Kaunas University of Technology
Faculty of Chemical Technology

Application of biofiltration for treatment of agricultural machinery rinse water

Master's Final Degree Project

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Kaunas, 2022

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Environmental Engineering (6211EX003)

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Kaunas, 2022. 57 pages.

Summary

The agriculture sector is one of the top 3 water polluters. Growing worldwide pressure on water quality, increasing number of water treating and reclamation technologies helps to reduce contaminants in many fields. But agricultural machinery rinse water doesn't have many options for easily accessible water treatment technologies. Usually, biofilters with biomixture are used for this type of water treatment. However, biofilters could be maintained for a limited time. And there is not much research on biomixture improvements. For this reason, research in this field was investigated.

The removal of pesticides using three types of biofilters rinsed with agricultural machinery to rinse wastewater was investigated in the operation of three parallel columns. Column 1 – biomixture; Column 2 – biomixture with activated carbon (AC) layer; Column 3 – biomixture + AC with slow sand filter layer (SSF).

The main purpose of the experiment was to determine the best performing column. To evaluate the conditions for the best microbiological performance, influent (from agriculture machinery rinse water) and effluent pH, Turbidity, ATP, Microbiological activity on agar plates and DOC were made. To evaluate the removal performance of pesticides, influent and effluent water samples were analyzed for pesticide concentrations. To evaluate biofiltration stability, influent water samples and 3 effluents of 4 months were analyzed.

Taken together, these findings suggest that column 2 had the best conditions for biological activity in biofilter, it also showed highest pesticides removal efficiency and the most robust conditions in overall comparing with other two columns.

Kilaitė Ugnė. Žemės ūkio technikos plovimo vandens valymas biofiltracijos metodu. Magistro baigiamasis projektas / vadovė doc. Inga Urniežaitė; Kauno technologijos universitetas, Cheminės technologijos fakultetas.

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Kaunas, 2022. 57 p.

Santrauka

Žemės ūkis yra vienas iš 3 labiausiai vandenį teršiančių sektorių. Pasaulyje didėjantis susirūpinimas vandens kokybe, sparčiai besiplečiančios vandens valymo ir regeneravimo technologijos, padeda mažinti teršą daugelyje sričių. Tačiau žemės ūkio technikos plovimo vandens valymo technologiniai sprendimai nėra plačiai prieinami. Paprastai tokio tipo vandens valymui naudojami biofiltrai užpildyti biologiškai aktyviu dirvožemio mišiniu. Tačiau biofiltrai efektyviai veikia ribotą laiką, o tyrimų apie biomišinio tobulinimą nėra daug. Dėl šios priežasties buvo atliekami šios srities tyrimai.

Ištirtas pesticidų šalinimas naudojant trijų tipų bioreaktorius, patalpintus į vienodo tipo cilindrines kolonėles. Kolonėlės buvo užpildytos tokiomis medžiagomis: Kolonėlė 1 – biologiškai aktyviu dirvožemiu; Kolonėlė 2 – biologiškai aktyviu dirvožemiu su aktyvintos anglies sluoksniu; Kolonėlė 3 – biologiškai aktyviu dirvožemiu su įmaišyta aktyvinta anglimi bei smėlio filtru kolonėlės apačioje.

Pagrindinis eksperimento tikslas buvo nustatyti geriausiai veikiančią kolonėlę. Siekiant įvertinti geriausiai mikrobiologinį efektyvumą atitinkančias sąlygas, atlikta įtekančio (žemės ūkio technikos plovimo vandens) ir ištekančio iš kolonėlės vandens analizės. Tirtas pH, drumstumo, ATP kiekis, mikrobiologinis aktyvumo agaro lėkštelėse ir ištirpusios organinės anglies (IOA) kiekis. Siekiant nustatyti pesticidų šalinimo efektyvumą, buvo ištirta pesticidų koncentracija įtekančiame ir ištekančiame vandenyje. Biofiltracijos stabilumui įvertinti buvo analizuojami įtekančio ir 3-jų ištekančių 4 mėnesių laikotarpio vandens mėginiai.

Apibendrinant galima teigti, kad 2-os kolonėlės biofiltre susidarė geriausios sąlygos biologiniam aktyvumui. Toje pačioje kolonėlėje nustatytas geriausias pesticidų šalinimo efektyvumas, taip pat stabiliausias pesticidų šalinimas, lyginant su kitomis dviejomis kolonėlėmis.

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List of abbreviations

ATP – adenosine triphosphate;

CE1 – effluent from column 1;

CE2 – effluent from column 2;

CE3 – effluent from column 3;

CFU – colony-forming units;

DOC – dissolved organic carbon;

DT_{50} – half life time value [days];

(G)AC – (Granular) Activated Carbon;

logKow – octanol-water partition coefficient;

SSF – slow sand filter.

Introduction

Climate change, increasing risk of water scarcity, results in developing water saving, treating, and reusing technologies. One-third of the water used in the world is related to agriculture. Attaining of sustainable agriculture makes to search wastewater treatment technologies in different and even specific farming activities. As a main concern in with agriculture related water is considered as pesticides contaminated wastewater. 70 % of agricultural pesticides reaching surface waters could be considered as a point loss by filling and cleaning of agricultural equipment sprayers [1]. When pesticides from point pesticides contamination sources plays a crucial role in leaching to groundwater, to avoid such a loses they shout be eliminated right on the farm.

Increased awareness of the environment pollution and continuous efforts to implement economically viable and environmentally safe agricultural practices raised desire to implement green biotreatment technologies. Biopurification systems excels of their relatively low prices, simple construction and maintenance and also effective way of eliminating wastewater contaminated with pesticides and in the decontamination of pesticide residues point sources, which are potentially harmful to water pollution.

Even though biopurification systems are already used in different countries, they still have some limitations related with fast biofilter degradation. Biopurification systems could be improved with different filtration effectivenes increasing materials. Activated carbon (AC) and slow sand filter (SSF) are well-known absorbents/technologies for wastewater treatment. But not so many research were made on combination bioactive soil with AC or SSF for pesticide removal.

Development of low-cost, simply applicable and long lasting biofiltration technologies for agriculture point source pesticides contamination could reduce groundwaters comtamination from agriculture machiniery rinse water. Findings on biofiltration can be applied to better understanding biomixture interaction with pesticides and also increase variety of biofiltration models used for pesticides contaminated water.

Object of the project

Biomixture filters, containing different types of materials:

- 1 – biomixture
- 2 – biomixture and activated carbon (AC)
- 3 – biomixture with AC and slow sand filter (SSF)

Objective

Investigate the effectiveness of biofiltration-based wastewater treatment technology in rinse water treatment.

Research methods and models applied in the project

For this research mixed research methods were used by making experiments and analyzing data.

Tasks

1. To present scientific literature review of:
   - water in agriculture;
   - pesticides properties and hazard;
   - water reclamation technologies by biofiltration;
2. To determine the biomixture that provides the best conditions for microbiological activity;
3. To establish relations on pesticides properties and pesticides degradability potential;
4. To evaluate the efficiency of different bioreactors' pesticide removal performance;
5. To determine if activated carbon has an impact on biofilter deterioration;
6. To select the most promising biofilter for pesticides degradation.

Hypothesis

- Activated carbon-improved biofiltration reactors could perform greater pesticide removal efficiency;
- Biomixture improvement by AC could reduce biofilter deterioration.

Publications

An article was published and presented on the student scientific conference 'Chemistry and Chemical Technology 2022' which was held on 13 May 2022 at Vilnius University, Faculty of Chemistry and Geosciences, Saulėtekio 3, LT-10257 Vilnius.

Funding

This experiment was carried out during an Erasmus internship in the Center of Expertise Water Technology (CEW) company located on the Water campus, Leeuwarden, The Netherlands. It is part of the 'Emission-Free Rinsing Place' project, where a new concept is being developed by implementing biofiltration for agricultural equipment rinse wastewater.

Structure

This paper has been divided into the following parts: literature review, materials and methods, results and discussion, conclusions.

1. Literature review

1.1. Agriculture water use

Some 1.1 billion people worldwide have water supply limitations, and 2.7 billion faces water scarce for one month per year. Many water systems, which maintain the health of ecosystems and feed the growing population, are already stressed. The current consumption rate will only exacerbate the situation. By 2025, two-thirds of the world's population is likely to be exposed to water shortages and global ecosystems will suffer even more [2].

Population centers, industrial and agricultural activities are the main sources of water pollution [3]. Agriculture uses more water than other sources, and most of it is wasted due to inefficiency. At present, agriculture accounts for around 70 % of freshwater withdrawals worldwide (and even more so, "consumptive water use" is caused by crop evaporation).

It is estimated that agriculture needs to expand by approximately 70% till 2050, in combination with increased consumption of more complex foods and beverages accompanied by income growth in developing countries [4]. Unfortunately, water pollution is an important challenge, both in developed and developing countries [3].

Sustainable water resource management and safe water and sanitation access are essential to unlock economic growth and productivity, subscribes Sustainable Development Goal 6 - Clean water and sanitation – United nations [5]. Different agricultural water managing practices are created (Fig. 1).

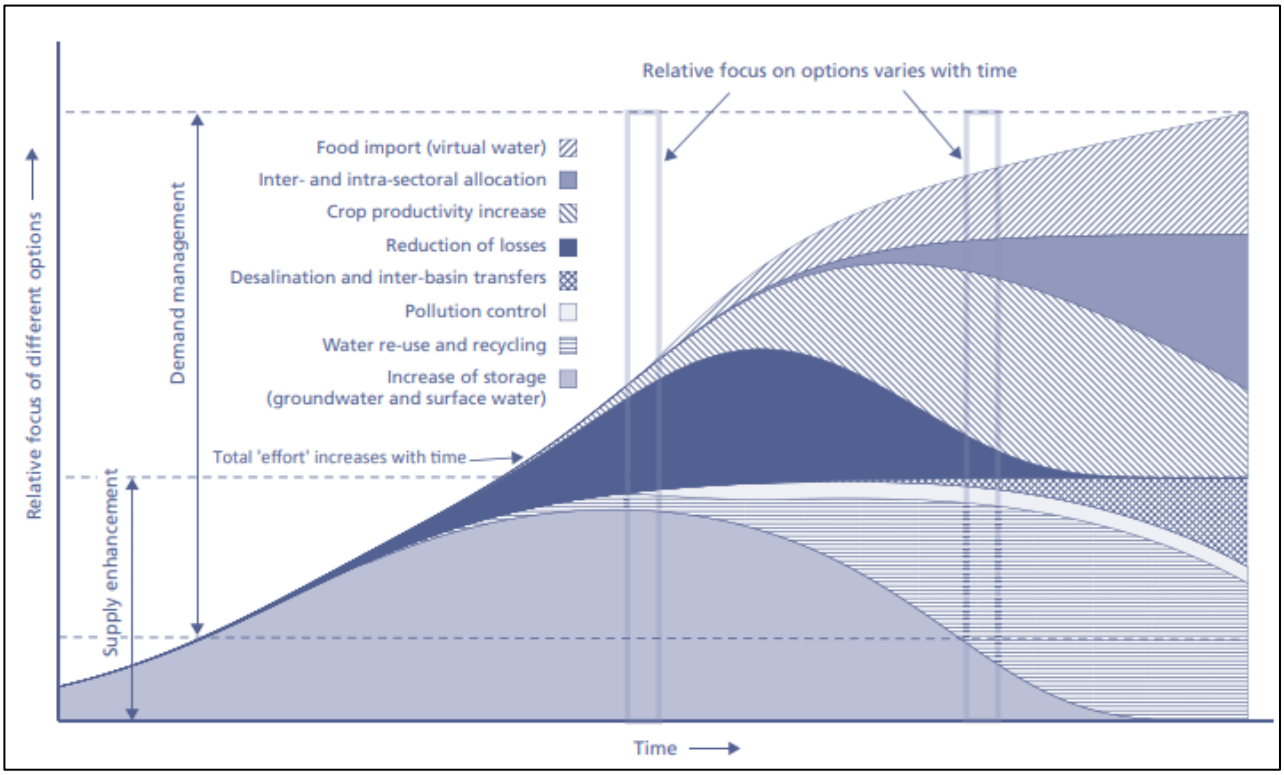

Fig. 1. A schematic representation of the relative importance of the different options to address the growing water scarcity in the agricultural sector over time **[6]**

It is expected that the 2030 Agenda for Sustainable Development will have a significant impact on future policies and strategies and will ensure that water pollution control is recognized as an international and national priority [3].

1.2. Pesticides properties and potential hazards

The use of pesticides is a vital element in modern agriculture. However, their production benefits contrast with the risks they pose due to the toxicity they cause to their environment and the direct or indirect exposure of living organisms [7]. Pesticide use has increased several times in recent decades. Approximately 6.1 billion EUR is used annually worldwide, and pesticides are estimated to be used annually [8].

Chemical-based pesticide classification is very complex. In general, modern pesticides are generally organic chemicals. These include synthetic and plant-derived pesticides. However, some inorganic compounds are also used as pesticides [8].

An overview of the situation in the Netherlands' groundwater and drinking water shows that 24 % of 771 samples, residues of pesticides have been found in groundwater bodies and 11 % exceed the Water Framework Directive (WFD) given limit of 1µg/l [9].

The pesticide pollution source in agriculture may be classified as a point source and a nonpoint source. Water contamination by pesticides is often related to points rather than nonpoint sources. For example, agricultural areas where pesticides are processed and filled with sprayers and areas where sprayers are cleaned are these points of source contamination [10].

2022 May 10 the survey was made to find out if there is a high risk of point sources contamination In Lithuania after agricultural machinery is rinsed. 56 farmers answered survey where they were asked to fill if they have places adapted to agricultural equipment rinsing and treating afterwards (Fig. 2).

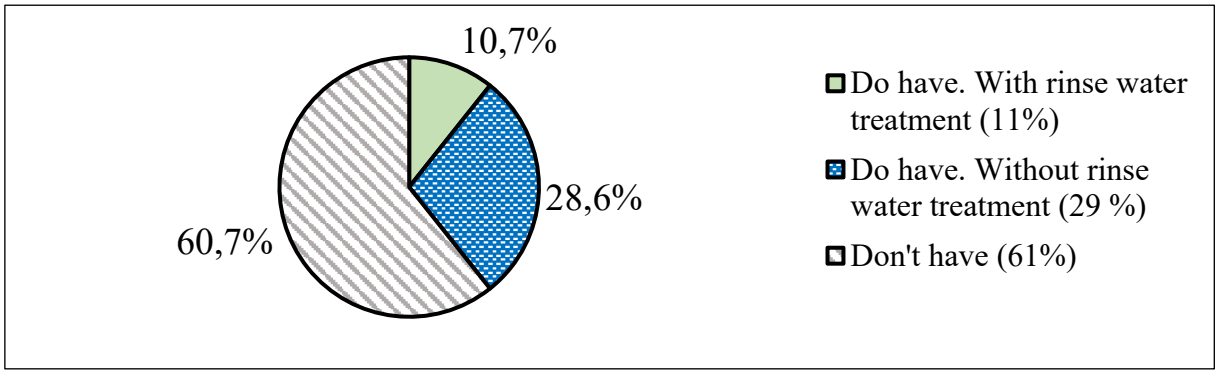

Fig. 2. Agriculture machinery rinsing place presence in Lithuania (2022)

It showed that 90 % of respondents discharge agriculture machinery rinse water to environment without treating. Knowledge about contamination of groundwater and surface waters exerts pressure on the use of pesticides, taking into account its management after being used.

1.2.1. Classification

There are many articles trying to find the best way for pesticide classification. But in general, there are three main features of pesticides that can be classified. These three commonly used pesticide classification methods include: classification according to pesticide function, classification according to entry mode and pesticides causing mural effect, and classification according to the chemical composition of pesticides [8]:

- assignment (could also be target group, type of pest) - e.g., fungicides, herbicides, insecticides etc.;
- Method of pesticide impact. Mostly, they are categorized to contact, systemic, or fumigants. Contact pesticides in some cases external action is to dry the pest's body or create a gas-close film that blocks the normal gas exchange or block the nervous system. Systemic pesticides penetrate easily through the barriers of the organism and affect all organs. Fumigants - chemical compounds inhaled into the body affect the blood flow, enzymes, and nervous systems of organisms [11].
- Chemical Properties - the most specific way to distinguish a complex compound from a multiclass and subclass that exhibits a variety of chemically different structures, as detailed in the British Crop Protection Council published Pesticide Manual. The most popular pesticides are divided into the following classes depending on the chemical structure: organophosphates, organochlorine, carbamates, chlorophenol, and synthetic pyrethroids pesticides [11] [12]. Organophosphorus pesticides are the most commonly used pesticides in agriculture because they are biodegradable and shorter-lived than organochlorine pesticides [12].

The information on chemical and physical characteristics of pesticides is very useful in determining the mode of application, precautions that need to be taken during application and the application rates [8]. But there is one more popular way to classify pesticides. The classification differentiates between more dangerous and less dangerous forms of pesticides, because it is based on the toxicity of chemical compounds and their formulations. Acording to EPA (United States Environmental Protection Agency) the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) is a global initiative to promote standard criteria for chemical classification according to the risks to health, health, and the environment [3]. This classification is based mainly on acute oral and skin toxicity in rats, as these determinations are the standard toxicology methods [13]. Table 1 shows the criteria recommended for classification.

Table 1. The classification of pesticides according to the WHO Hazard Classification 2009

Class		**LD50 (mg/kg body weight for the rat) coefficient**	
		Oral	**Dermal**
Ia	Extremely hazardous	<5	<50
Ib	Highly hazardous	5-50	50-200
II	Moderately hazardous	50-2000	200-2000
III	Slightly hazardous	Over 2000	Over 2000
U	Unlikely to present acute hazard	5000 and higher	

In recent years, the octanol and water partition coefficient (logKow) have become key parameters in research into organic chemical environmental fate. It was found that it is related to water, soil adsorption coefficients, and sediments, and biological concentration factors in aquatic life. LogKow is increasingly used to estimate other properties, and is considered a necessary property in problematic chemical compounds [14].

