Potential of bioash for removal of the herbicide terbuthylazine from the aquatic matrix

Maltašić, Gracijela

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MASTER'S THESIS

Gracijela Maltašić

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Gracijela Maltašić

Supervisor:

Prof Gabrijel Ondrašek, PhD

Assist Prof Sanja Stipičević, PhD

Zagreb, September 2021

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Agronomski fakultet

University of Zagreb
Faculty of Agriculture

STUDENT'S STATEMENT

ON ACADEMIC RECTITUDE

I, **Gracijela Maltašić**, JMBAG 0079047669, born on 10th of July 1992 in Zagreb, declare that I have independently written the thesis under the title of

Potential of bioash for removal of the herbicide terbuthylazine from the aquatic matrix

With my signature, I guarantee:

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REPORT

ON EVALUATION AND MASTER'S THESIS DEFENSE

Master's thesis written by **Gracijela Maltašić**, JMBAG 0079047669, under the title of

Potential of bioash for removal of the herbicide terbuthylazine from the aquatic matrix

Is defended and evaluated with the grade______________________________, on_____________.

Committee for thesis evaluation and defense:

1. Mentor Prof Gabrijel Ondrašek ______________________________

2. Co-mentor Assist Prof Sanja Stipičević ______________________________

3. Committee member Assoc Prof Klara Barić ______________________________

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ABSTRACT

Of the master's thesis – student **Gracijela Maltašić**, entitled

Potential of bioash for removal of the herbicide terbuthylazine from the aquatic matrix

Terbuthylazine (TBA) is one of the most used herbicides in agriculture, forestry, and transportation sectors, particularly since 2007 when widely used atrazine has been banned. Soon after its pre-emergence application, TBA residues can be spread from soil to the entire aquatic environment. Biodegradation of TBA in the soil is much faster than its abiotic degradation in water, where it is proved to be relatively persistent and harmful to a range of aquatic organisms. Therefore, the efficiency of fly ash (FA)-treatment of watercourse samples fortified with TBA was tested in laboratory conditions. Deciduous hardwood-derived FA was produced in a cogeneration plant facility and subjected to in-depth characterization using SEM and XRD methods. The results showed that the addition of 1% *(w/v)* of the finest FA fraction, with a nano-to-micro-sized structure and alkaline Ca/Mg/Si-enriched mineralogy, can reduce the TBA half-life in the watercourse sample by more than 30-fold. The TBA dissipation in FA-amended water was well described with the first-order kinetics ($R^2 > 0.98$). TBA and its environmentally relevant degradation product desethylterbuthylazine (DET) were not detected in the water sample 48 hours after the FA addition, whereas nearly 80% of TBA was found in the untreated sample. The XPS analysis of post-treated FA suggested a plausible involvement of O, Ca, K, Mg, and Si in TBA adsorption. A basis for the development of innovative FA-based polymers targeted to decontamination aquatic ecosystems loaded with TBA and other hazardous (agro)chemicals were proposed.

Keywords: Terbuthylazine, Surface water, Wood biomass, Fly ash, Dissipation

1. INTRODUCTION

Due to intensive pesticide consumption over the last several decades in agriculture, but also in forestry and transportation sectors, these compounds became ubiquitous environmental contaminants with long-term and still poorly known effects on non-target organisms (Nicolopoulou-Stamati et al., 2016). Shortly after application, a certain proportion of pesticide active components or its degradation product reaches the surface waters and groundwater through runoff and leaching processes in soil (Stipičević et al., 2017). Mobility of compounds is a result of its weak or reversible adsorption onto soil particles, therefore highly mobile pesticides can be frequently detected in aquatic bodies.

The herbicide terbuthylazine (TBA) has replaced atrazine in most EU countries, becoming one of the most used pesticides and, therefore, frequently detected in natural waters (Tasca et al., 2018). A relatively high affinity of TBA for soil organic matter suggests prolonged contamination. Degradation in the soil leads to the release of the metabolite desethylterbuthylazine (DET), which is a more hydrophilic compound than the parent, resulting in a higher associated risk for contamination of groundwaters.

According to the recent study on the occurrence of organic persistent pollutants in European groundwater, TBA was the most detected herbicide that often exceeded the European Union (EU) groundwater standard of 0.1 μg/L, while DET was the most detected TBA degradation product in surface waters showing negative effects on aquatic organisms. Since atrazine has been banned for use in Europe in 2004, TBA became one of the widely used pre-/post-emergent broad-spectrum herbicides for maize protection and a chemical for controlling slime-forming algae, fungi, and bacteria. In 2017 TBA was ranked the third most used herbicide in Croatia (Barić et al., 2019).

TBA is classified as medium mobile, while DET is a highly mobile compound. The persistence of TBA and DET in the soil is medium to high (65–167 days and 27–113 days, respectively, at 20 °C and soil moisture 11–36% *w/w*). Due to the combination of their persistence and toxicity to living organisms, several remediation techniques for the contaminated aquatic ecosystem have been proposed so far. Methods for removal of organic contaminants from aqueous matrices are mostly associated with chemical coagulation, sedimentation, filtration (Ahmaruzzaman et al., 2011), adsorption (Ghosh et al., 2013), redox processes (Álvarez et al., 2016), and various electrochemical and biodegradation mechanisms (Gikas et al., 2018), as well as photocatalytic mineralization (Tasca et al., 2018). Adsorptive capacities of synthetic and natural materials (Mines et al., 2017) are highly variable due to differences in particle size distribution, porosity, and total surface functionalities. Moreover, relatively high costs of production of some commercial adsorbents, as well as loaded material treatment, limit their wider utilization. Finally, although highly efficient, the degradation treatments are costly and difficult for onsite operation and can generate toxic co-products and waste (Trivedi et al., 2018).

Because of enforcing more sustainable activities and green policies for mitigating and controlling climate changes, in many countries energy generation has been shifted from the fossil C to C-neutral (bio)renewables, such as biomass. Biomass stands for all the biodegradable parts of products, waste, and remnants of biological origin from agriculture, forestry, and similar production fields, including fishing and aquaculture, as well as the biodegradable components of industrial and municipal waste and/or residues (Ondrasek et al., 2021). Biomass-fired mineral co-product – fly ash (FA) is classified as a potentially hazardous waste, which has been produced at increasing rates worldwide in the last decades. However, due to specific physicochemical characteristics, notably micro-to-nano sized and pH-reactive matrix capable of interacting with potentially toxic substances, bioash has been studied intensively over the last decades and is recognized as a valuable and eco-friendly resource in eco-engineering and environmental remediation (Ondrasek et al., 2021). Additionally, in some EU countries such as Croatia, biomass power plant facilities are fired exclusively by a virgin, certificated hardwood forest biomass, generating bioash forms enriched in alkali minerals and macronutrients (Ca, Mg, K, P), but low in metal (Hg, Pb, Ni, Cr, Cd), carbon (C) and sulfur (S) content. These properties make bioash suitable for the amelioration of acidic and nutrient-deficient agricultural and forest ecosystems and biochemical wastewater conditioning such as removal of P, color, fecal coliforms, and intestinal enterococci (Ivanković et al., 2014).

1.1. Hypothesis and aims

The hypothesis of this research is that low-C and metal-clean FA, as the finest and most reactive biomass ash fraction, can be effectively exploited for removal of TBA from contaminated by either accelerated alkaline degradation or adsorption onto a huge active FA surface.

The hypothesis is tested in a laboratory microcosm for 48 h by using hardwood-derived FA from a modern cogeneration biomass plant facility and TBA dissolved in a watercourse sample.

This study aims to:

(1) evaluate and compare the half-lives of TBA in watercourse sample treated with FA (1%, *w/v*) and in FA-free water sample

(2) provide an in-depth characterization of pre-treated and post-treated FA to estimate the possible effect of FA addition on TBA fate in aquatic media

(3) compare the basic physicochemical properties of water in FA-treated and FA-free samples during the study period.

2. LITERATURE REVIEW

2.1. Bioash and its implication in agricultural and forest ecosystems

2.1.1. Production and physicochemical characterization

The EU Directive 2009/28/EC on the promotion of the use of renewable energy sources, with the goals of controlling energy consumption, increasing energy efficiency, and reducing greenhouse gas emissions, is one of the proactive initiatives for mitigating global climate change impacts and ensuring EU climate neutrality. One of the proactive initiatives for mitigating global climate change impacts and ensuring EU climate neutrality is the EU Directive 2009/28/EC on the promotion of the use of renewable energy sources, with the goals of controlling energy consumption, increasing energy efficiency, and reducing greenhouse gas emissions (EP, 2019). Among renewable energy facilities, biomass-fuelled cogeneration plants, that produce both electricity and heat, account for a significant percentage (Figure 2.1.1.1.).

Figure 2.1.1.1. Biomass-fuelled cogeneration plant producing both electricity and heat, with bioash as a side product

Regardless of the various positive effects of using biomass for energy production, certain possible hazards and risks must be recognized and managed carefully. Furthermore, biomass facilities generate a by-product known as biomass ash, which can be used for a variety of purposes, including raw material in the construction industry and civil engineering, phytonutrient source in food/feed/fiber production, additive to improve nutrient utilization/bioavailability from waste materials, and phytostabilization. Biomass FA shows a strongly alkaline reaction with water (pH > 12.5, and it is rich in specific minerals and nutrients). Additionally, its fine structure, where some fractions are as small as 1 mm, provides a large reactive surface capable of interacting with potentially toxic elements such as Cd, Zn, and Cu. These characteristics make biomass fly ash very promising, as well as a very potent, material for the remediation of acidic and nutrient-deficient soils depleted in organo-mineral colloids for the sorption of potentially hazardous metals. Because of the various fuel sources and ash types, biomass ashes have complex and diverse matrices. In general, wood-derived (so-called C type) ashes have more Ca, Mg, Mn, and pH content than herbaceous/agro-derived (so-called K type) ashes, which are rich in K, P, S, and Cl. Different ash types (bottom *vs.* fly) obtained from the same plant facility and burned by the same woody biomass, on the other hand, might differ significantly in elemental composition, organic compounds, and metal-binding adsorption capacity (Ondrasek et al., 2021). Given the wide range of bioashes, a high caution is required for their usage as soil conditioners, particularly in food/feed agroecosystems, where they may enhance environmental hazards or have severe health consequences (Ondrasek et al., 2020b).

Biomass ash materials have a high potential for multi-purpose reuse in the industry (e.g., cement and concrete production), construction (e.g., replacing sand, gravel and other granulates in road construction), and/or land nutrient recovery (James et al., 2012; Carević et al., 2019; Tosti et al., 2019). Furthermore, specific bioash forms (i.e., FA) have the ameliorative potential for acidic, low-nutrient, and/or metal-contaminated soils (Ondrasek et al., 2018; Yu et al., 2019), and bottom ash also showed a potential to be re-used in land reclamation (Yin et al., 2020). Such ameliorative implications of biomass ash are mostly related to its: i) strong basic properties (pH > 13.0), ii) mineral (silica, lime, calcite, magnesia) and nutrient (P, K, Ca, Mg) enrichment, and iii) huge reactive interfaces for trace elements retention (immobilization) by chemosorption and precipitation reactions (Gupta et al., 2002; Seyfferth et al., 2019; Park et al., 2020). Immobilization is not a strategy for removing metal(s) from soils but is very effective in alleviating certain metal-related risks i.e., production of "toxic metal-clean" food and preservation of water resources and related biota from impacts of toxic metals (Liu et al., 2018; Lee et al., 2019).

2.1.2. Effects on soil quality

Biomass ashes have complex and diverse matrices due to different fuel sources (e.g., forestry hardwood *vs.* agro-waste of annual crops) and ash types (bottom *vs.* fly) (Tosti et al., 2019; Palansooriya et al., 2020). On the other hand, different ash types originated from the same plant facility (combustion technology) and fuelled by the same woody biomass can vary significantly in i) elemental composition (e.g., higher metal contents in fly *vs.* bottom ash) (Tosti et al., 2019), ii) organic compounds (e.g., a higher concentration of PAHs, C, and N in bottom *vs.* FA fraction)

(Park et al., 2020; Ondrasek et al., 2021), and iii) adsorption capacity for metal binding (e.g., more than 4-fold higher capacity of FA *vs.* bottom ash for sorption of Cd) (Park et al., 2020).

