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Innovative Biotic Symbiosis for Plastic Biodegradation to Solve their End-of-Life Challenges in the Agriculture and Food Industries

PATRIZIA CINELLI[1], NICCOLETTA BARBANI[1], SARA FILIPPI[1], GIOVANNA STRANGIS[1], MARCO SANDRONI[1], ANTONIO PRATELLI[1], MARIA J LOPEZ[2], PABLO BARRANCO[2], TOMAS CABELLO[2], PATRICIA CASTILLO[2], MARIE ALINE PIERRARD[3], MAURIZIA SEGGIANI[1]

[1]Department of Civil and Industrial Engineering, University of Pisa,
Largo Lucio Lazzarino 2, 56122,
ITALY
[2]Unit of Microbiology, Department of Biology and Geology, CIAIMBITAL, ceiA3,
University of Almeria, Almeria, 04120,
SPAIN
[3]IDELUX Environment, Drève de l'arc-en-ciel 98, 6700 Arlon,
BELGIUM

Abstract: - At present just about 30% of the waste plastic collected is efficiently recycled, while the rest is incinerated, disposed in landfills, or can end up in compost and be released in the environment, inducing a very negative effect on safety and health of flora and fauna. Sustainable management of hardly recyclable plastic waste generated by light weight single use packaging and agricultural films can be improved by applying biotechnological approaches, combining microorganisms, new enzymes, earthworms, and insects to work collaboratively, not only to promote the degradation of these plastics but also to obtain, by-products of the biodegradation process to be valorized as fertilizers, functional polysaccharides, etc.
In order to develop a feasible process, mapping and characterization of the most diffused agri-food waste plastic were conducted isolating the main types of plastic involved. Plastic waste in agriculture is mainly constituted by polyethylene (PE) both linear low density (LLDPE) and high density (HDPE), polypropylene (PP) and polystyrene (PS), whereas in food packaging polyethylene is still present together with a large presence of polypropylene, polystyrene and polyethylene terephthalate (PET). Combining plastic presence and availability of organisms for their degradability, representative samples of plastics (PE, PET, PS) were selected for analysis of deterioration and potential subsequent biodegradation by enzymes and organisms. To monitor the plastic degradability by enzymes, and larvae, methods for the plastic analysis were set, outlining some differences in virgin and post consumer plastic in particular after use in agriculture, assessing the possibility to monitor the degradability of plastic with time and different treatments, in particular, some evidence of polyethylene degradability from larvae of *Tenebrio molitor* was observed.

Key-Words: - Agri-food waste plastic, enzymes, biodegradation, larvae, biomass, end of life.

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1 Introduction

Plastics are important in our society, providing a range of benefits for human health and for the environment. For example, plastic packaging protects food and goods from getting wasted and/or contaminated, thereby saving resources. The light weight of plastic packaging compared to other materials, such as glass, saves fuel and decreases emissions during transportation, similarly low-density plastic materials, used as replacements for metals or ceramics in cars and aircraft, save fuel and decrease emissions. However, such diverse properties lead to a diverse waste stream, [1].

In 2018 over 360 million tons of plastic were produced worldwide, of which up to 40% and 3.5% are consumed by the packaging and agricultural industries, respectively, [2].

During their use for application, such as those in food packaging and in agriculture, plastic materials are contaminated by different impurities such as food, soil and agrochemical particles difficult to be removed, washed away, from the post consume plastic. These plastics end-of-life is generally

represented by incineration or landfilling when they are properly collected. Highly degraded or contaminated plastics that cannot be mechanically recycled can be successfully used as an alternative fuel in power plants or in cement factories. Unfortunately, abandonment and burning are practices still frequently used, although they are against the law in several European Countries, [3]. To encourage plastic reuse, the EU has adopted a recycling target of 55% by 2030 for household plastic packaging waste, complemented by voluntary commitments from the European plastics industry to recycle 70% (plastic packaging) and 50% (all plastic waste) by 2040. However, there is a lack of knowledge about possible tangible solutions to achieve these ambitions. The challenge is to increase recycling rates and change the unfavorable structure of plastic waste reuse. Currently, the energy recovery rate (41.6%) is even higher than the recycling rate (31.1%), and the recycling rate only slightly exceeds that of the landfill rate (27.3%), [4]. Poor segregation of waste at source, multilayers in plastic items and inefficient collection of recyclable materials are among the barriers to achieving a higher recycling rate.

