Czech University of Life Sciences Prague
Faculty of Agrobiology, Food and Natural Resources
Department of Agro-Environmental Chemistry and Plant Nutrition

Czech University
of Life Sciences Prague

Transfer of Antimicrobials from Soil to Plants

Diploma Thesis

Author: Bc. Agnessa Karavayeva
Supervisor: Ing. Filip Mercl, Ph.D.
Co-Supervisor: Ing. Milena Dulović

Declaration

I declare that the master's thesis was written by me under the supervision of my supervisor. In the text there are references to the sources that I used, they are presented in the section "References".

Prague, 2024

Agnessa Karavayeva

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Transfer of Antimicrobials from Soil to Plants

Summary

The mechanism of transfer of antimicrobials from soil to plants is a complex process involving various physicochemical and biological interactions. The interaction between plant roots and soil particles plays a key role in the transfer of antimicrobials from soil to plants. Some antimicrobials can form complexes with organic matter in soil, which may increase their resistance to transfer to plants. The influence of environmental factors such as soil temperature, moisture and pH can also have a significant impact on the mechanisms of antimicrobial transfer from soil to plants. Understanding the mechanisms of antimicrobial transfer from soil to plants is important for assessing the human health and environmental risks associated with the use of antimicrobials in agriculture.

The research examined the impact of two irrigation solutions: one containing a blend of antimicrobial compounds and preservatives (climbazole, clindamycin, triclosan, methylparaben, ethylparaben, propylparaben, and butylparaben), and the other with a mixture of antimicrobial compounds, preservatives, pharmaceuticals, and plasticizers on the zucchini *(Cucurbita pepo L.)* plant.

According to the results obtained, the application of biochar into the soil could prevent the uptake of antimicrobial substances, but not of additional pollutants, the uptake of which, on the contrary, biochar could enhance. Also, the application of biochar in the soil significantly increased the plant biomass and the amount of water consumed. The presence of additional micropollutants had little effect on the uptake of antimicrobials by the plant, as most of the antimicrobials were not found in the stems, leaves, and fruits. It can also be assumed that the application of pollutants and antimicrobials, without additional nutrient medium like biochar, has a strong effect on plant biomass, number of fruits and quality of the plant, significantly deteriorating its condition.

Keywords:
zucchini; biochar; triclosan; climbazole; parabens; absorption; bioaccumulation

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1 Introduction

Detection of many emerging chemicals of concern, including antimicrobials and steroid hormones, in the environment has increased in the past decade with the advancement of analytical techniques. There are several potential sources of these inputs, including municipal wastewater discharge, municipal biosolids, pharmaceutical production, and agriculture-related activities (Lee et al. 2007).

Some pollutants have been recognized as chemicals of emerging concern because they are widespread in the environment and have the potential adverse effects on non-target organisms and humans (e.g., endocrine disruption and preservation of antibiotic resistance) (Kumar et al. 2020).

Conventional wastewater treatment processes cannot effectively remove all pollutants from the influents, leaving the pharmaceuticals in the effluents at the levels of ng/L to low µg/L (Wydro et al. 2023) and in biosolids at µg/kg to low mg/kg (dry weight) (Mejías et al. 2021).

Sizeable amount of research has been conducted on the possible absorption of antimicrobials by plants from contaminated soil and water used for irrigation of crops. In most cases, pollutants are taken by roots and translocated into various tissues by transpiration and diffusion. Due to the plant absorption, the occurrence of pharmaceuticals in food sources such as vegetables is a public concern (Zhao et al. 2019).

Pollutants can be transferred from soil to plants through several mechanisms. In order for compounds to be absorbed and transported within the plant, various factors must be considered, including the physicochemical properties of the compounds, the physiology of the plant, and environmental factors (Su & Zhu 2007).

2 Scientific hypotheses and thesis objectives

2.1 Hypotheses

If biochar is utilized as a growing medium for zucchini plants, it is expected to decrease the absorption of antimicrobials by the plants.

The absorption of antimicrobials by plants is anticipated to be influenced by the presence of other micropollutants in irrigation water.

2.2 Experiment objectives

To investigate whether antimicrobials influence courgetti growth and to determine whether biochar favors the uptake of antimicrobials.

To investigate whether the presence of several micropollutants affects their accumulation in biomass and to assess the safety of biomass for consumption.

3 Literature Review

Agriculture is the world's largest consumer of the world's fresh water (Mishra 2023). The increase of the water use efficiency (WUE) in agricultural systems, defined as the yield obtained per unit of water applied, it is possible with appropriate irrigation scheduling (Shaheen et al. 2016).

Antimicrobials are substances employed to prevent, control, and treat infectious diseases across human, animal, and plant populations. This category encompasses antibiotics, fungicides, antiviral agents, and parasiticides. Additionally, disinfectants, antiseptics, various pharmaceuticals, and natural products may possess antimicrobial properties.

Pharmaceuticals and personal care products (PPCP) are compounds with special physical and chemical properties that address the care of animal and human health. Several pharmaceuticals and personal care products (PPCP) present potential concerns at environmentally relevant concentrations. Pharmaceuticals and personal care products (PPCP) mixtures may produce synergistic toxicity. Various methods have been used for the ecological risk assessment of pharmaceuticals and personal care products (PPCP) in aquatic systems. There are similarities in these methods, but no consensus has emerged regarding best practices for the ecological risk assessment of these compounds. Human health risk assessments of PPCP contamination in aquatic systems have generally indicated little cause for concern (Cizmas et al. 2015).

Pharmaceuticals and personal care products (PPCPs) have drawn wide attention in the past decade as a group of emerging organic contaminants due to their extensive use and potential harmful effects on human health and the environment. PPCPs include various prescription and non-prescription drugs for the treatment or prevention of human and animal diseases, fragrances, and disinfectants added to personal care products (such as soaps, shampoos, and sunblocks), as well as many other chemicals (Gros et al. 2010). These chemicals and their bioactive metabolites enter the environment through treated or untreated wastewater discharged from households, pharmaceutical factories, animal husbandry systems, aquaculture, and hospitals (Xie et al. 2019).

3.1 Environmental pollution from pharmaceuticals and personal care products (PPCPs)

Pharmaceuticals and personal care products (PPCPs) are a unique group of emerging environmental contaminants, due to their inherent ability to induce physiological effects in human at low doses. An increasing number of studies has confirmed the presence of various PPCPs in different environmental compartments, which raises concerns about the potential adverse effects to humans and wildlife (Ebele et al. 2017).

Regarding the environmental contamination with pharmaceuticals, the European Commission has admitted that "pollution of waters and soils with pharmaceuticals is an emerging environmental issue and also a critical concern for public health", but there are no standards regulating discharges of antibiotics from different sources (Polianciuc et al. 2020).

Antimicrobials are compounds utilized for their biological activity, frequently excreted without alteration and capable of entering the environment. In developed nations, pharmaceutical concentrations in aquatic environments typically fall within similar ranges (μg L−1 and below). Nevertheless, it remains uncertain whether this pattern extends to less-developed countries as well (Kümmerer 2010). In recent decades, the production and consumption of antimicrobials products have rapidly increased with the development of medicine. Approximately 3,000 compounds are used as pharmaceuticals, and the annual production quantity exceeds hundreds of tons (Carvalho & Santos 2016; Ortúzar et al. 2022).

Anti-inflammatory drugs, antibiotics, and analgesics are the most common drugs used around the world. Consequently, the emergence of water-soluble and pharmacologically active organic micropollutants or antimicrobials has gained much attention worldwide. Humans use a variety of these pharmaceuticals for their health in everyday life, but large quantities of these drugs are also used as veterinary medicine on farms around the world, to prevent and treat animal diseases and to increase economic benefits in intensive livestock (Guerra et al. 2014; Patel et al. 2019; Ortúzar et al. 2022).

Pollutants found in high concentrations in wastewater include non-steroidal anti-inflammatory drugs (NSAIDs), β-blockers and psychoactive compounds, analgesics, antibiotics, endocrine disruptors, antiretroviral drugs, and drugs to treat cancer. These are the pollutants most detected due to the analytical methods available and their resolution, although new methods for identifying these compounds are increasingly being developed (Ortúzar et al. 2022). PPCPs enter the soil through irrigation with contaminated water, sludge application, and animal manure. This contamination can affect soil microorganisms and plants, altering ecosystem functions (Ebele et al. 2017) . One of the critical concerns is the development of antibiotic-resistant bacteria due to the presence of antibiotics in the environment. This resistance can transfer to pathogenic bacteria, posing a significant public health risk (Polianciuc et al. 2020).

3.1.1 Sources of pollutants in the ecosystem

Environmental pollution from antimicrobials is a growing concern worldwide, impacting ecosystems and posing potential risks to human health. Antimicrobials pollution primarily originates from the disposal of unused or expired medications, industrial manufacturing processes, and excretion after human and animal use. Hospitals, households, and agricultural runoff are significant contributors (Wang et al. 2023).

Antimicrobials enter the environment and subsequently soil and plant systems through several pathways. Understanding these sources is crucial for managing and mitigating their impact (Xiao et al. 2023). More information about ways of contamination with pharmaceuticals can be seen in the Figure 1. Pollutants are often released into bodies of water, including rivers, lakes, and groundwater. Conventional wastewater treatment plants are unable to completely remove all contaminants, resulting in their release into treated water. Contaminant transfer may happen through various pathways such as irrigation water containing contaminants, the application of contaminated manure or biosolids as fertilizers, or direct deposition of dust particles onto plants. Additionally, contaminant transfer can occur between plants, either through pollen or direct contact (Samal et al. 2022; Aguilar-Aguilar et al. 2023).

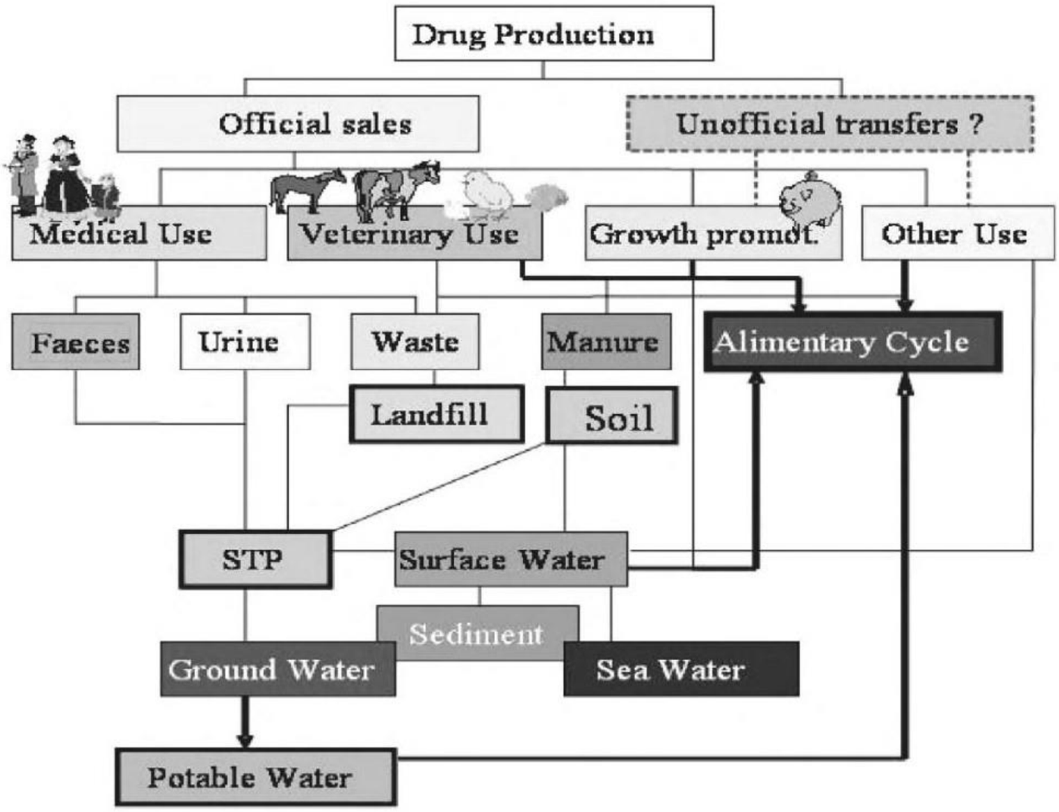


Figure 1 Routes of distribution of Antimicrobials into the environment (Kümmerer 2010).

After consumption, humans excrete antimicrobials and their metabolites, which enter the sewage system. Not all these compounds are fully metabolized in the body or removed during wastewater treatment, leading to their release into the environment (Kortesmäki et al. 2020).

