AN EVALUATION AND COMPARISON:

USING EGGSHELLS, COFFEE GROUNDS AND BANANA PEELS TO DEGRADE PETROL IN SOIL

A scientific investigation

ABSTRACT

The aim of this investigation was to investigate whether soil contaminated with petrol could be bio remediating using one of three common food wastes (banana peels, eggshells and coffee grounds). Also, the efficiency of each material was compared to investigate which of the three substances trialled were the most effective. It was hypothesised that the banana peels would bio remediate the polluted soil, within a period of 8 weeks, most efficiently as it would decompose quickest.

Soil was collected and divided into 15 equal parts. Three of the samples remained uncontaminated to act as a control and the remaining twelve were contaminated with equal amounts of petrol. These 12 contaminated samples were then split into four groups with three samples. Equal amounts of each food waste were added to the samples in three of the four groups while the fourth remaining group was left as a petrol only control. After the petrol was left to absorb for 2 weeks, 15 rocket seedlings were planted into each pot. The soil was then observed over the next 8 weeks. Photographs and seedling height was recorded every 2 weeks.

The conclusions of this investigation were promising and held potential for use in real life applications and in further investigations. It was concluded that all materials remediated the petrol-contaminated soil well but the eggshells were the most efficient bioremediators followed by the coffee grounds then the banana peels. This statement only stands true when the ratio of remediator to oil is 16:13 meaning that coffee grounds and banana peels may be very effective bioremediators but when used in a different material to oil ratio.

INTRODUCTION

The world relies on petrol. Petrol (or gasoline) is an important and common resource in today's society as many people use it frequently worldwide. But, it is well known that petrol can greatly damage the environment as a result of the pollution it creates. One of the most damaging yet poorly managed types of pollution caused by petrol is soil pollution which can be caused by a wide variety of factors such as accidental oil spills, industrial activity and improper disposal of petrol. Petrol pollution in soil can go on to severely impact plants, animals and humans. Removing pollutants from polluted soil can be difficult and often results in the pollution being worsened or moved elsewhere.

An effective, efficient and increasingly popular method of removing contaminants from polluted soil is bioremediation, a method that degrades pollutants with naturally occurring organisms. Unlike the commonly used methods this can permanently remove the pollution and can be very inexpensive. Naturally occurring organisms that can degrade pollutants can be stimulated by a wide variety of materials that can introduce more nutrients to the area and therefore stimulate more pollutant degrading organisms, as demonstrated in several studies. Many studies have investigated and confirmed the positive effects of bioremediation.

However, the possibility of using organic fertilisers which are known to introduce essential nutrients to the soil and often come in the form of unwanted food wastes has not been heavily researched though this solution holds a lot of potential as it could solve two significant global issues (pollution and food waste). Egg shells, banana peels and coffee grounds are common food wastes that usually serve no further purpose once the food is consumed. A bioremediation solution that utilises these materials could greatly help the environment.

BACKGROUND INFORMATION

WHAT IS PETROL?

Petrol is made from a fossil fuel called petroleum, also known as crude oil. Crude oil can be a black, green or clear liquid of varying consistencies. It is made up of different hydrocarbons (organic compounds containing carbon and hydrogen atoms) each containing different amounts of carbon. Crude oil is separated into useful oils through a process called fractional distillation. It is heated to a vapour and allowed to rise up a tower where they will condense. Since the hydrocarbons have different amounts of carbon atoms they have different boiling points so heavier molecules will condense lower in the tower. Petrol is one of the lightest of these layers as it is made of relatively light compounds. After the petrol has been separated, it undergoes further processes to improve the quality of the petrol. Petrol is complex mixture of more than 500 hydrocarbons with seven to eleven carbons. Its components vary but it usually contains the following:

General name	Examples	Percentage
Aliphatic - straight chain	Heptane	30-50
Aliphatic - branched	Isooctane	30-50
Aliphatic – cyclic	Cyclopentane	20-30
Aromatic	Ethyl benzene	20-30

EFFECTS OF OIL CONTAMINATION IN SOIL

Examples of oils that can contaminate soil includes diesel fuel, gasoline, motor oil and crude oil. Such oils can contaminate soil as a result of oil spills, refineries, garages, gas stations, engines, pipelines, transportation activities or the dumping of chemicals. Additionally, a study by scientists at the John Hopkins Bloomberg School of Public Health showed that, at gas stations, while amounts of oil evaporate, a relatively large amount seeps through the concrete, contaminating soil and water. Soil pollution can lead to water pollution if runoff reaches bodies of water and which can then go on to contribute to air pollution by releasing volatile compounds (compounds that evaporate rapidly) into the air.

Approximately 0.6% of gasoline is benzene, a chemical that is carcinogenic for humans. As well as this, it contains numerous other chemicals known to cause cancer in humans too. Contaminated soil can pose many health hazards to humans like reproductive disorders, cardiovascular disorders and blood disorders. Even if the soil isn't heavily polluted it can affect humans and animals indirectly through bioaccumulation. This describes plants growing in slightly polluted soil that absorb molecules from pollutants, which also stunts plant growth. Oil molecules can accumulate in plants growing in contaminated soils. These oil molecules then move up the food chain into organisms eat organisms that have accumulated the pollution.

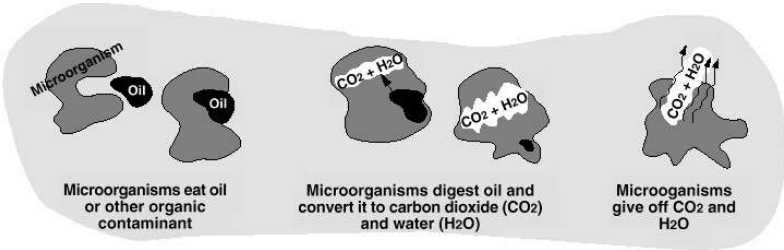
When humans eat such plants or animals they can be poisoned resulting in health issues like liver damage and nerve damage.

Oil contamination can also greatly impact flora. A study by researchers at the University of Benin showed that soil polluted with crude oil had increased organic matter, less phosphorous and that the soil inhibited growth of plants. Less growth in plants was also found to be occur because the oil makes essential nutrients like nitrogen and oxygen unavailable to plants and microorganisms since the oil concentrates in the plants, clogging up the plant cells resulting in the death of the plants.

Another study by researchers at the University of Lagos observed seeds planted in crude oil contaminated soil for a period of 15 days and found that crude oil pollution caused the germination rate of seeds to go from 100% in the control to 31-38% and greatly decreased seedling height. The study also found that contaminated soil treated with Pseudomonas putida had a germination rate and seedling height approximately the same as in the control.

