Czech University of Life Sciences Prague

Faculty of Environmental Sciences

Department of Land and Water Management


# Effects of Soil Properties on Plastic Degradation

MSc. THESIS

Author: Kelli McGarry

Supervisor: Ing. Martin Kočárek, Ph.D

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## Declaration

I certify that work presented in this thesis is, to the best of my knowledge and belief, original, except as acknowledged in the text, and that the material has not been submitted, either in whole or in part, for a degree at this or any other university.

Prague, April 19, 2014 Kelli McGarry

# Abstract

The production of synthetic polymers, or plastic, has been steadily increasing over the past few decades. Plastic is a favorable material due to its durability which provides opportunity for a production of a multitude of various products. Consequently, the demand for plastic products creates an immense output of waste where a majority is emitted into the environment ultimately causing detrimental environmental conditions (Sivan, 2011). To combat this issue, different methods of plastic removal and safe plastic degradation has been researched and applied. Biodegradation of plastic, is an especially promising method because it focuses on the natural process such as mineralization the products by microorganisms (Singh and Sharma, 2008). This work is an attempt at proving that soil factors as well as increased microbial activity of soil microorganisms can result in greater rates of plastic degradation in soil. Soil analyses as well as a microbial analysis were performed to study potential plastic degrading factors and features such as loss of plastic weight, microbial activity in soil and degradation ability comparison of plastic in different soil horizons were examined.



**Keywords:** Plastic degradation, soil microorganisms, synthetic polymers, soil properties

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# Index

# 1 Introduction

The discovery of synthetic polymers or more commonly known as plastics, during the early 20th century is said to be one of the most momentous turning points in chemical and material history. Since that time approximately 140 million tons of common plastic material has been produced and released into our world each year (Shimao, 2001). This concurrently, enabled the explosive growth of numerous synthetic polymer focused industries. Given the many beneficial characteristics in which plastics possess, such as its low-cost production price and diverse structural capabilities, this material creates the ideal foundation for production of thousands of everyday products that are essential to today's way of living. Although, within the past few decades, it has been found that the most preferred trait of plastic, durability, is paradoxically causing serious detriment to our environment (Sivan, 2011).

Plastic's durability allows for the material to remain in our world for hundreds of years, due to its ability to resist significant physical factors, which in turn creates a colossal negative impact on our lives, as well as marine and terrestrial habitats. To combat this issue, global efforts to find a solution to the problem have been being avidly conducted (Accinelli et al., 2012). One of the most promising methods being used today is biodegradation. In short, biodegradation can be constituted as a biochemical transformation of compounds in mineralization by microorganisms, which can be defined by change in surface properties of plastics, loss of tensile strength and/ or reduction in molecular weight (Singh and Sharma, 2008). Since the introduction of plastic biodegradation, many different approaches of this theme have been performed in hopes to yield the most efficient and environmentally safe results.

## 1.2 Aim of Study

The aim of this thesis work is to evaluate the affect of soil properties on degradation rate of polyethylene terephthalate (PET) and commercial bioplastics. Also, to evaluate the affect of inoculation of soil by fusarium and aspergilus fungi on the plastic degradation rate.

## 1.3 Hypothesis

My hypothesis for this thesis work is to prove that the degradation rate of plastics films is faster in soil inoculated by Fusarium and aspergilus fungi in compraision with non inoculated soil and also degradation rate of plastics films in topsoil is faster than in subsoil.

# 2 Literature Review

## 2.1 Plastics

Plastics are synthetic polymers which are highly resilient to microbial attack or deterioration (El-Shafei et al., 1997). What makes this man made material resistant to microbiological degradation, is its impressively stable long chain of polymeric molecules. Due to their diminutive time of presence in nature, evolution could not design new enzyme structures capable of degrading synthetic polymers (Shah et al., 2007). The plastics we use today are made from both inorganic and organic raw materials, i.e. carbon, silicon, hydrogen, nitrogen, oxygen and chlorine. Basic materials used for making plastics are extracted from crude oil, coal and natural gas (Seymour, 1989).

In addition to their chemical characteristics, synthetic plastic are considered favorable in the material world because of their lightweight nature, durability, generally low price and potential for various miscellaneous applications (Lee et al., 2013). Today, a significant fraction of those applications are made through a wide variety of petroleum-based synthetic polymers, which are produced worldwide to the extent of approximately 140 million tons per year (Shiamo, 2001). A particularly auspicious petroleum- based plastic to consumers, especially consumers of the EU is, polyethylene, with an average annual consumption of 100 billion units (Accineli et al., 2012). Through the years, synthetic plastics such as those used to create the plastic carrier bag in addition to others, have begun to rapidly replace natural materials in almost every area, resulting in plastics becoming an indispensable part of life. As described by Sabir, of The International News in the report, Plastic Industry in Pakistan, synthetic plastics are used in multitude of different everyday necessity,

"Synthetic plastics are extensively used in packaging of products like food, pharmaceuticals, cosmetics, detergents and chemicals. Approximately 30% of the plastics are used worldwide for packaging applications. This utilization is still expanding at a high rate of 12% per annum. In 1993, the total world demand for plastics was over 107 million tons and it was estimated about 146 million tons in 2000" (Sabir, 2004).

The most widely used plastics and generally most abundant used to produce the above products, are polyethylene (LDPE, MDPE, HDPE and LLDPE), polypropylene (PP),

polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PUR), poly (ethylene terephthalate) (PET), poly (butylene terephthalate) (PBT), and nylons (Table 1).

**Table 1** Uses of Synthetic Plastics (Vona et al., 1965).

| Plastic | Use |
|---|---|
| Polyethylene | Plastic bags, milk and water bottles, food packaging film, toys, irrigation and drainage pipes, motor oil bottles |
| Polystyrene | Disposable cups, packaging materials, laboratory ware, certain electronic uses |
| Polyurethane | Tyres, gaskets, bumpers, in refrigerator insulation, sponges, furniture cushioning, and life jackets |
| Polyvinyl chloride | Automobile seat covers, shower curtains, raincoats, bottles, visors, shoe soles, garden hoses, and electricity pipes |
| Polypropylene | Bottle caps, drinking straws, medicine bottles, car seats, car batteries, bumpers, disposable syringes, carpet backings |
| Polyethylene terephthalate (PET) | Used for carbonated soft drink bottles, processed meat packages peanut butter jars pillow and sleeping bag filling, textile fibers |
| Nylon | Polyamides or Nylon are used in small bearings, speedometer gears, windshield wipers, water hose nozzels, football helmets, racehorse shoes, inks, clothing parachute fabrics, rainwear, and cellophane |
| Polycarbonate | Used for making nozzles on paper making machinery, street lighting, safety visors, rear lights of cars, baby bottles and for houseware. It is also used in sky-lights and the roofs of greenhouses, sunrooms and verandahs. One important use is to make the lens in glasses |
| Polytetraflouro-ethylene (PTFE) | PTFE is used in various industrial applications such specialized chemical plant, electronics and bearings. It is met with in the home as a coating on non-stick kitchen utensils, such as saucepans and frying pans |

### 2.1.1 Thermoplastics and Thermosets

The synthesizing process of plastics can be conducted through polymerization (polyaddition or polycondensation) of small molecules and can be further classified into two groups i.e. Thermoplastics and Thermoset plastics. (Alauddin et al., 1995). Singh and Sharma (2008) elaborate on what constitutes a thermoplastic in their journal publication, Mechanistic implications of plastic degradation,

"Thermoplastics are linear chain macromolecules where the atoms and molecules are joined end-to-end into a series of long, sole carbon chains. The bi-functionality necessary to form a linear macromolecule from vinyl monomers can be achieved by opening the double bond and reaction proceeds by a free radical mechanism. Such type polymerization is known as addition polymerization"

As cited by Singh and Sharma (2008) in their above mentioned article; the way thermoset plastics differ from thermoplastics is by way of step-growth polymerization and cross-linkage of molecules. “Thermosets, under suitable condition’s allowing bi-functional molecules to condense inter-molecularly with the liberation of small by-products such as $H_2O$, HCl, etc. at each reaction step.” (Ghosh, 1990). During this time, the monomers experience some chemical changes upon heating and finally convert themselves into an infusible mass irreversibly (Zheng et al., 2005).

In regards to the physical properties of thermoplastics and thermosets, the main difference is heat and pressure resistance i.e. the ability to reshape and mold into another product. This ability or lack thereof ultimately dictates what type of product may be produced (Table 1.2 and 1.3).

**Table 1.2** Properties and uses of thermoplastics (BBC, 2014)

| Name | Properties | Principal uses |
|---|---|---|
| Polyamide (Nylon) | Creamy colour, *tough*, fairly *hard*, resists wear, self-*lubricating*, good resistance to chemicals and machines | Bearings, gear wheels, casings for power tools, hinges for small cupboards, curtain rail fittings and clothing |
| Polymethyl methacrylate (Acrylic) | Stiff, hard but scratches easily, durable, *brittle* in small sections, good electrical *insulator*, machines and polishes well | Signs, covers of storage boxes, aircraft canopies and windows, covers for car lights, wash basins and baths |
| Polypropylene | Light, hard but scratches easily, tough, good resistance to chemicals, resists *work fatigue* | Medical equipment, laboratory equipment, containers with built-in hinges, 'plastic' seats, string, rope, kitchen equipment |
| Polystyrene | Light, hard, stiff, transparent, brittle, with good water resistance | Toys, especially model kits, packaging, 'plastic' boxes and containers |
| Low density polythene (LDPE) | Tough, good resistance to chemicals, flexible, fairly soft, good electrical insulator | Packaging, especially bottles, toys, packaging film and bags |
| High density polythene (HDPE) | Hard, stiff, able to be sterilised | Plastic bottles, tubing, household equipment |

**Table 1.3** Properties and uses of thermoset plastics (BBC, 2014).

| Name | Properties | Principal uses |
|---|---|---|
| Epoxy resin | Good electrical insulator, hard, chemicals well | Casting and encapsulation, adhesives, bonding of other materials |
| Melamine formaldehyde | Stiff, hard, strong, resists some chemicals and stains | Laminates for work surfaces, electrical insulation, tableware |
| Polyester resin | Stiff, hard, brittle unless laminated, good electrical insulator, resists chemicals well | Casting and encapsulation, bonding of other materials |
| Urea formaldehyde | Stiff, hard, strong, brittle, good electrical insulator | Electrical fittings, handles and control knobs, adhesives |

Properties and uses of the thermoset plastics.

## 2.2 Synthetic Plastic Classification

The ability to categorize the numerous forms of synthetic plastics that exist today has become an imperative task for today's society. Due to present manufacturing technology, plastic products of completely different chemical make-up can look virtually identical. This can transpire many problems for producers and consumers during recycling phases, while trying to properly differentiate and sort plastics. In order to combat this issue, classification systems have been created to help bring greater holistic awareness to producers and consumers (Society of Plastic Industry, 2014).

### 2.2.1 ASTM D7611

One classification system that is used world-wide today is the ASTM D7611 sorting system. This system was created together by United States Plastics Trade Associations, Society of Plastic Industry (SPI) and The American Society for Testing and Materials (ASTM), a global leader in the development and distribution of international voluntary consensus standards. The system accounts for seven different kinds of synthetic plastics, further separating into a numbering system which pertain to the specific type of plastic resin a given product predominantly consists of. The foreground for this system was originally created by SPI alone, and was simply known as the Resin Identification code, RIC. 2008 was the year SPI and ASTM came together to enhance the RIC, and ultimately

in 2013, the current ASTM D7611 sorting system was born (Table 2) (ASTM International Guide, 2013).

**Table 2** ASTM D7611 sorting system (ASTM International Guide, 2013).

| Resin | Resin Indentification Code-Option A | Resin Indentification Code-Option B |
|---|---|---|
| Poly(ethylene terephthalate) | 1 PETE | 01 PET |
| High density polyethylene | 2 HDPE | 02 PE-HE |
| Poly(vinyl chloride) | 3 V | 03 PVC |
| Low density polyethylene | 4 LDPE | 04 PE-LD |
| Polypropylene | 5 PP | 05 PP |
| Polystyrene | 6 PS | 06 PS |
| Other resins | 7 OTHER | 07 O |

## 2.3 Persistence

Widespread applications of plastics are not only due to their favorable mechanical and thermal properties but also mainly due to their stability, durability and persistence against environmental factors (Rivard et al., 1995). The chemical structure of synthetic polymers are quite certainly the driving force as to what makes the material so stable. It's persistence to overcome various environmental influences lies within their individual constituents, forming multiple molecule linkages to create an incredibly stable configuration. Stability is directly related to the plastics degree of cross linking and molar mass weight. The higher the degree of cross linkages between molecules means the higher the molar mass weight resulting in a greater level of resistance to deformation through heat and/ or physical force the plastic obtains (Reusch, 1999).

