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THEME

Biodegradation of plastic by Bacillus megaterium

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IN FRONT OF THE JURY

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Dedicated to

My family…

Meriem

Dedicated to

My beloved family and friends
Thank you for the love, support and loyalty

Nesrine

List of abbreviation

Abs: Absorbance.

Bp: Base pair.

C: Cytosine.

CcpA: catabolite control protein A.

CCR: Carbon catabolite repression.

Cd: Cadium.

CH_4: Methane.

CO_2: Carbon dioxide.

COG: Clusters of Orthologous Groups.

Crh: Catabolite repression Hpr.

Cu: Copper.

DBP: Dibutyl phthalate.

DCHP: Dicyclohexyl phthalate.

IS: Insertion sequence.

g: gram

Kb: Kilo-base.

L: litter.

LDPE: Low density polyethylene.

M: mass.

Mb: Megabase.

ml: milliliter.

Mm: millimetre.

MPs: microplastics.

MT: metric tons.

MW: Microwave.

DEHP: Di (2-ethylhexyl) phthalate.

DHP: Dihexyl phthalate.

DNA: Deoxyribonucleic acid.

DOP: Dioctyl phthalate.

DOA: Dioctylphthalate.

G: Guanine.

GO: Gene onthology.

H_2O: Two hydrogen atoms and one oxygen atom; water.

H_2S: Hydrogen sulphide.

HPr: Histidine-containing protein.

HPrK: HPr kinase/phosphorylase.

IARC: International Agency for Research Cancer.

NH_3: Ammonia.

N.m: nanometer.

NO_2: Nitrogen dioxide.

NTP: National Toxicology Program

O_2: Oxygen.

PA: Phthalic acid.

PAEs: Phthalate acid esters.

PBS: Poly (butylene succinate).

PBT: Polybutylene terephthalate.

PE: Polyethylene.

PET: Polyethylene terephthalate.

PG: Peptidoglycan.

PGPR: Plant growth promoting rhizobacteria

PHB: Poly (hydroxybutyrate).

PO: Polyolefin.

PP: Polypropylene.

PRDs: Phosphoenolpyruvate–carbohydrate phosphotransferasesystem-regulatory domains.

PS: Polystyrene.

PTS: Phosphotransferase system.

PTT: Poly (trimethylene terephthalate).

PU: Polyurethanes.

PVC: Polyvinylchloride.

rDNA: Ribosomal DNA.

RNA: Ribonucleic acid.

SO_2: Sulfur dioxide.

UV: Ultraviolet.

V: volume.

Vs: Versus.

w/w:weight by weight.

^{o}C: Degrees Celsius.

°F: Fahrenheit

Abstract

Plastics in the ocean and in landfill are of great concern nowadays due to the problems they pose to the aquatic and terrestrial environment and their living organisms. The increase in the production of plastic leads clearly to its accumulation in the environment while only a very small portion is recycled. The aim of this work is to study the degradation of plastic by *B.megaterium*. The degradation capability of the *B.megaterium* was investigated by incubation in mineral medium containing two plastic types under two different conditions for three months. A significant reduce in plastic weight was recorded in both acidic and neutral media, while the average weight loss in brown and transparent plastic was 5.6% and 6% respectively. The results in the present work show that the *B.megaterium* has the ability to degrade plastic material under both neutral and acidic media, and utilise it as a carbon source.

Keywords: *Bacillus megaterium*, plastic, biodegradation, biodegradable plastic.

Résumé

Le plastique sur terre ferme ou dans les océans présente actuellement une source d'inquiétude en raison de ces conséquences nuisibles à l'environnement marin et terrestre en plus des organismes qui y vivent. La production élevée du plastique mène à son accumulation non négligeable dans l'environnement car seulement une modeste partie de ce dernier est destinée au recyclage. Le but de ce travail est d'étudier la dégradation du plastique par *B.megaterium* mais aussi d'explorer son pouvoir de dégradation par son incubation dans un milieu mineral comportant deux types de plastiques dans différentes conditions pour une période de trois mois. Un remarquable abaissement du poids fut enregistré à l'égard du plastique présent dans les deux milieux neutre et acide car le poids du plastique sujet à cette expérimentation a diminué en moyenne de 5.6% et de 6% pour le plastique marron et transparent respectivement. Les résultats indiqués dans ce travail prouvent que *B.megaterium* possède les compétences éxigées pour dégrader une matière plastique en dépit de la neutralité ou de l'acidité du milieu dans lequel elle s'y trouve au surplus de son utilisation du carbone comme source d'énergie.

Mots clés: *Bacillus megaterium*, plastique, biodégradation, plastique biodegradable.

الملخص

تعد المواد البلاستيكية في المحيطات و مكبات النفايات مصدر قلق كبير في الوقت الحاضر بسبب المشكلات التي تطرحها على البيئة المائية والبرية والكائنات الحية . تؤدي الزيادة في إنتاج البلاستيك إلى تراكمه في البيئة بينما يتم إعادة تدوير جزء صغير جدًا فقط. الهدف من هذا العمل هو دراسة تحلل البلاستيك بواسطة *Bacillus megaterium*. تم التحقق من قدرة *Bacillus megaterium* في تحليل البلاستيك عن طريق الحضن في وسط معدني يحتوي علي نوعين من البلاستيك تحت حالتين مختلفتين لمدة ثلاثة أشهر. تم تسجيل انخفاض ملحوظ في وزن البلاستيك في كل من الوسط المحايد و الحمضي, بينما كان متوسط انخفاض وزن البلاستيك البني والشفاف 5.6٪ و 6٪ على التوالي. نتائج هذا العمل تظهر ان *Bacillus megaterium* لديها القدرة علي تحليل مادة البلاستيك في كل من الوسط المحايد و الحمضي, واستخدامها كمصدر للكربون.

الكلمات المفتاحية: *Bacillus megaterium* , البلاستيك, التحلل الحيوي, بلاستك قابل للتحلل

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Duringthe past century, plastic became increasingly one of the most common manufacturing materials, replacing more traditional materials such as glass, aluminium, and natural fibers **(Andrady and Neal 2009).** Around the world, the use and production of polymers has grown exponentially **(Gross 2017)**, leading the plastic-based products entering the waste steam to increase **(Jambeck *et al* 2015).** A big amount of the plastic waste has accumulated infloating marine garbage patches, nevertheless about 80% comes from the continents **(Eriksen *et al* 2016)**.While plastic pollution research has earned considerable attention lately, it is yet to gain notability among terrestrial ecologists as a main reason of global change. Because of plastic's nature of manufacturing, and the longevity of the product, it remains in the environment for long periods of time making plastic pollution one of the most omnipresent contemporary threats to the world's coastal and marine environments **(Thompson *et al* 2009, Worm *et al*2017).** Because wide amounts of synthetic plastic remain non degradable, numerous microorganisms have the capacity to degrade multiple types of plastic, but owing to the hardness of these polymers and their non-solubility in water, biological decomposition is a slow process. This issue introduced a friendlier type of plastics, natural plastics which are made from plant and animal sources, or produced by a range of microorganisms **(Alshehri 2017)**. The biodegradation of polymer based plastics by means of *Bacillus megaterium* under various conditions have been reviewed in addition of the recreation of a biodegradable plastic made from plant sources and it's biodegradation process under different factors. The biodegradation of plastics using non-conventional methods promises a future free of accumulation of commodity plastics used in packaging and commercial polymers, which are the most abundant form of plastic wastes (**Muthukumar and Veerappapillai 2015).** That's why the aim of our project is to study the degradation process of two plastic samples by the *Bacillus megaterium* bacteria over a period of three months time from the 29th of February 2019 to the 19th of May 2019.

1 General properties of plastics

Plastic material offers numerous properties such as lightweight, resilience, resistance to corrosion, colour, transparency, and ease of processing, which makes them superior over other materials in many applications. A single product can necessitate a combination of properties to satisfy all of its requirements **(McKeen 2012).** Asplastic raw materials are combined to produce plastic parts such as films, sheets, profiles, and molded parts that offer different properties **(Shrivastava 2018).**

1.1 Chemical properties of plastics

Plastic materials interact with variety of chemicals during their processing and use. There are two basic mode of interaction between plastics and chemicals, first is the physical change which includes absorption of moisture or solvent that can cause dissolution, softening, or swelling, and second is the chemical attack on the chains which reduces physical properties, including oxidation. In several cases the chemical can cause depolymerization or degradation during processing **(Woishinis and Ebnesajjad 2012)**.

1.1.1 Chemical resistance

Almost all plastic parts are exposed to various chemicals which could affect the plastic properties either physically or chemically. Integrity of various applications is based on the resistance these plastics show to different chemicals **(Woishinis and Ebnesajjad 2012)**.
Chemical resistance largely depends on the chemical structure as shown in (Figure1) and (Figure 2) and composition of the polymer and the type of chemical it is exposed to. Weak links susceptible to chemical attacks are chemical defects in the chain, branching points, and polymer end groups **(Shrivastava 2018).** The extent of chemical resistance can be classified into four categories:
(1) None such as water toward polyethylene, (2) swelling/softening such as water to Nylon and acetone to poly (vinyl chloride), (3) dissolving such as poly (vinyl alcohol) in water, and (4) reacting such as nitric acid reacting with cellulose to form nitrocellulose **(Su 2013).**

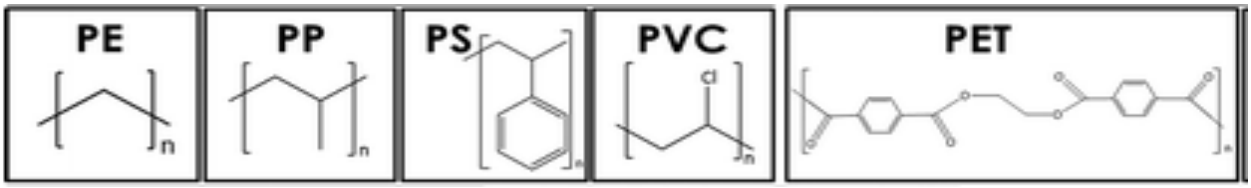

Figure 1: Chemical structure in polymer formula of commodity plastics (plasticeurope.org 2015).

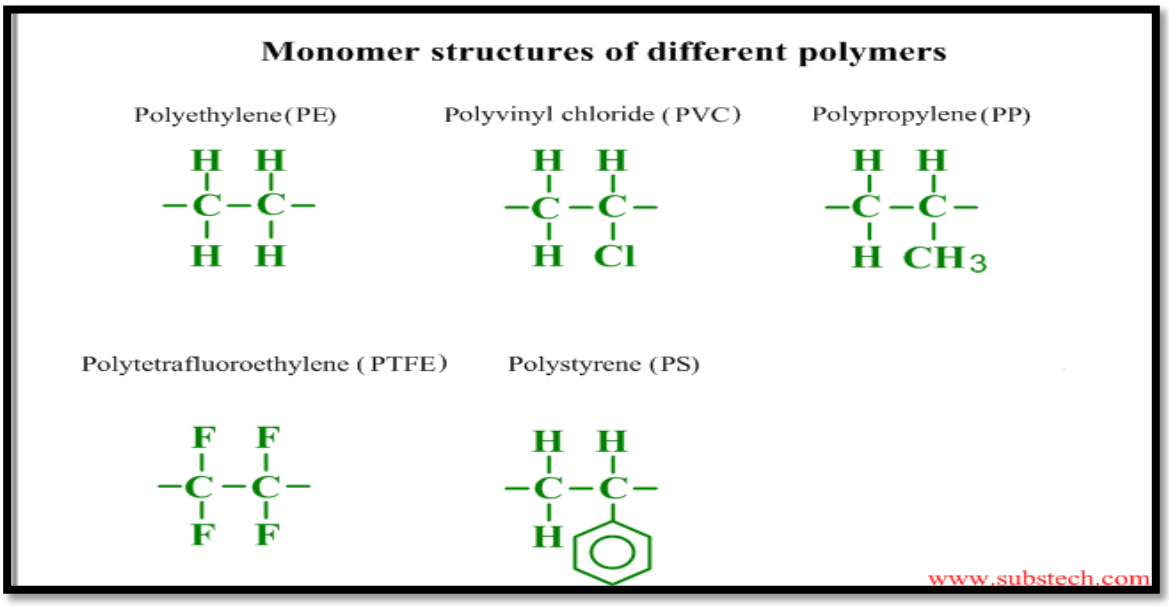

Figure 2: Chemical structure in monomer formula of commodity plastics (substech.com 2013).

1.1.2 Solubility

When solvents are added to the polymer, the polymer molecules experience the dispersion forces. If the polymer-polymer forces are lower than the polymer solvent force, then the polymer starts absorbing the solvent molecules. The absorption of solvent increases the polymer volume and begins to separate the neighboring chains thus, the dissolution occurs. Dissolution of polymers is slow process some of the commonly used solvents include toluene, tetra hydrofuran, dimethyl formamide, diethyl ether, acetone, and formic acid. In some cases, chloroethylene, ethyl acetate, ethanol, and water could be used **(Braun 2013).**

Polar polymer such as polyvinyl alcohol and poly (acrylic acid) are soluble in water, whereas non-polar polymers such as polyethylene, PP, PVC, PBT, and PET are insoluble in water.

Higher molecular weight polymer takes longer to dissolve compared to lower molecular weight **(Woishinis and Ebnesajjad 2012)**.

An important aspect of solubility is plasticization. Plasticizers are generally added to improve processing and impart flexibility. Depending on the type and quantity of plasticizer, the polymer could experience reduced resistance to temperatures, lower chemical resistance, and reduced hardness and stiffness **(Shrivastava 2018).**

1.1.3 Permeability

Permeability is defined as the passing of liquid, gases, or radiations through a solid material. The barrier properties of plastic materials are related to the effectiveness of restricting the permeability of various gases, lights, heat, and radiations that are to the integrity of products. Diffusion of molecules through a plastic material is dependent on the secondary bonding, higher crystallinity, packing of the molecular chains, pressures, and volatility of the diffusant, in general, higher free volume between the chains limits the barrier abilities of the plastic thus, at higher temperature the plastics become more permeable, hence a polymer with many polar groups is sensitive to a polar chemical, that same polymer would be permeable to a polar gas or liquid. Conversely, a non-polar polymer would be a barrier to polar gases and liquids. For instance, polyethylene has very low water permeation but relatively high oxygen permeation, hence a polymer with many polar groups is sensitive to a polar chemical **(Shrivastava 2018, Woishinis and Ebnesajjad 2012, Su 2013).**

1.2 Physical properties of polymers

An important physical property, density, is defined as mass (M) per unit volume (V) of plastic materials, additionally the crystallinity of the polymer also plays an important role in the mechanical strength of polymer, furthermore the mechanical properties of polymers can be expressed by stress-strain plot. When organic substances are heated to high temperatures they have a tendency to form aromatic compounds, moreover, polymers containing aromatic structure exhibit high temperature resistance and in general higher mechanical properties. Thermal stability of polymer is primarily determined by the bond energy of chemical bonds in the polymer chain. When the temperature increases to the point where vibration energy causes bond rupture, the polymer degrades **(Shrivastava 2018, Su 2013).**

2 Categories and classification of plastics

Because of the diversity of function and structure found in the field of macromolecules, it is advantageous to construct some scheme which groups these materials under convenient headings, (Figure 3) in addition to (Figure 4) is one such way of classifying polymers. Naturally occurring polymers usually have more complex structures than their synthetic counterparts. Elastomers or rubbers can be either natural or synthetic (man-made). While all polymeric materials, both natural and synthetic, can be produced in a cellular form, the majority of polymeric foam products are derived from synthetic polymers and elastomers **(Obi 2018).**

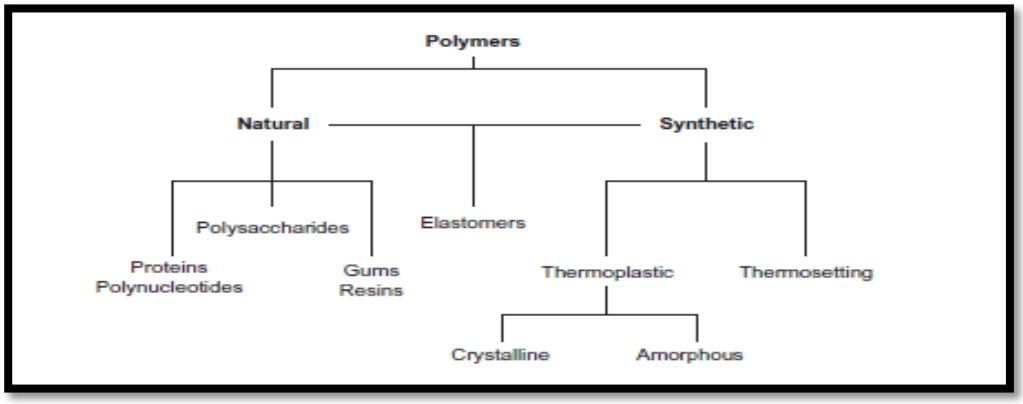

Figure 3: Classification of polymers **(Obi 2018).**

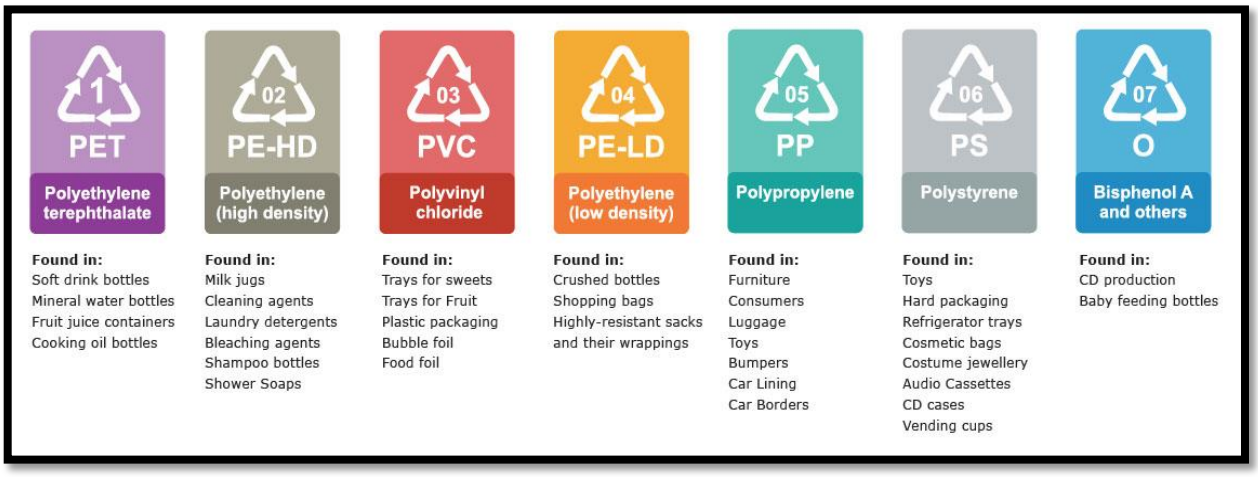

Figure 4: classification of multiple polymers (Semac-USA.com).