WHAT IS BIO-REMEDIATION?

Bioremediation a naturally occurring process where pollutants are removed from contaminated areas by microbes. These organisms break down the chemicals for food and energy leaving only harmless products like water and carbon dioxide. Given the right environment with suitable food supply, these microbes can reproduce and quicken the bioremediation process. Instead of dumping contaminated soil into landfills, as is often currently done, bioremediation is a simple and efficient solution to various types of pollution that makes use of naturally occurring organisms that consume and break down the pollutants. It was used to clean up the 2010 BP Deepwater Horizon and the 1989 Exxon Valdez spill. Bioremediation usually takes several weeks or months and can be carried out in two ways: by enhancing the growth of pollution-eating microbes present at the contaminated site (bio stimulation); or by introducing specialised microbes to clean up the site (bio augmentation).



Bio stimulation is a widely used approach that stimulates microorganisms – like bacteria, fungi and protists – by providing them with nutrients and other conditions that encourage their growth and encourage them to work to break down contaminant. The addition of inorganic or organic nutrients can quicken the process of bioremediation. Often this is done through nitrogen amendment. Many studies have demonstrated the positive effects of nitrogen amendment on microbial activity. Nitrogen and phosphorous are known to be the most important nutrients in encouraging hydrocarbon degrading bacteria to break down contaminants efficiently, so by adding material that is rich in these nutrients hydrocarbon degrading microorganisms can be encouraged to work more efficiently.

HYDROCARBON DEGRADING BACTERIA

Many microbes exist that can break down hydrocarbons. As soon as oil began to appear on Earth, they evolved to be able to use it as an energy source. Some are capable of breaking down the compounds while others simply transform the compounds into smaller chunks that other species will then further break down.

The efficiency of microbes in degrading hydrocarbons is heavily dependent on the chemical makeup of the contaminant and several environmental factors. For instance, temperature affects the rate of biodegradation since it affects the chemistry of the pollutants and the diversity of microorganisms. Generally, the rate of hydrocarbon degradation decreases as temperature decreases making the ideal temperature 30-40°c. The availability of nutrients also plays a large factor in the efficiency of microbes. Hydrocarbon degrading bacteria require the hydrocarbon they degrade for energy, certain amino acids/vitamins, nitrogen and phosphorous.

WHAT MAKES A GOOD BIOREMEDIATOR?

By introducing more nutrients such as nitrogen, phosphorous, carbon and oxygen to soil and controlling pH levels in polluted soil, microorganisms can be stimulated to degrade the pollutants. However, research has also indicated that excess nitrogen can decrease degradation of pollutants and can be detrimental for surrounding water bodies. This means that a substance that introduces too much of a certain nutrient will not be able to effectively remediate contaminated soil.

Many fertilisers, both organic and inorganic, are known to introduce these essential nutrients into the soil and are rated by an "NPK". The letters represent three different compounds, nitrogen, phosphorous and potassium. If the number in the ratio is higher then the concentration of the nutrient is higher. For instance, if a fertiliser has a 20-5-5 ratio than it has 4 times more nitrogen than the other two nutrients and a 20-20-20 fertiliser has twice the concentration of all nutrients than 10-10-10. While the levels of nitrogen, phosphorous and potassium effects the suitability of bioremediators, the efficiency is dependent on several environmental factors such as indigenous microbial populations, oxygen supply, pH, soil moisture, temperature, nutrient availability, organic matter, the quantity and quality of the soil and soil properties.

Organic fertilisers, which come from animals or plants, can be good bioremediators since as they decompose; they release many nutrients, which encourage hydrocarbon-degrading bacteria. A few studies have been conducted which utilise organic fertilisers to bioremediate soil contaminated with different types of oil and have concluded that they are simple, effective and cheap bioremediation solutions. Most studies have shown that types of animal dung (such as cow dung and chicken dung) are effective bioremediators. For example, researchers at the University of Port Harcourt found that by adding cow dung and palm kernel husk ash to crude oil contaminated soil, biodegradation of the soil increased.

A study by researchers at the University of Malaya used organic waste ammendments to biodegrade motor oil in soil. They utilised 10% of brewery spent grain, banana skin, and spent mushroom compost in soil contaminated with 5% and 15% lubricating oil. The final results after on observation period of 84 days were that brewery spent grain degraded oil best in 5% oil pollution and banana skin degraded oil best in the 15% oil pollution.

WHAT MAKES THE CHOSEN BIOREMEDIATORS EFFECTIVE?

Banana peels, egg shells and used coffee grounds are all known to be efficient fertilisers as they all contain substantial amounts of nitrogen, phosphorous and/or potassium. These materials are also all organic fertilisers, which means that they release important nutrients slowly and steadily over many years rather than in one quick rush that can be instantly washed away in the case of rain. Organic fertilisers release their nutrients faster as they decompose. Used in bioremediation, this can mean that as times goes on, the fertilisers will release more nutrients that are favoured by the microbes therefore encouraging more hydrocarbon degrading bacteria and increasing the rate of bioremediation.

Nitrogen encourages the growth of the green parts of the plant, also known as vegetative growth, however too much nitrogen can make the plant "leggy" (meaning the plant becomes very long and slender). Materials that are juicy or that come from animals, such as fresh fruit and coffee grounds, tend to be high in nitrogen. Phosphorous helps a plant to grow and supplies plants with energy. It supports root, fruit and flower growth and can also quicken maturity as it supports cell division and the development of new tissue. Usually plants that lack phosphorous are of an abnormal dark-green colour and are quite small. Potassium strengthens plant stems as it helps to form proteins. It is also a key nutrient involved in the uptake of CO_2 as it regulates the opening and closing of stomata in the plants. Also, it assists in regulating water (osmo-regulation) and helps to activate enzymes including Adenosine Triphosphate (ATP) which is an important energy source for many chemical processes in plants. Potassium deficiency can be seen in plants that have slow or stunted growth, poor resistance to temperature change and loss of leaves.

Banana peels have an NPK ratio of 0-3-42 meaning they are 42% potassium and are therefore one of the best sources of potassium for plants. They do not contain nitrogen but have small amounts of phosphorous and micronutrients like calcium, manganese, sodium, magnesium and sulfur. The NPK of coffee grounds is 2-0.3-0.3. Coffee grounds are a good source of nitrogen and have a close to neutral pH that is beneficial for most soils. The NPK of eggshells is 1-0.4-0.1. Eggshells are also rich in calcium, which is an important micronutrient and can make the soil less acidic.