1.2.2. Environmental issues

Environmental pollution is a worrying factor that accounts for global risks and outcomes on human health. Pesticides are designed to kill and are often used to kill or harm other organisms, including

humans, as their mechanisms are not specific to any one species. The World Health Organization estimates that 3 million people are infected with pesticides every year and that up to 220,000 people are killed, mainly in developing countries. Pesticides result in the production of reactive oxygen substances that, in turn, reduce antioxidant levels and their defense against oxidative damage in the tissue cells. Lipids, proteins, and nucleic acids are regulated by imbalance and affect cell signal pathways. Oxidative stress and the synthesis of oxygen species have long-term health effects, such as cancer, neurodegeneration, cardiovascular disease, respiratory disease, kidney disease, endocrine disease and reproductive disease. When pesticides disrupt the oxidation balance, they paved the way for these diseases [8].

In many cases, the application of pesticides is usually not very accurate. They are now found almost all over the world. Pesticides not only have a beneficial impact on crops, but also have serious environmental impacts. Excessive use of pesticides can result in the destruction of biodiversity. Many birds, aquatic organisms, and animals are endangered by harmful pesticides for survival. Pesticides are of great concern for the sustainability of the environment and for global stability. Pesticides are deliberately released toxic chemicals or biological agent mixtures into the environment to prevent, discourage, control, kill, and destroy insects, weeds, fungi, or other harmful pests [8].

Human-induced pesticides can also enter the water through surface discharges, leaks, and erosion. At the same time, drifting, evaporation and wind erosion can bring pesticide residues into the atmosphere. This can lead to flooding, soil, flora and fauna, often in places far from their origin [15].

Pesticides pose a problem in aquatic environments due to their harmful effects on aquatic life and human beings. The high toxicity and biological recalcitrant nature of pesticides and their associated environmental hazards also increase concerns about public health [16]. Given the fact that agrochemicals contribute to increasing agricultural production, they may damage the environment, including the ecosystem and human beings [8].

While the problems caused by the use of pesticides are more often associated with agricultural or forest practices, they are also common components of urban wastewater accumulating as a result of the treatment of weeds along roads and railway lines, and of garden, park and urban forest areas [15].

That leads to the conclusion that the uncontrolled use of pesticides reduces the number of species of animals and plants in the terrestrial and aquatic worlds.

1.3. Water reclamation technologies for pesticides removal

Rapid industrial and social development has caused intense environmental problems, such as soil and water pollution derived from industry (inorganic compounds: heavy metals, pigments, etc.) agricultural activities (spraying organic contaminants like pesticides, fertilizers, etc.). Wastewater treatment technologies that contain organic compounds belong to the following categories:

Non-destructive methods – held on the physical adsorption, removal, stripping processes, etc. Biological destructive methods – held on biological processes (by using active mud).

The destructive oxidative methods – held on the chemical oxidation process [17].

In developing countries, the main reason for the rapid use of pesticides and dependence on a wide range of pesticides is the rapid growth of the use of pesticides. The poor implementation of the rules and the limited awareness of farmers of the use of dangerous chemicals pose major challenges in

terms of safe and sustainable pesticide management [18]. Inaccurate handling of agricultural chemical residues poses an important environmental risk due to pollution from the point source [19].

Biopurification systems are used as biotechnology tools to reduce the impact of environmental pollutants containing agricultural wastewater on the environment. Although it is effective in removing various pesticides, very resistant compounds have shown that their elimination is low in the biopurification system [19].

The adsorption and degradation of the soil have shown that the amount of leaching of pesticides is significantly controlled. Since pesticide molecules dissolved in water or relates with organic matter, colloids move with the flow of water and determine the leakage behavior of soil matrix adsorption processes. The soil microorganisms play an important role in the degradation of pesticides, increasing the activity and biomass of microbials in plant roots, and increasing the rate of degradation in the soil interface increase the rhizosphere biological activity [20].

There are currently three ways of providing treatment that are included in the exemption of the environmental agency from the agricultural waste regulations: biobeds, biofilters and Phytobac [21].

The biological treatment system is a well or container filled with biological active mixtures (biomixtures) composed of soil, lignocellulosic material, and humid organic substrates. They are mixed in different volumes, and the aim is to treat point contamination. This biological active soil is characterized by high microbial activity and high concentrations of decomposition of pesticides and their metabolites [18].

These systems are currently widely distributed around the world, with over 10,000 units, mainly in European and South American countries. These systems have active biomixtures, absorb pesticides into organic matter, and increase microbial degradation. The most common biomixtures include soil, peat, and straw (25:25:50 Vol. %)—the original Swedish biobed [22].

The biomixtures used in the biotreatment systems must be periodically replaced every 5 to 8 years to maintain the overall effectiveness of the biotreatment system. After use, exhausted biomixtures may contain pesticide residues, so they need special treatment before being released into the environment [23].

In conclusion, there are three methods to eliminate pesticides, biological, chemical, and physical [12] [17]. The development of green technologies for the protection of environmental and human health is essential to minimize the contamination of natural water resources by pesticides [18]. Sorption and degradation are two basic characteristics of pesticides that prevent leakage [20].

1.3.1. Adsorption

Adsorption is used for two important water recovery applications: the continuous removal of organic materials and as an obstacle to the breakthrough of organic matter from other unitary processes. In some cases, the adsorption is used to control the precursor of toxic compounds formed during disinfection. In water purification, adsorption is used to remove solutions by accumulating solid phases. Adsorption is a mass transfer operation because the constituents are transferred from the liquid state to the solid state. Adsorbent is the substance removed from the interface phase of liquids or gases. Adsorbent is the phase of solids, liquids, or gases in which adsorbate accumulates [24].

Adsorption is used to remove resistant organic components, residual inorganic components such as heavy metals, sulfides, nitrogen, and aromatic compounds [24].

Types of adsorbents

The treatment process with adsorption material involves either the liquid passes through the absorbent material in the bed of the reactor or mixing the adsorbent material into a single process followed by sedimentation or filtering to remove the adsorbent that has been used. The most well-known adsorption cleaning practices are active carbon, silica gel, discolored soil, molecular sieves and cotton fibers [17], granular ferric hydroxide, and activated alumina [24].

Active carbon is the main adsorbent used in adsorbent products. It is known that activated carbon has low absorption sensitivity for low-molecular weight polar organic compounds. If the biological activity in carbon contacts and other biological unit processes is low, it can be difficult to remove low-molecular-polar organic compounds from active carbon. Carbon-based absorbents are most used for the absorption of recovered water due to their relatively cheap price [24].

Table 2. Comparison of various adsorbent materials [24]

Parameter	**Unit**	**Activated carbon**		**Activated alumina**	**Granular ferric hydroxids**
		Granular (GAC)	**Powdered (PAC)**		
Total surface area	m^2/g	700-1300	800-1800	300-350	250-300
Bulk density	kg/m^3	400-500	360-740	0.641-0.960	1.22-1.29
Particle density, wetted in water	kg/L	1.0-1.5	1.3-1.4	3.97	1.59
Particle size range	µm	100-2400	5-50	290-500	320-2000
Effective size	mm	0.6-0.9	na		
Mean pore radius	Â	16-30	20-40		
Iodine number		600-1100	800-1200		
Abrasion number	min value	75-85	70-80		
Ash	%	≤8	≤6		

The use of AC, polymer resin, clay, agricultural/sub-products and industrial/sub-products adsorption is increasing [25].

When GAC filters water containing mixtures with different adsorption parameters, the equilibrium concentration of the compounds is influenced by competition [25].

1.3.2. Biofiltration

In biofiltration, organic pollutants of the vapor phase, such as hydrocarbons, are transmitted through the soil bed, which binds to the surface of the soil and is destroyed by soil microorganisms. The filter can be filled with specific bacteria, which preferably degrade certain compounds [26]. There biodegradation processes appear.

Biodegradation is the process of decomposing organic materials into small components by enzymes produced by living microorganisms. Microorganisms transform substances through enzymatic or metabolic processes. In the biodegradation process, microbial organisms transform substances but

often their final products are carbon dioxide or methane. Organic matter can be decomposed by aerobic or anaerobic processes [26].

Some microorganisms have a wide range of degrading, transforming and accumulating compounds, including polychlorinated biphenyls (PCBs), radionuclides, metals, polyaromatic hydrocarbons (PAHs), and pharmaceutical substances [26].

Previously, biofilters were not able to treat chlorinated components. Recent demonstrations have shown that they can also be used to eliminate chlorinated compounds. In recent demonstrations, it has been demonstrated that they can also be used to remove chlorinated compounds [27].

The biological treatment system is a well or container filled with biologically active mixtures (biomixtures) composed of soil, lignocellulosic materials, and humid organic substrates. This biomixture is characterized by high microbial activity and high concentration decomposition of pesticides and their metabolites [18]. Different biofiltration systems is on the Figure 3.

Fig. 3. Configurations of different biofiltration systems

Biological systems are used to eliminate the contamination of pesticides from the point of origin during the cleaning and filling of spray equipment. In Sweden, in the 1990s, BPS was introduced as a simple, cheap construction designed to contain pesticide residues in agricultural farms [28]. The original biomix contains 50% straw, 25 % soil, and 25 % peat [18].

Biofiltration systems uses and improves microbial degradation capabilities (mainly for bacteria and fungi) and the absorbability of its components, to minimize the impact of pesticides on the environment [28].

The effectiveness of bofiltration systems depends on the biomixture's ability to degrade (biodegrade) and absorb large amounts of pesticides released into the system [28].

When it is disposed of in the biofilter, the pesticide interacts with the microbial community. The reaction to the loading of pesticides depends mainly on factors associated with pesticides and can be

expressed in two ways: as the growth and spread of microorganisms that degrade pesticides and can use pesticides as an energy source or the inherent toxicity of pesticides generally reduces the size and activity of some or all microbial communities [29]. Pesticide removal rates in biomixtures could average in 12 – 100 % [30].

Table 3. Pesticides degradation in different biomixtures after biofiltration [27]

Active ingredient	**Main removal results**
Azoxystrobin	68.1% - 81.5%
Imidacloprid	100%
Chlorpyrifos, Malathion, Linuron, Metalaxyl, Dimethomorph	100% metalaxyl and malathion; 20 -70 % chlorpyrifos; 10% dimethomorph
Glyphosate and its metabolite	85–99%
Diuron, Imidacloprid, Tebuconazole, Oxyfluorfen	58–100% diuron; 19–61% imidacloprid; 12–49% tebuconazole; 47–74% oxyfluorfen.
Ametryn	59% triazines, 68% organophosphates
2,4-dichlorophenoxyacetic acid (2,4-D) Atrazine Carbofuran Diazinon Glyphosate	>98% after 20 days
Imidacloprid Dimethoate Tebuconazole Diuron Oxyfluorfen	100% dimethoate; 80% imidacloprid; 73% tebuconazole; 75% diuron.
2,4-D, Bromoxynil, Thifensulfuron-methyl Tribenuron-methyl Pyrasulfotole Thiencarbazone-methyl Metsulfuron-methyl	100% 2,4-D, bromoxynil, and thifensulfuron-methyl, after 35 days; 93% tribenuron-methyl; 70% pyrasulfotole; 64% thiencarbazone-methyl; 34% metsulfuron-methyl
2,4-D Atrazine Carbofuran Diazinon Glyphosate	>99%
Atrazine Chlorpyrifos Iprodione	>95% after 5 days
Terbuthylazine Difenoconazole Diflufenican Pendimethalin	13–99 % after 120 days
Carbofuran	22–46% after 3 days 98.5% after 16 days

There are many studies made by trying to implement different agricultural waste depending on locally available materials like: rice straw and husk, coconut fiber, vermicompost of wet olive cake, olive tree pruning, mushroom substrate, sugarcane bagasse, wood chips and newsprint paper, banana stem, or eucalyptus chip and etc.

Biofilters have several advantages over conventional active carbon dioxide absorbers. Since the biofilter regenerates itself, it maintains maximum absorption capacity. Its main advantage is that it destroys pollutants rather than separates them [27].

After use, exhausted biomixtures may contain residual pesticide residues. The biomixture used in the biofilter must be replaced every five to eight years to maintain the overall efficiency of the biofiltration system [18].

Overall, various biological and physicochemical systems have been implemented to mitigate the effects of pesticide pollution. However, many of them are expensive or require the implementation of complex technologies, which limits their use on the farm. Due to these limitations, a simple system of pesticide biofiltration could be developed and implemented [28].

1.3.3. Activated carbon filtration (ACF)

Active carbon is generated by placing organic foundation materials such as coal, wood, almond, coconut, or hulls through a process of pyrolysis, followed by high-temperature exposure to oxidized gases such as CO_2 and steam. As shown in Figure 4, the carbon structure of the resulting carbon structure is porous and large in surface area [24].

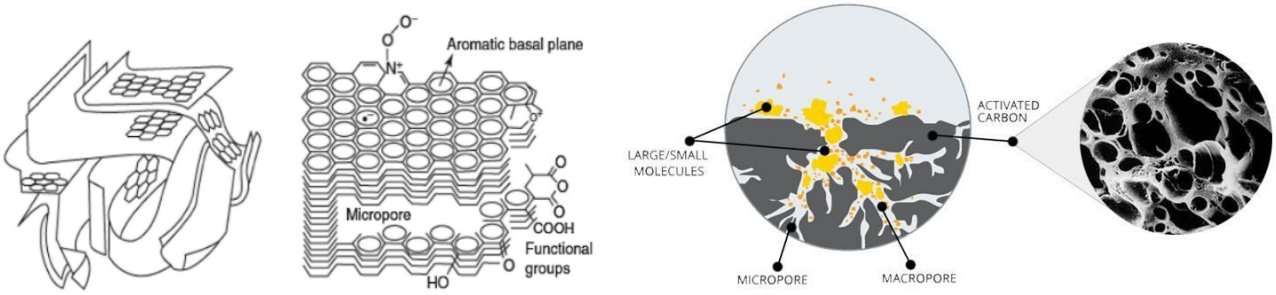

Fig. 4. AC structure **[31]**

The surface properties, the distribution of the pore size, and the resulting regeneration characteristics depend both on the raw material used and on the preparation process; let us proceed with many variations. The two types of activated carbons are powder activated carbon (PAC) and granular activated carbon (GAC). The diameter of PAC is usually less than 0.074 mm, and it is directly added to the active sludge process or the solid contact process. The diameter of GAC exceeds 0.1 mm and is used for pressure and gravity filtration [24].

Active carbon is an effective absorbent to eliminate water pollutants [32]. AC was reported for the first time for water treatment in the United States in 1930 [25]. For removal of organic contaminants, natural organic matter like humic and fulvic and biodegradable compounds granular activated carbon is usually used. GAC filters are also used for water treatment to remove unsuspected color, smell, or taste, pesticides, and other xenobiotics [33] - Table 4.

Table 4. Readily and poorly adsorbed organics on activated carbon [24]

Readily adsorbed organics		**Poorly adsorbed organics**
Aromatic solvents	Benzene Toluene Nitrobenzenes	Low-molecular weight ketones, acids, and aldehydes Sugars and starches Very-high-molecular weight or colloidal organics Low-molecular weight aliphatics
Chlorinated aromatics	PCBs Chlorophenols	
polynuclear aromatics	Acenaphthene Benzopyrenes	
Pesticides and herbicides	DDT Alden Chlordane Atrazine	
Chlorinated nonaromatics	Carbon tetrachloride Chloroalkyl ethers Trichloroethene Chloroform Bromoform	

Readily adsorbed organics		Poorly adsorbed organics
High molecular weight hydrocarbons	Dyes Gasoline Amines Humics	

The efficiency of GAC filtering is based not only on raw materials and production processes but also on the quality of the water flowing, the flow rate and the contact time. Regenerating activated carbon can restore the absorption capacity after breakthrough. The AC's ability to remove organic compounds from water is primarily due to the material's properties, particularly its high-specific surface (porous structure that allows adsorption of components). During GAC filtration, adsorption and biodegradation occur simultaneously [33].

The specific surface area is 400–1500 $m^2 g^{-1}$ due to the high porosity of the GAC. GAC particles used in the treatment of drinking water usually have a diameter of 0.4 to 2.5 mm. The shape, size, volume, surface area of pores and spatial distribution of carbon particles determine GAC porosity. These properties depend on the materials used to make carbon (wood coal, mud, lignite, coal, wood, coconut shells). In addition, bacterial adhesion can be achieved with a macropore radius of more than 500 nm (> 0.5 μm) [33].

Activated carbon filters are combined with physical and biological purification. Pesticide adsorption and decomposition occurs in activated carbon, and in addition microorganism decomposition may also occur [34].

1.3.4. The Slow Sand Filtration (SSF)

Slow sand filtration is a simple technology with low energy costs and high contamination removal. In Europe, slow-sand filtration facilities are used to supply water to large communities, such as London or Amsterdam [35].

Slow sand filter ecosystems include bacteria, protozoans such as rhizopods and ciliates, rotifers, copepods, and aquatic worms. A biological layer is created on the sand surface, known as schmutzdecke (German for ‘dirt layer’). Schmutzdecke is composed of mineral dust and colonized microorganisms, including bacteria, fungi, protozoa, and even some large eukaryotes [36].

There is some strain in the schmutzdecke and when water flows downwards, the schmutzdecke breaks down certain organic substances. As a result, schmutzdecke plays an important role in the removal of particles. Water enters the upper layer of the sand, physically pressing inert suspended particles, and biological action takes place. Particles are attached to the surface of sand particles [35].

The filter is generally used to remove turbidity, pathogenic microorganisms, and biodegradable compounds [37]. The highest layer is the supernatants water that is subject to filtration. The water column offers sufficient water-static pressure to be permeated by the filter system. Furthermore, the thick layer of the actual filter medium is the fine sand layer (0.15–0.3 mm). It is a cheap and durable filtering medium. Smaller particle sizes provide a large surface for filtration and biofilm formation, but smaller void sizes reduce flow (0.1–0.3 m/h) through the SSF. Because of its smaller particle size (0.15–0.3 mm) fine sand provides large surface area for filtration as well as for the formation of biofilm, however its small voids size decreases flow rate (0.1–0.3 m/h) through SSF [36].