Most plastics are non-biodegradable and when subjected to degradative environments slowly break down into smaller fragments, first as microplastics, then further as nano-plastics, [5].

Biotechnological approaches, combining microorganisms, enzymes, earthworms, and insects working collaboratively may promote sustainable management of hardly recyclable plastic waste generated by light weight single use packaging and agricultural films.

In the present paper, we report a mapping and characterization of the most diffused agri-food waste plastic, which brought to the selection of polyethylene (PE), polyethylene terephthalate (PET), and polystyrene (PS), as plastic substrate to be investigated for biodegradability, and a brief overview of recent biotic symbiosis approaches for plastic assimilation as well as the setting of analytical tools to monitor the possible degradation, in selected plastic samples by environment and by the action of larvae. The use of enzymes and microorganisms for plastic degradation and valorisation, [6], [7], is widely investigated as well the action of some worms and insects on plastic degradation has been proved and is under further investigation to be promoted on a real scale system. Thus, the degradation of usually recalcitrant plastic in the environment, and the possible approach to improve it, is a topic of growing interest, [8].

Indeed, slow polyethylene (PE) degradation was recorded after 4 to 7 months of exposure to the bacterium *Nocardia asteroides*, [9], and by bacterial strains [10], [11], from the guts of plastic-eating waxworms.

Bacteria are also reported to degrade PET, [12]. Thus, by screening natural microbial communities exposed to PET in the environment, a novel bacterium, *Ideonella sakaiensis* 201-F6, was isolated that is able to use PET as its major energy and carbon source. When grown on PET, this strain produces two enzymes capable of hydrolysing PET and the reaction intermediate, mono(2-hydroxyethyl) terephthalic acid. Both enzymes are required to enzymatically convert PET efficiently into its two environmentally benign monomers, terephthalic acid and ethylene glycol.

As well several papers report evidence of the degradation of PS, for example, mealworms (the larvae of *Tenebrio molitor*) from different sources chew and eat Styrofoam, a common PS product, [13], [14].

2 Biotic Symbiosis Approach

In order to conduct a systematic and specific study to promote the degradability of most representative plastic samples, generated by the food packaging and agricultural sectors, in the present study an inventory collection of plastic waste derived from food packaging and agriculture sectors was conducted. On the selected plastic types some representative samples of pre and post consumer plastic were analysed for morphology and structure, in order to estimate degradation undergone during use and to set a study on the possible degradability of these plastics by the action of microorganisms and insects.

Thus, for example, recently researchers in Spain and England claimed that the larvae of the greater wax moth can efficiently degrade polyethylene, which accounts for 40% of plastics waste, [11]. In this work macro-organisms such as yellow flour worms (*Tenebrio molitor*) (Figure 1) were tested to confirm their ability to chew and digest the LDPE, [13].

In our running experiments selected representative samples of plastic wastes will be pre-treated (chemical, enzymes, etc) to improve degradability and will undergo the action of microorganisms and insects to promote their biodegradation.

Fig. 1: *Tenebrio molitor*.

2.1 Selection of Plastic Samples

Analysis of mixed municipal plastic waste was done on samples collected by IDELUX Environnement, a municipal waste treatment plant located in the south of Belgium.

The analysis was conducted by selecting and characterizing the mixed plastic waste (Figure 2).

Fig. 2: Municipal solid waste selection at Idelux facilities.

Post consume samples were washed carefully with water and detergent, then rinsed with water and mechanical action. Selected samples were dried 48 hours at 80 °C, and analysed for weight distribution. The combined analysis of chemical structure (FTIR) and thermal properties by thermal gravimetric analysis (TGA) and Differential scanning calorimetry (DSC) allowed identifying the polymeric samples and drawing a distribution of the most present plastic types.

Linear low-density polyethylene (LLDPE), resulted to be one of the main polymers present in food packaging, and was selected for testing the effect of different abiotic treatments on the polymer structure and using it as a diet for insect breeding.

Thermal/thermomechanical, photo-oxidation and chemical/thermochemical treatments were applied to representative LLDPE samples.

2.2 Mealworms and Test Materials

Tenebrio molitor larvae (length: approximately 2 cm) were utilised to test LDPE ingestion and biodegradation. Larvae were fed with LDPE in four batches of 100 mass larvae with 100% plastic and 50 individualized larvae with doses of 90% and 100% of LDPE.

