Valorization of Coffee Waste for Wastewater Treatment and Fertilizer Products

by

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Abstract

Current mainstream fertilizers are disruptive to the environment. This includes pollution, soil quality degradation, and even air pollution. Alternative methods for supplying nutrients to plants is important, as there is a high demand for improving crop yields to sustain the growing population. Spent coffee grounds are rich in C, N, and K which makes them a viable option for fertilizer applications as they would improve soil quality through the addition of organic matter. Additionally, spent coffee grounds are in abundance due to the high consumption of coffee globally. This would lead to less waste coffee entering the landfill, which would in turn reduce greenhouse gas emissions by reducing the rate at which coffee grounds are sitting decomposing in a landfill. In addition to spent coffee grounds improving soil quality, minerals can be crystallized onto the surface of the coffee grounds in order to target the essential nutrients for plant growth. Struvite was selected, since it contains many elements that aid in photosynthesis in plants, but it is also a byproduct in wastewater treatment and management. Therefore, functionalizing the coffee grounds with PEI for metal ion removal, and GMAC for bacterial inactivation to aid in the treatment of wastewater enables a system to be created that starts with the decontamination of wastewater, and can act as a slow-release fertilizer.

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Chapter 1 – Introduction

The agricultural industry in Alberta is made up of many different sectors, including: cattle ranches, family farms, hunting, fishing, and beekeeping. Together, these sectors cover about one-third of the available land in Alberta [1]. The agricultural industry produces wastewater through: washing various products, using water for transport in processing plants, delivering nutrients to crops, canning goods, cooling off cattle, and much more. In addition to the agricultural industry, wastewater is generated by municipal households, businesses, and industries [2]. According to the Government of Canada, millions of cubic meters of waste water is produced daily [2]. Due to the volume of wastewater generated, there is interest in either: i) finding ways to reduce the volume of wastewater, or ii) finding ways to reuse the wastewater. Agricultural wastewater has commonly been used for the irrigation of crops, and this practice dates back to the Bronze Age [3]. However, when introducing wastewater to the environment it is important to reduce the toxic contaminants that are present. This is due to the adverse effects contaminants may have on human health and the ecosystem.

The contaminants found in wastewater can be divided into 4 categories: i) dissolved organic species, ii) dissolved inorganic species, iii) suspended organic materials, and iv) suspended inorganic materials [4]. Different treatment options are available depending on the type of contaminant present. An unfortunate drawback to current wastewater treatment strategies is that no single option is capable of removing all contaminants. This results in current treatment strategies utilizing multiple steps in order ensure the quality of the water is adequate.

Many natural materials have been investigated for water purification. The most common natural materials considered are: i) chitosan, ii) clay, iii) zeolites, and iv) wood [5]. Natural materials are considered for water purification due to their high availability, low cost, and adsorbent properties. Other natural materials have been investigated and show promise for wastewater treatment, such as coffee grounds. Waste coffee grounds have been shown to remove Cu(II) and Cr(VI) from aqueous solutions via adsorption [6].

1.1 Maximum Element Concentrations for Drinking Water and Crop Irrigation

Health Canada, World Health Organization (WHO), and the Food and Agriculture Organization (FAO) all defined standards for element concentrations in water to ensure it is safe for consumption or for crop irrigation. Depending on the application, different maximum concentrations are set for heavy metals due to humans and plants having differing nutrient needs and thresholds for toxic effects. According to Health Canada, the maximum acceptable concentration for heavy metals is determined based on available research related the impact of each metal on human health [7].

Table 1: Maximum allowable concentration of chemical pollutants for drinking water and crop irrigation according to Health Canada, WHO, and the FAO [7, 8] .

Chemical Pollutant	Maximum Concentration for Drinking Water	Maximum Concentration for Crop Irrigation	Health Concerns
Aluminum	N/A	5.0	No evidence showing human health concerns.
Arsenic	0.010	0.1	Human carcinogen, skin/vascular/neurological effects.
Beryllium	N/A	0.1	N/A
Cadmium	0.007	0.1	Kidney damage, decreased bone density.
Chromium	0.05	0.1	Hyperplasia of small intestine due to chromium (VI).
Cobalt	N/A	0.05	N/A
Copper	2	0.2	Long-term liver and kidney effects, short-term gastrointestinal.
Iron	N/A	5.0	No known health concerns.
Lead	0.005	5.0	Possible concerns: Behavioural effects in children, reduced cognitive function, increased blood pressure, and renal dysfunction.
Lithium	N/A	2.6	N/A
Manganese	0.12	0.2	Deficit in memory, attention, and motor skills.
Molybdenum	N/A	0.01	N/A
Selenium	0.05	0.02	Hair loss, tooth decay, weakened nails, and nervous system disturbances.
Zinc	N/A	2.0	Considered non-toxic high concentrations leave the water with a bad taste.

1.2 Methods for Wastewater Treatment

1.2.1 Chlorination for Water Purification and Disinfecting

Chlorination is a disinfection technique that is commonly used for the removal of harmful microbiological contaminants [9, 10]. This technique is simply the addition of chlorines or chloramines to the contaminated water. The addition of these compounds will act to inactive or completely destroy the microbiological contaminants. Chlorine has been used as an antimicrobial agent in many different industries due to its oxidizing potential [11]. Additional applications where chlorine is used for disinfecting include the food industry for the sanitization of raw food as well as food processing equipment [11]. Overall, chlorination has had a positive impact on society by reducing waterborne pathogens as well as foodborne pathogens [10, 11].

The chlorine used for chlorination acts to destroy the cell membranes of microorganisms [12]. The amount of chlorine needed for disinfection depends on the volume of wastewater being treated. It is important to use enough chlorine during disinfecting in order to ensure all microorganisms are inactivated [13]. In wastewater, *Escherichia coli* (*E. Coli*) is a common microorganism and has been known to cause gastrointestinal issues in humans [7]. At 5 mg/ml of chlorine, after 10 minutes there is no culturable *E. Coli* remaining [13]. This shows the effectiveness of chlorination as a disinfection method for wastewater treatment, as the final concentration of *E. Coli* follows the standards set by Health Canada [7].

Chlorination, although it has been a mainstream method for inactivating microorganisms in wastewater, there are several downsides to it. Mainly, chlorine has been known to react with matter present in the wastewater to produce by-products [10, 14]. Chlorine has been known to form trihalomethanes, haloacetic acids, and halogenated acetonitriles [10].

These formed compounds have been found to have adverse effects on human health, such as carcinogenic properties [10, 14]. Another limitation of chlorination is that it is only used to remove the microorganisms from the contaminated water. This means additional treatment steps are required to remove particles and ions that have adverse effects on human health in order for the quality of the water to comply with the standards set by the Health Canada, WHO, and the FEA (outlined in 1.1). Some additional steps would include screening of large particles, and removal of any remaining heavy metal ions. Finally, another drawback to chlorination is the fact that high concentrations of chlorine present in potable water may lead to an off-taste and odour. Conversely, insufficient concentrations of chlorine have been known to lead to the reactivation of microorganisms [13, 15].

1.2.2 Nanotechnology-based Purification of Water

The use of nanotechnology for water purification has not seen wide-spread commercial use. Some examples of how nanotechnology is used for water purification include the addition of nanoparticles, and through the use of carbonaceous nanostructures [16]. These methods have been identified as promising water purification due to the simultaneous filtering of large particles via adsorption while also eliminating microorganisms due to the inherent antimicrobial properties of some nanomaterials used [18]. Some common examples of nanomaterials used for water purification include: i) silver nanoparticles, ii) iron nanoparticles, iii) zinc nanoparticles, and iv) carbon nanotubes [16, 17]. This leads to a chlorine-free disinfection method for eliminating microorganisms while simultaneously removing heavy metal ions from solution [19].

Silver nanoparticles have been widely studied due to their antimicrobial properties, stability, and low toxicity [20]. It has been observed that silver nanoparticles cause permanent damage to the cellular membrane of microorganisms [21]. Silver nanoparticles are capable of inactivating microorganisms, but a method for removing any microorganism remains is still needed. This is obtained using two methods: i) functionalize silver nanoparticles onto some other material that is capable of acting as a filter, or ii) making a sheet of silver nanoparticles to act as a membrane for simultaneous bacterial inactivation and filtering [17, 20]. The low toxicity of silver to humans allows for the leaching of silver ions into solution while not being a large concern for water quality. Silver nanoparticles have been shown to remove 97.8-100% of *E. Coli* present in solution, which makes it a viable disinfection technique since Health Canada requires there to be no detectable *E. Coli* in 100 mL of water [17].

Iron nanoparticles have gathered interest in industrial wastewater purification due to their adsorption capacity, and low cost [23]. In addition, they can be easily separated due to their magnetic properties [23]. Many studies have shown the capabilities of iron nanoparticles for purifying wastewater, however not much is known about the environmental impact [24]. Iron nanoparticles have seen success due to their relatively stronger reduction ability compared to other metal nanoparticles (aluminum, zinc, silver) [17]. The strong reduction ability of iron nanoparticles assists with the removal of heavy metals, metalloids, and inorganic anions [16, 17].

Zinc nanoparticles have gained increased popularity in wastewater treatment, and currently see use in cosmetics, and UV-filtration [25]. As zinc becomes more prevalent in wastewater treatment, this means there will be more zinc nanoparticles in water and in the environment. Although zinc is not immediately toxic to humans, it leaves water with a poortaste [7]. Therefore, minimal zinc should be present after treating wastewater to produce potable water. Zinc nanoparticles have been reported to act as a heavy metal adsorbent due to its reduction ability, additionally the nano-scale particles have been known to penetrate bacterial walls therefore causing inactivation [26].

Carbon nanotubes have been studied for water treatment applications due to their high adsorption capacity [16, 17]. In addition, carbon nanotubes and other forms of nanostructured carbon materials have high strength, good thermal and electrical properties, and good separation characteristics [16]. Due to their hydrophobic surface, no agglomeration will occur which ensures adsorption remains plentiful [19]. Not only have carbon nanotubes been used for heavy metal ion adsorption, but they have been shown to cause membrane damage to bacteria [27]. Carbon nanotubes can be made into a membrane in order to filter solutions. It was shown that a carbon nanotube-based filter can have no bacterial colonies able to grow in the filtrate [27]. In addition, the bacterial inactivation could be further improved with the addition of silver nanoparticles.

Overall, nanotechnology has been used successfully in the treatment of wastewater. Although no widespread use has been observed in large-scale facilities, they have shown promise due to their adsorbent and antimicrobial properties. Furthermore, nanotechnology provides a chlorine-free option for inactivating bacteria which leads to no hazardous chlorination by-products forming. This makes nanotechnology a desirable option for wastewater treatment. Since not much is known about the long-term impacts of nanoparticles on the environment, further study needs to be performed to evaluate the eco-friendliness of nanoparticles.

1.2.3 Coagulation, Sedimentation, and Flocculation

Sedimentation is the act of particles settling under gravity. This is commonly used during water treatment in order to separate large particles from the water, and occurs in natural bodies of water such as lakes and rivers [28]. Coagulation and flocculation, in wastewater treatment, are often used alongside sedimentation [29]. The purpose of these two processes is to remove the charge from suspended particles, which serves to promote the attachment of particles to one another [29]. All three of these processes have seen use as a pretreatment in wastewater treatment, since other filtration techniques have a higher operating and maintenance costs [30].

Chemicals, known as coagulants, are added during coagulation in order to remove the charge from any suspended particles [29]. The most common coagulant used in water treatment is alum [31]. Alum is the common name for aluminum sulfate [32]. Aluminum compounds are used as they hydrolyze into cationic species that adsorb onto the negatively charged species in order to neutralize the charge [31, 32]. After the addition of the coagulant, the suspended particulate is stirred to encourage collisions, and therefore improve the rate of agglomeration [29, 33]. As the neutral particles start to collide and attach, the agglomeration of particles is known as a floc [29]. Finally, after floc formation, the larger particles can settle at the bottom of a large tank thereby separating the solid contaminants from solution [28].

One of the main benefits of using coagulation, flocculation, and sedimentation for wastewater treatment is that it does not require much additional operating as it is a gravity-based separation method. Therefore, as previously stated, using sedimentation can cut operating and maintenance costs [30]. Since it is a gravity-based method, the technique takes time in order for the large particles to settle on the bottom of the tank.

1.3 Example Wastewater Treatment Process from Alberta Capital Region Wastewater Commission Facility

Current wastewater treatment facilities use a combination of different steps in order to remove contaminants from the wastewater. The Alberta Capital Region Wastewater Commission facility based in Fort Saskatchewan starts the treatment process with screening [34]. In this stage, the wastewater flows through a screen which removes large suspended materials. Additionally, after the screening, the wastewater then enters and aerated grit chamber before entering the next step of the process [34]. This facility refers to the second step as primary clarifying. In this step, the water flows very slowly to allow fine solids time to settle at the bottom of the tank. In addition, any remaining large particles will float to the surface allowing them to easily be skimmed. All collects solids are referred to as sludge, and are further processed/treated separately from the remaining liquids [34]. The remaining wastewater contains mostly dissolved contaminants after the previously mentioned steps, and is sent for further processing.