Efficiency depends mainly on sand particles' size and filling height, filter speed and temperature. The slow filtration rate of the SSF allows for longer retention periods of the substrate water and water that permeates the bed, allowing for sufficient filtration and biological activity. The elimination efficiency is improved by the depth of the bed but is reduced by the temperature and the filtration rate [36].

Studies show that filter material effective particle sizes are 0.15-0.35 mm, with 1.5-2.0 mm non-uniformity coefficients that can improve filter performance [38].

SSFs are usually placed at the end of the treatment to remove turbidity, pathogen microorganisms, and biodegradable compounds as polishing steps. In normal operating conditions, the water flowing through the filter bed is introduced without disturbing the sand [35]. SSF effectively eliminates waterborne pathogens such as viruses, bacteria, and protozoan cysts. The elimination of pathogenic bacteria ranged from 99.0 % to 99.9 % [36].

Slow sand filtration technology (BSSF) is a low-energy, simple operation, and high-flow contaminant removal technology [38].

1.4. Literature summary

The most emerge environmental problem - climate change, requires quick attention on developing moderate in price wastewater treatment technologies, which would be easily acceptable for society.

The discharging of pesticides to the surface is dangerous to the surrounding environment. To prevent this kind of contamination, pesticides should be carefully degraded at leaching spots.

Stricter requirements for water quality in the future supposed to increase agriculture sector interest of different low-cost, easy applicable and implementable, complying with the requirements, technologies.

Biofiltration systems seems an appropriate start for this kind of technologies. Fast biofilter degradation or/and pesticide removal efficiency deplation over time could be improved with some inexpensive well-apsorbing and filtering components, like activated carbon and slow sand filtration.

2. Materials and methods

2.1. Project design

The project is composed of 3 main steps. The first step of the project is pilot columns testing pesticide removal under different conditions. This step covers artificial and real water treatment through the Biofilter. The biofilter has the following: biofiltration, evaporation, and slow sand filtration purification processes. Biofiltration includes two main processes: sorption and biological degradation of chemical pollutants.

The second step is the pilot in lab conditions which follows to confirm that the selected column design works in a bigger scale. In this step disinfection experiments must be done to find out the most efficient way to treat water out of the pathogenic microorganisms. For disinfection experiments, water ozonation, UV disinfection and ultrafiltration methods will be compared.

The last project step tests if pilot design works at the same efficiency in outdoor conditions and follows the main project goals: to degrade 95 % of pesticides and remove 100 % of pathogenic microorganisms from rinse water for secondary using.

In this report the main focus will be added to the first step results interpretation by trying to recognize the most effective column design.

Laboratory scale pilot construction

The Biofilters bioreactors were designed and manufactured on a laboratory scale. Three columns – bioreactors with different construction have been set-up:

- 1st column: 0.45 m layer of composed substrate;
- 2nd column: 0.45 m layer of composed substrate and 0.1 m granular activated carbon layer under the substrate;
- 3rd column: 0.20 m layer of composed substrate mixed with 0.05 m granular activated carbon layer and 0.2 m slow sand filter layer under the substrate mixture.

Schematic view of columns design is on the figure below (Fig. 5).

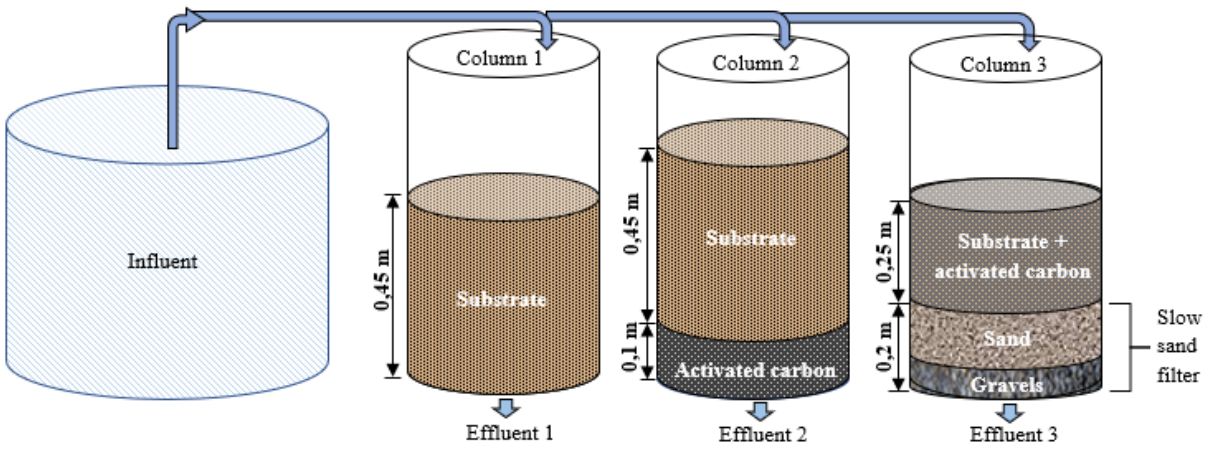

Fig. 5. Scheme of the laboratory pilot columns

From water tank with contaminated rinsing water, which is called influent water, by using peristaltic pump water is distributed of each column by using dripping system. Influent water flows through each column – bioreactor and ends up at the bottom part of reactor where goes out into the effluent tank. Here, water is collected and sampled every week. The flow rate settled to each column was 1,7 mL/min (pump set at 14 rpm), which corresponds to a flow rate of 15 m^3/day for a 450 m^2 filter.

Materials used to build columns:

- 3 purple transparent PVC columns;
- Granular Activated Carbon FA 100 (coal based);
- Gravel: 8.0 mm - 16.0 mm size AA5;
- Sand: Light weight filter media, Clack Crop Filter-AG A8014;
- Potting Soil;
- Parcel Soil;
- Straw;
- Drainage tubes;
- 4 water collecting buckets (3 buckets of 20 l for effluent and 1 bucket 50 l for influent);
- Peristaltic Pump Masterflex L/S® NO. 07528-10; with 3 Pump Head Materflex L/S ® 07519-75.

2.2. Pesticides analysis

Every seven days, four samples, one of influent and three effluents, were taken and once a month the samples were sent to WLN laboratory (Water Laboratorium Noord) to see which pesticides are present in the water and to analyze the performance of the columns in terms of pesticides degradation GC-MS and LC-MS analysis was performed for pesticide detection. For GC-MS, the WLN COW-42.1 method was used [39]. For LC-MS was used WLN-CO.W.40.1 method [40].

Method:

The supplied sample has been examined by means of LC-MS and GC-MS to determine various organic compounds. Upon entry, the sample smelled like gasoline/diesel. The sample was yellow in color and not completely transparent. Based on these observations, it was decided to dilute the sample 1,000 and 10,000 times [41].

GC-MS

The concentration equivalent is an indicative unit empty concentration using internal standard linuron-d6 for positive ionizing components, and bentazone-d7 for negative ionizing components. The area of the component is divided by the area of the internal standard and multiplied by the concentration of internal standard. The column “Category” shows the identification level according to the “Schymanski” method. In this method, the identification is assessed, and a number is given for each component. Figure 2 shows a schematic explanation of this method. Category 1 in this method is a target substance. This is equal to the target substance screening shown in table 1. Category 2 means that an unambiguous structure has been found. The difference with category 1 is that no pure substance was measured. In category 3, multiple structures may be possible, the given name reflects the best match of the possible structures. In category 4, the molecular formula is unambiguous. The given name is also the best match that fits the molecular formula. Finally, in category 5, where a component is found that is interesting, but cannot be identified higher [41].

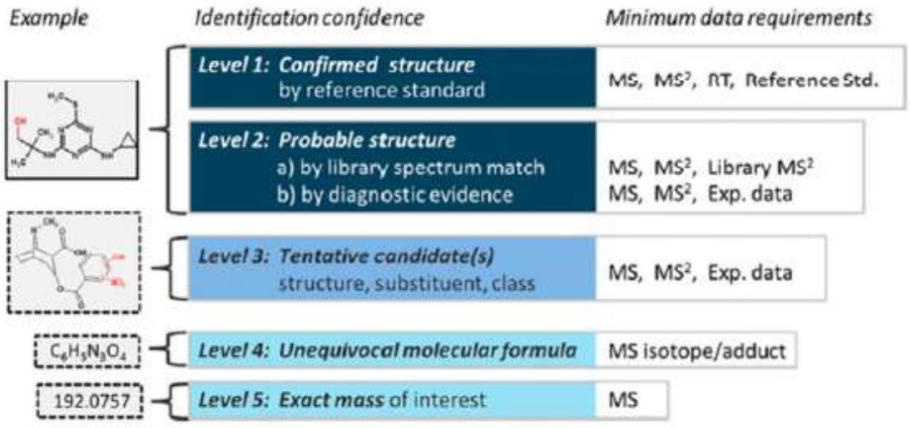

Fig. 6. According to Schymanski, levels/categories of identification.

GC-MS principle:

To the sample, NaCl, sodium titrate, methanol, extractor, and internal standards are added. After extraction, extract is examined through PTV injection, capillary gas chromatography, and mass selection detection (multiple quadruple). Identification and quantification are performed using as specific as possible mass transitions, comparing retention times and surfaces. Internal standards have been adjusted [41].

LC-MS principle:

The samples are injected after acidification and added to labeled internal standards and analyzed by high-pressure liquid chromatography and precise mass selective detection. The area under the peaks in the chromatogram is a measurement of concentration [41].

Removal efficiency was calculated of the concentration in the influent compared to the effluent concentrations by using this formula:

$$\text{Removal efficiency } [\%] = \frac{C_{inf.} - C_{eff.}}{C_{inf.}} \times 100 \qquad (2.2.1)$$

1. Preparing samples for pesticide analysis

The way samples are collected is important for analysis. Washing samples with acid is especially important to eliminate organic traces.

Materials used:

- 200 ml brown glass bottles;
- 2 % Nitric acid solution;
- Milli-Q;
- 105° C temperature oven.

Principle:

The glass bottles are soaked in nitric acid for 2 hours. Hereafter, they are rinsed with Milli-Q water. Bottles were dried in the oven. They were cooled down before picking water sample. Stored water is homogenized first after which bottles are half filled by sample. Bottles are labeled by sample name

and date. The bottles are frozen in a -19 °C temperature freezer. Selected samples were sent to WLN laboratory for pesticide analysis (paragraph 2.2. Pesticides analysis).

Fig. 7. sampling bottles disinfection

2.3. Effluent characteristics

2. The pH is measured to monitor neutrality. This is highly important for microbiological life so any changes in pH may indicate changes in the columns.

Materials used:

- Sample;
- Calibration buffers 4, 7 and 10 pH;
- Multimeter with pH sensor.

Principle:

After the standard sensor calibration of pH 4, 7 and 10 calibration buffers, the pH of each sample is measured.

Fig. 8. pH measurement

3. Turbidity - water turbidity measures the water light transmission properties. The test shows the quality of the water in relation to colloidal and residual suspended matter. The turbidity measurement is based on the comparison of the intensity of the light dispersed by the sample and the intensity of

the light dispersed by the reference suspension under the same conditions. Formazin suspension is used as a standard. Turbidity is measured by FNU with an infrared light source, which corresponds to European drinking water protocol (ISO 7027).

Materials used:

- Sample;
- Turbidimeter HACH 2100Q iS;
- Standard.

Principle:

The turbidimeter is adjusted according to different standards. Every solution should be well mixed. Place the sample in the sample cell and add a cap. The samples are homogenized before measuring turbidity. The measuring tubes are filled with sample and analyzed by a turbidimeter.

Fig. 9. Turbidity measurement

4. ATP tests is used to audit the quantity of bacteria to reveal differences within a process. They give fast and reliable results about the microbiological characteristics of the biofiltration process.

ATP is measured by firefly luciferase, when a sample with ATP is introduced into a solution with the luciferase enzyme, which naturally occurs in the tail of fireflies, it produces light. Light is detected as Relative Light Units (RLUs) by the light meter:

$$ATP + O_2 + luciferin \xrightarrow{Mg{+}{+}luciferase} \text{AMP} + \text{PPi} + \text{oxyluciferin} + \text{light} \qquad (2.3.1)$$

The kit used for the analysis uses a one-minute dilution analysis to measure a parameter called Total ATP (tATP™). tATP measurement shows the total living biomass of the sample, because ATP is a molecule that is present in living cells and surrounding cells. In this way tATP represents ATP from living and dead microorganisms suspended in a liquid and thus represents a plankton population [42].

The Luminometer displays the value of RLU (relative light unit). The following formula is used to convert the value to ng ATP/L.

$$tATP\ [\text{pg ATP/mL}] = \frac{\text{RLU}_{\text{tATP}}}{\text{RLU}_{\text{ATP1}}} \times 1000[\text{pg ATP/mL}] \qquad (2.3.2)$$

In this equation, tATP is the total ATP in ng/L, RLU_{tATP} is the total ATP sample value, RLU_{tATP1} is the calibration value [42].

Table 5. ALB tATP Interpretation Guidelines [42]

Application	Good Control (ng tATP/L)	Preventive Action (ng tATP/L)	Corrective Action (ng tATP/L)
Cooling & Process Water Oxidizing Biocides	<10	10 to 100	<100
Cooling & Process Water Non-Oxidizing Biocides or Non-Chemical Treatment	<100	100 to 1,000	<1,000
Papermaking Product Quality (Newsprint, Fine Papers)	<1,000	1,000 to 10,000	<10,000
Papermaking Odor Control (Paperboard, Recycle Water)	<10,000	10,000 to 100,000	>100,000

Materials used:

- Sample;
- Luminultra Acculight Basic Test Kit;
- Pipette 100 µL;
- Pipette 400 µL;
- Test tubes.

Principle:

The ATP analyzer device is turned on. Performed one UltraCheck 1 calibration into plastic tube. The analysis in the analyzer is performed to record calibration results. Homogenized sample is filled into plastic tube and 2 drops (100 µL) of UltraLyse Lite is added and mixed. After one minute, 400 µL of Luminase Lite is added and gently swirled. Tubes with sample were placed into the analyzer.

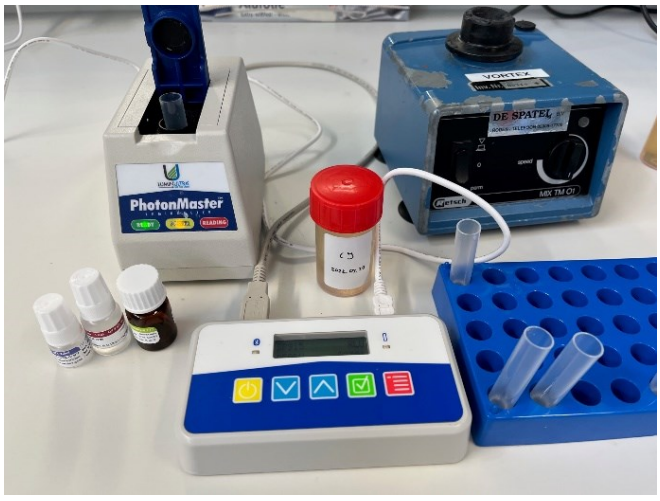

Fig. 10. ATP measurement

5. The dissolved organic carbon (DOC) is measured to give a measure of the carbon content of the influent and effluent. The measurement is important to know if there is enough of available carbon for pesticides degrading bacteria in each column [43].

Fig. 11. DOC measurement

Materials used:

- Sample;
- Glass tubes with a cover suitable for autosamplers;
- TOC analyzer;
- 1000 ppmC: 2.125 g/l Potassium hydrogen phthalate;
- 1000 ppmC: 3.497 g/l Sodium hydrogen carbonate; 4.412 g/l Sodium carbonate (anhydrous).

Principle:

Startup TOC analyzer 1 hour before analysis. Create the sample sequence in the program. Prepare the fabric liquid and fill the calibration liquid in the glass tube. The samples are filtered through a filter (black ribbon, Cellulose Paper Filter, grade 589[1], medium-fast, 20-30 µm particle retention) and filled into a glass tube. Start the program to analyze samples.

6. Presence of microbiology - to determine the number of bacteria present in the rinsing water, measurements were made by plating. This enables the comparison of bacteria between the three effluent columns and influents waters.

Fig. 12. Presence of microbiology

Materials used:

- Samples;
- Milli-Q water;

- Petri dishes (2 dishes for each sample);
- Agar (TSA Trypto-casein soy agar);
- Pipette;
- Glass sealable bottle;
- Incubator (37 degrees Celsius);
- Autoclave (121 degrees Celsius).

Principle:

To prepare agar, mix 40 grams of agar in 1 liter of milli-Q water. Put the petri dishes, pour the liquid agar into the dishes, until it is about full, then let the agar harden completely. Pipette the 100 µm of sample on the agar plates. Distribute it on agar plate and hermitize plate. Repeat it with other samples and remain agar plates for 24 hours in incubator. After 24 hours count colonies.

Table 6. Measurements frequency and used equipment.

Measurement	**Frequency**	**Equipment**	**Model**
DO	1 time a week	Multimeter HACH HQ40d: DO sensor	HACH LDOTM LDO101
pH	1 time a week	Multimeter HACH HQ40d: pH sensor	IntelliCALTM PHC101
Turbidity	1 time a week	Turbidimeter	HACH 2100Qis Portable Turbidimeter. Cat. No. 2100QIS01
tATP	1 time a week	Luminometer	LuminUltra PhotonMaster
DOC	1 time a week	TOC analyzer	Shimadzu TOC-L CPN 638-91110-48
Presence of microbiology	1 time per 3 months	-	-

Each measurement was repeated 2 times.

2.4. Statistical analysis

Identifying errors

Descriptive statistics data analysis was used to identify standard errors of pesticides removal efficiency. These calculations were made by using Microsoft® Excel® for Microsoft 365 MSO (Version 2204 Build 16.0.15128.20210) 64-bit. The software determines the margin of error with this formula:

$$ME = \mathrm{Z} \times \frac{\sigma}{\sqrt{n}} \quad (2.4.1)$$

ME – Margin of error

σ – Population of standard Deviation

n – sample size

Correlation

Linear regression determines the correlation between different water parameters (confidence level > 95%). These calculations were made by using Microsoft® Excel® for Microsoft 365 MSO (Version 2204 Build 16.0.15128.20210) 64-bit.