2.3 Experimental Methods

Differential Scanning Calorimetry (DSC) measurements were performed with a Pyris (Perkin Elmer Instrument, Waltham, MA, USA) equipped with a Perkin Elmer IntraCooler 1 as a refrigerating system. Dry nitrogen was used as purge gas at a rate of 25 mL/min. Samples of about 10 mg were analysed from room temperature to a temperature subsequent to the melting point at a heating rate of 10 °C/min in two runs alternated by cooling at the same speed, with an aluminium empty pan as reference.

Thermogravimetric analysis (TGA) was performed using a TA Q-500 (TA Instruments, Waters LLC, New Castle, DE, USA). About 15 mg of sample were placed into a platinum pan and heated from room temperature to 700 °C at 10 °C/min under nitrogen atmosphere.

FTIR technique has been used to obtain information on the functional groups that characterise the polymeric structure of the material allowing its identification. Infrared spectroscopic analysis was performed using an FTIR ATR Spectrum 400 Perkin-Elmer (germanium crystal) spectral sensitivity 4 cm^{-1}, in the frequency range of 4000-650 cm^{-1}.

Morphological and FTIR analysis of sample surfaces was also performed using the optical microscope combined with a FTIR Spotlight Chemical Imaging Perkin Elmer instrument (300X magnification).

3 Results and Discussion

Based on the results of the analysis of the mixed municipal plastic waste, and of the agri-waste provided by European farmers to Idelux was conducted a pre-selection of samples of agri-food packaging waste types, including in each case virgin plastics and post consume counterparts picked individually from Municipal solid waste (MSW) or agricultural films.

In food packaging waste, the main plastic types present in the non-recyclable fraction were multi-layers (20%), different polymers, and multi-materials including layers with aluminium (30%). Among raw polymers, polyethylene, both low density (LDPE) and linear low-density polyethylene (LLDPE), (17%) were the most present, followed by polypropylene (PP) (15%), polystyrene (PS) (8%), polyethylene terephthalate (PET) (5%), and high-density polyethylene (HDPE) (5%).

Concerning agricultural plastics, the most common polymers retrieved were PE, PP and PS in particular expanded PS.

PE and more specifically LDPE and LLDPE, PET, and PS were considered most representative and suitable for this study for both presence in MSW and the availability of enzymes, microorganisms and insects promising for their biodegradation.

The analysis of plastic multi layers outlined that most of them were based on PE and PET confirming the large presence of these polymers in plastic packaging waste. It is very common to associate the good moisture barrier properties of PE with oxygen barrier properties of PET, for food packaging film production. Figure 3 reported the DSC analysis of a multi-layer film that was composed of PE and PET. While figure 4 reports the FTIR of the upper and lower side of the film, proving it is made with PE and PET layers.

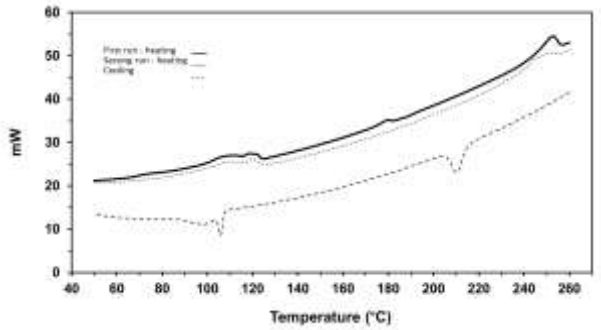

Fig. 3: Differential scanning calorimetry analysis of a PE/PET multi-layer in food packaging waste.

In the DSC graph, we can see the melting peak of PET at about 250 °C, and the melting peak of PE at about 110-120 °C, while in the cooling scan we can see a peak for crystallization of PET at 210°C, and one for PE at 105 °C, [15], [16].

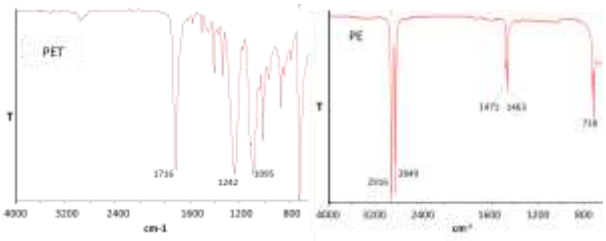

Fig. 4: FTIR of PET/PE bi-layer films, respectively side a) PET and side b) PE.