After the primary treatment to remove suspended solids, the wastewater then enters bioreactors. In this stage, the primary objective is to remove organic pollutants and nutrient pollutants [34]. This is obtained with the addition of microorganisms into the wastewater. The added microorganisms are not specifically disclosed, but they remove the pollutants through metabolizing them. This stage includes multiple cells which contain aerobic and anaerobic conditions for metabolizing different contaminants [34]. By the end of the biological treatment, over 95% of the organic pollutants are removed. Removal of the remaining organic pollutants is done via ultraviolet disinfection. Ultraviolet disinfection operates by penetrating through microorganisms and destroying their DNA. In this final stage, pathogenic microorganisms are inactivated before the effluent is finally discharged into the North Saskatchewan River [34].

The collected sludge from the primary treatment is fermented and the resulting liquid contains volatile fatty acids which are used in the bioreactor for the removal of phosphorus [34]. The remaining sludge and skimmed solids from primary treatment are sent to digesters with anaerobic microorganisms to break down some of the organic matter into methane and carbon dioxide. Remaining sludge at the end of digestion is disposed of.

1.4 Technical Challenges in Wastewater Treatment

Plastics have been a large environmental concern due to their inherently slow rate of degradation. In water, large sources of plastics can easily be removed. However, microplastics and nanoplastics are not as easily removed from the water [35]. Microplastics are commonly considered as any piece of plastic that is smaller than approximately 5 mm [35]. The removal of microplastics from water is important due to the impact they may have on marine and mammalian life. Ingestion of microplastics is the most common mode of exposure, followed by inhalation and dermal contact [36]. The small size of the particles makes them difficult to remove after ingestion or inhalation. This may lead to inflammation and discomfort, which may lead to an increased chance for cancer. Additionally, the particles have a large surface area. This will allow them to act as a carrier for other contaminants. Futhermore, the large surface area has been linked to cytotoxicity and causes oxidative stress [36]. Due to the associated health risks with exposure to microplastics, a method to safely separate and dispose of them is highly desirable. Current water treatment facilities do no commonly include stages targeted towards microplastic removal [35].

Additionally, current wastewater treatment methods utilize many steps in order to remove contaminants. The wastewater treatment process outlined in 1.3 demonstrates the complicated procedure required to remove all contaminants in order to make the wastewater safe to release back into the environment [34]. During nitrogen removal in wastewater treatment, nitrous oxide is produced as a byproduct [37]. Nitrous oxide emissions have been estimated to take up approximately 83% of the carbon footprint of wastewater treatment plants [37]. In addition, nitrous oxide is the third most abundant greenhouse gas, and is the most significant ozone-depleting substance [37]. Due to the impact of nitrous oxide on the environment, reducing emissions has been a growing concern in wastewater treatment facilities.

There has been a focus on resource recovery from various stages of the wastewater treatment process. Some possibilities of resources that could be recovered from wastewater treatment includes product recovery from sludge, phosphorus recovery, energy recovery, and nutrient recovery [38]. Resource recovery is often aimed at improving wastewater treatment plant operations [39]. In some cases, such as struvite precipitation, nutrients can be recovered and collected in the form on a mineral that can be repurposed as a slow-release fertilizer [39]. Recovering resources has been a large interest for wastewater treatment plants to reduce costs and as a step towards sustainability [40].

1.5 Natural Materials Used in Water Purification

Natural materials have been frequently studied as an alternative for water purification methods due to their availability, low-cost, and adsorbent properties [5,6]. Some examples of where natural materials can be used in water purification include: i) as an adsorbent to be added for removing heavy metal ions, ii) as an antimicrobial agent to inactivate bacteria from the wastewater, and iii) as a filter/membrane to separate large particulate from the remaining water [4,5]. The common natural materials that have been explored for wastewater treatment include chitosan, clay, zeolites, and wood [5].

Chitosan is a natural biopolymer that is derived from crustacean shells [5,42]. It has received attention for water purification due to its adsorbent properties, and high adsorption capacity [43]. In addition, it is one of the most abundant natural biopolymers and has a low cost [43]. Chitosan has free $–NH_2$ and $–OH$ bonds which have been known to contribute to adsorption of metals [44]. The amine groups present on chitosan make it cationic, which is the basis for the adsorption properties demonstrated [45]. Furthermore, the hydroxyl group aids in stabilizing the metal binding with the amine groups [45]. When using chitosan as an adsorbent for heavy metal removal, increasing available adsorption sites is important to maximize ion removal. This is commonly achieved through the fabrication of chitosan nanoparticles, since this increases the effective surface area and can therefore have more available free amine groups for metal ion binding [46].

Clay and clay minerals have been widely studied for water treatment due to its naturally occurring adsorbent properties [47]. Clay offers high adsorption capacity, low permeability, large surface area, and good chemical and mechanical stability [5]. These materials carry a permanent negative charge, which can be neutralized by nearby cations [48]. Therefore, making it a decent option for removing metal ions from solutions. In fact, clay and clay minerals have been shown to remove metal ions out of solution therefore making it a viable wastewater purification strategy [49]. However, a drawback to using clay for the adsorption of metal ions is at a pH above 6, precipitates form which lowers the adsorption capacity of clay [47].

Zeolites are natural materials based on volcanic sedimentary rock [50]. They are microporous aluminosilicates that are abundant in nature and low-cost [5]. The porous nature of them increases the available surface area for adsorption which has led to attraction for water purification applications [5]. Natural zeolites have gained an increase in popularity for wastewater treatment due to their ion-exchange and adsorption properites [5, 50, 51]. Additionally, the porous nature leads to sieve-type behavior allowing for filtration of large particulate alongside increasing surface area for adsorption [5].

Wood is a natural adsorbent that is very abundant [5]. It has been tested for its ability to remove pollutants from water and wastewater [5]. Wood has gained interest due to its natural porous structure alongside its low cost and biocompatibility [20]. Wood is made up of cellulose fibers and lignin to form a highly porous structure [52]. Similar to zeolites, the porous nature increased available surface area for adsorption and therefore improves metal ion removal for wastewater treatment. Additionally, the porous structure allows wood to act as a filter/membrane for a natural separation technique [20]. This allows wood to separate large particles from solution while removing metal ion contaminants.

Many of the discussed natural materials have excellent adsorbent properties. The good surface area alongside adsorbent properties make natural materials an efficient alternative for wastewater treatment. However, a common disadvantage is that no single natural material is capable of removing all contaminants from solution. For example, chitosan and clay are not porous and therefore do not inherently act as filters whereas zeolites and wood can effectively remove solid matter due to their porous nature. In all cases, increasing the available surface area will improve the adsorption capacity due to increasing the number of adsorption sites. To further improve the wastewater treatment process using natural materials, additional steps are needed. Microorganisms are still present in the wastewater due to none of the discussed materials having antibacterial properties. This makes natural materials a viable option for adsorption for dissolved species, but further treatment is required to ensure the water is safe for consumption, or environmentally safe. Therefore, more attention will need to be paid towards removing contaminants in order to achieve metal ion concentration and microorganism levels that comply with Health Canada and WHO standards.

1.6 Spent Coffee Grounds in the Agricultural Industry

One of the most widely consumed beverages is coffee, and as a result the coffee industry is responsible for plenty of organic waste in the form of spent coffee grounds. It is estimated that approximate one million tons of coffee grounds are produced each year [53]. Spent coffee grounds are commonly disposed of in the landfill, which results in the production of greenhouse gases [54]. Valorization of spent coffee grounds would help reduce the amount of waste that ends up in the landfill [55]. In addition to greenhouse gas production, the caffeine in spent coffee grounds has been discovered to be toxic to aquatic life [56]. In wastewater treatment effluents, caffeine concentrations of 0.03-9.5 μg/L were measured [56]. Additionally, caffeine and phenolics have been determined to be toxic to animals and plants [57]. The toxicity of caffeine to animals and plants has limited the use of spent coffee grounds in the agricultural industry. However, the adsorbent properties and porous nature of coffee grounds have made them a viable option for wastewater treatment as it has shown promise for the removal of dyes and heavy metals [58].

Coffee grounds have seen numerous uses in a variety of industries due to the popularity of coffee as a beverage. Spent coffee grounds have been experimented with in the energy industry as a fuel source for boilers [53]. Additionally, the oil in coffee grounds has been processed and converted into biodiesel [59]. Coffee grounds have been commonly used as a fertilizer in domestic gardens as they exhibit higher concentrations of N and K than other natural waste products (such as animal manure) [53]. The increased N and K content provided by coffee grounds acts to improve soil fertility and water retention, therefore acting as a viable option for a natural waste fertilizer [60]. In addition to the previous uses for spent coffee grounds, it has shown promise in wastewater treatment as it can effectively remove lead ions via adsorption [55]. It was determined that 0.6 mg of lead ions can be adsorbed per gram of spent coffee grounds [55].

Chapter 2 – Project Design and Hypotheses

Current wastewater treatment strategies implement multiple stages in order adequately remove all contaminants from solution. Spent coffee grounds are abundant as a natural waste material and contain many hydroxyl functional groups allowing for modification of the surface through chemical reactions. Through appropriate valorization of spent coffee grounds, a system can be created that is capable of treating wastewater in a single step. When designing the system, several additional factors were considered to improve the efficiency and uniqueness of the project. These factors include: i) incorporating antibiotic and microplastic removal into the system, ii) improving the ion removal capabilities for better decontamination, iii) incorporating bacterial inactivation, iv) determine a way to easily collect loose spent coffee grounds in a tank of water, and v) determine an alternative use for the nutrient-packed coffee grounds after wastewater treatment. Figure 1 shows a schematic for the proposed concept and all the expected outcomes.

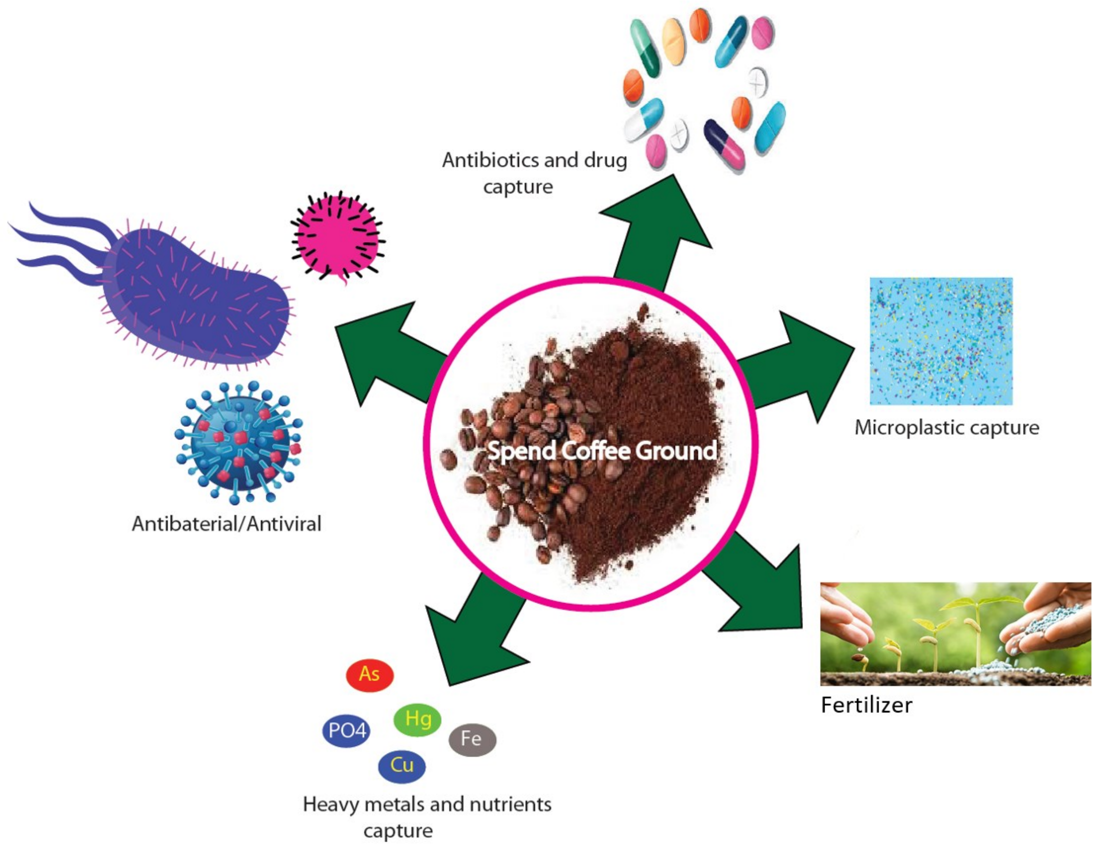


Figure 1. Illustration demonstrating the targeted outcomes for the proposed system [61].

Spent coffee grounds on their own have limited use for ion removal from solution. To improve the ion removal from solutions, it is proposed to functionalize the surface of the spent coffee grounds using polyethyleneimine (PEI). PEI has plenty of amino groups, which are used for highly efficient heavy metal ion removal (Figure 2a) [62]. Spent coffee grounds do not inherently have antibacterial properties, and therefore to reduce the presence of pathogenic microorganisms, modification to the spent coffee grounds must be done. The use of glycidal trimethylammonim chloride (GMAC) on other natural materials has been investigated and it has been seen to have an inhibitory effect on several microorganisms (Figure 2b) [63]. Some studies propose that GMAC has antimicrobial effects due to the quartenary ammonium salt being able to penetrate the wall of the microbes [64]. Figure 2 shows the chemical reaction schemes for PEI and GMAC with the spent coffee grounds.

