



Disintegration behaviour of bio-based plastics in coastal zone marine environments: A field experiment under natural conditions

D. Briassoulis ^{*}, A. Pikasi, Chr. Briassoulis, A. Mistriotis

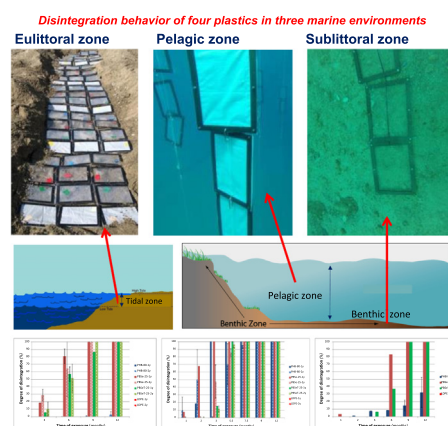
Department of Natural Resources & Agricultural Engineering, Agricultural University of Athens, Athens 11855, Greece



HIGHLIGHTS

- Biobased plastic films tested for disintegration in marine environments
- Field tests performed in East Mediterranean coastal zones (intertidal, pelagic, benthic)
- Degradation was measured by Image Analysis, FTIR-ATR, and mechanical testing.
- Disintegration rate depends on material, habitat, prevailing conditions.
- Measured degree of disintegration attributed predominantly to biodegradation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 7 June 2018

Received in revised form 7 June 2019

Accepted 8 June 2019

Available online 11 June 2019

Editor: Kevin V. Thomas

Keywords:

Bio-based plastics

Biodegradable plastics

Coastal zone

Marine environment

Disintegration in seawater

Bio-fouling

ABSTRACT

The accumulation of plastic wastes in the marine environment represents a steadily increasing global environmental threat. The replacement of conventional plastics with bio-based biodegradable materials may contribute to alleviating the problem in the long run. This work studies the disintegration behaviour of three bio-based plastic materials, namely Polyhydroxybutyrate (PHB), Polybutylene sebacate (PBSe), Polybutylene sebacate-co-terephthalate (PBSeT), in three different coastal zone marine environments under natural conditions. The three studied environments were: 1) the seashore zone which is periodically covered by the seawater due to waves or tide, called eulittoral or intertidal zone; 2) the water column zone of small depth (about 10 m), called pelagic zone; and 3) the interface zone between the water column and the seabed sediment at small depth (about 20 m), called sublittoral or benthic zone. The experiments took place in the Aegean Sea at the SW coast of Salamis Island. The results showed that disintegration, as an indicative measure of biodegradation, occurs in all three tested environments, even though the rate depends on the material, the habitat, and the prevailing during the testing period environmental conditions. The degrees of disintegration of all materials in the three environments exhibited significant differences: Benthic > Intertidal > Pelagic. The observed disintegration can be attributed to biodegradation since the negative reference Low-density Polyethylene (LLDPE) material did not disintegrate.

© 2019 Elsevier B.V. All rights reserved.

^{*} Corresponding author.

E-mail address: briassou@aua.gr (D. Briassoulis).

1. Introduction

Pollution due to plastics is a major problem of the marine environment. Since most plastics do not biodegrade, they accumulate over time (Acampora et al., 2014). Plastic items or fragments are found on the seafloor, floating in surface waters (Law et al., 2011) or buried in the sediment (Van Cauwenberghe et al., 2013). The plastic debris accumulation in the ocean, including a growing amount of micro-plastics (particles <5 mm), has been identified as an environmental hazard of global scale and importance.

Micro-plastics are found in various marine habitats such as the water column, the sediments, even the deep sea (Galgani et al., 2015). It is characteristic that the amount of micro-plastic beads litter in 2012 only in the EU was estimated at 4360 t (UNEA, 2016). UN Environment launched in February 2017 an unprecedented global campaign aimed at the elimination of “major sources of marine litter: micro-plastics in cosmetics and the excessive, wasteful usage of single-use plastics by the year 2022” (UN Environment, 2017). According to (Koulouvaris et al., 2015), the main component of the marine floating litter is plastic, comprising up to 95% of the total marine litter accumulated on the shorelines, floating on the ocean waters and sunk on the seabed.

Important steps have been taken by the EU to reduce plastic waste (e.g. Plastic Bags Directive: Directive (EU) 2015/720 (2015), banning single-use plastics). A European strategy for plastics was adopted in 2018 to ensure that all plastic packaging is reusable or recyclable in a cost-effective manner by 2030 (European Commission, 2018a). This circular approach allows for setting criteria for the feedstock selection, manufacturing, product use, and End-of-Life (EoL) recovery options.

Besides the need to reduce the use of plastics and its uncontrolled disposal to the environment and to implement a holistic design approach for sustainable plastic products, the development of alternatives, such as the substitution of conventional plastics by *biodegradable materials* could be helpful (UNEP-NOAA, 2011). Biodegradable products are materials completely degrading under the action of micro-organisms in a specific environment and within a given time frame. Biodegradable plastics may also be *bio-based*, namely wholly or partly derived from biomass (EN 16575, 2014). High biomass content does not necessarily imply biodegradability. Some bio-based plastics are biodegradable in specific environments (e.g. polylactic acid (PLA) is compostable under industrial composting conditions but does not biodegrade under natural soil conditions) (European Bioplastics, 2018). Other bio-based plastics are non-biodegradable (e.g. bio-polyethylene (Bio-PE)).

Although littering by any kind of plastics, including biodegradable plastics, should also be avoided, biodegradation could contribute to the reduction of the accumulated plastic debris volume with time. For this reason, determining the biodegradation behaviour of developing innovative bio-based plastics in the marine environment is important for characterizing the new materials and designing the relevant products in support of the emerging Bio-economy and the recirculation principle of the Circular Economy. It is stressed that apart from the inherent biodegradability of a material, the ability of microorganisms to biodegrade this material can vary widely depending on the environmental and biological conditions of the natural ecosystem the material is exposed to.

There is a large diversity with respect to marine habitats (e.g. coastal and open ocean habitats) and the corresponding environmental, biological and physicochemical conditions. The terminology for marine habitats is not defined and classified in a standardized way yet, but usually, habitats are defined non-systematically, or on a subjective ad hoc basis (Fraschetti, 2012). For the purpose of the present work the following definitions were adopted:

Coastal zone: A strip of varying width at the interface between sea and land, on both the land and sea sides, that depends on the nature of the environment, bio-geographical conditions and management needs (Lavalle et al., 2011).

Littoral zone: Operationally defined as the part of the coastal zone extending from the rarely submerged high water mark to the permanently submerged shoreline areas at a depth where a minimum of 0.1% of the incident irradiance penetrates the surface water, allowing for the growth of benthic (macro or micro) plants (Duarte, 2008).

Eulittoral zone: The intertidal zone (or foreshore or tidal), extending from the rarely submerged spring high tide line to the usually submerged neap low tide line. It represents the benthic zone falling between the sea water fluctuation level limits (Merriam-Webster, 2018).

Sublittoral zone: The sublittoral zone (approximately equivalent to neritic zone), is the benthic zone that starts immediately below the eulittoral zone and extends to depths covered permanently with seawater (photic zone approx. up to 200 m depth). Marine life is very rich in the sublittoral zone. Tidal flows, wave's energy dissipation, internal waves and flows, dominate the sublittoral zone's environment. However, the benthic zone in the sublittoral is much more stable as compared to the intertidal zone in terms of temperature, water pressure, sunlight (Salomon and Markus, 2018).

Pelagic zone: The water column of the open sea above the sublittoral zone. This is defined as the epipelagic subzone within the limits of the neritic zone (deep waters and sea waters beyond the littoral zone are not considered in the present work). This pelagic zone is an illuminated zone, like the sublittoral zone below, photosynthetically very active with high concentrations of plankton and floating seaweed and fish (Halstead and Basham, 2013).

Biodegradation in marine environments is studied by both field and laboratory experiments. Standard Methods for testing the biodegradation of plastic materials in the marine environment are still in an early stage of development (Marine sediment: ISO 18830 (2016), ISO 19679 (2016) and ISO/FDIS 220404 (2019); Marine field tests: ISO/DIS 22766 (2019); Seawater or sandy sediment: ASTM D6691 (2017) and ASTM D7991 (2015)). Laboratory tests are used to measure the biological decomposition of the tested materials since the respiration activity of the microorganisms, which aerobically decompose and assimilate the polymers, can be monitored (Pauli et al., 2017; Weber et al., 2018). Field experiments demonstrate the relevance of the laboratory results to the natural bio-assimilation of the tested materials. The comparison between field and lab results cannot be straightforward since in field experiments only the disintegration rate and degree of the materials are measured. Disintegration can be considered as an indication of biodegradation in combination with other complementary methods and taking also into account the abiotic degradation effects. Because of the large diversity of marine habitats, the production of reliable field data that could be used for the validation of new laboratory testing methods is very difficult. In many cases, abiotic factors such as solar radiation, waves, sea currents, abrasion by sea sediments, etc., contribute to physical degradation and disintegration of the samples. Therefore, the effect of biodegradation cannot be isolated, measured and monitored easily. Experiments performed in diverse natural marine zones have been reported recently in the literature. Field experiments attempt to detect biodegradation by measuring the disintegration of samples (i.e. fragmentation and/or deterioration of mechanical properties) as an indication of the materials biodegradation.

Pauli et al. (2017) examined the degradation of conventional polyethylene plastic (PE) and of biodegradable polymer blend (MaterBi®) carrier bags under field conditions (exposed to sublittoral and pelagic coastal habitats) over a period of 1 year. The mechanical properties of the samples were measured as an indicator of degradation and also of the change in the polymers molecular structure. The disintegration (%) of the films was determined photogrammetrically. Only MaterBi was found to disintegrate. The biodegradation of MaterBi® samples was confirmed by laboratory tests.

In another experimental study, the disintegration of polymer samples made of blended starch-poly(β -hydroxybutyrate-co- β -hydroxyvalerate) (PHBV) formulations under marine conditions was monitored for one year at four stations in three different coastal water

Table 1
Materials used in the experiments.

Code name	Material (film)	Thickness (μm)	Type	Producer
LDPE	Low-density polyethylene	25	Fossil-based hydrocarbon	Grade: LUPOLEN 2420 K Lyondell base II
PHB-80	Polyhydroxybutyrate	80	Bio-based (100%), biodegradable	Metabolix Grade: Mirel™ P5001
PBSe-25	Polybutylene sebacate	25	Aliphatic polyester Bio-based (>70%)	Novamont
PBSe-100	Polybutylene sebacate	100	Aliphatic polyester Bio-based (>70%)	Novamont
PBSeT-25	Polybutylene sebacate-co-terephthalate	25	Aliphatic-Aromatic co-polyester Bio-based (>30%)	Novamont
PBSeT-100	Polybutylene sebacate-co-terephthalate	100	Aliphatic-Aromatic polyester Bio-based (>30%)	Novamont

environments (coastline, reef, deep water) SW of Puerto Rico (Imam et al., 1999). The disintegration of the bioplastic was estimated by measuring the weight loss and the deterioration of mechanical properties. The correlation to biodegradation was analysed indirectly (but not measured) by studying the microbial growth by standard spread plate methodology using three different media. Imam et al. (1999) reported that the disintegration of polyhydroxyalkanoates (PHAs) occurs relatively rapidly in coastal marine mud, where the microbial population is dense and metabolic activity is intense.