To validate this explanation, Polymer scientists Krevelen and Nijenhuis (2009) elaborate on the structure of the polymer within their written works, Properties of Polymers, fourth edition,

"The polymer molecule consists of a "skeleton" (which may be linear or branched chain or a network structure) and peripheral atoms or atom groups. Polymers of a finite size contain so-called groups, which do not form part of the repeating structure proper. Their effect on the chemical properties cannot be neglected, but their influence on the physical properties is usually small at degrees of polymerization as used in practice. Sometimes use id made of these groups to increase molecular weight" (Figure 1) (Krevelen and Nijenhuis, 2009).

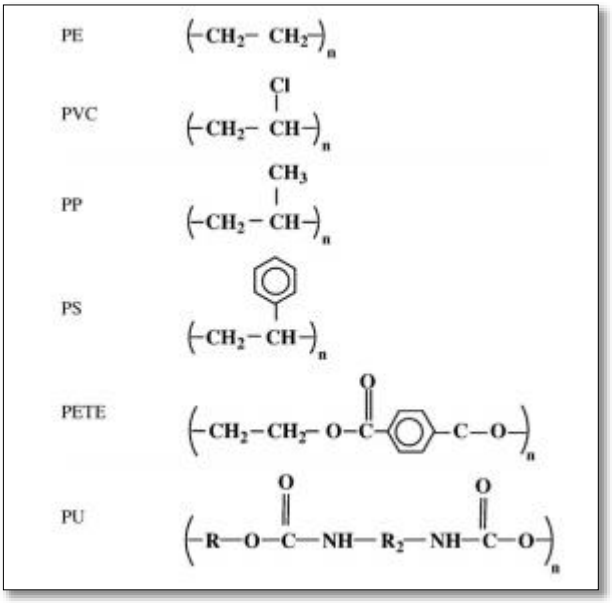


**Figure 1**. Chemical structures of conventional synthetic plastics (Shah et al., 2007).

## 2.4 Environmental Issues from Synthetic Polymers

The buildup of plastics in the environment is a matter of serious concern leading to long term environmental, economic and waste management problems (Singh and Sharma, 2007). Synthetic polymer products are extremely diverse, both in terms of chemical composition, properties and possible applications. Several hazardous substances may be released during the life cycle of a plastic product; and considering the large and growing global consumption of plastic products, and their negative effect on the environment, there is a greater need for assessment of these products (Pranamuda et al., 1997).

Methods to alleviate this issue have been conducted throughout the years but have failed to yield significant enough results to solve this massive problem. The most consumed synthetic polymer is polyethylene (PE) with a current global production of ca. 140 million tons per year. Due to lack of effective methods of safe disposal of plastic waste these synthetic polymers accumulate in the environment creating an increasingly dangerous ecological threat to terrestrial and marine life (Shiamo, 2001; Barnes et al., 2009). Accumulation of PE remnants has alone has created one of the most ongoing changes to the environment. According to Environmental scientists, Frias et al., 2010 and Teuten et al., 2009, in their written works, Organic pollutants in micro plastics from two beaches of the Portuguese Coast and Transport and release of chemicals from plastics to the environment and to wildlife, respectively,

"Within just a few decades, since mass production of plastic products has initiated, plastic debris has accumulated in the terrestrial and marine environments. These micro plastics can be ingested by various marine animals that, by mistake, identify the micro plastics as plankton. Thus, the ingested plastic debris is likely to penetrate and accumulate in the food chain, exerting multiple hazards that their outcome still have to be elucidated*"* (Frias et al., 2010; Teuten et al., 2009).

Another major driving force that also serves as a significant environmental problem is based on from what the synthetic polymers are created from. As mentioned earlier, some plastics are derived from raw materials such as crude oil, coal and natural gas and considering the amount of dependency our world has on synthetic plastics, there is a direct parallel with the need to obtain the materials needed for plastic production. Although some synthetic plastics are made from natural material, environmental issues such as resource depletion and resource extraction method effects are major consequences that must be carefully supervised. For example, natural gas fracking or otherwise known as, hydraulic fracturing in order to produce plastic has proven to be a highly detrimental process for the environment. Contamination of local water bodies, release of harmful VOC's into the atmosphere, scarcity of surrounding crops and death of nearby animals are all consequences of natural gas fracking used in producing synthetic plastic (Dong, 2013).

### 2.4.1 Bio-based and Biodegradable Bio-based Plastics

Out of the estimated 140 million tons of synthetic plastic being annually produced worldwide (Shiamo, 2001), there is also a constant rate of approximately 25 million of those tons, being directly or eventually amassed into our environment. Attributable to these estimates, there is a growing interest in the development of plastics that have less resistance against degradation or provide greater eco system services in comparison to that of conventional plastics (Lee et al., 1990). Bio-based and biodegradable bio-based plastics differ from conventional plastics because they are derived from renewable biomass feedstocks. The push to utilize and produce more plastic of these two categories comes from the desire to improve the sustainable use of the earth's resources, rather than exhaust the nonrenewable resources used in making conventional plastics (Gomez and Michel, 2013).

The use of bio-based plastic is shown to benefit the atmosphere because of its ability to lower $CO_2$ rates. As cited by Gomez and Michel (2013), the benefits of using plastic made from renewable biomass feedstock's and how the usage of this type of plastic yields advantage for the atmosphere is explained,

"These are called "bio-based plastics." On a balance this type of plastic offers a great potential to reduce greenhouse gases in the atmosphere by sequestering carbon. This is because atmospheric CO2 is fixed into the carbohydrates used as their feedstock. If the plastic is eventually landfilled, this carbon will become locked for millennia within the landfill and on balance reduce atmospheric CO2." (Weiss et al., 2012).

Biodegradable bio-based plastics are potential alternatives to non-renewable resource based plastics because of their ability to be incorporated back into organic recycling systems based on anaerobic digestion or composting (Gomez and Michel, 2013). Since these plastics possess the ability to be composted and/ or biodegraded, a completion of a pertinent missing link within the ecological cycle may be provided. An example from this group of plastics is (PHA), polyhydroxyalkanoates- based resin. An overview of PHA's and their physiological and engineering aspects has shown that PHA is suited for negative ecological impact products because of its good biodegradation properties (Reddy et al., 2003). Efforts to compensate for the apparent detriment caused by the persistence of conventional plastics in the environment through form of new plastics such as the above

bio-plastics are still being made. Overall positives and negatives are also continued to be postulated.

## 2.5 Degradation of Plastic

As referenced in the research paper, Biological degradation of plastics: A comprehensive review (Shah et al., 2007), Plastic degradation is defined as,

"Any physical or chemical change in polymer as a result of environmental factors, such as light, heat, moisture, chemical conditions or biological activity. Processes inducing changes in polymer properties (deterioration of functionality) due to chemical, physical or biological reactions resulting in bond scission and subsequent chemical transformations (formation of structural in homogeneities) have been categorized as polymer degradation*"* (Pospisil and Nespurek, 1997).

Attempts to produce, degradable, cost-effective plastic materials date back to the second half of the 20th century and their potential degradability and ultimate biodegradability began to be questioned during the early 1970's (Scott and Wiles, 2001).

One of the first controversial publications on this topic was written by Kavelman and Kendrick (1978), in their study, Degradation of a Plastic-Poly Epsilon-Caprolactone-by Hyphomycte, 1978, they begin by describing key preliminary hypothesis's regarding plastic degradation being thought out during this revolutionary age of polymer research,

"Natural polymers like cellulose, lignin, keratin and chitin are very subtle substances which do not break down when exposed to environmental extremes of temperature, radiation or moisture. Vertebrates, which needs these abundant compounds as carbon sources, have not evolved enzymes with which to digest cellulose and lignin, but must rely on bacteria and fungi to decompose these substances. Man, seeking as usual to improve on nature, is producing synthetic polymers in great profusion: about forty families of these giant molecules are now in widespread use. Synthetic polymers are organic materials, in many cases very similar to natural polymers. This means they could be susceptible to biodegradation." (Kavelman and Kendrick, 1978).

Fortunately, the foresights of Kavelman and Kendrick (1978) were proven to be accurate. Since the publication of their work, widespread studies on the degradation of plastics have been researched extensively. Countless studies have been performed and many have been

found to be successful through biological, chemical and physical methods. In addition, the methods for polymer degradation can now be further differentiated into different classifications, depending upon the nature of the degrading agents. These groups are photo-oxidative degradation, thermal degradation, ozone- induced degradation, mechanochemical degradation, catalytic degradation and biodegradation. From these base groups, discoveries of many other forms of degradation have branched. (Shiamo, 2001; Singh and Sharma, 2008).

### 2.5.1 Abiotic Degradation

Abiotic degradation includes the physical and/ or chemical processes that exerts intramolecular modifications in the polymer through forms such a hydrolysis, reduction or oxidation, to stimulate biodegradation (Sivan, 2011). For example, through the use of photo-oxidative degradation, low density polyethylene (LDPE) and polypropylene (PP) films can be activated using metal oxides as catalysts. Those materials require oxidative degradation in order to rude molar mass and to form oxygenated groups, which are more easily metabolized by microorganisms (Shawaphun et al., 2010; Koutny et al., 2006; Tuomela et al., 2002; Scott, 2000).

In a study conducted in 2003 by Stephen Bonhomme, abiotic degradation of environmentally degradable polyethylene was demonstrated. Abiotic oxidation was performed in an air oven, to simulate the effect of the compost environment and then in the prescience of selected organisms. Bonhomme (2003) elaborates his study in his written works, Environmental biodegradation of polyethylene, by stating,

"Through this study, it was determined that pre-ageing of either light or heat were proven to be crucial abiotic precursors to provide plastic bio-degradation. There was a clear reduction in molecular weight and abiotic peroxidation process is the rate determining step for biodegradation" (Bonhomme, 2003).

### 2.5.2 Mechanochemical Degradation

Breakdown of molecular chains under shear or mechanical force is often aided by a chemical reaction and is known as Mechanochemical degradation (Singh and Sharma, 2008). Mechanochemical degradation of polymers involves the degradation of polymer

under mechanical stress and by strong ultrasonic irradiations (Li et al., 2006). For example, as explained by (Ghosh, 1990),

"Mastication of rubber can lead to chain breakage and development of plasticity under shear. In the atmosphere of nitrogen at ordinary temperature, mastication of rubber does not change the plasticity and molecular weight appreciably, but in presence of oxygen, degradation occurs rapidly. This is due to the reason that the rubber molecule breaks into radicals, and oxygen as radical scavenger readily reacts with, leading to permanent chain breakage, whereas nitrogen is not a radical scavenger and thereby led to radical recombination."

The significant mechanism responsible for this occurrence has been explained by the interaction of ultra sound and component molecules. As cited by (Singh and Sharma, 2008), in Mechanistic implications of plastic degradation,

"Molecules, in a liquid and upon irradiation of ultrasound, have been exposed to alternate compression and expansion modes, by which bubbles are formed and inevitable collapse. On a molecular level, this is implication for a rapid motion of solvent molecules to which the macromolecules embedded in the solvent cannot be adjusted. Thus, friction is generated which causes strain and eventually bond rupture in the macromolecules" (Kim et al., 2002).

Lastly, it has been detected that at low temperatures, radicals from main- chain scission exist. Upon warming, the radicals attack the polymer matrix, creating more scission reactions by radical-rearrangement reactions (Figure 2) (Mark, 1986).

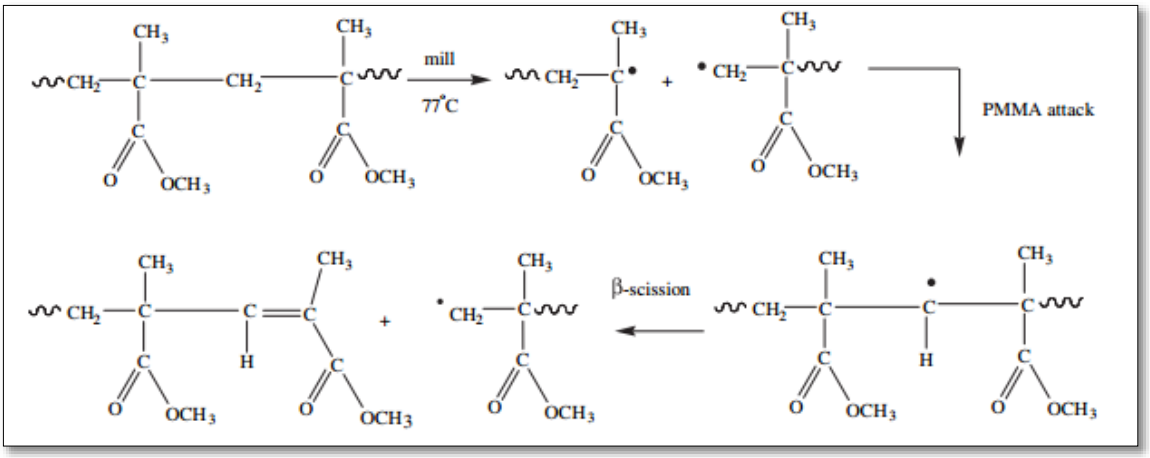


**Figure 2.** Radical-rearrangement reactions by carbon radicals (Mark, 1986).

### 2.5.3 Biodegradation

Biodegradation is a biochemical transformation of compounds in mineralization by microorganisms. Mineralization of organic compounds yields carbon dioxide under aerobic conditions, methane and carbon dioxide under anaerobic conditions (Singh and Sharma, 2008).

