

**Integrated study of chemical, hydrological
and biological aspects of impaired rivers to
support restoration strategies**



Federica Benvenuto

UNIVERSITA' DEGLI STUDI DI MILANO – BICOCCA
Facoltà di Scienze Matematiche, Fisiche e Naturali
Dottorato in Scienze Ambientali (XXII ciclo)



**INTEGRATED STUDY OF CHEMICAL, HYDROLOGICAL
AND BIOLOGICAL ASPECTS OF IMPAIRED RIVERS TO
SUPPORT RESTORATION STRATEGIES**

Tutor: Dott.ssa Valeria MEZZANOTTE

Dottoranda: Federica BENVENUTO

Matricola 596065

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CHAPTER I

Integrated study of chemical, hydrological and biological aspects of impaired rivers to support restoration strategies

1.1. Introduction

The human presence and activities affect the environmental quality of water bodies in different ways. Pressures concern various aspects of the overall environmental quality and their effects are cumulative and synergic. That's why an integrated approach is needed to understand and assess the importance of each kind of stressors and to define restoration strategies.

Excellent reviews of the rapidly expanding literature on streams draining urban areas have recently been published [1-2].

The human presence and activity generate on the one hand the input of various kinds of polluting loads and on the other one a series of morphological and hydrological alterations.

In general, pollutants can derive from point and nonpoint sources. Point source pollution is typically associated to domestic and industrial discharges, municipal waste treatment facilities, and confined animal feeding operations.

The input point can clearly be found and monitored and the setup of a load reduction strategy is possible, to a certain extent. Pollutants associated with treated urban wastewater include potentially pathogenic microorganisms, nutrients, oxygen-demanding compounds and, often disinfectant residues or by-products. Most urban wastewater treatment plants (WWTPs) also receive industrial wastewater, which can contain a variety of inorganic and organic pollutants and be toxic for the process biomass and the receiving water body, and soil runoff water carrying pollutants from the watershed surface. The nature of industrial discharges varies widely compared to discharges from municipal plants and effluent flow is not always a good measure of the significance of an industrial discharge. Based on process type they come from, industrial discharges can contain organic, heavy oxygen-demanding compounds, as in the cases of pulp and paper mills, chemical and food industries or tanneries. Large amounts of noncontact cooling water can also be discharged. Surface runoff from mining and quarrying operations, where the principal source of pollutants is the land-disturbing activity rather than the addition of any chemicals or organic material, can also enter the sewers, as well as metal contaminated wastewater deriving from metal finishing or plating industry.

Nonpoint source pollution is more difficult to quantify and, especially, to control. It includes, among others, the fertilizers and pesticides that farmers may use and all the pollutants deposited on soil which can reach surface water with rain run-off or be lost by erosion and transported by wind. Tillage and other soil-disturbing activities can promote erosion and loading of sediment to water bodies unless controlled by management practices. Nutrients contained in fertilizers, animal wastes, or natural soils may be

transported from agricultural land to streams in either sediment-attached or dissolved forms. Loading of pesticides and pathogens is also of concern for various agricultural operations. Solid transport is one of the most common phenomena resulting from agricultural operations. It consists mainly of the transport of mineral particles delivered by the soil erosion, but it can also concern crop debris and animal wastes. Excess sediment loads can damage aquatic habitat by smothering and shading food organisms, altering natural substrate, and destroying spawning areas. Runoff with elevated sediment concentrations can also scour aquatic habitat, causing significant impacts on the biological community. Excess sediment can also increase water treatment costs, interfere with the recreational uses of water bodies, create navigation problems, and increase flooding damage. In addition, a relevant fraction of nutrients lost from agricultural lands, particularly phosphorus, is transported attached to sediment. Many organic chemicals used as pesticides are also transported with particles. Actually, the phenomenon of land runoff is natural, but the amount and the kind of substances which are present on land surface and can thus be carried by rain runoff, are strictly related to human presence and activity.

Wet and dry deposition from atmosphere also generate non point polluting loads.

While a difference can easily be seen between point and non point source generation and control, the same difference cannot be seen for the kind of pollutants and, especially, for the environmental risks they involve.

Nutrients can reach surface waters by sewage or – less frequently – by industrial wastewater discharge as well as by land runoff. In Italy, analytical data on the influents to wastewater treatment plants show specific pro-capite contribution of total phosphorus (normally present as ortho-phosphate in wastewater) around 1 g/inhabitant per day. For nitrogen, found in wastewater as organic and ammonia form, pro-capite contribution can be estimated as 12 g/inhabitant per day. Agriculture can be a significant source of nutrients, which can lead to excess or nuisance growth of aquatic plants and depletion of dissolved oxygen. The nutrients of most concern from agricultural land uses are nitrogen (N) and phosphorus (P), which may come from commercial fertilizer or land application of animal wastes. Both nutrients assume a variety of chemical forms, including soluble ionic forms (nitrate and phosphate) and less-soluble organic forms. Less soluble forms tend to travel with sediment, whereas more soluble forms move with water. Nitrate-nitrogen is very weakly adsorbed by soil and sediment and is therefore transported entirely in water. Because of the mobility of nitrate-nitrogen, the major route of nitrate loss is to streams by interflow or to ground water in deep seepage. Phosphorus transport is a complex process that involves different components of phosphorus. Soil and sediment contain a pool of adsorbed phosphorus which tends to be in equilibrium with the phosphorus in solution (phosphate) as water flows over the soil surface. The concentrations established in solution are determined by soil properties and fertility status. Adsorbed phosphorus attached to soil particles suspended in runoff also equilibrates with the phosphorus in solution.

At the same time some typical atmospheric pollutants, such as Polycyclic Aromatic Hydrocarbons (PAHs) can be found in wastewaters, but also in

runoff water [3-6]. Another typical case is represented by heavy metal loads. Inputs of heavy metals are related to direct industrial discharges, but also to the weathering of building components, such as roofs, gutters and facades, to the wear and tear of tyres and brake pads and of the contact wires of electrical powered rail vehicles on the roads [7].

The concept of pollution cannot be considered as independent from background and final concentrations. Actually, some pollutants act and are considered as such because of the high concentration they reach, due to human impact, but are naturally present in the environment. It is the case of nutrients and of most metals. Some others are xenobiotics and their presence in the environment is strictly dependent on artificial synthesis and input. It is the typical case of organic micropollutants such as pesticides, PAHs and many others.

Pesticides applied in agricultural production can be insoluble or soluble and include herbicides, insecticides, miticides, and fungicides. They are primary transported directly through surface runoff, either in dissolved form or attached to sediment particles. Some pesticides can cause acute and chronic toxicity problems in the water or throughout the entire food chain. Others are suspected human carcinogens. In spite of their being banned since some years ago, some pesticides, such as atrazine and simazine, are still regularly found in both surface and groundwater as such or as degradation by-products [8-13]. The prevailing of the compounds themselves or of their by-products depends of the time passed from the pollution period. Urban and suburban land uses are also a potential source of pesticides and herbicides through application to lawns and turf, roadsides, and gardens and beds.

A further pollutant source, important, even if occasional, leading to point input of both point and non point source pollutants, is the discharge of effluents from sewer overflows [14]. In the case of separate sewers, rain is conveyed carrying the pollutants released from soil by leaching and runoff. The conveyed waters are then discharged and their impact depends on the polluting load they have carried, on their receptor and on their having been treated or not before discharge. In the case of combined sewers, which are the large majority in Italy, polluting load reaching surface water bodies can be much greater, due to the first flush [15]. The increased flow due to rain events causes the re-suspension of sediments in sewers, as well as the mixing of rain water to sewage, and the first effluents whose flow exceeds the discharge threshold are characterized by high pollutant concentrations. Of course, the importance of polluting loads depends on the extension and the features of the concerned area, in terms both of domestic and industrial loads collected by the sewer networks, and of surface area and land use and characters. In principle, storage tanks for the first flush should be present and focused at collecting them gradually to wastewater treatment plants when the flow decreases or at sending them to a specific treatment before discharge. However, most often, the discharge from overflows is direct and no first rain tank exists, so rain events become the occasion of a high impact also on river water quality, especially in the first minutes. Later on, the dilution of wastewater carried by sewers prevails, so that the load discharged by overflows decreases and, at the same time, the river flow

increases, diluting furtherly the discharged load. Even if the impact of overflows has a short duration, on annual scale the input load from overflows may be significant [16, 17].

Another important point related to urbanization is the increased rate of impervious areas leading on the one hand to increased runoff and on the other one to the loss of water which cannot infiltrate in soil. During rain events, this situation causes rapid increase of water velocity and of water flow in rivers and, thus, to frequent floods. When rain stops, no water has been stored and the river baseflow is often poor [18-20]. The rapid change from flood to drought is a source of severe impact on the biotic component of the river ecosystem and the scarce availability of water flow does not allow the dilution of the eventual input of polluting loads. That's why also residual loads from wastewater treatment facilities may be high enough to generate concentrations over acceptable levels in the receptors.

The more and more important problem of low flows is obviously relevant in determining high pollutants (and/or nutrient) concentrations in river water. The climate is a chief factor, as well as the soil properties (especially permeability). The intensive exploitation of water resource due to urbanization plays itself an important role. As a consequence, often the actual flow is much lower than the ecological flow defined as the minimum level of water allowing a balanced presence and development a well structured biotic community. Water losses may be relevant on the overall water balance and water withdrawal involves the construction of in-river dams and the alteration of natural conditions of the riverbed and of the riversides, leading to habitat impairment and to decrease habitat availability. Habitats are negatively affected also by other kinds of hydraulic interventions, theoretically conceived for protecting the surrounding lands from floods or for allowing more efficient exploitation of the rivers, whose effect are immediate but increase with time, affecting, first of all, solid transport and, thus, the natural balance between erosion and sedimentation. So, channel erosion and sometimes dramatic increases in channel width and depth (incision) may take place, disconnecting the river from its floodplain, decreasing sinuosity and homogenising its profiles [21, 22]. Leopold, Huppman & Miller described these hydrogeomorphic changes as part of the 'urbanization cycle' in small river basins. In the past, a leading objective has been the maintaining of rivers as 'unchanging in shape, dimensions and pattern' [23]. This desire for physical channel stability has led to highly simplified urban stream channels – in the most extreme cases urban streams are confined in concrete channels or routed through underground pipes. More commonly the banks of urban streams have been 'hardened' using over-sized boulders or rip-rap to prevent lateral channel migration and bank erosion. These hardened streams are far from physically stable in the traditional sense that there is no progressive adjustment in channel form [24, 25], yet urban stream channels often undergo progressive enlargement and erosion [22]. A highly impacted urban stream channel often has little variation in depth or the particle sizes of bed material. Downcutting or channel incision is a common feature of urban stream channels as a result of high volume scouring flows and lateral constraints to channel migration [24].

Ecological research has only recently begun to focus on understanding how urbanization affects ecological communities and ecosystem functions. Not surprisingly, given their flashy hydrographs, low habitat heterogeneity and high contaminant loads, this recent research has documented that urban fish and invertebrate assemblages are typically species poor [25-27].

During naturally severe conditions such as droughts or floods, any individual stressor, or group of stressors, may have more severe impacts to aquatic life than during normal climatic conditions. Thus, when municipal and industrial wastewater effluents are discharged to ephemeral or intermittent streams, effluents may comprise the majority of stream flows [28]. In effluent dependent water bodies, instream flows are entirely dependent on effluent discharges. Effluent-dominated and dependent streams [29] have unique water quality characteristics that, in most cases, are comparatively different from normal stream conditions upstream of the discharge or at regional reference sites [30]. Continuous flow augmentation of intermittent streams by effluent discharges can modify available habitat, temperature, dissolved oxygen regimes, nutrient and chemical constituent loadings, water quality, and instream toxicity. Such a continuous flow regime may decrease temporal and spatial variability of instream water quality and quantity, while simultaneously causing changes in riparian habitat form and function. Therefore, understanding the chemical, physical, and biological dynamics of these ephemeral or intermittent streams is critical for maintaining water quality and offers challenges in regulatory permitting, compliance, monitoring and water quality modeling.

1.2. Structure of the research

Impacts must be identified and quantified, discerned if combined, and assigned the correct weights. Most of all, the new tools should give answers to stakeholders and planners, in order to help decision making processes when impaired freshwater ecosystems are included in protection and restoration programs. Thus, water use will be granted also in the future, hopefully in a sustainable way.

The research work carried out in the last three years and shown in this thesis concerns impaired river ecosystems and includes, in particular:

- 1) Integrated study of chemical, hydrological and biological aspects of impaired rivers, especially those affected by multiple alterations in urban environments and, thus, WWTP effluent dominated. A case study (Lura stream) is presented.
- 2) Development of analytical methods for determining organic micropollutants (PAHs, triazines and their main transformation products) in a wide range of surface and wastewater samples (coming from different environments in Italy and Spain) and, consequently, for evaluating the contamination from WWTP discharges.

- 3) Evaluation of the organic micropollutant removal efficiencies of WWTPs and comparison of the concentrations measured in effluent samples to the environmental quality objectives fixed for surface waters, as most of the receptor flow is made by the discharged effluent itself.
- 4) Study of the performance of a demonstration scale constructed wetland in removing inorganic and organic contamination from WWTP effluents, as a possible strategy for impaired river restoration.

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CHAPTER II

Effect of multiple stressors on water quality and macroinvertebrate assemblages in an effluent-dominated stream

Sergio Canobbio¹, Valeria Mezzanotte¹, Umberto Sanfilippo², Federica Benvenuto¹

¹ Dipartimento di Scienze dell'Ambiente del Territorio, Università degli Studi di Milano-Bicocca, Piazza della Scienza 1, 20126 Milano, Italy.

² Dipartimento di Ingegneria Idraulica, Ambientale, Infrastrutture Viarie e Rilevamento, Politecnico di Milano, Piazza Leonardo da Vinci 32, 20133 Milano, Italy.

Abstract

Lura stream flows in the populated and industrialized conurbation North of Milan, Italy. The area suffers a sprawling urbanization which is leading to major alterations in water quality, hydrology and morphology of streams. These water bodies are known as *effluent-dominated streams*, because most of the baseflow is given by Wastewater Treatment Plant (WWTP) discharges. In this paper, a 5 year long assessment of Lura stream is presented and the collected data is discussed to understand overall ecological quality. Multivariate analysis carried out on macroinvertebrate assemblages and environmental variables suggests that invertebrate communities suffer severe alteration both upstream and downstream WWTP discharges. Results indicate that the high polluting loads coming from WWTP discharges affect seriously the stream water quality, but the most important cause of impairment are pulse perturbations related to the modified hydrology, causing droughts and flash floods, and to the spills of untreated sewage from overflows during rain events.

Keywords

Benthic macroinvertebrates, droughts, floods, pollution, urban streams, wastewater treatment plant effluents.

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2.1 Introduction

The present research was carried out in order to provide the basis for studying and comparing possible recovery strategies for Lura stream, a small water body in Northern Italy. Lura can be defined as *effluent-dominated stream* [1], a kind of watercourse greatly affected by the *urban stream syndrome* as described by Walsh *et al.* [2]. In such stream basins, land over-exploitation in urban areas stresses the flashiness of watercourses, decreasing their natural flows and leading to sudden floods and frequent droughts. Flows given by Wastewater Treatment Plant (WWTP) effluents become the major contribution to total baseflow.

Thus, this kind of streams presents a paradox [3] in that the flow needed to support the development of biotic communities is essentially provided by the discharge of WWTP effluents, involving the input of residual but conspicuous polluting loads which can affect in other ways the communities themselves. In this kind of watersheds further problems are due to the point input of untreated sewage, the occurrence of flash floods and the consequent input of high polluting loads from the overflows of combined sewers during rain events, the alteration of riparian and riverbed morphology. Brooks *et al.* [4] indicate various challenges in studying effluent-dominated streams: (1) absence of reference condition sites; (2) influence of site-specific conditions, which are difficult to point out, on water quality; (3) possible presence, as a worst-case scenario, of emerging contaminants; (4) difficult interpretation of data due to the influence of low flow and drought conditions on biological communities; (5) alteration of water quality given by effluents and stormwater; (6) conflict between the possible use of water [5] and the integrity of stream ecosystem. The assessment of such streams is both difficult, due to variability and confounding factors [6], and important, due to the high number of people living in urban areas and asking for good environmental quality.

Therefore, an increasing number of studies is investigating these ecosystems, and particular attention is given to macroinvertebrate assemblage structures [7, 8] and their interaction with environmental variables such as physico-chemical [9, 10], hydrological [11, 12], and morphological [13, 14] indicators.

Thus, streams like Lura need a better comprehension of the synergistic effects of multiple anthropogenic stressors, and are a priority within the goals of assessment and restoration programs [15].

This paper aims to assess the overall anthropogenic pressure of Lura stream basin, and to relate changes in biotic communities to alteration occurrence, basing on the results of a 5 year survey on water chemistry, environmental variables and macroinvertebrate assemblages. Lura stream is a valuable subject of study due to the uniformity of its small basin landscape, which makes stream morphology (width, depth, slope, reach order) quite homogeneous at watershed spatial scale. Thus, macroinvertebrate assemblages are less affected than normally by the high amount of variance due to substratum composition and hydraulic gradient [16-18] as well as to longitudinal ecological changes.

2.2. Materials and Methods

2.2.1. Study area

Lura stream is 45 km long and passes through 17 municipalities in Lombardy (Italy), north of Milan, as shown in Figure 1. Its catchment (about 130 km²) is long and narrow, as is the typical case of lowland streams in this area. Lura receives water from superficial groundwater, wet meadows and small creeks, both on right and left side, and merges into the Olona river at Rho, close to Milan. Impairment is due to both the widespread urbanization, generating high polluting loads and catchment imperviousness, and to the presence of several industrial settlements. The three most critical aspects can be summarized [19, 20] in (1) the increasing water scarcity, determining frequent droughts in the upper part of the stream; (2) the occurrence of flash floods along the whole watercourse; (3) the loss of habitat suitability due to destruction of riparian vegetation and river bed morphological diversity; (4) the regular input of great polluting loads from WWTPs. Starting from the first WWTP discharge, effluents constitute most, and sometimes the only, stream flow. Two of the existing WWTPs, Alto Lura and Lura Ambiente, about 150,000 Equivalent Inhabitants (EI) each, discharge directly into Lura, the other one (Livescia, about 40,000 EI) into a small tributary, called Livescia.

Thus, Lura stream can basically be divided in 3 sectors. The first one (about 7 km) can be considered as a “reference” sector, although already affected by anthropic presence. The surrounding area suffers from a sprawling urbanization, but natural base flow is still present for most of the year, though some droughts can occur in summer. Occasionally, some untreated sewage spills affect water quality.

The second sector, 7 km long, is subject to severe droughts, due both to changes in land use and to high river bed permeability. Water can be absent for many consecutive months, regardless of the season but with strict dependence from weather conditions.

The third sector, 31 km long, is effluent-dominated. The first WWTP (Alto Lura, AL-WWTP) is straightaway followed by the inflow of Livescia tributary (2nd WWTP). After the first WWTPs, the stream runs for 15 km in a wooden and farmed land, till the town of Saronno and the 3rd WWTP.

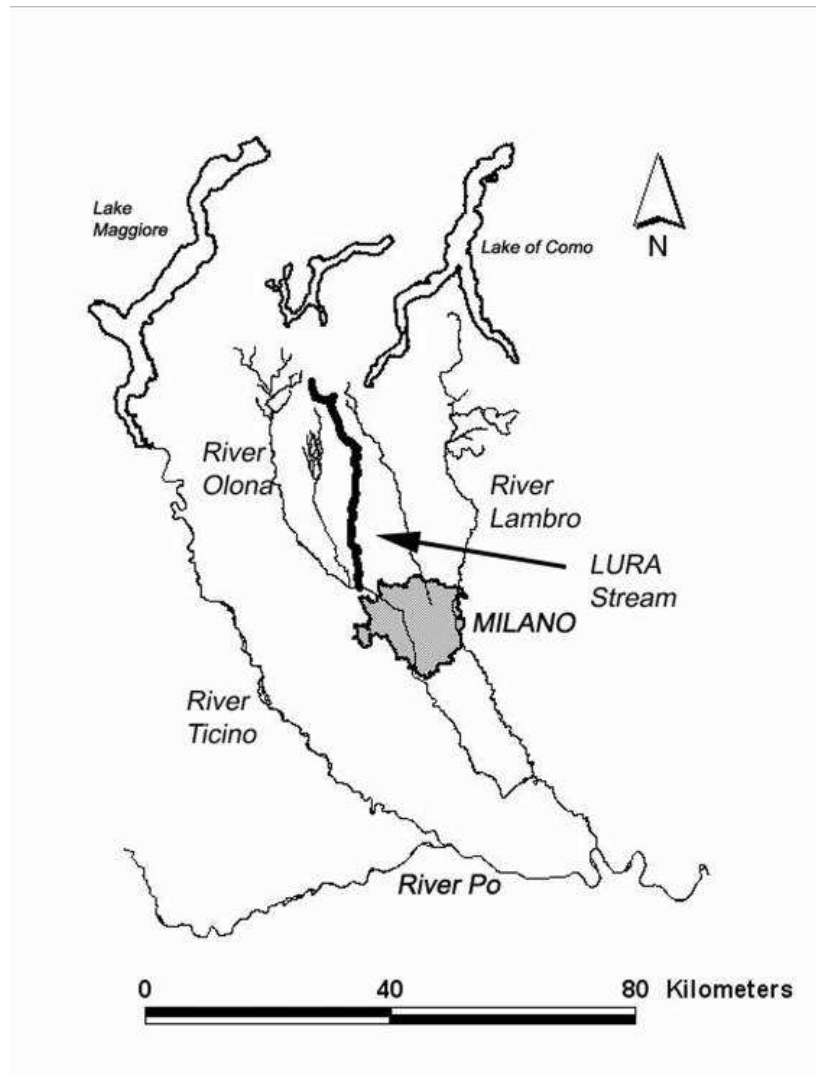


Fig. 1. Lura stream and the surrounding area

2.2.2. Data collection

To study the hydrologic regime of the Lura stream, a detailed and physically based model has been built on a MIKE 11 platform (<http://www.dhigroup.com/Software/WaterResources/MIKE11.aspx>), which is one of the most reliable and commonly used tools for river simulation. Rainfall data were collected continuously for two representative years of continuous rainfall data (2004 normal, 2005 dry) using 3 raingauges whose area of influence was estimated by the Thiessen method.

Basing on such data, hydrographs were made for 4 relevant stream sections (1 in the first "reference" sector, 1 in the second, upstream of AL-WWTP, and 2 in the longer effluent-dominated one, just downstream and 8 km after the discharge of AL-WWTP), corresponding to the 4 sampling sites chosen for monitoring macroinvertebrate assemblages. Then, flow-duration curves have been plotted for each one of the 4 river sections, in order to assess the hydrological regime of the Lura stream in terms of number of days in the year during which the river flow rate has been equal or above a given threshold value.

Water quality has been studied for 5 years (2001-2006), by monthly sampling and physico-chemical analyses. Water samples were collected in 11 sites: 1 in the first sector, 2 in the second, 8 in the third. Physico-chemical analyses included temperature, electric conductivity, dissolved oxygen (DO), pH, COD, total phosphorus (tot-P), total and ammonia nitrogen (tot-N and NH₄-N).

Macroinvertebrate assemblages were studied in 4 representative sites (as described above). Sampling campaigns have been carried out every 6 months, and every sampling campaign was done in very close days, with relatively homogeneous hydrological conditions. During each campaign, in each of the 4 sites 6 samples (covering 0.1 m²) have been collected with a 500µm mesh Surber sampler in different microhabitats, possibly along transects, thus trying to investigate the whole physical environment of the site. Invertebrates were stored in a final solution of 4% formaldehyde. In laboratory, macroinvertebrates were counted and taxonomically identified, generally to genus or family level. Invertebrates were identified using keys described in Sansoni [21].

Extended Biotic Index (EBI - Woodiwiss, [22], adapted to Italian watercourses by Ghetti, [23]), based upon DO sensitivity of taxa and their richness, was first determined, as well as Taxa Richness (S) and Shannon's Diversity Index (D). Environmental variables describing the macroinvertebrate habitats were estimated in field or in laboratory. Mean water depths and flow velocities for each site were recorded with replicate measurements at each sampling site.

Time from potential destructive flood and drought events was also recorded, and used to determine drought vicinace (quantified as the number of days from the last drought, if any, in 120 days), drought frequency (as the number of days of drought, if any, in 120 days) and flood vicinace (as the number of days from the last flood, if any, in 120 days). Finally, IFF, a river functionality index based upon RCE-2 [24], introduced in Italy by Siligardi and Maiolini [25] and adopted by the Italian Environmental Protection Agency (ANPA) [26], was used to attribute a qualitative value to four environmental variables: riparian vegetation, bankside structure, riverbed morphology and general biota conditions. These values were recorded as a ratio between obtained values and IFF given optimum (maximum obtainable value) for each of the four variables.