2.1Polymers used extensively in foams

Synthetic (man-made) polymers are predominantly used to produce foamed products. While almost all synthetic polymers could be transformed into cellular materials, there are a handful of polymers that find widespread applications in various foamed products (Figure 5). These polymers in order of their global volumetric consumption into foamed products are polyurethanes (PU), polystyrene (PS), polyolefin (PO), and polyvinyl chloride (PVC). These polymers are mostly transformed into foamed materials for major applications **(Obi 2018).**

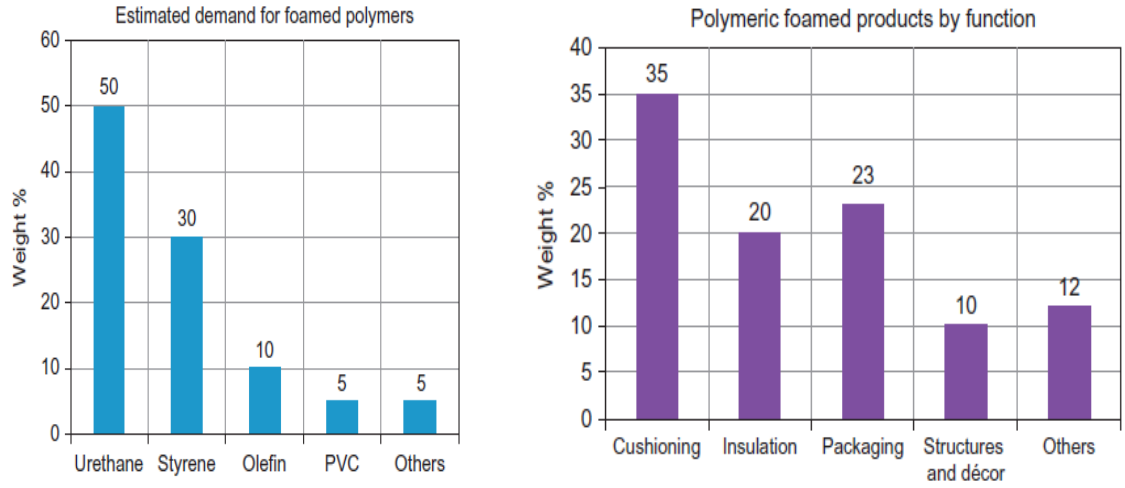

Figure 5: Relative demands and products by function for foamed polymers **(Plastemart.com 2013).**

2.1.1 Thermosets

Thermosetting polymers, are changed irreversibly from flow-able (melt-able), soluble products into highly intractable cross-linked (networked) resins which cannot be molded by flow. The resulting thermosetting polymers generally have superior dimensional stability compared with the thermoplastic polymers which have better flexural characteristics **(Obi 2018).**

2.1.2 Thermoplastic

Thermoplastic polymers are polymers that can be deformed on heating above melt temperatures, these polymers form and become flow-able and moldable. They can then be shaped into various forms unlike thermoset polymers. They return to their hardened state in the new shaped form on cooling below these transition temperatures **(Obi 2018).**

2.1.3 Elastomers

Rigid plastics and fibers are resistant to deformation and as such are characterized by very high modulus at very small deformations. Elastomers, on the other hand, can easily undergo very large deformations and can achieve very high levels of strain at relatively low stress levels. Elastomers have typically relatively lower modulus than rigid plastics and fibers as well as flexible plastics. They are typically characterized by a high degree of elasticity and resiliency **(Obi 2018).**

2.2 Organic compounds in plastic composition

In it's composition plastic contains a number of organic coumpounds weather used as stabilizers, plasticizers or dyes, to name few of them. Dibutyltin compounds are used preferably as stabilizers in colourless and/or transparent PVC plastic articles for example: containers, bottles and films (wrapping) in addition to organic flame retardants as phosphate esters, halogenated phosphate esters, halogenated hydrocarbons along with organic based pigments such as Cobalt (II) diacetate **(Hansen *et al* 2013) .**

2.3Bioplastics

Within the term bioplastics, we distinguish (a) bio-based and (b) biodegradable plastics, but a bioplastic can also fulfil both of these criteria. Bio-based plastics are typically made from renewable sources by the action of living organisms. They can be polysaccharides, proteins, or products of microorganisms as ; poly (hydroxyalkanoate) such as poly (hydroxybutyrate) (PHB) Furthermore, bio-based plastics can also be chemically synthesized from bio-derived products e.g.(PLA), poly (butylene succinate) (PBS), and poly (trimethylene terephthalate) (PTT) **(IfBB 2017, Song *et al* 2009).** Moreover, there is another fraction of plastics that isbio-based but not biodegradable that is called ‘‘drop-ins" (e.g., bio-PET, bio-PE, bio-PP) as shown in (Figure 6) with identical features as their petrochemical ancestors **(Batori *et al* 2018).**

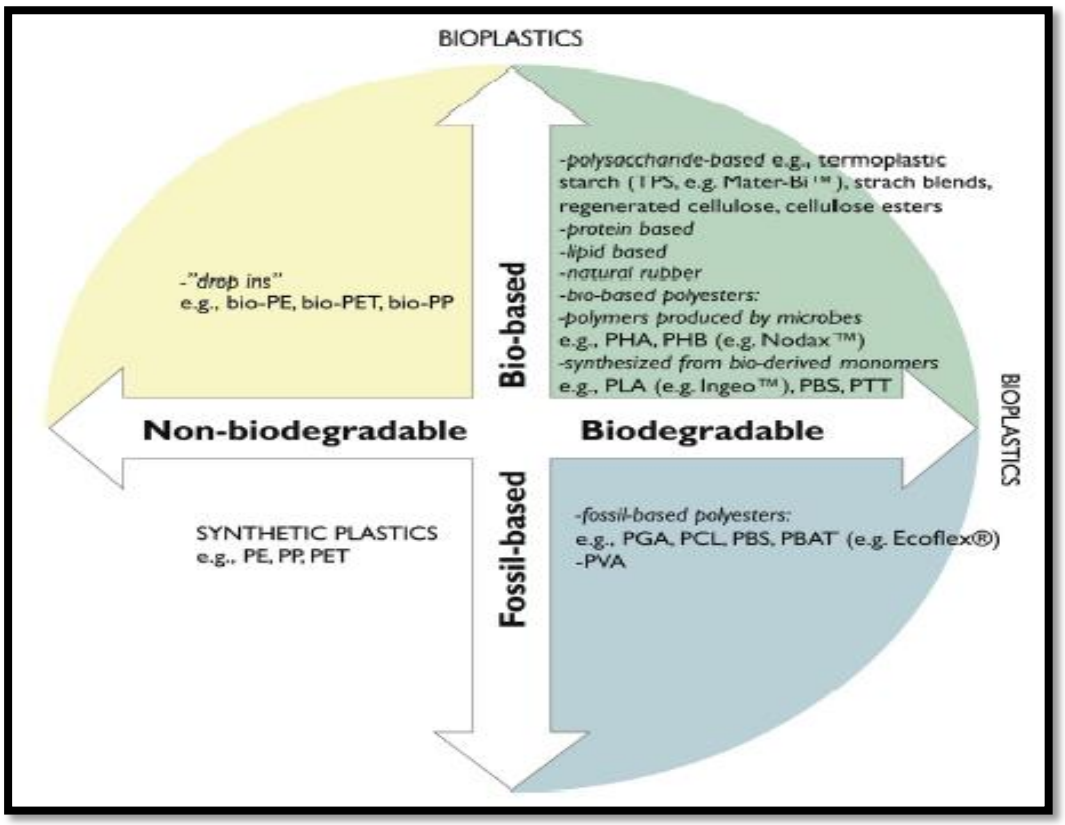

Figure 6: Classification of biodegradable and non-biodegradable plastics depending on their base **(Batori *et al* 2018).**

3 Uses of plastics

Across the world, the use and production of polymers has grown exponentially (Figure 7), and become increasingly common and convenient manufacturing material, surpassing other materials like glass, aluminium and natural fibres **(Critchell *et al* 2019)**, and it only dates back to 1950, the outstanding exceptions are used extensively in the construction sector, such as steel and cement, and are used in other sectors like electrical and electronic, transportation, industrial machinery **(Geyer *et al* 2017)**. Moreover they provide a wide range of benefits for human health and the environment. Like protecting food from contamination is assured by plastic packaging. Clean water is provided by plastic water supply systems and storage tanks, another important point is its light weight compared to other materials which saves fuel and decreases emissions during transportation. From a health and safety point of view Plastic products delivers important equipment such as blood pouches, tubing's, syringes and plastic protective clothing like fire proof materials, helmets and air bags to prevent injuries **(Hahladakisa *et al* 2018).**

The plastics demand in Europe is estimated to be 45.9 million tonnes in 2012. **(Galloway 2015)** While the Plastics' largest market is packaging (Table 1), which includes food and beverage packaging, the global shift from reusable to single-use containers accelerated its growth. Non-fiber plastics defined as resins plus additives contain 93% polymer resin and 7% additives by mass. Plasticizers, fillers, and flame retardants account for about three quarters of all additives, which will be detailed in the next section **(Geyer *et al* 2017).**

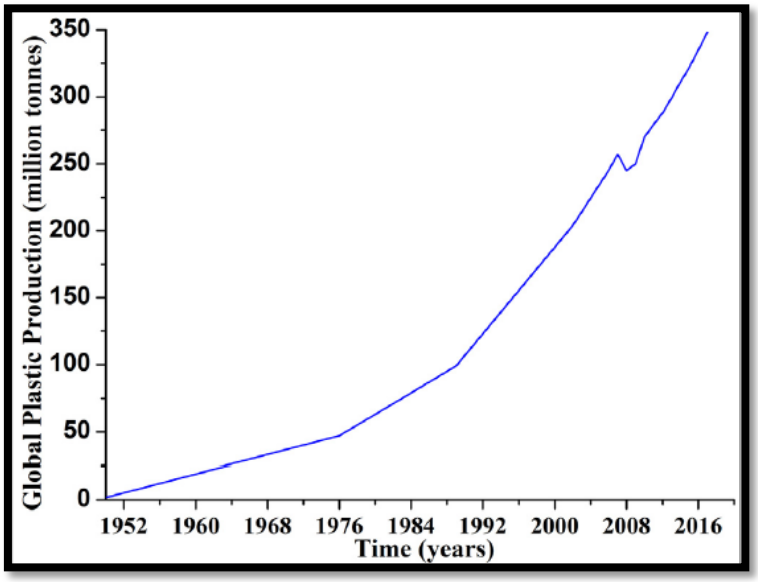

Figure 7: Global annual production of plastics (1950–2017) **(Radhana *et al* 2019).**

Table 1: Plastics demand by industry segment in Europe, 2012 **(Geyer *et al* 2017).**

Industry segment	Volume (millions of tonnes)	percentage of total
Packaging	18.1	39.4
Building and construction	9.32	20.3
Automotive	3.76	8.2
Electronics and electrical	3.03	6.6
Agriculture	1.93	4.2
Other (furniture, health and safety, sport, consumer and household appliances etc.)	10.3	22.4
Total (demand for 2012)	45.9	100

3.1**Migration of chemical substances present in plastics**

The migration of chemicals present in plastic product is potentially possible from the plastic to the medium in contact with the product and can also, slowly migrate within the plastic to the surface **(Hahladakisa *et al* 2018).** Migration of various chemical substances from plastic packaging materials were comprehensively reviewed by Bhunia *et al* during MW and conventional heating, under various storage conditions **(Bhunia *et al* 2013).** The rate at which chemical substances are released from the plastic is controlled by many factors, including the size of the additive, the permeability of the polymer itself (migration is greater for highly permeable polymers), and the temperature and pH of the surrounding medium (air, water, soil, body tissues) **(Galloway 2015).**

There are two types of migration, one that can be engineered and controlled which is a required process, the other type is an unwanted migration based on the liberating of additives in polymer which is the most common. A very dangerous type of chemical substances migration in food or medicine plastic packaging, as some of the migrating substances may be toxic or give an unpleasant taste to the food or affect the medicine or enhance the degradation of the active substances in the medicine **(Hahladakisa *et al* 2018).**

3.1.1 Plasticizers

As the phthalate plasticizers are not chemically bound to PVC, they can leach, migrate or evaporate into indoor air and atmosphere, foodstuff, other materials, etc. Consumer products containing phthalates can result in human exposure through direct contact and use, indirectly through leaching into other products, or general environmental contamination **(Ambrogi *et al* 2018).**

A study done by Fang *et al* determined that both DEHP and DBP had the highest migration under strong acidity (pH=3) from the polypropylene food containers, with a heating time of 0–5 min. importantly migration increased with prolonged heating time **(Fang *et al* 2013).** Making clear that PVC is not suitable for food-contact applications in a MW oven due to high migration of DOA **(Bhunia *et al* 2013).**

Plasticizers migration is influenced by food composition, contacting phase, the time temperature combination of exposure of the food to the packaging film, and the initial concentration of the migrant components in the film **(Bhunia *et al* 2013).**

National Toxicology Program (NTP) reported the concerns with phthalates which possess a risk to the human health. This showed that when rats and mice were fed diets with high levels of DEHP for nearly a year, developed liver tumours. The used amount of DEHP on rats and mice's is equivalent to a human taking a cup of liquid plasticizer per day for several years. The International Agency for Research Cancer (IARC) listed DEHP as "probably carcinogenic to humans" based on these results, and were banned from children's toys under the age of 12 **(Godwin 2017).**

3.1.2 Antioxidants

The migration of antioxidants and their degraded product were quantified by several studies under various conditions from different polymers, as an example Alin and Hakkarainen demonstrated that prolonged heating (1 h) in MW promotes degradation of antioxidant in food simulants compared to conventional heating using oil bath. The processing temperature was maintained constant (80 $^{\circ}$C) for both MW and conventional heating. High temperature caused more swelling of Polypropylene (PP) in isooctane during MW heating and increased the diffusion coefficient by factors of 100 to 1000. Aqueous soluble antioxidants migrate into aqueous food simulants was also reported **(Bhunia *et al* 2013).**

3.1.3 Flame retardants

Despite the affective role of the flame retardants as additives to polymers, some flame retardants belonging to halogen-based flame retardants, especially brominated, are considered harmful for the environment **(Ambrogi *et al* 2018).**

3.1.4 Dyes

Synthetic dyes cannot be commercialized or used within the polymers unless they pose no health risk under end-use conditions. For this purpose the compounds known to pose health risks should not be involved in the manufacturing of synthetic dyes, include a large group of

aromatic amines which are considered either established mutagens or cancer-suspect agents **(Ambrogi *et al* 2018).**

3.2 Environmental contamination by plastic waste

According to Geyer *et al* around 8300 million metric tons (MT) of virgin plastics have been produced to date (2017). For 2015 around 6300 Mt of plastic waste had been generated, around 9%of which had been recycled, 12% was incinerated, and 79% was accumulated in landfills or the natural environment. If the present production and waste management continues to increase, landfills and the natural environment will be full of roughly 12000 Mt of plastic waste by 2050. (Figure 8) illustrates the prediction of plastic waste generation and disposal based on previous years **(Geyer *et al* 2017).**