The industrial manufacturing of antimicrobials can result in environmental contamination, particularly when waste products are not properly treated prior to disposal. This encompasses both direct releases into water bodies and inadvertent leaks or spills. The disposal of unused or expired medications through household drains or toilets also contributes to pharmaceutical contamination. Such substances may circumvent wastewater treatment procedures and enter the environment directly (Musoke et al. 2021).

3.1.2 Adverse health and environmental consequences of pollutants

Antimicrobial contamination and antimicrobial resistance have become global environmental and health problems. Many antimicrobials are used in medical and animal husbandry, leading to the continuous release of residual antimicrobials into the environment. It not only causes ecological harm, but also promotes the occurrence and spread of antimicrobial resistance. The role of environmental factors in antimicrobial contamination and the spread of antimicrobial resistance is often overlooked (Wang et al.

2023). The Figure 2 provides information about all pharmaceuticals and personal care products (PPCPs) and their effect on the endocrine system. Their presence, even in trace amounts, can disrupt ecosystems and potentially pose risks to human health. And at low concentrations, pharmaceuticals in water bodies can harm aquatic organisms. They can disrupt endocrine systems, affect reproductive processes, and lead to antibiotic resistance in aquatic microorganisms (Letsoalo et al. 2023). Antimicrobials in water bodies can affect aquatic organisms. For example, exposure to hormones has been shown to disrupt the reproductive systems of fish. Antidepressants can alter fish behavior, and antibiotics can affect the growth and survival of algae and other microorganisms (Biswas et al. 2021). One of the most significant concerns is the contribution to the development of antibiotic-resistant bacteria. When antibiotics enter the environment, they can promote the evolution of resistance in bacterial populations, a major public health threat (Muteeb et al. 2023). Antimicrobials in soil can alter microbial communities, affecting soil health and fertility. This can have knock-on effects on plant growth and soil ecosystem services (De Souza Machado et al. 2019).

Antimicrobials can accumulate in the tissues of plants and animals. This bioaccumulation can lead to higher concentrations in organisms higher up the food chain, including humans, potentially affecting health. Certain pharmaceuticals in the soil can have phytotoxic effects, meaning they can inhibit plant growth and development. This can impact agricultural productivity and biodiversity (Carballo et al. 2022). Some antimicrobials act as endocrine disruptors. They can interfere with the hormone systems of wildlife, leading to problems such as decreased fertility and changes in behavior or development (Guarnotta et al. 2022). The presence of antimicrobials in the environment could potentially lead to decreased efficacy of these drugs for medical use, due to the development of resistance or other adaptive responses in humans and animals (Serwecińska 2020).

Addressing these impacts necessitates a multifaceted approach, encompassing enhanced pharmaceutical waste management practices, the advancement of eco-friendly pharmaceuticals, the implementation of advanced water treatment technologies, and raising public awareness and education regarding proper medication disposal methods.

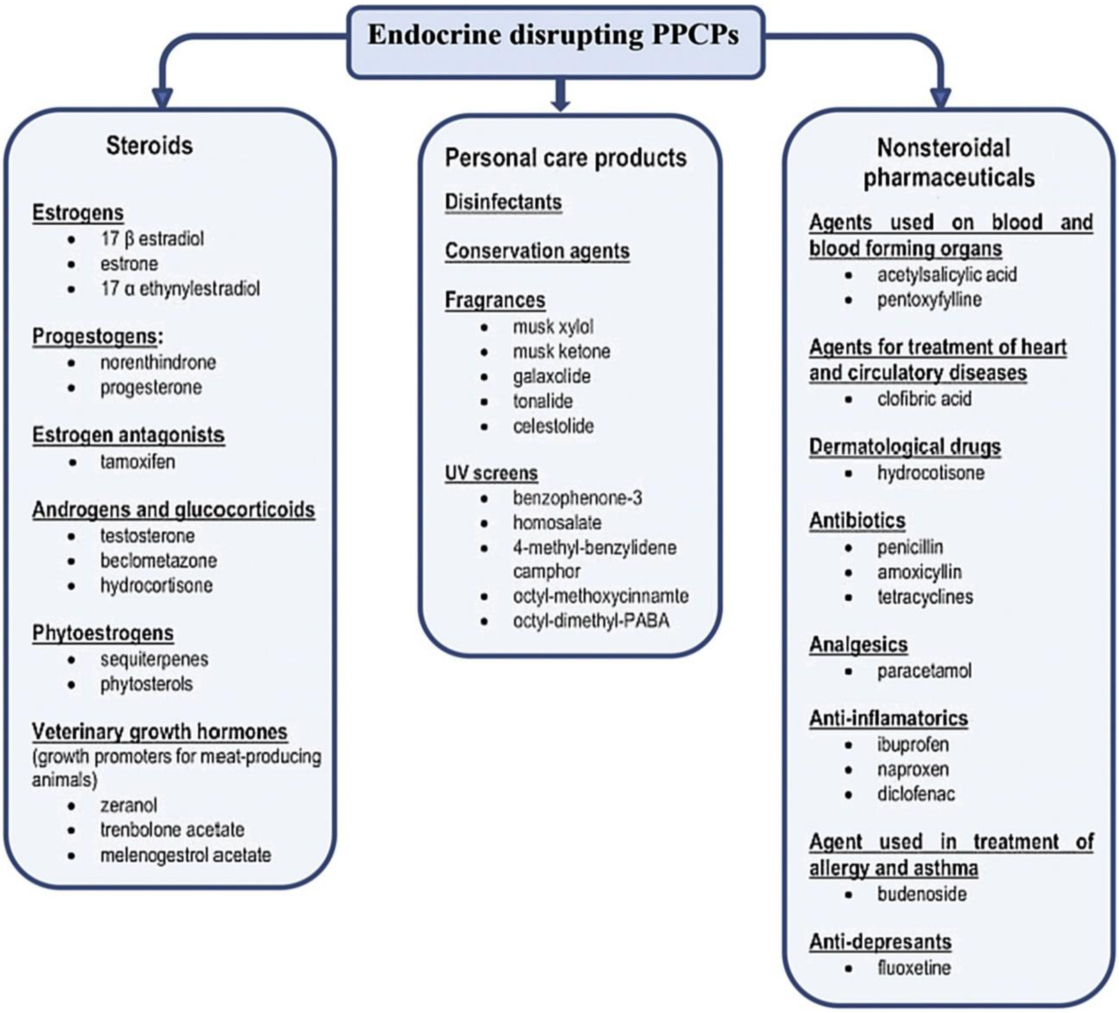


Figure 2 Summary of endocrine disrupting PPCPs (Ebele et al. 2017).

3.1.3 Types of pollutant and their effects

Pharmaceuticals encompass a wide range of substances, each with specific uses and potential effects on human health and the environment. Antibiotics used to treat bacterial infections, antibiotics can contribute to the development of antibiotic-resistant bacteria if overused or improperly disposed of, posing a significant public health risk (Adebisi 2023). Drugs like antidepressants, used to treat depression and anxiety, can affect the behavior and reproductive systems of aquatic organisms when they enter water bodies. Laboratory-based studies have reported effects across a wide range of fish species, showing that antidepressants are able to disrupt multiple biological processes in aquatic organisms, affecting gene expression, neurotransmitters, hormones, growth, locomotion, colour physiology, behaviour, feeding and reproduction (Gould et al. 2021). Hormones and Endocrine Disruptors, including birth control pills and hormone therapies, these can interfere with the endocrine systems of both humans and wildlife, potentially causing reproductive and developmental issues. Painkillers (analgesics) is commonly used for pain relief, these medications, especially opioids, can lead to addiction and abuse in humans. In the environment, they can affect the behavior and physiology of wildlife (Seyfried & Hester

2012). Anti-Inflammatories used to reduce inflammation and pain, can impact the kidney function and cardiovascular systems in humans if overused. In aquatic environments, they can harm fish and other organisms. The anti-inflammatory drug ibuprofen is considered to be an emerging contaminant because of its presence in different environments (from water bodies to soils) at concentrations with adverse effects on aquatic organisms due to cytotoxic and genotoxic damage, high oxidative cell stress, and detrimental effects on growth, reproduction, and behavior (Marmon et al. 2021). Sedatives and Anesthetics used to induce sleep or anesthesia, these drugs can cause side effects like dizziness and lowered blood pressure in humans. Their release into the environment can potentially affect the nervous systems of aquatic organisms (Tobias & Leder 2011).

Understanding the effects of these pharmaceuticals is crucial for their safe use and disposal, as well as for addressing their potential environmental impacts. Proper management and regulatory controls are essential to mitigate these effects on both health and ecosystems.

3.1.4 Pollutants and their Impact

Antimicrobial agents, including conservation agents, antibiotics, antivirals, antifungals, and antiparasitic, play a crucial role in treating infections. However, their use and misuse have significant impacts on both health and the environment (Nankervis et al. 2016).

Antibacterial agents like triclosan (TCS, 5-chloro-2-(2,4-dichlorophenoxy) phenol) and triclocarban (TCC, 3-(4-chlorophenyl)-1-(3,4-dichlorophenyl) urea are commonly found in everyday household and personal care products. Both compounds are widely distributed in the environment, acting as potential sources of contamination to ecological safety and human health problems such as algal growth-inhibiting effects; bioaccumulation in algae; endocrine-disrupting effects; development of microbial resistance, and formation of toxic degradable products. Environmentalists are increasingly concerned about the emergence of these micro-organic pollutants in the environment (Amigun Taiwo et al. 2022). TCC is an antimicrobial agent and also emerging endocrine disruptor that can cause immune dysfunction and affect human reproductive outcomes. Furthermore, TCC alters the expression of proteins related to binding and metabolism, skeletal muscle development and function, nervous system development and immune response. Accumulation of TCC in organisms depends on the lipophilicity and bioavailability of TCC in sediment and water. TCC was continuously detected in aquatic system (Vimalkumar et al. 2019).

The antibacterial soap additive triclocarban (TCC) is widely used in personal care products. TCC has a high environmental persistence (Schebb et al. 2011). Available data on the occurrence of triclosan (TCS) in various environmental media, in human body and in wildlife show that the compound is not well regulated. Its uncoordinated use and careless disposal may threaten lives and the ecosystem generally. Cell based studies have shown toxicity potentials of TCS in several cells. Results of epidemiological studies may have supported the in vitro study reports. Cell-based assays are short-term (hours–days) and cannot be used to directly address the effect of chronic exposures (Olaniyan et al. 2016). The halogenated antimicrobial triclocarban (TCC) has large production and consumption over last decades. Its extensive utilization in personal care products and insufficient treatment in conventional wastewater treatment plants (WWTPs) has led to its listing as one of emerging organic contaminants (EOCs) (Yun et al. 2020).

Climbazole is an antimycotic agent with a high in vitro and in vivo efficacy (Wigger-Alberti et al. 2001). Climbazole acts as a C14-demethylase inhibitor (DMI) fungicide and thus has a high efficacy against fungi, but knowledge of its potential environmental impact is lacking (Richter et al. 2013). It was found that the toxicity of climbazole is mostly similar to that of other DMI fungicides, whereas it proved to be particularly toxic to primary producers (Richter et al. 2013). Reduction of frond size in water lentils and shoot length in higher plants suggested an additional plant growth–retarding mode of action of climbazole. In addition, it was demonstrated here that for an ionizable compound such as climbazole, the soil pH can have a considerable influence on phytotoxicity (Richter et al. 2013).

Methylparaben, which is known to be an endocrine-disrupting chemical, is added to various personal care products, including cosmetics, and is also used as a food preservative and in pharmaceuticals. Furthermore, unlike other substances such as metals and pesticides, there is no regulation of levels or safe concentrations of methylparaben in soil ecosystems. We conducted acute bioassays on eight species within six taxonomic groups and chronic bioassays on five species within four taxonomic groups (Kim et al. 2018). The distribution of species-sensitive soil species is shown in Figure 3.