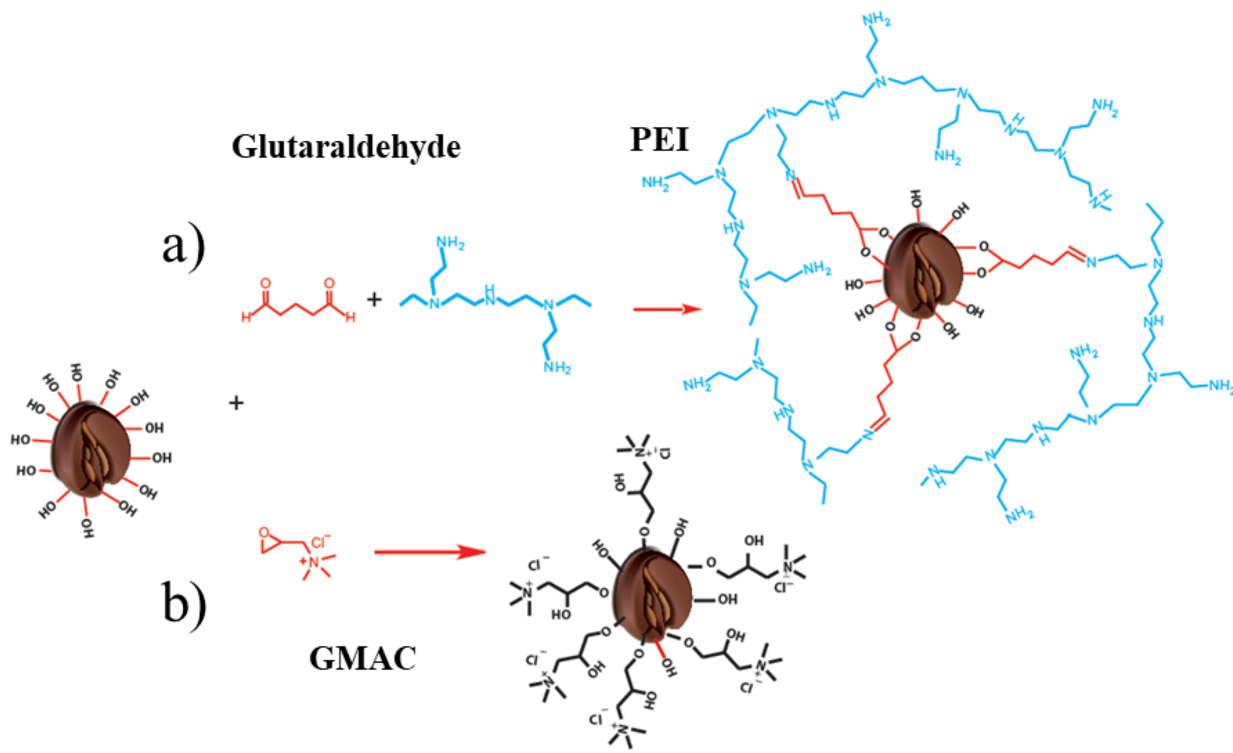


Figure 2. Chemical reactions between spent coffee grounds and a) PEI and glutaraldehyde and b) GMAC

Due to the porous nature of the spent coffee grounds, it should be able to capture microplastic particles suspended in solution. The pores increase the available surface area for adsorption, and as a result it makes the coffee grounds a viable option for a one-step wastewater treatment process. For the one-step wastewater treatment using GMAC and PEI functionalized spent coffee grounds, the coffee grounds can be added into a large tank containing wastewater. In order to easily collect the spent coffee grounds, iron nanoparticles can be functionalized onto the surface of the coffee grounds. This would allow the spent coffee grounds to be collected with the use of a magnetic field as shown in Figure 3.

Figure 3. Schematic of the spent coffee grounds being added to contaminated water, and then removed using a magnetic field.

After ion capture and bacterial inactivation, the collected coffee grounds will have nutrients captured on the surface. Farm wastewater has been known to contain Mg, N, and P which commonly forms a mineral known as struvite. Furthermore, struvite is a well-known slow release fertilizer and contains nutrients required for plant growth. Therefore, a byproduct of using spent coffee grounds to capture ions in farm wastewater is that it can be repurposed as a slow-release fertilizer after being collected with a magnetic field. Figure 4 shows the reaction on the spent coffee grounds that would result in the formation of struvite.

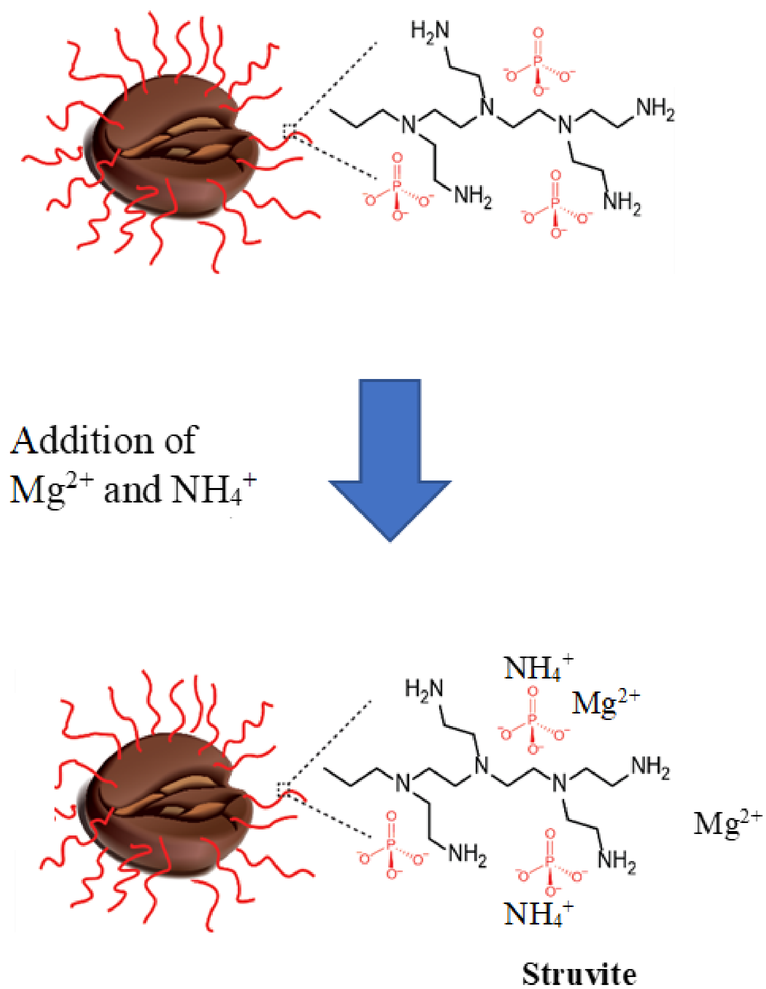


Figure 4. Basic reaction schematic showing the formation of struvite on the surface of spent coffee grounds.

The main hypothesis of this study is that using spent coffee grounds, a single-step wastewater treatment process can be made. This process will act as an eco-friendly alternative, as well as produce a byproduct that will be used as a slow-release fertilizer to improve crop yield.

2.1 Project Objectives

1) Develop magnetic coffee grounds functionalized with PEI and test its performance to remove ionic contaminants from contaminated water.
2) Develop magnetic coffee grounds functionalized with GMAC and test the bacterial inactivation performance.
3) Find conditions that enable struvite growth on the spent coffee grounds.
4) Test the release rate of nutrients from the struvite grown on the functionalized spent coffee grounds.

Chapter 3 – Materials and Methodology

3.1 Washing and Bleaching

Spent coffee grounds were collected from local coffee shops. Large and small coffee particles were separated during initial washing stages through filtering. All small particles were discarded and the large particles were collected. Next, the coffee was bleached to remove organic contaminants. To bleach the spent coffee grounds, 400 g of coffee grounds were added to a 4 L beaker. Afterwards, 3 L of water was added alongside 2 tablets of Bru-Clean TbC (Brulin Holding, Indianapolis, IN, USA). Everything was mixed using a magnetic stir bar. After 1 hour, all of the water was replaced and 2 new tablets of Bru-Clean TbC (Brulin Holding, Indianapolis, IN, USA) were added to the beaker. The was done 10 times in total, and the final product was washed 10 times and then left to fry in a Binder Series BF Avantgarde oven (Binder, Tuttlingen, Germany) at 45°C for 24 hours.

3.2 Spent Coffee Grounds Functionalization

3.2.1 Functionalization of Spent Coffee Grounds with Iron Nanoparticles

Iron nanoparticles are functionalized onto spent coffee grounds in order to give the coffee grounds magnetic properties. This allows for easy collection of the waste coffee through the use of an electromagnetic field. To functionalize spent coffee grounds with iron nanoparticles, 250 g of bleached spent coffee grounds were added to a round bottom flask alongside 200 mL of DI water. This was mixed using a magnetic stir bar. Next, nitrogen was bubbled into the flask for 20 minutes. After the nitrogen was bubbled in, 40.75 mmol of iron

(II) chloride tetrahydrate (Sigma-Aldrich, St. Louis, MO, USA) and 74 mmol of iron (III) chloride (Sigma-Aldich. St. Louis, MO, USA) was added to the round bottom flask. The pH of the mixture was increased to be between 9 and 12 through the addition of 60 mL of 28-30% aqueous ammonium hydroxide (Fisher Scientific, Ottawa, ON, Canada) solution. The round bottom flask was heated to 90°C in an oil bath and left stirring overnight. A rubber stopper was placed on the round bottom flask to prevent the ammonia from escaping. The final product was collected by vacuum filtering and was washed 7 times. After washing, the final product was place in a Binder Series BF Avantgarde oven (Binder, Tuttlingen, Germany) at 45°C for 24 hours to dry.

3.2.2 Functionalization of Spent Coffee Grounds with Polyethyleneimine

Polyethyleneimine (PEI) can successfully remove many contaminants from water. Therefore, PEI on spent coffee grounds can offer a low-cost solution for water purification. To functionalize spent coffee grounds with PEI, 250 g of bleached coffee grounds was mixed with 200 mL of DI water in a round bottom flask. This was mixed using a magnetic stir bar. Next, 25 g of PEI, branched, MW 70,000, 30% w/v (Polysciences, Warrington, PA, USA) was added to the round bottom flask alongside 6 g of glutaraldehyde (Sigma-Aldrich, St. Louis, MO, USA). All of the contents are heated using an oil bath to a temperature of 60°C. This is stirred constantly for 5 hours. Afterwards, the final product is washed with DI water 7 times and then dried in a Binder Series BF Avantgarde oven (Binder, Tuttlingen, Germany) at 45°C for 24 hours.

3.2.3 Functionalization of Spent Coffee Grounds with Glycidyl Trimethylammonium Chloride

Spent coffee grounds were functionalized with glycidyl trymethylammonium chloride (GMAC). To functionalize the spent coffee grounds with GMAC, 250 g of bleached coffee grounds were added to 200 mL of DI water in a round bottom flask. This was mixed using a magnetic stir bar. Air was removed from the pores of the bleached coffee grounds with 3 cycles of degassing in a vacuum chamber. Next, 20 mL of GMAC (Aldrich, St. Louis, MO, USA) was added to the flask. Using an oil bath, the flask was heated to 50°C. After heating, 0.5 mL of 1% acetic acid (Sigma-Aldrich, St. Louis, MO, USA) was added as a catalyst. This flask was left overnight. The final product was washed 7 times with DI water and left in a Binder Series BF Avantgarde oven (Binder, Tuttlingen, Germany) at 45°C for 24 hours to dry.

3.3 Struvite Growth on Functionalized Spent Coffee Grounds

Struvite is the mineral name for $NH_4MgPO_4 \cdot 6H_2O$. The formation of struvite crystals can be seen with the addition of NH_4^+, Mg^+, and PO_4^{3-} in a 1:1:1 mole ratio. Magnesium chloride (Sigma, St. Louis, MO, USA), ammonium chloride (Acros Organics, New Jersey, USA), and potassium phosphate monobasic (Sigma-Aldrich, St. Louis, MO, USA) were added to 300 mL of DI water until there was a concentration of 0.1 M for each of the previously mentioned ions. After the solution was made, 100 g of PEI functionalized spent coffee grounds were added to the solution. This was stirred using a magnetic stir bar. As the contents are stirring, 10 mL of 28-30% ammonium hydroxide solution (Fisher Scientific, Ottawa, ON, Canada) is slowly added. This was adjust the pH of the solution to be between 8 and 10, which are the favourable conditions for struvite growth. The mixture is lefting stirring for 4 days.

Afterwards, the final product is collected and washed 3 times using DI water. It is then placed in a Binder Series BF Avantgarde oven (Binder, Tuttlingen, Germany) at 45°C for 24 hours to dry.

3.4 Scanning Electron Microscopy

The surface morphology of the spent coffee grounds were characterized using scanning electron microscopy (SEM; FESEM S4800, Hitachi, Japan). Samples were prepared by placing the spent coffee grounds on double-sided carbon tape, and attaching the tape to an aluminum stub. The sample was then coated with an 8 nm layer of gold using the Denton Desk II (Denton Vacuum, Moorestown, NJ, USA), this was done to improve the conductivity in order to be properly imaged using an SEM. All images were taken as 15 kV (20 μA).

3.5 X-Ray Diffraction

The growth of struvite on the spent coffee was analyzed using X-Ray Diffraction (XRD; Rigaku Ultima IV, ON, Canada). By comparing the XRD spectra obtained with the known spectra for struvite, the formation of struvite on spent coffee can be identified. To collect the XRD spectra for the struvite grown on spent coffee, the struvite crystals were separated from the spent coffee. This is due to the amorphous nature of spent coffee grounds. The collected struvite crystals were compacted into the appropriate powder sample holder which was then placed into the ASC-10 stage of the XRD machine. The starting and ending scan angle were 15° and 50°, respectively. To obtain the XRD spectra, the Rigaku Ultima IV uses CuKα x-rays. The x-ray tube was operated at 40 kV and 44 mA.