The FTIR spectra of Figure 4, confirm the presence of typical functional chemical groups of respectively PET, and PE outlining the composition of the film as a bi-layer of a PE film and a PET film.

Considering the large amount of PE and PET present in both food packaging and agriculture plastic waste, these two polymers were selected as main ones to be studied for the degradation with enzymes and larvae. Considering that the films which will undergo degradation will be post-consumed plastic, we conducted an analysis to compare the post-consumed films with the raw ones, in order to estimate the degradation induced by the stress during use. Analysis of LDPE films coming from post-consumption in agriculture outlined some differences when compared to same LDPE films pre-use "virgin" material.

In a comparison between the spectra of samples LDPE films used in horticultural mulching in Spain, and same film as virgin material, main variations are due to the presence, in the used sample, of a widened band with a maximum of 1028 cm^{-1} (Figure 5) attributable to soil contamination as highlighted optical image (Figure 6).

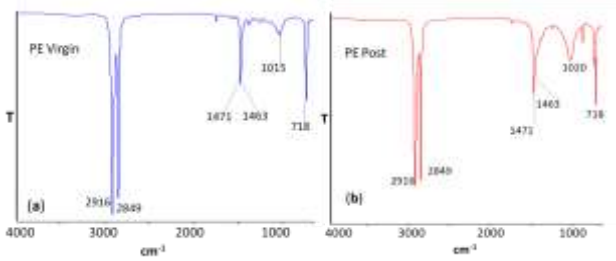

Fig. 5: FTIR of LDPE mulching film a) virgin and b) post consume.

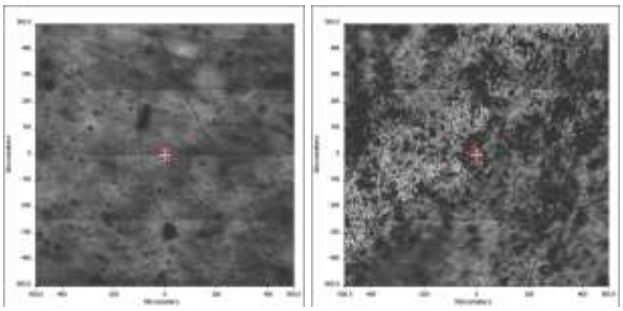

Fig. 6: Surface morphology of respectively LDPE virgin and LDPE used.

The ratio between the intensity of the band relative to the amorphous phase at 717 cm^{-1} and that relative to the crystalline phase at 730 cm^{-1} was measured, using the instrument software and was noted an increase in the superficial amorphous phase in the used sample compared to the virgin sample.

Similarly, for LDPE films we can observe some differences in thermal stability (Figure 7) between the virgin and the used samples.

In the samples of LDPE post use, there is an additional step of degradation at 620 °C that might

be attributed to the soil debris adhered to the film or to some oxidised form of PE.

Even for samples based on PS, used as germination trays, a difference in morphology between the virgin and used samples is reported in Figure 8. In the used samples, an increase in the superficial amorphous phase was compared to the virgin sample, and a rougher surface was also reported. The FTIR of the used PS samples was significantly different compared to the virgin PS (Figure 9).

These most significant differences in the used samples from plastic batches, in the comparison of virgin and used plastic in food packaging, are attributed to the use in agriculture and thus a more serious exposure to temperature and sunlight than what happens to polymers used in food packaging.

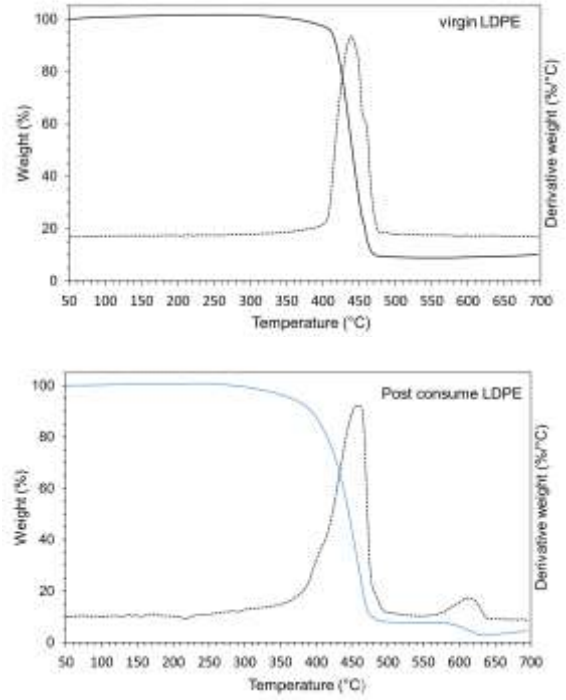

Fig. 7: Thermal gravimetric analysis on mulch film sample virgin LDPE and used LDPE.