Through time, an accumulation of many different characteristics that which properly constitute and embody the term, "biodegradation" have been formed. Some prominent examples of today for characterization of biodegradation are, loss of weight, molecular weight distribution, change in tensile strength, change in dimensions, change in chemical or physical properties, carbon dioxide production, bacterial activity (Kathiresan, 2003; Kiatkamjornwong, 2001). Detection of biodegradation can also happen, if any of the above mechanisms were to be combined with one another.

According to ASTM standard D-5488-94d biodegradation is defined as,

"A process which is capable of decomposition of materials into carbon dioxide, methane, water, inorganic compounds, or biomass in which the predominant mechanism is the enzymatic action of microorganisms, that can be measured by standard tests, in a specified period of time, reflecting available disposal conditions" (ASTM book of Standards, 2005).

Successful methods for plastic biodegradation lie within proper selection of test procedure taking into consideration the nature of the given plastic and the climatic conditions of the study environment. Some examples of past methods used to study the biodegradation of plastic are pure culture methods, which focus on the inoculation of specific microorganisms under study to biodegrade plastic. Compost methods, where biodegradation is measured based on the amount of material carbon converted to carbon dioxide. There are also aerobic degradation methods that use the presence of oxygen with microorganism to break out the given plastic, and of course, soil burial methods, which is usually preformed under natural conditions, or laboratory conditions to simulate natural conditions (Singh and Sharma, 2008).

During performance of the above methods, many different factors that administrate the process of plastic biodegradation must also be considered. Factors pertaining to the nature of pretreatment, organism type and polymer characteristics, such as the mobility,

crystallinity, molecular weight, tactility, etc. are all extremely dynamic features of biodegradation (Artham and Doble, 2008).

## 2.6 Methods for estimation of biodegradability

In past years, it was determined that estimation of plastic waste biodegradability is most favorable under natural conditions, i.e. in soil or seawater but unfortunately, under these conditions, reproduction of results are often difficult to duplicate (Orhan and Buyukgungor, 2000). Recently however, given the series of past studies focused on biodegradation and the knowledge gained from this research, notable figures of multiple relative fields have been able to focus on factors that have been shown to yield the most valuable results. Thus providing more precise controls for estimation of biodegradation rates of plastic. In addition to this, a multitude of guides and which allow for more controlled monitoring and detailed assessment for future projects regarding biodegradation of plastic have also been created.

### 2.6.1 ASTM D5988-12

One recent method for estimation of plastic biodegradation was created by The American Society for testing and materials in 2012, ASTM D5988, or otherwise known as the Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in Soil. ASTM's methods for assessing degradability of plastic has been evolving simultaneously with general knowledge of the topic for over a decade, with one of their old publications on the topic dating back to 1992, when focus on degradation was just only based on undesirable changes in appearance and physical properties of plastics (Yanannavar and Bartha, 1994).

The results of the current test method, as stated in ASTM D5988-12,

"Permits an estimation of the degree of biodegradability and the time period over which plastics will remain in an aerobic soil environment. This test method also determines the degree of aerobic biodegradation by measuring evolved carbon dioxide as a function of time that the plastic is exposed to soil" (ASTM, 2012).

## 2.7 Soil burial method for plastic degradation

Through the use of soil conditions, the number of biodegrading microbial species and their population may be determined. Which strongly affects overall biodegradation (Kimura et al., 1994).

Multiple studies on the biodegradation of plastic through use of the soil burial method have been executed in the past. In fact, it is noted to be one of the most frequently used methods for the determination of plastic biodegradability (Yang, 2005). As stated prior, depending of the given plastic type, for example natural polymer, synthetic polymer, plastic containing additives, bio-based plastics, etc., different methods and climatic conditions of study environment should be altered to fit given circumstances (Artham and Doble, 2008).

As referenced by (Singh and Sharma, 2008), Luis Llorca (1993) explains the standard methodology for the soil burial method, in his work, Study of biodegradation of starch plastic films in soil using scanning electron microscopy,

"In this method, biodegradation test is performed under natural conditions or laboratory conditions. Sample with definite weight and dimension is buried in specific depth of soil for different time intervals. After a specified time, sample is taken out of soil, thoroughly rinsed with distilled water following immersion in distilled water and after that dried at 50C for 24 h in a vacuum oven. Sample is allowed to equilibrate to ambient temperature and humidity for at least 24 h. before measurement" (LLorca, 1993).

In a study conducted by Kathiresan (2003) of Annamalai University, Parangipettai, India, the biodegradation of polyethylene bags and plastic cups within two different mangrove zones colonized by *Rhizophora* sp. and or *Avicennia* sp. were examined in situ. After, a further look at degrading microorganisms were studied and cross examined. A relative research paper also written by (Kathiresan and Bingham, 2001), explains why the mangrove soils act as an ideal study environment for plastic degradation,

"The mangrove soils maintain moisture by tidal water flood during high tide and the soil gets heated during low tide when exposed to sunlight as well due to exothermic reactions of biological compounds in the soil. Besides these abiotic conditions, microbial counts are also high, perhaps favoring the degradation of plastics."

Biodegradation of both plastic sources were allowed to degrade naturally in the soil for a period of 9 months and were sampled at the intervals of 2, 4, 6 and 9 months. Degradation was determined in terms of percent of weight loss of the materials over time. Kathiresan (2003) represents the results of this study in his written works, Polythene and Plastics-degrading microbes from mangrove soil. As shown in Table 3 and explained below.

**Table 3** Biodegradation of polythene bags and plastic cups buried for different duration under two mangrove zones (Kathiresan, 2003).

| Month of analysis | Biodegradation (% weight loss) | | | |
|---|---|---|---|---|
| | *Rhizophora* zone | | *Avicennia* zone | |
| | Polythene | Plastic | Polythene | Plastic |
| 2 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 |
| 6 | 1.98 ± 0.29 | 0 | 1.74 ± 0.12 | 0 |
| 9 | 3.77 ± 0.29 | 0.17 ± 0.02 | 4.21 ± 0.31 | 0.25 ± 0.03 |

Values between months of analysis are significant at 5%, but non-significant between mangrove zones.

"As shown, polythene bags were not found to be degraded until after 6 and 9 months of incubation in the soil and no results were yielded during months 2-4. Plastic cups were found degraded only after 9 months, but during months 2,4 and 6 of the analysis. The biodegradation of polythene was maximum of 3.77% and 4.42% respectively under Rhizophora and Avicennia zones after 9 months of analysis, and the corresponding values for biodegradation of plastics were only 0.25% and 0.17%."

This study represents a good example of the soil burial method for plastic degradation because it is a practical example that allows for greater knowledge of how plastics degrade naturally in an in situ condition. To conclude his study, Kathiresan (2003) states his claim that "The plastic materials have been degraded in the mangrove soil irrespectively of the mangrove zones. This reveals that the mangrove soil can be a source of factors responsible for the degradation of plastic materials."

### 2.7.1a Soil moisture and Soil Temperature

Two factors that are almost always analyzed but most importantly have been proven to be imperative influential soil factors in these studies are soil moisture content and soil temperature. Many investigators have stated that the linkage between soil moisture

content and temperature is the allowance for greater or less microbial activity. Within the study, Fungal communities Associated with Degradation of Polyester Polyurethane (PU) in soil (Cosgrove et al, 2007), makes a relevant point to justify the significant difference in maximum loss of tensile strength between PU pieces buried under laboratory conditions for 1.5 months and within a in situ condition for 5 months. Essentially, it was explained that although the in situ plastic pieces were buried for much longer then the laboratory pieces, much of the in situ burial period was during the winter and early spring months, when soil temperatures and fungal activity are low and are likely to retard degradation (Cosgrove et al., 2007).

Another study that was successful in finding a correlation between the two soil factors and plastic degradation was conducted by Accinelli (2012) of the Department of Agro-Environmental science and Technology at the University of Bologna, Italy. In his work, Deterioration of bio-plastic carrier bags in the environment and assessment of a new recycling alternative, 2012. The subject of this study was the currently manufactured Mater-Bi (MB) starch based Bioplastic carrier bags (BCB) and its deterioration process through soil burial method in the laboratory and in situ during a period of 3 months. Data expressing the deterioration of BCBs are summarized in Figures 3a, 3b and 4. Through these results, it was found that degradation of BCB's proceeded rapidly in soil samples incubated under constant soil moisture and temperature, especially during the last month of incubation (Accinelli et al., 2012).

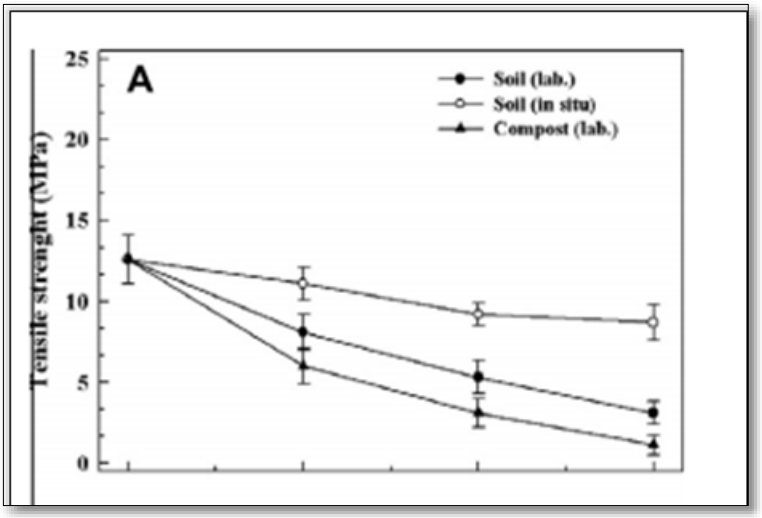


**Figure 3a** Tensile strength (Accinelli et al., 2012)

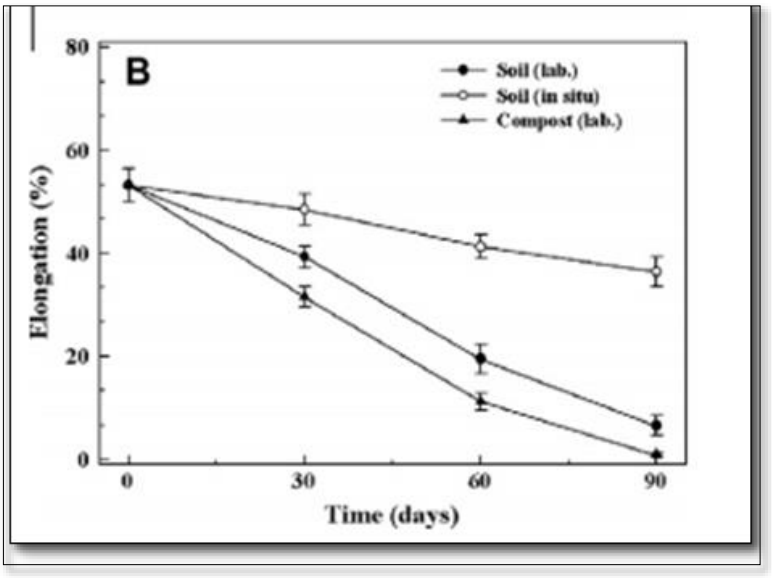


**Figure 3b** Elongation (Accinelli et al., 2012)

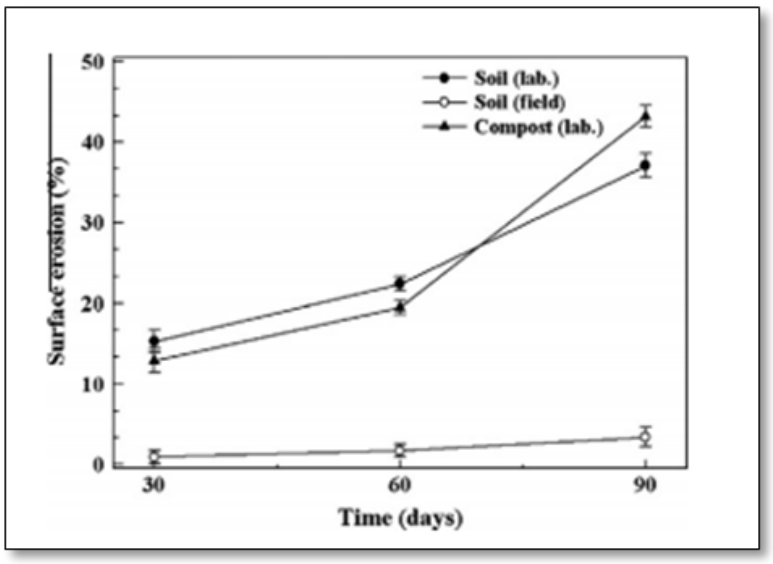


**Figure 4** Surface erosion (Accinelli et al., 2012)

## 2.7.2 Influential soil factors

Taking into considering the success and failure of past studies, there has been a noticeable trend of influential soil factors that have played a role in the manner and rate at which soil microorganisms degrade plastic during experimentation.