Tsuji and Suzuyoshi (2002a) tested poly(ϵ -caprolactone) (PCL), poly[(*R*)-3-hydroxybutyrate] (R-PHB) and poly(L-lactide) (PLLA) in large onshore tanks where natural static seawater and sediment were kept under controlled conditions (mesocosm test). The degradation of the aliphatic polyesters, monitored by measuring the molecular weight, the crystallinity, the melting temperature, and the tensile properties of the plastic material (no disintegration measurements reported), decreased in the order: PCL > R-PHB > PLLA. The same polymers were also tested in a mesocosm experiment involving mechanically driven water flow (Tsuji and Suzuyoshi, 2002b). The stresses due to the moving seawater caused additional mechanical damage or abiotic degradation of the films, accelerating the degradation process measured under static conditions. Another mesocosm experiment was performed by immersing fibres of three aliphatic polyesters, namely PCL, PHB/V, and poly(-butylene succinate) (PBS) in tanks containing deep-sea water collected from three different locations in Japan (Sekiguchi et al., 2011). The materials degradation was studied by means of mechanical properties measurements and surface morphology analysis. No information about disintegration is provided. Also, PCL-degrading bacteria were screened and isolated. The obtained results indicated that PCL and PHB/V were

degraded in deep-sea water, despite the low temperatures, and five PCL-degrading bacteria were isolated and characterized.

This work presents the analysis of the disintegration behaviour of three bio-based biodegradable plastic films in various natural marine environments of the coastal marine zone. The field experiments were planned and performed in the framework of the OpenBio (2016) project. In parallel laboratory tests were performed for measuring the biodegradation of the same plastics in inocula obtained from the corresponding marine environments (Tosin et al., 2016). The lab results will be presented separately due to space limitations. The parallel field and laboratory tests aimed at developing new laboratory testing methods for assessing the biodegradation of plastics in various marine environments. The disintegration results of the field experiments presented in this work are considered as an indication, but not as a measure, of the biodegradation rates of the three materials under natural conditions, taking also into account the accelerating abiotic degradation effects. The field disintegration/biodegradation test results were used for validating and calibrating the corresponding laboratory tests (Tosin et al., 2012).

2. Materials and methods

2.1. Materials

Four plastic films were exposed to three different natural coastal zone marine environments. Two materials were the bio-based plastic films with different degrees of bio-based content: Polybutylene sebacate (PBSe) (Siotto et al., 2013) and Polybutylene sebacate-co-terephthalate (PBSeT). In addition, the bio-based biodegradable



Fig. 1. Geographic details of the field test site at Salamis Island (location: 37° 53' 33" N, 23° 24' 30" E).

Table 2

Overview of the performed experiments.

Zone of exposure	Start date	End date	Materials	Frequency of sampling (months)
Eulittoral (S1)	8/7/2014	8/10/2015	PHB-80, PBSe-25, PBSeT-25, LDPE	3
Benthic	8/7/2014	8/10/2015	PHB-80, PBSe-25, PBSeT-25, LDPE	3
Benthic	22/12/2014	22/5/2015	PHB-80, PBSe-25	1
Pelagic	8/7/2014	8/10/2015	PHB-80, PBSe-25, PBSeT-25, LDPE	3
Pelagic	22/12/2014	22/5/2015	PHB-80, PBSe-25	1
Eulittoral (S1, S2)	15/10/2015	8/2/2017	PHB-80, PBSe-25, PBSeT-25, LDPE	3
Eulittoral (S2)	3/11/2015	3/11/2016	PBSe-100, PBSeT-100	3
Benthic	10/11/2015	23/11/2016	PHB-80, PBSe-25, PBSeT-25, LDPE, PBSe-100, PBSeT-100	3
Pelagic	10/11/2015	23/11/2016	PHB-80, PBSe-25, PBSeT-25, LDPE, PBSe-100, PBSeT-100	3

Polyhydroxybutyrate (PHB) film was used as the positive control (Emadian et al., 2017) and the fossil oil based Linear Low-Density Polyethylene (LDPE) film as the negative control. The technical characteristics of the materials are described in Table 1.

The expected disintegration of these materials in marine environment follows the order: LDPE \ll PBSeT < PBSe < PHB (Tosin et al., 2016; Siotto et al., 2013), where LDPE is considered as non-biodegradable.

2.2. Location

The field experiments took place at the southern coast of the Salamis Island (Fig. 1) near Athens, Greece (location: 37° 53' 33" N, 23° 24' 30" E). This location near the facilities of an aquaculture unit was selected for conducting the field tests. In this way, the installation of the experimental set-up, the monitoring of the testing installations, the condition of the environment and the sampling were facilitated as the experiments were carried out in a relatively controlled and safe area.

Samples of the materials described in Table 1 were placed in three different marine environments: the benthic (sublittoral), the pelagic and the intertidal (eulittoral). These three habitats are described as following:

- 1) *Eulittoral or intertidal zone* (Tosin et al., 2012): The sandy sediment in this zone usually has high levels of moisture content although it frequently remains above the sea level. Two nearby beaches marked in Fig. 1 as site S1 and site S2, were used for the exposure of the samples.
- 2) *Pelagic zone* (Tosin et al., 2012): In the present study the epipelagic subzone within the limits of the neritic zone, a well-aerated zone of the coastal seawater near the coastline at a depth range of 10–50 m below sea level was used as the broader field test location. The samples were installed at 10 m depth.
- 3) *Sublittoral or benthic zone* (Tosin et al., 2012): In the present study the benthic zone that starts immediately below the eulittoral zone within the limits of the neritic zone, near the coastline at a depth range of 10–50 m below sea level was used as the broader field

test location (i.e. the sublittoral zone below the pelagic zone). This is a biologically active zone, with very rich biodiversity (based on visual observations and measurements of TOC and N). During the present experiment, the samples were installed on the seabed at 20 m depth. The pelagic and benthic field test sites with the sample installations were at the same location in different depths (Fig. 1).

2.3. Experimental setup

Two main series of one-year experiments were performed in all three studied environments. However, certain materials were found to disintegrate faster than expected in specific marine habitats, so the sampling frequency was found to be too low to determine accurately their disintegration behaviour with time. For these cases, additional experimental series were organized with shorter sampling times in order to further clarify the disintegration process of each material in all studied environments. The complete overview of the performed experiments is presented in Table 2. In order to monitor the experiments more efficiently, samples were inspected more frequently than the originally planned recalls. During such intermediate inspections, less than three samples were photographed on site, without removing the samples, and analysed. The difficulty to photograph all sample repetitions (by recovering them and reinstalling back) on the site explains the lack of standard deviations in the measurements of the disintegration of the specific recalls.

Each test material sample was placed inside a plastic (PP) net supported by a plastic frame with internal dimensions 210 × 297 mm. The net mesh size was 7 × 7 mm and was fixed along its four sides on the frame so as to keep the test film flat (Fig. 2). For each experiment, five recalls were planned for each pair material–environment over an exposure period of one year. Each recall included three samples of each test material.

The test samples for both, the pelagic (water column) and the benthic (sublittoral) environment were suspended by ropes (Fig. 3). The three samples of each recall and material were supported as separate sets for the benthic and the pelagic zones, on a single rope. Thus, for the five recalls of the four different materials, twenty ropes were installed. The ropes were stretched in a vertical position by means of a



Fig. 2. Sample supporting system consisting of a rigid frame and plastic net with mesh size 7 × 7 mm.

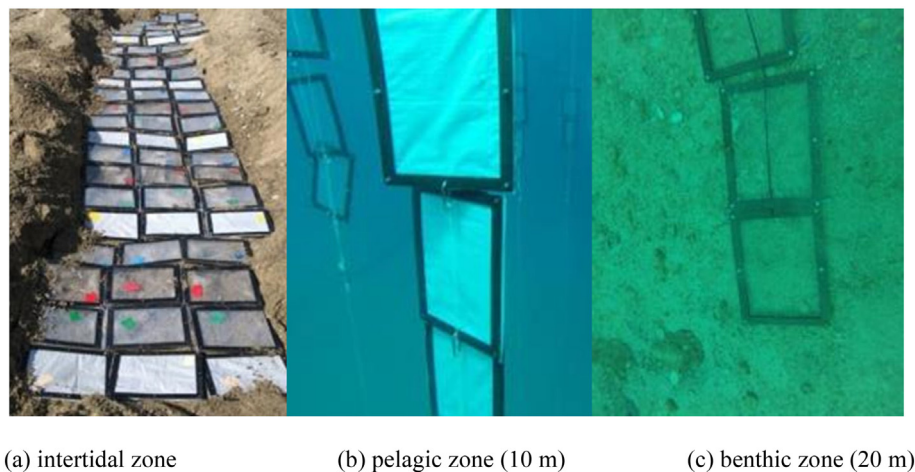


Fig. 3. Samples placed in the intertidal zone (a) and sample repetitions placed in the pelagic (b) and the benthic (c) zone.

free-floating pickup buoy and a cement block on the seabed acting as an anchor. The buoys were connected by ropes to a nearby aquaculture cage for security reasons (Fig. 4).

The ropes suspending the samples were placed in a row having a distance of approximately 1 m from each other. The three samples of each recall exposed to the benthic (sublittoral) zone were tied to the cement block anchor lying on the seabed at 20 m depth. The three samples of each recall exposed to the pelagic zone were tied to the vertical rope at approximately 10 m depth.

During the second year's test, based on the experience gained during the first year, the three samples per recall and material in the pelagic environment were covered by geotextile to prevent the growth of bio-fouling on the plastic film. A fourth sample not covered with geotextile was also added in each recall set for comparison reasons. In this way, the effect of bio-fouling on the disintegration could be studied.

The samples for the eulittoral test were placed inside a pit at an average 20 cm depth (Fig. 5) in two nearby beaches shown in Fig. 1 as Site-1 (S1) and Site-2 (S2). During the first year's series of experiments, samples were exposed only at site S1. During the second year of tests, an additional series of test materials, namely PBSe, PBSeT, and PHB were also placed at site S2. Additional recalls of these materials were installed because during the first trial it was found that they have fully disintegrated after a period of three months. A complete overview of the tests is given in Table 5.

2.4. Methods for monitoring the environmental conditions

The sea water and sediment conditions at the field test areas were measured in random time intervals during the experiment. The quality of the seawater was characterized by measuring oxygen content,

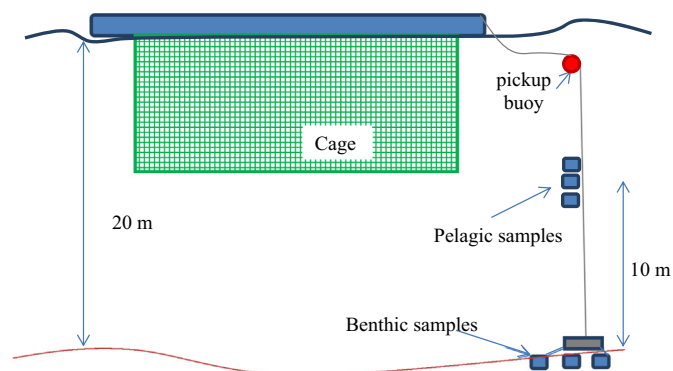


Fig. 4. Design of the experimental set-up for the benthic (sublittoral) and the pelagic zone.

turbidity, and salinity. Measurements for heavy metals were performed for the needs of the *OpenBio* (2016) project by R.J. Hill laboratories Limited, New Zealand. No contamination of any kind was detected.

The measurements for the dissolved oxygen were made by an OPTOD (Optical Dissolved Oxygen technology) luminescent waterproof sensor (Ponsel Mesure - Aqualabo (FR) OPTOD sensor) that complies with the ASTM International Method D888-05. The dissolved oxygen was measured in ppm, with a resolution of 0.01 ppm and an accuracy of ± 0.1 ppm. The OPTOD sensor was connected via a Modbus RS485 interface to a data-logger and it collected data every 10 s.

The turbidity was measured with an NTU (Nephelometric Turbidity) waterproof sensor (Ponsel Mesure - Aqualabo (FR) NTU sensor) complying with the ISO 7027 standards. The sensor was calibrated with a standard solution before being used. The turbidity was measured in NTU ($= 1$ mg/l) with a resolution of 0.01 mg/l and accuracy below 5%.

A C4E Digital waterproof sensor (Ponsel Mesure - Aqualabo (FR) C43 model) was used to measure salinity. The resolution fluctuated from 0.01 to 1 ppm with an accuracy of $\pm 1\%$.