3.6 Fourier Transform Infrared Spectroscopy

The growth of struvite was analyzed using Fourier Transform Infrared Spectroscopy (FTIR). To analyze struvite growth of spent coffee grounds, three different concentrations of NH_4^+, Mg^+, and PO_4^{3-} (0.01 M, 0.05M, and 0.1 M) were selected. FTIR spectroscopy was performed using a Nicolet 8700 (Thermo Fisher Scientific, Waltham, MA, USA). All scans were done in attenuated total reflection mode. The spectra were collected between 400 and 4000 cm^{-1}. Each spectra collected was done using 100 scans.

3.7 Microplastic Removal Experiment

In order to test microplastic removal of spent coffee grounds, plastic particles were created using a metal file on polyethylene (Polymer Shapes, Edmonton, AB, Canada), polypropylene (Polymer Shapes, Edmonton, AB, Canada), and polyethylene teraphthalate (Polymer Shapes, Edmonton, AB, Canada) sheets. The obtained particles were filtered and screened in order to remove any particles larger than 1 mm. Microplastic dispersions were prepared with 4 different concentrations of microplastics present (50, 25, 15, and 5 μg/mL). Next, 200 mL of 1 μg/mL Nile red (Sigma-Aldrich, St. Louis, MO, USA) dye in methanol (Sigma-Aldrich, St. Louis, MO, USA) was added to the dispersions and stirred for 30 minutes. Absorbance was measured using a Bio Rad iMark Microplate spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA) at a wavelength of 630 nm both before and after the addition of the spent coffee grounds. A computer using the Microplate Manager Software was used to measure the optical density and correct the pathlength to adjust for 150 µL volume in each well in the 96-well plate.

3.8 Bacterial Inactivation

Bacterial inactivation experiments were performed on 3 different bacterial strains: *Salmonella*, *E. Coli*, and *Campylobacter*. To prepare the *Salmonella* and *E. Coli* samples, the bacterial strains were inoculated on an agar plate overnight at 37 °C. A single colony was incubated at 37 °C overnight in 3 mL of MH broth (Becton Dickinsin and Company, Sparks, MD, USA) while be shaken at 200 rpm. The culture broth was diluted in a fresh broth medium and further incubated until the mid-log phase (3~4 hours) at 37 °C while be shaken at 200 rpm. The bacterial culture broth was harvested and washed twice with DI water in an Eppendorf Centrifuge 5810 R (Eppendorf, Hamburg, Germany) operating at 14,000 rpm or 5 minutes. The washed bacterial samples were adjusted to an optical density of 600 to 0.001 in DI water (10 mL total volume).

The Campylobacter samples were prepared by inoculating the bacterial strains on an agar plate at 42 °C in microaerobic condtions (5% O_2 and 10% CO_2). The bacteria colonies were scraped using a spreader, and placed in 1 mL of fresh MH broth (Becton Dickinsin and Company, Sparks, MD, USA). This was then transferred to a 1.5 mL tube. The bacteria strain was then washed twice with DI water in a centrifuge operating at 14,000 rpm for 5 minutes. The washed bacterial samples were then adjusted to an optical density of 600 to 0.001 in DI water (10 mL total volume).

To test the pathogen inactivation of the coffee samples, 500 μg of the spent coffee grounds were placed in 2 mL tubes. After adding the spent coffee ground, 1 mL of the diluted bacterial samples was added to the tube. This was done for each of the bacterial strains tested. These samples were incubated for 15, 30, 45, 60, 75, and 90 minutes at room temperature while be rotated. At each time point, 100 μL of the bacterial sample was taken from the tubes. The 100 μL of each bacterial sample taken was then serially diluted 10 fold with DI water and spotted on MH agar for CFU counting.

3.9 Antibiotic Removal

Antibiotic removal was determined using High Performance Liquid Chromatography (HPLC; Agilent 1100 series, Santa Clara, CA, USA). HPLC was used to determine the concentration of antibiotics removed by measuring the amount of antibiotic remaining over time and seeing the portion that is removed. This was done by combining 50 μg/mL of each antibiotic and 10 mg of spent coffee grounds. After 2, 6, and 10 hours the solution was extracted and ready to have the antibiotic concentration measured. The HPLC (Agilent 1100 series, Santa Clara, CA, USA) was pretreated using 30 mL of 2% sodium chloride (Sigma-Aldrich, St. Louis, MO, USA). The effluent used consisted of 35% DI water, 60% acetonitrile (Sigma-Aldrich, St. Louis, MO, USA), and 5% 0.2 M phosphate buffer.

3.10 Phosphate Release Test

The total phosphate released from the struvite grown on spent coffee grounds can be determined by placing a known quantity of the product in DI water. To do this, 20 mg of struvite on spent coffee grounds was placed in a 50 mL tube. After, 10 mL of a solution of known pH (5.5, 6.5, and 8) was added to the tube. For each pH condition, samples were collected after 2 hours, 4 hours, 8 hours, 1 day, 2 days, 4 days, 1 week, 2 weeks, 1 month, and 2 months. For each time, a different tube was used and all the solution was collected.

To measure the phosphate of the samples, 1 mL of the previously collected solution was placed in a 25 mL Erlenmeyer flask with 9 mL of DI water. Next, 200 µL of 11N H_2SO_4 and 200 µL of a 0.4 g/mL ammonium persulfate solution was added to each flask. Each flask was gently boiled using a hot plate for 30 minutes. After boiling, the samples were diluted to 10 mL. In each sample, 800 µL of ammonium molybdate-antimony potassium tartrate and 400 µL of ascorbic acid was added. The samples were mixed for 10 minutes, then 150 µL of each sample was placed in a 96 well plate. The optical density was measured at 595 nm in a Bio Rad iMark Microplate spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA). The optical density was processed using a computer with the Microplate Manager Software. This software was also used to correct the pathlength to account for the 150 µL volume in each well.

3.11 Experimental Plan for Tomato Plant Growth

To test the struvite effectiveness as a fertilizer on functionalized spent coffee grounds, it will be used to grow tomato plants. To do this, the tomato seeds will be germinated in topsoil. After the seedlings emerge, they will each be transferred to a 1L pot (one plant per pot). Table 2 shows a summary of how the experiment will be run using different portions of fertilizer.

Table 2: Summary of the experimental setup for comparing the effectiveness of struvite as a fertilizer compared to no fertilizer, spent coffee grounds, and a mixture of organic manure with coffee grounds.

Treatment	**Coffee ground % added to the pots**		**Number of pots**
Control (just topsoil)	0		30
Coffee ground mixed with topsoil	1%		30
	5%		30
	10%		30
Coffee ground plus organic manure mixed with topsoil	Coffee ground	Organic manure	30
	0%	1%	
		5%	30
		10%	30
	1%	0%	30
		1%	30
		5%	30
		10%	30
	5%	0%	30
		1%	30
		5%	30
		10%	30
	10%	0%	30
		1%	30
		5%	30
		10%	30
Our fertilizer for each species mixed with topsoil	0%		30
	1%		30
	5%		30
	10%		30

During plant growth, various physical parameters will be measured to assess the effectiveness of our system as a fertilizer. In 2 week intervals, the height and diameter of the plant will be measured. This will show how the fertilizer impacts plant growth, and compare it with the impact spent coffee grounds has on overall plant growth. The timing of fruit ripening will be recorded, alongside the number of fruits per plant and the weight of each fruit after harvesting.

Chapter 4 – Results and Discussion

4.1. Characterization of Functionalized Spent Coffee Grounds

After functionalizing the spent coffee grounds, the surface morphology was investigated. This includes how the morphology of the functionalized surface compares to that of the raw spent coffee grounds. The porous nature of the spent coffee grounds allows it to have a higher surface area than regular particles. Ensuring the porosity remains intact allows for better efficiency during ion/antibiotic removal, improves the available surface area for bacteria inactivation, as well as improves the available surface area for struvite to form allowing for more nutrients to be carried by a single particle. In addition, the porous nature of the spent coffee grounds increases the available adsorption sites which aids in it having a high adsorption capacity. In Figure 5 below, the morphology of raw spent coffee grounds can be seen at various magnifications. This shows and confirms the porous surface given by the spent coffee grounds and means that it is a suitable natural material for water purification.

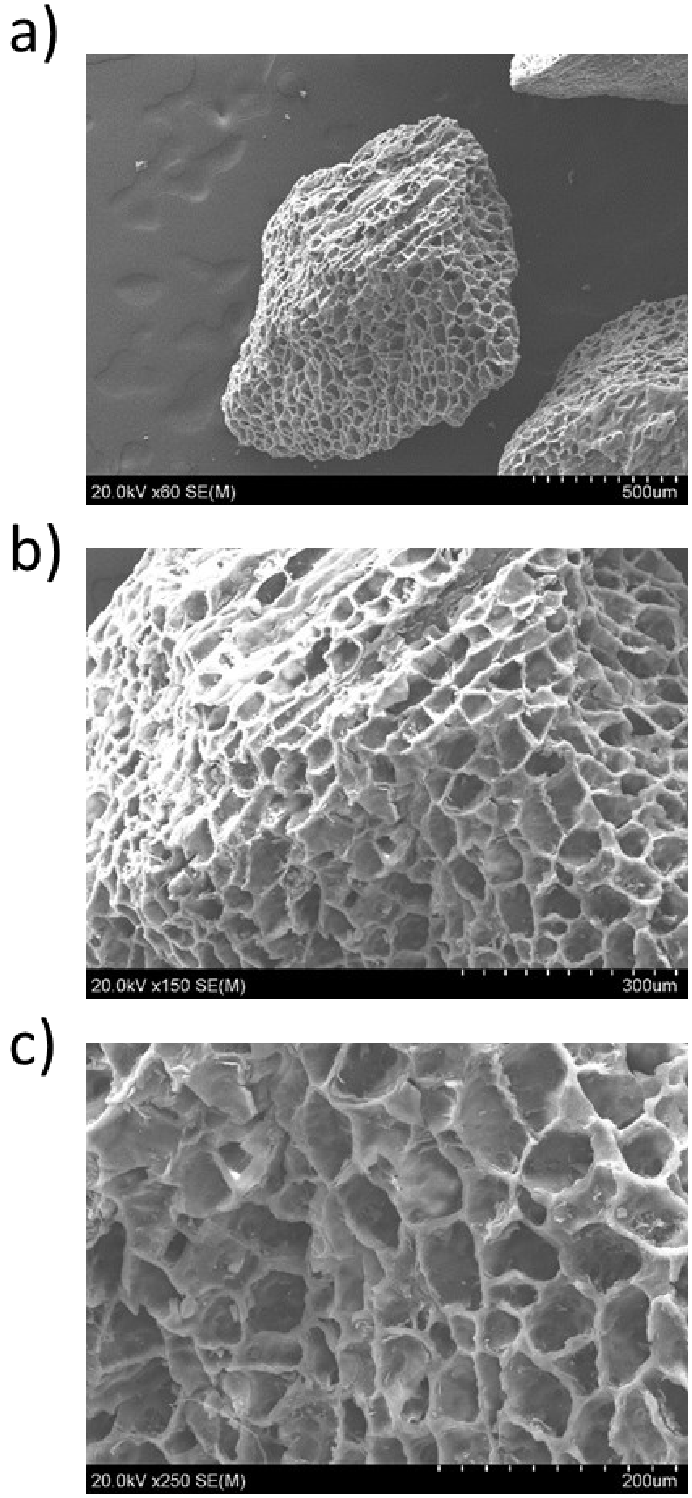
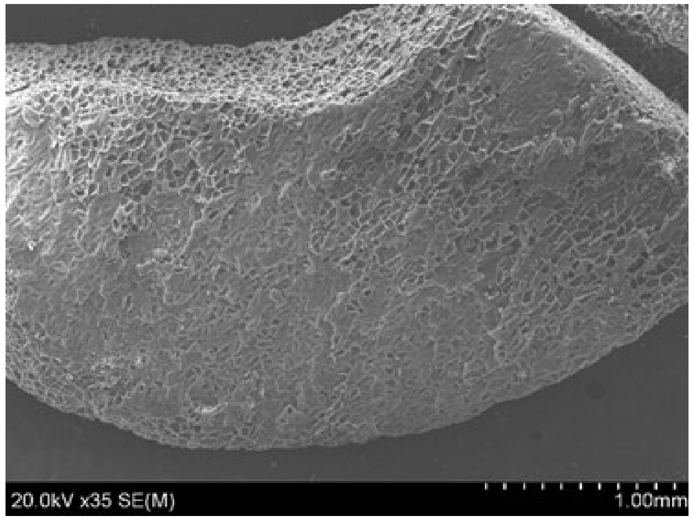


Figure 5. SEM images at 3 different magnifications to show the surface morphology and porous nature of the raw spent coffee grounds. a) low magnification, b) medium magnification , and c) high magnification.

