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Effect of agricultural microplastic and mesoplastic in the vermicomposting process: Response of *Eisenia fetida* and quality of the vermicomposts obtained☆

Z.E. Blesa Marco [a], J.A. Sáez [a], A.M. Pedraza Torres [b], E. Martínez Sabater [a], L. Orden [a,c], F.J. Andreu-Rodríguez [a], M.A. Bustamante [a,*], F.C. Marhuenda-Egea [d], M.J. López [e], F. Suárez-Estrella [e], R. Moral [a]

[a] *Centro de Investigación e Innovación Agroalimentaria y Agroambiental (CIAGRO-UMH), Universidad Miguel Hernández, Ctra. de Beniel Km 3,2, Orihuela, Alicante, 03312, Spain*

[b] *Laboratorio Ecotoxicologia, Instituto de Ciencias Ambientales (ICAM); Universidad de Castilla La Mancha, Avda. Carlos III, 45071, Toledo, Spain*

[c] *Estación Experimental Agropecuaria INTA Ascasubi (EEA INTA Ascasubi), Ruta 3 Km 794, 8142, Hilario Ascasubi, Buenos Aires, Argentina*

[d] *Department of Agrochemistry and Biochemistry, Multidisciplinary for Environmental Studies Ramón Margalef, San Vicent Del Raspeig, 03690, Alicante, Spain*

[e] *Unit of Microbiology, Department of Biology and Geology, CITE II-B, Agrifood Campus of International Excel-lence CeiA3, CIAIMBITAL, University of Almeria, 04120 Almeria, Spain*

A R T I C L E I N F O

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A B S T R A C T

This work evaluates the effect of agricultural plastic waste (APW) in two particle sizes, microplastic and film debris, and subjected to a pre-treatment by exposure to UV-C, in the development of the vermicomposting process. *Eisenia fetida* health status and metabolic response and the vermicompost quality and enzymatic activity were determined. The environmental significant of this study is mainly related to how can affect plastic presence (depending on plastic type, size and/or if it is partially degraded) not only to this biological process of organic waste degradation, but also to the vermicompost characteristics, since these organic materials will be reintroduced in the environment as organic amendments and/or fertilizers in agriculture. The plastic presence induced a significant negative effect in survival and body weight of *E. fetida* with an average decrease of 10% and 15%, respectively, and differences on the characteristics of the vermicomposts obtained, mainly related with NPK content. Although the plastic proportion tested (1.25% f. w.) did not induce acute toxicity in worms, effects of oxidative stress were found. Thus, the exposure of *E. fetida* to AWP with smaller size or pre-treated with UV seemed to induce a biochemical response, but the mechanism of oxidative stress response did not seem to be dependent on the size or shape of plastic fragments or pre-treated plastic.

Environmental implication

The main objective of this study was to evaluate the effect of agricultural plastic waste (microplastic and film debris) during the vermicomposting process. In addition, the plastic material was pre-treated by exposure to UV-C to simulate the natural weathering and partial degradation of plastic polymers in the environment. The environmental implication of this work is mainly related to how can affect the plastic presence, depending on the plastic type, the size and/or if it is partially degraded, to the biological process of degradation of organic wastes as vermicomposting and to the quality of the vermicomposts obtained, since these organic materials will be reintroduced in the environment as organic amendments and/or fertilizers in agriculture. Several previous works have reported the effect of microplastic during vermicomposting, but there is little information concerning how can affect to the process specific characteristics of the material on the whole, such as type of plastic, size and previous degradation on the vermicompost characteristics and on *Eisenia fetida* response and health status using indicators of the oxidative stress apart from survival and body weight.

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* Corresponding author.
E-mail address: marian.bustamante@umh.es (M.A. Bustamante).