For statistical correlation analyses between different water parameters, Spearman correlation was used to determine relationships between influent and effluent waters by using SPSS 21 statistical software at a confidence level > 95% ($p < 0.05$) and > 99% ($p < 0.01$).

Data distribution

In normal distributions, the data is distributed in a symmetrical way without skewed distribution. Most values are clustered around the central region, and the values are reduced when they move further away from the central region [44]. Box and whisker plots were used to determine how data is distributed.

3. Results and discussion

To determine which column-bioreactor has the highest performance of pesticide removal, not only should pesticide removal efficiency parameters be taken into consideration, but also optimal conditions for microorganism activity and biofilter degradability potential.

For this study, lab-scale columns with different biofilters were analyzed. As mentioned above, columns differ to each other by their construction (Figure 5). Mainly column 1 contains biosubstrate mixture; column 2 is improved by layer of AC above biosubstrate mixture and column 3 has AC in biosubstrate mixture with additional SSF layer above biosubstrate (Table 7).

Table 7. Parameters for biofiltration columns design

Parameter	**Column 1**	**Column 2**	**Column 3**
Diameter [m]	0.31		
Radius [m]	0.15		
Area [m^2]	0.073		
H [m]	0.45	0.55	0.45
V [m^3]	0.033	0.040	0.033
m [kg]	28.24	31.91	35.74
Flow rate [ml/min]	1.7	1.7	1.7
Biomixture* [%] *50% soil, 30% straw, 20% potting soil	100	81.8	45
AC [%]	-	18.2	10
SSF [%]	-	-	45

Each column was fed with rinse water, in this study called influent. And after filtration, each column effluent was analyzed by different parameters: from column 1 – effluent 1; from column 2 – effluent 2; from column 3 – effluent 3.

Experiment set up in a figure below. Column 1 stands on the left, column 2 in the middle, column 3 at the right. Above them there is tanks to collect effluent water for measurements.

Fig. 13. Lab-scale setup for biofiltration experiment

This experiment is part of the "Emission-free Rinsing Place" project, where a new concept is being developed by implementing biofiltration for agricultural equipment rinse wastewater with activated carbon and slow sand filtration. There are almost no studies related to concept like this even if AC is broadly used for wastewater contaminated by pesticides treatment.

Project main purpose is to build a circular wash area, which brings back 90 % of treated water for secondary reuse. And only 10 % of the water is lost due to the evaporation process. The schematic view of water reuse is in Figure 14.

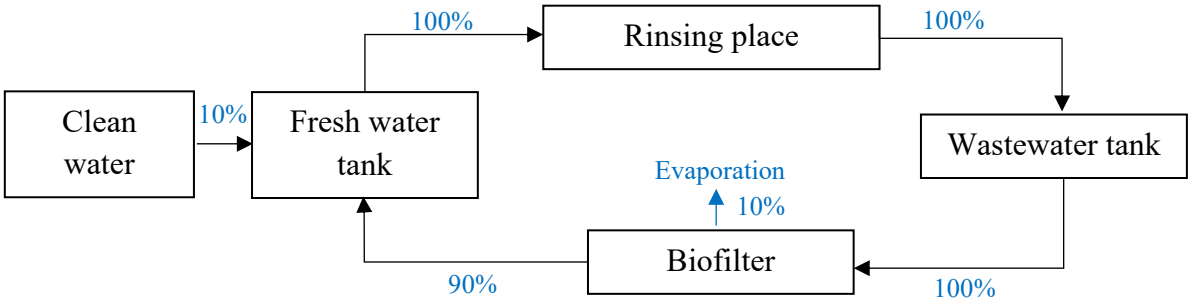

Fig. 14. Diagram of the reuse of rinse water with 90% reuse

This water wouldn't be discharged to sewed system or environment after treating. Therefore, the project "Emission-free rinsing place" is expected to help reduce the contamination of pesticides point source. And its emissions to surface and groundwater. Also increasing environmental quality and biodiversity in countryside areas, by moving farming to circular, more sustainable, 0 emissions economy. Obvious to reach proper quality of water pesticides, pathogens, soil and oil residues, color, odor should be eliminated. This study focuses only on the degradation of pesticides.

The first stage of the project is lab-scale experiments with pesticides removal. For it, three different biofilters were created. The technique of biological degradation by microorganisms is based on different filtration methods (evaporation, oxidation, sorption, and filtration). The biomixture with great microbial activity is characterized by high concentration degradation of pesticides and their metabolites [18].

3.1. Characterization of biofilter materials

Three different columns were build by implementing different purification techniques. Water is treated by a combination of biofiltration-based purification processes. The main layer for microorganisms activity is bio-mixed substrate layer. See constituent materials used in biofilters in Figure 15).

a b c d e f

Fig. 15. Constituent materials used in biofilters: a – straw; b – soil; c – potting soil; d – activated carbon; e – sand and f - gravels

3.1.1. Biomixture (substrate)

Most of the biofilter biological purification is performed on the substrate. Typical biomixture composition has soil, lignocellulosic materials and peat by specific proportions [29]. The volumetric ratio of the substrate consists of 50 % field soil/clay, 20 % potting soil and 30 % chopped straw according to the Phytobac recommendations for biomix proportions. Each component has a specific role in the biofiltration process. Soil plays the main role for suppling pesticide-degrading microorganisms. Because soil is from agricultural fields, it already contains specific bacteria that are already adapted to pesticide contamination and its degradation.

Straw is one of the lignocellulosic materials which promote pesticide degradation activity, increase the adsorption capacity of biological mixtures, and supply energy and carbon for microbiological activity. Even if the kind of lignocellulosic materials used for biofilters differ depending on crop cultivated in each different region, straw is one of the most popular lignocellulosic biofiltration materials [29].

Potting soil function is to regulate moisture content, decreases pH (to limit fungal ligninolytic activity) and increase the adsorption capacity of the biomixture. All three components must exist simultaneously to ensure maximum degradation capacity [29].

3.1.2. Activated carbon

The adsorption and decomposition of pesticides occurs in activated carbon, and the decomposition of microorganisms starts to biodegrade them. It also has physical treatment properties which is related of high AC active area and very porous surface. Thus, AC is a promising compound to increase pesticide removal efficiency. Granular activated carbon (steam activated, coal based) used for biofiltration was recommended for dichlorination and purification of potable and process water. Apparent density 500 ± 30 [g/L]. Active area 700-1300 [m^2/g]. Diameter > 0.1 mm [45].

3.1.3. Slow sand filtration

The slow sand filter (SSF) acts as a filter for the generation of a particles-free water from a column, which, acting as a drainage system, should protect the column from clogging. Another important reason for SSF is its ability to remove pathogens, which is important for the project itself.

The SSF consists of 2 layers. 75 % of SSF is sand on the top and 25 % are gravels on the bottom. Instead of sand for better performance, Ag. light weight filter media were used. Gravel size in filter variates between 8.0 mm to 16.0 mm [46].

SSF was added only at the bottom in column 3.

3.2. Rinsing water parameters (pH, turbidity, ATP, microbiological activity, and DOC)

Rinse water appears after treating agricultural equipment after different agricultural activities (especially spraying). To prevent the equipment from clogging, it should be well rinsed. This water could not be discharged into the environment without treatment. Because this water is highly contaminated by pesticides, the biomixture containing microbial communities is supposed to have a great microbial, pesticides adsorption and biodegradation of pesticides [18]. Biofilters columns performance has been monitored by multiple parameters of influent and effluent water. pH, turbidity, ATP, and DOC were measured weekly, and microbiological activity was measured once. These parameters were important in determining the optimal conditions for microbal pesticides degradation performance.

3.2.1. pH

Each bacterial species grows best in certain pH ranges, usually close to neutral pH 7 [47]. The amount that can be dissolved in the water (solubility) and the amount that can be utilized by aquatic life of chemical constituents (biological availability) could be determined by water pH. Different contaminant has the best solubility in different pH ranges [48] [49]. Some studies shown that lower pH increase of the pesticide's adsorption. Because of the existence of H^+ ions, the absorbent surface has a more positive charge, resulting in a high interaction between the absorbent surface and the P-electron cloud of the phenanthrene molecules [12]. However, the higher risk potential of corrosion is expected at lower water pH, which could have a negative impact on the system (faster pipe degradation, lower disinfection efficiency, formation of disinfection by-products) [50].

Between 2021 December 14 and 2022 March 29, pH in influent water and 1st, 2nd, and 3rd columns was monitored. After rinsing agricultural equipment, the total water pH varies around 8.3. The values can be seen in the graph below (Figure 16) by influent and effluent water. All values of pH could be seen in Appendix 1.

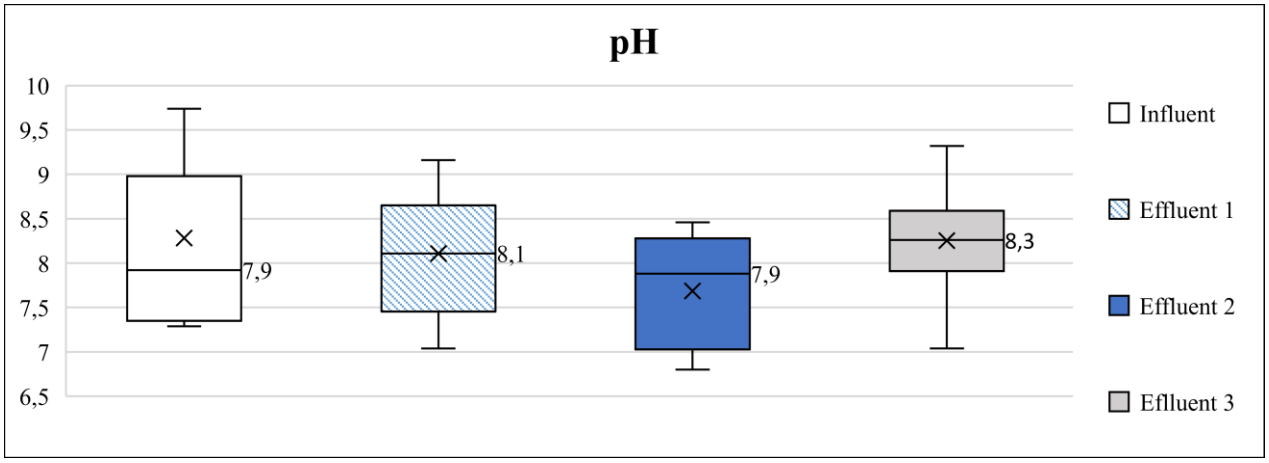

Fig. 16. Results of rinse water pH analyses

The pH value of the influent water ranged between 7.3 and 9.7 throughout the period. However, the most common values for influent water ranged from 7.4 to 9.0 and during all period it tended to be alkaline.

The pH of effluent 1 varied between 7.5 and 8.7 (min 7.3, max 9.7) with an average of 8.1. Effluent 2 pH most of the values varied between 7.0 to 8.3 (min 6.8, max 8.5) with an average of 7.7. The pH of effluent 3 varied between 7.9 and 8.6 (min 7.0, max 9.3) with an average of 8.3. It is important to mention that all effluents tended to be alkaline, which could be affected by influent water alkalinity.

Because biofiltration is based on microbiological activity, distinguishing the most appropriate pH conditions matching column could ensure proper functioning microorganisms' activity. pH analyzes showed that effluent water 2 is more likely to be neutral, with lower pH values compared to effluent water 1 and 3, which is the best suited to biological activity. Moreover effluent 2 values were steadier during experiment comparing with other two effluent waters.

3.2.2. Turbidity

Turbidity is one of the factors that determine water quality. It is a measurement of the relative clarity of water, to determine the volume of suspended particles in the water system (organic matter, algae, sediments) [51].

The elimination efficiency of contaminants from waterborne micropollutants, such as CBM, 2,4-D, BPA, TCB, and NP generally increased with increasing water turbidity. Articles show that the results of the adsorption of micropollutants leading to high turbidity water indicates that adsorption is an important mechanism for the elimination of micropollutants [52]. In many cases, turbidity may indicate the existence of potentially harmful substances [53]. The World Health Organization (WHO) states that drinking water turbidity should not exceed 5 NTU and should ideally be less than 1 NTU [54].

Between 2021 December 23 and 2022 March 29, turbidity in influent water and effluent in the 1st, 2nd and 3rd columns was monitored.

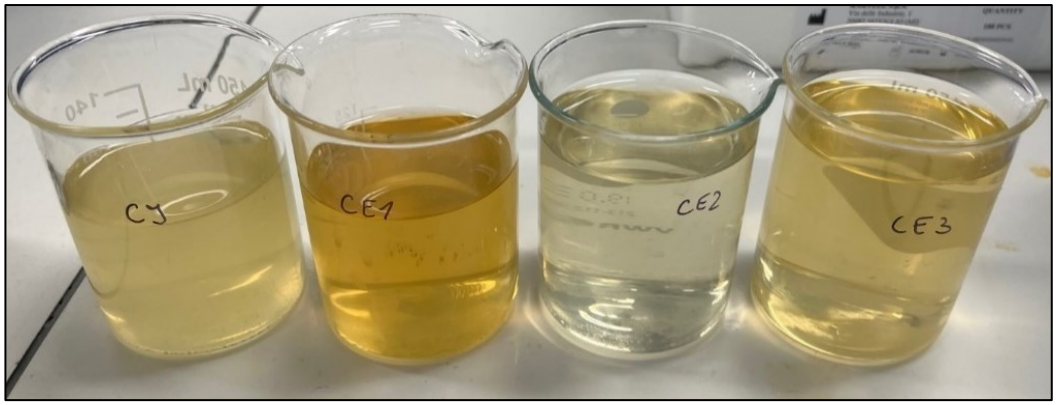

Fig. 17. Turbidity of waters used for experiment. CI - Influent water; CE1 – effluent from column 1; CE2 – effluent from column 2; CE3 – effluent from column 3

By visual observation, there are clear differences between water turbidity. Highest turbidity level monitored in influent water with average of 40.2 FNU which has a milky-yellow color. Influent water turbidity values varied between 4.3 to 80.3 FNU (min 3.1, max 117.0) (Figure 18).

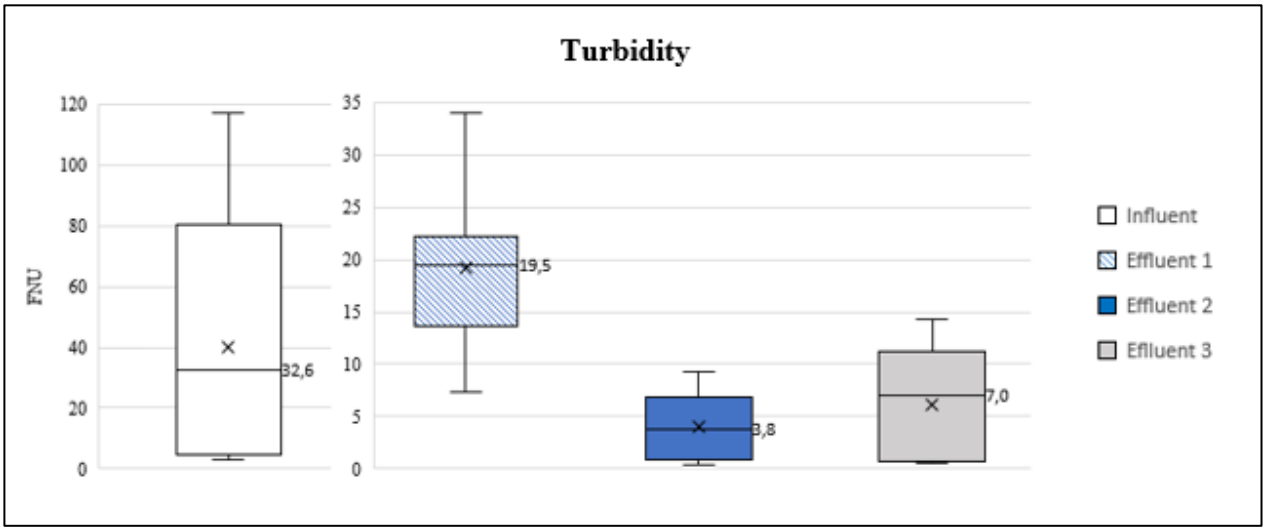

Fig. 18. Results of rinse water turbidity [FNU] analyses

The turbidity of the effluent 1 turbidity most of the values varied between 13.6 to 22.3 FNU (min 7.3, max 34.1 FNU) with an average of 19.3 FNU. Effluent 2 turbidity most of the values varied between 0.8 to 6.8 FNU (min 0.4, max 9.3 FNU) with an average of 3.8 FNU. Effluent 3 turbidity most of the values varied between 0.7 to 11.3 FNU (min 0.5, max 14.2 FNU) with an average of 6.1 FNU. All the turbidity values can be seen in Appendix 2.

Solid particles in the influent and effluent water may block the pipes and there is a risk for clocking, lower pesticides degradation efficiency, hazards related with water disinfection in becoming water treating steps. Submerged particles also help to attach many other toxic compounds like pesticides or heavy metals [51]. Lower turbidity in biofiltration means better performance. This study showed that different influent water turbidities did not have a high effect on effluents. The SSF in column 3 did not have an impact on turbidity as expected, it reduced turbidity, but the activated carbon layer in column 2 had the best performance in decreasing turbidity.

3.2.3. ATP

ATP is a molecule located in the cells of living organisms related to biological functions (food consumption, maintenance, reproduction), in other words, it indicates the level of biomass energy. The bioluminescence method of ATP (adenosine triphosphate) is used to determine the activity and concentration of microorganisms in biological wastewater filtration systems. Method is fast and accrue general microbiology analysis [33].

Between 2021 December 15 and 2022 March 29, ATP in the influent water and effluent in 1st, 2nd and 3rd columns were monitored. All ATP values could be seen in Appendix 3, and the experimental data distribution is in Figure 19.