AIM

To investigate whether food wastes containing different nutrients (ground up egg shells, banana peels and coffee grounds) can bio remediate petrol-contaminated soil and which material is the most efficient at doing so.

HYPOTHESIS

The banana peels will be the most efficient bio remediator in the given time frame as it decomposes quickest and contain a variety of micronutrients which will encourage hydrocarbon degrading bacteria.

EXPERIMENTAL DESIGN

Seeds are germinated before being planted to ensure that the all plants had an equal chance of growing so that the results from the experiment were not influenced by other factors. Soil is collected from the same location sieved to ensure the soil texture and properties remain the same throughout the soil. Sterile equipment must be used to ensure the soil and therefore results are not affected by other variables. Plants are checked every 2 weeks over a period of 8 weeks. This long period of time ensures that the results obtained are a broad representation of the true, long-term trend. Also, plants were checked every 2 weeks as trial experiments showed that measurable change occurred after this time frame. Three trials of each material were conducted to ensure results are reliable and consistent. Two control groups were set up, one without oil, one with only oil, so that the data collected from the groups with remediators added could be compared and a suitable conclusion made. These groups ensure that the environment does not affect the data obtained and the final conclusions. For instance, if the plants in the control group die then any decline in the other groups are to be concluded as the result of other factors, not the oil contamination or added materials.

VARIABLES

CONTROLLED: type and source of soil, type of oil, amount of seeds, type of seed, time of sowing, time of watering, environment the soil is kept in, type of containers used, amount of soil, amount of bio remediating material added, time intervals between observations, amount of water poured over seeds, source of water, temperature of water, depth the seeds are sown at, distance between seeds, scale used to weigh equipment

INDEPENDENT: type of bioremediator used

DEPENDENT: plant height, amount of seedlings that grow and stay alive, leaf area, leaf density

MATERIALS

- 15 x 640g soil (collected from 10 cm below ground at the same site)
- Scale
- Shovel
- 15 x canvas bags
- 2mm sieve
- 15 x clean plastic containers with drainage holes
- 12 x 7 mL petrol
- Safety goggles
- Face mask
- Gloves
- 10mL measuring cylinder
- Permanent marker
- 64g ground up eggshells (collected from same type of boiled chicken eggs, membrane still attached)
- 64g cut up banana peels (collected from same type of banana)
- 64g dried coffee grounds (collected from same type of coffee)
- 1 packet of rocket seeds
- Paper towels
- Plastic bag
- 75 x 15 mL water
- Camera
- Measuring tape

METHOD

Soil preparation

1. Collect 15 x 640 g in sterile canvas bags ensuring that it is collected with a sterile shovel from a depth of 10 cm and above
2. Sieve the soil with a 2mm sieve
3. Whilst wearing appropriate PPE (gloves and safety goggles), weigh 640g of soil into a clean plastic container with 4 holes drilled at the bottom. Repeat with all 15 containers
4. Air dry the soil in a well ventilated, undisturbed area for 24 hours
5. Whilst wearing appropriate PPE (gloves, face mask and safety goggles), measure 7mL of petrol with a measuring cylinder. Pour it evenly over 12 of the 15 soil samples and incorporate well by mixing with a sterile spade and shaking
6. Label the uncontaminated soil samples as the control and put aside
7. Leave all of the samples for 24 hours in an undisturbed, enclosed, well ventilated area so the soil can absorb the oil
8. Label three of the 12 contaminated soil samples as the "contaminated control"
9. Split the remaining 9 containers into 3 groups of 3 containers

Bioremediator preparation

10. Prepare banana peels by chopping up the different peels and chopping into 1cm wide slices
11. Prepare coffee grounds by air-drying in a well ventilated, undisturbed area for 3 days.
12. Prepare eggshells by sterilising the eggshells in an oven set to 50^{o}c for 30 minutes. Then, in a clean bowl, grind up the eggshells to an almost powder like size
13. Add 64g (10%) of eggshells to the first group of 3 containers. Incorporate well by mixing and turning the soil thoroughly with a sterile spade
14. Repeat step 13 with the banana peels and coffee grounds
15. Leave each sample in an undisturbed, enclosed and well ventilated area for 2 weeks
16. In the last three days of this 2 week period, germinate 80 seeds by placing them between two pieces of damp kitchen towel that are inside a plastic bag and leave this in a warm area (such as above a fridge) for 2 weeks. After 3 days, carefully remove the seeds that have germinated.
17. Sow 15 of the germinated seeds into each sample (spaced around 1 cm apart, 5 rows with 3 seedlings each) and water each sample with 15 mL of room temperature water
18. After 2 weeks record the number of seedlings, the percentage of seeds that have germinated compared to the percentage of seeds sown, seedling height and take images of each to evaluate leaf area
19. Water each sample with 15 mL of room temperature water
20. Repeat steps 18 and 19 every 2 weeks

MATERIAL COLLECTION

The soil was collected from a depth of 10cm and came from the Warrumbungle National Park in the north of New South Wales. It is a mixture of sandstone and volcanic soil. It was sieved, transported in canvas bags then allowed to air dry for 24 hours before being weighed into each container. The banana peels were obtained through a local smoothie store. Coffee grounds were collected from a local coffee store and left to air-dry indoors for several days. Eggshells were collected from eggs boiled at home, dried in a slightly warm oven to sterilise then crushed up using a mortar and pestle

Soil divided into containers

Soil, petrol and bio remediators in pots

Soil in canvas bags

RISK ASSESSMENT

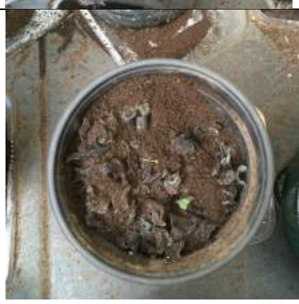
POTENTIAL HAZARD	LIKELIHOOD	HOW SEVERELY COULD IT HURT SOMEONE?	MANAGEMENT
Igniting a fire when filling the portable fuel container with petrol	Likely	High	Make sure the container is certified to be filled with flammable liquids by the government and made of plastic or metal. When filling the container, it must be on the floor in open air. Ensure all ignition sources are not present (e.g. smoking, phones, static electricity)
Petrol coming in contact with skin and causing irritation	Likely	High	When handling petrol (e.g. spraying it over soil), make sure to wear gloves and a long sleeved shirt or apron. If petrol spills on clothes, clean the area with cold water before cleaning to prevent a static electric spark
Inhaling petrol fumes	Likely	Low	When handling petrol make sure to wear a mask over mouth and nose and keep face a suitable distance from source of petrol and that you are working in a ventilated area
Getting petrol in eyes, causing irritation	Unlikely	Mild	Wear goggles when working with petrol and keep the petrol container a suitable distance from face while holding the container steadily and moving it slowly
Ingesting soil, causing issues for internal body parts	Unlikely	Low	Make sure to wear gloves when handling soil and wash hands thoroughly, with soap, afterwards
Getting soil in eyes, causing irritation	Unlikely	Mild	Keep face a suitable distance from soil and make sure to not throw the soil up or flick particles around by moving too abruptly or carelessly. Wear safety goggles whenever soil is being handled.