Given that wood ash has been used as a soil amendment for several decades, many studies have investigated its impact on the soil microbial communities that play a key role in nutrient cycling, plant growth, and carbon sequestration (Fierer, 2017). Ash amendments were shown to increase microbial activity as measured by soil CO_2 production (Saarsalmi et al., 2012), as well as microbial biomass turnover or growth rate and nutrient cycling. Moreover, ash addition can change the soil bacterial abundance. However, some of these effects were observed only after high application rates or repeated applications (Bååth et al., 1994).

In addition to stimulating microbial abundance and activity, the application of ash typically altered soil bacterial community structure (Mahmood et al., 2003) or total microbial community structure. However, these studies used fingerprinting techniques, such as Phospholipid fatty acids (PLFA) measurements, that provided only limited resolution of taxonomic and functional responses (Bang-Andreasen et al., 2020). In contrast, newer molecular approaches such as amplicon sequencing of 16S rRNA genes for bacteria and of ITS1/ITS2 region for fungi or shotgun metagenomics have greatly increased the ability to study microbial communities at an unprecedented level of detail, permitting the identification of the community members to the family/genus/species level or the assessment of functional roles of active microbial communities by deciphering expression of specific genes. For instance, 16S rRNA gene amplicon sequencing showed the shifts in the soil bacterial community composition after wood ash application with the enrichment of copiotrophic bacterial groups such as *Bacteroidetes* and a decline in oligotrophic phylum such as *Acidobacteria* (Noyce et al., 2016). Additionally, there was no difference in the bacterial community with the increasing ash addition from 0.7 to 5.7 t/ha. Another study found yet more pronounced effects with increasing ash addition rate from 5 t/ha (the current legislative threshold in Scandinavian countries) to 22 t/ha (Bang-Andreasen et al., 2017). However, detrimental effects on soil bacteria were observed only at an extreme and unrealistic rate of 167 t/ha with alkaliphilic genus *Alcalibacterium* and spore-forming bacteria dominating.

Opposed to the bacterial community, some studies showed that the addition of high rates of ash to soil increased fungal abundance, especially the abundance of fast-growing saprotrophic fungi such as the genera *Mortierella* and *Peziza*, as well as the order *Hypocreales* (Bang-Andreasen et al., 2020). Compared to free-living fungi, the impact of bioash on ectomycorrhizal (EM) and arbuscular mycorrhizal (AM) fungi (which in symbiotic association with plant roots can improve plant nutrient uptake) remained less clear. Some authors found that wood ash application had no effect on EM fungal biomass or species richness (Kjøller et al., 2017; Cruz-Paredes et al., 2019).

2.1.3. Effects on pesticide dissipation in soil and water

Conventional agri-food production is inconceivable without the use of pesticides. Cereal cropping is prevalent, occupying ~50% of the total cultivated area in the world, with the yields doubling in the last three decades with an increase in pesticide consumption from 1.54 to 2.63 kg/ha (Figure 2.1.3.1.). Hence, pesticides are ubiquitous chemicals in the environment, but their long-term effects on non-target organisms are still poorly known. Shortly after application, a certain proportion of pesticide active component/metabolite can be moved from the field to drains/channels (and eventually surface water bodies) in runoff (Medalie et al., 2020) or can be leached through the soil profile to groundwater (Stipičević et al., 2017). Sites with a high pesticide load (e.g., due to equipment cleaning, disposal of empty packaging or expired formulations, and release from plant/crop residues) can be hotspots for significant water contamination by pesticides.

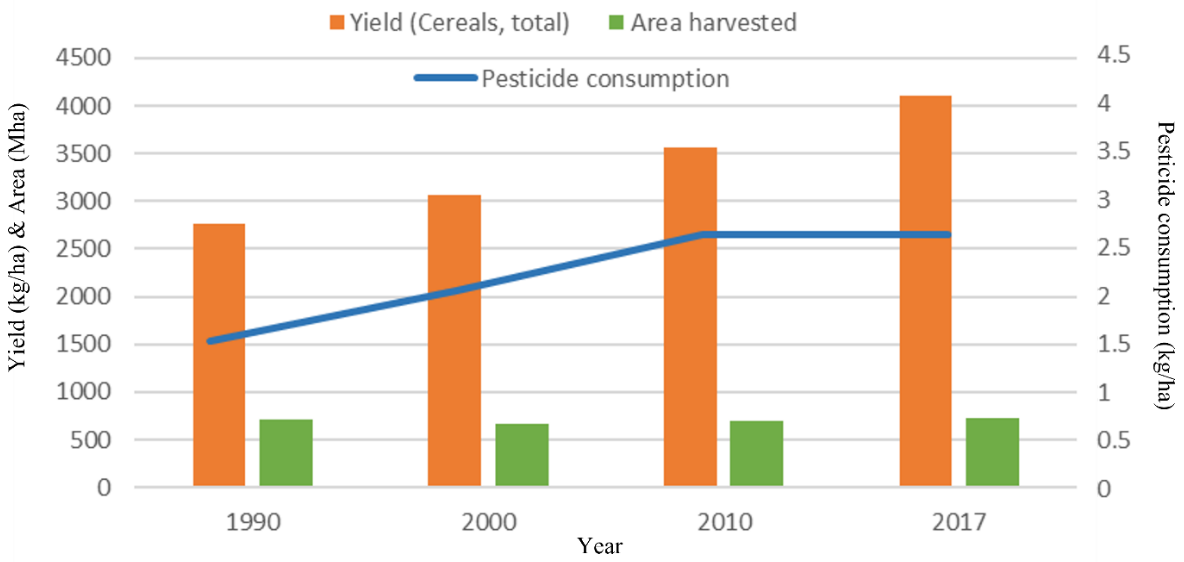


Figure 2.1.3.1. Global trends of pesticide consumption in cropland, total cereal yield, and harvested area over the three last decades (FAOSTAT, 2020)

Highly mobile pesticides can frequently be detected in aquatic bodies (Fingler et al., 2017). Most conventional pesticides have been formulated to be poorly water-soluble and highly persistent. Methods of their removal from aqueous solutions are typically based on chemical coagulation, sedimentation, and filtration (Ahmaruzzaman et al., 2011). However, after decades of intensive usage and due to a high potential to accumulate in soils and biota, most of the hydrophobic pesticides have been replaced by the polar and hydrophilic pesticides that are more susceptible to leaching than hydrophobic ones, emphasizing the need for protection or remediation of surface and groundwater resources. Field-scale methods for the removal of pesticides should be easily accessible, economic, highly efficient, and fast-acting, as well as eco-friendly. Adsorption by a range of materials seems to be an efficient measure for controlling organic contaminants such as pesticides in soil, but it proved to be rather not so practical. The degradation-based approaches are also highly efficient, but more costly and difficult for in situ application, and can generate toxic co-products and waste as well (Trivedi et al., 2018). Therefore, a practical, efficient, and cost-effective solution is in line to be proposed.

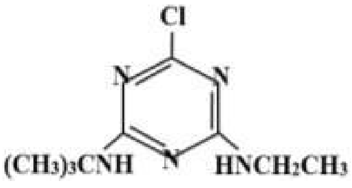
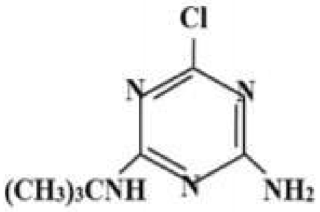
2.2. Triazine herbicide terbuthylazine

2.2.1. Physicochemical properties and environmental behavior

Terbuthylazine (TBA) is a broad-spectrum herbicide used in the pre- and post-emergent treatment of a variety of agricultural crops as well as roads, railways, and industrial areas. It belongs to a group of symmetric (*s*)-chlorotriazines, a chemical class of nitrogen-containing heterocycles. The *s*-chlorotriazine herbicides, mainly atrazine, are among the most widely used herbicides in the world for selective weed control (Tasca et al., 2018). However, the detection of atrazine and its dealkylated metabolites in aquatic ecosystems beyond the maximum allowable concentration limits along with its potential adverse impacts on different organisms and human health led to the ban of atrazine in 2007 in the European Union countries. Since then, TBA (introduced in 1967) has intensified its application in agriculture, mainly for maize protection, and it seems that TBA will be soon an emergent compound of similar concern as atrazine.

TBA is a hydrophobic compound that shows a good affinity to adsorption by soil organic matter (Table 2.2.1.1.). That is why it is moderately mobile through the soil horizon with an even high tendency for surface runoff (soil erosion). As a moderately persistent herbicide in soils, with average field half-lives of up to four months, TBA is approved for use in fields with crop rotation within a year. The hydrolysis of TBA in aqueous soil suspension at pH 7-9 is very slow (half-life >200 days), then its persistence relies mainly on biodegradation. DET is a relevant product of TBA biodegradation in soil. It is a more polar and more mobile compound, showing a higher leaching potential than the parent molecule. It shows no herbicidal effects but can stress the aquatic organisms (Tasca et al., 2018).

Table 2.2.1.1. Structure and selected physicochemical properties of herbicide TBA and its degradation product DET (PPDB: Pesticide Properties DataBase)

Property **(20-25 °C, pH 7)** **Structure**	**Terbuthylazine** **TBA** 	**Desethylterbuthylazine** **DET** 
Solubility in water (mg/L)	8.5	327
Vapour pressure (mPa)	0.12	0.35
$^{a}\log K_{\mathrm{ow}}$	3.4	2.3
$^{b}\mathrm{p}K_{\mathrm{a}}$	1.9	−
$^{c}K_{\mathrm{f}}$ (mL/g)	2.1−10.5	0.3−3.3
Aqueous hydrolysis pH 7-9	Stable	Stable

^{a}*n*-octanol-water partition coefficient reflecting the hydrophobicity; ^{b}dissociation constant representing the pH value of the system at which a molecule:ion ratio of the analyte corresponds to 50:50; Freundlich partition coefficient indicating the adsorption intensity; − no data

Persistence and behavior of TBA in a soil environment depend on several factors including chemical characteristics (such as hydrophobicity expressed as the *n*-octanol-water partition coefficient, K_{ow}, and ionization potential, expressed as the dissociation constant, pK_{a}), soil type (pH and humus/clay content), soil microbiome and soil history, meteorological conditions (precipitation, temperature), and crop management practice (herbicide use, field tillage, dose and frequency of application, equipment) (Udiković Kolić et al., 2007; Stipičević et al., 2015). TBA environmental fate can be only predicted according to data derived from adsorption and dissipation studies.

It was found that TBA residues persisted in the uppermost 60 cm of soils even after 17 months of application (Stipičević et al., 2015). Adsorption intensity, expressed mostly as the Freundlich partitioning coefficient K_{f} in certain soil-chemical systems, indicates the chemical (bio)availability for degradation, as well as the potential of chemical mobility through the soil horizon.

Dissipation comprises two main types of processes: transfer processes, such as volatilization, leaching, plant uptake, run-off, or erosion that transfer substances to different environmental compartments; and degradation processes, such as microbial degradation, hydrolysis, and photolysis that transforming substances into degradation products (Figure 2.2.1.1.).

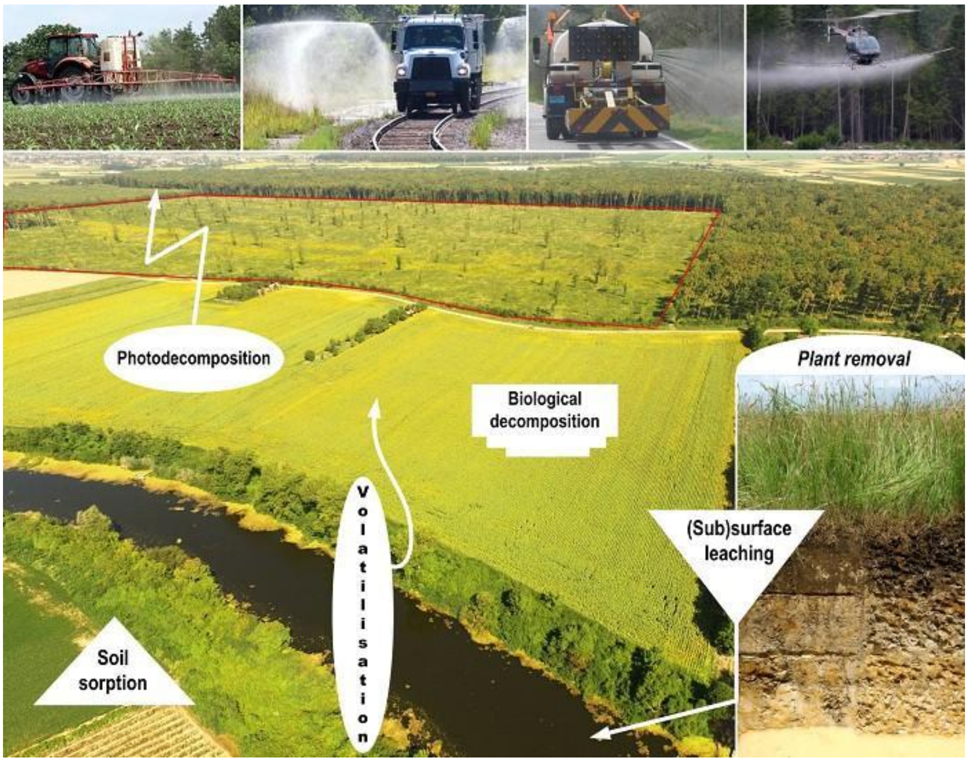


Figure 2.2.1.1. Herbicide pathways in agro-forest ecosystems after weed management application are depicted schematically. The red line encompasses a reforested area on the outskirts of the Spačva watershed, which serves as one of Croatia's most significant sources of certified deciduous forest biomass for power plant facilities (Ondrasek et al., 2021).

