100) 129(32) 92(20)
3-chloroaniline	9.47	127	127(100) 129(32) 92(22)
4-chloroaniline	8.61	127	127(100) 129(32) 92(21)
2-chloro-4-methylaniline	10.16	141	141(100) 106(93) 140(90)
3-chloronitrobenzene	10.14	111	75(100) 111(93) 157(88)
2-chloronitrobenzene	10.45	111	75(100) 111(93) 157(88)
4-chloronitrobenzene	10.49	111	75(100) 111(98) 157(87)
3-chloro-4-methylaniline	11.58	141	141(100) 140(83) 106(65)
4-chloro-2-nitrotoluene	11.81	89	89(100) 154(88) 126(57)
3,4-dichloroaniline	14.51	161	161(100) 163(63) 99(16)
1-chloro-2,4-dinitrobenzene	17.03	202	30(100) 202(55) 75(46)
4-chloro-2-nitroaniline	17.95	172	172(100) 126(83) 99(57)

*In commas the relative % abundances of the main considered ions.

2.2 Benzotrifluoride compounds determination

2.2.1 Reagents and standards

The tested benzotrifluoride compounds were purchased from Aldrich (Sigma-Aldrich, St. Louis, MO, US) as technical reagents for organic synthesis because no environmental standards were available on market. Aniline-d_5 used as internal standard and nitrobenzene-d_5 as surrogate, in concentration of 2000 μg/mL in dichloromethane, were purchased from Supelco (Sigma-Aldrich, St. Louis, MO, US). Stock primary solutions, in a range of 900-2000 mg/L for each compound, were prepared in 2-propanol. Working solutions for SPE extractions (5 levels in the range 8-1500 μg/L) were prepared by dilution of stock solutions with a mixture of methanol/ethylacetate 1:1. Working solutions of more volatile BTF for P&T extraction were prepared in 5 levels at each batch of analysis from stock solutions. Stock and working solutions were stored at 4°C, except aniline-d_5 solutions stored at -20°C. Aqueous solutions were prepared by spiking water with appropriate amount of working solution. Water for chromatographic purpose was purified by a Milli-Q system (Millipore®, Billerica, MS USA) and the organic solvents as methanol, ethylacetate, *n*-hexane, dichloromethane, acetonitrile and 2-propanol were all pesticide analysis grade (VWR Milan, Italy). The pH 9 buffer solution (sodium tetraborate/hydrochloric acid) used was Fluka Fixanal grade (Sigma-Aldrich, St. Louis, MO, US) and NaOH to prepare a solution of 10% w/w was purchased from Merck (Readingtong, NJ US). To obtain acidic pH of samples, a solution of HCl 1M were prepared from HCl 37% (Riedel-DeHaen, St. Louis, MO, US) and added drop wise for each specific purpose.

2.2.2 Sample collection

For each groundwater and surface water sample destined to the BTFs determination, different amounts were collected on the basis of the analytical technique: samples for SPE extraction and reservoir, were collected in 1L borosilicate amber glass bottles, samples for the P&T extraction and nitrogen speciation determination in 250mL borosilicate amber glass bottles and samples for the ecotoxicological testing in 500mL propylene sterilized bottles. The sample collection was carried out following EN ISO 5667-3: 2003(E) and wells/piezometers water collected after a purging of at least 10 minutes. For samples destined to P&T extraction the bottles were completely filled without any head space of air at the top. Samples were stored at 4°C and were analysed in the following days after the sampling. Samples for ecotoxicological determination were stored for a few days at -20°C before each test, while the ones for P&T and nitrogen speciation were used the same day or at least the day after. After the sampling, some parameters were measured with a multi-parameter Hydrolab Quanta G meter system (Corr-Tek Idrometria srl, Verona Italy) kindly lend by the territorial Staff of Venice Department of ARPAV. The pH, conductivity salinity and ORP were measured by the multi-parameter system.

Table 2.3: active principle and primary stock solutions of benzotrifluoride tested.

Compounds	CAS number	Producer	Purity %	Stock primary solution concentration [mg/L]*
SPE/GC-MS method				
aniline-*d*$_5$ (int standard)		Supelco	-	2000 μg/mL in CH_2Cl_2
3,4-dichlorobenzotrifluoride	328-84-7	Aldrich	97.0	1499.62
2,4-dichlorobenzotrifluoride	320-60-5	Aldrich	98.0	1517.04
nitrobenzene-*d*$_5$ (surrogate)		Supelco	-	2000 μg/mL in CH_2Cl_2
3-aminobenzotrifluoride	98-16-8	Aldrich	99.0	1369.17
3-nitrobenzotrifluoride	98-46-4	Aldrich	97.0	1449.18
3-amino-4-chlorobenzotrifluoride	121-50-6	Aldrich	98.0	1430.80
3-nitro-4-chlorobenzotrifluoride	121-17-5	Aldrich	97.0	1573.34
4-chloro-3,5-dinitrobenzotrifluoride	393-75-9	Aldrich	99.0	1219.68
P&T/GCMS method				
benzotrifluoride	98-08-8	Aldrich	100.0	1589.00
4-chlorobenzotrifluoride	98-56-6	Aldrich	98.0	1641.50
3,4-dichlorobenzotrifluoride	328-84-7	Aldrich	97.0	1907.02
2,4-dichlorobenzotrifluoride	320-60-5	Aldrich	98.0	1589.00

* Stock primary solutions prepared in 2-propanol.

2.2.3. Sample extraction and concentration

A strategy to determine volatile and non-volatile BTFs with two different and separate methods was decided after a qualitative determination of which pollutants occurred in the first two samples collected form the area of Vicenza province and after the assessment of the results obtained during CAs and CNBs method development. The non-volatile BTFs were extracted, purified and concentrated with the same procedure developed for CA and CNB compounds, by GS-MS analysis of the extract, while the more volatile BTF compounds were extracted by a Dynamic Head Space system (Purge&Trap) set on another GS-MS with single quadrupole detector system. 3,4-dichlorobenzotrifluoride and 2,4-dichlorobenzotrifluoride, semi-volatile compounds, were determined by both methods with a major recovery and more reliability with the P&T system. Results from the two methods were compared.

2.2.3.1 Solid Phase Extraction in basic conditions

The same SPE extraction procedure, used after the CA and CNB method development, was applied to extract, purify and concentrate the non-volatile BTF compounds.

For each sample, spiked or real one, an amount of 200 mL was extracted and prepared as described in paragraph 2.1.4. The real samples were all previously filtered with 0.45 μm filters (Sartorius, Goettingen Germany) and the pH was eventually adjusted to 9 adding NaOH 10% drop wise and controlled with the pHmeter. Before the analysis, a volume of 50 μL of internal standard aniline-d_5 was added to each sample obtaining a final volume of 0.55 mL into vials. The vials were stored in fridge until the GC-MS determination, if the analysis wasn’t performed just after the preparation procedure.

2.2.3.2 Dynamic Head Space extraction

The Dynamic Head Space system based on Purge&Trap is a method for the concentration of trace contaminants in water prior the analysis and is required by many official and standardized protocols (i.e. EPA methods for drinking water and wastewater analysis). A clean and inert gas is purged over and through the aqueous sample and blows up, causing the stripping of insoluble volatile and semi-volatile chemicals from the matrix. The stripped compounds are then “trapped” on an absorbent column (called trap or concentrator) at room temperature. The trap is then heated and the volatile compounds are thermally desorbed and directly addressed by a carrier gas stream to the injection system of the GC.

From the primary stock solutions, listed in table 2.3, two intermediate multiresidue solutions were prepared respectively 1:100 (A) and 1:10000 (B) in methanol. From these intermediate solutions, 6 levels of work solutions were prepared at each batch of analysis, following the scheme below and table 2.4:

LEVEL 1: 4 μL of solution B in 40 mL of Milli-Q water
LEVEL 2: 40 μL of solution B in 40 mL of Milli-Q water
LEVEL 3: 100 μL of solution B in 40 mL of Milli-Q water
LEVEL 4: 400 μL of solution B in 40 mL of Milli-Q water
LEVEL 5: 40 μL of solution A in 40 mL of Milli-Q water
LEVEL 6: 100 μL of solution A in 40 mL of Milli-Q water

Also aniline and nitrobenzene were added to the levels of standard tested to check their presence on real sample.

Table 2.4: levels of concentrations used for the calibration curve of volatile BTFs.

Analite	**lev 1 [μg/L]**	**lev 2 [μg/L]**	**lev 3 [μg/L]**	**lev 4 [μg/L]**	**lev 5 [μg/L]**	**lev 6 μg/L]**
BTF	0.016	0.158	0.397	1.589	3.973	15.890
4BTF	0.016	0.164	0.410	1.642	4.104	16.415
34DCBTF	0.018	0.191	0.477	1.907	4.768	19.070
24DCBTF	0.018	0.178	0.444	1.775	4.437	17.748
ANILINE	-	0.116	0.290	1.158	2.896	11.582
NITROBENZENE	-	0.138	0.345	1.378	3.445	13.780

The 40 mL vials used for the P&T system were purchased by Chromacol (Herts, UK). The environmental samples were transferred into the 40 mL vials with the wariness to close them immediately with a cap to avoid losses of volatile compounds. A Teledyne Aquatec Autosampler equipped with a Tekmar-Dohrmann Velocity XPT sample concentrator (Mason, OH US) was used and 1,2,3-trifluorobenzene was chosen as internal standard. The trap was a Supelco K VOCARB 3000 (Sigma-Aldrich, St. Louis, MO, US) filled with 10cm of Carbopack^{TM} B, 6cm of Carboxen^{TM}-1000 and 1cm of Carboxen^{TM}-1001 and it was directly connected to the GS-MS system. Also other chlorinated organic compounds were investigated in the real samples. The concentrations of these compounds, for a quantitative determination, were compared to two quality control solutions, daily used for their detection in routine analyses of environmental water with the same instrumentation (Table 2.5).

Table 2.5: concentrations in μg/L of other chlorinated organic compounds detected in the samples.

Compounds	QC 1 [μg/L]	QC 2 [μg/L]
TRICHLOROETHYLENE	0.27	1.31
TETRACHLOROETHYLENE	0.20	0.97
CHLOROBENZENE	0.28	1.33
1,4-DICHLOROBENZENE	0.16	0.76
1,2-DICHLOROBENZENE	0.16	0.79

2.2.3.3. Solid Phase Extraction in acid conditions

The SPE extraction method described in paragraph 2.2.3.1 was modified to extract acidic compounds from environmental water samples. The purpose of performing also this extraction on groundwater and surface waters already extracted at basic conditions, was a preliminary qualitative detection of other compounds, especially phenols BTFs derivatives. Modern analytical systems as HPLC coupled with a High Resolution Time of Flight Mass Spectroscopy were used for these purposes.

To each 200 mL of environmental sample at room temperature, 5 mL of methanol was added. The pH was adjusted to 2 adding HCl 1M drop wise and controlled with the pHmeter. The extraction technique was carried out using the Gilson Aspec XL Automatic Solid Phase Extraction Station. Oasis HLB^{TM} cartridges were used as well as for the basic extractions. The copolymeric poli(divinylbenzene-*N*-vinylpyrrolidone) sorbent resin is balanced for lipophylic substances as well as for hydrophylic compounds and can be suitable for the extraction of a wide series of organic compounds in a pH range between 2 and 13. Each cartridge was activated with 5 mL of methanol and equilibrated with 5 mL of Milli-Q water buffered at pH 2 drop wise at 3 mL/min. Avoiding the cartridge to dry, the amount of sample was passed thorough the conditioned cartridge at a flow-rate of 5 mL/min. After the extraction, the cartridge was rinsed with 3 mL of Milli-Q water at flow-rate of 3mL/min to remove possible impurities and co-retained interferences from the matrix. Afterwards, the cartridges were dried under vacuum for about 1 h. The elution was performed with 3 mL of methanol at 1mL/min and the eluted solvent collected in a 10 mL tube. To concentrate, a gentle steam of nitrogen was applied until the final sample volume of 0.5 mL. The sample volume was finally transferred in a vial and ready to be analysed. The elution with methanol was chosen for the compatibility of the solvent with the HPLC system.

2.2.4 Instrumental analysis

2.2.4.1 Gas chromatography /mass spectroscopy

A Perkin Elmer Clarus 500 (Waltham, MA US) gas chromatograph with single quadrupole mass detector was used for the volatile and semivolatile fraction. The GC-MS system was connected with the P&T system to permit a direct thermal desorption from the concentrator. A Restek RTX$^{\circledR}$-624 (Bellafonte, PA US) column (30 m, 0.25 mm ID, 1.4 µm df) operating at a max temperature of 240°C was utilized and injection was carried out in splitless modality.
The experimental parameters of the chromatographic run were:

Carrier gas: Helium
Injection temperature: 250°C
Injection volume: 1.0 µL
Split flow: 10 mL/min
Gas Flow
Equilibration time: 0.5 minutes
Oven program: 40°C hold time for 7 minutes
ramp of 10°C/min to 110°C
5 minutes hold time
ramp of 20°C/min to 250°C
3 minutes of hold time

The total run was of 29.00 minutes.
A unique mass spectrometer detection method for both volatile BTFs and other chlorinated organic compounds was developed with the mass described on table 2.6. Three detection SIR windows were used: 1) from 7 to 14 minutes, 2) from 14 to 22 minutes and 3) from 22 to 29 minutes. The final concentration of the internal standard 1,2,3-trifluorobenzene was 5.12 µg/L.

Table 2.6: the ions used for the qualitative (qual) and quantitative (quan) determination of the volatile analytes.

SIR Windows	Analite	Retention time [min]	SIR (quan) [amu]	SIR (qual) [amu]
1	TRICHLOROETHYLENE	10.51	130	130 132
1	1,2,3-TRIFLUOROBENZENE	10.79	132	132 134
1	BTF	11.15	146	96 127 146
1	TETRACHLOROETHYLENE	13.54	166	164 166 168
2	CHLOROBENZENE	15.10	112	77 112 114
2	4BTF	15.27	180	145 161 180 182
2	34DCBTF	20.46	214	195 214 216
2	1,4-DICHLOROBENZENE	20.94	146	111 146 148
2	24DCBTF	21.15	214	179 214 216
2	1,2-DICHLOROBENZENE	21.58	146	111 146 148
3	ANILINE	23.06	93	65 66 93
3	NITROBENZENE	23.09	77	50 77 123

For the non volatile BTFs, the same gaschromatograpic system used for the determination of CAs and CNBs was used, but with different separation and detection conditions. GC-MS analysis was carried out using a Thermo Finnigan Trace GC Ultra equipped with a DSQ single quadrupole mass detector (Walthman, MA US). A Varian (Palo Alto, CA US) VF-5MS column (30 m, 0.25mm ID, 0.25 μm film thicknesses) with 5% phenyl - 95% dimethylpolysiloxane was installed and a PTV injector in CT splitless modality was used for the investigations. The injector was maintained at 270°C with a split flow of 40 mL/min and a splitless time of 0.8 mins; 1 μL of sample was injected. The following oven conditions were used:

- an initial temperature of 75°C for 3 min,
- a ramp of 2.5°C/min from 75°C to 110°C,
- a ramp of 32°C/min until 300°C,
- a final hold time of 8 mins at 300°C.

Helium was used as carrier gas at constant flow of 1 mL/min for a total run of about 24 minutes. The ion source and the transfer line were kept at 250°C and 300°C respectively. From the spectrum of each compound one ion (not always the parent ion) to quantify the response in SIM mode and three ions for the qualitative determination were selected from each peak signal after an acquisition both in modality Full Scan (FS) and Total Ion Current (TIC) as shown in Table 2.7. The mass range selected for FS was between 25 and 350 and five SIM windows were chosen, The instrumental and processing methods were both managed by the Xcalibur® Thermo software.

Table 2.7: the ions used for the qualitative (qual) and quantitative (quan) determination of the non volatile analytes.

SIM WINDOWS	COMPOUND	RET TIME (min)	SIM (quan)	SIM* (qual)
1	aniline-d_5 (int standard)	5.38	98	98(100)71(42)70(34)
1	34DCBTF	5.61	214	214(100)216(64)179(33)
1	24DCBTF	6.40	214	214(100)216(63)179(39)
2	nitrobenzene-d_5 (surr.)		82	82(100) 54(76) 128(35)
3	3NBTF	7.42	145	145(100)191(428)95(21)
3	3ABTF	7.52	161	161(100)142(21)114(20)
4	3A4CBTF	11.59	195	195(100)197(32)176(13)
4	3N4CBTF	11.89	179	179(100)30(95)225(57)
5	4C35DNBTF	18.40	270	143(100)270(88)30(87)

*between comma the relative abundances of the main ions considered

2.2.4.2 HPLC-HRTOF/MS

The HPLC equipped with an ESI injection system and a Time of Flight High Resolution Mass Spectrometer (HPLC-HRTOF/MS) were used for the analysis of the acidic extracts. The sample extracts was inject an Agilent 1200 HPLC system using an Agilent (Agilent Technologies, PaloAlto CA, USA) using an Agilent G1329B autosampler. The chromatographic separation was performed using a Fusion column (Phenomenex, Torrance CA US) Extended C-18 stationary phase (2.5μm x 10cm x 2mm). The LC column temperature was thermostated at 30°C with an Agilent G1316A column heater and before the column a Fusion Phenomenex pre-column (Torrance, CA, USA) was installed. The mobile phase was a mixture of methanol (A) ultrapure quality from Romil (Dublin, Ireland) and water Milli-Q grade (B) both containing ammonium acetate 10 mM/L. The solution of ammonium acetate 10 mM/L was previously prepared from ammonium acetate (purity ≥ 99.0%) purchased from Fluka (Sigma-Aldrich, St. Louis, MO, US). Elution (flow: 0.05 mL/min) started with 40% of A which was linearly increased to 99% over 10 minutes. 10 μL of sample was injected for a total run of 26 minutes.

The detection and quantification ware performed using an Agilent G1969A LC/MSD TOF, via Electro Spray Interface (DualESI™) under negative conditions. With an ESI system, the mobile phase from the HPLC system is introduced in the ionization chamber at atmospheric pressure. A spray of charged drops is created and addressed to the cone of the electrode. The desolvatation and evaporation process of the spray is enhanced by a needle heating and a nitrogen flow. The result is the total consumption of solvent with a formation of desolved ions in gaseous phase characterized by unitary or multiple charge. The DualESI^{TM} source is characterized by a double nebulizator system that improves the mass accuracy with the simultaneous introduction with the sample of an internal reference standard for the instant mass axes calibration. This method permits the automatic calibration during each chromatographic run. It is possible to work both in positive and in negative ionization.

The HRTOF/MS parameters were initially identified to detect analytes by the use of a series of flow injection analysis (FIA) bypassing the separation system. The negative ionization mode was selected for the conditions of the target polar organic compounds.

Nebulizing gas was nitrogen kept at 60 psig and 350°C as well the drying gas at flow of 10 L/min. The optimized main parameters of the TOF detector were: Fragmentor: 170V, Skimmer: 60V, Capillary voltage: 4000V, Octopole RF peak: 200V. Identification of analytes in real samples was automatically by the Data Analysis software performed by retention time (± 0.5 min of the corresponding standard) and by compound mass/charge ratio (< 3 ppm).

Because of the absence of target standards for a qualitative analysis, the optimization of both the separation run and the mass conditions were performed on a solution in 2-propanol (Romil, Dublin Ireland) of m-cresol and 4-chloro-3-methylphenol (≈100 ppm) both purchased by Fluka (Sigma-Aldrich, St. Louis, MO, US) as technical reagents.

2.3 Ecotoxicological testing

The paragraph shows materials and methods used for the application of ecotoxicological tests for a preliminary assessment of toxicological effects on the environment. In literature there are no relevant ecotoxicological studies about BTF compounds and the aim of this section is to give a first investigation on the toxicological effects of these pollutants on the environment and a preliminary evaluation of acute and chronic effects of environmental waters as suggest on the indications expressed by Directive 2000/60.

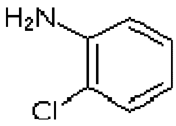
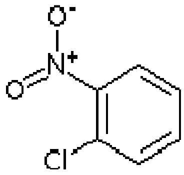
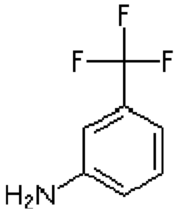
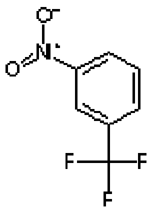
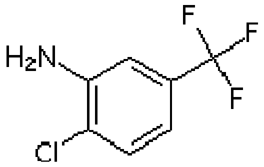
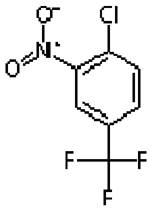
The use of acute and chronic aquatic toxicity data is underlined by risk assessment strategies to understand impact of dangerous and priority substances on the environment, but also is well explained on recent important pieces of legislation regarding properties, classification and labelling of substances *in primis* the REACh Regulation and CLP Regulation that transpose the GHS[95]. Acute toxicity, for purpose of classification of chemicals, refers to the intrinsic property of a substance to be injurious to an organism in a short-term exposure to them. Acute toxicity is generally expressed in terms of concentration or dose which is lethal to 50% of the test organism (LC_{50} or LD_{50}), cause a measurable adverse effect to 50% of the test organism (EC_{50}) or leads to a 50% reduction in test organism (treated organism) responses compared with control organism (untreated organism) responses. Substances with an acute toxicity determined to be less than 1 mg/L (1 ppm) are generally recognized as being very toxic. The handling, the use or the discharge into the environment of these substances pose a high degree of hazard and for a categorization for acute toxicity. In contrast those over 100 mg/L (>100 ppm) are regarded as practically non-toxic. Between 1 to 100 mg/L a range of concentration is not negligible for toxic effect on the organism tested. Chronic toxicity, always for purpose of classification, refers to the potential property of a substance to cause effects to aquatic system during an exposure on long-term in relation to the life-cycle of the organisms (UN definition)[96]. Chronic effects include a range of sub-lethal endpoints generally expressed in terms of NOEC (No Observed Effect Concentration) or an equivalent value express as EC_x. Observable endpoints include growth, reproduction, survival and development. The exposure duration of acute and chronic toxicity tests widely depend on the cycle-life of the organisms tested as well the endpoints measured.

For a preliminary ecotoxicological assessment three tests were chosen: the inhibition mobility test on *Daphnia magna Straus,* the inhibition test of light emission on *Vibrio fisheri* bacteria and the algal inhibition test on *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*). Six pure substances (pure active principles) were tested after dilution in Milli-Q water and the results compared. Subsequently the methods were also tested on some environmental groundwater samples collected in the area of Agno Valley during 2008 and 2009.

2.3.1 Preparation of solutions

The pure active principles of the substances investigated, were purchased Aldrich (Sigma-Aldrich, St. Louis, MO, US) as technical grade for organic synthesis as no environmental standard were available on the market. Only 2-chloroaniline and 2-chloronitrobenzene were analytical environmental standards and were purchased from Dr Ehrenstorfer (Augsburg, Germany). However all purity percentage were between 97.0 and 99.5 %. An amount of about 50-100 mg of each substance was dissolved on 3 mL of HPLC grade methanol (VWR Milan, Italy) and diluted to 1 L of Milli-Q water (Millipore®, Billerica, MS USA). The Milli-Q water used had a TOC of 4 ppb and a conductivity of 0.054 µS/cm. To facilitate the dissolution of the substances, the solution were sonicate for 10 minutes. The stock solutions obtained were stored in dark bottles at 4°C. Table 2.8 shows the substances considered and the concentrations of solutions obtained for the acute and for the chronic tests. The solutions used as blanks in the considered tests, also contained the percentage of methanol used to dissolve the pure substances. This was necessary to test the possible harmful or hormesis effects of the organic solvent used to dissolve the substances. Only one of the chemicals tested was in solid state in the pure form, the others were all in the liquid state.

Table 2.8: substances tested in the ecotoxicological experimental part.

Substance	CAS number	Physical state of the pure substance	Concentration in stock solution [mg/L]
2-chloroaniline 	95-51-2	Liquid	56.2* 60.6**
2-chloronitrobenzene 	88-73-3	Solid	44.4* 39.1**
3-aminobenzotrifluoride 	98-16-8	Liquid	65.3* 64.8**
3-nitrobenzotrifluoride 	98-46-4	Liquid	58.2* 71.8**
3-amino-4-chlorobenzotrifluoride 	121-50-6	Liquid	62.9* 71.0**
3-nitro-4-chlorobenzotrifluoride 	121-17-5	Liquid	55.5* 76.9**

*stock solution prepared for *Daphnia magna Straus* and *Vibrio fisheri* toxicity tests
**stock solutions prepared for *Selenastrum capricornutum* toxicity tests

2.3.2 Mobility inhibition of *Daphnia magna Straus*

Daphnia magna Straus (Cladocera, Crustacea) is a planktonic crustacean with size between 0.2 and 5 mm in length. It is used as indicator species particularly useful because of its short lifespan and for its reproductive capabilities. The procedure used for the determination of acute toxic effects of environmental waters (surface, drinkable, mineral, marine, groundwater and wastewater) was applied and conducted in environmental ecotoxicological control laboratory ARPAV in Mestre Venice. The method permits the determination of initial concentration of sample that produce the immobilization of 50% of organisms exposed to the polluted sample in 24h. This concentration, the effective inhibition concentration, is indicated as EC_{50}-24h. The procedure used follow the official EN ISO 6341:1999 method[97] based on OECD Guideline n 202 (2004)[98] and adopted by Italian institutions[99] (APAT IRSA CNR 8020 Man 29 2003).

Daphnia magna Straus organisms were cultured in an environmental chamber at 20 °C ± 2 °C with a photoperiod of 16h of light and 8h of dark under moderate aeration. For a correct test the organism used were younger than 24h. The rearing medium and the dilution water consisted of water with the following characteristics:

- 7.5 – 8.5 pH range
- 250 mg/L ± 25 mg/L hardness expressed as [$CaCO_3$]
- Ca/Mg ratio close to 4:1
- Na/K ratio = 10
- Dissolved Oxygen concentration >7 mg/L.

They were prepared adding 0.352 g of $CaCl_2 \cdot 2H_2O$ and 0.044 g of $NaHCO_3$to 2 L of commercial oligomineral drinkable water.

The real environmental samples were collected in 500 mL polyethylene bottle filled until the top to avoid the presence of air layers. The samples, if not analyzed in few hours after the sampling, were stored at 4°C for maximum 48h or at t<-20°C for longer periods.

The test was performed in two steps:

1- the screening test: to determine the concentration range for the final toxicity test. It gives an approximate EC_{50}-24h value.
2- the final test: to determine the definitive EC_{50}-24h in the range obtained from the preliminary test.

The screening test determined the concentration range for the final test and was used to verify the absence of toxicity of real samples. The sample (real or stock solution) was diluted with dilution water. The preliminary test was carried out in one replicate as shown in the following scheme:

Concentration	**Volume**	**n. *daphnia***
Blank	50 mL	5
100%	50 mL	5
50%	50 mL	5
25%	50 mL	5

The blank solution was dilution water for real environmental samples and Milli-Q water + 3‰ of methanol for the tests on pure substances. The flasks with test organisms for the screening were stored at dark in incubator (Memmert GmbH, Schwabach Germany) at 20°C ± 2°C for 24h. At the end of the test, the number of organisms immobilized was registered and the calculations for the final test were made.

The final test determined the EC_{50}-24h of studied samples. The sample was diluted to obtain 5 solutions in the dilution range of 10-90 % of the EC_{50}-24h preliminary value. Each dilution was tested in 4 replicates (total of 20 organisms for each dilution). The test flasks were stored as the same at dark in incubator at 20°C ± 2°C for 24h and at the end of the test, the number of organisms immobilized was registered for final calculation. A sequence of blank solutions was always determined to have a control of the organisms as well a sensitivity test with a refence toxicant (potassium dichromate). $K_2Cr_2O_7$ of analytical grade from Fluka (Sigma-Aldrich, St. Louis, MO, US) was therefore used for quality purpose and to test the performance of the method. For each batch of tests, a stock solution of 10 mg/L of $K_2Cr_2O_7$ was prepared in Milli-Q water and a definitive test on five dilution ranges (3.2 – 1.8 – 1.0 – 0.56 – 0.32 mg/L) carried out. For a correct quality control of the method, the EC_{50}-24h of $K_2Cr_2O_7$ needed to be in the range 0.6-1.7 mg/L as defined on OECD procedure. Immobility data, finally, were elaborated by Probits Analysis[100] with the Probfis2.exe software of CNR-IRSA.

2.3.3 Inhibition of light emission test of *Vibrio fisheri*

Vibrio fisheri is a gram-negative rod-shaped bacterium found globally in marine environments. Its bioluminescence properties are caused by transcription induced by population-dependant quantum sensing[101]. The 11348-3 EN ISO procedure used[102] for the determination of acute toxic effects of marine waters, following the OECD requirements[103], can be applied also for other environmental waters (surface, drinkable, mineral and groundwater). The principle of the method is to measure the emitted light of microorganisms in controlled conditions in contact with different diluted solutions of the samples for 30 minutes. The decrease of light emitted between initial and final conditions is proportional to the bacteria damage and is calculated considering a correction factor related to the variation of light intensity during the time. The result is a concentration-response curve useful to determine EC_{50} and EC_{20}. As for *Daphnia magna*, the procedure is carried out with a preliminary test and final test for the calculation of EC values. The samples, if not analyzed in few hours after the sampling, were stored at 4°C for maximum 48h or at t<-20°C for longer periods.
With the screening test the range for final test and the possible absence of toxicity of solutions was verified. The stock solutions and real samples were diluted with dilution water prepared with 2% of NaCl (Carlo Erba, Milan Italy) in Milli-Q waters. For analysis of non-marine waters, an osmotic adjustment was necessary with the addition of a further 22% NaCl in Milli-Q water solution. The test was carried out with a Microtox® Mod.500 Analyzer system (Strategic Diagnostics Inc, Newark DE US) operated at 490 nm with thermostatic cells at 15°C ± 1°C. The lyophilized *Vibrio fisheri* bacteria strain used were NRRL-B-11177 and stored in freezer at temperature of -20/-25°C. Each vial of bacteria was useful for two hours. For each cell an amount of 1 mL (900 μL of solution + 100 μL of bacteria reagents) was analyzed. After 30 minutes the final light emitted was recorded after and compared by the software MicrotoxOmni® with the initial one. The outputs of the software were the plots of the percentage of inhibition/effect versus concentration. The approximate EC_{50} obtained by extrapolation from the plot was used for determination of the concentration range for the final test. The difference between the screening test and the final test was only the number of dilution solutions used (10 solutions for the final test) in a range around the approximate values of EC_{50}. Validation criteria for the satisfactory test results were determined for each batch of analysis with the inhibition effects of bacteria on three reference toxicant solutions: 3.4 mg/L 3,5-dichlorophenol, 2.2 mg/L

of $ZnSO_4 \cdot 7H_2O$ and 18.7 mg/L of $K_2Cr_2O_7$ previously prepared from analytical grade reagents (Fluka, Sigma-Aldrich, St. Louis, MO, US). The results were plotted and elaborated to obtain the EC_{50} and EC_{20} final values.

2.3.4 Growth inhibition test with unicellular green algae

Selenastrum capricornutum (renamed *Preudokirchneriella subcapitata*) is a non-motile, unicellular, crescent-shaped (40-60 μm^3) green alga (Chlorophyceae) that is common in most fresh waters. Its uniform morphology makes it ideal for enumeration with electronic particle counter. Growth is sufficiently rapid to measure cell numbers after 72h and the species is moderately sensitive to toxic substances[104]. After 72h of incubation, algal density was determined by measuring the absorbance of the test solutions at 663 nm. The 72h *Selenastrum capricornutum* test can assess effects over several generations of organisms. The procedure followed the EN ISO 8692:2004[105] Inhibition was measured as a reduction on growth rate, relative to control cultures grown under identical conditions. The algal cultures were ATCC 22662 (American Type Culture Collection, Rockville Maryland US). The algal nutrients were prepared on four stock solutions in water according to the compositions given in Table 2.9. All chemicals used for the stock solution were of reagent grade quality and purchased by Riedel De Hahn and Fluka (Sigma-Aldrich, St. Louis, MO, US) and Merck (Darmstadt, Germany).

The apparatus for measuring algal cell density was a particle counter Beckman Coulter Z1 (Fullerton, CA US) equipped with a double threshold window set at 3-6 μm. The growth medium was prepared by adding appropriate volume of nutrient stock solutions (sol 1. = 10mL, sol 2. = 1mL, sol 3. = 1mL, sol 4. = 1mL) to 1 L of Milli-Q water and its pH was measured with a Crison pHmeter GLP 21 (Barcelona, Spain). A pre-culture was started two days before the beginning of the test with inoculation of growth medium at a sufficiently low cell density in order to maintain exponential growth until test starting ($5 \cdot 10^4$ cells/mL). This exponentially growing pre-culture was used as *inoculum* for the test and the cell density in the pre-culture was immediately measured before the use in order to calculate the required *inoculum* volume. A screening test, with three dilutions (1:10, 1:100; 1:1000) of each stock solution of the chemicals of table 2.8, was made. The test was carried out without adjustment of pH of the medium after addition of the sample. The test and control batches were after prepared by mixing the appropriate volumes of test sample or test sample growth medium and *inoculum* in the test vessels. Two replicates for the screening test were prepared. The Memmert (Schwabach Germany) incubation room was lighted and thermo-stated at 25 °C ± 1°C.The cell density of each test solutions was measured every 24 h and stopped at 72 h ± 2 h.

The final and definitive test was carried out at the same modality in five dilutions chosen after the result elaboration of each screening test.

Table 2.9: concentrations of nutrients used in the test solutions with *Selenastrum capricornutum*.

STOCK SOLUTION	NUTRIENT	CONCENTRATION IN STOCK SOLUTIONS	CONCENTRATION IN TEST SOLUTIONS
1: macro-nutrients	NH_4Cl	1.5 g/L	15 mg/L
	$MgCl_2 \cdot 6H_2O$	1.2 g/L	12 mg/L
	$CaCl_2 \cdot 2H_2O$	1.8 g/L	18 mg/L
	$MgSO_4 \cdot 7H_2O$	1.5 g/L	15 mg/L
	KH_2PO_4	0.16 g/L	1.6 mg/L
2 Fe-EDTA	$FeCl_3 \cdot 6H_2O$	64 mg/L	64 μg/L
	$Na_2EDTA \cdot 2H_2O$ (Titriplex III)	100 mg/L	100 μg/L
3: trace elements	H_3BO_3	185 mg/L	185 μg/L
	$MnCl_2 \cdot 4H_2O$	415 mg/L	415 μg/L
	$ZnCl_2$	3 mg/L	3 μg/L
	$CoCl_2 \cdot 6H_2O$	1.5 mg/L	1.5 μg/L
	$CuCl_2 \cdot 2\ H_2O$	0.01 mg/L	0.01 μg/L
	$Na_2MoO_4 \cdot 2H_2O$	7 mg/L	7 μg/L
4: bicarbonate	$NaHCO_3$	50 g/L	50 mg/L

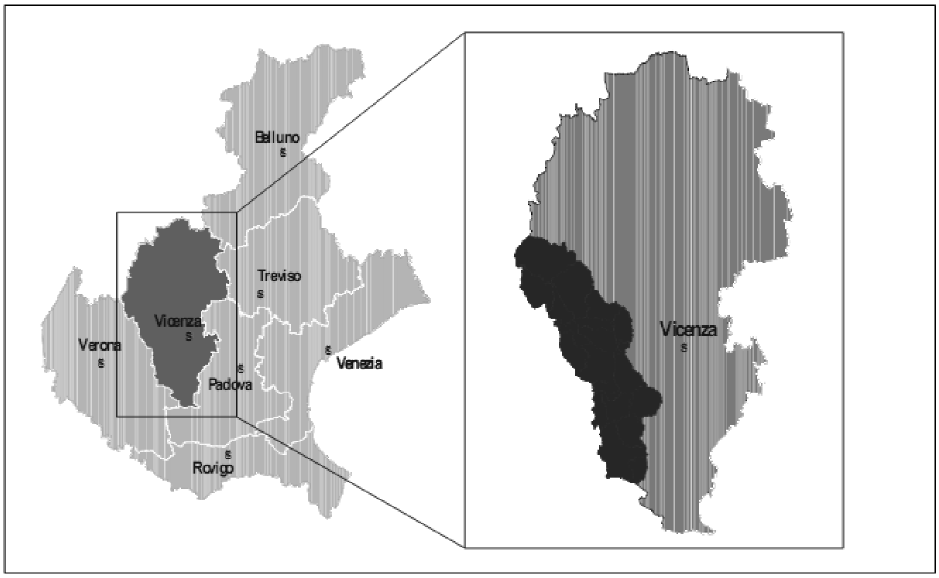
2.4 Historical trend contamination of a pollution accident: a study case

This part of the chapter *Materials and Methods* focuses the attention on an historical chemical contamination accident occurred in the second half of 1977 and involved the Rimar industry. The factory, located in the Vicenza Province area, produced fluorinated intermediates for the synthesis of a wide range of organic compounds used in several productive sectors. A territorial framing of the area with brief geological, hydrogeological and hydrochemical considerations is made with the purpose to understand the situation nowadays after a temporal interval of more than 30 years.

2.4.1 A territorial framing of the study case

The studied area is located in the Vicenza Province, at the north part of Veneto Region. The area is defined between the East Lessini Mountains and the contiguous plain in the part of Province at west between Verona Province and the Vicenza city district. The area corresponds to the catchment basins of two water bodies that flow from the surrounding mountains: Agno-Guà River and Chiampo Stream.

Figure 2.1: framing at regional level of the case study area.



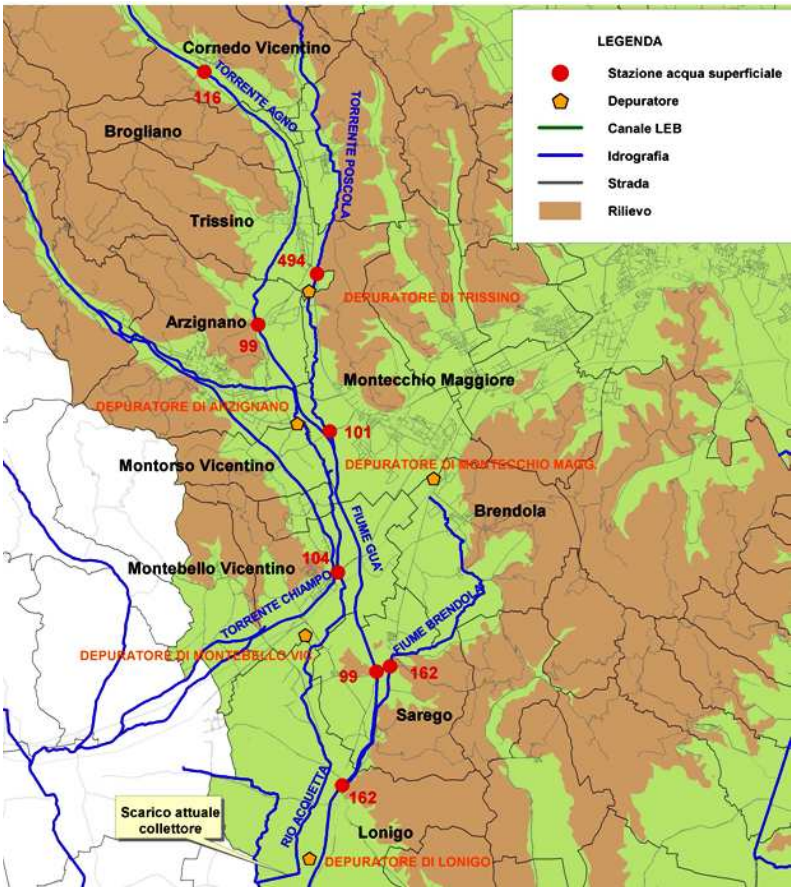
From the geographical point of view the northern part of the area is delimited by Recoaro Terme and Valdagno municipalities with the bottom of pre-Alps mountains

(Carega, Civillina, Faedo Mountains) where the two valleys of the catchment basins begin. The east part is bounded by small mountains (Torrebelvicino, Malo Mountain, Gambugliano) while the south part extend in the Padana Plain until Lonigo and Alonte municipalities grazing Berici Hills on SE direction. The area is mostly mountainous and hilly especially in the northern part while the south is preferably with a plain morphology. From the hydrographical point of view in the area two main water bodies shaped the valleys: Chiampo Stream and Agno-Guà River. Figure 2.2 shows the specific area with part of the municipalities involved. Figure 2.3 instead examined, with a geographical map, the main surface water bodies and relief, representing also the ARPAV surface monitoring stations and the WWTP of the area.

Figure 2.2: municipalities of the Chiampo and Agno-Guà valley.

Figure from: Progetto Giada “Acque sotterranee” Vicenza Province/ARPAV (2006 update)

Fig 2.3: geographical map of the area considered with the hydrography (blue lines), ARPAV surface monitoring stations (red dots) and WWTP (orange dots).



From: Mario Cecchetto: "Progetto di monitoraggio ambientale del bacino del Fratta-Gorzone" (ARPAV Vicenza Province Department)[106].

A deep investigation of geological and hydromorphological profiles of the Agno-Guà River and of Chiampo Stream catchment rivers is well described in the specific studies of Giada Project[118], a work financial supported by LIFE contribution of European Community and carried out by Giada Consortia with integrated collaboration of Arpav, Vicenza Province administration and Centro Idrico Novoledo. In the reports considered all the groundwater systems are well described with hydrochemical considerations.

2.4.2 Hydrogeological outlines[106,107,108]

In the specific, the examined area considers the low and medium-low part of Agno-Guà RBD. In geological terms, the upper superficial layers of the area are characterized by alluvial sediments of Quaternary period (pebbles, gravel and sand) with lens inclusions of fine sediments as silt and clay (5-15%). The thickness of this layer has variability between 70m and 100m. The formations below are of rock type of Miocene epoch with a total thickness ranging from 55m and 65m. The alluvial layer is the water-bearing stratum in these formations and is clearly defined by the hydraulic gradients of the surrounding areas. Most part of the alluvial materials were deposited by Agno River. The area, therefore, shows a porous material layer with weak silt-clay matrice elements. The alluvial layer of Agno-Guà Valley is characterized by a rich phreatic water table that, in the south part, is divided into a multi-aquifer system with weak piezometric differentiation[110]. The feeding of the aquifer is guaranteed by the strong dispersion of the bed river in the northern part (Cornedo Vicentino municipality), from direct precipitations and from dispersion of smaller water bodies of the area as Poscola Stream, Arpega Stream and Restena Stream. Studies[111] had demonstrated the active recharge of the phreatic groundwater due to hydrogeological characteristics strictly related to the hydrometric variations of Agno-Guà River. From the Recoaro municipality, Agno River turns in south direction with an initial NW-SE trend until Trissino from which start to flow mainly in NS direction. The contribution of this basin on the groundwater hydric system is higher than Chiampo Stream catchment basin for the bigger extension and for the reasons explained before. Finally it is to underline pluviometry considerations remembering that the area of pre-Alps, especially in Recoaro municipality zone, is an important regional area for rains (Figure 2.5). Moreover Chiampo Stream Basin in the northern part has a similar and parallel direction than Agno-Guà River with a NNW-SSE valley morphology until the Montebello municipality where it roughly turns in West direction with inflow to Adige River. Figure 2.4 shows in details the two River Basins considered with the drainage divide in the relief zone between them.

The Giada project made also an interesting first evaluation of the permeability and water penetration of the area. The grade of permeability of the lithological elements of the territory was considered and elaborated in a qualitative way or rather as potential of infiltration.