In the SEM images of the raw spent coffee grounds, the pores on the surface can be easily identified. After functionalizing the surface with PEI and iron nanoparticles, the surface morphology is expected to change. In Figure 6, the surface of the spent coffee grounds after functionalization can be seen. This shows that the surface morphology has changed, but pores and a rough surface is still present. After functionalization, the high surface area of the spent coffee grounds is still present which allows it to maintain its high adsorption capacity. Although the morphology was expected to exhibit minor changes after functionalization, it was expected that the spent coffee grounds would retain their porous nature. This is what was seen, the pores were still present which enables to spent coffee grounds to have their high adsorption capacity.

a)



b)

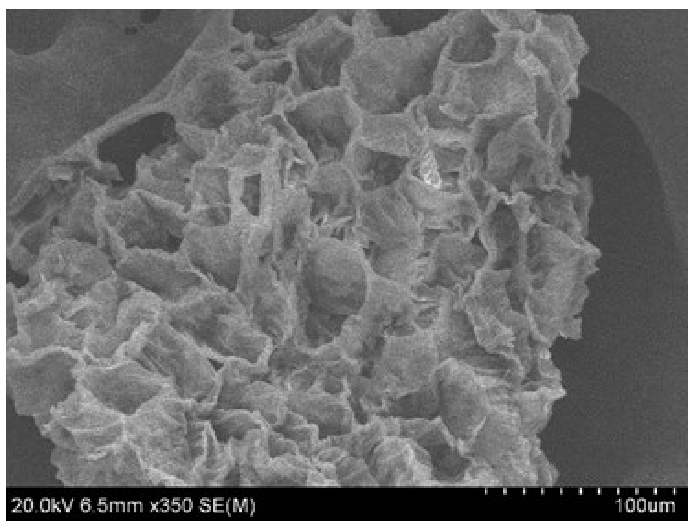


Figure 6. SEM images showing the surface morphology changes after functionalizing the surface with PEI and iron nanoparticles. a) Low magnification and b) high magnification.

After functionalization, and confirming the porous morphology was left intact, the next step was to perform EDX mapping to ensure the iron nanoparticles were sufficiently adhered to the surface. Chemical mapping shows the spent coffee grounds, and what chemicals can be identified along the surface. This clearly shows that spent coffee grounds are primarily composed of N, O, and C. In addition, a high concentration of Fe can be seen. This means the attachment of iron nanoparticles was successful, and they can be seen covering the surface of the spent coffee grounds. It was important to ensure the iron nanoparticles were appropriately attached to the spent coffee grounds, as this is what gives the magnetic properties. The magnetic properties are how the spent coffee grounds will be collected out of the tank of water after purification.

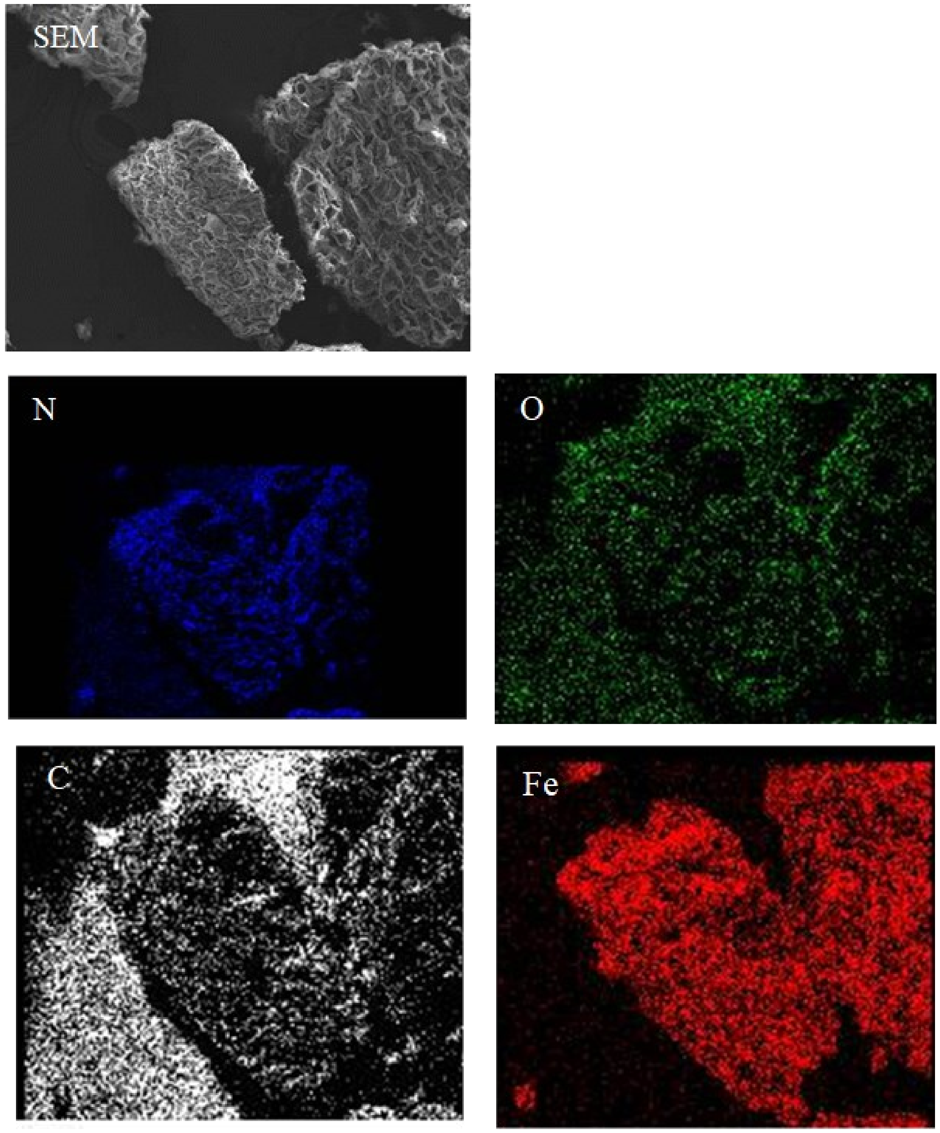


Figure 7. EDX chemical analysis to show the distribution of N, O, C, and Fe on the surface of the functionalized coffee grounds.

After EDX chemical analysis, it can be seen that Fe is distributed homogeneously across the surface of the spent coffee ground. This confirms that the iron nanoparticles have attached to the surface, thus allowing the spent coffee grounds to be collected with an electromagnetic field. The idea is to give magnetic properties to the spent coffee grounds. This allows the functionalized spent coffee grounds to be combined with contaminated water, and then later separated using a magnetic field. Figure 8 below shows a demonstration of how the coffee grounds can be collected with a magnet.

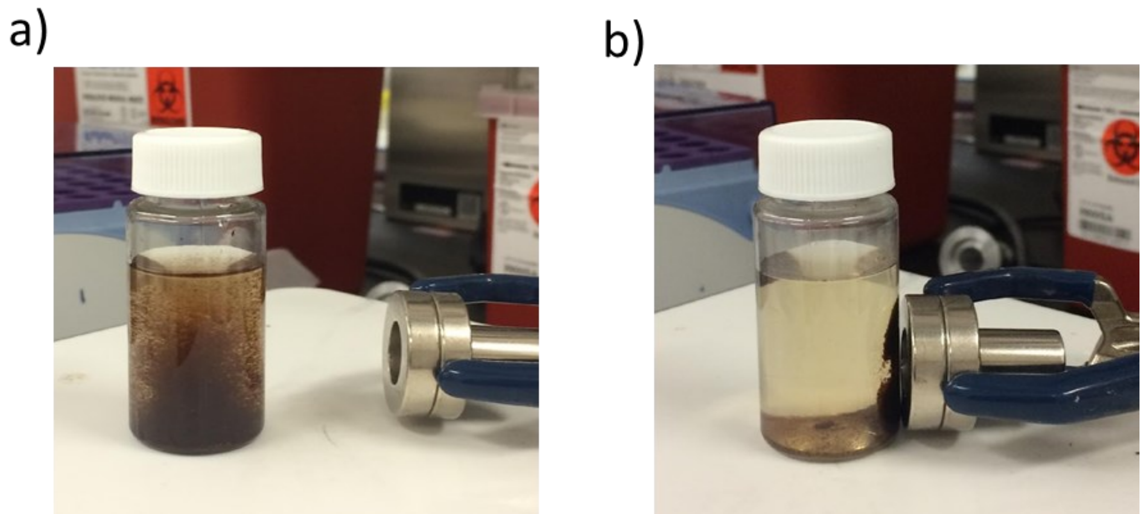


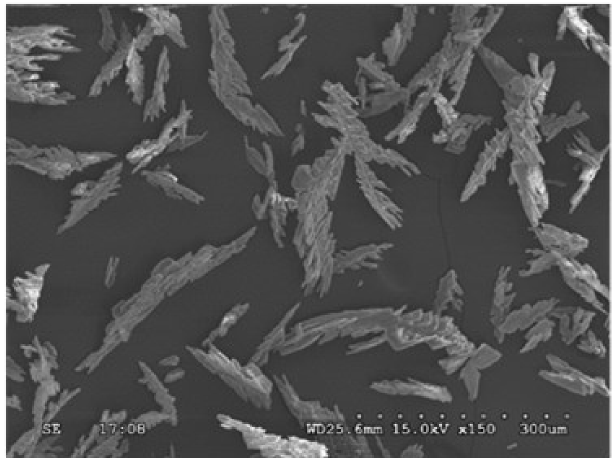
Figure 8. Demonstration of how the coffee grounds can be collected in the presence of an electromagnetic field. a) coffee grounds dispersed in solution without magnetic field, and b) with application of magnetic field.

In the above figure, the left image shows the spent coffee grounds suspended in water. This was done by gently shaking the vial containing the functionalized spent coffee grounds. After placing a magnet adjacent to the vial, it can be noted that all the suspended particles were attracted towards the magnet. This acts as proof of concept that after functionalization with iron nanoparticles the spent coffee grounds can be easily gathered. After being gathered, the spent coffee grounds can easily be separated using basic filtration. This allows for the system to be added to stationary contaminated water, such as a tank of water or a lake. After purification, the spent coffee grounds can be easily removed leaving treated water behind.

4.2. Characterization of Struvite Grown on Functionalized Spent Coffee Grounds

The spent coffee grounds functionalized with PEI were added to a solution containing magnesium chloride, ammonium chloride, and potassium phosphate monobasic. These compounds are naturally present in farm wastewater and have been known to form struvite. After the reaction, the first thing to do is confirm the compound that formed was struvite. Some on the crystals were separated from the spent coffee grounds for identification purposes. SEM images were taken of the formed crystals to visually identify what they look like. Figure 9 below shows SEM images of the crystals.

a)



b)

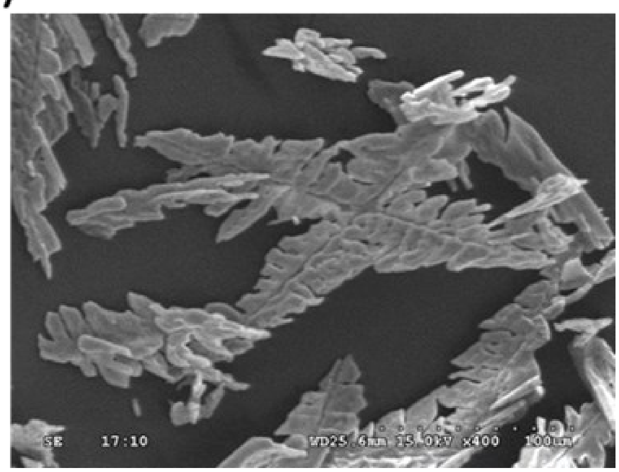


Figure 9. SEM images of the crystal after separating it from the spent coffee grounds. a) Low magnification image of the crystals, and b) high magnification image.

With the crystals separated, the exact crystallographic planes and existing bonds can be determined which will more accurately prove that struvite formed. By identifying XRD and FTIR peaks and comparing with known peaks, this will conclusively identify the compound that formed. Figure 10 shows the comparison between the obtained XRD spectra for the formed crystals and struvite.

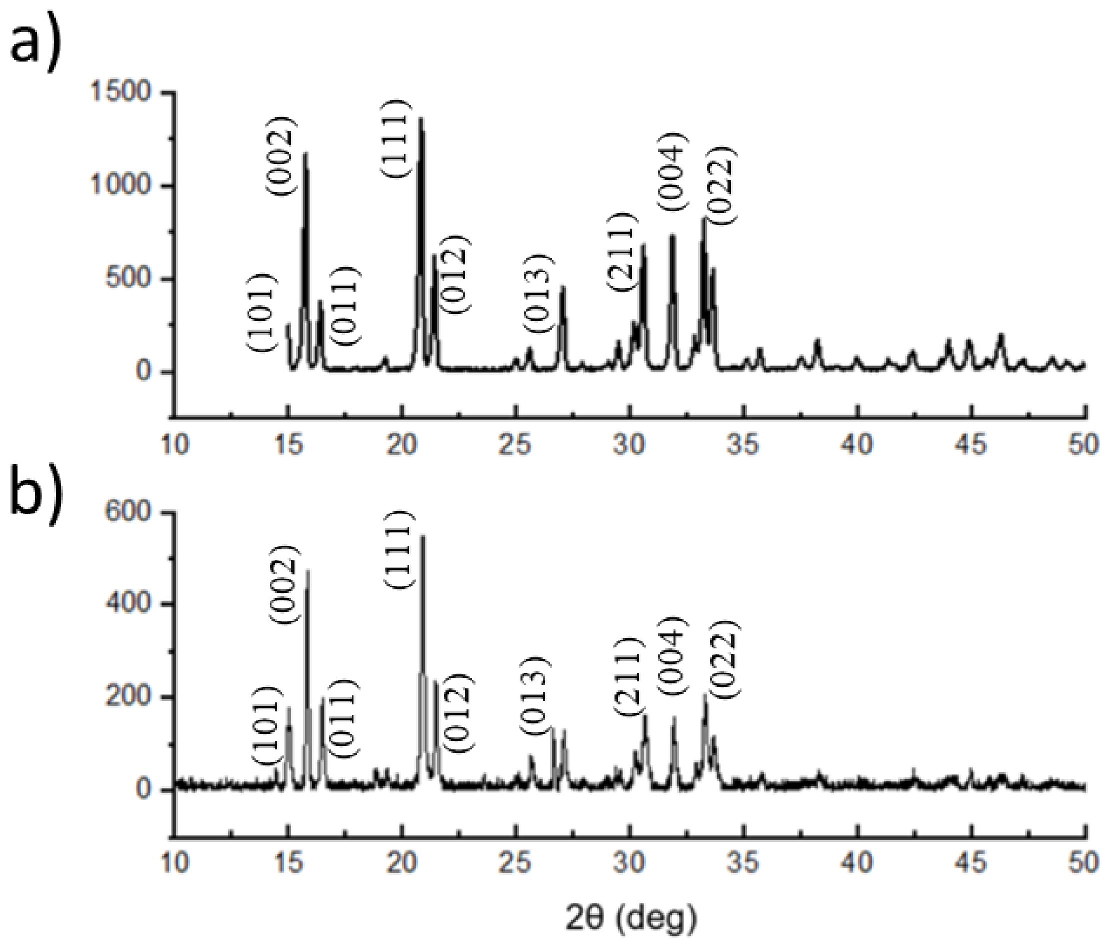


Figure 10. XRD spectra obtained from the crystals separated from the spent coffee. a) Spectra obtained from the grown crystals and b) reference spectra for struvite.