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1. Introduction

Currently, the use of plastics is extended in European agriculture, due to their contribution to the increase in the quality and the quantity of crop production, but their end-of-life management constitutes an environmental problem due to the usual incomplete recovery after the crop season. To produce flexible, semi-rigid and/or rigid materials, the main polymers used in agriculture are the polyethylene (PE) and the polyethylene terephthalate (PET), due to their high impact resistance, low cost, good workability, and optimal chemical resistance properties. Low-density polyethylene (LDPE) and linear low-density polyethylene (LLPDE) are principally used to obtain films (for greenhouses, low tunnels, mulching, and silage), due to their elasticity and high tear and impact strength (Dorigato et al., 2011). The most abundant and problematic types used in agri-food industries that comprise >60% of the agro-industrial sector, are still largely considered to be non-biodegradable (Inderthal et al., 2021). In 2019, the global production of plastic material was around 370 million tons, of which only 9% was recycled, 12% was incinerated and remaining left in the environmental or landfill (Kumar et al., 2021). Thus, one of the production sectors with higher mulching plastic consume is the agricultural sector. In this sense, Zhang et al. (2020) estimated that largest use of plastic mulch in agricultural system led to an accumulation of 550,800 tons of plastic residues in soil per year.

Plastic degradation of plastics comprises any change of the physical or chemical properties. It can occur because of biological, chemical or physicochemical processes and when this alteration is irreversible over time is known as ageing. In the environment, these changes can lead to microplastic formation by fragmentation of larger plastic (mesoplastic) by exposure to weather conditions (Jiang, 2018; Sobhani et al., 2022). Thus, due to their progressive fragmentation into smaller size particles, the plastic wastes from the agro-industrial sector have demonstrated to be a source of plastic debris in multiple environmental compartments, to be atmospherically long-transported, or even incorporated in the trophic chain (Allen et al., 2019). This increasing in dispersion of plastics in multiple ecosystems has led to their accumulation in bio-wastes of several streams, such as municipal solid waste (Li et al., 2022), sewage sludge (Gao et al., 2020), animal manure (Wu et al., 2021) or agricultural organic wastes and their derived compost (Weithmann et al., 2018). Currently, there is a lack of studies about the quantity and different type of plastic presence in organic wastes on a global scale. Thus, several local studies have demonstrated the accumulation of plastic debris of multiple size and shape in biowaste (Gui et al., 2021; Pathan et al., 2020).

Verrmicomposting, as a single treatment or combined with composting as pre-treatment, is an alternative widely used for the valorisation of a wide variety of organic wastes, which allows to obtain a stabilized organic with a fertilizer value, which can be used in agriculture as organic amendment. Several studies suggest that earthworms interact with microplastics in soil (Huerta Lwanga et al., 2016, 2018; Rillig et al., 2017; Hodson et al., 2017). However, these studies have been performed using soil-dwelling earthworms in terrestrial ecosystems (Rillig et al., 2017; Rodríguez-Seijo. et al., 2018; Li et al., 2018), and comparable interaction should be investigated in vermicomposting, with epigeic earthworm species such as *Eisenia fetida*. Moreover, the studies carried out to evaluate the effect of the presence of plastic material using these annelids have been focused on microplastic (MP) and nanoplastic (NP) size particles (Rodríguez-Seijo et al., 2018; Jiang et al., 2020; Lackmann et al., 2022). Although some studies have shown that during the fragmentation process various types of associated plastic chemical can be detected (Sobhani et al., 2022), often no attempt is made to evaluate the potential toxicity of large plastic such as film debris commonly defined as mesoplastic (fragments of 1 to <10 mm size). In addition, Pathan et al. (2020) found that non-compostable plastic material can be as a microhabitat, which is quickly colonized by microorganisms, forming the plastisphere, a dense biofilm on the surface of the plastic. Furthermore, Jing et al. (2014) reported a mechanism of passive protection of biofilm against plastic particles in soils, which are dependent on the biofilm matrix characteristics and capable of entrapping and binding chemical by-products into the biofilm surface layer.

Thus, this work aims to evaluate the influence of different agricultural plastic wastes, at different particle sizes (microplastics and mesoplastics) and subjected to a pre-treatment by exposure to UV-C (250h) to simulate environmental ageing, during the vermicomposting process to evaluate the potential adverse effects on the quality and enzymatic activity of the vermicompost obtained and on *Eisenia fetida* response and health status.

2. Material and methods

2.1. Feedstock and AWP characteristics and biofilm samples

The feedstock used in the experiment was an organic material from agro-industrial wastes pre-treated by a partial composting to degrade toxic compounds for earthworms, such as ammonium and considered in a previous experiment. This stabilized organic material was obtained after an aerobic turning process that lasted 96 days, from a mixture of four ingredients (khaki pruning waste, agri-food sludge from citric industries, goat manure and vineyard pruning) in a proportion of volume of 45:35:15:5, respectively. A more detailed description of the process can be found elsewhere (Sáez et al., 2022). This material was selected for the experiment due to the absence of plastic materials in the raw materials used and during the stabilization process.