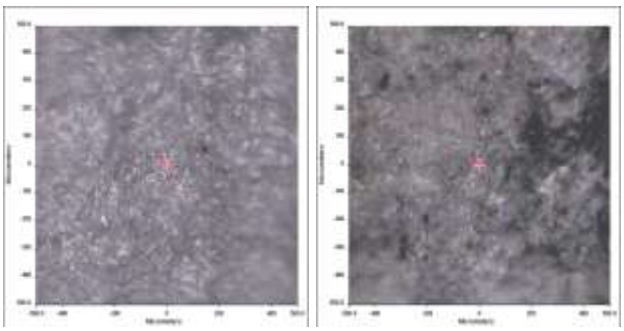

Fig. 8: Morphology of respectively sample virgin PS and used PS.

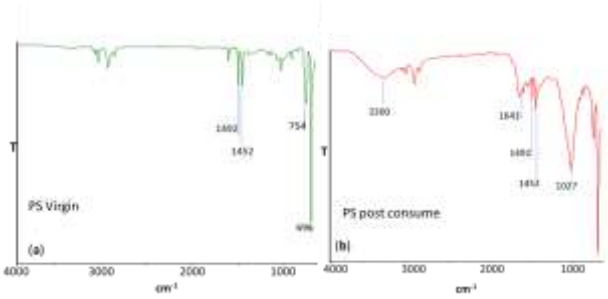

Fig. 9: Morphology of respectively sample virgin PS and post consume PS.

Selected samples of the virgin and post consumer plastic were used for feeding larvae of different, macro-organism. Preliminary tests have already allowed us to observe promising results in the case of larvae of *Tenebrio molitor*, tested for LDPE ingestion and biodegradation (Figure 10).

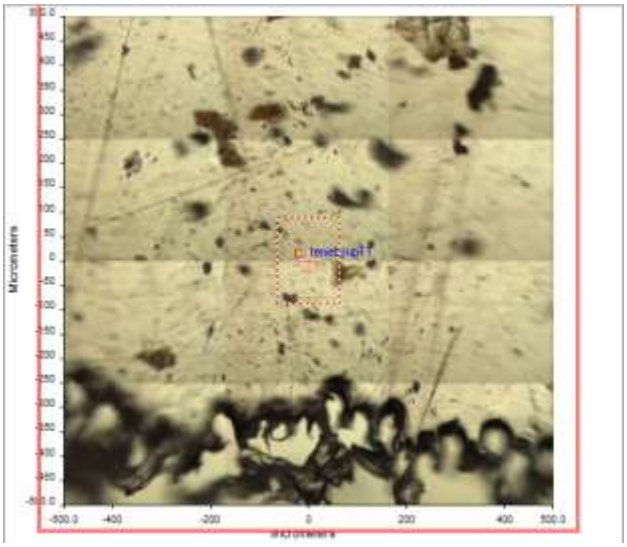

Fig. 10: LDPE film in contact with *Tenebrio molitor* larvae.

FTIR analysis (Figure 11) evidenced incorporated oxygen functional groups as can be observed at 3500-3100 cm^{-1}(-OH) and 1800–1500 cm^{-1}(C=O).