RESULTS

FIRST CHECK

	Number of Plants			Average plant height (cm)		
	Pot 1	Pot 2	Pot 3	Pot 1	Pot 2	Pot 3
Control	14	6	8	0.79	0.27	0.26
Oil	4	3	5	0.1	0.1	0.12
Coffee Grounds	4	2	4	0.75	0.2	0.3
Banana Peels	7	2	3	0.63	0.8	0.5
Eggshells	2	3	5	0.7	0.23	0.14

PHOTOS

Group	Pot 1	Pot 2	Pot 3
Control			
Oil			
Coffee Grounds			
Banana Peels			

Eggshells			

HEIGHT OF EACH PLANT

Group	Pot #	Individual Plant Height (cm)*														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control	1	2	1.6	1	2.1	0.3	0.6	0.2	0.1	0.7	0.4	0.8	0.6	0.3	0.3	0
	2	0.3	0.2	0.2	0.3	0.2	0.2	0.3	0.4	0	0	0	0	0	0	0
	3	0.2	0.2	0.3	0.4	0.2	0.3	0	0	0	0	0	0	0	0	0
Oil	1	0.1	0.1	0.1	0.2	0	0	0	0	0	0	0	0	0	0	0
	2	0.1	0.1	0.1	0	0	0	0	0	0	0	0	0	0	0	0
	3	0.2	0.1	0.1	0.1	0.1	0	0.9	0	0	0	0	0	0	0	0
Coffee Grounds	1	1	1	0.4	0.6	0	0	0	0	0	0	0	0	0	0	0
	2	0.2	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0.2	0.3	0.4	0.3	0	0	0	0	0	0	0	0	0	0	0
Banana Peels	1	1	1	0.6	0.8	0.4	0.4	0.2	0	0	0	0	0	0	0	0
	2	0.6	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0.1	0.1	1.3	0	0	0	0	0		0	0	0	0	0	0
Eggshells	1	0.4	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0.4	0.1	0.2	0.6	1	0	0	0	0	0	0	0	0	0	0
	3	0.1	0.3	0.2	0.2	0.1	0	0	0	0	0	0	0	0	0	0

SECOND CHECK

	Number of Plants			Average plant height (cm)		
	Pot 1	Pot 2	Pot 3	Pot 1	Pot 2	Pot 3
Control	14	15	13	1.86	1.98	1.95
Oil	7	4	7	0.74	1	1
Coffee Grounds	6	5	7	1.7	1.14	1.67
Banana Peels	3	4	5	1.5	1.05	1.7
Eggshells	2	5	3	1.5	1.92	2

PHOTOS

Group	Pot 1	Pot 2	Pot 3
Control			
Oil			
Coffee Grounds			
Banana Peels			
Eggshells			

HEIGHT OF EACH PLANT

Group	Pot #	Individual Plant Height (cm)*														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control	1	4	4	2	3.5	1	1.5	0.6	0.4	2	2	1.6	1.3	1.1	1	0
	2	4.2	1.1	1	1.2	2.8	3	2.3	3.6	0.8	1.7	1.4	1	1.3	3.8	0.5
	3	3.7	4	4.7	1	1.5	3	2	1.3	0.2	2	0.9	0.8	0.2	0	0
Oil	1	0.6	1	1.3	0.2	0.8	0.2	1.1	0	0	0	0	0	0	0	0

	2	1	1.2	1.2	0.6	0	0	0	0	0	0	0	0	0	0	0
	3	1.3	1	0.8	1.2	0.8	1	0.9	0	0	0	0	0	0	0	0
Coffee Grounds	1	3	2.5	2.5	1	0.6	0.6	0	0	0	0	0	0	0	0	0
	2	4	1	0.2	0.3	0.2	0	0	0	0	0	0	0	0	0	0
	3	5	4.5	1	0.2	0.3	0.4	0.3	0	0	0	0	0	0	0	0
Banana Peels	1	1	2	1.5	0	0	0	0	0	0	0	0	0	0	0	0
	2	1	1	1	1.2	0	0	0	0	0	0	0	0	0	0	0
	3	3	2	1	1	1.5	0	0	0		0	0	0	0	0	0
Eggshells	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	3	2	3	0.6	1	0	0	0	0	0	0	0	0	0	0
	3	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0

THIRD CHECK

	Number of Plants			Average plant height (cm)		
	Pot 1	Pot 2	Pot 3	Pot 1	Pot 2	Pot 3
Control	15	15	11	2	2.41	2.52
Oil	4	0	2	1	0	1.5
Coffee Grounds	8	9	6	1.9	1.34	1.32
Banana Peels	4	0	3	2.08	0	3.67
Eggshells	5	7	5	2.1	2.47	2.02

PHOTOS

Group	Pot 1	Pot 2	Pot 3
Control			
Oil			

Coffee Grounds			
Banana Peels			
Eggshells			

HEIGHT OF EACH PLANT

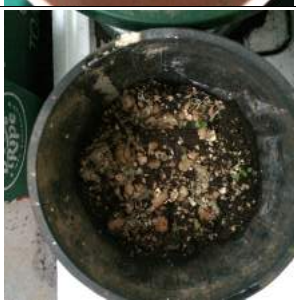
Group	Pot #	Individual Plant Height (cm)*														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control	1	3.5	3	4	4	3	4	2	2	2.4	1.7	1.9	3.5	2.5	2.3	2.3
	2	5.4	1.8	2	1.4	3	3.4	2.5	4	1	1.8	1.6	1	1.7	4.5	1
	3	4.5	4.2	5	1	1.6	3.2	2.4	1.8	0.4	2.3	1.3	0	0	0	0
Oil	1	3	1	0.5	0.4	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Coffee Grounds	1	3.1	4.6	3	0.3	2.8	1	0.3	0.1	0	0	0	0	0	0	0
	2	4.2	3.5	1	1	0.9	0.6	0.2	0.2	0.5	0	0	0	0	0	0
	3	1.6	3.2	0.9	0.3	1	0.9	0	0	0	0	0	0	0	0	0
Banana Peels	1	4	3.9	0.3	0.1	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	5	4.8	1.2	0	0	0	0	0		0	0	0	0	0	0
Eggshells	1	1	1.5	2	2	4	0	0	0	0	0	0	0	0	0	0
	2	2	3.8	3.6	2.6	4	1.3	1	1	0	0	0	0	0	0	0
	3	0.1	2	3.4	1	3.8	0	0	0	0	0	0	0	0	0	0