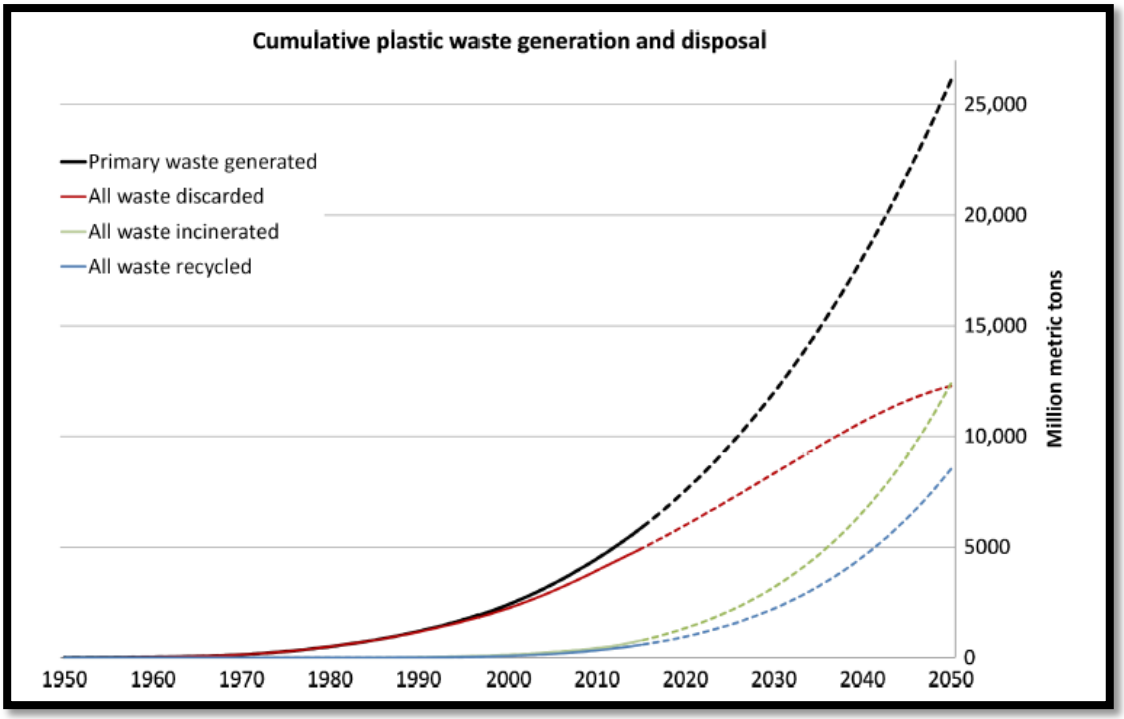

Figure 8: Cumulative plastic waste generation and disposal (in million metric tons). Solid lines show historical data from 1950 to 2015, dashed lines show projections of historical trends to 2050 **(Geyer *et al* 2017).**

Plastics are synthesised from monomers, which are polymerised to form macromolecular chains, during the manufacturing process of plastic, a range of additional chemicals are added such as stabilisers, plasticisers, flame retardants, pigments and fillers that can alter the nature of the final plastic. Some additives are not bound to the polymer matrix and because of their

low molecular weight, these substances can leach out of the plastic polymer into the surrounding environment, including into air, water, food or body tissues **(Galloway 2015).**

The release of the constituent monomers themselves may also pose a hazard, breaking of the chemical bonds in the polymer backbone leads to chain scission and depolymerisation due to different environmental conditions, e.g. temperature variations and oxygen, and proceed at different rates for different polymer types, for example with polyesters, polycarbonate and polyurethane more susceptible to depolymerisation for example than polyethylene or polypropylene **(Galloway 2015).**

The fundamental reason of the large volumes of plastic waste are generated due to the short lifespan of many plastic products, around 40% of plastic products have a service life of less than 1 month. This large waste develops a serious environmental problem. **(Hahladakisa *et al* 2018).** Roland Geyer *et al* combined plastic production data with product lifetime distributions for eight different industrial use sectors. The reason was to model how long plastics are in use before they reach the end of their lifetimes and are discarded. They assumed log-normal distributions with means ranging from less than 1 year, for packaging, to decades, for building and construction (Figure 9), **(Geyer *et al* 2017).**

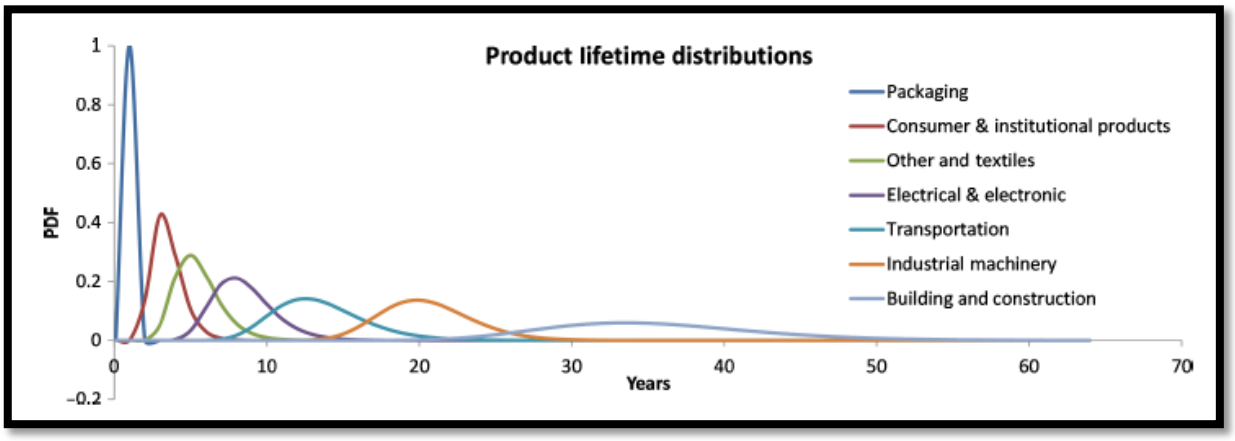

Figure 9: Product lifetime distributions for the eight industrial use sectors plotted as log-normal probability distribution functions (PDF) **(Geyer *et al* 2017).**

3.2.1 Environmental water contamination by plastics disposal

Around 4.8 - 12.7 million tons (MT) of plastic waste entering the ocean per year **(Hahladakisa *et al* 2018)** and 5.25 trillion plastic particles weighing 0.26 MT are floating at sea, the majority of which are considered to be microplastics (less than 5 mm in size), these plastic debris in the ocean are hazardous waste because plastic adsorbs toxic pollutants while they traverse through the environment (Figure 10) **(Wang *et al* 2019),** particularly exposing the marine environment and its living organisms to plastic waste contamination**. (Hahladakisa *et al* 2018).**

Figure 10: Floating plastic debris in coastal waters in The Bay Islands, Honduras (left) turtle entangled in a ghost net (right) **(Radhan *et al* 2019).**

Macroplastics are defined to be the large plastic debris on the marine environment (>25 mm in size). They pose a hazard for numerous marine industries. Besides, macroplastics can cause injury and death of marine wildlife as a result of plastic ingestion and entanglement. Macroplastics can fragment into meso-sized plastics (5-25 mm in size), which can further break down into microplastics. Microplastics or microliter are plastics pieces that measure less than 5 mm in diameter **(Wang *et al* 2019)**, makes them more easily ingested by marine organisms such as seabirds, mammals, sea turtles, fish, and a range of invertebrates, may thus

act as vectors for the chemical transfer of pollutants within the food chain.**(LI *et al* 2016)**, many marine fish species (>50) have ingested plastic debris and it is expected that there will be more plastic pieces than fishes in the oceans by 2050 **(Malizia *et al,* 2019).**

The microplastics are of two types primary and secondary. Primary microplastics are plastics manufactured to have a microscopic size, generated from industrial and domestic products including cosmetics, medicine, synthetic fibers, and raw materials used for plastic production directly enter the oceans as micro-sized particles, accumulate in seas and freshwater bodies **(LI *et al* 2016).** Whilst secondary microplastics are mainly derived from the fragmentation of large-sized plastics debris at sea and on land **(Wang *et al* 2019),** as a result of various physical, biological and chemical processes that minimize the structural integrity of plastic debris **(LI *et al* 2016).**

3.2.1.1 Distribution of plastic debris

The distribution of plastic debris in the sea depends on various mechanisms, including winds, currents, coastline geography and human factors such as urban areas, trade routes and fishing activities. Sedimentation explains areas with very low turbulence, producing an accumulation zone **(LI *et al* 2016).**

3.2.1.2 Effects of plastic debris on organisms

Plastic debris has been found in multiple species worldwide, including seabirds, turtles, crustaceans and fish, the pathway of plastic ingestion by different organisms is shown in (Figure 11). Physical hazards of macroplastics and microplastics to organisms if ingested, includes blockage of the intestinal tract, inhibition of gastric enzyme secretion, reduced feeding stimuli, decreased steroid hormone levels, delays in ovulation and failure to reproduce. Ingestion rarely causes immediate mortality in organisms. Hence, chronic effects have long-term consequences, it also might lead to a reduction in food consumption (Figure 12) **(LI *et al* 2016).**

Ryan (1988) investigated the potential effect of plastic ingestion by seabirds using domestic chicks. The chicks, which were fed with polyethylene pellets, had reduced food consumption due to reductions in their stomach storage volume, showing a negative correlation between the fitness of seabirds and the total mass of ingested plastics. The accumulation of plastic in

the gastrointestinal tract will eventually lead to gastrointestinal blockage or problems with feeding stimuli and activity levels **(LI *et al* 2016).**

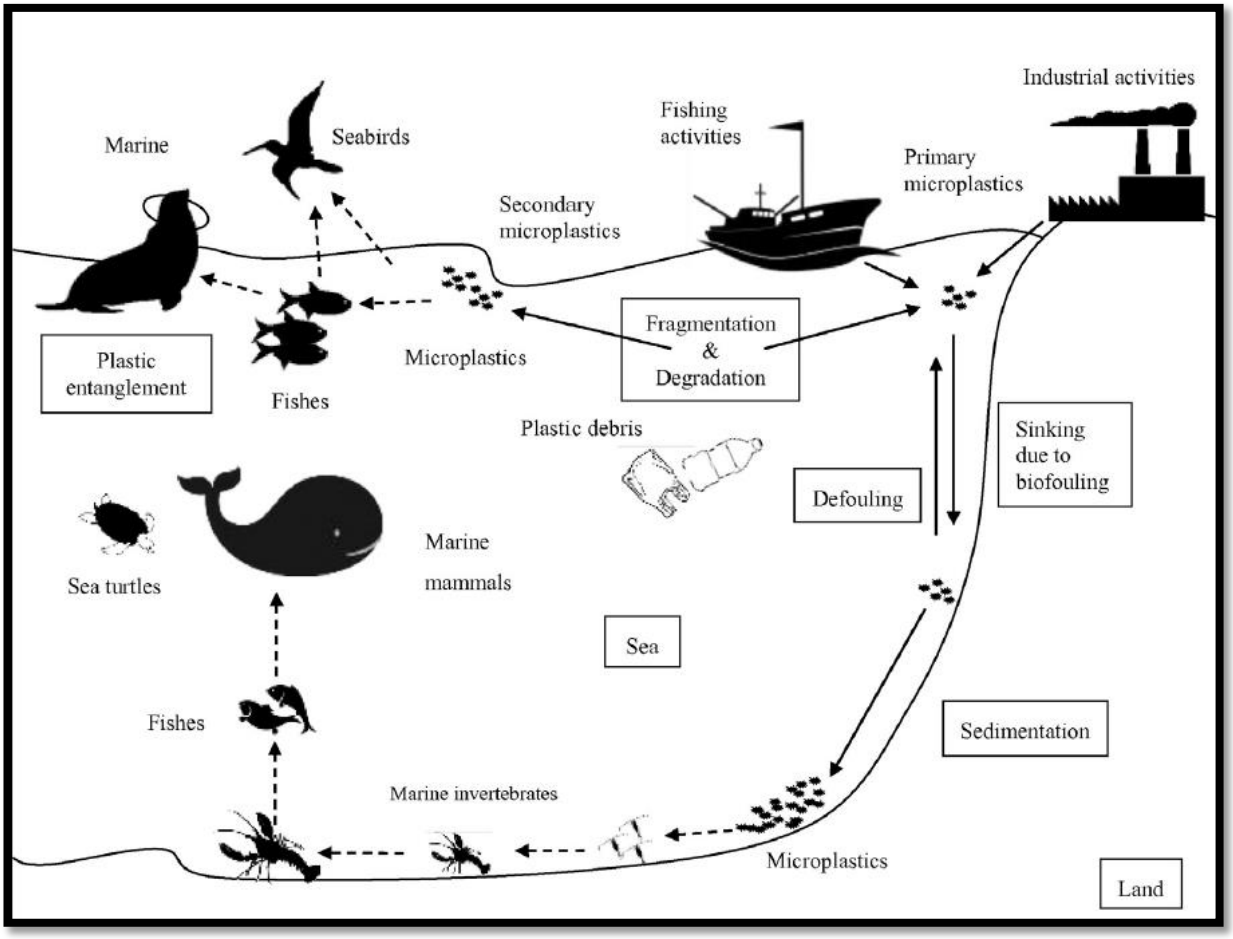

Figure 11: Potential pathways of plastic debris transportation and its biological interactions. Dashed lines show plastics ingestion **(LI *et al* 2016).**

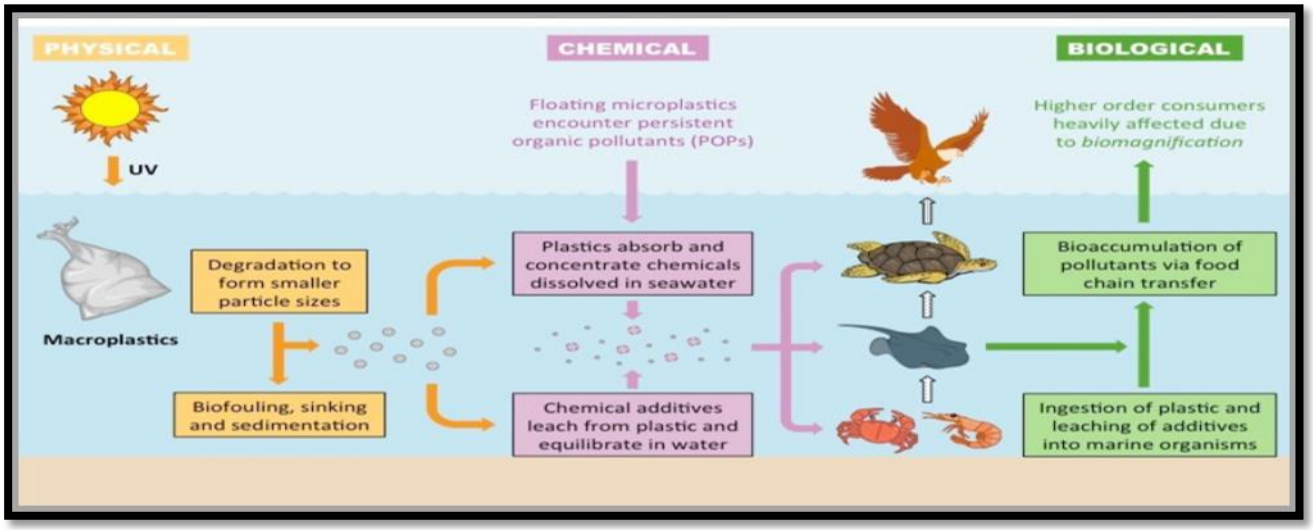

Figure 12: The impact of micro-plastics on the organisms **(http://ib.bioninja.com.au/_Media/plastic-debris_med.jpeg)**

3.3 Contamination of soil by plastics disposal

Plastics are produced on land. Landfills, urban surroundings, and agricultural fields are among the most contaminated terrestrial environments by plastic. The danger of small plastics in the soil and terrestrial ecosystems is due to their impact on soil organisms **(Malizia *et al* 2019).** Organisms including humans and many other relay on soil for their survival, and hence soil pollution and contamination is a serious factor, even affecting food safety for humans. While the industrial development has accelerated and manufacture and disposal of plastic have increased, concerns on plastic pollution are growing. **(Chae and An 2018).** Various plastics enter the soil environment via several sources, including domestic sewage, containing fibers from clothing and microplastic beads from personal care products, biosolids, fertilizers, landfills from urban and industrial centres, lake water flooding, and atmospheric particles transported over long distances. These various plastics enter the soil environment, settle on the surface, and penetrate into subsoil's. Moreover, these plastic pieces may adsorb organic contaminants from the surrounding water, these organic contaminants plus plasticizers can leach from waste disposal sites into groundwater or surface waters. They can also accumulate in soils of natural, at some point may have a direct effect on agricultural sustainability and affect the functioning and biodiversity of terrestrial ecosystems (Figure 13), **(Malizia *et al* 2019).**

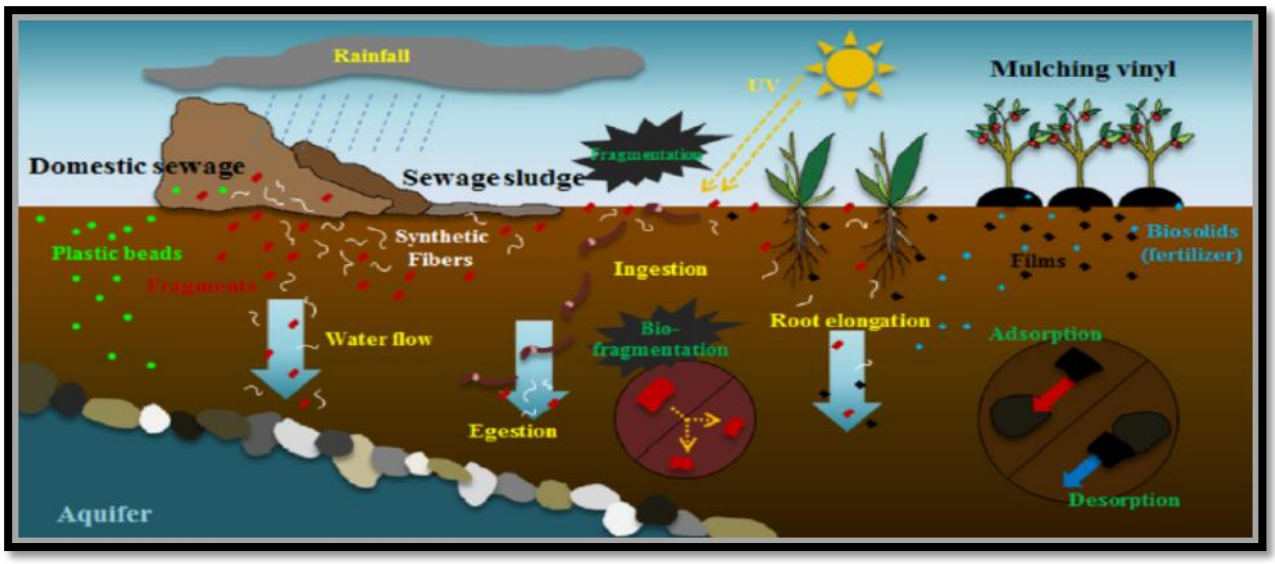

Figure 13: Schematic of the flow of plastic wastes in the soil environment and their distributions and fate in soil (**Chae and An 2018).**