Fig 2.4: map that shows the two River Basin considered with the hydromorfology of the area. (green area = relief, black outline = drainage divide). [from: Progetto Giada "Acque sotterranee" Vicenza Province/ARPAV (2006 update)]

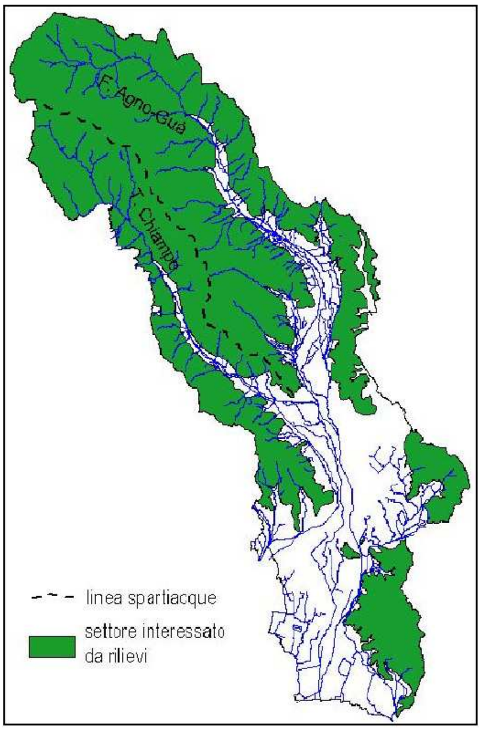
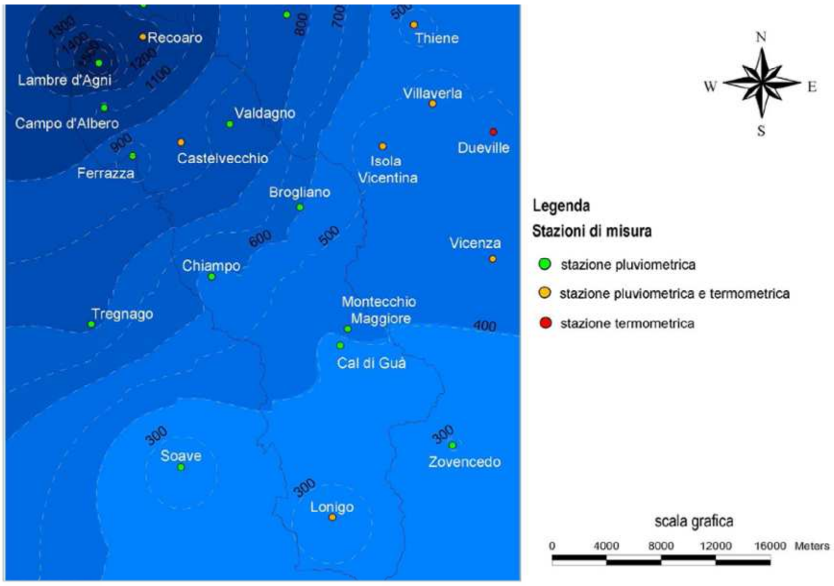
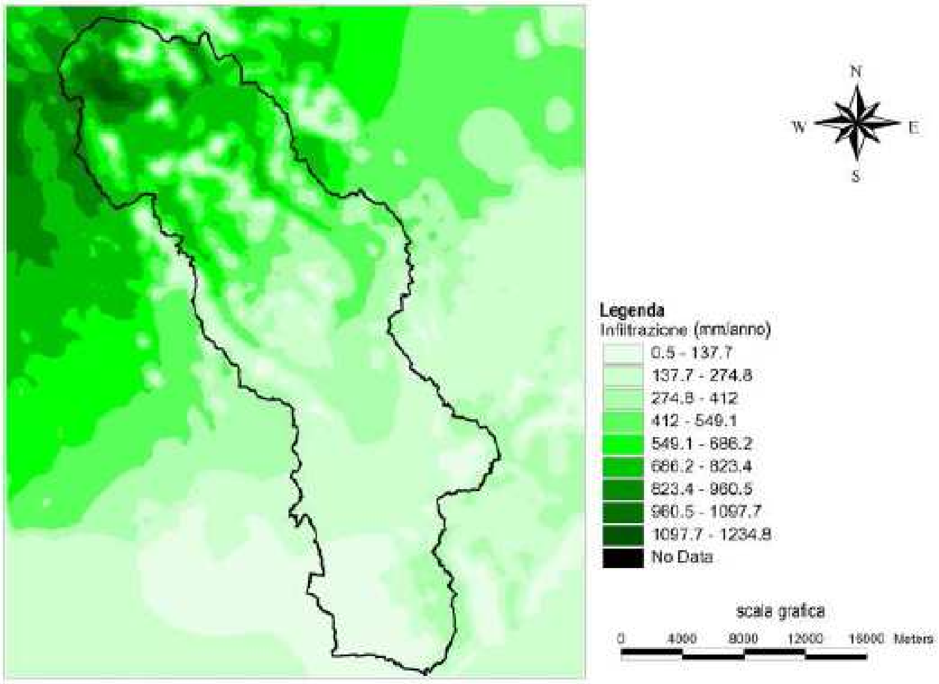


Figure 2.5: elaboration of 1985-1995 pluviometric data from Annali Idrologici del Magistrato delle Acque di Venezia [from: Progetto Giada "Acque sotterranee" Vicenza Province/ARPAV (2006 update)]



The analysis was based on the following elements: lithology of the territory, the tectonic movements of macro area considered, topographic elements to estimate the conformation and shape of the ground and finally the elaboration of pluviometric data on yearly base (Figure 2.6). The evaluation didn’t consider the human urbanized and industrialized area and all the anthropic effects that might affect the water penetration.

Figure 2.6: permeability and water penetration evaluation of Chiampo and Agno-Guà valleys. [from: Progetto Giada “Acque sotterranee” Vicenza Province/ARPAV (2006 update)]



The alluvial aquifer system is different in comparison with the other three systems of the Vicenza Province (Astico-Bacchiglione, Brenta, Colli Berici). Specifically the underground system shows the following aquifers:

- phreatic aquifer of sub-river bed of valley bottom of Chiampo Stream and Agno-Guà River;
- phreatic aquifer of the portion of plain between Alte of Montecchio Maggiore and Brendola municipalities;
- multilayer system under pressure of the medium plain of Lonigo-Almisano.

The intra-valley system characterized by alluvial deposit of claims and sands with variable layers, and the alluvial valley bottom area permit the formation of a monolayer aquifer with sub-river bed phreatic groundwater. This free groundwater is strictly connected with surface waters that helps the feeding and is widely influenced by ancient river beds totally buried. Agno River spread into the undifferentiated aquifer almost 100 L/s per Km in the 24 Km length between Cornedo Vicentino and Montebello Vicentino municipalities. The undifferentiated aquifer deposits of the northern part of Chiampo and Agno-Guà Valleys, with a good water power, represent one of the most important hydro-geological areas of Veneto as reservoir of recharge water for the medium plains aquifers (area of Lonigo and San Bonifacio). The transition from undifferentiated monolayer aquifer system to a multi-aquifer differential system occurs gradually in a narrow belt of territory of 5 Km corresponding the phreatic aquifer of the portion of plain between Alte of Montecchio Maggiore and Brendola municipalities. The differential system of the middle plains is constituted by:

1. a surface aquifer with different thickness from few meters to 30 m generally with free groundwater but locally confined by an aquitard in relation at the presence of silt and clay;
2. a system of aquifers under pressure composed of a three levels confined aquifer deep: 40-60m (1st aquifer under pressure), 70-80m (2nd aquifer under pressure) and 90-110m (3rd aquifer under pressure);
3. the water intake from the undifferentiated system of groundwater pressure of Almisano area operated by different consortia and Lonigo municipality (0.12 m^3/s, 4000000 m^3/y).

Other different detailed studies[112] during the nineties determined the natural intrinsic vulnerability to pollution present in the alluvial aquifer investigated. The natural vulnerability is the specific susceptibility of aquifers, in their various parts, of the bear the presence of a pollutant and the impact on them. The results defined six vulnerability classes with highest vulnerability on the bottom valley of Agno-Guà River and Chiampo Stream and the lowest in all the south part of the area considered.

The geological profile[107] of the area, close to the pressure point of contamination (Rimar factory) was studied and is proposed on figure 2.7. The presence of pebbles, sands and marl stones with inclusion of sandstones, limestones, silts and clays builds a first layer of alluvial materials from recent flooding of the valley floor with high permeability. A following layer intensely fractured of calcarenites (calcareniti di Castelgomberto) from Oligocene Epoch shows the most evident karst phenomena of all the area of Lessini Mountains. The lower layer finally is composed of marl clays (marne di Priabona), from the upper Eocene Epoch, well stratified and impermeable and represents the substrate and the bottom aquiclude. The Castelgomberto calcarenites, which constitute the backbone of the hill system, are overall a good reservoir linked to a past rich groundwater limited by the aquiclude. Figure 2.8 furthermore shows the profile of four wells located on the NS line direction along the Poscola River and close to the interested area. It is clear as a porous layer of different materials cover the overlying soil in all the valley area before to reach a layer of impermeable clay at about 55m from the surface at the Rimar level.

Figure 2.7: WE stratigraphic profile at the level of Rimar factory [from IRSEV study]

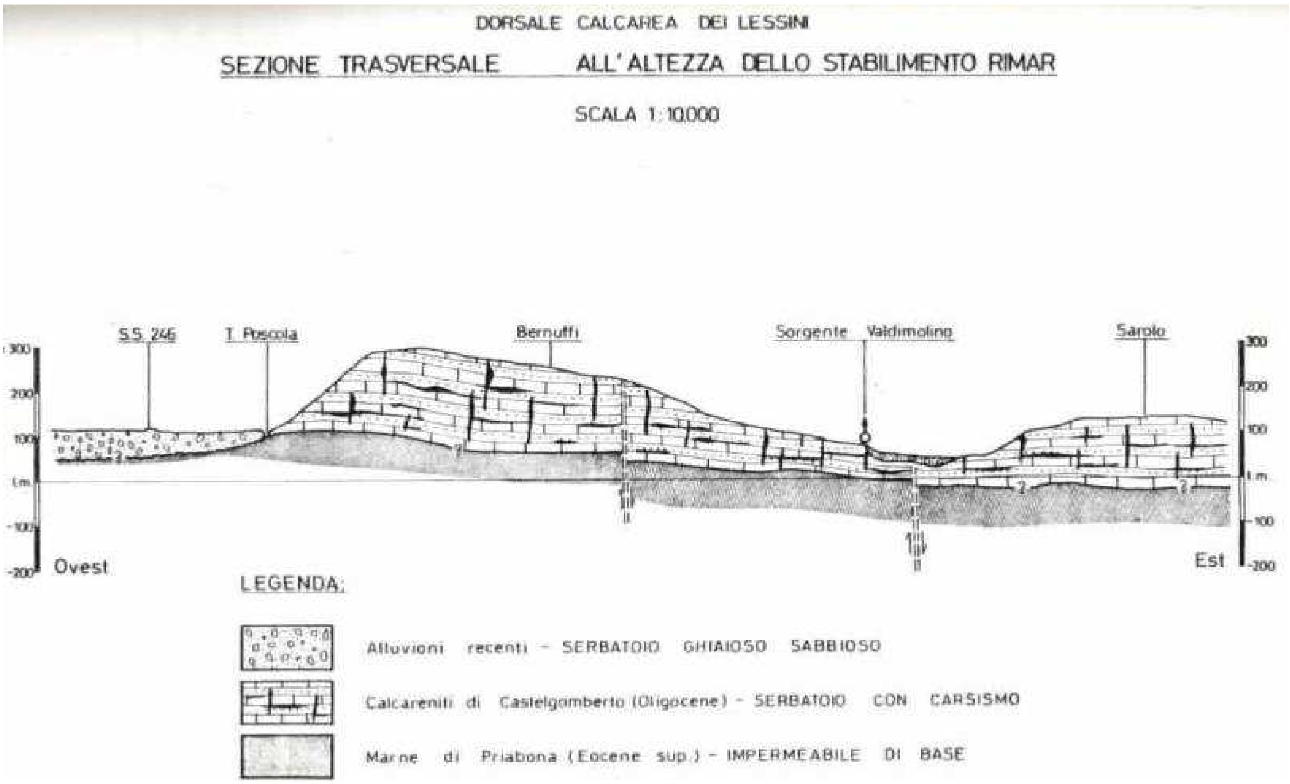
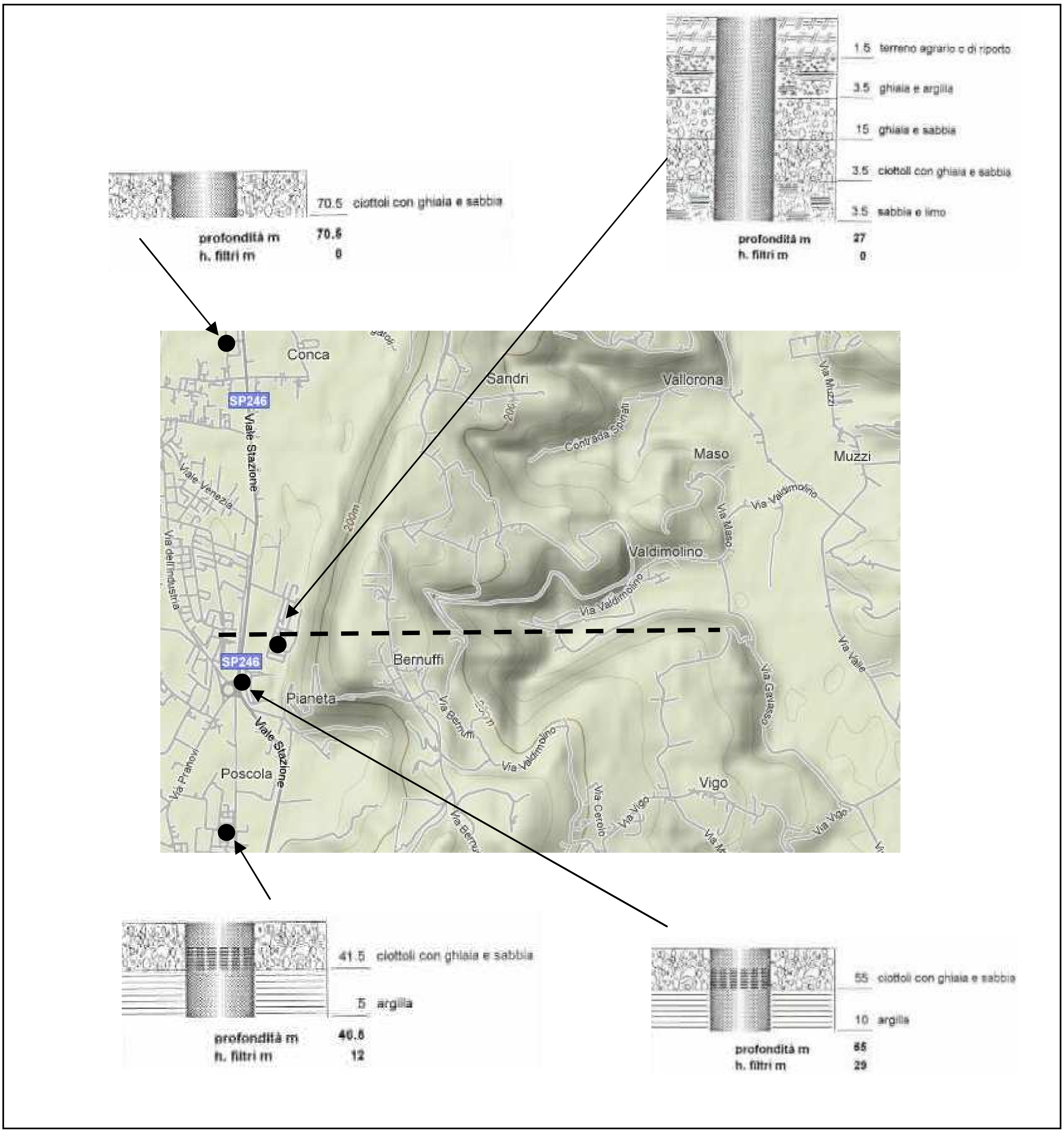


Fig 2.8: stratigraphic profiles of four wells on the direction NS following the Poscola River bed. The black dotted line shows the WE profile described on the previous figure.



2.4.3 Hydrochemical profile of the area

A connection between chemical state and pollution of groundwater and the productive and industrial activities is currently clear. Until ten years ago productive activities emissions were discharged directly in surface water bodies or, at least, not treated as nowadays. So from surface water bodies there was a continuous percolation and leaching to the aquifers of contaminants characterized by high water solubility and great chemical stability and persistence (i.e. Cr^{6+}, Cl^-, NO_3^-). In the considered area a single collector system (ARICA collector), built in 2000, collects most industrial wastewater from the WWTPs of the area and then transfers the final outflow to the south area, after the reservoir zone of Almisano water intake system. The water bodies classification, even if different during the last years due to different classification systems adopted can be synthesized in table 2.10.

Table 2.10: classification of ecological status of the water bodies considered (Vicenza Province Administration data source).

Water body	Municipality	Quality class	
		1986-1988	2000-2001
Chiampo	San Pietro Mussolino	Slightly polluted	Sufficient-good
	Chiampo	Polluted	-
Agno	Valdagno	Polluted	-
	Cornedo Vicentinp	Very polluted	Sufficient-good
Guà	Tezze di Arzignano	Very polluted	scarse
	Montebello Vicentino	Highly polluted	scarse
Acquetta	Montebello Vicentino	Highly polluted	scarse
	Lonigo	Highly polluted	-
Poscola	Castelgomberto	Not polluted	-
	Monteccho Maggiore	Highly polluted	-
Brendola	Brendola	Very polluted	sufficent
	Lonigo	Highly polluted	-

Although different evaluation criteria, it was possible to detect an improvement on the general water quality especially after the beginning of work of the collector.

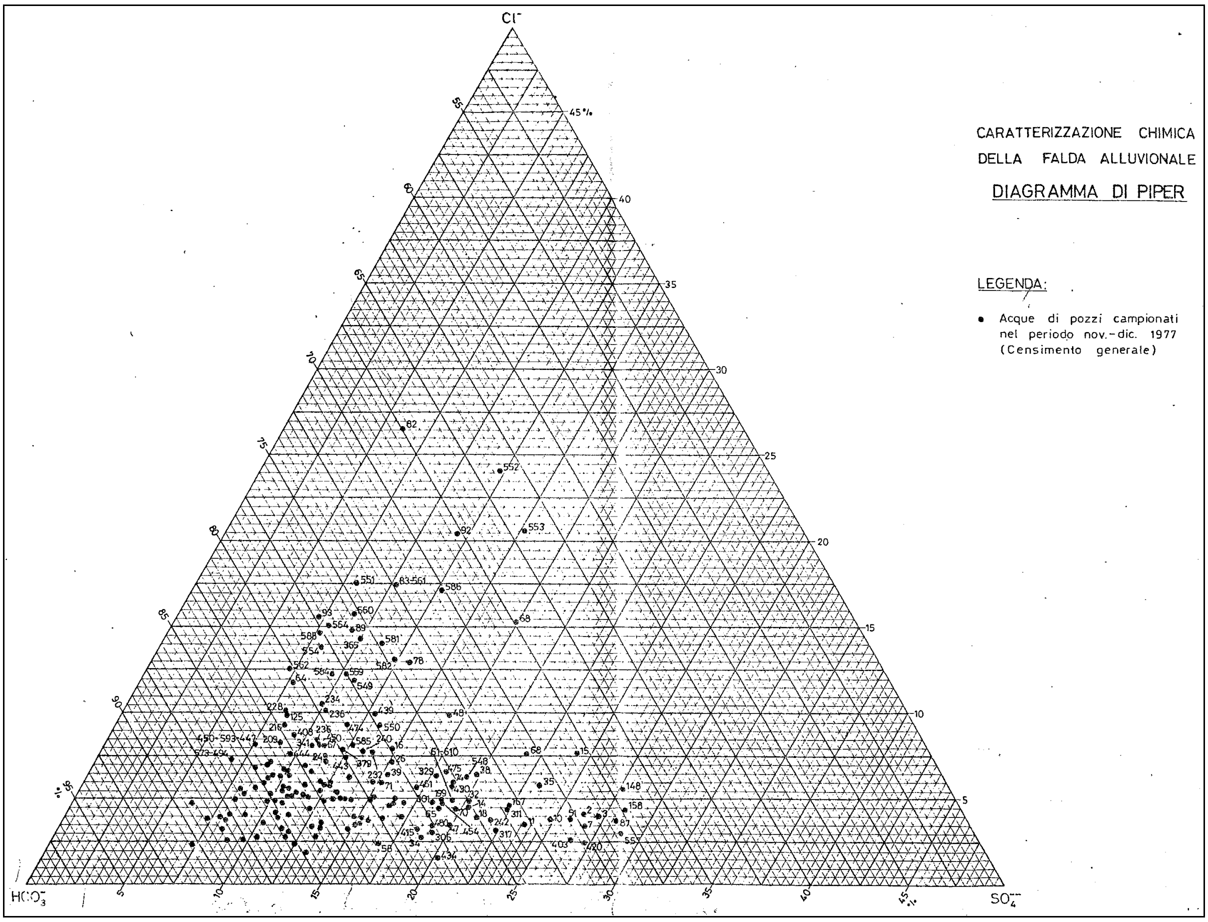
Another consideration is due to the reduction of piezometric levels in all the area (i.e. in the confined aquifer of Almisano the piezometric load was reduced of more than 5m in 60 years) with the consequence of drainage and abandonment of many private wells especially in the northern part of the area.

For a complete hydrochemical frame of the aquifers, results of datasets elaborations of monitoring campaigns from 1977 and 2003 were taken into account.

The IRSEV[107] (Istituto Regionale di Studi e Ricerche Economico-Sociali del Veneto) report elaborated a preliminary study of chemical parameters with the purpose to obtain a first hydrochemical characterization of groundwater of the area. Analysis of anions concentrations (Cl^-, SO_4^{2-}, HCO_3^-) was made on almost 200 wells and data were elaborated in a ternary plot (Piper diagram, Figure 2.9). These data were later confirmed and reported by other studies[108]. Most part of samples had shown a well defined chemical composition with:

- 70-90 % of HCO_3^-
- 2-15 % of Cl^-
- 8-30 % of SO_4^{2-}

Figure 2.9: ternary plot obtained from the analysis of sampling point of IRSEV study.



Only few groundwater samples reported Cl^- until 30% and more SO_4^{2-} concentrations in the area located close to the volcanic hills that delimit Agno-Guà valley in W direction. Summarizing, the alluvial groundwater seemed to belong at a unique hydro-chemical family with local variations in terms of Cl^- and SO_4^{2-}.

During the following three decades, other campaigns of groundwater analysis were carried out with the specific aim to understand the pollution situation. Analysis of qualitative and quantitative parameters in the years 1986, 1987, 1988, 1989 and 1994 were did by PMP Laboratories (now ARPAV) on 213 wells and in 2003 from Giada Project on 57 wells. The complete results from PMP Laboratories were 1082 controls so distributed:

- 266 sampling between June and November of 1986
- 513 sampling between January and December of 1987
- 189 sampling between March and September of 1988
- 52 sampling between February and march of 1989
- 62 sampling between November and December of 1994

The monitored parameters were:
specific conductivity at 20°C, total dissolved solids, pH, total hardness, Kubel oxidability, NH_4^-, NO_2^-, NO_3^-, alkalinity, PO_4^{3-}, Cd, total Cr, Cr^{6+}, Fe, Hg, Pb, Cu, Zn, phenols, MBAS, TOC, alachlor, atrazine derivatives and metabolites, simazine, chloroform, 1,1,1-trichloroethane, carbon tetrachloride, trichloroethylene, tetrachloroethylene, 4-nitrobenzotrifluoride (qualitative), 4-chloro-3-nitro-benzotrifluoride and total nitro-halogenated derivatives (qualitative).
This first dataset was elaborated for the most significant parameters by Giada Project work group with the following conclusions: 1) presence under the LODs of phenols, MBAS, PO_4^{3-} and alachlor and 2) presence of pollution phenomena due to:

- chromium (1986-1988) in Arzignano, Montecchio Maggiore and Brendola;
- organohalogenated compounds (1986-1988) in San Bonifacio and Arzignano;
- nitro-halogenated derivatives in Montecchio Maggiore;
- atrazine and derivatives in Lonigo and Sarego;
- cadmium in Arzignano, Trissino, Montecchio maggiore, Zermeghedo.

The limit of the study is the inhomogeneous dataset collected from the temporal and spatial profiles. Indeed sampling were spread in different periods and seasons during the campaigns and the number of sampling stations was decreased in the years because of the lack of water and for drainage of aquifers at certain levels.

Giada monitoring campaign (2003) was carried out on 57 of the 80 planned monitoring stations focusing the attention on wells strategic for a continuous use (i.e. for productive and industrial uses, for water intake) and wells with critic chemical values detected in the previous campaigns (i.e. presence of inorganic and organic micropollutants, concentration of nitrates > 25 mg/L). The sampling period, September and October 2003, was in a good quantitative situation with wells located on the sub-river bed aquifer of Agno-Guà River and Chiampo Stream, on the undifferentiated aquifer system and on confined Almisano aquifer.

The relevant results for this study regard: a) the sub-river bed aquifer of Agno-Guà River and b) the phreatic aquifer of the middle plain on Chiampo Stream.

a) 17 stations were considered with different deeps from 23 m and 97 m and good chemical parameters level in the northern part of the valley before Trissino center. This aquifer showed a high sulfate contents (≈ 60 mg/L) compared with other surrounding areas as the Chiampo Stream aquifer (25-27 mg/L). This is a confirmation of the previous IRSEV data analyzed. Other relevant consideration were made on Cr concentration, double in a comparison between the northern sub-river bed aquifer and the southern part in Montecchio Maggiore area (from 3 to 6 μg/L) and VOC organohalogenated compounds concentrations in the range of 1.3 and 1.7 μg/L. Finally a consideration was made in relation to the study case considered in this thesis with nitro-halogenated derivatives in two wells at concentrations of 3 and 9 μg/L. Table 2.11 shows the average chemical parameters values of the considered area obtained in the 2003 Giada project campaign.

b) The aquifer is located at south of industrial area of Arzignano (important area for tanning production) and it is without an upper aquiclude layer. 18 stations were considered in and the aquifer was divided in three sub-basins. The average deep of wells was 65 m. Most of chemical parameters decreased the concentrations in direction NS with higher contents of micropollutants in comparison with the Agno-Guà aquifer. Table 2.12 shows the average chemical parameters values of the second considered aquifer.

Table 2.11: the average values of chemical parameters of sub-river bed aquifer of Agno-Guà reported by 2003 Giada Project groundwater monitoring campaign.

Parameter	Type of parameter	Valdagno – Cornedo-Castelgomberto – Trissino (8 stations)	Montecchio Maggiore (9 stations)
pH	macro descriptor	7.6	7.7
Spec Conductivity [μS/cm]	macro descriptor	484	533
TDS [mg/L]	macro descriptor	328	359
Total hardness [°F]	macro descriptor	27.1	30.0
Cl^- [mg/L]	macro descriptor	5	8
NO_3^- [mg/L]	macro descriptor	10	16
SO_4^{2-} [mg/L]	macro descriptor	65	55
Cr_{TOT} [μg/L]	micropollutant	2.9	5.7
Fe_{TOT} [μg/L]	micropollutant	-	-
Ni_{TOT} [μg/L]	micropollutant	1.4	20.
Cu_{TOT} [μg/L]	micropollutant	4.4	2.2
Trichloroethylene [μg/L]	micropollutant	0.2	0.1
Tetrachloroethylene [μg/L]	micropollutant	1.5	1.0
Total VOCs [μg/L]	micropollutant	1.7	1.3

Table 2.12: the average values of chemical parameters of phreatic aquifer in the middle plain of Chiampo Stream reported by 2003 Giada Project groundwater monitoring campaign.

Parameter	Type of parameter	Montorso (6 stations)	Zermeghedo Montebello (4 stations)	Montecchio Maggiore (9 stations)
pH	macro descriptor	7.6	7.6	7.7
Spec Conductivity [μS/cm]	macro descriptor	601	749	667
TDS [mg/L]	macro descriptor	407	525	451
Total hardness [°F]	macro descriptor	31.4	36.2	33.2
Cl^- [mg/L]	macro descriptor	24	37	38
NO_3^- [mg/L]	macro descriptor	18	24	22
So_4^{2-} [mg/L]	macro descriptor	38	54	49
Cr_{TOT} [μg/L]	micropollutant	5.3	6.7	5.3
Fe_{TOT} [μg/L]	micropollutant	216	143	10
Ni_{TOT} [μg/L]	micropollutant	2	3	1.7
Cu_{TOT} [μg/L]	micropollutant	2	6	1.9
Trichloroethylene [μg/L]	micropollutant	0.4	0.1	0.3
Tetrachloroethylene [μg/L]	micropollutant	5.2	2.1	2.6
Total VOCs [μg/L]	micropollutant	5.8	2.3	3.7

3. RESULTS AND DISCUSSION

3.1 Chloroaniline and chloronitrobenzenes determination

3.1.1 Development of the method

The aim of this part of work was to develop an adequate, simple and fast new multiresidue method for the simultaneous analysis of 8 chloroanilines (CAs) and 6 chloronitrobenzenes (CNBs) as pollutant of environmental waters. The first part of the work was intended to search the best analytical conditions to obtain an easy and rapid, but at the same time rugged, method to apply in a routine/control environmental laboratory.

3.1.1.1 Comparison between different cartridges and different solvents

Initially a work of method's development was carried out with the aim to find the most suitable analytical process for both these two different classes of compounds. The development was assessed on Milli-Q water samples spiked with standard and working solutions of CAs and CNBs.

First of all the method was thought according with the literature through the comparison of different kinds of commercial solid phase adsorbents and through their elution with different organic solvents. 200 mL of Milli-Q water were spiked obtaining 1÷3 µg/L solution of each compound. The sorbents were activated with 5 mL and eluted with 10 mL of different solvents: (a) ethylacetate, (b) acetonitrile/dichloromethane 1:1, (c) *n*-hexane. Four different commercial sorbents for reversed SPE were assessed using cartridges of 6 mL volume filled with 500 mg of phase. The cartridges used, which their physical properties are explained in Table 3.1, were:

- ISOLUTE® C18(EC) (IST, Mid Glamorgan UK), a standard monomerically bondend silica derivatized with octadecyl hydrocarbon chains;

- Supelclean™ ENVI-18 (Supelco, Belafonte PA US), a polimerically bonded endcapped silica derivatized with octadecyl hydrocarbon chains;

- Supelclean^{TM} ENVI-Carb^{TM} Plus (Supelco, Belafonte PA US), a non porous and non bonded graphitized carbon-based sorbent;

- Oasis^{TM} HLB (Waters, Milford MA US) a copolymeric sorbent with hydrophilic-lipophilic balance.

Table 3.1: physical properties of the cartridges considered.

Cartridge	ISOLUTE® C18	Supelclean^{TM} ENVI-18	Supelclean^{TM} ENVI-Carb^{TM}	Oasis^{TM} HLB
specific surface area [m^2/g]	521	475	1149	810
average pore diameter [Å]	54	60	27,2	80
total pore volume [cm^3/g]	-	0,8	0,78	1,3
average particle diameter [μm]	30÷90	45	-	30÷60

Table 3.2 shows the comparison of recoveries between four different cartridges eluted with different organic solvents. The spiked samples were extracted in triple replicates obtaining Relative Standard Deviations (RSDs) values between 0.3 and 11.3.

Table 3.2: comparison of recoveries (%) between different solid-phase commercial sorbent extracted with different organic solvents: ethylacetate (a), acetonitrile/dichloromethane 1:1 (b) and hexane (c)

COMPOUND	C18 (a)	C18 (b)	HLB (a)	HLB (b)	HLB (c)	ENV Carb(a)	ENV Carb(b)	ENVI-18 (a)
aniline	19	21	27	54	-	58	16	-
nitrobenzene	0	2	79	37	-	81	37	-
2-chloroaniline	30	13	88	56	39	0	3	78
3-chloroaniline	30	25	98	92	-	2	3	-
4-chloroaniline	14	20	77	69	2	1	4	69
2-chloro-4-methylaniline	38	37	92	59	-	5	3	-
3-chloronitrobenzene	85	41	80	43	-	84	54	-
2-chloronitrobenzene	89	42	86	50	-	100	64	-
4-chloronitrobenzene	-	-	83	-	78	-	-	74
3-chloro-4-methylaniline	0	53	66	98	-	0	14	-
4-chloro-2-nitrotoluene	94	34	75	54	-	105	51	-
3,4-dichloroaniline	71	73	83	99	-	0	2	-
1-chloro-2,4-dinitrobenzene	71	0	62	0	1	7	0	115
4-chloro-2-nitroaniline	116	75	102	61	-	0	10	-

Table 3.2 shows that a copolymeric sorbent designed for the analysis of a wide range of pollutants in environmental samples give best compromise recoveries even of CAs and CNBs than other types of phases using ethylacetate as extraction solvent. Ethylacetate is more powerful in extraction than *n*-hexane and also than the mixture acetonitrile/dichloromethane 1:1.

Using HLB Oasis^{TM} *N*-vinylpirrolidone-divinylbenzene copolymeric sorbent with hydrophilic-lipophilic balance, unlike traditional SPE sorbents, undesirable irreproducible silanol activity and low recoveries were eliminated. The ability of the sorbent is to retain non-polar and polar compounds at the same time, even if the bed runs dry shown good performance for the development of the method.

Supelclean^{TM} ENVI-Carb^{TM} Plus cartridges gave best recoveries for CNB class, but recoveries of CAs were very low. However using a two-trap tandem system catridges, one containing a graphitized carbon black (Carbopack B), and the other one filled with a resin-based strong cation exchanger, as tested by Di Corcia *et al.*[86] the CAs recovery was improved, but otherwise this procedure cannot be used to determinate CNBs.

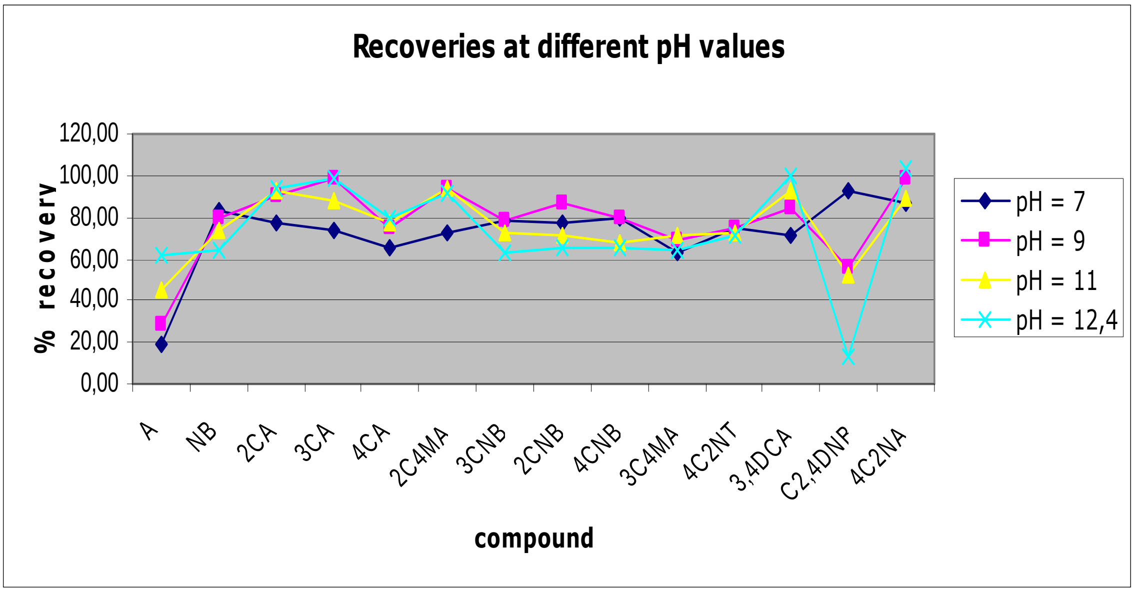
3.1.1.2 Influence of pH

A further step in the development of the SPE method was the adjustment of pH of the sample to obtain the optimum extraction and analytes retention. The best pH conditions were evaluated through the analysis of 200 mL Milli-Q samples at four basic different pH values spiked obtaining 1÷3 µg/L of each compound working solution. The pH of each solution was adjusted with NaOH 10% w/w drop wise and controlled by a pHmeter GLP 21 (Crison, Barcelona Spain). After the preparation, the samples were extracted with HLB cartridges, conditioned and eluted with ethylacetate. Results are showed on table 3.3 and figure 3.1. For the weak basic properties of CA as aromatic amines, due to the negative inductive effect of the ion pair on the nitrogen partially delocalised into the *п*-system of the benzene ring, only basic pH ranges were considered. CNB don’t show significant acid-basic properties so they were no affected by pH modifications. The evidence shows that the best pH compromise to operate for both CA and CNB is at pH=9. The results expressed in the table show the mean recovery of three replicate experiments (n=3) with a RSD range between 1.6÷11.0.

Table 3.3: comparison between extractions at different pH of the sample.

Analytes	pH = 7.0	pH = 9.0	pH = 11.0	pH = 12.4
aniline	19	**29**	45	62
nitrobenzene	83	**80**	73	64
2-chloroaniline	77	**91**	93	94
3-chloroaniline	73	**99**	88	98
4-chloroaniline	66	**75**	77	79
2-chloro-4-methylaniline	73	**94**	94	92
3-chloronitrobenzene	79	**79**	76	64
2-chloronitrobenzene	78	**86**	72	65
4-chloronitrobenzene	80	**80**	67	65
3-chloro-4-methylaniline	63	**69**	71	64
4-chloro-2-nitrotoluene	75	**74**	73	72
3,4-dichloroaniline	71	**85**	93	99
1-chloro-2,4-dinitrobenzene	93	**56**	55	13
4-chloro-2-nitroaniline	87	**99**	89	103

Figure 3.1: a graphic comparison between mean recoveries at different pH values for all the analytes considered.



3.1.1.3 Drying step

Drying of the cartridge after the sample extraction is a primary and essential step during all over the procedure. A correct drying time under vacuum or through a positive flow of nitrogen from the top of the cartridge, was necessary to eliminate possible water drops and aqueous residual traces held back into the sorbent phase. It was tested that 60 minutes is the minimum time under vacuum to have a complete dryness of HLB Oasis^{TM} cartridges. Nevertheless, to accelerate the procedure's step without considerable recovery loss, the possibility to insert a further step with anhydrous sodium sulphate (Na_2SO_4) was tested. A Bond Elut Jr. cartridge filled with 2.2 g of Na_2SO_4 (Varian, Harbour City CA, US) was placed in tandem after the final elution step with ethylacetate and a drying time of only 15 minutes under vacuum.

Table 3.4 and figure 3.2 explain the comparison of the procedure with and without the anhydrous sodium sulphate step in an extraction of 200 mL spiked Milli-Q water at pH=9 buffered solution. Adding this time-saver new step, a loss of total recovery was clear, but it doesn't compromise the total recoveries of analytes (except for 3-chloroaniline and 2-chloro-4-mathyaniline) and can be useful where the extraction of a large numbers of samples in the minimum time possible is necessary.

Table 3.4: comparison between % recoveries obtained with and without the Na_2SO_4 drying step (RSD values of 3 replicates).

Analytes	With Na_2SO_4 (%)	Without Na_2SO_4 (%)
aniline	24 ± 4	29 ± 1
nitrobenzene	75 ± 10	80 ± 6
2-chloroaniline	86 ± 4	91 ± 11
3-chloroaniline	79 ±5	98 ± 7
4-chloroaniline	74 ± 9	75 ± 5
2-chloro-4-methylaniline	79 ± 8	94 ± 4
3-chloronitrobenzene	77 ± 8	79 ± 6
2-chloronitrobenzene	84 ± 6	86 ± 9
4-chloronitrobenzene	-	86 ± 5
3-chloro-4-methylaniline	66 ± 9	69 ± 3
4-chloro-2-nitrotoluene	73 ± 7	74 ± 10
3,4-dichloroaniline	82 ± 3	85 ± 9
1-chloro-2,4-dinitrobenzene	51 ± 8	56 ± 8
4-chloro-2-nitroaniline	95 ± 7	99 ± 2

Figure 3.2: graphic comparison between % mean recoveries obtained with and without the Na_2SO_4 drying step.

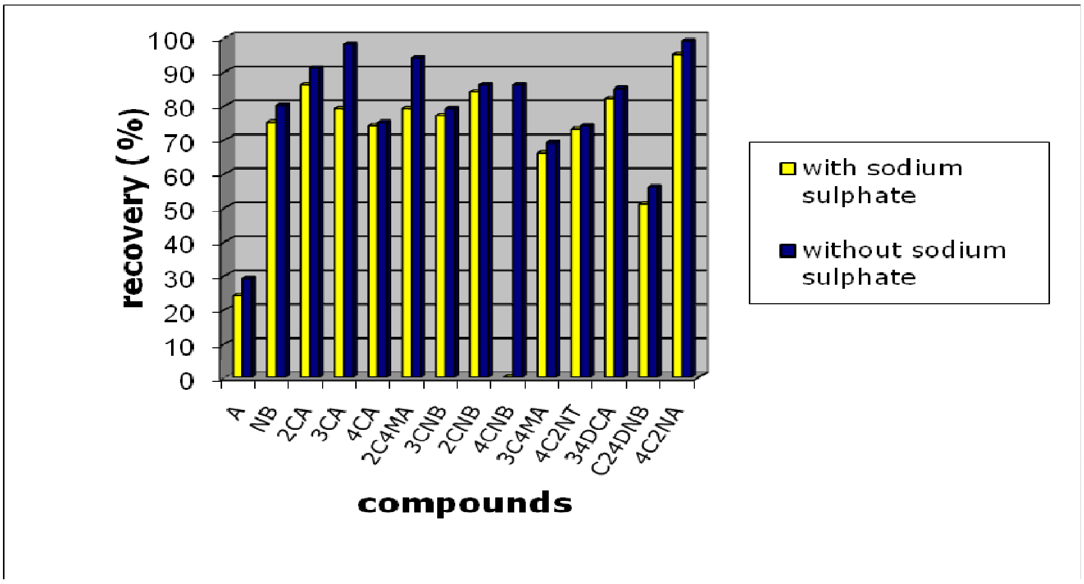


Table 3.5: comparison on recoveries with different amount of elution solvent (n=3).

Analytes	3 mL	6 mL	10 mL	15 mL
aniline	28	**29**	27	33
nitrobenzene	56	**80**	79	78
2-chloroaniline	75	**91**	88	90
3-chloroaniline	72	**98**	98	101
4-chloroaniline	56	**75**	77	75
2-chloro-4-methylaniline	70	**94**	92	89
3-chloronitrobenzene	75	**79**	80	79
2-chloronitrobenzene	82	**86**	87	89
4-chloronitrobenzene	-	**80**	83	-
3-chloro-4-methylaniline	47	**69**	66	67
4-chloro-2-nitrotoluene	73	**74**	75	74
3,4-dichloroaniline	48	**85**	83	85
1-chloro-2,4-dinitrobenzene	15	**56**	62	68
4-chloro-2-nitroaniline	-	**99**	102	-

3.1.1.4 Elution volume adjustment

A final elution volume adjustment was made to test the total extraction of the analytes held on the sorbent. After the extraction of 200 mL spiked Milli-Q water (around 4÷10 μg/L of each compound) buffered at pH=9, the elution with 15 mL, 10 mL, 6 mL and 3 mL of ethylacetate was compared.

Table 3.5 shows that 6 mL of organic solvent was the right amount to extract the total analytes absorbed on the cartridge. The advantage to work with low quantity of eluent is the more rapid time to concentrate, with a Turbovap concentration system or with a gentle nitrogen flow, at the final volume of 0.50 mL. It was also tested that is preferable work in two separate elution steps (3 mL + 3 mL) waiting 2 minute between them to allow a better and total wetting of the dry sorbent with ethylacetate, instead of an unique total elution of 6 mL. The results expressed in the table show the mean recovery of three replicate experiments (n=3) with a RSD range between 0.4 ÷ 11.4.

3.1.2 Validation of the method

A work of validation was necessary in support to the multiresidue developed method before its application on real samples from an environmental monitoring. The validation includes the checking of linearity range of the calibration curves, the precision (through the repeatability), the study of the trueness and recovery, the LOD and LOQ as well possible breakthrough effects. A preliminary calculation of uncertainty was also assessed using empirical equations. The use of empirical equations was supported by the acceptability of repeatability of the method and the verification of compatibility with results of proficiency tests carried out on waters contaminated by organic compounds.

3.1.2.1 Linearity of the calibration curves

With the conditions described above and on Paragraph 2.1 a series of six levels of standard multiresidue solutions were obtained at the linearity range described in table 3.6. The chromatogram is shown in Fig 3.3. In all cases, there was an excellent linear regression ($P<0.008$) except for the 1-chloro-2,4-dinitrobenzene with a $P=0.03$. The chromatogram shows a partial overlapping of 3-chloroaniline and 4-chloroaniline with the instrumental chromatographic conditions chosen, that was avoided with an adequate peak integration helped by Xcalibur® software. Even the overlapping troubles of 2-chloro-4-methyaniline with 3-chloronitrobenzene can be overcome using different SIM condition selected for the quantitation. The isomers 2-

chloronitrobenzene and 4-chloronitrobenzene were totally overlapped with a similar spectrum so with the selected condition it wasn't possible the separation with the column equipped. For this reason, when it was possible, a study of a 4-chloronitrobenzene single solution was considered without the other anilities for validation.

Table 3.6: conditions and linearity range obtained for the final adjust method.

Analytes	Retention time (min)	SIM	instr range µg/L (pg abs)	method range (µg/L)	R^2
aniline-d_5 (int std)	5.35	98	145.45	-	-
aniline	5.37	93	11,42 – 1756.33	0.03 – 4.83	0.9993
nitrobenzene-d_5 (surr)	7.25	82	145.45	0.40	0.9997
nitrobenzene	7.27	77	15.27 – 1392.70	0.04 – 3.83	-
2-chloroaniline	8.10	127	8.14 – 1359.98	0.02 – 3.74	0.9990
3-chloroaniline	9.47	127	19.42 – 2545.41	0.05 – 7.00	0.9999
4-chloroaniline	9.61	127	17.23 – 1603.61	0.05 – 4.41	0.9997
2-chloro-4-methylaniline	10.16	141	10.17 – 2636.32	0.03 – 7.25	0.9996
3-chloronitrobenzene	10.14	111	17.32 – 2196.32	0.05 – 6.04	0.9977
2-chloronitrobenzene	10.45	111	20.73 – 1829.06	0.06 – 5.03	0.9925
4-chloronitrobenzene	10.49	111	16.58 – 1683.61	0.05 – 4.63	0.9941
3-chloro-4-methylaniline	11.58	141	12.57 – 2272.69	0.04 – 6.25	0.9993
4-chloro-2-nitrotoluene	11.81	89	28.52 – 2141.78	0.08 – 5.89	0.9913
3,4-dichloroaniline	14.51	161	7.26 – 2752.68	0.02 – 7.57	0.9995
1-chloro-2,4-dinitrobenzene	17.03	202	90.82 – 3570.85	0.25 – 9.82	0.9709
4-chloro-2-nitroaniline	17.95	172	22.54 – 1567.24	0.06 – 4.31	0.9915

The concentration factor of the described method was 363.6 as 200 mL were concentrated to a final volume of 0.50 mL and after 50 µL of aniline-d_5 (internal standard) addition.