All three sensors were integrated into one measurement device. Measurements were made at 2 m above seabed (18 m) to obtain a more realistic measurement of turbidity.

A CAMPBELL CR800 portable edition with 3 digital inputs and a regulated 12 V power supply via NiMH battery was used as a data-logger. All the sensors were connected via a Modbus RS485 interface which measured every 10 s. Silicon protected; zero-ohm cables were used to connect the sensors to the data-logger. The final stored data were collected on average every 3 min.

The sediments in both the coastal (intertidal) and the benthic sites were characterized by measuring soil quality characteristics. These included organic carbon and nitrogen content and texture. The total organic matter and organic carbon were measured by the Walkley-Black method (Walkley and Black, 1934). The nitrogen content was measured by the Kjeldahl Method (Bremner and Mulvaney, 1982). The pH of the sediment pore-water was measured by using an InoLab pH-meter according to ISO 10390 (2005). The seawater analysis was performed by the Laboratory of Agricultural Hydraulics of the Agricultural University of Athens according to the following methods (APHA, 2005): Nitrite: Diazotization Method; Nitrate: Cadmium Reduction Method; Phosphate: Ascorbic Acid Method; Ammonium: Nessler Method.

2.5. Methods for the analysis of recovered samples

Measurement of the disintegration of the recovered samples was the only way to evaluate the evolution of their biodegradation in the natural environment, taking also into account the contributing abiotic degradation effects. The disintegration rate of the samples with the exposure



(a) Intertidal site S1

(b) Intertidal site S2

Fig. 5. Samples placed in the intertidal (eulittoral) environment (sites S1 (a) and S2 (b)).

time was used as an indication (but not as a measure) of the biodegradation rate. The abiotic degradation factors accelerating disintegration were evaluated by monitoring the abiotic degradation of the negative reference samples of LDPE. Biodegradation of the samples in the same inocula of the three marine environments (seawater and sediment) was measured in the laboratory by means of the developed testing methods (OpenBio, 2016). The field disintegration/biodegradation test results were compared and verified against the laboratory test results (Tosin et al., 2016).

The physical condition of all the samples was inspected in-situ during the planned time intervals for recovery of samples. Images of the samples were taken by using a camera GoPro Hero3 HD. Thereafter, the targeted samples were recovered for laboratory analysis.

The degree of disintegration of the recovered samples was analysed in the laboratory by several physical and chemical methods:

- 1) *Image analysis*: An easy to use, efficient technique for studying samples even at high disintegration levels is the use of digital images for measuring the remaining surface of the film specimens. Digital photographs of 12Mp resolution were analysed with the help of Adobe Photoshop CS5 software. In this way, the disintegrated area of each sample was measured.
- 2) *Measurement of thickness*: The thickness of the samples was measured with the help of a Byko-test 4500 Fe/NFe coating thickness gauge (digital thickness meter) range 0–999 μm (accuracy $\pm 2 \mu\text{m}$). Before measurements, the surface of the samples was cleaned with ethanol.
- 3) *FTIR-ATR*: The IR transmission spectrum of the remaining fragments of the samples was studied to analyse chemical alterations of the polymer. The analysis was performed by FTIR Bruker Tensor 27 instrument with the help of OPUS 5.5 60 software. Before running the measurements, the instrument and the samples were cleaned with ethanol and left to dry.
- 4) *Tensile properties*: During the first recalls, when the condition of the recovered samples allowed, tensile properties were tested by using an INSTRON 4004 dynamometer.

2.6. Method for the statistical analysis of the disintegration results

For each of the three environments, a factorial ANOVA was performed (StatSoft, 2013) using the angularly transformed value (%) of

the disintegrated area as the dependent variable and the time, polymer type, experiment (year) and thickness as explanatory factors, analysed in various combinations as presented in the next section and the Supplementary information (SI), section C. Additional factors investigated are the sites for the intertidal trials, geotextile protection for the pelagic trials, and the comparison of the three environments. The appropriate angular transformation of the values (%) of the degree of disintegration was applied by the formula: transformed value = arc sine ($\sqrt{\text{value}(\%)}$). Following the ANOVA analysis, Duncan's test was used for post-hoc comparisons of the angularly transformed means (Steel and Torrie, 1980).

3. Results and discussion

3.1. Characterization of the environmental conditions

The measured seawater parameters describing the conditions in the field test site (pelagic and benthic site shown in Fig. 1) during the experiment are presented in Table 3. The dissolved oxygen (DO) concentration, measured at 2 m above seabed (18 m), was close to saturation (at least 90%) and its value depended on the water temperature. Other weather conditions such as wind and wave size may also influence DO concentration. Turbidity was measured low in most observations and the results showed no sensitivity to this quantity. Salinity was found lower than the typical values in the Saronic Bay (3.7–3.9%). This was a result of the small distance of the experimental site from the coast, so it was influenced by the flow of nearby gullies draining rainwater to the sea and underground fresh water particularly during winter and spring.

The properties of the sediments of the sand beach as well as of the seabed were determined and are summarized in Table 4. The particle size distributions of the sediments from the two intertidal sites (S1 and S2) and the benthic sediment are presented in Fig. 6. The most relevant measured parameters regarding the performed disintegration tests were the concentrations of organic carbon (TOC) and nitrogen. Nitrogen and TOC levels were particularly low in the two intertidal (beach) sites. The lowest values were measured in Site-1. On the other hand, the concentrations measured in the benthic (seabed) sediment were the highest as expected in general for the benthic zone, possibly indicating a small influence also from the nearby aquaculture unit. The porosity (and permeability) was not measured as the sand sediment

Table 3

Conditions of the seawater in the field test site during the experiment at 18 m depth.

Date	Dissolved oxygen (DO)		Water temperature (°C)	Turbidity mg/l	Salinity ppt
	(mg/l)	(%) of DO saturation concentration			
8/10/2014	6.7 \pm 0.1	100	24.8 \pm 0.1	0.0	36.0 \pm 0.1
23/1/2015	6.8 \pm 0.1	90	17.9 \pm 0.1	4.0 \pm 0.4	32.0 \pm 0.1
2/4/2015	6.7 \pm 0.1	96	21.0 \pm 0.1	1.8 \pm 0.3	31.9 \pm 0.1
16/2/2016	6.8 \pm 0.1	90	16.9 \pm 0.1	4.0 \pm 0.4	30.1 \pm 0.1
10/5/2017	6.7 \pm 0.1	93	20.9 \pm 0.1	2.8 \pm 0.3	30.9 \pm 0.1

Table 4
Properties of sediments of the sand beach and the sea bed.

Properties	Intertidal S1	Intertidal S2	Benthic	Testing method
Soil textural class	Sand	Sand	Sand	USDA (2017) ^a
Particle size distribution (%), (Clay, Silt, Sand)	2.4; 2.2; 95.4	3.6; 2.0; 94.4	3.6; 7.2; 89.3	Sieving ^b and sedimentation ^c
pH	7.7		7.5	ISO 10390 (2005)
Total N (% wt/dry wt)	0.018 ± 0.004	0.025 ± 0.001	0.05 ± 0.02	Kjeldahl ^d
Total Organic Mater (% wt/dry wt)	0.10 ± 0.02	0.15 ± 0.01	0.9 ± 0.3	Walkley-Black ^e
Total Organic C (% wt/dry wt)	0.052 ± 0.009	0.076 ± 0.002	0.4 ± 0.2	Walkley-Black
C/N	3	3	8	
Water holding capacity (% water wt/dry wt)	19	22	29	Den Biggelaar (2004)
Porosity		23.07 ± 2.63	20.21 ± 2.46	Gravimetric method ^f
Permeability		N/A	N/A	Free flow of water through the samples

^a Based on Soil Texture Triangle.

^b ASTM D6913/D6913M (2017).

^c ASTM D7928 (2017).

^d ISO 11261 (1995).

^e Schumacher (2002).

^f Danielson and Sutherland (1986).

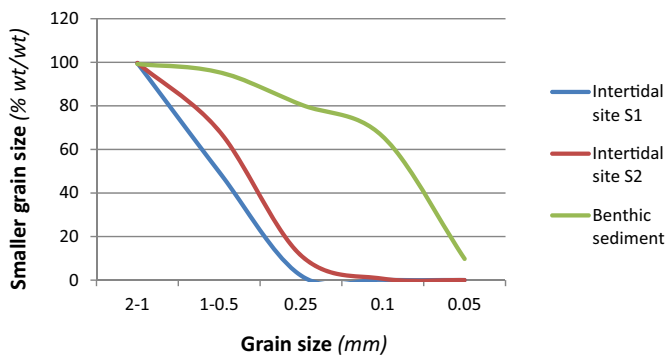


Fig. 6. Particle size distribution of the sediments from the two intertidal sites (S1 and S2) and the benthic sediment.

was under a continuous change of the grain arrangements (density) under the action of the strong sea water currents (benthic zone) or the waves and the wind action along the beach (S1, S2 intertidal locations) and so it was not possible to get undisturbed samples representative of typical porosities of the sites.

The pH of the seawater was measured at 7.4. The seawater in the pelagic zone was measured by the Laboratory of Agricultural Hydraulics of the Agricultural University of Athens to be poor in nutrients. Specifically, the concentrations of ammonia and nitrate were determined to equal to 0.32 mg/l, and 2.37 mg/l respectively, while the concentrations of nitrite and orthophosphate were below the detection level. The concentrations

of ammonia, nitrate, and orthophosphate in the pore-water of the intertidal zone were measured equal to 0.05 mg/l, 3.97 mg/l, and 0.087 mg/l respectively, while the concentration of nitrite was below the detection level. The results for nitrates may be compared for illustrative purposes against the measurements reported in (European Commission, 2018b): In saline waters, average annual values of nitrate concentrations are lower than 2 mg/l in 76% of the measurement stations and higher than 25 mg/l in 0.7% of the stations. The value of 2.37 mg/l of the seawater in the pelagic experimental site is slightly higher but within the standard deviation.

No antibiotics or pesticides were used by the aquaculture unit (certified by an independent agency through the fish quality control).

3.2. Image analysis

3.2.1. Intertidal (eulittoral) environment

Fig. 7 presents three recovered samples after six months of exposure in the intertidal environment at site S1. The thin (25 µm) samples PBSe-25 and PBSeT-25 showed strong signs of disintegration, while the 80 µm PHB-80 samples remained intact.

In Fig. 8 the degree of disintegration versus time of exposure for the materials exposed to the two different intertidal (eulittoral) environments (Site-S1 and Site-S2) are presented and compared. Average values and standard deviations of the recalls are shown in all figures of this work. It is noted that photographs were taken also on site on one sample of each group at selected intermediate intervals between recalls in order to monitor the evolution of the disintegration process without however removing the sample. This was the only possible approach after 7 months of exposure when the remaining samples

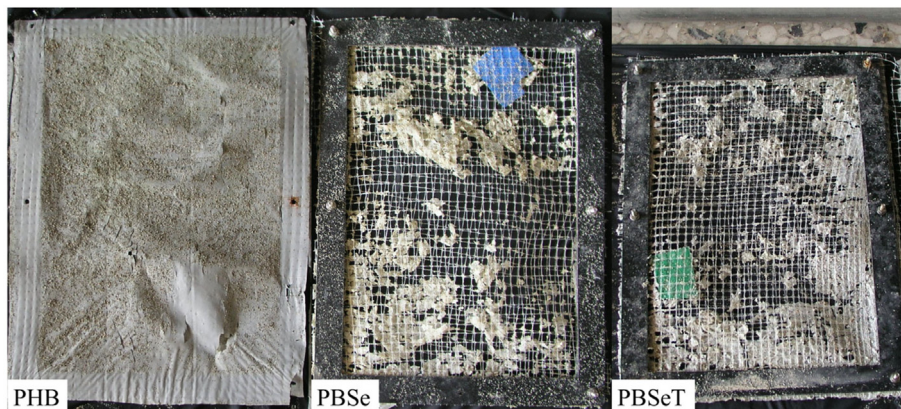


Fig. 7. Samples recovered after 6 months from site S1 (intertidal environment) – 1st year series.