The ATP value in the rinse water of agricultural machinery is highly different from the ATP concentration in the distributed water in the Netherlands, which is on average distributed between 0,3 and 28 ng/L. Unchlorinated water could have two times higher concentration values of ATP [55]. In influent rinse water most of the values varied between 2874 to 24340 ng ATP/L (min 1428, max 50512 ng ATP/L) with an average of 11728 ng ATP/L.

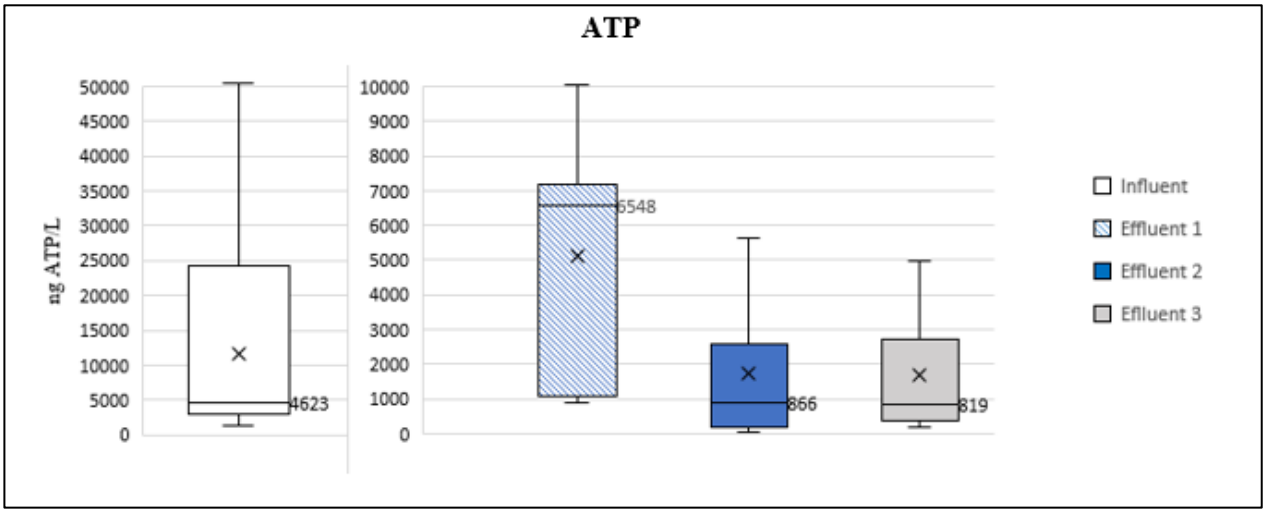

Fig. 19. Results of rinse water ATP analyses

The majority of the values varied between 1056 and 7184 ng ATP/L (min 896, max 10057 ng ATP/L) with an average of 5112 ng ATP/L. Effluent 2 turbidity most of the values varied between 204 to 2577 ng ATP/L (min 36, max 5649 ng ATP/L) with an average of 1725 ng ATP/L. Effluent 3 turbidity most of the values varied between 368 to 2729 ng ATP/L (min 167, max 4956 ng ATP/L) with an average of 1686 ng ATP/L.

Because the biodegradability efficiency process depends on the concentration and activity of the biomass of microorganisms and their nutritional properties [33],, high ATP values in influent water could be affected by the rinsed nitrogen and phosphorus of agricultural machinery that induce microbiological activity. Influent and effluent 1 values are indicated as requiring in corrective action (>1000 ng ATP/L) Table 5. During the period, ATP values were growing and stabilizing in each biofilter (column 1, 2 and 3). Higher ATP values did not increase ATP values in effluent waters, but effluents 1 and 3 followed slightly the ATP concentration curve of influent water. The results also show that AC biofilters (2 and 3) have lower ATP values which by 50% of the values indicated as preventive corrective action (100 to 1000 ng ATP/L) require values (Table 5). Only effluent water 2 corresponded to good control values (<100 ng ATP/L) but only at the beginning of the measuring period. The high concentrations of biomass in the waters can be explained as an indicator of biodegradable compounds of high load [33].

3.2.4. Microbiological activity

In a biofilter, a microbial community is formed that degrades pesticides composed mostly of bacteria and fungi. The main bacterial phyla in different biofilters were determined of: *Proteobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Verrucomicrobia, Firmicutes,* and *Gemmatimonadetes*. Fungal groups were mostly dominant by the class of *Dothideomycetes, Hypocreales* and *Sordariales*. [56] In this study, exact bacterial and fungal colonies were not determined. The concentrations of bacteria and microbiological changes in water treatment and distribution are mainly monitored by determining the number of heterotrophic plates in the solid-agar medium [55].

2022 March 11, microbiological activity in the influent water and effluent in 1st, 2nd and 3rd columns was monitored on solid agar media (see Figure 20).

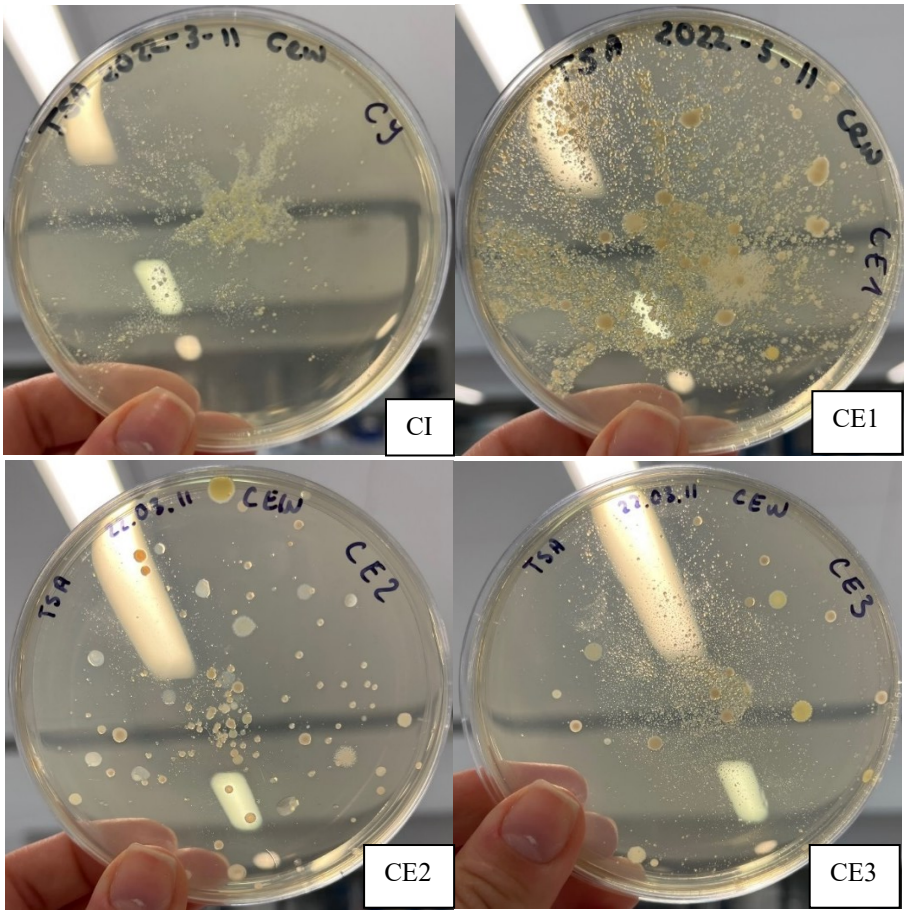

Fig. 20. Results of rinse water microbiological analyses

The growth of biofilms was visible and confirmed in heterotrophic agar plates, where the results matched the liquid phase measurements of ATP [57]. Differences could be explained by AC and SSF media under the substrate layers in column 2 and 3. Filtration media ensures the cessation of bacterial penetration.

3.2.5. DOC

DOC - dissolved organic carbon – measurement that could be used to define the biological stability threshold levels is an important focus of the experiment [33]. Changes in DOC are usually used as substrate-independent indicators to verify the final biodegradation of chemical substances [58].

In water recycling, DOC is a problem when it provides a precursor to the formation of disinfection by-products or when it provides a source of carbon/energy for microorganisms and biofilms. In treated effluent waters DOC is a measurement of the organic compounds that remain after consuming easily biodegradable compounds. DOC also produces many by-products by chemical reactions with disinfectants. At biofilters, it is important to ensure for bacteria (especially those that live in biofilms) carbon and energy [59].

Determined water DOC values of surface freshwater at lakes were found from 1.6 to 7.5 mg C/L at creeks from 7.0 to 15 mg C/L [60].

Between 2021 December 30 and 2022 March 29, DOC in the influent water and effluent in 1st, 2nd and 3rd columns were monitored. Because of DOC device fault between 2022 Jan 20 - 2022 Feb 07 no measurements were made for more than 2 weeks. All DOC values can be seen in Appendix 4, and the experiment data distribution is shown in Figure 21.

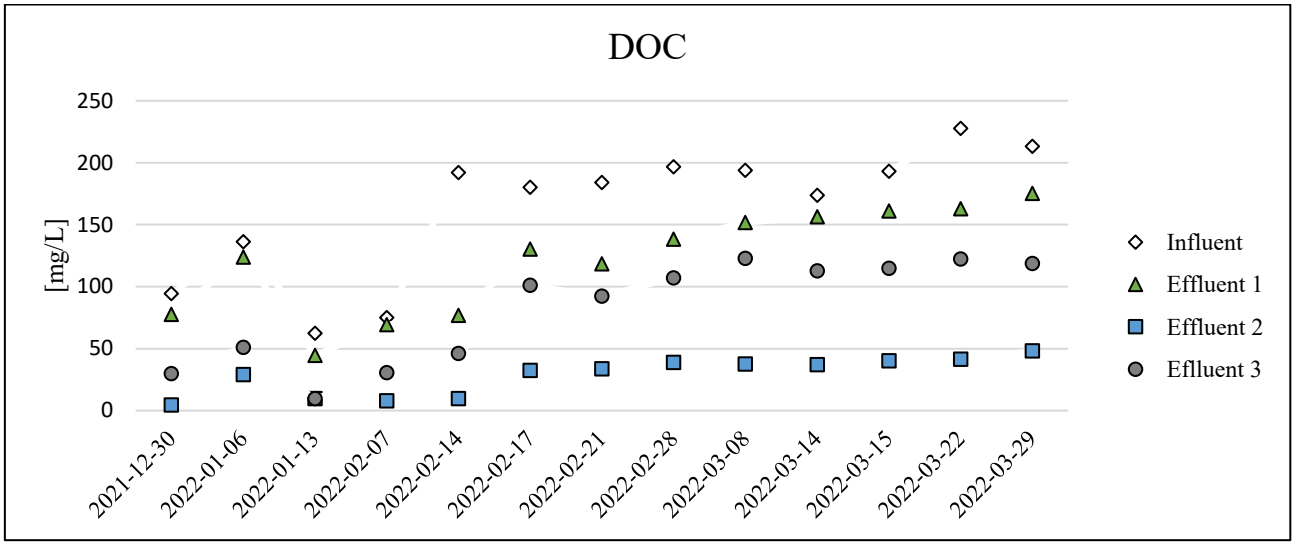

Fig. 21. Results of rinse water DOC analyses values [mg/L]

The influent concentration of DOC varied between 62.2-227.7 mg/L. Effluent 1 from 44.5 to 175.2 mg/L; Effluent 2 - 4.5-48.0 mg/L; Effluent 3 - 9.6-122.8 mg/L. There is a tendency achieve higher DOC values in effluent waters when influent has a higher value.

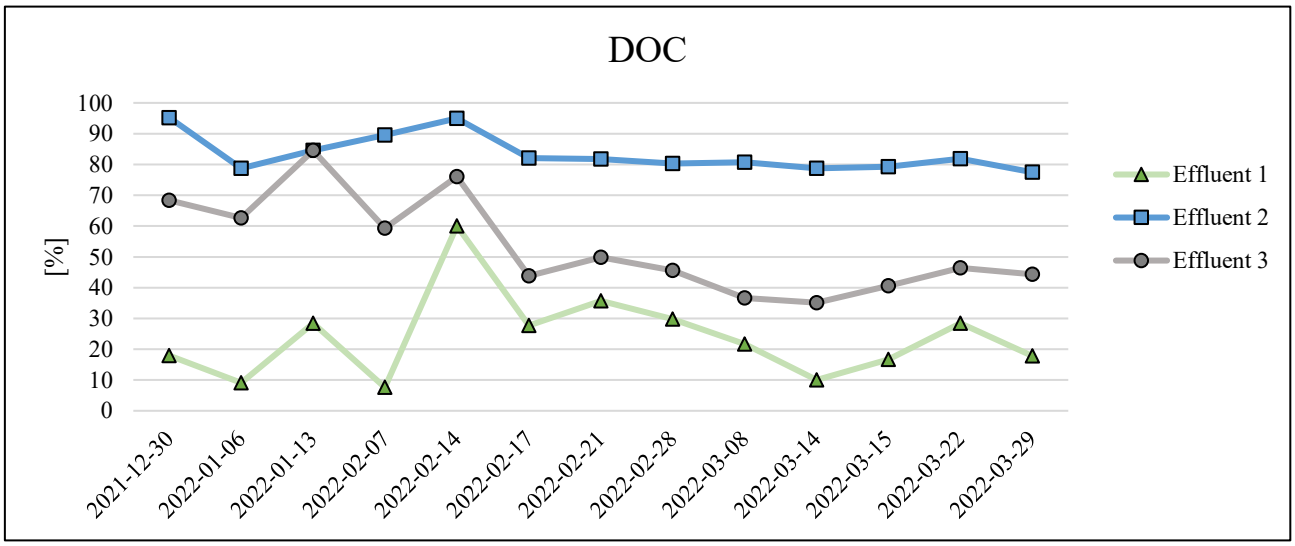

Fig. 22. Results of rinse water DOC removal efficiency [%]

DOC removal efficiency shown (Figure 22), that in column 2 with AC layer dissolved organic carbon removal is highest so microbiological activity in this column is greatest by its usage of oxygen. It is normal, because studies have shown that activated carbon increases DOC removal [61] [62] [59]. AC filters achieve much greater DOC elimination than sand filters, due to the wide surface area of AC with the ability of DOC to bind organic compounds until they are biodegraded [59].

3.2.6. Correlation of biofilter parameters

The correlations between the measured parameters were compared. Some relations between influent water parameters with effluent water were found in each column.

Strong correlation between Influent and effluent 1 water were found on: Influent pH and effluent pH; Influent turbidity and DOC between effluent DOC. Additionally, a medium correlation was detected - the pH of effluent 1 was affected by Influent ATP and DOC. There were also found correlation

between inner effluent 1 water parameters between effluent pH and its DOC measurements (see Figure 23, more information is in Appendix 5).

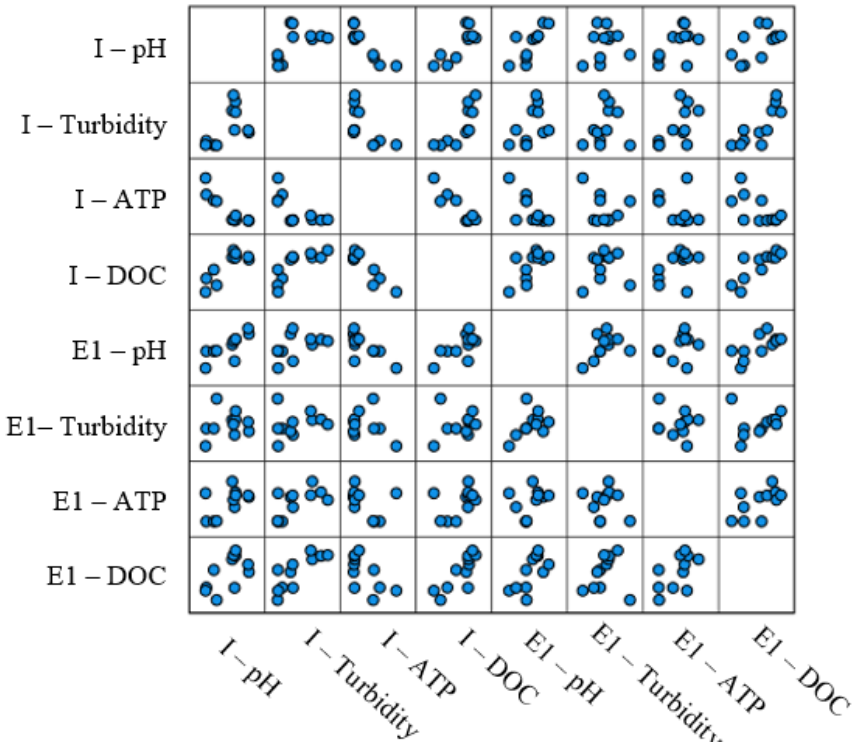

Fig. 23. Relations between influent and effluent 1 water parameters

The medium correlation between influent pH and effluent 2 turbidity, ATP, and DOC was determined. Likewise, a medium correlation was found between effluent 2 pH with influent ATP and DOC.

Influent turbidity significantly strongly correlated with effluent 2 turbidity, ATP, and DOC. There was also strongly significant difference recorded between influent DOC parameters and effluent 2 turbidity, ATP, and DOC parameters as well as between influent and effluent pH.

Also, it was noticed that in general in column 2 measured parameters were more dependent on each other. Medium correlation on pH and turbidity and strong correlation on pH and DOC; Turbidity and ATP, DOC; ATP and DOC (see Figure 24, more information is in Appendix 5).