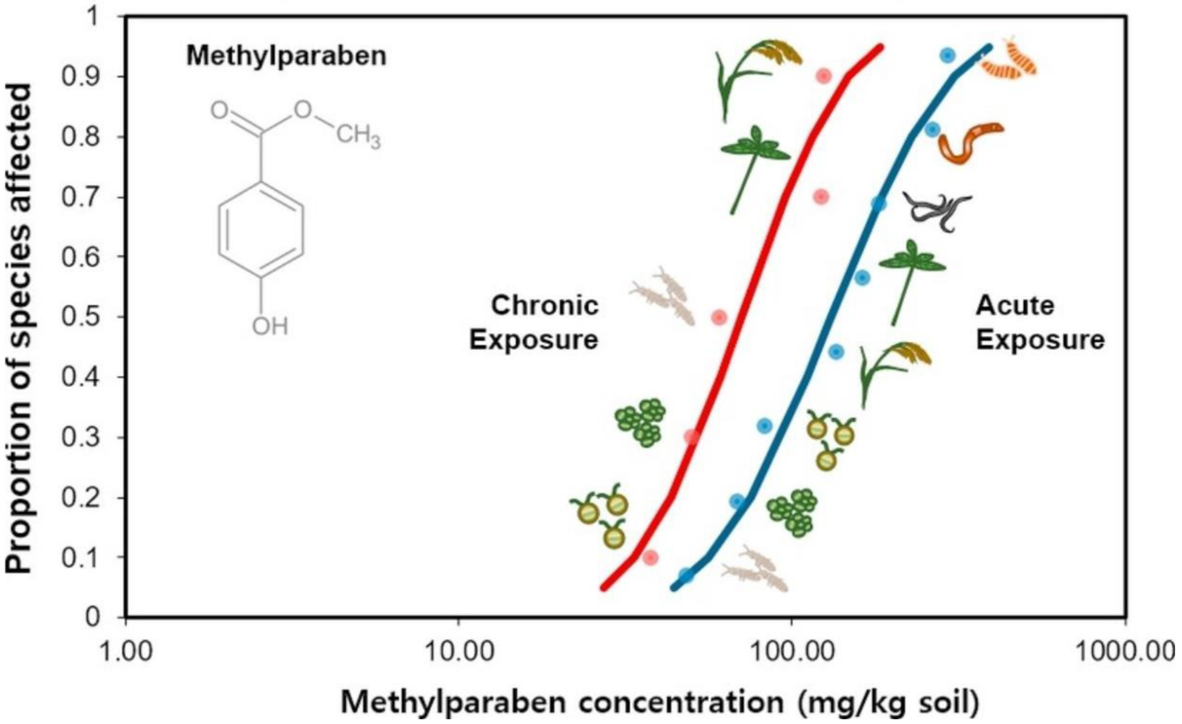


Figure 3 Species sensitive distribution for soil species (Kim et al. 2018).

Propylparaben is widely used as a preservative in pharmaceuticals and personal care products and is ultimately excreted by the human body. Thus, propylparaben reaches sewage and enters the soil environment by sludge fertilization and wastewater irrigation. However, there are few existing studies on the toxicity and risks of such chemicals in terrestrial environments (Kim et al. 2020). Disposal of personal care and pharmaceutical products containing propylparaben can lead to its presence in landfill sites, from where it can leach into the soil (Osuoha et al. 2023). In soil, propylparaben's behavior is influenced by factors such as soil pH, organic matter content, and temperature. These factors affect its mobility, degradation, and bioavailability. Propylparaben can bind to soil particles, which may reduce its mobility but can also lead to long-term persistence in the soil (Maia et al. 2023). There is a possibility of bioaccumulation in soil organisms, which could have further implications for the food chain, although more research is needed in this area (Alengebawy et al. 2021).

Ethylparaben, like other parabens, is a widely used preservative in various cosmetic, pharmaceutical, and food products. Its presence in soil, primarily through anthropogenic activities, raises environmental concerns (Atli 2022). Like other parabens, ethylparaben tends to bind to soil particles, which can affect its mobility and persistence in the soil. Its behavior in soil is influenced by factors such as soil pH, organic matter content, and the presence of microorganisms. Ethylparaben can be subject to biodegradation by soil microbes, but its persistence can vary depending on the environmental conditions. There is potential for bioaccumulation in soil organisms, which could lead to broader ecological impacts, including effects on the food chain (Alengebawy et al. 2021).

Butylparaben and benzylparaben, used as preservatives mainly in cosmetic products, have been found to be weakly estrogenic (Yamamoto et al. 2007). Butylparaben, part of the paraben family of chemicals, is commonly used as a preservative in many personal care products, pharmaceuticals, and foods. In the soil, butylparaben may bind to soil particles, affecting its mobility and bioavailability. In the soil, butylparaben may bind to soil particles, affecting its mobility and bioavailability. Ethylparaben may undergo photodegradation under sunlight and microbial degradation, but its half-life in soil can vary, influencing its overall environmental impact (Frontistis et al. 2017).

3.2 Absorption of pollutants by plants in the ecosystem

The absorption of antimicrobials by plants in the environment is a complex and emerging area of environmental science. This process involves various ways in which plants can absorb antimicrobials compounds that are present in the soil, water, or air (Wei et al. 2023). Medications display various physicochemical characteristics that vary from one to another.

Antimicrobials can enter the environment through various routes. Common sources include effluent from wastewater treatment plants, runoff from agricultural lands where livestock manure is used, and improper disposal of medications (Manyi-Loh et al. 2018).

Plants can absorb antimicrobials through their roots when these compounds are present in the soil or water. Some compounds can also be absorbed through the leaves if they are present in the air (Hlihor et al. 2022). Different plants have varying abilities to absorption and accumulate pharmaceuticals. The extent of absorption depends on the plant species, the type of pharmaceutical, and environmental conditions (Wei et al. 2023).

Studies have shown that the re-use of wastewater effluent with pollutants constituents for irrigation of crops could result in the absorption of pollutants by plant roots (Christou et al. 2017). This could result in the occurrence of pharmaceuticals in food sources such as vegetables which may cause threats to the health of the consumer. On the other hand, the absorption of pharmaceuticals by plants can be an advantage because it could assist in the reduction of the pollutant load in surface water (Cui et al. 2014). Constructed wetlands have been investigated for their ability to remove pharmaceuticals from water through the usage of certain plants (Zhang et al. 2011). Accumulation of antimicrobials in plants as documented in literature lead to growth suppression and reduction in photosynthetic pigments among other side effects (Karlický et al. 2021).

A study was conducted on the fate and absorption of pharmaceuticals (carbamazepine, diclofenac, fluoxetine, propranolol, sulfamethazine) and personal care product (triclosan) in soil-plant systems using radish *(Raphanus sativus)* and ryegrass *(Lolium perenne)*. Five of the six chemicals were detected in plant tissue. The results demonstrate the ability of plant species to accumulate pharmaceuticals from soils with absorption apparently specific to both plant species and chemical (Carter et al. 2016). While only a few studies have explored the occurrence of active pharmaceutical ingredients (APIs) in the soil environment, available data indicate that a range of API classes, including nonsteroidal anti-inflammatory drugs, antidepressants, anticonvulsants, and antibacterial agents do occur in soils in concentrations up to the low mg/kg level (Kinney et al. 2006; Durán-Alvarez et al. 2009; Vazquez-Roig et al. 2010).

3.2.1 Root absorption

In the process of nutrient uptake, plants can also take up pharmaceutical compounds present in the soil and in the environment. This uptake mechanism can lead to the accumulation of pharmaceutical compounds in various parts of the plant, including fruits and leaves (Wei et al. 2023).

The presence of pharmaceutical compounds in soil can be a source of uptake by plants, especially if water treatment is inadequate or if contaminated fertilizers are used. However, not all pharmaceutical compounds can be taken up by plants, and the mechanisms of uptake may differ depending on the chemical properties of the compounds and the type of plant. It is also important to consider the influence of environmental factors such as soil type, climatic conditions, and pollution levels on the uptake of pharmaceutical compounds by plants. Ensuring clean water and soil, as well as the application of bioremediation techniques, can help to reduce the level of pharmaceutical compounds available for uptake by plants (Carter et al. 2016).

Vegetables can absorption many PPCPs when exposed to these chemicals, but different PPCPs show significant differences in root absorption potential and subsequent translocation. Of the 20 PPCPs considered in the study, triclocarban, fluoxetine, triclosan and diazepam accumulated in roots at higher levels than other PPCPs (Wu et al. 2013).

The research investigated the plant absorption potential of 13 pharmaceutical chemicals and examined the relationship between their accumulation patterns in plants and their physicochemical properties. Pea and cucumber plants were exposed to an aqueous solution containing the pharmaceutical chemicals. Among the tested compounds, ten were detected in the leaves and stems of the plants. Analysis of the plant absorption characteristics and octanol-water partition coefficient of the pharmaceutical chemicals revealed that compounds with intermediate polarity, such as carbamazepine and crotamiton, were readily transported to the plant shoots.

Further research in this area will allow a better understanding of the mechanisms of uptake of pharmaceutical compounds by plants and the development of effective strategies to reduce the risks of adverse effects.

3.2.2 Factors influencing the uptake of pollutants by plants.

The processes impacting the absorption of antimicrobials by plants are complex and influenced by numerous factors associated with the antimicrobial compounds, the plant species, and the surrounding environment.

The physicochemical characteristics of antimicrobials, such as molecular size, polarity, solubility, and stability, significantly influence their absorption by plants (Vaou et al. 2021). Lipophilic (fat-soluble) and small molecules are generally more readily absorbed compared to hydrophilic (water-soluble) and larger molecules. The primary route of absorption is through the plant roots (Moiseev et al. 2019).

After absorption, antimicrobials can be translocated to different parts of the plant. This process depends on the plant's vascular system and the properties of the antimicrobials. Some compounds may accumulate in specific plant tissues, while others may be more uniformly distributed (Zhang et al. 2023). The properties of the soil, including pH, organic matter content, and texture, can affect the bioavailability of antimicrobials to plants. For example, soils with high organic matter may bind certain pharmaceuticals, reducing their availability for root absorption (Song et al. 2023).

Environmental factors such as temperature, moisture, and light exposure can also impact the absorption of pharmaceuticals by plants. For instance, higher temperatures might increase the solubility and hence the bioavailability of certain antimicrobials in the soil (Ponce-Robles et al. 2022).

Once inside the plant, antimicrobials can be metabolized, which can alter their chemical structure and potentially their toxicity. Some plants host endophytes - microorganisms that live inside plant tissues. These endophytes can influence the plant's ability to absorption and metabolize pharmaceuticals (Gouda et al. 2016).

Comprehending these mechanisms holds paramount importance for evaluating the environmental risks posed by antimicrobials, administering contaminated sites, and guaranteeing the safety of agricultural products. Continual research in this domain is indispensable for enhancing our comprehension and devising effective strategies to alleviate the potential hazards linked to pharmaceutical absorption by plants.

3.3 Biochar for mitigating the ingress of pollutants into plants

Biochar is a sustainable and environmentally friendly material, offering a green solution to the issue of antimicrobials pollution. The use of biochar as a medium to reduce the penetration of pharmaceuticals into plants presents an interesting application in environmental management and agriculture. Antimicrobials in the environment, particularly in soil and water, pose a risk to plant health and the broader ecosystem (Abukari et al. 2021). Biochar, with its porous structure and large surface area, can adsorb a wide range of organic compounds, including pharmaceutical residues (Jagadeesh & Sundaram 2023).

The adsorptive properties of biochar are attributed to its surface chemistry and pore structure, which can trap and immobilize antimicrobials molecules. By adsorbing antimicrobials, biochar can effectively reduce their bioavailability in the soil, thereby minimizing their absorption by plants (Bocșa et al. 2023). When used as a soil amendment, biochar can improve soil quality while simultaneously acting as a sink for antimicrobial contaminants. Beyond soil, biochar can also be used in water filtration systems to remove pharmaceuticals from contaminated water sources, further protecting plant life (Doran & Zeiss 2000).

Biochar is an organic material produced via the pyrolysis of C-based feedstocks (biomass) and is best described as a ‘soil conditioner’. Despite many different materials having been proposed as biomass feedstock for biochar (including wood, crop residues and manures), the suitability of each feedstock for such an application is dependent on several chemicals, physical, environmental, as well as economic and logistical factors. Evidence suggests that components of the carbon in biochar are highly recalcitrant in soils, with reported residence times for wood biochar being in the range of 100s to 1.000s of years, i.e. approximately 10-1.000 times longer than residence times of most soil organic matter (SOM) (European Commission. Joint Research Centre. Institute for Environment and Sustainability. 2010).

Given the prevailing concern regarding climate change mitigation and the irreversible nature of biochar application to soil, it is imperative to conduct a thorough evaluation of biochar stability in the environment and its impacts on soil processes and functioning.