Five different APW materials frequently used in different agricultural practices were considered: low-density polyethylene (LDPE), linear low-density polyethylene (LLDPE), LLDPE + LDPE, polyethylene terephthalate (PET) and polystyrene (PS). LLDPE and LLDPE + LDPE were provided by two Spanish private companies (Repsol S.A. and Solplast S.A.), while the rest of plastics were provided by a public entity (University of Pisa, UNIPI). Additional information regarding the characteristics of the APW selected are shown in Table S1. The variables considered, apart from the type of plastic type, were: a) particle size of the APW material (two different particle sizes were assessed and selected as representative of the size of mulching film pieces found in the environment after natural ageing, such as microplastic-MP (1–1000 μm) and film debris-mesoplastic (fragments 1 to <10 mm) with pieces of approx. 1 cm^2); b) a pre-treatment based on the exposure of the plastics to ultraviolet light type C for 250h in order to simulate the natural exposure to the weathering conditions (natural ageing). For the UV pre-treatment, a close chamber (0.25 m^2) with reflective walls and 2 UV-C lamps at 253 nm (Philips TUV T8 F17 1SL/25) were used, where the APW material was exposed to irradiation for 250 h at 137 W/m^2. Also, continuous ventilation was installed in order to maintain the temperature in a range from 15 to 25 °C.

The mesoplastic pieces were obtained using scissors to cut the AWP material into small pieces with irregular shape, whereas for the microplastic (MP) size, the plastic in pellet format were mechanically grinded in a rotor paddle Mill (RETSCH Mill SK100 Comfort) and then sieved. In order to discriminate the affectation of biofilm formation in the microbial community, further enzymatic activities have been also measured. For the obtaining of the biofilm samples, the small APW pieces were carefully separated from the substrate and scraped with a spatula. Later, the sample was homogenized and 0.1 g were suspended with 15 ml of distilled water ratio 1: 15 (w/v), being kept in refrigerator at 4 °C until determination of the enzymatic activities.

2.2. Experimental design

In order to reproduce the conditions of the vermicomposting process of agro-industrial organic wastes with plastic presence at laboratory scale, five different APW materials, pre-treated or not with UV light and at different sizes (mesoplastic and microplastic) were used. The

Table 1
Effect of the type of AWP material and *Eisenia fetida* presence in the physico-chemical and chemical parameters of the organic material obtained at the end of the bioassay.