3.1.2.2 Study of trueness and precision

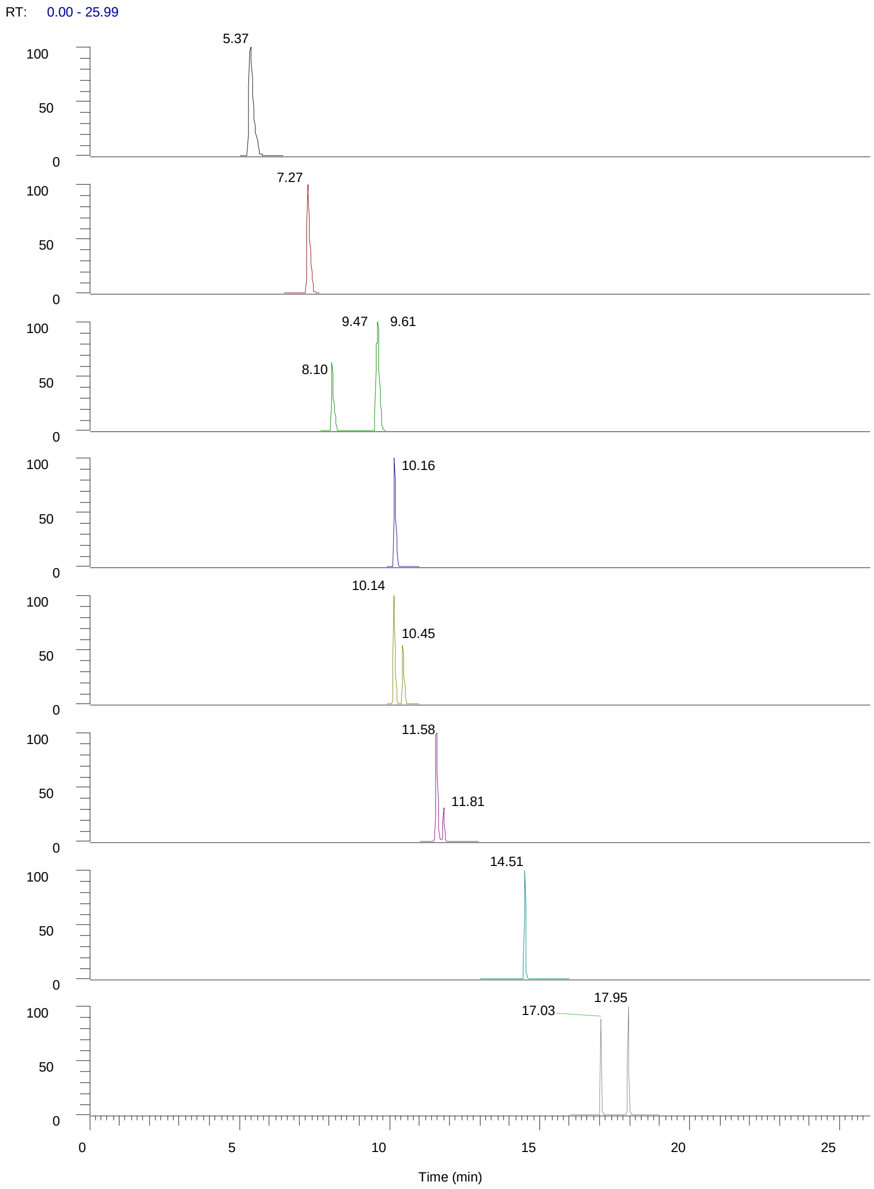
Mean recoveries in different matrixes and at different concentrations levels were assessed to test the trueness of the method. Table 3.7 and 3.8 shows the recoveries after the extraction and analysis of 200 mL of water spiked with different standard levels. The recoveries were evaluated at three different concentration levels for Milli-Q spiked water and for real samples of river waters and wastewater from input of WWTPs. Recoveries results with their standard deviation were compared to the known spiked concentrations taken as reference value. The recoveries were stable at different concentrations tested on the same matrixes except for 4-chloro-2-nitrotoluene with variables values at lower concentrations. The recoveries decreases comparing the tested Milli-Q water with real matrixes, but the GC-MS SIM quantitative determination can select specific ions avoiding most part of the undesirable matrices effects.

Table 3.7: recoveries % on Milli-Q waters at different concentration levels

Analytes [µg/L]	**lev 2* [0.1÷0.3]**	**lev 5# [1÷3]**	**lev 6* [4÷10]**
aniline	19 ± 6	29 ± 1	27 ± 2
nitrobenzene	78 ± 7	80 ± 6	77 ± 8
2-chloroaniline	63 ± 9	91 ± 11	94 ± 3
3-chloroaniline	82 ± 6	98 ± 7	91 ± 2
4-chloroaniline	73 ± 8	75 ± 5	82 ± 5
2-chloro-4-methylaniline	85 ± 5	94 ± 2	92 ± 4
3-chloronitrobenzene	79 ± 6	79 ± 6	83 ± 8
2-chloronitrobenzene	78 ± 5	86 ± 6	81 ± 8
4-chloronitrobenzene	83 ± 8	86 ± 5	85 ± 5
3-chloro-4-methylaniline	64 ± 6	69 ± 3	73 ± 4
4-chloro-2-nitrotoluene	75 ± 9	74 ± 10	78 ± 7
3,4-dichloroaniline	82 ± 7	85 ± 3	89 ± 7
1-chloro-2,4-dinitrobenzene	-	56 ± 8	50 ± 14
4-chloro-2-nitroaniline	70 ± 7	99 ± 2	107 ± 5

*:three replicates; #:six replicates

Fig 3.3: chromatogram of a standard multiresidue solution



Precision, as the degree of correlation between results obtained in an independent test conditions, was estimated in terms of repeatability. Six independent samples of Milli-Q water spiked with the multiresidue working solution (1÷3 μg/L) were extracted with the same procedure conditions (same operator, same instrumentation and same day of analysis) and results are included on Table 3.7.

Table 3.8: recoveries % on river (r) and waste (w) water at different concentration levels (n. replicates =3)

Analytes [µg/L]	lev 2 (r) [0.1÷0.3]	lev 5 (r) [1÷3]	lev 6 (r) [4÷10]	lev 2 2 (w) [0.1÷0.3]	lev 5 (w) [1÷3]	lev 6 (w) [4÷10]
aniline	15 ± 2	31 ± 4	26 ± 1	14 ± 7	28 ± 6	28 ± 4
nitrobenzene	73 ± 3	77 ± 7	75 ± 5	65 ± 3	65 ± 8	68 ± 1
2-chloroaniline	77 ± 1	80 ± 4	89 ± 5	71 ± 6	74 ± 11	75 ± 3
3-chloroaniline	75 ± 9	76 ± 10	83 ± 6	-	66 ± 11	76 ± 4
4-chloroaniline	69 ± 5	62 ± 9	71 ± 5	49 ± 7	54 ± 10	56 ± 11
2-chloro-4-methylaniline	59 ± 11	84 ± 4	89 ± 3	66 ± 3	76 ± 9	71 ± 5
3-chloronitrobenzene	74 ± 13	76 ± 4	100 ± 6	67 ± 5	74 ± 7	69 ± 4
2-chloronitrobenzene	71 ± 9	75 ± 7	77 ± 8	74 ± 8	68 ± 15	69 ± 4
4-chloronitrobenzene	-	-	-	67 ± 7	62 ± 6	-
3-chloro-4-methylaniline	55 ± 7	69 ± 2	72 ± 3	55 ± 4	61 ± 10	63 ± 3
4-chloro-2-nitrotoluene	135 ± 12	72 ± 10	72 ± 3	-	66 ± 8	65 ± 5
3,4-dichloroaniline	71 ± 11	77 ± 3	76 ± 7	72 ± 5	76 ± 7	75 ± 6
1-chloro-2,4-dinitrobenzene	-	56 ± 12	56 ± 13	-	36 ± 10	41 ± 12
4-chloro-2-nitroaniline	64 ± 13	76 ± 10	100 ± 9	57 ± 16	73 ± 6	81 ± 7

3.1.2.3 Breakthrough

Breakthrough volume is the maximum amount of water that can be percolated through a cartridge without any considerable loss of recovery. The eventual breakthrough volumes were tested on tap water spiked obtaining 1÷3 µg/L solutions and results are presented in the table 3.9. After adding 10% of buffer and 2.5% of methanol, 50, 100, 200, 500 and 1000 mL of sample were percolated through the cartridges and the extraction performed in triple replicate (except for 1000 mL volume). No breakthrough was observed for most the analytes, however for aniline low recovery values are observed with the increase of water volume showing a 30-40% decrease in recovery when more than 200 mL are extracted. It is expected that lower breakthrough values will be encountered in more complex environmental samples as WWTPs or industrial effluents. For dirty matrixes SPE efficacy and performance are reduced for the obstruction of the active sites of the sorbent and the consequent increasing of breakthrough troubles and the appearance of more interferents on the final chromatogram. The water volume pre-concentrated is always a compromise between LOD and matrices effects[113].

Table 3.9: recoveries % at different tap water volumes extracted and spiked at 0.6÷1.3 μg/L of each analytes (n=3 replicates except for 1000 mL).

Analytes	50 mL n=3	100 mL n=3	200 mL n=3	500 mL n=3	1000 mL n=1
aniline	58.1	38.9	28.7	25.6	20.1
nitrobenzene	11.8	45.9	79.7	100.8	103.2
2-chloroaniline	18.2	50.0	90.7	91.4	89.6
3-chloroaniline	28.5	79.1	98.4	81.6	91.8
4-chloroaniline	11.8	44.6	75.3	71.3	78.2
2-chloro-4-methylaniline	20.8	55.7	93.9	99.5	99.0
3-chloronitrobenzene	16.7	42.4	78.5	76.0	74.0
2-chloronitrobenzene	19.7	56.1	86.4	95.2	95.4
4-chloronitrobenzene	-	-	86.0	-	-
3-chloro-4-methylaniline	21.6	31.7	68.5	77.7	86.1
4-chloro-2-nitrotoluene	15.3	42.5	74.4	85.0	87.1
3,4-dichloroaniline	21.6	51.1	84.7	80.3	87.3
1-chloro-2,4-dinitrobenzene	11.7	28.7	56.3	108.9	103.9
4-chloro-2-nitroaniline	16.7	51.0	99.1	90.3	96.1

Table 3.10: LODs and LOQs values determined for direct injection of the standard solution and for extraction of milli-Q, river and waste water (n=3 replicates).

	inj. of standard[*]			**milli-Q**[*]	**river**[*]	**waste***
COMPOUND	LOD [μg/L]	[pg]	LOQ [μg/L]	LOQ [μg/L]	LOQ [μg/L]	LOQ [μg/L]
aniline	0.008	[2.78]	0.03	0.19	0.19	0.30
nitrobenzene	0.030	[11.02]	0.04	0.06	0.06	0.15
2-chloroaniline	0.006	[2.15]	0.02	0.06	0.15	0.15
3-chloroaniline	0.012	[4.30]	0.05	0.11	0.11	0.28
4-chloroaniline	0.019	[7.02]	0.04	0.07	0.07	0.17
2-chloro-4-methylaniline	0.011	[4.18]	0.03	0.11	0.11	0.29
3-chloronitrobenzene	0.014	[5.00]	0.04	0.24	0.24	0.48
2-chloronitrobenzene	0.028	[10.00]	0.06	0.20	0.20	0.40
4-chloronitrobenzene	0.034	[12.36]	0.05	0.23	-	0.43
3-chloro-4-methylaniline	0.010	[3.61]	0.03	0.25	0.25	0.41
4-chloro-2-nitrotoluene	0.056	[20.30]	0.07	0.47	0.47	0.93
3,4-dichloroaniline	0.009	[3.20]	0.02	0.03	0.12	0.12
1-chloro-2,4-dinitrobenzene	0.111	[40.26]	0.27	0.78	0.78	3.89
4-chloro-2-nitroaniline	0.047	[17.23]	0.06	0.07	0.17	0.34

[*] in commas the instrumental LOD concentration detected for the direct injection of the standards that correspond at pg absolute injected (Vol inj.=1μL) with a dilution factor of 363.6.

3.1.2.4 LODs and LOQs

Detection and quantification limits differed substantially for the various compounds analysed. LODs were calculated by a comparison of the signal-to-noise ratio (*S/N*) of the lowest detectable concentration to a value of *S/N*=3. At the same time LOQs were calculated considering *S/N*=10 values and it roughly corresponded to the lowest point of the calibration curve.

LOQs values, however, were determined for different matrixes: Milli-Q water that shown very similar values as groundwater, river waters and waste waters from an input effluent of a WWTP.

3.1.3 Environmental samples

To examine the feasibility of the developed method, the application at real water samples was made. The method was tested on real environmental samples collected during the years 2007, 2008 and 2009. 62 surface river water samples were monthly procured from the catchment river basin of Venice lagoon from the Monitoring Plan of ARPAV and from other specific and critical areas of Veneto Region close to industrial districts and interested by I.S.PER.I.A. Project. The further samples collected were: 13 piezometers from three different contaminated sites of the industrial area of Porto Marghera (Venice), 7 leachates from landfills and 24 samples of wastewater from input and output of different WWTPs. The positive (>LOD) samples after the extraction of CAs and CNBs are shown in Table 3.11 and 3.12. The results are in µg/L and not corrected for the recovery values. Into the river waters collected aniline was detected in one monitoring station for the period October 2008 - December 2008 in a range of 0.3÷0.5 µg/L (Table 3.12). The sampling station considered was from Fratta-Gorzone River in an area (Cavarzere, Valcerere Dolfina) located after an highly industrialised zone of Vicenza province. Before and after this three months period other traces of aniline were not detected so it may be possible to refer it at a punctual episode limited at that period of time. It is reasonable to underline that no CAs and CNBs were detected in the river samples at the LOD of the method. The same table represents also the presence of aniline and some chloroanilines on a piezometer from a polluted contaminated site in Porto Marghera (Venice) area detected in November 2008 and on two different leakages of a landfill situated in the Venice province in January 2009. Table 3.12, instead, shows the positive results of 24 samples of wastewater from six different WWTP. Presence of aniline was detected in five samples around a value of 1 µg/L except for the input of the Montebello WWTP were an amount of 25.68 µg/L was detected and where a dilution of the sample was necessary to determine it in the linearity range of the validated method. It is also clear that the treatment plant is efficient to hold back the amount of aniline of the input effluent. Also 2-chloroaniline, 4-chloroaniline, 3-chloro-4-methylaniline, 3,4-dichloroaniline and 4-chloro-2-nitrotoluene were detected in levels >LODs with a maximum value of 6.56 µg/L for 3-chloro-4-methylaniline.

Table 3.11: concentrations (µg/L) of analytes detected in real environmental samples (values not corrected by the recoveries).

Fratta-Gorzone River	June 07 – Sept 08	Oct 08	Nov 08	Dec 08	Jan 09 – May 09
aniline	<LOQ	0.44	0.36	0.34	<LOQ

P. Marghera piezometer	Nov 08
aniline	0.28
3-chloroaniline	0.23
4-chloroaniline	0.44
4-chloro-2-nitrotoluene	<LOQ
3,4-dichloroaniline	0.18

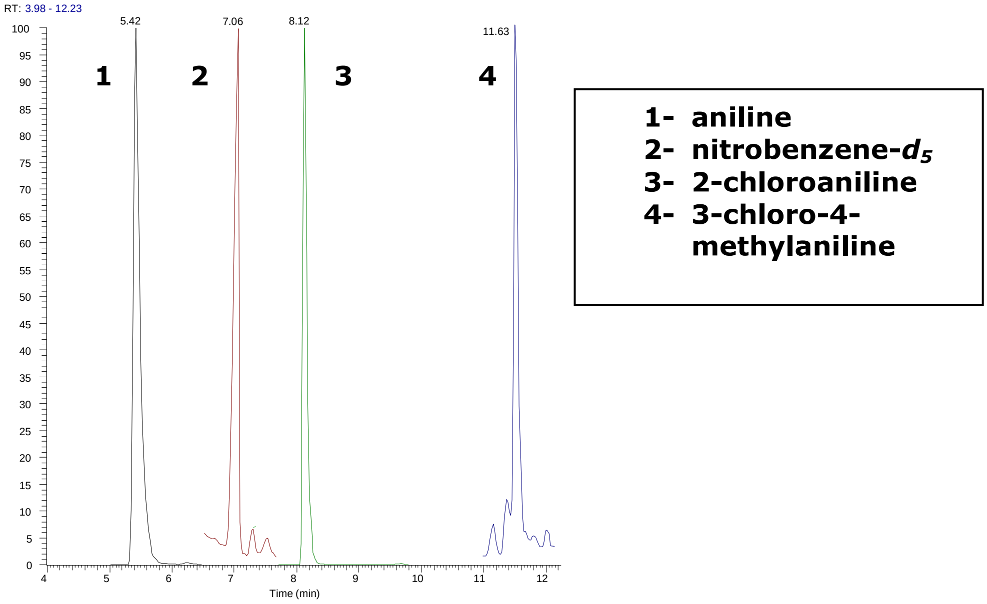
Leachates from landfill	Jan 09 1	Jan 09 2
aniline	2.64	1.24
4-chloroaniline	0.41	0.73

Table 3.12: concentrations (µg/L) of analytes detected in samples from WWTP.

COMPOUNDS	out PG 1	in TR 1	in TR 2	out TR 1	out TR 2	in MB 1	out MB 1	out MB 2
aniline	0.40	1.01	0.96	0.30	0.35	25.68	0.72	0.65
nitrobenzene						<LOQ		
2-chloroaniline		0.21	0.19	<LOQ	<LOQ	0.32	<LOQ	<LOQ
4-chloroaniline		<LOQ	<LOQ			<LOQ		
3-chloro-4-methylaniline		0.43	0.47	<LOQ	<LOQ	6.56	<LOQ	<LOQ
4-chloro-2-nitrotoluene						<LOQ		
3,4-dichloroaniline						<LOQ		

Values not corrected for the recoveries, samples collected between January and February 2009 from input and output of: PG - Portogruaro (Venice) WWTP, TR - Trissino (Vicenza) WWTP, MB - Montebello (Vicenza) WWTP. 1 and 2 refer to different extraction of the same sample.

Figure 3.4: portion of the chromatogram of a sample collected from the input of Trissino WWTP (feb 2009)



3.1.4. Uncertainty estimation.

Given the fact that the true value of the measurement is and remains unknown, the measurement process provides only an estimation of the true value. Therefore the analytical result is not complete, neither comparable with a reference value, if it is not related with an estimation of measurement uncertainty. The uncertainty, as the parameter that characterizes the dispersion of the value, can be estimated in several methods for an internal validated method. The metrological approach is the more strictness and considers all the contributors that affect the uncertainty. However on a chemical measure the random contributions are prevalent than systematic errors and the approach is difficult to apply due to different weight that each contribution give to the measure. Simplified approaches, like holistic or based on empirical equations, find more application on chemical and microbiological analysis if the measurement system is under QA/QC requirements. These simplified approaches included: measures on CRM, comparison with alternative methods and participation to collaborative trials. If no one of these approaches is feasible, because of lack of CRM or proficiency test with the considered substances, the empirical formulae could be used with the proper precautions. Horwitz relation approach, with modifications proposed by Thomson for environmental analysis, is based on the supposition that the random errors give the most evident contribution and are inversely proportional to concentration

(EURACHEM/CITAC). Empirical formulae relate analyte's concentration with the RSD and are argued after the study of several collaborative trials. However the approach is an useful tool for a first estimation of uncertainty in a development of a method especially if quality rugged data are not yet available. The Horwitz equation modified from Thompson for trace chemical environmental analysis (values under 120 μg/L) is:

$$\sigma_R = 0.22 \cdot C \qquad (1)$$

with σ_R as reproducibility standard deviation and C concentration of the analyte.
σ_R can be used as uncertainty values with a cover factor of 2 giving the extended uncertainty of:

$$U(y) = 2 \cdot \sigma_R \qquad (2)$$

The applicability of the equation requires that the measures are under QA/QC control with the following condition (HORRAT – Horwitz Ratio):

$$1/2 \;\leq\; \sigma_r / \sigma_R \;\leq\; 2/3 \qquad (3)$$

using σ_r as experimental standard deviation of repeatability. If (3) is confirmed for all analytes, then (2) can be used as estimation of the uncertainty for the applied analytical method. Besides, if the method repeatability is better than the lower extreme of the equation (3), it is necessary to justify the laboratory decision to use empirical formulae on the calculation (i.e. the use of efficient extraction techniques or modern analytical instrumentation that enhances the reproducibility of the method).

Using repeatability values of level 5 (6 replicates) shown on Table 3.7, the (2) and (3) relations were applied obtaining the uncertainty values shown on follow:

aniline	1.52	±	0.67
nitrobenzene	1.22	±	0.54
2-chloroaniline	1.18	±	0.52
3-chloroaniline	2.22	±	0.98
4-chloroaniline	1.40	±	0.62
2-chloro-4-methylaniline	2.30	±	1.01
3-chloronitrobenzene	1.92	±	0.84
2-chloronitrobenzene	1.60	±	0.70
4-chloronitrobenzene	2.10	±	0.92

3-chloro-4-methylaniline	1.98	±	0.87
4-chloro-2-nitrotoluene	1.86	±	0.82
3,4-dichloroaniline	2.40	±	1.06
1-chloro-2,4-dinitrobenzene	3.12	±	1.37
4-chloro-2-nitroaniline	1.37	±	0.60

In support to the use of empirical formulae, a comparison between results of different Unichim proficiency tests to which ARPAV has partecipated over the years 2007-2008 was made. Precision data explained on the Unichim final reports are useful for the large number of laboratories that partecipate to the exercises and for the σ_R and CV% results divided for different extraction/analytical methods. The analytes of the proficiency tests considered were: haloforms and related compounds in drinkable water and wastewater (3÷7 µg/L, 2007), benzene in drinkable water (1 µg/L, 2008) and chlorinated pesticides in drinkable water (80-110 µg/L, 2008). The reproducibility standard deviations of the proficiency tests were compared with the empirical standard deviations calculated with Thompson approach obtaining agreeable results.

3.2. Benzotrifluoride compounds traceability

This paragraph of the *Results and Discussion* part considers more in details the traceability of benzotrifluoride (BTFs) derivatives compounds on environmental waters. First of all the development and validation of methods for the analytical determination of two classes of BTFs was considered. The BTFs considered using two different analytical methods were the volatile BTFs and the non-volatile BTFs fractions. The hydroxy-benzotrifluoride derivatives (phenols BTFs) were also evaluated but at this stage only in a qualitative way using sophisticated analytical technology as HPLC-HRTOF/MS. After the validation work, analyses from real environmental samples were carried out considering a specific study case: a past groundwater contamination event occurred during the seventies in the Vicenza Province. Therefore the historical situation was weighted with a deep investigation of the data available about the episode. These data were then elaborated. In specific the dataset available were assessed with a reconstruction of the spatial and temporal modification of the plume in the period subsequent the episode. The past situation was finally compared to the actual situation through a first monitoring of the area and the determination of possible BTFs residues still detectable in trace.

3.2.1 Development of methods for benzotrifluorides traceability

3.2.1.1 Determination of volatile fraction of benzotrifluorides

The method developed with the materials used for the extraction and determination of the volatile BTFs fraction is described on the *Materials and Methods* chapter. For volatile and semivolatile compounds the Dynamic Head Space method was considered using a Purge & Trap system for extraction and concentration. The P&T was connected with a GC equipped with a single quadrupole mass detector system. With the conditions described on Paragraph 2.2, the linearity of the calibration curves were readily studied and demonstrated on a broad range of concentration, from 0.005-0.006 μg/L to 100-200 μg/L, greater than the working range of table 2.4. The LOLs of BTF, 4BTF, 34BTF and 24BTF compounds are described on table 3.13 considering the area values of the peaks after normalization with the internal standard 1,2,3-trifluorobenzene.

Table 3.13: linearity range, LOD and LOQ of the volatile BTFs considered

Compounds	low LOL [μg/L]	high LOL [μg/L]	LOD [μg/L]	LOQ [μg/L]
BTF	0.005	158.90	0.002	0.005
4CBTF	0.005	164.15	0.002	0.005
34BTF	0.01	190.70	0.004	0.01
24BTF	0.01	177.48	0.005	0.01

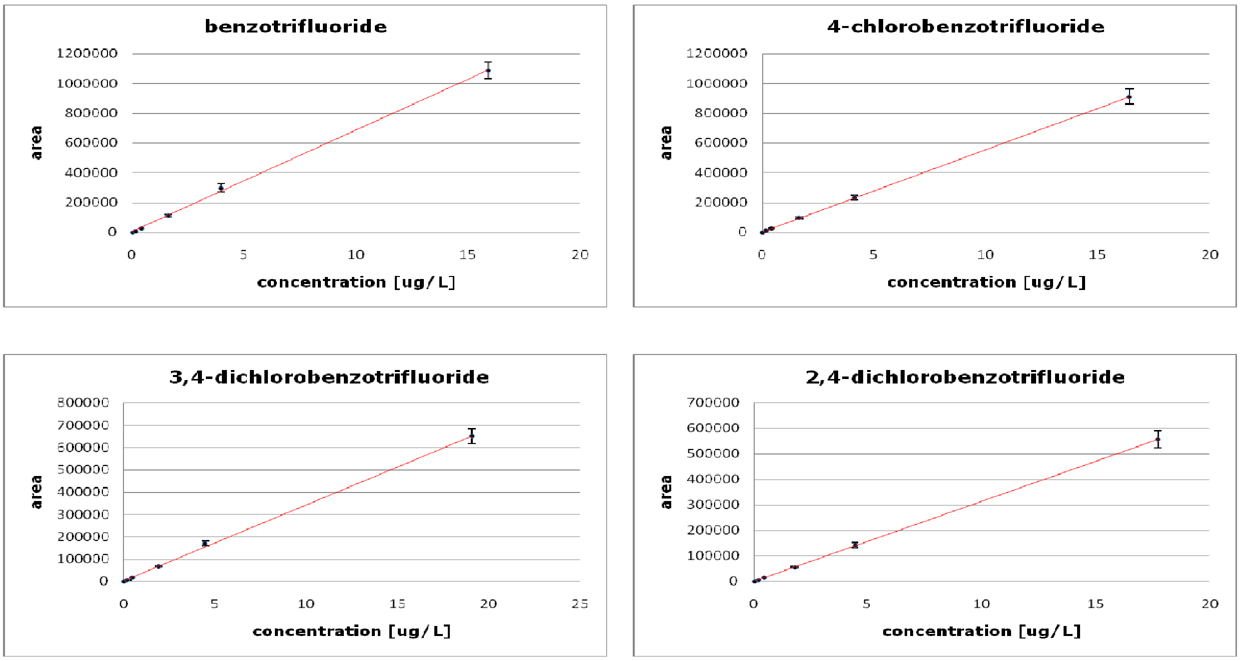
The LODs were calculated as three times the ratio between the lower signal compared with the noise calculated for a blank sample extracted (*S/N* =3). The LOQs were as the same calculated with the ratio *S/N* =10. The LOQs were different from the low calibration standard points, however because shows comparable values, they were considered as the lower points of the linearity curve (LOL). Table 3.13 shows also LOD and LOQ values.

Table 3.14: values of LOQ and LOD calculated as *S/N* ratio.

Compounds	linearity equation	R^2
BTF	y = 71649x-331	0.997
4CBTF	y = 60455x-265	0.999
34BTF	y = 35506x-267	0.998
24BTF	y = 31195x-167	0.999

For the quantification of environmental samples, six levels (see table 2.4) were considered because the wide linearity range was not useful for trace determination. Figure 3.5 shows the calibration curves used for the determination of volatile BTFs while table 3.14 the linearity equations calculated on three replicates for each level. From the data obtained the RSDs calculated directly on the area values ranged from 8÷16 % of level 1 (0.016÷0.018 μg/L) to 4÷6 % of level 6 (15.89÷19.07 μg/L).

Figure 3.5: calibration curves for determination of volatile BTFs.



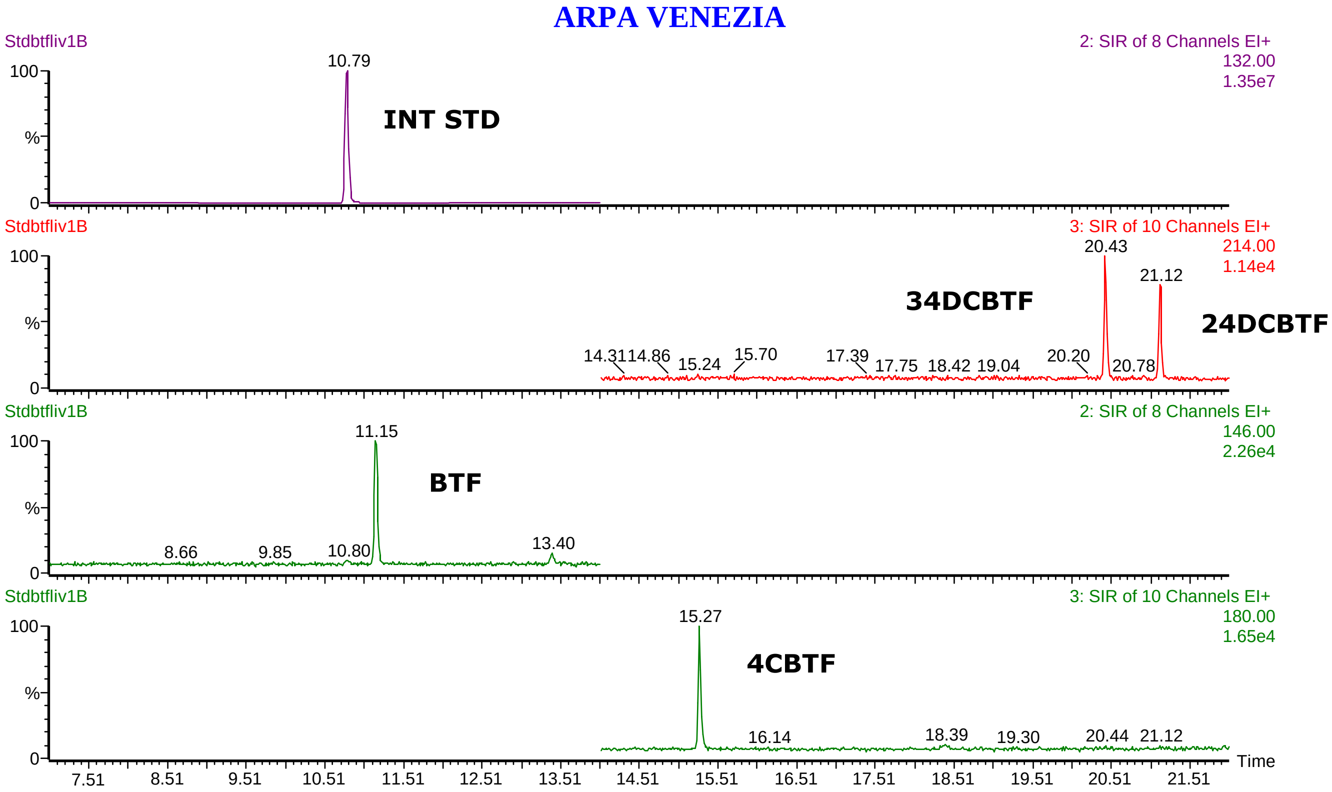
benzotrifluoride

4-chlorobenzotrifluoride

3,4-dichlorobenzotrifluoride

2,4-dichlorobenzotrifluoride

Figure 3.6: chromatogram of standard level 1 (≈0.02 μg/L) of volatile BTFs (1,2,3-trifluorobenzene = internal standard).



The calculation of recoveries was not-essential because of the complete treatment and extraction of all levels of the multiresidue standard solutions as sample. With this method all the concentrations from the standard solutions were directly compared with the real samples trough the calibration curves. Furthermore the use of 1,2,3-trifluorobenzene as internal standard, with a final concentration of 5.12 μg/L, gave back a good stability and ruggedness to the method also at very low levels of concentration on the environmental samples (figure 3.6).

3.2.1.2 Determination of non-volatile fraction of benzotrifluorides.

The non-volatile fractions of BTFs compounds were extracted with the SPE method at pH=9. The method applied was the same developed for CAs and CNBs using the Thermo GC/MS with quadrupole detector as instrumentation. The method was described in details at paragraph 2.1.4 and 2.2.3.1.

The linearity range was calculated on the five standard levels reported on table 3.15. Two multiresidue solutions in methanol were prepared from the stock primary solutions previously described on table 2.3. From these two solutions (level 3 and level 5) further three levels were prepared and stored at 4°C for a maximum period of 3 months.

3,4-dichlorobenzotrifluoride and 2,4-dichlorobenzotrifluoride, being semivolatile compounds, were also tested with the method for the volatile BTFs and the results compared with the SPE extractions.

The linearity resulted on the five standard levels and the curves were explained on table 3.16.

Table 3.15: level of standard prepared for the non-volatile BTF determination.

Compounds	lev 1 [μg/L]	lev 2 [μg/L]	lev 3 [μg/L]	lev 4 [μg/L]	lev 5 [μg/L]
34DCBTF	15.00	74.98	149.96	749.81	1500.00
24DCBTF	15.17	75.85	151.70	758.52	1520.00
3NBTF	14.49	72.46	144.92	724.59	1450.00
3ABTF	13.69	68.46	136.92	684.59	1370.00
3N4CBTF	15.73	78.67	157.33	786.67	1570.00
3A4CBTF	14.31	71.54	143.08	715.40	1430.00
35DN4CBTF	-	60.98	121.97	609.84	1220.00

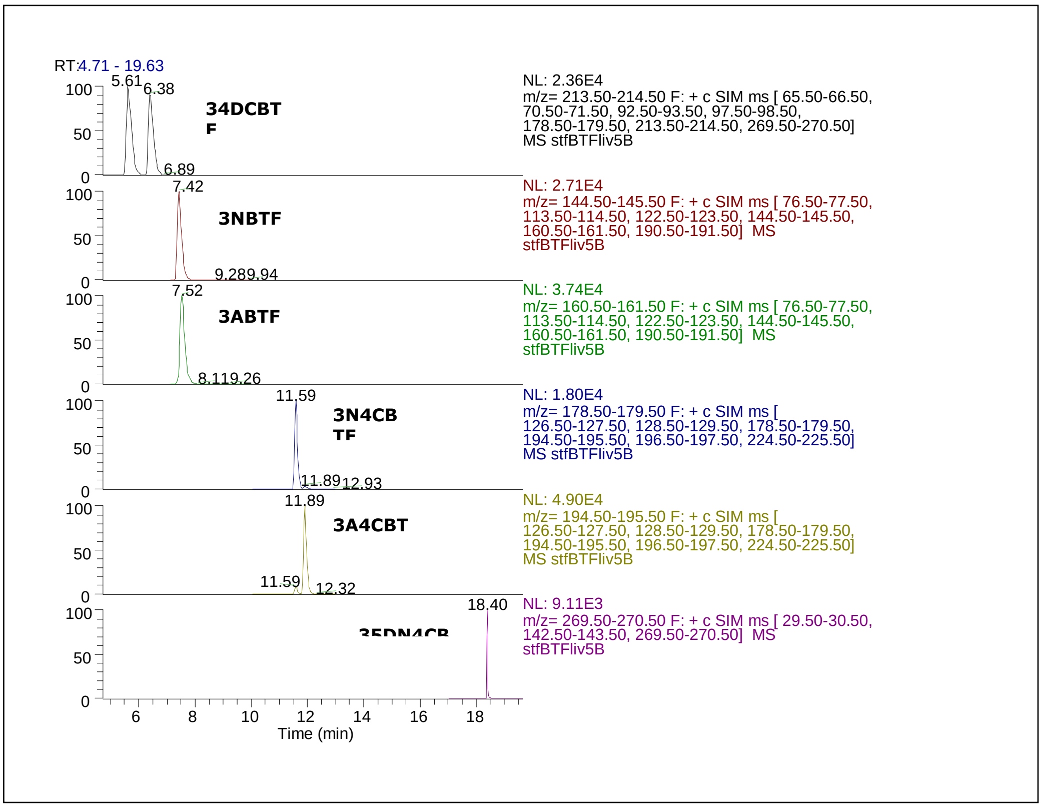
Table 3.16: conditions and linearity range obtained for non-volatile BTF method.

Compounds	**Retention time (min)**	**SIM**	**instr range μg/L (pg abs)**	**method range (μg/L)**	**R^2**
aniline-d_5 (int std)	5.38	98	145.45	-	-
34DCBTF	5.64	214	15-1500	0.03-4.13	0.9980
24DCBTF	6.40	214	15.17-1520	0.03-4.18	0.9982
nitrobenzene-d_5 (surr)	7.10	82	145.45	0.40	0.9997
3NBTF	7.42	145	14.49-1450	0.03-3.99	0.9996
3ABTF	7.52	161	13.69-1370	0.03-3.77	0.9999
3N4CBTF	11.59	179	15.73-1570	0.03-4.31	0.9991
3A4CBTF	11.92	195	14.31-1430	0.03-3.93	0.9997
35DN4CBTF	18.40	270	12.20-1220	0.02-3.36	0.9983

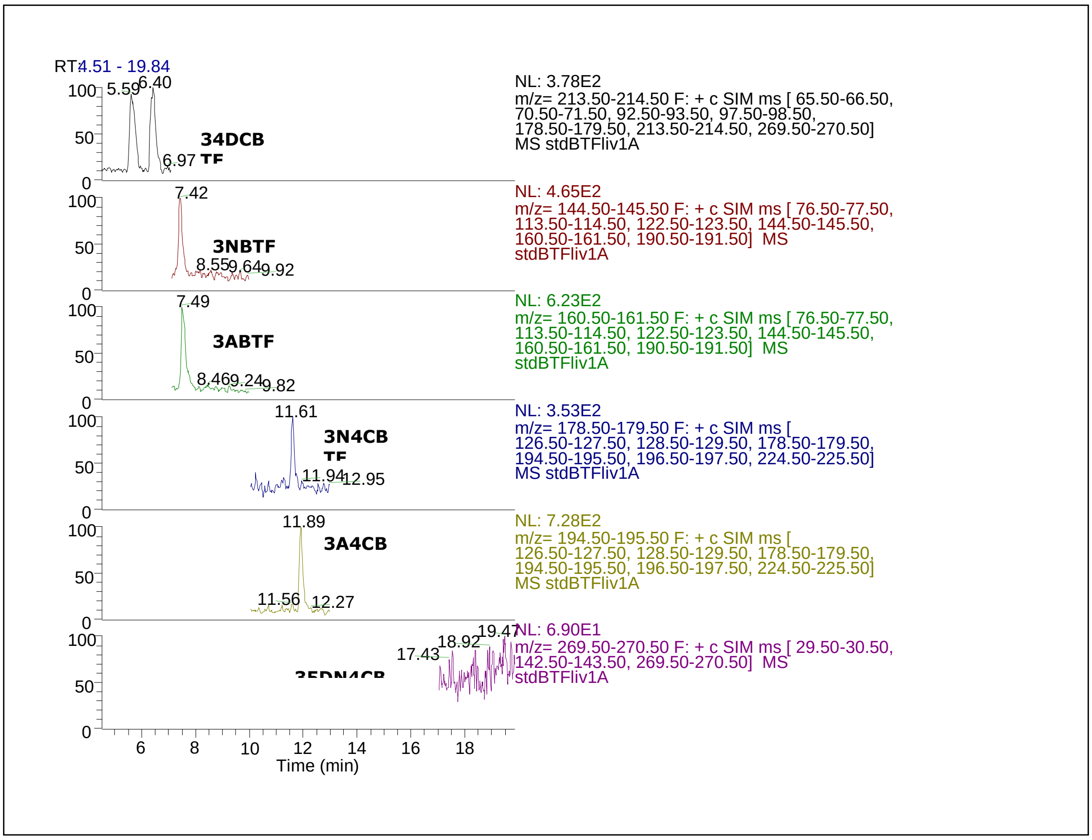
The overlapping of 3NBTF and 3ABTF peaks and of 3N4CBTF and 3A4CBTF peaks on the chromatograms were overcome with the different masses considered in the SIM modality used for the quantitation of areas' peaks. For each level, a triplicate injection was made for the calculation of the RSDs. The concentration factor of the described method was 363.6 as 200 mL were concentrated to a final volume of 500 μL and then added 50 μL of aniline-d_5 (internal standard). Nitrobenzene-d_5, as surrogate, was used for the recovery control of the entire process (77 % ± 3 %). The concentrations of standard levels, in the final calculation on the real samples, already considered the dilution due to the addiction of the internal standard as programmed in the processing method created with the Xcalibur® Thermo software. Figure 3.7, instead, shows the chromatograms obtained of standard level 5 and standard level 1.

Figure 3.7: chromatogram of standard level solutions (lev 5: ≈1500μg/L; lev 1: ≈15 μg/L) of non-volatile BTF.

LEVEL 5



LEVEL 1



Detection and quantification limits don’t differ a lot between the various compounds analysed (table 3.17). As the same for the volatile BTFs method, LODs were calculated by a comparison of the signal-to-noise ratio (*S/N* = 3) of the lowest detectable concentration to the average value of noise, while LOQs were calculated considering *S/N*=10 values. LOQs roughly corresponded to the lowest point of the calibration curves. LOQs values, however, were determined in milli-Q water that, as previously clarified, showed similar values, after the extraction process, compared with groundwater.

Table 3.17: values of LOQ and LOD calculated as *S/N* ratio.

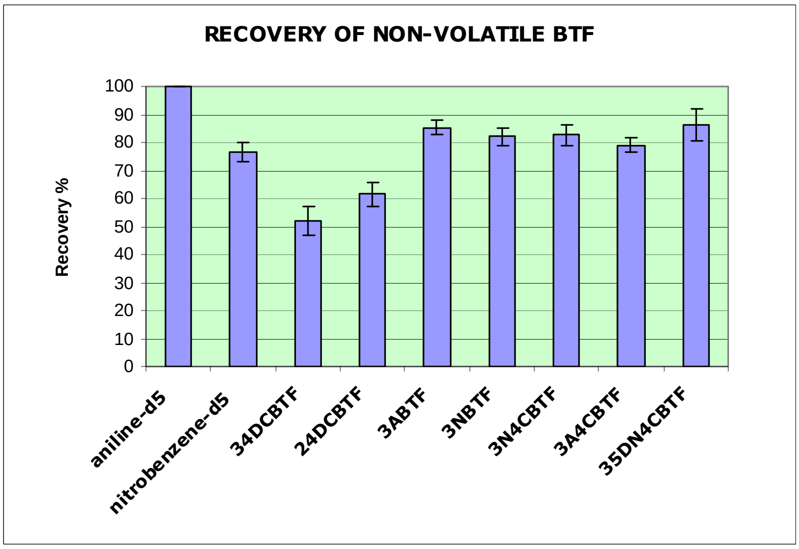
Compounds	LOD	low LOL [µg/L]	LOQ
34DCBTF	0.01	0.03	0.03
24DCBTF	0.01	0.03	0.03
3NBTF	0.01	0.02	0.02
3ABTF	0.01	0.02	0.02
3N4CBTF	0.02	0.03	0.03
3A4CBTF	0.01	0.02	0.02
35DN4CBTF	0.07	0.1	0.1

The trueness was estimated with the recoveries determination. The recoveries were calculated spiking with standard level 2 (≈ 0.2 µg/L final concentration) 200 mL of Milli-Q water extracted in triplicate. The results of the extractions are listed on the following table 3.18 and on figures 3.8.

Table 3.18: recovery extraction of 200 mL milli-Q water spiked with lev 2 (0.2 µg/L)

n=3	**1**	**2**	**3**	**Mean REC**	**SD**
aniline-*d5*	100,0	100,0	100,0	**100**	0
nitrobenzene-d5	75,7	73,9	80,6	**77**	3
34DCBTF	57,9	50,3	47,7	**52**	5
24DCBTF	65,4	62,1	57,0	**61**	4
3ABTF	84,8	82,9	87,9	**85**	3
3NBTF	85,0	82,6	78,4	**82**	3
3N4CBTF	85,6	78,5	83,7	**83**	4
3A4CBTF	80,0	76,0	81,4	**79**	3
35DN4CBTF	87,4	80,1	91,7	**86**	6

Figure 3.8: chart representation of the recoveries on non-volatile BTFs extractions (n=3).



The recoveries range from 77±3 to 86±6 for the non-volatile BTFs, while recoveries of 34DCBTF and 24DCBTF were around 50-60 %. For these reasons the method for volatile BTFs was also tested for these compounds obtaining better results. Being these two compounds more volatile, they were also well extractable with the P&T method.

3.2.2 The study case: a groundwater pollution episode

3.2.2.1 The past situation

The 29 August 1977 the presence of an unidentified pollutant in water from some wells of Altavilla, Sovizzo and Montecchio Maggiore municipalities was notified at Veneto Regional Council level. The interested area is located in the Vicenza Province, at the north part of Veneto Region.

The chemical factory (ex-Rimar, from 1996 aquired by Miteni) involved on the pollution episode was specialized in the production of fine organic chemicals, majority of which were fluorinated. Most part of these chemicals were used as intermediate for the production of agrochemicals and pesticides, pharmaceuticals and drugs, colorants and dyes, additives for rubber and polymeric products. The main processes used included electrofluorination, chlorination, fluorination and nitration and in the seventies the company employed about 500 local workers[114]. The three most important productions of the industry were:

- intermediates for crop protection chemicals;
- intermediates for pharmaceuticals products;
- perfluorinated derivatives.

It was estimated that the annual production of those intermediates was risen from 200 t/year in 1973 to 2500 t/year in 1980.

The factory was located on the lower part of Agno-Gua River Basin in the south part of Trissino in Vicenza province. The Poscola Stream, a small tributary of Agno River flows along the factory perimeter and at that time received the process waste waters after their purification. The streams flows into Guà River, a tributary of Gorzone River, which flows finally in the Adriatic Sea (figure 3.9).