FOURTH CHECK

	Number of Plants			Average plant height (cm)		
	Pot 1	Pot 2	Pot 3	Pot 1	Pot 2	Pot 3
Control	15	9	4	3.49	3.78	3.75
Oil	0	0	0	0	0	0

Coffee Grounds	5	7	1	2.96	2	2.2
Banana Peels	0	0	0	0	0	0
Eggshells	4	2	4	2.85	2.75	2.63

PHOTOS

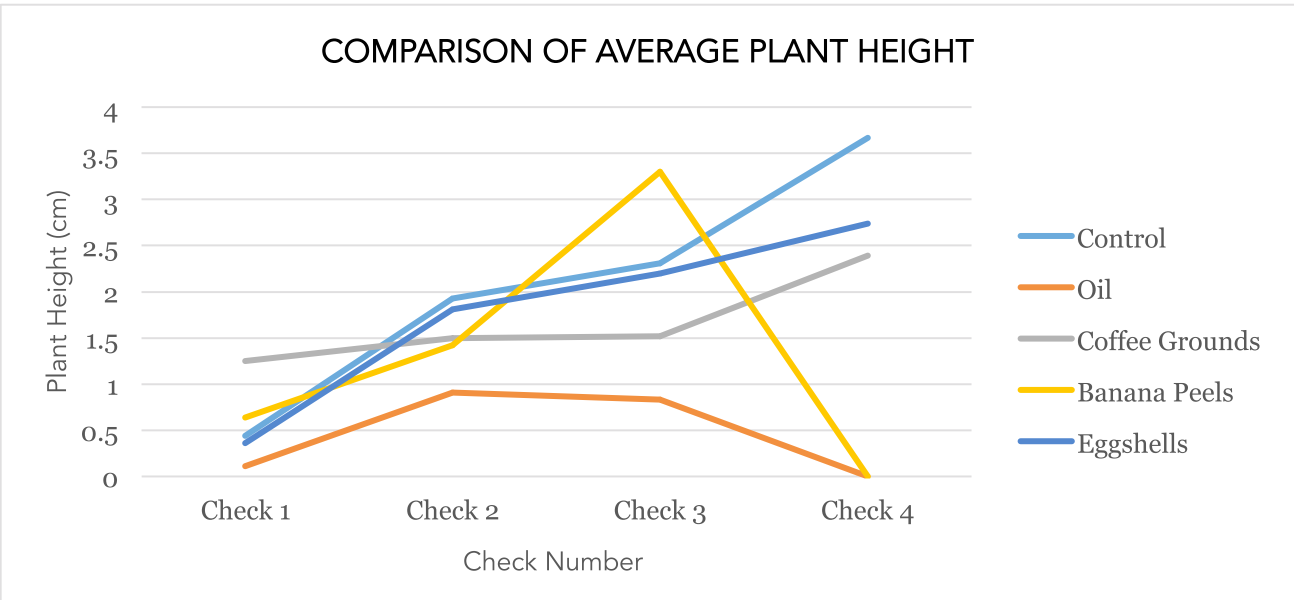
Group	Pot 1	Pot 2	Pot 3
Control			
Oil			
Coffee Grounds			
Banana Peels			
Eggshells			

HEIGHT OF EACH PLANT

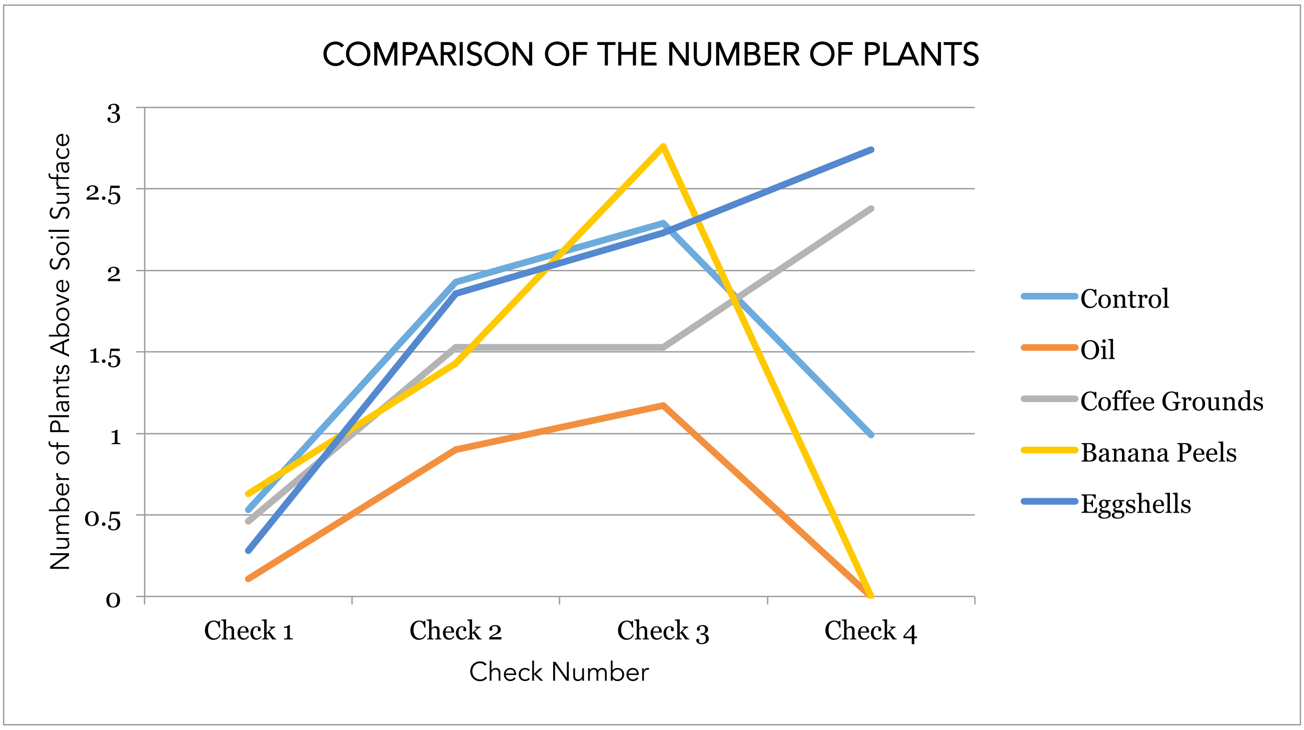
Group	Pot #	Individual Plant Height (cm)*														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control	1	0.2	0.1	0.1	0.1	0.1	0	0	0	0	0	0	0	0	0	0
	2	0.3	0.2	0.2	0.3	0.2	0.2	0.3	0.4	0	0	0	0	0	0	0
	3	0.2	0.2	0.3	0.4	0.2	0.3	0	0	0	0	0	0	0	0	0