When calculating the dissipation rate and corresponding half-lives of chemicals in ground and surface waters, single first-order kinetics can be used when the fit with experimental data is acceptable (correlation coefficient very close to 1). The first-order kinetic model is a simple two-parameter exponential equation that is commonly applied to a unimolecular reaction whose dissipation rate depends on the concentration of only one reactant (Figure 2.2.1.2.).

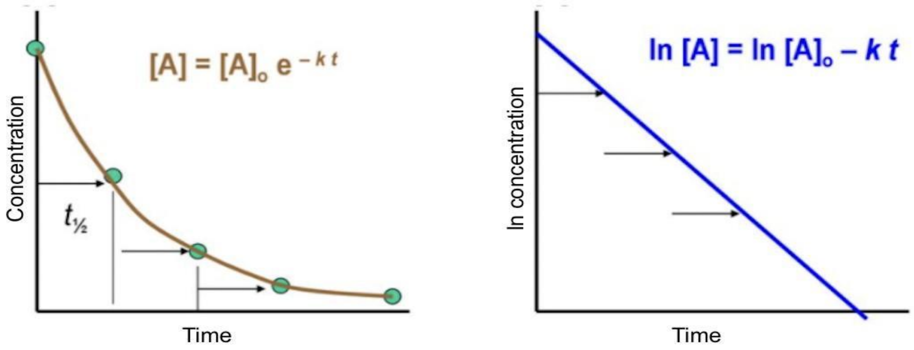


Figure 2.2.1.2. Concentration *vs.* time of the single first-order kinetics: integrated (exponential) equation form (left side) and logarithmic (linear) equation form (right side). $[A]$ and $[A_0]$ are TBA concentrations found in water at time t and at zero time, respectively, and k is a rate constant.

It assumes that the amount of pesticide molecules is small compared to the population of degrading agents (e.g., microbiome enzymes in the case of biodegradation or the number of water molecules in the case of hydrolysis). As a result, the rate of change in pesticide concentration is directly proportional to the actual concentration in the system at that given moment. Seasonal changes in temperature and moisture can affect the degradation rate and cause deviations from first-order kinetics (e.g., degradation rate may decrease in winter due to lower temperatures and in summer due to drier conditions) (FOCUS 2006).

In single first-order kinetics, the time for a specific percentage decline in concentration is constant throughout the experiment and independent of the pesticide's initial concentration. A half-life (DT_{50}) is the time taken for a substance to halve its quantity:

$$DT_{50} = \ln(2)/k \approx 0.693/k$$

Single first-order kinetics have until recently been the preferred model for estimating DT_{50} values for several reasons:

- many biotic and abiotic processes in environmental compartments, such as soil, effectively follow single first-order kinetics (exponential decay)
- the equation is simple and has only two parameters that are independent of concentration and time
- it is easy to fit the equation to experimental data
- DT_{50} values are easy to calculate
- first-order kinetics have frequently been used to describe leaching and degradation processes in pesticide fate models (FOCUS, 2006).

2.2.2. Occurrence and persistence in watercourses

The occurrence of TBA residues and its degradation products in water resources, as the result of its extensive agricultural and non-agricultural use over the last few decades, has been recognized as a serious problem worldwide (Fingler et al., 2017). The persistence (average half-life) and mobility (sorption and solubility) determine TBA leaching potential to groundwater.

Seasonal variations in TBA concentration have been observed in surface waters, because of patterns in pesticide application and rainfall. In Greece, the highest TBA concentrations in the Volvi, Doirani, and Kerkini lakes were detected in spring (Kalogridi et al., 2014), but it also can be in winter (Koal et al., 2003). Monitoring of 2382 water samples, collected in the period August 2010 to December 2012 from 102 sampling sites located on rivers, streams, and canals of the Pinios River Basin (Greece), showed that TBA was among the most detected pesticides, posing a relevant risk for the environment, with 436 positive findings, out of 95 exceeded the ‘predicted no effect concentration’ (Tsaboula et al., 2016). In Switzerland, the highest levels of TBA were found in April during the 4-month monitoring of the Sion-Riddes canal, which corresponded to the period of TBA application on crops (Schopfer et al., 2014). Monitoring of surface waters conducted in Croatia showed maximum TBA and DET levels in a period from April to August, shortly after the TBA application. In groundwater, TBA was detected in 26% of samples and up to 25 ng/L (Fingler et al., 2017).

In 2008, Lombardy was the Italian region with the highest number of positive findings of TBA and DET in surface waters, detected from 89% of monitoring points; 59% (TBA) and 36% (DET) of the measured values were above the limit (0.1 μg/L) (Bottoni et al., 2013). Pollutant pathways of the Swist river basin (Germany) were investigated from 2012 to 2015. TBA and chloridazon were the two chemicals most often found in landscape runoff from cropland. TBA and DET were previously detected in effluent streams located immediately prior to the outlets of four wastewater plants, as well as in watercourse discharges; 20% and 25% of measurements were positive (above 0.2 μg/L) between July 2010 and August 2011 (Christoffels et al., 2016). From April 2009 to November 2010, 83 surface water samples collected in the south-eastern province of Jaen, a region with four natural parks, reservoirs, wetlands, and a high olive oil production, showed TBA and DET to be two of the most detected chemicals (Robles-Molina et al., 2014). Nowadays, TBA is a ubiquitous compound in the natural waters of Spain, as a consequence of its increased use in recent years (Herrero-Hernandez et al., 2017), while TBA and DET concentrations over 0.1 μg/L have recently been reported for numerous river basins (Ccanccapa et al., 2016).

Triazines and their metabolites are the most ubiquitous and abundant polar pesticides found in European groundwaters (Loos et al., 2010), with TBA and DET often exceeding the threshold of 0.1 µg/L (Le Cunff et al., 2015). Due to slow biodegradation rate, low temperatures, and anaerobic conditions, groundwater contamination can be more persistent than surface water pollution (Kim et al., 2017).

The Vistrenque shallow alluvial aquifer (France) was tested between 2011 and 2013, where TBA was found in 59% of the 66 samples collected, up to 40 ng/L (Sassine et al., 2017). DET was also the most frequently detected compound in Maribor groundwaters (Slovenia) collected in 2010, with 77% of the 56 samples analyzed showing the presence of the compound, with concentrations of DET higher than those of TBA. The highest levels of TBA (up to 25.7 ng/L) were observed during the spring and the DET/TBA ratio ranged from 1.4 ng/L to 2.9 ng/L (Korosa et al., 2016) suggesting diffuse contamination with biodegradation as a dominant dissipation process. In contrast, the point-source pollution has a high potential for faster TBA leaching to groundwater, without a significant herbicide interaction with soil constituents, resulting in DET/TBA ratio lower than unity (Bozzo et al., 2013).

In 2008, TBA and DET levels were monitored in samples of the most affected aquifers of the Piedmont region of Italy (ISPRA 2011). TBA and DET were detected in 36% and 8% of the samples, respectively. Furthermore, 28% of the positive samples contained TBA at levels above 0.1µg/L, while DET levels exceeded the same limit in 14% of samples (Bottoni et al., 2013). Monitoring of groundwaters in the Lombardy region was carried out from 2005 to 2009. Concentrations of TBA and DET exceeded the limit for drinking water in 9 and 11 sampling points of the Adda-Oglio basin, respectively. The analysis did not indicate seasonal variation in contamination levels, while the DET/TBA ratio was higher than 1 suggesting significant interaction with the soil particles (Guzzella et al., 2006). High levels (> 5 µg/L) of TBA have recently been found in groundwater samples of LaRioja where TBA and DET were the most detected compounds in 12 surface waters monitored between September 2010 and September 2011. In September 2010, concentrations of both chemicals were above 0.1 µg/L in 10 samples which followed the time of herbicide application (Herrero-Hernandez et al., 2017).

The threshold limit for pesticides in drinking water was also exceeded for TBA during a 4-years monitoring program conducted by the Catalan Water Agency on 29 aquifers of Catalonia (Kock-Schulmeyer et al., 2014). TBA concentrations were several times higher than the critical limits for water quality in the Guadalquivir River Valley during the winter season, coinciding with the principal application in olive orchards (Hermosin et al., 2013) and high rainfall, typical for the Mediterranean climate (Calderon et al., 2015). Herbicide loss through runoff and soil erosion were enhanced by low olive tree density and on slopes ranging from 10% to 30%

(Calero et al., 2009). Groundwater analyses were performed on samples from the Jucar River Basin during spring and fall monitoring from 2010 to 2013 (Menchen et al., 2017). TBA was the most frequently detected pesticide in 314 analyzed samples, which levels occasionally exceeded0.1 µg/L. The herbicide concentration in other Spanish river basins was recently also found to be above 1.2 µg/L (Jurado et al., 2012). A two-year monitoring program (2009–2011) conducted in Gran Canaria detected TBA in 90% of reclaimed waters and 50% of volcanic aquifers, at levels up to 0.62 µg/L (Estevez et al., 2016).

2.2.3. Removal technologies and mitigation techniques

Currently, the removal of herbicides and their derivatives from the environment is one of the worldwide environmental alarms. Due to the wide use of different types of herbicides, it is very difficult to develop a single universal method for their removal. Basically, three groups of methods are used for remediation: biological, chemical, and physical (Figure 2.2.3.1.). Biological remediation results in the disintegration of organic compounds into small molecules such as CO_2 and H_2O. These methods are low-cost and eco-friendly, compared to the physical and chemical remediation methods. In chemical remediation, herbicides are converted into compounds due to reaction with selected agents/catalysts. Chemical treatment is usually endorsed with physical remediation processes. However, the costs of these compatible treatments are very high and vary depending on the matrix. Physical remediation is based mostly on the process of adsorption, which is one of the most used methods for water purification because of its capacity, efficiency, and applicability on a large scale (Andrunik et al, 2021).

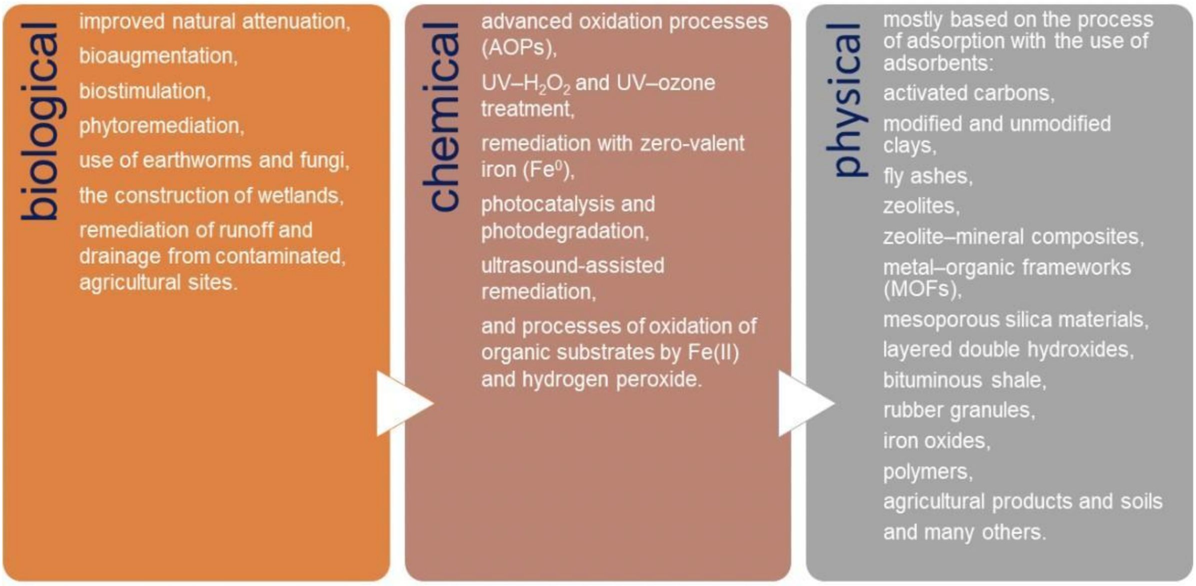


Figure 2.2.3.1. Methods used for water remediation (https://doi.org/10.3390/ma14133532)

The addition of biochar to agricultural soils is a valid in-situ technique capable to reduce the dissipation of organic pollutants. However, mineral particles could cover the reactive surface of the carbon-rich material, suppressing its sorption capacity (Kookana, 2010).