However, it has also been determined that soil factors which play part in the biodegradation of soil vary depending on the given plastic type (Artham and Doble, 2008). Therefore, it is important from the viewpoint of soil microbiology to determine

the biodegradability of plastics and microorganisms responsible for it in each soil condition for reaching the general conclusion on its biodegradability (Orhan et al, 2003).

### 2.7.3 Successful soil microorganisms

The discovery of influential soil factors during the study of soil burial methods of both laboratory and in situ conditions were able to provide significant knowledge in regards to successful soil microorganisms and their plastic degrading abilities. Studies that have focused on soil temperature and soil moisture were able to demonstrate that these two factors exert significant effects on the degradation of the plastics, and that microorganisms were mainly responsible for the degradation of plastics in soil, such as the study performed by Nishdie et al. (1999), the effects of soil conditions such as temperature and moisture, as well as anaerobiosis on degradation of biodegradable plastics PHB/ HV, PCL, PBSA, PBS were recorded. His results showed that due to alteration of different soil conditions (i.e. soils with a temperature of 30°C and soils at 52°C in both aerobic and anaerobic conditions) a significant change in fungal existence took place. This study suggested that fungi were mainly responsible for the degradation of the plastics PHB/HV, PCL, PBS and PBSA in soil, and that microorganisms responsible for degradation were different depending on the kinds of plastics and most importantly, soil conditions (Nishide et al, 1999). A list of strains are represented by Table 4 and the degradation rate of the plastic in terms of percentage of weight loss is represented by Figure 5.

**Table 4** Relative abundance of fungi on degraded plastics and identification of the plastic-degrading microorganisms (Nishide et al., 1999).

| Plastics | Temperature | Microscopic observation | Identified microorganisms as plastic degraders |
|---|---|---|---|
| PHB/HV | 30°C | Fungi+++* | *Mucor* sp. |
| | 52°C | Fungi+ | *Talaromyces* sp., an actinomycete strain |
| PCL | 30°C | Fungi++ | *Paecilomyces* sp. |
| | 52°C | Fungi++ | *Thermomyces* sp. |
| PBSA | 30°C | Fungi++ | *Cunninghamella* sp., *Aspergillus* sp. |
| | 52°C | Fungi++ | *Thermomyces* sp. |
| PBS | 30°C | Fungi++ | not isolated |
| | 52°C | Fungi++ | not isolated |

*Relative amount of fungal mycelia on the films. +: present, +++: abundant.

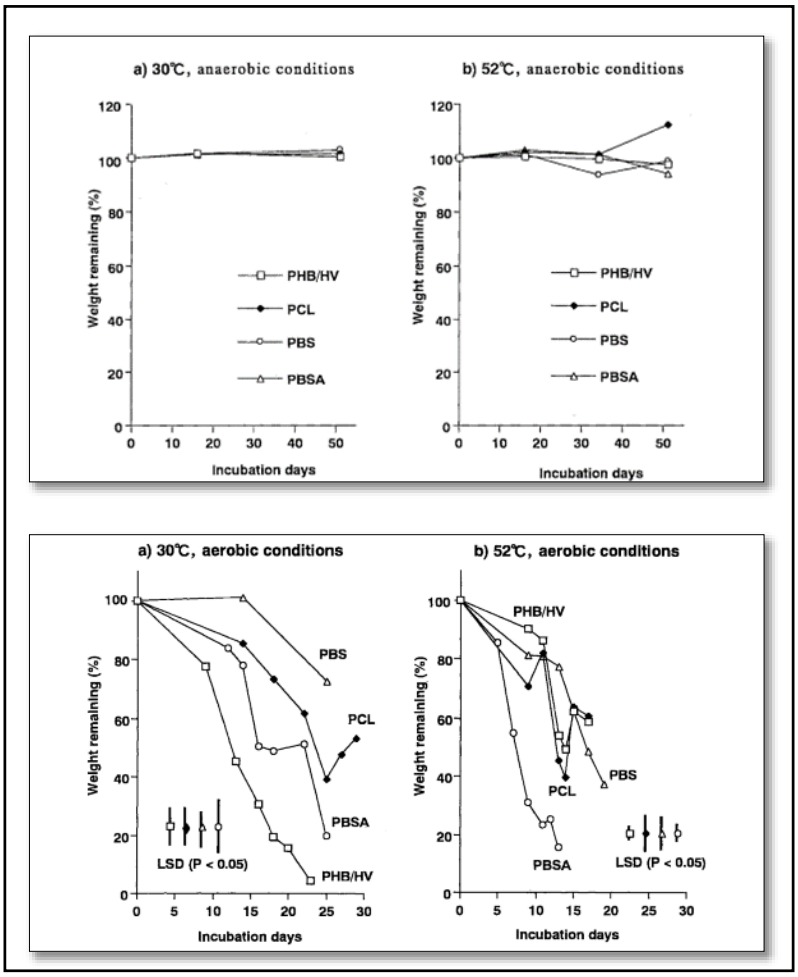


**Figure 5** Degradation of plastic under aerobic and anaerobic conditions (Nishide et al., 1999).

In other studies, some strains of bacteria such as Pseudomonas aeruginosa, Pseudomonas fluorescens and fungi Penicillium simplicissimum have been reported as the most commonly use organisms for plastic degradation (Norman et al, 2002; Tadros et al, 1999).

For example, according to Shimao, 2001, polyvinyl alcohol (PVA), a polymer with some special features in its structure and characteristics, is the only vinyl polymer known to be biodegradable and almost all reported strains able to degrade PVA belong to the Pseudomonas Genus. The pathway was proposed to degrade PVA first by the action of dehydrogenase to yield polyvinyl ketene or carboxylate termini (Figure 6) (Shiamo, 2001).

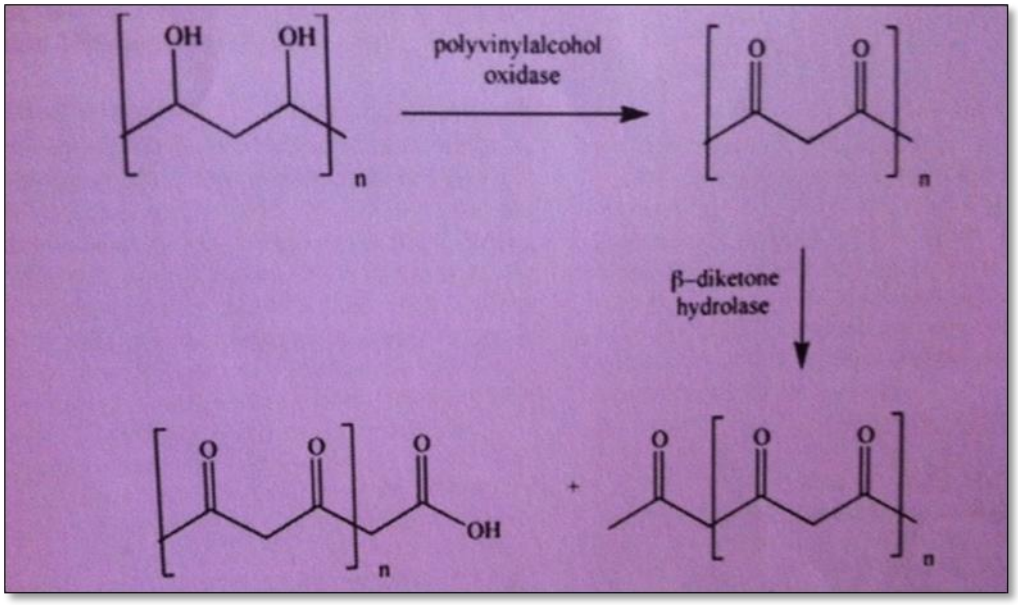


**Figure 6** PVC degradation of Pseudomonas (Eubeler, 2010).

# 3 Methodology

## 3.1 Soil collection

Soil chosen for this thesis work was Haplic chernozem soil, which consists of 24.4% sand 56.3% silt and 19.3% clay and also possess a ρs (g cm−3) of 2.52 (Kodešová et al., 2011), which was retrieved from the Czech University of Life Sciences practice field, in Praha suchdol on September 23, 2013. The conscious decision to incorporate chernozem into this project was due to its range of soil organic matter content, which could potentially increase the speed of microbial degradation on the plastic. The soil was then separated into topsoil (horizon 0.00-0.30 m) and subsoil (0.31-0.60 m) and then dispersed evenly into 50, 23cm Vulcanic Milano ceramic planter pots, model 2343J, purchased from Pirson Keramika Service, Czech Republic. 25 vessels were used to hold subsoil and the remaining 25 vessels were used to hold topsoil.

## 3.2 Plastic weighing

This project utilized two different types of synthetic plastics. The focal point plastic being Low density poly ethylene (LDPE) and a commercial bio plastic to compare potential rates of degradation. For the plastic weighing portion, the Mettler Toledo XS105 scale was used to find precise unit weight of the plastics. The Low density polyethylene as well as the commercial bio plastic were cut into one-inch square pieces, then separated accordingly to which predestined vessel the plastic pieces were to be placed.

## 3.3 Plastic placing into vessels

For each vessel, six plastic pieces were placed. Three being LDPE and three being Bio plastic. Each piece was strategically placed approximately five inches deep into the vessel and approximately one-inch apart. Pieces were then buried and placements were marked onto the vessel for future retrieval for post burial plastic weighing.

## 3.4 Microorganism collection

The microorganisms used during this project were bacteria CCF 2967 *Fusarium solani* and fungus CCF 3264 *Aspergillus niger*. Both samples were retrieved from the Culture Collection of Fungi at Charles University, Prague. These microorganisms are well recognized soil microorganisms, which possess different species within each genius that have shown to be influential factors in past projects relative to plastic degradation i.e. (Kavelman and Kendrick, 1978, El-Shafei et al, 1997, Kathiresa, 2003, etc.). Thus, the reasoning behind this particular selection.

### 3.4.1 Cultivation of Microorganisms

For the cultivation of microorganisms, the use of agar medium HIMEDIA Oatmeal Agar for cultivation of *Fusarium solani* and the use of HIMEDIA Potato Dextrose agar for cultivation of *Aspergillus niger*. 40 Petri dishes of cultivated microorganisms were produced. 20 of *Fusarium solani* and 20 of *Aspergillus niger*.

### a) Petri dish preparation and inoculation

For the preparation of both agars, the necessary materials needed to complete this process is represented in the table below.

| Materials | Oatmeal | Potato dextrose |
|---|---|---|
| Distilled $H_2O$ | 400 mL | 400 mL |
| Given Medium | 29 g | 15.6 g |
| Agar | 2 g | 2 g |

As for the process, the components for each agar were combined within two 500 mL glass Erlenmeyer flasks, one for each agar. Solutions for mixed for approximately 5 minutes and then sterilized in autoclave at 100kPa for 20 minutes. After sterilization, 15-20 mL of cooled agar solution (at approximately 40-50°C) was poured into the forty Petri dishes and were left in room temperature for solidifying.

### 3.4.2 Inoculation of soil

As mentioned prior, the soil used in this project were separated and dispersed into 50 vessels, 25 vessels containing topsoil and 25 containing subsoil. Out of the 25, vessels were than divided once more, 8 serving as control, 8 as vessels inoculated with *Fusarium solani* and 8 inoculated with *Aspergillus niger*. Due to unfortunate events, the first attempt of soil inoculation was only able to provide two vessels with microbial inoculum, thus validating the prior scheme of soil separation.

For the inoculation of soil, each Petri dish with cultivated microorganisms was washed with 15 mL of saline with Tween 80. Approximately 1 Petri dish was used as source of inoculum per one vessel, which received 10 mL of microorganism inoculum. Next, the layer of soil above plastic pieces within the vessel was removed and separated into two parts. One part was saturated with 7 mL of microorganism inoculum and then placed back onto the buried plastics. The remaining part of soil, was saturated with 3 mL of inoculum, then replaced subsequently over the first layer of soil.