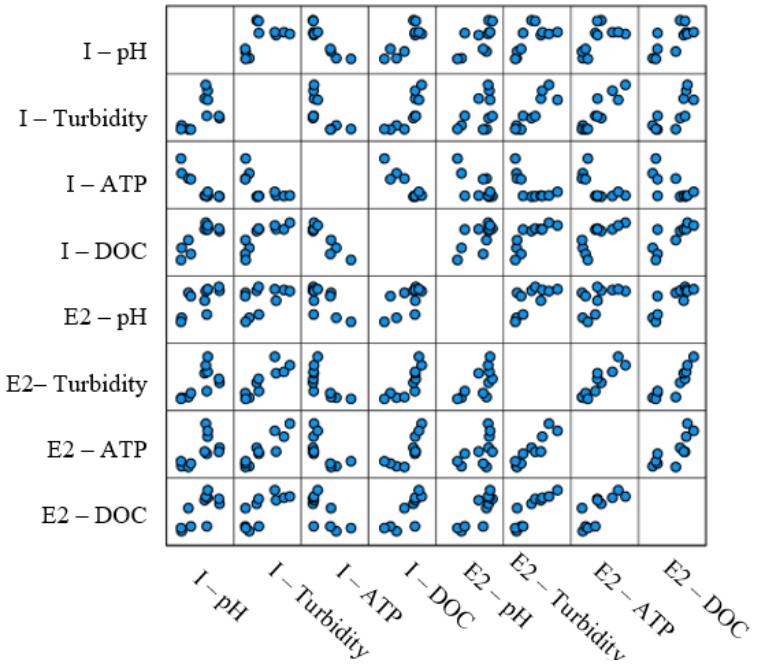

Fig. 24. Relations between influent and effluent 2 water parameters

Medium correlation between Influent and effluent 3 water were found on: influent pH and effluent pH, turbidity, ATP values. Furthermore, medium relations were recognized on influent turbidity and DOC with effluent ATP. Influent ATP had medium negative correlations with effluent pH and ATP. Significantly strong relations were determined between Influent turbidity and effluent 3 turbidity, DOC and vice versa, including influent and effluent DOC ‘s.

Internal water parameters in column 3 showed medium correlation between ATP and DOC. Significantly strong relations between turbidity and ATP, DOC (see Figure 25, more information is in Appendix 5).

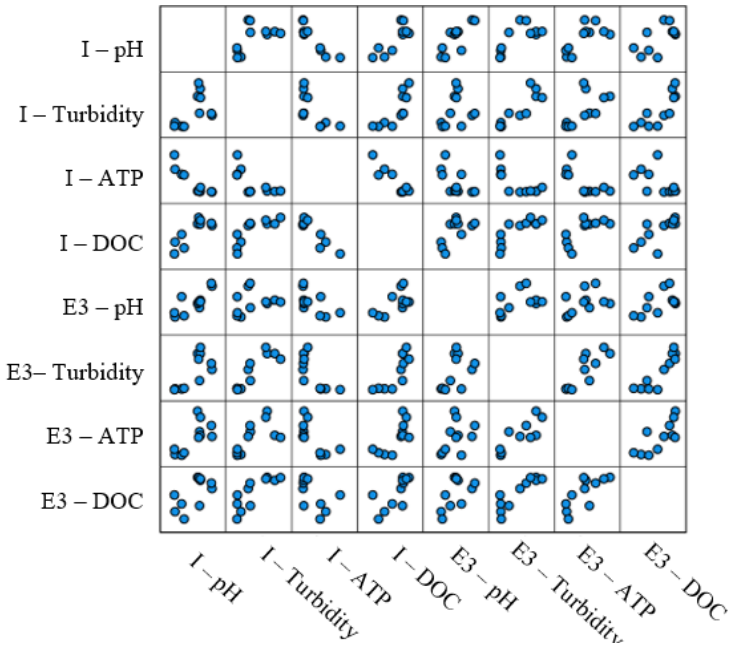

Fig. 25. Relations between influent and effluent 3 water parameters

Not only did the different water parameters better match the conditions for the microorganisms optimal conditions in column 2, but also the correlation analysis showed that the relationships between the parameters are stronger and more dependent on each other in column 2.

It is captured, that effluent DOC in all three columns were significantly high affected of influent turbidity and DOC levels.

In general column 2 and 3 shows the best conditions for microorganism activity. They are more stable in all measured parameters, which gives more stable abient for biodegradation processes.

Could be noted that in all effluent water correlations with effluent high relations were captured in influent pH with influent ATP; Influent turbidity and influent DOC; Effluent turbidity and efluent DOC; Influent turbidity and efluent DOC; Influent DOC and efluent DOC. According to these correlations, decisions for future measurements necessity could be disscused.

3.3. Pesticides characteristics

133 different pesticides were analyzed in rinse wastewater. Representative concentration results of 14 pesticides were determined in influent and effluent waters that significantly exceed the detection limits to receive representative results. These pesticides were: Metolachlor, 2,6-dichlorobenzamide, Boscalid, Dimethenamid, Epoxiconazole, Fluopicolide, Flutolanil, Lenacil, Mesosulfuron-methyl, Tebuconazole, Fluxapyroxad, Metsulfuron-methyl, Pyroxsulam and Flonicamid [63]. See those pesticides parameters in the table below.

Table 8. Properties of the detected pesticides in rinse water

No.	Pesticide name	Molecular formula	DT_{50} (days)	log Kow	Type	Ecotoxicity
1	Metolachlor	$C_{15}H_{22}ClNO_2$	15	3.13	Herbicide	Moderately
2	2,6-dichlorobenzamide	$C_7H_5Cl_2NO$	1194	0.38	Metabolite	Moderately
3	Boscalid	$C_{18}H_{12}Cl_2N_2O$	484	2.96	Fungicide	Moderately
4	Dimethenamid	$C_{12}H_{18}ClNO_2S$	13	2.20	Herbicide	Moderately
5	Epoxiconazole	$C_{17}H_{13}ClFN_3O$	354	3.30	Fungicide	High
6	Fluopicolide	$C_{14}H_8Cl_3F_3N_2O$	271	2.90	Fungicide	Moderately
7	Flutolanil	$C_{17}H_{16}F_3NO_2$	400	3.17	Fungicide	Moderately
8	Lenacil	$C_{13}H_{18}N_2O_2$	50	1.69	Herbicide	Moderately
9	Mesosulfuron-methyl	$C_{17}H_{21}N_5O_9S_2$	44	-0.48	Herbicide	Moderately
10	Tebuconazole	$C_{16}H_{22}ClN_3O$	365	3.70	Fungicide	High
11	Fluxapyroxad	$C_{18}H_{12}F_5N_3O$	183	3.13	Fungicide	High
12	Metsulfuron-methyl	$C_{14}H_{15}N_5O_6S$	23	-1.87	Herbicide	Moderately
13	Pyroxsulam	$C_{14}H_{13}F_3N_6O_5S$	3	-1.01	Herbicide	Moderately
14	Flonicamid	$C_9H_6F_3N_3O$	1	-0.24	Insecticide, Aphicide	Moderately

Metolachlor, an organic herbicide that is used for weed monitoring in various areas. Emulsifiable concentrate usually is an active ingredient.

2,6-dichlorobenzamide - the product of chemical transformation.

Boscalid is a fungicide that acts against a wide range of plant pathogens, including vegetables and other plants. Usually supplied as a soluble granule mixed with water and spray.

Dimethenamid - a herbicide used in a crop to control a variety of broad-leaved grasses and weeds.

Epoxiconazole, a broad spectrum fungicide that is effective against Ascomycetes, Basidiomycetes, and Deuteromycetes-causing diseases. A soluble concentrate that is mixed with water and applied as a spray is a supply method.

Fluopicolide, a fungicide that is effective against downy mildew and blight or other Oomycete diseases. Supplied as dispersible granules that were mixed with water and applied as spray.

Flutolanil, a fungicide that is effective against a broad spectrum of pathogens including Rhizoctonia spp. in rice and other crops. Often supplied as concentrates that are mixed with water and used as a spray or as dry powder and used for treating potato tubers and other seeds.

Lenacil - a herbicide used in beet and other fields to control weed. Wettable powder and soluble concentrate are the main supply methods.

Mesosulfuron-methyl - A herbicide used after germination to control grasses and weed in grain. An oil suspension is the main supply method.

Tebuconazole, a fungicide used to treat cereals and other field crops from various foliar diseases. An oil in water emulsion or concentrate merged with water and used as a spray is the main supply method.

Fluxapyroxad is an active substance of pesticides used to control a wide spectrum of fungal pathogens in food crops, especially cereals or as a seed dressing. Applied as a foliar spray and supplied as an emulsifiable concentrate.

Metsulfuron-methyl - an organic herbicide that is useful for weed and some grass control in cereals or temporarily unused lands. Wettable or water-soluble granules and dry flowable formulation are its supply methods.

Pyroxsulam, an herbicide that is used after germination to control broad-leaved and annual grasses in cereals. Often supplied as water dispersible granules or oil dispersions.

Flonicamid - an insecticide that effective controls whitefly, aphids, and thrips in various environments such as glasshouses. Available in wettable granules and are mixed with water and used as a spray.

3.3.1. Degradability potential of pesticides

LogKow and DT_{50} were selected to determine pesticides degradability potential.

LogKow is the n-octanol water-participation coefficient of a two-phase system composed of n-octanol and water. LogKow is a concerned indicator of the tendency to adsorb organic compounds into soil and living organism fat. LogKow generally has reverse relations to solubility in water and is directly proportional to the molecular weight of the substance [64]. LogKow for pesticides degradation was taken because some positive correlations were determined in few articles of pesticides degradation [65] [66].

Values of logKow usually are between -3 (highly hydrophilic or soluble in water) and +10 (highly hydrophobic) [67]. Substances with logKow >2.0 are considered hydrophobic. With logKow >5.0 - highly hydrophobic, but high hydrophobility potential could be taken if logKow values are equal or exceed 3. It could be noted that hydrophobic pollutants with great logKow values (>5.0) should be considered of their higher potential bioconcentrate in living organism fatty tissues [68].

Pesticides: 2,6-dichlorobenzamide, Lenacil, Mesosulfuron-methyl, Metsulfuron-methyl, Pyroxsulam and Flonicamid were determined to have low solubility potential (highly hydrophobic logKow ≥ 3). To moderate the solubility potential (logKow >2.0) were categorized: Boscalid, Dimethenamid, Fluopicolide. Metolachlor, Epoxiconazole, Flutolanil, Tebuconazole and Fluxapyroxad were categorized as having high potential of solubility (Hydrophylic logKow < 2.0).

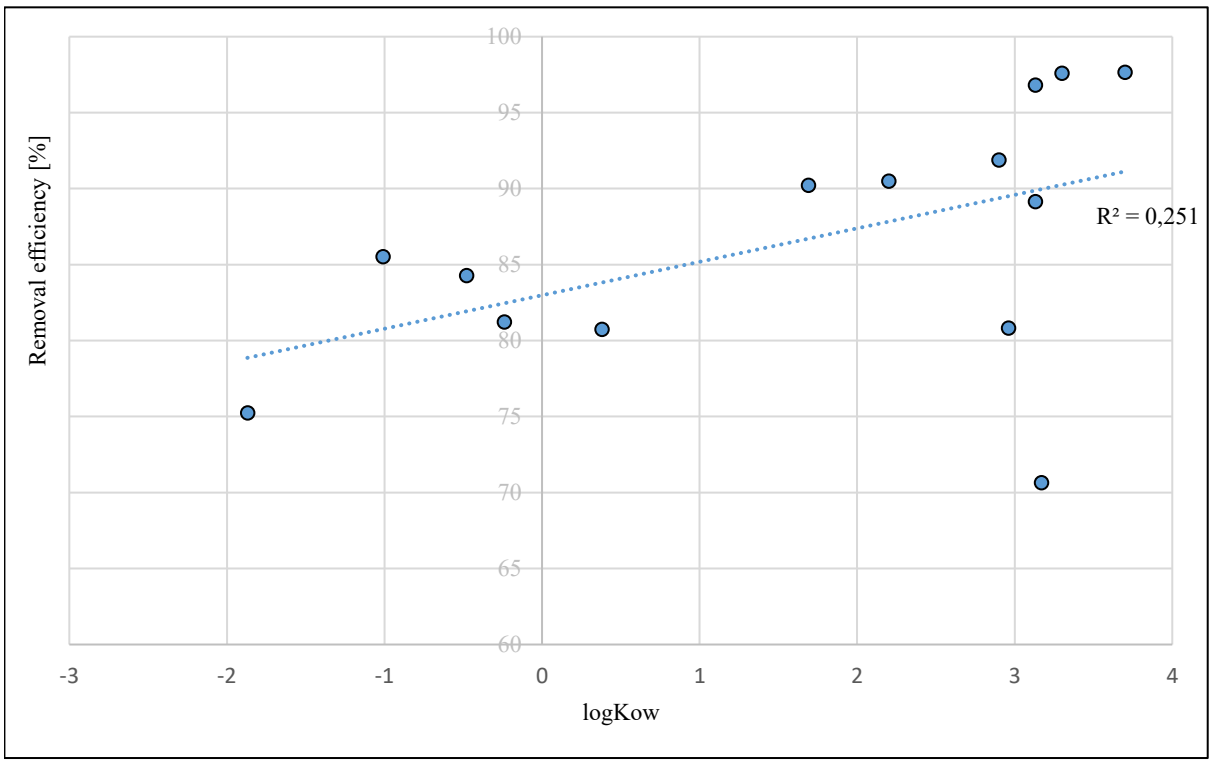

Fig. 26. Relations between pesticides removal efficiency [%] and logKow

Linear regression (Figure 26) did not based on a significant correlation for removal efficiency and hydrophobic or hydrophylic properties of the substance. This was also observed by other authors. In this experiment, future analysis logKow should not be considered as an appropriate parameter to determine the possible degradability level in respect of solubility.

DT_{50} is the time needed for concentration to drop by 50% the original value [69]. The half-life of the chemical substance will help estimate the duration of the chemical substance's life in the aquatic environment [64]. For this experiment DT_{50} for lab at 20 °C in aerobic soil conditions was taken. Because these conditions corresponded with the experimental conditions.

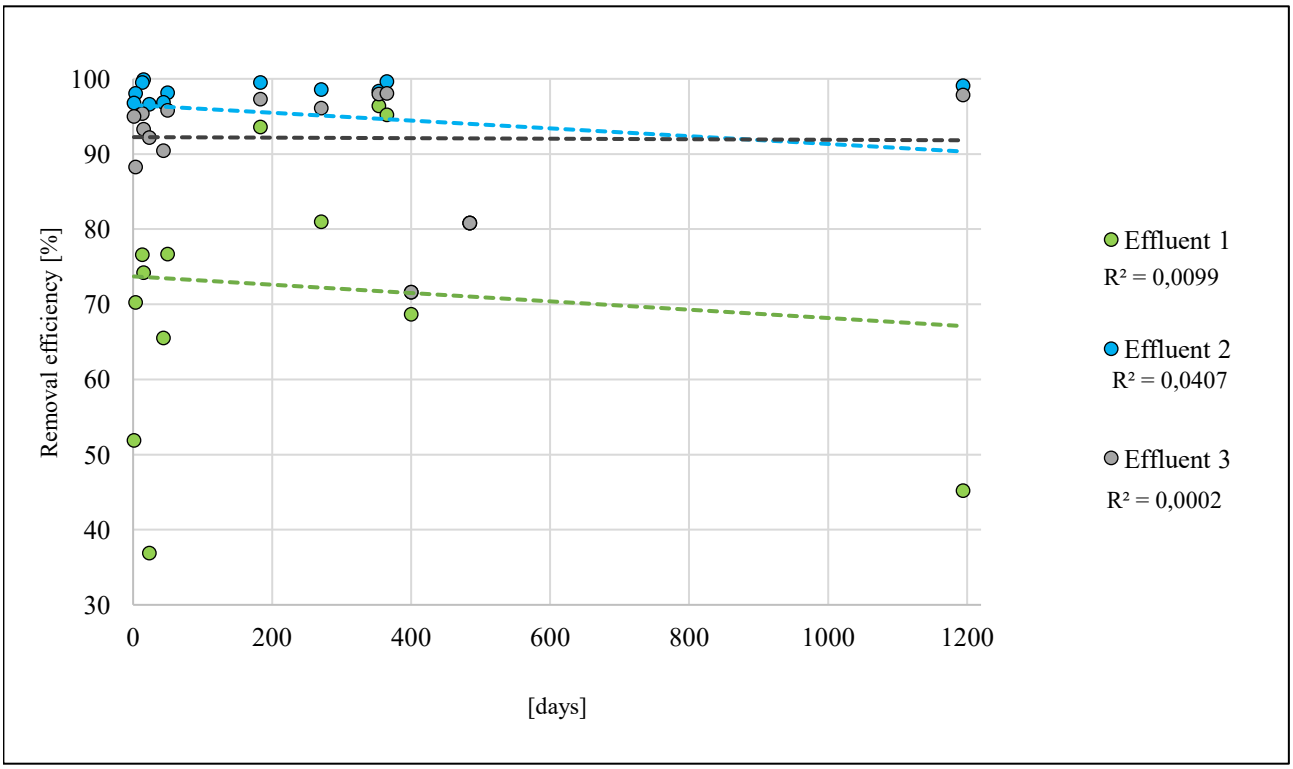

Fig. 27. Relations between pesticides removal efficiency [%] and DT_{50}

In literature reported DT_{50} values did not correspond with an experiment (Figure 27). As also other authors observed, DT_{50} values for all analyzed pesticides in soil were higher than actual pesticides degradation DT_{50} values [18] [70] [20]. This consequence caused by efficient microbial activity, AC and SSF. AC adsorption process in combination of biodegradation had a highest impact on pesticides removal efficiency, which reduced expected DT_{50} values. This study does not identify DT_{50} values in the biomixture, but could be a useful study to have an approximate view on the degradation of contaminants in bioactive soil.

3.3.2. Removal performance of pesticides

Influent water used for the experiment was rinse wastewater from local farm, having typical vegetable crop agricultural contamination. Effluent water from each column was collected every week for month and the most representative samples in each month were taken for pesticides removal performance analysis on the dates of (YYYY-MM-DD): 2021-10-15; 2021-11-11; 2021-12-16 and 2022-01-13.

133 pesticides were detected in rinse wastewater. When the compounds were lower than the method reporting limit (MRL), for effluent instead of zero the reporting limit value was used. However, for further analysis, pesticides with MRL in influent and effluent values were removed. 14 representative pesticides were detected. Removal efficiency of each pesticide reported in Appendix 8.