3.3.1Microplastic pollution in the soil environment

Horton et al, 2017 suggested that plastics fragmentation can occur in the surface soil by UV radiation and elevated temperature. These little fragmentized plastic particles can be MPs of small sizes (<5 mm), the plastics on the soil surface can be incorporated into the deep soil by burrowing activities of earthworms, later they can be further transported to deeper layers of the soil by the activities of soil organisms such as, insects, and plants **(Malizia *et al* 2019).**

Microplastics could alter physical and chemical properties of the soil, For example, plastic particles following exposure in the environment are extremely slow to decompose and thus likely to accumulate in soils in carbon stocks as persistent pollutants, not only in natural areas but also in agricultural lands **(Malizia *et al* 2019).**

3.3.2 Impacts of microplastics on soil organisms

Once plastic particles are in the soil, they can be ingested and transferred to soil organisms, leading to unwanted effects on their bodies **(Chae and An 2018),** microplastics may be ingested by micro and meso fauna, such as worms, and accumulated in soil detrital food network **(Malizia *et al* 2019).**

Gaylor *et al* (2013) simulated the exposure of brominated flame retardants that are considered harmful for the environment to earthworm *Eisenia fetida*. They found that the additive leached from the polymer used and were accumulated in the bodies of earthworms, showing that chemicals derived from MPs can enter the soil ecosystem and be accumulated in soil invertebrate organisms **(Chae and An 2018).**

Huerta Lwanga *et al* (2016) exposed earthworm *Lumbricus terrestris* to low-density polyethylene (LDPE) MPs (<150 mm) for 60 days, and investigated their mortality, growth, tunnel formation, and MP ingestion after 14 and 60 days of exposure. suggesting that the health of earthworms was affected when they were exposed to high concentrations of MPs (28, 45, and 60% w/w microplastics in litter, or MPs have the potential of being preferentially retained in the earthworms and transferred to other organisms in the soil ecosystem through the food chain **(Chae and An 2018).**

The reason from using earthworms as test species is that they are sufficiently large for easy identification, convenient for conducting experiments. They also directly ingest plastic wastes

in the soil media and the adverse effects of ingesting these plastic wastes can be easily assessed **(Chae and An 2018).**

The ecological effects of this plastic pollution are alarming and resulted in 59% of the seabird's species in the world ingesting some type of plastic by 2012. By 2050, estimations of seabird's species that will have consumed plastic reach up to 99% **(Malizia *et al* 2019).**

1 Definition of biodegradation

Biodegradation can be defined as the decay or breakdown of materials that occurs, when Micro-organisms such as Bacteria and fungi, including yeasts and molds use an organic substance as a source of carbon and energy, whereas Speight defined the process of biodegradation (biotic degradation, biotic decomposition), as the chemical degradation of contaminants by bacteria or other biological means. Furthermore, Organic material can be degraded aerobically (in the presence of oxygen) or anaerobically (in the absence of oxygen). In addition, Speight has set a number of conditions leading to a successful biotic degradation of contaminants which are : first the presence of metabolically capable and sustainable microbial populations , second a suitable environmental growth conditions, such as the presence of oxygen; but also a temperature, which is an important variable keeping a substance frozen or below the optimal operating temperature for microbial species, can prevent biodegradation; most biodegradation occurs at temperatures between 10°C and 35°C (50°F and 95°F) as well as the presence of water along with appropriate levels of nutrients and contaminants; and finally favourable acidity or alkalinity **(Speight 2017, Poznyak *et al* 2019).**

2 Biodegradation of plastics

Biodegradation of plastics is defined as any physical or chemical change in the material caused by microorganisms such as bacteria, fungi and actinomycetes involved in the degradation of both natural and synthetic plastics which are usually biodegraded aerobically in nature or anaerobically in sediments and landfills and partly aerobically in compost and soil **(Ishigaki *et al* 2004).**

2.1 Aerobic degradation

During the aerobic biodegradation process the microorganisms utilize the polymer as a carbon and energy source and produce carbon dioxide and water as the main degradation by products in addition to the remaining part, which is called compost as shown in (Figure 14), also known as aerobic respiration, it is an important part of the natural attenuation of

contaminants in many hazardous waste sites as aerobic microbes use oxygen as an electron acceptor.

$$\text{Organic matter} + S + O_2 \rightarrow CO_2 + H_2O + NO_2 + SO_2 + \text{Heat} + \text{Compost}$$

(Nayak and Tiwari 2011, Mohee *et al* 2008).

2.2 Anaerobic degradation

Anaerobic biodegradation is the breakdown of organic contaminants by microorganisms during the absence of oxygen, **(Gu 2003)** and the digestion occurs when the anaerobic microbes are dominant over the aerobic microbes **(Poznyak *et al* 2019).** In the absence of oxygen, the organic matter goes through a conversion leading to the formation of methane gas, carbon dioxide, water, hydrogen sulphide, ammonia and hydrogen, which results in a sequence of metabolic interactions by different groups of microorganisms **(Mohee *et al* 2008).** The remaining part is called the digestive residue as demonstrated in (Figure 14), during this process Microorganisms are unable to transport the polymers directly through their outer cell membranes, into the cells where most of the biochemical processes take place, due to their large size and not water-soluble so In order to use such materials as a carbon and energy source, microbes developed a strategy in which they excrete extracellular enzymes that depolymerize the polymers outside the cells **.** During degradation, microbial exo-enzymes break down complex polymers, yielding short chains or smaller molecules like oligomers, dimers and monomers that are small enough to be water-soluble, and can pass through the semi-permeable outer bacterial membranes to be used as carbon and energy sources **(Gu 2003).**

$$\text{Organic matter} + H_2O + \text{Nutrients} \rightarrow \text{Digestive residue} + CO_2 + CH_4 + NH_3 + H_2S + \text{Less heat}$$

During the process of aerobic degradation, the energy stored in organic matter is released in the form of heat. On the other side, in anaerobic degradation, the energy stored in organic matter is mainly released as methane, and due to the lack of oxygen in the process, less heat and less microbial biomass are produced **(Batori *et al* 2018).**

3 Microorganisms with plastic degrading abilities

Microorganisms such as bacteria, fungi and actinomycetes share an important role for both synthetic and natural plastic degradation **(Gu *et al* 2000)**.

Microbial degradation of plastics is a result of oxidation or hydrolysis using microbial enzymes that lead to chain cleavage of the large compound polymer into small molecular monomer by the metabolic process **(Kumar *et al* 2013)**, in addition, several factors comprising the availability of water, redox potential, temperature carbon and energy source have an impact on the microorganism's growth **(Sand 2003).**

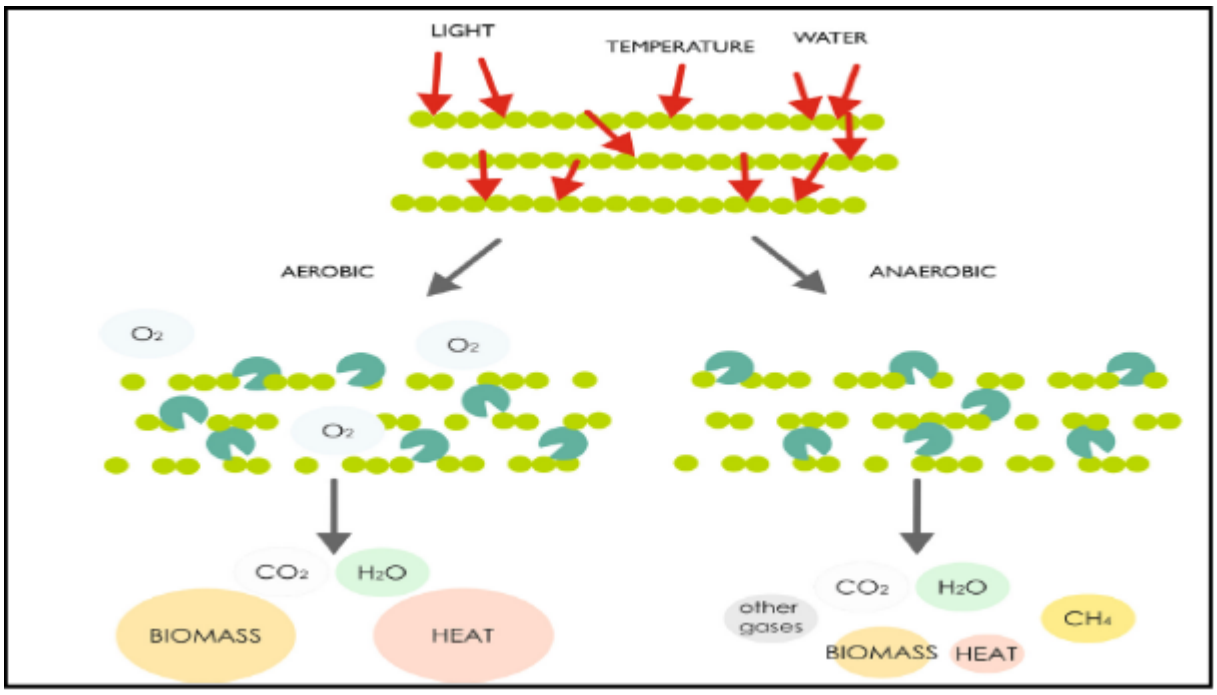

Figure 14: Biodegradation of polymers, aerobic vs anaerobic degradation **(Batori *et al* 2018).**

3.1 *Bacillus megaterium*

In 1872 , Cohn identified the genus bacillus for the first time **(Zhang *et al* 2016)**, the members belonging to this genus are Gram-positive , rod shaped and endospore as demonstrated in (Figure 15),moreover they have the ability to occupy a variety of ecological niches and have been isolated from diverse environmental regions **(Liu *et al* 2016).** The bacteria owes it's name to it's large size "*megatherium*" (Greek for big animal) of 1.5 by 4 µm, this microorganism is the largest of all bacilli **(Epinger *et al* 2011).**

Found in multiple habitats, *B.megaterium* is mainly considered as a soil bacterium and has the ability to exploit diverse carbon sources and grow at a vast temperature range (from 3 °C to 45 °C). It furthermore possesses the capacity of promoting plant growth activity as instance bio-control ability against plant pathogens **(Vary *et al* 2007, Kildea 2008, Nguyen *et al* 2011).**

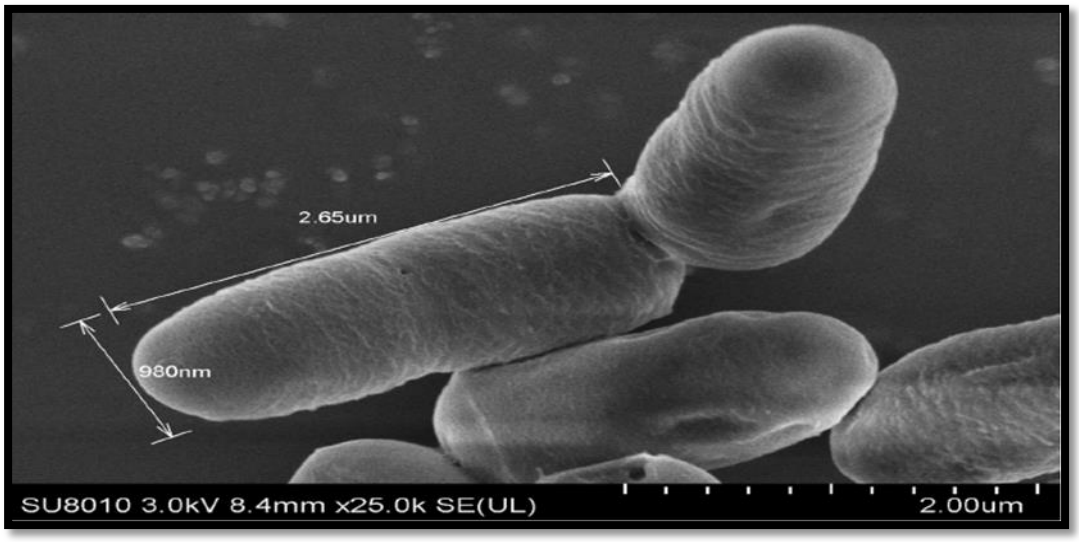

Figure 15: *Bacillus megaterium* cell with dimensions of 0.98 µm×2.65 µm observed using field emission scanning electron microscopy **(Huang *et al* 2019)**

3.2 Genome sequencing and architecture of *Bacillus megaterium*

The chromosomes of *B.megaterium* strain QM B1551 are a circular shaped molecule of 5,097,129 Bp as shown in (Figure 16). The chromosome of this strain contains 5,284 genes and disposes a high level of genome conservation, over and above , the chromosomes show an average G-C content of 38.2% and a higher G-C content compared to the plasmids (38.2 vs 33.0 to 36.5) displayed in (Table 2), besides strain QM B1551 contains seven indigenous plasmids pBM100 to pBM700, with sizes from 5.4 kb to over 164 Kb. Eppinger *et al* cataloged a total of 300 isolate-specefic genes, and found that the gene insertions in the chromosome are rare in a 2-Mb region around the origin of replication (ori) Compared to the function of genes common to the strain, strain-specific genes are increased in functions affecting interactions with the environment ,cell envelope, transport, signal transduction, and gene regulation, in contrast, relatively fewer strain-specific genes are associated with basic cellular processes, such as amino acid, nucleotide, or cofactor biosynthesis, central

intermediary metabolism, fatty acid metabolism, protein synthesis or degradation, or transcription **(Eppinger *et al* 2011).**

Table 2: Genomic properties of *B.megaterium* compared to related species.

Species	Strain	Genome size (Mbp)	% GC	No. of protein-coding genes	No. of tRNAs	No. of rRNA operons
B. megaterium	QM B1551	5.1	38.2	5,130, plus 499 on plasmids	120, plus 19 on plasmids	11, plus 1 on pBM400
B. megaterium	DSM319	5.1	38.2	5,124	115	11
B. subtilis	168	4.2	43.5	4,176	85	10
B. amyloliquifaciens	FZB42	3.9	46.5	3,813	86	10
B. licheniformis	ATCC 14580	4.2	46.2	4,192	72	7
B. pumilus	SAFR-032	3.7	41.3	3,678	69	7
B. cereus	ATCC 10987	5.2	35.6	5,602	97	12
B. anthracis	AO465	5.2	35.4	5,040	95	11
B. thuringiensis	Al Hakam	5.3	35.4	4,736	104	14
B. weihenstephanensis	KBAB4	5.3	35.6	5,155	109	14
B. halodurans	C-125	4.2	43.7	4,065	78	8
B. clausii	KSM-K16	4.3	44.8	4,096	74	7
Oceanobacillus iheyensis	HTE 831	3.6	35.7	3,500	69	7
Listeria monocytogenes	EGD-e	2.9	38.0	2,846	67	6

3.2.1 *Bacillus megaterium's* plasmid analysis

In regards to the QM B 1551, the plasmids make up 11% of the genome , as mentioned previously, the strain harbors seven indiginous plasmids as shown in (Table 3) with a plasmid copy numbers varying from 1 to 18 copies, the plasmids elevate a variety of genes, including genes for sporulation, germination, regulation, transport, and antibiotic synthesis, as well as erythromycin and rifampin resistance in conjunction with genes for fatty acid metabolism, cell wall hydrolysis, sigma factors, and cell division, as well as integrons, sequence (IS) elements, and transposons see (Figure 17). Because *B.megaterium* is frequently found with *Pseudomonas* species in contaminated environments, it has long been suspected of having the capability of metabolizing unusual substrates of possible bioremedial use **(Kunnimalaiyaan *et al* 2005, Scholle *et al* 2003).** Likewise, *B.megaterium* plasmids carry genes for heavy metal resistance, including Cu and Cd, and genes such as styrene monooxygenase are present, numerous metabolic genes on the larger plasmids are organized to be functional operon structures and may enable the strain to survive in abnormal and unconventional habitats.