3.3.1 Properties of biochar

Biomass thermochemical processes result in a common byproduct char. Effective utilization of biochar is critical for improving economic viability and environmental sustainability of biomass thermochemical technologies (Qian et al. 2015). In addition to this, biochar sequestration, in combination with sustainable biomass production, can be carbon-negative and therefore used to actively remove carbon dioxide from the atmosphere, with potentially major implications for mitigation of climate change. Biochar production can also be combined with bioenergy production using the gases that are given off in the pyrolysis process (Lehmann & Joseph 2012). Biochar is highly porous, which is a fundamental characteristic contributing to its large surface area. This porosity is crucial for its ability to absorb and retain water, nutrients, and pollutants (Amalina et al. 2022). The pores in biochar are categorized based on their size. Micropores (less than 2 nm), mesopores (2-50 nm), and macropores (greater than 50 nm) each contribute differently to its adsorption properties and interaction with soil and water (see Figure 4) (Guo et al. 2022).

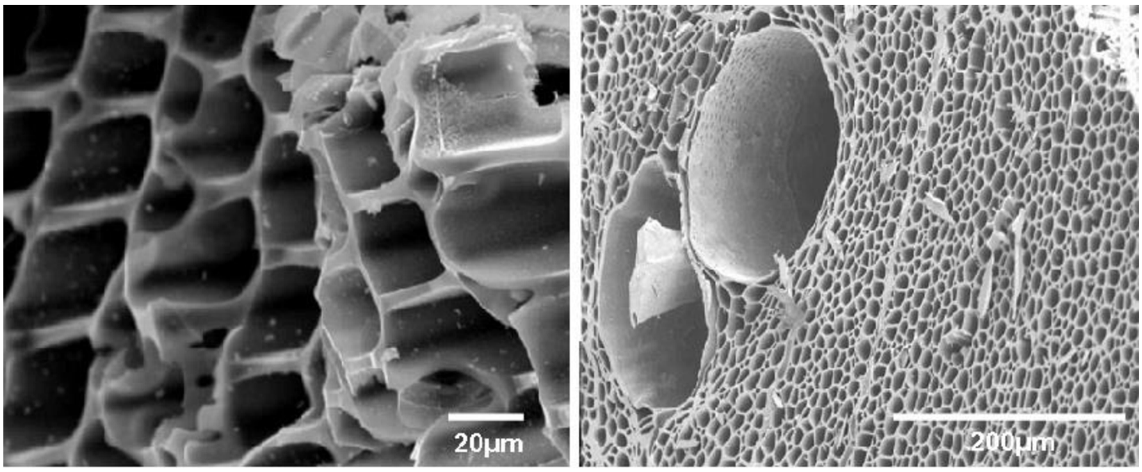


Figure 4 The porous structure of biochar (Thies & Rillig 2009).

The physiochemical property of biochar varies with the types of feedstocks. Biochar can be modified by acid, alkali, oxidizing agents, metal ions, carbonaceous materials, steam, and gas purging (Wang & Wang 2019). The selection of modification methods depends on the environmental application fields. The biochar has been used for soil remediation and amelioration, carbon sequestration, organic solid waste composting, decontamination of water and wastewater, catalyst and activator, electrode materials and electrode modifier, which were discussed in detail (Amalina et al. 2022).

The application of biochar in the carbon sequestration should be further investigated at similar experimental conditions to obtain the consistent results. The effect of biochar on soil microbes should be further investigated to elucidate the dominant reason for the improvement of soil fertility based on different soil and feedstock. In addition, more attention should be paid to the release of heavy metals and polycyclic aromatic hydrocarbons (PAHs) from biochar to the environment when biochar is practically used for the environmental remediation (Wang & Wang 2019). Depending on the feedstock and pyrolysis conditions, it can either raise or lower soil pH, which can be important for growing certain types of crops (Kinney et al. 2012). Its structure increases the water holding capacity of the soil, benefiting plants during periods of drought. Biochar can reduce nutrient leaching, keeping more nutrients in the soil and available to plants. The chemical properties of biochar, such as its cation exchange capacity, play a crucial role in nutrient absorption and retention.

Biochar is thermally stable, which makes it resistant to change under varying environmental conditions. Biochar is improving soil aeration, which is beneficial for root growth and health and can help in insulating the soil, maintaining more stable soil temperatures, its use in soil can reduce emissions of nitrous oxide and methane, two potent greenhouse gases (Bo et al. 2023). It has shown promise in the remediation of heavy metal-contaminated soils due to its adsorption capabilities. Biochar-based adsorbents have shown great promise for removing toxic heavy metals, such as Pb, Zn, Cd, Cu, U, Cr, and others. The adsorption performance of biochar in removing heavy metals is influenced by a variety of factors, including water environmental conditions and biochar characteristics (Hama Aziz & Kareem 2023). More information about the use of biochar in industry can be seen at Figure 5.

The multifaceted properties of biochar render it an exceptionally versatile material, well-suited for diverse applications ranging from enhancing soil health and agricultural productivity to environmental remediation and carbon sequestration.

Figure 5 How can Biochar be applied (Hama Aziz & Kareem 2023).

3.3.2 Biochar for decreasing the uptake of pollutants by plants.

The efficacy of biochar in mitigating the uptake of antimicrobials by plants has garnered increasing attention, particularly within the realms of environmental contamination and agricultural safety.

To evaluate the soil enhancement potential of biochar, a study was conducted involving lettuce *(Lactuca sativa L.)* and carrots *(Daucus carota)*, aimed at reducing the absorption of antimicrobial agents such as ciprofloxacin, triclocarban, and triclosan. They found that the use of biochar resulted in a 67% reduction in ciprofloxacin and triclocarban levels in lettuce leaves and a significant 67% reduction in triclosan levels in carrot root.

Biochar is known for its high adsorption capacity due to its large surface area and porous structure. This property allows it to adsorb and immobilize pharmaceutical compounds present in the soil, reducing their availability to plants. As shown in Figure 6, the removal of pharmaceutical contaminants by biochar mainly involves adsorption pathway and degradation pathway via persulfate-based advanced oxidative processes (AOPs) (Ambaye et al. 2021).

Each of these pathways is of practical importance for the removal of pharmaceutical contaminants.

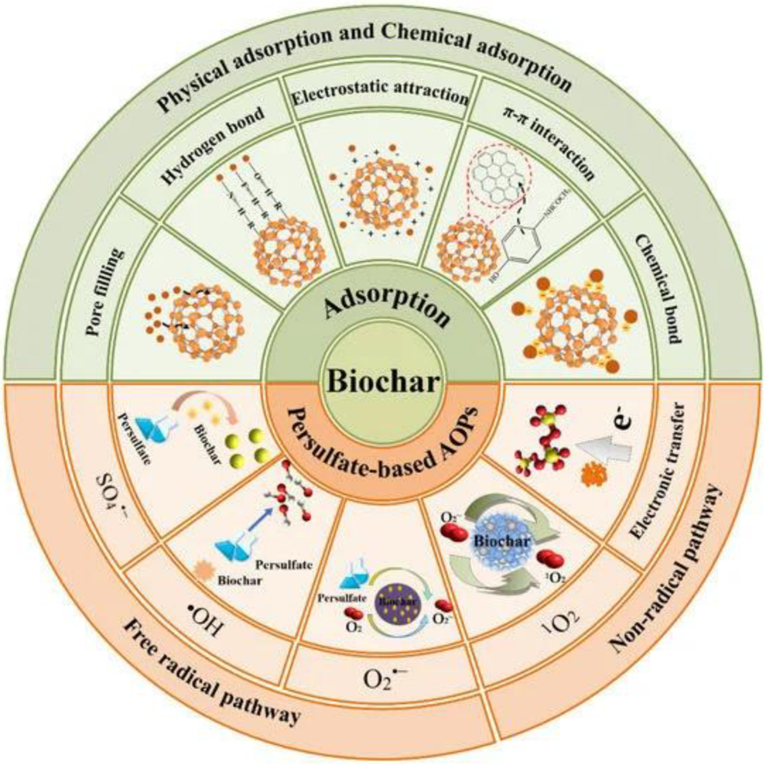


Figure 6 Removal mechanisms and main interactions of pollutants by biochar (Kang et al. 2022).

Due to the porous structure of biochar's surface, biochar can be used for the adsorption and removal of pharmaceutical pollutants. It has the advantages of convenient operation and a simple structure design, and it has more economic benefits than reverse osmosis, ion exchange, and electrolysis (Kang et al. 2022). Advanced oxidation processes (AOPs) provide a means to degrade pharmaceutical pollutants by inducing the production of reactive oxygen species (ROS) (Guo et al. 2022). In general, there are many advanced oxidation processes, including ozone oxidation, Fenton oxidation, persulfate oxidation, etc. Among them, persulfate AOPs have better pH adaptability and selective oxidation and have become an efficient strategy for degrading pharmaceutical pollutants (Tian et al. 2022). The degradation of pharmaceutical pollutants by persulfate based AOPs mainly includes a free radical pathway and a nonradical pathway. The nonradical pathway can be divided into electron transfer and the application of singlet oxygen (O2) (Kang et al. 2022). The removal efficiency of paracetamol using a spherical biochar derived from pure glucose and a non-spherical biochar from pomelo peel waste has also been demonstrated (Tran et al. 2020).

One study focused on determining the most effective biochar for pharmaceutical removal. Biochar made from renewable materials demonstrates 100 per cent recovery of pharmaceutical contaminants. Unlike other adsorbents, biochar can be reused up to 8 times with very little reduction in efficiency. The highest recovery of pharmaceutical contaminants using biochar was 1163 mg/g for tetracycline using biochar from Eucommia ulmoides; 400 mg/g for sulfamethoxazole using biochar from sugarcane cake; 596 mg/g for naproxen using biochar from peanut shells and 698.6 mg/g for norfloxacin using biochar from corn cobs (Monisha et al. 2022).

In the following study, two different types of NaOH activated pharmaceutical precipitates were used to prepare biochar. The characteristics of biochar prepared by impregnation method and dry mixing method were analysed, including N2 adsorption-desorption isotherms, surface functional group analysis and micromorphological observations. The results showed that both types of biochar had high adsorption efficiency of tetracycline and excellent pH adaptability. Biochar made by dry mixing activation method had the best adsorption performance (379.78 mg/g, 25 °C). Regeneration experiments showed that the adsorbent had a stable efficiency in tetracycline adsorption (Liu et al. 2021).

The article reviews show the latest progress on biochar-based adsorption removal of pharmaceutical compounds (PCs) from aqueous environment. Additionally, this article attempts to address different adsorption routes and theoretical kinetic models available to design efficient treatment process for different classes of PCs, viz., antibiotics, analgesics, NSAIDs, salicylates, antibacterials, and anti-infectives. Clearly, biochar facilitates PCs removal via π–π interactions among π electrons of biochar and aromatic portion of the PCs, the formation of hydrogen bonds between surface hydrogen on biochar and more electronegative functional groups present on the surface of the PCs for further adsorption. Physicochemical properties like specific surface area (SSA) and surface charge govern adsorption by pore entrapment and electrostatic interactions, respectively. Further, the pH of the system governs the surface charge of the biochar and speciation of the target PCs.

3.4 Zucchini *(Cucurbita pepo L.)*

Zucchini *(Cucurbita pepo L.)* is an economically important vegetable crop. The Cucurbitaceae family is the second most large horticultural family in terms of economic importance after Solanaceae (Andolfo et al. 2017). This family also includes cucumbers, melons, and pumpkins. Zucchini is considered a low-calorie vegetable with health-promoting properties. The fruits contain biologically active compounds including lutein, β-carotene, and folic acid, as well as vitamins and minerals (Kopczyńska et al. 2020).

Zucchini fruits have gained significant importance not only on the fresh food market but also as a raw material for various kinds of vegetable-based processed food items, especially in Mediterranean and European countries (Lust & Paris 2016). Zucchini is thought to have originated in Mesoamerica and has been part of human diets for thousands of years. It's now cultivated worldwide due to its adaptability to various climates and growing conditions (Landon, 2008). Zucchini is normally grown in soil extensively during the summer season and intensively under greenhouse conditions during the fall and winter seasons for national and international markets (Contreras et al. 2020). Zucchini production is typically intensely managed with high inputs of fertilisers and irrigation water, which increases the risk of groundwater contamination (Zotarelli et al. 2008).

It's particularly useful for studies on plant growth, disease resistance, and root absorption properties, as well as in genetics due to its well-documented genome. Zucchini's combination of agricultural importance, ease of cultivation, and biological characteristics makes it a valuable plant in both practical and research contexts (Wang et al. 2021).