From the data of that period[114], the liquid effluents from the factory included two major fractions:

1) treatment wastewaters from the nitration process which, until 1976, were partially neutralized and only in a second period discharged. Their compositions is shown on table 3.19;
2) waste acids from the nitration processes were formerly diluted and neutralized with lime. The resulting sludge was collected in a trench sited inside the perimeter of the factory, while the liquid fraction was discharged in the same manner as the treatment waters. The waste acids contained approximately 1-2% of organic compounds of which 80% was 4-chloro-3,5-dinitrobenzoic acid and the remain 20% formed by 4-chloro-3-nitrobenzotrifluoride and 4-chloro-3,5-dinitrobenzotrifluoride.

Figure 3.9: the catchment area framing from Poscola Stream to Adriatic Sea. [from: Progetto Giada - "Surface water" report - Vicenza Province/ARPAV (2006 update)]

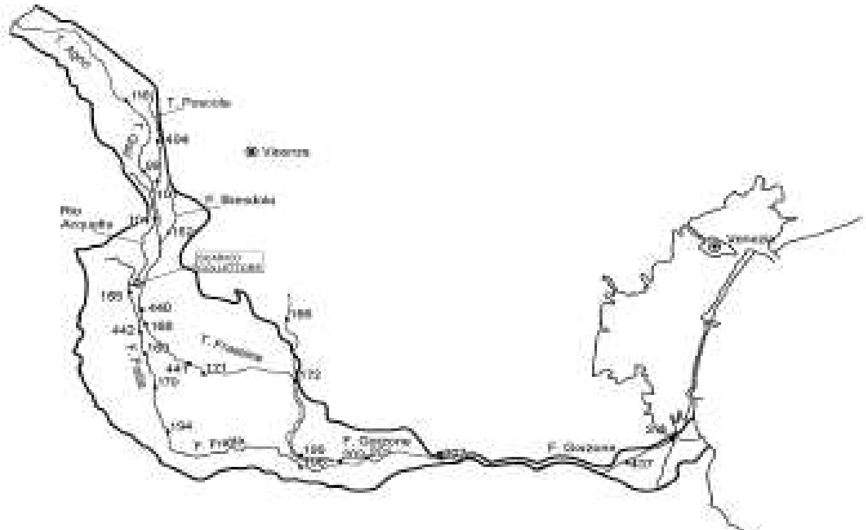


Table 3.19: approximately composition of treatment waters from nitration process of fluorinated compounds.

Pollutant	Concentration discharged [g/L]
Fluorides	≈ 0.1 – 0.2
Sulfates	≈ 0.5 – 1.0
Nitrates	≈ 1.0 – 5.0
Chlorides	≈ 1.0 – 5.0
4-chlorobenzotrifluoride + 3-nitrobenzotrifluoride derivatives + 3,5-dinitrobenzotrifluoride derivatives	≈ 0.1 - 0.2
4-hydroxy-3,5-dinitrobenzotrifluoride + 4-chloro-3,5-dinitrobenzoic acid	≈ 0.5 – 1.5

The pollution episode dated back at the summer of 1977, when it was realized that water from some wells had been polluted by halogenated aromatics compounds, subsequently identified as: 4-chlorobenzotrifluoride, 4-chloro-3-nitro-benzotrifluoride, 4-chloro-3,5-dinitrobenzotrifluoride, trichloroethylene and tetrachloroethyene.
People lived in the area remember at that time the weakly yellow colour of waters from some wells and an aromatic-like smell in the air of surrounding area. When pollution was recognized and the source of pollutants well localized, local authorities immediately prohibited the use of groundwater for drinking purpose and new sources of water supply for the aqueducts were arranged. It is important, instead, to remark that no restrictions placed on the use of contaminated water for other purpose such as irrigation, industrial uses and consumption by farm animals.

Simultaneously, for economic reasons, at the end of August 1977 the production of 4-chloro-3,5-dinitrobenzotrifluoride was ceased and authorities decided to stop its production until conditions that effective treatment plants were constructed, to ensure proper purification of liquid effluents. It was asked to the factory to take actions to contain the damage and to prevent further contaminations both in normal and abnormal process conditions. In the following years (1978-1980) the necessary improvements were introduced in the factory. In particular, all the plants and surrounding areas had been enclosed in an impermeable perimeter with raised edges so possible spills and associated washing water were drained in a collection tank and pumped in a new liquid effluent incineration plant. The incineration plant operated at

1000°C and received also the liquid fraction from the acid neutralization process as well the nitration treatment waters. Besides an activated carbon filtration system was installed to treat the residual waters from scrubbing of exhausted gases after neutralization. It was tested that, in the worst case, concentrations of chloro-nitrobenzotrifluoride compounds not filtered were below 5 µg/L. Finally lime from neutralization plant, in form of 80% as calcium fluoride but with 0.2-0.5% of organic compounds (mainly 4-chlorobenzotrifluoride), were, after the improvements, collected in a special impermeable tank inside the factory[114].

For the following two years, factory workers had been given haematological and clinical examinations based on six-months periods and no significant effects were observed. Furthermore epidemiological studies were made on random samples of population lived in the polluted area. The studies showed that only a limited portion of the population had previously used well-water for drinking purpose and there was no toxic effect on them. For these reasons, and in view of the fact that odour and colour of water were unpleasant when 4-chloro-3-nitro-benzotrifluoride was present in concentration up to 50 µg/L, the studies concluded that the risk for population from drinking contaminated water, before its use was forbidden, was very low.

For a monitoring of the polluted area outside the factory perimeter, a network of sampling points was established based on hydrogeological parameters and conditions. After the episode, analysis had been carried out on samples from many wells and on streams and rivers of the area in order to determine the distribution and the concentration of pollutants and to understand more clearly the nature of the impact on the surface water and groundwater. Quantitative analysis had been restricted for the entire area to 4-chloro-3-nitro-benzotrifluoride considered, at that time, to be the pollutant present in the highest concentration (i.e. from 5 to 1000 µg/L). The complete data set of the analysis on 4-chloro-3-nitro-benzotrifluoride in the wells was published by IRSEV[107] in June 1979 and is repurposed in ANNEX II. The IRSEV report described the geological and chemical studies conducted in the years 1977-1979 by a technical committee of experts nominated by Region Veneto. Although an approximate spatial distribution to define the polluted area was made, a real temporal time-trend of the pollutants distribution was not considered in comparison with different zones of the polluted area during the period 1978-1979 or rather the time period of the first monitoring plan.

The study described the 13 months campaigns conducted between February 1978 and February 1979 with measures of flows and concentrations of 4-chloro-3-nitro-benzotrifluoride (3N4CBTF). The total pollutant load was calculated as well as the multiplication between flow and concentration of the contaminant measured. For surface waters only few monitoring stations were chosen. Three selected station were on Poscola Stream, one in the northern part of the factory involved, a second one in the southern and the third before the inflow of Poscola Stream into Guà River. There was no contamination on the first station while it was not relevant in the other two. Four stations, instead, were decided for the monitoring of Retrone River. The Retrone River area considered is sited in the east part of the Agno-Guà Valley and apparently there are no connections with the interested zone. It is a resurgences zone where 4-chloro-3-nitro-benzotrifluoride was determined in considerable amount for reasons that will be explain later.

For groundwater more than 80 stations were chosen after the study of the morphology of the aquifers. IRSEV report described that there was a N-S direction distribution of the pollutants for the first part close to the point source. The flow followed two main directions: Agno Rivers underground axis with groundwater that run in direction of the multi-aquifer system of Lonigo Almisano and Poscola Stream underground axis which, in the in the area of Canova - Alte Ceccato, change direction to E-NE. This flexion is the result of an high piezometric level in south of Alte Ceccato and the critic threshold is determinant for an inversion of groundwater flow direction with a semi-circular propagation in the area of Creazzo, Sovizzo and Altavilla municipalities where the Retrone River resurgences are present. Chemical pollution data indeed shown clearly that no contamination was detectable in the area of Brendola-Montebello but there was propagation through Montecchio Maggiore detected especially in Sovizzo and Creazzo wells.

The thickness of the alluvial deposit was variable from 120 m under Trissino until 320 m in correspondence of Guà River, while less thickness was checked in the Retrone River basin (from 35 m close to Alte Ceccato until 120m in Sovizzo-Olmo area). It was composed, as already mentioned in paragraph 2.4, by porous material as pebbles, gravel and sand. The hydraulic gradient measured in November-December 1977 was about 6.5‰ in Agno-Guà valley at Montecchio Maggiore along Poscola Stream axes and decreased to 1‰ after to enter in Sovizzo-Altavilla-Creazzo plain. It is important to notice that during the period of sampling and the studies, the general geometry of the groundwater flow didn’t change although the piezometric level oscillation was great in some wells. It means that the drainage divides maintained their own position. The maximum high piezometric level was measured in March-April while a decrease from May to a minimum in December was recorded.

During that monitoring campaign, surface waters and groundwater from sampling stations were collected in 1L glasses bottle and stored in fridge before the analysis. The liquid-liquid technique was used for extraction of 50-500 mL of sample with 5-1 mL of *n*-hexane as solvent. The different volumes chosen depended from the average concentration of contaminant, previous tested on screening samples from different areas. The samples were extracted after the addition of an acid buffer to avoid the precipitation of calcium and magnesium salts. The organic part was concentrated and analysed by GC with Electronic Capture Detector (ECD). With the analytical techniques of that period, a packed column was used with the GC system because of capillary columns weren’t yet on commerce. Anyway an LOD of 0.1 μg/L for 4-chloro-3-nitro-benzotrifluoride in groundwater was possible to reach. All the dataset is repurposed on ANNEX II and elaborated in the next paragraphs.

The following groundwater monitoring campaigns, from 1986 to 2003, considered marginally the nitro-halogenated compounds. During the second trance of analysis only in 1986 and 1987 campaigns some nitro-halogenated compounds were determined as explained on table 3.20. It is clear that a no deep investigation was made with only a few number of stations controlled, most of them in a qualitative way (attendance or non-attendance of benzotrifluoride compounds in the sample).
Only for two stations in Montecchio Maggiore, stations n. 96 (via Lombardi, 50 m deep) and n. 128 (viale Europa, 52 m deep) some quantitative data are available.
The concentrations detected are shown below on table 3.21.
More data are available with the last campaign (Project Giada) with 12 stations analyzed during October 2003 and quantitative analysis on: benzotrifluoride, 4-chlorobenzotrifluoride, 3-nitrobenzotrifluoride, 4-chloro-3-nitrobenzotrifluoride and concentration given as total nitro-halogenated benzotrifluoride (table 3.22). The method used was the liquid-liquid extraction with *n*-hexane on a 250 mL volume sample and reaching LODs of 0.1 μg/L. Complete dataset with other parameter analysis is shown on ANNEX III and published with gently permission of Giada Consortia.

Table 3.20: summary of the samples collected during the 1986-1987 campaigns for the determination of BTF.

		stations	samples
1986	total	**12**	**13**
	qualitative on total nitro-halogenated derivatives	9	9
	qualitative on 3-nitrobenzotrifluoride	1	1
	quantitative on 3-nitrobenzotrifluoride	2	3
	qualitative on 4-chloro-3-nitrobenzotrifluoride	1	1
	quantitative on 4-chloro-3-nitrobenzotrifluoride	2	3
1987	total	**4**	**5**
	qualitative on nitro-halogenated derivatives	2	2
	qualitative on 3-nitrobenzotrifluoride	1	2
	quantitative on 3-nitrobenzotrifluoride	0	0
	qualitative on 4-chloro-3-nitrobenzotrifluoride	0	0
	quantitative on 4-chloro-3-nitrobenzotrifluoride	2	3

Table 3.21:concentrations of 3NBTF and 4C3NBTF detected on samples during the campaigns in the years 1986 and 1987.

data	# station	3NBTF [μg/L]	4C3NBTF [μg/L]
09/06/86	96	2.20	0.50
09/06/86	128	1.70	0.25
25/08/86	128	2.00	0.40
23/02/87	96	2.00	qual
19/03/87	96	2.00	qual
18/09/87	128	1.60	-

3.2.2.2 Elaboration of the historical trend

A first comparison between the different campaigns data was made. A preliminary work of identification of the sampling stations was necessary because the ID numeration between IRSEV (1978-1979) study campaigns and the PMP (1986-1987) and Giada (2003) campaigns were different. This work has been forward by a georeference action of the IRSEV stations with the geographical information system (GIS) ArcView® GIS 3.2 (ESRI, Redlans CA US) and a comparison with the Giada Project stations, already located with exact geographical coordinates. From this first analysis only few monitoring stations were comparable because of during the long range of time, from 1978 to 2002, many wells were abandoned or drained and because during the last campaigns the concentration of BTFs was monitored only in few points focusing on other pollutants. Moreover, as already explained, during the IRSEV study only concentrations of 3N4CBTF were monitored. The elaborated map with the IRSEV sampling station is illustrated on figure 3.10.

Figure 3.11, instead, shows the simulation of the spatial propagation of the groundwater plume after the elaboration of IRSEV historical dataset. In figure, the black continuous line delimited the contamination area. A drastic change of direction of the plume from NS at the high piezometric level sited in Alte Ceccato is clearly evident with the flow turning from SW to NE until the resurgences area of Sovizzo and Creazzo. The involved area was about 25 Km^2.

Table 3.22: concentrations of BTFs detected during the Giada Project campaign (2002)

well #	municip.	well deep	BTF	4BTF	3NBTF	3N4CBTF	35DN4CBTF	total BTFs
		m	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
37	Trissino	65	< 0,1	**2,1**	< 0,1	**0,7**	< 0,1	**2,8**
93	Montecchio Maggiore.	55	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5
128	Montecchio Maggiore.	52	< 0,1	**6,1**	**0,3**	**2,7**	< 0,1	**9,1**
131	Montecchio Maggiore.	40	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5
144	Montecchio Maggiore.	67	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5
145	Montecchio Maggiore.	88	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5
154	Montecchio Maggiore.	97,5	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5
162	Montecchio Maggiore.	40	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5
163	Montecchio Maggiore.	64	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5
175	Montecchio Maggiore.	38	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5
179	Montecchio Maggiore.	23	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5
204	Brendola	115	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5

Figure 3.10: map with the location of the IRSEV sampling stations (see ANNEX II for details).

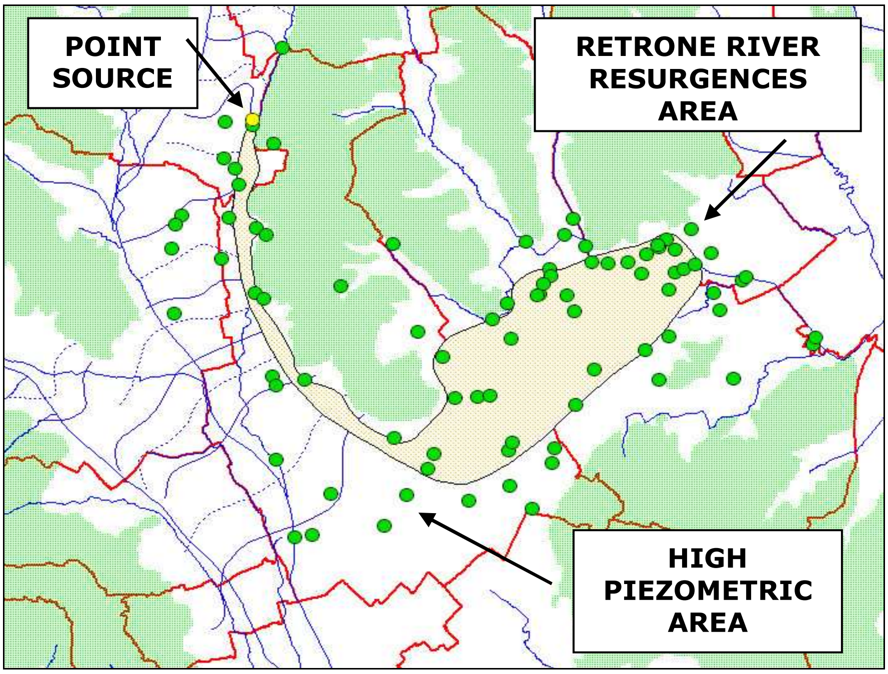
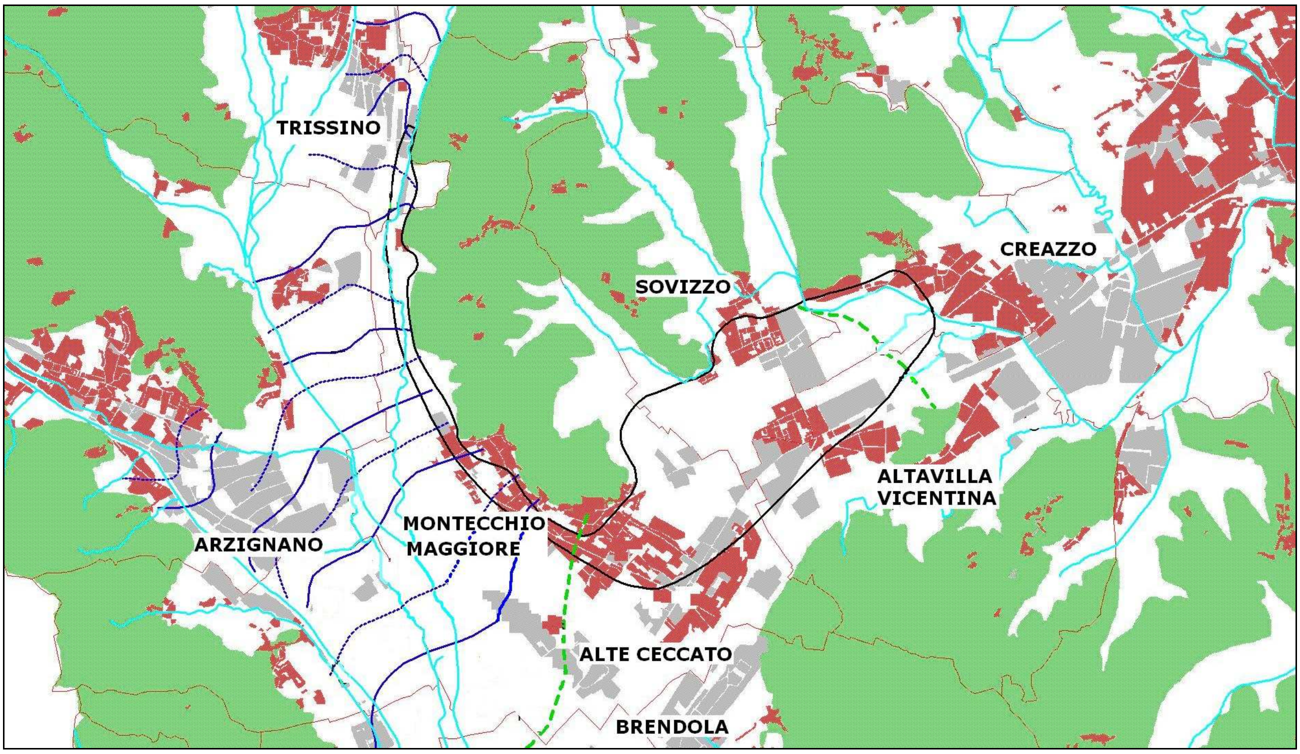


Fig 3.11: spatial elaboration of the plume contamination after the analysis of IRSEV data of 3N4CBTF concentrations (elaboration with ArcView® software, thanks to dott. Altissimo of Centro Idrico Novoledo).

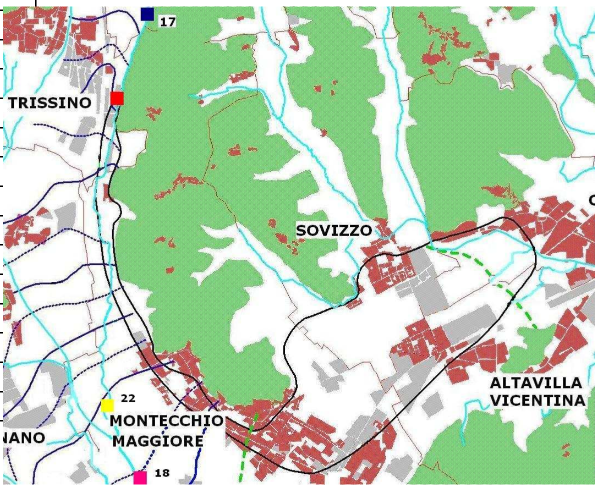


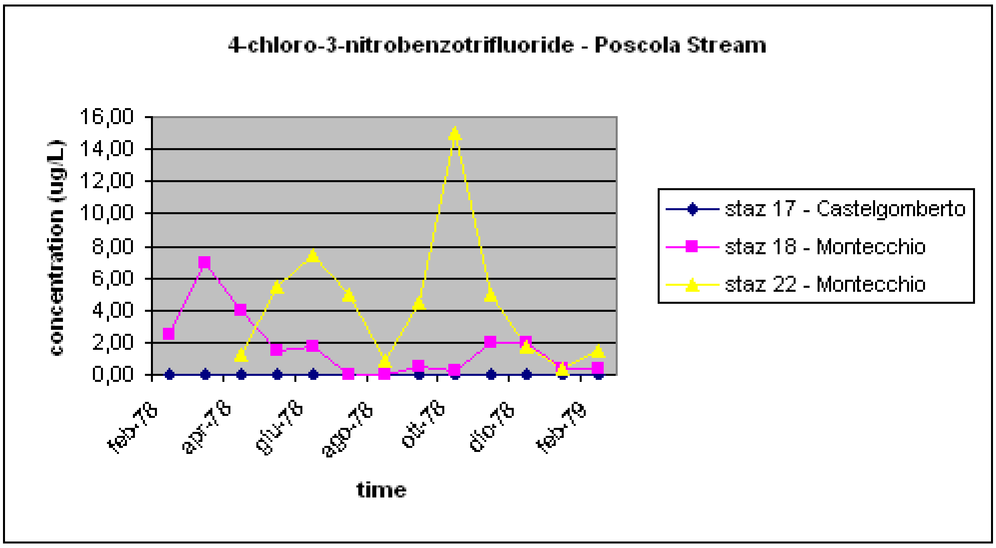
From the dataset a preliminary elaboration of 3N4CBTF concentration trend on surface water samples of Poscola Stream was made as explained on figure 3.12.

Figure 3.12: IRSEV data of 3N4CBTF on the three surface stations on Poscola River.

Concentration of 4-chloro-3-nitrobenzotrifluoride
POSCOLA STREAM SURFACE STATIONS
IRSEV 1979 STUDY

	Poscola Stream (ug/L)		
	C.GOMBERTO	MONTECCHIO	MONTECCHIO
#.STAZ	17	18	22
date			
Feb-78	<LOD	2.50	-
Mar-78	<LOD	7.00	-
Apr-78	<LOD	4.00	1.2
May-78	<LOD	1.50	5.5
Jun-78	<LOD	1.70	7.5
Jul-78	<LOD	<LOD	5.0
Aug-78	<LOD	<LOD	0.9
Sep-78	<LOD	0.50	4.5
Oct-78	<LOD	0.30	15.0
Nov-78	<LOD	2.00	5.0
Dec-78	<LOD	2.00	1.7
Jan-79	<LOD	0.40	0.4
Feb-79	<LOD	0.40	1.5





Station 17 (Castelgomberto), sited at north of the point source, was used as blank reference point. Since there were no information about the flow conditions of Poscola River during the sampling as well the meteorological situations, only a visual description of the trend, without further considerations, is possible to make for surface station stations on Poscola River.

Further trends can be obtained considering some groundwater sampling stations. A graphic elaboration of the concentration trend of 3N4CBTF in stations close to the source point (Trissino), in stations in Montecchio Maggiore and in station close to the Retrone River resurgences (Creazzo) are shown below on Figures 3.13, 3.14 and 3.15.

Figure 3.13: 3N4CBTF concentration trend during the period 1978-79 in stations P66 (Conceria Valleagno, Trissino), P64 (Meggiolaro private well, Trissino) and P786 (ORMEC, Montecchio Maggiore). See ANNEX II for stations details.

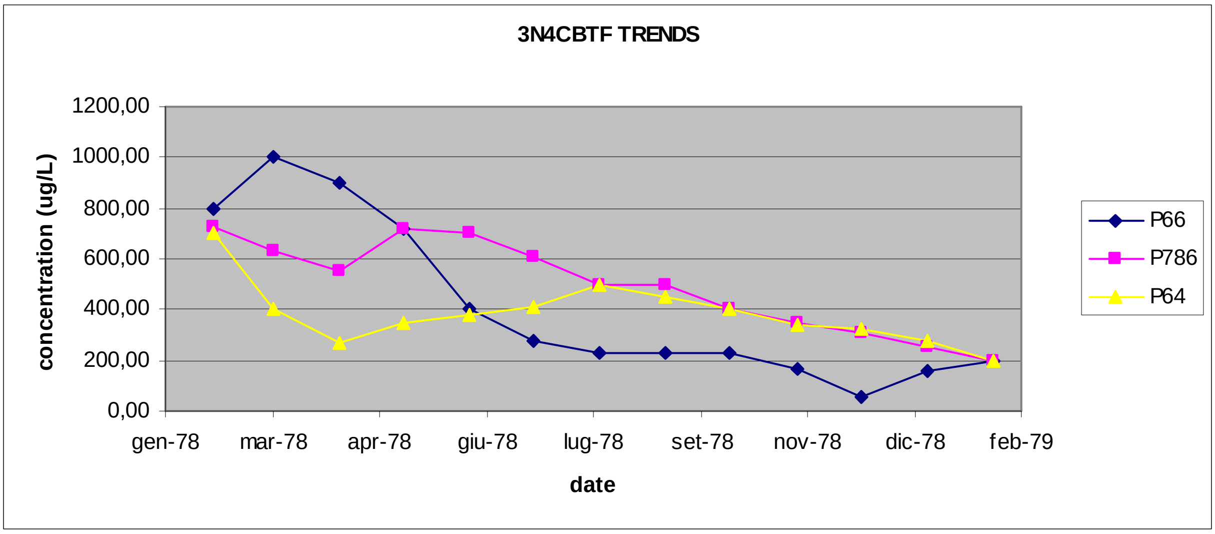


Figure 3.14: 3N4CBTF concentration trend during the period 1978-79 in stations P31 (Confezioni Castelli, Montecchio Maggiore), P29 (Ospedale civile, Montecchio Maggiore) and P63 (Bomitali private well, Montecchio Maggiore). See ANNEX II for stations details.

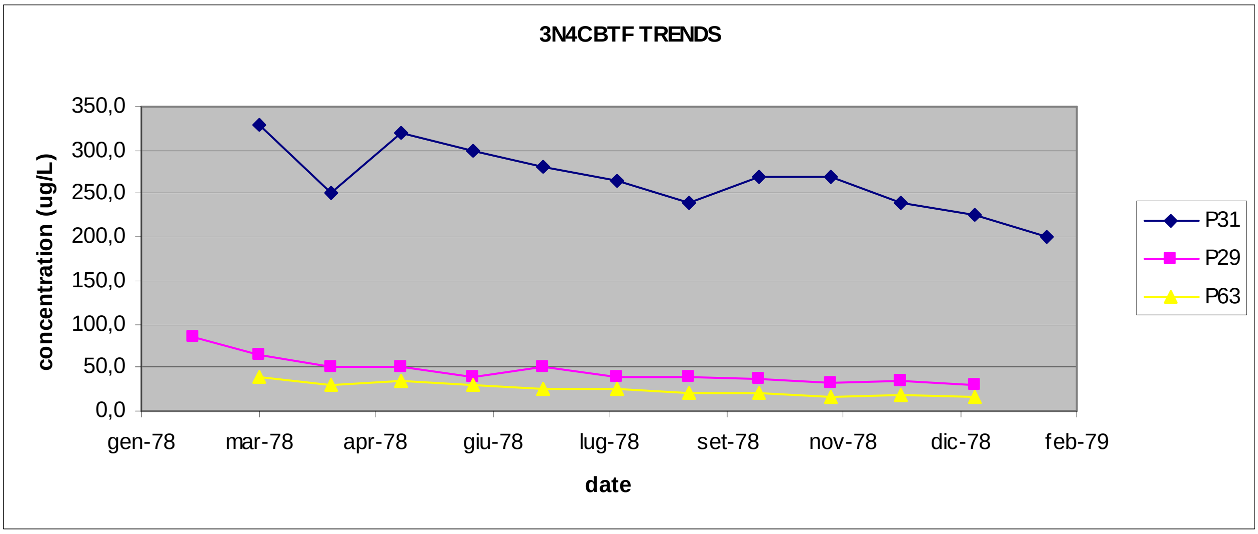
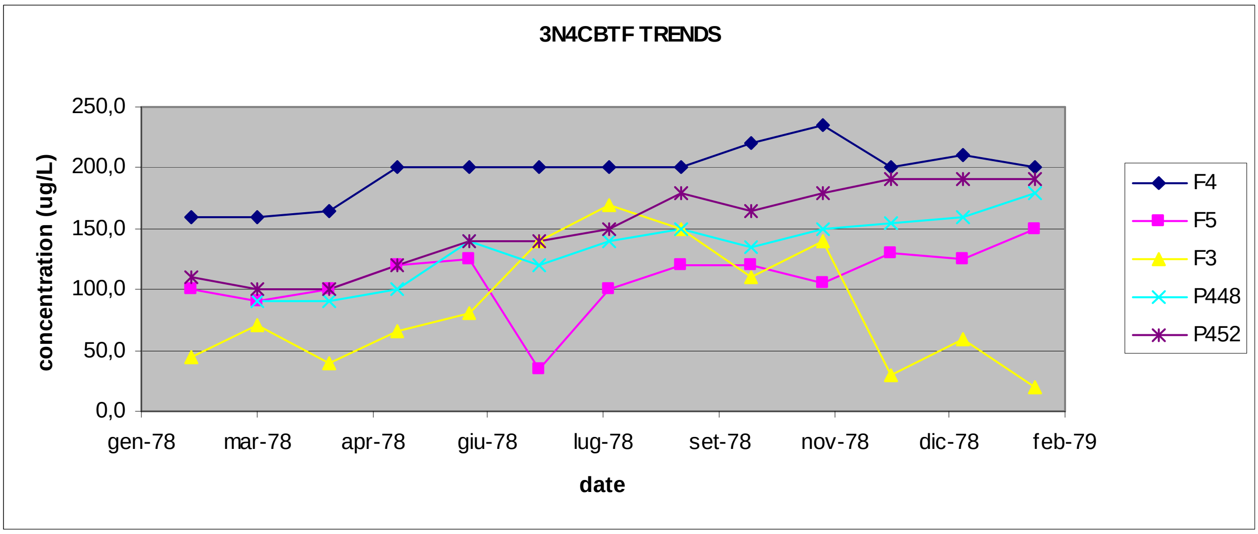


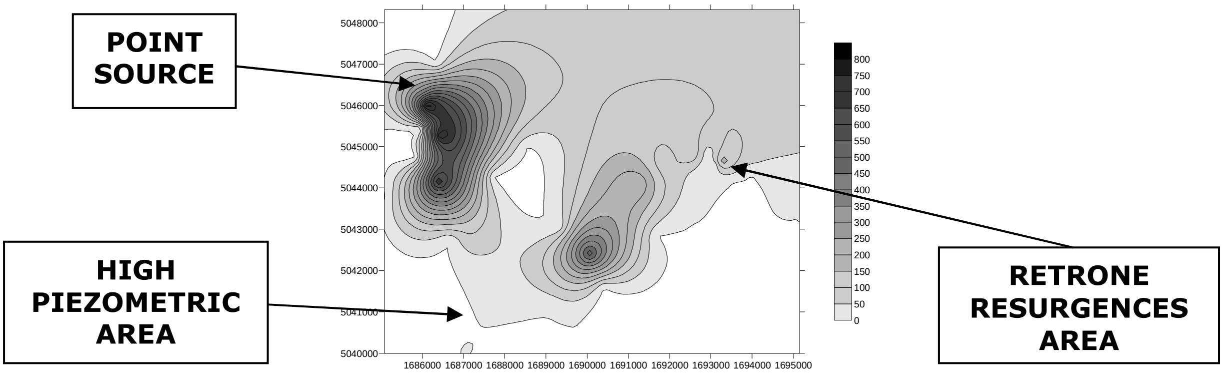
Figure 3.15: 3N4CBTF concentration trend during the period 1978-79 in three surface water stations F3 (Retrone River, Creazzo), F4 (Risorgiva Spino, Creazzo), F5 (Roggia Spino, Creazzo), and in two groundwater stations P448 (Casarotto private well, Creazzo) and P452 (Gobbi private well, Creazzo). See ANNEX II for stations details.



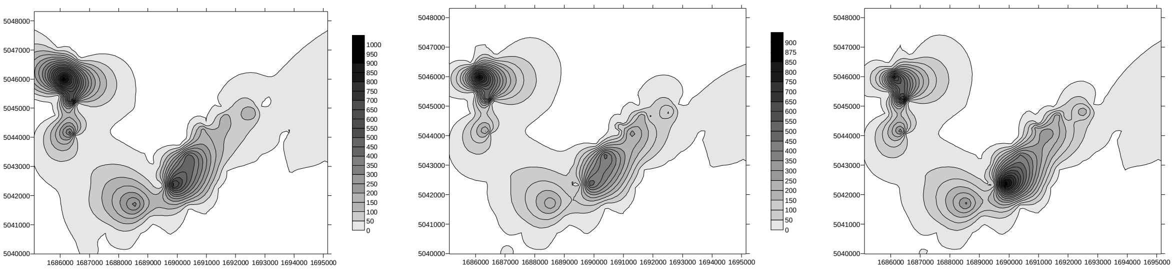
From the charts a substantial decrease of concentration is clear close to the point source of pollution with minimum values in Montecchio Maggiore area to an increment of concentration at the end of the plume, in the area of resurgences.

The temporal and spatial trends of 3N4CBTF concentration, considering the whole sampling stations of ANNEX II (84 stations), were assessed with Surfer® 8.0 Surface Mapping System simulation software (Golden Software Inc., Golden CO US). The georeferenced Gauss-Boaga coordinates (X and Y) of each sampling point were compared with the monthly concentration of 3N4CBTF (coordinate Z) in a grid using the Kriging Gridding Method of the software. The monthly elaborations allow obtaining the contour maps of figure 3.16 representing the pollution episode propagation from February 1978, when the first 3N4CBTF monitoring data were collected, until February 1979. The diffusion of the contaminant plume is clearly evident during the first year after the contamination episode and the stop of 3N4CBTF production. The simulation draws the plume qualitatively described on figures 3.10 and 3.11 with the limitation that doesn’t consider the geographical conformation of the territory.

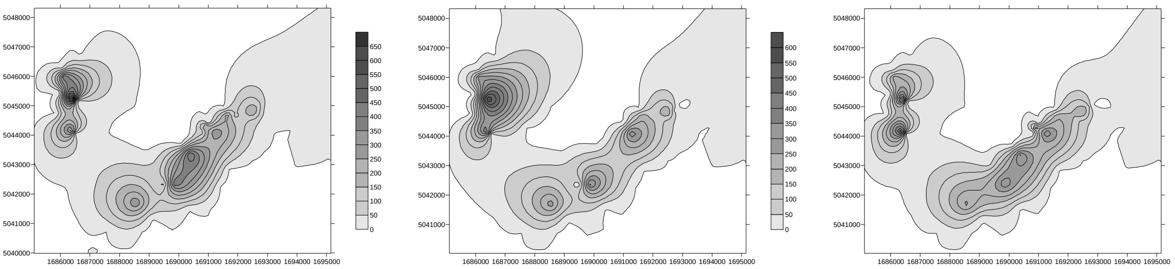
Figure 3.16: Surfer® simulation of the contamination based (X= X coordinates Gauss-Boaga, Y = Y coordinates Gauss-Boaga, Z = concentration of 3N4CBTF in μg/L).



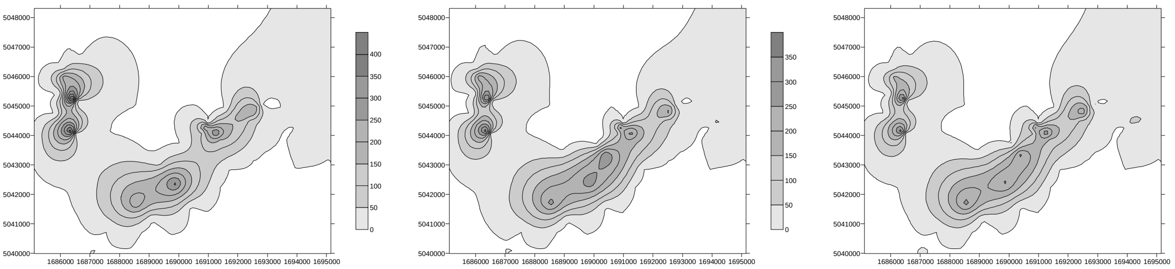
February 1978



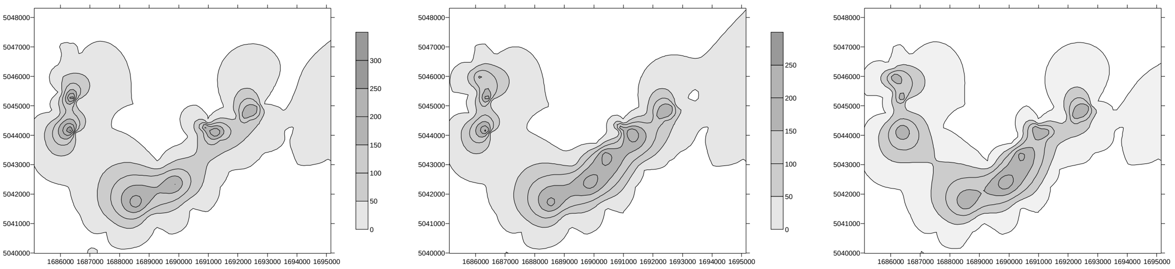
March 1978 April 1978 May 1978



June 1978 July 1978 August 1978



September 1978 October 1978 November1978



December1978 January 1979 February 1979

3.2.2.3 Environmental sample: the current situation

An investigation of the current situation of the contamination from BTFs compounds was made trying to understand if some residues were still detectable on groundwater of Vicenza Province and in particular of Agno-Guà Valley area. The last monitoring campaign on BTFs in 2002 (GIADA project) determined BTF, 4BTF, 3NBTF, 3N4CBTF and 35DN4CBTF on 12 wells. The analytical panel was extended also to the analytes of paragraph 3.2.1.1 and 3.2.1.2. and the methods developed in this work were applied.
A first individuation of the wells was made with the following criteria:

- BTFs determination on the most representative and past contaminated wells of the area;
- detection of residues on the surrounding of the plume contamination of Figure 3.11;
- availability of authorizations to collect groundwater from the stations.

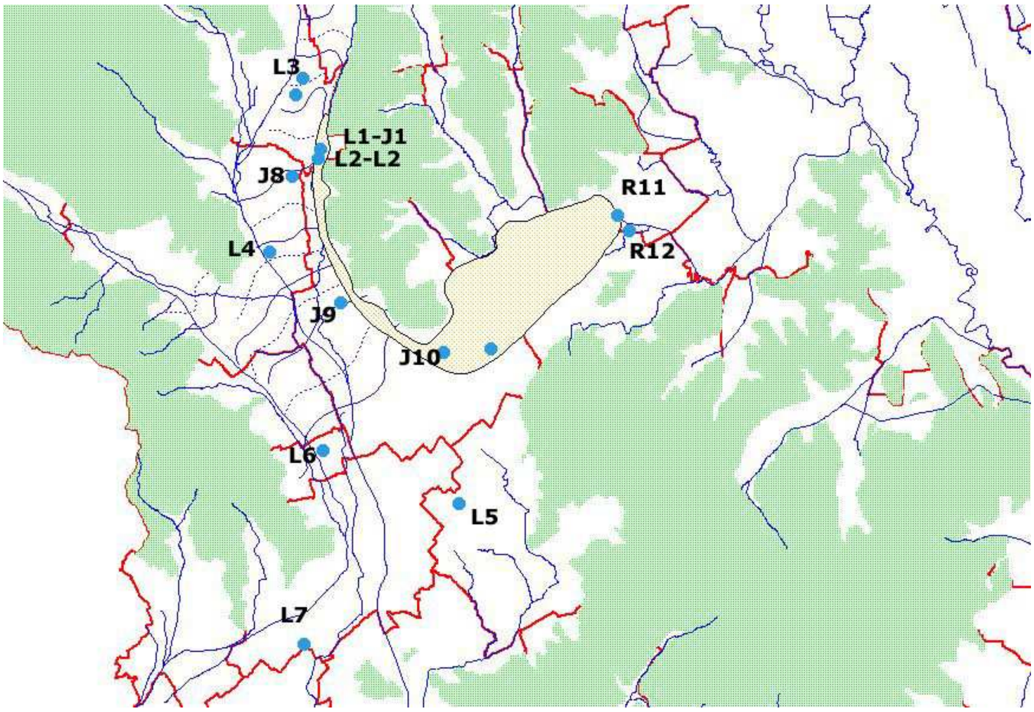
The selected sampling stations are explained on table 3.23. In the table are also represented the details of the location of sampling, the deep of the sampling point and the most important physicochemical properties of the samples measured after the sampling. In a second moment the stations were geo-referenced with the ArcView® GIS software. (Figure 3.17). The samples were collected in four different periods between September 2008 and August 2009 from 12 wells of a total of 15 selected. Two of the planned monitoring wells were closed during these last years (in Lonigo and in Montecchio Maggiore) and one, in Trissino, was found completely drained. Two more samples from surface water (Retrone River) were collected in the last period of the activity. The samples L3, from a private well in the centre of Trissino municipality, was used as blank sample because the sampling point was sited at north of the contaminated area.

The most critical physicochemical properties and the speciation of nitrogen (NO_3^-, NO_2^- and NH_4^+) were tested. These values were compared with the same obtained during the past campaigns (see Annex III). From the values obtained, especially from ORP, [NO_3^-] and [NH_4^+], no particular reductive behaviour was detected. Furthermore, from the lithology of the territory (porous materials and gravel formations), as already explained, particular reductive processes at groundwater levels were excluded.

Table 3.23: list of the sampling collected during the period 2008-2009 with the most important phisico-chemical parameters.

Sample	Municipal.	well ID	date	deep m	pH	conductiv. (20°C) µS/cm	salinity PSS	ORP mV	NH_4^+ mg/L	NO_2^-, mg/L	NO_3^- mg/L
Conceria Valleagno	Trissino	**L1**	Sep-08	65	7,65	516	0,27	+185	0,10	<0,01	15,00
Conceria Basmar	Trissino	**L2**	Sep-08	48	7,48	557	0,29	+197	<0,02	<0,01	17,70
Private well	Trissino	**L3**	Sep-08	30	7,51	509	0,26	+174	<0.02	<0.01	16,94
Private well	Trissino		well drained	15							
Municipality waterworks	Arzignano	**L4**	Nov-08		7,57	387	0,18	+205	<0,02	<0,01	11,00
Groundwater well close the WWTP	Montebello Vic.	**L7**	Nov-08		7,49	544	0,26	+203	<0,02	<0,01	17,30
ARPAV station	Brendola	**L5**	Nov-08	42	7,38	587	0,29	+157	<0,02	<0,01	17,70
Pozzo captazione	Zermeghedo	**L6**	Nov-08	17	7,40	650	0,31	+208	<0,02	<0,01	24,30
Private well	Lonigo		well closed								
Conceria Basmar	Trissino	**J2**	Jun-09	48	7,25	467	0,23	+139	<0,02	<0,01	23,23
Conceria Valleagno	Trissino	**J1**	Jun-09	65	7,73	578	0,28	+123	<0,02	0,04	24,77
FIAMM	Montecchio M.	**J10**	Jun-09	52	7,26	649	0,32	+142	<0,02	<0,01	20,43
Materiali Edili Concato	Arzignano	**J8**	Jun-09	35	7,45	553	0,27	+146	<0,02	<0,01	16,68
Municipality waterworks	Montecchio M.	**J9**	Jun-09	55	7,39	545	0,26	+150	<0,02	<0,01	26,39
Zarantonello Car Washing	Montecchio M.		well closed								
Retrone River	Creazzo	**R11**	Ago-09	sup	7,38	741	0,36	+168	-	-	-
Roggia Poletto	Creazzo	**R12**	Ago-09	sup	7,27	688	0,33	+178	-	-	-

Figure 3.17: location of the sampling station collected during the 2008-2009 period.



The volatile BTFs fraction was firstly detected on the samples collected. The 40 mL vials used for P&T extraction were directly filled with the samples at room temperature without any head space or air layer on the top. The samples were extracted and analyzed on the same day or at maximum on the day after, of the date of sampling. Results were clearly explained on the following Tables 3.24 and on Figure 3.18, 3.19, 3.20 and 3.21.