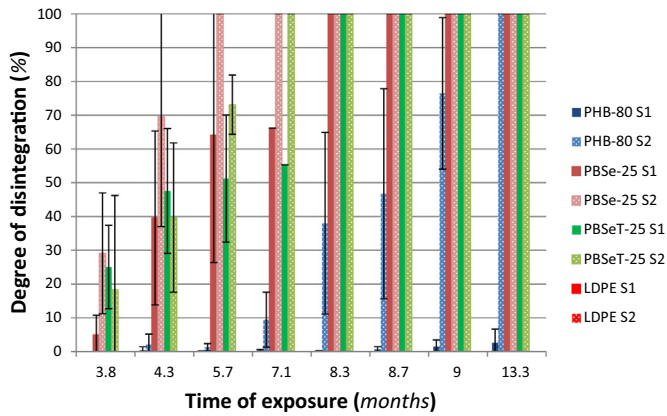


Fig. 8. Comparison of the degree of disintegration (%) of the bio-based and reference materials exposed at the two intertidal sites, S1 and S2 (Figs. 1, 5) - (2nd year experiment).

(repetitions of some materials) were lost during a storm. In these cases, no standard deviations are presented. Thus, only one sample of PBSe-25 and PBSeT-25 was photographed and analysed in month 7 at Site-S1.

These results, which have been obtained through the second experimental series (Table 2), indicated that the environmental conditions (Table 4) have a strong impact on the disintegration rate. The PHB samples exposed at the first location (Site-S1) had not substantially disintegrated even after 13.5 months of exposure. On the other hand, PHB-80 samples of the same 80 μm thickness exposed in the second environment (Site-S2) started disintegrating after 8 months of exposure and completely disintegrated after month 13. Faster disintegration rates at the site S2 were also observed for the other two materials (PBSe-25 and PBSeT-25 of 25 μm thickness) although the differences are smaller. The slower disintegration of PHB-80 compared to PBSe-25 and PBSeT-25 in both sites S1 and S2 is also indicated by the statistical analysis shown in Table 5. The significantly faster disintegration observed in site S2 (Table 5) probably correlates to its increased content of nutrients as it is shown in Table 4. The sediment at site S2 is richer in TOC and more importantly in nitrogen (+40%), so its additional

Table 5
Statistical analysis results for the degree of disintegration of the tested materials in various marine environments.

Case tested	Figure ^a	Variables	Statistics [*]	Degree and rates of Disintegration: significant differences ^{**}
Intertidal environment: Sites S1 and S2 (experiment 2)	1	Materials	F(2,123) = 620.18 P = 0.0000	PHB-80 < PBSeT-25 < PBSe-25
	2	Sites vs. materials	F(2,123) = 25.228 P = 0.0000	Differences of materials between sites S1, S2: PHB-80 > PBSe-25 > PBSeT-25
	3	Site S1 vs. S2	F(1,123) = 90.615 P = 0.0000	S1 < S2
	4	Materials vs. Time	F(14,123) = 9.080 P = 0.0000	PHB-80 < PBSeT-25 < PBSe-25
Intertidal environment: Site S2 (experiment 2)	5	Thickness	F(1,79) = 175.62 P = 0.0000	Last 5 months PBSe = PBSeT = full disintegration: PBSe-25, PBSeT-25 > PBSe-100, PBSeT-100
Intertidal environment: Site S1 (experiments 1–2)	6	Materials vs. experiments	F(2,233) = 29.145 P = 0.4773	PHB-80, PBSe-25, PBSeT-25: no significant differences between the two experiments
	7	Materials vs. Time for both experiments	F(20,206) = 44.26 P = 0.0000	PHB-80 < PBSe-25 ≈ PBSeT-25 Last 5 months PBSe = PBSeT = full disintegration:
Pelagic environment: (Experiment 2) ^b	8	Experiments vs. Time for all Materials	F(10,217) = 0.523 P = 0.8732	Experiments: 2nd year ≈ 1st year
	9	Materials (geotextiles, thin)	F(1,7) = 0.12076 P = 0.7384	PBSe-25 ≈ PBSeT-25
Pelagic environment: (Experiment 1)	10	Materials (geotextiles, thin) vs. Time	F(2,7) = 13.124 P = 0.0043	PBSe-25 ≠ PBSeT-25: increase with time in an inconclusive manner
	11	Thickness (geotextiles)	F(1,18) = 3.4463 P = 0.0798	PBSe-25, PBSeT-25 ≈ PBSe-100, PBSeT-100
	12	Materials (no geotextiles)	F(10,21) = 15.947 P = 0.0000	PHB-80 < PBSeT-25 < PBSe-25 Last 3 months PBSe = PBSeT = full disintegration
Pelagic environment: (Experiment 1–2) ^b	13	Geotextile or not (thin)	F(1,15) = 0.0421 P = 0.8402	PBSe-25, PBSeT-25 without geotextile ≈ PBSe-25, PBSeT-25 with geotextile
	14	Geotextile or not (thin) for both experiments	F(2,150) = 0.5501 P = 0.5780	PBSe-25, PBSeT-25 without geotextile ≈ PBSe-25, PBSeT-25 with geotextile; also for PHB for recorded values no significant differences
	15	Geotextile vs. Time for all materials and experiments	F(7,140) = 0.7287 P = 0.6479	No significant difference with or without geotextile
Benthic environment: (Experiment 2)	16	Materials	F(2,42) = 0.31975 P = 0.7281	PHB-80 ≈ PBSeT-25 ≈ PBSe-25
	17	Materials vs. Time	F(12,42) = 5.9865 P = 0.0001	PHB-80 ≠ PBSeT-25 ≠ PBSe-25; all reach full disintegration after the 7th month; disintegration of PBSe, PBSeT starts after 2 months of exposure PBSe-25, PBSeT-25 > PBSe-100, PBSeT-100
Benthic environment: (Experiment 2)	18	Thickness	F(1,80) = 4281.5 P = 0.0000	
Benthic environment: (Experiments 1–2)	19	Materials vs. experiments	F(2,107) = 0.2651 P = 0.7676	PHB-80, PBSe-25, PBSeT-25: no significant differences between the two experiments
	20	Materials vs. Time for both experiments	F(12,92) = 1.8886 P = 0.0458	PHB-80, PBSe-25 > PBSeT-25 during the first 3 months of exposure
	21	Experiments vs. Time for all Materials	F(6,99) = 7.9995 P = 0.0000	Experiments: 1st year > 2nd year; full disintegration is reached after 7.5 months of exposure in both experiments
	22	All materials vs. Environment	F(2,320) = 154.04 P = 0.0000	Benthic > Intertidal > Pelagic
All environments (Experiment 2)	23	Materials vs. Environment	F(3,360) = 8.7521 P = 0.0001	Intertidal: PHB-80 < PBSe-25 ≈ PBSeT-25; Benthic: PHB-80 ≈ PBSe-25 ≈ PBSeT-25; PBSe-25 ≈ PBSeT-25; benthic ≈ Intertidal > pelagic
	24	Environment vs. time (all materials)	F(30,320) = 2.138 P = 0.0070	Benthic > Intertidal > Pelagic consistently with time
	25	Materials vs. time (all environments)	F(14,242) = 1.245 P = 0.2438	PBSe-25 ≈ PBSeT-25 consistently with time

* Precision of p-values to four digits.

** Highlights of the statistically different groups based on the ANOVA (p-values <0.05) and the Duncan post-hoc test results; the behaviour is shown in the corresponding Figures and Tables of the ANOVA results (refer to SI).

^a Supplementary information, section C.

^b Missing data for PHB after 6 months for the second experiment (lost samples).

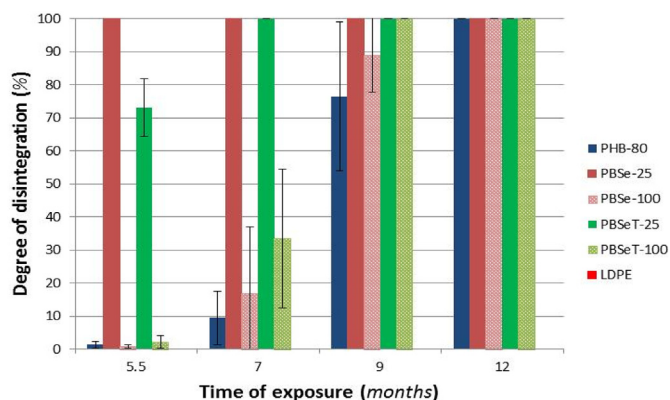


Fig. 9. Effect of sample thickness on the degree of disintegration (%) in the intertidal environment (site S2 – 2nd year series).

nutrients enhance biodegradation resulting in faster disintegration of the samples. Moreover, the samples at site S2 were kept wet for longer and more frequent periods since they were buried closer to the seashore line. The higher moisture content of the sediment combined with higher nutrients concentration facilitates biodegradation and disintegration of the samples.

The slower disintegration of PHB-80 compared to PBSe-25 and PBSeT-25 in both experimental sites (S1 and S2), which is confirmed by the statistical analysis shown in Table 5, is attributed to the larger thickness of the PHB-80 samples (approximately 80 μm). As Fig. 8 shows, the effect of thickness of a given material becomes important when the main biodegradation/disintegration factors (nutrients and water) are limited. As confirmed by the analysis shown in Table 5, thickness appears to influence significantly the disintegration of the thick (80 μm) PHB-80 samples at site S1, which was poor in nutrients in contrast to the fast disintegration of the thin PBSe-25 and PBSeT-25 samples.

The effect of the thickness was further investigated by testing PBSe and PBSeT samples of different thickness (25 μm vs. 100 μm) at the same intertidal environment (site S2) during the second experimental series. The results are presented in Fig. 9. Only one sample of PBSeT-100 was photographed without removing it in month 7. The increase of the thickness affects adversely the time to reach complete disintegration/biodegradation by delaying the initiation of disintegration, as expected (Table 6). This conclusion is statistically confirmed by the data shown in Table 5. Thickness does not affect however the final degree of disintegration.

In Fig. 10, the reproducibility of the test is assessed. The degrees of disintegration measured during the 1st and the 2nd year experiments for all three materials exposed at site S1 were compared. The disintegration behaviour of all materials was found to be approximately the same despite the fact that the exposure took place during different seasons (Table 2). Specifically, the 1st year test began in the summer of 2014 and the second in late autumn 2015. Fig. 10 shows that disintegration progresses faster during the summer period. However, the seasonal influence on the disintegration of the tested samples was found weak (Fig. 10). The statistical analysis results (Table 5, SI section C) indicate no significant difference between the two experimental series of years 1st and 2nd. Intense disintegration appeared for the thin (25 μm)

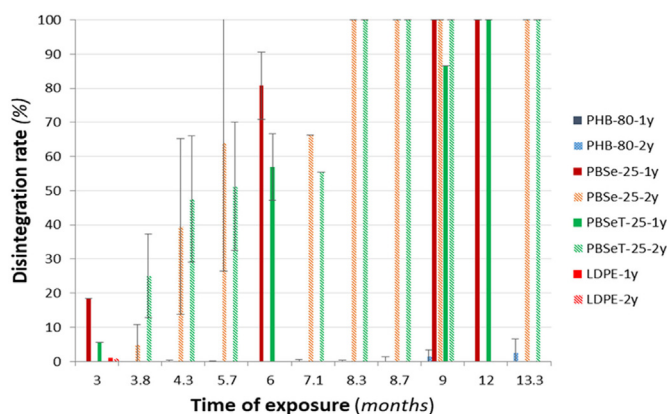


Fig. 10. Comparison between 1st and 2nd year experiments in the intertidal environment (site S1).