2.2.3. Data Analysis

Macroinvertebrate assemblages and related environmental variable data sets were analyzed using CANOCO 4.0 [27]. Specific ordination techniques (Legendre and Legendre, 1998) were used to examine patterns in the macroinvertebrate data (presence/absence of taxa) and to identify variables that were most closely associated to invertebrate distribution. Preliminary detrended correspondence analysis (DCA) on the taxa data revealed a gradient length <3 SD, indicating that most exhibited linear response [28] to environmental variations, thus justifying the use of linear multivariate analysis. Redundancy Analysis (RDA) was carried out with a forward selection of environmental variables. 999 Monte Carlo permutations were performed to determine which variables were significantly related ($P \leq 0.05$) on invertebrate distribution. The non significant variables were excluded from the analysis. Next, the Variance Inflation Factors (VIFs) of included variables were inspected. Variables with $VIF > 20$, indicating strong multicollinearity, were excluded.

2.3 Results and discussion

2.3.1. Hydrological parameters

On the whole, the MIKE 11 model confirmed the differences among sites and the partition of stream in three sectors presenting different hydrological conditions, as shown in figure 2.

Comparing the 2004 and 2005 curves, the ephemeral regime of the stream, due to the lack of groundwater contribution to the river flow, can be observed from the trends in Sites 1 and 2. In 2005 (representative of scarce rainfall years, $\text{rain/year}=544$ mm), water was completely absent for 75 and 230 days in Sites 1 and 2 respectively. In 2004, when rainfall was higher ($\text{rain/year}=839$ mm), a baseflow was always present in Site 1 and in 210 days in Site 2. The difference between the two sites is chiefly related to the high permeability of the riverbed in site 2 and to the consequent water loss.

In sites 3 and 4 flow, in dry periods, is made essentially of the effluents discharged by WWTPs, while the direct rainfall-runoff raises the stream flow for no more than one day after the event. Site 3 receives the effluent from AL-WWTP while site 4 receives also the input from Livescia (the most important tributary), whose flow, in dry periods, is completely made of the effluent from Livescia WWTP.

The flashiness of floods is clearly shown in figure 2 by the short duration of higher flows. In agreement with the greater amount of rain fallen in 2004, peak flows were higher than in 2005, furtherly confirming the strict dependence of flow from meteorological events.

2.3.2. Physico-chemical parameters

Trends of physical and chemical factors in low flow (site 2 dry), moderate flow (site 2 with a base flow) and flood peak conditions are shown in figure 3. In low and moderate flow conditions, the most noticeable event

was always the input of the effluent from AL-WWTP (site 3), which strongly affected the values of all parameters.

Water temperature was usually higher after WWTP discharges (indicated by arrows in figure 2) and decreased downstream, due to exchange with air temperature, especially in low flow conditions. When a base flow was present upstream the AL-WWTP discharge, temperature peaks were controlled by the immediate dilution. With higher flows (flood condition) temperature was almost homogeneous in the entire stream, due to the very little contribution of WWTP effluents to the total streamflow.

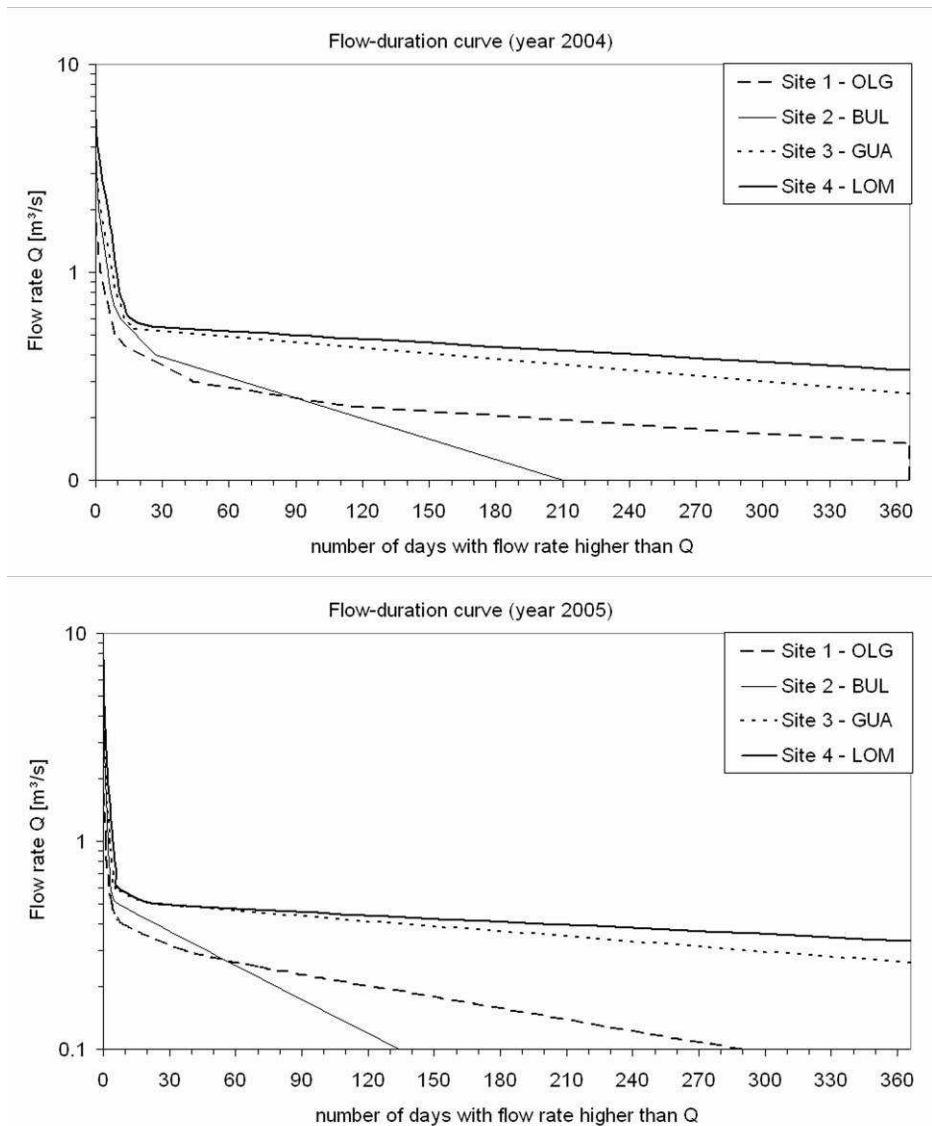


Fig. 2. Flow-duration curves for 4 Lura sections in years 2004 and 2005

Though the stream receives WWTP effluents which commonly have low DO saturation, drops in DO saturation were not significant as expected. This is due to the fact that AL-WWTP effluent presents high DO saturation itself, mainly because of the use of ozone as the main disinfectant. Thus, mean DO saturation was never below 80%, even downflow the effluent discharges. Drops in DO saturation were almost cancelled with the presence of significative base flow. During floods, starting from site 2 the action of various sewage overflows, pouring in high loads of ammonium nitrogen and organic matter, caused oxygen saturation to decrease.

In low flow conditions, $\text{NH}_4\text{-N}$ concentrations measured at Site 1, though considered as a reference site, were high (average > 1 mg/l), probably because of uncontrolled inputs in rural areas. After AL-WWTP discharge (site 3), mean conductivity was about 1500 $\mu\text{S/cm}$, COD 60 mg/l, total P 1,5 mg/l and total N 17 mg/l. With moderate flows trends were similar but concentrations were lower. However, some spills in urban areas originated other peaks. During floods, peaks in COD and total N and, especially, $\text{NH}_4\text{-N}$ were already present in site 2 due to the already cited sewage overflows. At site 2, during floods, ammonia concentration raised to about 2 mg/l (as mean flood value), leading to estimate polluting load as very high, taking into account the high diluting flows (7.555 m^3/s in November 2004 sampling campaign, which is Q_1 flow for site 2 in figure 2).

2.3.3. Macroinvertebrate assemblages

45 taxa were identified in the 4 monitored sites. First, Taxa Richness (S), Shannon's Diversity Index (D) and EBI were calculated in order to obtain a simple ranking tool. Results are reported in Table 1. Site 1, representing the first "reference" reach, is characterized by benthic invertebrate assemblages of higher quality. Both the highest Taxa Richness (from 10 to 16, mean $S=12.3$) and the most sensitive taxa were detected, including Plecoptera (Leuctridae and Nemouridae) and various Ephemeroptera. Stoneflies disappeared completely in the other sampling sites, while the only mayflies detected downstream were *Baetis* spp. In site 2 *Baetis* spp. and some Diptera (especially Chironomidae and Simuliidae) were usually dominant, while in site 3 and 4 Trichoptera Hydropsichidae was the most noticeable taxon. *Baetis* almost disappeared in site 3 but was always found in site 4. In these two sites, Chironomidae were ubiquitous and extremely abundant. An occasional presence of Gastropoda, sometimes abundant, could be observed.

At site 1, EBI values were comprised between 7 and 8, corresponding to Class II-III (of V), while Shannon's Diversity Index (D) had a mean value of 1.62. In Site 2 water quality was still acceptable, but, as already stated, the watercourse suffered from important hydraulic fluctuations, with long droughts and sudden floods. Such situation led S to 6-11 (mean value 7.9) and EBI and D to lower values. At site 3 the AL-WWTP effluent influence brought to highly variable EBI values (2 to 6, corresponding to Class III to V). Such variability, involving also S and D values, was mainly related to flow conditions. At site 4, better riverbed morphology and a residual self-purification ability of the stream (which can buffer pulse disturbances and

polluting load peaks) led to a slightly better situation: EBI raised to 5-6, ranking site 4 in Class III-IV, while D was comprised between 0.85 and 1.80.

Table 1
Synthesis of macroinvertebrate sampling results and EBI classification

Site	N. sampling campaigns	Taxa richness (S)	Abundance of individuals (n/0.1m ²)	Shannon's Diversity Index (D)	EBI value
		min – max (mean ± SD)	min – max (mean ± SD)	min – max (mean ± SD)	min – max (mean ± SD)
1 – Olgiate	10	10 – 16 (12.3 ± 2.0)	194-580 (375 ± 149)	1.49 – 1.88 (1.62 ± 0.13)	7 – 8 (7.8 ± 0.4)
2 – Bulgaro	8	6 – 11 (7.9 ± 1.9)	53-1082 (516 ± 296)	0.94 – 1.60 (1.30 ± 0.28)	5 – 6 (5.4 ± 0.5)
3 – Guanzate	10	4 – 11 (7.8 ± 2.4)	119-2601 (1092 ± 697)	0.65 – 1.33 (1.04 ± 0.22)	2 – 6 (5.1 ± 1.3)
4 – Lomazzo	10	6 – 10 (7.8 ± 1.1)	100-844 (503 ± 238)	0.85 – 1.80 (1.31 ± 0.26)	5 – 6 (5.8 ± 0.4)

Mean values for environmental variables observed during macroinvertebrate sampling are presented in table 2, as well as standard deviations, minimum and maximum values. Water depth was comparable between sites, and stream width increased with flow. Mean velocity values were similar in site 1, 3 and 4, but much lower in site 2. This variable was related to drought frequency and drought proximity. The values observed for physico-chemical parameters during macroinvertebrate sampling campaigns were comparable to the values measured over the whole period of monitoring of water quality (see figure 3). Site 1 had the best vegetation (with willows, alders and cottonwoods as valuable riparian elements within the black locust woods) and biota, because of normal periphytic cover and availability of coarse particulate organic matter (CPOM).

Higher values of rainfall were related to the proximity of flood events but lower values were not related to droughts, because droughts involved only site 2 (and partially site 1). Thus, collinearity between scarce rainfall and droughts could only be observed for site 2. Droughts were recorded also at site 1 but only before autumn sampling campaigns (years 2003 and 2005).

Table 2
Environmental variables at macroinvertebrate sampling sites during monitoring campaigns; mean values \pm SD (min. – max.)

Environmental variables		1 - Olgiate	2 - Bulgaro	3 - Guanzate	4 – Lomazzo
Depth (mean)	cm	13 \pm 2 (10 - 18)	20 \pm 4 (15 - 26)	18 \pm 3 (15 - 23)	21 \pm 2 (18 - 26)
Width	m	3.1 \pm 0.1 (2.9 - 3.3)	3.9 \pm 0.3 (3.5 - 4.4)	5.5	6.3 \pm 0.1 (6.3 - 6.7)
Flow Speed (mean)	m/s	0.34 \pm 0.05 (0.27 - 0.42)	0.15 \pm 0.04 (0.09 - 0.21)	0.39 \pm 0.05 (0.34 - 0.51)	0.32 \pm 0.05 (0.28 - 0.45)
T	°C	8.2 \pm 5.8 (1.5 - 16.5)	12.8 \pm 2.8 (9.4 - 17.5)	17.5 \pm 6.0 (11.2 - 26)	14.0 \pm 5.8 (5.8 - 22.1)
DO	%	98.1 \pm 10.6 (82.1 – 118.5)	105.2 \pm 36.8 (61.0 - 186.1)	98.0 \pm 15.9 (73.8 - 124.1)	97.4 \pm 10.9 (81.7 - 114.0)
pH	pH u.	7.81 \pm 0.29 (7.27 - 8.21)	8.04 \pm 0.31 (7.56 - 8.56)	7.47 \pm 0.27 (6.93 - 7.8)	7.86 \pm 0.29 (7.18 - 8.22)
Conductivity	mS/cm	441 \pm 175 (254 - 815)	419 \pm 56 (309 - 506)	1320 \pm 506 (418 - 1876)	1341 \pm 461 (398 - 1764)
COD	mg/l	9 \pm 3 (5 - 17)	19 \pm 19 (5 - 60)	53 \pm 17 (35 - 83)	52 \pm 12 (31 - 72)
P-tot	mg/l	0.174 \pm 0.108 (0.047 - 0.370)	0.217 \pm 0.187 (0.056 - 0.632)	1.359 \pm 0.682 (0.325 - 2.618)	1.567 \pm 0.858 (0.537 - 2.782)
N-tot	mg/l	5.017 \pm 1.640 (2.740 - 8.590)	4.599 \pm 2.295 (2.300 - 9.170)	17.449 \pm 5.626 (6.950 - 24.750)	16.420 \pm 5.630 (5.830 - 23.735)
NH4-N	mg/l	0.632 \pm 0.835 (0.082 - 2.580)	0.592 \pm 1.021 (0.000 - 2.465)	2.966 \pm 4.901 (0.029 - 15.950)	1.213 \pm 2.446 (0.045 - 7.950)
Riparian Vegetation Condition	IFF value / IFF optimum	0.65	0.37	0.53	0.42
Riparian Structure Condition	IFF value / IFF optimum	0.55	0.13	0.56	0.67
River Bed Condition	IFF value / IFF optimum	0.41	0.42	0.44	0.55
Biota Condition	IFF value / IFF optimum	0.53	0.22	0.18	0.20
Rain	mm (120 days)	265 \pm 124 (64 - 420)	269 \pm 135 (80 - 517)	283 \pm 133 (129 - 568)	278 \pm 110 (167 - 567)
Droughts (vicinace)	1-(days from dry/120 days)	0.152 \pm 0.251 (0.000 - 0.625)	0.590 \pm 0.407 (0.000 - 1.000)	0.000	0.000
Droughts (frequency)	days of dry /120 days	0.171 \pm 0.299 (0.000 - 0.750)	0.498 \pm 0.398 (0.000 - 1.000)	0.000	0.000
Floods (vicinace)	1-(days from event/120 days)	0.352 \pm 0.330 (0.000 - 0.833)	0.190 \pm 0.297 (0.000 - 0.833)	0.413 \pm 0.380 (0.000 - 0.900)	0.335 \pm 0.341 (0.000 - 0.778)

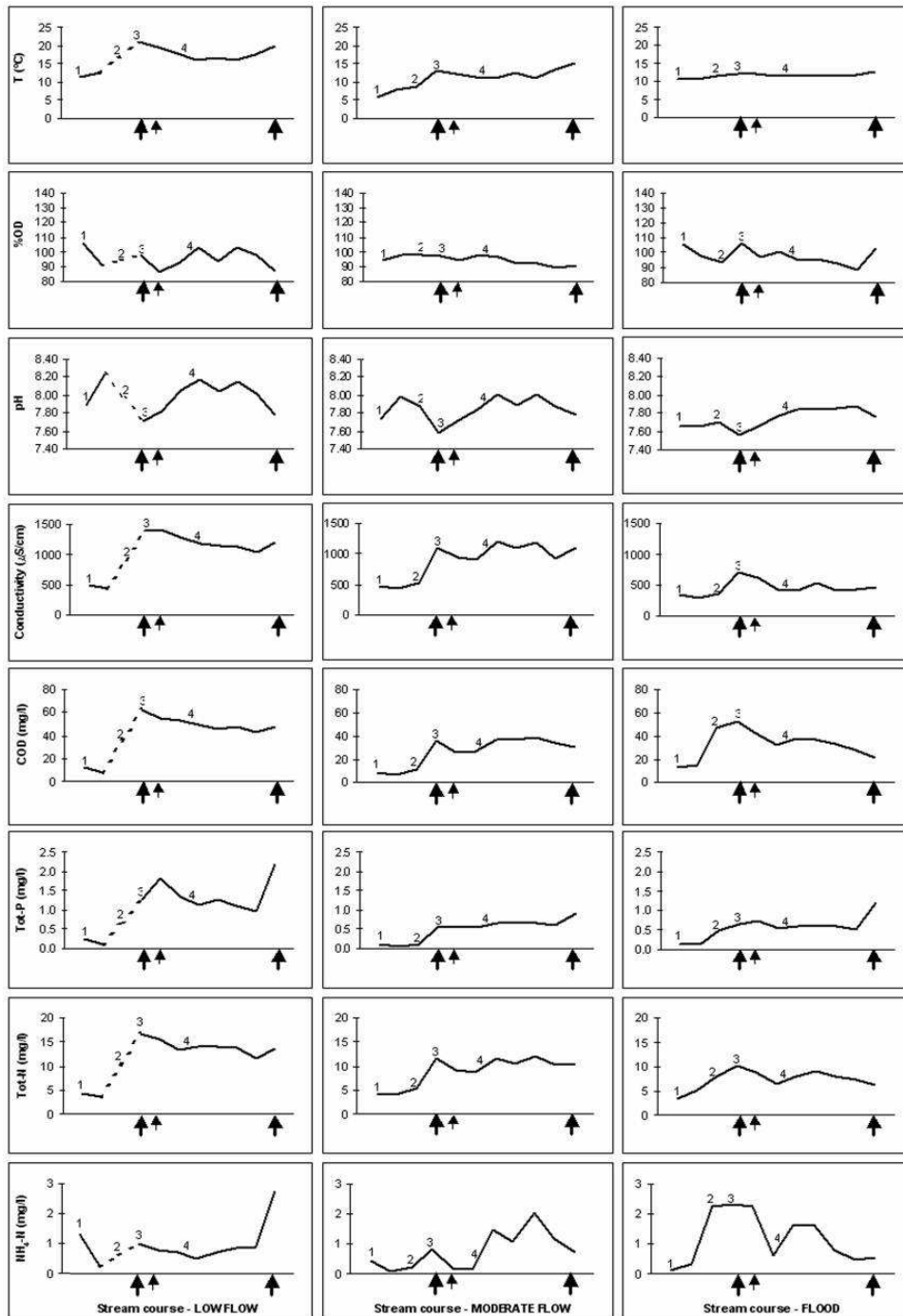


Fig. 3. Mean values of physico-chemical parameters in low flow (column A), moderate flow (column B) and flood (column C) conditions along the stream course during 2001-2006. Numbers indicate macroinvertebrate sampling sites, arrows indicate WWTP discharges.

Redundancy Analysis was applied to macroinvertebrate community data and associated environmental variables as shown in figure 4 and 5. In figure 4, different colors indicate EBI classes for each site in each sampling campaign. Site 2 is represented by 8 scores only, because in spring/summer 2003 and in autumn/winter 2005 (samples 2.04 and 2.09) droughts covered the whole period and sampling was not possible. Site scores are separated along the two axis that account for most of the variability based on benthic invertebrates assemblage structure. For this RDA, the first two axes showed 26.8% of macroinvertebrate variability and 51.5% of species-environment relation variability. The test of significance for both the first axis and overall RDA was highly significant ($P = 0.001$).

Position of site scores indicates a clear division within the first axis among sites upstream and downstream the AL-WWTP in all seasons. Occasionally, sites downstream the effluent discharge (4.05; 3.03, 3.05, 3.06) were more similar to upstream site 2. This was directly related with floods event vicinity and, thus, with higher base flow in the upstream sites and higher diluting flow downstream. This, however, brought to the lowest EBI values (3.03 and 3.06) due to flood destructivity and sewage overflow spills. WWTP upstream sites (sites 1 and 2) are distinctly separated by vertical Axis 2, as site 2 is mainly affected by drought events.

Environmental variable vectors represent gradients through the community data, with the arrow pointing the area of higher value for each variable, but the gradient extends through the whole set. Axis 1 is positively related with droughts, pH and biota quality, and negatively related with temperature, ammonia nitrogen, conductivity and riverbed morphology (the latter presents low values along all the stream). These variables are also collinear with other, omitted chemical parameters and they are all related with the effluent dominated sector of the stream. Worse EBI results for sites downstream AL-WWTP discharge occur in two clusters near highest value of $\text{NH}_4\text{-N}$ and floods. Site 2 scores are related with both droughts and (along Axis 2) floods, and are defined by low EBI values. On the other hand, site 1 scores are the best of the entire set and are only slightly related with droughts, but strongly related with good riparian vegetation and biological quality.

Taxa distribution is represented in figure 5. Environmental variables were omitted from graph to make it clearer, but their gradients are reported in figure 4. The greatest diversity is positively related with Axis 1 and negatively related with Axis 2, and is thus in the lower-right quadrant where site 1 scores are clustered (presenting negative relationship with both pollution on axis 1 and droughts on axis 2). All sensitive taxa are here, including all stoneflies and mayflies. Some taxa presented positive relationship with droughts, particularly Crustacea (both Gammaridae and Asellidae) and some Gastropoda (*Ancylus*). Caddisflies of Rhyacophilidae family were found only in one sample at site 2, thus being allocated in the same area. Some taxa were extremely tolerant about pollution, preferring sites 3 and 4 which, on the other hand, ensure to invertebrate communities a constant base flow. Such characteristics were preferred by Odonata, some Diptera, Gastropoda (*Lymnaea* and *Physa*) and Trichoptera Hydropsichidae. Both Oligochaeta Tubificidae and Diptera Chironomidae were completely ubiquitous and are centered in the origin of the graph.

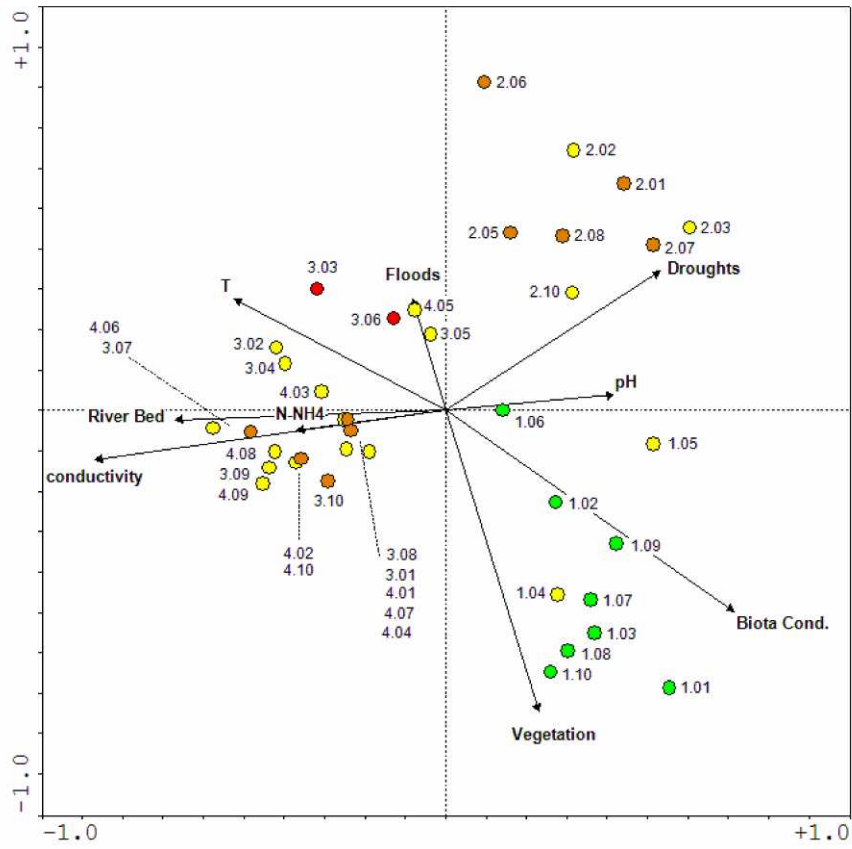


Fig. 4. RDA biplot graph indicating relationships between environmental variables (arrows) and macroinvertebrate communities sampled in the 4 sites. Labels show also a number indicating the period of sampling, every 6 months from fall/winter 2001 (.01) to spring/summer 2006 (.10). Site scores are indicated with different colors showing their EBI value (red: 2 to 4; orange: 5; yellow: 6-7; green: 8).