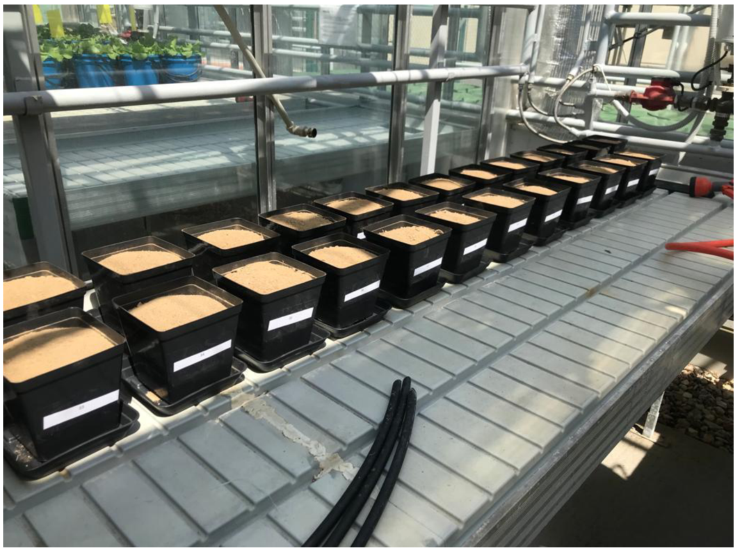
Through these characteristics, zucchini plants offer a practical and effective way to study root absorption properties, contributing valuable insights in areas like plant physiology, agricultural science, and environmental biology.

4 Material and Method

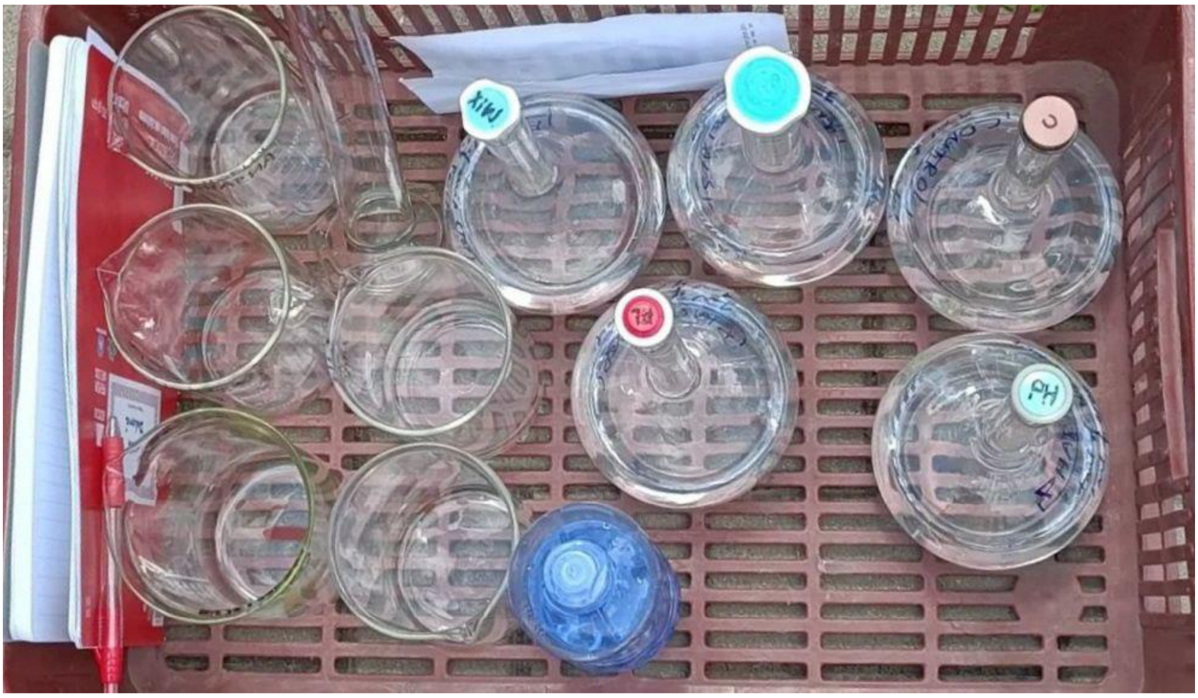
4.1 Experimental desing

The study began with the preparation of all materials and samples. The experiment involved 60 pots arranged in a row. During the experiment, the pots were moved to other tables as the plants grew. Zucchini seeds *(Cucurbita pepo L.)* were used in the experiment. The entire process took place in the greenhouse of the Department of Agro-Environmental Chemistry and Plant Nutrition. Pictures 1,2 and 3 are presented to provide a more detailed view of the components of the experiment.

Picture 1 Zucchini seed packet.

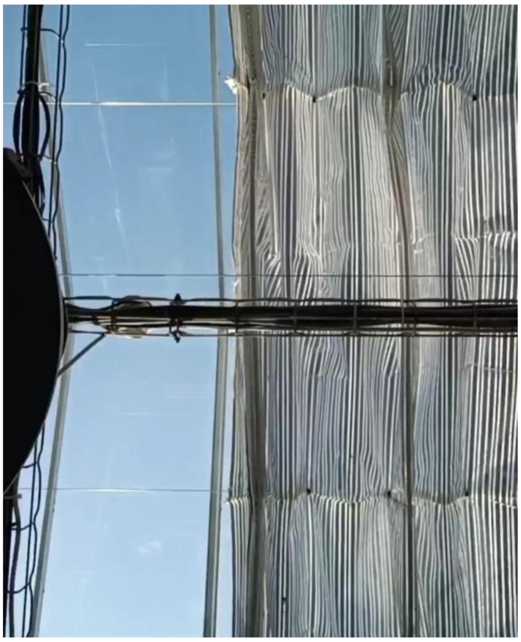


Picture 2 Arrangement of pots in the greenhouse.



Picture 3 Prepared solutions for irrigation of plants.

In the greenhouse, there was adjustable natural sunlight provided through special blinds (see Picture 4).



Picture 4 Blinds in greenhouse.

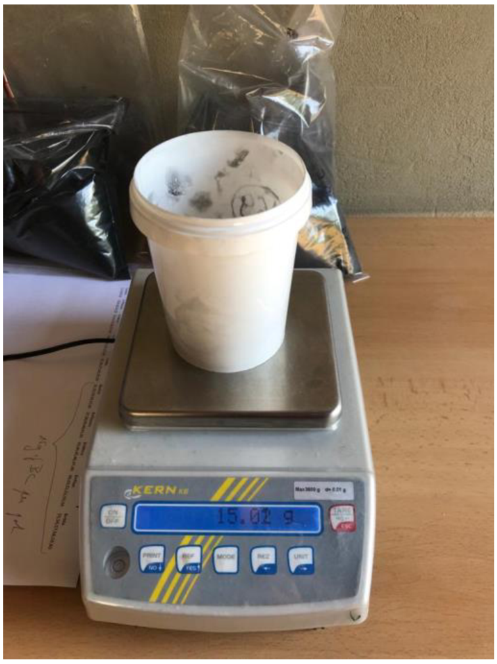
The experiment encompassed three distinct treatments: control, micro, and a mix of micropollutants. Zucchini seeds were planted and allocated in accordance with a fully randomized design, with six replicates to ensure robustness in the results. In the experiment, sixty pots were employed, each marked with a unique number. The initial thirty pots, numbered from 1 to 30, utilized soil exclusively as their growth medium, with a uniform amount of 1500 grams per pot (as illustrated in Picture 6). The subsequent set of pots, numbered 31 through 60, incorporated a blend of soil and an additional substance called biochar. Specifically, each of these pots contained 1% biochar mixed into the soil (as depicted in Picture 5). Further details regarding the experimental design can be found in Appendix 1.

Two seeds were planted in each pot and the seeds were sown 5-7 cm deep (Picture 8). The temperature inside the facility was maintained at 18 °C throughout the day.

Regular watering was carried out everyone, two, or three days to maintain optimal conditions for growing zucchini.

To supplement the growth process, nutrients were introduced to the pots in the form of fertilizer solutions. These solutions consisted of ammonium nitrate (NH4NO3) and potassium hydrogen phosphate (K2HPO4), as depicted in Picture 7.

Watering frequency varied depending on several factors, including prevailing weather conditions, the capacity of plants to absorb water, and the pace of plant development. Moreover, the decision to water was influenced by the need to maintain soil moisture at approximately 60% of its total water-holding capacity.



Picture 5 The amount of biochar applied to the pot.

Picture 6 The amount of soil used for each pot.

Picture 7 1L of fertilizing solution.

Picture 8 Zucchini seeds.

4.2 Type of biochar and soil

The application rates of the produced biochar were in accordance with established standard guidelines for its incorporation into agricultural soil (Wang & Wang 2019). Biochar derived from sewage sludge was synthesized by the Department of Agro-environmental chemistry and Plant nutrition at the Czech University of Life sciences in Prague. The process involved fast pyrolysis conducted in a fixed bed reactor, operating at a temperature of 700°C. After production, the biochar underwent milling to attain a particle size suitable for passage through a 2 mm sieve. The selection of the pyrolysis temperature was deliberate, aiming to optimize both the maximum energy yield and the stability of the resulting biochar.

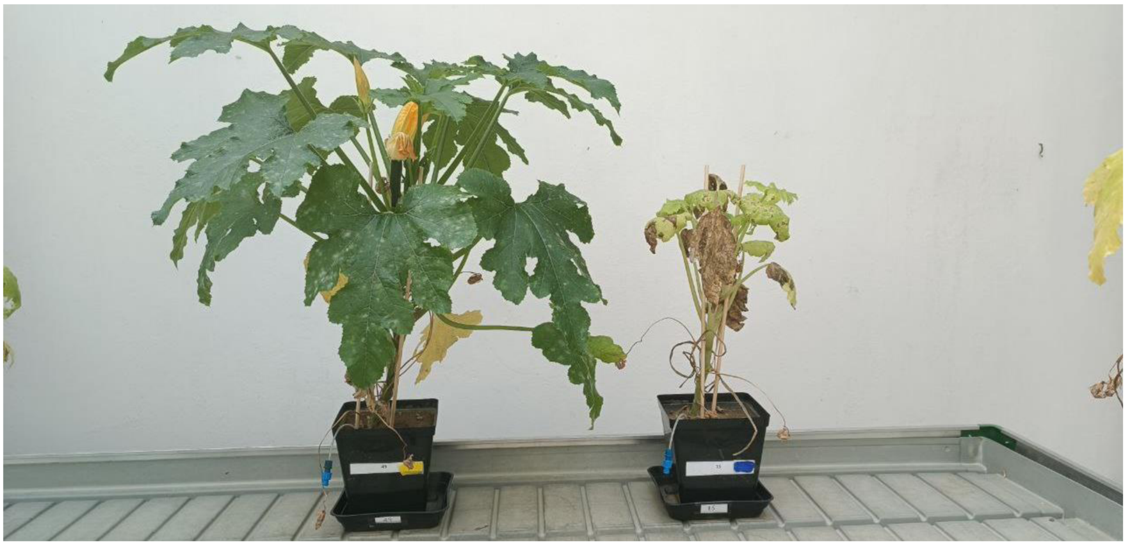
The experimental investigation utilized soil obtained from a field located in Humpolec, Czech Republic. The soil from this site possesses a sandy loam texture and typically has a pH range spanning from 4.5 to 6.6.

4.3 The process of growing

One week after sowing, plants were monitored every day to follow seed germination. The plants started to germinate 2 weeks after sowing (Picture 9), and in the interval excess seedlings were removed. The plants reached their full maturity after 77 days of the experiment (Picture 10,11).

Picture 9 Sprouting zucchini seeds after 2 weeks.

Picture 10 Grown zucchini.



Picture 11 Difference between pots with biochar (left) and without biochar (right).

4.4 Watering solution

Demineralized water was added to each of the 6 flasks, then certain solutions were added: 1ml of control and 1ml of EtOH for the control solution, 1ml of antimicrobials mix and 1ml of EtOH for the micro solution, and finally, 1ml of mixed solution along with 1 ml of paraben mix for the Mix solution. Each flask was filled with demineralized water up to the indicated line, resulting in a total volume of 1 liter for each irrigation solution.

For the antimicrobial treatment solution, each micropollutant, such as ciprofloxacin, climbazole, clindamycin, ofloxacin, triclosan, triclocarban, methylparaben, ethylparaben, propylparaben, and butylparaben, was dissolved in methanol.

The mixture treatment solution consisted of the same micropollutants used in the antimicrobial treatment, with the inclusion of amisulpride, carbamazepine, citalopram, metoprolol, propafenone, sertraline, tiapride, tramadol, trospium, venlafaxine, bisphenol a, bisphenol f, and bisphenol s. All prepared preparations were stored in a refrigerator at -42 degrees Celsius (Picture 12).