Hydrophobic interactions and π-π dispersive forces have been suggested as major mechanisms of TBA adsorption on activated carbon (AC). The removal efficiency of 60% was achieved by using 10 mg/L of powdered AC on natural water containing TBA at the initial concentration of 1.024 ug/L. Testing the granular form of sorbents provides great insight into material performance within a filter configuration; performances of AC granular samples were recently verified in batch and column tests. Granular AC exhibited a higher uptake in batch for TBA than multiwalled carbon nanotubes (MWCNT), probably due to the higher surface area of the AC used. However, adsorption was faster on MWCNT than on AC, and this was attributed to the easier diffusion of TBA molecules into the more developed mesoporous structure of MWCNT materials. Experimental data of AC was described by a Freundlich adsorption isotherm model. Performance of AC samples was also studied in rapid small-scale column tests, performed with an aqueous solution of TBA at 5 mg/L flowing through a glass column with an empty bed contact time of 1.3 min. Around 40% breakthrough was achieved at the beginning of the run, while 50% breakthrough was achieved at ~10,000 bed volumes, and saturation was reached at ~30,000 bed volumes (Tasca et al., 2018).

Chlorination is a commonly used purification method for drinking water but while its high effectiveness has been proven on widely used herbicides such as glyphosate, oxidation carried out with NaClO on natural water removed only 30% of TBA (initial concentration:1.024 ug/L) (Jonsson et al., 2013). No improvements have been observed with the additional use of $Al_2(SO_4)_3$ as a coagulant, while the combined treatment with chlorine and activated carbon reached 70% of removal efficiency (Ormad et al., 2008).

TBA does not react appreciably with H_2O_2. Hydrogen peroxide alone (15 mg/L) was found to be ineffective in the oxidation of 10 μg/L of the herbicide in groundwater samples. In photolytic (UV) and UV/H_2O_2 treatments, the abatement of TBA is mainly due to direct photolysis or oxidation by hydroxyl radicals, generated from the photolysis of H_2O_2. Direct photolysis of 5 mg/L of TBA in a 250 mL reactor did not produce significant degradation, while the removal rate increased significantly in the UV_{254}/H_2O_2 combined process. An average fluency rate of 2.9 mW was applied to 400 mL of ultrapure water containing 5 mg/L of the herbicide. The first-order kinetics for TBA removal from the water was proposed and the required doses for 90% TBA removal were 1404 to 535 mJ, with initial H_2O_2 concentrations of 5 and 50 mg/L, respectively. The UV dose required to reach the same efficiency without H_2O_2 was 2002 mJ. These doses are significantly higher than those commonly applied in drinking water disinfection.

The enhanced efficiency due to increasing UV doses, as well as the main contribution of direct photolysis to the removal of TBA via UV/H_2O_2 process, was recently confirmed by the oxidation of groundwater samples containing 10 μg/L of TBA. Up to 52% removal yield was obtained with a UV_{254} dose of 1,200 mJ. When H_2O_2 was added, the UV fluence increased from 1,200 to 2,000 mJ, enhancing herbicide degradation by 10–30%, while an increase of H_2O_2 concentration from 5 to 10 mg/L at fixed UV doses did not affect the efficiency significantly (Tasca et al., 2018).

Photocatalytic processes have been widely focused on the removal of many pesticides and endocrine-disrupting chemicals not readily degraded by common treatments (Le Cunff et al., 2015). These advanced oxidation processes (AOPs) are based on oxidation-reduction reactions involving excited electrons (e^-) and the generated holes (h^+) at the valence bands. Electrons are promoted from the valence to the conduction band by the excitation of a semiconductor through the absorption of photons, having energy greater than their bandgap (Fujishima et al., 2000).

The microbial potential to degrade TBA can be evaluated by comparing the half-life (DT_{50}) in microbiologically active microcosms with those that have previously been sterilized. The DT_{50} value of 1.5 mg TBA/kg soil decreased from 82 to 22 days when sterile and active soils were used, respectively. DT_{50} of TBA (0.1 mg/L) in sterile 25 groundwater was 224 days, with decreasing trend to 151 days when using non-sterile groundwater. The degradation patterns were confirmed to fit first-order kinetics (Caracciolo et al., 2013).

Biopurification systems can be used to reduce the contamination of soil and water sources. These systems are based on mixtures, with a composition depending on the local availability of agro-industrial wastes. The effect of increasing microbial activity, due to agricultural compost amendment, on TBA removal from soil was recently confirmed (Kravvariti et al., 2010). The potential of biomass to degrade xenobiotics was also tested in a laboratory bioreactor on an aqueous solution of TBA, acetochlor, chlorpyrifos, and metalaxyl. The filtration column (biofilter) was a mixture of 35% soil and 65% compost. After 35-40 days, the percentage of TBA remaining was the highest among the pesticides analyzed, greater than 26%. No significant effect of the environmental temperature was observed, but practical issues 26 encountered when replacing the biomass need to be solved to promote the applicability of such systems (Suciu et al., 2013).

Wetlands can collect and retain many chemicals, improving the sustainability of agricultural production. Sedimentation, photolysis, hydrolysis, and other degradation processes are strictly connected with the hydrochemistry and changes of runoff regime. TBA concentrations were monitored to evaluate the efficiency of a 0.32 ha constructed surface flow wetland (CSFW), located at the outlet of a 6-ha agricultural basin. The wetland was flooded with 33 m^3 of water containing TBA (2.3 mg/L) and S-metolachlor (3.8 mg/L) and with pure water after 21 days, and again after 65 days. After the first initial flood, the reduction in TBA was more than 97%, perhaps due to reversible adsorption on plants, plant residues, and soils. Indeed, the second and third floods partially mobilized the herbicide. However, no herbicides bypassed the wetland and entered 27 of the surface waters outside the basin. Complete TBA runoff mitigation was also demonstrated by samples collected from the inlet, the sediment deposition zone, and the outlet of a 319 m^2 stormwater wetland, collecting the runoff of a vineyard catchment. The wetland consisted of a sediment deposition pond, collecting the suspended solids, and a gravel filter (saturated hydraulic conductivity: 10−3 m/s), which increased the hydraulic retention time. Results indicated 100% of TBA removal during the months of pesticide application, with no significant transfer of the herbicide from the water column to the bed sediments. Biofilms, sediment, root complexes, and electron donors, provided by roots and organic matter, likely contributed to the degradation of the herbicide via reductive dechlorination (Tasca et al., 2017).

Phytoremediation can play an important role in pollutant abatement operated through constructed wetlands or buffer strips. This technique takes advantage of the ability of some plants to remove and/or metabolize pollutants. A plant-based biotest (RHIZOtest) was performed using *Lolium multiflorum* and TBA at aqueous concentrations of 0.5, 1.0, and 2.0 mg/L (Chaignon et al., 2003; Bravin et al., 2010). The plant was able to remove 38%, 42%, and 33% of the initial concentrations, respectively, in 240 h. Over that period the the capacity of plants for adsorption of herbicide at 0.5 mg/L increased from 1.58 to 3.50 μg/g of fresh weight. Glutathione S-transferase activities increased in response to all treatments (Mimmo et al., 2015). This enzyme is involved in the response to the overproduction of reactive oxygen species (Del Buono et al., 2011).

2.2.4. Methods of determination in water

Several methods for herbicide extraction from aqueous samples have been developed, but liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are among them mostly used (Bruzzoniti et al., 2006). The LLE is an “old-school” extraction technique that has several significant disadvantages: a large solvent consumption, vigorous shaking/mixing, waiting for layers to separate, phase emulsions, and poor efficiency for polar chemical accumulation. SPE is a more popular extraction method over the LLE due to the ability to use all solvents and to work with large volumes of samples, good reproducibility, the lower risk of extract contamination, and a range of sorbents of various polarity and functionalities such as C18, graphitized activated carbon, and copolymer sorbents. C18 is one of the most used sorbents for the removal of non-polar to moderately polar pesticides from water. Commercially available C18 sorbent columns may differ from one another, resulting in a different accumulation efficiency from water.

General SPE procedure includes four main steps (Figure 2.2.4.1.):

- Conditioning: Solvent is passed through the sorbent to wet the bonded functional groups for consistent interaction and to treat the sorbent with a solution that is similar (in polarity, pH, etc.) to the sample matrix aiming to maximize retention
- Sample addition: Introduction of the sample (analyte of interest are bound/extracted onto the sorbent)
- Washing: Selectively remove unwanted interferences co-extracted with the analyte without prematurely eluting analytes of interest
- Elution: Removing analytes of interest with a solvent that overcomes the primary and secondary retention interactions.

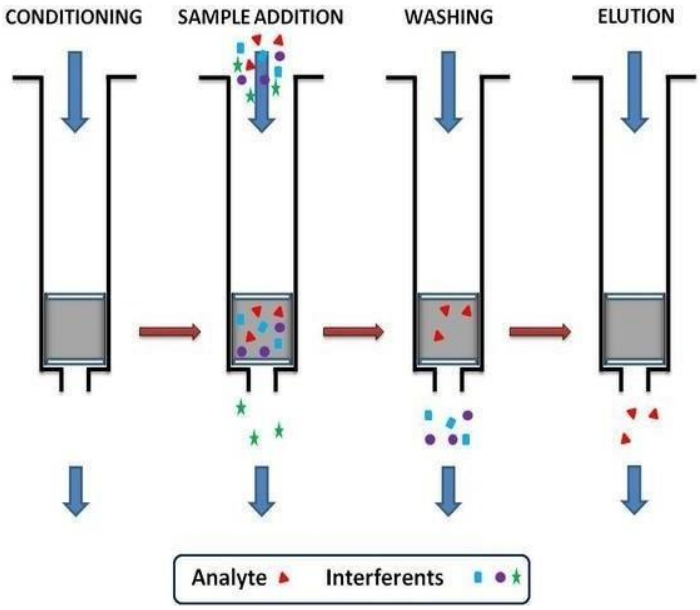


Figure 2.2.4.1. Basic solid-phase extraction (SPE) steps (https://www.waters.com)

SPE proved to be a reliable and cost-effective technique for selective isolation and concentration of a wide range of analytes and matrices (Figure 2.2.4.2.). Some of the common applications for SPE include environmental trace enrichment of organic pollutants, extraction of pesticides and growth promoters from foods, purification of peptides, drug analysis in pharmaceutical dosage forms, and clinical applications for drugs in physiological matrices (Wells, 2013).

