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Transfer of Pharmaceuticals from Soil to Plants

Master´s thesis

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Declaration

I confirm that my master's thesis Transfer of Pharmaceuticals from Soil to Plants was completed with the assistance of my advisor while following proper research protocol. Relevant sources have been cited throughout the text and listed in the references section. I also certify that all third-party copyrights have been respected and no infringement was committed.

In Prague on 14 April 2024 _____

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Transfer of Pharmaceuticals from Soil to Plants

Summary:

The mechanism of pharmaceutical transfer from soil to plants has garnered significant attention in recent years due to its potential impact on the environment as well as human health. The impact of irrigation practices on pharmaceutical distribution in crop-production agricultural systems is a significant concern for both environmentalists and farmers. The intensive use of water resources for irrigation, coupled with the application of various agrochemicals, has led to increased pharmaceutical prevalence in the crops.

This experiment was conducted from June to August (approx. 77 days) analysing the results of the process on Zucchini plants (*Cucurbita Pepo L.*). The study evaluated the effects of two irrigation solutions: Pharma, containing pharmaceutical compounds, and Mix, containing pharmaceutical compounds and a few micropollutants. Focusing on aspects such as the concentration of pharmaceutically active compounds (PhACs), the absorption of PhACs, and the bioaccumulation factor (BAF) of PhACs. One crucial aspect is the uptake of these compounds by zucchini plants, which then translocate to different parts of the plant, including the edible portions such as fruits. Understanding these complex interactions provides valuable insights for mitigating potential risks and ensuring a safe and sustainable agricultural future.

According to the research conducted, additional micropollutants in irrigation water do not significantly affect zucchini plants' absorption of pharmaceutical substances. Therefore, the coexistence of various micropollutants in irrigation water has no significant effect on the uptake of pharmaceutical compounds by zucchini plants. Also, incorporating biochar into the soil does not hinder the absorption of pharmaceuticals. Using pharma versus mix treatment groups, no significant differences were observed in the amount of accumulation of various PhACs in plants biomass. The concentration of PhACs was significantly lower in the soils with biochar compared to those without, as indicated by the results. Plants were able to grow more in size due to the increased nutrient content in biochar, resulting in increased water consumption and lower concentrations of PhACs.

Keywords:

biochar; zucchini; soil solution; carbamazepine, pharmaceuticals, micropollutants, uptake, bioaccumulation

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1 Introduction

The environment is regularly exposed to pharmaceuticals, which are regarded as developing pollutants. Pharmaceuticals have the potential to be potent sources of healing when used properly, but when misused, they can have negative environmental effects. When pharmaceuticals are released into the environment, they can accumulate in the soil and plants (Carter et al., 2014). Research has shown that a common antibiotic, namely ciprofloxacin, can be taken up into plants from soil (Lillenberg et al., 2010) and that the concentration of the antibiotic in the plant increases as the concentration in the soil increases (Bassil et al., 2013). Globally, more than 600 pharmaceutical substances are estimated to be present in the environment (Küster & Adler, 2014). A further concern is that pharmaceuticals may infiltrate groundwater and surface water, where they may adversely affect the ecosystem (Nikolaou et al., 2007). For instance, antibiotics, hormones, and antidepressants, among other active pharmaceutical components, have been found in both surface and groundwater (Roberts & Thomas, 2006). Therefore, it is crucial to comprehend the processes and mechanisms by which pharmaceuticals are transmitted from soil to plants as well as any potential environmental effects.

There are several ways pharmaceuticals can pollute the environment, including wastewater discharge and agricultural practices (Daughton & Ternes, 1999). Manufacturing plants, hospitals and households can discharge pharmaceuticals into the environment (Cardoso et al., 2014). Further, drugs used in agriculture may contaminate aquatic habitats through bioaccumulation by leaching into soil and streams (Santos et al., 2010). Pharmaceuticals can also bioaccumulate in the food chain, increasing health risks for humans (Clarkson, 1995). Some organisms may become resistant to antibiotics as a result of the potential toxic effects on the environment (Kümmerer, 2009).

Pharmaceutical active compounds (PhACs) are transferrable from soil to plants in a number of different ways. In order for compounds to be absorbed and translocated in the plant, multiple parameters must be present such as the physicochemical properties of the compounds, the physiology of the plant, and the environmental factor (Bigott et al., 2020). The risk of PhACs crop contamination can be decreased by using a variety of techniques. For the elimination of micropollutants, efficient biological techniques have been invented (Santos et al., 2022). Farmers can also utilize soil testing to detect whether there are any drugs in their soil. Furthermore, other measures such as phytoremediation, soil amendments, and the use of alternative crops can be employed to further reduce the risk of crop contamination (Oseni et al., 2020).

2 Scientific Hypotheses and Objectives of the Thesis

2.1 Hypotheses

- If biochar is used as a medium for growing zucchini plants, then it will reduce the uptake of pharmaceutical active compounds (PhACs) by the plants.
- The uptake of pharmaceuticals by plants will be affected by the presence of other micropollutants in irrigation water.

2.2 Objectives of the Experiment

- To examine the impact of pharmaceutically active compounds (PhACs) on zucchini growth and determine whether biochar hinders the uptake of PhACs.
- To determine whether the combination of multiple micropollutants influences their accumulation in biomass and to assess the safety of the biomass for consumption.

3 Literature Review

Pharmaceutical active compounds (PhACs) are a collection of chemicals - of both natural and synthetic origin - that are utilized for curative, diagnostic, or preventive purposes. PhACs have increasingly been observed in the surrounding environment where they pose a significant health hazard to both terrestrial and aquatic organisms (Nikolaou et al., 2007). These substances are very resistant to most traditional wastewater treatments, thus leading to their accumulation in surface and groundwater (Jelic et al., 2011). This pollution has severe implications for ecosystems and human health. The contamination arises from the introduction of these chemicals into the environment in ways that are harmful to the environment and the people. Such pollutants have a long-lasting effect on the environment and may be difficult to remove due to their tendency to bioaccumulate in the food chain (Mishra et al., 2019). Therefore, preventive measures need to be enforced to mitigate the adverse impacts of pharmaceutical pollution.

Preventative measures include reducing the number of pharmaceuticals that are released into the environment through waste management, improved wastewater treatment, better monitoring and control of pharmaceuticals in the environment (Michael et al., 2013). Additionally, research needs to be conducted to understand the long-term effects of pharmaceuticals on ecosystems and human health. According to (Kümmerer, 2009) the presence of antibiotics in the environment can reduce the effectiveness of antibiotics in the treatment of human diseases.

3.1 Pharmaceutical Contamination in Soil-Plant Systems

Pharmaceuticals in soil can enter plants through the roots and contaminate the edible parts (Boxall et al., 2006). This can lead to health risks for humans and animals when contaminated food is consumed. These effects can be compounded by the presence of other contaminants in the environment. This is why it is important to monitor and analyze the levels of pharmaceuticals in soils and plants, to minimize any potential health risks and to ensure that the soil and plants are safe for consumption. In two different studies, the presence of antibiotics in soil was found to reduce the growth and development of lettuce plants and was suspected to be a main cause of food contamination (Pan & Chu, 2017; Ye et al., 2016). A soil contaminated with antibiotics can adversely affect the growth, development, and health of plants, as well as humans who consume those plants (Pan & Chu, 2017).

In nearly 90% of cases, older adults regularly take at least one prescription drug, and in almost 80% of cases, they take at least two prescription drugs. The rates are even higher when over-the-counter and dietary supplements are taken into account (Ruscin & Linnebur, 2021). The physical, chemical, and biological characteristics of pharmaceuticals determine how they behave and where they end up in soil. These compounds can either be kept in the topsoil or leached into groundwater and flow towards surface water, depending on the physicochemical characteristics of

medicines and soil characteristics (Doruk et al., 2018; Jałowiecki et al., 2019; Yang et al., 2012). Table 1 shows the concentration of certain drugs found in soils across several countries.

Substance	Country	Measured concentrations [ng/g]	Average integrated pharmaceutical masses (total ng) in soils normalized to soil organic carbon content
Triclocarban	China	0.3–51.8	-
Salicylic acid	China	1.0–7.3	-
Oxytetracycline	China	3.3–139	-
Tetracycline	China	1.9–17.4	-
Acetaminophen	USA	-	5.42–33.2
Trimethoprim	USA	-	1.22–2.22
Warfarin	USA	-	4.48–23.9
Sulfamethoxazole	USA	-	9.13–42.6
Erythromycin	USA	-	108.3–210
Carbamazepine	USA	-	16.9–23.5
Doxycycline	Malaysia	62.6–728.4	-
Norfloxacin	Malaysia	< MQL-95.7	-
Trimethoprim	Malaysia	< MQL-60.1	-
Progesteron	Malaysia	< MQL-24.2	-
Acetaminophen	Spain	n.d.-5.95	-
Diclofenac	Spain	n.d.-5.06	-
Carbamazepine	Spain	0.08–1.36	-
Flumequine	Spain	n.d.-5.31	-
Hydrochlorothiazide	Spain	0.38–1.20	-

Table 1 The concentration of selected pharmaceuticals in soils in different countries(ng/g) (Gworek et al., 2021)

3.1.1 Sources of Pharmaceuticals in the Environment

There are numerous ways for pharmaceuticals to get into the environment. Figure 1 depicts the potential sources and routes by which pharmaceutically active compounds could enter the environment. A deeper understanding of how pharmaceuticals are absorbed into plants and accumulated by them is vital to understanding the potential risks associated with the transfer process from soil to plant. The transfer can occur through the uptake of contaminated irrigation water, the use of contaminated manure or biosolids as fertilizers, or the direct deposition of dust particles onto the plant. Transfer of pharmaceuticals can also take place from plant to plant, either through pollen or direct contact. (Boxall et al., 2006; Prosser & Sibley, 2015; Smith & Jones, 2000; X. Wu et al., 2015).

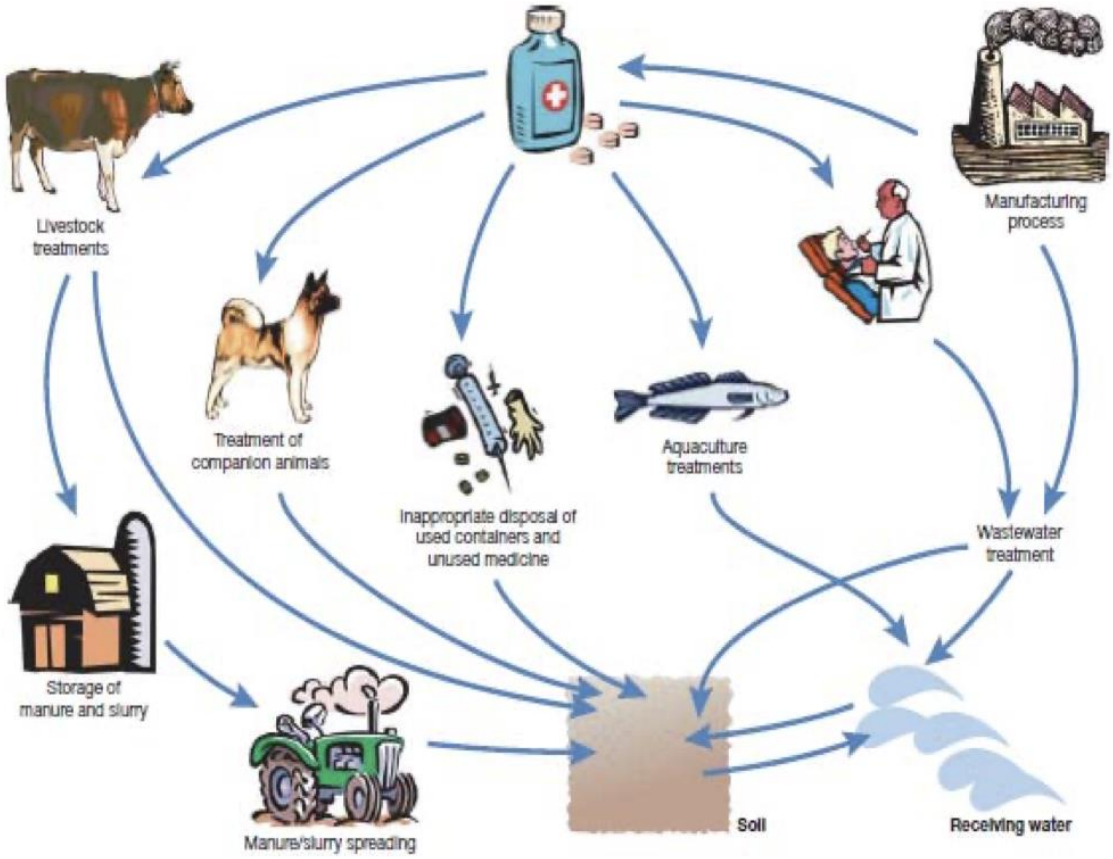


Figure 1 Pharmaceuticals' environmental entry points (Boxall, 2004)

Most of the time, contamination is transferred via contaminated irrigation water (Wu et al., 2014). The reason for this is that pharmaceuticals can accumulate in the soil, and when water is added to the soil, the pharmaceuticals become dissolved and can be absorbed by the plants (Boxall et al., 2006; Carter et al., 2014; Li, 2014). When contaminated manure or biosolids are used as fertilizers, pharmaceuticals can also enter plants through the soil and be absorbed by roots (Prosser & Sibley, 2015). Particles containing pharmaceuticals can also settle on the plant, allowing them to be transferred (Smith & Jones, 2000). Pharmaceuticals found in contaminated soil include antibiotics, antimicrobials, and other drugs (McEachran et al., 2016). It has been shown that contaminated soil leads to health problems in humans (Berman & Lali, 2022).

Another main source of pharmaceuticals and personal care products (PPCPs) in the environment is from wastewater treatment plants (WWTPs) (Daughton & Ternes, 1999; Liu & Wong, 2013). Daughton & Ternes also indicate that WWTPs were developed to manage human waste predominantly composed of natural substances, utilizing the acclimatized degradative capabilities of microorganisms. Over time, metabolic efficiency of a particular drug can improve due to enzyme induction and cellular adaptation. Additionally, coagulation and flocculation of suspended solids are employed, and on occasion, tertiary treatments such as chemical or ultraviolet (UV) oxidation are incorporated. However, the fate of most anthropogenic chemicals

introduced alongside this conventional waste remains uncertain (Daughton & Ternes, 1999).

Table 2 shown that PPCPs consist of a range of organic compounds including antibiotics, hormones, anti-inflammatory drugs, antiepileptic drugs, blood lipid regulators, β-blockers, contrast media, and cytostatic drugs for pharmaceuticals. Additionally, they include antimicrobial agents, synthetic musks, insect repellant, preservatives, and sunscreen UV filters for personal care products.

	Subgroups	Representative compounds
Pharmaceuticals	Antibiotics	Clarithromycin
		Erythromycin
		Sulfamethoxazole
		Sulfadimethoxine
		Ciprofloxacin
		Norfloxacin
		Chloramphenicol
	Hormones	Estrone (E1)
		Estradiol (E2)
		Ethinylestradiol (EE2)
	Analgesics and anti-inflammatory drugs	Diclofenac
		Ibuprofen
		Acetaminophen
		Acetylsalicylic acid
	Antiepileptic drugs	Carbamazepine
		Primidone
	Blood lipid regulators	Clofibrate
		Gemfibrozil
	β-blockers	Metoprolol
		Propanolol
	Contrast media	Diatrizoate
		Iopromide
	Cytostatic drugs	Ifosfamide
		Cyclophosphamide
Personal Care Products	Antimicrobial agents/Disinfectants	Triclosan
		Triclocarban
	Synthetic musks/Fragrances	Galaxolide (HHCB)
		Toxalide (AHTN)
	Insect repellants	N,N-diethyl-m-toluamide (DEET)
	Preservatives	Parabens (alkyl-p-hydroxybenzoates)
	Sunscreen UV filters	2-ethyl-hexyl-4-trimethoxycinnamate (EHMC)
		4-methyl-benzilidine-camphor (4MBC)

Table 2 Classification of PPCPs (Liu & Wong, 2013)

3.1.2 Pharmaceuticals Active Compounds (PhACs) in the Environment

A growing number of PhACs are being found in soil, such as antibiotics and antimicrobials. Mass-introduction of antibiotics occurred after 1945 where they were used to prevent and treat disease, protect plants, preserve food, and promote animals' growth (Kirchhelle, 2020). Over-prescription of pharmaceuticals and irrigation runoff can all contribute to the production of these compounds in soil. The presence of these compounds poses a serious environmental concern, as they can cause antibiotic resistance, changing of soil ecology, and contaminate food and water sources (Chaturvedi et al., 2021). Approximately 1.27 million people worldwide die each year due to antibiotic resistance (Thompson, 2022).

According to (Buxton & Kolpin, 2004), the amount of PhACs entering the sewage system has increased because of population growth, rising prosperity, and easier access to medications. When land is irrigated with treated, improperly disposed, or untreated effluents and sewage biosolids, some medicines may accumulate in the soil in those areas (Lees et al., 2016).

Table 3 shows the variety of pharmaceutical concentrations in soil that have been found from different literature obtained from (Verlicchi & Zambello, 2015). Different concentration ranges were discovered for trimethoprim, carbamazepine, and triclosan by (Kinney et al., 2008) and (Li, 2014), confirming that a variety of circumstances may affect their prevalence. These variables include the frequency and rate of sludge application, soil characteristics and conditions, chemical and biological

properties of the compound (Butler et al., 2011), the interval between sludge application and soil sampling (Jones et al., 2014), and rainfall and runoff (Kladivko & Nelson, 1979).

In the first eight months after the sludge application in three different soil types, Butler et al. (2011) observed a minor attenuation of triclosan in soil (initially 0.8-1 mg/kg). After one year of application, the reduction was around 80%. They explain this decrease by the biodegradation of triclosan to methyl triclosan, which was reported to have a concentration of roughly 0.4 mg/kg. Norfloxacin and ciprofloxacin levels in the topsoil eight months after sludge application were measured by Golet et al. in 2002. For norfloxacin, they discovered 0.29–0.32 mg/kg dry matter, and for ciprofloxacin, they found 0.35–0.40 mg/kg dry matter. They also observed a modest decline in the antibiotic levels in the sludge-amended soil after 21 months, indicating that fluoroquinolone residues remain and may accumulate in the terrestrial environment following sludge application.