Also other volatile parameters were tested (Table 3.25) with the same method described on Paragraph 2.2.3.2. The LOQ values of the others parameters detected were:
TrCE = 0.03 μg/L   TeCE = 0.05 μg/L   CB = 0.03 μg/L   14DCB = 0.02 μg/L
12DCB = 0.02 μg/L   AN = 0.1 μg/L   NB = 0.1 μg/L

Table 3.24: volatile BTFs values of sample collected between 2008 and 2009.

well ID	date	BTF [μg/L]	4BTF [μg/L]	34DCBTF [μg/L]	24DCBTF [μg/L]
L1	Sep-08	0.098	11.982	6.44	0.22
L2	Sep-08	0.005	0.165	0.14	<LOQ
L3	Sep-08	<LOQ	<LOQ	<LOQ	<LOQ
L4	Nov-08	<LOQ	0.015	<LOQ	<LOQ
L5	Nov-08	<LOQ	0.047	0.02	<LOQ
L6	Nov-08	<LOQ	0.023	0.02	<LOQ
L7	Nov-08	<LOQ	<LOQ	0.01	<LOQ
J1	Jun-09	0.041	9.963	5.21	0.37
J2	Jun-09	<LOQ	0.051	0.05	0.02
J8	Jun-09	0.009	0.026	0.06	0.05
J9	Jun-09	0.005	0.02	0.04	0.08
J10	Jun-09	0.017	2.629	1.40	0.08
R11	Ago-09	0.181	4.544	3.45	0.92
R12	Ago-09	0.439	1.069	2.41	2.46

Table 3.25: other volatile and semivolatile organic compounds detected on the samples collected between 2008 and 2009.

well ID	date	TrCE [μg/L]	TeCE [μg/L]	CB [μg/L]	14DCB [μg/L]	12DCB [μg/L]	AN [μg/L]	NB [μg/L]
L1	Sep-08	0.14	0.36	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
L2	Sep-08	0.36	0.75	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
L3	Sep-08	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
L4	Nov-08	0.04	0.85	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
L5	Nov-08	0.14	2.99	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
L6	Nov-08	0.06	3.04	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
L7	Nov-08	0.59	6.99	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
J1	Jun-09	0.09	0.33	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
J2	Jun-09	0.08	0.60	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
J8	Jun-09	0.11	0.48	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
J9	Jun-09	0.05	0.61	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
J10	Jun-09	0.06	0.45	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
R11	Ago-09	0.11	0.98	<LOQ	<LOQ	<LOQ	<LOQ	0.3
R12	Ago-09	0.04	1.75	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

TrCE = trichloroethylene, TeCE = tetrachloroethylene, CB = chlorobenzene,
14DCB = 1,4-dichlorobenzene, 12DCB = 1,2-dichlorobenzene, AN = aniline,
NB = nitrobenzene

Figure 3.18: BTF concentration in μg/l of the environmental samples tested (two different scales).

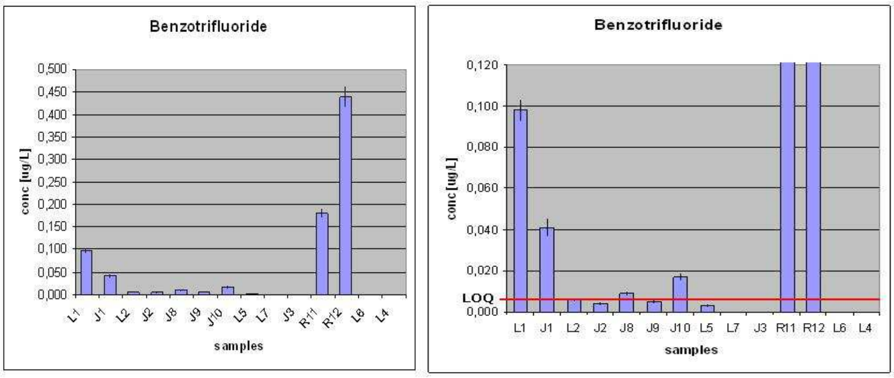


Figure 3.19: 4CBTF concentration in μg/l of the environmental samples tested (two different scales).

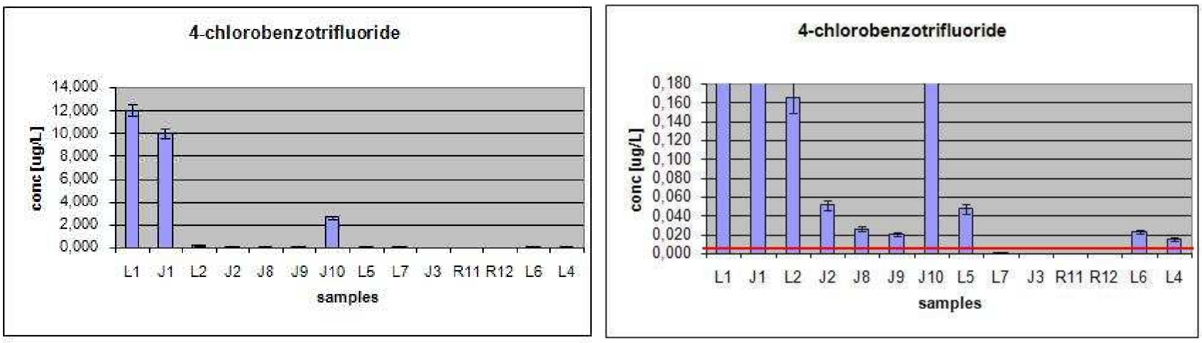


Figure 3.20: 34DCBTF concentration in μg/l of the environmental samples tested (two different scales).

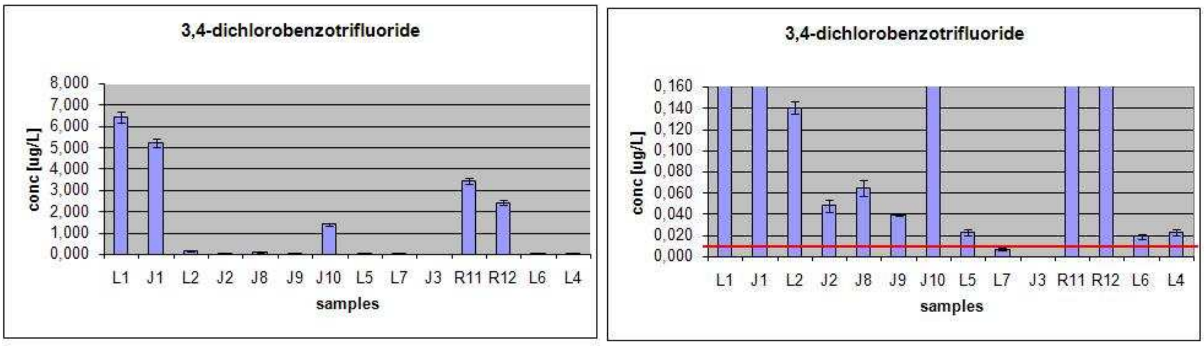
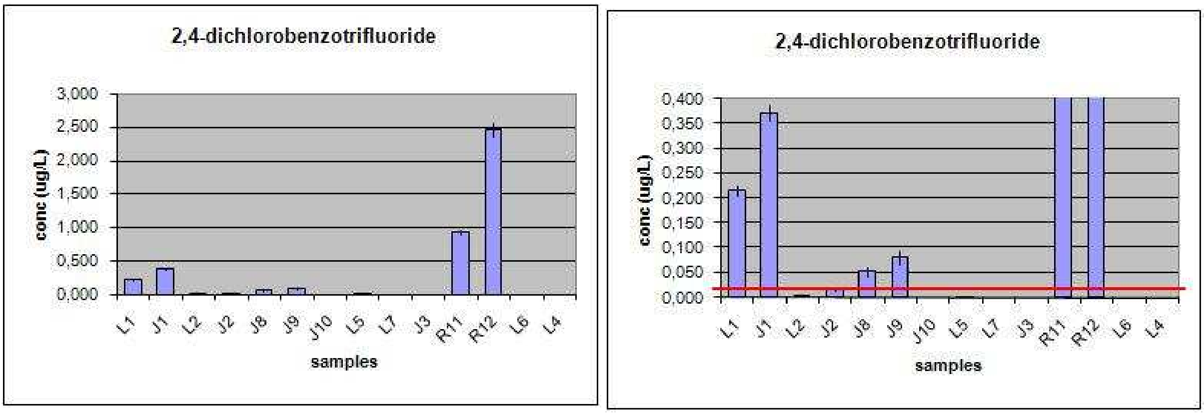


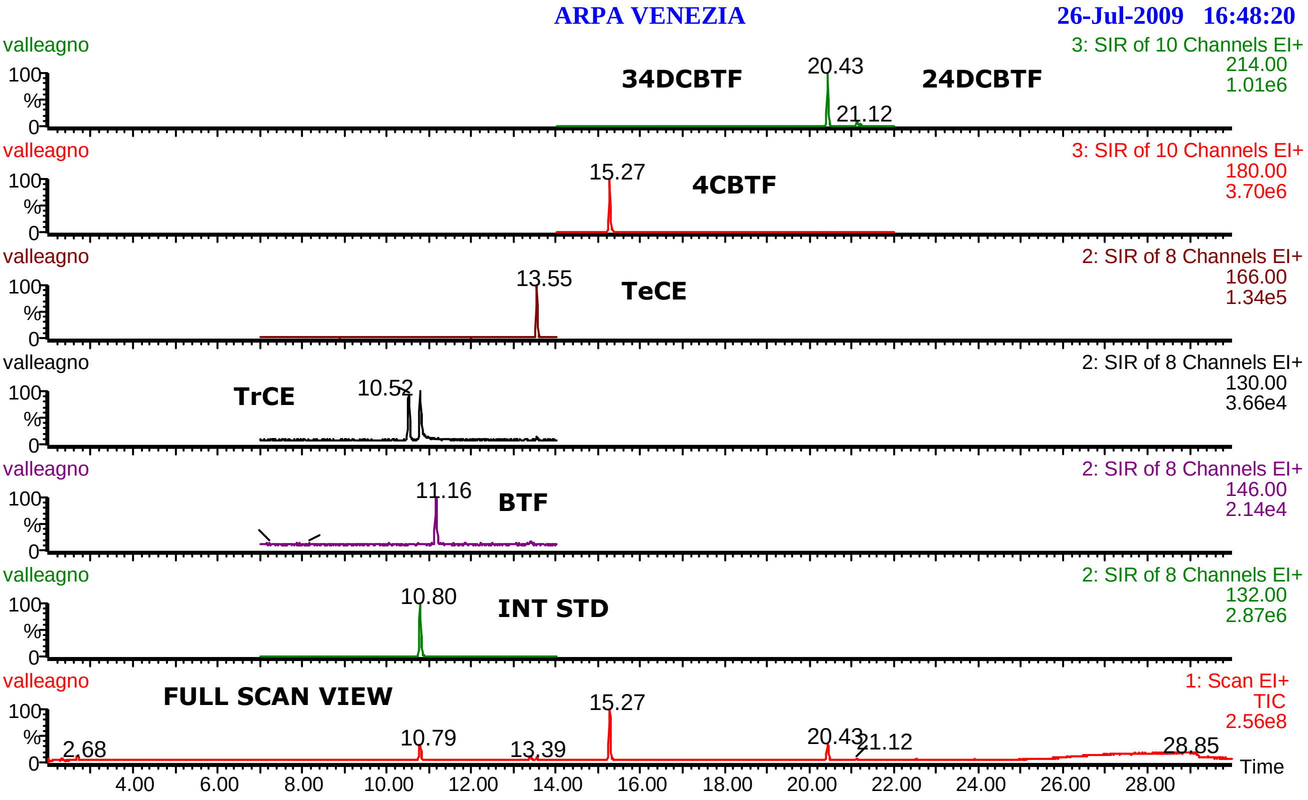
Figure 3.21: 24DCBTF concentration in μg/l of the environmental samples tested (two different scales).



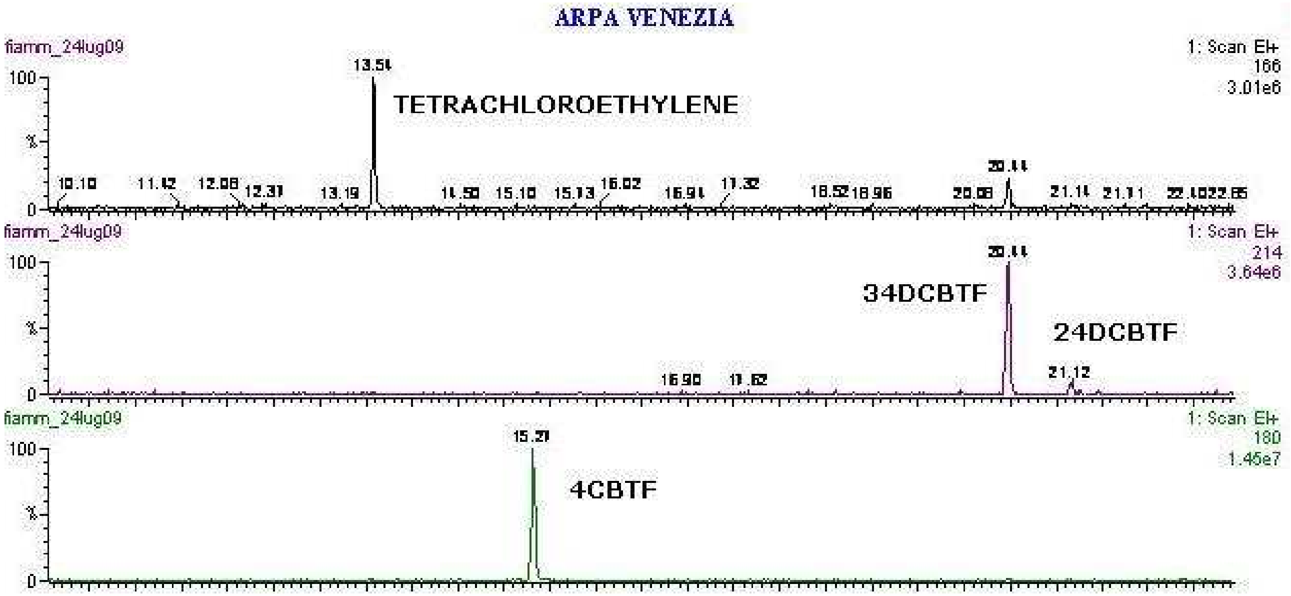
In figure 3.22 are also represented the chromatograms of volatile BTFs determination of some real samples analysed.

Figure 3.22: chromatograms of samples: L1, J10 and R12.

L1 (Trissino – Conceria VALLEAGNO)



J10 (Montecchio Maggiore – FIAMM)



R12 (Creazzo – Roggia Poletto)

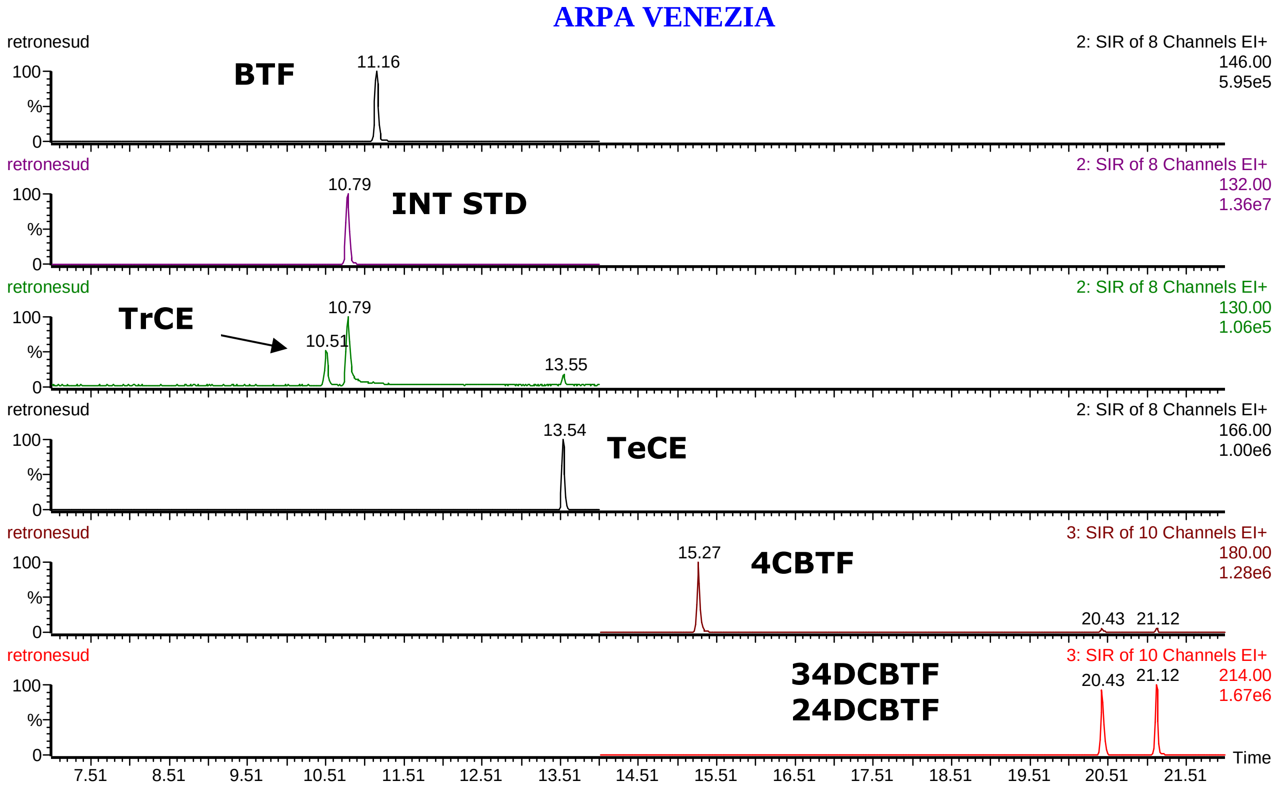
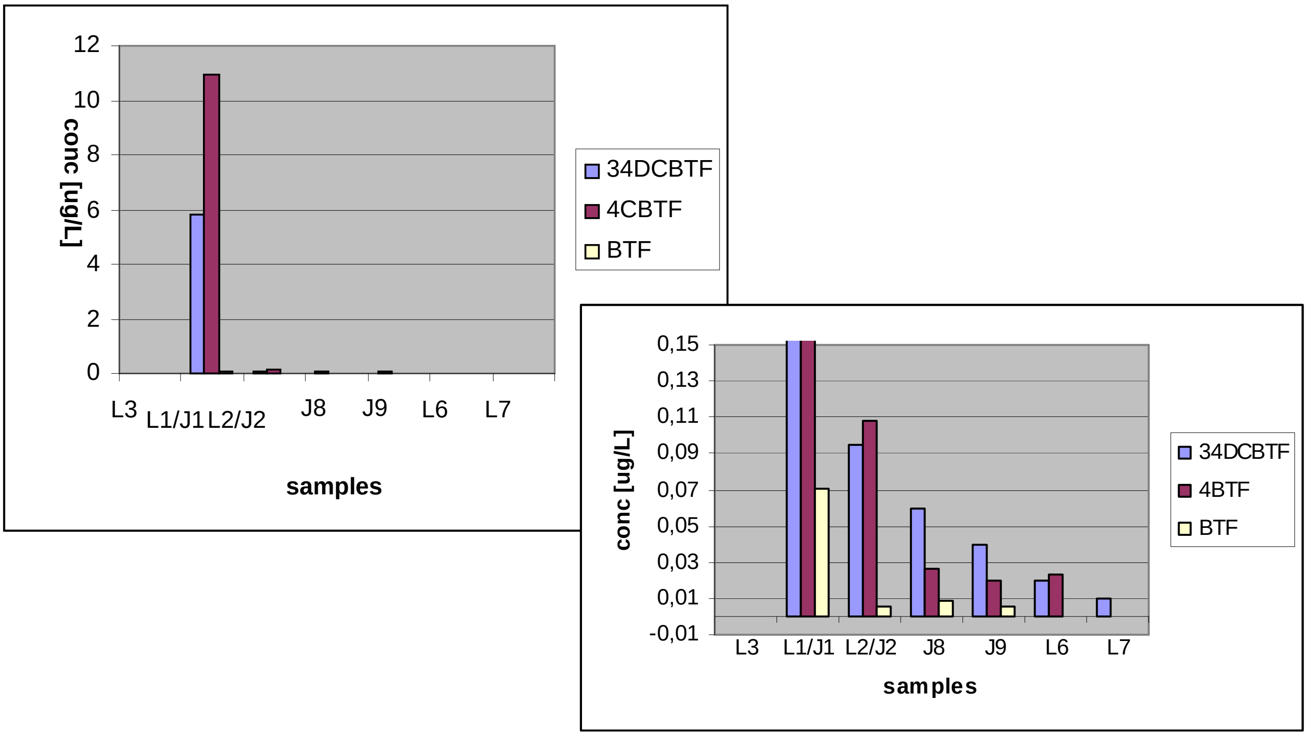


Table 3.24 shows variable concentrations of volatile BTFs from values close to the LOQs up to 10 µg/L. The samples with highest concentrations were L1/J1 collected in an area close to the pressure point (conceria Valleagno), J10 collected in the city

centre of Montacchio Maggiore (FIAMM) and R11/R12 (surface waters of Retrone River). While the highest concentrations of 4CBTF and 34DCBTF were registered for L1/J1, the highest concentrations of BTF and 24DCBTF were recorded at level of Retrone River resourgences. L1 and L2 were collected during the summer 2008 while J1 and J2 in summer 2009 from the same sampling points. On some samples considerable quantities of tetrachloroethylene were also detected (table 3.25).

Considering the sampling stations from North to South along Poscola Stream Valley and then Guà River, a decrease of BTF, 4CBTF and 34DCBTF concentrations values was detectable. The lower LODs and LOQs values of BTF and 4CBTF together with the addition of 34DCBTF in the analytical panel, showed that the more volatile fraction of BTFs, after contamination of the groundwater, moved on the NS line over the plume contour obtained after elaboration of the IRSEV data (Figures 3.10 and 3.11). This is represented in Figure 3.23 where the values of L1 and J1 and of L2 and J2 (two different samples collected in two different periods from the same station) were considered as the average concentration value.

Figure 3.23: comparison of 34DCBTF, 4CBTF and BTF concentration (2008-2009) along the NS direction of groundwater contamination.



The non-volatile extraction method with SPE in basic conditions was applied at the same samples with the results of table 3.26.

Table 3.26: non-volatile BTFs values of sample collected between 2008 and 2009.

well ID	**34DCBTF [µg/L]**	**24DCBTF [µg/L]**	**3ABTF [µg/L]**	**3NBTF [µg/L]**	**3A4CBTF [µg/L]**	**3N4CBTF [µg/L]**	**4C35DCBTF [µg/L]**
L1	2.88	0.42	0.58	0.43	2.61	5.91	<LOD
L2	0.02	<LOD	<LOD	<LOD	0.01	0.04	<LOD
L3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
L4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
L5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
L6	0.08	0.01	<LOD	<LOD	<LOD	<LOD	<LOD
L7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
J1	2.29	0.32	0.51	0.49	2.47	7.17	<LOD
J2	0.02	<LOD	<LOD	<LOD	0.01	0.02	<LOD
J8	ND	ND	ND	ND	ND	ND	ND
J9	<LOD	<LOD	<LO D	<LOD	<LOD	<LOD	<LOD
J10	0.50	0.05	0.07	0.07	0.32	0.98	<LOD
R11	0.72	0.07	0.02	0.15	0.675	0.30	ND
R12	0.03	<LOD	<LOD	0.01	0.03	0.03	<LOD

ND: not determined

Values obtained from analysis of 34DCBTF and 24DCBTF were readily compared with the same obtained with the P&T method, considering that the recovery SPE method for these semi-volatile compounds were respectively 52 ± 5 % and 61 ± 4 %. Different results were recorded for R12 with data not totally comparable.

Some examples of chromatograms obtained were shown on figures 3.24, 3.25 and 3.26.

Figure 3.24: chromatogram of sample L3 (Trissino, city centre) extracted with basic SPE.

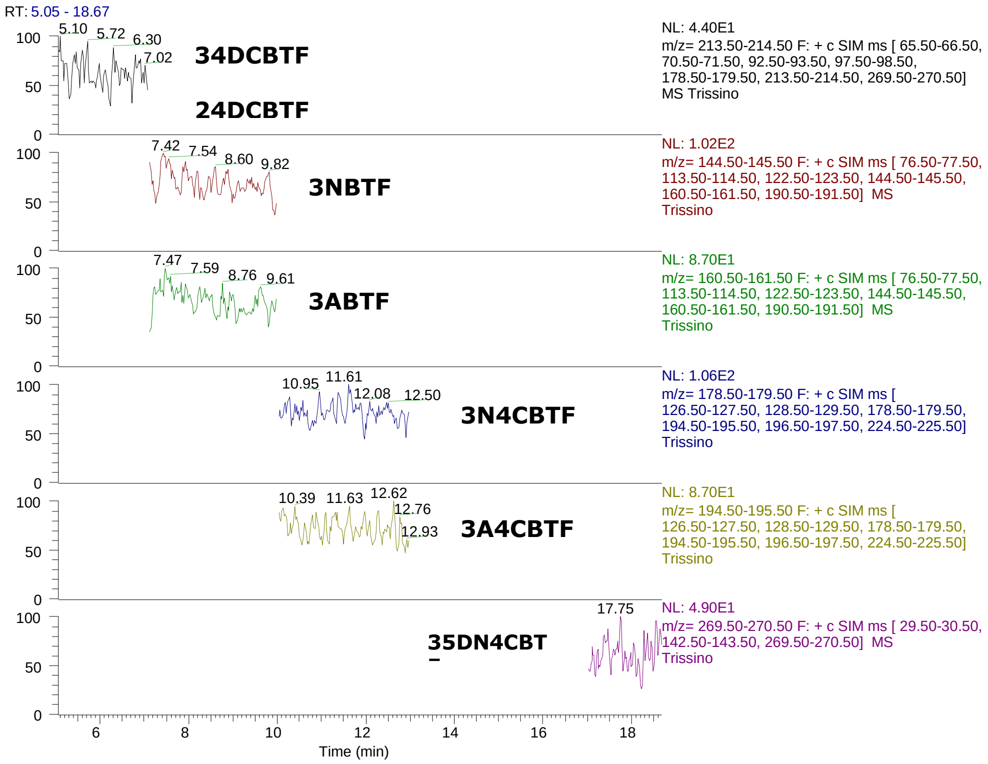


Figure 3.25: chromatogram of sample J1 (Trissino, Conceria Valleagno) extracted with basic SPE.

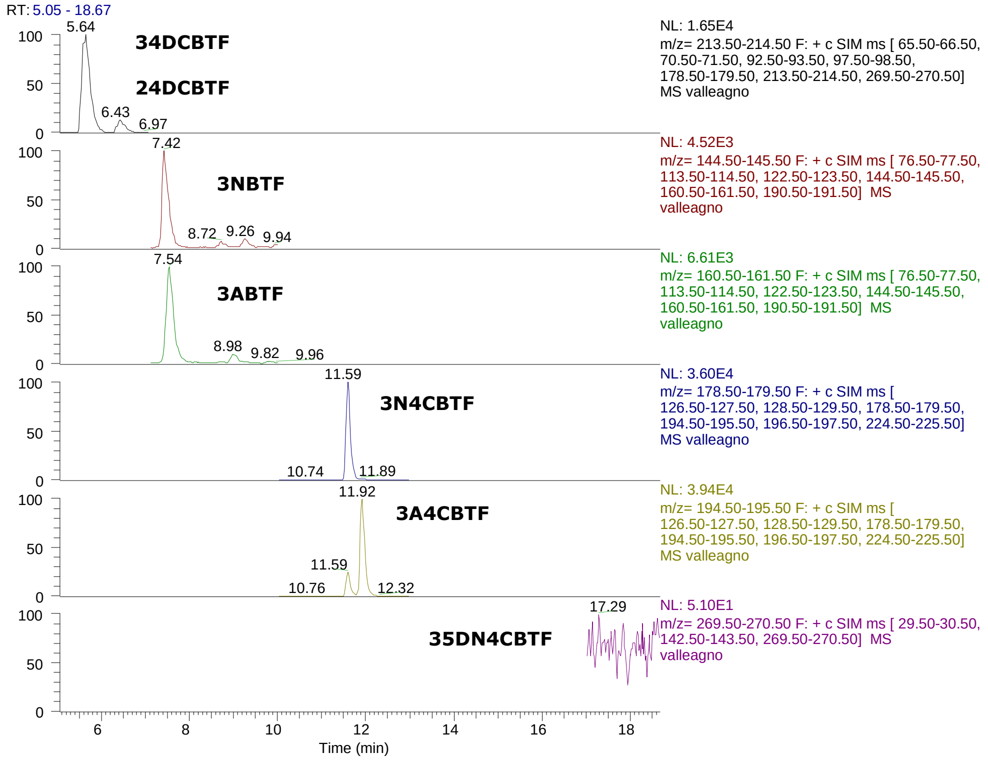
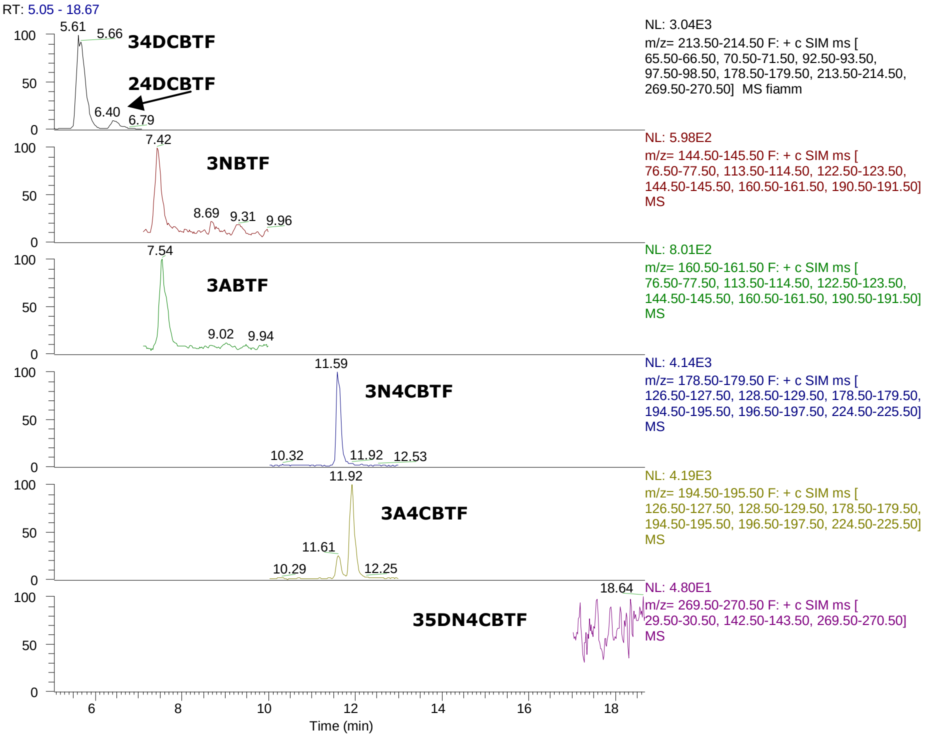


Figure 3.26: chromatogram of sample L10 (Montecchio Maggiore, FIAMM) extracted with basic SPE.

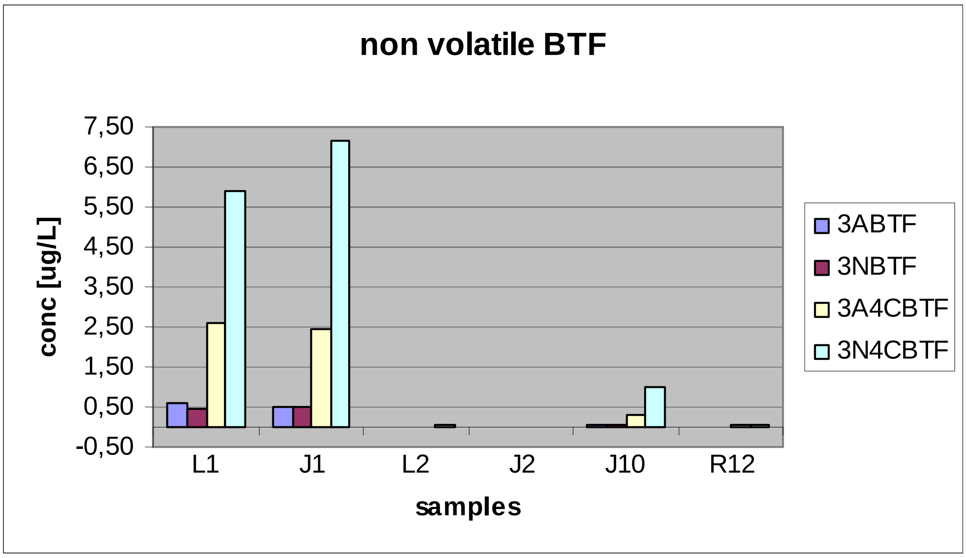


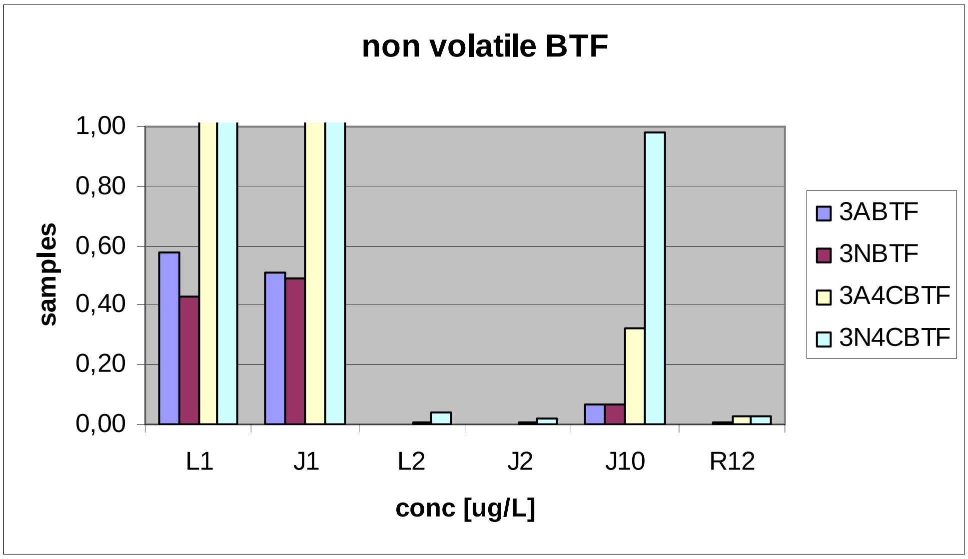
The non-volatile BTFs results confirmed analysis with P&T that the most polluted samples were L1/J1, J10 and R11. The maximum concentration detectable of 3A4CBTF was in L1 with 2.61 µg/L and of 3N4CBTF was in J1 with 7.17 µg/L.

From figure 3.27 it is possible to have a visual comparison between the amount the non-volatile BTFs and the ratio of 3ABTF/3NBTF and of 3A4CBTF/3N4CBTF in the samples collected. In particular the concentration of 3ABTF is comparable with the concentration of 3NBTF while the amount of 3N4CBTF seems to be almost three time the concentration of 3A4CBTF in different point of the plume described. The data cannot be compared with other obtained in the past campaigns because the detection of ammino-BTFs derivatives was never reported. Despite a similar trend between the same amino/nitro BTFs derivatives, conclusions about the residential time and propagation are difficult to draw because of a discrepancy of historical data that give reason of a sediment accumulation of BTF contaminants which varies considerably with the load of water during the different sampling campaigns.

No concentration of 4-chloro-3,5-dinitrobenzotrifluoride was detected in the 12 samples analysed although from the historical data available was considered one of the major organic contaminants discharged.

Figure 3.27: comparison of the concentrations of non-volatile BTFs on samples extracted.





3.2.2.4 SPE acidic extraction and HPLC-HRTOF/MS determination

The HPLC technique coupled with the HR Time of Flight Mass Detector can be used for empirical formulae determination and for the confirmation of both naturally-occurring and synthetic molecules. The advantage of using Full Scan (FS) modality with HRTOF/MS was that is not a target analysis acquisition. Therefore, additional compounds can be detected without modifying the acquisition method or developing and optimizing specific MS parameters. In the specific case the presence of some hydroxy-benzotrifluoride (trifluoromethyl phenols) derivatives compounds was tested at the moment only in a qualitative way. In 1977, during the episode pollution, some hydroxy-benzotrifluoride derivatives were discharged and in particular a losses of 4-hydroxy-3,5-dinitrobenzotrifluoride was reported. It is expected that these compounds, with a hydroxyl function, were more soluble in aqueous samples.

The SPE acid extraction procedure was performed on two samples (L1, J1) and the extracts on methanol stored on fridge. The aim was to check the eventual presence on the extracts of the following molecules (Figure 3.28):

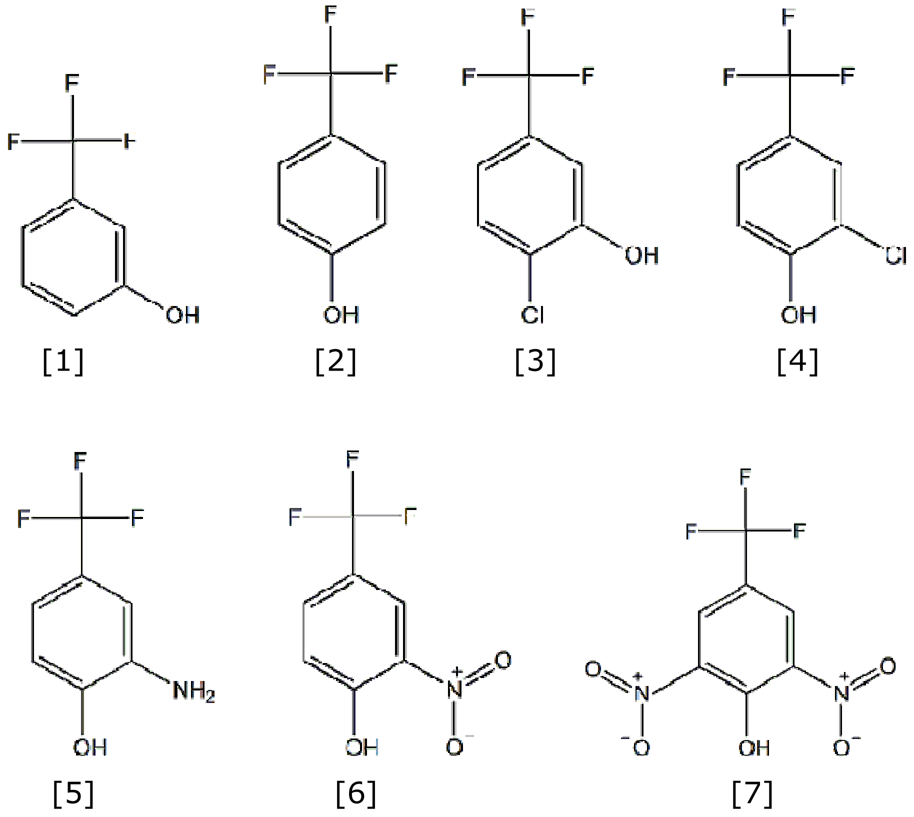
3-hydroxy-benzotrifluoride ($C_7H_5F_3O$) [1]
4-hydroxy-benzotrifluoride ($C_7H_5F_3O$) [2]
4-chloro-3-hydroxy-benzotrifluoride ($C_7H_4F_3ClO$) [3]
3-chloro-4-hydroxy-benzotrifluoride ($C_7H_4F_3ClO$) [4]
3-amino-4-hydroxy-benzotrifluoride ($C_7H_6F_3NO$) [5]
3-nitro-4-hydroxy-benzotrifluoride ($C_7H_4F_3O_3N$) [6]
3,5-dinitro-4-hydroxy-benzotrifluoride ($C_7H_3F_3O_5N_2$) [7]

L1 and J1 sample extracts were injected in the HPLC equipped with an HRTOF/MS detector with the condition described on Paragraph 2.2.4.2. Four of the molecules (and isomers) were detected in a qualitative way on the samples. Table 3.27 and figure 3.29 shows results on the L1 sample extracted. The amount of the signal shown that a relevant presence of 3,5-dinitro-4-hydroxy-benzotrifluoride is still detected on the samples extracted.

Table 3.27: conditions for FS detection of hydroxyl-BTFs compounds searched (sample L1).

Compound	Detected pseudo-molecular ion	Exact mass of pseudo-molecular ion [amu]	Retention time (min)
$C_7H_4F_3ClO$	$[M\text{-}H]^-$	194.98300	5.65
$C_7H_6F_3NO$	$[M\text{-}H]^-$	176.03287	-
$C_7H_4F_3O_3N$	$[M\text{-}H]^-$	206.00705	5.85
$C_7H_3F_3O_5N_2$	$[M\text{-}H]^-$	250.99213	6.09
$C_7H_5F_3O$	$[M\text{-}H]^-$	161.02197	6.90

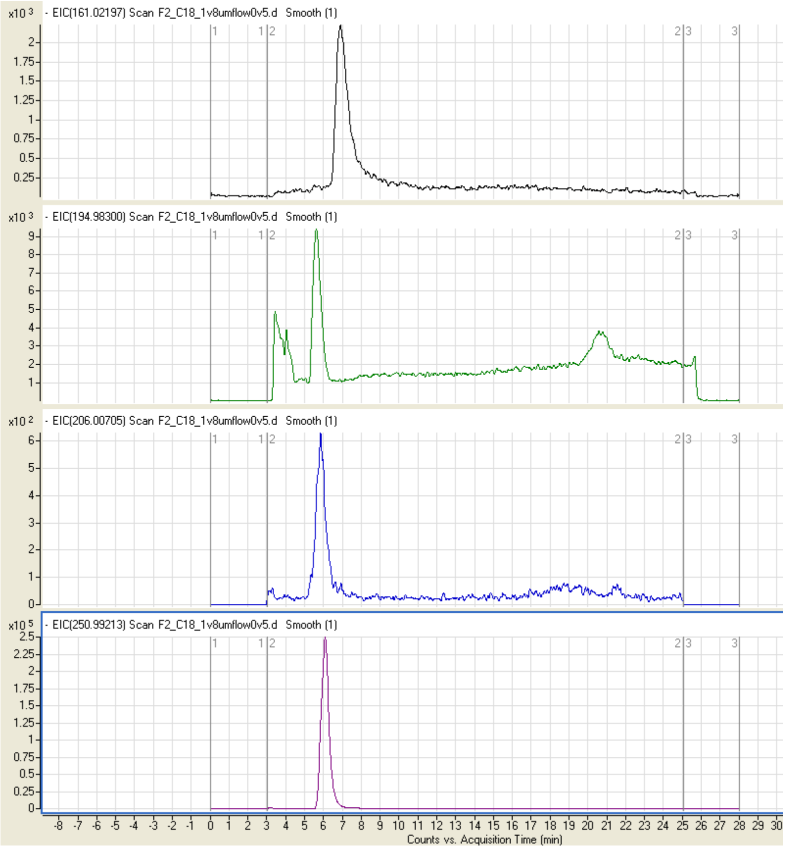
Figure 3.28: contaminants checked on the SPE extracts in acidic conditions.



[1] [2] [3] [4]

[5] [6] [7]

Figure 3.29: first chromatogram of the L1 extracted fraction.



3.3 Preliminary assessment of ecotoxicological effects

In this section the results of a preliminary assessment of toxicological effects on the environment of some dangerous substances considered in the previous paragraphs are discussed. In literature there is a lack about ecotoxicological data and laboratory test related to the chemicals considered, with few works mainly on chloroanilines and chloronitroaromatics[115,116]. No specific works examinated ecotoxicological experiments on benzotrifluoride derivatives. In this section, the results of toxicity tests on the mobility inhibition of *Daphnia magna Straus*, on the light inhibition of *Vibrio fisheri* and on the growth inhibition of *Selenastrum capricornutum*, of substances listed in table 2.8, are examined and discussed. For all the substances considered, a preliminary screening test was necessary to identify the more suitable range to conduct the final tests. Finally some results from real groundwater and surface water samples collected in the area of Agno-Guà Valley during 2008 and 2009 were presented to evaluate the possible persistence of pollutants effect on the aquifer involved in the 1977 pollution episode.

3.3.1 Mobility inhibition of *Daphnia Magna Straus* test results

The results of the effects of substances considered in table 2.8, tested on the ability of *Daphnia magna* to swim, are shown and discussed. The final results were plotted and elaborated after a preliminary screening test for each chemical. EC_{50} values were calculated for each substance after a 24h test period with the Probits Analysis method and the Probfis2.exe software (CNR-IRSA owned registered). The relation between concentrations and observed dead organisms was evaluated considering also the linear correlation between the logarithm of concentration and the observed dead organisms. Table 3.28 summarizes the results obtained. The effect of Milli-Q water with 3‰ of methanol was also tested on each testing batch showing that no damage or hormesis effects were detectable on 24h contact with the organisms.

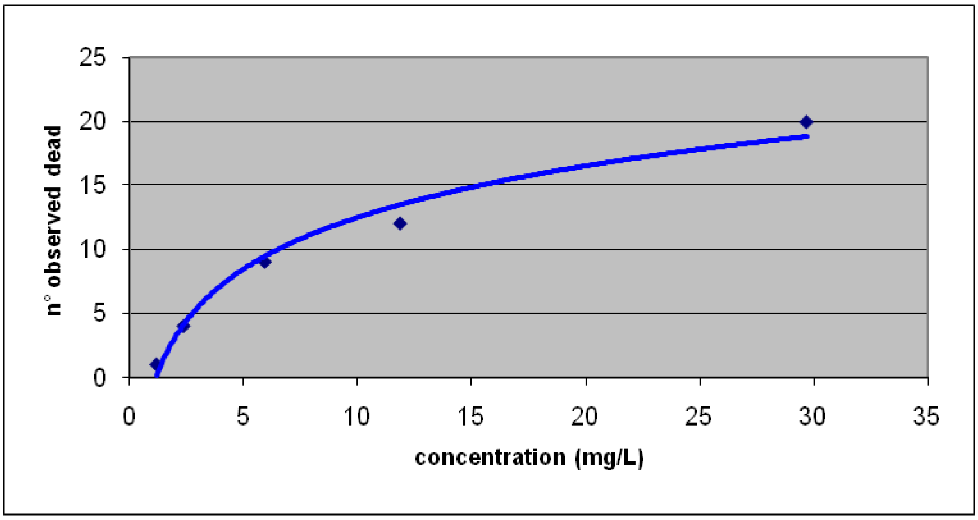
Figure 3.30: data elaboration of *Daphnia magna* test on 2-chloroaniline.