Picture 12 The extract of irrigation solution stored at -42°C.

Table 1 provides complete information on the concentration of micropollutants that were introduced into each pot. Each 1 liter watering flask contains 10 µg of contaminants and 100 µg of parabens.

Pollutants	**Concentration of pollutant in irrigation water (µg/l)**	**Total amount of pollutants per pot (µg/pot)**			
		Control groups		**Biochar groups**	
		micro	**mix**	**micro**	**mix**
ciprofloxacin	10	30.62	30.62	53.66	53.66
climbazole	10	30.62	30.62	53.66	53.66
clindamycin	10	30.62	30.62	53.66	53.66
ofloxacin	10	30.62	30.62	53.66	53.66
triclosan	10	30.62	30.62	53.66	53.66
triclocarban	10	30.62	30.62	53.66	53.66
methylparaben	100	306.2	306.2	536.6	536.6
ethylparaben	100	306.2	306.2	536.6	536.6
propylparaben	100	306.2	306.2	536.6	536.6
butylparaben	100	306.2	306.2	536.6	536.6
amisulpride	10	x	30.62	x	53.66
carbamazepine	10	x	30.62	x	53.66
citalopram	10	x	30.62	x	53.66
metoprolol	10	x	30.62	x	53.66
propafenone	10	x	30.62	x	53.66
sertraline	10	x	30.62	x	53.66
tiapride	10	x	30.62	x	53.66
tramadol	10	x	30.62	x	53.66
trospium	10	x	30.62	x	53.66
venlafaxine	10	x	30.62	x	53.66
bisphenol a	10	x	30.62	x	53.66
bisphenol f	10	x	30.62	x	53.66
bisphenol s	10	x	30.62	x	53.66

Table 1 Concentration of pollutants in irrigation water.

4.5 Water consumption

To water the plant, the exact amount of irrigation water for each pot must be determined. The Maximum Water Holding Capacity (MWHC) is calculated considering the amount of soil, pot, sump, and bagging. For a 1500 gram per pot, 255 g was calculated for a 40% MWHC. In the initial stages, the MWHC was between 40-60%. The average amount of irrigation water is presented in table 2. Detailed calculation can be found in Appendix 2.

4.6 Harvest

The zucchini was harvested on 15 August (77 days after the start of the experiment). The biomass was separated from the roots (Picture 16). The biomass was carefully cut into smaller pieces (picture 13), the plant segments were washed with demineralized water, air-dried with filtered paper, and then placed in aluminum foil for storage (Picture 14). The weight of each sample was varied and recorded. The samples were stored in a freezer at -42 degrees Celsius and the fruits were separated and put into special jars for further analysis (Picture 15).

Picture 13 The plant was cut up into small pieces.

Picture 14 The biomass was wrapped in aluminum foil.

Picture 15 Fruits in the glass container prepared for freeze dryer.

Picture 16 Roots from separated biomass.

4.7 Extraction of pollutants from plant biomass and fruits

To prepare for the extraction, the soil, zucchini biomass, and zucchini fruits were freeze-dried before they were processed for extracting. When it was time for harvest, samples of fruits and soil were placed in pre-weighed glass bottles, weighed again, and then stored in the freezer. Using the same foil in which the biomass was collected, the biomass was dried the same way it was collected. After 7-8 days, the samples were taken out, reweighed, and the weight of dried soil, plant biomass, and fruit samples was calculated by subtracting the weight of the glass bottles from the weight after freeze-drying. A laboratory electric mill was used to pulverize the zucchini biomass and fruits after which the results were analyzed (Picture 17,18).

Initially, 0.1 grams of freeze-dried zucchini biomass was accurately measured and transferred into a 15 mL Falcon tube. Subsequently, 2 mL of Milli-Q water was added to the tube, followed by vigorous vortexing and cooling for 10 minutes. After this, 1mL of acetonitrile (MeCN) was introduced into the samples and vortexed for one minute, followed by five minutes of sonication, followed by 1 mL of acetonitrile (MeCN) being introduced into the samples. It was then added 0.65 grams of a sample preparation technique that vastly simplifies the analysis of pesticide residues. It's quick, easy, cheap, effective, rugged, and safe (QuEChERS) salts to the solution and stirred for an additional minute before being centrifuged at 4°C for the remainder of the process. It was centrifuged for a maximum of 10 minutes at 4500 rpm for the samples to be analyzed.

The resulting supernatant was meticulously transferred into a new 1.5 mL Eppendorf tube using a Pasteur pipette. Subsequently, 700 µl of the supernatant was carefully transferred to a solid phase extraction (SPE) salt using a micropipette. The SPE salt comprised 150 mg MgSO4 (removes excess water), 50 mg of primary and secondary amine exchange material (PSA) (removes sugars, fatty acids, organic acids, and anthocyanine pigments), and 20 mg of graphitized carbon black (GCB) (removes pigments, sterols, and nonpolar interferences). Following this, the samples underwent vortexing and centrifugation at 14,000 rpm for an additional 10 minutes at 4°C. Finally, the supernatant was transferred to a 400 µL from liquid chromatography and tandem mass spectrometry method (LC-MS/MS) vial using a Pasteur pipette, ensuring that the presence of salt did not disturb the process.

Following these steps, 40 µl of internal standard solution was added to all treated samples. Control samples underwent various analyses, including those without sample addition, with the addition of 40 µl of internal standard solution, and with the addition of 40 µl of internal standard solution combined with 16 µl of STD (125 ppb). A total of six samples were used: two blank samples with 40 µl of internal standard solution added and four complete blank samples.

After the extraction procedure, liquid chromatography-tandem mass spectrometry (LC-MS/MS) was employed to assess the concentration of the drug and its derivatives. Each analyte was identified based on retention time and the availability of multiple reaction monitoring (MRM) mode for quantitative and confirmatory analysis.

Picture 17 Biomass grinding.

Picture 18 The biomass was ground using an IKA A11 basic analytical mill.

4.8 Absorption of pollutants and bioaccumulation coefficient

The absorption of antimicrobial compounds in the biomass is calculated based on the difference in compound concentration before and after exposure, taking into account factors such as sample mass and volume.

Biomass content of antimicrobials (ng) = Biomass production (g) x Concentration of antimicrobials (ng/g).

Once the data regarding the absorption of antimicrobial active compounds in biomass are collected, they are utilized in a subsequent equation to calculate the bioaccumulation coefficients (BF). These coefficients provide insights into the uptake and accumulation of antimicrobials and their transformation by-products in zucchini plants.

Bioaccumulation coefficient, BAF (%) = (Content ofantimicrobial in biomass/ Amount of antimicrobial applied) x 100%.

4.9 Statistical Method

The data analysis involved the use of one-way analysis of variance (ANOVA) to calculate mean values and standard deviations. This analysis also evaluated factors such as absorption and bioaccumulation. Subsequently, Tukey post hoc analysis was carried out to identify significant differences between mean values. For statistical computations, the Microsoft Excel program was employed. Significance levels of $P \leq 0.05$ was adopted to determine statistically significant differences between mean values.

5 Results

5.1 Water intake

Throughout the experiment, each plant was watered with water to accurately estimate water consumption. The daily water consumption of each plant was recorded to identify any discrepancies between groups and to assess the efficiency of water use.

	Water volume (ml)	**Pollutants (ml)**
Control	5416	X
Biochar	8110	X
Micro	2346	3072
Micro+B	2887	5396
Mix	2239	3112
Mix + B	2755	5336

Table 2 The volume of water and substances dissolved in it.

The highest water consumption was in the Micro + B group - 8283 ml, the lowest value was in the Mix group – 5351 ml. The water consumption of the pots with biochar was 50% higher than that without biochar.

5.2 Plant biomass yield

Graph in Figure 7 illustrates the average biomass production of the six different treatments as well as the corresponding standard deviations after growing and drying. Table 3 shows the mean mass of dried biomass and standard deviation.

	Average (Dry (g)
Control	6.39 ± 0.44
Biochar	18.7 ± 1.17
Micro	6.06 ± 0.37
Micro + B	18.8 ± 1.13
Mix	6.05 ± 0.55
Mix + B	19.2 ± 1.14

Table 3 - Plant Biomass yield. Each group had 6 replications.

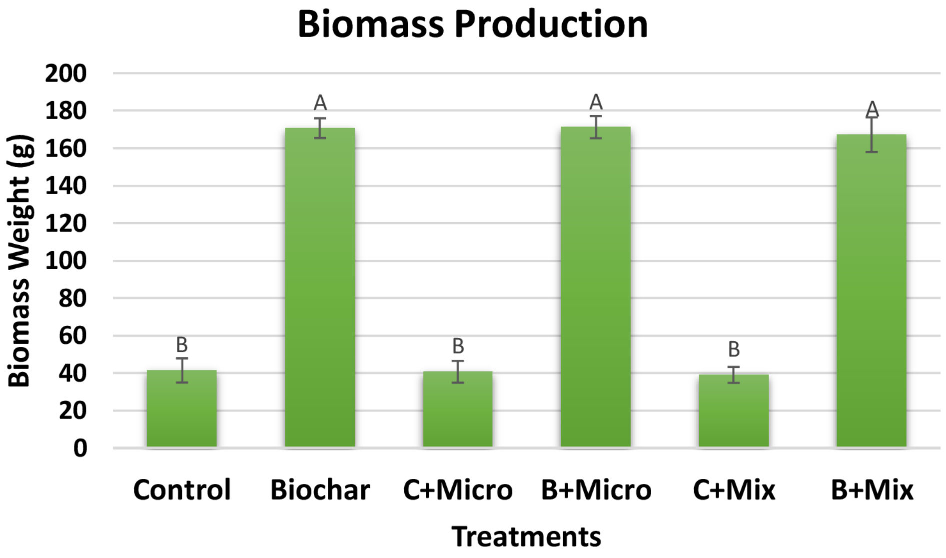


Figure 7 – Average wet biomass per pot for different treatments. Each group had 6 replications.

Treatments Control, C + Mix and C + Micro had the lowest average biomass production. All groups with biochar had the highest rates. In Figure 7, there is a clear statistical difference between groups A and B. Highest value in group B + Micro is 171 ± 5.91 gram. Lowest value in group C + Mix is 39 ± 4.27 gram.

5.3 Concentration of pollutants in biomass

Table 4 presents a comprehensive analysis of the mean and standard deviation values for various pollutants found in zucchini biomass samples for every treatment groups. Specifically, the pollutants include triclosan, climbazole, clyndamycin, methylparaben, ethylparaben, propylparaben and butylparaben. Notably, the concentration metric is denoted as ng/g, signifying nanograms of pollutants per each gram of dried biomass.

Concentration in Biomass (ng/g)							
	Triclosan	**Climbazole**	**Clindamycin**	**MeP**	**EtP**	**BuP**	**ProP**
Control	<MLOD	<MLOD	<MLOD	16.3 ± 1.30	2.15 ± 0.34	0.31 ± 0.07	0.68 ± 0.08
Biochar	<MLOD	<MLOD	<MLOD	17.5 ± 3.78	1.78 ± 0.15	0.08 ± 0.03	0.06 ± 0.06
C+Micro	<MLOD	<MLOD	<MLOD	16.6 ± 1.87	2.31 ± 0.17	0.54 ± 0.07	0.94 ± 0.26
B+Micro	<MLOD	<MLOD	0.34 ± 0.20	19.1 ± 4.72	1.90 ± 0.48	0.76 ± 0.22	0.83 ± 0.37
C+Mix	<MLOD	<MLOD	<MLOD	19.2 ± 5.13	2.45 ± 0.45	0.66 ± 0.15	1.14 ± 0.47
B+Mix	<MLOD	<MLOD	0.39 ± 0.21	15.02 ± 2.51	1.80 ± 0.43	0.75 ± 0.47	0.66 ± 0.47

Table 4 - A comparison of the average and standard deviation of concentration of each pollutants in the vegetative biomass samples with and without application of biochar.

5.4 Amount of pollutants found in biomass

According to Table 4 triclosan and climbazole were not detected or were below the detection limit (<MLOD) in plant biomass. Clindamycin was detected only in the two study groups Micro + B and Mix + B. Parabens were detected in every control group.