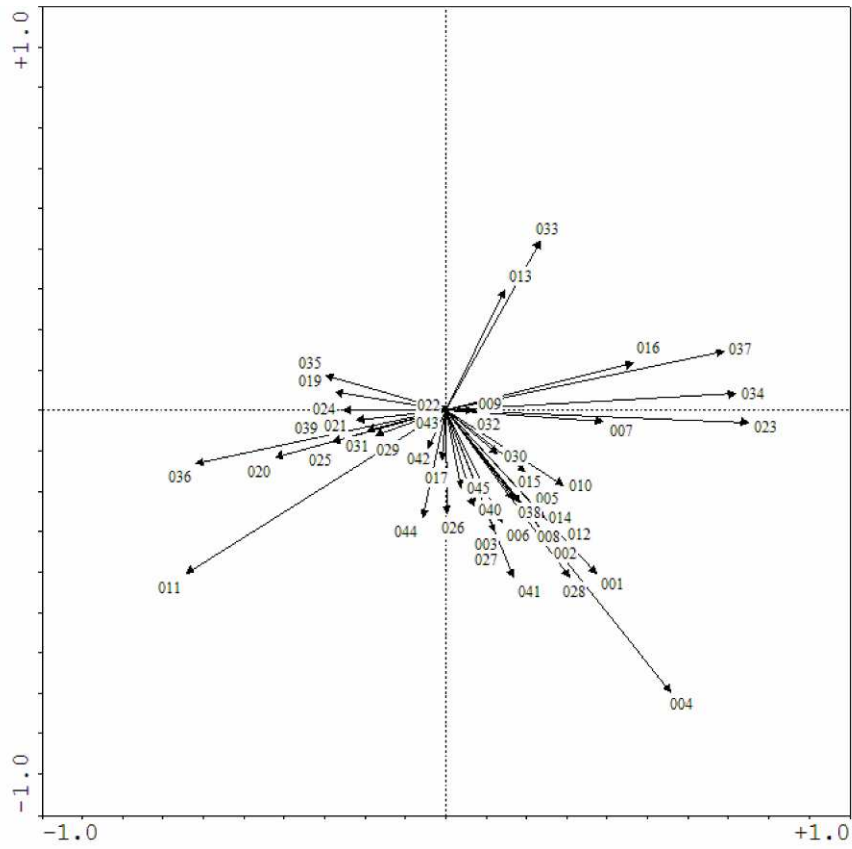


Fig. 5. Scatter diagram of macroinvertebrate taxa found in Lura Stream. Scores are related to environmental variables and sites as shown in figure 4. Legend:

<i>n.</i>	<i>Taxa</i>	<i>n.</i>	<i>Taxa</i>	<i>n.</i>	<i>Taxa</i>	<i>n.</i>	<i>Taxa</i>
1	<i>Leuctra</i>	13	Rhyacophilidae	25	Tabanidae	37	<i>Ancylus</i>
2	<i>Anphinemura</i>	14	Philopotamidae	26	Tipulidae	38	<i>Planorbis</i>
3	<i>Nemoura</i>	15	Goeridae	27	Psychodidae	39	<i>Haemopis</i>
4	<i>Ecdyonurus</i>	16	Dytiscidae	28	Dixidae	40	<i>Dina</i>
5	<i>Rithrogena</i>	17	Elminthidae	29	Rhagionidae	41	Naididae
6	<i>Habrophlebia</i>	18	Hydraenidae	30	Athericidae	42	Lumbricidae
7	<i>Baetis</i>	19	<i>Onychogomphus</i>	31	Anthomyidae	43	Tubificidae
8	<i>Procleon</i>	20	<i>Orthetrum</i>	32	<i>Nepa</i>	44	Lumbiculidae
9	<i>Habroleptoides</i>	21	<i>Crocothemis</i>	33	Gammaridae	45	Mermithidae
10	<i>Paraleptophlebia</i>	22	Chironomidae	34	Asellidae		
11	Hydropsichidae	23	Simuliidae	35	<i>Lymnaea</i>		
12	Limnaephilidae	24	Limonidae	36	<i>Physa</i>		

2.4. Conclusions

According to the results of physico-chemical monitoring, the most important factor affecting water quality of Lura stream is Alto Lura WWTP discharge. Effluents input continuously a too high polluting load (especially organic matter and nutrients) which cannot be diluted enough by the scarce or void baseflow which is usually present. Hydrologic regime, which is heavily affected by the basin intense urbanization, has a key role in influencing WWTP effluent effects on stream water quality. Collected data shows that very different physico-chemical conditions occur when the three hydrologic regimes (low flow, moderate flow and flood) alternates. The best conditions, with low pollutant concentrations, were generally observed during moderate flow periods, due to the stream diluting capability. However, hydrological parameters analysis shows that such periods are short, especially in years presenting scarce rainfall. Low flow is a more standard condition in effluent-dominated streams, usually influenced by their basin land use and, thus, presenting ephemeral regime. Flash floods are another hydrological distinctiveness of the stream, and even if they present a low frequency, as shown by hydrological parameters analysis, they strongly influence the stream overall ecological quality. During floods, spills of untreated wastewater occur along the stream, balancing the very high diluting capability of such high flows with greater polluting loads. The main consequence is that some peaks in pollutant concentrations (especially ammonia nitrogen and organic matter) can be found, affecting both the stream sector upstream WWTP discharges and the effluent-dominated one. Moreover, this kind of perturbation occur when the mechanical action of floods already affects biotic communities.

Thus, the altered hydrologic cycle and the consequent alternance of drought and flood events have the greatest impact on the ecology of Lura stream. This happens both upstream the WWTP discharges, where water scarcity originates destructive droughts preventing a balanced development and survival of invertebrate assemblages [30], and after them, where the effect of floods, taking place frequently, becomes dominant [31]. As expected [3], variation in macroinvertebrate assemblages is associated with various classes of variables. Multivariate analysis divides macroinvertebrate communities upstream and downstream WWTP discharges along Axis 1, as shown also in Couceiro *et al.* [32], but the worst EBI and Shannon's D Index values, indicating the poorer biotic communities, are confined in clusters influenced by the simultaneous presence of the worst water quality [33], given by ammonia nitrogen spills, and of recurring floods. Thus, while the effluent-dominated section of the stream obviously presents a worse macroinvertebrate assemblage structure if compared with the reference site (lower Taxa Richness, Shannon's D Index and EBI values), the worst impacts affecting the stream ecology are not given by WWTP treated effluents, but are caused by untreated discharges (mainly due to sewer overflows), according to literature [34, 35], and altered hydrology. Samples related to such extreme conditions show not only a worse quality in taxa sensitivity (no Plecoptera, only tolerant Ephemeroptera and Tricoptera),

typical for all the samples in the effluent-dominated portion of the stream, but also a significantly lower taxa richness, caused by the absence of even some tolerant taxa, and density. The relationship between macroinvertebrate taxa and environmental variables given by multivariate analysis shows that there are very few taxa positively related with the presence of hydrological perturbations (both floods and droughts), while there are various taxa (mainly tolerant ones) that show a positive relationship with pollution indicators of the effluent-dominated sector of the stream. Tolerant macroinvertebrates can use the stream environment downstream the WWTP discharges as a stable living habitat (due to the continuous presence of water), but communities are greatly affected by episodic perturbation such as droughts, floods and spills of untreated wastewater. Moreover, the overall quality of the stream morphology is low and this causes an increase in all the other alteration effects, due to low habitat availability and absence of refugia for biotic communities [36].

On the basis of the disturbance characteristics and the way they occur in the stream, perturbations affecting Lura can be divided in two kinds, that can be called *press* and *pulse* [37]. Actually, *press* impacts (WWTP discharges) showed a more evident effect on water quality but *pulse* ones (droughts, floods, spills) presented worse effects on biota, due to the low resistance of the stream ecosystem. Paradoxically, WWTP discharges grant a stable baseflow to biotic communities and, thus, macroinvertebrate assemblages in the effluent-dominated sector, when no other perturbation occur, show high densities of individuals and intermediate quality. Such quality is significantly lower if compared with reference site but much better than the quality of assemblages affected by *pulse* perturbations, both upstream and downstream WWTP discharges. In site 2, where water quality is generally good but *pulse* perturbances occur, values of Taxa Richness, Shannon's D Index and EBI can drop far below the mean values of the effluent-dominated sites, if perturbations like droughts, floods and/or sewage overflows take place. On the other hand, in the effluent-dominated sector of the stream, the worst ecological quality of the whole stream is found when *pulse* perturbances (floods, untreated wastewater discharges) sum their effects to the continuous *press* disturbance given by WWTP discharges.

In effluent-dominated streams like Lura, WWTP effluents can be perceived as the greater impact. It is obvious that, if compared with available reference sites, the water quality and biotic communities of effluent-dominated sites result greatly altered. However, it must be considered that the same basin features (especially sprawling urbanization) that normally lead to a conspicuous amount of effluent discharges, originate other kinds of perturbations, both *press* (such as reduced baseflow and altered morphology) and *pulse* (droughts, flash floods and spills of untreated wastewater) that are both difficult to assess and greatly impacting. *Pulse* perturbations do not allow the ecosystem to regulate itself to the new conditions, while *press* perturbations concur in making the ecosystem resistance and resilience weak. Thus, all the mentioned alterations have great influence and involve themselves reciprocally. Environmental studying and restoration planning in effluent-dominated streams should not be limited to water quality, but should consider, in example, measures for increasing

habitat quality, providing acceptable flow conditions [38] and improving morphology.

Acknowledgements

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CHAPTER III

Removal of polycyclic aromatic hydrocarbons and triazine herbicides in wastewater treatment plants

Abstract

Samples of raw and treated wastewater were collected between May 2008 and January 2009 at two wastewater treatment plants (WWTPs) located in Northern Italy (Como Province). The collection sites are two activated sludge WWTPs receiving domestic and industrial wastewaters and provided with ozonation as final treatment step. Analysis of simazine, atrazine, terbuthylazine and fifteen types of Polycyclic Aromatic Hydrocarbons (PAHs) were performed by coupling High Performance Liquid Chromatography (HPLC) with UV-diode array detection (UV-DAD) and fluorescence detection (FLD) after a pre-concentration step based on solid-phase extraction (using OASIS HLB cartridges). Satisfactory recoveries (60-110%) and relative standard deviations (<10%) were obtained for almost all compounds in Milli-Q water samples spiked at 0.3 µg/L. Only for simazine significant losses in this process were observed in all the tested water samples.

The two investigated WWTPs showed similar removal rates, around 70 % and 50%, for Σ triazines and Σ PAHs, respectively. Ozonation did not appear to have significant role in the removal of the analyzed micropollutants. The additional removal due to ozonation ranged between <1 and 35% in both WWTPs.

Finally, the concentrations measured in the effluent samples were compared to the Environmental Quality Standards for surface waters, as the treated wastewaters contribute most of the receptor flows.

Keywords

Polycyclic Aromatic Hydrocarbons, triazine herbicides, wastewater treatment, activated sludge process, ozonation, removal efficiency.

Working in progress

3.1. Introduction

Due to the increased public concern over possible exposure to pollutants in water, increased attention has been given to the fate of pollutants introduced to wastewater treatment plants (WWTPs), especially those serving both urban and industrial areas. If these pollutants are not completely mineralized within a treatment system then it is possible for some fraction of the pollutant involved to be released into the environment as part of the final effluent discharge or as a component of the sludges produced or, indeed, or to be volatilized directly to the atmosphere.

The effective operation of WWTPs plays an important role in minimizing the release of organic pollutants into the aquatic environment. However, nearly all systems now in operation have been designed for reducing the values of some non-specific analytical parameters, such as the Biochemical oxygen demand (BOD), the Chemical Oxygen Demand (COD), and Total Suspended Solids (TSS) [1, 2] or for removing nutrients (N and P). These parameters are useful in defining the efficiency of WWTPs for the pollutants; however present trends indicate that effluent standards will soon be based not only on such parameters but on individual toxic priority pollutants as well [3, 4]. Moreover, often the receiving water bodies cannot provide any dilution of the residual load input, due to their low flow, and the discharge of effluents from WWTPs, even if well treated can therefore be a significant source of impact and, often, prevent to comply with quality standards for river waters [3].

The removal of micropollutants from wastewater during treatment may occur by abiotic occurs through abiotic transformation, biological degradation and/or sorption. Among these mechanisms, sorption to suspended solids and biodegradation were reported to play predominant roles [5, 6]. Nevertheless, mechanisms of removal do not follow a general rule since their relative contribution depends on the physicochemical properties of the specific micropollutant, the origin and composition of the wastewater, and the operational parameters of the wastewater treatment facility. Many of the organic micropollutants found in wastewater are hydrophobic compounds. Hydrophobicity is the main property, which leads to sorption to the sludge, fat and particulate matter during the wastewater treatment [7, 8].

Micropollutants can sorb to suspended solids and subsequently be removed via the withdrawal of the excess sludge during the wastewater treatment. Sorption of micropollutants to the solid phase can be estimated using the K_{ow} values, representing the partitioning of the organic solute between the organic phase, i.e., octanol and the water phase [9, 10]. High K_{ow} is characteristic for hydrophobic compounds, poor hydrosolubility and high tendency to sorb on organic material of the sludge matrix. For compounds with $\log K_{ow} < 2.5$, the sorption to activated sludge is not expected to contribute significantly to removal via excess sludge withdrawal via. Between $\log K_{ow}$ 2.5 and 4 moderate sorption is expected and values higher than 4.0 are synonyms to high sorption potential.

Advanced WWTPs combine physical, chemical and biological treatment steps to remove solids and nutrients, perform flocculation and sedimentation, and precipitate phosphates to reduce the risk of

eutrophication for the receiving surface waters. However, most conventional sewage treatment plants throughout Europe adopt a system of 3 different steps made of a primary clarifier, an activated sludge basin, and a secondary clarifier (Figure1).

In the primary clarifier, suspended solids are settled and build up a first sludge fraction. In the activated sludge basin, nutrients and organic matter are removed by biological mineralization and nitrification/ denitrification. In the biological oxidation step some inorganic and organic recalcitrant compounds are also removed by sludge sorption. Phosphates are partly removed by biomass uptake and, in specifically designed WWTPs, their removal is enhanced by providing optimal conditions to polyphosphate-accumulating bacteria. When the effluent is discharged into a lake or in risk areas, phosphate removal can also be obtained by chemical precipitation. The secondary clarifier removes sludge and absorbed chemicals. Sludge from the primary clarifier and the surplus sludge from the activated sludge system are treated in separate units and finally disposed [5].

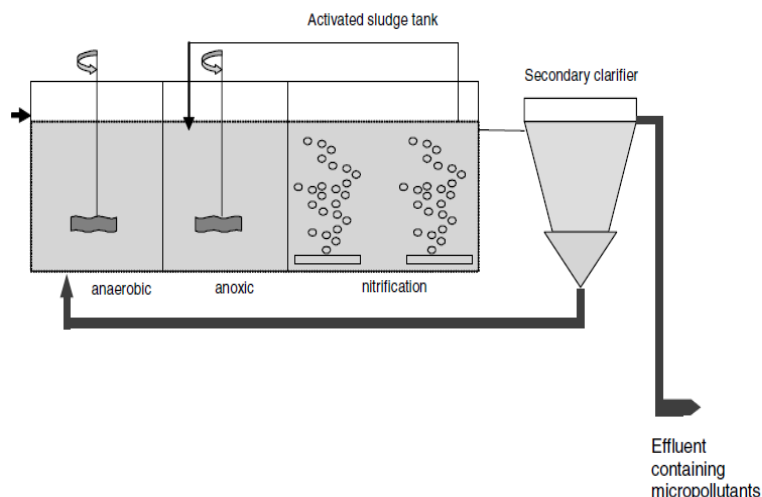


Fig. 1. State of the art of wastewater treatment (modified after Siegrist et al. 2004). From left to right: Primary clarifier for sedimentation of solids; Activated sludge basin for microbial decomposition of nutrients and pollutants; Secondary clarifier for removal of fines and chemicals by absorption processes.

In order to improve the removal efficiencies of organic micropollutants within WWTPs, discussion has focused on upgrading the overall treatment process with additional treatment steps [11, 12]. Activated carbon and ozonation are processes bearing the potential to significantly lower the micropollutant load and are also feasible with regard to energy consumption and costs [12].

Ozonation of drinking and wastewater for disinfection purposes has a long tradition, but its benefits for micropollutant removal during wastewater treatment have only been discussed recently [13-18].

Ozone is a selective oxidant with second-order rate constants for the reaction with organic compounds ranging over approximately 10 orders of

magnitude, from no reaction up to almost 10^{10} M^{-1} [19]. It is particularly reactive toward functional groups with high electron density such as double bonds, activated aromatic systems, nonprotonated secondary and tertiary amines, and reduced sulfur species. The major uncertainty of ozonation is related to the formation of oxidation by-products. That's why the applications of ozone as a final treatment step for the removal of specific compounds is not so widespread. When high ozone doses are required, the risks of generating undesirable by-products increase, as well as, of course the treatment cost [20].

The micropollutants which can be found in wastewater include include triazines herbicides and polycyclic aromatic hydrocarbons (PAHs), which are regulated by the European Directives for their potential toxicity [3]. Their chemical structures and physical properties are shown in Tables 1 and 2.

Triazines, herbicides with high efficiency for weed control, have been largely used in agriculture over the past years. However, they are also in the list of chemical pollutants that need to be carefully monitored due to their toxicity, persistence and accumulation in the environment and to their effects on the environment and human health [4].

Triazines and their degradation products are very toxic and highly resistant. They may persist even many years in the soil [21], water [22], plants, and animals [23]. That's why most triazinic compounds have been banned in many countries, including Italy, where the use of simazine and atrazine is no more allowed since 2004. In spite of this, due to their persistence, it is still common to find them in surface- and, especially, in ground waters [24, 25]. In the European Community, the upper limit for the presence of an individual pesticide in drinking water is set at $0.1 \mu\text{g/L}$ and the limit is $0.5 \mu\text{g/L}$ for the total pesticide content [26]. In surface water, these limits are about an order of magnitude higher [27]. In 2009 the Italian legislation has established specific limits for pesticides, considering the toxicity of each one: quality objectives of 1, 0.6 and $0.5 \mu\text{g/L}$ for the concentration of simazine, atrazine and terbuthylazine respectively are fixed [28].

PAHs are ubiquitous environmental pollutants with carcinogenic and mutagenic properties, entering the environment via natural and anthropogenic pathways [29, 30]. Human activities are significant sources in the environment, since PAHs are formed in all processes involving incomplete combustion of organic compounds. Anthropogenic sources include emissions from vehicle exhausts, asphalt pavements, unvented radiant and convective kerosene space heaters, and heating appliances; natural sources include all incomplete high-temperature combustion reactions and pyrolytic processes involving fossil fuels, e.g. peat, coal, and petroleum.

PAHs may enter a treatment plant either as part of an industrial discharge, or as a component of domestic effluent drainage into the sewerage system and urban runoff, including fossil fuel spillages and atmospheric deposition [31-34]. Due to their lipophilic and hydrophobic nature, PAHs enter the wastewater treatment systems notably adsorbed onto particulate organic matter, and because of this affinity for particulates, they are effectively removed during the primary and secondary sedimentation of wastewaters to the sludge stream [35]. Furthermore, mass balance investigations have

showed substantial losses (>40%) for the lower molecular weight PAHs during biological treatment, thus suggesting removal due to air stripping and/or biodegradation [32].

Because of their mutagenic, carcinogenic, toxic nature and environmental ubiquitous occurrence, 16 of them have been selected by the US Environmental Protection Agency (EPA) as US-EPA and EU priority pollutants and consequently their concentrations need to be controlled in treated wastewater effluents.

The aim of the present work was to improve knowledge on the removal of PAHs and some triazine herbicides during the wastewater treatment process. For this purpose, organic micropollutants, were determined in wastewater samples collected from various points along two WWTPs, including ozonation as final treatment step. Removal efficiencies were calculated from actual field data of a year measuring campaign. The concentrations measured in effluent samples were compared to the Environmental Quality Standards (EQSs) for surface waters, as the treated wastewaters represent the majority of stream baseflows.

Table 1

Properties and molecular structure of triazine herbicides considered in this study from Worthing, and Hance (1991)

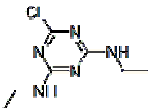
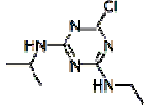
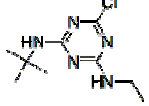




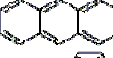

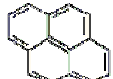
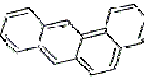
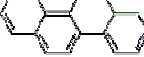
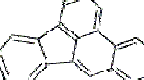
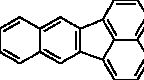
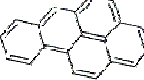
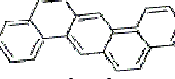
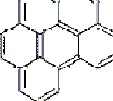
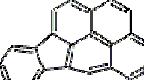
Compounds	Chemical Structure	M (g/mol)	Water solubility at 20°C (mg/L)	Log K _{ow}
Simazine (SMZNE)		186.64	35	2.10
Atrazine		215.68	30	2.69
Terbutylazine		229.71	8.5	3.20

Table 2

Properties and molecular structure of PAHs considered in this study from Young and Cerniglia (1995)

Compounds	Chemical Structure	M (g/mol)	Water solubility at 25°C (mg/L)	Log K _{ow}
Naphthalene		128.17	30	3.36
Acenaphthene		152.21	1.93	3.98
Fluorene		166.2	1.98	4.18
Phenanthrene		178.20	1.20	4.46
Anthracene		178.23	0.045	4.50
Fluoranthene		202.25	0.206	4.90
Pyrene		202.30	0.132	4.88
Benzo(a)anthracene		228.29	0.0094	5.63
Chrysene		228.30	0.0018	5.63
Benzo(b)fluoranthene		252.30	0.0015	6.04
Benzo(k)fluoranthene		252.31	0.0080	6.21
Benzo(a)pyrene		252.32	0.0016	6.06
Dibenzo(a,h)anthracene		278.35	0.0050	6.86
Benzo(ghi)perylene		276.34	0.0007	6.78
Indeno(1,2,3-cd)pyrene		278.35	0.0002	6.58

3.2. Experimental

3.2.1. Sampling points and sample pre-treatments

The 24-h composite samples (municipal and industrial wastewater influent and effluent) were collected on weekly basis between May 2008 and January 2009 from two wastewater treatment plants (WWTPs) in Northern Italy (Como Province).

The concerned plants, Alto Lura (AL WWTP) and Alto Seveso (AS WWTP), receive approx. 7.000 m³/day of domestic wastewater from the major neighbouring residential districts and approx. 18.000 m³/day of industrial wastewater, chiefly deriving from textile dyeing industry. As the sewer networks are combined, the WWTPs also treat part of the local runoff water, carrying urban pollutants, such as atmospheric deposition and traffic emissions deposited on the road surface, and non point loads from cultivated and non cultivated lands.

The studied WWTPs are based on activated sludge, include nitrification/denitrification and a final polishing treatment by ozone. At Alto Lura the removal of residual suspended solids before ozonation is performed by sand-filtration, at Seveso Depur by lamella settling, after coagulation.

Samples were collected from the following points along the WWTPs:

- 1) In AL WWTP (influent);
- 2) In AL WWTP's Ozone treatment;
- 3) Out AL WWTP's Ozone treatment (treated effluent);
- 4) In AS WWTP (influent);
- 5) In AS WWTP's Ozone treatment;
- 6) Out AS WWTP's Ozone treatment (treated effluent).

Samples were filtered with glass fiber membranes (0.45 µm, Whatman (Maidstone, UK)), and stored at 4°C in amber brown glass bottles prior to extraction. All chemical analyses were performed within 48 hours after sampling.

3.2.2. Reagents and materials

Acetonitrile, n-hexane, dichloromethane, methanol, 2-propanol and ultrapure water of HPLC grade were purchased from J.T. Baker (Denver, Netherlands).

The method was optimized and validated with simazine (SMZNE), atrazine (ATZNE), terbutylazine (TBZNE) and 15 of the EPA-PAHs (except acenaphthylene): naphthalene (NAPH), acenaphthene (ACE), fluorene (FLU), phenanthrene (PHEN), anthracene (ANTH), fluoranthene (FLT), pyrene (PYR), benzo[a]anthracene (BaA), chrysene (CHRY), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DiahA), benzo[ghi]perylene (BghiP) and indeno[1,2,3-cd]pyrene (INPY). Standards were purchased from Restek (Bellefonte, PA, USA) as mix solution of 2000 mg/L (purity >99% for each compound) in methylene chloride.

Individual PESTANAL analytical triazine standards (simazine, atrazine and terbuthylazine) were delivered by Sigma-Aldrich (St. Louis, MO, USA). Independent 100 mg/L stock solutions of triazines were prepared by dissolving 10 mg of the different triazines in a small amount of methanol and diluting to 100 mL with the same solvent. From these stock solutions of triazines and PAHs, intermediary solutions of 20 mg/L were prepared into methanol for LC analysis. Mixed working solutions of pesticide and PAHs mixtures used for spiking water samples and for preparation of the aqueous calibration standards were prepared from intermediate solutions at different concentrations by appropriate dilution with HPLC-grade water.