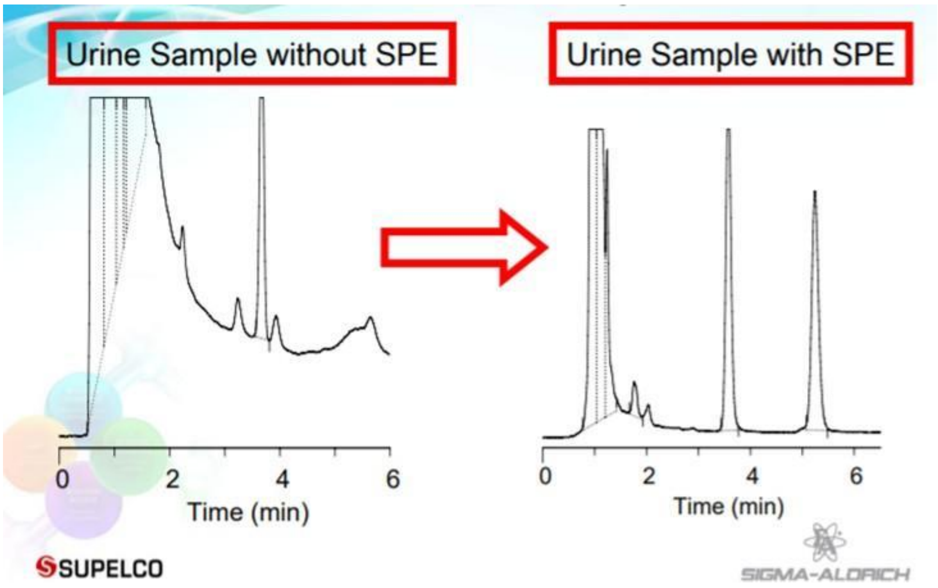


Figure 2.2.4.2. The improved selectivity of chromatographic analysis by using SPE

https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/marketing/global/documents/407/572/t4070 4 8h.pdf

The SPE sorbent can be placed into plastic or glass tube (cartridge) format or can be embedded between two poly (tetrafluoroethylene) or fiberglass membranes to form a disk. Fiberglass disks are thicker and firmer than membranes, allowing faster sample flow without the loss of accumulation efficiency. Disks are primarily used to short the extraction time of large sample volumes. A sample preparation system using SPE can be performed separately from the final chromatographic system or can be automated as an integral part of the analytical system. Because there are no losses due to sample evaporation, the SPE automated version provides more accurate results. Furthermore, the whole volume of sample extract, rather than just a portion of it, is injected into the chromatographic column. However, due to the device's high cost and complexity, this technique is not widely used (Sabik et al., 2000).

Bruzzoniti et al. (2006) tested the efficacy of accumulation of triazines and their degradation products from 1000 mL of water on several different sorbents in columns: octadecyl silicon dioxide (C18), styrene-divinylbenzene (SDB), HF Bond Elut, Discovery DSC-Ph, Supelclean Envi-Carb, and mix-mode sorbent C18 + SDB. The mass concentration of each triazine compound in the water sample was 0.1 µg/L. The authors also compared the efficacy of methanol and acetone as conditioning and eluting solvents. TBA and DET were accumulated most effectively on C18 using acetone for sorbent conditioning and acetone followed by methanol for final elution. The extraction recovery was 103% for TBA and 111% for DET.

Recently has been developed the Solid-Phase Microextraction (SPME), a highly effective method for extraction from water without the use of solvents. Adsorption of the analyte from the aqueous medium by immersing the SPME fiber in the sample, followed by thermal desorption of the analyte from the polymer layer into the carrier gas stream in the gas chromatograph injector. Small sample volumes (2 to 5 mL) are required for analysis using this quick and simple technique. The compounds are extracted and accumulated on the fiber directly from the sample. The sample preparation time was significantly reduced, and the detection limits were improved. It is mostly used in conjunction with gas chromatography with selective detectors, such as a mass spectrometer, but liquid chromatography analysis is also possible.

Chromatography is a powerful technique for the efficient separation of components of a complex mixture. The overall chromatographic principle is to dissolve the mixture in the mobile phase (gas or solvent) and carry it through a stationary phase (sorbent) (Figure 2.2.4.3.)

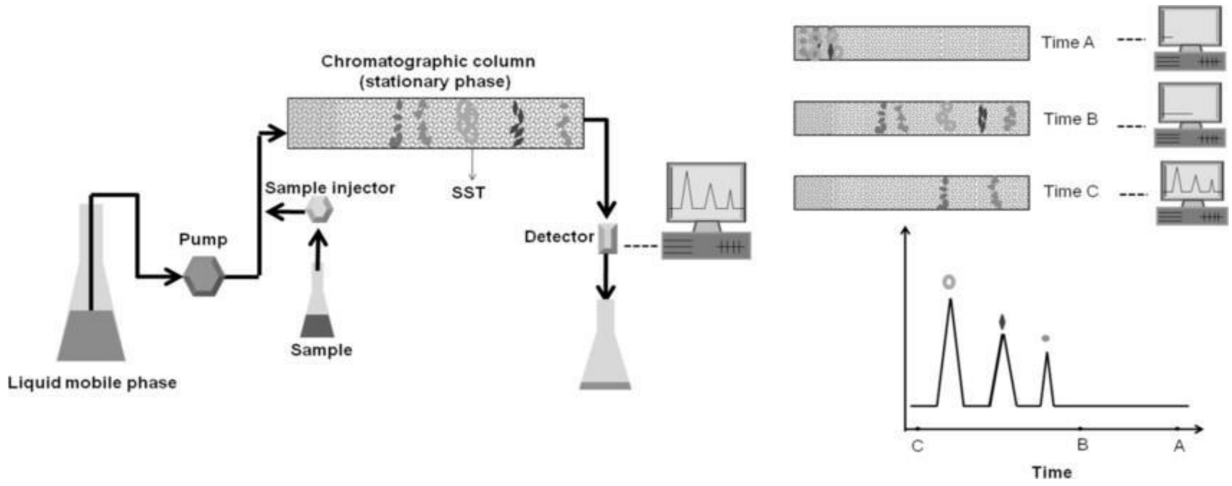


Figure 2.2.4.3. High-performance liquid chromatography workflow

(https://www.researchgate.net/figure/Fig-2-Schematic-illustration-of-high-performance-liquid-chromatog r aphy-HPLC-for_fig2_276363502)

Gas chromatography (GC) using capillary columns is suitable for the determination of volatile and non-polar compounds dissolved in organic extracts, whereas a preferred method for the determination of polar and thermolabile compounds in aqueous extracts is high-performance liquid chromatography (HPLC) (Kuster et al., 2009). Both types of chromatography can be coupled with a mass spectrometer (MS) as a universal detector, as well as the other specific detectors such as electron capture detector (ECD) and nitrogen sensitive detector (NPD) for GC and diode array detector (UV-DAD) for HPLC (Stipičević et al, 2003).

Liquid chromatography with tandem mass spectrometry (LC-MS/MS) is a method for detecting pesticides in natural and treated waters with good sensitivity and selectivity. The stationary phase is often octadecyl silicon dioxide or octyl silicon dioxide (C18 or C8), whereas the mobile phase is typically methanol or acetonitrile (Kuster et al., 2009). A more efficient Ultra Performance Liquid Chromatography (UPLC) is based on the use of columns filled with porous particles smaller than 1.7 µm and applied pressures higher than in HPLC for a better resolution, higher sensitivity, and shorter analysis time. Aguera and colleagues applied the UPLC-MS/MS technique to determine pesticides in groundwater following the accumulation on Oasis HLB sorbent and elution with methanol (Tariba, 2009). The accumulation efficiency of TBA was 110%.

UPLC combined with tandem mass spectrometry is an excellent technique for the determination of compounds such as pesticides in the aquatic environment. European regulation on drinking water requires that the limit of determination of analyzed compounds should be 0.025 ug/L, which is 4-fold lower than maximum permitted concentrations of pesticides in these waters. Marín et al. (2009) determined different classes of pesticides in groundwater and surface waters. A volume of 100 ml of the water sample, in which the concentration of test compounds was 0.025 µg/L, was passed through an Oasis HLB column. The compounds were eluted with acetone and the extracts were analyzed by UPLC-MS/MS. The efficiency of TBA accumulation was 95% from the surface water sample and 93% from the groundwater sample.

3. MATERIALS AND METHODS

3.1. Chemicals and equipment

Standards, solvents, and reagents:

- terbuthylazine (TBA), IUPAC name:
  2-N-tert-butyl-6-chloro-4-N-ethyl-1,3,5-triazine-2,4-diamine (99.1%), Pestanal, Riedel-de Haën, Seelze, Germany
- desethylterbuthylazine (DET), IUPAC name:
  2-N-tert-butyl-6-chloro-1,3,5-triazine-2,4-diamine (99.8%), Pestanal, Riedel-de Haën, Seelze, Germany
- acetonitrile, LiChrosolv, Merck, Darmstadt, Germany
- methanol, SupraSolv, Merck, Darmstadt, Germany
- ethyl acetate, for Organic Residue Analysis, J. T. Baker, Deventer, Netherlands
- Milli-Q water, Millipore, Bedford, MA, USA
- Sep-Pak C18 cartridge (360 mg), Waters, Milford, MA, USA

Instruments and accessories:

- High-Performance Liquid Chromatography (HPLC) equipped with a solvent delivery unit ProStar 230 SDM, an injector unit ProStar 410, and a UV diode array detector unit Prostar 330 (UV-DAD), all Varian, Walnut Creek, CA, USA, and a Gemini C18 analytical column (250 mm x 4.6 mm) with a Gemini C18 pre-column (4 mm x 3 mm), both 5 µm particle size, Phenomenex, Torrance, CA, USA
- Inductively Coupled Plasma - Optical Emission Spectrometer – ICP-OES, Vista MPX, Varian
- Microwave digestion, MARSXpress, CEM, Charlotte, NC, USA
- X-ray Diffraction (XRD), Shimadzu XRD6000, Kyoto, Japan
- Scanning Electron Microscopy (SEM), Jeol JSM-7800F, Tokyo, Japan
- Energy dispersive X-ray (EDX) spectrometer, X-Max 80, Oxford Instruments, Abingdon, United Kingdom
- X-ray Photoelectron Spectroscopy (XPS), Phoibos MCD 100, SPECS GmbH, Berlin, Germany
- CHNS Analyser, Thermo Fisher Scientific, Waltham, MA, USA

- digital dual channel pH/EC, Mettler-Toledo MPC 227, Columbus, OH, USA
- mechanical incubator shaker, KS 4000i control, and vortex MS3 control, both IKA, Staufen, Germany
- oven Memmert IPP 400, Buechenbach, Germany
- cellulose syringe filter, pore size 0.45 µm, Millex, Belgium
- PTFE Minispike syringe filter, pore size 0.2 µm, Waters, Milford, MA, USA
- blue ribbon filter paper, Munktel, Sweden
- pipette Eppendorf ep T.I.P.S., Hamburg, Germany
- analytical scale, Mettler-Toledo AB104, Schwerzenbach, Swiss.

3.2. Standard solutions

Stock solutions were separately prepared by dissolving 1 mg/mL of each pure compound in acetonitrile. Suitable dilutions of the stock solution with Milli-Q water were carried out to obtain: i) four calibration solutions containing TBA and DET used for their HPLC-UV/DAD quantitative analysis (Table 3.2.1.) and ii) the spike solution containing 10.62 µg/mL of TBA used for contamination of watercourse samples to reach a final concentration of 25.5 ng/mL. Concentrations of TBA and DET in watercourse samples after the kinetic experiment were calculated using the calibration curves. All validation steps of the analytical method were performed following the international guidelines (SANTE 2021). A recovery test was performed in triplicate for watercourse samples spiked with TBA and DET at three different levels: 50, 100, and 150 ng/L. During the study, all standard solutions were kept cold at +4 °C.

Table 3.2.1. Standard solutions of TBA and DET in Milli-Q water used for external calibration of HPLC-UV/DAD method

Calibration solution	**Mass concentration (ng/mL)**	
	TBA	**DET**
Level 1	42	47
Level 2	106	118
Level 3	212	237
Level 4	531	592

3.3. Chromatographic conditions

The mobile phase was acetonitrile (A) and Milli-Q water (B) at a flow rate of 1 mL/min. A high chromatographic performance (the adequate peak shapes, suitable retention times, and high selectivity) were carried out by gradient elution according to the program shown in Table 3.3.1. The sample injection volume was 100 µL. Detection and data monitoring were conducted at wavelengths of maximum UV absorption of analytes that were 215 nm for DET and 222 nm for TBA. Data analysis was carried out using Varian Star software (Varian, Walnut Creek, CA, USA).

Table 3.3.1. Chromatographic performance (HPLC-UV/DAD)

Time (min)	**Mobile phase composition (%)**		**Elution**
	A	**B**	
0−8	40	60	isocratic
8−20	40 → 60	60 → 40	gradient
20−22	60 → 40	40 → 60	gradient
22−25	40	60	isocratic

3.4. Watercourse sample

In late August 2019, five liters from the natural Bliznec watercourse were collected using the accredited method HRN ISO 5667-6 downstream from Medvednica Mt. in Maksimir Park, Zagreb, Croatia (45°82′N, 16°02′E). The selected watercourse represents a relevant aquatic matrix that drains surface waters from complex agricultural, forest, urban, and native park/forest ecosystems (Figure 3.4.1). A watercourse sample was filtered using blue ribbon filter paper prior to the kinetic experiment.