PBSe-25 and PBSeT-25 samples after 6 months of exposure, when the degree of disintegration exceeded 50% for both series of experiments. After month 9, the disintegration had reached almost 100% in both 1st and 2nd year's tests. Therefore, it was concluded that the disintegration of thin PBSe-25 and PBSeT-25 films started before month 3 and was accomplished approximately after 9 months of exposure in the intertidal (eu littoral) environment. The PHB-80 samples did not exhibit detectable disintegration up to 12 months of exposure in both one-year tests which took place at site S1. No disintegration was observed in any LDPE sample in the intertidal environment during the two years of experiments. The statistical analysis results confirm a significant difference between the materials PHB vs. PBSe and PBSeT (Table 5, SI section C).

3.2.2. Benthic (sublittoral) environment

All tested materials (PHB-80, PBSe-25, PBSeT-25) with the exception of the negative reference (LDPE) disintegrated fully in the benthic environment (Fig. 11). The disintegration results in the benthic environment of the 1st and the 2nd year of field tests are compared in Fig. 12. As in the case of the intertidal test, seasonal weather effects are limited and the disintegration of the materials progressed in a similar pattern as during the first year. This fact was also confirmed statistically as shown in Table 5 (and SI section C). The recall of the PHB-80 corresponding to two months of exposure exhibited a much higher disintegration rate during the 2nd year compared to the 1st year. In contrast, the disintegration of the PBSe-25 samples for the same period of exposure (first 2 months) was considerably higher during the 1st year of field tests. The significant differences in the disintegration rates with the time between the materials in both experiments or between the two experiments for all materials was also confirmed with the statistical analysis results (Table 5 and SI section C). These variations are explained by the fact that the samples on the seabed may or may not be covered by sediment depending on random seabed conditions, namely currents of seawater inducing sand transport and deposition at the seabed level. This uncertainty with respect to the covering conditions of the samples concerned mainly the first period of exposure since, after a few months of exposure, a layer of sediment usually covered most of the samples. The appearance of biofilm (fouling) on the upper surface of some recovered samples indicates that they were not fully covered by the sediment. After 3 months of exposure, the PHB-80 and PBSe-25 samples fully disintegrated during the 1st year while the disintegration of the PBSeT-25 samples was in progress and was found fully disintegrated in month 6. Similarly, during the 2nd year, all materials were fully disintegrated after 5 months of exposure. No disintegration was observed in any LDPE sample in the benthic environment during the two years of experiments.

Table 6
Time of exposure (months) for the complete disintegration of three samples exposed in the intertidal environment (Site S2).

Material	Disintegration time (mo)		
	25 μm	100 μm	80 μm
PHB-80			13.3
PBSe-25	5.5	12.0	
PBSeT-25	7.0	9.0	

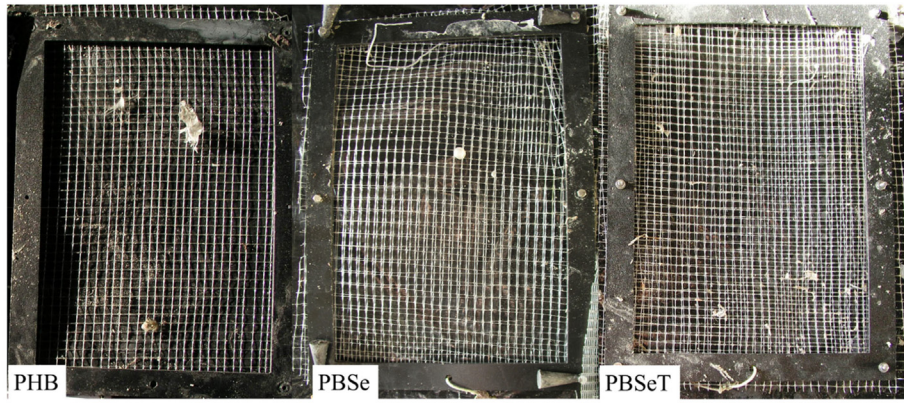


Fig. 11. All three bio-based materials disintegrated fully in the benthic environment after 6 months of exposure (1st year series).

The effect of the thickness of the film was investigated during the 2nd year experimental series. Samples of PBSe and PBSeT films of two different thicknesses, namely 25 μm and 100 μm were exposed in the benthic environment. The results presented in Fig. 13 and Table 5 indicate that the increase of the thickness slows down the disintegration/biodegradation rates as expected, similarly to the results for the intertidal environment. The thick samples PBSe-100 and PBSeT-100 remained intact in terms of their apparent solid area for >10 months. On the opposite, the PHB-80 samples disintegrated fast in the benthic zone, despite their thickness, at a rate similar to the thin PBSe-25 and PBSeT-25 films. Therefore, the thickness is not the only parameter influencing the disintegration/biodegradation rate of each polymer in a given environment. The biodegradation behaviour is mainly an inherent property of the material (OECD, 2006). For example, PHB-80 was found to biodegrade faster than PBSe-25 and PBSeT-25 provided that the biodegradation conditions (e.g. nutrients and humidity) are not inhibiting the process (as in the intertidal zone).

3.2.3. Pelagic environment

In this experiment, the samples were suspended floating between the sea surface and seabed at about 10 m depth. This is a biologically active zone where an ecosystem of many species of marine flora and fauna develops.

Fig. 14 presents the results of the 1st year’s experiment. Disintegration became detectable after 6 months of exposure and it was faster for the thinner films (PBSe-25 and PBSeT-25) (Table 5). PHB-80 also disintegrated in a period of about one year and fully decayed soon after 12 months of exposure. In the pelagic environment, which is a well-aerated habitat with high levels of solar radiation the growth of a biofilm that gradually develops into biofouling is common (Pauli et al.,

2017). Samples were usually covered totally by bio-fouling (Fig. 15). Consequently, the analysis of the results became difficult since the tested plastic film was not easily accessible. For this reason, less than three samples were analysed for several recalls. Standard deviation marks are missing at the corresponding columns of Fig. 14. Moreover, the effect of bio-fouling on the biodegradation and the related disintegration of the plastics was not clear and required further investigation.

The effect of bio-fouling was studied during the 2nd year by protecting the samples by permeable covers made of plastic geotextile. The used geotextile was woven PP material made of tape threads. Its weight was 100 g/m² and its optical porosity was 0% (mesh size zero). Its water flow rate was 900 L/min/m² (information provided by the producer Thrace Plastics S.A.). In this way, the bio-fouling effect on the samples was eliminated (Fig. 16).

Three of the samples shown in Fig. 16 had been covered with geotextile during their exposure. A fourth unprotected sample was also added for comparison. No bio-fouling could be detected on the samples that were covered with geotextile, while bio-fouling started growing on the unprotected one.

In Fig. 17 the disintegration of samples with and without geotextile covering during the 2nd year is presented. The samples protected with geotextile covers do not show a significant difference in their degree of disintegration. During the 2nd year, the samples protected with geotextile covers exhibited slightly faster disintegration, although this effect was not found statistically significant (Table 5). The effect of geotextiles in both experiments of the first and second year also showed no statistically significant effect. It should be noted that no samples with geotextile protection were tested in the first year, while PHB samples in the 2nd year were

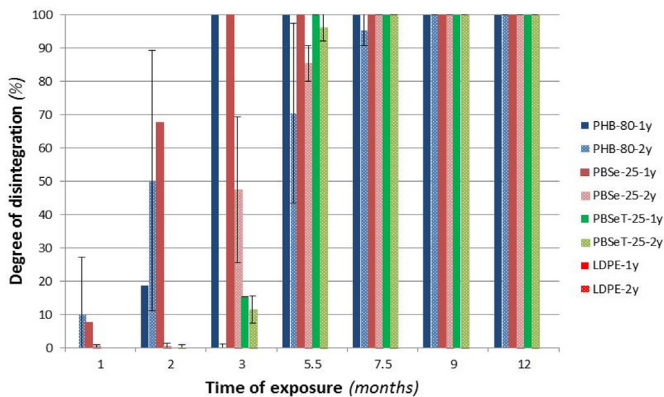


Fig. 12. Comparison between 1st and 2nd year experiments in the benthic (sublittoral) environment.

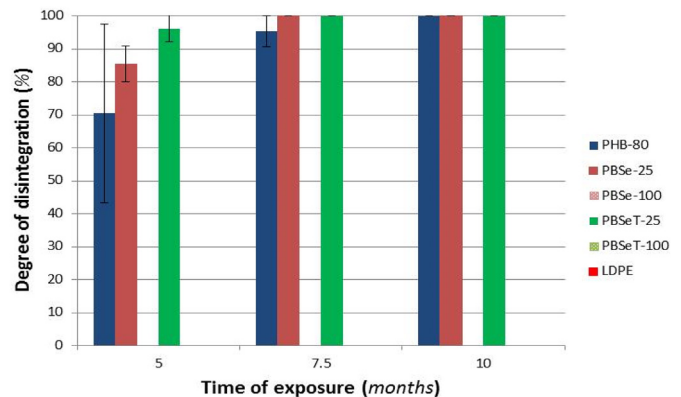


Fig. 13. Effect of the thickness on the degree of disintegration of the bio-based materials in the benthic environment (2nd year test).

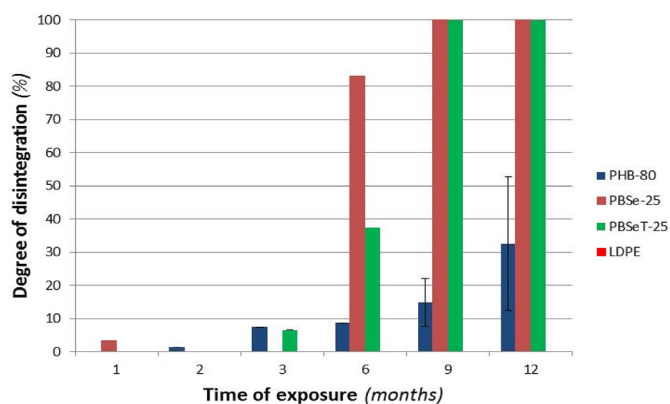


Fig. 14. Degree of disintegration (%) in the pelagic environment during the 1st year's experimental series.

lost after the 6th month. In any case, the effect of biofouling regarding biodegradation needs to be investigated systematically.

During the 2nd year experiments, the effect of the thickness on the disintegration of the films was studied as with the other two environments. For this reason, samples of the thick PBSe-100 and PBSeT-100 films, protected by geotextile covers to avoid bio-fouling, were also exposed following the same experimental protocol as for the thin materials. No disintegration of the thick PBSe-100 and PBSeT-100 and PHB-80 samples could be detected by Image Analysis at six months of exposure in the pelagic zone. Unfortunately, the PHB samples were lost after the period of six months due to an unknown event (most possibly by storm; Fig. 18). After 10 months of exposure, an average disintegration degree of 25% for PBSeT-100 and no disintegration for PBSe-100 were detected. In contrast, the disintegration of the thin films exceeded 70% in a period of 12 months (Fig. 17). Therefore, thickness influences the disintegration rate similarly to the eu littoral environment (Table 5).

The benthic environment was found to be the most active of the three tested habitats (Table 5). This fact is partially explained by the higher levels of total nitrogen in the benthic zone compared to the intertidal and the pelagic environments (Table 4).

3.2.4. Negative reference - polyethylene films

The sample of thin (25 μm) LDPE film, which was used as a negative reference, did not exhibit any signs of disintegration in any of the three tested environments. The LDPE samples showed only a partial loss of clarity (ASTM D1003, 2013) in the intertidal environment probably due to abrasion, and were fully covered by bio-fouling in the pelagic zone (when not protected by geotextile).

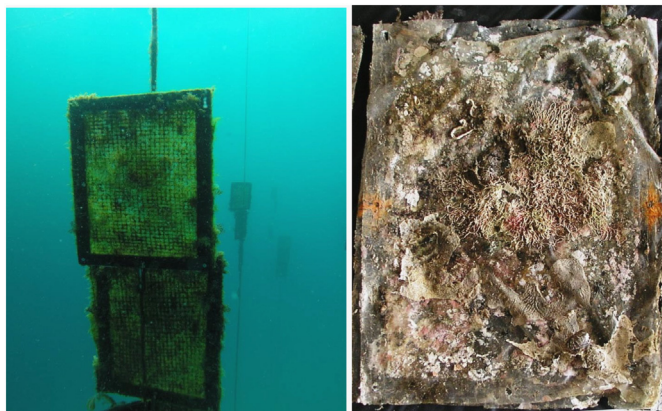


Fig. 15. Biofouling developed on pelagic-zone samples.