		pH	EC (dS m^{-1})	OM (%)	TOC (%)	TN (%)	P (%)	K (%)	WSC (g kg^{-1})	C_{FA} (%)	C_{HA} (%)
Main effects											
***E. fetida* presence**	Yes	7.47 a	4.66 b	53.8 a	26.5 a	2.36 a	0.74 a	1.16 a	8.19 a	2.52 a	3.23 a
	No	7.86 b	3.88 a	54.6 b	26.9 a	2.34 a	0.75 a	1.17 a	9.57 b	2.54 a	3.49 b
Type of AWP	LDPE	7.68 b	4.44 b	53.0 ab	28.4 b	2.35 a	0.72 b	1.19 b	7.66 ab	2.34 bc	3.06 b
	LLDPE	7.67 b	4.22 ab	53.8 ab	27.4 ab	2.33 a	0.76 bc	1.22 b	9.29 bc	2.75 b	3.96 b
	PET	7.49 a	4.23 ab	54.3 b	25.0 ab	2.40 a	0.61 a	0.78 a	7.61 ab	1.62 a	1.56 a
	PS	7.48 a	4.23 ab	54.0 ab	25.0 ab	2.35 a	0.63 a	0.79 a	7.18 a	1.58 a	1.88 a
	LDPE + LLDPE	7.73 bc	3.84 a	53.9 ab	24.6 a	2.31 a	0.81 c	1.21 b	9.09 bc	2.91 c	3.75 b
***E. fetida* presence**	**Type of APW**										
Yes	Control without AWP	7.48 ab	5.07 d	52.0 a	25.3 a	2.41 ab	0.76 a	1.24 a	8.22 a	2.93 a	3.75 a
	LDPE	7.50 ab	4.84 cd	52.1 a	27.8 a	2.44 ab	0.72 a	1.19 a	7.22 a	2.27 a	3.04 a
	LLDPE	7.52 b	4.42 bc	53.8 ab	27.3 a	2.30 a	0.74 a	1.22 a	8.83 a	2.64 a	3.70 a
	PET	7.27 a	4.66 c	53.2 ab	24.7 a	2.45 ab	0.64 a	0.81 a	7.36 a	1.64 a	1.28 a
	PS	7.42 ab	4.37 bc	52.9 ab	24.8 a	2.39 ab	0.60 a	0.73 a	6.82 a	1.71 a	1.64 a
	LDPE + LLDPE	7.37 ab	4.45 bc	55.0 b	25.2 a	2.29 a	0.72 a	1.24 a	8.35 a	2.65 a	3.47 a
No	Control without AWP	7.88 bc	4.17 bc	55.3 b	27.3 a	2.53 b	0.72 a	1.40 a	8.85 a	2.57 a	3.16 a
	LDPE	7.85 c	4.04 ab	54.0 ab	29.1 a	2.27 a	0.72 a	1.19 a	8.11 a	2.42 a	3.08 a
	LLDPE	7.82 bc	4.01 ab	53.9 ab	27.5 a	2.36 ab	0.78 a	1.28 a	9.73 a	2.86 a	4.21 a
	PET	7.71 bc	3.90 ab	55.4 b	25.3 a	2.31 a	0.59 a	0.75 a	7.86 a	1.61 a	1.85 a
	PS	7.53 b	4.08 b	55.1 b	25.3 a	2.31 a	0.65 a	0.85 a	7.54 a	1.43 a	2.11 a
	LDPE + LLDPE	8.10 c	3.22 a	52.8 ab	24.1 a	2.32 a	0.81 a	1.18 a	9.82 a	3.17 a	4.11 a
Statistical significance											
	E. fetida presence	***	***	*	ns	ns	ns	ns	*	ns	*
	Type of APW	***	***	***	***	ns	**	**	***	***	***
	E. fetida x APW	***	***	***	ns	**	ns	ns	ns	ns	ns

EC: Electrical conductivity, TOC: Total organic carbon, OM: Total organic matter, WSC: water-soluble carbon, C_{FA}: fulvic acid-like C; C_{HA}: humic acid-like C. n.s.: not significant $P > 0.05$; *, **, ***: significant at $P \leq 0.05$, 0.01 and 0.001, respectively. Average values in a column followed by the same letter are not significantly different at $P < 0.05$ (Tukey-b post-hoc test).

earthworms used belong to the specie *Eisenia fetida,* selected for this study due to this epigeic specie is commonly used for vermicomposting approaches (OECD, 2016). The procedure of obtaining and preparation of the earthworms for the bioassay are detailed in a previous work (Sáez et al., 2022). Considering the main factors established in the experiment (plastic type, pre-treatment or not and particle size, *E. fetida* presence and AWP presence), three different types of experimental devices with three replicates each were prepared: a) feedstock + AWP without earthworms; 2) feedstock + earthworms without AWP presence; c) feedstock + APW + earthworms.

The bioassay consisted in an incubation during 45 days in Petri dishes (15 cm ø) (Domínguez, 2018) using 80g of feedstock adjusted with distilled water to 70% of moisture content. Then, 1g of APW material was added per replicate (1.25% f. w. proportion) (Sáez et al., 2022). The incubation containers were kept into isolated chambers under controlled conditions (20° ± 2 °C and darkness). After 7, 21, 30 and 45 days of exposure, survival and body weight variation were determined. For this, earthworms were carefully extracted from the Petri dish of each replicate by hand sorting, counted for survival, weighted and recorded to obtain the mean body weight of each treatment. During the exposure time of bioassay, when mortality was observed, the worms were immediately removed from the Petri dish. At the end of bioassay, the final materials obtained, after the bioassay without earthworms (hereinafter referred to substrate) and with earthworms (vermicompost) from each replicate were homogenized and the samples were divided in two subsamples: one was used immediately for moisture determination and freeze at −80 °C until enzyme activity determination and the other were partially air dried in oven equipped with forced aeration at 60 °C. To obtain a dust particle size the sample was ground using an agate ball mill (RESTCH mod. MM400) and dried at 105 °C for further determinations. In addition, microscope images were obtained using a Camera MOTICAM S3 connected to Trinocular Microscope MOTIC® SMZ 140/143to compare the effect of the bioassay in the plastic size and shape (images display in S3 as Supplementary Material).