Figure 3.4.1. Bliznec watercourse
([https://odgovorno.hr/pilot-projekt-procjena-ekoloskog-zdravlja-urbanih-potoka-grada-zagreba-dobiveni - su-prvi-rezultati/](https://odgovorno.hr/pilot-projekt-procjena-ekoloskog-zdravlja-urbanih-potoka-grada-zagreba-dobiveni-su-prvi-rezultati/))

3.5. Wood fly ash

Biomass FA was provided by the cogeneration (combined net electrical and heat power of ~26 MW) biomass facility Viridas Biomass, Babina Greda, Croatia (45°11′09″N; 18°51′17″E), fuelled by the deciduous hardwood chips mixture (oak 85%, hornbeam 10% and ash 3% as the major species) with the dominant size fraction (~96%) of 3.2–32 mm and composition (*w/w*): 48% C, 45% O, 5.3% H, 0.2% N, and 0.05% S. The cogeneration plant facility uses a fluidized bed combustion process whereby an exogenous addition of silica (SiO_2) sand fraction is blown in with the aim to enhance burnout efficiency of the biomass. The final mineral combustion product is separated into two main fractions: FA and bottom ash.

Physicochemical properties of biomass FA were analyzed after microwave-assisted digestion (HNO_3:$HClO_4$:HF, 2:5:2, *v*/*v*/*v*) using ICP-OES. Mineralogy was examined by XRD analysis, acquiring scans above finely powdered specimens for two theta angles between 10 degrees and 90 degrees with a step size of 0.05 degrees. Losses by ignition (at 550 °C and 1100 °C) were established by a gravimetric approach. Total C and N contents were determined after dry combustion using a CHNS Analyser, and pH and EC of the ash-water suspension were determined as described in detail by Ondrasek et al. (2021). Advanced microscopic/spectroscopic measurements of the biomass FA were performed at the University of

Rijeka, Department of Physics, Croatia. A scanning electron microscope SEM coupled with an energy dispersive X-ray EDX spectrometer was employed to determine the surface morphology and elemental composition of representative biomass FA specimens with the beam acceleration voltage of 5.0 kV and the working distance of 10 mm. Prior to SEM analyses, the examined samples were attached to a Si substrate by a conductive carbon tape and coated in a Gatan PECS system with a 4-nm layer of platinum in order to prevent the surface charging during the electron beam irradiation (Ondrasek et al., 2020a). The XPS spectral footprint, before and after the 48-h experiment, was used to determine the surface elemental composition and binding energy to explain some of the possible sorption mechanisms that occurred at the outermost surface of the tested bioash matrix.

Figure 3.5.1. Cogeneration biomass plant facility Viridas Biomass, Babina Greda, Croatia (https://bioenergyinternational.com/heat-power/27145; https://seenews.com/news/croatias-uni-viridas-extends-biomass-power-plant-om-deal-with-valmet-644172)

3.6. Kinetic study design

Three watercourse samples of 500 mL were prepared in glass Schott bottles and fortified with TBA spike solution (10.62 µg/mL) to achieve an initial contamination level of 25.5 ng/mL. Two samples were treated with 5 g of FA (1%, *w/v*), while the third sample was FA-free and used as a control sample (Figure 3.6.1.). All treatments were put on a mechanical shaker (300 rpm) to prevent ash sedimentation and run at 25°C with no additional aeration. A series of 12 ml-aliquots of spiked samples were taken from each treatment in duplicate on times: 0, 1, 2, 3, 6, 12, 24, 32, 48, 56, and 72 hours following the addition of FA. The first set of aliquots was used for pH and EC measurement, after that the aliquots were returned into the bottle to continue shaking. The second set of aliquots was filtered through a 0.45 µm cellulose syringe filter and stored at −18°C until the extraction procedure. At the end of the experiment, the ash in the FA-treated sample was precipitated and the supernatant was removed. The wet FA matrix was dried in an oven at 60 °C to constant weight and subjected to XPS analysis.

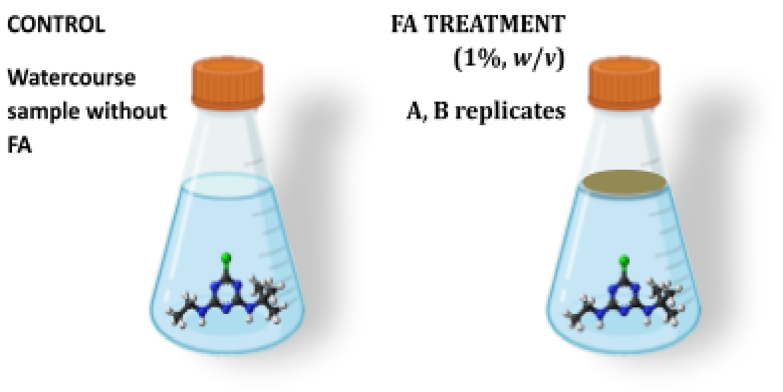


Figure 3.6.1. Experimental setup for kinetic study of the FA effect on TBA persistence in surface water

3.7. Extraction procedure

The extraction of each water sample was carried out using the SPE technique. SPE SepPak C18 columns were used for water sample clean-up and enrichment. Each sample of 10 mL was applied to the SPE column, which was previously activated with 2 mL of methanol and 5 mL of Milli-Q water using positive air pressure and the flow rate of 2-5 mL/min. The analyte was eluted from the column with 5 mL of ethyl acetate. The eluent was evaporated to dryness under the gentle stream of nitrogen and the dry residue was reconstituted with a 1,0 mL of methanol: Milli-Q water, 50:50 (*v/v*) to obtain final extract for HPLC-UV/DAD analysis. Extracts were filtered through a PTFE Minispike disc of 0.2 µm pore size prior to final analysis using the external calibration method for analyte's quantification.

3.8. Data analysis

Data processing and graphic presentation were performed with a Microsoft Office Excel 2019 (Microsoft Inc.). Experimental data were interpreted according to the first-order kinetic model (Section 2.2.1.).

4. RESULTS AND DISCUSSION

4.1. Chromatographic performance and method validation

A quantitative assay method for triazine compounds in water samples, using solid-phase extraction with a SepPak C18 cartridge followed by HPLC-UV/DAD, was adopted from the work of Tariba (2009). The selected validation parameters were tested in actual laboratory conditions in terms of linearity (calibration curve regression coefficient, R^2), sensitivity (limit of detection, LOD), repeatability (relative standard deviation, RSD), and accuracy (extraction recovery test).

The linearity was tested by analysis of calibration standards in MQ-water. The calibration curves indicated excellent linearity with a very high regression coefficient (> 0.999) in the range of 42 − 531 ng/mL for TBA and 47 − 592 ng/mL for DET (Figure 4.1.1.).

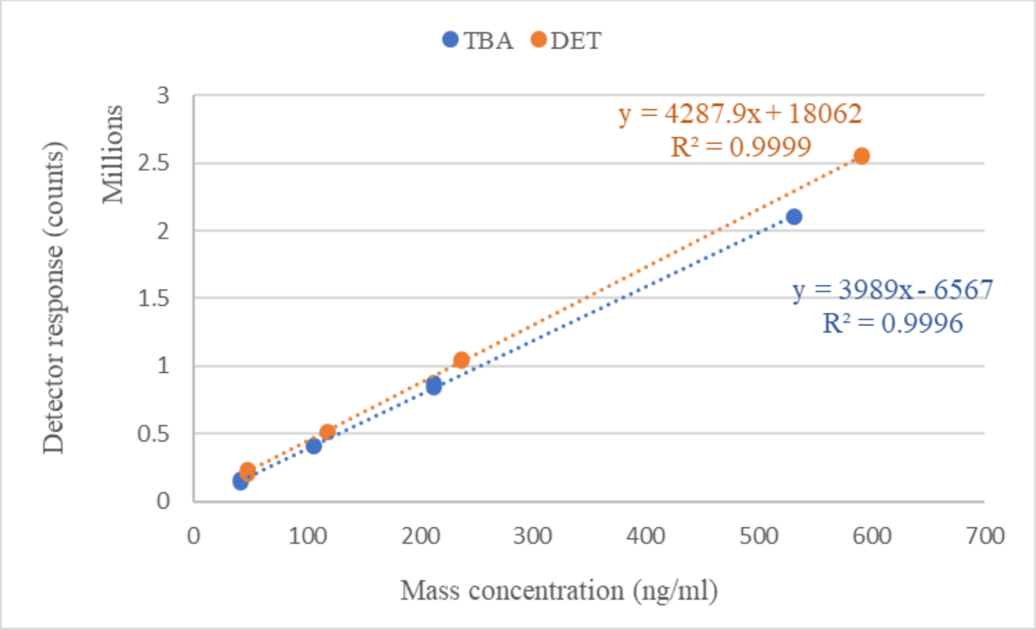


Figure 4.1.1. Calibration curves of TBA and DET in Milli-Q water

Average extraction recovery from fortified water samples was quantitative (higher than 90%) for both compounds with a very good precision (RSD ≤ 5%) (Table 4.1.1.). C18 sorbents have been demonstrated to be suitable for the extraction of hydrophobic chemicals (log $K_{ow} > 2$). The efficiency of several common solvents was tested, and it was shown that acetonitrile and ethyl acetate were the best eluents for triazine compounds (Tariba, 2009).

The method sensitivity expressed by the limit of detection (LOD) was 10 ng/L in water for both compounds. UV spectra showed wavelengths of absorption maximum (222 nm for TBA and 214 nm for DET) that were used for the highest detection sensitivity (Figure 4.1.2.).

Table 4.1.1. Selected method validation parameters for quantification of TBA and DET in water samples

	TBA	**DET**
Linearity / mass concentration range (ng/mL)	42 − 531 (R^2 = 0.9996)	47 − 592 (R^2 = 0.9999)
Extraction recovery, n = 9 (%)	95 ± 4	90 ± 4
Repeatability (%)	4	5
Limit of detection (ng/L)	10	10

R^2 linear regression coefficient of determination; n number of samples

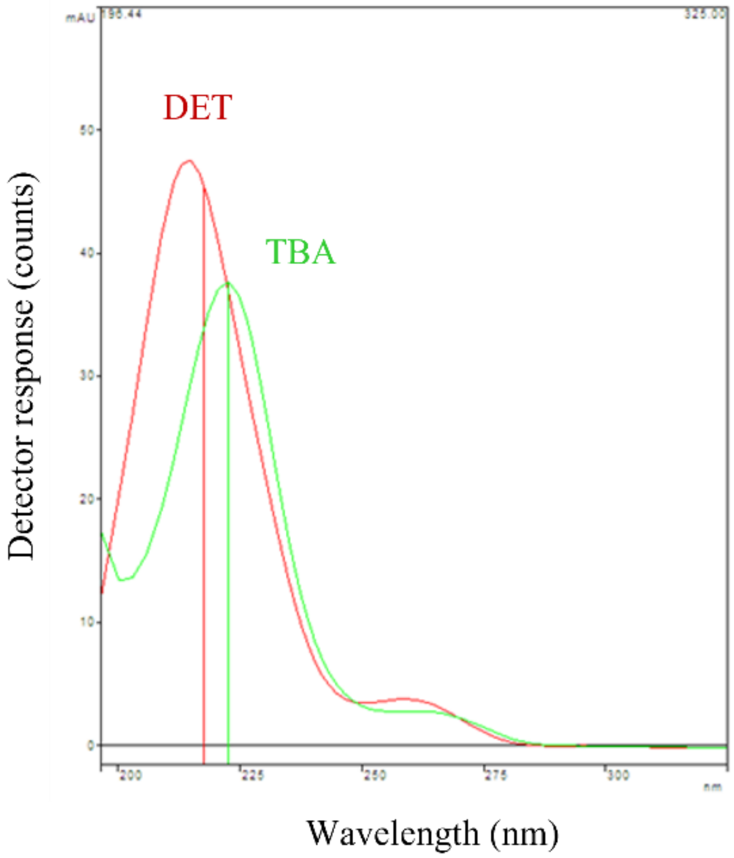


Figure 4.1.2. UV spectra of TBA and DET in Milli-Q water

TBA and DET were efficiently separated on the Gemini C18 column using gradient elution with acetonitrile and water (Section 3.3.). For many years, octadecyl silicon dioxide has been utilized as a universal sorbent for the removal of nonpolar to medium polar chemicals from water. The principal mechanism of a chemical accumulation from an aqueous medium on this nonpolar sorbent is the hydrophobic interaction between analyte and sorbent alkyl chains. Interference-free HPLC chromatograms of TBA and DET in calibration solution and watercourse samples showed high column selectivity (Figure 4.1.3.). Retention times were 18.45 and 8.82 min for TBA and DET, respectively. DET was not detected in any of the watercourse samples indicating that it is not a relevant TBA degradation product of alkaline hydrolysis or may be adsorbed by polar active sites on FA surface.