It can be noted that both the sample and the reference spectra contain the same peaks. This is a strongly leads to the conclusion that struvite was the compound that formed, as both of the materials have the same crystallographic planes present. While acquiring the XRD spectra of the sample, the crystals had to be separated from the spent coffee due to the amorphous peak that was present due to the coffee grounds. Due to this, the measured peaks were only the crystals after separation from the coffee grounds, otherwise no notable peaks could be discerned. In addition to XRD, FTIR spectroscopy was performed in order to further confirm the formation of struvite. Figure 11 below shows the obtained FTIR peaks for the struvite grown on functionalized spent coffee grounds.

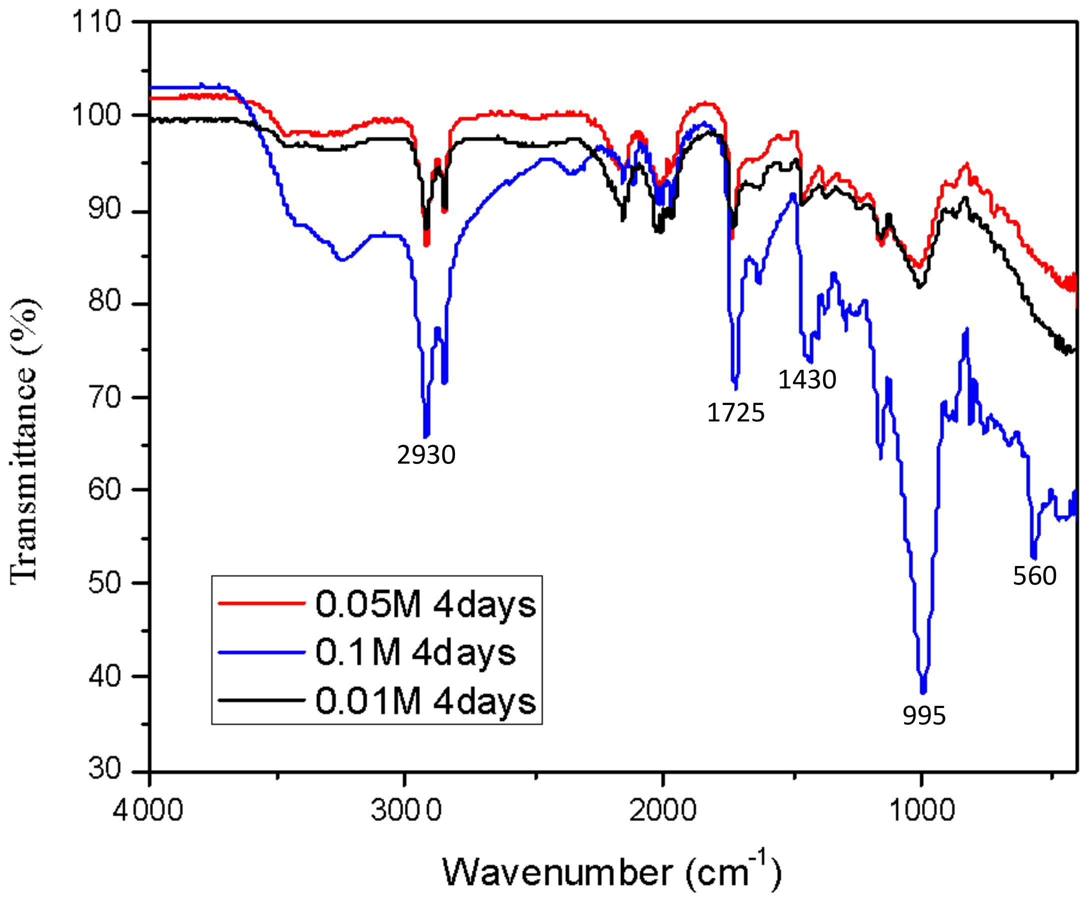


Figure 11. FTIR spectra obtained for struvite grown on PEI functionalized spent coffee grounds. The samples were collected after 4 days of reacting in the solution containing 1:1:1 of NH_4^+, Mg_2^+, and PO_4^{3-}.

Based on a literature search of others who examined the FTIR spectra of struvite, it appears that the small peak seen around 500 cm^{-1} is due to Mg-O bonding [65]. D. Sidorczuk et al. found that the prominent peak that can be observed at 1000 cm^{-1} is a result of PO_4^{3-}, whereas the peaks at 1625 and 1400 cm^{-1} correspond to H-N-H bonding [65]. All of these peaks show the presence of magnesium, ammonium, and phosphate which are the main components that make up struvite. Therefore, the FTIR spectroscopy results suggest the possibility of the formation of struvite. The data obtained from FTIR spectroscopy and XRD both suggest the formation of struvite. It can be safely concluded that since both methods point to the same conclusion, then struvite was likely formed as expected.

4.3. Water Treatment Using Spent Coffee Ground-based System

4.3.1. Inorganic Ion Removal

As a part of water treatment, contaminants in the form of unwanted ions must be removed from solution. Many regions have different policies regarding sufficient quality for potable and non-potable water. Being able to adequately reduce the ion concentration from solution is a very important step in water purification. In this work, a known starting concentration of various ions was placed in solution. The PEI functionalized spent coffee grounds was mixed with the solution, and the inorganic ions would adsorb onto the surface on the spent coffee. This would effectively remove the contaminants from solution. To test this, the initial concentration of each metal ion was set to be 100 ppm. This initial concentration was present in a 5 mL tube, and then 100 μg of PEI functionalized coffee was mixed in order to remove the contaminants in solution. By measuring the concentration at specific time points, the adsorption capacity for the metal ions can be determined. Adsorption capacity can be determined with $q = (C_0 - C_t)/m_q$. Where q is the amount of metal ion adsorbed per unit mass of the adsorbent at a given time t; C_0 is the initial concentration; C_t is the concentration at time t; and m_q is the mass of the adsorbent. Figure 12 below shows the results of investigating the adsorption capacity of the metal ions.

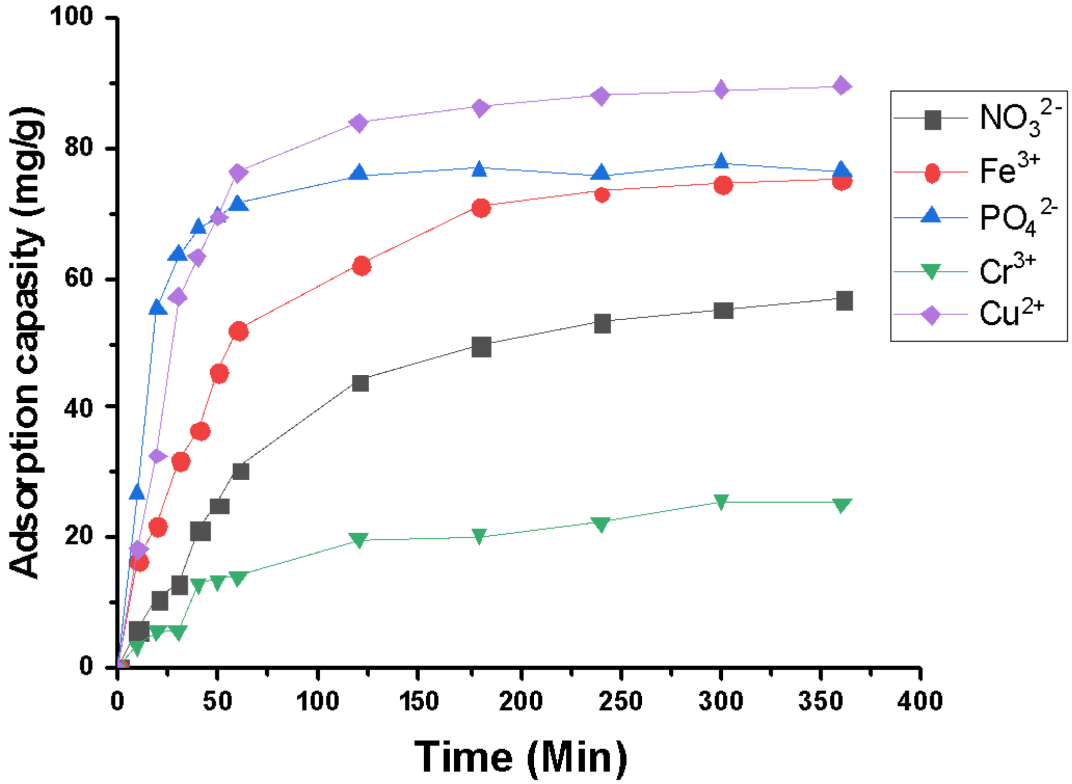


Figure 12. Ion adsorption capacity of PEI functionalized spent coffee grounds for NO_3^{2-}, Fe^{3+}, PO_4^{2-}, Cr_3^{+}, and Cu^{2+}.

Another way to view the ion removal due to the functionalized spent coffee is to determine the capture efficiency. Prior to treating with coffee, 100 ppm of each ion was present in the solution. After mixing with raw spent coffee grounds and PEI functionalized spent coffee grounds, the new concentration of each ion present in solution can be measured. With this concentration determined, the percentage of each ion removed from solution can be calculated. Figure 13 shows the reduction in ion concentration after treatment with raw spent coffee, as well as after contact with PEI functionalized spent coffee grounds. The main observation is that PEI functionalized spent coffee grounds are capable of removing over 90% of the ionic contaminants in solution. This is due to several factors: i) spent coffee ground have inherent adsorption properties, ii) spent coffee grounds have a high surface area due to their porosity which further increases their adsorption capabilities, and iii) PEI further improves the adsorption seen by spent coffee grounds.

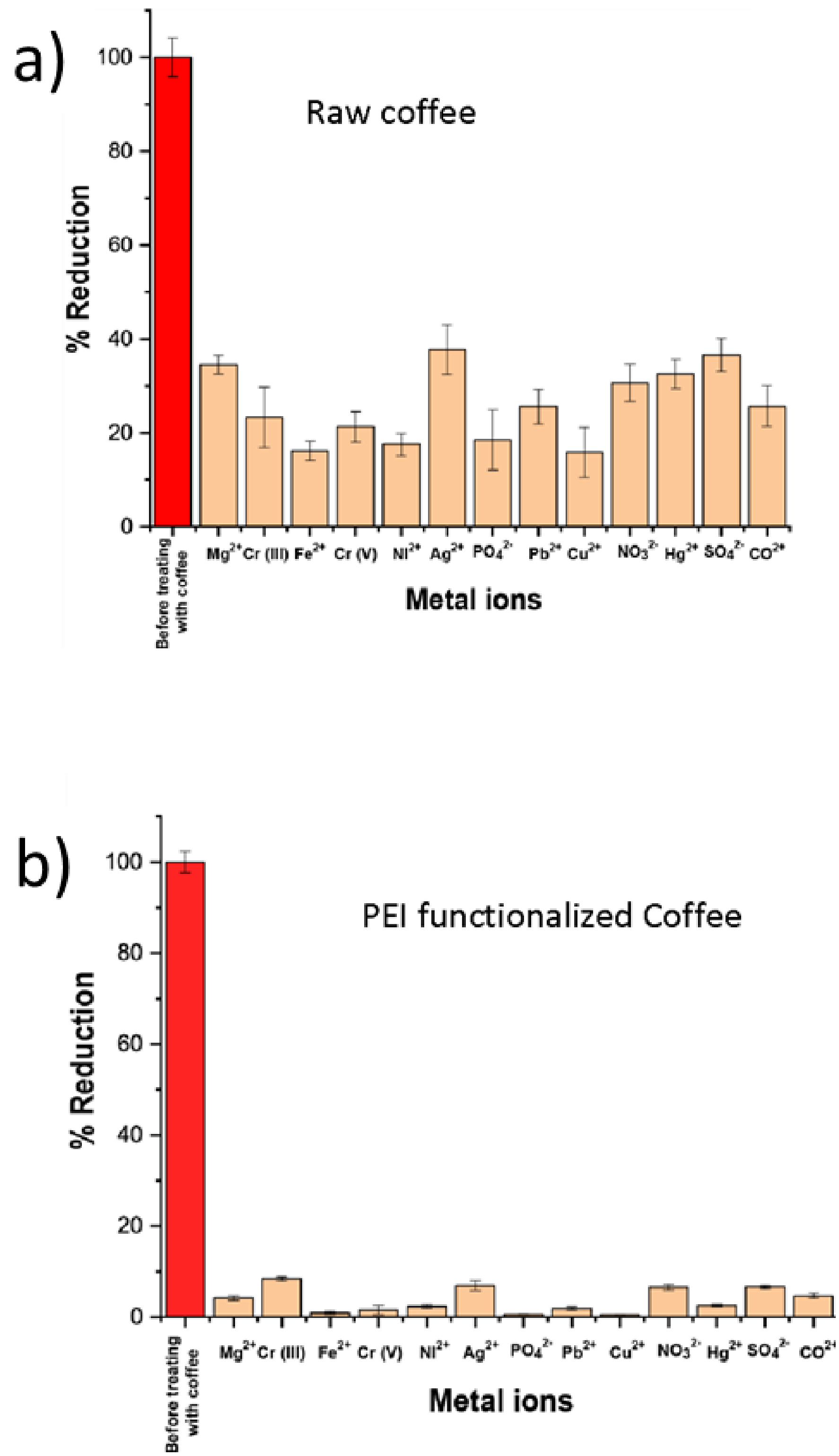


Figure 13. The comparison of inorganic ion capture efficiency of a) raw spent coffee grounds and b) PEI functionalized magnetic spent coffee grounds.