Compound	Measured concentrations [ng/g]	References
Diclofenac	n.d.[a] — 1.16	Li (2014)
Ibuprofen	n.d. — 5.03	Li (2014)
Ciprofloxacin	350–400 after 8 months	Golet et al. (2002)
	280–270 after 21 months	
	450 (2.5 cm depth)	Golet et al. (2003)
Norfloxacin	320–290 after 8 months	Golet et al. (2002)
	270–300 after 21 months	Golet et al. (2002)
	350 (2.5 cm depth)	Golet et al. (2003)
Sulfadiazine	n.d. — 3.82	Li (2014)
Trimethoprim	0.64	Kinney et al. (2008)
	n.d.	Kinney et al. (2008)
	n.d. — 60.1	Li (2014)
Diphenhydramine	n.d.	Kinney et al. (2008)
	n.d.	Kinney et al. (2008)
Carbamazepine	n.d.	Kinney et al. (2008)
	n.d.	Kinney et al. (2008)
	0.02–7.5	Li (2014)
Caffeine	n.d.	Kinney et al. (2008)
	n.d.	
Triclosan	833	Kinney et al. (2008)
	96; 160	Kinney et al. (2008)
	n.d. — 16.7	Li (2014)
	774–949	Butler, Whelan, Ritz, Sakrabani and Van Egmond (2011)
Galaxolide (HHCB)	633	Kinney et al. (2008)
	1050; 2770	
Tonalide (AHTN)	113	Kinney et al. (2008)
	287; 773	Kinney et al. (2008)
NP1EO	n.d.	Kinney et al. (2008)
	n.d.	Kinney et al. (2008);
NP2EO	n.d.	Kinney et al. (2008)
	n.d.	Kinney et al. (2008)

Table 3 Measured concentrations of pharmaceutical compound in soil (Verlicchi & Zambello, 2015)

3.1.3 Environmental and Health Impacts

Pharmaceuticals and personal care products (PPCPs) that contain a variety of organic compounds, such as synthetic musks, hormones, antibiotics, and antimicrobial agents, among others, have caused significant concern in recent years due to their ongoing use and potential threat to both human health and the environment (Liu & Wong, 2013). Studies have examined the biological impacts on fish of some frequently observed pharmaceuticals in aquatic ecosystems, including nonsteroidal anti-inflammatory drugs (NSAIDs), fibrates, β-blockers, selective serotonin reuptake inhibitors (SSRIs), azoles, and antibiotics. A review of the literature reveals that laboratory findings on the biological consequences in fish tend to be consistent with the documented effects of these pharmaceuticals in mammalian species (Corcoran et al., 2010).

Various substances can be absorbed by plants through photosynthesis, stored in their tissues, and even transferred to humans when consumed (McElrone et al., 2013). According to a study conducted by Peralta-Videa et al., arsenic (As), which can be acquired, for example, through the ingestion of As-contaminated rice, is known to induce bladder, lung, and skin cancer. Along with harming the female reproductive system, cadmium can also damage the kidney, liver, and bones. Humans can also be exposed to chromium, which can cause cancer, by smoking and eating plants high in the metal. Well-known neurotoxins, lead and mercury are found in seafood, vegetables, and rice (Peralta-Videa et al., 2009).

3.2 Uptake of Pharmaceuticals by Plants in the Environment

Generally, clay-type soil is more likely to hold certain pharmaceuticals, such as antibiotics, resulting in greater uptake (Carter et al., 2016). On the other hand, organic compounds are more attracted to organic matter, resulting in lower levels of uptake (Schroll & Scheunert, 1992), in other words, different types of soil can affect the availability and uptake of pharmaceuticals by plants. Plants take up organic pollutants through their roots, leaves, (Zhang et al., 2017) and stems. Pharmaceuticals are taken by roots and translocated into various tissues by transpiration and diffusion (Madikizela et al., 2018). Additionally, the chemistry of the pharmaceutical can also influence its uptake. The pH level of the soil can also play a role in the uptake of pharmaceuticals. Soils with higher pH levels tend to bind pharmaceuticals more strongly, resulting in a decreased uptake, whereas soils with lower pH levels tend to allow higher uptake of pharmaceuticals. Furthermore, the type of organic matter present in a soil can also influence the uptake of pharmaceuticals, with organic matter with higher levels of hydrophobicity and adsorption capacity typically resulting in higher levels of pharmaceutical uptake (Hari et al., 2005).

Medications display various physicochemical characteristics that vary from one to another. Table 4 shows some properties of the listed pharmaceuticals. As shown in the table, diclofenac, tylosin and glyburide are amongst the medications that are largely insoluble in water. This can quickly cause their adsorption into sludge and sediments and make them easily accessible for plants to absorb (Madikizela et al., 2018).

Therapeutic group	Pharmaceuticals	Molecular weight (g/mol)	Water solubility (mg/L)
NSAIDs	Ibuprofen	206	58
	Naproxen	230	44
	Diclofenac	296	2.37
	Ketoprofen	254	51
	Acetaminophen	151	14,000
Antibiotics	Sulfamethazine	277	1500
	Sulfamethoxazole	253	610
	Sulphadimethoxine	310	343
	Sulfadiazine	250	77
	Penicillin G	334	210
	Trimethoprim	290	400
	Oxytetracycline	460	313
	Norfloxacin	319	1,78E05
	Ciprofloxacin	331	3E04
	Minocycline	457	5.2E04
	Tetracycline	444	231
	Chlortetracycline	479	288
	Lincomycin	407	927
	Tylosin	916	5
Antibacterial	Carbadox	262	1755
β-Blockers	Propanolol	259	62
	Atenolol	266	9.54×10^5
Calcium channel blocker	Verapamil	454	4.47
Antiepileptics	Carbamazepine	236	18
	Dilantin	252	32
	Primidone	218	500
Steroid hormones	17α-Ethinylestradiol	296	11.3
Antidepressant	Fluoxetine	309	50,000
Antidiabetic	Metformin	129	1380
	Gliclazide	323	–
	Glyburide	494	4
Antihistamine	Diphenhydramine	255	363
Antineoplastic agent	Cyclophosphamide	260	1–5E04
Anti-itch	Crotamiton	203	–
X-ray contrast agent	Iopromide	791	–
Lipid-lowering agents	Atorvastatin	6.36	0.00112
	Gemfibrozil	250	11
Benzodiazepines	Diazepam	285	50
Tranquilizers	Meprobamate	218	4700

Table 4 Physicochemical properties of pharmaceuticals that have been detected in plant tissues (Madikizela et al.,2018)

3.2.1 Root Uptake

The absorption of harmful substances or pharmaceutically active compounds by plant roots is a multifaceted process, which has been extensively examined in environmental science. Numerous factors contribute to this process, such as water and nutrient absorption by roots, the existence of root hairs and mycorrhizal fungi, and the compound's chemical properties (Gianinazzi-Pearson & Gianinazzi, 1983).

Two primary mechanisms for toxic materials or pharmaceutically active compounds to be absorbed by roots are passive diffusion and active transport (Orita, 2012). Passive diffusion occurs when the compound travels from an area of high concentration to one of low concentration across a semi-permeable membrane (Briggs et al., 1987). Active transport, on the other hand, requires energy and involves

molecules moving against their concentration gradient (Orita, 2012). Facilitated diffusion is akin to passive diffusion but necessitates a carrier protein to assist in moving molecules across the membrane (Ebel, 1985).

Once roots absorb these materials, they can be transported throughout the plant via the xylem and phloem systems (White, 2012). Factors such as the compound's solubility and mobility in water, chemical properties, and soil concentration influence this transportation process (McGechan & Lewis, 2002).

It is crucial to recognize that plants exhibit varying susceptibility levels to harmful substances or pharmaceutically active compounds' root uptake. Certain plants have evolved mechanisms to either exclude or neutralize these compounds, while others might accumulate them in their tissues (Carter et al., 2014). Soil characteristics like pH level, organic matter content, and microbial activity can also impact root uptake (Barber, 1984).

Numerous studies have explored root uptake effects on plant growth and development concerning toxic materials or pharmaceutically active compounds. For instance, research has revealed that exposure to heavy metals like lead and cadmium can result in stunted plant growth and diminished yield. In the same vein, exposure to pharmaceutical agents such as antibiotics and anti-inflammatory medications can disrupt plant physiology and curtail crop productivity (Nagajyoti et al., 2010).

In summary, root absorption of toxic materials or pharmacologically active compounds from soil is an intricate process with notable implications on plant growth and development. Further investigation is required to enhance our comprehension of the underlying mechanisms and generate methods for counteracting these compounds' detrimental effects on plant health.

3.2.2 Phytoremediation and the Limitation

The soil provides nutrients to plants, including both beneficial and toxic substances. Plants can absorb and store harmful substances from the soil, which can then be transferred to humans through consumption. Thus, Phytoremediation plays a crucial role in safeguarding our food supply (Arthur et al., 2005; Oladoye et al., 2022). The concept of phytoremediation was first introduced in 1991 by Ilya Raskin of Rutgers University. Raskin coined the term in a grant proposal to the Superfund Program of the US Environmental Protection Agency (EPA) (Beans, 2017). Some of the most effective plants for phytoremediation are aquatic species such as hyacinth, azolla, duckweed and cattail. This is due to their high accumulation of heavy metals, high tolerance, fast growth, and high biomass production (Yan et al., 2020).

Although phytoremediation is a natural process that can be used to clean up contaminated soil, it is not a perfect solution. One of the main problems with phytoremediation is that it can take a long time for the plants to break down the contaminants especially for soils with high levels of contamination or complicated pollutant combinations (EPA, 2012). It is also not as effective as other methods of

remediation of polluted soils in situ (Koptsik, 2014). This can be a problem if the contaminants are causing immediate harm to humans or the environment.

3.2.3 Importance of Understanding the Mechanisms Affecting Plant Uptake of Pharmaceutical

The amount of medical waste that is produced in the United States each year is estimated at more than 5.9 million tons based on the 33 pounds of waste produced by each staffed bed per day (Overstreet S., 2018). Pharmaceuticals can be hazardous to soil and water ecosystems if not properly managed (Caracciolo et al., 2015). To ensure the safe disposal of these substances, we need to understand how plants absorb these substances. By researching the mechanisms of plant uptake, we can develop methods of disposal that will not affect the environment in a negative way. Furthermore, this knowledge can be applied to develop drug delivery methods that are more effective and less harmful to the environment (Hatefi & Amsden, 2002;George et al., 2019). It can also help us to identify potential risks associated with the release of pharmaceuticals into the environment.

Table 5 shows the negative effects of pharmaceuticals recorded in some of the literature. For instance, the antidepressant fluoxetine has been shown to reduce root growth and asexual reproduction in the *Lemna minor* plant when exposed to 323 nmol/L for 21 days (Amy-Sagers et al., 2017). Similarly, when carbamazepine levels in soil surpass 4 mg/kg, it leads to burnt edges, white spots, and a decrease in photosynthetic pigments in *Cucurbita pepo*'s mature leaves (Carter et al., 2015). Moreover, oxytetracycline has been found to hinder shoot and root growth in alfalfa (*Medicago sativa L.*) by up to 61% and 85% respectively (Kong et al., 2007), causing leaves to turn light green or even yellow as the dosage increases. In *Zea mays* seedlings, irrigation with water containing a blend of salbutamol, atenolol, lincomycin, cyclophosphamide, carbamazepine, bezafibrate, ofloxacin, and ranitidine resulted in reduced root length without impacting germination (Marsoni et al., 2014). Additionally, an extensive investigation into ten antibiotics' impact on seed germination and root elongation in lettuce (*Lactuca sativa*), alfalfa (*Medicago sativa*), and carrot (*Daucus carota*) found that they hindered root length and overall plant growth (Hillis et al., 2011).

Therapeutic group	Pharmaceuticals	Plant species	Effect on plants
Antidepressant	Fluoxetine	*Lemna minor*	Decreased root growth
Antiepileptic	Carbamazepine	*Cucurbita pepo*	The mature leaves suffered from burnt edges, white spots and reduction in photosynthetic pigments
Antipyretic	Acetaminophen	*Brassica juncea* L. Czern	Oxidative stress as well as increasing amounts of bleaching and dot-like lesions occurred on adaxial side of the leaf, and necroses.
Antidiabetic	Metformin	*Daucus carota* cvs. Napoli and Amagar	Reduced growth and biomass production
Antimicrobial	Sulphadimethoxine Mixture*	*Hordeum distichum* L.	Growth suppression
		Zea mays	Decreased in root length
Antibiotics	Chlortetracycline and oxytetracycline	*D. carota* and *M. sativa*	Decreased in root length
Antibiotic	Oxytetracycline	*Medicago sativa* L.	Leaves colour changed. Also, leaves and roots biomass decreased
Antibiotic	Sulfadiazine	*Salix fragilis* L. and *Zea mays* L.	Stress developed in *Salix fragilis* L. (e.g., reduced C/N ratio and total chlorophyll content) and death of *Zea mays* L.

Table 5 The negative effects of pharmaceuticals in plants (Madikizela et al. 2018)

3.2.4 Mechanisms for Remediation of Pharmaceuticals in the Environment

Different mechanisms include physical processes such as adsorption and desorption, chemical processes such as hydrolysis and oxidation, and biological processes such as biodegradation and bioaccumulation. These processes can affect the availability of pharmaceuticals in the environment. An example of a process that can degrade pharmaceuticals in the environment is biodegradation. This process lowers the number of medicines that are accessible for plant uptake (Maeng et al., 2011).

Physical treatment techniques in WWTPs encompass adsorption (Boudrahem et al., 2019; de Andrade et al., 2018; Ndoun et al., 2021), coagulation-flocculation (Alazaiza et al., 2022; Kooijman et al., 2020), electrocoagulation (Ensano et al., 2017), and reverse osmosis processes (Gholami et al., 2012). Out of all remediation methods, adsorption can effectively remove a broad range of pharmaceuticals from wastewater. This approach is highly adaptable because of its numerous benefits, such as the simplicity of operation (Srivatsav et al., 2020), lack of harmful waste, expandability, and affordability (Maged et al., 2021). Moreover, this technique possesses the ability to absorb various pharmaceuticals onto the adsorbent's surface. All types of adsorbents can potentially serve as appropriate sites for attracting pharmaceuticals. Nonetheless, the adsorbent should be economically viable and possess a high capability for eliminating contaminants from wastewater. The process of pollutant removal is straightforward, in which the adsorbate, like pharmaceuticals, moves and attaches to a fitting reactive site on the adsorbent's outer boundaries, consequently eliminating it from the water-based solution.

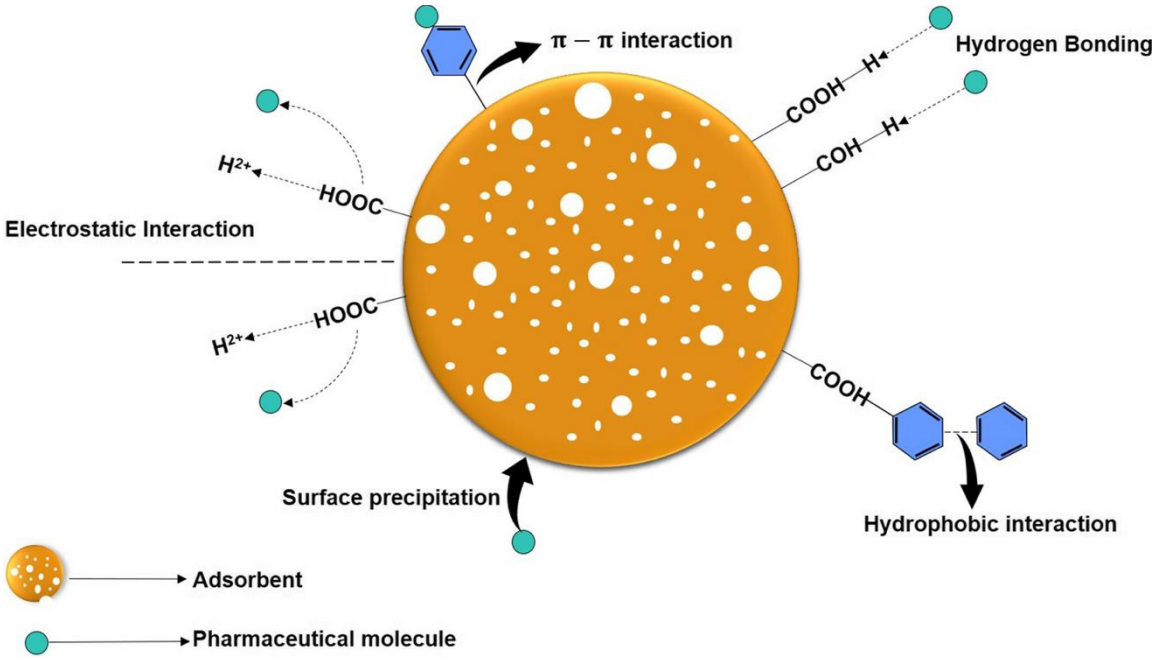


Figure 2 Typical mechanism for adsorption of pharmaceuticals (Chauhan et al., 2022)

Generally, adsorbents retain pollutants through π–π interactions, electrostatic connections, hydrophobic bonds, surface precipitation, and van der Waals attractive forces, as depicted in Figure 2. Over time, in the natural sorption process, a dynamic equilibrium state is reached when adsorption and desorption rates become equal. At this point, the adsorbate's capacity to attach to the preferred location diminishes significantly, indicating that the adsorption process has reached its maximum capacity or peak adsorption. Adsorption isotherms help comprehend adsorption equilibrium conditions by describing adsorption data. The main goal of adsorption isotherms is to clarify the relationship between solute molecule concentrations on solid surfaces and their equilibrium concentration in liquid phase under specific temperature and pressure conditions. Reaction kinetics aid in determining the adsorption rate by optimally selecting the adsorbate and adsorbent. Consequently, it is crucial to select materials for pollutant adsorption so as not to adversely impact the environment. Factors such as pH level, adsorbate concentration, nutrients, and media temperature determine the appropriateness of this process (Chauhan et al., 2022).

3.3 Biochar as a Medium to Reduce the Pharmaceutical Uptake to the Plant

3.3.1 Biochar and its Properties

According to Lehmann and Joseph, (2009) biochar is the carbon-rich byproduct of heating biomass, such as wood, manure, or leaves, in a closed container with little to no available air. In the technical terms, biochar is created by the process of organic material being thermally decomposed at low temperatures (about 700°C) with a constrained oxygen (O_2) supply. Biochar is growing in popularity as a sustainable product that may help lessen the demand for fertilizers while simultaneously lowering

carbon emissions (Glaser et al., 2001). Biochar also could increase microbial activity, increase nutrient availability, and decrease aluminum (Al^{3+}) toxicity, all of which can help in improving soil fertility (Kookana et al., 2011).

Research suggests that biochar may be able to assist the soil retain nutrients because of its charged surface and high surface area due to its porous structure (see Figure 3), which enable it to adsorb nutrients (Glaser et al., 2002) like nitrogen, phosphorous, and carbon. Study by Kookana et al., (2011) has demonstrated that biochar has an impact on a variety of soil chemical properties, and that it can cause quick changes in nutrient availability, pH, and electrical conductivity.