Oil	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coffee Grounds	1	2.5	2.8	2.6	2.8	3.1	0	0	0	0	0	0	0	0	0	0
	2	4.5	3.6	1.2	1	1.1	1.5	1.1	0	0	0	0	0	0	0	0
	3	2.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Banana Peels	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0		0	0	0	0	0	0
Eggshells	1	2	6	2	1.4	0	0	0	0	0	0	0	0	0	0	0
	2	3.5	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	1.3	4.8	3.2	1.2	0	0	0	0	0	0	0	0	0	0	0

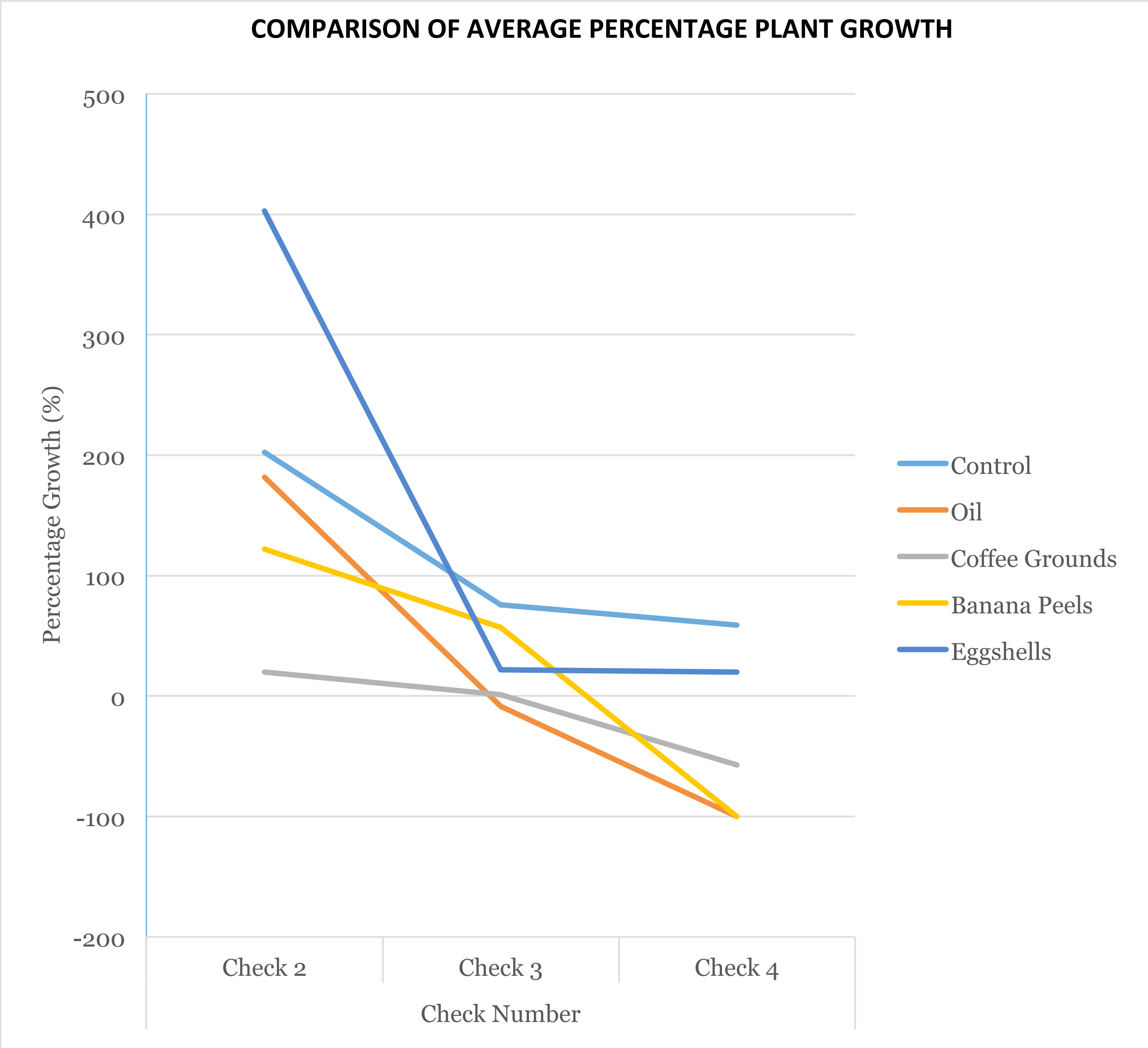
*The numbers above the plant measurements to not indicate a specific plant in each pot



Graph 1


COMPARISON OF THE NUMBER OF PLANTS
Number of Plants Above Soil Surface
3
2.5
2
1.5
1
0.5
0
Check 1
Check 2
Check 3
Check 4
Check Number
Control
Oil
Coffee Grounds
Banana Peels
Eggshells

Graph 2



Graph 2

Average Percentage Growth Rate

Group	Average Growth Rate (%)		
	Check 2	Check 3	Check 4
Control	202.27	75.94	58.87
Oil	181.82	-8.79	-100
Coffee Grounds	20	1.3	-57.24
Banana Peels	121.88	56.97	-100
Eggshells	402.78	21.55	19.71

DISCUSSION

The results of this experiment were almost the opposite to the hypothesis, that the banana peels would remediate the soil best, since the banana peels were one of the least effective bioremediators and the eggshells proved to be the most effective bioremediator. However, the data from this experiment can be used to develop ways to use banana peels or coffee grounds to bioremediate soil more efficiently.