Deionized water used for preparing spiking water samples was obtained by a Milli-Q water purification - system (Millipore, Bedford, MA, USA).

The SPE was performed with OASIS HLB cartridges (divinylbenzene/*N*-vinylpyrrolidone copolymer, 60 mg, 3 mL) from Waters ((Milford, MA, USA). A VAC ELUT SPS 24 (Varian, Palo Alto, CA, USA) was used to simultaneously process up to 24 SPE cartridges. The Vacuum Pump V-700 with Vacuum Controller V/850 (BUCHI, Milano, Italy) was optimally suited for work with the vacuum manifold. With a flow rate of 1.8 m³/h and an ultimate vacuum below 10 mbar, it was used to dry up to 24 SPE cartridges at one time.

3.2.3. Recovery experiments in spiked Milli-Q water

Aliquots of 2-propanol (i.e. 10, 15, 20 and 25%, v/v) were added to Milli-Q water, and the samples were shaken and then spiked at a concentration of 0.3 µg/L for all the compounds.

The cartridges were conditioned by passing 5 mL dichloromethane, then 5 mL 2-propanol and finally 5 mL of a solution of Milli-Q water: 2-propanol proportional to the content of 2-propanol previously added into the water sample (e.g. Milli-Q water: 2-propanol 90:10 (v/v); 85:15 (v/v); 80:20 (v/v), 75:25 (v/v) respectively).

The aqueous samples (200 mL) were passed through the cartridges at 2.5 mL/min flow rate.

Sample loading was followed by flushing a solution of Milli-Q water and 2-propanol (5 mL; 90:10 (v/v); 85:15 (v/v); 80:20 (v/v), 75:25 (v/v) respectively) to remove interferences from sorbing material.

The cartridges were dried under vacuum in the manifold system at maximum pressure (15 mbar) for 30 min.

The analytes were subsequently eluted with 5 mL of a solvent mixture consisting of *n*-hexane:dichloromethane (50:50, v/v). The volume of eluate was reduced to 100 µL under a gentle stream of nitrogen.

The final extracts were diluted to 2 mL with acetonitrile before HPLC analysis.

3.2.4. Environmental samples

An organic modifier, 2-propanol 15% (v/v), was added to 200 mL of wastewater samples and the solutions were mixed thoroughly.

The OASIS HLB cartridges were conditioned at a low flow rate with sequential elutions of dichloromethane (5 mL), 2-propanol (5mL) and a solution of Milli-Q water:2-propanol (5 mL; 85:15, v/v).

The aqueous samples were passed through the cartridges at a flow rate of 2.5 mL/min.

The cartridges were rinsed with a solution of Milli-Q water and 2-propanol (5 mL; 85:15, v/v) and then dried under vacuum in a manifold system for 30 min. Finally, elution and treatment of extracts were performed as described in Section 3.2.3.

Concurrently with wastewater samples, Milli-Q water (200 mL) was extracted as a procedural “blank”.

3.2.5. Instrumental analysis

The HPLC analyses of the sample extracts were performed on an Agilent 1100 HPLC system (Palo Alto, CA, USA) including G1311A Quaternary Pump, G1313A Autosampler, G1322A Vacuum Degasser, Solvent Module, HP Chemstation. A Hypersyl Green PAH column (150 × 4.6 mm I.D., 5 μm) protected by two C18 guard columns (4mm × 3 mm) supplied by Thermo Scientific, was used for the chromatographic separation of the compounds.

The analytical column temperature was set at 25°C in order to achieve a better separation between the compounds analyzed.

The mobile phase was a gradient prepared from acetonitrile and water. At the beginning of a chromatographic run the composition was kept at acetonitrile–water, 30:70 (v/v), for 6.5 min. It was then changed to 100% acetonitrile over the next 26 min, kept at 100% acetonitrile for 5 min, and finally returned to the initial conditions in 8 min. The mobile phase flow rate was 2 mL/min and the sample injection volume was 20 μL.

The PAHs were analyzed using an Agilent 1100 series multi-wavelength Fluorescence Detector and the excitation (λ_{exc}) and emission (λ_{ems}) wavelength were optimized, setting the following program: time 0 min λ_{exc} 280, λ_{ems} 330; time 19.0 min λ_{exc} 250, λ_{ems} 380; time 21.2 min λ_{exc} 240, λ_{ems} 460; time 22.1 min λ_{exc} 240, λ_{ems} 390; time 24.0 min λ_{exc} 280, λ_{ems} 410; time 25.7 min λ_{exc} 270, λ_{ems} 410; time 27.8 min λ_{exc} 260, λ_{ems} 420; time 31.0 min λ_{exc} 295, λ_{ems} 420; time 32.8 min λ_{exc} 305, λ_{ems} 500.

The triazine herbicides were monitored at 220 nm with an Agilent G1315A Diode Array Detector.

The linearity of the method was investigated by analyzing calibration standards in triplicate at seven concentration levels, ranging from 1 to 250 μg/L. The integrated area data of the selected quantification masses were used to construct the curves and satisfactory linearity was assumed when the correlation coefficient (r) was higher than 0.99 based on analyte peak areas measurements.

Instrumental limits of detection (LODs) and instrumental limits of quantification (LOQs), defined as the concentration of the analyte that produced a signal-to-noise ratio of three and ten times the baseline noise respectively, were estimated from the chromatograms of samples spiked at

low concentration levels, ranging from 0.1 to 1 µg/L. Identification of analytes in the chromatograms was based on retention times, combined with structural confirmation, which was performed by matching the FLD and the UV-DAD analyte spectra with spectra from the reference library. The recovery percentages obtained from spiked WWTP samples were corrected by subtracting the contributions attributed to PAHs in “blank” samples.

3.3. Results and discussion

3.3.1. *Optimization of SPE procedure in Milli-Q water: effect of organic modifier*

In order to avoid adsorption of spiked analytes onto glass surfaces of equipment used during sample processing, aliquots of 2-propanol (i.e. 10, 15, 20 and 25%, v/v) were added to Milli-Q water. The SPE procedure employed is described in detail in Section 3.2.3 and 3.2.4.

Pure 2-propanol and methanol were not tested because of poor percentage recoveries achieved for the high molecular weight PAHs [36]. The results of these experiments are summarised in Table 3, where the percentage recovery and the corresponding RSD for each triazine and US EPA PAH are reported.

The concentration of organic modifier was found to be a key factor in the efficient recovery of the entire suite of PAHs and pesticides, as described by Busetti et al [37]. This was demonstrated by the decrease in the percent recoveries of the analytes that were obtained using 10, 15, 20 and 25% of 2-propanol in spiked Milli-Q water samples. The lowest concentration of 2-propanol (10%, v/v) promoted the recovery of pesticides and two to three ring PAHs, while 2-propanol at the highest concentration (25%, v/v) promoted the recovery of five to six aromatic ring compounds. No significant differences in the percentage recoveries were observed for the three to four aromatic ring PAHs (approx. from ANTH to BkF), and these compounds appeared to be the least sensitive to the amount of organic modifier added to the water samples.

As shown in Table 3, the percent recoveries decreased consistently with increasing analyte molecular weight. These results were as expected, based on the increasing hydrophobic properties of these compounds with increasing molecular weight. Our observations are consistent with those reported by many authors who observed that the concentration of the organic modifier is a critical parameter influencing the quantitative recovery of PAHs from spiked aqueous samples [38-40].

When the amount of 2-propanol added to water samples was set at 10% (v/v), the recoveries for the High Molecular Weight PAHs (HMW-PAHs) decreased because of their low water solubilities, leading to irreversible adsorption of these compounds onto glass surfaces during sample processing. In contrast, when the amount of 2-propanol added to water samples was set at 25% (v/v), the observed recovery yields increased for the HMW-PAHs, while a decrease in the percentage recoveries was observed for the Low Molecular Weight PAHs (LMW-PAHs). This was due to

the higher sample elutrophic strength at high modifier concentrations, which resulted in lower breakthrough volumes for the two to three aromatic rings PAHs.

Therefore, an organic modifier strength of 15% of 2-propanol was chosen as a good compromise, since this would adequately prevent losses due to surface adsorption of the pesticides and HMW-PAHs, without excessive losses of LMW-PAHs due to breakthrough of the SPE phase. As Table 3 shows, recoveries were satisfactory (between 60 and 110%) for all compounds at the spiking level (0.3 µg/L) with the exception of simazine, which reported the highest losses in this step. The method presented satisfactory precision and sensitivity with all RSD values below 10% and instrumental limits of detection ranging in the order of ng/L.

Table 3

Recoveries and % RSDs (n = 4) obtained processing 200 mL of spiked Milli-Q water (0.3 µg/L spiking level) containing 10, 15, 20 and 25% of 2-propanol as organic modifier contents

Analyte	LOD (ng/L)	% 2-propanol							
		10%		15%		20%		25%	
		Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)
SMZNE	2.58	55	14	45	6	32	8	20	10
ATZNE	3.09	82	9	60	6	49	8	32	3
TBZNE	2.81	127	10	110	7	93	7	90	6
NAPH	2.75	114	11	109	5	96	5	89	2
ACE	3.15	96	8	80	5	78	4	72	5
FLU	2.75	94	10	84	5	87	5	64	4
PHEN	2.80	115	7	106	4	96	9	81	2
ANTH	2.75	83	8	77	3	78	9	80	3
FLT	3.15	95	9	95	6	101	10	98	3
PYR	2.55	98	4	106	6	99	8	104	3
BaA	3.11	67	3	83	5	87	9	92	8
CHRY	3.22	78	8	87	9	74	5	92	7
BbF	3.29	82	5	80	8	78	6	88	4
BkF	2.74	80	5	78	7	92	7	89	4
BaP	2.99	60	4	78	6	86	7	98	8
DiaA	3.17	58	4	72	3	82	4	96	2
BghiP	3.31	60	3	70	2	80	3	98	4
INPY	3.36	55	3	76	2	88	5	95	3

Abbreviations: see tables 1-2.

3.3.2. Removal of organic micropollutants

The individual concentration of triazines and PAHs and their mean sum in wastewater from the two investigated sites are summarized in Table 4.

Table 4

Average PAH and pesticide concentrations in $\mu\text{g/L}$ in raw wastewater (influent), treated wastewater after ozonation and effluent samples from the two investigated WWTPs

	AL WWTP			AS WWTP		
	Influent	In O ₃	Out O ₃	Influent	In O ₃	Out O ₃
	($\mu\text{g/L}$)	($\mu\text{g/L}$)	($\mu\text{g/L}$)	($\mu\text{g/L}$)	($\mu\text{g/L}$)	($\mu\text{g/L}$)
SMZNE	1.27 ± 1.19	0.73 ± 0.60	0.62 ± 0.59	0.84 ± 0.80	0.69 ± 0.85	0.48 ± 0.63
ATZNE	0.51 ± 0.49	n.d.	n.d.	0.79 ± 1.12	n.d.	n.d.
TBZNE	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
NAF	1.11 ± 0.38	0.48 ± 0.14	0.50 ± 0.05	1.31 ± 0.49	0.56 ± 0.15	0.49 ± 0.18
ACE	0.32 ± 0.19	0.15 ± 0.01	0.12 ± 0.01	0.28 ± 0.12	0.14 ± 0.04	0.13 ± 0.04
FLU	0.75 ± 0.25	0.16 ± 0.03	0.15 ± 0.05	0.47 ± 0.17	0.18 ± 0.05	0.17 ± 0.03
PHEN	0.36 ± 0.12	0.34 ± 0.11	0.28 ± 0.13	0.48 ± 0.19	0.27 ± 0.10	0.24 ± 0.12
ANT	0.12 ± 0.03	0.11 ± 0.02	0.09 ± 0.12	0.12 ± 0.01	0.11 ± 0.02	0.09 ± 0.02
FLT	0.20 ± 0.04	0.14 ± 0.05	0.12 ± 0.06	0.26 ± 0.05	0.19 ± 0.01	0.17 ± 0.03
PIR	0.26 ± 0.13	0.20 ± 0.08	0.16 ± 0.10	0.22 ± 0.14	0.14 ± 0.07	0.13 ± 0.05
BaA	0.20 ± 0.12	0.15 ± 0.07	0.12 ± 0.06	0.22 ± 0.18	0.16 ± 0.04	0.16 ± 0.03
CRI	0.12 ± 0.01	0.09 ± 0.06	0.08 ± 0.06	0.13 ± 0.07	0.09 ± 0.07	0.08 ± 0.04
B(b)F	0.16 ± 0.07	0.14 ± 0.03	0.11 ± 0.09	0.17 ± 0.03	0.13 ± 0.07	0.11 ± 0.05
B(k)F	0.15 ± 0.04	0.13 ± 0.03	0.10 ± 0.07	0.15 ± 0.04	0.14 ± 0.07	0.13 ± 0.01
BaP	0.18 ± 0.07	0.15 ± 0.07	0.12 ± 0.07	0.15 ± 0.004	0.11 ± 0.09	0.11 ± 0.08
DiaA	0.21 ± 0.02	0.13 ± 0.08	0.09 ± 0.10	0.25 ± 0.13	0.15 ± 0.12	0.12 ± 0.11
BghiP	0.27 ± 0.05	0.15 ± 0.09	0.15 ± 0.14	0.26 ± 0.10	0.14 ± 0.15	0.12 ± 0.10
INPI	0.09 ± 0.05	0.04 ± 0.01	0.05 ± 0.02	0.16 ± 0.09	0.09 ± 0.07	0.08 ± 0.09
ΣPest	1.81 ± 1.40	0.73 ± 0.60	0.63 ± 0.59	1.92 ± 1.18	0.69 ± 0.85	0.48 ± 0.63
ΣPAHs	3.46 ± 0.99	2.24 ± 0.60	2.04 ± 0.61	3.35 ± 0.88	1.80 ± 0.30	1.52 ± 0.24

Abbreviations: Not detectable (n.d.); Alto Lura (AL WWTP); Alto Seveso (AS WWTP).

The observed concentrations were not significantly different between the two studied WWTPs.

For most triazines and PAHs concentrations decreased along wastewater treatment. The sum of triazines decreased from 1.81 and 1.92 $\mu\text{g/L}$ in AL and AS WWTPs influent samples to 0.63 and 0.48 $\mu\text{g/L}$ in the final effluent samples, with 65% and 75% removal through wastewater treatment. The highest average concentration of PAHs, expressed as the sum of the 15 compounds, was found in influent wastewater samples from AL WWTP (3.46 $\mu\text{g/L}$) with similar values at the other site (3.35 $\mu\text{g/L}$). These values

decreased to 2.04 and 1.52 $\mu\text{g/L}$ in the effluent wastewater samples from AL and AS WWTPs respectively, with 41 and 54% overall removal (Table 4).

The mean removal efficiencies calculated for each treatment step are shown in Figure 2. In the case of individual triazines and PAHs, the average overall removal rates in both WWTPs varied approximately from 22 to 100 % but concentrations were highly variable, as shown by standard deviations. In several cases the removal efficiency could not be calculated, as the concentrations measured were higher in the effluent than in the influent samples. This was probably due to a consistent matrix effect, strongly affecting the overall sensitivity of the analytical method which had been setup.

As Figure 2 shows, the two investigated WWTPs showed similar removal rates concerning the primary and secondary treatments. In this case, mean removals ranged approx. between 6 and 74% for the two to three ring PAHs, and between 30 and 48% for the 5- and 6-ring PAHs. No significant removal was observed for the three to four aromatic ring PAHs (approx. from ANTH to BkF), which appeared to be the least sensitive to the primary and secondary treatments, with the exception of FLT and B(a)A for AL WWTP and PIR and B(a)A for AS WWTP.

Atrazine was removed completely in both WWTPs during the first step, probably due to the adsorption on sludge produced through wastewater treatment, whilst the removal of simazine in the first stages ranged from 15 to 45 % in AS and AL WWTPs, respectively. This is likely to depend on the lower affinity of simazine for sludge, shown by the lower K_{ow} value.

Ozonation did not appear to have a significant role in the removal of triazines and PAHs. The removal due to ozonation ranged between <1 and 35% in both WWTPs. In the specific case, it is likely that the applied ozone dose was depleted for removing other kinds of residual pollutants (residual organic matter, dyestuffs, non ionic surfactants, suspended solids, bacteria) and was thus not enough for oxidizing the analyzed micropollutants.

On the other hand, it can be seen that for most PAHs and for the two investigated herbicides, the removal efficiency of the primary and secondary treatments was positively correlated with the influent concentration and, of course, the same correlation applies to the overall treatment including ozonation which, as previously observed, played a very limited role in the overall removal. Moreover, the removal should probably be attributed to the hydrophobic nature of these compounds which are mainly removed from wastewater through sorption on sludge particles.

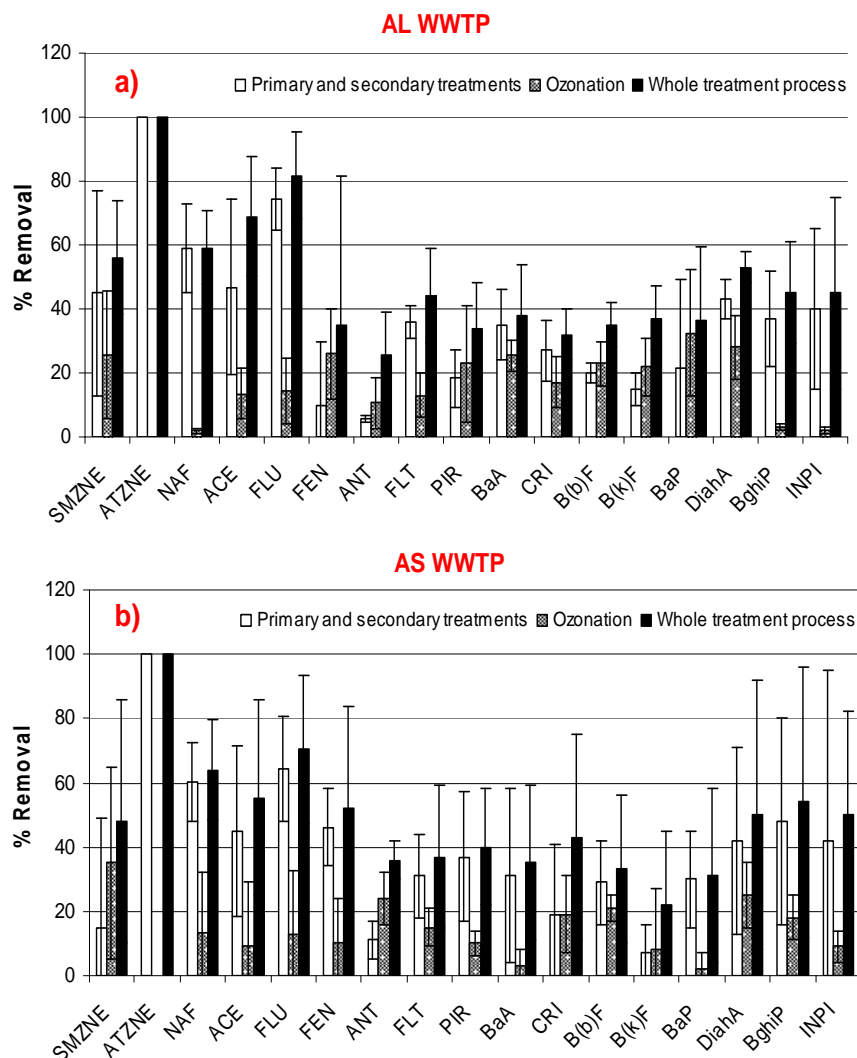


Fig.2. Experimental removal percentages of triazines and PAHs in the different treatment stages (mean \pm SD) at (a) AL WWTP and (b) AS WWTP.

The examined compounds belong to different chemical classes and have thus different properties. Actually, due to the complex nature of wastewater and of all the possible interactions among the various components, the two above mentioned factors (i.e. hydrophobicity and starting concentration) are surely important. In particular, removal seems to be correlated to the product of $\text{Log } K_{ow}$ and starting concentrations for PAHs, whilst for pesticides $\text{Log } K_{ow}$ seems to be more important. Figure 3 presents the overall removal efficiencies of individual PAHs as a function of the product of $\text{Log } K_{ow}$ and

concentrations and the concentrations measured in influent samples. A linear relationship, significant at the $P < 0,01$ level, was observed. R^2 value is low, but this is related to the different factors which can interfere with removal from wastewater.

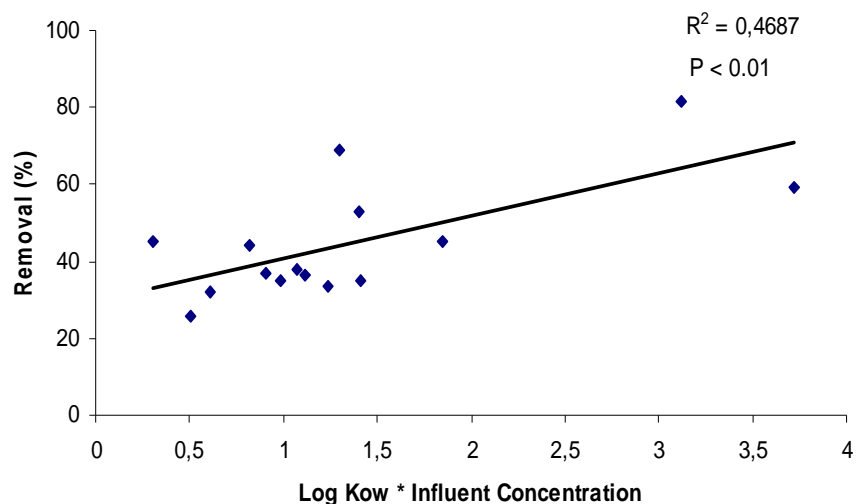


Fig. 3. PAHs removal efficienciens in the whole treatment vs. the product of Log Kow and starting concentrations.

3.3.3. Comparison to surface water quality criteria

The enforcement of the European Water Framework Directive (WFD, 2000/60) [3] has probably been the most significant step for many years, in the field of water protection at international scale. The Directive takes a broad view of water management, setting the prevention of any further deterioration of water bodies and the protection and enhancement of the status of aquatic ecosystems as its key objectives. The overriding objective of the policy is the achievement of “good status” in all water bodies. Its implementation should result in an intensification of monitoring of aquatic ecosystems and increased control of contaminants.

Article 16 of the WFD sets out the European Union (EU) strategy for protecting water bodies from the pollution by chemical substances. According to the provisions of this article, a list of 33 priority substances, which represent a significant risk to or via the aquatic environment at the EU level, has been established [4] and will have to be re-examined by the European Commission (EC) at least every four years. Most of the pollutants included in the current list are organic contaminants (e.g., hydrocarbons, organochlorine compounds, organic solvents, pesticides, and chlorophenol). According to the WFD, good chemical status for a water body is obtained when the concentrations of the priority substances in water, sediment or

biota are below the Environmental Quality Standards (EQSs): this is expressed as “compliance checking”. The EC set the EQSs with the agreement of Member States after in-depth discussions between experts, who used the risk-assessment approach described in the Technical Guidance Document [41, 42]. In order to reach the “good status” for all European water bodies, Member States have to implement river-basin-management plans, including water-monitoring programs, and take measures when results do not comply with the EQSs.

To implement appropriate water-quality-monitoring programs and to ensure the comparability of the data on chemical contamination of the aquatic environment, the EC has recently issued the Directive 2008/105 [27], which defines specific EQSs for each substances. In Italy this Directive has been enacted by Decree 2009/56 fixing the same EQSs of the EU Directive [28]. The EQSs fixed for the compounds analyzed in this study are shown in Table 5. Note that ACE, FLU, FHEN, PYR, BaA, CHRY and DiahA are not currently regulated.

Table 5
EQSs for the 18 compounds investigated in this study (EU and Italian guidelines)

Compounds	AA-EQS	MAC-EQS
	Inland SW	Inland SW
	µg/L	µg/L
SMZNE	1.0	4,0
ATZNE	0.6	2.0
TBZNE	0.5	n.a
NAF	2.4	n.a
ANT	0.1	0.4
FLT	0.1	1.0
B(b)F	Σ=0.03	n.a
B(k)F		
BaP	0.05	0.1
BghiP	Σ= 0.002	n.a
INPI		

Abbreviations: Not applicable (n.a.); surface water (SW); annual average (AA); maximum allowable concentration (MAC).

Figure 4 reports the average concentrations in the influent and effluent samples collected from the two WWTPs. In both WWTPs, all substances were at least partially removed, but the average concentrations in effluent samples for FLT and for the sum of concentrations of BbF and BkF and BghiP and INPI, even if complying with the standards for discharge, were higher than the EQSs for surface waters (Table 5). The effluent from the two investigated WWTPs contributes most of the receptor flow and practically no dilution of the discharged effluents can occur. That’s why the compliance of the limits for discharge is not enough to ensure that the river water quality is

acceptable according to EQSs for surface waters, while these ones are the only reasonable reference values.