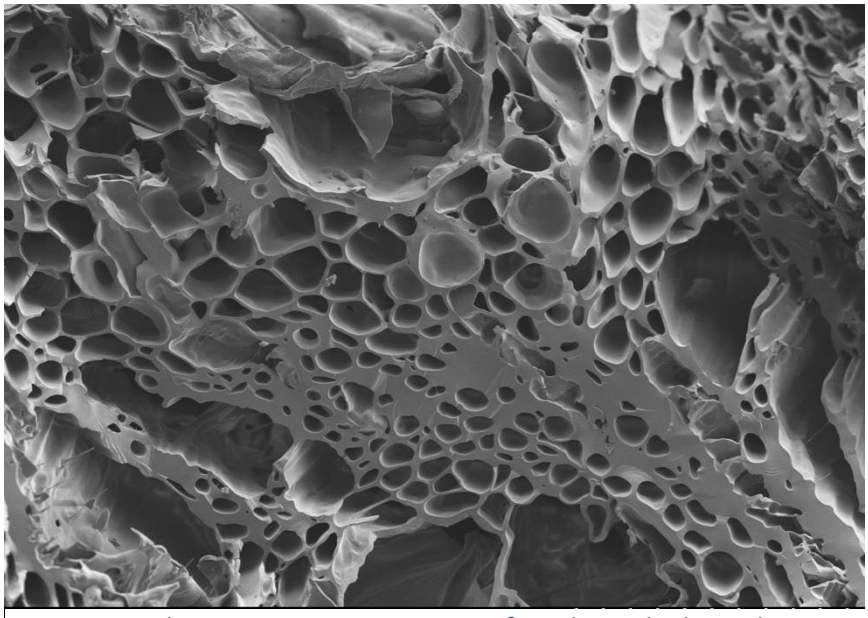


Figure 3 Electron microscope images of Biochar in high-resolution (Image credit: Dr Jocelyn, biocharproject.org)

The biochar production process consists of three phases: pre-pyrolysis, primary pyrolysis, and the creation of carbon-rich soil products (Lee et al., 2017). The temperature of pyrolysis has a significant connection with alterations in the composition and physicochemical characteristics of biochar (Asadullah et al., 2007; Chen et al., 2008; Jindo et al., 2014). The biochar's physicochemical attributes, such as surface area, pH, and functional groups were significantly impacted by the pyrolysis temperature, which in turn influenced its effectiveness as a soil amendment (Ding et al., 2014). A rise in pyrolysis temperature led to an expansion in surface area, carbonized portions, pH levels, and volatile substances, while concurrently reducing cation exchange capacity (CEC) and the concentration of surface functional groups (Tomczyk et al., 2020).

Research has shown that elevating the temperature of pyrolysis leads to alterations in biochar's surface area and porosity (Bonelli et al., 2007). The decomposition of organic substances and the creation of micropores may be the primary reasons for this phenomenon, as suggested by (Katyal et al., 2003). Furthermore, the breakdown of aliphatic alkyls and ester groups, along with the unmasking of the aromatic lignin core at elevated pyrolysis temperatures, could contribute to an enlarged surface area, according to Chen & Chen, (2009) study. Ghani et al. (2013) demonstrated that below 500°C, lignin does not transform into a water-repellent polycyclic aromatic hydrocarbon (PAH), leading to a more water-attracting biochar. However, when temperatures exceed more than 650°C, biochar becomes thermally stable and its hydrophobic properties increase.

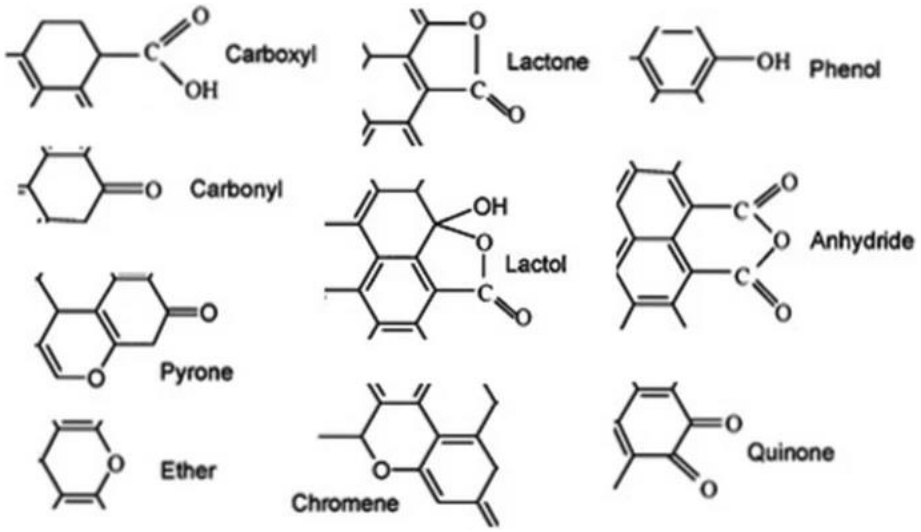


Figure 4 Biochar surface functional acidic groups (Tomczyk et al., 2020)

Figure 4 illustrates sample configurations of the graphene sheets' outer surface (Harris, 1997) and the associated pores (Van Zwieten et al., 2010). When biomass is heated to temperatures between 350-650 °C, the chemical bonds within it are broken and rearranged, resulting in the formation of new functional groups such as carboxyl, lactone, lactol, quinine, chromene, anhydride, phenol, ether, pyrone, pyridine, pyridone, and pyrrole (Mia et al., 2017).

3.3.2 Study Findings on Biochar's Effectiveness in Reducing Pharmaceutical Uptake

Numerous research studies have delved into the efficacy of biochar as a means to mitigate the absorption of pharmaceuticals. For instance, Bair et al., (2020) investigate the effectiveness of biochar as a soil enhancer to decrease the absorption of antimicrobial substances (ciprofloxacin, triclocarban, and triclosan) in lettuce (*Lactuca sativa L.*) and carrot (*Daucus carota*). Through the application of biochar, a decrease in the concentration of ciprofloxacin and triclocarban within lettuce foliage

was observed, while a notable 67% reduction in triclosan levels was found in the roots of carrot plants. Additionally, when combining biochar with biosolids, there was a notable increase in soil pH levels and overall nutrient content, which directly correlated with an enhancement in the biomass of lettuce shoots. The findings from (Bair et al., 2020) study highlight the promising effectiveness of utilizing walnut shell biochar as an adsorbent for pharmaceutical pollutants present in the soil, all without causing detrimental effects to the growth and development of plants.

Similarly, a study by Li et al. (2020) assessed the impact of biochar amendment on the absorption of 15 pharmaceuticals in radish (*Raphanus sativus*) cultivated in sandy loam at two amendment levels (0.1 and 1% w/w). In comparison to the unamended soil, the presence of acetaminophen, carbamazepine, sulfadiazine, sulfamethoxazole, lamotrigine, carbadox, trimethoprim, oxytetracycline, tylosin, estrone, and triclosan in radishes grown in soil supplemented with 1.0% biochar was significantly reduced by 33.3-83.0%. The diminished absorption of pharmaceuticals by plants was primarily attributed to the decreased levels in pore water due to biochar's presence. The researchers observed that the calculated daily consumption figures indicate that incorporating biochar might potentially reduce overall human exposure to a combination of pharmaceuticals.

The research papers examined in this article employed a variety of biochar types and experimental methodologies. Bair et al. (2020) utilized walnut shell biochar, while (Li et al., 2020) opted for forest pine wood biochar. Both investigations conducted greenhouse experiments to assess the efficacy of biochar in minimizing the absorption of pharmaceuticals.

Current studies indicate that biochar successfully decreases the absorption of pharmaceuticals in plants. This holds significant consequences for lessening the effects on both the environment and human health caused by these substances. More investigation is required to ascertain the best application of biochar in order to minimize pharmaceutical absorption, but the preliminary results show great potential.

3.4 Zucchini (*Cucurbita Pepo L.*)

Zucchini, also known as courgette (*Cucurbita Pepo L.*), is a type of summer squash that belongs to the Cucurbitaceae family. This annual plant yields green or yellow fruits that are typically long, cylindrical, and have a smooth skin. In addition to being a rich source of dietary fiber and vitamins A and C, zucchini is low in calories and fat, making it a nutritious supplement to any meal plan (Cervoni & Valdez, 2022). This vegetable is well-liked in many regions across the globe.

The roots of zucchini plants extend up up to 4 feet (1.2 m) deep in the soil (Richard et al., 2023). This allows zucchini plants to absorb more nutrients and minerals from deeper layers of soil, making them hardy and resilient. Zucchini plants also have shallow root systems that spread out, allowing them to better absorb moisture and nutrients from the surface soil. The combination of long and shallow roots makes

zucchini plants very efficient in collecting and utilizing the available resources in the soil. The deeper roots provide access to a wider range of minerals and nutrients, while the shallow roots allow for more efficient absorption of water and other surface-level nutrients (Rouphael et al., 2004).

In the study by Tamez et al. from 2019, where they wanted to determine the transport capability and immediate exposure impact of copper-based nanoparticles and substances within a complex soil environment, they discovered that after three weeks, copper (Cu) levels in the roots, stems, and leaves of zucchini plants were significantly higher compared to the control group. Nonetheless, the increased Cu concentration did not negatively influence plant growth or chlorophyll production (Tamez et al., 2019).

Zucchini plants are suitable for experiments for several reasons. They grow quickly, are easy to care for, and produce a large harvest. Their life span is brief, as most types reach maturity in around 50-60 days. According to Postma & Lynch, (2012), the unique root structures of squash create a beneficial interaction, leading to better nutrient absorption than in single plant cultures. These plants are also highly versatile and can be grown under a variety of conditions. Furthermore, as they are relatively small plants, they are perfect for experiments in small spaces, such as greenhouses.

Finally, zucchini plants are also useful for studying root uptake properties. As mentioned earlier, zucchini roots have a moderate uptake rate, which can be useful for studying the dynamics of nutrient and water uptake in plants. Additionally, the shallow root system of zucchini plants makes it possible to study the distribution of roots in the soil and their interactions with other organisms in the soil, such as mycorrhizal fungi (Heijden & Horton, 2009).

In summary, zucchini is a versatile and useful vegetable for experimental purposes, particularly for studying root uptake properties and other aspects of plant physiology.

4 Material and Method

4.1 Experimental Design

The study was designed as a pot-based experiment and was conducted under controlled conditions in the greenhouse facility at the Department of Agro-Environmental Chemistry and Plant Nutrition. The temperature of the room was maintained at 18 °C for day and night. Direct sunlight was suitably regulated through shading. The plant species utilized in the study was Zucchini (*Cucurbita Pepo. L.*), specifically the Pumpkin variety. The plants were cultivated in black plastic pots with drip trays.

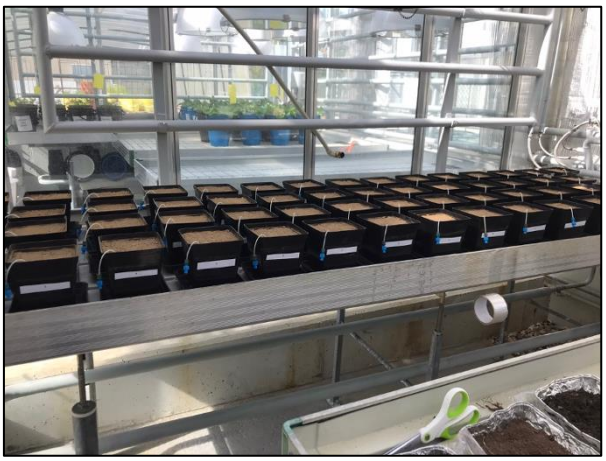


Figure 5 (on the left) The seeds of Zucchini
Figure 6 The experimental arrangement in the greenhouse

It was carefully planned that the experiment would involve zucchini plants planted in soils not amended with biochar and soils amended with biochar. It was complete with three separate treatments comprising of Control, Pharmaceuticals, and a Mix of PhACs and micropollutants. Zucchini seeds were placed in pots and arranged in a fully randomized design with six replications. In both the biochar treatment and the treatment without biochar, six replications were conducted. In order to achieve this, individual pots were accurately weighed and about 1500 grams of dry soil were added to each pot. Furthermore, the group that included Biochar (specifically pots numbered 31 through 60) had an additional 15 grams of Biochar incorporated into their respective soil mixtures. For more information about the Experimental Design, see Appendix 1.

To maintain optimal growing conditions throughout the experiment, all pots were subjected to a regular irrigation schedule every two to three days in the early phase of experiment, but the frequency increased as the plant grew, ensuring they

attained 60% of soil water holding capacity. In addition to this essential watering routine, each pot was also nourished with a nutrient-dense fertilizing solution consisting of ammonium nitrate (NH_4NO_3) and di-potassium hydrogen phosphate (K_2HPO_4). This solution was thoroughly combined with the soil within each pot so as to facilitate the best possible environment for growth and development of the zucchini plants.

Figure 7 The soil were weighed and distributed

Figure 8 1L of fertilizing solution

4.2 Type of Soil and Biochar

The soil used in the experimental study was obtained from a field located in Humpolec, Czech Republic, which is a part of an extensive ongoing long-term field fertilization research project that was initially established back in 1979. Situated at a distance of 102 kilometers towards the southeast of the city of Prague, the Humpolec region can be easily pinpointed on the map. The weather conditions in this particular area are characterized by an average annual precipitation rate of about 589 millimeters and an average yearly temperature measuring around 7.0 degrees Celsius. The geological elements that primarily dominate this region include various forms of metamorphic and igneous rocks, making it a significant part of the Moldanubian Zone. According to the Food and Agriculture Organization's (FAO) classification framework, the soil found in the Humpolec vicinity has been duly identified as Haplic Cambisol, which is notably situated upon a paragneiss substrate, as mentioned in Žigová et al. study conducted in 2013. Furthermore, the soil at this specific site boasts a sandy loam texture with pH between 4.5 to 6.6.

Figure 11 Mixing process in the bucket

Figure 12 Transferring to the pot

The Department of Agro-Environmental Chemistry and Plant Nutrition (CZU, Prague) produced biochar from sewage sludge by utilizing fast pyrolysis in a fixed bed reactor at 700 °C. Following the production process, the biochar was milled to pass through a 2mm sieve. The chosen temperature for biochar production ensured the maximum energy exploitation and stability of the end product. The rates of application fall under the standard scope for biochar incorporation in agricultural soil (Wang et al., 2019).

4.3 Crop Cultivation

After a period of one week following the sowing of plant seedlings, germinated seed counts were conducted for a duration of six days, with the exception of the third day on which a gap was maintained. Subsequently, once at least one zucchini seeds had successfully germinated, which typically occurred around the 14-day mark after initially sowing them, that zucchini seedling was retained while any surplus seedlings were carefully removed. The zucchini plants were closely monitored, and upon reaching their harvest stage after approximately 77 days from the initial sowing, they were harvested accordingly.

Figure 9 Two seeds were planted in each pot

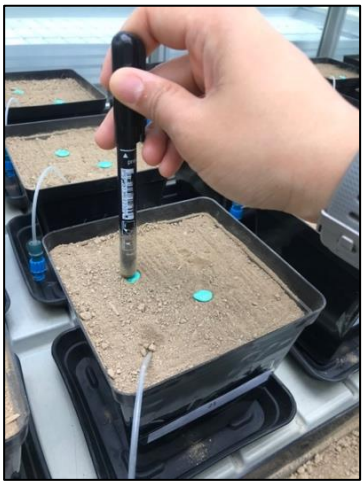


Figure 10 The seeds were sown 10cm deep

4.4 Soil Solution Extraction

The process of attempting to obtain pore water samples was carried out 7 times, due to the fact that some of these attempts were unsuccessful in securing the desired soil samples, thus resulting in the need to repeat the procedure. The soil solution was carefully obtained from specifically assigned pots for this purpose. To elaborate, a 5mL Syringe manufactured by Braun was attached to a Rhizon soil water sampler located in the pot to facilitate the collection of pore water samples. In order to maintain the vacuum within the syringe, a wooden spacer was utilized to hold the plunger in its appropriate position, as demonstrated by (Dickens et al., 2007; Seeberg-Elverfeldt et al., 2005).

Figure 13 The extraction of soil solution

To ensure that an adequate volume of pore water had been collected, the syringe was left undisturbed overnight. Once 18 to 24 hours passed, the pore water samples were deemed ready for collection. The initial acquisition of these samples took place on July 4th, approximately one or two hours after the plant had been treated with demineralised. The final collection of soil water samples was carried out on August 10th, just before the harvesting process commenced.

Upon completion of this procedure, these samples were transferred into designated vial storage containers and subsequently stored in a controlled environment kept at a frigid temperature of (-42°C) in order to ensure their preservation for future use and analysis. However, as the time constraint was not conducive to a thorough examination of the samples, they could not be included in the analysis.

4.5 Irrigation Solution

The irrigation solution was prepared according to the following protocol, which outlines the steps for creating various irrigation solutions: First, add a small amount of demi water into each of the 1-liter volumetric flasks. Next, use a micropipette to add the stock solutions according to the instructions - 1ml of control and 1ml EtOH for the Control solution, 1ml pharma mix and 1ml EtOH for the Pharma solution, and finally, 1ml of mixed solution along with 1 ml paraben mix for the Mix solution. Lastly, fill each flask with demi water up to the marked line to achieve a total volume of 1 litre for each irrigation solution.

The solution for pharmaceuticals treatment was prepared by dissolving each micropollutant which includes amisulpride, carbamazepine, citalopram, metoprolol, propafenone, sertraline, tiapride, tramadol, trospium, and venlafaxine in powder form into methanol. For the mixture treatment solution, it was prepared utilizing the same powdered micropollutants as in the pharmaceutical treatment. However, this solution included the addition of ciprofloxacin, climbazole, clindamycin, ofloxacin, triclosan, triclocarban, methylparaben, ethylparaben, propylparaben, butylparaben, bisphenol *a*, bisphenol *f*, and bisphenol *s*. The treatment mixture was then stored at -42°C throughout the duration of the experiment.

Table 6 presented includes information on both the concentrations of micropollutants found in irrigation water and the total amount of micropollutants applied to each pot. This data is essential in understanding the potential impacts of micropollutants on crops and the environment. In each one-litre portion of this irrigation solution, there is an inclusion of 10 µg of these pollutants. Conversely, when it comes to the paraben family—namely methylparaben, ethylparaben, propylparaben, and butylparaben—a total concentration of 100 µg for each pollutant is present within the mixture.