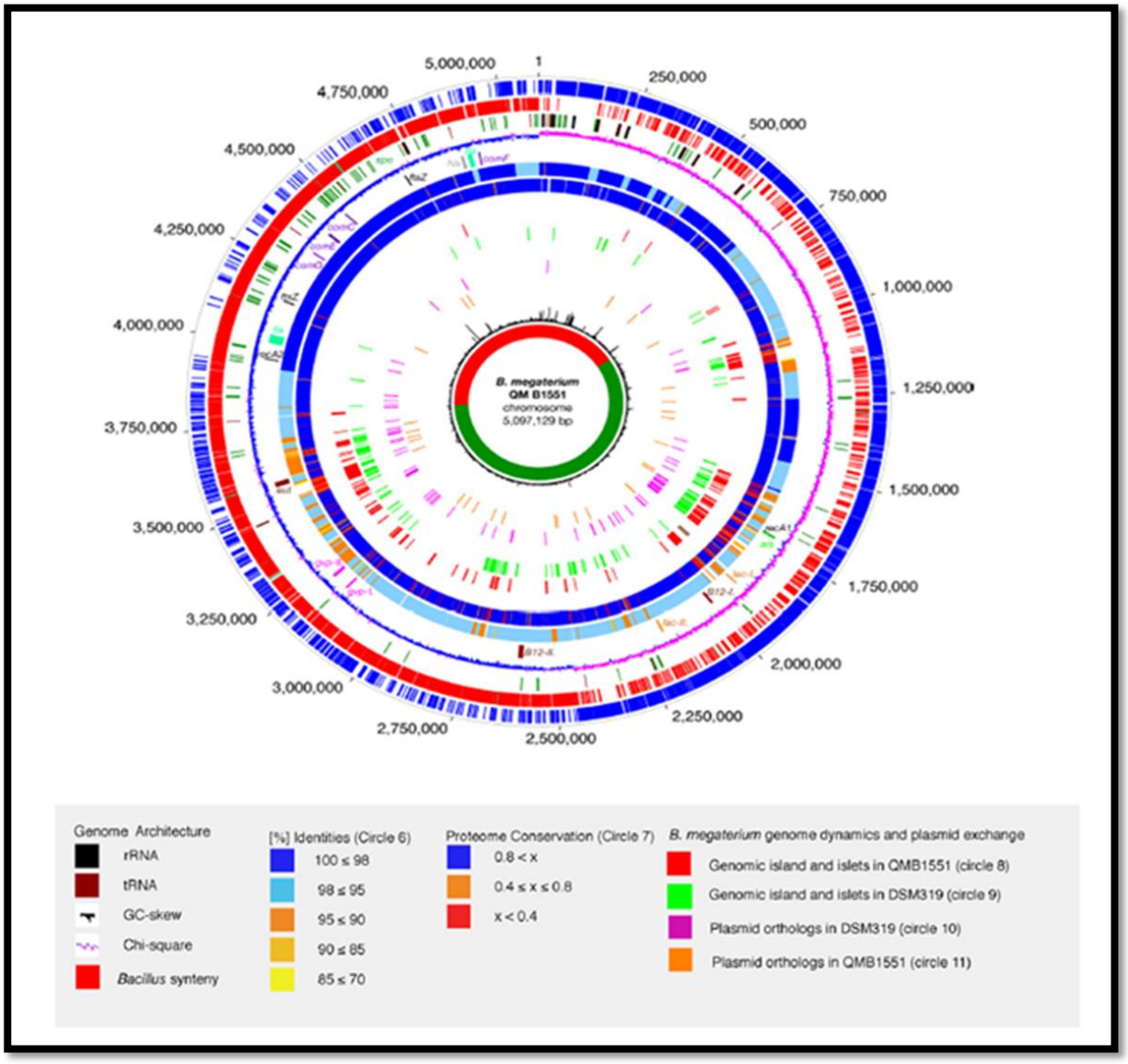

Figure 16: Circular representation of the *B. Megaterium* chromosome. Circles (numbered 1 to 13, from outer to inner circle): circles 1 and 2,predicted open reading frames encoded on the QM B1551 plus (circle 1, blue) and minus (circle 2, red) strands; circle 3, predominant genes in conserved *Bacillus* region, showing tRNAs (brown), rRNA clusters (black), and sporulation and germination (dark green); circle 4, GC skew; circle5, chromosomal regions of interest, including gas vesicle operons I and II (gvp; magenta), vitamin B12 operons I and II (B12; brown), flagellar operons (fla; aqua), RecA genes (rec; black), arsenate resistance operons (ars; green), beta-galactosidase genes (lac; orange), ethanolamine utiliazation (eut; brown), histidine biosynthesis (his; gray), and competence operons CEFG (com; purple); circles 6 and 7, comparative analysis of the QM B1551 chromosome identities (circle 6) and proteome (circle 7) to DSM319; circles 8 and 9, non-random distribution of genomic islands and islets in QM B1551 (circle 8) and DSM319 (circle 9); circles 10 and 11, chromosomal genes with QM B1551 plasmid-borne orthologs in QMB1551 (circle 10) and DSM319 (circle 11); circle 12, chi-square values to show GC deviations; circle 13, region of conserved synteny (red) neighboring the *ori* identified by the comparative study of genome .

Table 3: Plasmid content of *B.megateriums* train QM B1551

Plasmid	Size (bp)	Read count	Read coverage	Copy no.	CDS	rRNA	tRNA	% GC
pBM100	5,428	253	40X	3	8	0	0	34.8
pBM200	9,098	1,826	169X	14	13	0	0	34.5
pBM300	26,587	7,052	224X	18	38	0	0	35.5
pBM400	53,865	7,849	123X	10	60	3	18	36.5
pBM500	66,985	5,163	65X	5	94	0	0	33.9
pBM600	99,694	2,334	20X	2	111	0	0	33.0
pBM700	164,406	2,365	12X	1	175	0	1	33.5

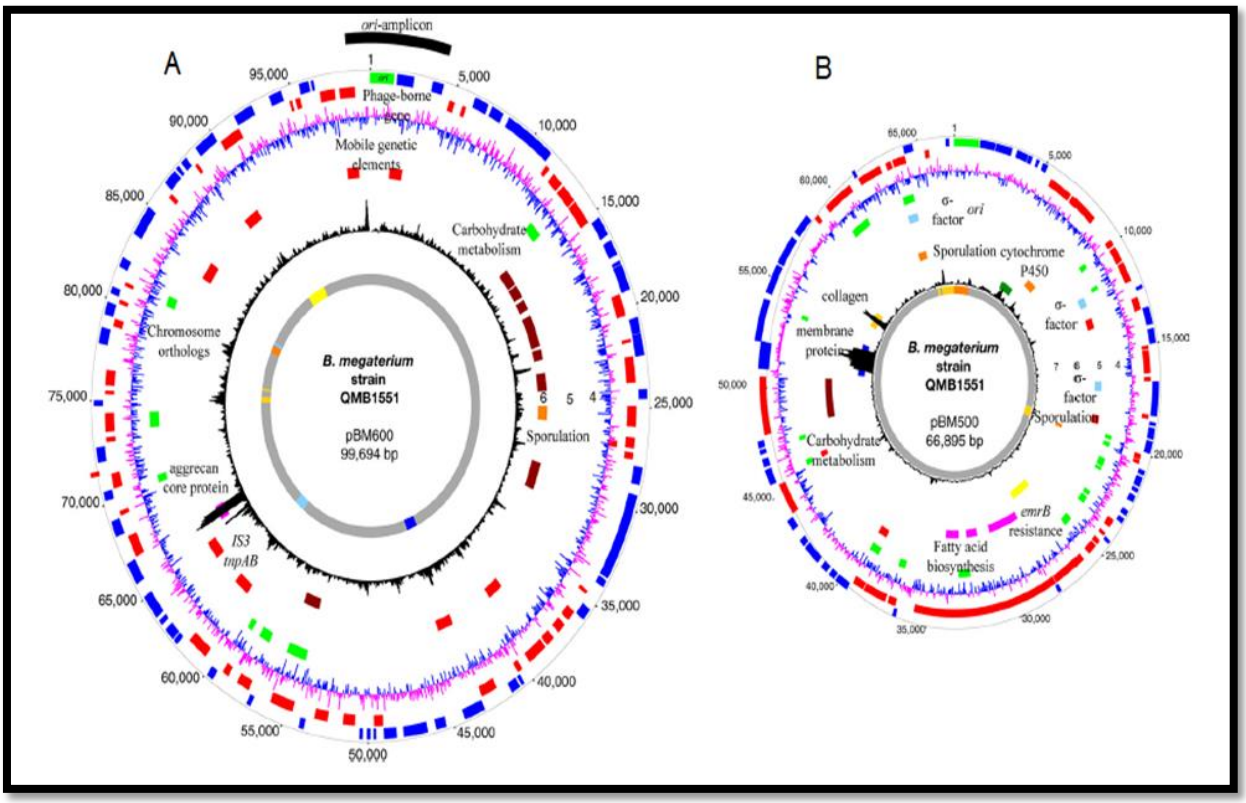

Figure 17: Circular maps of the *B.megaterium's* QMB1551 plasmids pBM 600 and Pbm500. (A) Regions and genes of interest in pBM600. The origin amplicon is shown in black, with the identified replication gene in green. Circle 5, mobile genetic elements (red). Circle 6, carbohydrate metabolism (brown) and sporulation genes (orange). (B) Regions and genes of interest in pBM500. Circle 5, regulatory functions, including transcription regulators (red) and three sigma factors (light blue); circle 6, metabolism features, including carbohydrate metabolism (brown) and fatty acid biosynthesis (magenta); circle 7, sporulation (orange) and *erm* (B) macrolide resistance (yellow); circle 8, regions of deviating GC content, membrane protein (blue), collagen-like protein (gold), and cytochrome P450 (green) **(Eppinger *et al* 2011).**

4 Metabolitic and physiological capabilities of *Bacillus megaterium*

4.1 Extracellular enzymatic depolymerisation

Carbon sources are generally used in a hierarchical manner. The ability of one carbon source to repress the utilization of another is called catabolite repression that is to say one carbon source is used preferentially over others **(Plumbridge and Deutscher 2014),** for the uptake of Carbon Sources from the medium, the utilization operons generally encode for the enzymes that transform the more exotic starting materials into one of the compounds of the central carbon degradation pathway requiring a specific outer membrane porin **(Figueroa-Bossi *et al* 2009).** While Dang *et al* made a study about the degradation of plastic they found that those actif extracellular enzymes were hydrolase enzymes counting CMCase, protease, chitinase, xylanase, and lipase **(Dang *et al* 2018).** A signal transduction pathway senses the external presence of the compound and turns on the expression of the required genes. Detection of a signal outside the bacteria provokes the auto-phosphorylation of a histidine sensor kinase, which in turn phosphorylates and activates a second protein, a response regulator **(Wright *et al* 2000).**

4.2 Intracellular carbon metabolism by *B. megaterium*

CCR revolves mainly around the HPr protein of the PTS**(Warner and Lolkema2003),** only another cofactor will replace the Hpr protein when the bacteria is found in a media with complex carbon sources, since intracellular sensors detect radical physiological changes, global cellular rearrangements are initiated **(Lengeler 2013).** By growing Crh in wild-type cells in minimal medium supplemented with multiple carbon sources Landmann *et al* found that Crh appeared higher when cells utilized unfavourable carbon sources **(Landmann *et al* 2011),** and unfavourable substrates led to an efficient phosphorilation of Crh by 80 % unlike Hpr **(Singh *et al* 2008),** and it is therefore deliberated that Crh is implicated in signalling the nutrient status of the cell. Because Crh is a homologue of Hpr , it replaces it in carbon metabolism though it does not contain a His15 residue and features an exclusively regulatory function that depends on its HPrK mediated phosphorylation of Ser46, thus the Crh protein is phospohrilated by HPr kinase/phosphorylase at Ser46, the resulting serine-phosphorilated Crh binds to CcpA allowing it to bind to specific operator sites (*cre*) which is mostly located in the transcription-initiation regions or overlap with the promoter consensus sequence, due to the lack of His15 residue, the affinity of the phosphorilated Crh to CcpA is much weaker than

it's homologue leading the complex not to bond efficiently to the cre repressing the metabolism of class b carbon sources until the depletion of class a carbon sources **(Gork and Stulke 2008, Nessler 2003, Bruckner and Titgemeyer 2002, Schumacher *et al* 2006).**

5 Aerobic degradation of Phthalate in gram positive bacteria

The phthalate esters represent a class of chemicals which are very little volatile and not very soluble in water. They are widely used as plasticizers for polyvinyl chloride in a wide range of domestic and industrial applications **(Saillenfait and Laudet-Hesbert 2005),** and are found in several household products such as food packaging, furniture and toys. Due to the abundance of plastic in our society, the exposure to phthalates is ubiquitous. Human are exposed to phthalates through different ways such as inhalation, ingestion and dermal contact, which then leads to serious health risks **(Muscogiuri and Colao 2017).**

Bacterial degradation of phthalate is efficient as these compounds are either biotransformed or mineralized completely. Both aerobic and anaerobic routes are possible for the degradation of the phthalate isomers. The degradation of phthalate esters as the sole carbon source has been reported by many bacterial strain. Proteins that are involved in the transport and degradation are induced by the presence of phthalate in the bacterial medium, longer chain phthalate esters are difficult to degrade, **(Krishna and Phale 2008),** such as dicyclohexyl phthalate (DCHP), dihexyl phthalate (DHP), dioctyl phthalate (DOP), and di- 2-ethylhexyl phthalate (DEHP) **(Liang *et al* 2008).**

Esterase hydrolyses the phthalate esters to their respective isomers first, and then the phthalate isomers are transported inside the cell by specific permease, which in turn induces specific phthalate 3,4-dioxygenase, present mostly in Gram positive bacteria. This enzyme catalyzes the initial step of incorporation of two hydroxyl groups on the phthalate rings to yield phthalate dihydrodiols. Action of phthalate 3,4-dioxygenase produces *cis*-3,4-dihydroxy-3,4-dihydrophthalate, which is converted to 3,4-dihydroxyphthalate and subsequently decarboxylated to yield 3,4-dihydroxybenzoate by 3,4-dihydroxyphthalate decarboxylase. By the ortho pathway the enzyme protocatechuate 3,4-dioxygenase ring-cleaves the 3,4-dihydroxybenzoate to yield β-carboxy-cis,cis-muconic acid ,which is further oxidized to succinyl-CoA and acetyl-CoA via β-ketoadipate (Figure 18) **(Krishna and Phale 2008).**

The 'upper pathway' constitutes the metabolic steps and the enzymes responsible for the conversion of phthalate esters to 3,4-dihydroxybenzoate, while the 'lower pathway' is referred to the steps responsible for the conversion of 3,4-dihydroxybenzoate to central carbon pathway. The metabolic diversity in microorganisms is mostly observed in the lower pathway **(Krishna and Phale 2008).**

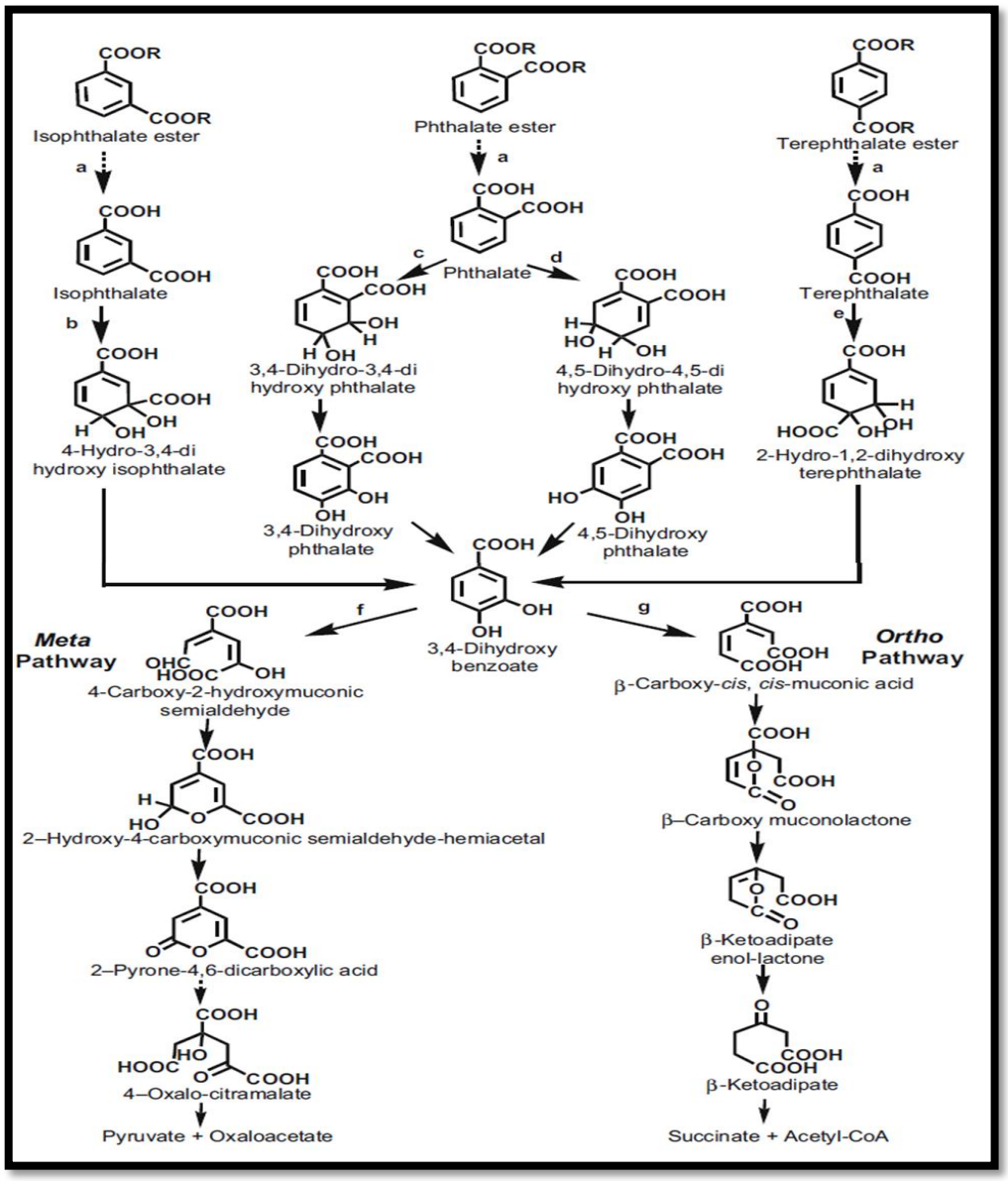

Figure18: Metabolic pathway of phthalate esters. Few key enzymes of the metabolic pathway are: a, esterase; b, isophthalate 3,4-dioxygenase; c, phthalate 3,4-dioxygenase; d, phthalate 4,5 dioxygenase; e, terephthalate 1,2-dioxygenase; f, protocatechuate 4,5- dioxygenase; g, protocatechuate 3,4-dioxygenase **(Krishna and Phale 2008).**