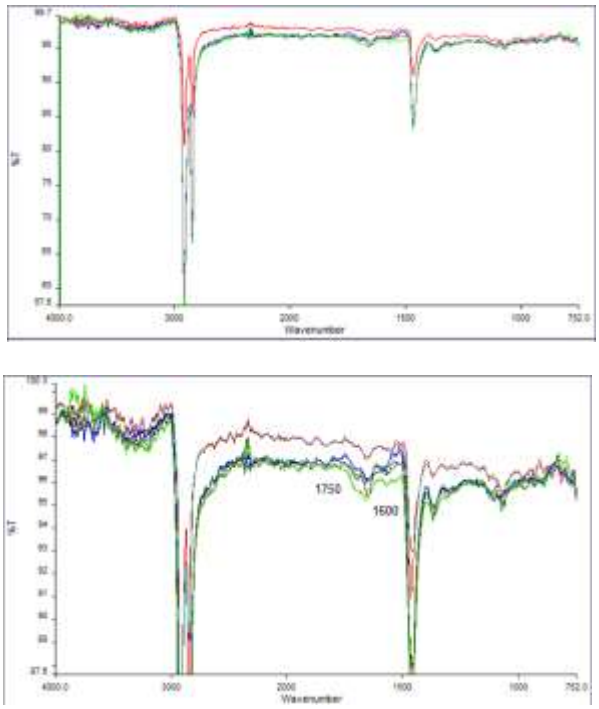

Fig. 11: LDPE film surface after being fed to *Tenebrio molitor* larvae.

The presence of spherical particles with a diameter range of 150 to 300 μm in the larvae feces suggests the presence of polymer residues (Figure 12).
The FTIR spectra acquired in the selected area in Fig.12, revealed the presence of bands indicating the presence of LDPE residues presenting sign of depolymerisation (Figure 13).

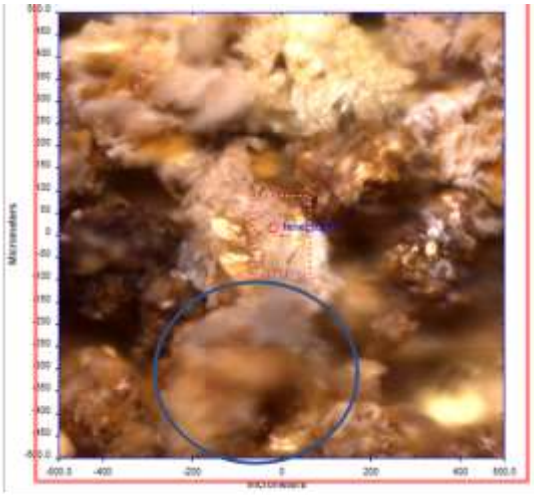

Fig. 12: *Tenebrio molitor* feces with spherical particles.

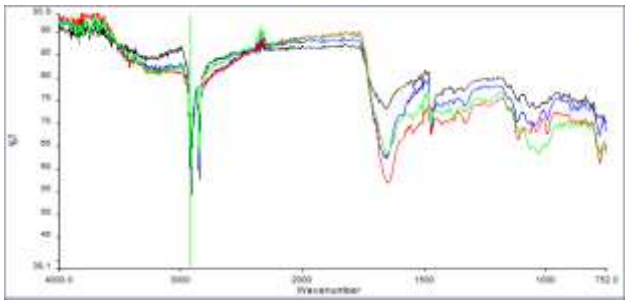

Fig. 13: FTIR of the selected area in *Tenebrio molitor* feces.

The appearance of peaks at 1654 cm $^{-1}$ (C=C stretch) suggests de-polymerization of LDPE.

4 Conclusion

At present, a low amount of plastic used in the agricultural and food packaging sector is recycled and most of it ends up in landfill or goes to thermal valorisation. An analysis was performed in these plastic streams evidencing that the main polymers present were PE, PP, PET and PS. A selection of polymers was conducted in order to apply biotic approaches to improve recalcitrant plastic waste biodegradation, considering both plastic presence in the waste stream and availability of enzymes and organisms to attempt their degradation. Considering all these factors we decided to focus further research on LDPE, LLDPE, PET and PS. Representative samples of these polymers, both virgin and used (recycled stream) were acquired and investigated to observe the degradative effect due to use. The post-consume samples presented some evidence of degradation versus the virgin material due to the stress of real application, evident in particular in PET and PS. The polymers were subjected to attack with enzymes and larvae. In preliminary tests with *Tenebrio molitor* larvae the same evidence of assimilation was observed for LDPE films. On the basis of these positive results, more tests are currently running on the degradation of selected plastic samples with larvae and adults of insects and worms.

References:

[1] Hahladakis, J.N., Velis, C.A., Weber, R., Iacovidou, E., Purnell P. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. *J. Hazard. Materials*, Vol. 344, 2018, pp.179-199.

[2] Plasticseurope, *Plastic the Fact*, plasticseurope.org/wp-content/uploads/2021/10/2019-Plastics-the-facts.pdf, access 11.10.2022

[3] Scarascia Mugnozza, G., Sica, C., Russo, G., Plastic materials in European agriculture: actual use and perspectives. *J. Agric. Engin*,.Vol. 42, No3, 2011, pp. 15-28.