2.3. Analytical methods

2.3.1. Physico-chemical and chemical characteristics of the substrate and vermicompost

The physico-chemical and chemical parameters in the substrate (after the bioassay without earthworms) and vermicompost samples were determined according to the methods described by Sáez et al. (2022). Briefly, water-soluble extracts (1/10, w/v) were analysed for the physico-chemical parameters (pH and electrical conductivity); total organic carbon (TOC) and total nitrogen (TN) were determined using an automatic elemental micro-analyser; total organic matter (OM) was evaluated by loss on ignition (430 °C for 24 h). Moisture content was determined by drying at 105 °C for 24 h, whereas macronutrients and micronutrients (P, K, Ca, Cu, Mg, Fe, Mn and Zn) and toxic heavy metals (Cr, Ni, Cd, Hg and Pb) were determined in the extract obtained after the acid digestion (HNO_3/H_2O) (1:1 v/v) using a microwave by ICP-OES. The 0.1 M NaOH-extractable organic carbon (Cex), fulvic acid-like carbon (C_{FA}), humic acid-like carbon (Cha) and water-soluble C were determined with an automatic carbon analyser for liquid samples (TOC-V CSN Analyser).

2.3.2. Enzymatic activity of substrate, vermicompost and biofilm and earthworm biomarkers

The enzymatic activities (carboxylesterase (CbE), catalase and dehydrogenase (DHE)) were determined using the microplate-scale format protocols described in detail by Sáez et al. (2022) in an aqueous suspension 1:50 (w/v) of the substrate and vermicompost samples, respectively, while for the biofilm, the sample separated from the substrate or vermicompost was previously homogenized in the water-soluble ratio 1:10 (w/v).

For the determination of the biomarkers (total protein content, CbE determination and lipid peroxidation), six earthworms randomly selected from each replicate were selected and 24 h without feedstock in order to eliminate their gut content. Later, the body tissue of the earthworms was homogenized to obtain the post-mitochondrial fraction which was aliquoted and stored at −80 °C until analysis. This procedure