3.2.5. Comparison with lab tests

A comparison between field disintegration results and laboratory biodegradation data for the tested materials in the pelagic environment is presented as Supplementary data for illustrative purposes.

3.2.6. Summary of disintegration results

The results presented analytically in the previous sections and confirmed by the statistical analysis shown in Table 5 and the SI section C are summarized below:

Intertidal: The degree of disintegration of the thick material PHB-80 was much slower than that of the two thin materials PBSe-25 and PBSeT-25 in both sites S1 and S2, and in general lower in the site S1 than in site S2 (2nd experiment). During the 2nd experiment, the rates of disintegration of the materials with time were found to differ significantly following the order $\text{PHB-80} < \text{PBSeT-25} < \text{PBSe-25}$. The thick materials PBSe-100 and PBSeT-100 exhibited much slower degrees of disintegration than their thin counterparts. Concerning the degree of disintegration and the disintegration rates of the materials with time in the two experiments (1st and 2nd year), it was confirmed that $\text{PHB-80} < \text{PBSe-25} \approx \text{PBSeT-25}$ for both experiments with small differences between the two experiments.

Pelagic: In the 2nd year (experiment 2) the degree of disintegration of the two materials PBSe-25, PBSeT-25 protected with geotextiles did not differ while the disintegration rate with time appears to be relatively higher for PBSe-25 (no data were recorded for PHB-80 after the 6th month of exposure as the samples were lost due to a storm). The thick materials PBSe-100 and PBSeT-100 exhibited much slower degrees of disintegration than their thin counterparts as in the intertidal environment. The influence of bio-fouling on biodegradation was investigated by comparing the data of 1st year series (no geotextile) with those of the 2nd year experiment, although some PHB data are missing. The effect of geotextile protection was not found to be statistically significant.

Benthic: In the 2nd year (experiment 2) the degree of disintegration of the materials was found to be similar while the rate of disintegration was different between PHB-80 and the thin films PBSeT-25, PBSe-25 during the first 3 months of exposure. The thick materials PBSe-100 and PBSeT-100 exhibited much slower degrees of disintegration than their thin counterparts. Concerning the disintegration rate of the materials with time in the two experiments (1st and 2nd year) it was confirmed that $\text{PHB-80} \approx \text{PBSe-25} > \text{PBSeT-25}$, especially during the first 3 months of exposure. All materials disintegrate after 5 months of exposure. The degree of disintegration of the three materials was not found to be significantly different between the two experiments. The disintegration rates were found to be higher during the first experiment.

All environments (2nd experiment): The degrees of the disintegration of all materials in the three environments exhibited significant differences: Benthic > Intertidal > Pelagic. The tested materials showed different degrees of disintegration in each environment: $\text{PHB-80} < \text{PBSe-25} \approx \text{PBSeT-25}$ in intertidal vs. $\text{PHB-80} \approx \text{PBSe-25} \approx \text{PBSeT-25}$ in benthic vs. $\text{PBSe-25} \approx \text{PBSeT-25}$ in pelagic. Especially for PBSe-25, PBSeT-25: benthic \approx intertidal > pelagic. The disintegration rates with the time of all materials vs. environment showed consistent behaviour: Benthic > Intertidal > Pelagic. The disintegration rates of the materials with time for all environments confirmed $\text{PBSe-25} \approx \text{PBSeT-25}$ (PHB could not be compared due to missing data in the pelagic environment).

3.3. FTIR analysis

3.3.1. Disintegrated samples from field tests

FTIR analysis was applied to selected exposed samples in order to detect chemical changes due to biodegradation at the early stages of exposure. In Fig. 19, the FTIR ATR analysis of PHB-80 exposed to the pelagic environment for different times is presented, representative of the benthic environment as well (the inset presents the PHB-80 exposed to the

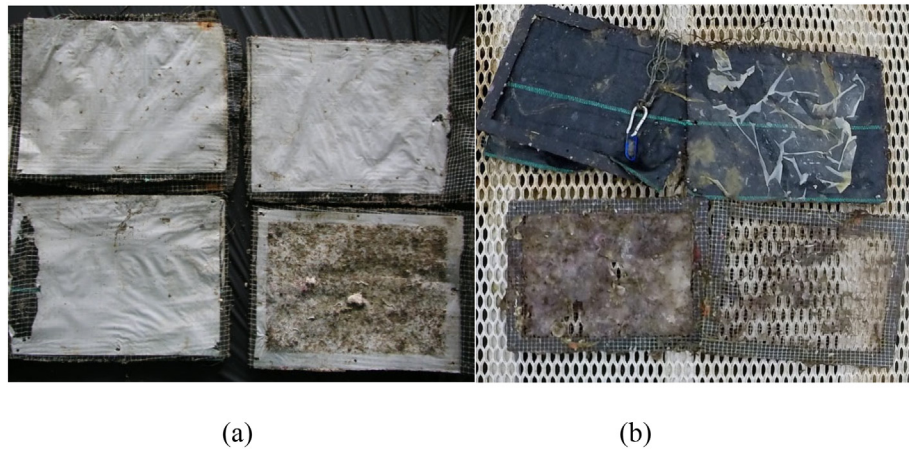


Fig. 16. a) Three PHB-80 samples protected by geotextile and one not covered (control) exposed in the pelagic environment for 6 months; b) PBSeT samples protected by geotextile and one unprotected exposed in the pelagic environment for 10 months (2nd year series).

intertidal environment for different times). Similarly, in Fig. 20, the FTIR ATR analysis of PBSe-100 and PBSeT-100 (of 100 μm) which were exposed to the benthic environment is presented.

The spectra show that two additional peaks develop at approximately 1650 (possibly amides) and 1540 cm^{-1} (possibly nitro compounds) for the PHB-80, PBSe-100, PBSeT-100 samples exposed to the marine environments predominantly to the benthic and pelagic zone which were not present in the spectra of the initial materials. Moreover, a broad peak appeared at approximately 3300 cm^{-1} . FTIR TR (transmission) analysis was also performed for all samples and the results were in accordance with the ATR analysis. The peaks at 1650 and 1540 cm^{-1} may have a contingency with groups associated with biological effects. These groups can be possibly amides (Humbert and Quilès, 2011) present in the biofilm formed and grown with the time of exposure on the samples. The broad peak at 3300 cm^{-1} represents OH^- groups (broad, strong O—H absorption of alcohols and phenols; O—H Stretch 3550–3200) most probably produced by the hydrolysis of the material (e.g. by PHB depolymerase enzyme; Roohi, et al, 2018), possibly including also amine groups (medium amine N—H Stretch 3500–3300), (Merlic and Strouse, 2000). No changes were observed by FTIR for the LDPE films and this is an additional indirect indication of biodegradation of the bio-based films.

Despite the fact that the LDPE films showed signs of abiotic degradation of mechanical properties, no carbonyls were detected after several months of exposure in the three marine environments. Specifically, the tested LDPE samples were exposed to: a) intertidal environment for a

period of 3 months (October 2014 to January 2015) and for 28 months (October 2015 to February 2018: covered by sediment while they were not subjected to thermal degradation; b) pelagic environment for two periods of 6 months (July 2014 to January 2015) and 9 months (July 2014 to April 2015): covered by biofouling and so they were not subjected to UV radiation (no photo-degradation); c) benthic environment for a period of 9 months (July 2014 to April 2015): covered by sand or biofouling and so they were not subjected to UV radiation (no photo-degradation).

Any carbonyls possibly developed due to photo-oxidation or thermal degradation of the LDPE samples exposed to the three marine environments were not detectable. The explanation for this behaviour was given by Briassoulis et al. (2015). The carbonyl species formed in samples of two different LDPE films (20 μm and 16 μm) after their exposure to UV radiation, were found to decrease during their exposure in soil-water dilution. After the exposure of the samples in the dilution for 32 days, the CI was decreased by 69% and 57%, respectively. This behaviour is attributed by Hakkarainen and Albertsson (2004) to the low molecular weight carboxylic acids migration into the water phase during the exposure of the degraded LDPE film for a long period in soil or water.

According to these results, FTIR ATR analysis comprises a method that can be used as a complementary tool for the early detection of on-going biodegradation in field experiments.

3.3.2. FTIR ATR comparative analysis of samples from lab and field tests disintegrated samples from field tests

The comparison of FTIR ATR analysis results obtained from samples tested under controlled conditions in the laboratory and samples exposed to natural marine environments during the field tests is presented in Fig. 21. The spectra of the materials, from the lab tests as well as those from the field tests, are qualitatively similar. More specifically, in Fig. 21(a), the spectrum of the PBSeT-25 material exposed to the pelagic lab conditions presents the same peaks with the sample exposed for 6 months to the pelagic field environment. The broad peaks at approximately 3300 cm^{-1} in both spectra confirm hydrolysis as they represent OH^- groups, possibly combined with biological phenomena as they can also be associated with amine groups. The peaks at approximately 1650 cm^{-1} , which appear in both spectra, are possibly associated with biological effects. The results of the FTIR ATR analysis for the PBSeT-100 material (Fig. 21(b)) show a similar qualitative agreement. The sample exposed to the intertidal lab test presents exactly the same peaks with the material exposed to the benthic field test for 5 months (no samples were available from lab tests in benthic conditions as they biodegraded quickly). An additional peak in both spectra of PBSeT-100 material, at approximately 1540 cm^{-1} , appears which is associated as well with biological effects.

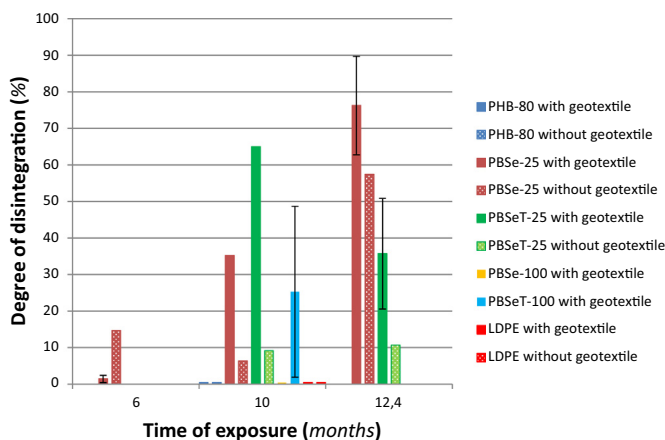


Fig. 17. Effect of the geotextile cover on the degree of disintegration (%) of samples exposed for 10 and 12.4 months to the pelagic environment (2nd year experiment).



Fig. 18. Samples exposed to the pelagic environment were destroyed during the 2nd year due to storms (PHB samples were lost). Samples exposed to the benthic environment were displaced along with their anchor several meters from their initial position by forces applied by the waves through the buoys and water currents.

3.4. Mechanical properties

Testing of the mechanical properties of the exposed materials was also considered as an alternative method for assessing the progress of degradation before visual disintegration is observed. In Fig. 22, the elongation at break (ϵ_{br} %) in the transverse direction of non-disintegrated samples, recovered at different exposure times from different marine environments, is presented. Only samples protected with geotextile were taken into account in the pelagic zone, so their surface was kept clear of bio-fouling, and their mechanical properties can be measured.

It is shown that the PHB-80 samples exhibited sharp and intense deterioration with respect to their mechanical properties (expressed by ϵ_{br} %) following the order: benthic > intertidal > pelagic. Image analysis of samples exposed in the intertidal site S2 confirms this order regarding the disintegration rates of PHB-80 in the three tested environments (Fig. 8). In contrast, the samples exposed in the intertidal site S1 resisted disintegration for >12 months, indicating that the biodegradation of PHB-80 was slower in this intertidal zone when compared to the PHB-80 samples in the pelagic environment (see for example the Figs. 8 and 14). Nevertheless, their mechanical behaviour was already downgraded after 3 months of exposure probably due to abiotic degradation (Fig. 22).