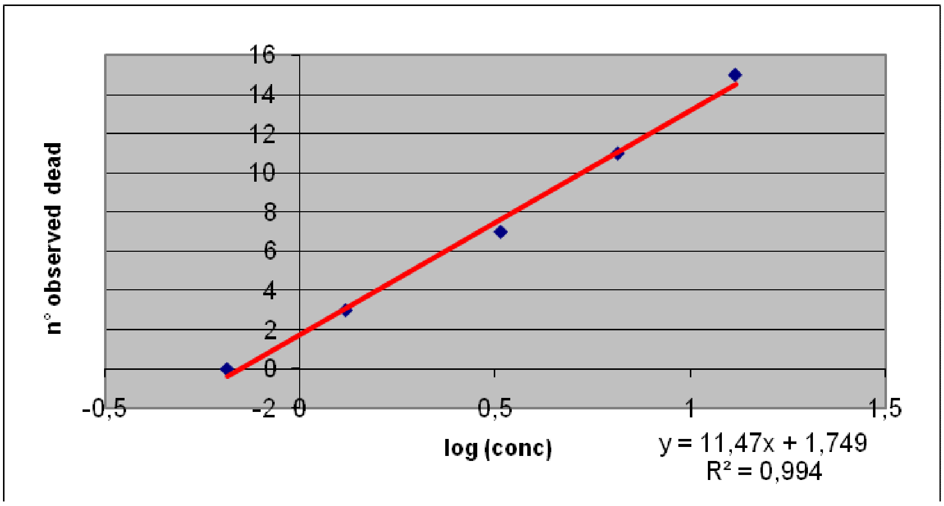
DAPHNIA MAGNA STRAUS ACUTE TOXICITY TEST

final test

2-chloronitrobenzene **88-73-3**

conc	log(conc)	n° organism	n° obs dead org	n° estim dead org
1,19	0,07555	20	1	1,43
2,38	0,37658	20	4	3,57
5,94	0,77379	20	9	8,38
11,88	1,07482	20	12	12,64
29,7	1,47276	20	20	17,09
Controllo 1		20	0	0
Controllo 2		20	0	0





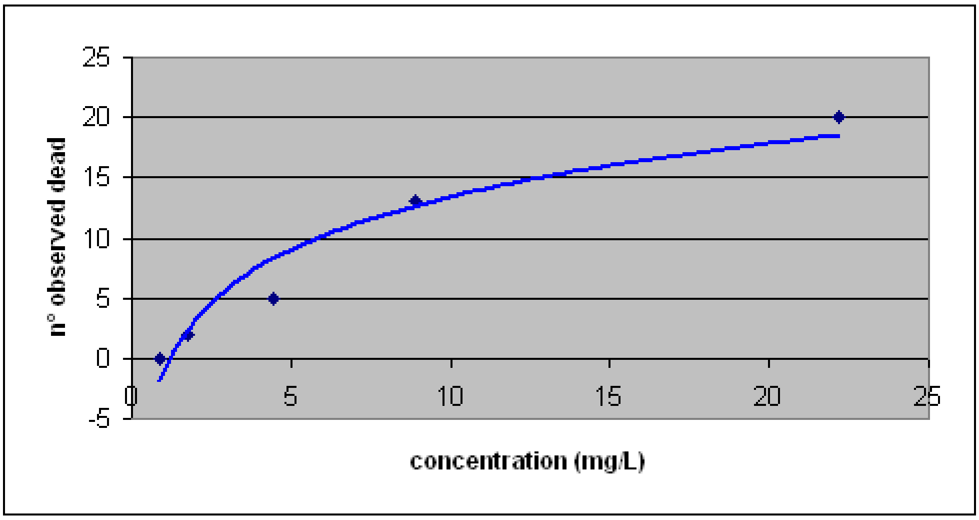
y = ponderated probits y=a+bx
x = ponderated log(conc)

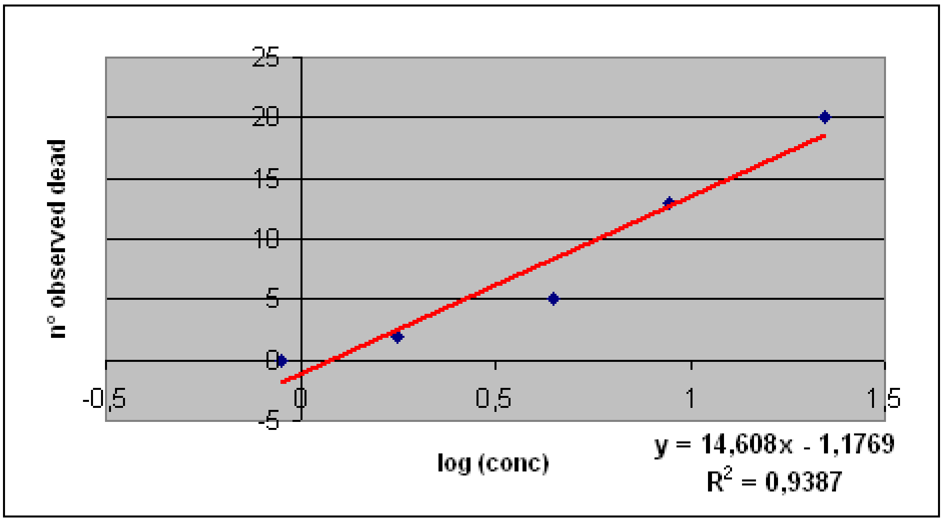
ENDPOINT	CONC	CONFIDENCE RANGE (95%)	
		upper	lower
EC50	**6,6877**	4,9057	9,1619

Figure 3.31: data elaboration of *Daphnia magna* test on 2-chloronitrobenzene.

DAPHNIA MAGNA STRAUS ACUTE TOXICITY TEST
final test
2-chloronitrobenzene **88-73-3**

conc	log(conc)	n° organism	n° obs dead org	n° estim dead org
0,89	-0,0506	20	0	0,28
1,78	0,24944	20	2	1,49
4,44	0,64738	20	5	6,46
8,88	0,94841	20	13	12,26
22,2	1,34635	20	20	17,98
Controllo 1		20	0	0
Controllo 2		20	0	0





y = ponderated probits   y=a+bx
x = ponderated log(conc)

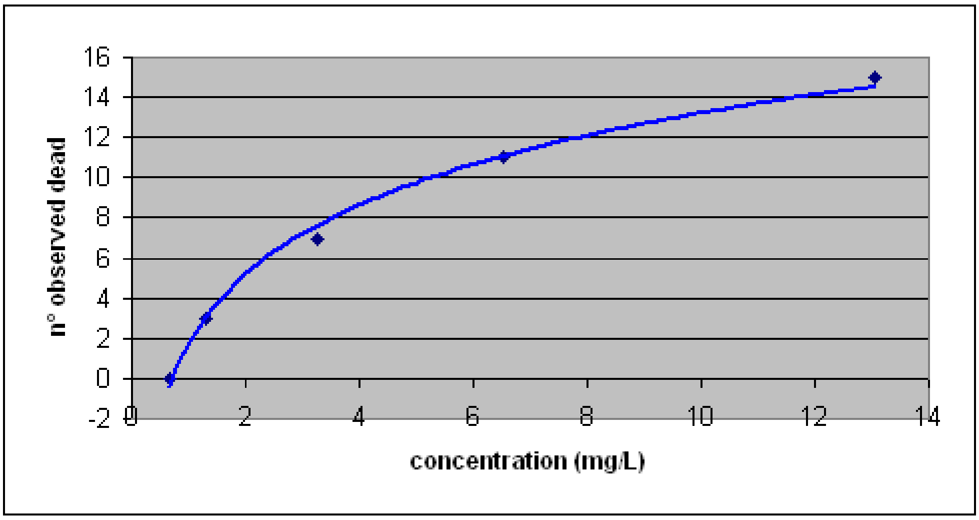
ENDPOINT	**CONC**	**CONFIDENCE RANGE (95%)**	
		upper	**lower**
EC50	**6,2596**	4,8747	8,1026

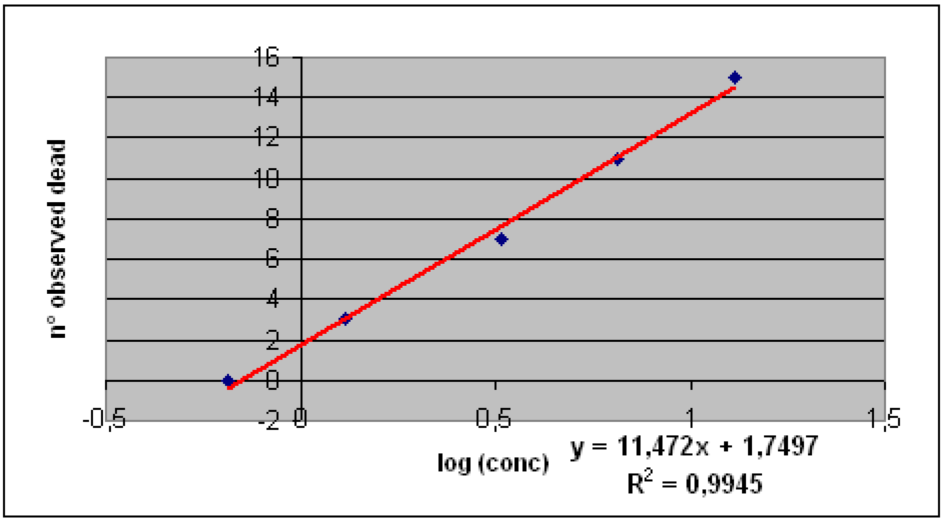
Figure 3.32: data elaboration of *Daphnia magna* test on 3-aminobenzotrifluoride.

DAPHNIA MAGNA STRAUS ACUTE TOXICITY TEST

3-aminobenzotrifluoride **final test**
98-16-8

conc	log(conc)	n° organism	n° obs dead org	n° estim dead org
0,65	-0,1871	20	0	1,13
1,31	0,11727	20	3	2,89
3,27	0,51455	20	7	7,07
6,53	0,81491	20	11	11,12
13,06	1,11594	20	15	14,9
Controllo 1		20	0	0
Controllo 2		20	0	0





y = ponderated probits y=a+bx a=3.5633 b=1,9366
x = ponderated log(conc)

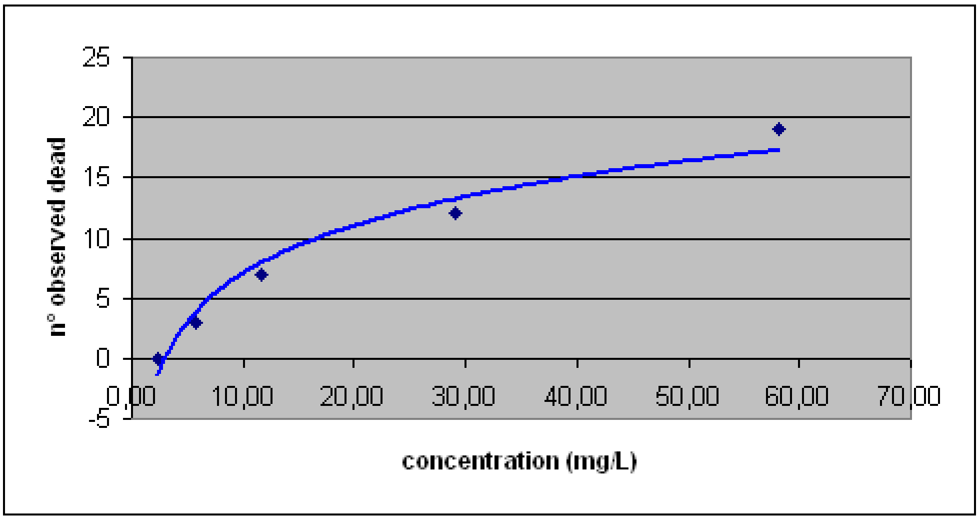
ENDPOINT	**CONC**	**CONFIDENCE RANGE (95%)**	
		upper	**lower**
EC50	**5,5191**	3,9531	8,4887

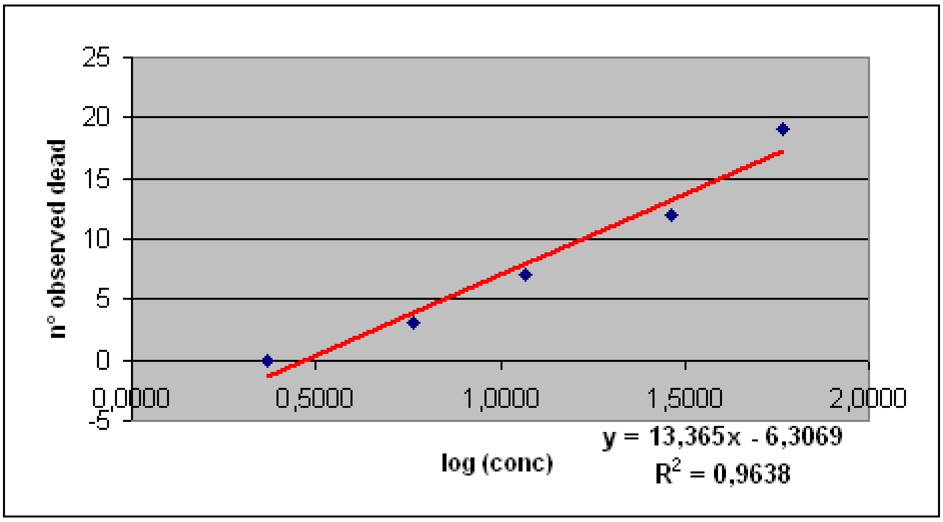
Figure 3.33: data elaboration of *Daphnia magna* test on 3-nitrobenzotrifluoride.

DAPHNIA MAGNA STRAUS ACUTE TOXICITY TEST

3-nitrobenzotrifluoride **final test** **98-46-4**

conc	log(conc)	n° organism	n° obs dead org	n° estim dead org
2,33	0,3674	20	0	0,41
5,82	0,7649	20	3	2,64
11,64	1,0660	20	7	6,74
29,1	1,4639	20	12	13,83
58,2	1,7649	20	19	17,69
Controllo 1		20	0	0
Controllo 2		20	0	0





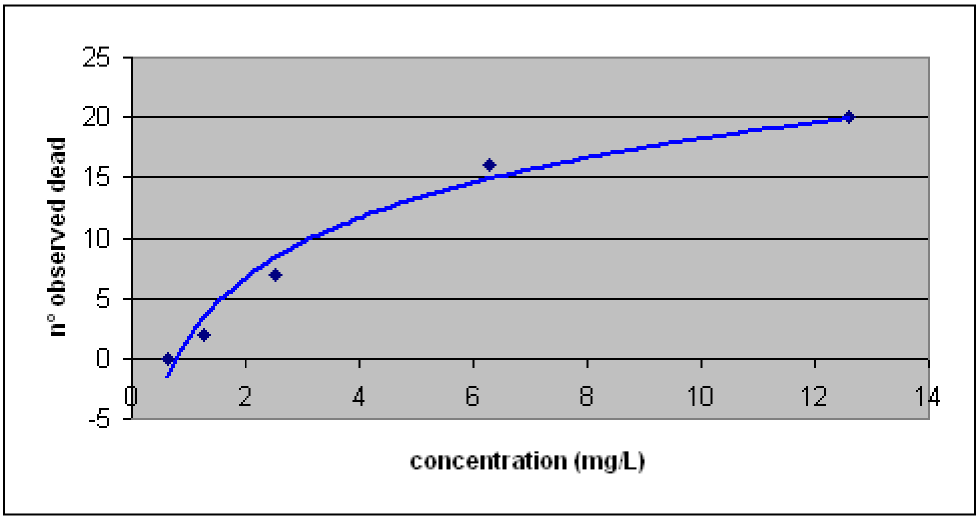
y = ponderated probits y=a+bx
x = ponderated log(conc)

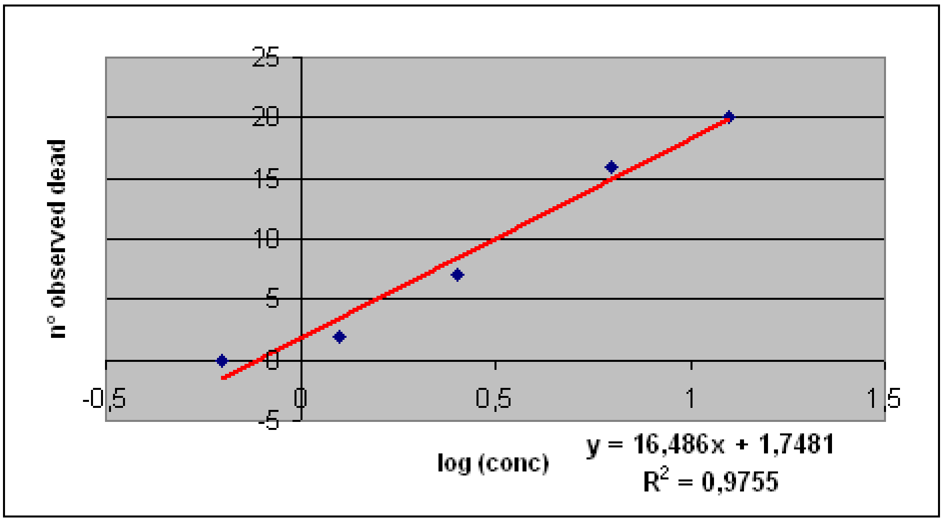
ENDPOINT	CONC	CONFIDENCE RANGE (95%)	
		upper	**lower**
EC50	**17,7425**	13,3032	24,1845

Figure 3.34: data elaboration of *Daphnia magna* test on 3-amino-4-chlorobenzotrifluoride.

DAPHNIA MAGNA STRAUS ACUTE TOXICITY TEST
final test
3-amino-4-chlorobenzotrifluoride 121-50-6

conc	log(conc)	n° organism	n° obs dead org	n° estim dead org
0,63	-0,2007	20	0	0,26
1,26	0,10037	20	2	1,92
2,52	0,4014	20	7	7,06
6,29	0,79865	20	16	16,03
12,58	1,09968	20	20	19,24
Controllo 1		20	0	0
Controllo 2		20	0	0





y = ponderated probits y=a+bx
x = ponderated log(conc)

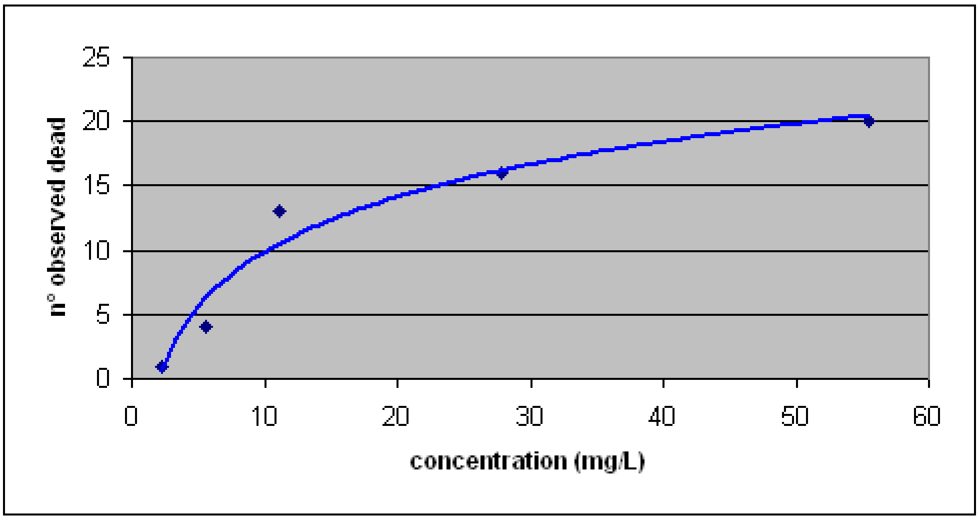
ENDPOINT	**CONC**	**CONFIDENCE RANGE (95%)**	
		upper	**lower**
EC50	**3,2552**	2,5764	4,1295

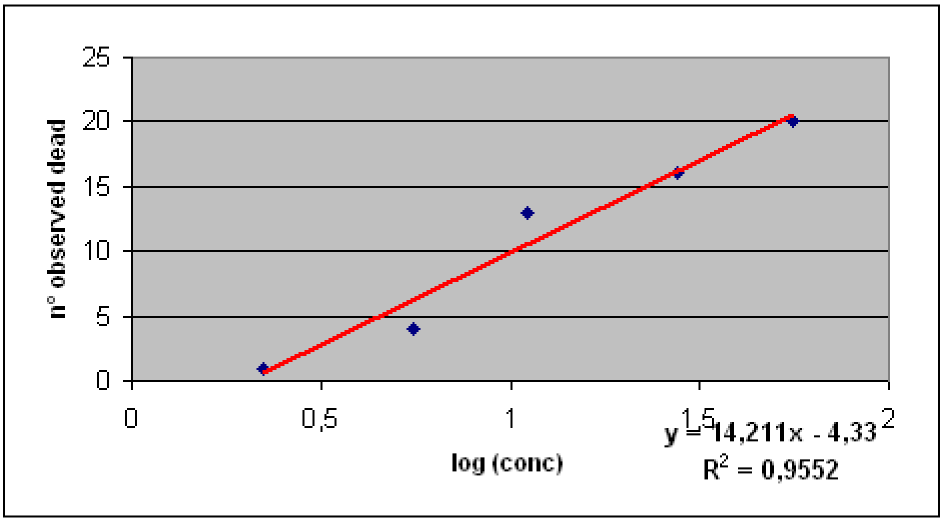
Figure 3.35: data elaboration of *Daphnia magna* test on 3-nitro-4-chlorobenzotrifluoride.

DAPHNIA MAGNA STRAUS ACUTE TOXICITY TEST

3-nitro-4-chlorobenzotrifluoride **final test** **121-17-5**

conc	log(conc)	n° organism	n° obs dead org	n° estim dead org
2,22	0,34635	20	1	1,08
5,55	0,74429	20	4	5,15
11,1	1,04532	20	13	10,57
27,75	1,44326	20	16	16,95
55,5	1,74429	20	20	19,2
Controllo 1		20	0	0
Controllo 2		20	0	0





y = ponderated probits y=a+bx
x = ponderated log(conc)

ENDPOINT	CONC	CONFIDENCE RANGE (95%)	
		upper	lower
EC50	**9,9950**	7,4529	13,2015

The EC_{50} results on *Daphnia magna* tests are summarized on table 3.28.

Table 3.28: EC_{50} values obtained from *Daphnia magna* tests.

SUBSTANCE / CAS	ENDPOINT	CONC mg/L	LOWER CONF. LIMIT (95%)	UPPER CONF. LIMIT (95%)
2-chloroaniline 95-51-2	**EC_{50}**	**6.69**	4.91	9.16
2-chloronitrobenzene 88-73-3	**EC_{50}**	**6.26**	4.87	8.10
3-aminobenzotrifluoride 98-16-8	**EC_{50}**	**5.52**	3.95	8.49
3-nitrobenzotrifluoride 98-46-4	**EC_{50}**	**17.74**	13.30	24.18
3-amino-4-chlorobenzotrifluoride 121-50-6	**EC_{50}**	**3.26**	2.58	4.13
3-nitro-4-chlorobenzotrifluoride 121-17-5	**EC_{50}**	**10.00**	7.45	13.20

Data obtained were compared with Kuhn results[115] that found an EC_{50} value of 4.2 mg/L (P 95%: 2.4 ± 7.5) for 2-chloroaniline and EC_{50} of 6.6 mg/L (P 95%: 2.6 ± 17) for 2-aminobenzotrifluoride.

The EC_{50} shows values in the 3.3÷ 17.7 mg/L range with an outward greater toxicity of amino compounds than the corresponding nitro-substituted.

Daphnia magna were tested on three groundwater samples collected from the Agno Valley area (L1, L2, L3). Five organisms were tested on each environmental sample and on their dilution 1:2. The procedure was replicated four times with the control of blank solutions and $K_2Cr_2O_7$ solutions as described on paragraph 2.3.2. For all the samples tested, no organisms' death or mobility inhibition was recorded. Finally no effects were detectable for the presence of 3‰ of methanol in the blank solution.

3.3.2 Inhibition of light emission of *Vibrio fisheri* test results

The elaboration of results for *Vibrio fischeri* tests of substances listed in table 2.8 permitted to obtain a concentration/response curve useful to extrapolate the threshold parameters of EC_{50} and EC_{20} for acute toxicity evaluation. A preliminary screening for all stock solutions was made before the final definitive experiments.

Correction factors (*f*) were calculated using the equation (1) based on 30 minutes exposition time:

$$f = \frac{I_{kt}}{I_0} \quad (t = 30 \text{ mins}) \qquad (1)$$

where I_{kt} is the light intensity measured in luminescence relative units on the control and I_0 is the luminescence measured before the addition of control solution. With more control samples, the average value of them was considered. The initial luminescence (I_0) of each cell was corrected using formula (2) obtaining I_c value:

$$I_C = I_0 \cdot f \qquad (2)$$

The percentage of inhibition (*H*) for each point was, afterwards, calculated as:

$$H = 100 \cdot \frac{I_C - I_t}{I_C} \qquad (3)$$

where I_t was the relative light intensity in luminescence relative units measured for each dilution tested after 30 minutes of exposition. To estimate the effect/concentration correlation, the *H* values were transformed in gamma values (Γ) using (4) equation:

$$\Gamma = \frac{H}{100 - H} \qquad (4)$$

The Γ value was not calculated for *H* values of 0% and 100% and normally values in the 10-90% range were used for the calculation of the concentration/effect correlation. The gamma value (Γ) was correlated with the concentration percentage (*c*) using the linear equation (5):

$$Log\ (c) = b \cdot Log\ \Gamma + Log\ a \qquad (5)$$

With *b* as slope of the toxicity curve and *a* intercept with x axes.

The EC values with the relative confidential range were calculate with the minimum square method:

$$c = EC_{50} \quad \text{with} \quad \Gamma = 1.00 \qquad (6)$$

$$c = EC_{20} \quad \text{with} \quad \Gamma = 0.25 \qquad (7)$$

Blank samples were carried out with dilution water and with Milli-Q water + 3‰ of methanol to assess the effect of the solvent used in the initial stock solutions. A weak hormesis effects was notable in Milli-Q water with addition of methanol with gamma values <0.00 (Γ = -0.1521 ± 0.004, n=6) and an average % effect of -17.93% of inhibition.

The results of the effects of substances tested with *Vibrio fisheri* are shown on the figures below and table 3.29 summarize the EC_{20} and EC_{50} values extrapolated.

Table 3.29: summary of EC_{50} and EC_{20} results obtained from *Vibrio fisheri* tests

SUBSTANCE / CAS	Concentration 100% [mg/L]	EC50 %	EC50 (EC20) [mg/L]	LOWER CONF. LIMIT (95%)*	UPPER CONF. LIMIT (95%)*
2-chloroaniline 95-51-2	56.20	29.34	**16.49 (4.99)**	16.40	17.2
2-chloronitrobenzene 88-73-3	22.20	22.77	**5.05 (1.30)**	4.82	5.45
3-aminobenzotrifluoride 98-16-8	32.65	38.63	**12.61 (3.83)**	11.41	13.06
3-nitrobenzotrifluoride 98-46-4	58.20	28.62	**16.66 (4.50)**	16.35	17.33
3-amino-4-chlorobenzotrifluoride 121-50-6	6.29	34.21	**2.15 (0.62)**	2.14	2.26
3-nitro-4-chlorobenzotrifluoride 121-17-5	55.50	50.68	**28.13 (5.93)**	26.33	30.13

* the lower and upper confidential limits are in mg/L and referred to EC50 value

For the validation criteria the controls with toxicant references (3,5-dichlorophenol, $ZnSO_4 \cdot 7H_2O$ and $K_2Cr_2O_7$ solutions) in the concentrations described on paragraph 2.3.3, were performed with each batch of test.

Figure 3.36: data elaboration of *Vibrio fisheri* test on 2-chloroaniline.

Sample: 95-51-2 2-chloroaniline

Calculation on 30 mins data

Sample	Conc %	Conc mg/L	Io	Ic=Io*f	It	H=100*(Ic-It)/Ic	Gamma	%effect
Control	0		91,92	130,61832	130,58	0,0293	0,0002935	0,0293
1	8,18	4,59716	90,61	128,75681	105,11	18,3655	0,224972	18,3655
2	16,36	9,19432	92,86	131,95406	86,46	34,4772	0,5261862	34,4772
3	24,54	13,79148	91,47	129,97887	72,15	44,4910	0,8015089	44,4910
4	32,72	18,38864	89,71	127,47791	59,28	53,4978	1,1504371	53,4978
5	40,9	22,9858	94,14	133,77294	54,83	59,0126	1,4397764	59,0126
6	49,09	27,58858	93,77	133,24717	48,92	63,2863	1,723777	63,2863
7	57,27	32,18574	92,25	131,08725	41,65	68,2273	2,1473529	68,2273
8	65,45	36,7829	85,64	121,69444	37,09	69,5220	2,281058	69,5220
9	73,63	41,38006	88,16	125,27536	34,15	72,7401	2,6683854	72,7401
10	81,81	45,97722	90,29	128,30209	32,24	74,8718	2,9795934	74,8718

100% = 56,2 mg/L

Correction factor (f): 1,421

y = a ln(x) + b

y=50 à x=EC50

a = 25,081

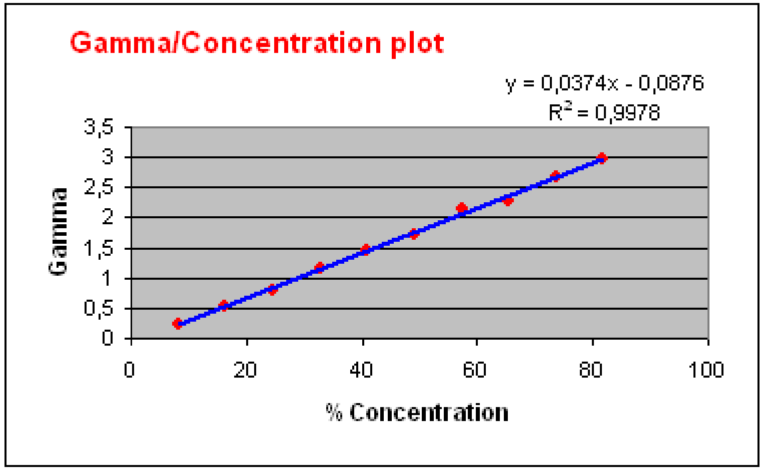
b = -34,748

ln(x) = 3,3789721

x = 29,340597

EC50 = 29,34 of 56,2 mg/L

EC50 = 16,49 mg/L **16.49**



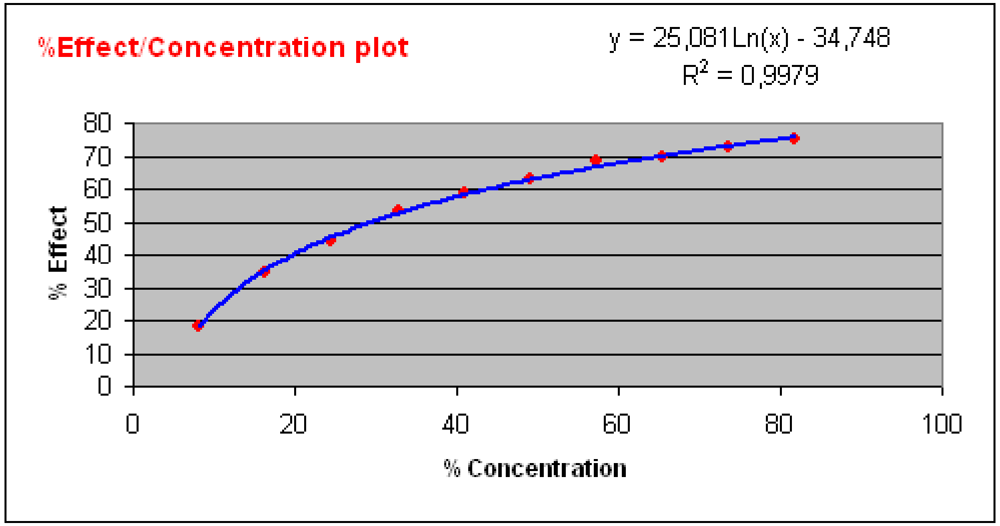


Figure 3.37: data elaboration of *Vibrio fisheri* test on 2-chloronitrobenzene.

Sample: 88-73-3 2-chloronitrobenzene

Sample	Conc %	Conc mg/L	Io	Ic=Io*f	It	H=100*(Ic-It)/Ic	Gamma	%effect
Control	0		96,98	105,61122	105,58	0,0296	0,0002957	0,0296
1	8,18	1,81596	94,8	103,2372	77,55	24,8817	0,331234	24,8817
2	16,36	3,63192	95,1	103,5639	59,07	42,9628	0,7532402	42,9628
3	24,54	5,44788	93,38	101,69082	46,4	54,3715	1,1916125	54,3715
4	32,72	7,26384	92,9	101,1681	40,61	59,8589	1,4912115	59,8589
5	40,9	9,0798	94,72	103,15008	38,17	62,9957	1,7023862	62,9957
6	49,09	10,89798	95,53	104,03217	33,17	68,1156	2,1363331	68,1156
7	57,27	12,71394	91,93	100,11177	29,94	70,0934	2,3437465	70,0934
8	65,45	14,5299	90,85	98,93565	26,58	73,1341	2,722184	73,1341
9	73,63	16,34586	91,92	100,10088	25,12	74,9053	2,9849076	74,9053
10	81,81	18,16182	98,01	106,73289	25,26	76,3334	3,2253717	76,3334

Calculation on 30 mins data

100% = 22,2 mg/L

Correction factor (f): 1,089

y = a ln(x) + b

y=50 à x=EC50

a = 22,109

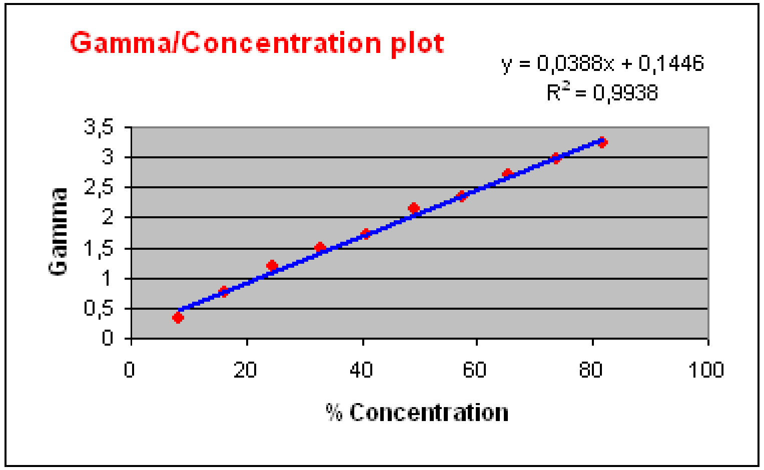
b = -19,098

ln(x) = 3,1253336

x = 22,767488

EC50 = 22,77 of 22,2 mg/L

EC50 = 5,05 mg/L 5.05



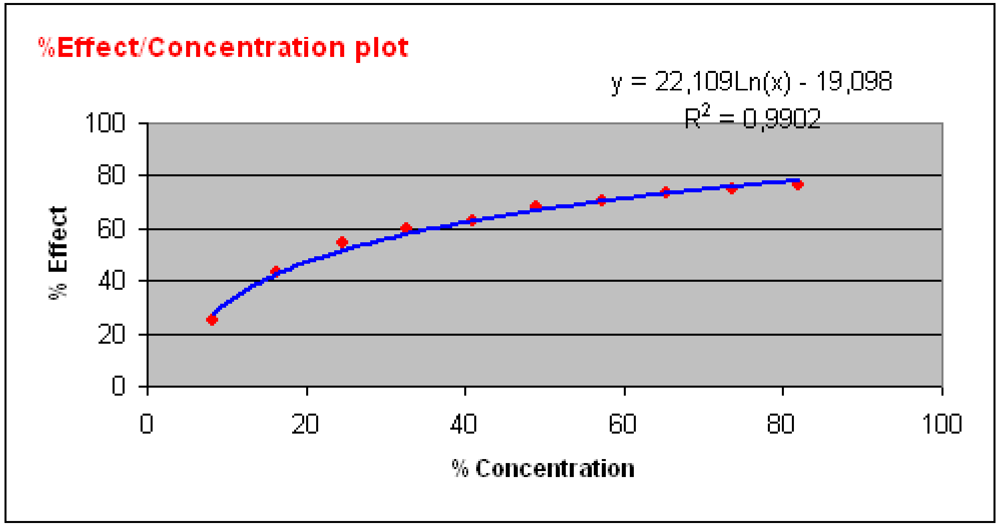


Figure 3.38: data elaboration of *Vibrio fisheri* test on 3-aminobenzotrifluoride.

Sample: 98-16-8 3-aminobenzotrifluoride

Sample	Conc %	Conc mg/L	Io	Ic=Io*f	It	H=100*(Ic-It)/Ic	Gamma	%effect
Control	0		102,11	113,95476	117,78	-3,3568	-0,0324778	-3,3568
1	8,18	2,67077	93,93	104,82588	93,59	10,7186	0,1200543	10,7186
2	16,36	5,34154	87,5	97,65	69,89	28,4281	0,3971956	28,4281
3	24,54	8,01231	91,52	102,13632	62,16	39,1402	0,6431197	39,1402
4	32,72	10,68308	91,48	102,09168	57,4	43,7760	0,7786007	43,7760
5	40,9	13,35385	94,81	105,80796	50,35	52,4138	1,1014491	52,4138
6	49,09	16,027885	92,41	103,12956	44,51	56,8407	1,3169975	56,8407
7	57,27	18,698655	84,87	94,71492	37,73	60,1647	1,5103345	60,1647
8	65,45	21,369425	90,86	101,39976	34,47	66,0058	1,9416815	66,0058
9	73,63	24,040195	88,25	98,487	33,89	65,5894	1,9060785	65,5894
10	81,81	26,710965	88,26	98,49816	33,09	66,4055	1,9766745	66,4055

Calculation on 30 mins data

100% = 32,65 mg/L

Correction factor (f): 1,116

y = a ln(x) + b

y=50 à x=EC50

a = 25,145

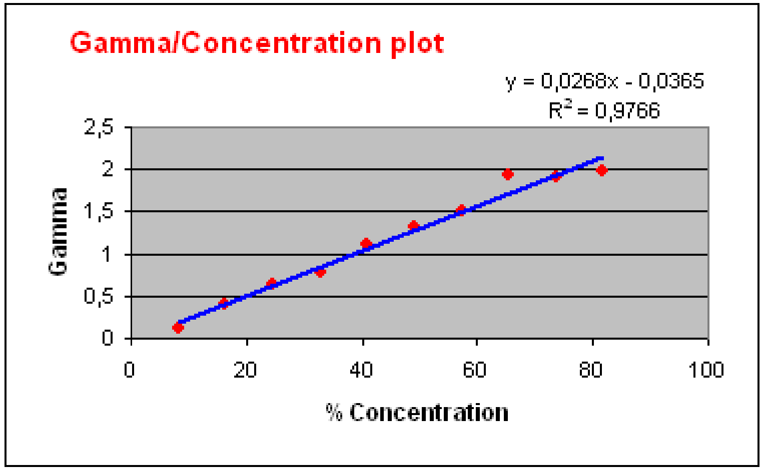
b = -41,879

ln(x) = 3,653967

x = 38,627598

EC50 = 38,63 of 32,65 mg/L

EC50 = 12,61 mg/L 12.61



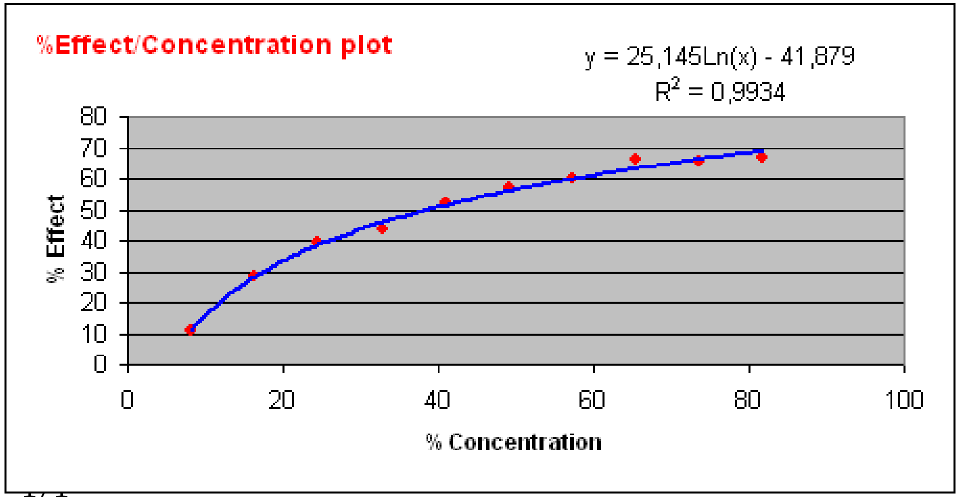


Figure 3.39: data elaboration of *Vibrio fisheri* test on 3-nitrobenzotrifluoride.

Sample: 98-46-4 3-nitrobenzotrifluoride

Sample	Conc %	Conc mg/L	Io	Ic=Io*f	It	H=100*(Ic-It)/Ic	Gamma	%effect
Control	0		96,2	114,5742	114,54	0,0298	0,000299	0,0298
1	8,18	4,76076	96,91	115,4198	88,46	23,3580	0,304768	23,3580
2	16,36	9,52152	97,23	115,8009	75,13	35,1214	0,541341	35,1214
3	24,54	14,28228	94,56	112,621	61,49	45,4009	0,831533	45,4009
4	32,72	19,04304	94,72	112,8115	54,18	51,9730	1,082162	51,9730
5	40,9	23,8038	95,93	114,2526	47,44	58,4780	1,408361	58,4780
6	49,09	28,57038	96,59	115,0387	42,14	63,3688	1,729917	63,3688
7	57,27	33,33114	91,2	108,6192	37,02	65,9176	1,934068	65,9176
8	65,45	38,0919	92,97	110,7273	33,94	69,3481	2,262442	69,3481
9	73,63	42,85266	91,98	109,5482	30,54	72,1219	2,587039	72,1219
10	81,81	47,61342	87,25	103,9148	27	74,0172	2,848694	74,0172

Calculation on 30 mins data

100% = 58,2 mg/L

Correction factor (f): 1,191

y = a ln(x) + b

y=50 → x=EC50

a = 22,912

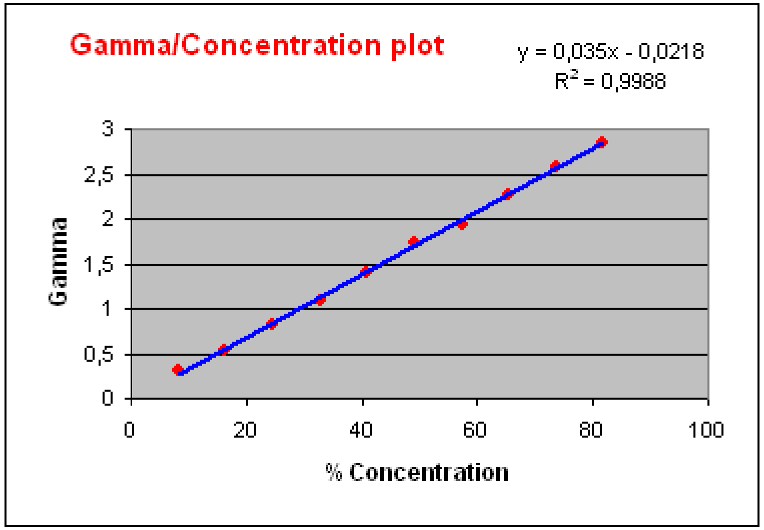
b = -26,852

ln(x) = 3,354225

x = 28,62341

EC50 = 28,62 of 58,2 mg/L

EC50 = 16,66 mg/L 16.66



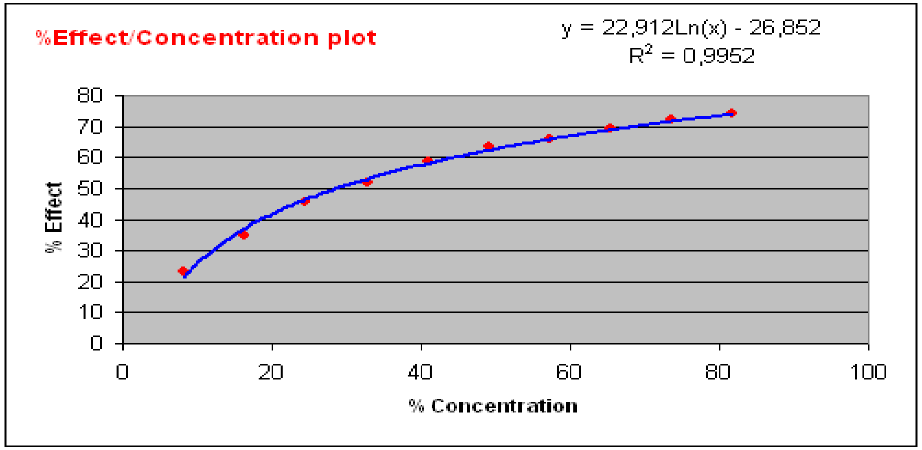


Figure 3.40: data elaboration of *Vibrio fisheri* test on 3-amino-4-chlorobenzotrifluoride.