### 3.4.3 Plastic removal from vessels

Plastic removal from vessels took place approximately 4 months after soil inncoulation. Six pieces of plastics (three pieces of LDPE and three pieces of bioplastic) from each mediums topsoil and subsoil horizon were taken out of two vessels from each medium, resulting in 36 plastic pieces taken for weighing. All Pieces were washed thoroughly by hand with distilled water and then dried in autoclave for 24 hours at 50°C.

## 3.6 Soil analysis

All analysis of soil used in this project were performed at the Czech University of Life sciences (CZU) within the Department of Agrobiology Soil Science and Soil Protection. Soil pH, soil salinity, soil organic matter, soil organic carbon, particle density and soil texture were determined. Both inoculated soil and non-inoculated soil (control) were subject to analysis and then compared and preformed 3x to yield sufficient results, excluding particle density and soil texture determination, that which only analyzed control soil. Prior to all analysis, soil samples were air dried and sieved through their respective sized sieve, according to the analysis being performed.  All soil analysis

methods used were based on the laboratory methods of past analysis. Additional knowledge was also obtained by colleagues at CZU.

### 3.6.1 Soil pH

pH determination was examined by analyzing soils pH_ $H_2O$ and pH_KCL. Determination of pH_ H2O began by placing 10 g ± 0.05 g soil sample (fine earth < 2mm) into a 50 ml beaker and then adding 20mL of deionized water into the beaker as well. Deionization of the water was achieved by boiling and then cooling to room temperature to rid water of $CO_2$ . Solution was than mixed for 5 minutes with glass stick and finally, pH readings were measured using the inoLab pH Level 1 Meter. Determination for pH_KCl also used 10 g ± 0.05 g soil sample (fine earth < 2mm) but was instead placed into 50mL plastic bottles, to be placed in centrifuge. 20mL of 1 mL KCl was added to the bottles and then was shook for 60 minutes on the shaker. After 60 minutes, bottles were then centrifuged for 5 minutes and pH measurements of the solution were also gained using the inoLab pH Level 1 Meter (ISO 10390, 1994).

### 3.6.2 Electric conductivity

To determine electric conductivity of soil samples, 10 g ± 0.05 g soil sample (fine earth < 2mm) were placed into 100 mL plastic bottles and then 50 mL of 50% of metanol was added. Solution was then shook on shaker for 120 minutes, after time elapsed, electrical conductivity of solution was measured using the inoLab Conductivity meter (Hendershot et al. 1993).

### 3.6.3 Soil organic carbon (SOC)

In order to determine SOC, 0.2-0.4 g of soil sample (fraction <0.25 mm) was placed into 50 mL beaker, and combined with 10 mL solution of dichromate + sulphuric acid. For factor determination, 3 blank samples were also made. All samples were placed into oven for 45 minutes at 125°C. After baking, samples were allowed to cool down and then were carry out by the process of titration.

Calculation used for factor determination by titration was:

$$f = \frac{40}{a}$$

Where $a$ was an average consumption on the blank samples.

The oxidable organic matter content was counted by the following equation:

$$Cox = (12 - 0.3\ S\ f)\ \frac{100}{N}$$

Where S is consumption on soil sample and N is the soil sample weight [mg] (Skjemstad and Baldock, 2008).

### 3.6.4 Soil organic matter quality

SOM was determined spectrophotometrically by the use the spectrometer, from the labs within the Department of Agrobiology Soil Science and Soil Protection at CZU. To start, 2 g (± 0.05 g) of soil sample were placed into 50 mL plastic bottles and then combined with 40 ml 0.05M of tetrasodium pyrophosphate ($Na_4P_2O_7$). Solution was then shook for 60 minutes on shaker followed by centrifuge of 11 000 RPM for 3 minutes. Liquid was then poured into glass tubes and used for the determination of black. Blank solution was used to determine baseline (threshold) for the measurement, if following samples exceeded the threshold, sample was diluted with additional ($Na_4P_2O_7$) and then measured again. Absorbance (A) at 400nm and 600nm was observed and later expressed mathematically as:

$$Q\ 4/6 = \frac{A400}{A600}$$

The quality was then expressed as humic acids (Ha) and fulvic acids (FA), which was represented as:

$$HA/FA = 17.2\ Q4/6^{-2.19}$$

(Looppert and Suarez, 1996).

### 3.6.5 Particle density

To find particle density, 10 g ± 0.001g of soil sample were placed into metal containers and combined with distilled water. Solution was then boiled on hot plate and mixed with glass stick until soil particles were sufficiently dissolved. As soil was being boiled, six glass pyknometers were being prepped for the determination. First, the pyknometers were filled with water and placed into a water bath with no stopper to equilibrate for approximately 20 minutes at 20°C. After 20 minutes, stoppers were placed into corresponding pyknometers and weighed. Water was then removed from pyknometer and quantitatively filled with the boiled soil sample solution, making sure there were no losses to ensure that all particles were included. Pyknometers filled with soil sample were then allowed to equilibrate without stopper in water bath for approximately 20 minutes. After 20 minutes, stoppers were once again placed into corresponding pyknometer and weighed again. Calculation of particle density of the soil sample is represented as:

$$P = \frac{n}{n + pwater - psoil}$$

Where, $n$ is the soil sample weight, pwater is the weight of pyknometer filled with water and $p\ soil$ is the weight of the pyknometer filled with soil sample (Flint & Flint 2002).

### 3.6.6 Soil texture

Soil texture determination was found by weighing approximately 50-70 g of soil sample and placing into metal containers. 10 mL of distilled water mixed with 10mL of HMP was added to every 10g of soil sample and then solution was boiled for 30 minutes, until sufficient soil particles were dissolved. Solution was allowed to cool down and was then added to 1000mL glass graduated cylinders. One filled with topsoil solution and the other with Subsoil solution.

With use of the hydrometers borrowed from the Department of Soil science and Soil Protection at CZU, readings as well as temperature after 30 seconds, 1 minutes, 2 minutes, 5 minutes, 15 minutes, 45, minutes, 2 hours, 5 hours and finally 24 hours were taken and then compared (Gee & Or 2002).

## 3.7 Microbial analysis: Colony Forming Units determination

Colony Forming Units, CFU, is an estimate of viable bacterial or fungal numbers. Unlike direct microscopic counts where all cells, dead and living, are counted, CFU estimates viable cells (Wikipedia, 2014). Determination of CFU was performed in order to realize how abundant the microorganisms of bacteria *Fusarium solani* and fungi *Aspergillus niger* remained in the test soils, even under undesirable conditions. Analysis was performed on Topsoil, Subsoil and control soil and preformed times to obtain more solid results, totaling in 18 samples. All knowledge of analysis were based on personal accounts gained from Prof. Ing. Karel Vorisek, CSc. of the Department of Microbiology, Nutrition and Dietetics in the Fakulta agrobiology, at the Czech University of Life Sciences, Prague.

### 3.7.1 Preparation of materials

During the preparation stage of the analysis, three mediums were created; NaCL for dilution rate, medium for fungi, and medium for total bacteria count to be used for 18 samples. Commercial product, Thornthon agar, Producer: Léčiva Praha, Czech Republic used in creation of medium for total bacteria count and Potato Dextrose Agar, Producer: HiMedia Mumbai, India used in the creation of medium for fungi count, were both obtained from the Department of Microbiology, Nutrition and Dietetics in the Fakulta agrobiology, at the Czech University of Life Sciences, Prague. Materials and method are represented by Table 5.

**Table 5** Materials and Methods

| Media | Materials | Method |
|---|---|---|
| Dilution rate | a. NaCL solution<br>b. 16 sterilized Erl. flasks (volume 200-300mL)<br>c. 80 sterilized glass tubes | a. 2500mL dist. water + 22.5 NaCL<br>b. 90mL of NaCL solution added to each flask + glass pearls+ cellulose stopper<br>c. 10 ml of NaCL solution added to each glass tube |
| Medium for Fungi | a. 5 Erl. flasks (Volume 500mL)<br>b. Distilled water (300ml)<br>c. Streptomycin (3mg/100mL of medium) | a. 11.7g of Potato Dextrose agar added to each flask + aluminum cover<br>b. Added to gar<br>c. Added to medium later |
| Medium for total bacteria count | a. 5 Erl. flasks (volume 500mL)<br>b. Distilled water (300mL) | a. 4.1g of Thornton agar + 1.5. agar base +aluminum cover<br>b. Added to agar later |

## 3.7.2 Preparing of the media

The two main components that made up the microbial analysis was first, the process of soil dilution and second, inoculating of the petri dishes. However, prior to preforming these tasks, it was necessary to complete the procedure of preparing of media. This process began with weighing the recommended amount of medium for each sample. Second, adding the appropriate amount of distilled water and then mixing thoroughly. Next, pH was controlled and adjusted to appropriate level, followed by sterilization of the medium at 100kPa for 20 minutes. Last, medium was allowed to cool 50°C before pouring into petri dishes.

**a) Soil Dilution**

For soil dilution, it is necessary to first sterilize the mixing spoon and weighing vessel with ethanol. I then weighed 10 g of the given soil sample and moved the soil carefully

into its respective flask with 90 mL of saline. It was then shook on the shaker for 20 minutes. Next was the marking of the glass tubes with symbols from x/2 to x/6, there x was the number of the soil sample. For example, for soil sample number 1, test tubes were marked as: 1/2, 1/3, 1/4, 1/5, 1/6. Following this step, 1 mL of solution from flask was taken via pipette and aseptically transferred to the first glass tube (x/2), the tube was then mixed for approximately 30 seconds. Using a new pipette, 1 mL from dilution x/2 was transferred into second glass tube (x/3), this tube was then also shaken for approximately 30 seconds. This procedure was repeated until dilution x/6 was completed and performed for the rest of the 17 soil samples.

**b) Petri dish inoculation**

For inoculation of petri dishes, sterilization of workspace with ethanol was made. Next, 14 petri dishes for each soil sample was prepared; 8 for bacteria and 6 for fungi determination. I then signed 4 petri dishes with symbols x/3 – x/4 – x/5 and 2 petri dishes with x/6 for each respective soil sample. After the marking, I then pipetted 1 mL of dilution rate into the petri dishes (beginning with dilution x/6, then x/5, x/4, etc.). Once all dilutions were pipetted into correlating dishes, agar was poured into its corresponding microorganism dish, mixed thoroughly and then allowed to solidify. For the Thornton agar, 8 petri dishes were used (x/3 to x/6) and 2 repetitions for each dilution was conducted. For potato agar, 6 petri dishes were used (x/3 to x/5) and 2 repetitions for each dilution was also conducted. Finally, solidified petri dishes were placed bottom up into metal holding container. Containers were placed into thermostat for cultivation, bacteria dishes were cultivated at 30°C and fungi dishes at 25°C for approximately 5-7 days and quickly examined after 4 days.

### 3.7.3 Counting of microorganisms

To count number of microorganisms, colonies were signed with permanent marker on bottom of petri dish, and if it was possible, colonies were marked from two consecutive solutions. During this process, two types of colonies were than determined. The first type being mealy or fuzzy colonies, otherwise known as actinomycetes and the second type was considered as others or true bacteria." It was noted that the optimum amount of colonies per petri dish should be ≤ 300 for bacteria dishes and for fungus dishes ≤ 100. Calculations needed to determine colony forming units per 1 gram of original soil were

count of microbe calculation, dry matter percentage and CFU/1g of dry matter soil and can be represented below,

**a) Count of microbe calculation**

(For one dilution)

$((n1a + n1b)\ x\ f1)/2$

Where,

n1a = the number of colonies at the 1st Petri dish from the chosen solution

n1b= the number of colonies at the 2nd Petri dish from the chosen solution

f1= dilution factor of the lowest dilution

(For two consecutive dilutions)

$((n1a + n1b + 10n2a + 10n2b)\ x\ f1)/4$

Where,

n1a = the number of colonies at the 1st Petri dish from the chosen solution

n1b= the number of colonies at the 2nd Petri dish from the chosen solution

n2a= the number of colonies at the 1st Petri dish from the chosen solution

n2b= the number of colonies at the 2nd Petri dish from the chosen solution

f1= dilution factor of the lowest dilution

**b) Dry matter percentage calculation**

$$DM = \frac{weight\ of\ dry\ soil - weight\ of\ empty\ weighing\ vessel}{weight\ of\ wet\ soil - weight\ of\ empty\ weighing\ vessel} x100$$

**c) CFU/1g of dry matter soil percentage calculation**

$$\frac{count\ of\ microbe}{DM/\ 100}$$

# 4 Results

## 4.1 Soil Analysis results

Results for all soil analyses can be represented by figures 7-10. For clarification, soil type and repetition were marked as follows:

| | |
|---|---|
| A 1-3 | Vessels filled with topsoil, and it's number of repetition |
| B 1-3 | Vessels filled with subsoil, and it's number of repetition |
| FA 1-3 | Vessels filled with topsoil inoculated with *Fusarium solani* |
| FB 1-3 | Vessels filled with subsoil inoculated with *Fusarium solani* |
| AA 1-3 | Vessels filled with topsoil inoculated with *Aspergillus niger* |
| AB 1-3 | Vessels filled with subsoil inoculated with *Aspergillus niger* |