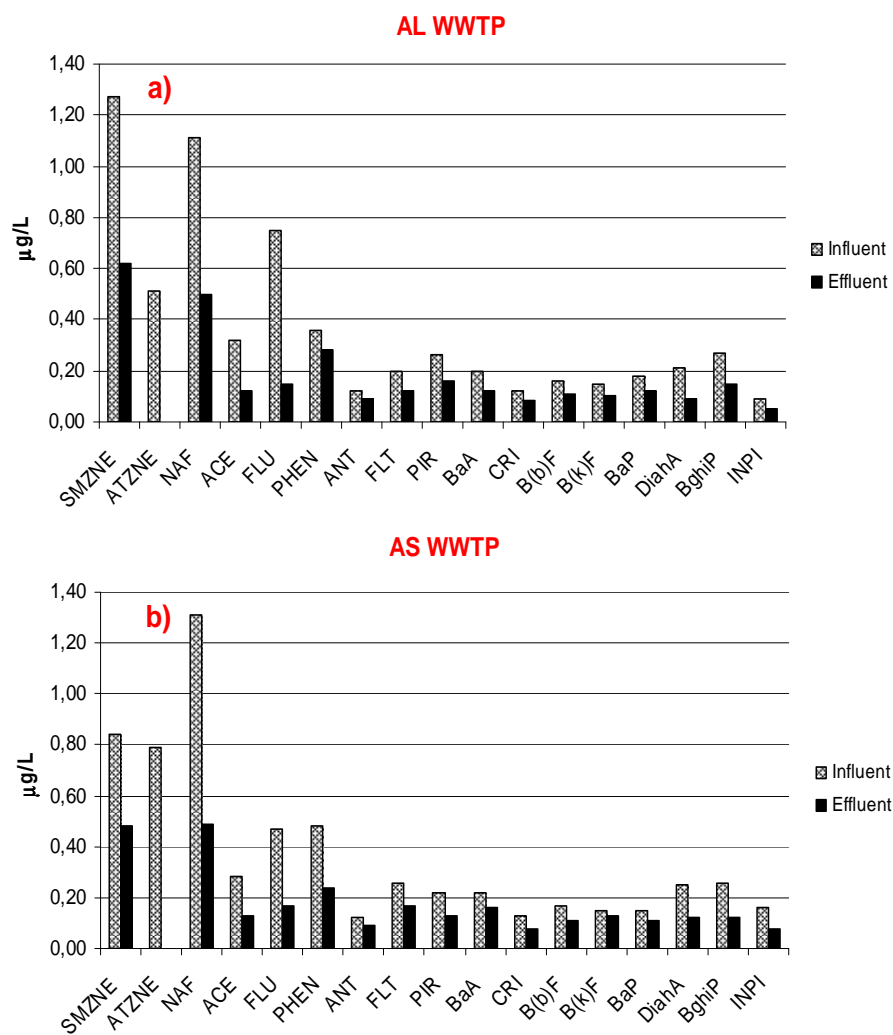


Fig.4. Bar charts showing the average concentrations of triazines and PAHs in influent and effluent wastewater samples at (a) AL WWTP and (b) AS WWTP.

3.4. Conclusion

The removal of triazine herbicides (atrazine, simazine and terbuthylazine) and Polycyclic Aromatic Hydrocarbons (PAHs) from municipal wastewater was examined in two conventional activated sludge wastewater treatment plants providing with ozonation as final treatment step. To this purpose, analyses were performed by coupling HPLC with UV-DAD and fluorescence detection (FLD) after a pre-concentration step based on solid-phase extraction (using OASIS HLB cartridges).

For each analyzed compound, removal efficiency was calculated for the whole treatment process sequence and, separately, for primary+secondary treatment and for ozonation. The latter appeared as scarcely affecting the overall removal efficiency. On the contrary, linear correlations were observed between the removal efficiency and the product $\text{Log } K_{ow}$ and influent concentrations for each compound. Such trend needs to be confirmed by further data, also because, as previously mentioned, in some cases the removal efficiencies could not be calculated as the concentrations in influent samples were lower than in the effluents. This could be explained by a high matrix effect, more relevant in the influent than in the effluent, leading to consistent SPE losses, especially in the influent samples.

The two investigated WWTPs discharge their effluents in receptors which can be defined as effluent-dominated streams, as their total baseflow is practically constituted by effluent wastewater samples. For this reason, the average concentrations of each compound were compared to the EQSs for surface waters. The results showed that the WWTPs were effective in reducing the concentration of all compounds below the EQSs, with the exception of fluoranthene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene and indeno(1,2,3-cd)pyrene.

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Chapter IV

Simultaneous determination of triazines and their main transformation products in surface and wastewater samples by ultra-high-pressure liquid chromatography-tandem mass spectrometry

Federica Benvenuto ¹, José M. Marín ², Juan V. Sancho ², Sergio Canobbio ¹, Valeria Mezzanotte ¹, Félix Hernández ²

1. *Department of Environmental Sciences, University of Milano-Bicocca, P. za della Scienza, 1, 20126, Italy*
2. *Research Institute for Pesticides and Water, University Jaume I, Avda. Sos Baynat, E-12071 Castellón. Spain,*

Abstract

This work describes the optimization, validation and application to real samples of an ultra-high-pressure liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method for the quantification and confirmation of 11 compounds (atrazine, simazine, terbuthylazine, terbumeton, terbutryn and many of their main transformation products) in surface and wastewater samples. Most of these analytes are included in the list of priority substances in the framework on European Water Policy.

To efficiently combine UHPLC with MS/MS, a fast-acquisition triple quadrupole mass analyzer was used. Working in selected reaction monitoring mode, up to three simultaneous transitions by compound were acquired allowing a reliable identification at ng/L levels. The proposed method includes a pre-concentration step based on solid-phase extraction (using OASIS HLB cartridges). Satisfactory recoveries (70-120%) and relative standard deviations (<20%) were obtained for all compounds in different water samples types spiked at two concentration levels (0.025 and 0.1 µg/L). The optimized method was found to have excellent sensitivity with instrumental detection limits as low as 50 fg.

In addition, the influences of the matrix constituents on ionization efficiency and extraction recovery have been studied in different types of Italian and Spanish surface and wastewater samples. Signal suppressions were observed for all compounds, especially for influent wastewater. The use of isotope-labelled internal standards was found to be the best approach to assure an accurate quantification in all matrix samples.

Keywords

Pesticide transformation products, ultra-high-pressure liquid chromatography, tandem mass spectrometry, matrix effects, surface and wastewater; triazines.

Article submitted to Analytical and Bioanalytical Chemistry

4.1. Introduction

One of the most important problems closely related to water resources concerns the presence in surface and drinking water of a wide variety of organic micropollutants. Many of these substances, generated by the main human activities, are characterized as being toxic and dangerous for both aquatic system and human health. Wastewater treatment plants (WWTPs), especially those serving both urban and industrial areas, consistently receive significant loads of these compounds. Therefore, it's important to evaluate to the impact of micropollutants discharged with treated effluents and sewers overflows on the receiving waterbody. The problem arises from the fact that WWTPs remove them to a certain (limited) extent, but river flows are in most cases too small to dilute the residual loads discharged and concentrations in the receiving waters increase, often much over the quality objectives defined for the river water quality.

Groups of compounds present in these mixtures include triazine herbicides, which are generally included in monitoring programs, as a result of their widespread presence and inclusion in the Water Framework Directive (2000/60/EC) [1]. This Directive was amended by the Decision 2455/2001/EC [2], which published a list of 33 priority substances, including 10 pesticides, selected on the basis of the risk to or via the aquatic environment. In 2000, the Spanish legislation fixed quality objectives of 1 µg/L for the concentration of simazine (SIMA), atrazine (ATRA) and terbuthylazine (TBZNE) in continental surface waters (SW) [3] whilst in 2009 the Italian legislation established specific limit for each of these compounds at 1, 0.6 and 0.5 µg/L respectively [4]. Regarding to drinking water, the European authorities fixed quality standards of 0.1 µg/L for pesticide individual concentration and 0.5 µg/L for the sum of all pesticides [5]. Triazines are used worldwide as selective pre and post emergence herbicides for the control of both grasses and broadleaf weeds in many agricultural crops like corn, maize, sorghum, peaches, and apple as well as for non-agricultural purposes such as soil sterilization and road maintenance [6]. Due to their higher mobility in the soil-water environment, during and after the herbicide application to the farming land, triazines may be found in both ground and surface water [7, 8]. In water and soil, parent molecules are subjected to degradation processes such as photolysis, oxidation, hydrolysis, and biodegradation, leading to dealkylation of the amine groups, dechlorination, and subsequent hydroxylation [9, 10]. The main transformation products (TPs) in ground and surface waters via biotic mechanism are the dealkylated chloro metabolites, such as deisopropyl-atrazine (DIA), desethyl-atrazine (DEA), desethyl-terbuthylazine (DETbzne) and desethyl-terbumeton (DETer) [11]. Hydroxy-atrazine (HA), hydroxy-simazine (HS) and hydroxy-terbuthylazine (HTbzne) are the major abiotic degradation product in water and soil.

Not much information is available on environmental impact of triazine TPs, which can be also toxic [12] and are normally more polar than parent compounds. Due to their high mobility in the soil-water environment, TPs can reach water bodies more easily. The presence of metabolites when

investigating the effect of herbicide application and its influence on the aquatic environment is relevant and the impact due to herbicides tends to be underestimated when samples are analyzed for the parent compounds only [13, 14]. Therefore, the most relevant TPs should be incorporated into current analytical methods to obtain a more realistic knowledge of water quality regarding pesticide contamination [15, 16].

Due to the prevailing of combined sewer systems, most runoff water, carrying pollutants from non point sources, is collected and reaches WWTPs, so that non point loads enter the receptors as point loads [17]. Conventional (biological) wastewater treatment processes are considered as ineffective in reducing the concentrations of triazine compounds with removal efficiencies consistently below 40 % [18] or even considered as non biodegradable [19]. However, other advanced processes, such as ozonation, have been reported to produce abundant triazine degradates [20]. Therefore, in practical situations, the presence of TPs has to be taken into account. Besides, primary waters contaminated by pesticides, may already contain TPs, which can be consequently found in effluent wastewater.

Most of analytical methods for residues of pesticides and their TPs in water are based on gas chromatography and/or liquid chromatography coupled to mass spectrometry. In recent years the application of tandem mass spectrometry in LC-MS/MS based methods has given and increased selectivity and sensitivity, minimizing or even removing many interferences when working in Selected Reaction Monitoring (SRM) mode making this technique highly suitable for polar pesticides and TPs in aqueous matrices [21-25]. Thus, several LC-MS/MS methods have been reported for the determination of triazines. The most recent papers have reported methods using ultra-high-pressure liquid chromatography (UHPLC) coupled to MS/MS for ultra fast separations and sensitive determination of these compounds [26-29]. UHPLC provides higher peak capacity, greater resolution, increased sensitivity and high speed of analysis by the use of stationary phases of particle size (<2 μm) smaller than conventional HPLC. To reach an efficiently combination UHPLC with MS/MS, fast-acquisition triple quadrupole mass analyzers must be used. It can allow the acquisition of more than two transitions to obtain reliable identifications, without resolution or sensitivity losses [29]. Therefore, UHPLC-MS/MS can offer not only good sensitivity but also high confidence on confirmation of compounds detected in samples, allowing to easily reach more than 3-4 identification points (IPs), as established in EU guidelines [30, 31].

According to European legislation on drinking water [5], pesticide limits of quantification (LOQs) of 0.025 $\mu\text{g/L}$, four times lower than the maximum allowed (0.1 $\mu\text{g/L}$), are required. Usually, a SPE pre-concentration step is necessary to reach this low concentration levels in trace analysis. In spite of SPE methods are typically fast, efficient and widely applied, matrix interferences are also pre-concentrated in SPE cartridges affecting to resulting sometimes in analyte ionization enhancement or suppression. This undesirable matrix effects can be considered as one of the main LC-MS/MS drawbacks, mainly when an electrospray ionization source (ESI) is used [32]. To ensure an accurate measurement of compounds, different methods are applicable in order to compensate for the matrix effects [33-39]. A simple

method is the dilution of the sample [35]. However, this is limited by the required limits of detection for the target compounds. The use of matrix-matched samples for calibration [36], is not much useful in environmental samples, as their composition vary in a broad range, and obtaining a blank of similar composition to sample is not easy. The standard addition method can provide accurate results [37], but in practice it needs both a time-consuming sample preparation and evaluation of the obtained results and therefore is not suitable for fast routine analysis. The use of appropriate internal standards is one of the best approaches to compensate for matrix effects, especially when using analyte isotope labelled internal standard (ILIS), as one expects that the internal standard is affected by matrix effects in the same way than the analyte [29, 38, 39]. Apart from the high cost, the main drawback of using an internal standard is that one isotope standard is, in principle, required for each analyte and that stable isotope standards are not generally available for all compounds to be analyzed. When ILIS is not available, other compounds eluting at similar retention times or being structurally analogues could be tested, but no satisfactory data are always assured [29].

Normally, TPs are not included (or only a few are included) in multiresidue methods for several reasons: main of them are still not well known; the number of potential analytes to be investigated in water would increase drastically; the commercial availability of reference standards is rather limited; their higher polarity in relation to the parent compound makes extraction/preconcentration more difficult than the usual SPE approaches [22]. This work is focused on the development of a rapid, selective and sensitive analytical method for the quantification and confirmation of triazine herbicides commonly used in Italy and in the Mediterranean coast of Spain, as well as their main TPs, which structures are shown in Table 1. Analytical methodology developed is based on a solid-phase extraction step (using OASIS HLB cartridges, 0.2 g) followed by UHPLC-MS/MS determination. Method has been validated for the determination of 11 compounds (ATRA, SIMA, TBZNE, terbumeton (TER), terbutryn and 6 of their main TPs) in surface and wastewater samples. It has been applied to the determination of those herbicides in small volumes of surface waters and polluted WWTP aqueous samples (Italian and Spanish 24-h composite influents – IWW - and effluents - EWW).

Three SRM transitions were acquired for each analyte to give more confident in the identification of the analytes in these complex matrices. In addition, the influence of matrix constituents on ionization efficiency and SPE extraction recovery has been studied in the types of water samples analyzed, testing different ILIS to assure the accurate quantification in all matrix samples.

Table 1
Chemical structures of the analyzed compounds

Compound	Structure	Compound	Structure
DIA		Htbzne	
DEA		ATRA	
HA		TER	
DETer		TBZNE	
SIMA		TBTYN	
DETBzne			

Abbreviations: DIA (desisopropylazine); DEA (desethylatrazine); HA (2-hydroxy-atrazine); DETer (desethylterbumeton); SIMA (simazine); DETBzne (desethylterbutylazine); Htbzne (2-hydroxy-terbutylazine); ATRA (atrazine); TER (terbumeton); TBZNE (terbutylazine); TBTYN (terbutryn).

4.2. Experimental

4.2.1. Reagents and materials

Pesticide reference standards obtained from Sigma-Aldrich (St. Louis, MO, USA). Stock standard solutions were prepared dissolving 50 mg, accurately weighted, in 100 mL of acetone obtaining a final concentration of 500 mg/L. From these solutions of triazines an intermediary solution of around 50 mg/L was prepared in methanol. Mixed working solutions of pesticide mixtures used for spiking water samples and for preparation of the aqueous calibration standards were prepared from intermediate solutions at different concentrations by appropriate dilution with HPLC-grade water.

Isotopically labelled compounds used were [²H₆]dimethoate (dimethoate-d₆), [²H₅]terbutylazine (terbutylazine-d₅), and [²H₆]thiabendazole (thiabendazole-d₆) purchased from Dr. Erhenstorfer. A mix of all isotopically labelled compounds at 100 µg/L was prepared by dilution of individual stock solutions of 1 mg/L in methanol. Further dilutions of this mix were prepared. To prepare calibration curves, working mix solutions of pesticides and isotopically labelled compounds were prepared in acetonitrile:water (10:90, v/v). In order to prevent photochemical degradations, standard solutions and sample extracts, were stored in brown glass vials at 4°C.

HPLC-grade methanol, HPLC-grade acetonitrile and acetone for residue analysis were purchased from Scharlau (Barcelona, Spain). HPLC-grade water was obtained by purifying demineralised water in a Milli-Q Gradient A10 (Millepore, Bedford, MA, USA). Formic acid (HCOOH, content > 98%) was supplied by Scharlau.

Cartridges used for SPE optimization were Oasis HLB (200 mg, 6 mL) and MCX (150 mg, 6 mL) from Waters (Milford, MA, USA). Oasis SPE polymer cartridges are built of a balanced mixture of hydrophilic and lipophilic (HLB) monomers, whilst Oasis MCX is a strong cation-exchange mixed mode polymeric sorbent built upon HLB copolymers. A VAC ELUT SPS 20 (Varian, Palo Alto, CA, USA) was used to simultaneously process up to 20 SPE cartridges.

4.2.2. Ultra-high-pressure liquid chromatography

LC separations were performed in a Waters Acquity UPLC system (Waters), using an Acquity UPLC HSS T3 column, 1.8 µm, 100 mm × 2.1 mm I.D. (Waters). The column was kept at 40°C and the sample manager was maintained at 5°C. Mobile phase was composed of a Solvent A (water) and a Solvent B (methanol) at a constant flow rate of 0.3 mL/min. The gradient was programmed to increase the amount of methanol from an initial 5-95% in 6 min, returning to the initial conditions (5% A) in 0.1 min. This condition was maintained until 7 min. The sample volume injected in UHPLC system was 20 µL.

4.2.3. Mass spectrometry

TQD tandem mass spectrometer with an orthogonal Z-spray-electrospray interface (Waters) was used for UHPLC analysis. Typical interface conditions were optimised for maximum intensity of precursor ion as follows: the cone gas and desolvation (drying gas) N₂ flows were set at 60 L/h flow and 1000 L/h, respectively. For operation in MS/MS mode, collision gas was Argon 99.995% (Carbueros Metalicos, Valencia, Spain) with a pressure of 2×10^{-3} mbar in the T-Wave cell. Capillary voltages of 3.5 kV were used in the positive ionization mode. The interface temperature was set to 500°C and the source temperature to 120°C. Dwell times of 0.1 s/scan were selected.

All data were acquired and processed using MassLynx v 4.1 software.

4.2.4. Sample preparation

Water samples were centrifuged at 4500 rpm for 5 min, only when suspended particulate matter was observed. Then, 100 mL of surface water, or ten and twenty times diluted effluent and influent wastewater respectively, containing 1 mL of concentrated HCOOH, were spiked with 100 µL of I.S. mixture solution, giving a final concentration of 0.05 µg/L for each ILIS.

Oasis HLB (200 mg) cartridges were conditioned with 5 mL of methanol, 5 mL of acetone, 5 mL of methanol and 5 mL of acidified water (1% HCOOH). After the conditioning step, aliquots of 100 mL of aqueous sample were loaded through the cartridges by gravity. Then, the sorbents were dried under vacuum in a manifold system for 40 min and the analytes were subsequently eluted with 5 mL of acetone. The extract was evaporated to dryness under a gentle nitrogen stream (40°C) and finally reconstituted with 1 mL of acetonitrile–water (10:90, v/v). Analyses were performed by injecting 20 µL of the final extract in the UHPLC–MS/MS system.

4.2.5. Validation studies

The performance characteristics of the method were established by a validation procedure following SANCO guidelines [31] using standard solutions prepared in acetonitrile–water (10:90, v/v) and spiked samples. Linearity of the method was investigated by analyzing calibration standards in triplicate at seven concentration levels, ranging from 1 to 100 µg/L. Satisfactory linearity, using weighed (1/X) least squares regression, was assumed when the correlation coefficient (*r*) was higher than 0.99 based on analyte peak areas measurements, and the residuals lower than 30%. Accuracy (expressed as recovery, in %) and precision (repeatability expressed as relative standard deviation, in %) were evaluated by analyzing surface water samples spiked at two concentration levels each (0.025 and 0.1 µg/L). In the case of influent and effluent wastewaters, these levels were twenty and tenfold higher, as they were diluted with HPLC water before sample treatment and analyses. All experiments were performed in quintuplicate (*n* = 5).

The limit of quantification (LOQ) was estimated for a signal-to-noise ratio of ten from the chromatograms of SW, IWW and EWW samples spiked at the lowest concentration level, using the confirmation transition q_1 . The instrumental limit of detection (LOD) was estimated for a signal-to-noise ratio of three from the SRM chromatograms (using the quantification transition Q) of low-concentration standards, ranging from 0.01 to 0.1 $\mu\text{g/L}$.

4.2.6. Application to real samples

The method was applied to a number of 32 water samples (SW, IWW and EWW) collected in different sites of Italy and Spain between April and September 2009. Samples were stored in the dark at $<-18^\circ\text{C}$ in plastic (high density polyethylene) containers until required for residue analysis levels. SW samples were collected in 14 sampling sites in Spain and Italy, representing a wide range of freshwater ecosystem (streams, rivers, marshes and lagoons) as well as different impairment conditions (Table 2).

Table 2
Surface water samples

<i>Surface water samples</i>	<i>Origin</i>	<i>Typology</i>	<i>Basin / Water body Description</i>
(1) Ebro	ES	River	Largest in Spain
(2) Ebro	ES	Delta	Natural
(3) Pego-Oliva	ES	Marsh	Natural, threatened by urbanization
(4) Almenara "estany"	ES	Lagoon	Natural
(5) Massalavés Riu Verd	ES	Wetland	Natural
(7) Borriana Clot	ES	Wetland	Natural
(8) Lura	IT	Stream	Effluent dominated
(9) Lura Albiolo	IT	Stream	Agricultural landscape
(10) Gorgonella	IT	Stream	Urbanized
(11) Seveso	IT	River	Natural
(12) Livescia	IT	Stream	Golf course drainage

Municipal and industrial IWW and EWW samples were collected in two sampling campaigns in April and June 2009, from two WWTPs in Northern Italy. Triazines fluxes in the sewers were calculated using measurements of the real 24 h influent flows on the sampling days. The concerned plants, Alto Lura (AL-WWTP) and Alto Seveso (AS-WWTP), receive approx. 7.000 m^3/day of domestic wastewater from the major neighbouring residential districts and approx. 18.000 m^3/day of industrial wastewater, chiefly deriving from textile dyeing industry. As the sewer networks are combined, the WWTPs also treat part of the local runoff water, carrying urban pollutants,

such as atmospheric deposition and traffic emissions deposited on the road surface, and non point loads from cultivated and non cultivated lands.

Three Spanish activated sludge WWTPs

Spanish 24-h composite wastewater samples were collected in April and June 2009 from three WWTPs (Castellon (CS), Burriana (BU) and Beniassim (BE)) of the province of Castellón de la Plana (Eastern Spain), which receive approx. 44.024, 16.805 and 8.250 m³/day respectively of urban and industrial wastewater.

In every sequence of analysis, water sample SPE extracts were injected by duplicate between two calibration curves (from 1 to 100 µg/L) and additionally two quality controls (QCs) were analysed together with each batch of samples. QCs consisted of a blank water (previously analyzed) fortified at two different levels, 0.025 µg/L and 0.1 µg/L. QC recoveries for every analyte were considered satisfactory if they were in the range 70–120%, thus assuring the quality of the analysis.

Confirmation of positive findings was carried out by calculating the peak area ratios between the quantification (Q) and confirmation (q₁ and q₂) transitions and comparing them with ion-ratios obtained from a reference standard. To consider a finding as an actual positive, the experimental Q/q_i ratios should fit with those of reference standards with a maximum deviation ranging from 20 to 50% depending on the relative ion intensities, in the line of EU Decision 2002/657/EC [30].

4.3. Results and discussion

4.3.1. MS/MS optimization

In order to optimize full-scan MS and MS/MS spectra of parent pesticides and TPs, infusion experiments were performed using the built-in syringe pump, directly connected to the interface. Thus, individual standard solutions at 1 mg/L in methanol/water (50:50, v/v) were infused at a flow rate of 10 µL/min.

All analytes were measured in positive ionization mode presenting an abundant [M+H]⁺, which was selected as precursor ion. The presence of halogenated atoms (Cl) in several compounds (ATRA, SIMA, TBZNE and DETbzne) allowed us to use two different precursor ions (corresponding to the ³⁵Cl and ³⁷Cl isotopes, respectively) which produced abundant product ions. Thus, the confirmation of these compounds would be feasible at similar concentration levels. Non-specific transitions, e.g. loss of water, were avoided as possible in order to minimize the risk of false positives [33].