4.3.2. Antibiotic Removal

To test the efficacy of PEI functionalized spent coffee grounds for antibiotic removal, two different antibiotics were selected. The selected antibiotics were amoxicilline, and monensin. These two were selected due to their presence in water. In this study, the antibiotic removal was at ambient temperature in air. This experiment was done using 50 ppm of amoxicilline and monensin, which was added to the functionalized spent coffee gounds. The mixture was stirred using a rotating shaker at 100 rpm for various times. The supernatant was collected and measured with HPLC at a wavelength of 220 nm. Figure 14 below shows the obtained HPLC results showing the ability of PEI functionalized spent coffee grounds to remove antibiotics from solution.

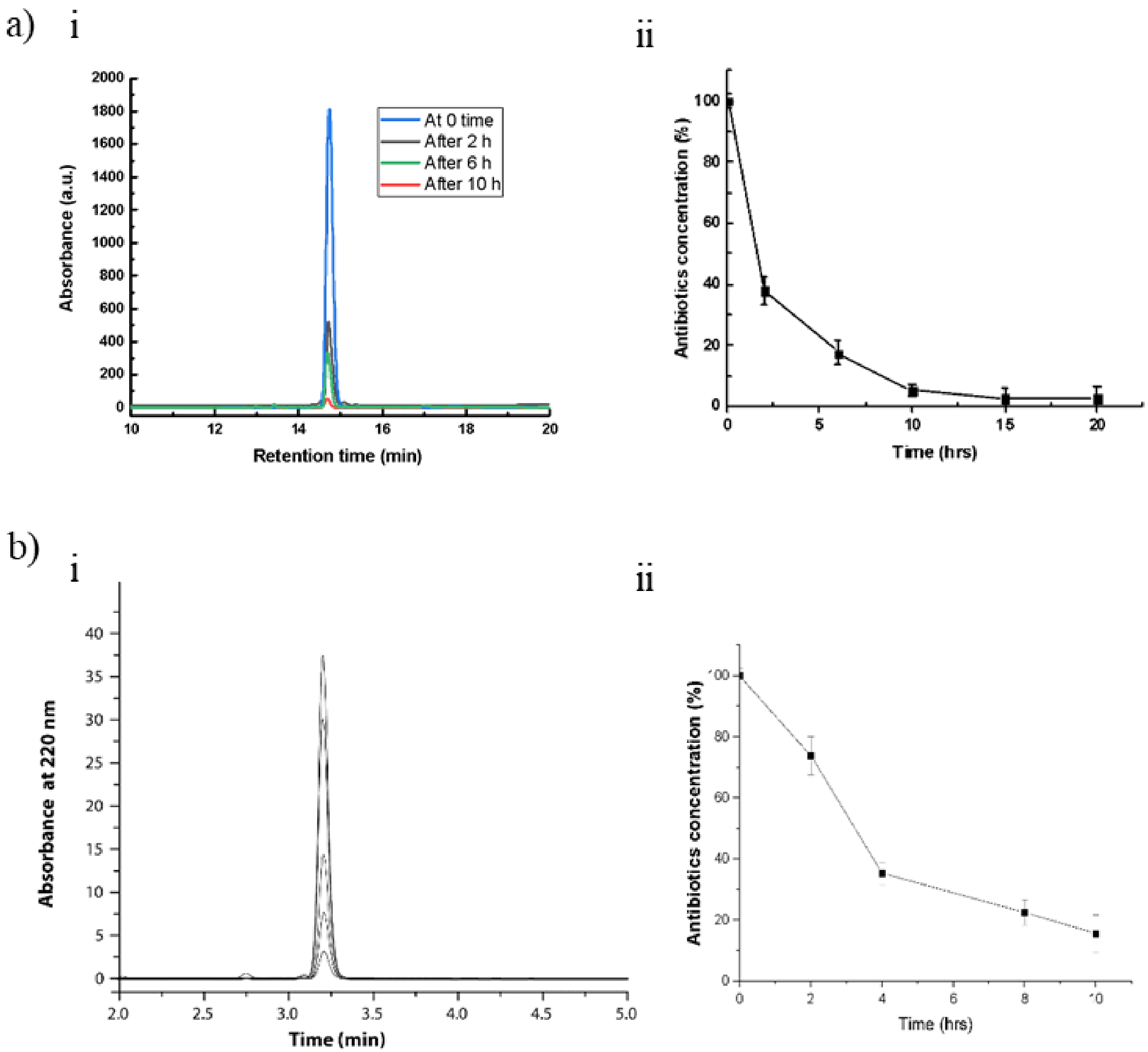


Figure 14. Antibiotic removal using HPLC for a) amoxicilline and b) monensin (i: absorbance spectra, ii: analysis).

Through HPLC analysis, it was determined that nearly all of the amoxicilline was removed after 20 hours of mixing. After 10 hours of mixing the monensin solution with PEI functionalized spent coffee grounds, 80% of the monensin was removed from the solution. This shows that any unwanted antibiotics that find their way into wastewater could effectively be removed using spent coffee grounds functionalized with PEI.

4.3.3. Bacterial Inactivation

Bacteria is commonly present in wastewater and can lead to sickness if consumed. Ensuring proper treatment to reduce the bacteria concentration present in the water can decrease the number of water consumption-related sickness. GMAC has been previously reported to inactivate several strains of bacteria. In this study, GMAC was functionalized onto bleached spent coffee grounds to create a sustainable method capable of reducing bacteria concentration in wastewater. In Figure 15 below, the bacterial inactivation of raw spent coffee, bleached spent coffee, and GMAC functionalized spent coffee were explored for *E. Coli*, *Salmonella*, and *Campylobacter*.

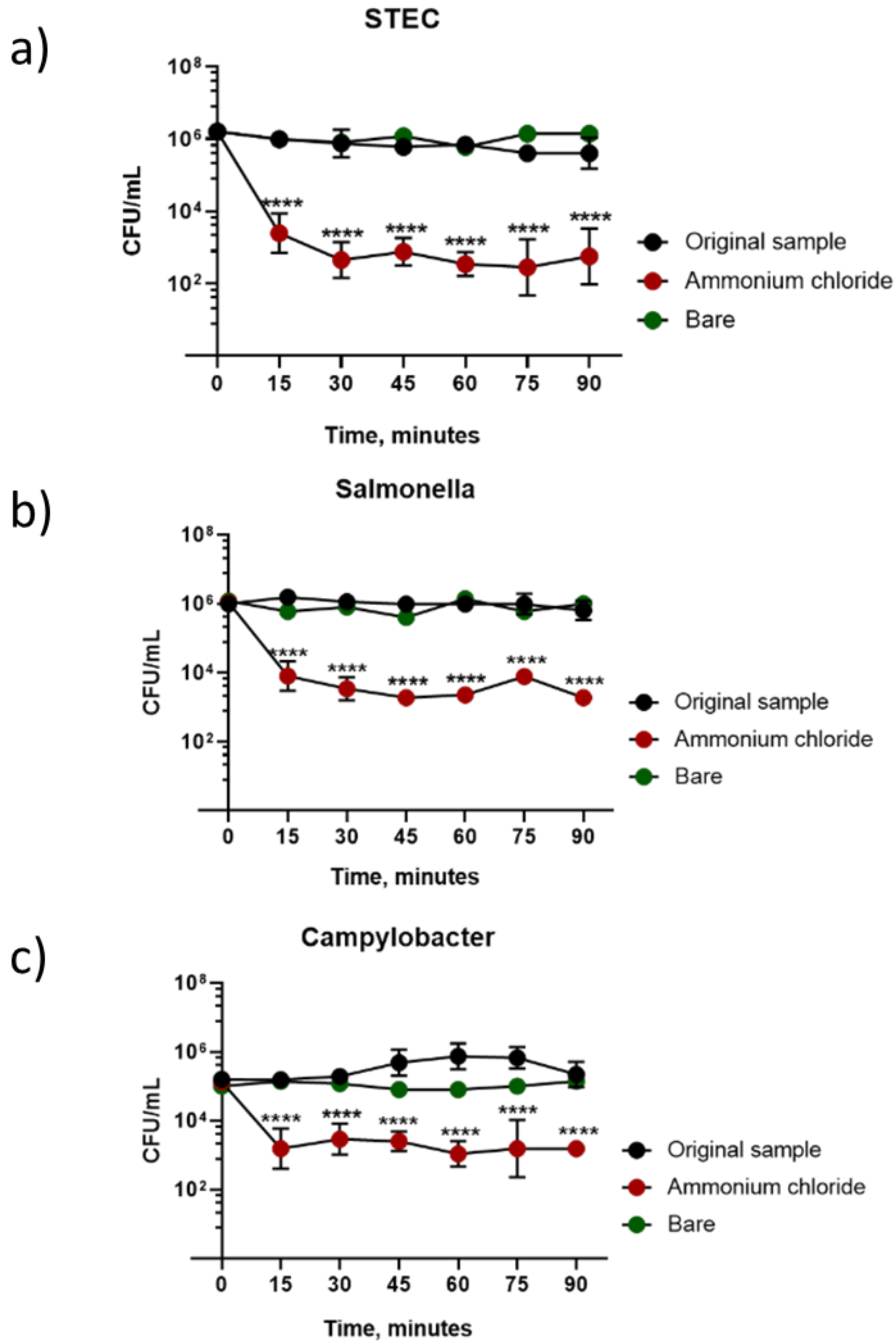


Figure 15. Bacterial inactivation of raw spent coffee grounds (original sample), bleached spent coffee grounds (Bare), and GMAC functionalized spent coffee (Ammonium chloride) for a) *E. coli*, b) *Salmonella*, and c) *Campylobacter*.

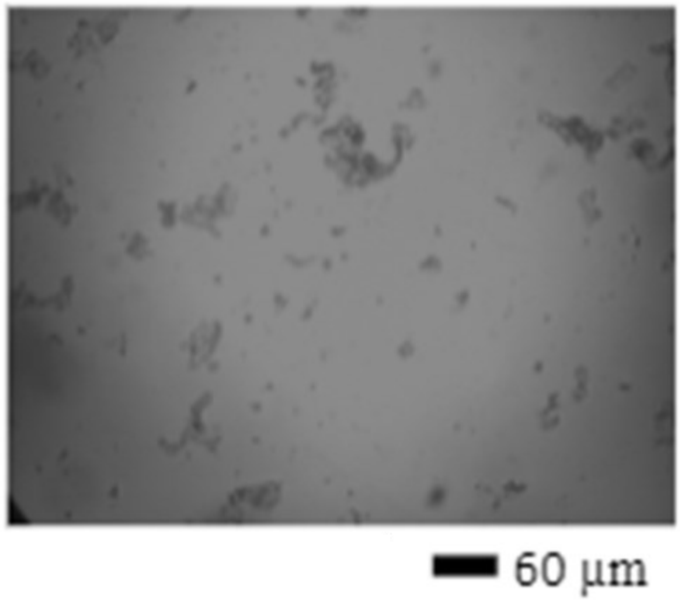
The GMAC functionalized spent coffee grounds exhibited bacterial inactivation for all strains tested. In the case of *E. Coli*, a 3-log reduction in the CFU/mL was observed. *Salmonella* and *Campylobacter* demonstrated a 2.2-log reduction and 2.8-log reduction, respectively. Whereas the raw spent coffee and bleached spent coffee exhibited no noticeable bacterial activation.

These bacterial inactivation experiments were done with high initial concentrations of bacteria. Higher than what would be found in typical wastewater. Therefore, the observed CFU reduction would be more than sufficient for the purpose of wastewater treatment.