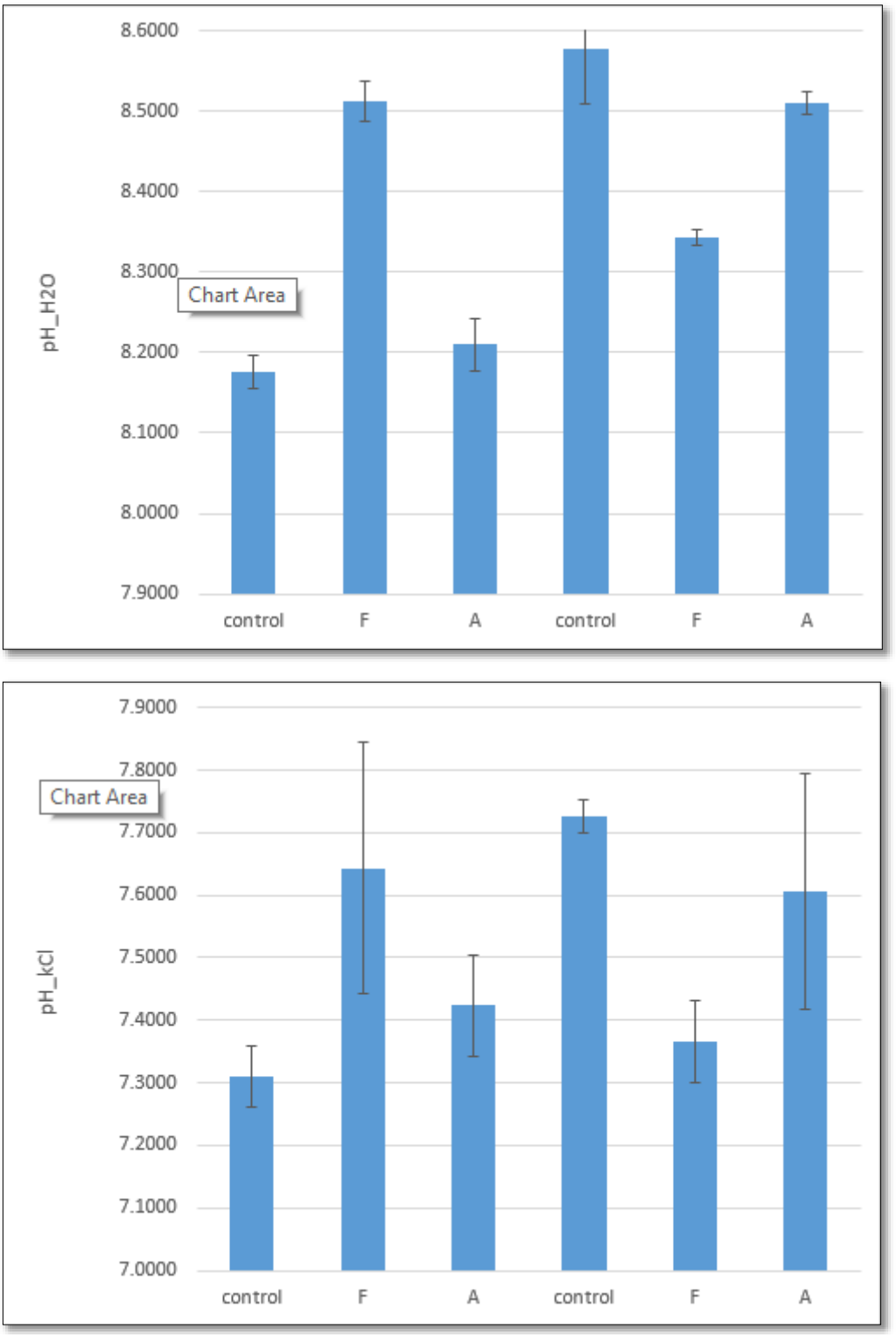


**Figure 7** Soil pH ($H_2O$ Top) (KCl bottom)

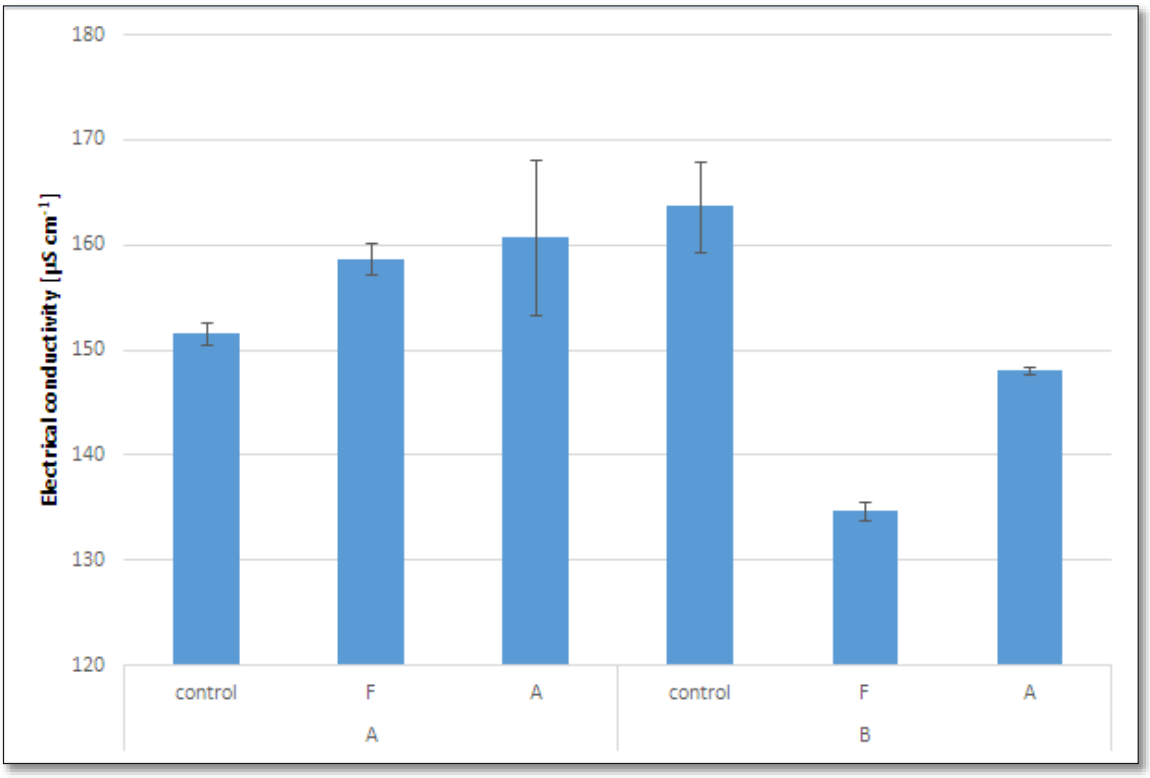


**Figure 8** Electric conductivity

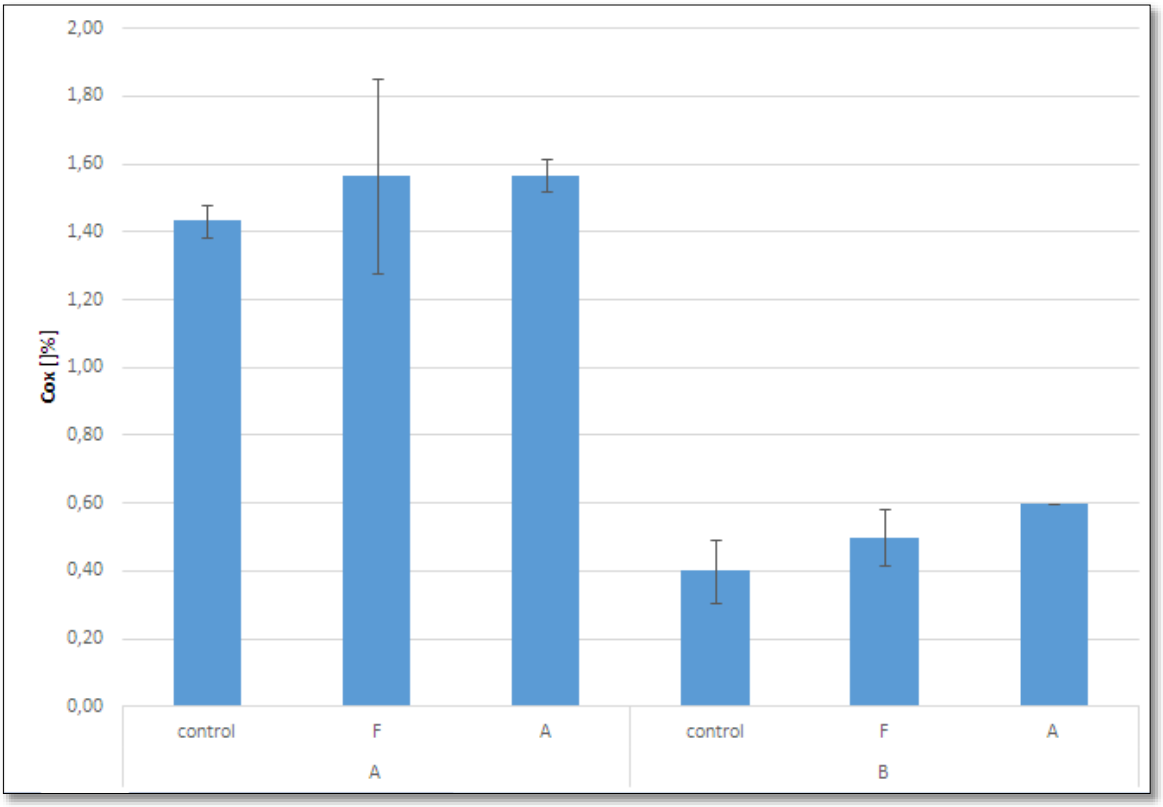


**Figure 9** Soil organic carbon (SOC)

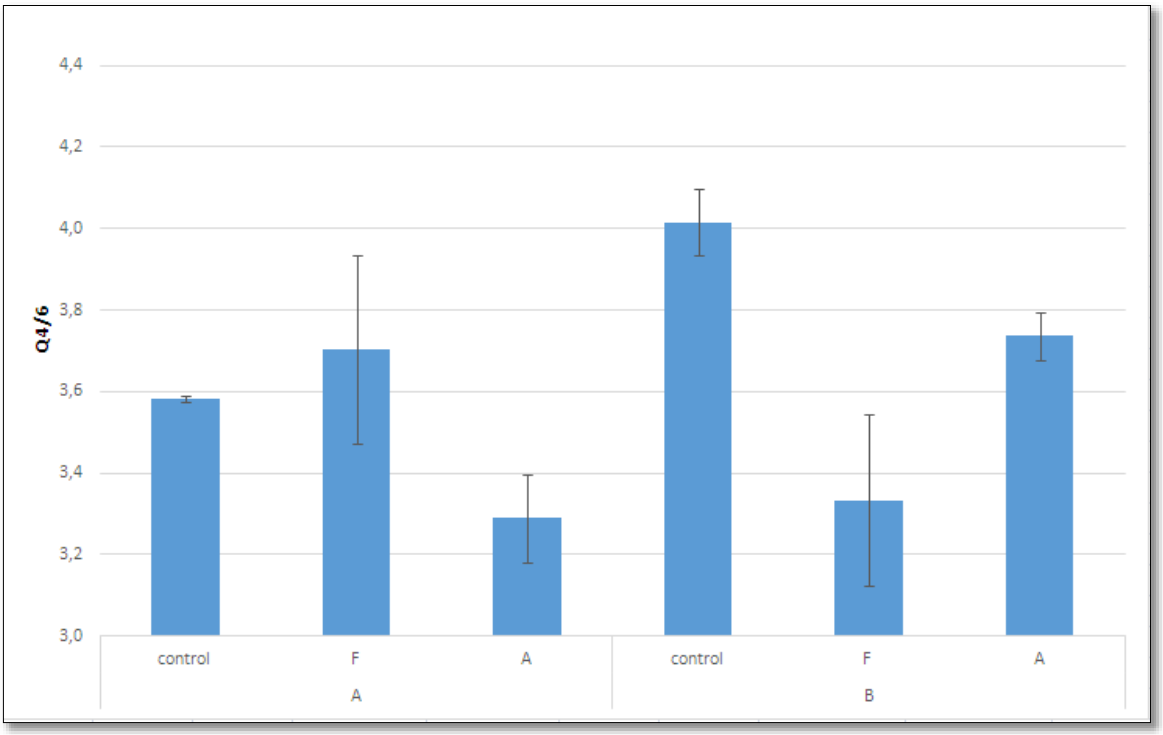


**Figure 10** Soil organic matter quality

## 4.2 Microbial analysis results

The following tables (Table 6 and Table 7) represent the results from count of microbes for bacteria and fungi for CFU / 1g original soil sample and also the dry matter percentage of soil. Samples with an x number of microbes signifies an error in the CFU process, yielding no colonies on the Petri dish.