5.1 Genetics of phthalate degradation

Genes associated with the metabolism of phthalate isomers are present either on the plasmids, chromosome, insertion elements, or both on the plasmid and chromosome. Genes are organized in operon(s). They are well characterized with respect to their organization and regulation. The organism that has the ability to degrade all phthalate isomers effectively as the carbon and energy source is considered to be the most precious **(Krishna and Phale 2008).**

5.2 Biodegradation of di-n-butyl phthalate (DBP) by *Bacillus megaterium*

One of the most important phthalate acid esters (PAEs) is DBP. DBP are phthalate with a short ester chain. Which are primarily used to improve the flexibility and workability of plastic, as environmental hormone, PAEs can affect reproduction and induce genetic aberrations in humans even at low concentrations, that way resulting in an increasing environmental concern. DBP is not chemically bonded to the plastic polymer like other (PAEs) and can be easily released into the environment during the manufacture, use, and disposal of plastics, leading to ubiquitous occurrence in various environmental forms. **(Feng *et al* 2018).**

Feng *et al* research showed that a novel endophytic strain YJB3could utilize a wide range of PAEs as the sole carbon and energy sources for cell growth, the endophyte was isolated from Canna indica root tissue. It was identified as *B.megaterium* based on morphological characteristics and 16S rDNA sequence homology analysis. YJB3 incubation in mineral salt medium containing di-nbutyl- phthalate (DBP) showed its degradation capability. The toxic organic contaminants accumulated in plant tissues may be mineralized in *planta* using endophytic bacteria, which is consider being beneficial. Results showed that the DBP was degraded more and more efficiently with the growth andbiomass accumulation of the strain YJB3, giving rise to a linear correlation between the degradation rate of DBP and the biomass of the strain YJB3 **(Feng *et al* 2018).**

5.3 Plant growth promotion by *Bacillus megaterium*

The role of microorganisms has becomes extremely important as they are eco-friendly, safe means which can replace, or supplement to a great degree, the use of chemicals such as fertilizers, insecticides and fungicides in agriculture **(Chakraborty *et al* 2006).**

The free living microorganisms that have the ability to aggressively colonize plant root and stimulate growth of plants including disease control are referred to as plant growth promoting rhizobacteria (PGPR). One type of these bacteria's is the *B.megaterium,* its ability to promote plant growth and cause disease reduction in plants was tested after its isolation from tea rhizosphere by Chakraborty *et al*. The application of the bacterium to the soil showed a remarkable reduce of *Fomesl amaoensis* which causes brown root rot compared to before application of *B.megaterium* **(Chakraborty *et al* 2006)**, as well as the activation of plant defence responses and secretion of plant growth-regulating substances such as auxins, cytokinins and bacterial volatiles. The control of growth involves phyto-hormones in almost every important developmental process in plants. Phyto-hormones secretion by bacteria can impact root architecture by overproduction of root hairs and lateral roots and subsequently increase nutrient and water uptake, thus contributing to growth **(Ortíz-Castro *et al* 2008).**

6 *Bacillus megaterium's* strategies to maintain membrane integrity in a low pH media

B.megaterium's growth characteristics are known to be highly influenced when found in acid enviroments, despite that, the neutralophilic microorganism developed multiple ways to adapt to the enviromental stress conditions **(Foster 2004)**. One of the basic and essential strategies being the maintenance of the cell wall / membrane integrity **(Krulwich *et al* 2011, Mols and Abee 2011, Kanehisa *et al* 2016)**, as the cell wall is composed of peptidoglycan (PG) to protect the cell against lysis due to the osmotic pressure which is polymerized and modified by the penicillin-binding proteins in order to build the morphology of the peptidoglycan exoskeleton together with cytoskeleton proteins that regulate septum formation and cell shape **(Pophan and Young 2003),** with the help of polysaccharide deacetylase which has been confirmed to be associated with lateral PG synthesis, biogenesis of PG during cell division and elongation, including polysaccharide modification **(Balomenou *et al* 2013)** led to the stabilization of the cell wall and aids in adaptation to high salt stress **(Arnaouteli *et al* 2015),** and with sporulation and germination **(Fukushima *et al* 2002)**. After a study made by Goswami *et al*, all genes of these membrane proteins and proteins involved in peptidoglycan

assembly showed a differential transcriptome and increased gene expression signifying a possibility for *B.megaterium* to remodel it's membrane structure for maintaining membrane integrity under acid stress alongside the activation of the gene Spo0a associated sporulation helping the bacteria to surmount the stress conditions **(Hamon and Lazazzera 2002, Goswami *et al* 2018).**

This study concentrates on the biodegradation of plastic by means of microorganism as in this case the *Bacillus megaterium* bacteria, this bacteria was handed to us conserved in a Eppendrof tube after being isolated from a contaminated plastic sample collected at the Ouled Boughalem by Hadda et al, 2018 and then identified via catalase test, gram staining test and finally an API 20E test.

1 Instruments used:

Agitators /Shakers, Autoclave, Beakers, Bunsen burner, Boiling flask, Cuvettes, Electronic balancer, Erlenmeyer flasks, Freezers / refrigerator, Funnel, Glass bottles, Glass jars, Graduated pipette, Graduated cylinder, Incubators, Inoculating Loop, Laboratory Mixers / Stirrers, Magnetic bar, Magnetic Stirrers, Measuring cup, Microscope glass Slide, Microscopes, Pasteur pipettes, Petri dishes, PH Meters, Spatula, Spectrophotometer, Spectrophotometer Cuvettes, Thermometers.

2 Protocol:

2.1 Culture media:

Three culture media were prepared, ingredients are mentioned in the annex.

2.2 Nutrient broth and nutrient agar

300 ml of distilled water were measured using a graduated cylinder, as well as weighing out the desired amount of nutrient broth ingredients that are going to be utilised, using a pre formulated dry powder, for this procedure a paper is used when weighing the chemicals to protect the balance, all weighed ingredients are then introduced into a flask, 300 ml of distilled water were added to the same flask as well as the stirring magnet bar following its placement on the stir plate.

The solution was stirred continuously until all of the visible clumps were broken down to obtain a uniform suspension. When dissolved, using a funnel, 200 mL of the solution was removed to another flask that includes the right amount of agar powder for the corresponding solution for the preparation of nutrient agar, this solution was well agitated, because this compound requires heat for a total decomposition. Diluted acid and base are used to adjust the desired PH in both mediums, for broth media pH resulted to 7.65 and 6.78 after adding 3 drops of diluted acid.

Both the Broth and the agar medias were dispensed into glass bottles (Figure 19), and are tightly closed, the two culture mediums will need to be sterilized to kill any contaminants, this procedure lasts for 20min under 121^{o}C and 20 PSI using an autoclave, later the mediums were allowed to cool prior use, to sustain and grow organisms.

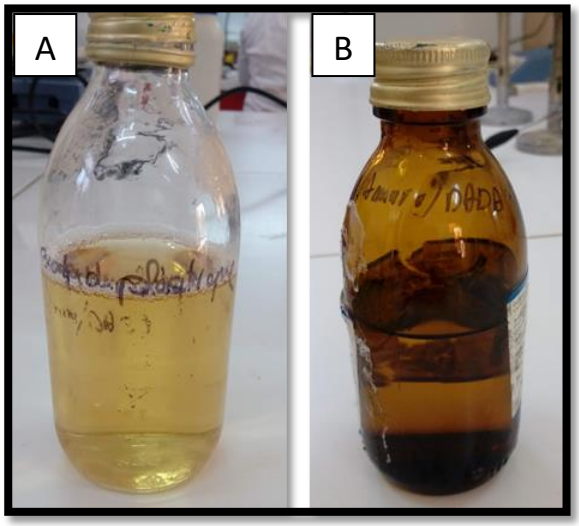

Figure 19: Prepared nutrient agar (A) and nutrient broth (B).

2.3 Mineral medium

2.5 L of distilled water was measured and only 2 L was introduced into a 3 L flask as well as the stirring bar. The mineral mediums ingredients were weighed and introduced into a 3L flask, the rest of 0.5 L of distilled water was poured down the sides of the flask to collect any loose powder from the sides of the flask. The flask was then placed onto the magnetic stirrer (Figure 20), and stirred continually to achieve a completely dissolved solution. When finished, the flask is removed from the magnetic stirrer.

Using a funnel, 150 ml of the mineral medium is poured into each glass jar, 8 glass jars were needed for this experiment, which were previously washed and cleaned. The jars are labelled and introduced to the autoclave for sterilization, once the autoclave has completed its cycle, the sterilized media is removed and let to cool.

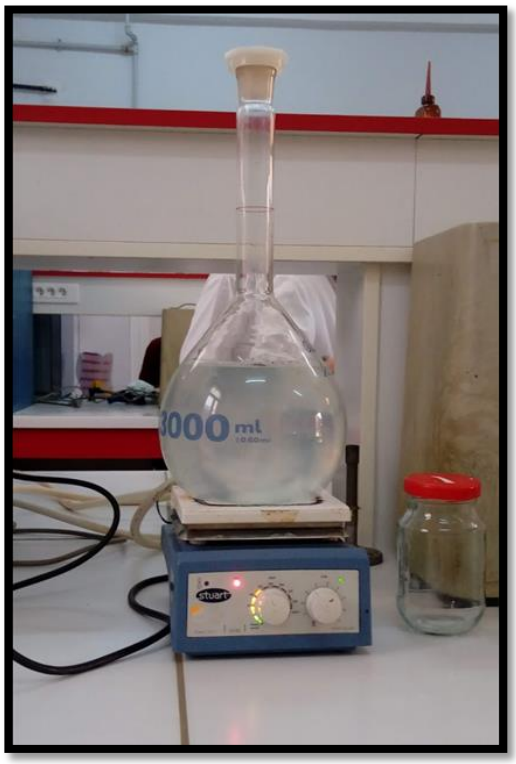

Figure 20: The placement of the boiling flask on the magnetic stirrer

2.4 Bacterial sample preparation

Melting the pre-prepared agar media and letting to cool to room temperature, media is poured into 3 Petri dishes covering the surface, once media has cooled it turns opaque, Petri dishes were then turned upside down to prevent moisture from condensing on the agar surface. Meanwhile, opening the Eppendrof tube containing the conserved strain and tapping the lid by a sterilized loop in order to ensure the loops coolness, following the collection of a small portion of the culture, the Epperndrof tube is then capped. Tapping the inoculum at the edge of the plate making a uniform smear, plate streak is preformed to cover the plate with bacteria. The agar plates are then turned upside down and placed in the incubator under 37^{o}C for 24h.

By placing the agar plate containing the bacteria culture upside down so it's easier to lift the agar section of the plates when selecting a single colony from a pure culture before inoculating. It is important to make sure that the inoculating loop is sterilized by passing it through the flame until red-hot and cooled by tapping it on an empty space as the side of the agar plate before grabbing a single isolated colony.

It is important when inoculating liquid culture to grab a single well isolated colony because once the bacteria is grown on a liquid culture it is difficult to see if there's any contamination.

Once a single colony is selected, by removing the lid of the glass bottle containing the broth media and flaming the neck of the bottle to remove any dust priori inoculating the loop into the media, following the shake of the loop into the liquid media. Once the loop is removed, the neck of the bottle is re-flamed and capped. The liquid culture is then placed in the incubator under 37^{o}C for 24h.

5ml of the liquid culture is dispensed in the six glass jars containing 150 ml of mineral media, Diluted acid is used to adjust the desired pH in to two jars to obtain an acidic medium (pH= 4.5).

2.5 Plastic sample preparation:

Two types of plastic were used in the present work brown plastic cups and transparent plastic bottles, the plastic were previously cut into slightly big pieces (Figure 21), and placed under UV light for 3 months. The reason for the plastic to be in big pieces rather than small pieces is that the smaller pieces showed a lower degradation rat compared to the larger pieces **(Yagi *et al* 2012).**

The resulting plastic sample were weighted and submerged in the previously prepared jars containing the mineral media. Plastic weight and jar content are provided in (Table 4).

The 8 glass jars are capped in a way to allow air to pass, and then placed on an agitator under 130rpmin an incubator under 37^{o}C, the reason for a continuous shaking is to release the energy stored in organic matter in form the heat during aerobic degradation. There for the agitation allows it to release some of this heat for a healthy microbial community **(Bátori *et al* 2018).**

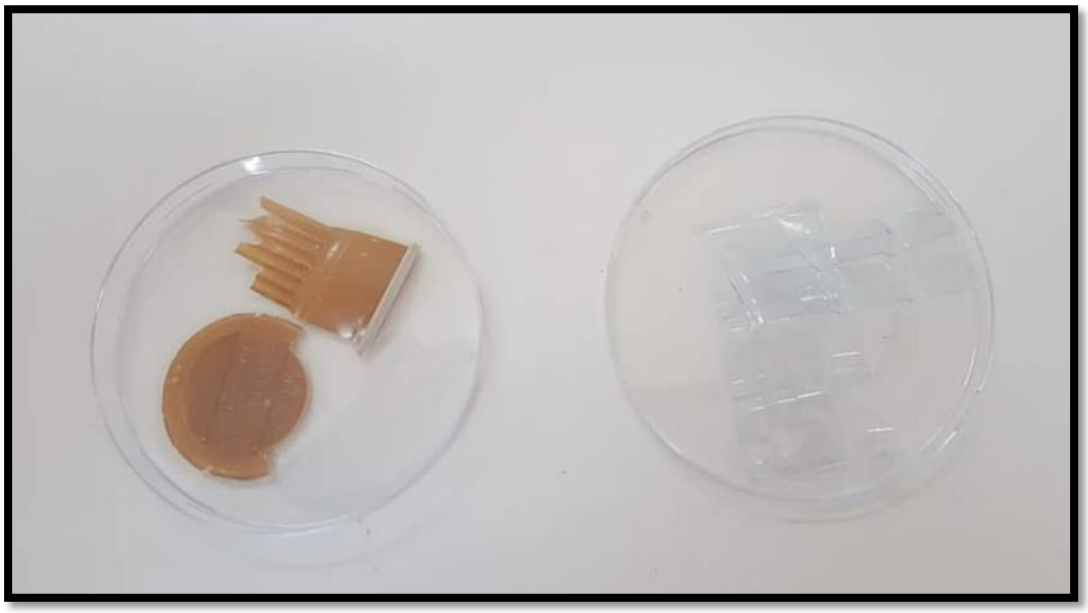

Figure 21: Plastic pieces used, brown plastic cups (A) and transparent plastic bottles (B).

Table 4: The initial weight of the plastic samples and the content of the glass jars.

Jar	Amount of mineral media	Brown plastic added (grams)	Transparent plastic added (grams)	Bacterial culture added (ml)	pH
1	150	2.534	1.575	/	7
2	150	2.517	1.509	/	7
3	150	/	/	5	7
4	150	/	/	5	7
5	150	2.509	1.440	5	7
6	150	2.524	1.477	5	7
7	150	2.505	1.587	5	4.5
8	150	2.507	1.553	5	4.5

2.6 Spectrophotometry of bacterial suspensions

Using a spectrophotometer to test the biomass of the bacterial suspension in every glass jar, the following procedure was applied ever week for 3 months continuously.

The jars were removed from the agitator to the working bench next to a lighting Bunsen burner, with the help of a Pasteur pipettes, 1 ml of each jar is inoculated in a separated Cuvettes, the Pasteur pipettes are dispensed after each use, the occupied Cuvettes are then

placed in a spectrophotometer chamber for measurements and the correct wavelength is set, in our case 600n.m is the appropriate wavelength for measuring bacterial growth in broth. The excitation of each Cuvette are then displayed on the screen and recorded.

2.7 Plastic weigh

Plastic samples were removed from each glass jar separately and rinsed with distilled water and let to dry under absorbent paper (Figure 22), and were blow dried to ensure full dryness (Figure 23). The plastic samples were weighed using an electronic balance.

Figure 22: Air drying the plastic over absorbent paper.

Figure 23: Drying the plastic by a hair dryer.

3 Conservation of bacterial strains

3.1 Long-term conservation

The nutrient agar was poured in to 2 Petri dishes covering the surface, once media has cooled, it turns opaque. Meanwhile, opining the Eppendrof tube containing the strain, using a sterilized loop, a small portion of the culture was collected. The Eppendrof tube was then capped, and plate streak was performed to cover the plates with bacteria. The agar plates were then turned upside down and placed in the incubator under 37^{o}C for 24h.

After 24h of incubation the agar plate containing the bacterial culture were taken out of the incubator, and one single colony was collected using a sterilized loop and inoculated in a pre-prepared nutrient broth tube, the tube was then capped and agitated using a vortex, it was then incubated under 37^{o}C for 24h.

After incubation, 1ml of the culture was inoculated in a sterilized Eppendrof tube, following the addition of 0.5 ml of glycerol, finally vortexing the tube and introducing it into a freezer under -17^{o}C.

4 Biodegradable plastic construction

The biodegradable plastic ingredients, mentioned in the annex were poured in a beaker and mixed using a spatular while its still cold making sure all starch clumps are dissolved , the beaker was then placed on a heated magnatic stirrer, the mixture started out as a white milky mixture and through the continuse mixing it started to get thiker and opaque, the mixture was removed away from heat when it stared to bubble up, it was then spreaded out thinly on a previosly laid aluminum foil, the thin layer of biodegradable plastic was left for 24h to dry.