Sample: 121-50-6 3-amino-4-chlorobenzotrifluoride

Sample	Conc %	Conc mg/L	Io	Ic=Io*f	It	H=100*(Ic-It)/Ic	Gamma	%effect
Control	0		94,95	97,9884	97,98	0,0086	8,57E-05	0,0086
1	8,18	0,514522	88,39	91,21848	75,64	17,0782	0,205956	17,0782
2	16,36	1,029044	87,29	90,08328	61,19	32,0740	0,47219	32,0740
3	24,54	1,543566	89,94	92,81808	55,41	40,3026	0,675114	40,3026
4	32,72	2,058088	85,6	88,3392	45,75	48,2110	0,930911	48,2110
5	40,9	2,57261	88,55	91,3836	42,04	53,9961	1,17373	53,9961
6	49,09	3,087761	84,3	86,9976	36,91	57,5735	1,35702	57,5735
7	57,27	3,602283	85,93	88,67976	33,34	62,4040	1,659861	62,4040
8	65,45	4,116805	75,83	78,25656	26,12	66,6226	1,99604	66,6226
9	73,63	4,631327	77,63	80,11416	24,37	69,5809	2,287409	69,5809
10	81,81	5,145849	70,46	72,71472	20,84	71,3401	2,48919	71,3401

Calculation on 30 mins data

100% = 6,29 mg/L

Correction factor (f): 1,032

y = a ln(x) + b

y=50 → x=EC50

a = 24,054

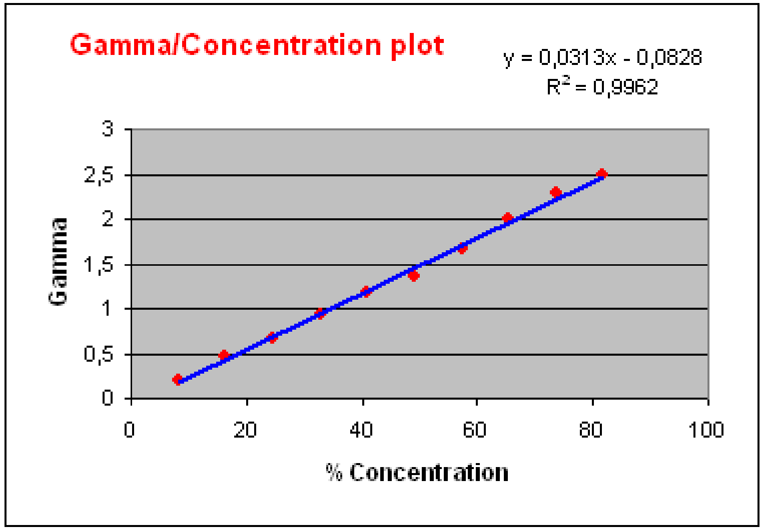
b = -34,969

ln(x) = 3,532427

x = 34,20689

EC50 = 34,21 of 6,29 mg/L

EC50 = 2,15 mg/L 2.15



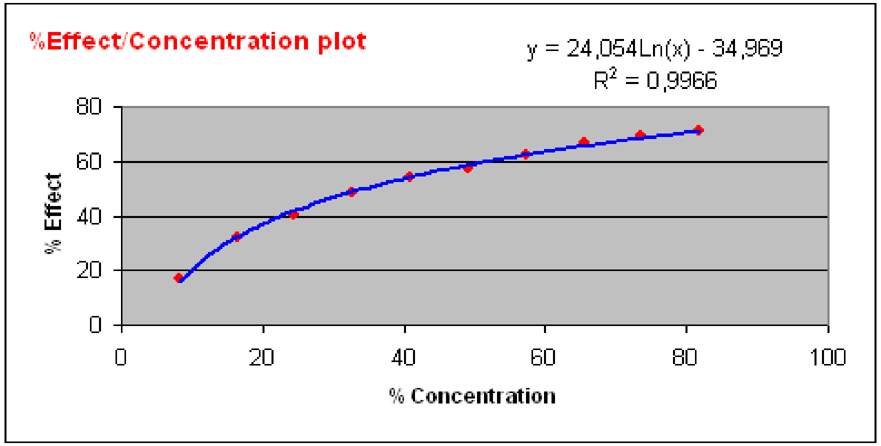


Figure 3.41: data elaboration of *Vibrio fisheri* test on 3-nitro-4-chlorobenzotrifluoride.

Sample: 121-17-5 3-nitro-4-chlorobenzotrifluoride

Sample	Conc %	Conc mg/L	Io	Ic=Io*f	It	H=100*(Ic-It)/Ic	Gamma	%effect
Control	0		96,91	102,9184	102,92	-0,0015	-1,5E-05	-0,0015
1	8,18	4,5399	93,31	99,09522	82,96	16,2825	0,194494	16,2825
2	16,36	9,0798	91,22	96,87564	68,75	29,0327	0,4091	29,0327
3	24,54	13,6197	90,93	96,56766	63	34,7608	0,53282	34,7608
4	32,72	18,1596	90,13	95,71806	60,22	37,0861	0,589473	37,0861
5	40,9	22,6995	98,21	104,299	57,09	45,2631	0,826923	45,2631
6	49,09	27,24495	93,39	99,18018	47,7	51,9057	1,079249	51,9057
7	57,27	31,78485	92,33	98,05446	46,87	52,2000	1,092052	52,2000
8	65,45	36,32475	91,01	96,65262	43,19	55,3142	1,237847	55,3142
9	73,63	40,86465	89,56	95,11272	40,05	57,8921	1,374849	57,8921
10	81,81	45,40455	96,3	102,2706	41,05	59,8614	1,491367	59,8614

Calculation on 30 mins data

100% = 55,5 mg/L

Correction factor (f): 1,062

y = a ln(x) + b

y=50 → x=EC50

a = 19,276

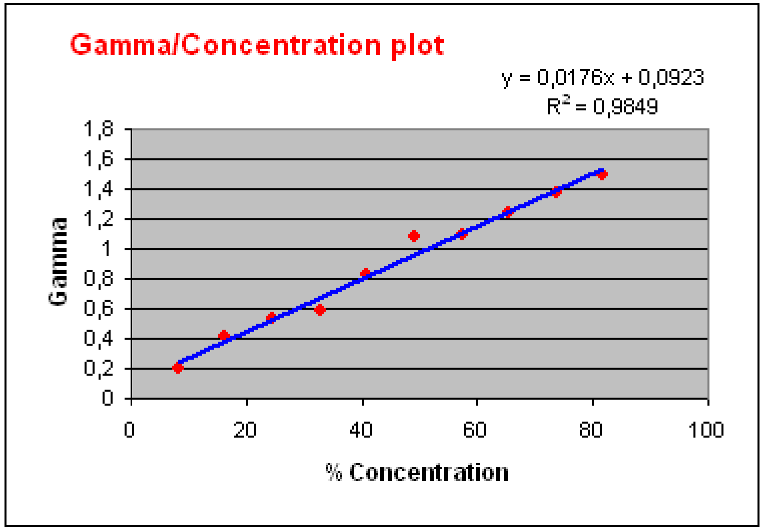
b = -25,669

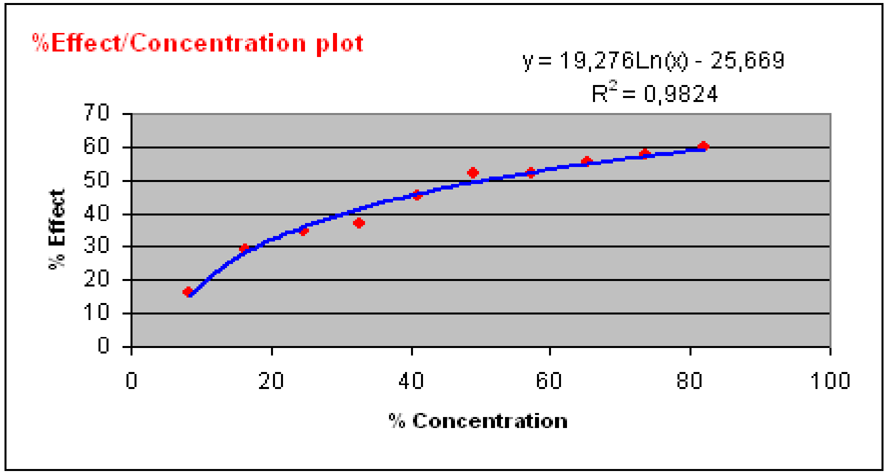
ln(x) = 3,925555

x = 50,6812

EC50 = 50,68 of 55,5 mg/L

EC50 = 28,13 mg/L 28.13





The tests were under QA/QC control when the inhibition effect of the toxicant references were in the range of 20÷80% at the following concentrations: 3.4 mg/L (3,5-dichlorohenol solution), 2.2 mg/L ($ZnSO_4 \cdot 7H_2O$ solution) and 18.7 mg/L ($K_2Cr_2O_7$ solution). Moreover also the EN ISO 11348-3:2001 procedure quality criteria, as correction factor *f* in the range 0.6÷1.8 and $R^2 \geq 0.95$ of the curves, were verified for all the six compounds.

The EC_{50} values extrapolated were in the range of 2-30 mg/L. For BTFs substances, as in *Daphnia magna* tests, the amino-compounds were more toxic than the correspondent nitro-compounds; while the toxicity on Vibrio of 2CNB is four time greater than the corrispective 2CA.

The procedure was tested also on three environmental groundwater samples.

The screening test was carried out with samples: L1, L2 and L3. All the samples were from Trissino area. Sample L3 was used as blank for the chemical analysis because collected at north of the area interested by the pollution episode. Sample L1 was the most polluted sample monitored with concentration of volatile BTF and non-volatile BTF explained in Paragraph 3.2. The screening tests on these solutions didn’t show any inhibition effect on the values of light emitted by Vibrio. These results can be explainable because the chemical contamination doesn’t exceed 15 μg/L for single contaminant in the most polluted sample. The concentrations of chemicals on the environmental samples were not comparable with the EC_{50} levels of pure substances in range between 2-30 mg/L.

An additional test at the EC_{50} values of concentration of 3-amino-4-chlorobenzotrifluoride (EC_{50} = 2.16 mg/L) and 3-nitro-4-chlorobenzotrifluoride (EC_{50} = 28.13 mg/L) was made to check possible synergetic effects of their mixture. Three tests on solutions of mixture of 3A4CBTF and 3N4CBTF in the ratio 1:1, 1:2, and 1:3 of their EC_{50} values were performed recording the final EC_{50} values obtained for each mixture after the tests. The results were in the range of 50±7 % of effects showing a simple additional effect of the two compounds mixed without any synergetic effect when combined together.

3.3.3 Growth inhibition test with unicellular green algae results

The *Selenastrum capricornutum* test on 72h can assess effects over several generations of the unicellular green algal species. It is possible to determine the value of EC_{50}-72h as well, with a statistical elaboration, the median parameter NOEC-72h. The EC_{10}-72h and EC_{20}-72h meaning as % of growth inhibition after 72h compared to the initial control can also used instead of NOEC or LOEC values. Recent scientific developments have led to a recommendation of abandoning the concept of NOEC replacing it with regression based point estimates EC_x. However an appropriate value for x has not been established for algal test[117]. A range of 10 to 20 % appears to be appropriate and preferably both the EC_{10} and EC_{20} should be reported.

A screening test, with three dilutions (1:10, 1:100; 1:1000) of each stock solution of principle active, prepared as described in table 2.8, was performed to find the best operative range. From these preliminary tests on the six substances considered was deducted that the best operative range for EC_{50} values was between the stock solutions concentrations and their 1:10 dilutions. Subsequently a definitive test was made for each chemical. The dilution considered were five: 1 (100%), 3/4 (75%), 1/2 (50%), 1/4 (25%) and 1/10 (10%) of each stock solution. After the preparation of the reagent and media solutions, the nutrients solutions, the growth medium and the pre-culture, the definitive tests with their control batches were carried out for the six substances. The tests were carried out in triplicate. The cell density of each test solutions was measured every 24h and stopped at 72h ± 2h. Cell density measurements were tabulate according to the concentration of test sample and the time of measurement. The testing method describes the growth rate as response variable to evaluate the effects. The average specific growth rate is the response variable calculated on the basis of the logarithmic increase of biomass during the test period, expressed per day.

A growth curve for each test concentration and control was plotted as graph of the logarithm of the mean call density against time (n=3). A linear growth curve indicated exponential growth whereas a levelling off indicated that cultures entered the stationary phase. First of all the average specific growth rates μ, for each test culture, was calculated using equation (8):

$$\mu = (\ln x_L - \ln x_0) / (t_L - t_0) \qquad (8)$$

where t_0 was the time of test start , t_L was the time of test termination (or the time of the last measurement within the exponential growth period in the control cultures), x_0 was the nominal initial cell density and x_L was the measured cell density at the t_L time. The mean value of μ was calculated for each test and control batch replicate in day^{-1} units. From these values, the inhibition percentage for each test batch replicate was determined from equation (9).

$$I_\mu = 100 \cdot (\mu_C - \mu_i) / \mu_C \qquad (9)$$

where I_μ was the percentage inhibition (growth rate) for the test concentration i, μ_i was the mean growth rate for test concentration $i$and μ_C was the mean growth rate of the control.

The results of the effects of substances considered, about the growth inhibition of *Selenastrum capricornutum*, are shown on the figures below.

Figure 3.42: results of the 72h growth inhibition of 2CA on *Selenastrum capricornutum*.

2-CHLOROANILINE 95-51-2

intensità luce (lux)	7000	qualità luce
temperatura (℃)	18-22	
metodo di misurazione della concentrazione cellulare		contaglobuli Coulter Z1

Risultati	Tempo (giorni)	n. cellule n.x10^3/mL			
		replica 1	replica 2	replica 3	
controllo	1	27	28	24	
	2	82	86	90	
	3	378	410	413	
		replica 4	replica 5	replica 6	media
	1	26	26	26	26
	2	78	81	84	84
	3	380	395	395	395
		replica 1	replica 2	replica 3	media
conc. 10 %	1	26,00	28,00	24,00	26
	2	76,00	80,00	89,00	82
	3	237,00	210,00	217,00	221
conc. 25 %	1	21,00	24,00	23,00	23
	2	77,00	80,00	77,00	78
	3	194,00	210,00	198,00	201
conc. 50 %	1	24,00	26,00	24,00	25
	2	71,00	76,00	76,00	74
	3	160,00	135,00	149,00	148
conc. 75 %	1	20,00	21,00	19,00	20
	2	30,00	37,00	37,00	35
	3	60,00	74,00	62,00	65
conc. 100 %	1	15,00	15,00	16,00	15
	2	21,00	20,00	20,00	20
	3	51,00	50,00	45,00	49

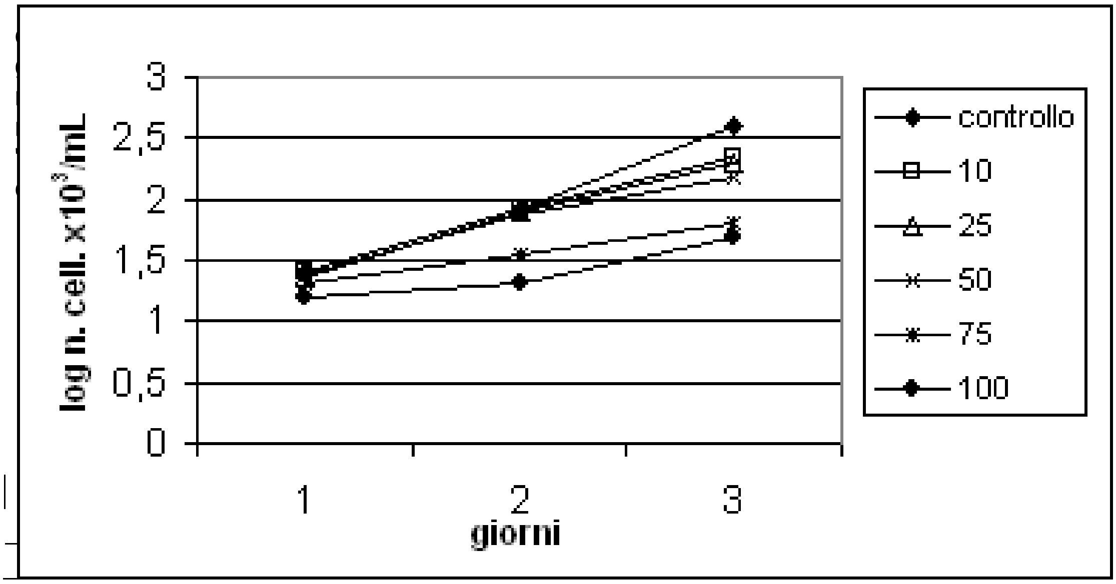


Figure 3.43: results of the 72h growth inhibition of 2CNB on *Selenastrum capricornutum*.

2-CHLORONITROBENZENE 88-73-3

intensità luce (lux)	7000	qualità luce
temperatura (℃)	18-22	
metodo di misurazione della concentrazione cellulare		contaglobuli Coulter Z1

Risultati	Tempo (giorni)	n. cellule n.x10^3/mL			
		replica 1	replica 2	replica 3	
controllo	1	27	28	24	
	2	82	86	90	
	3	378	410	413	
		replica 4	replica 5	replica 6	media
	1	26	26	26	26
	2	78	81	84	84
	3	380	395	395	395
		replica 1	replica 2	replica 3	media
conc.	1	26,00	28,00	26,00	27
10	2	90,00	97,00	92,00	93
%	3	398,00	410,00	380,00	396
conc.	1	26,00	23,00	24,00	24
25	2	95,00	94,00	82,00	90
%	3	367,00	365,00	340,00	357
conc.	1	24,00	24,00	23,00	24
50	2	90,00	87,00	80,00	86
%	3	250,00	300,00	280,00	277
conc.	1	19,00	23,00	22,00	21
75	2	50,00	52,00	50,00	51
%	3	200,00	187,00	204,00	197
conc.	1	12,00	16,00	18,00	15
100	2	24,00	28,00	18,00	23
%	3	40,00	44,00	50,00	45

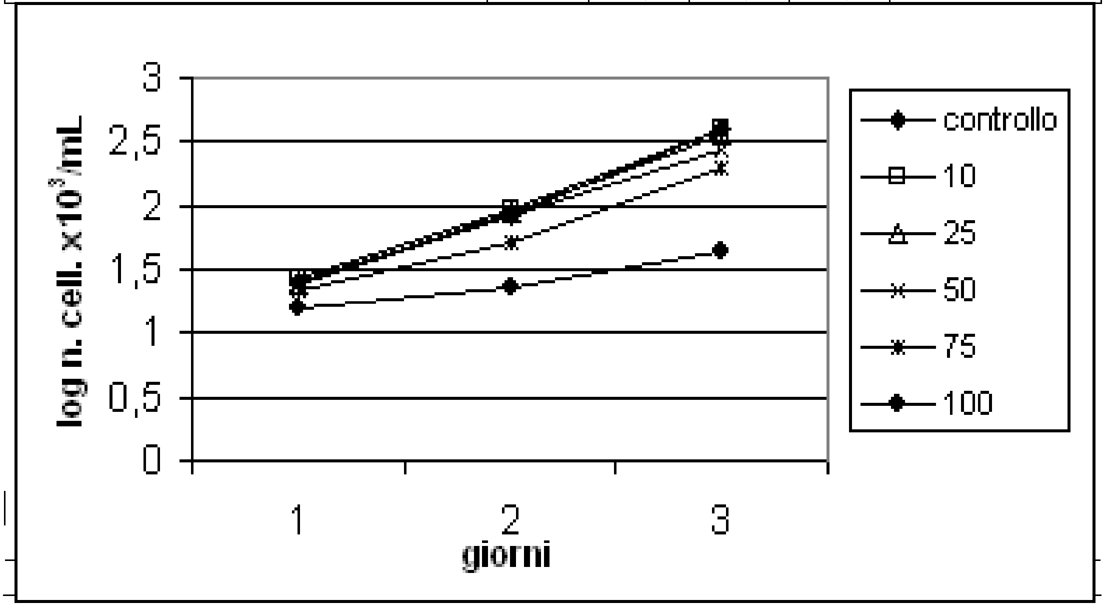


Figure 3.44: results of the 72h growth inhibition of 3ABTF on *Selenastrum capricornutum*.

3-AMINOBENZOTRIFLUORIDE 98-16-8

intensità luce (lux)	7000	qualità luce
temperatura (℃)	18-22	
metodo di misurazione della concentrazione cellulare		contaglobuli Coulter Z1

Risultati	Tempo (giorni)	n. cellule n.x10^3/mL			
		replica 1	replica 2	replica 3	
controllo	1	22	25	21	
	2	46	49	46	
	3	330	323	301	
		replica 4	replica 5	replica 6	media
	1	24	20	23	23
	2	36	39	43	43
	3	298	313	299	311
		replica 1	replica 2	replica 3	media
conc. 10 %	1	27,00	23,00	22,00	24
	2	44,00	38,00	40,00	41
	3	368,00	352,00	361,00	360
conc. 25 %	1	22,00	28,00	23,00	24
	2	52,00	58,00	56,00	55
	3	226,00	213,00	190,00	210
conc. 50 %	1	21,00	18,00	18,00	19
	2	37,00	33,00	33,00	34
	3	37,00	33,00	33,00	34
conc. 75 %	1	17,00	13,00	13,00	14
	2	31,00	28,00	30,00	30
	3	29,00	28,00	32,00	30
conc. 100 %	1	22,00	20,00	17,00	20
	2	26,00	31,00	30,00	29
	3	28,00	28,00	31,00	29

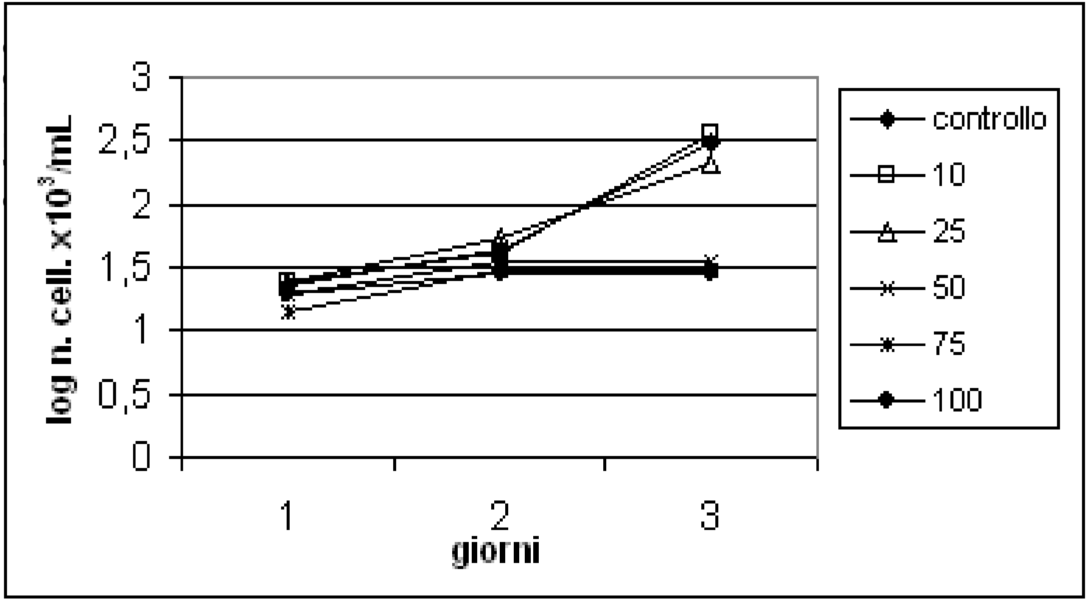


Figure 3.45: results of the 72h growth inhibition of 3NBTF on *Selenastrum capricornutum*.

3-NITROBENZOTRIFLUORIDE 98-46-4

intensità luce (lux)	7000	qualità luce
temperatura (℃)	18-22	
metodo di misurazione della concentrazione cellulare		contaglobuli Coulter Z1

Risultati	Tempo (giorni)	n. cellule n.x10^3/mL			
		replica 1	replica 2	replica 3	
controllo	1	22	25	21	
	2	46	49	46	
	3	330	323	301	
		replica 4	replica 5	replica 6	media
	1	24	20	23	23
	2	36	39	43	43
	3	298	313	299	311
		replica 1	replica 2	replica 3	media
conc.	1	22,00	24,00	20,00	22
10	2	42,00	48,00	40,00	43
%	3	291,00	325,00	338,00	318
conc.	1	23,00	18,00	17,00	19
25	2	40,00	45,00	44,00	43
%	3	281,00	289,00	286,00	285
conc.	1	18,00	19,00	19,00	19
50	2	30,00	33,00	30,00	31
%	3	166,00	175,00	145,00	162
conc.	1	19,00	19,00	19,00	19
75	2	20,00	28,00	28,00	25
%	3	80,00	50,00	50,00	60
conc.	1	20,00	19,00	19,00	19
100	2	29,00	20,00	21,00	23
%	3	43,00	60,00	40,00	48

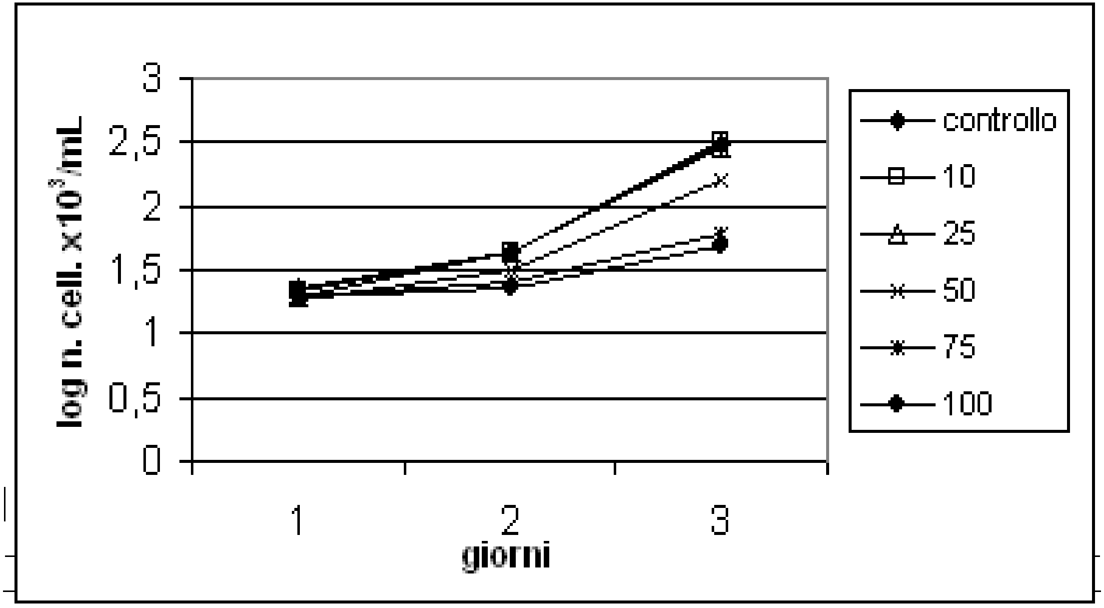


Figure 3.46: results of the 72h growth inhibition of 3A4CBTF on *Selenastrum capricornutum*.

3-AMINO-4-CHLOROBENZOTRIFLUORIDE 121-50-6

intensità luce (lux)	7000	qualità luce
temperatura (℃)	18-22	
metodo di misurazione della concentrazione cellulare		contaglobuli Coulter Z1

Risultati	Tempo (giorni)	n. cellule n.x10^3/mL			
		replica 1	replica 2	replica 3	
controllo	1	22	25	21	
	2	46	49	46	
	3	330	323	301	
		replica 4	replica 5	replica 6	media
	1	24	20	23	23
	2	36	39	43	43
	3	298	313	299	311
		replica 1	replica 2	replica 3	media
conc. 10 %	1	25,00	24,00	24,00	24
	2	52,00	60,00	62,00	58
	3	395,00	380,00	397,00	391
conc. 25 %	1	25,00	23,00	24,00	24
	2	40,00	53,00	51,00	48
	3	289,00	320,00	300,00	303
conc. 50 %	1	20,00	22,00	21,00	21
	2	32,00	34,00	30,00	32
	3	75,00	78,00	74,00	76
conc. 75 %	1	23,00	24,00	23,00	23
	2	24,00	25,00	26,00	25
	3	51,00	48,00	41,00	47
conc. 100 %	1	22,00	25,00	27,00	25
	2	25,00	23,00	32,00	27
	3	32,00	34,00	40,00	35

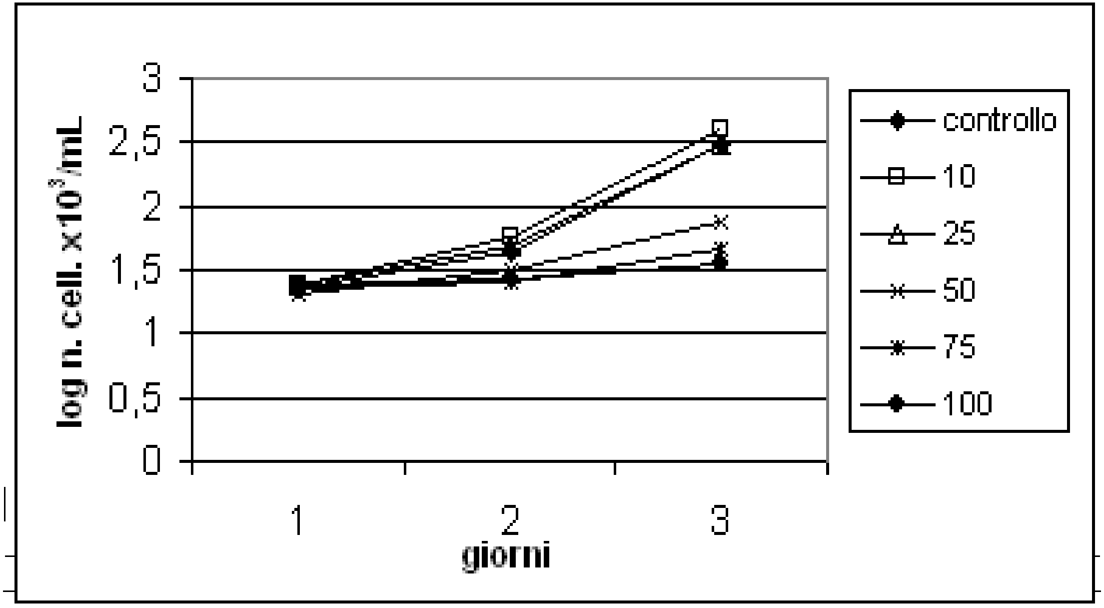
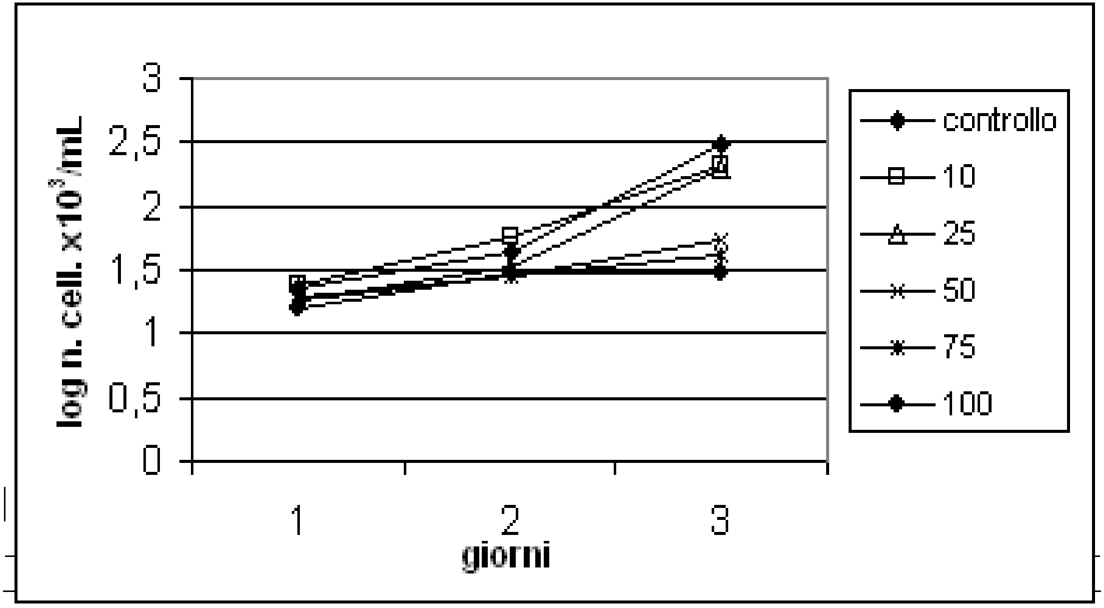


Figure 3.47: results of the 72h growth inhibition of 3N4CBTF on *Selenastrum capricornutum*.

3-NITRO-4-CHLOROBENZOTRIFLUORIDE 121-17-5

intensità luce (lux)	7000	qualità luce	
temperatura (℃)	18-22		
metodo di misurazione della concentrazione cellulare			contaglobuli Coulter Z1

Risultati	Tempo (giorni)	n. cellule n.x10^3/mL			
		replica 1	replica 2	replica 3	
controllo	1	22	25	21	
	2	46	49	46	
	3	330	323	301	
		replica 4	replica 5	replica 6	media
	1	24	20	23	23
	2	36	39	43	43
	3	298	313	299	311
		replica 1	replica 2	replica 3	media
conc.	1	24,00	26,00	24,00	25
10	2	50,00	55,00	63,00	56
%	3	220,00	212,00	201,00	211
conc.	1	18,00	18,00	20,00	19
25	2	35,00	32,00	33,00	33
%	3	192,00	195,00	210,00	199
conc.	1	18,00	19,00	18,00	18
50	2	27,00	29,00	29,00	28
%	3	55,00	56,00	48,00	53
conc.	1	20,00	22,00	17,00	20
75	2	25,00	31,00	28,00	28
%	3	50,00	34,00	41,00	42
conc.	1	16,00	17,00	15,00	16
100	2	28,00	32,00	30,00	30
%	3	31,00	31,00	28,00	30

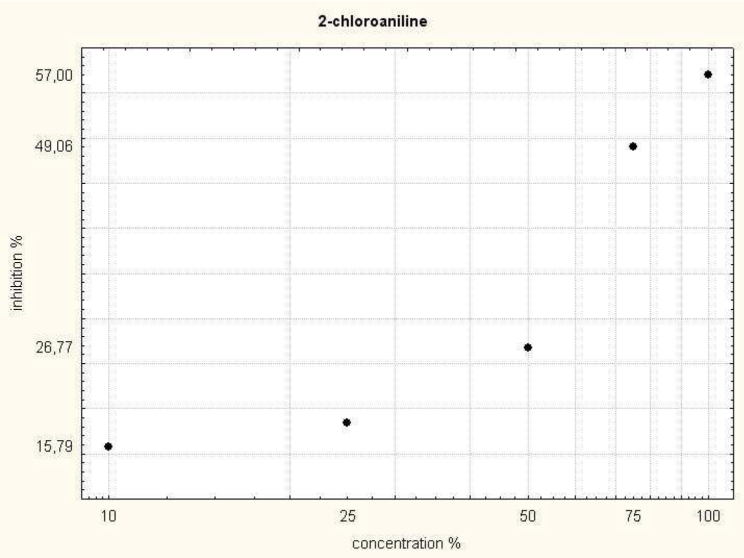


In the figures, the cell density measurements in the test media were tabulated according with the concentration of the test sample and the time of measurement. The result was the plots of growth curves. The logarithmic scales were useful also for the better presentation of variations in growth pattern during the test period. The exponential growth produces a straight line when plotted on a logarithmic scale so the slope of the line indicated the specific growth rate. A levelling off indicated that cultures entered in the stationary phase.

The concentration-effect curves for each substance, based on the percentage of inhibition of the growth rate I_{μ}, are plotted below.

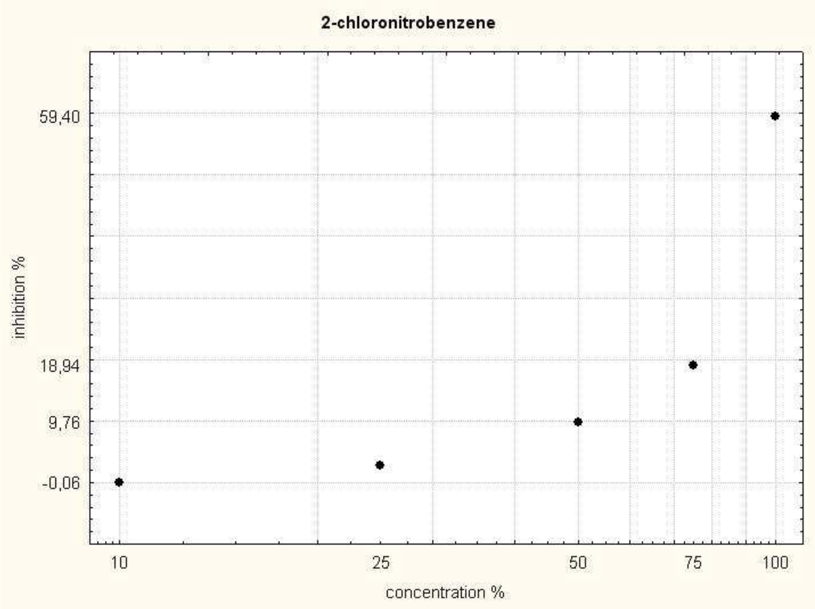
95-51-2 2-chloroaniline

concentration %	inhibition %
10	15,79
25	18,43
50	26,77
75	49,06
100	57,00



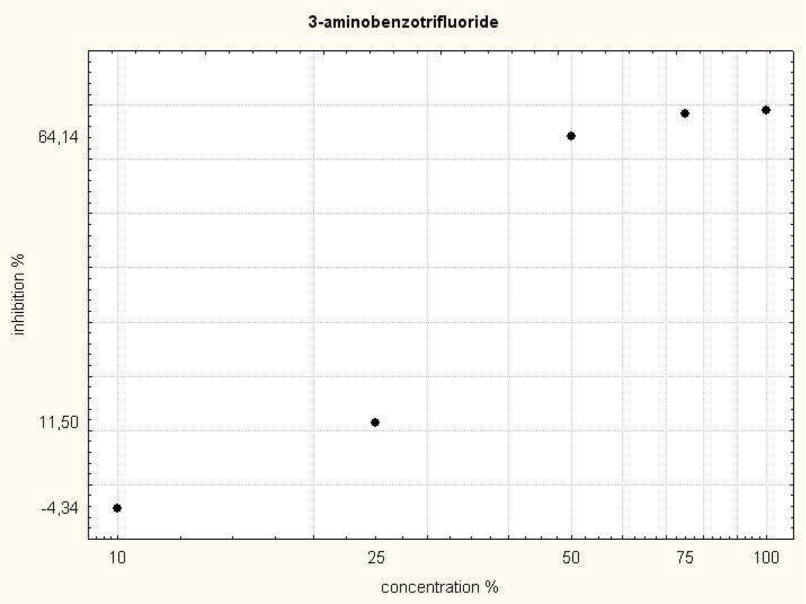
88-73-3 2-chloronitrobenzene

concentration %	inhibition %
10	-0,06
25	2,74
50	9,76
75	18,94
100	59,40



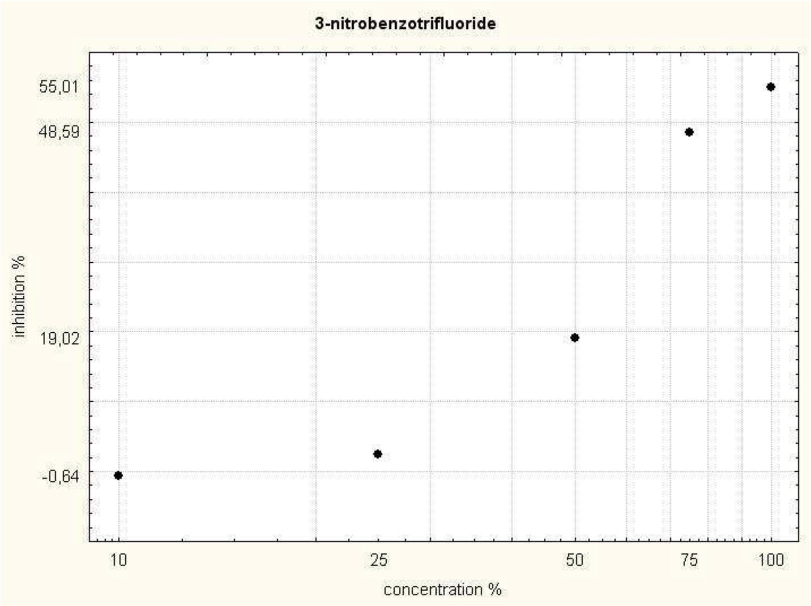
98-16-8 3-aminobenzotrifluoride

concentration %	inhibition %
10	-4,34
25	11,50
50	64,14
75	68,39
100	69,04



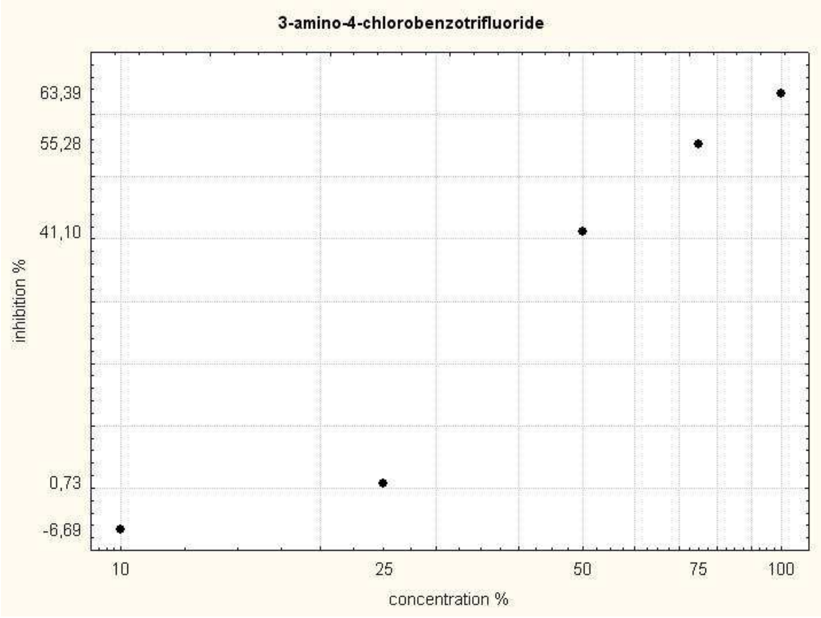
98-46-4 3-nitrobenzotrifluoride

concentration %	inhibition %
10	-0,64
25	2,46
50	19,02
75	48,59
100	55,01



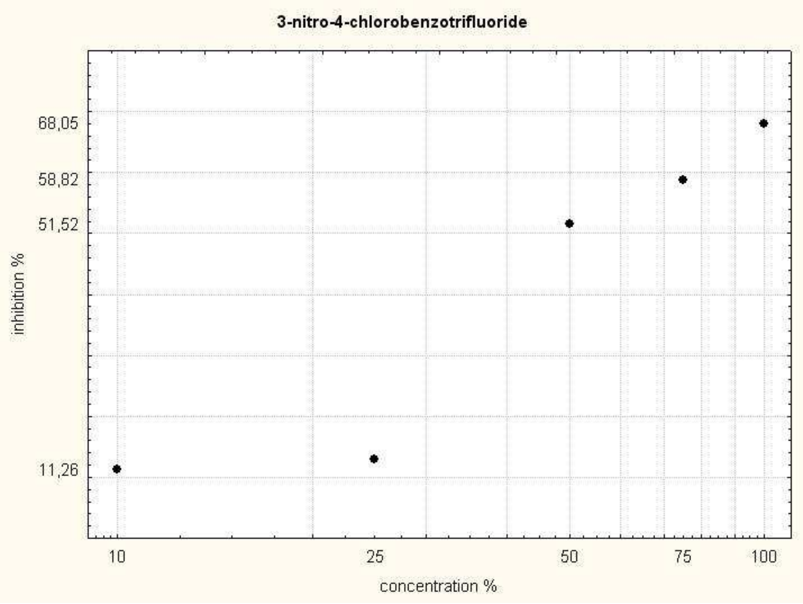
121-50-6 3-amino-4-chlorobenzotrifluoride

concentration %	inhibition %
10	-6,69
25	0,73
50	41,10
75	55,28
100	63,39



121-17-5 3-nitro-4-chlorobenzotrifluoride

concentration %	inhibition %
10	11,26
25	12,97
50	51,52
75	58,82
100	68,05



The concentration-effect curves, based on the percentage of inhibition of the growth rate I_{μ}, shows clearly the effect of the chemical concentration of each compound on the inhibition of the growth rate I_{μ}. An interpolation of the curves can give also an indicative EC_{50} value for each substance. The indicative values are shown on table 3.30.

Table 3.30: indicative EC_{50} values obtained by interpolation of concentration-effect curves.

Substances.	**% conc**	**Conc mg/L (100%)**	**EC_{50}**
2CA	86.18	52.2	**45.01**
2CNB	94.71	39.1	**37.03**
3ABTF	62.41	64.8	**40.44**
3NBTF	89.57	71.8	**64.31**
3A4CBTF	74.71	71.0	**53.04**
3N4CBTF	47.09	76.9	**36.21**

Data obtained with the test on *Selenastrum capricornutum* were, at this level of the work, only preliminarily assessed and no effects of the media solution (Milli-Q water with 3‰ of methanol) in comparison with other suitable solution were accurately studied at the moment.