5.4.1 Antimicrobial agents

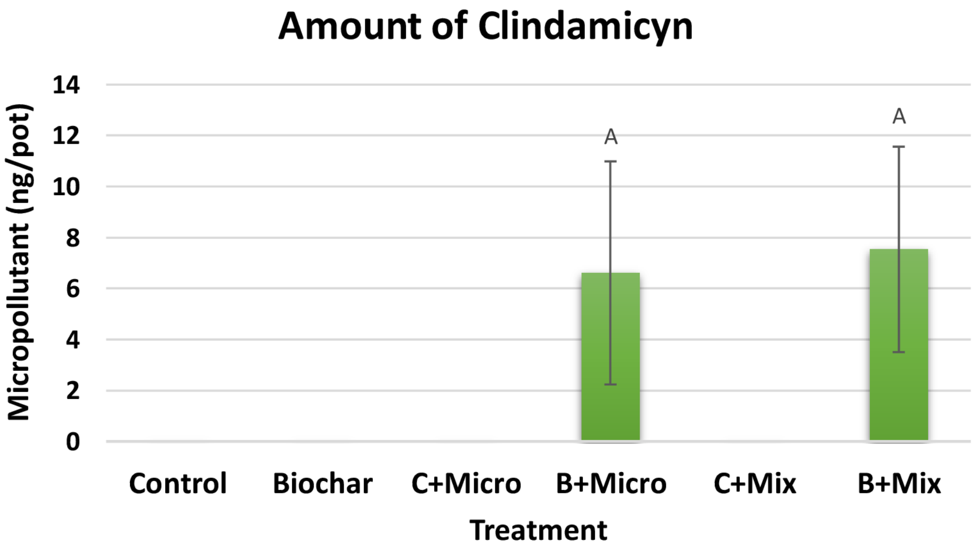


Figure 8 Amount of clindamycin per pot in different treatments.

In Figure 8, there are no significant differences between the groups.

5.4.2 Parabens

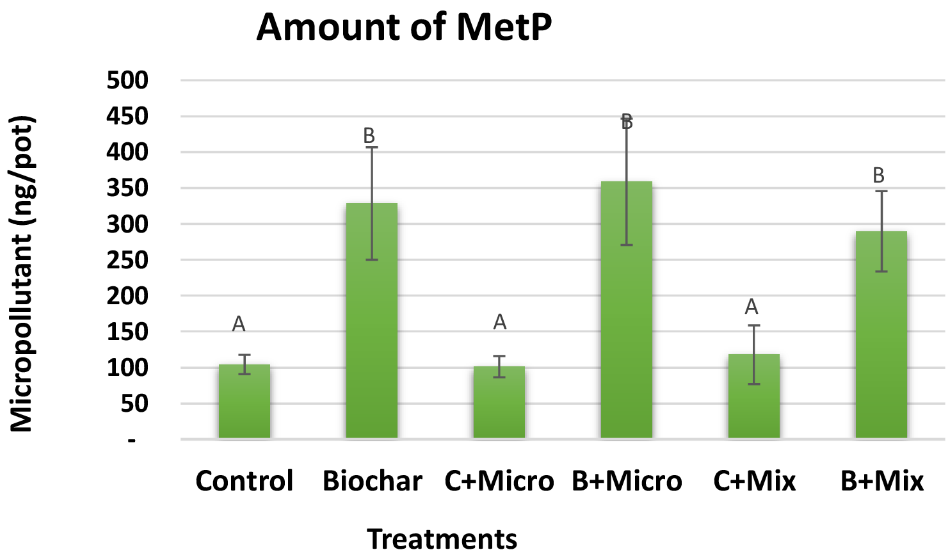


Figure 9 Amount of methylparaben (MetP) per pot in different treatments.

In Figure 9, there is a clear statistical difference between groups A and B. Highest value in group B + Micro is 358.3 ± 87.9 ng/pot. Lowest value in group C + Micro is 101.2 ± 14.8 ng/pot.

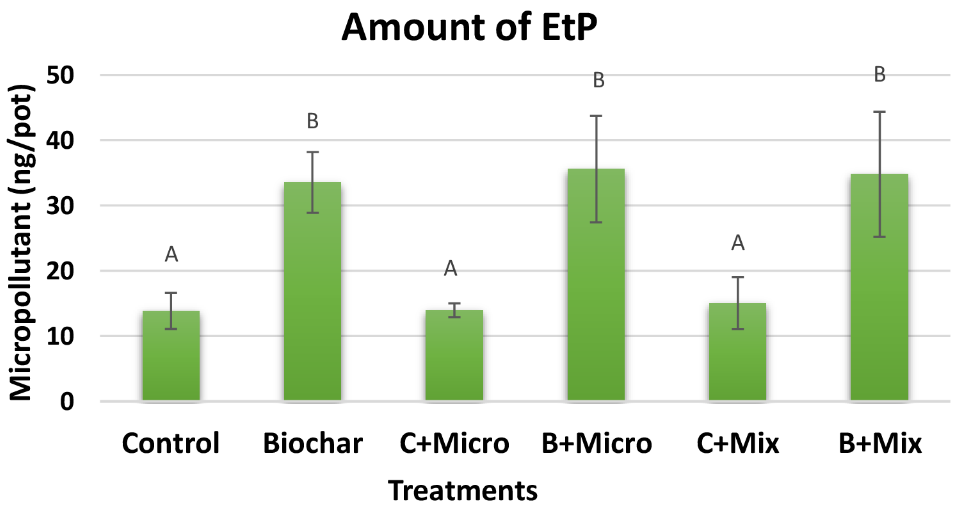


Figure 10 Amount of ethylparaben (EtP) per pot in different treatments.

In Figure 10, there is a clear statistical difference between groups A and B. Highest value in group B + Micro is 35.6 ± 8.16 ng/pot. Lowest value in group Control is 13.8 ± 2.75 ng/pot.

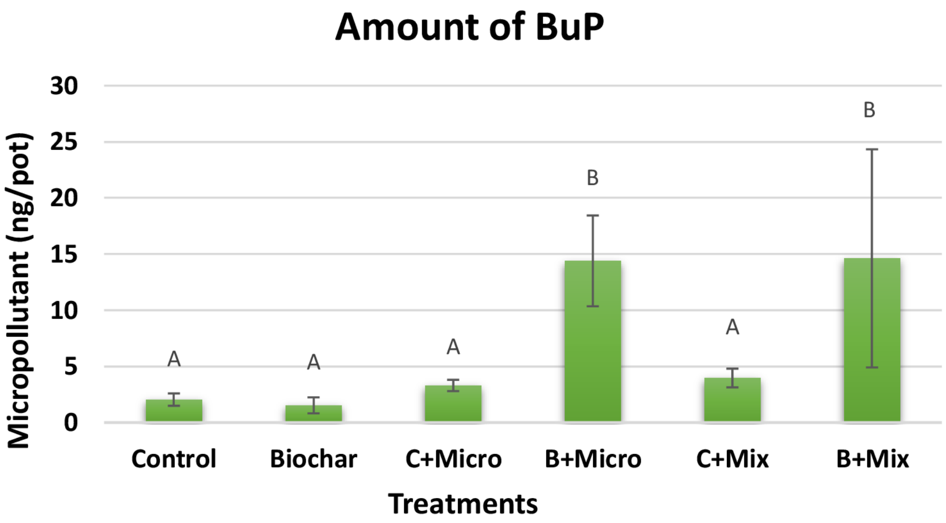


Figure 10 Amount of butylparaben (BuP) per pot in different treatments.

In Figure 10, there is a clear statistical difference between groups A and B. Highest value in group B + Mix is 14.6 ± 9.71 ng/pot. Lowest value in group Biochar is 1.53 ± 0.71 ng/pot.

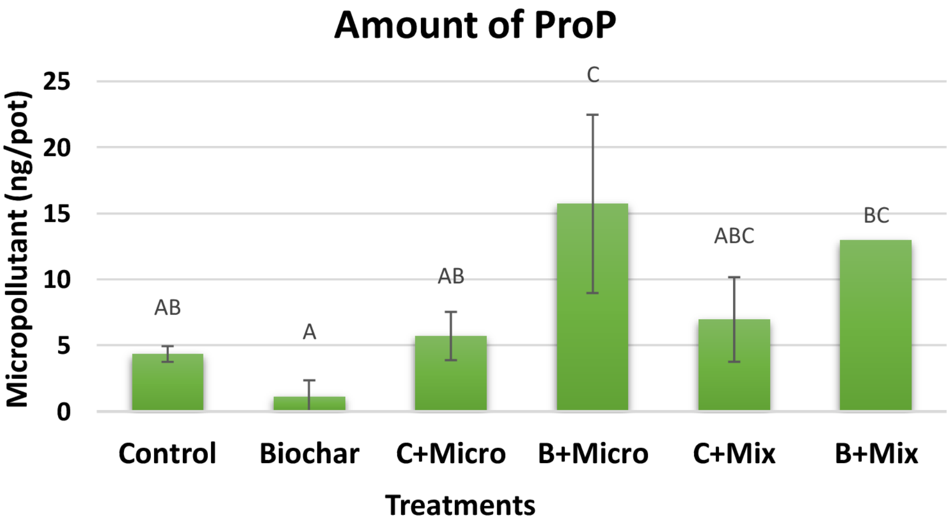


Figure 11 Amount of propylparaben (ProP) per pot in different treatments.

In Figure 11, there is a clear statistical difference between groups A and C, A and BC, C and AB. There is no clear statistical difference between the groups AB and BC, AB and ABC, A and AB.   Highest value in group B + Micro is 15.7 ± 6.75 ng/pot. Lowest value in group Biochar is 1.12 ± 1.24 ng/pot.

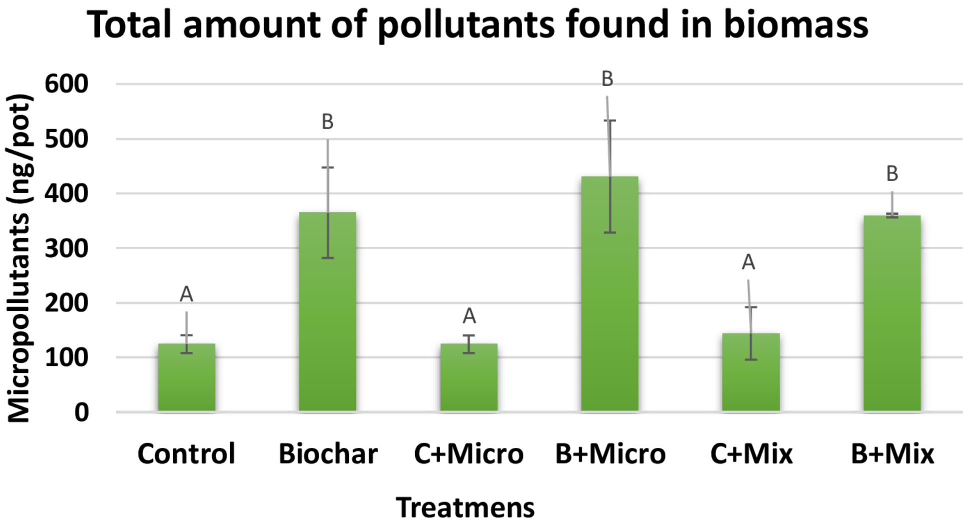


Figure 12 The mean amount of the pollunants absorption by vegetative dry biomass in ng/pot.

In Figure 12, there is a clear statistical difference between groups A and B. Highest value in group B + Micro is 430 ± 102 ng/pot. Lowest value in group C + Micro is 124 ± 16.1 ng/pot.

5.5 Bioaccumulation coefficient (BAF)

	C+Micro	B+Micro	C+Mix	B+Mix
Triclosan	0	0	0	0
Climbazole	0	0	0	0
Clindamycin	0	12.3 ± 8.15	0	14.0 ± 7.51
MeP	33.0 ± 4.82	66.8 ± 16.4	38.5 ± 13.4	53.9 ± 10.4
EtP	4.56 ± 0.34	6.63 ± 1.52	4.91 ± 1.29	6.48 ± 1.78
BuP	1.08 ± 0.16	2.68 ± 0.75	1.29 ± 0.27	2.72 ± 1.81
ProP	1.87 ± 0.59	2.93 ± 1.26	2.27 ± 1.05	2.41 ± 1.79

Table 5 The bioaccumulation coefficient value of pollutant in vegetative dry biomass (%) for each group in the soil.

Highest value in group B + Micro is 66.8% (MeP). Lowest value in group C + Micro is 1.08% (BuP).

6 Discussion

6.1 Water intake and biochar

During the experiment, the groups with added biochar required more water than the groups without biochar. In appendix 2 you can see more precise water consumption for each individual group. On average, the groups with biochar required 20% more liquid. The average amount of water consumed throughout the experiment varied from 5 to 8 liters per pot.