Another sample of the biodegradable plastic was prepared, in this case a food coloring was added, the sample was then left to dry for 24h, but the was left out in air to test the degradation time requied for this sample.

1. Macroscopic observation of *B.megaterium* culture:

The (Figure 24) shows the results of *B.megaterium* streak on agar plate, while (Table 5) demonstrates some of the bacterial characteristics.

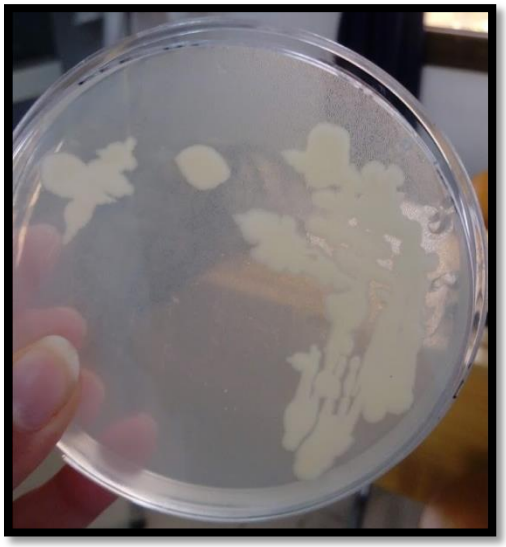

Figure 24: *B.megaterium's* colonies on an agar plate.

Table 5: Macroscopic aspect and characteristics of *B.megaterium* colonies.

colony characteristics	*bacillus megaterium*
Texture	dull
colour	Cream
diameter	3-5 mm
Shape	Irregular
Form	round
Surface	smooth

2. Spectroscopic analysis of absorbance

The biodegradation of plastic by the *B.megaterium* bacteria required to be studied on four sets of jars, each set composed of two jars complemented by a weekly photometric measurement as it is used to measure the concentration of organic compounds in the media by determining the absorbance of a 600 n.m wavelength of light.

The first two sets are witnesses as any obtained photometry result of the biomass would be evaluated in comparison to them. The first set of jars containing only plastics in a mineral

media called jar one and jar two registered both 0.103 abs and 0.109 abs on week zero while the second set of the witnesses named jar three and jar four containing only bacteria have shown 0.126 abs and 0.113 abs respectively.

Moving to the first week the absorbance of the biomass in the first jar remained stable while the second jar's absorbance showed a slight increase, moreover the third and fourth jars registered a moderate increase of 0.076 abs and 0.164 abs leading us to believe that the bacteria has entered the exponential phase where it starts to multiply due to it's insertion to the mineral media accompanied with a small quantity of nutrient broth since it was inoculated into it for 24 h. In addition the biomass of the bacterial suspension in the remaining four glass jars (jar 5, 6, 7and 8) recorded 0.342 abs, 0.388 abs, 0.253 abs and 0.258 abs respectively as shown in (Table 6). During the second week the photometric results revealed a minimal increase of 0.002 abs and 0.001 abs regarding the samples taken from the first and second jar, followed by an obvious absorbance augmentation in regards of the third and fourth jar as a sign of continuous exponential phase only to drop at the third week demonstrating it's entrance to the death phase after the depletion of carbon sources and experiencing a continuous decrease during the fourth week reaching 0.109 abs and 0.098 abs, furthermore constant absorbance elevation with regard to the first set of the jars containing only plastics until the fifth week going up to 0.155 abs and 0.137 abs only to decrease the following week to reach 0.147 abs and 0.131 abs respectively. Likewise the second set of jars absorption underwent a boost until the sixth week as it gained 0.033 abs and 0.070 abs for both the third and fourth jars during the fifth and sixth week, both being unexpected results.

As shown in the (Figure 38), positive results were measured concerning the jars number 5, 6, 7 and 8 owing to the uninterrupted amazement of the bacterial biomass reaching 0.815 abs, 0.888 abs, 0.613 abs and 0.522 abs by the sixth week meaning that the microbial biomass has grown since the first week with an average of 0.486 abs for the biomass exposed to the neutral pH and 0.312 abs for the biomass exposed to the low pH by reason of the bacteria's utilization of the carbon found in the polymer based plastic as a unique source of nutrient.

Until the ninth week the absorbance of the first and second sets of jars continued to drop to reach 0.127 abs, 0.117 abs, 0.105 abs and 0.104 abs successively for jars 1, 2, 3 and 4.

As highlighted in both (Table 6), and (Figure 25), the microbial biomass present in jars number 5, 6, 7 and 8 endured a permanent multiplication counting from week 6 increasing by

an average of 0.078 abs in regards to the fifth and sixth jar and average of 0.086 abs regarding the remaining set of jars for the similar reason expressed previously.

During the tenth week, the first and second jars in addition to the fourth jar‘s biomass‘s absorbance increased due to external factors as it is demonstrated in (Table 6), with an exception for the third jar as the absorbance level decreased by 0.008 abs whereas during the week 11 the registered absorbance from the first jar dropped by 0.020 abs, along with jar number one besides the unceasing death stage the bacteria went through in the jar number 3. Finally the bacterial biomass in jars number 5, 6, 7 and 8 experienced a settle growth since the ninth week counting an average growth of 0.010 abs and 0.110 abs for the biomass in a neutral pH media and low pH media respectively by reason of the bacterial senescence and accumulation of the remaining dead bacteria in the media.

The acquired results concerning the biomass in the neutral media (pH=7) were compared with those obtained by Hadda *et al* in the process of the same study, both biomass absorbance results demonstrated a noticeable growth only in our study an increase of 164.8% was registered in respect of Hadda *et al's* work furthermore, concerning the biomass in the acid media (pH=4.5) Goswami *et al* led a study on the subject in 2018 and found multiple up-regulation of certain genes that encode protease and peptidase in addition of the gene encoding glutamate decarboxylase during acid stress implicating it's involvement in intracellular pH homeostasis under acid stress in *B.megaterium* as decarboxylase is a helping factor for the bacteria to survive, moreover Up-regulation of the gene encoding Crp/Fnr family transcription regulators were observed under acid stress in *B. megaterium.*

Members of the Crp/Fnr family transcription regulators typically function as transcriptional activators that are involved in responses to a variety of intracellular or extracellular signals, such as anoxia, carbon monoxide, temperature, nitric oxide, and oxidative and nitrosative stress **(Goswami et al 2018).**

These results are the ultimate proof that the *B.megaterim* has the ability to degrade polymer based plastics by means of exploiting the plastic as a carbon source leading to it's deterioration over time and mainly the bacteria's resistance to the low pH through multiple mechanisms to keep it's membrane integrity making it an excellent choice for the reduction of the plastic wastes as it proved itself to survive not only under optimum conditions but also in acid stress circumstances setting a trend of a new safer way to dispose of the plastic leavings.

Table 6: Results of the biomass absorbance in 12 week time.

	Only plastic (1)	Only plastic (2)	Only bac (3)	Only bac (4)	Plastic+bac PH=7 (5)	Plastic+bac PH= 7 (6)	Plastic+bac PH=4.5 (7)	Plastic+bac PH=4.5 (8)
Week 0	0.103 abs	0.109 abs	0.126 abs	0.113 abs				
Week 1	0.103 abs	0.112 abs	0.202 abs	0.277 abs	0.342 abs	0.388 abs	0.253 abs	0.258
Week 2	0.105 abs	0.113 abs	0.312 abs	0.280 abs	0.533 abs	0.417 abs	0.367 abs	0.342 abs
Week 3	0.120 abs	0.116 abs	0.159 abs	0.128 abs	0.557 abs	0.518 abs	0.517 abs	0.440 abs
Week 4	0.147 abs	0.122 abs	0.109 abs	0.098 abs	0.758 abs	0.698 abs	0.583 abs	0.466 abs
Week 5	0.155 abs	0.137 abs	0.121 abs	0.140 abs	0.806 abs	0.787 abs	0.587 abs	0.507 abs
Week 6	0.147 abs	0.131 abs	0.142 abs	0.168 abs	0.815 abs	0.888 abs	0.613 abs	0.522 abs
Week 7	0.142 abs	0.120 abs	0.126 abs	0.139 abs	0.821 abs	0.941 abs	0.616 abs	0.532 abs
Week 8	0.138 abs	0.119 abs	0.109 abs	0.116 abs	0.859 abs	0.945 abs	0.763 abs	0.613 abs
Week 9	0.127 abs	0.117 abs	0.105 abs	0.104 abs	0.905 abs	0.953 abs	0.685 abs	0.622 abs
Week 10	0.128 abs	0.119 abs	0.097 abs	0.110 abs	0.909 abs	0.955 abs	0.690 abs	0.662 abs
Week 11	0.108 abs	0.134 abs	0.083 abs	0.117 abs	0.918 abs	0.960 abs	0.783 abs	0.743 abs

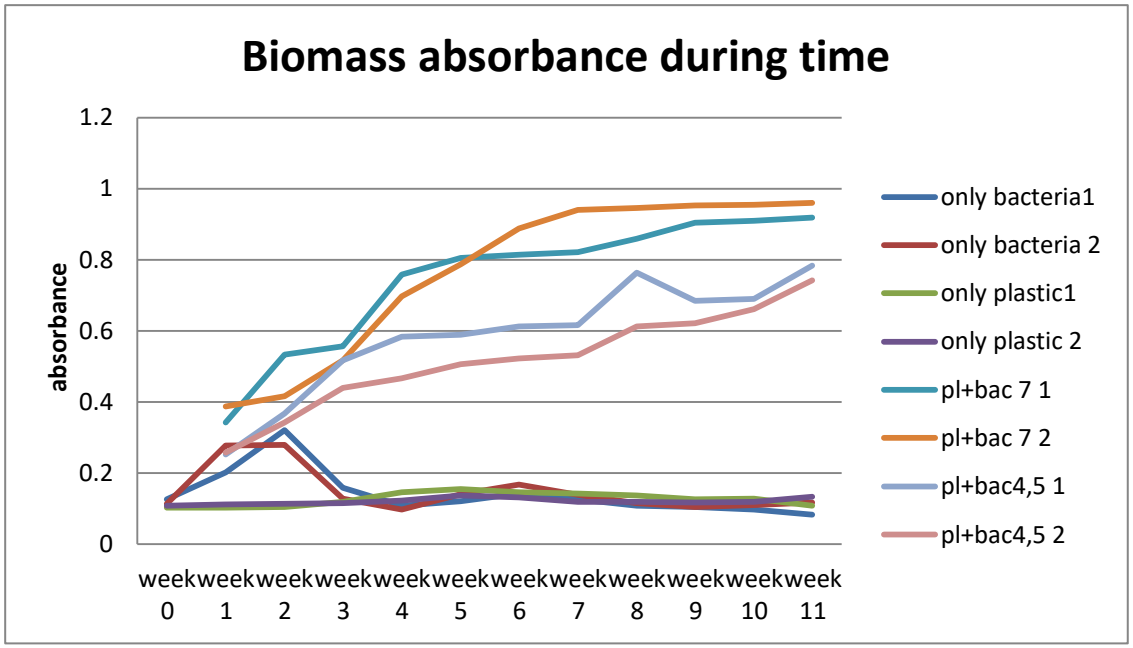

Figure 25: Graph representing the increase in the absorbance of biomass in the 8 jars during 12 weeks.

3. Mechanical alterations:

The weight of the two plastic types used at the end of the experiment are included in (Table 7), the difference in plastic weight before and after the action of *B.megaterium* is also represented as graphs in (Figure 26) and (Figure 27). Both the brown and the transparent plastic samples present in jar 1 and 2 showed no change in their weight, as the weight kept constant throughout the experiment period, due to the absence of *B.megaterium* inoculation in the mineral media while the plastic samples present in jar 5, 6 ,7 and 8 showed a significant weight reduce with 5.6% and 6% average weight loss (degradation) in brown and transparent plastic respectively, despite the acidic media in jar 7 and 8, the plastic's degradation process also showed considerable decrease slightly similar to the plastic present in the neutral pH media, with 2.5% and 3.1% average weight loss of brown plastic in pH=7 and pH=4.5, respectively, as well as 3.3% and 2.7% average weight loss of transparent plastic in pH=7 and pH=4.5 respectively. In a previous work done by Hadda *et al* the coloured and transparent plastic showed average weight reduce of 4.2% and 2.7% respectively **(Hadda et al 2018).** These positive results were obtained by dint of the initial large size of the plastic fragments to be degraded in addition of their pre exposure to UV lights supporting the results of a study that showed that small piece of plastic gets stuck together reducing the total surface area in contact with the sludge **(Yagi *et al* 2012)**, furthermore it is assumed that pre-modification with UV lights of plastic lead to an increase in

it's biodegradation, which makes it sensitive to subsequent microbial attack **(Gogotov and Barazov2012)**, this indicates that *B.megaterium* is able to engage in the degradation process in the acidic media as well as in the normal condition.

Table 7: Results of plastic weight after 12 weeks of degradation.

	Brown plastic Initial weight (grams)	Brown plastic Resulted weight(grams)	Transparent plastic Initial weight(grams)	Transparent plastic Resulted weight(grams)
Only plastic(1)	2.534	2.534	1.575	1.575
Only plastic(2)	2.517	2.517	1.509	1.509
Only bac(3)	/	/	/	/
Only bac(4)	/	/	/	/
Plastic+bac, PH=7(5)	2.509	2.497	1.440	1.416
Plastic+bac, PH=7(6)	2.524	2.511	1.477	1.468
Plastic+bac, PH=4.5(7)	2.505	2.489	1.587	1.573
Plastic+bac, PH=4.5 (8)	2.507	2.492	1.553	1.540

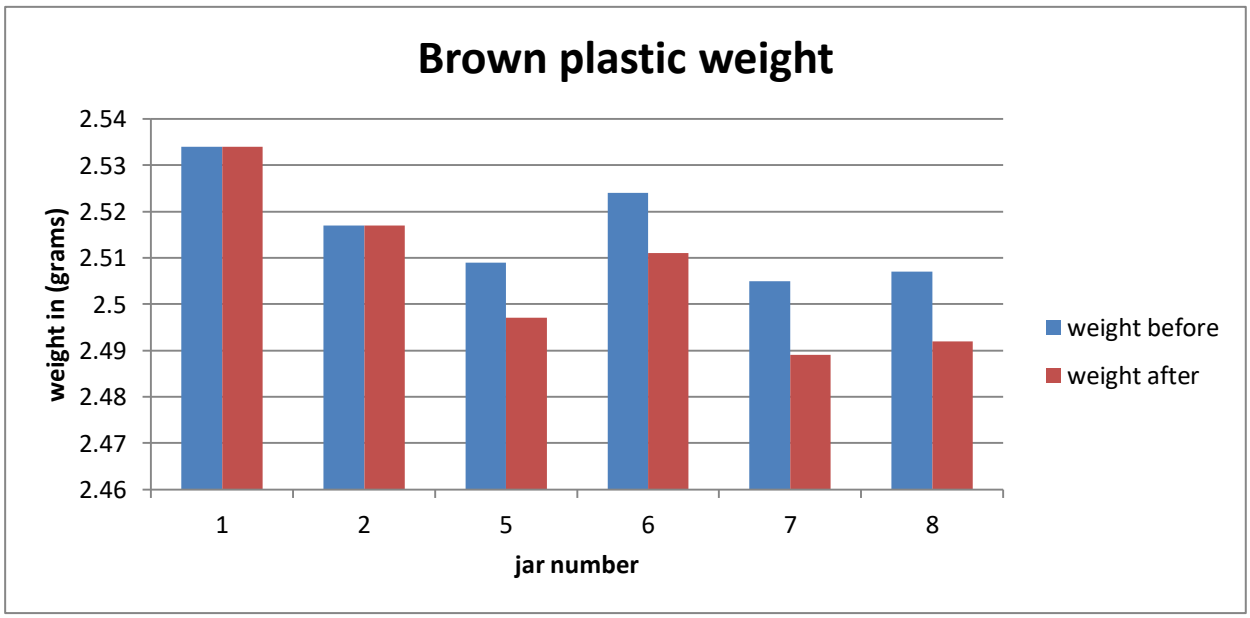

Figure 26: Graph representing the difference in brown plastic weight before and after degradation.

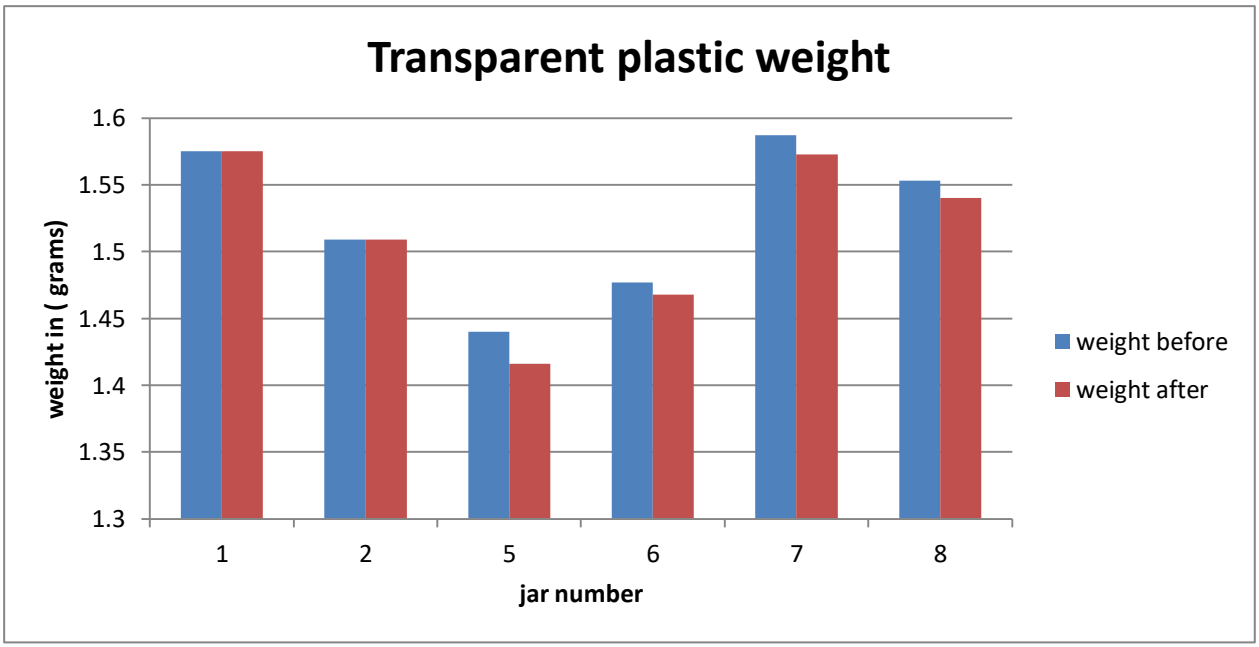

Figure 27: Graph representing the difference in transparent plastic weight before and after degradation.