PhACs/ Micropollutants	Concentration of micropollutant in irrigation water (µg/l)	Total amount of applied PhACs/micropollutants per pot (µg/pot)			
		Pot without Biochar		Pot with Biochar	
		pharma	mix	pharma	mix
amisulpride	10	30.62	30.62	53.66	53.66
carbamazepine	10	30.62	30.62	53.66	53.66
citalopram	10	30.62	30.62	53.66	53.66
metoprolol	10	30.62	30.62	53.66	53.66
propafenone	10	30.62	30.62	53.66	53.66
sertraline	10	30.62	30.62	53.66	53.66
tiapride	10	30.62	30.62	53.66	53.66
tramadol	10	30.62	30.62	53.66	53.66
trospium	10	30.62	30.62	53.66	53.66
venlafaxine	10	30.62	30.62	53.66	53.66
ciprofloxacin	10	x	30.62	x	53.66
climbazole	10	x	30.62	x	53.66
clindamycin	10	x	30.62	x	53.66
ofloxacin	10	x	30.62	x	53.66
triclosan	10	x	30.62	x	53.66
triclocarban	10	x	30.62	x	53.66
methylparaben	100	x	306.2	x	536.6
ethylparaben	100	x	306.2	x	536.6
propylparaben	100	x	306.2	x	536.6
butylparaben	100	x	306.2	x	536.6
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 6 Concentration of micropollutants in irrigation water and total amount of applied micropollutants per pot

4.6 Water Consumption

At the initial stages of the experiment, the pot was irrigated to reach 40% to 60% of MWHC on each pot. The Maximum Water Holding Capacity (MWHC) was calculated by considering the values of the pot, plate, soil within the pot, and a piece of sackcloth used to prevent soil from escaping through the bottom of the pot. For a 1500g pot, it was determined that the MWHC accounted for 40% is equal to 255g.

The formula to determine the Water Consumption is based on the following equation:

Water Consumption *= MWHC - The weight of the pot before irrigation*

The results were then recorded and the average consumption for the entire experiment was calculated and tabulated in Chapter 5. Detailed calculation can be found in Appendix 5.

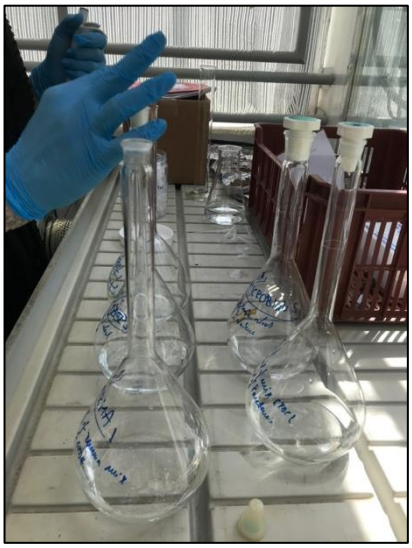


Figure 14 The prepared irrigation solution

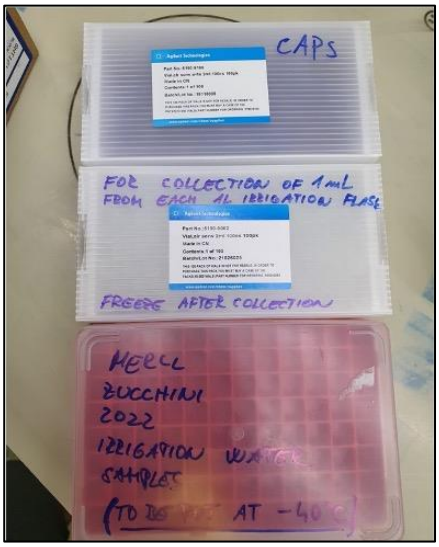


Figure 15 The extract of irrigation solution stored at -42°C

4.7 Harvest

The harvesting of zucchini took place on the 15th of August, after completing a lengthy 77-day period of vegetative growth. Following this procedure, the plant's biomass was meticulously separated from its root system and fruits were separated in glass bottles. Each individual component of the plant was then cautiously cut into smaller sections, thoroughly washed with demineralised water, and air-dried using filter paper. Upon completion of these steps, the weight and characteristics of each specimen were documented. Subsequently, the plant samples were cautiously wrapped in aluminium foil to ensure preservation. These samples were stored in a

freezer maintained at an extremely low temperature of -42°C before being ground into fine powders for further analysis.

Figure 16 The zucchini after 2 months of experiment

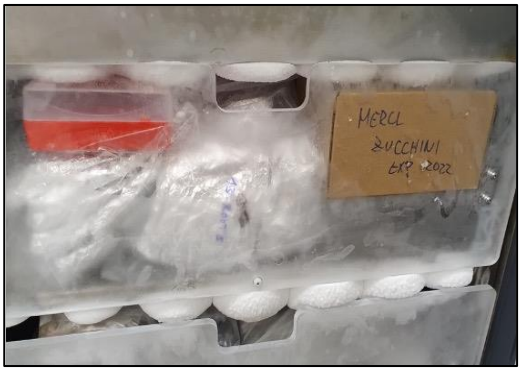


Figure 17 The placement of the biomass in the freezer

Figure 18 The plant were cut into smaller parts

Figure 19 The biomass were wrapped in the aluminium foil

4.8 Pharmaceuticals Extraction from Vegetative Biomass and Fruits

Prior to extraction, soil, zucchini biomass, and zucchini fruits required freeze-drying. During harvest, fruits and soil samples were placed into pre-weighted glass bottles and re-weighed before storing them in the freezer. To prepare them for freeze-drying, the glass bottles were covered with paper towels before placing them in the freeze-dryer. The biomass was freeze-dried in the same foil it was harvested in. After a duration of 7-8 days, samples were removed and weighed. The mass of freeze-dried soil samples, vegetative biomass, and fruits was calculated by deducting the weight of the glass bottles from the post-freeze-dried weight. Zucchini biomass and fruits were then milled using a laboratory electric mill.

Figure 20 The biomass was milled using IKA A11 basic analytical mill

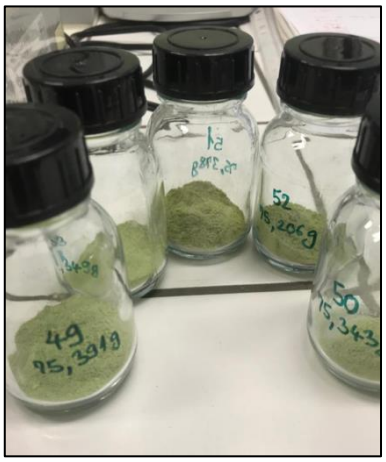


Figure 21 The biomass stored in the glass container

The extraction of vegetative biomass and zucchini fruits was performed using an adapted QuEChERS method as described by (Chuang et al., 2015). Initially, 0.1 g of lyophilized zucchini biomass was carefully weighed and placed into a 15 ml Falcon tube. Next, 2 ml of Milli-Q water was added to the tube, vortexed, and then refrigerated for 10 minutes. Following this, 1 ml of acetonitrile (MeCN) was introduced to the samples and vortexed for one minute before being sonicated for five minutes. Subsequently, 0.65 g of QuEChERS salts were added, vortexed for an additional minute, and placed in the centrifuge at a temperature of 4°C. The samples were then centrifuged at 4500 RPM for 10 minutes.

The supernatant was carefully transferred to a new 1.5 ml Eppendorf tube with the help of a Pasteur pipette. From there, 700 µl was moved to SPE salts using a micropipette. The SPE salts consisted of 150 mg $MgSO_4$, 50 mg PSA, and 20 mg GCB. Afterward, the samples were vortexed and centrifuged again at 14,000 RPM with a temperature set at 4°C for another ten minutes. Finally, the supernatant was cautiously transferred into an LC-MS/MS vial with a 400 µl insert using a Pasteur pipette without disturbing any salts present.

All treatment samples have been spiked with 40 µl of internal standard solution. The control samples are spiked in the following manner: unspiked, spiked with 40 µl of internal standard solution, and spiked with 40 µl of internal standard solution along with a 16 µl STD (125ppb). A total of six blanks were utilized, including two blanks spiked with 40 µl of internal standard solution and four complete blanks.

Following the extraction process, the concentrations of pharmaceuticals and their derivatives were evaluated using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The identification of each analyte was confirmed based on the retention time and the presence of both quantification and confirmation multiple reaction monitoring (MRM) mode.

4.9 Recovery Value

The reliability and accuracy of this analytical technique were established by evaluating the recovery rates of a known quantity of analyte integrated into the sample matrix. High Performance Liquid Chromatography was employed to examine the samples, while the spiked samples were produced by incorporating a specific amount of analyte into the matrix. The recovery rates were determined as the percentage of the introduced analyte that was retrieved from the sample. The average recovery rate was discovered to be 93.5%, accompanied by a standard deviation of 6%. These findings indicate that our approach is both accurate and precise for examining the analyte within the sample matrix.

4.10 Uptake of PhACs and Bioaccumulation Factor

The method to determine the absorption of pharmaceutically active compounds in biomass is based on the subsequent equation:

Uptake of PhACs (ng) *= Biomass production (g) x Concentration of PhACs (ng/g)*

The acquired data is subsequently utilized in the subsequent equation to determine the Bioaccumulation factors (BF) for pharmaceutical active compounds (PhACs) and their corresponding transformation byproducts within zucchini plants.

$$\textbf{\textit{Bioaccumulation Factor, BAF (\%)}} = \frac{Uptake\ of\ PhACs}{Amount\ of\ PhACs\ applied} \times 100$$

4.11 Statistical Method

One-way analysis of variance (ANOVA) was used to compute mean values, standard deviations, uptake of PhACs, bioaccumulation as well as to conduct variance analyses and ascertain significant distinctions among means ($P \leq 0.05$) and ($P \leq 0.001$), Tukey post hoc analyses and the MS Excel for Mac Version 16.71 was utilised.

5 Results

5.1 Germination Rate

The representation of percentage comparisons for seed germination across various treatment scenarios can be observed in Figure 22. A comprehensive analysis of the acquired data is displayed in the Table located in Appendix 2, which also includes information on standard deviations for better understanding and interpretation.

Upon examining the data showcased in the chart, it becomes evident that each distinct series experiences a unique germination progression over a period of time. Commencing at a 25% germination rate on the 9th day, the Pharma series witnesses a steady rise to attain 100% by the 12th day, where it subsequently maintains this percentage until the 15th day. Similarly, the Pharma + Biochar series kicks off with a germination rate of 33.33% on the 9th day and gradually ascends to reach its peak of 100% by the 13th day; this value then remains consistent until the 15th day.

As for the Mix series, an initial germination rate of 16.67% on day 9 is observed, followed by an increase that culminates in a complete 100% germination rate by day 13; such a rate then persists until day 15. Lastly, with regard to the Mix + Biochar series, an initial germination rate of a mere 8.33% commences on day 9 before escalating to a notable high of 100% by day 12—this percentage is then maintained until day 15.

The Control group saw only 50% of its zucchini seeds germinate on the 9th day, but by day 10, that rate increased to 75%. Days 12 to 15 saw a consistent germination rate of 75.12%, but escalated to 91.67% by the end of day 15, nearly all of the zucchini seeds in the Control group had successfully sprouted. The Control + Biochar group saw a slightly lower germination rate of 16.67% on day 9, but by day 12, almost all of the seeds had germinated, with a germination rate of 91.67%. From day 13 onwards, all of the seeds had germinated, with a germination rate of 100%.

The data depicted within the graph indicates that integrating biochar into soil composition results in an enhanced germination percentage for all Control, Pharma and Mix series – with more prominent effects observed specifically for Control and Mix series, which demonstrated slower germination in early stages. Nonetheless, discerning biochar's impact on germination rates for all Biochar-amended group is not as evident or straightforward.

The graphical representation exhibits the progression of germination rates for each series over a span of time. Initiating with a uniform allocation of two seeds per pot on the 13th day after sowing, these rates continue to retain their terminal value until the ultimate observation is documented on the 15th day. Each sequence maintains a distinct germination rate throughout time, but now the presence of error bars signifies the data's variability. These error bars are depicted by the standard

deviation. The length of these error bars conveys the variability extent within the data for every replication.

The chart reveals a diminishing level of data variability over time, as demonstrated by the progressively shorter error bars. This implies that a greater consistency in germination rates emerges as seeds continue to develop and mature. In addition to this observation, the chart also reveals noteworthy discrepancies in germination rates between various series, particularly at earlier stages.

After analysing the data in the graph through statistical methods, it has been concluded that none of the observed values achieve statistical significance at the $p<.001$ level. This result implies that no significant or noticeable connection exists between the number of germinated seeds and type of treatment, thereby preventing any meaningful interpretation or inference about their relationship.

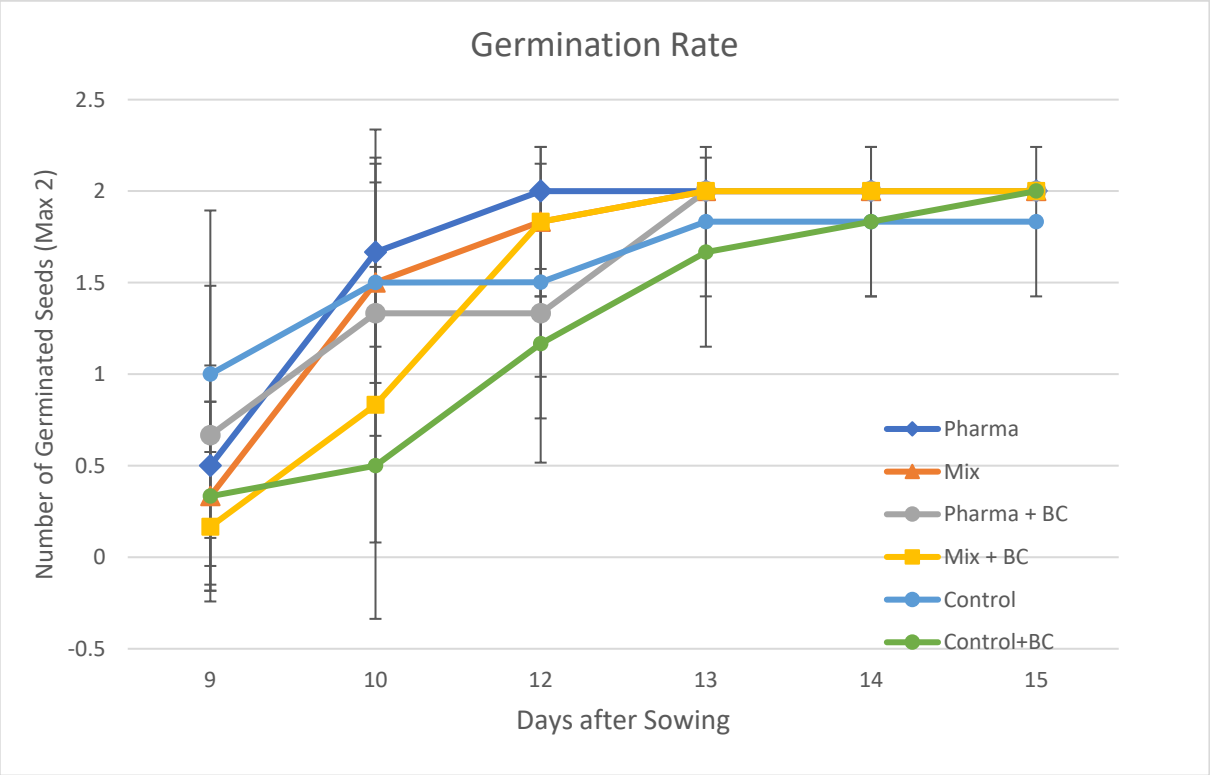


Figure 22 Number of germinated zucchini seeds treated with different PhACs. Maximal possible number of germinated seeds is 2. All of the variants had 6 replications.

5.2 Water Consumption and Amount of Each PhACs Applied

In this particular experiment, the consumption of water served as a crucial factor, directly influencing the growth and overall well-being of the plants being evaluated. These plants were subjected to irrigation both using water containing micropollutants compounds and demineralised water. The experiment was carried out with or without the addition of biochar to the soil. The main objective behind this

approach was to investigate whether incorporating biochar could potentially alleviate any adverse effects exerted by the pharmaceuticals on the plant subjects.

Water was provided to each individual plant throughout the experiment in order to accurately assess water consumption. Each plant's daily water usage was recorded, observing any discrepancies across groups and evaluating the effectiveness of the water use.

	Average of Water Consumption (ml)	Amount of Micropollutant Solution Applied (ml)
Ctrl	5416.01	x
Ctrl + BC	8110.65	x
Pharma	2346.32	3062
Pharma + BC	2887.95	5366
Mix	2239.23	3062
Mix + BC	2755.81	5366

Table 7 Amount of water consumption and chemicals applied in the zucchini. Each variance has 6 replications.

The findings of this experiment highlighted significant differences in water usage among various groups. Table 7 shows that some groups require more water than others. In particular, it was discovered that the group with biochar-enriched soil demonstrated a considerably increased need for water compared to the group without biochar amendments. The irrigation amounts varied in June, with no notable difference between the biochar and non-biochar plants. However, as the biochar plants grew larger and had increased transpiration, they needed more water. Consequently, the increased water supply contributes to larger plant size, leaf dimensions, and fruit yield, which leads to a greater need for water due to higher transpiration rates. The non-biochar plants appeared lighter in colour, implying they were nutrient deficient, specifically in nitrogen, hindering their growth. Although biochar may have had some impact, nutrient deficiency was likely the main contributing factor.

As the growing period approached week 11, the group that had been treated with biochar required additional irrigation due to the onset of wilting. Wilted plants occurred due to insufficient watering. The pots used were too small for the plants which led to the soil not retaining enough water for the plants' daily needs. As a result, we had to water them more frequently. This led to an increase in both water consumption and subsequently the application of irrigation solution in these particular groups, making their overall usage higher.

5.3 Biomass Production

The stacked-column graph in Figure 23 illustrates the mean biomass production across six distinct treatments, accompanied by their respective standard deviations after 77 days of vegetation period and drying process. The vertical axis of the graph showcases biomass production expressed in arbitrary units, while the horizontal axis displays the various treatments.