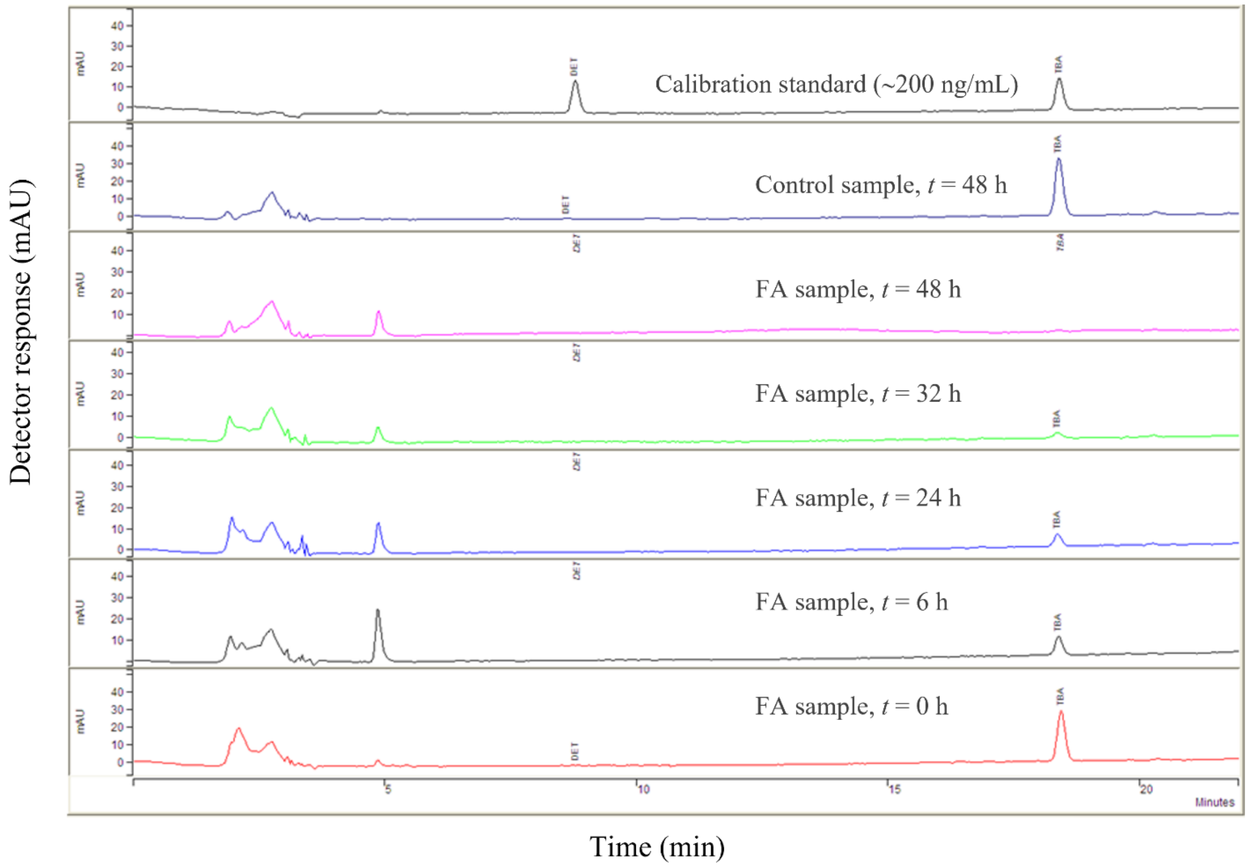


Figure 4.1.3. HPLC-UV/DAD chromatograms of watercourse extracts: control sample without FA, analyzed at the end of the study; and FA-treated samples, analyzed 0, 6, 24, 32, and 48 h after the addition of FA (1%, *w*/*v*), compared to the corresponding calibration standard chromatogram

4.2. Characterization of watercourse matrix

The addition of wood fly ash to a nearly neutral pH watercourse matrix (Table 4.2.1.) strongly increased pH reaction (by more than 5.0 pH units) (Figure 4.2.1.A). Such powerful pH alterations under fly ash exposure can be explained by a release and dissolution of alkaline constituents, mostly K/Na/Ca/Mg-containing minerals from the bioash matrix.

Table 4.2.1. Physicochemical properties of the watercourse Bliznec

Parameter	**Bliznec watercourse**[a]
pH	7.80
EC	0.49 mS/cm
Total N	0.920 mg/L
Total P	0.095 mg/L
Total Cu	1.283 µg/L
Total Zn	10.41 µg/L
Total Cd	0.01 µg/L
Total Cr	0.870 µg/L
Total Ni	7.758 µg/L
Total Pb	0.902 µg/L
Total Hg	0.01 µg/L
Total phenols	0.002 mg/L
Total PCBs[b]	0.01 µg/L

[a] National water quality monitoring program, Hrvatske vode, National water management agency

[b] Polychlorinated biphenyls (PCBs); EC Electrical conductivity

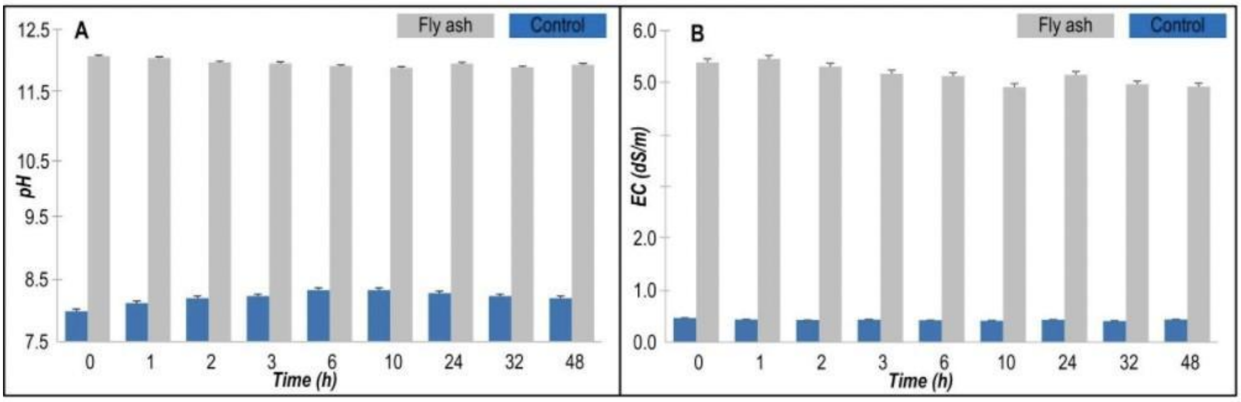


Figure 4.2.1. Dynamics of pH (A) and EC (B) in the watercourse matrix treated with FA (1% *w*/*v*) and control (without FA) during the 48-h period

During the 48-h period of study, in both watercourse matrices oscillation of pH reaction was in quite a narrow range: control (pH 7.99−8.33) and FA (pH 11.89−12.08) (Figure 4.2.1.A). Electrical conductivity (EC), as a measure of the total concentration of dissolved ions, also increased significantly after FA addition, salinizing the watercourse sample by more than 10-fold (4.91−5.45 dS/m) in comparison to the control treatment (Figure 4.2.1.B).

With respect to some negative environmental implications due to increased salinity and alkalinity, some of the possible solutions for such generated water matrix could be: i) re-using it as alkali disinfectant for municipal/industrial wastewaters or landfill leachates (e.g., Ivanković et al., 2014) or ii) their conditioning (pH neutralization) or mixing/dilution (desalination) prior to discharge and usage (Ondrasek et al., 2021). Furthermore, as demonstrated for some sorbents with a porous network structure, such as 3D graphene with adsorption efficiency 20-86 times greater than its mass, the benefits associated with the application of the tested fly bioash at lower rates are realistic and would likely induce modest pH/salinity changes; however, specifically designed experiments with a wider range of ash rates are needed to elucidate these potential benefits.

4.3. Characterization of tested fly ash

Bioashes exhibit diverse particle size morphometry and morphology from amorphous or rounded crystalline to highly complex porous agglomerations with narrow inter-particle, micro-to-nano-sized porosities (Figure 4.3.1.), resulting in a large active interface for physicochemical adsorption (Ondrasek et al., 2021b, 2021a). Applied FA was dominantly inorganic (>99%) bioash depleted in total N (<0.06%), C (<0.3%), S (<0.2%), and metals (Cd, Co, As, Hg, V, each <1.0 mg/kg), but enriched in alkaline (Ca, K, Mg, P) minerals and nutrients (Table 4.3.1.).

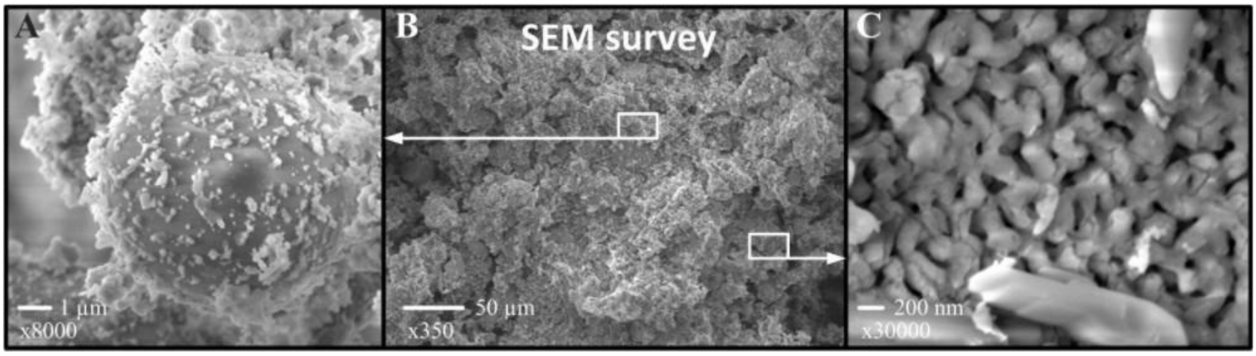


Figure 4.3.1. Surface characterization of tested FA matrix by SEM analysis

Table 4.3.1. Physicochemical properties of FA

Parameter	Fly ash
pH	12.6(±0.3)
EC	27(±0.2) mS/cm
Solubility in H_2O	215(±5) g/L
Solubility in 0.1 M HCl	675(±7) g/L
Solubility in 1.0 M HCl	750(±3) g/L
Density	0.645(±1) g/cm^3
Total N	0.5 g/kg
Total P	20(±3) g/kg
Total K	65 g/kg
Total C	2.9 g/kg
Total S	1.9 g/kg
Total Si	105(±1) g/kg
Total Ca	315(±3) g/kg
Total Mg	48(±2) g/kg
Total Cu	91(±3) mg/kg
Total Zn	78(±4) mg/kg
Total Cd	0.98(±0.1) mg/kg
Total Cr	36.5(±2) mg/kg
Total Ni	37.5(±2) mg/kg
Total Pb	19.5(±2) mg/kg
Total Hg	0.25(±0.1) mg/kg
Total Co	<0.01 mg/kg
Total As	<0.008 mg/kg
Total V	<0.3 mg/kg
Total PAHs[a]	<0.01 mg/kg

[a]Polycyclic aromatic hydrocarbons (PAHs); EC Electrical conductivity

The ionic strength of the media showed no measurable effect on the adsorption of neutral molecules. Elemental analysis (Table 4.3.1) and surface structure of the tested ash matrix showed that several active sites could contribute to the TBA adsorption under alkaline conditions: i) a high micro-to-nano porosity of complex surface agglomerates and rhombohedral cavities of calcite (Figure 4.3.1.) that can trap small-diameter molecules such as TBA, as well as ii) a high content of silanol sites derived from quartz and hydroxyl groups originated from portlandite (Figure 4.3.2.) that promote interactions by forming the hydrogen bonds.