4. Physical alterations:

The colour of the brown plastic material showed an interesting shift, the brown plastic present in jar 1 and 2 looked exactly same (Figure 28) (A), that is due to the same conditions they were exposed to and that's because no bacteria was inoculated in the jars, so the plastic was not affected, while the brown plastic present in jar 5, 6, 7, 8 showed a colour shift and some deep holes shown in (Figure 28) (B), due to the action of the bacteria, the difference in colour between the plastic present in jar 1 and 6, 1 and 7 is shown in (Figure 29). Despite the present of plastic in jar 7 in an acidic media, the results showed some similarity to a certain extent with the plastic present in jar 6 which was present in a neutral pH media. In a previous work done by Hadda *et al* plastic under similar conditions showed reflection or refraction of the plastic material after 2 months of agitation **(Hadda *et al* 2018).**

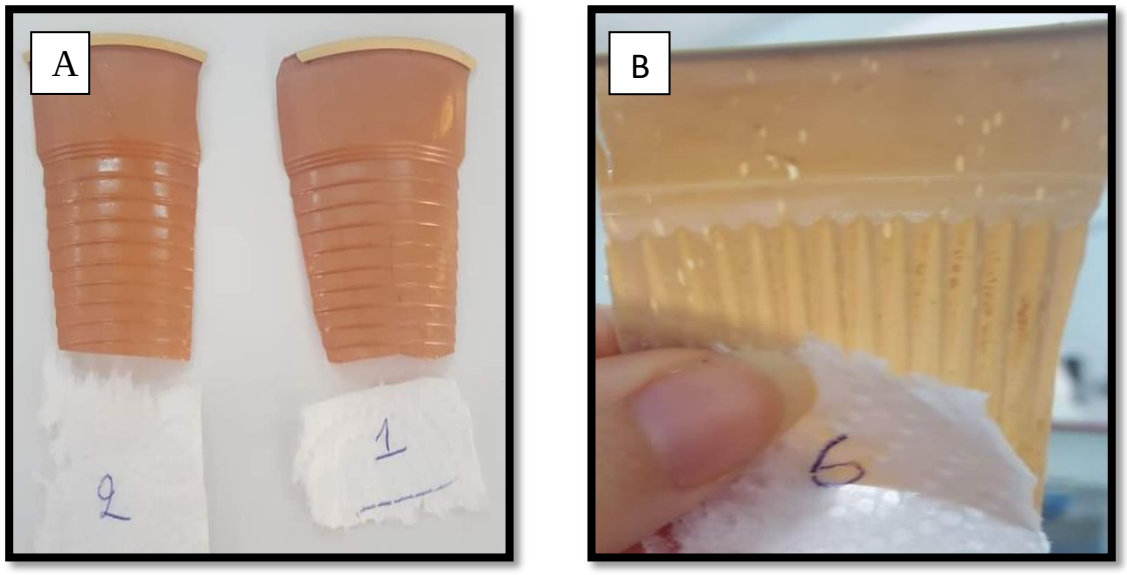

Figure 28: Similarity between plastic in jar 1 and 2 (A), deep holes in the plastic material demonstrating the physical alteration of plastic from jar number 6 due to bacterial exposure (B).

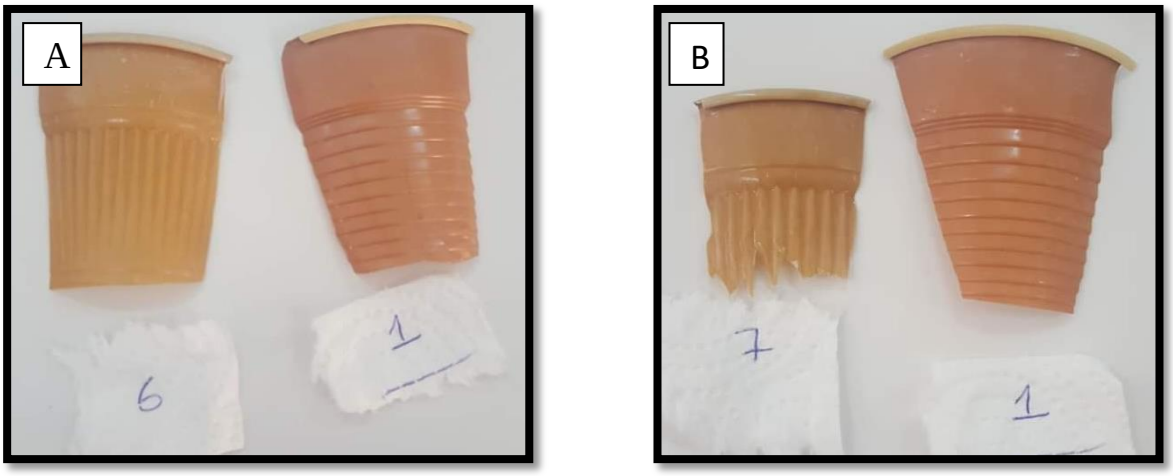

Figure 29: Comparison ofcolour modification between plastic from jars 6 (A), and 7 (B) to the one from jar 1.

5. Biofilm formation:

Microorganisms universally attach to surfaces and produce extracellular polysaccharides, resulting in the formation of a biofilm. Its starts by surface attachment to cell division, form micro-colonies, and produce the extracellular polymers that define a biofilm **(Donlan 2001).** (Figure 30) shows the two plastic types in jar 6 with biofilm on its surface and a closer view

of the biofilm on a transparent plastic from jar 6, giving evidence of a good bacterial growth, therefore a perfect bacterial degradation process.

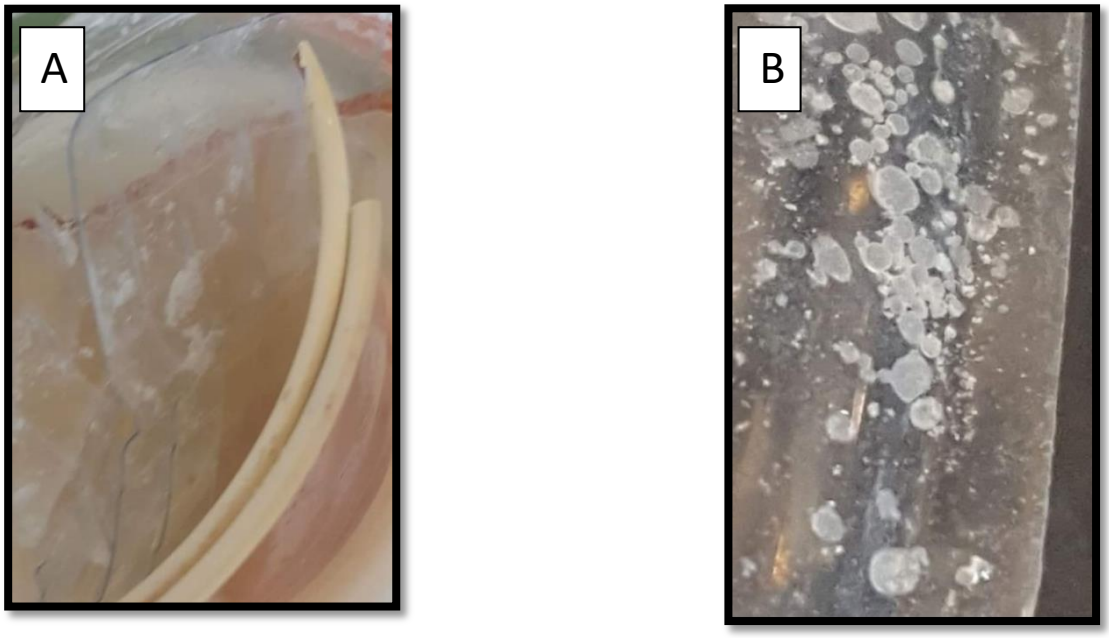

Figure 30: Illustration of biofilm on two plastic types (A), and a close view of biofilm on transparent plastic (B).

6. Biodegradable plastic construction:

As the optic density of the previous experiment kept increasing, we had the ambition to recreate a substitution to polymer based plastics known as « biodegradable plastic », this environment friendly plastic is made of molecules easier to break down and won't represent any harm to neither fauna nor flora. In this section we will discuss the biodegradable plastic's properties starting from it's physical changes, texture, flexibility and finally it's weight alteration when influenced by environmental factors as heat. (Figure 31) shows the result of the laid biodegradable plastic on an aluminium foil after preparation. Macroscopic identification of physical and mechanical changes after it's conception, the biodegradable plastic was exposed to air at a temperature of 37 ° in a dry environment. After a week considerable physical modifications were noticed as it was broken into several pieces as shown in (Figure 32).

Figure 31: biodegradable plastic laid out on an aluminum foil.

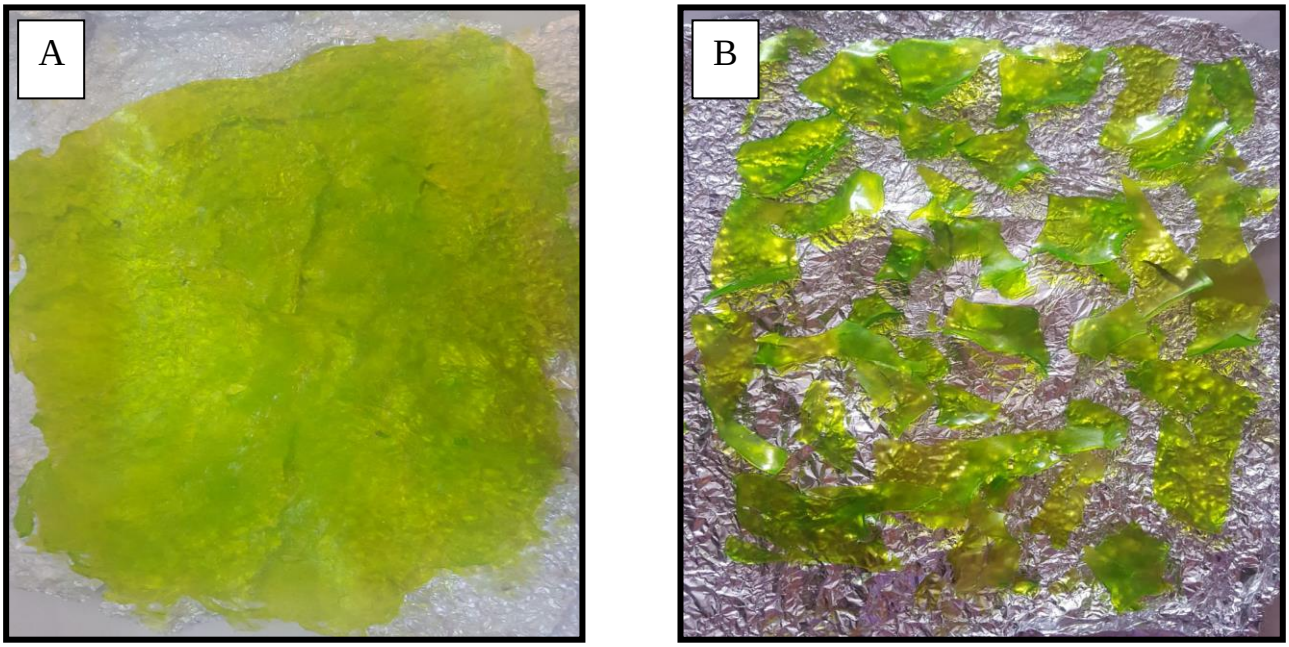

Figure 32: Comparison of visual modifications of physical properties of the biodegradable (B) during one week after it's realisation (A).

Furthermore, the eco-friendly plastic appeared to have lost it's resistance to pressure and flexibility as it would tear up instantly after being lightly stretched in addition of perceptible dryness and shrinkage of the little remaining pieces. Along with the physical and mechanical modifications, an impressive weight loss was registered only one week after the

biodegradable plastic was made as it weighted at week zero 24.653 grams to weight 19.851 grams at week one representing a loss of 4.802 grams during the first week, additionally it weighted 19.303 grams during the second week. According to the average of weight reduction, it is expected that the eco-friendly plastic reached a weight of 18.449 grams by the third week, while it dropped to 17.784 g by the fourth week as shown below in (Table 8), and represented as a graph in (Figure 33). All of these transformations related to either weight, physical or mechanical properties are all only a logical consequence of the air and heat exposure as these two factors led the liquid substances contained in the biodegradable plastic to evaporate.

Table8: Results of the weekly biodegradable plastic's weight loss.

	Biodegradable plastic's weight
Week zero	24.653 g
Week one	19.851 g
Week two	19.303 g
Week three	18.449 g
Week four	17.794 g

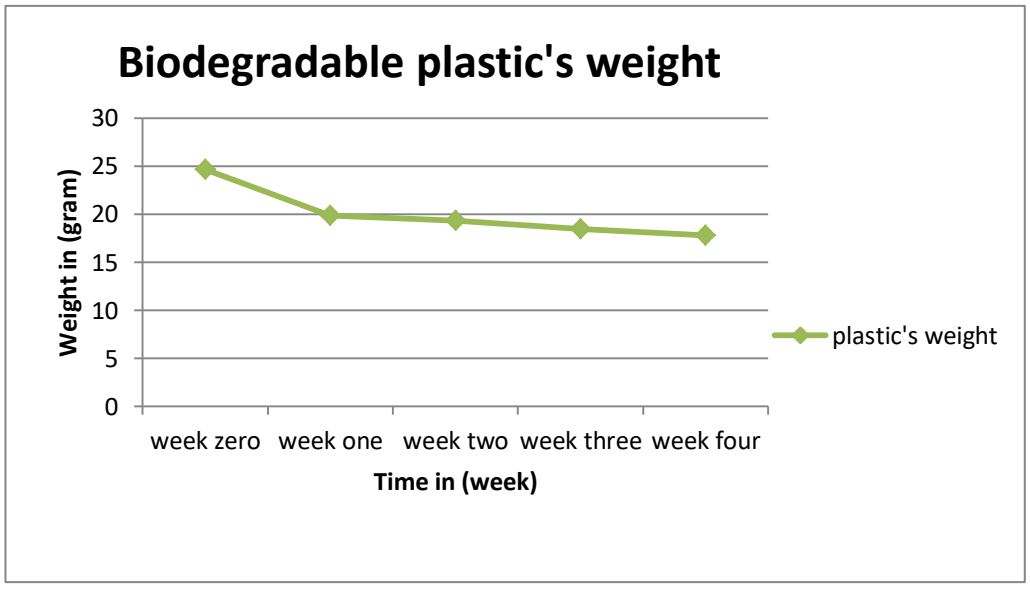

Figure 33: Graph representing weight loss of biodegradable plastic

The distribution of plastics and its debris is increasing exponentially around the world. Plastics are not immediately perishable unlike other kinds of organic waste generated by industrial activities, the assimilative capacity of plastics in the ocean is almost nil contaminating the marine environment and its living organisms, along with the ecological effects of plastic pollution and the high exposure of its living organisms to it **(Radhan *et al* 2019).** The ability of *B.megaterium* to degrade two plastic types under various conditions showed interesting results in the present work, as the plastic pieces were incubated with the bacterial culture in a mineral media for 3 months under continues agitation. The average amount of degraded plastic in both pH=7 and pH=4.5 showed considerable decrease, indicating the ability of the *B.megaterium* to degrade plastic material in the mentioned conditions. Despite the recorded results, the interest of this work was to study the degradation of plastic by means of *B.megaterium*, but another central point is that we should "reform the way we live rather than tweak the choices we make" **(Stafforda and Jones 2019).**

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Composition of culture media

Nutritious broth:

- Meat extract……1g
- Yeast extract……...2.5g
- Peptone ……..5g
- NaCl……5g
- Distilled water……1L
- PH……7

Nutrient agar:

- Meat extract……1g
- Yeast extract……...2.5g
- Peptone ……..5g
- NaCl……5g
- Distilled water……1L
- Agar……18g
- PH……7

Mineral medium

The mineral medium of Bushnell-Hass <BH> **(Atlas, 2005).** Prepared for 2.5L.

- $KH_2 PO_4$…….....2.5g
- $K_2 HPO_4$……..2.5g
- $NH_4 NO_3$…….2.5g
- $MGSO_4 7H_2O$…….0.5g
- Fe Cl_3 ……..0.125g
- Ca Cl_2 $2H_2O$……0.05g
- PH ……7

Biodegradable plastic:

- Water ……..59.12ml
- Glycerine ……....4.9ml
- Vinegar ……...4.9ml
- Starch ……..14.3g

Placement of the jars on the agitator in the incubator