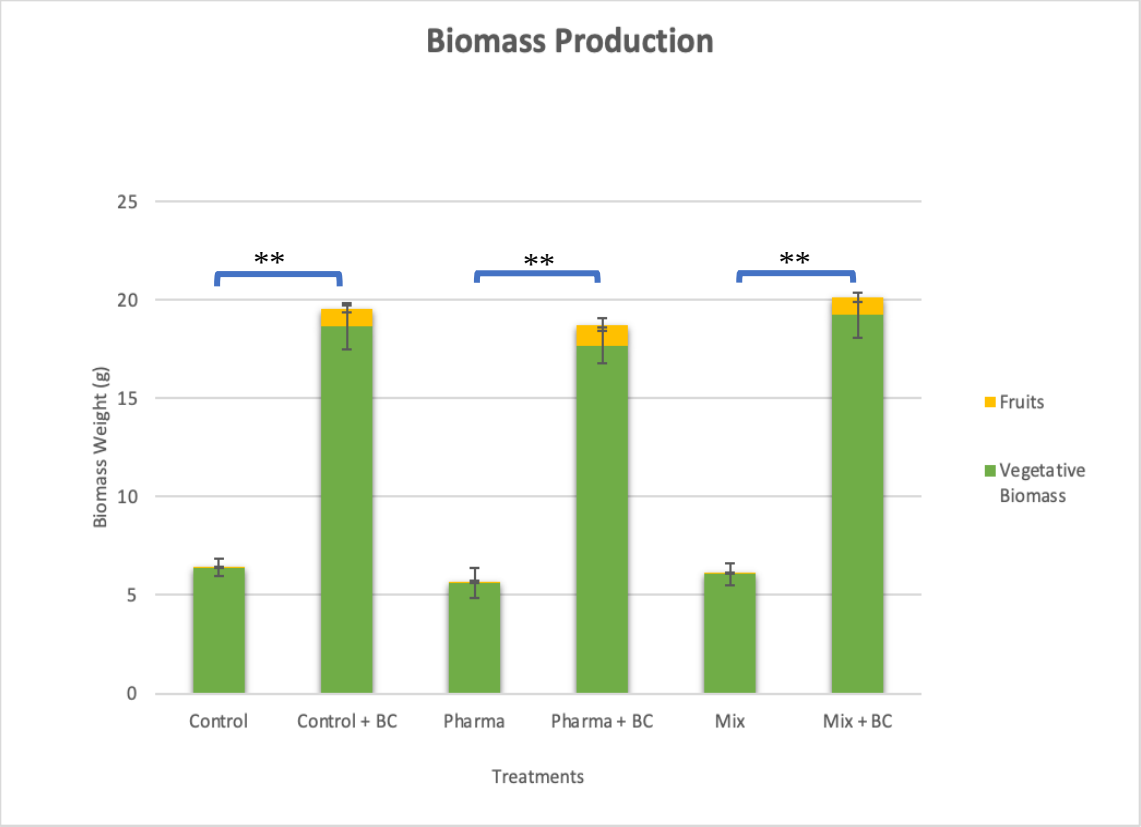
The average biomass production of each treatment is depicted by a horizontal bar within the graph, where the height of the bar is representative of the mean biomass production value. Moreover, the standard deviation specific to each treatment is portrayed as an error bar placed above each bar. The extent of this error bar exemplifies the degree of variability present in the data pertaining to each particular treatment.

The Control treatment and Pharma treatment had the lowest average biomass production while the Mix treatment had slightly better performance but was still inferior to Control + BC, Pharma + BC, and Mix + BC which showcased the highest average biomass production. The graph includes error bars which presents that data associated with the Control, Pharma, and Mix treatments displayed less variability than those corresponding to the Control + BC, Pharma + BC, and Mix + BC treatments. This pronounced variability in biochar-related treatments implies that biochar application had a noteworthy influence on biomass production and may have engaged in intricate interactions with other aforementioned treatments.

According to the graph, all biochar-amended soil samples show a p-value of less than 0.001, which implies that the observed differences between the two groups are statistically significant and not likely due to chance. As a result, we can have confidence in the study's findings, as they are not merely attributed to random variations. All of the Biomass, Fruits, and Biomass+Fruits series are marked with ** and to show the significance. When comparing between Control, Pharma and Mix treatment or Control+BC with Pharma+BC and Mix+BC, no statistically significant were found in these 3 groups.

To sum up, the graphical representation provided effectively illustrates disparities between average biomass production levels and variability amongst different treatment groups. This clear and concise visual aid allows for a swift yet comprehensive evaluation of differences between various treatments being analysed. This highlights the benefits of employing Biochar as a vital component in various soil treatment procedures.

Figure 23 Average vegetative dried biomass and average dried biomass per pot after vegetation period for different treatments. Number of replications is 6.



** indicated significance in statistical comparison of Biomass, Fruits and Biomass+Fruits where ($p \leq .001$)

5.4 Concentration of PhACs in Biomass

5.4.1 Pharma Treatment Group

Table 8 presents a comprehensive analysis of the mean and standard deviation values for various PhACs found in zucchini biomass samples for Pharma treatment groups. Specifically, the PhACs includes carbamazepine, tramadol, citalopram, metoprolol, propafenone, sertraline, tiapride, amisulpride, trospium, and venlafaxine. The initial column enumerates the PhACs by their chemical nomenclature. Subsequent columns 2 and 3 presents the average concentration with the standard deviation of each PhACs in the biomass samples, with absent and present of Biochar, respectively. Notably, the concentration metric is denoted as ng/g, signifying nanograms of PhACs per each gram of dried biomass.

Table 8 - A comparison of the average and standard deviation of concentration of each PhACs in the vegetative biomass samples with and without application of Biochar in Pharma treatment. Each group had 6 replications.

		Pharma	**Pharma+BC**
Concentration of micropollutants in Biomass (ng/g)	*carbamazepine*	214.379 ± 14.688[b]	72.715 ± 16.413[a]
	tramadol	46.693 ± 8.618	35.259 ± 6.959
	citalopram	0.297 ± 0.077	0.136 ± 0.036
	metoprolol	9.538 ± 1.998[b]	3.095 ± 0.414[a]
	propafenone	0.809 ± 0.105	0.381 ± 0.033
	sertraline	0.640 ± 0.149	0.363 ± 0.139
	tiapride	5.924 ± 1.212[b]	2.193 ± 0.668[a]
	amisulpride	2.220 ± 0.417[b]	0.881 ± 0.267[a]
	trospium	1.185 ± 0.183	0.665 ± 0.1137
	venlafaxine	3.521 ± 0.566	2.887 ± 0.4998

[a-b] Means in the same row with different lowercase letter are significantly different ($p < 0.05$)

By juxtaposing these findings with those acquired for biomass devoid of biochar incorporation (as showcased in the table), it becomes evident that the mean concentrations for the majority of PhACs are markedly diminished in biomass samples containing biochar compared to those pot with no application of biochar. Nevertheless, some compound such as carbamazepine and tramadol demonstrate higher average concentrations persisting within biochar-treated biomass samples. These observations imply that incorporating biochar into zucchini biomass has the potential to induce advantageous effects concerning mitigating concentration levels and fluctuations associated with specific PhACs compounds.

Among the PhACs assessed in table 8, carbamazepine, metoprolol, tiapride and amisulpride showed p-values under or equal to 0.05. Due to this, we can have

confidence in the study's results since they are not merely the result of random fluctuations.

5.4.2 Mix Treatment Group

Table 9 A comparison of the average and standard deviation of concentration of each PhACs in vegetative biomass samples with and without application of Biochar in Mix treatment. Each group had 6 replications.

		Mix	Mix+BC
Concentration of micropollutants in Biomass (ng/g)	*carbamazepine*	218.530 ± 29.666[b]	84.844 ± 25.050[a]
	tramadol	48.960 7.668	41.530 ± 11.206
	citalopram	0.395 ± 0.121	0.510 ± 0.574
	metoprolol	9.532 ± 1.508[b]	3.907 ± 1.006[a]
	propafenone	0.865 ± 0.0834	0.589 ± 0.424
	sertraline	0.554 ± 0.109	0.351 ± 0.316
	tiapride	5.137 ± 1.016[b]	2.385 ± 0.629[a]
	amisulpride	2.072 ± 0.450[b]	1.029 ± 0.716[a]
	trospium	1.302 ± 0.275	0.998 ± 0.804
	venlafaxine	3.780 ± 0.692	3.358 ± 0.750

[a-b] Means in the same row with different lowercase letter are significantly different ($p < 0.05$)

Table 9 delineates the mean and standard deviation values of pharmaceutical active compounds (PhACs) in the context of zucchini biomass with the Mix treatments applied. The PhACs enumerated within the table encompass carbamazepine, tramadol, citalopram, metoprolol, propafenone, sertraline, tiapride, amisulpride, trospium, and venlafaxine. The utilized unit of measurement for the concentrations is portrayed as nanograms per gram (ng/g) - the amount of PhACs in nanograms found in each gram of dried vegetative biomass.

Similar to Pharma treatments, upon examining the results in relation to biomass without biochar in the Mix Group, as illustrated in the table above, it is palpable that the average concentrations of a majority of pharmaceutical active compounds (PhACs) appear to be lower when biochar is incorporated into the biomass. These findings indicate that the inclusion of biochar in zucchini biomass could potentially yield positive outcomes in regard to mitigating concentration levels and minimizing variability for specific PhACs.

In this observation of various pharmaceuticals groups, carbamazepine, metoprolol and tiapride demonstrates significant outcomes (with p-values less than 0.05) when compared to their mixed counterparts. A statistical analysis was also conducted on the data between Pharma and Mix treatment groups, Pharma+BC versus Mix+BC treatment groups, none of the observed values showed statistical significance at the $p<.05$ level. It is difficult to interpret any link between the types of treatments since they do not differ significantly from one another.

The data presented illustrates that the findings represent a mean value derived from six replications. As a result, this experiment has been repeated six times on integrated groups, and for each pot, concentrations of carbamazepine, tramadol, citalopram, metoprolol, propafenone, sertraline, tiapride, amisulpride, trospium, and venlafaxine were measured extensively. These measurements were then averaged to obtain the documented concentrations presented in the study.

5.5 Uptake of PhACs

5.5.1 Uptake from Pharma Treatment

Table 10 presented a detailed look into the total uptake of PhACs, observed in distinct treatment scenarios. Considering the Pharma treatment exclusively, we can observe that the uptake of carbamazepine stands at 1199.461 ng while tramadol amounts to 258.797 ng for their total uptake, and citalopram registers at lowest among others which is 1.666 ng. As a result of determining biomass production and concentration, the uptake amount for PhACs within the zucchini biomass is determined, highlighting the differences that exist between PhACs. Tramadol and venlafaxine show significant results with p-values less than 0.001 when compared to their pharma-without-biochar counterparts in this study.

Table 10 The mean amount of the PhACs uptake by vegetative dry biomass in nanogram(ng); side-by-side comparison of Pharma treatment group and Pharma treatment with BC group.

		Pharma	**Pharma+BC**
Amount of the micropollutants uptake from biomass (ng)	*amisulpride*	12.408 ± 2.885	15.478 ± 4.137
	carbamazepine	1199.461 ± 165.089	1283.842 ± 291.496
	citalopram	1.666 ± 0.522	2.434 ± 0.707
	metoprolol	52.873 ± 9.811	54.787 ± 8.139
	propafenone	4.52 ± 0.741	6.738 ± 0.710
	sertraline	3.618 ± 1.075	6.393 ± 2.379
	tiapride	33.209 ± 8.491	38.358 ± 10.241
	tramadol	258.797 ± 38.696[b]	625.003 ± 134.387[a]
	trospium	6.655 ± 1.537	11.719 ± 1.778
	venlafaxine	19.563 ± 2.793[b]	51.209 ± 9.787[a]

[a-b] Means in the same row with different lowercase letter are significantly different (p <.001)

Analysing and comparing the absorption of pharmaceutically active compounds (PhACs) across a variety of treatment settings can give us a deeper understanding of the effect of biochar on their uptake. When examining the Pharma treatment in combination with biochar-enhanced soil, we can see that most PhACs show a slightly higher uptake than those in the Pharma treatment without biochar. As a result of this observation, it is possible that biochar could enhance the ability of certain compounds to get absorbed. It appears there is a consistent pattern indicating that Biochar enhances the absorption of pharmaceuticals.

Statistical analysis conducted on the data between Pharma and Mix treatment groups, as well as the Pharma+BC versus Mix+BC treatment groups, did not yield any significant results at the p<.001 level, similar to the concentration results. The lack of significant difference between the treatment types makes it challenging to draw conclusions regarding the uptake of compounds.

5.5.2 Uptake from Mix Treatment

Table 11 The mean amount of the PhACs uptake by vegetative dry biomass in nanogram(ng); side-by-side comparison of Mix treatment group and Mix treatment with BC group.

		Mix	Mix+BC
Amount of the micropollutants uptake from biomass (ng)	*amisulpride*	12.529 ± 2.630	20.071 ± 14.706
	carbamazepine	1329.011± 248.430	1630.092 ± 491.196
	citalopram	2.356 ± 0.582	9.982 ± 11.592
	metoprolol	57.773 ± 10.749	75.241 ± 20.456
	propafenone	5.256± 0.760	11.464 ± 8.722
	sertraline	3.332 ± 0.577	6.874 ± 6.463
	tiapride	31.146 ± 6.360	46.038 ± 13.248
	tramadol	297.066 ± 54.864[b]	801.576 ± 240.202[a]
	trospium	7.807 ± 1.240	19.477 ± 16.485
	venlafaxine	22.988 ± 5.192[b]	64.872 ± 16.828[a]

[a-b] Means in the same row with different lowercase letter are significantly different (p <.001)

Table 11 shows the total amount of the micropollutants uptake from biomass for both Mix treatment with and without Biochar in soil. Similar to Pharma treatment, Mix solution treatment recorded carbamazepine's highest uptake with value of 1329 ng in total absorption and tramadol's highest uptake at 297 ng, while citalopram's uptake was the lowest at 2.356 ng. The data indicate that the value of Mix treatment is higher than that of Pharma treatments. As demonstrated in the table, tramadol and venlafaxine showed notable findings with p-values less than 0.001 same as in Pharma treatment uptake.

5.6 Bioaccumulation Factor (BAF)

5.6.1 BAF for Pharma Treatment Group

Table 12 The bioaccumulation factor value of PhACs in vegetative dry biomass (%) for Pharma treatment with and without Biochar in the soil. Each variance has 6 replications.

		Pharma	Pharma+BC
Bioaccumulation factor (%)	*amisulpride*	0.042 ± 0.009	0.029 ± 0.008
	carbamazepine	3.917 ± 0.539	2.393 ± 0.543
	citalopram	0.005± 0.002	0.004 ± 0.001
	metoprolol	0.172 ± 0.032[b]	0.102 ± 0.015[a]
	propafenone	0.015 ± 0.002	0.013 ± 0.001
	sertraline	0.012 ± 0.0035	0.012 ± 0.004
	tiapride	0.109 ± 0.030	0.072 ± 0.019
	tramadol	0.845 ± 0.126	1.165 ± 0.250
	trospium	0.022 ± 0.005	0.022 ± 0.003
	venlafaxine	0.064 ± 0.009	0.095 ± 0.018

[a-b] Means in the same row with different lowercase letter are significantly different (p <.001)

Table 12 shows bioaccumulation factor of PhACs in dried biomass. Under both conditions, carbamazepine and tramadol show significantly higher bioaccumulation factors than other pharmaceuticals, with values of 3.9% and 0.8% in Pharma and 2.4% and 1.2% in Pharma + Biochar soil, respectively. In contrast, certain pharmaceuticals, such as citalopram, have low bioaccumulation factors in both situations, at about 0.005% in pharmaceuticals and less than 0.004% in pharmaceuticals + biochar soil. It is also likely that biochar has an effect on specific pharmaceutical bioaccumulation factors. Pharma + Biochar soil treatment shows notably lower bioaccumulation factors for amisulpride and propafenone, ranging from 0.04% to 0.03% and 0.015% to 0.013%, as compared with Pharma condition.

In comparison to other investigated PhACs, carbamazepine and tramadol are more likely to accumulate within zucchini biomass. In contrast, amisulpride and propafenone are less likely to accumulate due to their lower BAF. When compared with the pharmaceutical equivalents of various pharmaceutical groups, only Metoprolol exhibits significant results (with p-values below 0.001).

5.6.2 BAF for Mix Treatment Group

Table 13 The bioaccumulation factor of PhACs in vegetative dry biomass (%) for Mix treatment with and without Biochar in the soil. Each variance has 6 replications.

		Mix	**Mix+BC**
Bioaccumulation factor (%)	*amisulpride*	0.041 ± 0.009	0.037 ± 0.027
	carbamazepine	4.340 ± 0.811	3.038 ± 0.915
	citalopram	0.008 ± 0.002	0.019 ± 0.021
	metoprolol	0.189 ± 0.035	0.140 ± 0.038
	propafenone	0.017 ± 0.003	0.021 ± 0.016
	sertraline	0.011 ± 0.002	0.013 ± 0.012
	tiapride	0.102 ± 0.021	0.086 ± 0.025
	tramadol	0.970 ± 0.179	1.494 ± 0.448
	trospium	0.026 ± 0.004	0.036 ± 0.031
	venlafaxine	0.075 ± 0.017	0.121 ± 0.031

There is no significant different for the value in this table

As depicted in Table 13, the Mix treatment's exhibits similarities to the Pharma treatment's BAF findings. Likewise for Mix treatments, it becomes apparent that adding biochar to soil may affect the bioaccumulation of specific pharmaceuticals in zucchini biomass. For example, the carbamazepine bioaccumulation factor is lower in

the Mix with Biochar soil condition than in the Mix condition (3.03% vs 4.34%), which suggests that the presence of biochar could decrease carbamazepine accumulation in zucchini biomass.

Similarly, tiapride's bioaccumulation factor decreases in the Mix with Biochar soil condition compared to the Mix condition (0.086% vs 0.1%), hinting that biochar presence could also reduce tiaprides accumulation within zucchini biomass. On the other hand, some pharmaceuticals exhibit higher bioaccumulation factors in the Mix with Biochar soil condition compared to the Mix condition, such as tramadol at 1.49% versus 0.97%. This observation implies that biochar presence might boost tramadol accumulation within zucchini biomass.

The statistical analysis conducted on the data between Pharma and Mix treatment groups, as well as Pharma+BC and Mix+BC treatment groups, revealed no significant differences at the $p<.001$ level. Therefore, it is challenging to establish any apparent correlation between the types of treatments and the bioaccumulation of the compounds since there are no significant variations between them.

6 Discussion

6.1 Germination Rate

Germination rate refers to the percentage of seeds that successfully sprout and grow into healthy plants. According to Wallace, (1960) if the moisture content of the soil is too high, the seeds may rot before they have a chance to germinate, thereby resulting in a low germination rate. The chart in Figure 22 depicts that incorporating biochar into the soil structure may leads to improved germination percentages for both the Pharma and Mix series. This is followed by more noticeable effects seen in the Mix series, which initially exhibited slower germination. In the early stage of cultivating the seeds, there's no pharma or mix treatments used for irrigation.

In a study conducted by (Free et al., 2010) it was discovered that no interactions existed between the type or rate of biochar and soil type. The consistent effects of biochar were observed on different soil types, regardless of the type or amount of biochar utilized. Furthermore, the germination or initial growth of maize seeds, including root and coleoptile length as well as dry weight, was not significantly impacted by biochar. A similar study conducted by Kamara, (2014) supports this notion. Their findings revealed that the germination of maize and rice seeds was not negatively impacted when planted in soil treated with biochar created from their respective crop residues. Determining the influence of biochar on germination rates for the Pharma + Biochar and Mix + Biochar series is not as clear-cut or simple. None of the observed values obtain statistical significance at the $p<.001$ level, indicating no significant relationship between the number of germinated seeds and the type of treatment.

Chemical elements that encourage germination also positively impact emergence and seedling development (Hilhorst & Karssen, 2000). Temperature, moisture, oxygen and light are all important environmental factors that influence germination and seedling growth. The availability of nutrients and minerals in the soil also play an important role in successful germination and growth. Germination is the first step in the life cycle of a plant, so it is important that the conditions are right for the seed to emerge and grow.

To ensure optimal conditions for this experiment, the greenhouse temperature was set at 18 degrees Celsius to maintain proper humidity levels. The germination rate was deemed successful, as all seeds achieved 90-100% growth rate after 15 days.