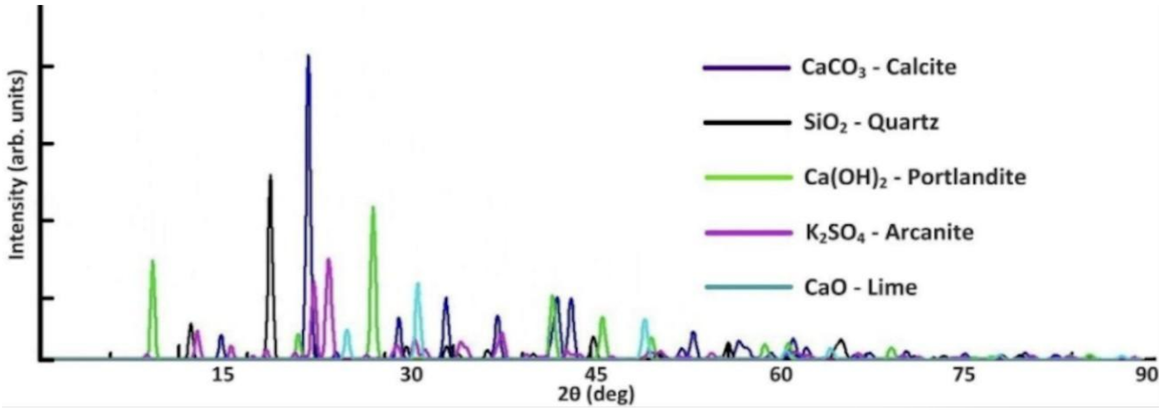


Figure 4.3.2. Surface characterization of tested FA matrix by XRD analysis

Several studies have highlighted the Si-C ratio as a critical variable in predicting bioash capacity for pesticide adsorption, with the smaller the ratio, the higher the adsorption capacity and equilibrium time for non-polar chemicals. Si-rich adsorbents have a predominantly negative surface charge in an aqueous solution starting at pH >2 and so facilitate cation retention. Si-rich bioash matrices, such as those formed from rice husk, can contain up to 46% Si, which is more than four times the amount found in the hardwood FA investigated here (Table 4.3.1.), and hence serve as precursors for the development and manufacturing of nanostructured zeolites. The use of wood FA may have a comparative advantage for the adsorption of both polar and non-charged molecules such as TBA in neutral and alkaline conditions.

4.4. Fly ash facilitates dissipation of TBA in the watercourse

TBA was not detected in watercourse samples 48 h after the FA addition (Table 4.4.1.). At the same time, its level in the control sample (without FA) decreased by less than 20% from its initial value (Figure 4.4.1.A).

Table 4.4.1. The TBA levels in watercourse samples during the 48 hours of the kinetic experiment: control sample without FA and FA-treated samples (A and B)

Time	**Control**		**A**		**B**		**Average A, B**
h	**Concentration or fraction in extract**						
	ng/mL	**%**	**ng/mL**	**%**	**ng/mL**	**%**	**%**
0	282	100	193	68	229	81	75
1	274	97	159	56	190	67	62
2	283	100	136	48	152	54	51
3	260	92	118	42	123	44	43
6	250	89	59	21	121	43	32
10	239	85	79	28	89	32	30
24	275	98	40	7	53	19	13
32	266	94	23	8	31	11	10
48	231	82	nd	0	11	4	2

nd not detected (< 0,01 ng/mL)

Experimental data were successfully fitted to the first-order kinetic model (Figure 4.4.1.B) that is commonly applied to a unimolecular reaction whose dissipation rate depends on the concentration of one reactant. The regression coefficient for TBA dissipation in water treated with FA was very high ($R^2 = 0.97$), with a rate constant $k = 0.068$ h^{-1}. In the control treatment, the regression was rather poor ($R^2 = 0.28$) and its rate constant $k = 0.002$ h^{-1} can be used for comparison only approximately. The half-lives (DT_{50}) calculated for TBA dissipation in the FA and control treatments were 10 h and 14 d, respectively, indicating that the addition of 1% (*w/v*) FA can reduce TBA level in selected watercourse sample more than 30 times faster compared to its persistence in water without FA (Figure 4.4.1.B).

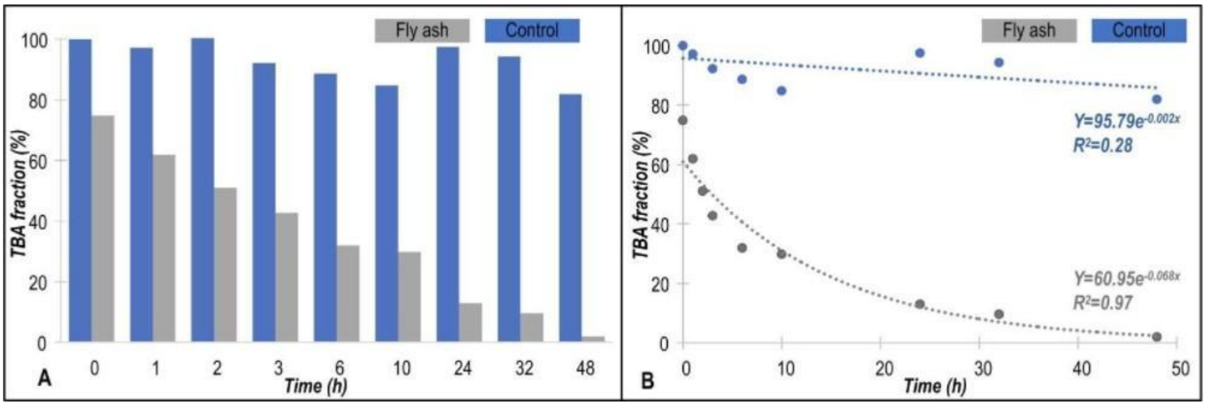


Figure 4.4.1. Dynamics of TBA fraction (%) in the watercourse matrix treated with FA (1%, *w/v*) and control sample (without FA) during the 48-h period (A), with data fitted to the first-order kinetics (B)

The XPS spectral footprint, performed before and after the 48-h experiment, was used to determine the surface elemental composition and binding energy to explain some of the possible sorption mechanisms that occurred at the outermost surface of the tested bioash matrix (Figure 4.4.2.). The XPS spectra clearly showed there was a shift in the position of some peaks and changes in the peak's intensity for several elements, notably O, Ca, K, Mg, and Si. The rising of the elemental peak's intensity after 48 h suggested that these elements participated in the adsorption of TBA and its removal from the watercourse sample. In addition, after adsorption, the O1s peaks were shifted to higher binding energies of 533.2, 533.8, and 534 eV, implying the formation of new bonds between O and TBA.

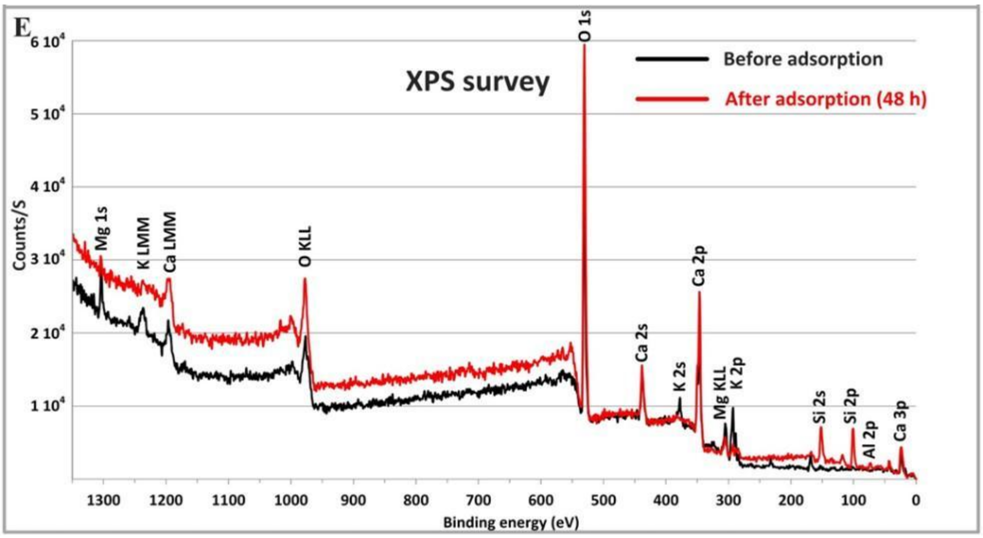


Figure 4.4.2. Surface characterization of tested FA matrix by XPS analysis

Most herbicides are sensitive to alkaline environments. TBA was found to be both hydrolytically and photolytically stable in aqueous solutions at pH 5−9 (DT_{50} = 86 to >200 days), although it degraded faster in severely acidic (DT_{50} = 8 days at pH 1) and alkaline (DT_{50} = 12 days at pH 13) environments. Despite being conducted in an extremely alkaline environment, our experiment revealed that TBA was detected in water two days after FA addition. According to the literature, no significant degradation of TBA could have been expected in such a short time. Furthermore, DET, a significant TBA metabolite, was not detected in any of the analyzed watercourse samples, showing that the removal process could be rather an adsorption than alkaline hydrolysis.

Bioash has previously been shown to be an effective adsorbent for the rapid clean-up of pesticide-contaminated water. The adsorption equilibrium of herbicide 2,4-D by rice husk, wheat straw, and bagasse fly ashes, for example, was reached in 15, 120, and 240 minutes, respectively. In comparison, activated C adsorption of 2,4-D took 96 hours. However, ionizable herbicides such as 2,4-D exhibited pH-dependent adsorption efficiency, which might be attributed to a repulsive contact between the anionic herbicide and the negative Si-based adsorbent surface under these conditions. In contrast, the bulk solution pH had no effect on the binding mechanism of non-ionizable herbicides, whose adsorption is dependent on non-electrostatic interactions, and hence their adsorption was stronger in alkaline aqueous solutions.

TBA can also be considered a non-ionizable herbicide in alkaline conditions, as in the FA treatment in this work, due to its very weakly basic character, which excludes the possibility of polar Coulomb interactions with the ash constituents. TBA is a chlorotriazine having ethylamino and tert-butylamino side chains that are connected to a heteroatomic aromatic ring with extremely reactive, delocalized electrons. As a result, hydrophobic Van der Waals interactions, hydrogen bridging, and reactions of delocalized electrons are plausible adsorption mechanisms for TBA in alkaline conditions. These interactions are primarily related to hydrophobic and aromatic organic compounds.

Mineral surfaces have a modest but significant number of adsorption sites for uncharged chemicals, which are dominated by their high porosity and specific surface area, and affinity for silanol (=SiOH). The ionic strength of the media has no discernible effect on neutral molecule adsorption. Elemental analysis and the surface structure of the tested FA revealed many active sites that could contribute to TBA adsorption under alkaline conditions. A high micro-to-nano porosity of complex surface agglomerates and rhombohedral cavities of calcite capable of trapping small-diameter molecules like TBA a large concentration of silanol sites obtained from quartz and hydroxyl groups derived from portlandite, which encourage interactions by generating hydrogen bonds. Finally, the XPS analysis suggested the contribution of O, Ca, K, Mg, and Si to the interaction between TBA and the FA surface (Figure 4.4.2.). Selected FA was low in C content, but its structure was certainly changed under combustion conditions, from native to condensed or even polymerized form, which might enable reactions involving delocalized electrons existing in aromatic centers and within the layers. Therefore, in the FA-amended watercourse, it can be expected TBA dissipation by rapid adsorption into FA rather than TBA degradation by alkaline hydrolysis, but an adsorption batch equilibrium experiment in the TBA-FA system has to be done. The results from this study showed a promising adsorption potential of the small addition of industrial low-cost by-products such as wood FA to watercourse matrix, aiming to reduce surface water and groundwater contamination with TBA from different sources, but further studies are priorly needed to confirm applicability.

4. CONCLUSIONS

Thorough characterization of deciduous hardwood FA revealed its highly developed structure and alkaline nature, capable of reducing the half-life of TBA in the watercourse matrix by nearly 34-fold after 48 h versus control without fly ash. The first-order kinetics of TBA dissipation with fly ash amendment was well described, and XPS analysis revealed plausible involvement of Si, O, Ca, K, and Mg in TBA adsorption. The study found that the tested fly ash had a promising potential for removing TBA from contaminated surface water bodies. More research is required to investigate this effect for other commonly used pesticides as well as the physicochemical properties of decontaminated waters. The results presented here serve as a foundation for future research into the development of new bioash-based polymers that can be used for chemical amelioration and decontamination of natural ecosystems contaminated with (agro)chemicals and their metabolites.

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5.1. List of tables

5.2. List of figures

Curriculum Vitae

Gracijela Maltašić was born on the 10^{th} of July 1992 in Osijek, Croatia. Until 2011 she attended III. gymnasium, a high school with an extended science and math curriculum. After that, she has enrolled in the Faculty of Agriculture at the University of Zagreb, where she finished a bachelor's study called Animal science. In 2018, she enrolled in the master study program in English called INTER-EnAgro (Environment, Agriculture and Resource Management) at the same University. Other than the English language for which she has a C1 certificate, she is actively using the german language in speaking and writing.