From graph 1 it can be seen that the plants growing in the contaminated soil where eggshells were mixed in had approximately the same average height as the control plants and grew mostly at the same rate. The plants in the eggshells also grew much faster and taller than those in the oil only group. This means that the eggshells bioremediated the soil excellently. The eggshells may have been the most effective as they contain high levels of calcium, which may be very good at stimulating hydrocarbon-degrading bacteria. Another reason why the eggshells were the most effective in remediating the soil may have been because they made the soil less acidic and therefore more comfortable for microbes to live in. Also, the eggshells had less potassium than both other materials, which may indicate that hydrocarbon-degrading bacteria do not want high levels of potassium. This is supported by the fact that the plants grown in banana peels, which are high in potassium, did not perform very well. The eggshells and the coffee grounds, the two most effective remediators, contain almost 10% less phosphorous than the banana peels which performed poorly meaning hydrocarbon-degrading bacteria may not be greatly stimulated by the addition of phosphorous. Banana peels were also the only material that didn't contain nitrogen, which may indicate that nitrogen is a key nutrient needed to stimulate microbes to degrade pollutants.

The plants grown in the eggshells grew by 1.45cm (403%) in the second check (4th week) but grew much slower in the following checks (0.39cm in the third check (22%) and 0.54cm in the fourth check (25%)). In the fourth check the plants in the eggshells grew by a smaller percentage than the plants in the control. This may have been because the eggshells released nutrients quickly in the first few weeks and gradually slowed as time went on, therefore by the fourth check the eggshells did not provide enough nutrients to encourage hydrocarbon-degrading bacteria to continue degrading the remaining petrol, which might have then started to accumulate in the plants and slow their growth. This could mean that for bioremediation of petrol polluted soil with eggshells to be effective; more eggshells would have to be added.

Another reason for the decreased growth rate could be that the eggshells supplied too much of a certain nutrient by the third of fourth checks causing the plants to grow much slower or the microbes to be unwilling to continue degrading the petrol. Too much of a nutrient is more likely the cause than too little of a nutrient as the control plants grew much more in the fourth check than the plants in the eggshells. Since this shows that in the control group there was enough nutrition for the plants to grow well and the only difference between the two groups was the addition of eggshells that supply additional nutrients, it is much more likely that a slowed growth rate was due to excess nutrients rather than too few nutrients. This suggests that there may have been a lot of microbial activity in the soil that would have caused the eggshells to decompose faster and therefore release a more nutrients in the two weeks between checks.

The images taken of the plants on the first check compared to those on the third and fourth check support this idea, as there are noticeably fewer, smaller eggshell pieces in the photos from the third and fourth check. There is also a more significant difference between the photos of the eggshells in the third and fourth check than the first and second which corresponds with the time periods where the growth of plants in the eggshells slowed down. This suggests that the eggshells decomposed faster in the time period between the third and fourth check meaning they released more nutrients and most likely excess nutrients that slowed the plant growth rate. If this is true, there is a possibility that the eggshells are still stimulating hydrocarbon degrading microbes and working efficiently to bio remediate the soil despite the fact that the growth rate of the plants has slowed. Another reason for the dramatic change in growth rate may have been due to the changing weather as this experiment began during the start of winter so the temperature became colder as the weeks went on which could have made it harder for the plants to grow. This possibility means that further trials need to be conducted to confirm that what was observed was only a result of the eggshells.

The coffee grounds also remediated the soil quite well, since the average plant height throughout the 8 weeks was only a small percentage less than the control. During the first 6 weeks the plants in the coffee grounds grew relatively slowly (as shown by the relatively flat line in graph 1), growing by about 34% of the average seedling height in the first check compared to 425% in the control. In the fourth check, as shown in graph 1, the plants in the coffee ground samples grew significantly more than in previous checks (by about

57% as compared to 20% in the second check and 1% in the third check). This may have been because coffee grounds decompose slower than the other two materials meaning it might have only begun to release nutrients after the third check. The sudden increase in growth rate after 8 weeks may indicate that coffee grounds are in fact very good bioremediators but only after around 6 weeks as the coffee grounds may only start to decompose and release nutrients after that time period. However, this conclusion can only be verified by doing further tests as the results may have been affected by other conditions and it is unknown if the coffee ground will continue this trend after 8 weeks.

Surprisingly the banana peels were not very effective bioremediators. After the 8-week period the average plant height of the seedlings grown in the soil with banana peels was the same as those grown in the soil with only oil. However, this may not necessarily mean that banana peels are ineffective bioremediators since in the first 6 weeks the average plant height of the plants grown in the soil with banana peels increased quickly and was substantially more than those grown in the soil with only oil. The plants grew very quickly in the two weeks between the second and third check (growing by 132% as compared to 122%).

The average height of the plants growing in the banana peels decreased dramatically in the fourth check (by 100%) making the average height equal to that of the plants growing in oil. A possible reason for this may be because the banana peels were relatively large and may have blocked the plant roots and prevented the growing plants from extending their roots to support their growth. The banana peels may have also made drainage difficult and for the plants to access water through their roots. To conclude whether this is true or not, more repeats of this experiment where the banana peels are cut smaller need to be conducted. Another reason for this dramatic decrease may be because the banana peels decomposed quickly and released too much of a certain nutrient therefore killing the plant. Following the same logic as discussed previously with the eggshells, this may mean that there were high levels of microbial activity and therefore hydrocarbon-degrading microbes in the soil meaning the banana peels did help to remediate the soil despite the fact that the average plant height was not consistently higher than those in the oil group. This means that the banana peels may work more efficiently in soil contaminated with a higher percentage of oil or that they would work more efficiently in this experiment if a smaller quantity were added or if they were removed after a shorter amount of time. However, more testing needs to be done to confirm whether the data collected was due to other, irrelevant factors (such as weather) and whether the trend will be the same after 8 weeks.

An interesting pattern to note is that in the fourth check, both the average plant height and the amount of plants increased or decreased substantially for all groups. The number of plants decreased for all groups in check 4. This suggests that this trend may have been due to environmental factors such as the cold, wind or rain, which all occurred in the time period between the third and fourth check. It is unlikely that this trend occurred as a result of the materials added to remediate the soil, as the nutrients available for the plants in each group were very different meaning the change in the number of plants would have been different for each. This means that the data collected from the fourth check may have been invalid, as other factors such as the weather might have caused the results. Since external factors may have affected the data collected in the fourth check, the data in graph 1 for the fourth check may be invalid too. If the data from the fourth check is disregarded as a result, the banana peels can be ruled as the second most effective bioremediator followed by the coffee grounds. However, once again, for this conclusion to be confirmed more trials need to be carried out.