6.2 Water Consumption and Amount of Each PhACs Applied

Water is a precious resource that is essential for agriculture, but excessive water consumption can have negative impacts on the environment and human health (Lewis & Bamforth, 2006). Zucchini is a popular summer squash that is grown in many

parts of the world. However, it requires a significant amount of water to grow and produce a high yield (Thomas, 2023). According to a study by the University of California Cooperative Extension, zucchini requires approximately 1.2335 million litres of water per hectare during the growing season. This means that farmers must rely on irrigation systems or natural rainfall to meet this demand (Molinar et al., 2005). However, excessive water consumption can lead to soil erosion, nutrient depletion, and reduced water availability for other uses.

The total irrigation solution consumption of Zucchini in this experiment ranging between 6 to 8 litres per pot, where 5.4 litres was for pots without Biochar and 8.3 litres for pots with Biochar. The increased water consumption can be attributed to the higher nutrient content present in biochar. As a result, the plants experienced enhanced growth, which subsequently led to a higher demand for water in order to sustain their development.

The research conducted by (Baronti et al., 2014) regarding the effects of biochar on plant water relations in grapevines demonstrates that biochar effectively increases soil moisture levels and reduces plant water stress. Similarly, a study by W. Wu et al., (2022) found that using biochar improved soil hydrological properties and increased crop water use efficiency. These findings are in line with the experiment's results, indicating a consistent pattern when using biochar.

Results from this Zucchini experiment indicated that biochar application rate could explain an increase of 35% in solution consumption throughout the experiment, consistent with Table 7. Additionally, the results do not confirm the theory that other micropollutants in irrigation water could affect pharmaceutical uptake by plants. However, the test showed a positive effect of the 1% biochar application rate on zucchini roots and shoot biomass.

6.3 Biomass production

Biomass production is an important aspect of this experiment to determine the concentration, uptake and accumulation of PhACs in zucchini plants. Using the freeze-drying process, the amount of biomass produced was determined after the biomass was dried.

In a study conducted by (Rooni et al., 2017), the researchers employed a freeze-drying technique to process barley. The results of their experiment demonstrated that utilizing this freeze-drying method led to a significant increase in hydrolysis efficiency, highlighting the effectiveness of this approach. For this zucchini experiment, the biomass was freeze-dried in -42℃ temperatures for a week before milling and undergoing LC-MS/MS procedure.

The total average of dried vegetation biomass produced by the Control, Pharma and Mix treatments group that did not have Biochar in their soil amounted to 18.06 grams. And the total average of 0.11 grams of fruits are produced in this group. The group treated with Biochar amended soil produced a total average of 55.6 g of

died vegetation biomass and 2.8 g of fruits. It is safe to say that Biochar amended soil yielded more than 30 % for biomass production. In a manner quite alike to the investigations carried out by (Abiven et al., 2015), it was observed that there was a notable enhancement in crop yields and the development of root systems subsequent to the application of biochar amendments. As such results in this zucchini experiment can be observed in Figure 23.

6.4 Concentration of PhACs in Biomass

The existence of pharmaceutical substances in vegetation has become an increasing issue for both human and ecological well-being. Due to the prevalent usage of pharmaceuticals in contemporary medicine, the discharge of these chemicals into the environment via wastewater and agricultural overflow has led to their build-up in soil and water resources, where they can be absorbed by plants. The results from LC-MS/MS were used in this experiment to investigate the concentration of PhACs in zucchini biomass.

In both the Pharma and Mix treatment groups in Table 8 and 9, it has been observed that carbamazepine demonstrated the highest concentration levels amounted to 214.37 $\pm$ 14.68 ng/g for Pharma and 218.58 $\pm$ 29.66 ng/g for Mix treatment. In the soil with Biochar, more than 60% of these values are reduced with amount of 72.7 $\pm$ 16.41 ng/g in Pharma and 84.8 $\pm$ 25.1 ng/g in Mix treatment. The fact that carbamazepine is found the highest in plants is not unfamiliar since few studies have been conducted on the persistence of it. The results of the study by (Riemenschneider et al., 2017) found that over 80% of the entire spiked quantity of carbamazepine was absorbed by the tomato plants and mostly retained in their leaves after 35 days of exposure. (Kodešová et al., 2019) also mentioned that carbamazepine was easily absorbed, accumulated, and metabolized by plants. It was found that carbamazepine sorption coefficients were negatively correlated with concentrations of carbamazepine in radish roots, lamb's lettuce roots, and spinach roots.

Second highest PhACs found is tramadol, in the soil without Biochar the value is at 46.7 $\pm$ 8.6 and 48.9 $\pm$ 7.6 ng/g in the Pharma and Mix treatments, respectively. In a study conducted by Kostanjevecki et al. (2019), it was found that tramadol was not does not show any toxicity to algae. In this zucchini experiment, there were a number of PhACs found to have a lower concentration than 10 ng/g such as metoprolol, amisulpride, tiapride, trospium and venlafaxine. While the rest of it namely sertraline, propafenone and citalopram had even lower concentrations than 1 ng/g even after almost 3 months of treatment with micropollutant solution.

It is important to note that even if individual PhACs have low concentrations in zucchini plants, its cumulative effect may still be harmful to human health. The long-term effects of consuming these PhACs through contaminated plant products are not fully understood, and further investigation is warranted. Also, it is important to

consider the impact PhACs may have on non-target organisms in the environment, including bacteria and wildlife.

6.5 Uptake of PhACs

Pharmaceutically active compounds (PhACs) in the environment have raised concerns about their potential impact on ecosystems and human health. Among the various environmental compartments, the uptake of these compounds by crops is particularly relevant, as it represents a direct pathway for human exposure. In this zucchini experiment, the uptake was determined by multiplying the amount of biomass with the concentration of the PhACs in the biomass.

Due to the fact that the uptake was derived from the concentration, the values are closely related to each other. We can see that the highest uptake also from Carbamazepine with 1199.461 ± 165.089 ng in Pharma treatment soil and 1283.842 ± 291.496 ng in Pharma with Biochar-amended soil. The same can be observed in Mix treatment with other micropollutants existent where it is 1329.011 ± 248.430 ng and much higher in Biochar-amended soil with 1630.092 ± 491.196 ng. In the same study by Riemenschneider et al. (2017) where they investigate the uptake, translocation, and transformation of carbamazepine in hydroponically grown tomato plants, they found that there was 33% of carbamazepine taken up in comparison of 11 transformation products (TP) measured by LC-MS/MS.

A study by Gworek et al., (2021) found that transpiration was responsible for carbamazepine movement within the plant. Carbamazepine showed no negative effects on ryegrass' growth, either individually or jointly. Therefore, the study suggests that the fate of pharmaceuticals in the environment is influenced by various factors, including their mobility within plants.

Uptake of citalopram is the lowest in this zucchini experiment. This could be due to the fact that citalopram is lypophilic (Schmiedjell, 2022) thus it is not solubilised in water compared to other PhACs. The minimal absorption of citalopram has the potential to exhibit the least amount of accumulation within the zucchini plant when compared to the effects of carbamazepine.

6.6 Bioaccumulation Factor (BAF)

The bioaccumulation factor (BAF) serves as an indicator of a substance's capability to build up within a living organism in comparison to its concentration present in the surrounding milieu. In the context of pharmaceuticals found within plants, the BAF offers insights concerning the likelihood of these chemical compounds to accumulate in consumable vegetation such as zucchini and the subsequent potential hazards posed to human well-being.

The BAF concerning pharmaceuticals in plant life may exhibit significant variability, contingent upon aspects like the physicochemical characteristics of the

compound, soil conditions, and the specific plant species involved (Lesmeister et al., 2021). Generally, lipophilic substances like certain antibiotics and antifungal agents have been demonstrated to possess higher BAFs in plants in comparison to hydrophilic compounds (Arnoldi & Merlini, 1990).

This research ascertained those certain substances, including carbamazepine and tramadol, exhibited relatively elevated BAFs within zucchini, signifying a potential for accumulation within edible portions of the plant. Citalopram has been observed to possess the lowest BAF, indicating that its accumulation within zucchini plants is relatively minimal compared to other substances.

Nevertheless, it is crucial to acknowledge that the BAF constitutes merely one element when determining the possible risks posed by pharmaceuticals present within plants. Other contributing factors such as the toxicological impacts of these compounds, dose-response associations, and exposure frequency must be taken into account. Furthermore, it is vital to guarantee that any potential hazards are evaluated against the advantages conferred by employing these chemical compounds medicinally while implementing suitable measures aimed at mitigating environmental contamination and human exposure.

7 Conclusion

The research conducted suggests that the presence of additional micropollutants in irrigation water does not have a significant impact on the absorption of pharmaceutical substances by zucchini plants. This means that even if there are various micropollutants present in the water used for irrigation, it does not affect the degree to which zucchini plants take up pharmaceutical compounds at tested concentrations. Furthermore, incorporating biochar into the soil does not hinder zucchini plants' pharmaceutical absorption. When comparing the accumulation of various pharmaceutically active compounds (PhACs) in plant biomass based on Pharma versus Mix treatment groups, no significant discrepancy was observed in terms of accumulation amount. However, there was a notable difference in the concentration of PhACs in soil with and without biochar. Soil with biochar had significantly lower concentrations of PhACs than the soil without biochar.

Based on these findings, it can be concluded that biochar may be a useful tool for reducing the concentration of pharmaceutical compounds in soil. While there was no significant difference in the accumulation amount of PhACs in plant biomass between the two treatment groups, the use of biochar resulted in lower concentrations of these compounds in soil. This suggests that incorporating biochar into soil could be an effective strategy for reducing the environmental impact of pharmaceutical compounds.

For the safety of consumption, as our experiment produced very low volume of biomass from the zucchini plant, we cannot assess the safety of biomass for the consumption.

References

Abiven, S., Hund, A., Martinsen, V., & Cornelissen, G. (2015). Biochar amendment increases maize root surface areas and branching: a shovelomics study in Zambia. *Plant and Soil*, *395*(1–2), 45–55. https://doi.org/10.1007/s11104-015-2533-2

Alazaiza, M., Albahnasawi, A., Ali, G., Bashir, M., Nassani, D., Al Maskari, T., Amr, S., & Abujazar, M. (2022). Application of Natural Coagulants for Pharmaceutical Removal from Water and Wastewater: A Review. *Water*, *14*(2), 140. https://doi.org/10.3390/w14020140

Amy-Sagers, C., Reinhardt, K., & Larson, D. M. (2017). Ecotoxicological assessments show sucralose and fluoxetine affect the aquatic plant, Lemna minor. *Aquatic Toxicology*, *185*, 76–85. https://doi.org/10.1016/j.aquatox.2017.01.008

Andrew J. McElrone, Brendan Choat, Greg A. Gambetta, & Craig R. Brodersen. (2013). Water Uptake and Transport in Vascular Plants. *Nature Education Knowledge*, 4(5)-6.

Arnoldi, A., & Merlini, L. (1990). Lipophilicity-antifungal activity relationships for some isoflavonoid phytoalexins. *Journal of Agricultural and Food Chemistry*, *38*(3), 834–838. https://doi.org/10.1021/jf00093a052

Arthur, E. L., Rice, P. J., Rice, P. J., Anderson, T. A., Baladi, S. M., Henderson, K. L. D., & Coats, J. R. (2005). Phytoremediation—An Overview. *Critical Reviews in Plant Sciences*, *24*(2), 109–122. https://doi.org/10.1080/07352680590952496

Asadullah, M., Rahman, M. A., Ali, M. M., Rahman, M. S., Motin, M. A., Sultan, M. B., & Alam, M. R. (2007). Production of bio-oil from fixed bed pyrolysis of bagasse. *Fuel*, *86*(16), 2514–2520. https://doi.org/10.1016/j.fuel.2007.02.007

Bair, D. A., Anderson, C. G., Chung, Y., Scow, K. M., Franco, R. B., & Parikh, S. J. (2020). Impact of biochar on plant growth and uptake of ciprofloxacin, triclocarban and triclosan from biosolids. *Journal of Environmental Science and Health, Part B*, *55*(11), 990–1001. https://doi.org/10.1080/03601234.2020.1807264

Barbie Cervoni, & Johnathan Valdez. (2022, December 12). *Zucchini Nutrition Facts and Health Benefits*. Verywellfit Website. https://www.verywellfit.com/summer-squash-and-zucchini-nutrition-facts-4114725

Baronti, S., Vaccari, F. P., Miglietta, F., Calzolari, C., Lugato, E., Orlandini, S., Pini, R., Zulian, C., & Genesio, L. (2014). Impact of biochar application on plant water relations in Vitis vinifera (L.). *European Journal of Agronomy*, *53*, 38–44. https://doi.org/10.1016/j.eja.2013.11.003

Barra Caracciolo, A., Topp, E., & Grenni, P. (2015). Pharmaceuticals in the environment: Biodegradation and effects on natural microbial communities. A review. *Journal of*

Pharmaceutical and Biomedical Analysis, *106*, 25–36. https://doi.org/10.1016/j.jpba.2014.11.040

Bassil, R. J., Bashour, I. I., Sleiman, F. T., & Abou-Jawdeh, Y. A. (2013). Antibiotic uptake by plants from manure-amended soils. *Journal of Environmental Science and Health, Part B*, *48*(7), 570–574. https://doi.org/10.1080/03601234.2013.774898

Beans, C. (2017). Phytoremediation advances in the lab but lags in the field. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(29), 7475–7477. https://doi.org/10.1073/PNAS.1707883114/ASSET/7205B89C-647C-4212-9366-0C78640B956D/ASSETS/GRAPHIC/PNAS.1707883114FIG01.JPEG

Bigott, Y., Khalaf, D. M., Schröder, P., Schröder, P. M., & Cruzeiro, C. (2020). *Uptake and Translocation of Pharmaceuticals in Plants: Principles and Data Analysis* (pp. 103–140). https://doi.org/10.1007/698_2020_622

Bonelli, P. R., Buonomo, E. L., & Cukierman, A. L. (2007). Pyrolysis of Sugarcane Bagasse and Co-pyrolysis with an Argentinean Subbituminous Coal. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, *29*(8), 731–740. https://doi.org/10.1080/00908310500281247

Boudrahem, F., Yahiaoui, I., Saidi, S., Yahiaoui, K., Kaabache, L., Zennache, M., & Aissani-Benissad, F. (2019). Adsorption of pharmaceutical residues on adsorbents prepared from olive stones using mixture design of experiments model. *Water Science and Technology*, *80*(5), 998–1009. https://doi.org/10.2166/wst.2019.346

Boxall, A. B. A., Johnson, P., Smith, E. J., Sinclair, C. J., Stutt, E., & Levy, L. S. (2006). Uptake of Veterinary Medicines from Soils into Plants. *Journal of Agricultural and Food Chemistry*, *54*(6), 2288–2297. https://doi.org/10.1021/jf053041t

Briggs, G. G., Rigitano, R. L. O., & Bromilow, R. H. (1987). Physico-chemical factors affecting uptake by roots and translocation to shoots of weak acids in barley. *Pesticide Science*, *19*(2), 101–112. https://doi.org/10.1002/ps.2780190203

Butler, E., Whelan, M. J., Ritz, K., Sakrabani, R., & van Egmond, R. (2011). Effects of triclosan on soil microbial respiration. *Environmental Toxicology and Chemistry*, *30*(2), 360–366. https://doi.org/10.1002/etc.405

Buxton, H. T., & Kolpin, D. W. (2004). Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams. In *Water Encyclopedia* (pp. 605–608). Wiley. https://doi.org/10.1002/047147844X.gw2107

Cardoso, O., Porcher, J.-M., & Sanchez, W. (2014). Factory-discharged pharmaceuticals could be a relevant source of aquatic environment contamination: Review of evidence and need for knowledge. *Chemosphere*, *115*, 20–30. https://doi.org/10.1016/j.chemosphere.2014.02.004

Carter, L. J., Harris, E., Williams, M., Ryan, J. J., Kookana, R. S., & Boxall, A. B. A. (2014). Fate and Uptake of Pharmaceuticals in Soil–Plant Systems. *Journal of Agricultural and Food Chemistry*, *62*(4), 816–825. https://doi.org/10.1021/jf404282y

Carter, L. J., Ryan, J. J., & Boxall, A. B. A. (2016). Effects of soil properties on the uptake of pharmaceuticals into earthworms. *Environmental Pollution*, *213*, 922–931. https://doi.org/10.1016/j.envpol.2016.03.044

Carter, L. J., Williams, M., Böttcher, C., & Kookana, R. S. (2015). Uptake of Pharmaceuticals Influences Plant Development and Affects Nutrient and Hormone Homeostases. *Environmental Science & Technology*, *49*(20), 12509–12518. https://doi.org/10.1021/acs.est.5b03468

Chaturvedi, P., Shukla, P., Giri, B. S., Chowdhary, P., Chandra, R., Gupta, P., & Pandey, A. (2021). Prevalence and hazardous impact of pharmaceutical and personal care products and antibiotics in environment: A review on emerging contaminants. *Environmental Research*, *194*, 110664. https://doi.org/10.1016/j.envres.2020.110664

Chauhan, S., Shafi, T., Dubey, B. K., & Chowdhury, S. (2022). Biochar-mediated removal of pharmaceutical compounds from aqueous matrices via adsorption. *Waste Disposal & Sustainable Energy*. https://doi.org/10.1007/s42768-022-00118-y

Chen, B., & Chen, Z. (2009). Sorption of naphthalene and 1-naphthol by biochars of orange peels with different pyrolytic temperatures. *Chemosphere*, *76*(1), 127–133. https://doi.org/10.1016/j.chemosphere.2009.02.004

Chen, B., Zhou, D., & Zhu, L. (2008). Transitional Adsorption and Partition of Nonpolar and Polar Aromatic Contaminants by Biochars of Pine Needles with Different Pyrolytic Temperatures. *Environmental Science & Technology*, *42*(14), 5137–5143. https://doi.org/10.1021/es8002684

Chuang, Y.-H., Zhang, Y., Zhang, W., Boyd, S. A., & Li, H. (2015). Comparison of accelerated solvent extraction and quick, easy, cheap, effective, rugged and safe method for extraction and determination of pharmaceuticals in vegetables. *Journal of Chromatography A*, *1404*, 1–9. https://doi.org/10.1016/j.chroma.2015.05.022

Claas Kirchhelle. (2020). *Pyrrhic Progress: The History of Antibiotics in Anglo-American Food Production*. Rutgers University Press.