With the aim that the method was also for confirmatory purposes, the acquisition of at least two specific transitions for each compound was required. As TQD mass spectrometer is a fast-acquisition triple quadrupole mass analyzer that allows decreasing dwell times and ionization mode switching time, without apparent sensitivity losses, this gave us the possibility of acquiring up to three SRM transitions per compound at 10 ms dwell time. Therefore, acquiring three SRM transitions, and using two

different precursor ions for several compounds, led to a satisfactory number of IPs, required for a safe confirmation [30]. Optimum MS source and analyzer conditions for SRM determination of each compound are listed in Table 3. Average

Table 3

MS/MS optimized conditions for selected compounds. For labelled internal standards, only typically the quantification related transition was acquired

Compound	Rt (min)	Precursor ion (m/z)	Cone (V)	Col. Ener. (eV)	Product ion (m/z) ^a	Q/q ratio (RSD%)
DIA	3.54	174.1	30	20	96.1	
				25	68.0	0.9 (3)
				20	132.1	1.9 (3)
DEA	3.86	188.1	20	20	146.1	
				25	110.1	6.3 (3)
				25	104.0	2.4 (3)
HA	3.94	198.2	35	20	156.2	
				25	86.1	1.2 (5)
				35	69.0	1.8 (5)
DETer	4.26	198.2	30	15	142.1	
				25	86.1	2.3 (5)
				30	57.1	3.8 (10)
SIMA	4.26	202.1	45	20	132.1	
		204.1		20	134.1	3.1 (5)
		204.1		25	96.1	3.2 (11)

Continued

Compound	Rt (min)	Precursor ion (m/z)	Cone (V)	Col. Ener. (eV)	Product ion (m/z) ^a	Q/q ratio (RSD%)
HTbzne	4.30	212.2	30	15	156.2	
				25	97.1	5.6 (7)
				30	86.1	2.6 (3)
ATRA	4.58	216.3	45	20	174.3	
				20	176.3	3.6 (4)
				25	96.0	1.7 (5)
TER	4.98	226.1	45	20	170.1	
				30	75.1	6.7 (5)
				25	128.1	21.5 (7)
TBZNE	5.02	230.1	45	15	174.1	
				15	176.1	2.9 (3)
				30	96.1	4.6 (5)
TBTYN	5.39	242.1	45	25	91.1	
				35	71.1	0.9 (3)
				25	158.1	3.8(7)
Dimethoate-d ₆	3.66	236.0	40	10	205.0	
Thiabendazole-d ₆	4.05	208.2	55	25	180.2	
Tbzne-d ₅	5.0	235.1	45	15	179.1	

^a The first transition (top) was used for quantification and the second transitions (bottom) were used for confirmation. *Abbreviations*: retention time (Rt); collision energy (Col. Ener). See table 1 for compound abbreviations.

4.3.2. LC optimization

In spite of the great selectivity provided by triple quadrupole detectors, in LC-MS/MS an efficient chromatographic separation can be necessary to avoid or minimize the undesirable matrix effect. Besides, an adequate mobile phase selection can also be important to enhance the detector response.

In this paper, methanol and acetonitrile, with different HCOOH and NH₄Ac contents, were tested as organic solvents during chromatographic optimization looking for a compromise between peak shape and sensitivity. All compounds presented better peak shape and ionization yield when methanol without additives was used as organic modifier due to its protic character.

4.3.3. SPE recoveries

In order to evaluate the efficiency of SPE process, two different sorbents were tested: Oasis HLB and Oasis MCX. To this aim, 100 mL of Milli-Q water spiked at a concentration of 0.05 µg/L were loaded onto the cartridges by gravity (triplicate analysis). Marked differences were observed in the performance of the two stationary phases tested, both in the recovery of analytes and in the reproducibility of the results.

OASIS HLB cartridges were chosen due to their ability to retain both non polar and polar compounds, obtaining the highest percentage recoveries (99–121%) and the lowest relative standard deviations (ranging from 6 to 9 %) in comparison to MCX sorbents (recoveries ranging from 30 to 109% with relative standard deviations in the range of 5–28%).

Efficiency and robustness on analytes pre-concentration process was tested in SW, IWW and EWW samples. Influent and effluent samples were 20 or 10 times diluted, respectively, previously to SPE pre-concentration to decrease their high organic matter content and viscosity. Response obtained for samples spiked before SPE step (X) (e.g. typically at 0.05 µg/L level) and for sample extracts spiked after SPE step (Y) (e.g. 5 µg/L) were compared ($n = 3$). The ratio $(X/Y \times 100)$ was taken as SPE absolute recovery [39]. "Blank" samples, without spiking, were also processed to subtract the levels of target compounds that might be present in the samples.

As can be seen in Figure 1, in the case of surface water, SPE absolute recoveries were slightly lower (68–103%) compared to Milli-Q water, influent and effluent wastewaters (84-121%). SW samples were loaded through the cartridges without dilution step and the losses may be due to particle blockage and small cross-sectional area. Only TER and TBZNE showed similar losses in all type of samples tested; in the case of TBZNE these losses could be corrected when adding the own ILIS as surrogate, as it will be discussed later.

However, satisfactory recoveries were obtained for almost all compounds (68-121%) and the use of ILIS as surrogates was not strictly necessary to correct losses in the SPE step.

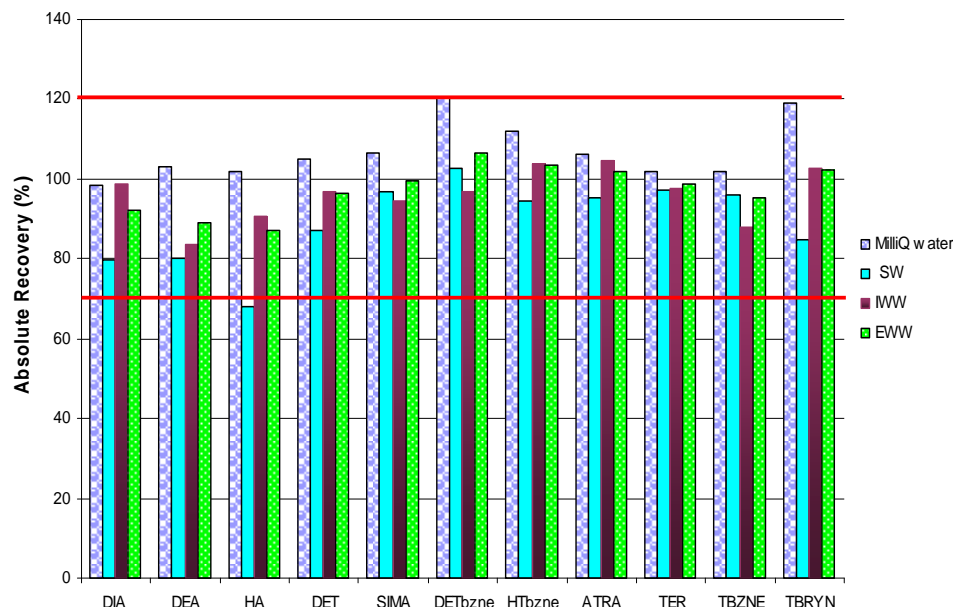


Fig. 1. Absolute recoveries of pesticides and TPs in Milli-Q, surface water (SW), influent (IWW) and effluent (EWW) wastewaters using HLB cartridges. *Abbreviations:* see tables 1.

4.3.4. Matrix effect

Preliminary experiments were performed on surface and wastewater samples by spiking SPE extracts in order to evaluate signal suppression or enhancement due to co-eluting matrix constituents also present in the samples extracts. Thus, SPE blank extracts for each type of sample were spiked at 5 µg/L of each individual pesticide and labelled I.S. used (equivalent to 0.05 µg/L in sample) and matrix effects were evaluated for each compound calculating the absolute (without internal standard correction) and relative (with internal standard correction) responses in comparison to those of reference standards in solvent at 5 µg/L [39].

As can be seen in Figure 2, matrix effects were not much noticeable for those pesticides and TPs determined in SW samples. Only for DIA and DEA, a remarkable signal suppression was found, whilst tolerable enhancement was revealed for TER and TBTYN. However, the effect of the sample matrix was more evident in wastewater samples, especially for IWW samples where notable signal suppression was observed for almost all compounds (22-63%) with the exception of TER and TBTYN. In addition to these compounds, also HTbzne showed a similar behaviour (84%) in effluent wastewater samples.

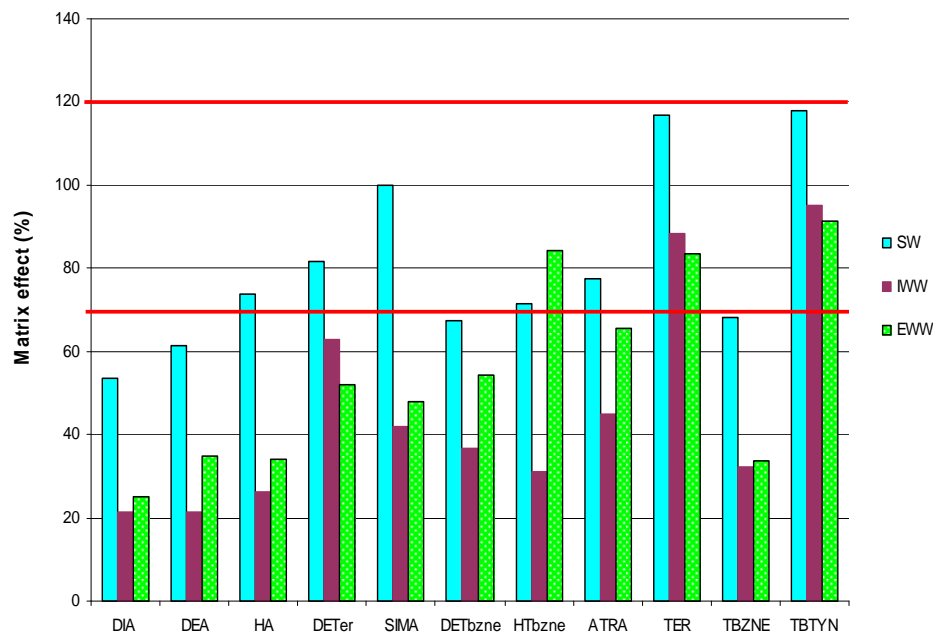


Fig. 2. Matrix effect for all selected analytes at 0.05 µg/L level in surface water (SW), influent (IWW) and effluent (EWW) wastewaters. *Abbreviations:* See tables 1.

Several approaches are typically applied to deal with matrix effects in quantitative LC-MS/MS analysis: improvement of the sample pre-treatment (clean-up) and/or the chromatographic separation, matrix-matched standards calibration, sample dilution or the use of stable-isotopically labelled internal standards, the latest being widely accepted to be the most satisfactory approach. The ideal situation would be to have each analyte corrected by its own isotope-labelled molecule, but this problematic is multi-residue analysis due to the commercial unavailability of reference standards for several compounds (e.g. some TPs) and the high cost of acquiring a large number of isotope labelled reference standards. An option normally applied within the environmental field, the use of only a few ILIS [29], has been explored in this work for correction of matrix effects (3 labelled compounds were tested for analytes correction). Analytes were divided into three groups as a function of their retention time with the objective of performing correction with the ILIS of the nearest retention time. As expected (Figure 3), satisfactory corrections were observed when terbuthylazine-d₅ was applied to TBZNE in all water samples tested. The results show how the use of the labelled molecule allowed to compensate the strong matrix effect obtained for IWW and EWW to a correct value around 100% allowing a right quantification of this compound. However, in spite of belonging to the same chemical class, when terbuthylazine-d₅ was

used to correct for matrix effects of other triazines and TPS, unsatisfactory results were obtained in several cases (Figure 3a).

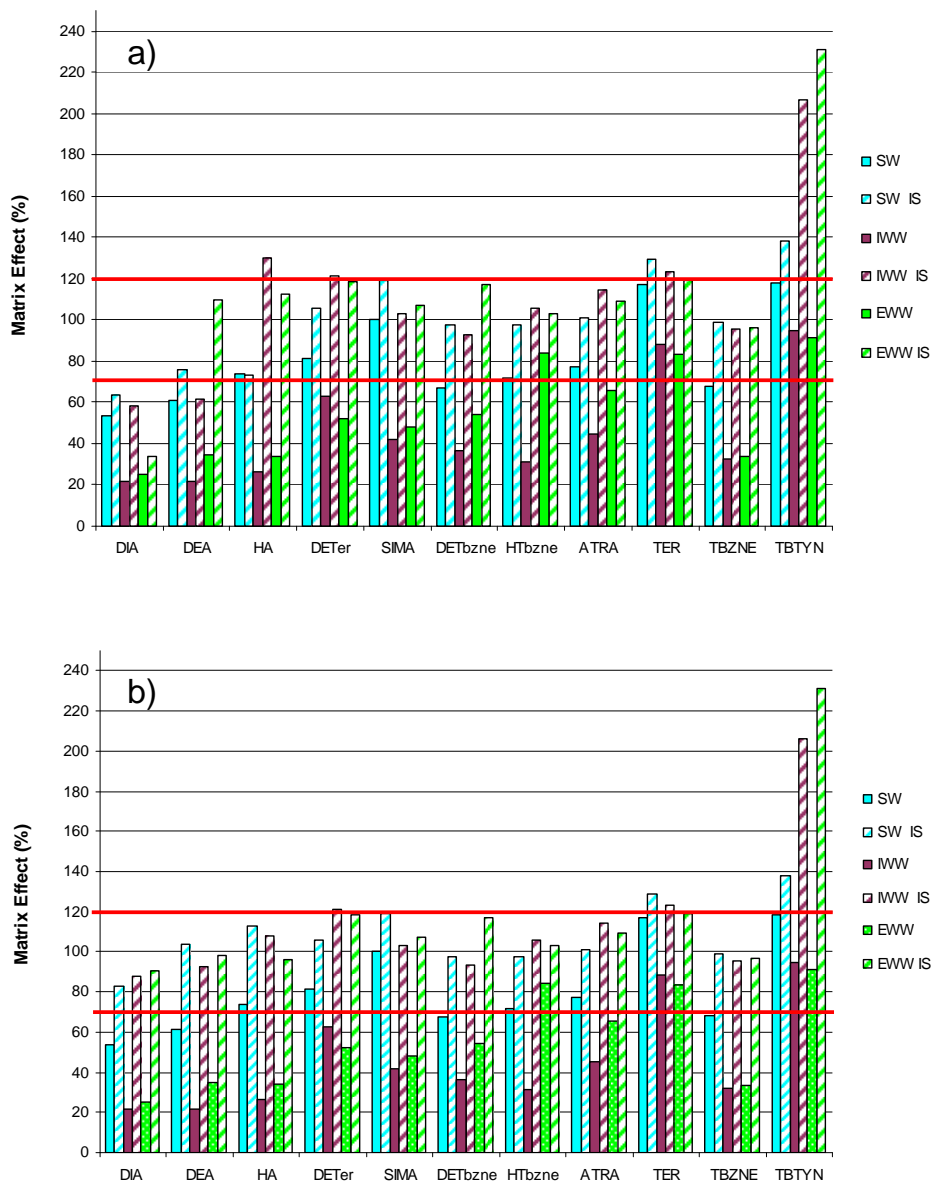


Fig 3. Matrix effects in different water samples (SW, IWW and EWW) for all selected analytes with and without correction of internal standards. (a) All compounds corrected by $tbzne-d_5$. (b) Correction with the nearest retention time labelled analyte. DIA corrected by dimethoate- d_6 ; DEA and 2-OH-atrazine corrected by thiabenzadole- d_6 and all the other compounds by $tbzne-d_5$.

Thus, undesirable enhancements were observed for TER and TBTYN in all samples, making the use of this ILIS inadvisable. Despite TER was almost co-eluting with TBZNE, matrix effect for all samples tested increased from around 88% (WW) and 117% (SW), without ILIS correction, up to around 130% when using the analogue ILIS. On the contrary, for compounds eluting rather separate to terbuthylazine-d₅, like DETer, SIMA, DETbzne, HTbzne and ATRA, matrix effect were notably corrected in all samples.

Matrix suppression for DIA was not correctly compensated by ILIS use in none of the samples, whilst DEA and HA were properly corrected in only EWW samples. In order to compensate matrix effect of the first three eluting TPs (DIA, DEA and HA), other ILIS were tested: dimethoate-d₆ and thiabendazole-d₆. In this case, the use of ILIS structurally different from triazines but eluted at similar retention time was found a satisfactory approach for quantification (Figure 3b). Dimethoate-d₆ was able to correct matrix effect on DIA, compensating the suppression from around 25% (WW) and 50% (SW) to a correct value around 90%. For the other two compounds, thiabendazole-d₆ was found more suitable, allowing a right quantification (around 100%).

Therefore, in order to compensate for errors associated to matrix effect, with the exception of TER and TBTYN, which were not affected significantly, each compounds was corrected by ILIS as follows: dimethoate-d₆ (DIA); thiabendazole-d₆ (DEA and HA); terbuthylazine-d₅ (for the rest of compounds). Matrix effects correction in real-world samples (SW, IWW and EWW) was performed as shown in Table 3.

4.3.5. Method validation

The whole analytical procedure was satisfactorily validated for linearity, precision, accuracy, sensitivity and specificity, in different type of water samples (SW, IWW and EWW) spiked with the compounds investigated in this work. These blank samples were previously analyzed and positive findings were subtracted from the spiked samples.

The linearity of the method was evaluated by linear regression analysis at seven concentrations. A seven point calibration curve, in the range from 1 to 100 µg/L, was generated by injecting in triplicate mixed standard solutions with a fixed amount of mixed internal standard solution. Good linearity was achieved for all of the analytes as indicated by correlation coefficients greater than 0.99.

Precision and accuracy were evaluated by spiking "blank" water samples at two concentration levels (0.025 and 0.1 µg/L), and analyzing five replicates of each spiked samples. It is worth to mention that three SRM transitions could be acquired also at low level for all analytes, making the reporting data highly confident from a quantitative point of view. As Table 4 shows, recoveries (between 70 and 120%) and precision (< 20%) were satisfactory for all compounds at both fortification levels. TER and TBTYN could be quantified without ILIS correction with acceptable recoveries (81-113%) and precision (3-14%).

The excellent sensitivity of the method is illustrated by instrumental LODs, which were in the range from 0.03 to 0.78 pg (Table 4). Regarding LOQs

values, it must be taken into account that they were estimated from the most sensitive confirmation transition (q1). This means, that analyse could be quantified (using Q transition) but also confirmed at the same level (using q1 transition). As table 4 shows, analytes could be quantified and their identity confirmed in a reliable way at levels as low as 0.9 ng/L in SW, 6 ng/L in IWW and 3 ng/L in EWW.

The specificity of the method was evaluated by analysis for possible interferences at the corresponding retention time for each substance. For that reason several blank water samples were analyzed with the proposed method and indicated no interfering peaks at the expected retention times of the analytes. Therefore, the method's ability to efficiently separate the analytes from interferences indicates the specificity of the developed assay. As an example, Figure 4 shows selected chromatograms for blank, a standard and a SW spiked at the LOQ level. As can be seen, the high sensitivity of the developed method allowed to a correctly quantify all selected pesticides and TPs at this low level. Additionally, the method was found to be highly specific as no relevant signal was observed in the blank.

Table4

Method validation for surface water (SW), influent (IWW) and effluent (EWW) wastewaters. Recovery (%) and relative standard deviation (RSD, %) for five replicates, instrumental limit of detection (LOD) and estimated limit of quantification (LOQ)

Compound	Rt (min)	LOD (pg)	SW						IWW				EWW				I.S. used	
			LOQ (ng/L)	0.025 µg/L (n=5)		0.1 µg/L (n=5)		LOQ (ng/L)	0.025 µg/L (n=5)		0.1 µg/L (n=5)		LOQ (ng/L)	0.025 µg/L (n=5)		0.1 µg/L (n=5)		
				Rec (%)	RSD (%)	Rec (%)	RSD (%)		Rec (%)	RSD (%)	Rec (%)	RSD (%)		Rec (%)	RSD (%)	Rec (%)		RSD (%)
DIA	3.54	0.29	6	77	5	83	4	20	77	14	80	9	8.0	77	7	73	9	Dimethoate-d ₆
DEA	3.86	0.12	5.9	83	7	92	6	8.7	70	9	82	11	14	72	8	73	6	Thiabendazol-d ₆
HA	3.94	0.78	1.4	98	6	88	6	22	109	9	112	4	9.0	88	14	70	12	Thiabendazol-d ₆
DETer	4.26	0.07	1.3	93	8	101	3	29	93	4	109	10	19	72	6	76	7	Tbzne-d ₅
SIMA	4.26	2.60	20	118	9	116	17	150	108	8	101	6	58	106	19	102	11	Tbzne-d ₅
DETBzne	4.29	0.19	2.3	100	12	106	4	25	101	10	111	8	20	101	14	85	12	Tbzne-d ₅
HTbzne	4.15	0.03	1.4	79	13	89	12	14	71	8	83	13	12	71	8	87	9	Tbzne-d ₅
ATRA	4.58	0.24	3.9	96	5	99	4	27	121	6	116	10	12	101	11	89	11	Tbzne-d ₅
TER	4.98	0.05	0.9	90	8	100	8	6.0	86	6	81	5	3.0	95	8	105	14	-
TBZNE	5.02	0.15	2.0	89	4	104	7	23	108	5	91	5	12	103	3	101	3	Tbzne-d ₅
TBTYN	5.39	0.25	1.3	97	6	104	8	22	83	5	99	3	11	102	3	113	6	-

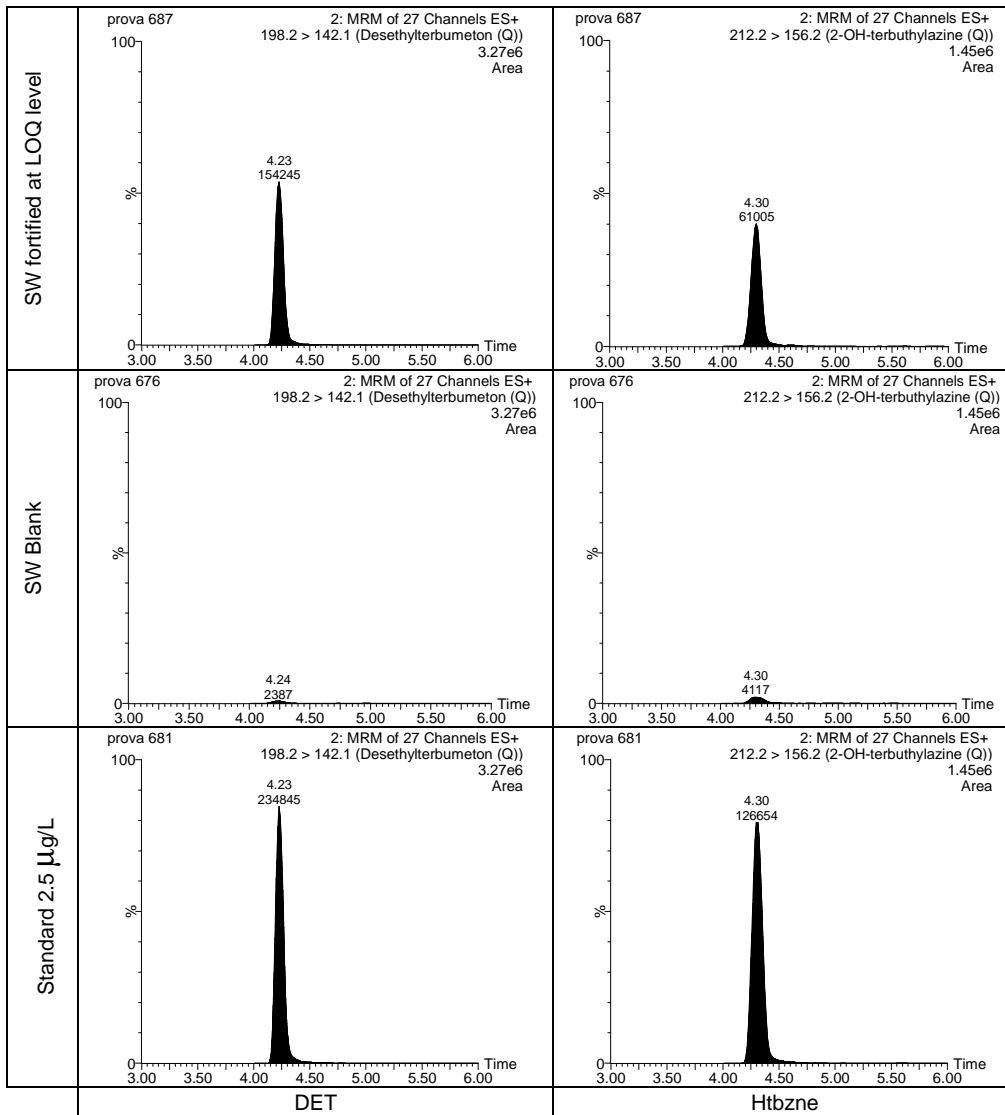


Fig. 4. Selected LC-MS/MS chromatograms corresponding to standard at 2.5 µg/L, blank surface water and surface water spiked at LOQ (0.025 µg/L).