Column 1, column 2 and column 3 performance was evaluated individually.

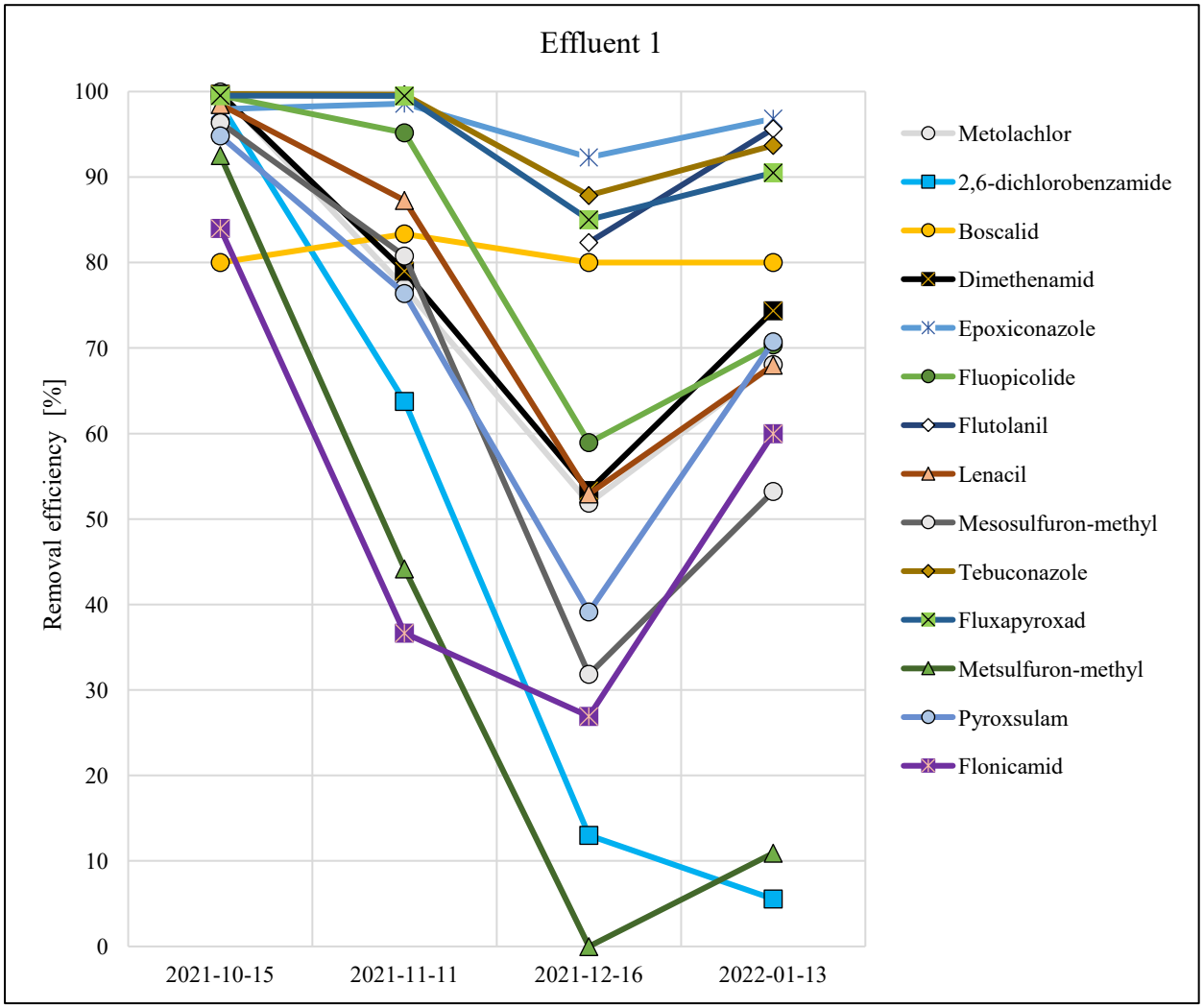

Fig. 28. Column 1 pesticides removal performance [%] by date

The worst average performance removal was detected on Metsulfuron-methyl pesticide at around 37 % (Figure 28). Comparing with other articles in similar conditions its removal was found of 34 % [19]. It could also be noted that it had the lowest logKow value (-1.87) which was expected to be soluble. It is also the only one of the compounds analyzed that had a 0% removal efficiency (on the sampling date).

At the beginning 4 compounds reached 100 % elimination: Dimethenamid, Fluopicolide, Tebuconazole, Fluxapyroxad. Later pesticides removal slightly decreased because of biofilter degradation during time.

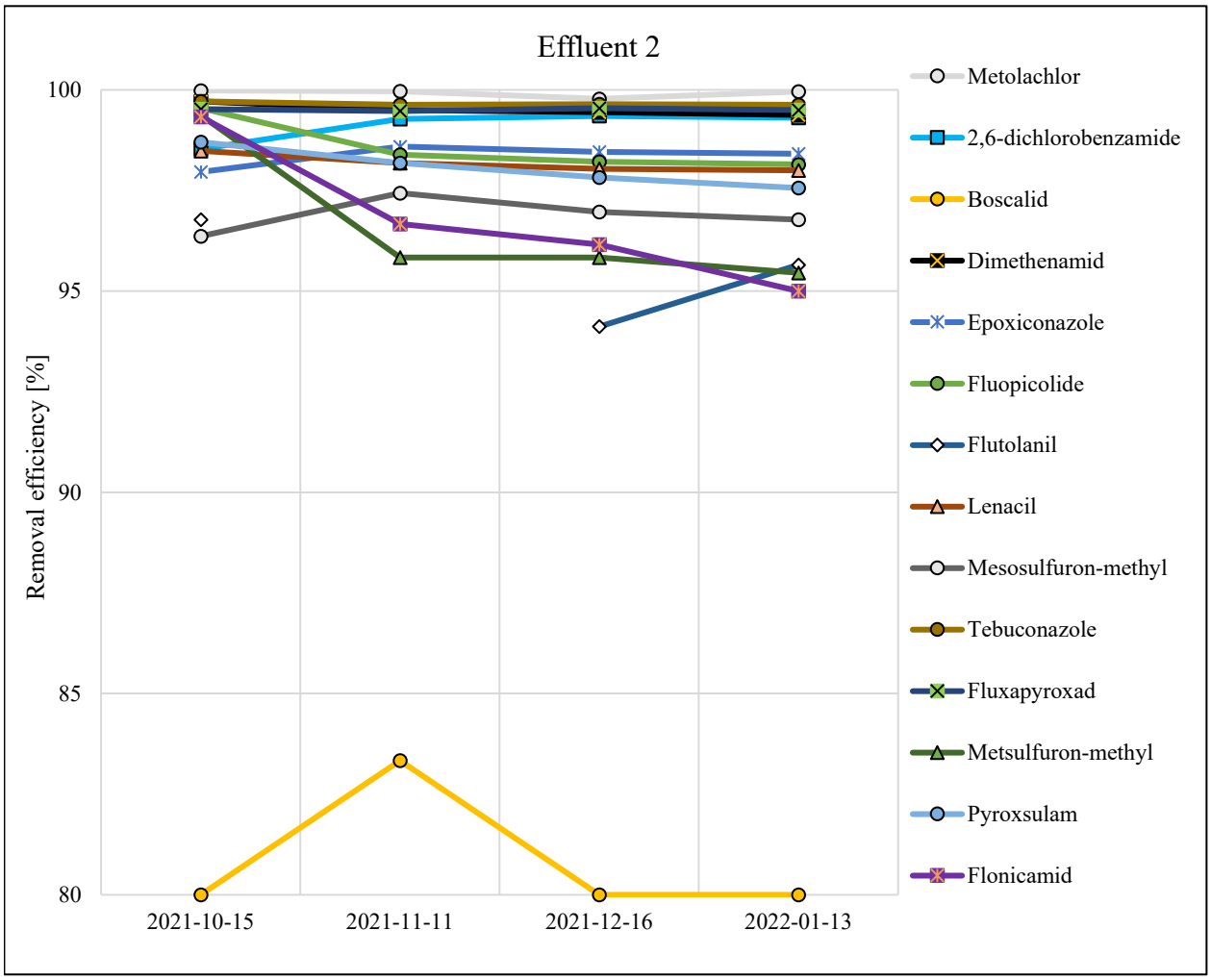

Fig. 29. Column 2 pesticides removal performance [%] by date

In column 2 (Figure 29) pesticides removal varied between 94 and 100 %, with the exception of the Boscalid fungicide. In all 3 columns, it had an average of 81 %. This means that specifically for this compound AC had no influence. Metolachlor and Tebuconazole all the period was removed by 100 % so biofilter degradability had no influence for these pesticides' removal efficiency.

Better column 2 performance is associated with biofilter improvement of the AC layer which absorbs most of the contaminant leftovers after bioactive soil filtration and stops their discharging with effluent water.

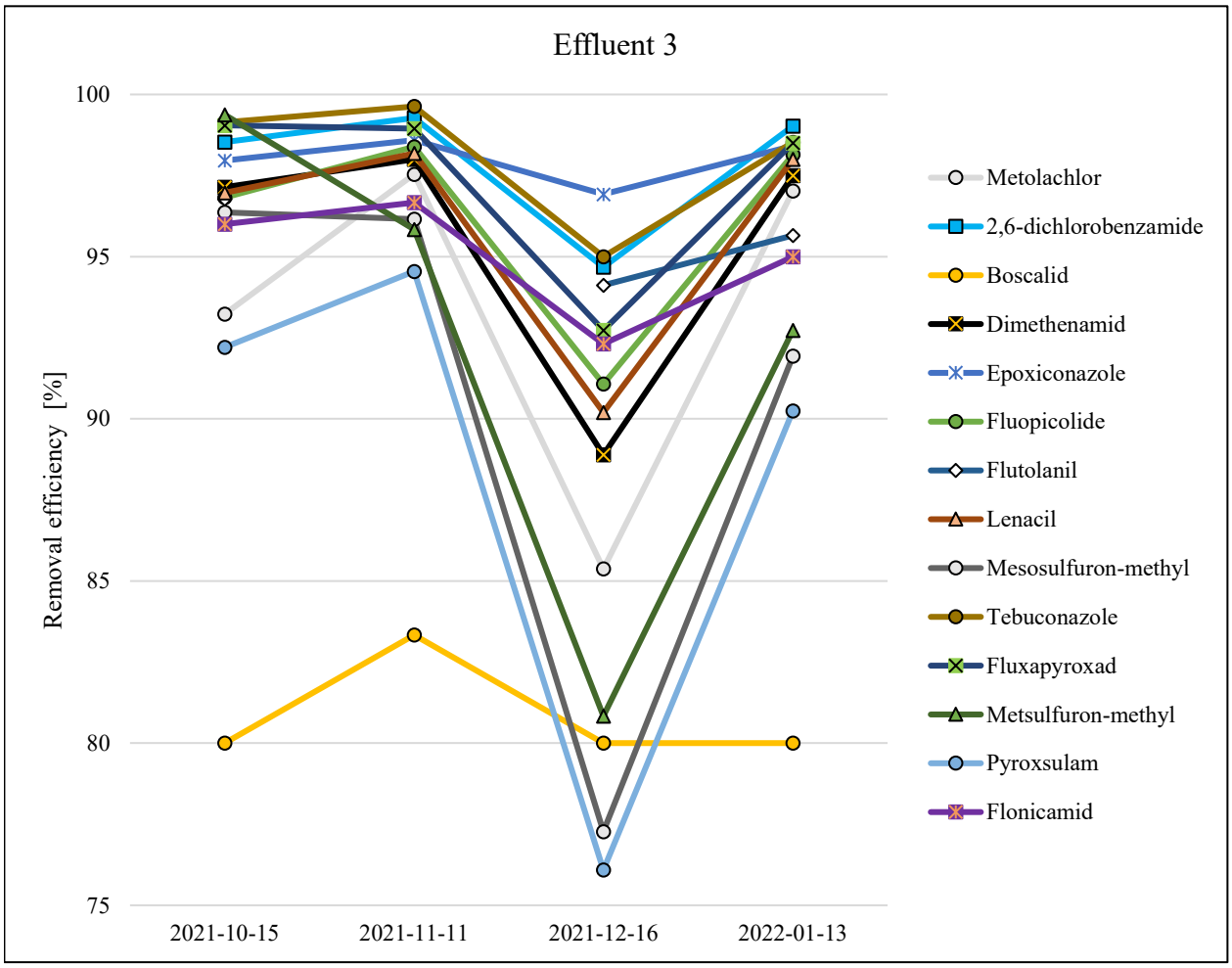

Fig. 30. Column 3 pesticides removal performance [%] by date

Comparison with column 1 and 2, column 3 is the only one which almost never (only once with Tebuconazole) reached removal efficiency of 100 %. The average removal efficiency variated from 88 to 98 %, again with the exception of Boscalid (81%) – Figure 30.

So, results shown that column 3 had a better pesticides removal efficiency than column 1, but lower than column 2.

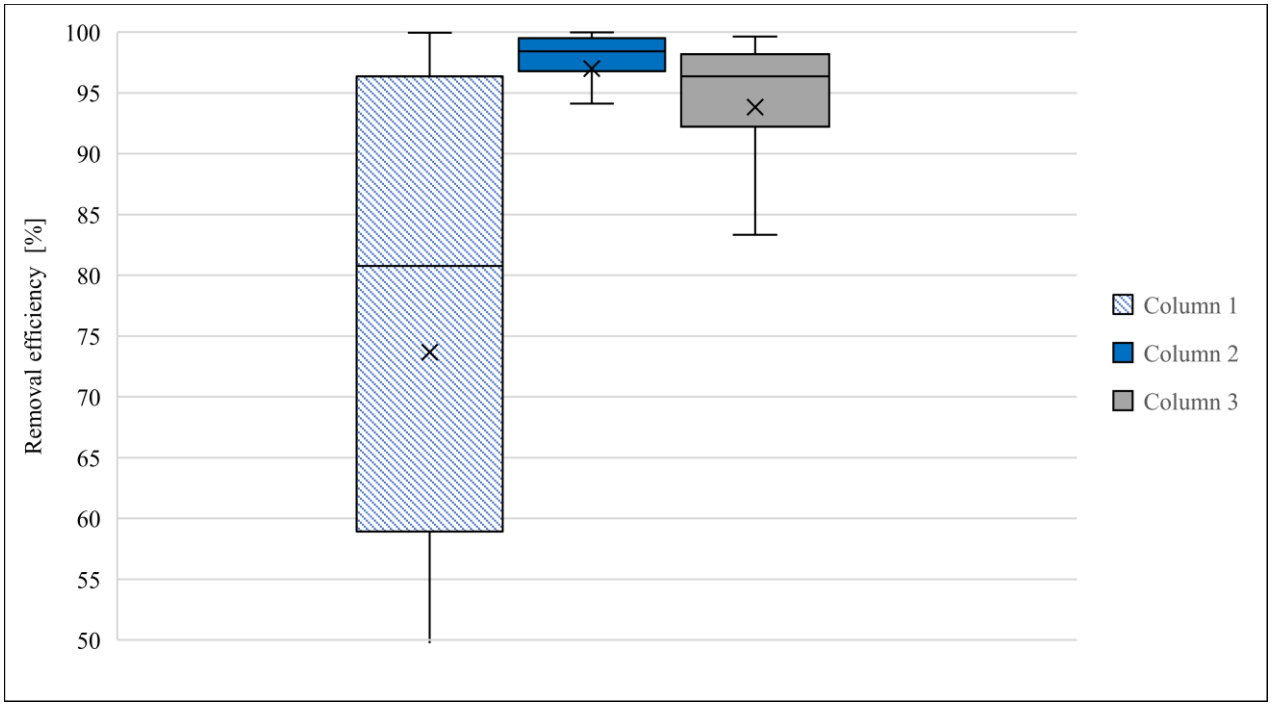

Fig. 31. Column 1, 2 and 3 pesticides removal values [%] distribution

Columns 2 and 3 have different comparable distributions (Figure 31). Half of the results of a lower removal efficiency were distributed differently in all columns. In column 1 between 59 to 81 %, column 2 - 97 and 98 %, column 3 between 92 to 96 %.

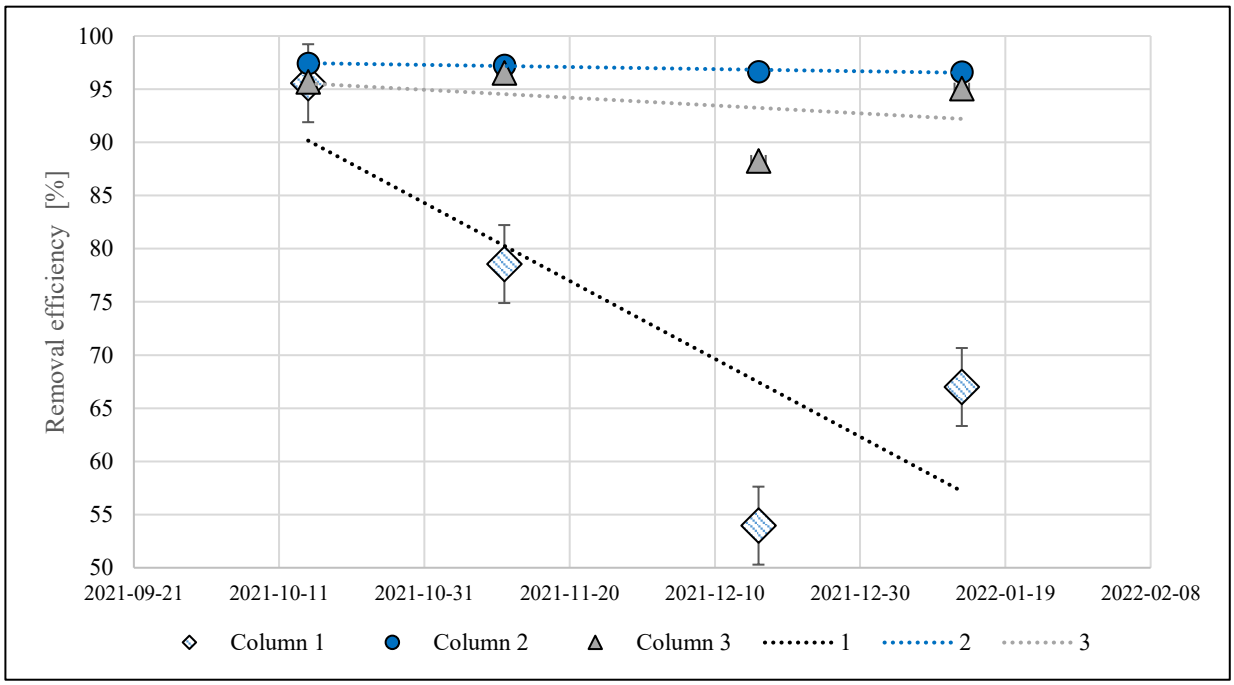

Fig. 32. Biofiltration pesticides degradation tendencies

Column 1 biofilter loses during time that the pesticides removal efficiency decreases faster compared to the other two columns. Even if there is no statistically significant difference between the removal efficiency in columns 2 and 3 at the beginning of the experiment, during the time column 3 the biofilter degrades faster than column 2 (Figure 32).