Clarkson, T. (1995). Environmental contaminants in the food chain. *The American Journal of Clinical Nutrition*, *61*(3), S682–S686. https://doi.org/10.1093/ajcn/61.3.682S

Corcoran, J., Winter, M. J., & Tyler, C. R. (2010). Pharmaceuticals in the aquatic environment: A critical review of the evidence for health effects in fish. *Critical Reviews in Toxicology*, *40*(4), 287–304. https://doi.org/10.3109/10408440903373590

Daughton, C. G., & Ternes, T. A. (1999a). Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environmental Health Perspectives*, *107*(suppl 6), 907–938. https://doi.org/10.1289/ehp.99107s6907

Daughton, C. G., & Ternes, T. A. (1999b). Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environmental Health Perspectives*, *107*(suppl 6), 907–938. https://doi.org/10.1289/ehp.99107s6907

de Andrade, J. R., Oliveira, M. F., da Silva, M. G. C., & Vieira, M. G. A. (2018). Adsorption of Pharmaceuticals from Water and Wastewater Using Nonconventional Low-Cost Materials: A Review. *Industrial & Engineering Chemistry Research*, *57*(9), 3103–3127. https://doi.org/10.1021/acs.iecr.7b05137

Dickens, G. R., Koelling, M., Smith, D. C., & Schnieders, L. (2007). Rhizon Sampling of Pore Waters on Scientific Drilling Expeditions: An Example from the IODP Expedition 302, Arctic Coring Expedition (ACEX). *Scientific Drilling*, *4, March 2007*. https://doi.org/10.2204/iodp.sd.4.08.2007

Ding, W., Dong, X., Ime, I. M., Gao, B., & Ma, L. Q. (2014). Pyrolytic temperatures impact lead sorption mechanisms by bagasse biochars. *Chemosphere*, *105*, 68–74. https://doi.org/10.1016/j.chemosphere.2013.12.042

Doruk, A. Y., Goker, H., & Cihangir, N. (2018). Biodegradation of diclofenac with fungal strains. *Archives of Environment Protect*, *44*(1), 55–62.

dos Santos, C. R., Lebron, Y. A. R., Moreira, V. R., Koch, K., & Amaral, M. C. S. (2022). Biodegradability, environmental risk assessment and ecological footprint in wastewater technologies for pharmaceutically active compounds removal. *Bioresource Technology*, *343*, 126150. https://doi.org/10.1016/j.biortech.2021.126150

Ebel, W. (1985). Carrier facilitated diffusion. *Journal of Mathematical Biology*, *21*(3), 243–271. https://doi.org/10.1007/BF00276225

Ensano, B., Borea, L., Naddeo, V., Belgiorno, V., de Luna, M., & Ballesteros, F. (2017). Removal of Pharmaceuticals from Wastewater by Intermittent Electrocoagulation. *Water*, *9*(2), 85. https://doi.org/10.3390/w9020085

Free, H., McGill, C., Rowarth, J., & Hedley, M. (2010). The effect of biochars on maize (Zea mays) germination. *New Zealand Journal of Agricultural Research*, *53*(1), 1–4. https://doi.org/10.1080/00288231003606039

George, A., Shah, P. A., & Shrivastav, P. S. (2019). Natural biodegradable polymers-based nano-formulations for drug delivery: A review. *International Journal of Pharmaceutics*, *561*, 244–264. https://doi.org/10.1016/j.ijpharm.2019.03.011

Ghani, W. A. W. A. K., Mohd, A., da Silva, G., Bachmann, R. T., Taufiq-Yap, Y. H., Rashid, U., & Al-Muhtaseb, A. H. (2013). Biochar production from waste rubber-wood-sawdust and

its potential use in C sequestration: Chemical and physical characterization. *Industrial Crops and Products*, *44*, 18–24. https://doi.org/10.1016/j.indcrop.2012.10.017

Gholami, M., Mirzaei, R., Kalantary, R. R., Sabzali, A., & Gatei, F. (2012). Performance evaluation of reverse osmosis technology for selected antibiotics removal from synthetic pharmaceutical wastewater. *Iranian Journal of Environmental Health Science & Engineering*, *9*(1), 19. https://doi.org/10.1186/1735-2746-9-19

Gianinazzi-Pearson, V., & Gianinazzi, S. (1983). The physiology of vesicular-arbuscular mycorrhizal roots. In *Tree Root Systems and Their Mycorrhizas* (pp. 197–209). Springer Netherlands. https://doi.org/10.1007/978-94-009-6833-2_20

Glaser, B., Haumaier, L., Guggenberger, G., & Zech, W. (2001). The 'Terra Preta' phenomenon: a model for sustainable agriculture in the humid tropics. *Naturwissenschaften*, *88*(1), 37–41. https://doi.org/10.1007/s001140000193

Glaser, B., Lehmann, J., & Zech, W. (2002). Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal - a review. *Biology and Fertility of Soils*, *35*(4), 219–230. https://doi.org/10.1007/s00374-002-0466-4

Golet, E. M., Strehler, A., Alder, A. C., & Giger, W. (2002). Determination of Fluoroquinolone Antibacterial Agents in Sewage Sludge and Sludge-Treated Soil Using Accelerated Solvent Extraction Followed by Solid-Phase Extraction. *Analytical Chemistry*, *74*(21), 5455–5462. https://doi.org/10.1021/ac025762m

Gworek, B., Kijeńska, M., Wrzosek, J., & Graniewska, M. (2021). Pharmaceuticals in the Soil and Plant Environment: a Review. *Water, Air, & Soil Pollution*, *232*(4), 145. https://doi.org/10.1007/s11270-020-04954-8

Hari, A. C., Paruchuri, R. A., Sabatini, D. A., & Kibbey, T. C. G. (2005). Effects of pH and cationic and nonionic surfactants on the adsorption of pharmaceuticals to a natural aquifer material. *Environmental Science & Technology*, *39*(8), 2592–2598. https://doi.org/10.1021/es048992m

Harris, P. J. F. (1997). Structure of non-graphitising carbons. *International Materials Reviews*, *42*(5), 206–218. https://doi.org/10.1179/imr.1997.42.5.206

Hatefi, A., & Amsden, B. (2002). Biodegradable injectable in situ forming drug delivery systems. *Journal of Controlled Release*, *80*(1–3), 9–28. https://doi.org/10.1016/S0168-3659(02)00008-1

Hilhorst, H. W. M., & Karssen, C. M. (2000). Effect of chemical environment on seed germination. In *Seeds: the ecology of regeneration in plant communities* (pp. 293–309). CABI Publishing. https://doi.org/10.1079/9780851994321.0293

Hillis, D. G., Fletcher, J., Solomon, K. R., & Sibley, P. K. (2011). Effects of Ten Antibiotics on Seed Germination and Root Elongation in Three Plant Species. *Archives of*

Environmental Contamination and Toxicology, *60*(2), 220–232. https://doi.org/10.1007/s00244-010-9624-0

J. Mark Ruscin, & Sunny A. Linnebur. (2021, July). *Aging and Medications*. Merck & Co., Inc., Rahway, NJ, USA.

Jałowiecki, Ł., Płaza, G., Ejhed, H., & Nawrotek, M. (2019). Aerobic biodegradation of norfloxacin and ofloxacin by a microbial consortium. *Archives of Environment Protect*, *45*(4), 40–47.

Jelic, A., Gros, M., Ginebreda, A., Cespedes-Sánchez, R., Ventura, F., Petrovic, M., & Barcelo, D. (2011). Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. *Water Research*, *45*(3), 1165–1176. https://doi.org/10.1016/j.watres.2010.11.010

Jindo, K., Mizumoto, H., Sawada, Y., Sanchez-Monedero, M. A., & Sonoki, T. (2014). Physical and chemical characterization of biochars derived from different agricultural residues. *Biogeosciences*, *11*(23), 6613–6621. https://doi.org/10.5194/bg-11-6613-2014

Johannes Lehmann, & Stephen Joseph. (2009). *Biochar for Environmental Management* (First Edition). Earthscan. https://www.css.cornell.edu/faculty/lehmann/publ/First%20proof%2013-01-09.pdf

Jones, V., Gardner, M., & Ellor, B. (2014). Concentrations of trace substances in sewage sludge from 28 wastewater treatment works in the UK. *Chemosphere*, *111*, 478–484. https://doi.org/10.1016/j.chemosphere.2014.04.025

Kamara, A. (2014). Effects of Biochar Derived from Maize Stover and Rice Straw on the Germination of their Seeds. *American Journal of Agriculture and Forestry*, *2*(6), 246. https://doi.org/10.11648/j.ajaf.20140206.12

Katyal, S., Thambimuthu, K., & Valix, M. (2003). Carbonisation of bagasse in a fixed bed reactor: influence of process variables on char yield and characteristics. *Renewable Energy*, *28*(5), 713–725. https://doi.org/10.1016/S0960-1481(02)00112-X

Kinney, C. A., Furlong, E. T., Kolpin, D. W., Burkhardt, M. R., Zaugg, S. D., Werner, S. L., Bossio, J. P., & Benotti, M. J. (2008). Bioaccumulation of Pharmaceuticals and Other Anthropogenic Waste Indicators in Earthworms from Agricultural Soil Amended With Biosolid or Swine Manure. *Environmental Science & Technology*, *42*(6), 1863–1870. https://doi.org/10.1021/es702304c

Kladivko, E. J., & Nelson, D. W. (1979). Surface Runoff from Sludge-Amended Soils. *Water Pollution Control Federation*, *51*(1), 100–110.

Kodešová, R., Klement, A., Golovko, O., Fér, M., Nikodem, A., Kočárek, M., & Grabic, R. (2019). Root uptake of atenolol, sulfamethoxazole and carbamazepine, and their

transformation in three soils and four plants. *Environmental Science and Pollution Research*, *26*(10), 9876–9891. https://doi.org/10.1007/s11356-019-04333-9

Kong, W. D., Zhu, Y. G., Liang, Y. C., Zhang, J., Smith, F. A., & Yang, M. (2007). Uptake of oxytetracycline and its phytotoxicity to alfalfa (Medicago sativa L.). *Environmental Pollution*, *147*(1), 187–193. https://doi.org/10.1016/j.envpol.2006.08.016

Kooijman, G., de Kreuk, M. K., Houtman, C., & van Lier, J. B. (2020). Perspectives of coagulation/flocculation for the removal of pharmaceuticals from domestic wastewater: A critical view at experimental procedures. *Journal of Water Process Engineering*, *34*, 101161. https://doi.org/10.1016/j.jwpe.2020.101161

Kookana, R. S., Sarmah, A. K., Van Zwieten, L., Krull, E., & Singh, B. (2011). *Biochar Application to Soil* (pp. 103–143). https://doi.org/10.1016/B978-0-12-385538-1.00003-2

Koptsik, G. N. (2014). Problems and prospects concerning the phytoremediation of heavy metal polluted soils: A review. *Eurasian Soil Science*, *47*(9), 923–939. https://doi.org/10.1134/S1064229314090075

Kostanjevecki, P., Petric, I., Loncar, J., Smital, T., Ahel, M., & Terzic, S. (2019). Aerobic biodegradation of tramadol by pre-adapted activated sludge culture: Cometabolic transformations and bacterial community changes during enrichment. *Science of The Total Environment*, *687*, 858–866. https://doi.org/10.1016/j.scitotenv.2019.06.118

Kümmerer, K. (2009). Antibiotics in the aquatic environment – A review – Part I. *Chemosphere*, *75*(4), 417–434. https://doi.org/10.1016/j.chemosphere.2008.11.086

Küster, A., & Adler, N. (2014). Pharmaceuticals in the environment: scientific evidence of risks and its regulation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1656), 20130587. https://doi.org/10.1098/rstb.2013.0587

Lee, X. J., Lee, L. Y., Gan, S., Thangalazhy-Gopakumar, S., & Ng, H. K. (2017). Biochar potential evaluation of palm oil wastes through slow pyrolysis: Thermochemical characterization and pyrolytic kinetic studies. *Bioresource Technology*, *236*, 155–163. https://doi.org/10.1016/j.biortech.2017.03.105

Lees, K., Fitzsimons, M., Snape, J., Tappin, A., & Comber, S. (2016). Pharmaceuticals in soils of lower income countries: Physico-chemical fate and risks from wastewater irrigation. *Environment International*, *94*, 712–723. https://doi.org/10.1016/j.envint.2016.06.018

Lesmeister, L., Lange, F. T., Breuer, J., Biegel-Engler, A., Giese, E., & Scheurer, M. (2021). Extending the knowledge about PFAS bioaccumulation factors for agricultural plants – A review. *Science of The Total Environment*, *766*, 142640. https://doi.org/10.1016/j.scitotenv.2020.142640

Lewis, M. J., & Bamforth, C. W. (2006). Water and Energy. In *Essays in Brewing Science* (pp. 143–160). Springer US. https://doi.org/10.1007/0-387-33011-9_13

Li, W. C. (2014). Occurrence, sources, and fate of pharmaceuticals in aquatic environment and soil. *Environmental Pollution*, *187*, 193–201. https://doi.org/10.1016/j.envpol.2014.01.015

Li, Y., He, J., Qi, H., Li, H., Boyd, S. A., & Zhang, W. (2020). Impact of biochar amendment on the uptake, fate and bioavailability of pharmaceuticals in soil-radish systems. *Journal of Hazardous Materials*, *398*, 122852. https://doi.org/10.1016/j.jhazmat.2020.122852

Lillenberg, M., L. S. V., N. L., R. M., & S. K. (2010). Enrofloxacin and ciprofloxacin uptake by plants from soil. *Agronomy Research*, 807–814.

Liu, J.-L., & Wong, M.-H. (2013). Pharmaceuticals and personal care products (PPCPs): A review on environmental contamination in China. *Environment International*, *59*, 208–224. https://doi.org/10.1016/j.envint.2013.06.012

Madikizela, L. M., Ncube, S., & Chimuka, L. (2018). Uptake of pharmaceuticals by plants grown under hydroponic conditions and natural occurring plant species: A review. *Science of The Total Environment*, *636*, 477–486. https://doi.org/10.1016/j.scitotenv.2018.04.297

Maeng, S. K., Sharma, S. K., Abel, C. D. T., Magic-Knezev, A., & Amy, G. L. (2011). Role of biodegradation in the removal of pharmaceutically active compounds with different bulk organic matter characteristics through managed aquifer recharge: Batch and column studies. *Water Research*, *45*(16), 4722–4736. https://doi.org/10.1016/j.watres.2011.05.043

Maged, A., Dissanayake, P. D., Yang, X., Pathirannahalage, C., Bhatnagar, A., & Ok, Y. S. (2021). New mechanistic insight into rapid adsorption of pharmaceuticals from water utilizing activated biochar. *Environmental Research*, *202*, 111693. https://doi.org/10.1016/j.envres.2021.111693

Marsoni, M., De Mattia, F., Labra, M., Bruno, A., Bracale, M., & Vannini, C. (2014). Uptake and effects of a mixture of widely used therapeutic drugs in Eruca sativa L. and Zea mays L. plants. *Ecotoxicology and Environmental Safety*, *108*, 52–57. https://doi.org/10.1016/j.ecoenv.2014.05.029

McEachran, A. D., Shea, D., Bodnar, W., & Nichols, E. G. (2016). Pharmaceutical occurrence in groundwater and surface waters in forests land-applied with municipal wastewater. *Environmental Toxicology and Chemistry*, *35*(4), 898–905. https://doi.org/10.1002/etc.3216

McGechan, M. B., & Lewis, D. R. (2002). SW—Soil and Water. *Biosystems Engineering*, *83*(3), 255–273. https://doi.org/10.1006/bioe.2002.0125

Mia, S., Singh, B., & Dijkstra, F. A. (2017). Aged biochar affects gross nitrogen mineralization and recovery: a 15N study in two contrasting soils. *GCB Bioenergy*, *9*(7), 1196–1206. https://doi.org/10.1111/gcbb.12430

Michael, I., Rizzo, L., McArdell, C. S., Manaia, C. M., Merlin, C., Schwartz, T., Dagot, C., & Fatta-Kassinos, D. (2013). Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: A review. *Water Research*, *47*(3), 957–995. https://doi.org/10.1016/j.watres.2012.11.027

Mishra, S., Bharagava, R. N., More, N., Yadav, A., Zainith, S., Mani, S., & Chowdhary, P. (2019). Heavy Metal Contamination: An Alarming Threat to Environment and Human Health. In *Environmental Biotechnology: For Sustainable Future* (pp. 103–125). Springer Singapore. https://doi.org/10.1007/978-981-10-7284-0_5

Molinar, R. H., Yang, M., Klonsky, K. M., & De Moura, R. L. (2005). *SUMMER SQUASH SAN JOAQUIN VALLEY-South*. http://coststudies.ucdavis.edu,

Nagajyoti, P. C., Lee, K. D., & Sreekanth, T. V. M. (2010). Heavy metals, occurrence and toxicity for plants: a review. *Environmental Chemistry Letters*, *8*(3), 199–216. https://doi.org/10.1007/s10311-010-0297-8

Ndoun, M. C., Elliott, H. A., Preisendanz, H. E., Williams, C. F., Knopf, A., & Watson, J. E. (2021). Adsorption of pharmaceuticals from aqueous solutions using biochar derived from cotton gin waste and guayule bagasse. *Biochar*, *3*(1), 89–104. https://doi.org/10.1007/s42773-020-00070-2

Nikolaou, A., Meric, S., & Fatta, D. (2007). Occurrence patterns of pharmaceuticals in water and wastewater environments. *Analytical and Bioanalytical Chemistry*, *387*(4), 1225–1234. https://doi.org/10.1007/s00216-006-1035-8

Oladoye, P. O., Olowe, O. M., & Asemoloye, M. D. (2022). Phytoremediation technology and food security impacts of heavy metal contaminated soils: A review of literature. *Chemosphere*, *288*, 132555. https://doi.org/10.1016/j.chemosphere.2021.132555

Orita, N. (2012). *Root Uptake of Organic Contaminants into Plants: Species Differences* [Graduate Studies]. Utah State University.