Continued

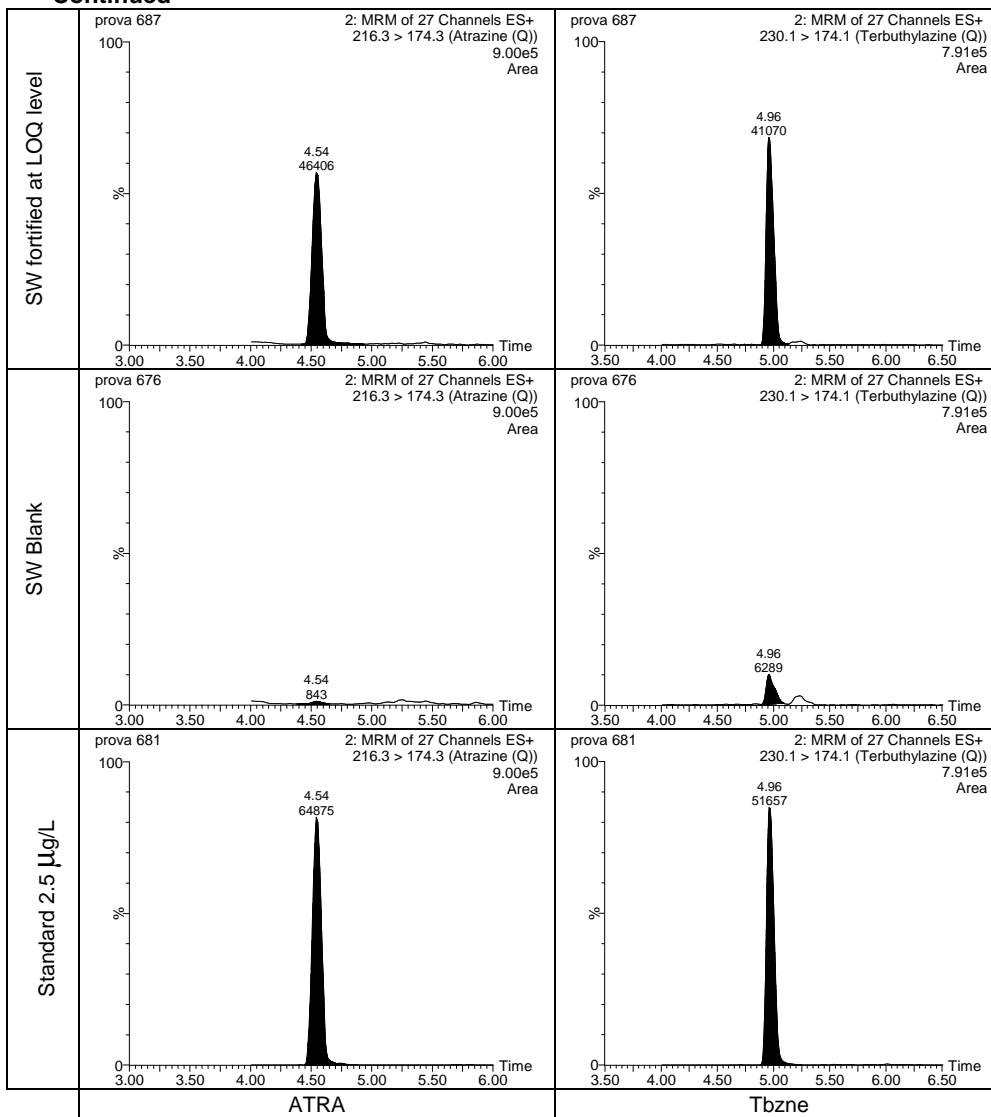


Fig. 4. Selected LC-MS/MS chromatograms corresponding to standard at 2.5 µg/L, blank surface water and surface water spiked at LOQ (0.025 µg/L)

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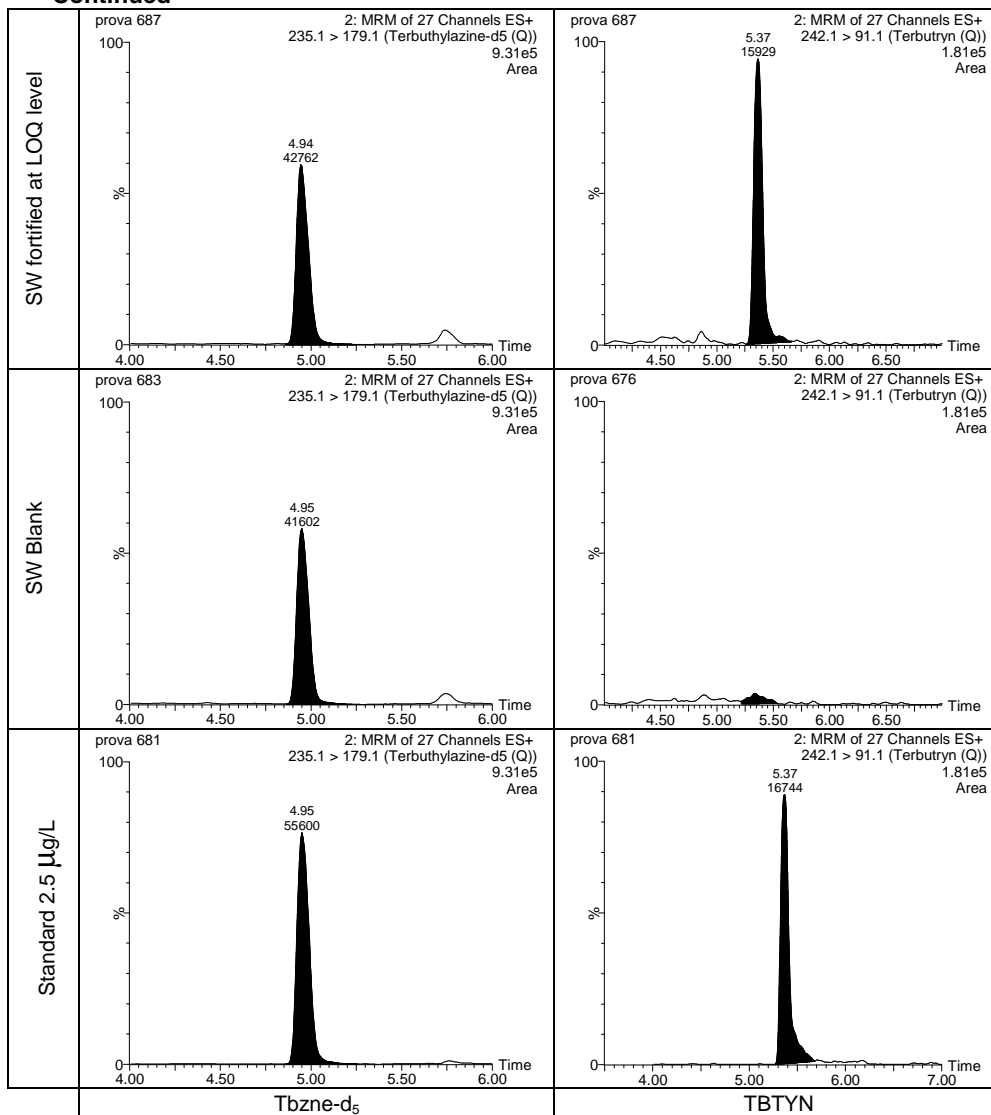


Fig. 4. Selected LC-MS/MS chromatograms corresponding to standard at 2.5 µg/L, blank surface water and surface water spiked at LOQ (0.025 µg/L).

4.3.6. Monitoring of pesticides and TPs in environmental waters

The developed method was applied 32 water samples (12 SW, 10 IWW and 10 EWW) collected in different sites of Italy and Spain in April, June and September, 2009. The results of the analysis are summarized in Table 5 (SW) and 6 (WW). All the selected compounds were detected at least once, and one of them (TBZNE) was detected in all the samples (at concentrations higher than 0.025 µg/L in around 23% of 378 the water analyzed). Other herbicides like TBTYN and SIMA were also frequently detected, as well as the TPs HTbzne, DETbzne, DIA and DETer. At least three compounds were present in every sample, but in only a few cases the levels were above 0.1 µg/L. These cases were dominated (>90%) by TPs (HTbzne).

Regarding SW, samples from Spain showed a higher number of positive findings (~90%) than from Italy (~50%). The highest level found (0.787 µg/L) corresponded to a TP (HTbzne) in Ebro delta. TBZNE and its dealkylated and hydroxy TPs (DETbzne, DIA and HTbzne) were found in almost all analyzed samples. It is interesting to point out, when comparing positive findings for unchanged triazines and for their TPs, that TPs levels were higher than the parent herbicides ones, in several analyzed samples, highlighting the interest of their inclusion in multiresidual methods for monitoring. Such situation was observed for ATRA, TBZNE and TER and their metabolites. Figure 5 shows SRM chromatograms for two SW samples (Ebro and Verd rivers) positives for ATRA, TBZNE and their main TPs. The high sensitivity of the method allowed the reliable confirmation of positive findings at very low concentration levels. For example, the three SRM transitions acquired for ATRA and TBZNE were reliably used for their identification at 0.009 µg/L. It is interesting to emphasize the lower concentration found for ATRA and TBZNE in comparison to their TPs, DEA (0.080 µg/L), HA (0.095 µg/L), HTbzne (0.112 µg/L) and DETbzne (0.080 µg/L). This fact illustrates how the effect of herbicide application and its influence on the environment is underestimated when samples are analyzed for the parent compounds only.

In relation to WW samples, TBZNE and HTbzne were found in all samples analyzed, and TBTYN and DETer in more than 70%. For these compounds, the highest levels found were in IWW at 0.21 µg/L. As expected, concentrations of parent herbicides in EWW were usually lower than in IWW. However, an increase was observed for TPs levels in EWW samples, probably related to the processes involved in the WWTP. This fact could not be confirmed in the case of TBTYN as no specific TP was selected. Figure 6 shows illustrative chromatograms for IWW and EWW. In spite that DIA or any of their precursors (ATRA, SIMA and TBzne) were not normally present in the Italian IWW, we found significant levels of DIA in the related EWW samples. Although this fact needs to be confirmed by further data, it is likely that some release of the removed and/or transformed compounds from sewage sludge or from exhausted activated carbon occurs within the treatment plants. Other possibility for DIA findings in EWW could be the

presence in the influent of other precursor triazines like cyanazine or sebutylazine, not included in the developed multiresidual method.

Table 5

Concentrations of triazines and their TPs obtained along the monitoring of these analytes in surface water from 14 sampling sites located in Italy (urbanized area north of Milan) and Spain (the Mediterranean Valencian area). Samples were collected in June and September, 2009

Compound	SW ($\mu\text{g/L}$)											
	June							September				
	1	2	3	4	5	6	7	8	9	10	11	12
DIA	0.017	0.008	d	0.009	0.010	-	0.015	-	0.018	d	d	d
DEA	0.080	d	-	0.009	0.009	-	0.020	-	-	-	-	-
HA	0.095	0.151	0.038	d	d	-	-	-	-	d	-	-
DETer	0.006	0.005	0.003	0.025	0.042	-	0.014	-	-	-	-	-
SIMA	0.038	d	-	0.028	0.026	-	0.160	d	d	-	0.025	-
DETBzne	0.008	0.016	d	0.070	0.080	-	0.040	0.016	0.008	0.015	0.013	-
HTbzne	0.105	0.787	0.317	0.087	0.112	d	0.068	-	-	d	-	-
ATRA	0.009	d	-	d	0.004	-	-	d	d	d	-	-
TER	0.009	d	0.002	0.002	0.009	-	d	-	-	-	d	-
TBZNE	0.026	0.004	0.003	0.002	0.009	-	0.053	0.008	0.009	0.026	0.005	0.015
TBTYN	0.003	0.008	0.002	-	-	-	0.030	0.003	0.003	-	0.022	0.004

d: detected at concentration level <LOQ, -: not detected.

Abbreviation: Rivers monitored: 1: Ebro; 2: Ebro Delta; 3: Pego-Oliva marsh; 4: Almenara; 5: Verd river; 6: Cecilia; 7: Clot Burriana; 8: Lurate; 9: Guanzate; 10: Gorgonella; 11:Seveso; 12: Livescia

Table 6

Concentration of triazines and their main metabolites in 24-h composite influent and effluent wastewater of five WWTPs placed in Spain and Italy, during two sampling campaigns in April and June, 2009

Compound	IWW ($\mu\text{g/L}$)										EWW ($\mu\text{g/L}$)									
	April					June					April					June				
	CS	BU	BE	AL	AS	CS	BU	BE	AL	AS	CS	BU	BE	AL	AS	CS	BU	BE	AL	AS
DIA	-	-	-	-	-	-	-	-	-	-	-	-	-	0.070	0.035	-	0.035	-	0.230	0.195
DEA	-	-	-	-	-	-	-	-	-	-	-	d	-	-	-	-	0.025	-	-	-
HA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DETer	d	d	0.030	-	-	d	d	0.030	-	-	d	0.024	0.041	d	d	d	0.020	0.044	-	-
SIMA	-	-	-	-	-	-	d	-	-	-	-	d	-	-	-	-	d	-	-	-
DETBzne	-	-	-	-	-	-	0.050	-	-	-	d	0.025	d	d	d	-	d	d	d	d
HTbzne	0.110	0.140	0.210	0.050	0.060	0.080	0.180	0.170	0.050	0.020	0.063	0.076	0.060	0.057	0.073	0.050	0.076	0.084	0.055	d
ATRA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TER	-	d	d	-	-	-	0.060	d	-	-	-	d	d	-	-	-	d	d	-	-
TBZNE	d	d	d	d	d	d	0.210	d	d	d	0.027	0.060	0.016	d	0.020	d	0.029	0.015	d	d
TBTYN	0.061	0.052	0.040	0.041	d	0.030	0.082	0.043	d	-	0.012	0.046	0.028	-	-	0.019	0.015	0.015	d	d

d: detected at concentration level $>\text{LOD}$ and $<\text{LOQ}$, -: not detected

Abbreviations: WWTPs: CS (Castellon); Bu (Burriana); BE (Benicassim); AL (Alto Lura); AS (Alto Seveso).

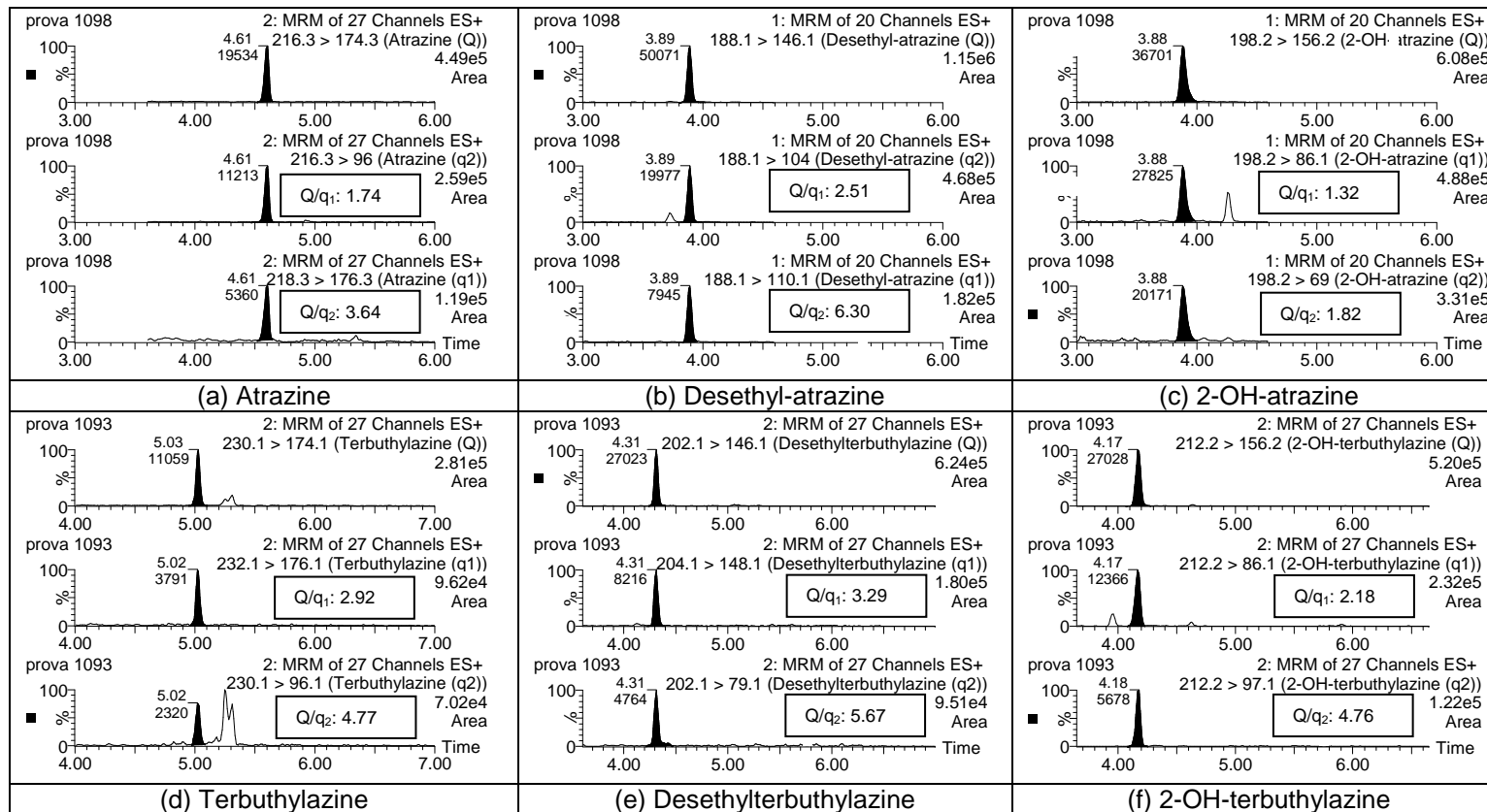


Fig 5. . Selected UHPLC-MS/MS chromatograms for two SW samples from the Ebro and Verd Rivers. Concentrations (a) 0.009 $\mu\text{g/L}$ (b) 0.095 $\mu\text{g/L}$. (c) 0.080 $\mu\text{g/L}$ (d) 0.009 $\mu\text{g/L}$ (e) 0.112 $\mu\text{g/L}$ (f) 0.080 $\mu\text{g/L}$. (Q) quantification transition; (q₁) and (q₂) confirmation transition.

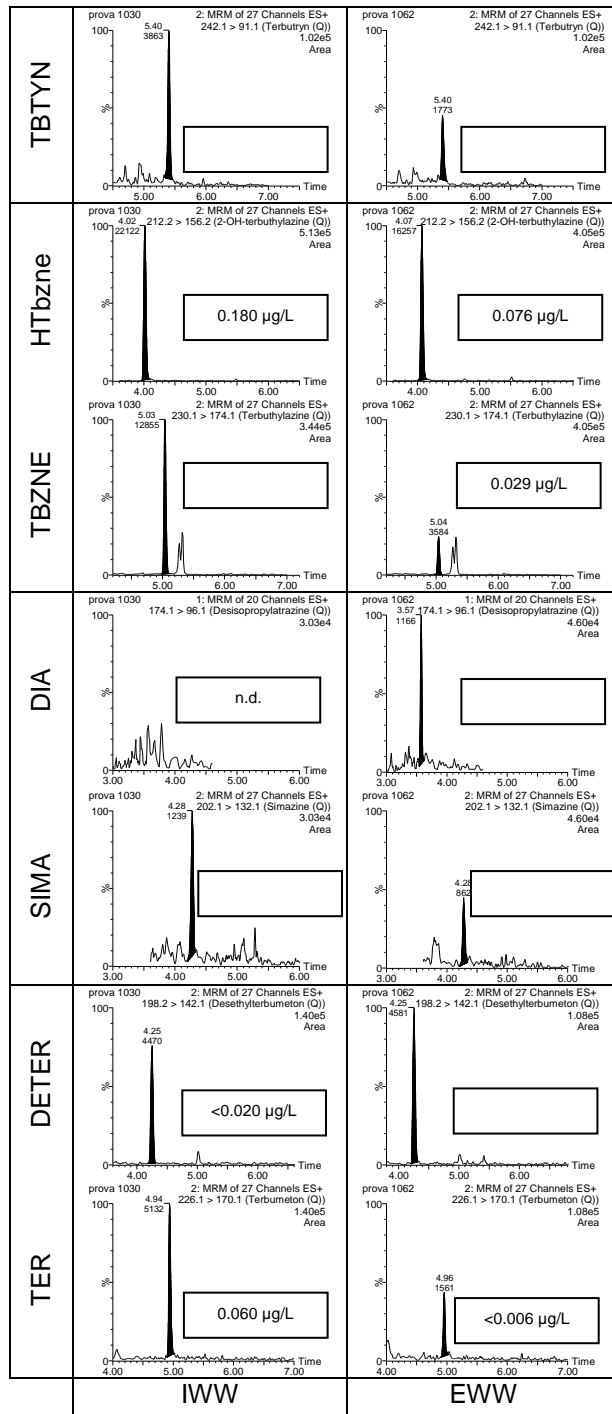


Fig 6. Selected UHPLC-MS/MS chromatograms for Burriana IWW and EWW samples collected in June 2009. (Quantification transition). *Abbreviations:* See tables 1-4.

4.4. Conclusions

This work describes the development and validation of a multi-residue UHPLC-MS/MS method for the quantification and confirmation of 11 triazine-related compounds (ATRA, SIMA, TBZNE, TER and TBTYN and their main transformation products) in surface and wastewater samples at ng/L levels. Losses associated to SPE process and matrix effects of signal suppression or enhancement due to co-eluting matrix constituents have been carefully evaluated. With the exception of TER and TBTYN, which did not require matrix effects correction, satisfactory results were obtained when using ILIS in all water samples tested. The overall analytical method has been validated in surface, influent and effluent wastewater at 0.025 and 0.1 µg/L, obtaining satisfactory recoveries and precision. Confirmation of the analyte identity was granted by acquiring 3 SRM transitions and the accomplishment of the ion ratio deviations in all analyte/matrix combinations, even at concentrations below 0.025 µg/L. The results obtained in this work after application of the method to real samples show the interest of including the most relevant TPs into current analytical methods to obtain a more realistic knowledge on water quality regarding pesticide contamination, as concentrations levels of TPs and detection frequency were normally higher than for parent herbicides.

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CHAPTER V

Short retention constructed wetlands for polishing treated effluents: first results of a demonstration scale research

Abstract

The performance of a horizontal subsurface flow constructed wetland installed at Livescia wastewater treatment plant (Como, Northern Italy) has been monitored in one year monitoring campaign.

The demonstration scale plant was designed to be fed by the effluent of an activated sludge wastewater treatment plant receiving mixed sewage, including domestic and industrial flows, mostly deriving from textile dyeing industries.

The constructed wetland, operated at 2 days retention time, was based on two parallel sectors, which presented different sizes of filling material (gravel) and were cropped with *Phragmites australis* and *Typha latifolia*. Average 24-h samples of the influent were collected weekly, whilst the effluents from the two sectors were collected 2 days after the inlet one, to account for the hydraulic retention time. The analyses of the main reference parameters (pH, electric conductivity, COD, Total N, N-NO₃, N-NO₂, N-NH₄, Total P, Total Suspended Solids) and of some heavy metals (copper, lead and zinc) showed unsatisfactory results regarding plant activity in reducing the investigated organic and inorganic pollutants, while a filtration effect can clearly be observed. The demonstration plant has to be still considered in the starting phase, even if more that one year has passed from the starting of operation.

Keywords

Constructed wetlands, horizontal subsurface flow, macrophytes, effluent wastewater, removal efficiency, polishing treatment.

Working in progress

5.1. Introduction

The problem of low environmental quality of rivers is often related not only to polluting loads in themselves, but also to other kinds of anthropic pressures. In Italy, a very important one is the low and variable flow characterizing most of the watercourses, due to the intensive exploitation of water resources and also related to the climate change. Considering the river as a complex ecosystem, where morphological, hydrological and biological aspects contribute to the overall environmental quality, it is easy to understand the negative effects of poor and variable flows. On the other hand, wastewaters coming from wide areas are normally collected in combined sewage networks and fed to large size, centralized wastewater plants, in order to optimize their treatment. As a consequence, high flows, resulting from both treated effluents and, when it rains, rainwater and run-off water, are discharged in a single site. Even if the plant performance is good, the residual presence of low concentration of pollutants may generate high polluting loads. In the end, as the available dilution in the receptor is often low, the effect of the input of any polluting load results in a fast increase of polluting concentration. As the difference between the standards in force for discharge and for river water quality is often great, in many cases a polishing treatment is needed in order to restore an acceptable environmental quality, especially in the cases known as “effluent dominated streams” [1].

When land availability is enough, constructed wetlands (CW) are interesting options for polishing, as their operation costs are low and their presence involves a positive environmental impact contributing to the overall biodiversity of the area, at the same way as natural wetlands.

Constructed wetlands have many advantages compared to conventional treatment techniques. They:

- have significantly lower total lifetime costs and often lower capital costs than conventional treatment systems.
- tolerate fluctuations in flow and pollutant concentrations,
- are capable of treating multiple or mixed contaminants,
- provide flood protection,
- have lower air and water emissions and secondary wastes,
- can be built to fit into the landscape,
- provide habitat for plants and wildlife,
- provide recreational and educational opportunities, and
- are environmentally friendly and are viewed favorably by the general public and regulators [2, 3].

Constructed wetlands can be of different kinds, essentially belonging to two categories: extensive (Surface Flow Systems - SF) and intensive (Sub-Surface Flow systems – SSF) ones [4-8]. In Europe SSF systems outnumber SF systems. In general, SF wetlands require more land to achieve the same pollution reduction as SSF wetlands, but are easier and cheaper to construct, operate and maintain [3, 9].