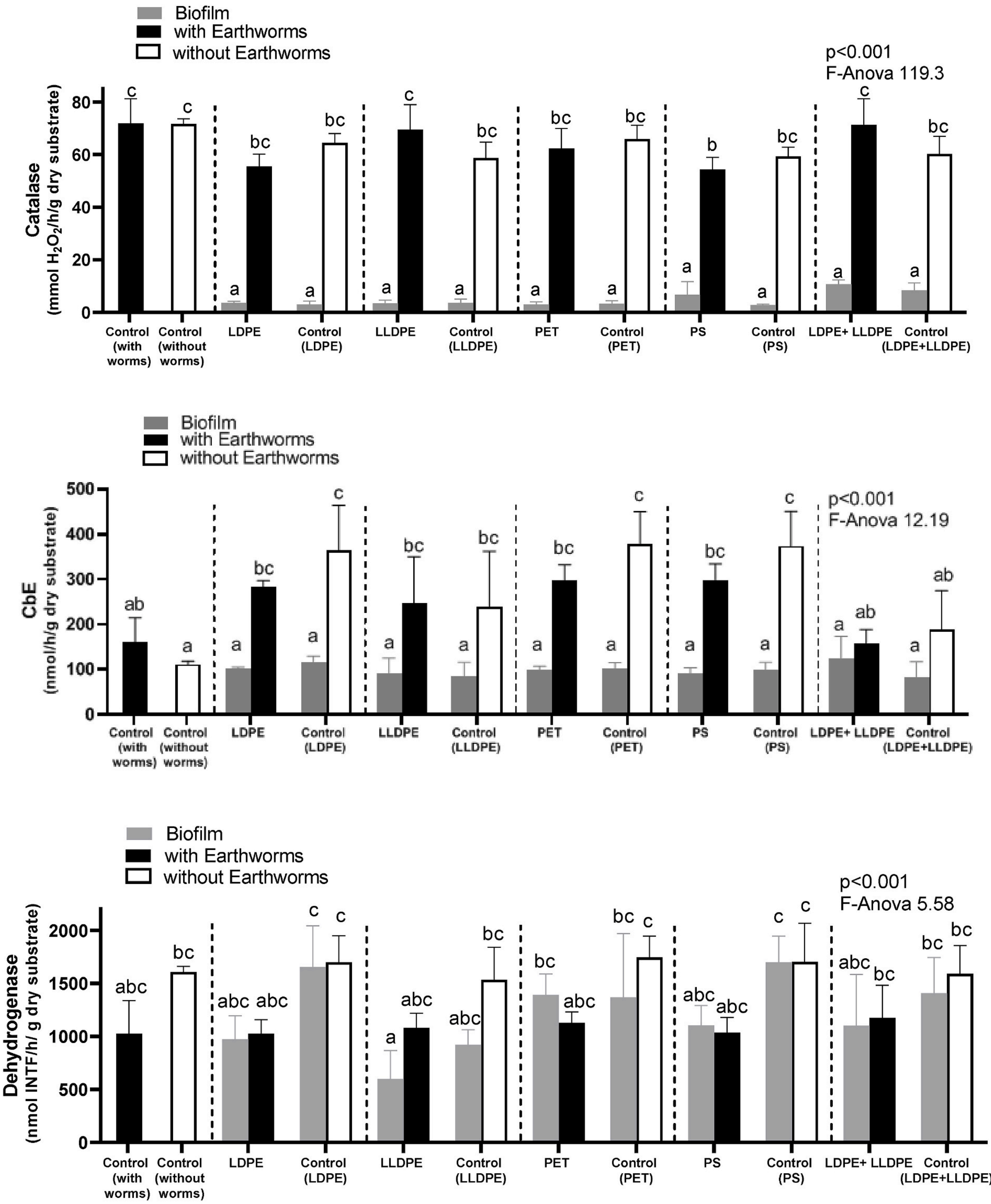

Fig. 1. Enzyme activities (catalase, carboxylesterase (Cbe) and dehydrogenase) measured in biofilm, substrate without earthworms and vermicompost. Different letters indicate significant statistical differences ($P < 0.05$).

and those for the determinations of the biomarkers can be found elsewhere (Sáez et al., 2022).

2.4. Statistical analysis

The statistical analyses were based on an ANOVA analysis to study the effects of the treatments in the environments with and without earthworms and the multivariate general linear model (GLM), to assess the effects of the five variables considered (*E. fetida* presence, APW type, APW presence, APW size and pre-treatment). In both statistical analyses, the Tukey-b was used as post-hoc test. The statistical analyses were conducted using the IBM SPSS Statics V.28 software package.

3. Result and discussion

3.1. Effect of the AWP type and/or E. fetida presence on the chemical characteristics of the end-products obtained

After 45 days of bioassay, the pH values were significantly lower in the vermicompost and with APW compared to those with APW and without earthworms (Table 1). This could be due to the release of volatile fatty acids as a consequence of the higher organic matter degradation in these treatments. On the other hand, *E. fetida* mucus is added to the ingested materials, leading to neutralize the substrate (Pérez-Godínez et al., 2017). Moreover, earthworms have shown an excellent pH neutralization efficiency due to their calciferous glands (Mubeen and Hatti, 2018) and the ability to regulate the release of organic acids depending on the characteristics of the starting feedstock (Angst et al., 2019). The type of AWP also showed a significant effect on the pH, showing the plastics PET and PS the lowest pH values. However, in the substrate without earthworms, compared with the initial substrate, no differences were found induced by the plastic presence.