OSENI, O. M., DADA, O. E., OKUNLOLA, G. O., OLOWOLAJU, E. D., AKINROPO, M. S., AFOLABI, A. M., & AKINLABI, A. A. (2020). Phytoremediation technology, plant response to environmental contaminants and the need for soil augmentation. *Notulae Scientia Biologicae*, *12*(3), 486–499. https://doi.org/10.15835/nsb12310737

Pan, M., & Chu, L. M. (2017). Fate of antibiotics in soil and their uptake by edible crops. *Science of The Total Environment*, *599–600*, 500–512. https://doi.org/10.1016/j.scitotenv.2017.04.214

Peralta-Videa, J. R., Lopez, M. L., Narayan, M., Saupe, G., & Gardea-Torresdey, J. (2009). The biochemistry of environmental heavy metal uptake by plants: Implications for the food chain. *The International Journal of Biochemistry & Cell Biology*, *41*(8–9), 1665–1677. https://doi.org/10.1016/j.biocel.2009.03.005

Postma, J. A., & Lynch, J. P. (2012). Complementarity in root architecture for nutrient uptake in ancient maize/bean and maize/bean/squash polycultures. *Annals of Botany*, *110*(2), 521–534. https://doi.org/10.1093/aob/mcs082

Prosser, R. S., & Sibley, P. K. (2015). Human health risk assessment of pharmaceuticals and personal care products in plant tissue due to biosolids and manure amendments, and wastewater irrigation. *Environment International*, *75*, 223–233. https://doi.org/10.1016/j.envint.2014.11.020

Richard Molinar, Jose Aguiar, Mark Gaskell, & Keith Mayberry. (2023). *Summer Squash Production In California - Small Farms*. Univ. of California Website. https://sfp.ucanr.edu/crops/squash1/

Riemenschneider, C., Seiwert, B., Moeder, M., Schwarz, D., & Reemtsma, T. (2017a). Extensive Transformation of the Pharmaceutical Carbamazepine Following Uptake into Intact Tomato Plants. *Environmental Science & Technology*, *51*(11), 6100–6109. https://doi.org/10.1021/acs.est.6b06485

Riemenschneider, C., Seiwert, B., Moeder, M., Schwarz, D., & Reemtsma, T. (2017b). Extensive Transformation of the Pharmaceutical Carbamazepine Following Uptake into Intact Tomato Plants. *Environmental Science & Technology*, *51*(11), 6100–6109. https://doi.org/10.1021/acs.est.6b06485

Robby Berman, & Ferdinand Lali. (2022, July 11). *Pollution and health: Contaminated soil may lead to heart disease*. https://www.medicalnewstoday.com/articles/pollution-and-health-contaminated-soil-may-lead-to-heart-disease

Robert B. Thomas. (2023, March 24). *Zucchini, Summer Squash*. Yankee Publishing, Inc.

Roberts, P., & Thomas, K. (2006). The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. *Science of The Total Environment*, *356*(1–3), 143–153. https://doi.org/10.1016/j.scitotenv.2005.04.031

Rooni, V., Raud, M., & Kikas, T. (2017). The freezing pre-treatment of lignocellulosic material: A cheap alternative for Nordic countries. *Energy*, *139*, 1–7. https://doi.org/10.1016/j.energy.2017.07.146

Rouphael, Y., Colla, G., Battistelli, A., Moscatello, S., Proietti, S., & Rea, E. (2004). Yield, water requirement, nutrient uptake and fruit quality of zucchini squash grown in soil and closed soilless culture. *The Journal of Horticultural Science and Biotechnology*, *79*(3), 423–430. https://doi.org/10.1080/14620316.2004.11511784

S. A. Barber. (1984). Soil Nutrient Bioavailability: A Mechanistic Approach. *Environmental Science*.

Santos, L. H. M. L. M., Araújo, A. N., Fachini, A., Pena, A., Delerue-Matos, C., & Montenegro, M. C. B. S. M. (2010). Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. *Journal of Hazardous Materials*, *175*(1–3), 45–95. https://doi.org/10.1016/j.jhazmat.2009.10.100

Sarah Overstreet. (2018, January 8). *INFOGRAPHIC: 10 Things to Know About Medical Waste Compliance*. https://blog.sharpsinc.com/10-things-to-know-about-medical-waste-compliance#

Schmiedjell Bsc, D. (2022). *SCREENING FOR METABOLITES OF VENLAFAXINE AND CITALOPRAM IN EDIBLE PLANTS Chemistry and chemical technology* [JOHANNES KEPLER UNIVERSITÄT LINZ]. www.jku.at

Schroll, R., & Scheunert, I. (1992). A laboratory system to determine separately the uptake of organic chemicals from soil by plant roots and by leaves after vaporization. *Chemosphere*, *24*(1), 97–108. https://doi.org/10.1016/0045-6535(92)90571-8

Seeberg-Elverfeldt, J., Schlüter, M., Feseker, T., & Kölling, M. (2005). Rhizon sampling of porewaters near the sediment-water interface of aquatic systems. *Limnology and Oceanography: Methods*, *3*(8), 361–371. https://doi.org/10.4319/lom.2005.3.361

Smith, K. E. C., & Jones, K. C. (2000). Particles and vegetation: implications for the transfer of particle-bound organic contaminants to vegetation. *Science of The Total Environment*, *246*(2–3), 207–236. https://doi.org/10.1016/S0048-9697(99)00459-3

Srivatsav, P., Bhargav, B. S., Shanmugasundaram, V., Arun, J., Gopinath, K. P., & Bhatnagar, A. (2020). Biochar as an Eco-Friendly and Economical Adsorbent for the Removal of Colorants (Dyes) from Aqueous Environment: A Review. *Water*, *12*(12), 3561. https://doi.org/10.3390/w12123561

Tamez, C., Hernandez-Molina, M., Hernandez-Viezcas, J. A., & Gardea-Torresdey, J. L. (2019). Uptake, transport, and effects of nano-copper exposure in zucchini (Cucurbita pepo). *Science of The Total Environment*, *665*, 100–106. https://doi.org/10.1016/j.scitotenv.2019.02.029

Thompson, T. (2022). The staggering death toll of drug-resistant bacteria. *Nature*. https://doi.org/10.1038/d41586-022-00228-x

Tomczyk, A., Sokołowska, Z., & Boguta, P. (2020). Biochar physicochemical properties: pyrolysis temperature and feedstock kind effects. *Reviews in Environmental Science and Bio/Technology*, *19*(1), 191–215. https://doi.org/10.1007/s11157-020-09523-3

United States Environmental Protection Agency. (2012, September). *A Citizen's Guide to Phytoremediation What Is Phytoremediation?*

https://www.epa.gov/sites/default/files/2015-04/documents/a_citizens_guide_to_phytoremediation.pdf

van der Heijden, M. G. A., & Horton, T. R. (2009). Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *Journal of Ecology*, *97*(6), 1139–1150. https://doi.org/10.1111/j.1365-2745.2009.01570.x

Van Zwieten, L., Kimber, S., Morris, S., Chan, K. Y., Downie, A., Rust, J., Joseph, S., & Cowie, A. (2010). Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. *Plant and Soil*, *327*(1–2), 235–246. https://doi.org/10.1007/s11104-009-0050-x

Verlicchi, P., & Zambello, E. (2015). Pharmaceuticals and personal care products in untreated and treated sewage sludge: Occurrence and environmental risk in the case of application on soil — A critical review. *Science of The Total Environment*, *538*, 750–767. https://doi.org/10.1016/j.scitotenv.2015.08.108

Wallace, H. A. H. (1960). FACTORS AFFECTING SUBSEQUENT GERMINATION OF CEREAL SEEDS SOWN IN SOILS OF SUBGERMINATION MOISTURE CONTENT. *Canadian Journal of Botany*, *38*(3), 287–306. https://doi.org/10.1139/b60-030

Wang, J., Odinga, E. S., Zhang, W., Zhou, X., Yang, B., Waigi, M. G., & Gao, Y. (2019). Polyaromatic hydrocarbons in biochars and human health risks of food crops grown in biochar-amended soils: A synthesis study. *Environment International*, *130*, 104899. https://doi.org/10.1016/j.envint.2019.06.009

White, P. J. (2012). Long-distance Transport in the Xylem and Phloem. In *Marschner's Mineral Nutrition of Higher Plants* (pp. 49–70). Elsevier. https://doi.org/10.1016/B978-0-12-384905-2.00003-0

Wu, W., Han, J., Gu, Y., Li, T., Xu, X., Jiang, Y., Li, Y., Sun, J., Pan, G., & Cheng, K. (2022). Impact of biochar amendment on soil hydrological properties and crop water use efficiency: A global meta-analysis and structural equation model. *GCB Bioenergy*, *14*(6), 657–668. https://doi.org/10.1111/gcbb.12933

Wu, X., Conkle, J. L., Ernst, F., & Gan, J. (2014). Treated Wastewater Irrigation: Uptake of Pharmaceutical and Personal Care Products by Common Vegetables under Field Conditions. *Environmental Science & Technology*, *48*(19), 11286–11293. https://doi.org/10.1021/es502868k

Wu, X., Dodgen, L. K., Conkle, J. L., & Gan, J. (2015). Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: a review. *Science of The Total Environment*, *536*, 655–666. https://doi.org/10.1016/j.scitotenv.2015.07.129

Yan, A., Wang, Y., Tan, S. N., Mohd Yusof, M. L., Ghosh, S., & Chen, Z. (2020). Phytoremediation: A Promising Approach for Revegetation of Heavy Metal-Polluted

Land. In *Frontiers in Plant Science* (Vol. 11). Frontiers Media S.A. https://doi.org/10.3389/fpls.2020.00359

Yang, Y.-Y., Gray, J. L., Furlong, E. T., Davis, J. G., ReVello, R. C., & Borch, T. (2012). Steroid Hormone Runoff from Agricultural Test Plots Applied with Municipal Biosolids. *Environmental Science & Technology*, *46*(5), 2746–2754. https://doi.org/10.1021/es203896t

Ye, M., Sun, M., Wan, J., Feng, Y., Zhao, Y., Tian, D., Hu, F., & Jiang, X. (2016). Feasibility of lettuce cultivation in sophoroliplid-enhanced washed soil originally polluted with Cd, antibiotics, and antibiotic-resistant genes. *Ecotoxicology and Environmental Safety*, *124*, 344–350. https://doi.org/10.1016/j.ecoenv.2015.11.013

ZHANG, C., FENG, Y., LIU, Y., CHANG, H., LI, Z., & XUE, J. (2017). Uptake and translocation of organic pollutants in plants: A review. *Journal of Integrative Agriculture*, *16*(8), 1659–1668. https://doi.org/10.1016/S2095-3119(16)61590-3

Žigová, A., Šťastný, M., & Kodešová, R. (2013). DEVELOPMENT OF SOILS ON PARAGNEISS AND GRANITE IN THE SOUTHEASTERN PART OF BOHEMIA. In *Acta Geodyn. Geomater* (Vol. 10, Issue 1).

Supplementary Material

Appendix 1 – Experimental Design

Treatment on soil without Biochar	Pot
	1
	2
	3
	4
	5
Control	6
	7
	8
	9
	10
	11
Pharmaceuticals	12
	25
	26
	27
	28
	29
Mix	30

Treatment on soil amended with Biochar	Pot
	31
	32
	33
	34
	35
Control	36
	37
	38
	39
	40
	41
Pharmaceuticals	42
	55
	56
	57
	58
	59
Mix	60

Appendix 2 – Germination Rate

	Pharma		Pharma+BC	
Days of Sowing	*Avg*	*Std*	*Avg*	*Std*
9	0.50	0.55	0.67	0.82
10	1.67	0.52	1.33	0.82
12	2	0	1.33	0.82
13	2	0	2	0
14	2	0	2	0
15	2	0	2	0

	Mix		Mix+BC	
Days of Sowing	*Avg*	*Std*	*Avg*	*Std*
9	0.33	0.52	0.17	0.41
10	1.50	0.84	0.83	0.75
12	1.83	0.41	1.83	0.41
13	2	0	2	0
14	2	0	2	0
15	2	0	2	0

Appendix 3 – Biomass Yield

	Average (Wet) (g)	Std Deviation (Wet)	Average (dry) (g)	Std Deviation (dry)
Control	36.4020071	6.46404956	6.39033333	0.44815831
Control + BC	170.775	5.25095325	18.6895	1.17532561
Pharma	37.95	8.93772678	5.60416667	0.75752371
Pharma + BC	159.701667	8.34383465	17.693	0.8835268
Mix	39.0033333	4.27208458	6.05883333	0.5520295
Mix + BC	167.226667	9.25302041	19.2455	1.1416556

Appendix 4 - Concentration of PhACs

		Pharma		Pharma + BC	
Concentration in Biomass (ng/g)		Average	Standard Deviation	Average	Standard Deviation
	carbamazepine	214.3791	14.6877	72.7152	16.4130
	tramadol	46.6931	8.6181	35.2590	6.9588
	citalopram	0.2966	0.0768	0.1364	0.0355
	metoprolol	9.5381	1.9980	3.0952	0.4137
	propafenone	0.8092	0.1049	0.3807	0.0326
	sertraline	0.6396	0.1487	0.3631	0.1396
	tiapride	5.9241	1.2118	2.1932	0.6679
	amisulpride	2.2198	0.4169	0.8816	0.2673
	trospium	1.1853	0.1832	0.6647	0.1137
	venlafaxine	3.5210	0.5662	2.8869	0.4998

		Mix		Mix + BC	
Concentration in Biomass (ng/g)		Average	Standard Deviation	Average	Standard Deviation
	carbamazepine	218.5305	29.6662	84.8443	25.0503
	tramadol	48.9593	7.6681	41.5306	11.2062
	citalopram	0.3950	0.1207	0.5104	0.5735
	metoprolol	9.5324	1.5075	3.9071	1.0064
	propafenone	0.8653	0.0834	0.5891	0.4243
	sertraline	0.5541	0.1085	0.3508	0.3155
	tiapride	5.1368	1.0161	2.3849	0.6287
	amisulpride	2.0721	0.4496	1.0293	0.7160
	trospium	1.3016	0.2751	0.9983	0.8035
	venlafaxine	3.7805	0.6924	3.3582	0.7497

Raw Data for PhACs Concentration on Each Pot

Sample											
Data File	Comment	Final Conc.	Final Conc	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.
RECOVERY (%)		93.01572	100.24	94.3859	90.951	83.3744	92.47058	96.50352	95.24085	103.0458	96.04165
	ng/g in dry biomass										
15 7.d	Pharmaceuticals	2.588777	233.26	0.370993	12.18975	0.896837	0.699273	6.902818	62.1968	1.282046	4.506961
23 8.d	Pharmaceuticals	2.751802	215.3	0.388724	9.907555	0.813674	0.796125	7.503158	46.56958	1.500796	3.300792
35 9.d	Pharmaceuticals	2.350085	216.93	0.237581	11.49602	0.911203	0.751406	6.581165	48.3509	1.171114	3.594451
49 10.d	Pharmaceuticals	1.647884	195.81	0.209248	7.20652	0.656683	0.556347	4.832033	37.04468	0.992693	2.954283
57 11.d	Pharmaceuticals	2.005169	225.96	0.332115	8.310613	0.868144	0.645984	4.947449	44.97758	1.067475	3.715666
71 12.d	Pharmaceuticals	1.975182	199.02	0.240828	8.11811	0.708388	0.388486	4.778136	41.01908	1.097771	3.054029
19 37.d	Pharmaceuticals+BC	0.910316	65.202	0.128523	3.053632	0.393132	0.537518	2.541169	31.15043	0.661495	2.403525
31 38.d	Pharmaceuticals+BC	0.58098	91.525	0.15886	3.611703	0.437636	0.266946	1.826733	44.01193	0.515403	3.254577
39 39.d	Pharmaceuticals+BC	0.806324	71.712	0.131268	3.354464	0.355682	0.200436	2.389662	40.01218	0.622926	3.210495
53 40.d	Pharmaceuticals+BC	0.711729	90.533	0.158962	3.307501	0.383888	0.254936	2.200104	40.14637	0.610449	3.480858
66 41.d	Pharmaceuticals+BC	0.918982	48.098	0.168824	2.505845	0.348346	0.436311	1.120968	27.87447	0.730748	2.710519
74 42.d	Pharmaceuticals+BC	1.361453	69.222	0.071896	2.738066	0.365527	0.48223	3.080374	28.35836	0.847009	2.261343
18 25.d	MIX	2.903203	172.39	0.56069	11.09833	0.969683	0.660737	6.861075	58.73579	1.846327	4.084207
25 26.d	MIX	1.907268	207.44	0.342428	8.295136	0.864994	0.439931	4.836025	46.04791	1.151096	3.631385
38 27.d	MIX	1.83802	244.34	0.376883	9.733659	0.874666	0.39876	4.672168	51.15892	1.141417	4.297904
52 28.d	MIX	1.860568	207.96	0.216949	8.072681	0.879816	0.57738	5.182456	41.10421	1.186344	2.782174
65 29.d	MIX	1.671181	223.88	0.496092	8.452201	0.713566	0.628128	3.80505	40.52265	1.323275	3.249221
73 30.d	MIX	2.252272	255.17	0.377078	11.54268	0.889285	0.619918	5.464103	56.18619	1.161123	4.638326
22 55.d	MIX+BC	2.436731	65.171	1.605531	3.570484	1.445193	0.989375	3.463452	29.44607	2.621806	2.854887
34 56.d	MIX+BC	1.080453	77.789	0.689614	5.011615	0.511317	0.232508	2.845363	45.25502	0.894067	3.499021
48 57.d	MIX+BC	0.539141	110.35	0.236984	3.944961	0.471239	0.1832	1.988773	40.83131	0.634683	3.221885
56 58.d	MIX+BC	0.730939	59.913	0.138931	2.764913	0.333732	0.249796	1.989114	30.66466	0.639476	2.728024
70 59.d	MIX+BC	0.808618	74.614	0.165581	2.987255	0.385772	0.279706	2.076249	42.80662	0.638657	3.061387
82 60.d	MIX+BC	0.579789	121.23	0.225464	5.163607	0.387609	0.170381	1.94641	60.18009	0.560989	4.784173

Appendix 5 - Treatment and Irrigation

Amount of Demineralised Water Treatment

	MWHC/Date	Ctrl	Ph	Mix	Ctrl+BC	Ph+bc	Mix+bc
Amount of Irrigation (ml)	**40% 31.5**	176.32	174.18	184.53	185.03	184.02	178.18
	40% 3.6	110.94	114.60	120.46	121.41	117.88	115.79
	40% 6.6	105.63	110.81	111.80	106.00	108.06	103.32
	40% 8.6	81.78	87.71	91.87	88.09	89.41	83.81
	40% 11.6	111.09	107.16	102.32	96.97	104.26	106.40
	40% 13.6	112.01	110.47	104.86	96.62	104.91	107.00
	60% 27.6	167.00	159.00	103.00	94.00	189.00	124.00
	80% 1.7	303.00	329.00	286.00	320.00	340.00	305.00
	60% 4.7	47.17	65.83	58.00	53.50	54.00	60.83
	70% 11.7	177.33	177.00	149.33	202.83	198.00	203.50
	73% 12.7	157.09	146.41	156.73	190.53	167.42	189.49
	73% 18.7	228.83	220.00	227.50	242.50	248.00	239.83
	80% 25.7	168.33	156.50	160.00	204.33	215.00	222.17
	80% 1.8	163.83	153.67	153.17	197.00	199.67	175.83
	60% 5.8	29.50	27.33	21.33	90.17	102.33	88.50
	80% 8.8	214.17	206.67	208.33	298.67	298.50	296.50
	90% 9.8				157.00	167.50	155.67
	TOTAL	2354.01	2346.32	2239.23	2744.65	2887.95	2755.81
	Std	69.3382	70.0638	65.7713	77.6511	77.2164	73.3477

Amount of Irrigated Chemicals

	Average Water Consumption (ml)	Average of Chemicals Solution Applied (ml)	Standard Deviation
Ctrl	5416.01	X	69.338
Ctrl + BC	8110.65	X	77.651
Pharma	2346.32	3062	70.064
Pharma + BC	2887.95	5366	77.216
Mix	2239.23	3062	65.771
Mix + BC	2755.81	5366	73.348