Conclusions

1. Three different biofilters were compared during an experiment. Biofilter 1 contained biomixture, biofilter 2 was improved by activated carbon layer under the bioactive soil, biofilter 3 had an activated carbon in biomixture and slow sand filter layer under the soil. The results reported in this study confirm that the best conditions for microbiological activity (pH, turbidity [FNU], DOC [mg/L]) were reached with biomixture with activated carbon layer on the bottom (biofilter 2). The ATP values [ng ATP/L] did not differ much between the biomixture with the activated carbon layer on the bottom (biofilter 2) and the biomixture with the activated carbon and slow sand filter layer under the soil (biofilter 3) with averages of 1725 ng ATP/L in biofilter 2 and 1686 ng ATP/L in biofilter 3.
2. Biofiltration efficiency of pesticides removal was improved by implementation of complex biodegradation and adsorption processes in bioreactors.
3. The biomixture systems with an activated carbon layer at the bottom (biofilter 2) and the biomixture with an activated carbon and a slow sand filter layer under the soil (biofilter 3) systems were more robust in the removal of pesticides than the biomixture (biofilter 1). The experimental measurements shows that biofilter with activated carbon layer (biofilter 2) was more effective in pesticide removal from agriculture equipment rinse wastewater than activated carbon in active biomixture (biofilter 3).
4. The biomixture with an activated carbon layer at the bottom (biofilter 2) has the highest and most stable pesticide removal efficiency over a period of 4 months with an average of 95.2 ± 0.65 %, while the biomixture containing the biofilter (biofilter 1) has an average pesticide removal efficiency of 71.9 ± 3.67 % and the biomixture with activated carbon and a slow sand filter layer under the soil (biofilter 3) – 92.1 ± 0.85 %.
5. Activated carbon helps to prevent biofilter from degradation. Filters with activated carbon in a 4-month period degraded on average 0.4 – 2.7 %, while filter without activated carbon degraded on 21.8 %.
6. The aim of this work is to broaden our knowledge of the effect of biomixtures on the elimination of pesticides, by implementing them with activated carbon and slow sand filtration. Investigations of this type of biofiltration technologies have started, for further research in the future.

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Appendices

Appendix 1. pH values

Table 9. Influent and Effluent 1, 2 and 3 water pH values

	Date	Influent	Effluent 1	Effluent 2	Effluent 3
pH	14-12-2021	7.3	7.1	6.9	7.4
	16-12-2021	7.5	7.0	6.8	7.0
	22-12-2021	7.4	7.8	7.3	8.0
	30-12-2021	7.3	-	7.2	7.9
	06-01-2022	7.8	8.1	8.2	8.7
	13-01-2022	7.9	8.1	8.1	7.9
	20-01-2022	7.8	-	7.0	8.0
	27-01-2022	7.3	7.3	7.0	8.0
	14-02-2022	8.9	7.6	7.3	8.3
	21-02-2022	9.7	8.9	8.3	9.2
	28-02-2022	9.7	9.2	8.5	9.3
	08-03-2022	8.8	8.4	7.9	8.4
	15-03-2022	9.0	8.6	8.3	8.6
	22-03-2022	8.9	8.6	8.3	8.5
	29-03-2022	9.0	8.7	8.4	8.5

Appendix 2. Turbidity values

Table 10. Influent and Effluent 1, 2 and 3 water turbidity values [FNU]

	Date	Influent	Effluent 1	Effluent 2	Effluent 3
Turbidity [FNU]	23-12-2021	3.1	12.6	0.8	0.7
	30-12-2021	13.8	-	0.6	0.7
	06-12-2022	4.1	17.2	0.8	0.7
	13-01-2022	3.5	34.1	1.6	0.9
	20-01-2022	5.7	-	8.0	7.8
	27-01-2022	4.6	7.3	0.4	0.5
	14-02-2022	38.2	13.6	2.0	3.4
	21-02-2022	32.6	15.6	3.8	7.0
	28-02-2022	37.5	20.9	4.6	9.0
	08-03-2022	82.0	22.0	5.8	12.2
	15-03-2022	102.0	22.3	6.1	12.2
	22-03-2022	117.0	19.5	7.5	10.4
	29-03-2022	78.7	27.1	9.3	14.2

Appendix 3. ATP values

Table 11. Influent and Effluent 1, 2 and 3 water ATP values [ng ATP/L]

	Date	Influent	Effluent 1	Effluent 2	Effluent 3
Concentration total ATP [ng ATP/L]	15-12-2021	1428	1016	71	439
	22-12-2021	4623	896	36	368
	30-12-2021	31728	-	227	247
	06-01-2022	25134	1006	134	167
	13-01-2022	24340	1095	574	435
	20-01-2021	5694	-	204	208
	27-01-2022	50512	7408	866	819
	03-02-2022	6613	6826	818	575
	14-02-2022	3076	4244	1824	2729
	21-02-2022	2188	6548	2577	2211
	28-02-2022	2584	6885	2071	3393
	08-03-2022	3426	10057	2098	4956
	15-03-2022	2874	7630	3994	2335
	22-03-2022	3505	5883	5649	2110
	29-03-2022	8199	6961	4732	4292

Appendix 4. TC, IC and DOC values

Table 12. Influent and Effluent 1, 2 and 3 water TC, IC and DOC values [mg/L]

	TC - total carbon [mg/L]				IC - inorganic carbon [mg/L]				DOC - dissolved organic carbon [mg/L]			
	I	E1	E2	E3	I	E1	E2	E3	I	E1	E2	E3
2021-12-30	144.9	151.8	74.9	81.2	50.5	74.4	70.4	51.4	94.4	77.4	4.5	29.8
2022-01-06	180.7	190.0	94.9	96.0	44.5	66.2	65.9	45.1	136.2	123.9	29.0	50.9
2022-01-13	110.5	128.1	69.2	58.8	48.3	83.6	59.6	49.2	62.2	44.5	9.6	9.6
2022-02-07	113.2	124.0	70.2	70.0	38.3	55.1	62.4	39.5	74.9	69.2	7.8	30.5
2022-02-14	598.3	237.9	201.8	271.0	406.4	161.2	192.1	225.1	191.9	76.7	9.7	45.9
2022-02-17	550.7	441.1	392.4	423.3	370.6	310.9	360.1	322.2	180.1	130.2	32.3	101.1
2022-02-21	522.9	384.4	388.4	383.6	339.0	266.1	354.8	291.4	183.9	118.3	33.6	92.2
2022-02-28	563.6	454.1	439.9	441.3	366.9	315.9	401.1	334.2	196.7	138.2	38.8	107.1
2022-03-08	576.5	517.7	464.2	517.7	382.8	366.0	426.8	394.9	193.7	151.7	37.4	122.8
2022-03-14	443.3	539.7	434.2	483.3	269.6	383.4	397.2	370.6	173.7	156.3	37.0	112.7
2022-03-15	591.0	555.1	474.9	500.4	397.9	394.2	434.9	385.7	193.1	160.9	40.0	114.7
2022-03-22	637.0	561.1	477.9	517.2	409.3	398.2	436.6	395.1	227.7	162.9	41.3	122.1
2022-03-29	616.1	575.5	479.2	466.1	402.9	400.3	431.2	347.4	213.2	175.2	48.0	118.7

I – influent; E1 – effluent 1; E2 – effluent 2; E3 – effluent 3

Appendix 5. Correlations between influent and effluent 1

Table 13. Influent and Effluent 1 water parameter correlations

			I_pH	I_Turbidity	I_ATP	I_DOC	E1_pH	E1_Turbidity	E1_ATP	E1_DOC
Spearman's rho	I_pH	Correlation Coefficient	1	0.473	-0.900**	0.636*	0.852**	0.241	0.305	0.482
		Sig. (2-tailed)	.	0.142	0	0.035	0.001	0.474	0.361	0.133
	I Turbidity	Correlation Coefficient	0.473	1	-0.5	0.845**	0.419	0.219	0.556	0.791**
		Sig. (2-tailed)	0.142	.	0.117	0.001	0.199	0.518	0.076	0.004
	I_ATP	Correlation Coefficient	-0.90**	-0.5	1	-0.536	-0.724*	-0.123	-0.342	-0.336
		Sig. (2-tailed)	0	0.117	.	0.089	0.012	0.719	0.304	0.312
	I_DOC	Correlation Coefficient	0.636*	0.845**	-0.536	1	0.651*	0.378	0.36	0.882**
		Sig. (2-tailed)	0.035	0.001	0.089	.	0.03	0.252	0.277	0
	E1_pH	Correlation Coefficient	0.852**	0.419	-0.724*	0.651*	1	0.498	0.333	0.620*
		Sig. (2-tailed)	0.001	0.199	0.012	0.03	.	0.119	0.316	0.042
	E1_ Turbidity	Correlation Coefficient	0.241	0.219	-0.123	0.378	0.498	1	0.205	0.428
		Sig. (2-tailed)	0.474	0.518	0.719	0.252	0.119	.	0.544	0.189
	E1_ATP	Correlation Coefficient	0.305	0.556	-0.342	0.36	0.333	0.205	1	0.428
		Sig. (2-tailed)	0.361	0.076	0.304	0.277	0.316	0.544	.	0.189
	E1_DOC	Correlation Coefficient	0.482	0.791**	-0.336	0.882**	.620*	0.428	0.428	1
		Sig. (2-tailed)	0.133	0.004	0.312	0	0.042	0.189	0.189	.

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Appendix 6. Correlations between influent and effluent 2

Table 14. Influent and Effluent 2 water parameter correlations

			I_pH	I_Turbidity	I_ATP	I_DOC	E2_pH	E2_Turbidity	E2_ATP	E2_DOC
Spearman's rho	I_pH	Correlation Coefficient	1	0.473	-0.900**	0.636*	0.818**	0.691*	0.664*	0.682*
		Sig. (2-tailed)	.	0.142	0	0.035	0.002	0.019	0.026	0.021
	I_ Turbidity	Correlation Coefficient	0.473	1	-0.5	0.845**	0.382	0.836**	0.855**	0.764**
		Sig. (2-tailed)	0.142	.	0.117	0.001	0.247	0.001	0.001	0.006
	I_ATP	Correlation Coefficient	-0.900**	-0.5	1	-0.536	-0.636*	-0.564	-0.555	-0.527
		Sig. (2-tailed)	0	0.117	.	0.089	0.035	0.071	0.077	0.096
	I_DOC	Correlation Coefficient	0.636*	0.845**	-0.536	1	0.673*	0.918**	0.800**	0.909**
		Sig. (2-tailed)	0.035	0.001	0.089	.	0.023	0	0.003	0
	E2_pH	Correlation Coefficient	0.818**	0.382	-0.636*	0.673*	1	0.727*	0.582	0.818**
		Sig. (2-tailed)	0.002	0.247	0.035	0.023	.	0.011	0.06	0.002
	E2_ Turbidity	Correlation Coefficient	0.691*	0.836**	-0.564	0.918**	0.727*	1	0.900**	0.955**
		Sig. (2-tailed)	0.019	0.001	0.071	0	0.011	.	0	0
	E2_ATP	Correlation Coefficient	0.664*	0.855**	-0.555	0.800**	0.582	0.900**	1	0.855**
		Sig. (2-tailed)	0.026	0.001	0.077	0.003	0.06	0	.	0.001
	E2_DOC	Correlation Coefficient	0.682*	0.764**	-0.527	0.909**	0.818**	0.955**	0.855**	1
		Sig. (2-tailed)	0.021	0.006	0.096	0	0.002	0	0.001	.

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Appendix 7. Correlations between influent and effluent 3

Table 15. Influent and Effluent 3 water parameter correlations

			I_pH	I_Turbidity	I_ATP	I_DOC	E3_pH	E3_Turbidity	E3_ATP	E3_DOC
Spearman's rho	I_pH	Correlation Coefficient	1	0.473	-0.900**	0.636*	0.727*	0.679*	0.636*	0.418
		Sig. (2-tailed)	.	0.142	0	0.035	0.011	0.022	0.035	0.201
	I_ Turbidity	Correlation Coefficient	0.473	1	-0.5	0.845**	0.255	0.825**	0.664*	0.818**
		Sig. (2-tailed)	0.142	.	0.117	0.001	0.45	0.002	0.026	0.002
	I_ATP	Correlation Coefficient	-0.900**	-0.5	1	-0.536	-0.673*	-0.583	-0.609*	-0.391
		Sig. (2-tailed)	0	0.117	.	0.089	0.023	0.06	0.047	0.235
	I_DOC	Correlation Coefficient	0.636*	0.845**	-0.536	1	0.5	0.852**	0.682*	0.800**
		Sig. (2-tailed)	0.035	0.001	0.089	.	0.117	0.001	0.021	0.003
	E3_pH	Correlation Coefficient	0.727*	0.255	-0.673*	0.5	1	0.378	0.318	0.455
		Sig. (2-tailed)	0.011	0.45	0.023	0.117	.	0.252	0.34	0.16
	E3_ Turbidity	Correlation Coefficient	0.679*	0.825**	-0.583	0.852**	0.378	1	0.802**	0.788**
		Sig. (2-tailed)	0.022	0.002	0.06	0.001	0.252	.	0.003	0.004
	E3_ATP	Correlation Coefficient	0.636*	0.664*	-0.609*	0.682*	0.318	0.802**	1	0.682*
		Sig. (2-tailed)	0.035	0.026	0.047	0.021	0.34	0.003	.	0.021
	E3_DOC	Correlation Coefficient	0.418	0.818**	-0.391	0.800**	0.455	0.788**	0.682*	1
		Sig. (2-tailed)	0.201	0.002	0.235	0.003	0.16	0.004	0.021	.

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Appendix 8. Pesticides removal efficiency [%]

Table 16. Pesticides removal efficiency in Effluent 1 by each experiment month [%]

	Compound	2021-10-15	2021-11-11	2021-12-16	2022-01-13
1	Metolachlor	100.0	77.0	51.9	68.1
2	2,6-dichlorobenzamide	98.5	63.8	13.0	5.6
3	Boscalid	80.0	83.3	80.0	80.0
4	Dimethenamid	99.7	79.0	53.3	74.4
5	Epoxiconazole	98.0	98.6	92.3	96.8
6	Fluopicolide	99.5	95.2	58.9	70.4
7	Flutolanil	96.8	0.0	82.4	95.7
8	Lenacil	98.5	87.3	52.9	68.0
9	Mesosulfuron-methyl	96.4	80.8	31.8	53.2
10	Tebuconazole	99.7	99.6	87.9	93.7
11	Fluxapyroxad	99.5	99.5	85.0	90.5
12	Metsulfuron-methyl	92.5	44.2	0.0	10.9
13	Pyroxsulam	94.8	76.4	39.1	70.7
14	Flonicamid	84.0	36.7	26.9	60.0

Table 17. Pesticides removal efficiency in Effluent 2 by each experiment month [%]

Compound	2021-10-15	2021-11-11	2021-12-16	2022-01-13
Metolachlor	100.0	100.0	99.8	100.0
Epoxiconazole	98.0	98.6	98.5	98.4
Flutolanil	96.8	0.0	94.1	95.7
Tebuconazole	99.7	99.6	99.6	99.6
Fluxapyroxad	99.5	99.5	99.5	99.5
Boscalid	80.0	83.3	80.0	80.0
Dimethenamid	99.7	99.5	99.4	99.4
Fluopicolide	99.5	98.4	98.2	98.1
2,6-dichlorobenzamide	98.5	99.3	99.4	99.3
Lenacil	98.5	98.2	98.0	98.0
Mesosulfuron-methyl	96.4	97.4	97.0	96.8
Metsulfuron-methyl	99.4	95.8	95.8	95.5
Pyroxsulam	98.7	98.2	97.8	97.6
Flonicamid	99.3	96.7	96.2	95.0

Table 18. Pesticides removal efficiency in Effluent 3 by each experiment month [%]

Compound	2021-10-15	2021-11-11	2021-12-16	2022-01-13
Metolachlor	93.2	97.5	85.4	97.0
Epoxiconazole	98.0	98.6	96.9	98.4
Flutolanil	96.8	0.0	94.1	95.7
Tebuconazole	99.1	99.6	95.0	98.5
Fluxapyroxad	99.0	98.9	92.7	98.5
Boscalid	80.0	83.3	80.0	80.0
Dimethenamid	97.1	98.0	88.9	97.5
Fluopicolide	96.8	98.4	91.1	98.1
2,6-dichlorobenzamide	98.5	99.3	94.7	99.0
Lenacil	97.0	98.2	90.2	98.0
Mesosulfuron-methyl	96.4	96.2	77.3	91.9
Metsulfuron-methyl	99.4	95.8	80.8	92.7
Pyroxsulam	92.2	94.5	76.1	90.2
Flonicamid	96.0	96.7	92.3	95.0