4.3.4. Microplastic Removal

Microplastics are simply defined as plastic particles that are smaller than 5 mm. Due to their small nature, they can easily by ingested and cause health problems. Therefore, when considering wastewater treatment, it is important to ensure microplastic removal is considered. There has been an increasing concern on the impact of microplastics on the environment due to their small size and the fact that current wastewater treatment do not actively remove them from water. In this study, a combination of polyethylene, polypropylene, and polyethylene terapthalate was used. Functionalized spent coffee grounds were then added and stirred in order to capture and remove the microplastics. Figure 16 below visually represents how the concentration of microplastics gets reduced by the addition of spent coffee grounds. One reason for the removal of microplastics by spent coffee grounds could be due to the size and porous nature of the coffee grounds. They act by physically adhering to the microplastics, thereby entrapping them allowing for removal.

a)



b)

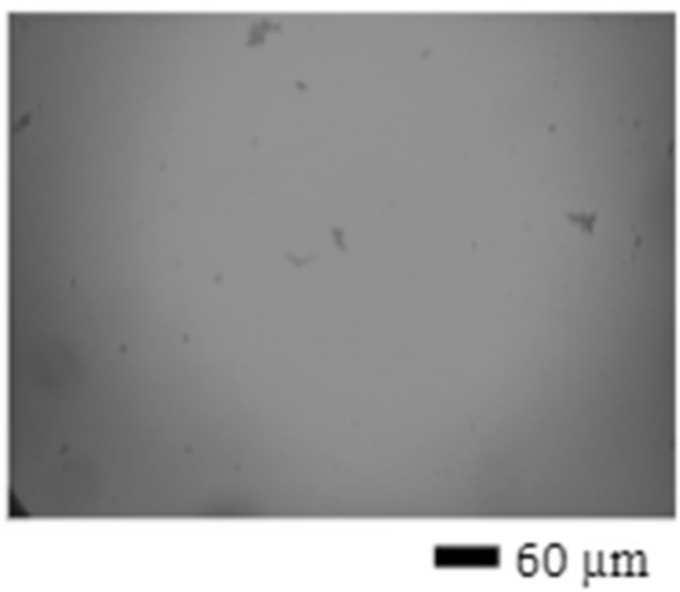


Figure 16. Optical microscope images showing the microplastic concentration present before (a) and after (b) the addition of spent coffee grounds.

Other than visual evidence of microplastic removal, quantification is possible. One method includes simply counting the particles present in the optical microscope images from before and after the addition of spent coffee grounds. Another method would be to stain the particles using Nile red dye. The absorbance can then be measured before and after the addition of the spent coffee grounds by using a spectrophotometer at a wavelength of 630 nm. In this case, the Nile red dye acts as a fluorescent label for the microplastics. Figure 17 below shows the microplastics dyed with Nile red.

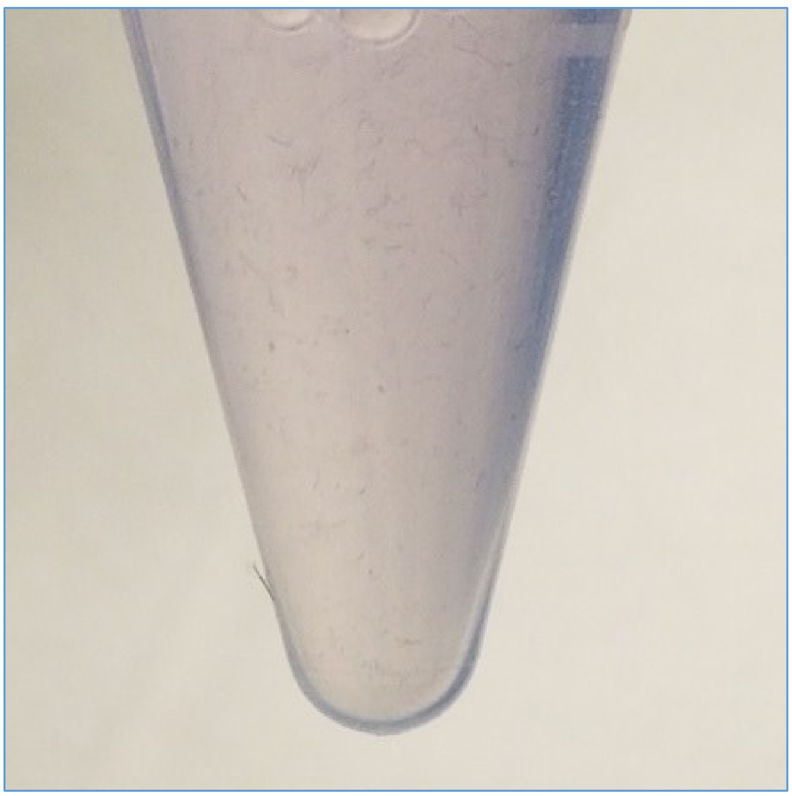


Figure 17. A 2 mL tube containing microplastics that were dyed using Nile red.

After the microplastics were dyed using Nile red, the absorbance was measured to determine the concentration of microplastics after the addition of spent coffee. This gives a quantifiable number to how much of the microplastics can be effective removed from solution by the spent coffee grounds. Table 3 below shows the initial concentration of the solution and the determined concentration of particles remaining in solution after the addition of spent coffee grounds.

Table 3: Microplastic removal efficiency of functionalized spent coffee grounds.

	Concentration of microplastic particles			
	a	**b**	**c**	**d**
Before the addition of functionalized coffee ground (µg/mL)	5	15	25	50
After the addition of functionalized coffee ground (µg/mL)	1.1	3.6	6.75	11.6
Removal Efficiency	88%	76%	73%	77%

While determining the removal efficiency of the spent coffee grounds, 4 different initial conditions were used. It can be seen that functionalized spent coffee grounds removed between 73% and 88% of the microplastic particles in solution. This shows that the method has promise for removing micrroplastics from wastewater.

4.4. Slow-Release Profile for Struvite as a Fertilizer

Struvite is a well-known slow-release fertilizer as it contains Mg, N, and P which are all needed nutrients for optimal plant growth. Additionally, struvite has been found to naturally form in wastewater as it has all the components required for struvite formation. Due to this, the impact that the spent coffee ground has on slow-release of nutrients from struvite was investigated. In order to determine the concentration from the measured absorbance, a standard must be made using known concentrations of PO_4^{3-}. Figure 18 below shows the determined standard over a P concentration range of 1 ppb to 25,000 ppb.

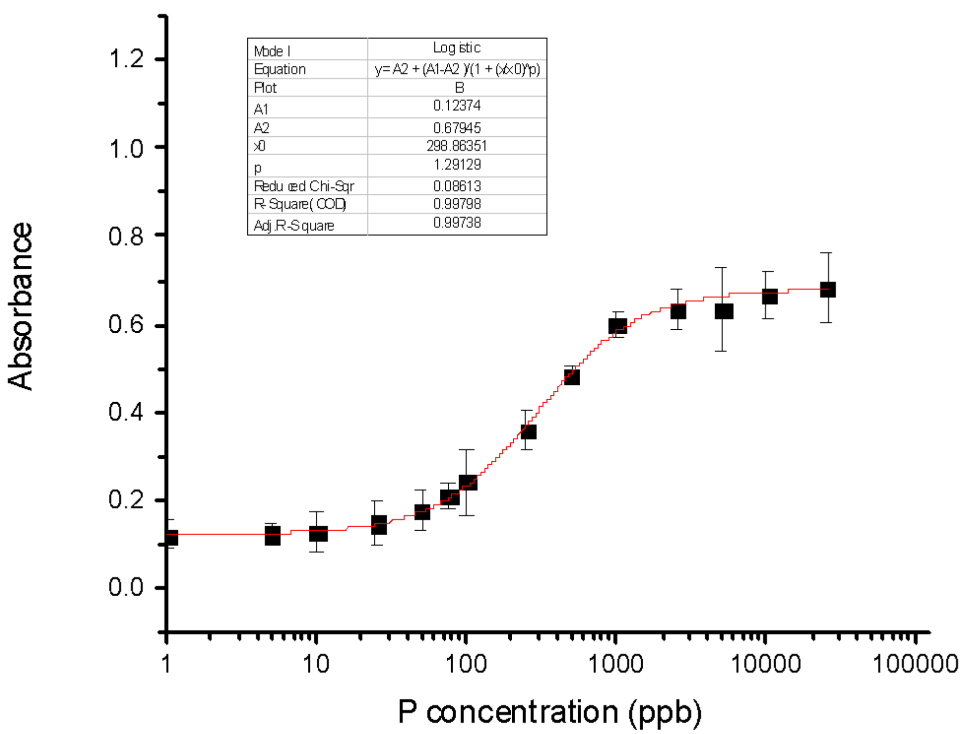


Figure 18. Standard curve relating absorbance values to P concentration.

After determination of the standard curve, the incubated samples with unknown PO_4^{3-} levels were able to be measured for absorbance. Using the standard, the absorbance of the phosphate-release tests can be related to specific concentrations. Thus allowing for the determination of the amount of PO_4^{3-} that was leached into solution. The amount of PO_4^{3-} present in DI water after set amounts of time was measured. This would show how the struvite was capable to slow releasing nutrients into the surround medium over time. Therefore, allowing nutrients to be present for plants during all stages of the plants life cycle. Figure 19 below shows the measured concentration of PO_4^{3-} measured over time.

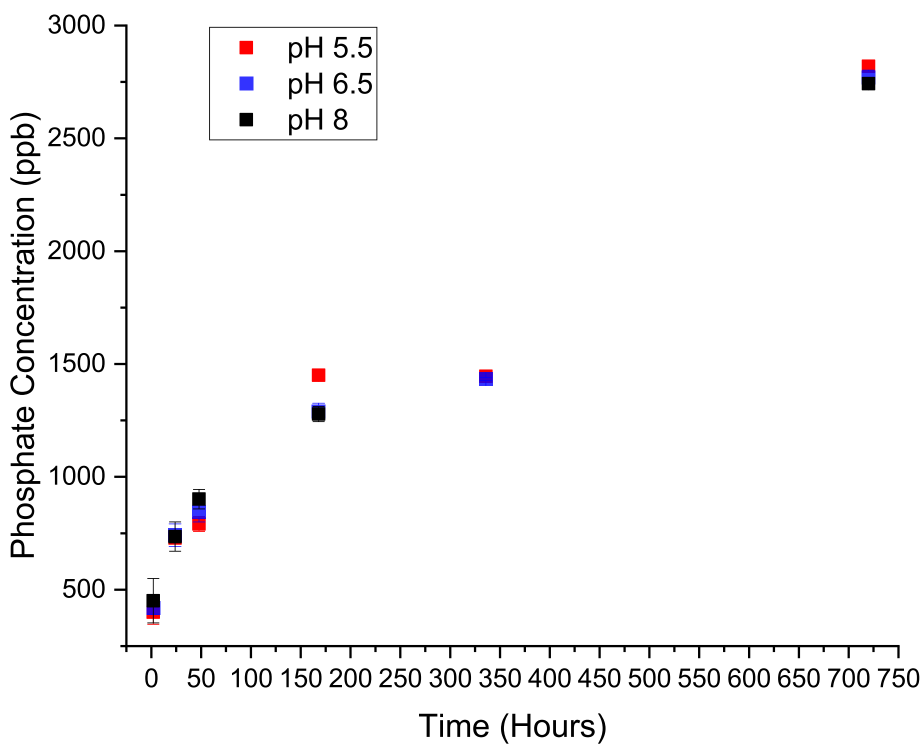


Figure 19. Concentration of PO_4^{3-} measured over the course of 1 month for 3 different pH levels (5.5, 6.5, and 8).

Chapter 5 – Conclusion and Future Work

In this work, a novel idea for valorizing natural waste in order to provide a sustainable method for wastewater purification and to create a slow-release fertilizer was investigated. Current wastewater treatment processes utilize multiple steps in order to fully treat the water and bring it in line with water quality standards set by Health Canada. Using a functionalized surface, the waste coffee can have its properties modified to effectively remove contaminants from wastewater. For contaminant removal, PEI and GMAC were considered since they exhibited ion capturing and bacterial inactivation, respectively. After functionalization onto the surface of the spent coffee, it was determined that more than 90% of the metal ion contaminants were removed from solution. Additionally, the CFU levels of *E. Coli*, *Salmonella*, and *Campylobacter* experienced a 3-log, 2.2-log, and 2.8-log reduction, respectively. This system was also proven effective as capturing microplastics present in wastewater (73-88% removal efficiency).

Struvite is a common by-product that forms in farm wastewater due to the high Mg, NH_4, and PO_4 concentrations present. This mineral has been seen to act as a slow-release fertilizer. In addition, spent coffee grounds have been commonly used as a fertilizer. Combining both allows for the slow-release of essential nutrients during the entire plant life-cycle, as well as the benefits of increasing the organic matter content in the soil due to the spent coffee grounds. After 1 month of incubation, 2,500 ppb of PO_4^{3-} was released from 20 mg of struvite crystallized on spent coffee grounds.

Future work related to the project would include scaling up the water treatment and fertilizer experiments. In practice, there will likely be a continuous input of contaminated water. To test this, a tightly packed cartridge can be filled with the PEI and GMAC functionalized spent coffee. Water can then continuously flow through, and the resulting water can be collected and tested to see ion removal efficiency and bacterial inactivation. In addition, a large-scale experiment should be done to demonstrate the capabilities of iron nanoparticles attached to PEI functionalized spent coffee. This would show the ease of collection by using a electromagnetic field to attract and collect the spent coffee grounds after ion removal and bacterial inactivation. The fertilizer study can be further improved by testing the nutrient release in soil at various pH, as well as using the spent coffee and struvite in practice to grow a tomato.

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