Although the LDPE film (negative reference) did not disintegrate in all three studied environments, its mechanical properties deteriorated as it is indicated in Fig. 22. LDPE material showed a measurable decline with respect to its mechanical properties even in the short period of

3 months exposure in the intertidal environment. The degradation followed the order PE intertidal > PE benthic > PE pelagic. However, LDPE samples resisted disintegration for the full duration of the experiment, with no signs of biodegradation in any environment. The abiotic degradation of the LDPE samples, as their mechanical performance indicated (Fig. 22), was the result of the combined actions of various weathering factors, such as the mechanical stress due to waves and sea currents, and the abrasion caused by the moving coarse and fine grains of the beach sediment in the intertidal zone, and sediment fine grains at the seabed. This confirmed the order of abiotic degradation measured by the decrease of the elongation at break of the LDPE samples in the three zones.

Only a few bio-based samples could be tested for mechanical properties because of brittleness and disintegration. The tested bio-based materials also suffered abiotic degradation contributing to biodegradation, which led to the disintegration of the samples. The abiotic factors were not sufficient to cause disintegration by themselves, as it has been shown in the case of the negative reference material (LDPE). Therefore, the disintegration of bio-based materials in a short finite time frame, determined by image analysis techniques, is considered as a good indication of biodegradation (Fig. 22).

The thickness of the samples was also measured as a complementary indication of their disintegration. The results show that the measurement of thickness may be a useful means for characterizing slow disintegrating thick films (e.g. PHB-80 in the intertidal environment). In the case of thin films such as PBSe-25 and PBSeT-25, the analysis is

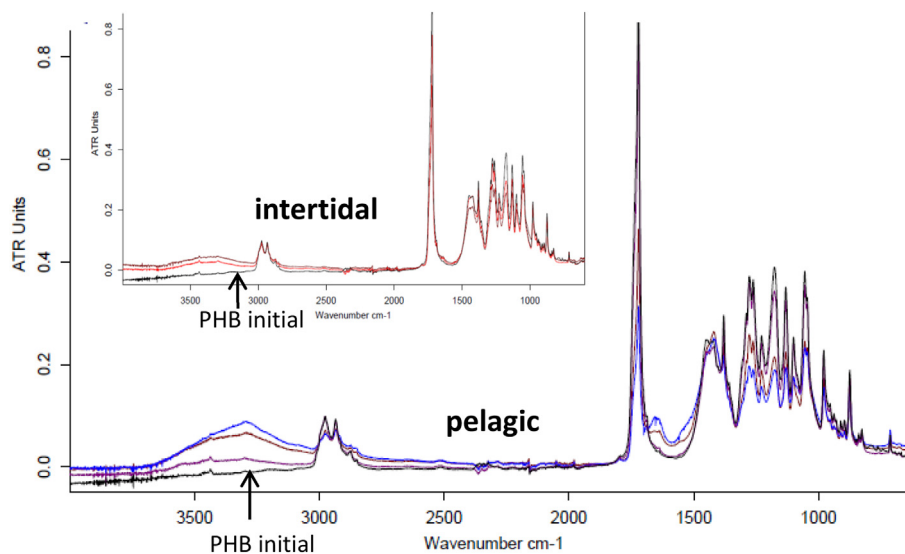


Fig. 19. FTIR ATR spectra of PHB-80 exposed to the pelagic environment: initial (black) and after being exposed to the pelagic environment for 1 (purple), 6 (brown) and 9 (blue) months. The inset figure presents PHB-80 exposed to the intertidal environment for 3 (red) and 6 (brown) months.

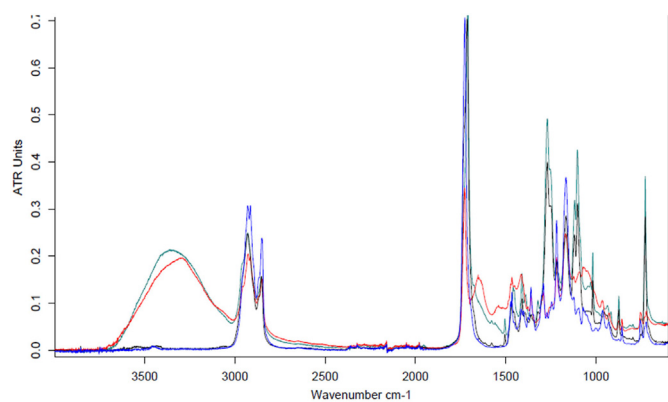


Fig. 20. FTIR ATR spectra of PBSe (100 μm) and PBSeT (100 μm): initial (blue and black respectively) and after being exposed to the benthic environment for 5 months (red and light blue for the two materials respectively).

more difficult since biofilm growth and sediment deposition interfere with the thickness measurements. The detailed results are shown in the Supplementary data file.

4. Conclusions

Four plastic films (PHB, PBSe, PBSeT, and LDPE) were tested in three different marine environments (intertidal, pelagic and benthic zones). Two of these plastic films, namely the bio-based PBSe and PBSeT films are known to biodegrade in soil but their behaviour in marine environments was unknown. The other two materials, namely the bio-based PHB and the LDPE films were used as positive and negative references respectively. The field experiment took place at the south coast of Salmis Island, Greece.

It has been shown by various measuring techniques, namely Image Analysis, FTIR-ATR spectroscopy and testing of mechanical properties, that all bio-based materials disintegrated in all three considered marine environments at different rates and degrees, depending on the marine environment, the prevailing conditions, the polymer type, and the film thickness. Only the negative reference material, LDPE film, did not disintegrate in any of these three marine environments, although its mechanical properties were degraded due to the combined abrasive actions of sediments, waves, and seawater currents. The FTIR analysis has shown changes in the chemical structure of the bio-based materials

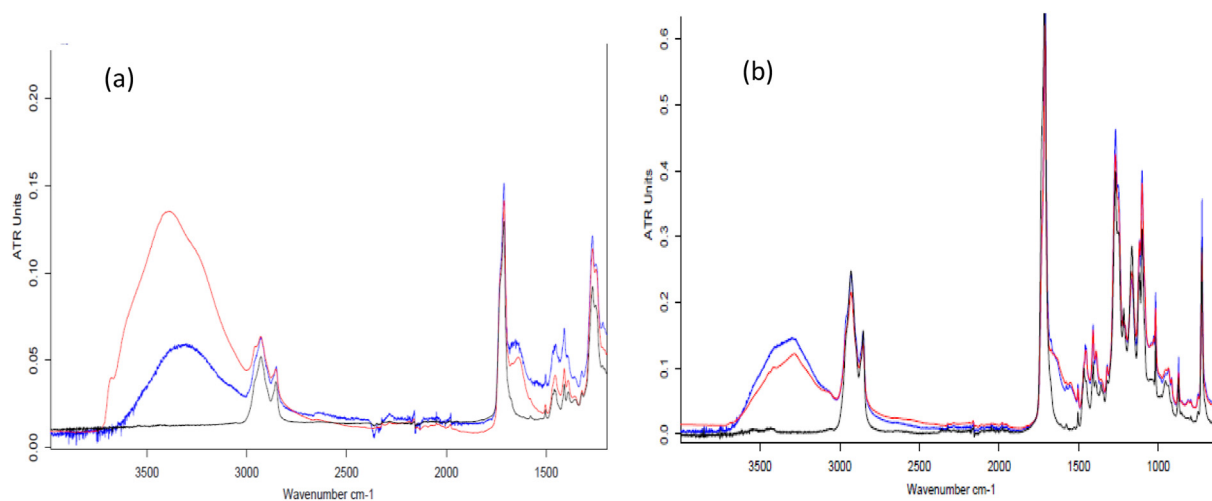


Fig. 21. FTIR ATR spectra of (a) PBSeT-25 (black: initial, red: pelagic lab test (7 months), blue: pelagic field test (6 months)), (b) PBSeT –100 (black: initial, red: intertidal lab test (5 months), blue: benthic field test (5 months)).

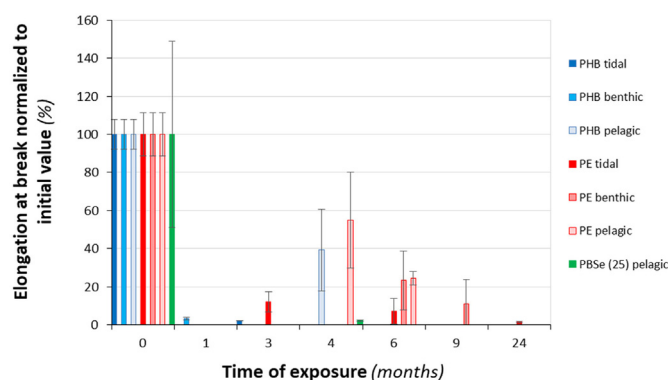


Fig. 22. Evolution of the elongation at break (normalised to initial values, 100%) in the transverse direction of various samples recovered after exposure in the three tested environments.

suggesting the possible presence of biofilm and hydrolysis of the material. Therefore, it can be assumed that the main cause of the disintegration of the three tested bio-based materials, PHB, PBSe, and PBSeT was bio-assimilation by marine microorganisms.

Particular attention was given to the effect of the bio-fouling growing on the samples in the pelagic environment. However, the present results were inconclusive and further research is needed to analyse the effect of bio-fouling on the biodegradation of plastics.

Laboratory tests for biodegradation of the same films under conditions simulating the three marine zones are in progress. The results will be published soon.

The present work presents strong indications that biodegradation of polymers is possible in various coastal environments although the rate varies depending on the material, the marine environment, and the local conditions. The current study was limited with respect to the geographical area, the marine zones and the number of samples. A broader investigation aiming to collect systematically more data over a wider spatial distribution of samples exposed to various marine environments and conditions is necessary. This would allow for analyzing in depth the biodegradation behaviour of specific commercially important bio-based materials and correlating their behaviour with key environmental and ecosystem parameters and conditions by statistical analysis. Expanding this research to other marine zones (e.g. mesopelagic) is another environmentally important objective. All field tests should be accompanied by parallel lab tests to verify the biodegradation behaviour of the tested materials.

Acknowledgements

The research leading to these results has received funding from the European Union 7th Framework Programme (Grant Agreement: 613677). The work has been conducted within the EU project OpenBio: “Opening bio-based markets via standards, labelling and procurement” (www.open-bio.eu). We also thank Argosaronikos Fishfarms S.A. for permitting the use of their installations for the needs of these experiments and supporting the technical work. We particularly thank Dr. A. Giannoulis for the technical work regarding Image Analysis, the AUA Laboratory of Soil Science & Agr. Chemistry for performing soil analysis and the AUA Laboratory of Agricultural Hydraulics for providing water analysis. Special thanks are due to Prof. P. Panagakis (Lab of Farm Structures) and H. Briassoulis (Aegean University, Dept. of geography) for their advice on the statistical analysis.

Appendix A. Supplementary Material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2019.06.129>.