3.3.4 Summary of ecotoxicological tests results

The results obtained from the first ecotoxicological assessment on three different organisms' tests, even in a preliminary way, can be compared.

The molecules chosen for the tests differ for the functional groups linked to the aromatic ring. The following derivatives of aniline were considered: 1) with a –Cl group in *o*-position, 2) with a $-CF_3$ group in *m*-position and 3) with both a –Cl group in *o*-position and a $-CF_3$ group in *m*-position. The same comparison was made with the nitro group instead of the amino group linked on the aromatic ring obtaining three derivatives of nitrobenzene.

The EC_{50} values calculated for all the three typology of test can be compared for the six different substances.

A first investigation on the effects shows the following EC_{50} results ranges:

Vibrio fisheri: 2.15 ÷ 28.13 mg/L

Selenastrum capricornutum: 36.21 ÷ 64.31 mg/L

Daphnia magna: 3.26÷17.74 mg/L.

The complete results are summarized below on table 3.31.

It is difficult, at this preliminary level of the study, to relate the toxicological data obtained with the functional group linked to the aromatic rings.

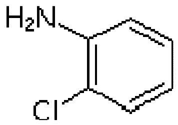
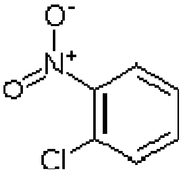
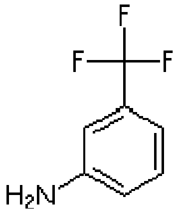
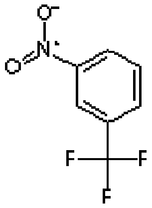
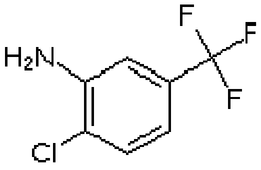
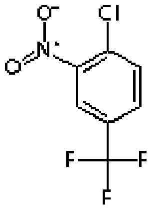
A preliminary evaluation can consider that the for *Selenastrum capricornutum* the toxicity is comparable for the six substances, with a weakly more toxic effect of –Cl substituent than - CF_3 group in the nitroderivatives.

For *Vibrio fisheri* tests, the nitroderivatives seems to be less toxic than the correspondent amino derivatives except for 2CA and 2CNB. The same conclusion on *Vibrio fisheri* can be made for *Daphnia magna* results. So a general tendency of a greater toxicity of the amino derivatives than the nitro derivatives is found especially considering substances with a group -CF_3 for which the difference of EC_{50} on the same organism can be one order of magnitude lower.

The additional experiment on the mixture of 3A4CBTF and 3N4CBTF at their EC_{50} values showed an additional effect of the two compounds without any synergetic effect when they are combined together.

As conclusion, considering EC_{50} values that range between 1-100 mg/L for pure substances, a not negligible toxic effect is given to the chemicals tested according with the EU suggestions.

Table 3.31: summarized results of the EC_{50} values obtained for the three different tests.

Substance	**Test**	**EC_{50} [mg/L]**
2-chloroaniline 	*Vibrio fisheri* *Selenastrum capricornutum* *Daphnia magna*	**16.5** **45.0** **6.7**
2-chloronitrobenzene 	*Vibrio fisheri* *Selenastrum capricornutum* *Daphnia magna*	**5.1** **37.0** **6.3**
3-aminobenzotrifluoride 	*Vibrio fisheri* *Selenastrum capricornutum* *Daphnia magna*	**12.6** **40.4** **5.5**
3-nitrobenzotrifluoride 	*Vibrio fisheri* *Selenastrum capricornutum* *Daphnia magna*	**16.7** **64.3** **17.7**
3-amino-4-chlorobenzotrifluoride 	*Vibrio fisheri* *Selenastrum capricornutum* *Daphnia magna*	**2.2** **53.0** **3.2**
3-nitro-4-chlorobenzotrifluoride 	*Vibrio fisheri* *Selenastrum capricornutum* *Daphnia magna*	**28.1** **36.21** **10.0**

4. CONCLUSIONS

The adoption of the Water Framework Directive (WFD 2000/6/EC) at EU level underlines the need to investigate through scientific criteria, before a technical application, on the impact on the environment, especially on the delicate aquatic ecosystem, of any anthropic activity. The target of this thesis work, into specific, addresses the attention on the approach to the control of dangerous/priority chemical substances considered as pollutants of the aquatic environment and to the concept of *good chemical status* outlined by the directive.
A deep knowledge of the territory, from the geographical and hydrochemical conformations to the localized human settlements and activities, is essential for the adjustment of environmental monitoring plans and for a better control of the pressure sources. Simultaneously, an improving of the competence of analytical control laboratories, as the ARPAV laboratories, is necessary to conform them to the determination of new priority and dangerous substances as well to reach the environmental quality standards established by the legislation.

The requests set by ARPAV were satisfied with the development of a multiresidue method for the simultaneous determination of aniline, seven chloroanilines, nitrobenzene and five chloronitrobenzenes in environmental waters. A suitable method, based on SPE extraction in basic condition and analysis with GC-MS, was developed as a compromise between the best performance and its application on a routine/control laboratory that manage a great number of samples everyday. The decision to work with an amount of 200 mL of sample with a simple and quick extraction procedure and with common analytical instrumentations avoiding any kind of derivatization, was a critical point for the everyday application of the method in a non-research laboratory. However a validation, supported by statistical data, was necessary to demonstrate the applicability and the ruggedness of the method. The method was validated in terms of linearity, repeatability, recovery, breakthrough, LODs and LOQs on spiked environmental samples, including first uncertainty estimation based on empirical formulae. The method was applied to real environmental waters from groundwaters, rivers, piezometers of contaminated land, leachates from landfill and from wastewater collected in a period of about two years. The results showed a confirmation of the applicability of the analytical method to quantify the analytes considered under the QA/QC requirement established for the EU purpose on dangerous/priority substances determination at trace levels. At the same time the presence of aniline and some chloro- derivatives was detected at regional level. Chloronitroaromatics weren't detected in any environmental sample analysed. Their non-presence as pollutants, at the LODs of the method tested, is reasonable after the comparison of the analytical data with the industrial processes analysis (territorial analysis) carried out by other ARPAV working groups involved in the ISPERIA Project. At the other hand, if the study explained a good application of the method for the simultaneous analysis of chloroanilines and chloronitrobenzenes, the quantitative determination of aniline gave low recoveries suggesting its determination on trace through other methods more suitable for volatile and semivolatile compounds.

Simultaneously, the attention on new emerging pollutants and other classes of organic contaminants was discussed. Part of the thesis work was aimed on similar organic substances: the benzotrifluoride derivatives (BTFs). In literature no methods were described for the specific determination of BTFs as environment contaminants. However their presence in the area of Valleagno, in Vicenza Province, was checked on the basis of a past chemical pollution episode dated 1977. The reconsideration of the case was the starting point for the further development of the work. The historical data available were discussed and elaborated with the tools available nowadays (GIS) and a more precise framing of the contamination episode was presented including an investigation of the spatial and temporal contaminant trend occurred in the first years after the event. At the same time the development of new analytical methods for the determination of volatile BTFs and of non-volatile BTFs, at basic and acidic conditions, was successfully studied using SPE and P&T as methods for extraction followed by GC-MS and HPLC-HRTOF/MS for the determination. The current situation of the contaminated area was investigated with a first monitoring campaign to assess the distribution of these substances. The presence nowadays of relevant concentrations (up to 12 μg/L) of several BTFs compounds on groundwaters and surface waters, collected from the Valleagno area and the Retrone River, suggests about their high persistence in the environment. Some of the BTFs detected, as dichloro- (24DCBTF, 34DCBTF) and amino- (3ABTF, 3A4CBTF) BTFs derivatives, were never mentioned as contaminants in the literature on the specific case. These results were argued concluding that, for the hydrogeological conformation of the territory, no significative reactions/transformations at subsoil level occurred and the attendance of new contaminants was possible because of a deep development of most suitable analytical methods for BTFs with modern instrumentation. The dataset available during the few past campaigns (1978-80, 1986-87, 2003) are difficult to compare with results obtained from this thesis work due to the non homogeneous and incomplete historical outcomes. However a comparison of data from the different campaigns showed different amounts of some BTFs (4CBTF, 3NBTF, 3N4CBTF) that cannot described a clear trend of the contamination and traceability in the groundwater during the three decades, as well a residence time study of the load of contaminants. Realistically is thought that when the episode was occurred, no drainage of the contaminated land was performed but only a limited containment of the damage localised around the pressure point. For these reasons, it is assumed that considerable loads of BTFs are still present in the area and that raising episodes of groundwater, due for example to intensive meteorological events, can lick and flush the contaminated land carrying the pollutants along the water aquifer until the resurgences area of Retrone River.

These considerations on the BTFs derivatives as environmental pollutants were the hint for the application of ecotoxicological tests with the aim of a preliminary assessment of toxicological effects on the environment of these substances. The tests performed compared the results on six compounds (one chloroaniline, one chloronitroaromatic and four benzotrifluoride derivatives) examined at three trophic levels (bacteria, algae, crustacean). A first investigation on the toxicological effects due to the different substituent of the molecules and a preliminary evaluation of effects, as suggested on the indication expressed by the WFD, consider EC_{50} values that range between 1-100 mg/L, concluding that a not negligible toxic effect is given to the chemicals tested.

This thesis work would give a contribution, with a multidisciplinary approach, to understand better the problem of hazardous and priority substances discharged into the aquatic ecosystem suggesting hints for the promotion of a sustainable use of water resources and for a better control of the environment at Veneto Region level.

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ANNEX I

SUMMARY OF THE ANALYTICAL METHOD IN LITERATURE REVIEWED ON PARAGRAPH 2.1.1

REFERENCES	POLLUTANTS	EXTRACTION TECNIQUE	SEPARATIVE TECNIQUE	COLUMN	MATRIX + SAMPLE VOLUME	EXTRACTION SOLVENT	LOD
EPA 8131 (1996)	2-CA 3-CA 4-CA 4Cl-2-NA 3,4-DCA	LLE + GPC cleanup if necessary	GC-NPD	SE-54 fused silica SE-30 fused silica	water in general waste water (1L)	toluene/esane/ethylacetate	0.7-5 μg/L
Kristenson (2004)	2-CA 3-CA + other CA	PLE (miniaturised pressurised LE)	GC-MS (LV-OC and PTV injection)	DB-XLB Agilent (LV-OC) ZB-5 Phenomemex (PTV)	soil (50 mg)	acetonitrile	1-35 (LV-OC) 0.2-25 (PTV) ng/g soil
Lacorte (1999)	2-CA 3-CA 4-CA 3,4-DCA + other CA	SPE (comparison 5 cartridges)	LC-ED 20mM lithium perclorate/ACN (70:30)	Interchim Nucleosil C_{18}	industrial waters with high TOC (200mL)	acetonitrile	range 10-40 till 1.0 μg/L
Lambropoulou (2000)	fungicides Dicloran (CA)	SPME (comparison 4 fibers) PA/PDMS/CW-DVB/PDMS-DVB	GC-ECD GC-MS	DB-1 J&W (ECD) DB-5-MS J&W (MS)	ground, sea, river, lake waters (3mL)	methanol	1-60 ng/L
Yang (2001)	2-CA 4-CA 2,4-DCA 2,5-DCA 3,4-DCA 3,5-DCA	SPME	IR hollow waveguides (suitable for organics with low volatility)	-	aqueous sol.		0-50 mg/L (linearity range)
Marengo (1999)	2-CA 3-CA 4-CA 2,3-DCA 2,4-DCA 2,5-DCA 3,4-DCA 3,5-DCA	-	optimisation of 6 sperimental parameters for HPLC–UV det Water/ACN (65:35)	end-capped Supersphere 100-RP Merks + Lichrosper RP-18 precolumn double layer, used IIR	water in general		4-20 μg/L
Muller (1997)	4-CA 2,4-DCA 2,5-DCA 2,5-DCA 3,4-DCA 3,5-DCA	SPME (CW-DVB fiber) no derivatization	GC-MS (SIM)	PTA-base deactivated Supelco	Ground water of polluted areas (10mL)	methanol	0.05-5 μg/L
Rodriguez (1999)	Diflubenzuron + metabolities (4-CA)	SPE (aminopropyl cartridges) with CAN	LC-DAD LC-EC (amperometric method)	Spherisorb C_{18} OD52 Tracer	Forestry matrix (pine needles) (3g)		2-25 (DAD) 0.07-1.8 (ED) 2.8 μg/Kg 4-

REFERENCES	POLLUTANTS	EXTRACTION TECNIQUE	SEPARATIVE TECNIQUE	COLUMN	MATRIX + SAMPLE VOLUME	EXTRACTION SOLVENT	LOD
							CA
Huang (1997)	aniline and nitroaniline, but not chlorinated	SPME (PA and PDMS fibers)	GC-FID	CBP-10 Shimadzu	environmental water (3mL)	aceton	5-10 µg/L
Peng (2004)	2-CA 4-CA 3,4-DCA 2,4-DCA	HS-LPME with an ionic liquid (IL)	HPLC-DAD	C_{18} ODS-P Inertsil GL Science	tap, river, waste water (20mL with 10µL ofIL)	acetonitrile	0.5-1.0 µg/L
Jen (2001)	2-CA aniline	microdialysis	HPLC – UV det	Supelcosil RP C_{18} Supelco	polymer/ind. Water (50mL)		0.1-0.5 mg/L
Zhu (2002)	4-CA + other AA	-	Ion chr.(IC)- Amp. Det.	Ion Pack CS12	waste w.(25 µL)		2-25 µg/L
Di Corcia (1999)	phenylurea herbicides + degradation products	SPE with GCB Carbograph 4 cartridges	LC + ES MS	C_{18} RP Alltech	drinking, ground river water	methanol	3-750 ng/L
Di Corcia (1990)	14 CA + DCA	(Carbopack B + resin based strong cation exchanger)	LC-UV	LC-18DB rev. phase (Supelco)	env. waters (200mL)	methanol	<0.1 µg/L 9-50 ng/L
Weigel (2001)	2-CA 2,5-DCA + many other pollut.	SPE in large volume (GCB,RP C_{18},S-DVB,PA cartridges)	GC-MS ion trap det	DB-5-MS (J&W)	sea water (>10L)		0.002-0.2
Berrada (2004)	Phenylurea herbicides + 4-CA 3,4-DCA	SPME (PA fiber)	GC-NPD	BP10 SGE column	vegetables (2mL of mix vegetable juice-water)		µg/Kg depends herbicide and vegetable
Schmidt (1998) Less (1998)	2-CA 3-CA 4-CA 3,4-DCA + other 49 AA	SPE (S-DVB) comparison 7 cartridges	GC-ECD HPLC-DAD UVD	DB5 J&W (GC) RP-18 (HPLC)	water waste water (250 mL)		0.5-20 µg/L
Zhao (2002)	3-CA 4-CA 3,4-DCA + other AA	LLLME with hollow fiber	HPLC-UV det.	Chrompack Inertsil ODS-2	water in general (4mL)		0.08-0.1 µg/L
Zhou (2004)	2-CA 2,4-DCA + other AA	MMLLE membrane	Capillary electrophoresis	-	water		0.16-7.5 µg/L

REFERENCES	POLLUTANTS	EXTRACTION TECNIQUE	SEPARATIVE TECNIQUE	COLUMN	MATRIX + SAMPLE VOLUME	EXTRACTION SOLVENT	LOD
		extraction system					
Patsias (2000)	4-CA 2,3-DCA 3,4-DCA 2,5-DCA + other	SPE (Hysphere-GP Hysphere-SM, PRP-1, PLPR-S)	HPLC-DAD/ED	Nucleosil 100-S C_{18} RP Macherey-Nagel	aqueous matrix (5-150mL)	methanol	0.05-0.2 µg/L
EPA 8091 (1996)	C2,4DNB C2NB C4NB 2C6NT 4C2NT 4C3NT DCNB	LLE + GPC cleanup if necessary	GC-ECD (confirm GC-MS)	DB-5, RTX-5, SPB-5 or equivalent column DB-1701, RT_x-1701 or equivalent column	ppb in water/soil ppm in waste (1L)	isoctane/esane	100 µg/L
Gatermann (1995)	CNB	L-L + allumina column (esane solvent)	GC-AFID/ECD (GC-MS confirm)	NB 54 DB5-MS	sea water 10 L		0.02-0.6 ng/L
Bacaloni (2007)	2-CA 3-CA 4-CA 34DCA	SPE Amberchrom CG161	LC/APPI-MS	Cyano propyl methyl silyl LUNA	tap, river, waste water		0.03-0.07 ng absolute
Chiang (2008)	AN 4-CA 3C4MA	DLLLE + derivatiz	GC-MS	HP-5MS	waste water		0.04-0.09 ug/L
Sun (2008)	AN 4-CA	CZE (electrophoresis)			water		
Nishikawa (1995)	NB 2CNB 3CNB 4CNB	L-L	GC-MS	DB-17	River water (1L), fish, sediments	hexane	0.12-0.23 ng/mL
Fromberg (1996)	3CNB 4CNB 2CNB 4C2NA 3,4DCNB 2,5DCNB	HS-SPME (PolyAcrilate fiber)	GC-ECD GC-MS	HT-8 column (SGE)	soil (5g)		0.5-250 µg/L (range not linear)

ANNEX II

COMPLETE DATASET OF IRSEV STUDY MONITORING (FEBR 1978- FEB 1979) WITH CONCENTRATION OF 4-CHLORO-3-NITROBENZOTRIFLUORIDE MEASURED AND THE GEOREFERENCE WORK OF ALL MONITORING STATIONS

TITOLARE_P	COMUNE_PZ	X_GAUSS_BO	Y_GAUSS_BO	COD_IRSEV
FIAMM SPA	Montecchio Maggiore	1689176,71	5041409,65	
FIS spa pozzo 2	Montecchio Maggiore	1689720,72	5040616,18	P 65
Castagnero Luigi	Montecchio Maggiore	1688748,03	5040712,87	P 811
Gasparotto Giandomenico	Montecchio Maggiore	1685848,58	5044736,86	P 6
Dal Maso Bruno	Montecchio Maggiore	1687560,19	5040727,30	P 20
Fratelli Faedo	Montecchio Maggiore	1685964,68	5045433,42	P 823
Acquedotto comunale (presa)	Montecchio Maggiore	1686645,58	5042729,35	P 3
Lowara spa	Montecchio Maggiore	1689861,85	5042369,98	P 48
Lowara spa	Montecchio Maggiore	1690053,69	5042403,59	P 33
Acquedotto comunale	Montecchio Maggiore	1686716,50	5042579,50	P 18
Acquedotto comunale	Montecchio Maggiore	1688401,85	5040174,28	P 14
Parise Severo	Montecchio Maggiore	1687280,09	5040023,95	P 18
Rimar 2	Trissino	1686340,93	5047014,33	P 814
Rimar 1	Trissino	1686336,98	5047103,17	P 817
Veneta Conciaria ValleAgno	Trissino	1686122,49	5045995,94	P 66
Conceria Basmar	Trissino	1686062,42	5046264,74	P 95
ORMEC	Montecchio Maggiore	1686400,50	5045254,15	P 786
Meggiolaro Antonio	Trissino	1686375,44	5044149,07	P 64
Ospedale civile	Montecchio Maggiore	1687156,68	5042669,18	P 29
Sorgente Ghisa	Montecchio Maggiore	1686566,69	5045148,34	S 3
Sorgente Carbonara	Montecchio Maggiore	1687724,31	5044274,02	S 7
Sorgente Strabusene	Sovizzo	1688545,39	5044991,55	S 8
Confezioni Castelli	Montecchio Maggiore	1688552,40	5041683,69	P 31
Pelosato	Montecchio Maggiore	1689092,21	5041156,20	P 62
Villa Cordellina	Montecchio Maggiore	1689509,07	5042349,89	P 819
Urbani Antonio	Montecchio maggiore	1688933,04	5043480,93	P 72
Fratelli Lovato	Sovizzo	1690092,11	5043707,24	P 821
Marchezzolo Girolamo	Montecchio Maggiore	1689311,74	5043053,57	P 37
Cracco Augusto	Sovizzo	1690379,81	5043363,55	P 571
Chiericati Luciano	Sovizzo	1690337,65	5043969,58	P 570
Acquedotto Comunale	Sovizzo	1690838,10	5044129,96	P 572
Acquedotto Comunale	Sovizzo	1690804,38	5044107,47	P 573
Dall'Ora Adriana	Sovizzo	1690614,09	5045022,36	P 822
Pieropan Ettore	Sovizzo	1690994,47	5044558,78	P 598
Pieropan Desiderio	Sovizzo	1691000,09	5044449,17	P 595
Finetti Renato	Sovizzo	1690893,29	5044307,23	P 645
De Mani Almerino	Creazzo	1691381,66	5043830,02	P 818
Ristorante tre scalini	Creazzo	1692820,52	5045055,52	P 474
Acquedotto Cons. Crosara	Creazzo	1692946,98	5044887,98	P 451
Acquedotto Com. Pistocche	Creazzo	1692952,06	5044503,44	P 445
Zanotto Bruno	Creazzo	1692434,77	5044472,50	P 486
Gobbi Angelo	Creazzo	1692221,91	5044666,75	P 452
Tirapelle Girolamo	Creazzo	1693209,72	5045245,08	P 490
Gruppach Gianfranco	Creazzo	1693525,52	5044824,00	P 454
Ditta Sadi	Altavilla Vicentina	1693658,70	5043861,98	P 341
Central Panta Market	Altavilla Vicentina	1692478,50	5043184,59	P 309
Fonderia Dalli Cani	Altavilla Vicentina	1691688,57	5042850,64	P 377
Ranzato Pietro	Altavilla Vicentina	1692860,70	5043412,65	P 346
Centro Altavilla	Altavilla Vicentina	1692700,87	5042675,34	P 406
Peotta Renzo	Montecchio Maggiore	1690716,92	5040472,71	P 61
Trattoria Melaro	Montecchio Maggiore	1691029,85	5041247,34	P 41
Pellizzaro	Montecchio Maggiore	1691074,53	5041494,83	P 59
Ditta Ciscato	Montecchio Maggiore	1690372,88	5040859,80	P 36
Torrente Poscola	Montecchio Maggiore	1686998,10	5039991,96	F 18
Ditta Marzotto O	Montecchio Maggiore	1690341,03	5041468,05	P 51

TITOLARE_P	COMUNE_PZ	X_GAUSS_BO	Y_GAUSS_BO	COD_IRSEV
Ditta Sorelle Ramonda	Montecchio Maggiore	1690397,95	5041594,85	P 54
Ditta Safas	Altavilla Vicentina	1691401,90	5042238,89	P 403
Torrente Valdiezza	Sovizzo	1691367,57	5045412,10	F 19
Fiume Retrone	Creazzo	1691548,43	5044951,73	F 20
Risorgiva Retrone	Creazzo	1691647,08	5044680,45	F 21
Torrente Onte	Sovizzo	1691230,70	5045138,48	F 2
Risorgiva Spino	Creazzo	1692506,57	5044803,07	F 4
Fiume Retrone	Creazzo	1691908,10	5044645,23	F 3
Roggia Spino	Creazzo	1692700,76	5044928,24	F 5
Risorgiva Pisocche	Creazzo	1692853,69	5044211,30	F 6
Fosso Pisocche	Creazzo	1693099,71	5044564,09	F 7
Fosso Pisocche	Creazzo	1693266,32	5044637,75	F 8
Risorgiva Ceregata	Creazzo	1693555,69	5044151,95	F 9
Fosso Ceregata	Creazzo	1694006,80	5044369,46	F 11
Fiume Retrone	Creazzo	1694069,65	5044425,58	F 12
Risorgiva Riello	Altavilla Vicentina	1693858,51	5042680,55	F 13
Fossa Riello	Altavilla Vicentina	1695119,04	5043281,22	F 15
Fiume Retrone	Altavilla Vicentina	1695151,92	5043384,26	F 16
Petrin Giovanni	Arzignano	1685231,71	5045468,47	P 13
Marchi Giacinto	Arzignano	1685139,64	5045319,40	P 12
Meggiolaro Adelino	Trissino	1685891,57	5046446,20	P 9
Torrente Poscola	Montecchio Maggiore	1686721,87	5041314,76	F 22
Gattazzo Antonio	Arzignano	1685076,96	5044906,70	P 5
Faccio M&M	Arzignano	1685109,66	5043794,57	P 90
Bomitali Boschetti	Montecchio Maggiore	1686520,57	5044056,32	P 63
Casarotto Ferruccio	Creazzo	1692683,58	5044962,83	P 448
Sorgente Pianeta	Montecchio Maggiore	1686667,65	5046694,50	S 2
Vaccaro Gino	Trissino	1685911,42	5047059,29	P 824
Sinico Giovanni	Sovizzo	1691254,58	5044101,39	P 594
Torrente Poscola	Castelgomberto	1686800,57	5048319,45	F 17

COD_IRSEV	feb-78	mar-78	apr-78	may-78	jun-78	jul-78	aug-78	sep-78	oct-78	nov-78	dec-78	jan-79	feb-79
P 65	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
P 811		0,7	1,1	0,7	0,6	0,3	0,0	0,0	0,3	0,3			0,4
P 6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
P 20		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
P 823	1,7	1,5	0,8	0,6	0,3	0,3	0,7	0,5	0,6	0,3	0,5	0,6	0,7
P 3	0,0												
P 48		650,0	500,0	950,0	400,0	370,0	340,0	320,0	280,0	260,0	210,0	240,0	250,0
P 33	540,0												
P 18		0,0	0,0	0,0	0,0		0,0	0,0	0,0	0,0	0,0	0,0	0,0
P 14		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
P 18		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
P 814	0,5	1,0	0,0	0,3	0,0	0,0					3,0	0,5	1,2
P 817	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
P 66	800,0	1000,0	900,0	720,0	400,0	280,0	230,0	230,0	230,0	165,0	55,0	160,0	200,0
P 95	430,0			85,0	50,0	20,0	20,0	30,0	47,0	28,0	6,0		40,0
P 786	730,0	630,0	550,0	720,0	700,0	610,0	500,0	500,0	400,0	350,0	310,0	250,0	200,0
P 64	700,0	400,0	270,0	350,0	380,0	410,0	500,0	450,0	400,0	340,0	325,0	280,0	200,0
P 29	85,0	65,0	50,0	50,0	40,0	50,0	40,0	40,0	37,0	32,0	35,0	30,0	
S 3		0,0	0,0	0,0	0,0		0,0	0,0	0,0	0,0	0,0	0,0	0,0
S 7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
S 8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
P 31		330,0	250,0	320,0	300,0	280,0	265,0	240,0	270,0	270,0	240,0	225,0	200,0
P 62	11,0	7,5	8,0	7,5	0,0	4,5	3,5	2,5	5,0	2,0	5,0	5,0	5,0
P 819		20,0	40,0	50,0	40,0	25,0							
P 72	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
P 821		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
P 37	10,0	4,0	40,0	18,0	70,0	75,0	25,0	30,0	130,0	100,0	0,0	27,0	0,0
P 571		450,0	480,0	400,0	450,0		355,0		300,0	260,0		230,0	220,0
P 570		0,0	0,0	0,0	0,0	0,0	0,0		0,0		0,0	0,0	0,0
P 572			0,0										
P 573				150,0	80,0	85,0	1,8	100,0	85,0	90,0	80,0	0,0	
P 822			0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0		0,0
P 598		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
P 595		1,5	2,0	4,0	5,5	6,0	9,0	9,0	7,5	6,0	6,5	5,0	4,0
P 645		170,0	180,0	220,0	220,0		250,0	260,0	235,0	225,0	230,0	200,0	175,0
P 818		120,0	220,0	240,0	230,0		255,0	140,0	165,0	130,0	95,0	210,0	65,0
P 474		0,0	0,0	0,0		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

COD_IRSEV	feb-78	mar-78	apr-78	may-78	jun-78	jul-78	aug-78	sep-78	oct-78	nov-78	dec-78	jan-79	feb-79
P 451	18,0	17,0	15,0	20,0	25,0	7,0	15,0	12,0	30,0	30,0	32,0	50,0	50,0
P 445	2,0	1,8	2,0	2,0	2,5	2,5	3,0	2,5	3,5	3,0	3,5	4,5	6,0
P 486	60,0	70,0	75,0	80,0	85,0	85,0	85,0	75,0	75,0	80,0	75,0	85,0	100,0
P 452	110,0	100,0	100,0	120,0	140,0	140,0	150,0	180,0	165,0	180,0	190,0	190,0	190,0
P 490		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0			
P 454							0,0	0,5	0,2	0,0	0,0	0,0	0,0
P 341		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0			
P 309	2,5	2,0	2,0	1,6	1,5	1,5	1,5	1,5	1,8	2,5	2,5	2,5	2,5
P 377	1,0	1,0	0,8	0,8	0,7	0,9	0,5	1,0	1,0	1,5	1,4	1,3	1,0
P 346		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
P 406	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
P 61		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
P 41		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
P 59		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
P 36						0,0							
F 18	2,5	7,0	4,0	1,5	1,7			0,5	0,3	2,0	2,0	0,4	0,4
P 51	1,5	1,2	1,2	1,6	2,0	2,0	2,0	1,8	2,0	2,0	2,0	2,0	2,0
P 54	2,5	0,0	0,0	0,0		4,0	4,5	1,5	3,0	2,5	4,0	4,0	2,5
P 403		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
F 19		0,0	0,0										
F 20		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
F 21		160,0	200,0	250,0	230,0	190,0	190,0						
F 2		0,0	0,0										
F 4	160,0	160,0	165,0	200,0	200,0	200,0	200,0	200,0	220,0	235,0	200,0	210,0	200,0
F 3	45,0	70,0	40,0	65,0	80,0	140,0	170,0	150,0	110,0	140,0	30,0	60,0	20,0
F 5	100,0	90,0	100,0	120,0	125,0	35,0	100,0	120,0	120,0	105,0	130,0	125,0	150,0
F 6	27,0	25,0	22,0	27,0	15,0	30,0	25,0	25,0	28,0	30,0	35,0	30,0	35,0
F 7	20,0												
F 8	185,0	15,0	15,0	23,0	25,0	15,0	15,0	17,0	17,0	17,0	18,0	20,0	22,0
F 9	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0
F 11	0,2	0,3	0,4	0,4	0,4	0,0	0,4	0,5	0,5	0,5	0,5	0,8	0,6
F 12	30,0	40,0	20,0	45,0	40,0	45,0	50,0	50,0	55,0	58,0	23,0	35,0	20,0
F 13	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
F 15	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
F 16	25,0	35,0	25,0	35,0	30,0	35,0	35,0	35,0	35,0	38,0	19,0	30,0	17,0
P 13	0,0												
P 12	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
P 9			0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0		0,0	0,0

COD_IRSEV	feb-78	mar-78	apr-78	may-78	jun-78	jul-78	aug-78	sep-78	oct-78	nov-78	dec-78	jan-79	feb-79
F 22			1,2	5,5	7,5	5,0	0,9	4,5	15,0	5,0	1,7	0,4	1,5
P 5	0,0												
P 90	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
P 63		40,0	30,0	35,0	30,0	25,0	25,0	20,0	20,0	15,0	18,0	15,0	
P 448		90,0	90,0	100,0	140,0	120,0	140,0	150,0	135,0	150,0	155,0	160,0	180,0
S 2		0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
P 824		0,0	0,0	0,0				0,0	0,0	0,0	0,0	0,0	0,0
P 594	250,0		280,0	315,0	300,0	340,0	345,0	310,0	280,0	290,0	280,0	260,0	200,0
F 17	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

ANNEX III

COMPLETE CHEMICAL ANALYSIS DATA SET OF GROUNDWATER MONITORING STATIONS INVOLVED BY PRESENCE OF BENZOTRIFLUORIDE DERIVATIVES DURING THE GIADA PROJECT CAMPAIGN OF 2002

#well	date sampling	Acquifer	Municipality	well owner	Address	well depth m	K spec. a 20 °C µS/cm	Turbidity NTU	pH	tot. hardness ° Fr	solid matter mg/l	Kubel Oxid mg/l	Cl^- mg/l
37	23/10/2003	A-G	Trissino	Veneta Conc. Valle Agno	Via della Stazione	65	550	< 2	7,60	33,0	370	< 0,50	8
93	09/10/2003	A-G	Montecchio Magg.	Acq.tto Comunale	Via Longa	55	525	0,5	7,60	29,5	355	< 0,50	4
128	28/10/2003	MP	Montecchio Magg.	FIAMM Spa	Via Europa	52	580	< 2	7,60	33,5	390	< 0,50	9
131	14/10/2003	MP	Montecchio Magg.	Pellizzaro Umberto	Via Melaro, 49	40	560	< 2	7,90	32,0	375	< 0,50	12
144	20/10/2003	MP	Montecchio Magg.	Ditta CECCATO	Via Battaglia	67	535	< 2	7,70	31,5	360	< 0,50	7
145	28/10/2003	MP	Montecchio Magg.	FIS Spa	Viale Milano, 26	88	505	< 2	7,70	29,5	340	< 0,50	6
154	09/10/2003	MP	Montecchio Magg.	Acq.tto Comunale	Via Natta - Z.I.	97,5	500	0,3	7,70	27,7	340	< 0,50	4
162	13/10/2003	MP	Montecchio Magg.	Parise Severo	Via Pagliarina, 14	40	520	< 2	7,60	28,0	350	< 0,50	10
163	14/10/2003	MP	Montecchio Magg.	FIAMM Spa	Via Gualda	64	530	< 2	7,90	30,0	355	< 0,50	9
175	13/10/2003	MP	Montecchio Magg.	Biasiolo Vittorio	Via Paglierina, 32	38	700	< 2	7,50	28,0	470	< 0,50	34
179	13/10/2003	MP	Montecchio Magg.	Spina Linda	Via Gualda	23	540	< 2	7,60	28,5	365	< 0,50	14
204	09/10/2003	MP	Brendola	Acq.tto Comunale	Via Madonna dei Prati	115	550	0,4	7,55	30,3	375	< 0,50	11

#well	date sampling	NO_3^- mg/l	$SO_4^=$ mg/l	NH_4^+ mg/l	NO_2^- mg/l	Cd µg/l	Cr tot µg/l	Fe µg/l	Ni µg/l	Pb µg/l	Cu µg/l	Hg µg/l	MC µg/l
37	23/10/2003	16	50	< 0,05	< 0,02	< 0,1	5	3	3	< 1	1	< 0,2	0,1
93	09/10/2003	14	56	< 0,05	< 0,02	< 0,1	5	5	< 1	1	2	0,1	< 0,1
128	28/10/2003	21	55	< 0,05	< 0,02	< 0,1	7	< 2	2	1	2	< 0,2	0,3
131	14/10/2003	16	55	< 0,05	< 0,02	< 0,1	8	< 2	3	< 1	7	< 0,2	0,1
144	20/10/2003	14	50	< 0,05	< 0,02	< 0,1	2	1900	3	< 1	2	< 0,2	0,1
145	28/10/2003	16	65	< 0,05	< 0,02	< 0,1	10	< 2	1	< 1	1	< 0,2	0,1
154	09/10/2003	13	57	< 0,05	< 0,02	< 0,1	5	< 5	< 1	1	1	0,1	0,3
162	13/10/2003	14	45	< 0,05	< 0,02	< 0,1	4	13	2	< 1	< 1	< 0,2	0,1
163	14/10/2003	14	55	< 0,05	< 0,02	< 0,1	5	< 2	4	< 1	2	< 0,2	< 0,1
175	13/10/2003	18	65	< 0,05	< 0,02	< 0,1	12	2	6	< 1	2	< 0,2	0,1
179	13/10/2003	22	55	< 0,05	< 0,02	< 0,1	5	3	2	< 1	2	< 0,2	0,1
204	09/10/2003	16	57	< 0,05	< 0,02	< 0,1	7	5	1	1	2	0,2	0,2

#well	date sampling	TeCE µg/l	TeCE µg/l	VOCs µg/l	Atrazine µg/l	Simazine µg/l	Terbutilaz. µg/l	des-etil atrazine µg/l	Alachlor µg/l	Metolaclor µg/l	other herbicides µg/l	tot. herbicides µg/l
37	23/10/2003	0,1	1,0	1,3	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,10
93	09/10/2003	0,1	1,6	1,7	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02	< 0,10
128	28/10/2003	0,1	1,4	2,0	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,10
131	14/10/2003	< 0,1	0,4	0,5	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,10
144	20/10/2003	0,1	1,6	1,9	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,10
145	28/10/2003	0,1	1,3	1,5	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,10
154	09/10/2003	0,5	1,0	1,8	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02	< 0,10
162	13/10/2003	< 0,1	0,5	0,6	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,10
163	14/10/2003	< 0,1	0,3	< 0,5	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,10
175	13/10/2003	< 0,1	1,5	1,6	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,10
179	13/10/2003	0,1	1,2	1,4	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,03	< 0,10
204	09/10/2003	0,2	1,0	1,5	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02	< 0,10

#well	date sampling	BTF µg/l	4CBTF µg/l	3NBTF µg/l	3N4CBTF µg/l	4C35DNBTF µg/l	BTF tot µg/l
37	23/10/2003	< 0,1	2,1	< 0,1	0,7	< 0,1	2,8
93	09/10/2003	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5
128	28/10/2003	< 0,1	6,1	0,3	2,7	< 0,1	9,1
131	14/10/2003	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5
144	20/10/2003	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5
145	28/10/2003	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5
154	09/10/2003	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5
162	13/10/2003	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5
163	14/10/2003	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5
175	13/10/2003	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5
179	13/10/2003	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5
204	09/10/2003	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,5

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Un infinito GRAZIE!!!

Estratto per riassunto della tesi di dottorato

Studente: ***LAVA ROBERTO*** *matricola:* ***955022***

Dottorato: ***SCIENZE AMBIENTALI***

Ciclo: ***XX°CICLO***

Titolo della tesi[1] *:*

PRIORITY CHEMICAL SUBSTANCES IN ENVIRONMENTAL WATERS UNDER THE IMPLEMENTATION OF THE WATER FRAMEWORK DIRECTIVE

Abstract (IT):

Il lavoro di tesi, finanziato dall'Agenzia per la Protezione e Prevenzione Ambientale della Regione Veneto (ARPAV) attraverso il Progetto I.S.PER.I.A., ha affrontato problematiche inerenti l'implementazione della Direttiva Quadro sulle Acque (2000/60/CE) da parte di organismi istituzionali tecnici in relazione al controllo delle sostanze pericolose immesse nell'ambiente acquatico della Regione Veneto. L'attenzione è stata posta innanzitutto sulla definizione di *buono stato chimico* dall'ambiente acquatico, descritto dalla legislazione nazionale che recepisce ed implementa quella europea, e ai conseguenti concetti di "*inquinante*" e "*sostanza pericolosa* e/o *prioritaria*". Questi concetti sono legati alle proprietà di tossicità, persistenza e bioaccumulo delle sostanze in questione con le relative conseguenze dovute alle interazioni una volta immesse nell'ambiente idrico. Sono stati presi in considerazione criteri per l'aggiustamento dei piani di monitoraggio, per l'adeguamento dei laboratori analitici di controllo e per una caratterizzazione del territorio con lo scopo di aver una maggior conoscenza degli effettivi problemi ambientali. Di conseguenza un'approfondita consapevolezza delle esigenze necessarie, per un maggiore controllo delle fonti di pressione d'inquinamento, diventa obiettivo primario di protezione ambientale.

Nello specifico, le tematiche affrontate dal lavoro di tesi hanno riguardato in un primo momento l'aspetto prettamente chimico-analitico, seguito da una indagine territoriale calata nella realtà regionale.

Innanzitutto è stato sviluppato un metodo multiresiduale rapido ed efficiente per la determinazione simultanea di anilina, nitrobenzene, e loro derivati clorurati (cloroaniline e cloronitrobenzeni), considerati dalla legislazione nazionale sostanze pericolose per l'ambiente idrico. Il metodo adotta l'estrazione SPE in ambiente basico con conseguente analisi in GC-MS senza alcun tipo di derivatizzazione. Una validazione della procedura in termini di linearità, recupero, ripetibilità e robustezza, nonché una preliminare valutazione di stima dell'incertezza, si sono rese necessarie per l'applicazione del metodo a livello di agenzia di controllo e laboratorio di analisi di routine su matrici ambientali. Lo stesso metodo è stato testato su matrici ambientali

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[1] Il titolo deve essere quello definitivo, uguale a quello che risulta stampato sulla copertina dell'elaborato consegnato.

reali per verificarne la fattibilità e per indagare sulla presenza di queste sostanze come inquinanti dell'ambiente acquatico della regione.

L'esigenza data dalla Direttiva Quadro di ottenere un *buono stato chimico*, sia per acque superficiali che per acque sotterranee, ha spinto allo sviluppo di ulteriori indagini legate alla problematica di nuovi inquinanti organici che in qualche modo influenzano la qualità dell'ecosistema acquatico. L'attenzione si è quindi concentrata su una classe di sostanze affine, per caratteristiche chimiche, a quelle già considerate: derivati azotati dei benzotrifluoruri (BTFs). La conoscenza in letteratura di un episodio di inquinamento della falda idrica dovuta ad alcune di queste di sostanze nella zona del Vicentino e risalente alla fine degli anni settanta, ha permesso di riconsiderare l'evento per verificare la situazione attuale nelle zone interessate dalla passata contaminazione. Le tecnologie d'indagine odierne, sia chimico-analitiche che territoriali, hanno permesso di riprendere il caso e studiarlo in modo maggiormente approfondito partendo dai pochi e disomogenei dati di cui si era in possesso. E' stato quindi possibile elaborare in modo maggiormente dettagliato i dati storici disponibili e quindi successivamente è stato possibile sviluppare specifici metodi di determinazione analitica partendo da alcune modifiche della procedura già considerata per l'analisi di cloroaniline e cloronitrobenzeni. I risultati hanno permesso di confrontare la situazione passata con quella attuale, di individuare la presenza di ulteriori contaminanti non descritti dalla letteratura come inquinanti ambientali e di identificarne una loro tracciabilità nel territorio. La persistenza ambientale di tali sostanze, riscontrabili in alcuni casi in considerevole quantità anche a distanza di decenni, e la propagazione di queste sostanze a livello di falda acquifera nella zona della Valleagno, pone la questione di quanto sia importante rivalutare e non trascurare eventi passati poiché il territorio può fungere da "archivio" per contaminazioni chimiche dell'ambiente.

Infine il lavoro di tesi ha affrontato problematiche connesse alla tossicità ambientale di alcune di queste sostanze indagando, seppur in modo preliminare attraverso test ecotossicologici effettuati su tre livelli trofici diversi, sulla reale pericolosità intrinseca delle sostanze pure.

Abstract (EN):

The thesis work, financially supported by the Environmental Protection Agency of Region Veneto (ARPAV) through the I.S.PER.I.A. Project, has addressed issues related to the implementation of the Water Framework Directive (2000/60/EC) by the institutional technical organisms in relation to the control of dangerous substances in the aquatic environment of Veneto Region. Attention was firstly focused on the definition of *good chemical status* of the aquatic environment, described by the national legislation that transpose and implement the EU legislation, and the concepts of "*pollutant*" and "*hazardous* and/or *priority substance*". These concepts are related to toxicity, persistence and bioaccumulation properties of the substances and connected with the interactions if discharged into the aquatic environment. Criteria for the adjustment of monitoring plans and of analytical control laboratories and for a characterization of the territory were considered with the target of a better knowledge of effective environmental problems. Consequently, a deep

awareness of necessary requirements for a better control of pressure sources of pollution in the territory become the primary goal of environmental protection.

Into specific, the issues addressed by the thesis focused in a first moment purely on chemical-analytical aspects, following by a territorial survey of the Region.

First of all a simple and efficient multiresidue method for the simultaneous determination of aniline, nitrobenzene and their chlorinated derivatives (chloroaniline and chloronitrobenzenes), considered hazardous substances by Italian legislation, was developed. The method is based on SPE in basic conditions and GC-MS analysis without any derivatization. Afterwards the method was validate in terms of linearity, repeatability, recovery and ruggedness including a first assessment of uncertainty estimation, with the aim to adopt the procedure in a routine/control environmental laboratory. The same procedure was tested on environmental water samples to check the feasibility and to investigate the real presence of these substances as pollutants of the aquatic system in the Region.

The requirement given by the Water Framework Directive to reach a *good chemical status*, both for surface waters and groundwaters, prompted the development of further investigations related to the new emerging organic pollutants that influence the aquatic ecosystem. The attention was focused on a class of substances very similar, for chemical properties, to the other already considered: nitrogen derivatives of benzotriflurides. The knowledge of a past pollution episode of groundwater due to some of these substances in the Vicenza Province area and dated back to the Seventies, allowed to reconsider the event and to related it with the current situation in the same area. The modern technology survey, both analytical and territorial, allowed to investigate the study case in more details starting from the few and not homogeneous past data held. Hence the elaboration and assessment of historical available data was performed following by a development of suitable analytical methods for the determination of BTFs, based on the previous developed method for chloroanilines and chloronitrobenzene. The result obtained helped to compare the current situation with the past one, to detect new pollutants never described on literature and to identify their traceability at groundwater level. The environmental persistence of these substances, detected in some cases in considerable amount even after decades, and their propagation in the groundwater of Valleagno area, raise the question of how important it is to reassess and not neglect past pollution events, as the territory can be considered a sort of chemical contamination “archive”.

Finally the thesis work addressed the issues to the intrinsic environmental toxicity of some of these substances with a preliminary assessment of ecotoxicological test based on three different trophic levels.

Firma dello studente