All of the groups that were contaminated with oil, including those with materials added as bioremediators, did not have as many plants as in the control group. This may be because the seeds were added to the oil contaminated soil when the materials had not been given time to remediate the soil so many of the seedlings that grew from the seeds died before they grew above the surface. As a result, comparing the different groups using the data in graph 1 may be better and more accurate than comparing the different groups using data from graph 2. This trend may also mean that none of the materials removed all of the oil so many of the plants still died from the pollution. In the future, this investigation could be replicated with different percentages of oil and remediators to evaluate which ratio of remediator and oil works the best.

By comparing the photos taken and the observations of the plants growing in the control group and the plants in the other group it can be seen that the plants in the control group generally had a larger leaf area and, in the fourth check, more stems than those in the other groups. The plants growing in the eggshells were the only plants that grew multiple stems by the fourth check. This could indicate that by the fourth check (8^{th} week) the plants grown in the eggshells had enough nutrients to grow more stems meaning that by the 8^{th} week the eggshells had remediated a substantial amount of the oil. All of the plants grown in oil-contaminated soil had relatively smaller and fewer leaves than the plants grown in the control group. This suggests that petrol pollution in soil has an effect on the size and amount of leaves in plants. It also suggests that in each group where oil and a bioremediator was added to the soil, there was still oil remaining at the end of the 8^{th} week which prevented the leaves of each plant from growing as large as those in the control but not so much oil that the plants died like those grown in the soil with only oil did. Based on this information it can be concluded that all 3 materials remediated the contaminated soil to a certain degree.

The experiment I conducted was reliable as I repeated trials of each different material in the soil three times and in all of the checks each pot followed the same trend as the others in the same group and had seedlings of approximately the same average height. For example, the average plant height in all coffee ground pots in the third check increased by around 50% in the fourth check. I also checked the seedlings four different times at regular intervals and the trend across these four weeks remained constant. Also, by observing the plants over a long period of time (8 weeks) the data produced is more trustworthy as it allows us to see whether the observed trends will remain the same or change. For example, the average plant height of plants grown in the banana peels dramatically decreased in the fourth check. If the fourth check had not been conducted it would be assumed that the banana peels would continue to make the average plant height increase as it had done in previous weeks.

This investigation may have been slightly invalid and inaccurate as plants were used to reflect the efficiency of bioremediators instead of proper soil tests, which were too costly and inaccessible. The plants may have been affected by other environmental factors, such as weather, meaning the obtained data was not reflective of the bioremediators added but due to other factors. However, since all groups were put in the same environmental conditions, invalidity can be overcome by comparing the results from each group to other groups instead of simply analysing the raw data.

A difficulty that was encountered was finding a way to ensure all seeds that were planted were viable so that all plants had the same chances of growing. To overcome this, the seeds were germinated for a period of three days and only the germinated seeds were planted. By doing this, the results are more valid because it was ensured that if seedlings were observed to not grow it was not due to the independent variable, not that the seeds were not viable. Another difficulty was the effects of the winter weather on the plants. If the weather killed the plants then it would not be possible to obtain results. To overcome this, the weather forecast was frequently checked and appropriate changes were made to ensure the plants were protected. For instance, when there was rain or wind the plants were moved undercover and when the temperature became too low the plants were covered to help them maintain warmth. However, the plants were still partially exposed to cold, wind and rain as such conditions replicate the conditions of real life applications and ensure that the tested bioremediation methods are still viable in real life applications where weather cannot be controlled. Another difficulty was ensuring that the measurements taken of each plant were accurate as it was difficult to manipulate the measuring type to be inside the pot and to follow the same shape as the plant then measure each correctly. To overcome this, each sprout was measured multiple times to ensure the measurements were correct and it was ensured that the measuring tape began at the 0cm mark and that each plant was measured to the same point (the highest point of the leaves).

There are various improvements that can be made to this investigation. In this experiment, plants were used to show how effective the materials were at remediating soil since the required soil testing was very expensive and due to safety laws, inaccessible. Carrying out Total Petroleum Hydrocarbon (TPH) tests, soil nutrient tests and analysing the microbial activity in the soil in a laboratory would make this investigation more accurate and valid since the observations made of the plants may not have been solely due to the soil and the materials added. With such data the relationship between soil nutrients and microbial activity can be further investigated. Also, the soil could be kept in a greenhouse where environmental conditions are monitored and kept constant so that the results obtained are valid and not a result of external factors. Another improvement could be to conduct this experiment both in the summer and winter and compare the results from both trials as this could allow us to eliminate or identify where environmental factors affect the data. It would also allow further investigation on the effects of temperature on bioremediation rates.

This investigation could also be improved if more accurate and advanced equipment is used to measure the seedlings. By using digital, computer operated measuring tools, instead of a measuring tape, the plants can be correctly measured without there being chance that the measurements are incorrect as a result of the measuring tape being held incorrectly (e.g. the starting point of measurement not being 0cm) and ensures that the soil and plants are tampered with less in an attempt to correctly hold up the measuring tape to the plant. This also reduces the chance that the measurement may be read or interpreted incorrectly. Also, using measuring equipment with a smaller limit of reading could ensure the data collected is more accurate.

This investigation has highlighted many areas that require further investigation. In general, more repeats of this experiment need to be carried out over a longer period of time to ensure that the data obtained was accurate and reliable since the trends that were seen from this investigation may not be the same in repeated experiments as other factors like the weather could have affected the data. Further investigations could include changing the relative amount of oil and the material to see which ratio of oil to bioremediator is the most efficient. The data obtained of the average plant height of plants grown in coffee grounds indicate that it may be more efficient and effective to use higher amounts of coffee grounds while the results obtained from the plants grown in banana peels indicate that it may be more effective to use fewer banana peels. Further investigations could also investigate whether combining two or more of the materials are more or less effective. Also, these materials could be trialled on different types of soil contaminated with different types of oil so as to investigate whether the materials work efficiently on all types of oil contamination in soil.

The results from this investigation could be used to support future investigations on the possibility of using banana peels, eggshells and coffee grounds to bioremediate oil polluted soil. Also, these results could be used to investigate the effects of different types of nutrients on the rates of bioremediation. The materials investigated in this investigation could be used in real life applications to remediate polluted soil however more research needs to be done to evaluate and fine tune the most effective ratio of oil to material. Based on the data, eggshells can efficiently bioremediate petrol contaminated soil (for instance, near gas stations) with the ratio of eggshells to oil being 16:13. This investigation could also be used to inspire other investigations using similar, organic fertilisers to bioremediate soil.

CONCLUSION

Based on what that has been observed and discussed above it can be concluded that banana peels, eggshells and coffee grounds can all be used to bioremediate petrol contaminated soil. The material most efficient at remediating oil in the ratio used in this experiment (material to oil, 16:13) is eggshells followed by coffee grounds and banana peels.

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