References

- Acampora, H., Schuyler, Q.A., Townsend, K.A., Hardesty, B.D., 2014. Comparing plastic ingestion in juvenile and adult stranded short-tailed shearwaters (*Puffinus tenuirostris*) in eastern Australia. *Mar. Pollut. Bull.* 78, 63–68.
- APHA, 2005. Standard Methods for the Examination of Water and Wastewater. 21 ed. American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC.
- ASTM D1003, 2013. Standard Test Method for Haze and Luminous Transmittance of Transparent Plastics. American Society for Testing and Materials, ASTM International, West Conshohocken, PA.
- ASTM D6691, 2017. Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in the Marine Environment by a Defined Microbial Consortium or Natural Sea Water Inoculum. American Society for Testing and Materials, ASTM International, West Conshohocken, PA.
- ASTM D6913/D6913M, 2017. Standard Test Methods for Particle-Size Distribution (Gradation) of Soils Using Sieve Analysis. American Society for Testing and Materials, ASTM International, West Conshohocken, PA.
- ASTM D7928, 2017. Standard Test Method for Particle-Size Distribution (Gradation) of Fine-Grained Soils Using the Sedimentation (Hydrometer) Analysis. American Society for Testing and Materials, ASTM International, West Conshohocken, PA.
- ASTM D7991, 2015. Standard Test Method for Determining Aerobic Biodegradation of Plastics Buried in Sandy Marine Sediment Under Controlled Laboratory Conditions. American Society for Testing and Materials, ASTM International, West Conshohocken, PA.
- Bremner, D.C., Mulvaney, J.M., 1982. Total nitrogen. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), *Methods of Soil Analysis*. Number 9 Part 2, Am. Soc. of Agron.
- Briassoulis, D., Babou, E., Hiskakis, M., Kyrikou, I., 2015. Analysis of long term degradation behaviour of polyethylene mulching films with pro-oxidants under real cultivation and soil burial conditions. *Environ. Sci. Pollut. Res.* 22, 2584–2598.
- Danielson, R.E., Sutherland, P.L., 1986. Porosity, in *Methods of Soil Analysis Part 1. Physical and Mineralogical Methods*, Agronomy Monograph No. 9. Soil Science Society of America, Madison, WI, USA, pp. 443–461.
- Den Biggelaar, C., 2004. Soil Sampling, Agroecology Lab Manual and Readings. Appalachian State University <http://www.appstate.edu/~denbiggelaar/>.
- Directive (EU) 2015/720, 2015. Directive (EU) 2015/720 of the European Parliament and of the Council of 29 April 2015 amending Directive 94/62/EC as regards reducing the consumption of lightweight plastic carrier bags. *Off. J. Eur. Union L 115/11* (6.5.2015).
- Duarte, C.M., 2008. Littoral zone. In: Chen, Chen-Tung Arthur, Jacques, C., Nihoul, J. (Eds.), *Oceanography – Volume II*. EOLSS Publications, p. 360 2009.
- Emadian, S.M., Onay, T.T., Demirel, B., 2017. Biodegradation of bioplastics in natural environments. *Waste Manag.* 59, 526–536.
- EN 16575:2014, 2014. Bio-Based Products – Vocabulary, European Standard. European Committee for Standardization, Brussels, Belgium.
- European Bioplastics, 2018. *Bioplastics—Industry Standards & Labels: Relevant Standards and Labels for Bio-based and Biodegradable Plastics*. European Bioplastics (Fact Sheet, March 2018).
- European Commission, 2018a. Communication From the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions, A European Strategy for Plastics in a Circular Economy, Brussels, 16.1.2018, COM(2018) 28 Final.
- European Commission, 2018b. Report From the Commission to the Council and the European Parliament on the Implementation of Council Directive 91/676/EEC Concerning the Protection of Waters Against Pollution Caused by Nitrates From Agricultural Sources Based on Member State Reports for the Period 2012–2015 (SWD (2018) 246 Final), Brussels, 4.5.2018 COM(2018) 257 Final.
- Fraschetti, S., 2012. Mapping the marine environment: advances, relevance and limitations in ecological research and in marine conservation and management. *Biol. Mar. Mediterr.* 19 (1), 79–83.
- Galgani, F., Hanke, G., Maes, T., 2015. Global distribution, composition and abundance of marine litter, chapter 2. In: Bergmann, M., et al. (Eds.), *Marine Anthropogenic Litter*, XVIII, pp. 29–56 https://doi.org/10.1007/978-3-319-16510-3_2 (447 pp.).
- Hakkariainen, M., Albertsson, A.C., 2004. Environmental degradation of polyethylene. *Adv. Polym. Sci.* 169, 177–199.
- Halstead, A., Basham, H., 2013. Ocean pelagic Zone, oceanic pelagic zone biology biome project. <https://www.slideshare.net/hbsham1/oceanic-pelagic-zone-biology-biome-project>.
- Humbert, F., Quilès, F., 2011. *Microbiology book series. Science Against Microbial Pathogens: Communicating Current Research and Technological Advances*. vol. 3 in press. www.formatex.info/microbiology3/book/889-895.pdf.
- Imam, S.H., Gordon, S.H., Shogren, R.L., Tosteson, T.R., Govind, N.S., Greene, R.V., 1999. Degradation of starch–poly(β-hydroxybutyrate-co-β-hydroxyvalerate) bioplastic in tropical coastal waters. *Appl. Environ. Microbiol.* 65, 431–437.
- ISO 10390, 2005. Soil Quality – Determination of pH. International Organization for Standardization, Geneva, Switzerland.
- ISO 11261, 1995. Soil Quality – Determination of Total Nitrogen – Modified Kjeldahl Method. International Organization for Standardization, Geneva, Switzerland.
- ISO 18830, 2016. Plastics – Determination of Aerobic Biodegradation of Non-floating Plastic Materials in a Seawater/Sandy Sediment Interface – Method by Measuring the Oxygen Demand in Closed Respirometer. International Organization for Standardization, Geneva, Switzerland.
- ISO 19679, 2016. Plastics – Determination of Aerobic Biodegradation of Non-floating Plastic Materials in a Seawater/Sediment Interface – Method by Analysis of Evolved Carbon Dioxide. International Organization for Standardization, Geneva, Switzerland.
- ISO/FDIS 22404, 2019. (under development). Plastics – Determination of the Aerobic Biodegradation of Non-floating Materials Exposed to Marine Sediment – Method by Analysis of Evolved Carbon Dioxide. International Organization for Standardization, Geneva, Switzerland.
- ISO/DIS 22766, 2019. (under development). Plastics – Determination of the Degree of Disintegration of Plastic Materials in Marine Habitats Under Real Field Conditions. International Organization for Standardization, Geneva, Switzerland.
- Koulouvaris, E., Hanke, G., Papatthanassiou, E., 2015. Policy brief, policy-oriented marine environmental research in the southern European seas. Perseus FP7 project n° 287600, December 2015 <http://www.perseus-net.eu/assets/media/PDF/5570.pdf>.
- Lavalle, C., Gomes, R.C., Baranzelli, C., Batista e Silva, F., 2011. Coastal Zones–Policy Alternatives Impacts on European Coastal Zones 2000–2050. European Commission, Joint Research Centre, Institute for Environment and Sustainability, JRC (64456, March 2011).
- Law, K.L., Moret-Ferguson, S., Maximenko, N.A., Proskurowski, G., Peacock, E.E., Hafner, J., Reddy, C.M., 2011. Plastic accumulation in the North Atlantic subtropical gyre. *Science* 329, 1158–1188.
- Merlic, C.A., Strouse, J., 2000. WebSpectra-Problems in NMR and IR Spectroscopy. Cambridge Isotope Laboratories and the UCLA Department of Chemistry and Biochemistry, University of California <https://webspectra.chem.ucla.edu/index.html>.
- Merriam-Webster, 2018. Unabridged dictionary. <https://www.merriam-webster.com/dictionary/>.
- OECD, 2006. Revised Introduction to the OECD Guidelines for Testing of Chemicals, OECD Guidelines for the Testing of Chemicals, Section 3. OECD Publishing, Paris <https://doi.org/10.1787/9789264030213-en>.
- OpenBio, 2016. Opening bio-based markets via standards, labelling and procurement, FP7-KBBE-2013-613677 (2013–2016). <http://www.biobasedeconomy.eu/projects/open-bio/>.
- Pauli, N.C., Petermann, J.S., Lott, C., Weber, M., 2017. Macro-Fouling Communities and the Degradation of Plastic Bags in the Sea: An In Situ Experiment. Royal Society Open Publishing <http://rsos.royalsocietypublishing.org/>.
- Roohi, Zaheer, M.R., Kuddus, M., 2018. PHB (poly-β-hydroxybutyrate) and its enzymatic degradation. *Polym. Adv. Technol.* 29, 30–40. <https://doi.org/10.1002/pat.4126>.
- Salomon, M., Markus, T., 2018. *Handbook on Marine Environment Protection: Science, Impacts and Sustainable Management*. Springer, p. 1024 (ISBN 3319601563, 9783319601564).
- Schumacher, B.A., 2002. Methods for the Determination of Total Organic Carbon (TOC) in Soils and Sediments. United States Environmental Protection Agency, Environmental Sciences Division, National Exposure Research Laboratory (EPA/600/R-02/069, NCEA-C-1282 ERASC-001).
- Sekiguchi, T., Saika, A., Nomura, K., Watanabe, T., Fujimoto, Y., Enoki, M., Sato, T., Kato, C., Kanehiro, H., 2011. Biodegradation of aliphatic polyesters soaked in deep seawaters and isolation of poly(ε-caprolactone)-degrading bacteria. *Polym. Degrad. Stab.* 96, 1397–1403.
- Siotto, M., Zoia, L., Tosin, M., Degli Innocenti, F., Orlandi, M., Mezzanotte, V., 2013. Monitoring biodegradation of poly(butylene sebacate) by Gel Permeation Chromatography, 1H-NMR and 31P-NMR techniques. *J. Environ. Manag.* 116, 27–35.
- StatSoft, 2013. *Statistica*, Ver. 12. TIBCO Software, Inc. <https://www.tibco.com/products/data-science/statistica-now-part-tibco-data-science>.
- Steel, R.G.D., Torrie, J.H., 1980. *Principles and Procedures of Statistics*. McGraw-Hill, New York, N.Y.
- Tosin, M., Weber, M., Siotto, M., Lott, C., Degli Innocenti, F., 2012. Laboratory test methods to determine the degradation of plastics in marine environmental conditions. *Front. Microbiol.* 3, 225.
- Tosin, M., Pognani, M., Degli Innocenti, F., De Wilde, B., Mortier, N., Briassoulis, D., Mistriotis, A., Pikasi, A., Schuman, E., Van Eekert, M., Biedermann, E., Van der Zee, M., Jame, P., Bulete, A., 2016. Marine degradation test of bio-based materials at laboratory and mesocosm scale, OpenBio project deliverable D5.7, publishable summary. http://www.biobasedeconomy.eu/app/uploads/sites/2/2017/07/Open-BioD5.7_public-summary.pdf.

- Tsujii, H., Suzuyoshi, K., 2002a. Environmental degradation of biodegradable polyesters 1. Poly(ϵ -caprolactone), poly[(R)-3-hydroxybutyrate], and poly(L-lactide) films in controlled static seawater. *Polym. Degrad. Stab.* 75, 347–355.
- Tsujii, H., Suzuyoshi, K., 2002b. Environmental degradation of biodegradable polyesters 2. Poly(ϵ -caprolactone), poly[(R)-3-hydroxybutyrate], and poly(L-lactide) films in natural dynamic seawater. *Polym. Degrad. Stab.* 75, 357–365.
- UN Environment, 2017. Clean Seas, Turn the Tide on Plastic. United Nations Environment Programme <http://www.cleanseas.org/>.
- UNEA, 2016. Marine Plastic Litter and Microplastics, Second Session of UN Environment Assembly of the UN Environment Programme 23–27 May 2016. UNEP headquarters, Nairobi, Kenya.
- UNEP-NOAA, 2011. The Honolulu Strategy - A Global Framework for Prevention and Management of Marine Debris. p. 30.
- USDA, 2017. Soil Texture Calculator, Natural Resources Conservation Service–Soils. United States Department of Agriculture https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/?cid=nr142p2_054167.
- Van Cauwenberghe, L., Vanreusel, A., Mees, J., Janssen, C.R., 2013. Microplastic pollution in deep-sea sediment. *Environ. Pollut.* 182, 495–499.
- Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* 37, 29–38.
- Weber, M., et al., 2018. Assessing marine biodegradability of plastic-towards an environmentally relevant international standard test scheme. *Proceedings of the International Conference on Microplastic Pollution in the Mediterranean Sea*. Springer, Cham, pp. 189–193.