SF wetlands consist of shallow basins partially filled with soil, peat or any other media that will support plant roots. These wetlands generally have a soil bottom, emergent vegetation, and a water surface above the substrate (Fig. 1). The water moves slowly through the wetland above the substrate.

The near-surface layer of water is aerobic while the deeper waters and the substrate are usually anaerobic. Plants, mostly cattails, reeds, sedges, and rushes, in these SF systems can tolerate continuously saturated soil conditions and the resulting anaerobic soils [2, 3].

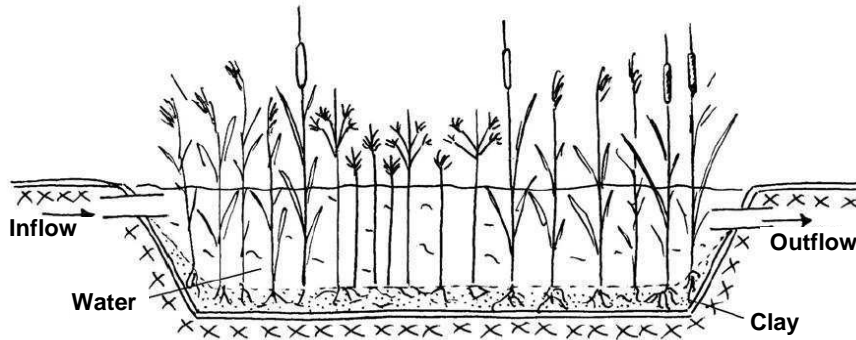


Fig. 1. Typical cross section of a SF wetland Cell (EPA , 2000).

SSF wetlands are generally constructed with a porous material (e.g. soil, sand, or gravel) as a substrate for growth of rooted wetland plants in addition to various microbes. These wetlands are designed so that water flows horizontally or vertically through the substrate and below the ground surface [2, 3]. Distinct aerobic and anaerobic treatment zones become established, which improves wastewater treatment. The emergent vegetation, mostly bulrush, reeds, and sometimes cattails, supplies oxygen to the substrate and allows biological growth to accumulate on its roots [10, 11]. Bacteria and beneficial fungi live in the substrate as biofilm attached to the substrate particles [3]. SSF wetlands can be classified into two basic flow systems: horizontal flow and vertical flow, which have similar contaminant removal mechanisms, but different hydraulics (Fig.2).

In horizontal subsurface flow wetlands, the wastewater flows only through the substrate. SSF wetlands should have no continuous free surface water or the flow will shortcircuit, moving quickly across the surface of the wetland and resulting in incomplete treatment. Vertical systems are constructed so that water moves uniformly down or up through the substrate. Water is generally ponded on the surface to help maintain anaerobic conditions in the substrate and to provide hydraulic head to encourage flow.

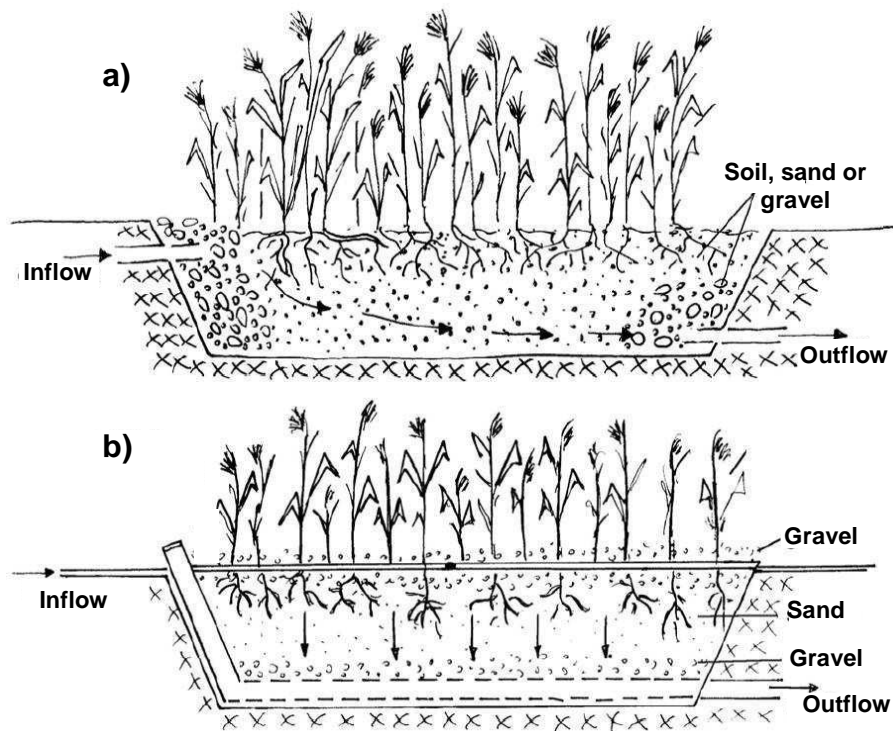


Fig. 2. Typical cross section of (a) a horizontal SSF wetland Cell and (b) a vertical SSF wetland Cell (EPA, 2000).

In both SF and SSF systems, microbial activity can exploit oxygen transferred by the roots to the substrate and the removal of pollutants is due to the synergy of physico-chemical and mechanical separation, of plant uptake and of biological activity in the substrate. In SSF systems, filtration plays also an important role.

Land availability is definitely a crucial point and can affect dramatically the investment costs, as the suggested hydraulic retention time is normally of some days and the specific surface requirement for polishing is around 1 m^2 per EI [4, 5]. Moreover, performance of CW may be less consistent than in conventional treatment; constructed wetlands depend on climate and, thus, may have reduced efficiencies during colder seasons. Finally, anaerobic conditions in SF wetlands might produce disagreeable odors associated with natural biological functions [2, 3].

The present paper deals with the results of a one year research at demonstration scale. The demonstration scale plant was designed to be fed by the effluent of a centralized wastewater treatment plant receiving mixed sewage, including domestic and industrial flows, mostly deriving from textile dyeing industries. So, the effluent, in spite of the satisfying performances of the treatment process, still contains non ionic surfactants and is visibly coloured. The case is representative of the above mentioned situation: the

flow of the receptor is practically only made of the flow of three wastewater treatment plants and practically no dilution of the discharged effluents can occur [12, 13]. The aim of the work was to assess the system efficiency, with particular reference to the kind of treated effluent, and to verify the possibility of obtaining good performance at short hydraulic retention time.

5.2 Materials and methods

The demonstration constructed wetland was installed at Livescia wastewater treatment site (Como, Northern Italy) and fed on the effluent from the plant. Figure 3 reports the scheme and a picture of the constructed wetland.

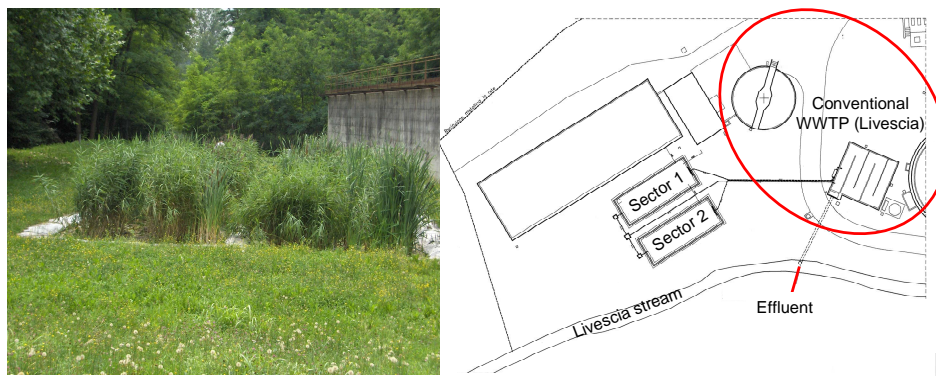


Fig. 3. Picture and scheme of the demonstration plant.

The demonstration plant is a horizontal subsurface flow wetland based on two parallel sectors, whose details are reported in Table 1. Basing on the inlet flow (200L/h), each sector provided the treatment for 24 EI. The filling material is gravel, 20 to 30 mm diameter in sector 1 and 5 to 15 mm diameter in sector 2, except from the inlet and the outlet area where the gravel diameter is larger in both sectors (about 50 mm). Hydraulic conductivity (K_f) is $10^{-3} - 10^{-2}$ m/s. The wetland was cropped with *Phragmites australis* and *Typha latifolia*.

During the experimental period, aiming at providing data for designing a full scale plant in a similar context, the constructed wetland was operated at 2 days retention time, quite short with respect to the standard conditions [4], but interesting in order to analyze the possibility of working at full scale in a quite densely urbanized area.

Table1

Main features of each sector of the constructed wetland

Length (m)	15.60
Width (m)	4.60
Area (m ²)	71.76
Inlet depth (m)	0.75
Outlet depth (m)	0.90
Slope (%)	1

Average 24-h samples of the influent were collected weekly. The effluents from the two sectors were collected 2 days after the inlet one, to account for the hydraulic retention time. The analyses concerned the main reference parameters (pH, electric conductivity, COD, Total N, N-NO₃, N-NO₂, N-NH₄, Total P, Total Suspended Solids) and were carried out according to Standard Methods [14]. Non ionic and anionic surfactants were determined by Hach-Lange kits LCK 332 and LCK 333.

Zinc, copper and lead were analysed weekly by atomic absorption spectrometry with graphite furnace as atomizer on samples acidified by HNO₃ (0.2% v/v) immediately after collection, kept at 4°C till analysis, according to USEPA.

Basing of the different degree of the macrophyte growth, four sub-periods have been defined within the whole experimental period, as reported in Table 2.

Table 2

The whole experimental period expressed in function of macrophyte growth

Sub-period	Macrophyte development	Sampling dates
Starting (St)	Presence of rhizomes, no epigeal development	11/03/08 – 17/04/08
2008 Vegetative season (08Veg)	Presence of living epigeal parts (> 20cm high)	24/04/08 – 26/11/08
2008 Rest period (2008 Quies)	Dead epigeal parts	12/12/08 – 08/04/09
2009 Vegetative season (09Veg)	Presence of living epigeal parts (> 20cm high)	16/04/09 – 15/11/09

Two ways ANOVA and Tukey test showed that no significant difference existed between the performances of the two sectors, so, their performance

was evaluated as a function of the average inflow and outflow concentrations.

5.3 Results and discussion

Electric conductivity showed no statistically significant variations over the whole experimental period, while pH showed a slight but significant decrease, leading its values around 7.3, while they were around 8 at the inlet. Apparently, the parameters which were more efficiently removed were Total Suspended Solids (76%), COD (23%) and Nitrates (57%), as reported in Figure 4.

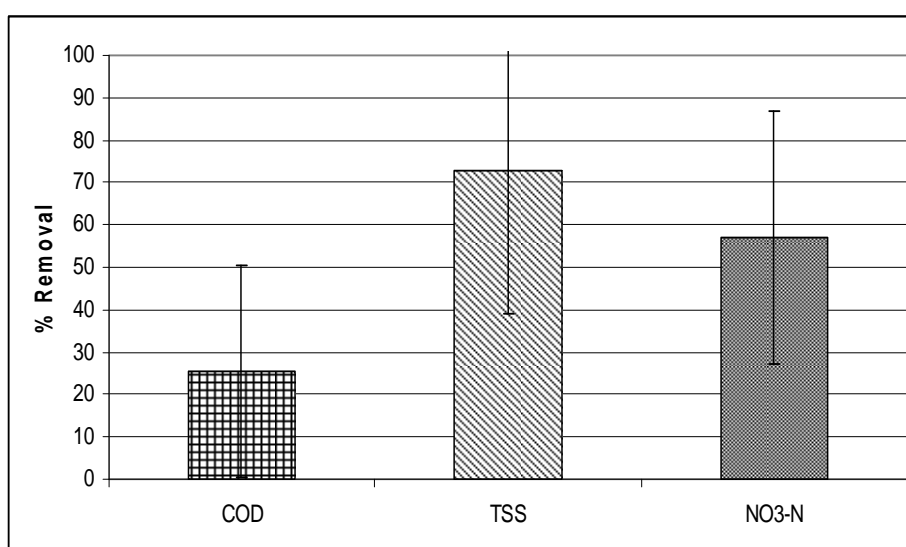


Fig. 4 . Percent removal of Total COD, TSS and nitrate-N in the constructed wetland in the experimental period.

However, the effect of filtration seems to be prevailing in COD removal, as the analysis on filtered samples showed no variations between the inlet and the outlet concentration and, moreover, percent removal obtained in the quiescency period was even higher (even if, due to the high variability of data, average values were not statistically different) than in the vegetative periods. Moreover, the performances obtained in the two monitored vegetation periods were comparable, as reported in Figure 5, while the plant development was very different from the first year to the following one.

On the contrary, nitrate removal seems to be very interesting, as the whole of residual nitrogen load outflowing an aerobic activated sludge plant is normally present as nitrate, and this is the case in the *Livescia* plant and, consequently, in the treated effluent fed as influent to the demonstration CW. However, in the present case, nitrate nitrogen has not been removed by plant uptake, but, rather, by denitrification and by chemical reduction, as

after one year from the operation start, no oxygen was still detected within the CW and the effluent was in clearly anoxic conditions. This statement is confirmed by the trend of ammonia nitrogen, which increased in most cases during the CW retention, as shown in Figure 6, and, consequently, of TKN. Even assuming that ammonia nitrogen is released by ammonification of organic nitrogen in the peat (and, eventually, in some sludge residues in the effluent), it can clearly be seen that the availability of oxygen in the CW was not enough to allow nitrification and plant uptake of the generated nitrogen. In global terms, residual nitrogen was not a problem in the present case study, as concentrations were anyway compliant with the quality objectives for surface waters.

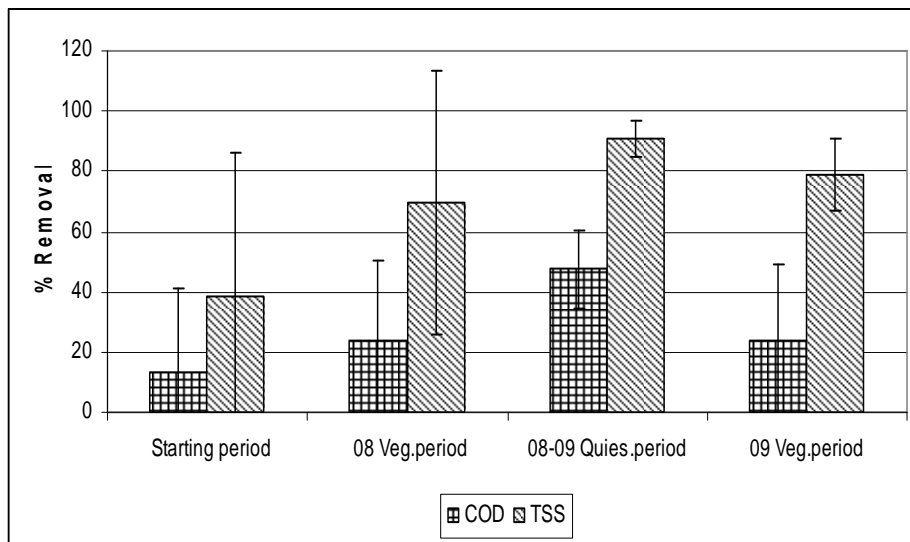


Fig. 5. Comparison among the removal efficiencies observed in the different experimental periods

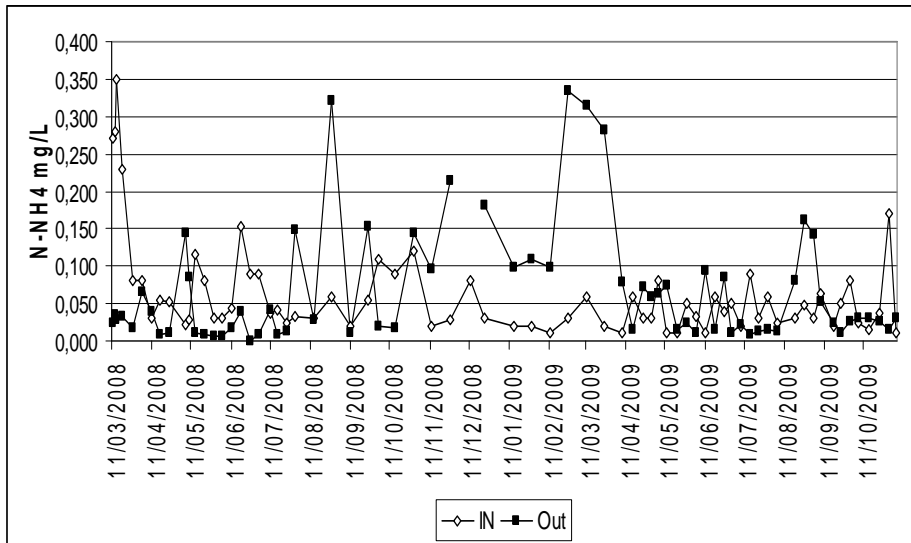


Fig. 6. Ammonia nitrogen concentration in the influent and in the effluent of the demonstration CW.

Copper, lead and zinc removal from CW was quite satisfactory, in spite of the high variability of the data, as shown in Figure 7. In this case, results were elaborated considering separately the performances in the two sectors, in order to see how the different size of the gravel bed could affect the removal efficiency, as this was chiefly due to filtration effect. The results, as average values, seem to be different. However, considering standard deviations, also metal removal appears to be comparable in the two sectors.

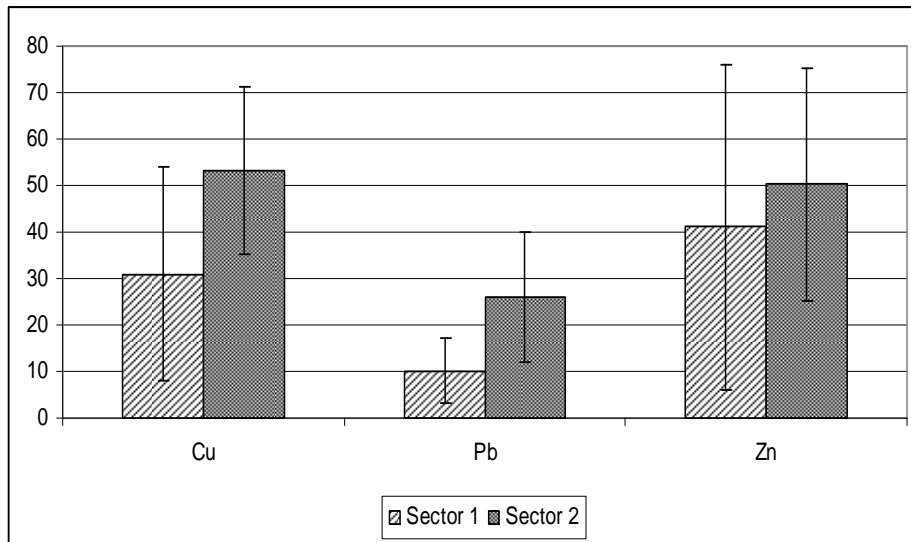


Fig. 7. Average percent removal of Copper, Lead and Zinc during the experimental period.

5.4. Conclusions

Based on the obtained results, the demonstration plant has to be still considered in the starting phase, as, even if more than one year has passed from the starting of operation, the presence of plants has not yet given the expected results. In spite of this, the simple filtration of effluent through the gravel bed has provided some results in terms of removal of COD, suspended solids and the analyzed heavy metals.

Till now, plant activity seems negligible and, consequently, macrophytes do not transfer oxygen to the rhizosphere and aerobic biodegradation processes cannot occur. Plant uptake is also negligible and cannot contribute to the overall removal of residual pollutants. The real operation phase will take place when oxygen will be regularly found in the gravel bed and ammonia nitrogen will be no more released.

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CHAPTER VI

Conclusions

The present research was carried out in order to provide the basis for studying and comparing possible recovery strategies for impaired streams, which can be included in a recently classified typology of streams, known as *effluent-dominated* [1].

The specific case of Lura stream is presented considering the integrated study of its chemical, hydrological and biological aspects. The situation of Lura stream is representative of most small watercourses in Lombardy, with a strongly anthropically modified basin, where, anyway, some interesting natural areas are still present. Human influence concerns the widespread urbanization, whose immediate effects are the generation of important polluting loads, the impermeabilization of wide land areas and the industrial density. This, in association with natural loss of water through the riverbed, causes droughts and greatly increases WWTP effluent negative effects. Changes in basin morphology cause also an increase in flood flashiness and, potentially, magnitude. Disturbance is given not only by the mechanical action of water, but also by sewage overflow spills associated with such events. The overall quality of the stream morphology is low and this causes an increase in all the other alteration effects, due to low habitat availability and absence of refugia for biotic communities [2].

According to the results of physico-chemical monitoring, the most important factor affecting the ecology of Lura stream is surely WWTP discharge. Effluents input continuously a too high polluting load which cannot be diluted enough by the scarce or void base flow which is usually present.

The modified hydrologic cycle and the consequent alternance of drought and flood events have a great impact on the entire stream course. This happens both before the WWTP discharges, where water scarcity originates destructive droughts preventing a balanced development and survival of invertebrate assemblages (as described by Lake [3]), and after them, where the effect of floods, taking place frequently, becomes dominant [4]. As expected [5], variation in macroinvertebrate assemblages is associated with various classes of variables and the worst EBI values are measured in traits characterized by the simultaneous presence of the worst water quality and of recurring floods.

The results obtained for Lura stream are in agreement with the need for a holistic approach suggested by the European Water Framework Directive, calling for the integration of morphology (habitat), water quality and water quantity. This is particularly evident in the case of effluent-dominated streams, where all of these factors have great influence and involve themselves reciprocally and leads to assess that recovery cannot be limited to water quality, but must involve measures for increasing the habitat quality, providing acceptable flow conditions [6] and improving morphological characters.

In order to extend the knowledge on the quality of water bodies, which are strongly impacted from WWTP discharges, analytical methods for the

determination of organic micropollutants, usually detected in effluent wastewater samples [7-10] as directly consequence of the human activities, were developed. These validated methods have allowed to monitor the presence in surface and wastewater samples of PAHs and triazines herbicides, detected in several cases at concentration levels higher than the environmental quality objectives for surface waters. WWTPs are normally designed to remove conventional organic pollutants (organic matter and nutrients); thus, the overall removal efficiencies calculated for most of substances were not sufficient to reduce the organic micropollutant loads in water bodies constituted by scarce base flows. Moreover, the PAHs and triazine removal rates were affected by analytical errors associated to losses in SPE processes due to the complex matrix investigated.

One part of the research carried out in the last year has shown how the environmental contamination can be caused not only by synthetic organic compounds but also by their recalcitrant transformation products. Several instances have been reported where single transformation products are present in higher concentrations or detected more frequently than their parent compound. Important examples include pesticides such as triazines [11, 12]. To this aim, a rapid, selective and sensitive method has been validated for the determination of 11 compounds (atrazine, simazine, terbutylazine, terbutometon, tebutryn and their main TPs) in surface and wastewater samples and then applied to the determination of those herbicides in a wide range of surface and wastewater samples (coming from different environments in Italy and Spain). The results have confirmed that TPs should be included in monitoring protocols, since they can be similarly or even more mobile, persistent, or toxic than their parent compounds [13]. In several analyzed samples, TPs concentration levels were higher than their parent compounds and this can show the effect of herbicide application and its influence on the environment tends to be underestimated when samples are analyzed for the parent compounds only.

An important part of the work has concerned the performances of wastewater treatments in the removal of inorganic and organic micropollutants.

Aiming at defining a frame of the potential and limits of the various, conventional and non conventional, sequences of treatments which can be adopted, a survey has been carried out on two existing WWTPs including ozonation as final polishing step and on a demonstration scale constructed wetland.

For organic micropollutants, the removal observed in conventional WWTPs appeared related chiefly to adsorption, rather than to ozonation or to biodegradation. For metals, whose removal in conventional WWTPs is well known, the survey has been concerned CW, where removal has been attributed to the substrate filtration and sorption, as for the other monitored parameters (COD, TSS, etc.). However, it was soon clear that the plant colonization in the CW was too recent for allowing the development of a biological activity effectively related to the rhizosphere, the plant development and the related microorganisms. Actually, the demonstration plant was still to be considered in the starting phase, even if more than one year has passed from the starting of operation.

The work carried out till now has answered to some questions but has also pointed out some aspect which will have to be considered and which will actually complete the obtained results, such as:

- ✓ The study of possible relationships between macroinvertebrate taxa and organic and inorganic micropollutants ;
- ✓ the development of a reliable analytical method for the simultaneous determination of PAHs and triazines in real wastewater samples, considering also the SPE losses which can occur in this type of samples and not only the recoveries in ideal conditions, as calculated in Milli-Q water samples;
- ✓ laboratory tests by ozonation on single parent compounds in order to extend the knowledge about transformation products, eventually also discovering new substance;
- ✓ the ongoing monitoring of the demonstration constructed wetland in order to estimate its performances with respect to macro and micropollutants and to evaluate the trend of the progressing operation

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