This difference is since biochar contains a high amount of nutrients, which favours the development and growth of the plants and its root system. In a study that investigated the effect of biochar added to tomato plants under drought conditions increased the stability of membranes and water content of tomato leaves. Biochar application can also increase the amount of moisture in grapes by about 30 per cent and increase available soil water by 35 per cent. Biochar also improves soil properties, namely its hydrological properties and increases its efficiency.(Wu et al. 2022).

In zucchini, it was externally very easy to determine the difference between the groups with and without biochar. The groups with biochar had much more fruit, the trunk height was twice as high, the leaves were well grown, had a regular shape, no dry parts and had a deep green color. The groups without added biochar, on the contrary, were much shorter, leaf color varied from yellow to light green, there were practically no fruits, many dry parts of the plant, which indicates a lack of nutrients.

Along with previous studies, one study that investigated the effect of added biochar in the soil for growing apple trees showed no significant difference between trees, except for the trunk girth of trees that were not treated with biochar (Eyles et al. 2015). But this can be explained by the fact that perennial horticultural systems require significantly more nutrients than zucchini and they may not respond to biochar.

In this way it can be said that biochar can influence plant growth and development, plant size, fruit size and root system, but has limited effect on the plant. In doing so, biochar significantly increases water consumption, which can create a problem when it is restricted.

6.2 Amount of biomass

The ongoing study shows very clearly the difference in biomass of the two groups: the group with biochar and the group without biochar. Before determinate the exact amount of biomass, freeze drying was carried out. The average amount of dried biomass of groups with added biochar (biochar, micro + b, mix + b) is 55.7 grams and 2.9 grams of fruit, but the average amount of dried biomass of groups without added biochar (control, micro + c, mix + c) decreases noticeably in the values of 18.5 grams and 0.16 grams of fruit.

The noticeable difference is due to the incorporation of biochar into the soil, which has the properties of maintaining the growing medium and improving the passage of nutrients through the roots to all parts of the plant (Jiang et al. 2024). Based on the results of the biomass obtained, it is safe to say that the addition of biochar has a positive effect on productivity, plant and fruit growth.

Biochar derived from renewable biomass feedstock is widely used as environmentally friendly adsorbents for pharmaceuticals due to their large surface area, large pore volume and well-defined pore structure (Ouyang et al. 2020).

6.3 Concentration of pollutants in biomass

In this experiment, the LC-MS/MS results were utilized to examine the concentration of pharmaceuticals in zucchini biomass.

According to Table 5, triclosan and climbazole were not detected in any of the studied groups. Only in two groups was Clindamycin detected is B + Micro 0.345 ± 0.204 ng/g and B + Mix 0.391 ± 0.207 ng/g. These results could be due to lower concentrations of the drug added to the other groups (C + Micro and C + Mix), which may have influenced the degradation rate.

In addition, according to the study, the toxicity of multiple compounds may be enhanced (i.e., synergistic effect) or reduced (i.e., antagonistic effect), or a combination of these effects may occur (Schmidtova et al. 2022).

In our study, triclosan and climbazole were not detected in plants, but in one extensive 20-year study, TCS was consistently detected in plant roots but not always confirmed in aboveground tissues. For example, it was not detected in lettuce and asparagus shoots lettuce and spinach leaves and in aboveground tissue of corn (Wu et al. 2013; Chuang et al. 2019; Pérez et al. 2022; Fernandes et al. 2024).

Parabens were detected in all monitored groups, even though parabens were only additionally added to 2 out of 6 groups. The detection of parabens can be explained by their presence in regular soil. Parabens accumulate in the root system, stems, and are intensively transported and metabolized in various plant segments, indicating an issue of paraben accumulation in soil and plants specifically (Kim et al. 2018; Karki & Philip 2024).

Regardless of the results obtained, it is worth noting that parabens, antimicrobials, and other pollutants may individually have low concentrations in plants, but their mutual influence may increase their negative effects on humans and nature.

6.4 Absorption of pollutants

The results indicate that methylparaben has the highest absorbance: 358.3 ± 87.9 ng/pot in soil treated with group B + Micro and 328.35 ± 78.3 ng/pot in soil with Biochar added. We also detected parabens in the Control, Biochar, B + Micro, and C + Micro groups, where parabens were not intentionally added. Their presence in plants can be explained by insufficient wastewater treatment methods for paraben removal. Studies also indicate a tendency towards toxicity to living organisms (Maia et al. 2023).

Triclosan and climbazole were not detected in any of the studied groups. Only in two groups was clindamycin detected. According to the study, antimicrobial agents have the property of accumulating in plant roots. Climbazole is biotransformed into reduced and oxidized products (Sochacki et al. 2021).

Thus, the study suggests that the fate of pharmaceuticals in the environment is influenced by various factors including their mobility in plants. Once in the topsoil, both physicochemical properties of preparations (stereochemical structure, redox potential, water solubility etc.) and internal characteristics of soils (clay and moisture content, pH, etc.) will change, which will determine their mobility and ability to leach or, on the contrary, their tendency to adsorb on solid particles (García-Galán et al. 2013).

6.5 Bioaccumulation coefficient (BAF)

Depending on a number of factors, contaminants may remain in root tissues (bioaccumulation) or, if they are able to reach the plant vascular system, be transported (i.e. translocated) by transpiration to other parts of the plant, where accumulation may also occur (Miller et al. 2016).

The table 6 shows the bioaccumulation coefficient of pollutants in dried biomass. Bioaccumulation indicates the percentage accumulation of pollutants in plants in this case. Some antimicrobial agents were not detected, but clindamycin was found in two groups with biochar, showing 12.3% and 14.0% bioaccumulation. Parabens demonstrate a high percentage of bioaccumulation, with Methylparaben being the highest at 66.8% in group B + Micro and the second highest at 53.9% in group B + Mix. Overall, comparing groups with biochar yields a higher percentage of bioaccumulation than those without it. Ethylparaben ranks second, with 6.63% in group B + Micro and a lower rate of 6.48% in group B + Mix. The lowest percentages of bioaccumulation are shown in group C + Micro. Additionally, parabens were detected in Control, Biochar, C + Micro, B + Micro groups, even though we did not add them additionally to these groups.

According to the findings in the study, parabens have quite high bioaccumulation and accordingly they tend to accumulate in plant parts. Butylparaben and propylparaben showed the lowest bioaccumulation and their accumulation in plants is minimal.

It is known that physicochemical properties of organic compounds, such as hydrophobicity and ionization, significantly affect their accumulation in organisms (Shahriar et al. 2021; Gao et al. 2022; Li et al. 2022). Several previous studies have reported a positive linear relationship between hydrophobicity and root accumulation of neutral xenobiotics in different plant species (Miller et al. 2016; Wu et al. 2022; Li et al. 2022).

However, it is important to recognize that BAF is just one element in determining potential risks associated with pharmaceuticals present in plants.

7 Conclusion

The extensive study on the absorption of pharmaceuticals, hormones, and parabens suggests that the likelihood of micro-pollutants entering agricultural crops under normal farming conditions is low, as no micro-pollutants were consistently detected (Sabourin et al. 2012).

In fact, limited data are available on the fate of pollutants in field studies because several processes compete for their dissipation, including sorption and formation of non-extractable residues, leaching, and biotransformation.

It should be noted that the experiments in this paper were conducted under simplified conditions. Under field conditions, there are more processes and variables involved in the soil-plant system and their interactions are likely to determine the ultimate fate and risks of a chemical.

The experiment shows that biochar does not hinder the absorption of pollutants compared to regular soil, but crop yield significantly increases with biochar. Biochar could be a valuable tool for plant development and growth without negative factors with further exploration. The presence of parabens in groups where they were not intentionally added only underscores the issue of wastewater filtration, pollutants removal, and their continued circulation, confirming the extent of this problem. Further investigation of this issue requires more data and comprehensive analysis.

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Supplementary Material

Appendix 1 – The numbering of each pot

	Treatments					
	Control	**Biochar**	**Micro**	**Micro+B**	**Mix**	**Mix + B**
Number of pot	1	31	13	43	25	55
	2	32	14	44	26	56
	3	33	15	45	27	57
	4	34	16	46	28	58
	5	35	17	47	29	59
	6	36	18	48	30	60

Appendix 2 – Demineralised water volume (ml)

Control	Biochar	Micro	Micro+B	Mix	Mix+B	Maximum water holding capacity (%)
176	185	174	184	184	178	40
110	121	114	117	120	115	40
105	106	110	108	111	103	40
81.4	88.6	87.1	89.0	91.0	83.2	40
111	96	107	104	102	106	40
112	96	110	104	104	107	40
167	94	159	189	103	124	60
303	320	329	340	286	305	80
47.7	53.3	65.8	54.4	58.7	60.2	60
177	202	177	198	149	203	70
157	190	146	167	156	189	73
228	242	220	248	227	239	73
168	204	156	215	160	222	80
163	197	153	199	153	175	80
29.5	90.2	27.3	102	21.3	88.5	60
214	298	206	298	208	296	80
	157		167		155	90
2354 ± 69.3	2744 ± 77.6	2346 ± 70.1	2887 ± 77.2	2239 ± 65.7	2755 ± 73.3	**Total number for each group**

Appendix 3 – Original data regarding the concentration of pollutants

Compound		**Triclosan**	**Climbazole**	**Clindamycin**	**MeP**	**EtP**	**BuP**	**ProP**
Recovery (%)		109	107	96	92	94	100	99
Pot no.		ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
1	Control	<MLOD	<MLOD	<MLOD	16.0	1.59	0.19	0.80
2		<MLOD	<MLOD	<MLOD	16.6	2.21	0.31	0.63
3		<MLOD	<MLOD	<MLOD	17.0	2.53	0.40	0.74
4		<MLOD	<MLOD	<MLOD	14.9	2.08	0.30	0.56
5		<MLOD	<MLOD	<MLOD	19.0	2.49	0.36	0.69
6		<MLOD	<MLOD	<MLOD	14.3	2.05	0.34	0.65
31	Biochar	<MLOD	<MLOD	<MLOD	20.7	1.96	0.14	0.16
32		<MLOD	<MLOD	<MLOD	14.3	1.75	0.08	0.02
33		<MLOD	<MLOD	<MLOD	22.6	1.81	0.05	0.01
34		<MLOD	<MLOD	<MLOD	18.8	1.88	0.05	0.09
35		<MLOD	<MLOD	<MLOD	15.3	1.81	0.09	0.02
36		<MLOD	<MLOD	<MLOD	13.2	1.52	0.07	0.05
13	C+Micro	<MLOD	<MLOD	<MLOD	19.8	2.60	0.69	1.15
14		<MLOD	<MLOD	<MLOD	15.9	2.23	0.51	1.08
15		<MLOD	<MLOD	<MLOD	15.7	2.25	0.56	1.17
16		<MLOD	<MLOD	<MLOD	17.5	2.07	0.46	0.46
17		<MLOD	<MLOD	<MLOD	14.3	2.39	0.51	0.87
18		<MLOD	<MLOD	<MLOD	16.7	2.31	0.55	0.94
43	B+Micro	<MLOD	<MLOD	0.24	19.7	2.68	0.74	1.09
44		<MLOD	<MLOD	0.74	16.8	1.67	0.59	0.68
45		<MLOD	<MLOD	0.30	28.1	2.26	1.20	1.46
46		<MLOD	<MLOD	0.18	17.2	1.63	0.64	0.72
47		<MLOD	<MLOD	0.27	18.1	1.81	0.63	0.49
48		<MLOD	<MLOD	0.35	14.5	1.35	0.82	0.58
25	C+Mix	<MLOD	<MLOD	<MLOD	16.5	2.42	0.73	1.32
26		<MLOD	<MLOD	<MLOD	29.4	3.29	0.77	1.94
27		<MLOD	<MLOD	<MLOD	15.7	2.14	0.55	0.60
28		<MLOD	<MLOD	<MLOD	17.5	2.42	0.46	0.81
29		<MLOD	<MLOD	<MLOD	16.9	1.97	0.86	1.23
30		<MLOD	<MLOD	<MLOD	19.1	2.52	0.59	0.97
55	B+Mix	<MLOD	<MLOD	0.38	17.8	2.52	1.65	1.53
56		<MLOD	<MLOD	0.65	13.7	1.82	0.76	0.85

57		<MLOD	<MLOD	0.39	14.5	1.46	0.54	0.43
58		<MLOD	<MLOD	0.10	16.9	1.87	0.57	0.33
59		<MLOD	<MLOD	0.24	10.9	1.25	0.72	0.59
60		<MLOD	<MLOD	0.58	16.1	1.86	0.27	0.27