[4] Baran, B. Plastic waste as a challenge for sustainable development and circularity in the European Union. *Ekonomia i Prawo. Economics and Law*, Vol. 19, No 1, 2020, pp.7-20.

[5] Laskar N., Kumar U., Plastics and microplastics: A threat to environment, *Environmental Technology & Innovation* Vol.14, No9, 2019, https://doi.org/10.1016/j.eti.2019.100352

[6] Padovani G., Carlozzi P., Seggiani M, Cinelli P., Vitolo S., Lazzeri A, PHB-Rich Biomass and BioH2 Production by Means of Photosynthetic Microorganisms, *Chem. Eng.Trans*. Vol.49, 2016, pp. 55-60, DOI:10.3303/CET1649010

[7] Seggiani M., Cinelli P., Geicu M., Popa Mona E., Puccini M., Lazzeri A., Microbiological Valorisation of Bio-composites Based on Polylactic Acid and Wood Fibre, *Chem. Eng.Trans*. Vol.49, 2016, pp. 127-132.

[8] Hamitouche F., Lardjane N., Grohens Y., Habchi Laribi H., Belhaneche Bensemra N., "Natural Weathering of Plasticized Polyvinyl Chloride (PVC) Stabilized with Epoxidized Sunflower Oil," WSEAS *Transactions on Environment and Developmen*t, Vol.18, 2022, pp. 417-426.

[9] Bonhomme S., Cuer A., Delort A.M., Lemaire J., Sancelme M., Scott C., Environmental biodegradation of polyethylene., *Polym. Degrad. Stab*. Vol. 81, 2003, pp. 441-452.

[10] Yang J., Yang Y., Wu W.M., Zhao J., Jiang L., Evidence of polyethylene biodegradation by bacterial Strains from the guts of plastic-eating waxworms", *Environ. Sci. Tech*.; Vol. 48: 2014, pp.13776-13784.

[11] Bombelli P., Howe C.J., Bertocchini F., 2017, Polyethylene bio-degradation by caterpillars of the wax moth Galleria mellonella, *Current Biology*, Vol. 27, No 8, pp. R292-R293.

[12] Yoshida S., Hiraga K., Takehana T., Taniguchi I., Yamaji H., Maeda Y., Toyohara K., Miyamoto K., Kimura Y., Oda K., A bacterium that degrades and assimilates poly(ethylene terephthalate), *Science*, Vol. 351, 2016, pp. 1196-1199.

[13] Billen P., Khalifa L., Fenno Van Gerven, Tavernier S., Spatari S., Technological application potential of polyethylene and polystyrene biodegradation by macro-organisms such as mealworms and wax moth larvae, *Science of The Total Environment*, Vol. 735, No. 139521, 2020, , 10pp.

[14] Yang Y., Yang J., Wu W.M., Zhao J., Song Y., Gao L., Yang R., Jiang L., Biodegradation and Mineralization of Polystyrene by Plastic-Eating Mealworms: Part 1. Chemical and Physical Characterization and Isotopic Tests, *Environ. Sci. Technol.,* Vol.49, No.20, 2015, pp. 12080-12086.

[15] Li D., Zhou L., Wang X., Young X., Effect of Crystallinity of Polyethylene with Different Densities on Breakdown Strength and Conductance Property, *Materials*, Vol. 12, 2019, pp 1746-1759; doi:10.3390/ma12111746.

[16] Woo E. M., Ko T.Y., A differential scanning calorimetry study on poly(ethylene terephthalate) isothermally crystallized at stepwise temperatures: multiple melting behavior re-investigated, *Colloid and Polymer Science*, Vol. 274, 1996, pp 309–315.

Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

Patrizia Cinelli, Maurizia Seggiani conceptualised plastic analysis and data organisation; Antonio Pratelli coordinated logistic for plastic management, Niccoletta Barbani, Sara Filippi, Giovanna Strangis, Marco Sandroni, performed chemical, thermal, morphological analysis and samples preparation.
Marie Aline Pierrard coordinated plastic collection and analysis of waste plastic.
Maria Jose Lopez, Pablo Barranco, Tomas Cabello, Patricia Castillo performed plastic pre-treatment and test on larvae feeding with plastic.

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