**Table 6** Count of microbes for bacteria and fungi – CFU / 1g original soil sample

| **Count of microbe in real numbers** | | |
|---|---|---|
| **For bacteria determination:** | | |
| Control subsoil | Fusarium topsoil | Aspergillus topsoil |
| 1b= 1,650,000 | 7b= 800,000 | 13b= 3,795,000 |
| 2b= 2,300,000 | 8b= 2,300,000 | 14b= 2,450,000 |
| 3b= 1,800,000 | 9b= 3,850,000 | 15b= 1,570,000 |
| **average= 1,916,667** | **average= 2,316,667** | **average= 2,605,000** |
| Control topsoil | Fusarium subsoil | Aspergillus subsoil |
| 4b= 4,450,000 | 10b= x | 16b= 3,100,000 |
| 5b= 1,747,500 | 11b= 2,305,000 | 17b= 2,875,000 |
| 6b= 2,900,000 | 12b= 21,300,000 | 18b= 2,975,000 |
| **average= 8,275,000** | **average= 11,802,500** | **average= 2,983,334** |
| | | |
| **For fungi determination:** | | |
| Control subsoil | Fusarium topsoil | Aspergillus topsoil |
| 1f= 17,000 | 7f= 5,500 | 13f= 115,000 |
| 2f= 13,000 | 8f= 13,500 | 14f= 150,000 |
| 3f= 8,500 | 9f= 4,500 | 15f= 155,000 |
| **average= 12,833** | **average= 7,667** | average= 140,000 |
| Control topsoil | Fusarium subsoil | Aspergillus subsoil |
| 4f= 10,500 | 10f= x | 16f= 19,055 |
| 5f= 13,500 | 11f= 12,000 | 17f= 10,000 |
| 6f= 14,000 | 12f= 13,000 | 18f= 15,500 |
| **average= 8,467** | **average= 19,000** | **average= 14,852** |

**Table 7** Dry matter percentage of soil

| Dry matter percentage of soil | | |
|---|---|---|
| control subsoil | Fusarium topsoil | Aspergillus topsoil |
| 1= 98.8% | 7= 98.3% | 13= 98.4% |
| 2= 99.1% | 8= 87.6% | 14= 98.2% |
| 3= 98.8% | 9= 94.4% | 15= 98.1% |
| control topsoil | Fusarium subsoil | Aspergillus subsoil |
| 4= 98.1% | 10b= x | 16= 97.5% |
| 5= 98.2% | 11= 98.9% | 17= 98.5% |
| 6= 98.4% | 12= 98.9% | 18= 98.2% |

As you can see from the results of the microbial anaylsis, there is an abundance of microrganisms even in the extremely dry soil. In regards to the two miccrorganisms in of study, *Fusarium solani* (pink colonies) and *Aspergillus niger* (black colonies), both were present within the analysis but a surprisingly large amount of *Aspergillus niger* colonies were present within this analysis and can be viewed in the image below:

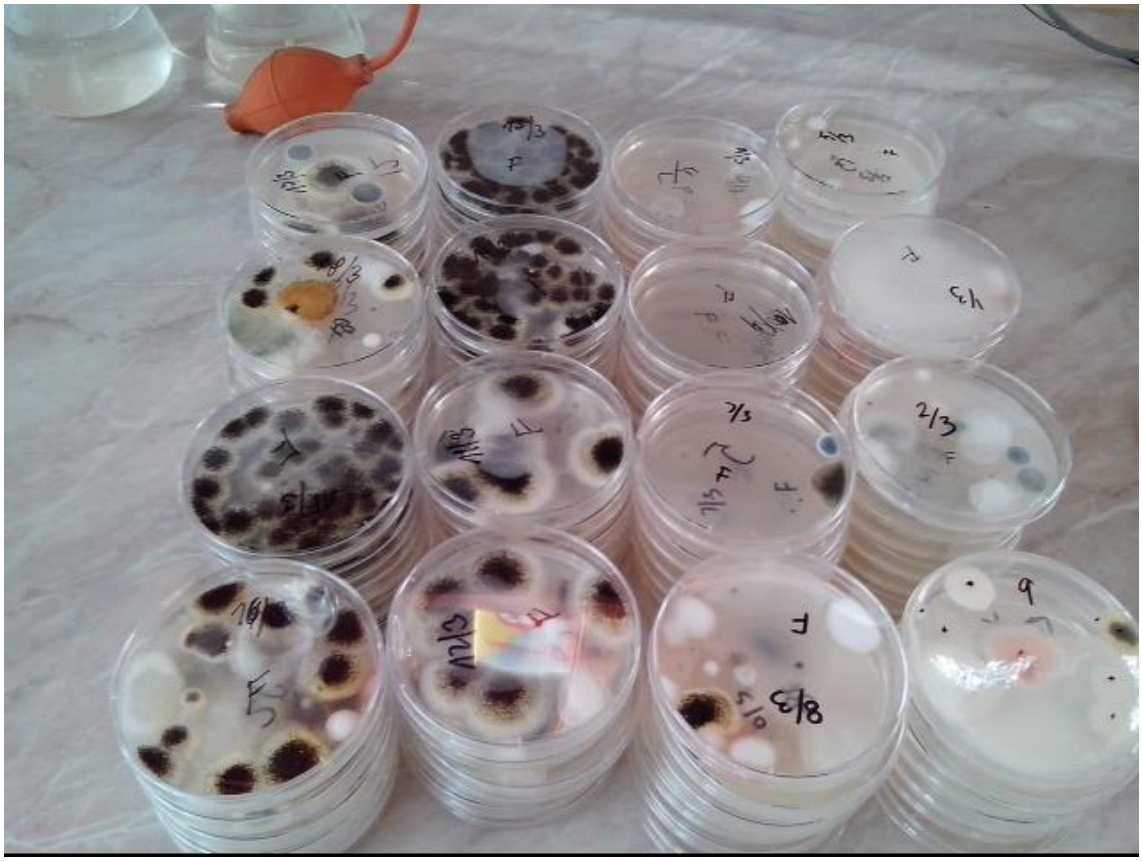

**Image 1** Soil fungus colonies found during microbial analysis

## 4.3 Pre and post burial plastic weights

For the post plastic piece weighing portion of this study, two vessels comprised of soil inoculated with *Fusarium solani*, two vessels comprised of *Aspergillus niger* and two control vessels, which all contained three pieces of LDPE and three pieces of Bio plastic each were taken and prepared for weighing. Although the results of this study were not peunominal, a difference in plastic weights, although miniscule were still observed, giving a slight recognition to my initial hypothesis, that soil factors as well as influential soil microorganism can potentially enhance the process of plastic degradation. Comparative weights of plastic pieces before and after burial are represented by Table 8.

| |
|---|
| units= grams |
| V=Vessel s=Sample |
| LDPE= Low Density Polyethylene |
| BIO=Bioplastic |
| 2 control vessels: 1 top soil 1 sub soil |
| 2 Fusariam vessels: 1 top soil 1 sub soil |
| 2 Aspergillus vessels: 1 top soil 1 sub soil |

**Table 8** Soil weights of pre and post burial plastic pieces

| **Weight of plastic pre burial plastic pieces** | | | | **Weight of plastic post burial plastic pieces** | | | |
|---|---|---|---|---|---|---|---|
| V26(Asp. Top) | | V23(Asp. Sub) | | V26(Asp. Top) | | V23(Asp. Sub) | |
| LDPE | BIO | LDPE | BIO | LDPE | BIO | LDPE | BIO |
| s1-14.19 | 18.04 | s1-16.72 | 20.95 | s1-10.01 | 17.5 | s1-15.32 | 19.23 |
| s2-11.58 | 16.66 | s2-13.60 | 20.69 | s2-11.56 | 16.32 | s2-12.57 | 20.32 |
| s3-11.90 | 17.38 | s3-13.85 | 19.58 | s3-11.10 | 16.02 | s3-13.26 | 18.74 |
| V45(Con. Top) | | V4(Con. Sub) | | V45(Con. Top) | | V4(Con. Sub) | |
| LDPE | BIO | LDPE | BIO | LDPE | BIO | LDPE | BIO |
| s1-10.68 | 19.81 | s1-9.05 | 19.35 | s1-9.98 | 19.12 | s1-8.32 | 19.33 |
| s2-12.21 | 22.16 | s2- 10.73 | 18.77 | s2-11.51 | 21.87 | s2- 10.04 | 18.06 |
| s3-10.63 | 20.31 | s3-9.25 | 17.76 | s3-10.02 | 20.12 | s3-9.19 | 17.01 |
| V36(Fus. Top) | | V15(Fus. Sub) | | V36(Fus. Top) | | V15(Fus. Sub) | |
| LDPE | BIO | LDPE | BIO | LDPE | BIO | LDPE | BIO |
| s1-13.25 | 17.75 | s1-12.23 | 18.02 | s1-13.10 | 17.21 | s1-11.57 | 17.89 |
| s2-14.20 | 18.37 | s2-12.33 | 18.23 | s2-13.78 | 18.02 | s2-11.54 | 17.98 |
| s3-10.63 | 17.43 | s3-12.15 | 19.38 | s3-10.02 | 17.25 | s3-12.02 | 18.84 |

## 4.4 Statisical analysis results

The above pre and post burial plastic weights were then subject to statistical anaylses, through use of program, The Statistic advisor. These weights show that there is a significant weight loss between the intital weights of both Bio plastic and LDPE plastics before and after soil burial. The affect of soil layer was not abile to be proven, therefore the affect of soil properties was not possible to study by statistical method. However through the anaylsis, the use of P-values were computed to yield the probablitly of the test statistics. Results with a P-value < 0.05% was considered to have a 95% confidence level, holding no significant difference amoungst samples. For example, it was found that there was a greater decrease in weight of the LDPE than the Bio plastic, possessing a P-value of 0.022, meaning that LDPE has a faster degradation rate than the commercial bioplastic (Figure 10). Also, in regards to weight loss in plastics according to different soil horizons, there were no significant effects in LDPE, with a P-value of 0.455305 (Figure 11) or in Bioplastic, with a P-value of 0.784784 (Figure 12). Results from The Statistic Advisor also showed that there was no significant soil treatment effects from the miccroorganisms on the degradation of both plastic pieces. For LDPE, a P-value of 0.4905 was obtained (Figure 13) and for Bioplastic, a P-value of 0.0728 was given. (Figure 14).

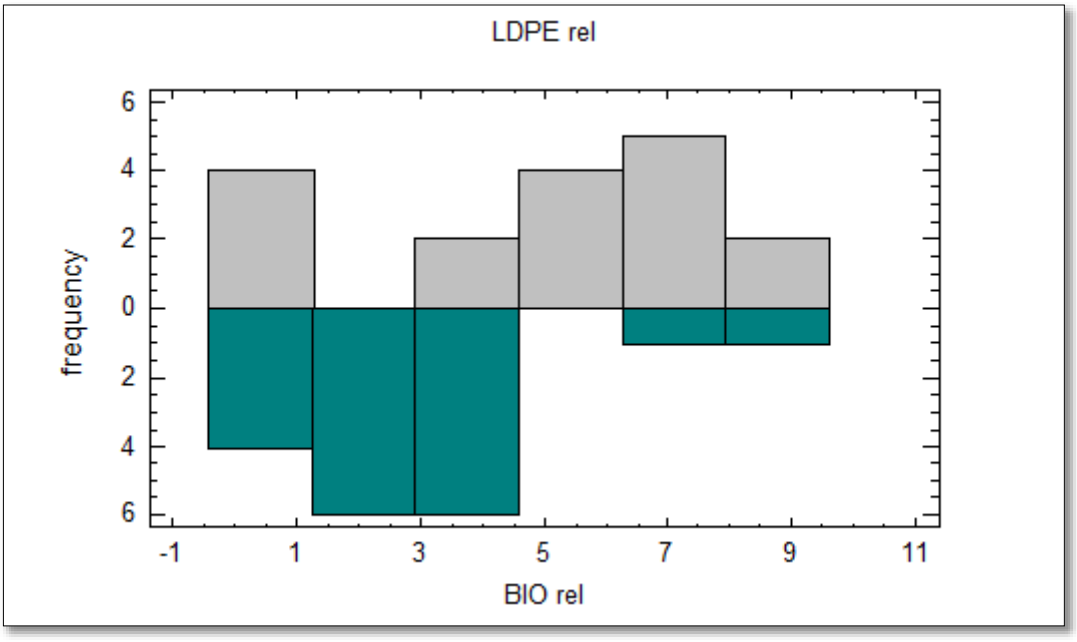


**Figure 10** Decompostion rate of LDPE is greater than bioplastic

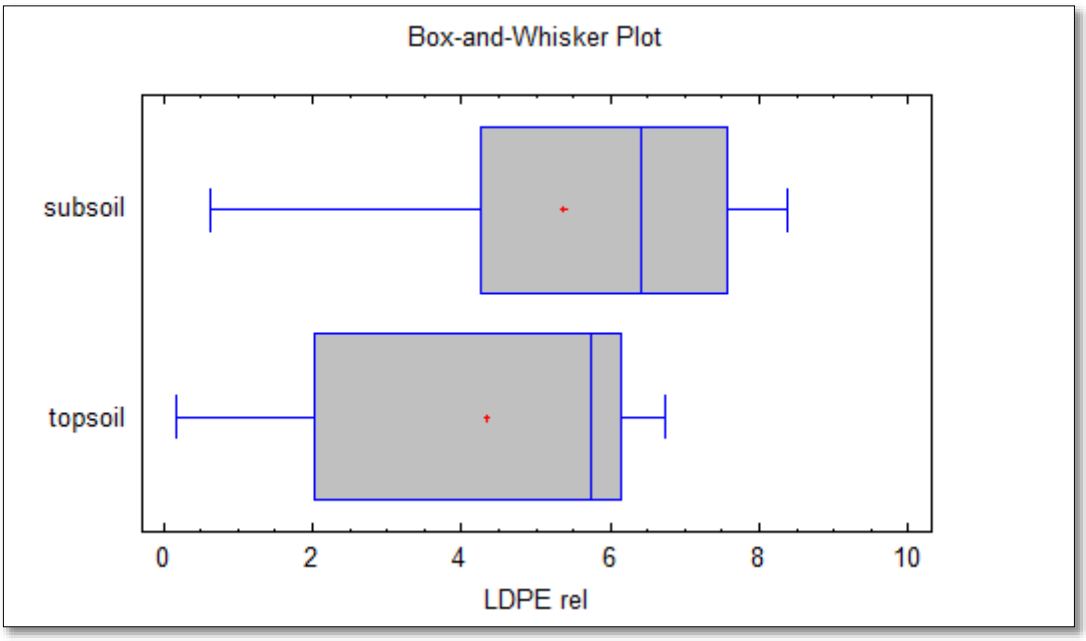


**Figure 11** Weight loss in LDPE according to soil horizons

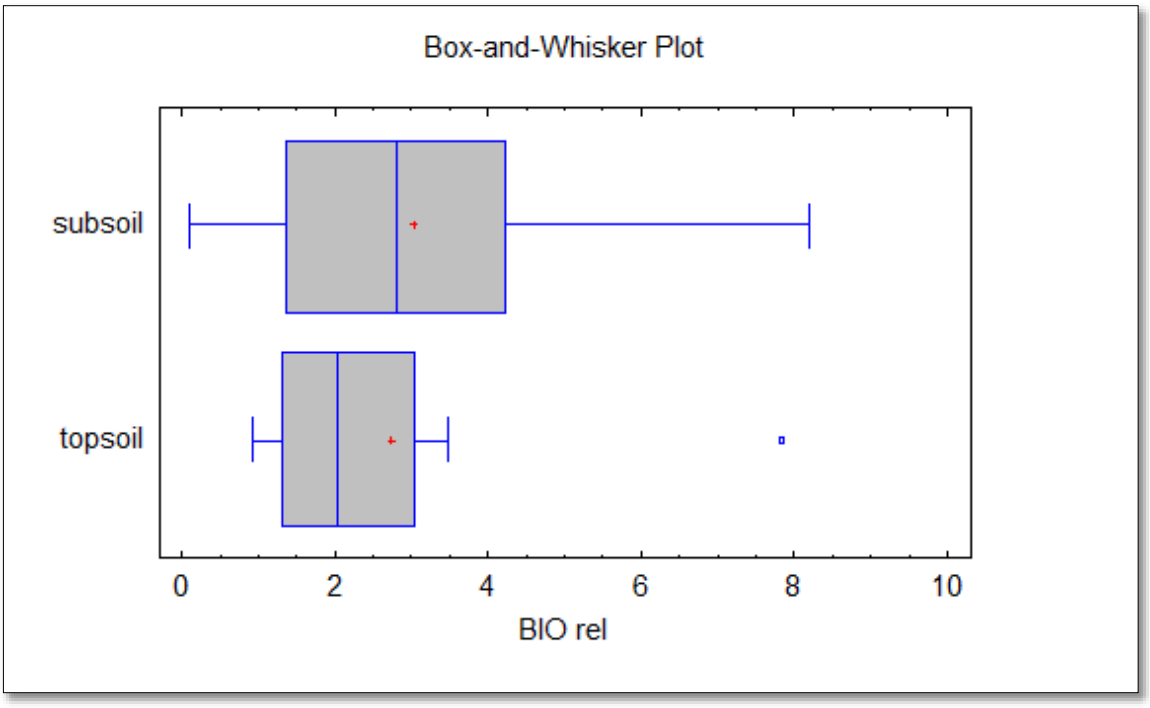


**Figure 12** Weight loss in Bioplastic according to soil horizons

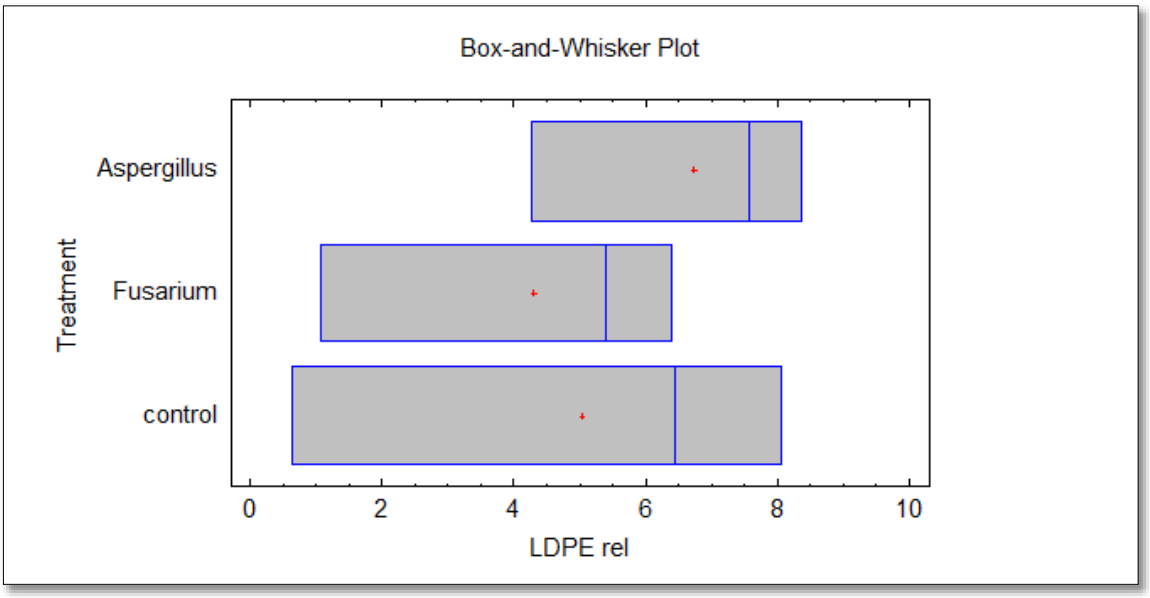


**Figure 13** Effect of treatment on LDPE

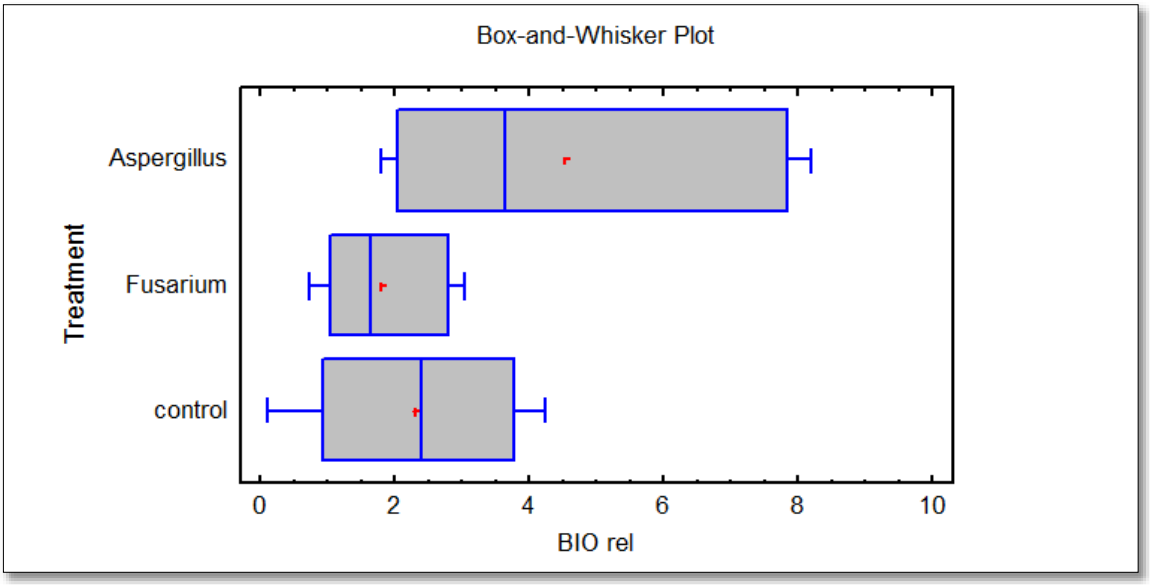


**Figure 14** Effect of treatment on Bioplastic

Results of this outcome prove that the hypothesis was not confirmed. Therefore stating that the exisitnce of microorganisms in the soil does not inhance the ability to degrade plastic in soil.

# 5 Discussion

Completion of this thesis project provided much personal insight into the study of plastic degradation by soil factors, including influential microbial factors. Although this project faced unfortunate circumstances in regards to ideal soil moisture, results are not entirely inconclusive. In regards to the results from the pre and post soil burial plastic weights, a significant decrease in weight after being buried in innoculated soil and also in non inoculated soil was found and than weights of Bioplastic were actually heavier compared to weights of LDPE pieces. Given this information, the statistical anaylsis determined that the degradation of LDPE was faster in comparison to that of bioplastic. This opens discussion for certain theories. It can be speculated that this peculiar result could be due to the fact that bioplastic pieces possess a generally rougher material surface, allowing soil particles from burial phase to remain on the pieces even after washing. Another theory is that the bioplastic was inhibitated by microorganisms as a suitable substrate, which could have been facilited by a rougher material surface. Therefore, the final weight of the bioplastics was influced by the additional weight of the microorganisms. Although, the first of the two theories seem to be the most sensible, results from the microbial anaylsis could be a sensible validation for the second theory. Through the determination of dry matter percentage during this analysis, it was found that even with the unusually dry soil conditions, there was still an abundance of of exisiting fungal colonies, specifically colonies of one of the microorganisms of study, *Aspergillus niger*. This finding provides a greater probability for validating the hypothesis that bioplastic pieces were inhabited by microoragnims, causing greater weights in comparions to the weights of LDPE but unfortunately, proof of this hypothesis is nonexisitant at the moment. The findings within the microbial analysis also open discussion for potential microbial activity in the future when water in restored to the inoculated substrate.

# 6 Conclusion

In conclusion, this study was conducted to determine whether soil properties as well as soil inoculated by microorganisms, has some effect on the degradation of Low density polyethylene and commercial bioplastic films. By performing an in situ plastic degration experiment, which included the inoculation of the study soil, plastic pieces were weighed and than allowed to remain in soil for approximately 4 months. After 4 months had elapsed, pieces were weighed once more and a decrease in the weights of the plastic pieces was witnessed. A soil anaylsis, a microbial anaylsis as well as a statistical anaylsis of study soil and plastics was also performed in attempts to validate the decrease in plastic weight. Through the use analyses performed on the soil of study, chernozem, determination of soil factors such as pH, electric conductivity, soil organic carbon, soil organic matter quality, particle density and soil texture was obtained. This thesis project proved a loss of plastic weight for both PET and Bioplastic. The statistitical alanylsis showed an unusual outcome, stating that LDPE held a faster degradation rate then that of bioplastic. Also during this research, it was discovered that there was no significant parameter which effected plastic degradation and lastly, no affect of soil inoculation was proven as well as no affect of soil properties was proven. Through the completion of microbial analysis, it was found that even in unusually dry soil conditions, the microorganisms of study, *Fusarium solani* and *Aspergillus niger,* especially *Aspergillus niger* can still exisit in abundance. It was speculated that this particular finding could validate the unusal outcome of the statistical anaylsis but unfortunately, this hypothesis was in need of further and longer term research.

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