E. fetida presence induced higher electrical conductivity mean values, also observing higher EC values in the treatments with AWP and earthworms (Table 1). The EC values obtained in all treatments with earthworms exceeded the threshold of 4 dS m^{-1}, limiting value for plant cultivation in soilless crops (Lasaridi et al., 2006), but within the recommended ranges for their use as organic amendments. On the other hand, EC values below 8 dS m^{-1} are adequate for earthworm growth and development (Rahimi and Karimi, 2016). This EC increase in vermicomposting processes has been reported in previous works (Khalil and Sanaa, 2009; Fernández-Gómez et al., 2010). The higher EC values observed in the treatment with worms can be attributed to the increased organic matter mineralization, which releases ions (cations and anions), unavailable nutrients in more available forms and the production of salts, ammonium and inorganic (soluble salts) (Bernal et al., 2009). This behaviour coincides with the greater decrease in the organic matter concentrations observed in the treatment with worms, which might also be explained by the ability of the worms to promote some hydrolytic enzymes, not only linked to the C cycle (β-glucosidase or carboxylesterase), but also related with other macronutrients, such as the phosphorus cycle (phosphatase) that remove phosphate groups from organic matter (Nogales et al., 2008) or related with N mineralization (urease, protease). The type of AWP also influenced this parameter, showing the treatments with the mixture of LDPE + LLDPE the lowest salinity mean values.

The strong reduction in the OM and TOC contents observed at the end of bioassay can imply an accelerated mineralization of nutrients bound to organic matter. However, the presence of earthworms and the combined factors (*E. fetida* and AWP presence) did not affect the evolution in TOC content, the opposite effect being observed for OM. In addition, with the presence of APW was observed a lower TOC reduction, especially in LDPE + LLDPE, this could be indicative of a slight antimicrobial effect on substrate. The type of AWP had a significant effect, showing PET the highest OM mean contents at the end of the bioassay, probably due to the different structure of this polymer, which could favour the retaining of substances with an organic matrix (Fadare et al., 2019).

In general, the total nitrogen (TN) tended to increase in all treatments compared with the initial feedstock, with and without earthworms, only founding a significant effect of the combined factors (*E. fetida* and AWP presence). In other studies of vermicomposting, an increase in TN has been reported due to bioconversion process of decomposition of waste by earthworms (Cynthia and Rajeskhumar, 2012). Also, trough microbial mediated nitrogen transformation results in further increase in nitrogen (Suthar and Singh, 2008).

Regarding to P and K contents, no statistical differences were found when the treatments with and without earthworms were compared, as in the case of the combined effect of *E. fetida* and AWP. However, the differences in the P and K contents seem to be related to the type of APW. Total K and P levels were significantly lower in PET and PS. As it has been previously observed in the OM, the type of structure of these polymers could have influence OM degradation, since it has been reported the capacity of polystyrene-based plastics to remain bound and retain substances with an organic matrix (Fadare et al., 2019), which avoids their decomposition and released of nutrient bond. Thus, the results obtained seem to indicate that the differences observed in NPK contents could be mainly related with the type and composition of APW.

In the WSC contents, a significant effect of *E. fetida* and of AWP presence was observed, but this effect was not significant when both factors were combined. The presence of the earthworms significantly reduced the WSC contents, this result being previously reported by other authors, since the gut associated processes and microorganisms consume labile forms of organic matter as carbon source in their tissue formation, resulting in a reduction in WSC content during vermicomposting (Yadav and Garg, 2011). Concerning the effect of the type of AWP, in general, the treatments with LLDPE polymer in their composition presented higher values of WSC. The same behaviour was observed in the treatments with and without earthworm presence for LLDPE. Thus, one potential reason for this is that the polymer backbone of LLDPE partially retains and bounds with organic matrix, avoiding its consumption, as it was found by Chen et al. (2018) in a study about the interaction of MP with the aromatic structure of DOM. The type of AWP had a significant effect on the humic and fulvic acid-like C contents, but the *E. fetida* presence only affected significantly C_{HA}. In both parameters (C_{FA} and C_{HA}), the combination of *E. fetida* and AWP presence did not show a significant effect, while the treatments with PET and PS showed again the lowest values. This reduction in humic compounds contrasts with that would be expected in vermicomposting process. However, other studies about plastic presence in soil have reported a decrease in humic acid-like compounds due to strong adsorption capability to polystyrene nanoplastic particles (Velzeboer et al., 2014; Cai et al., 2018).

3.2. Effect of APW presence on the substrate, vermicompost and biofilm enzyme activity

Catalase enzyme plays a key role in antioxidant system to response to oxidative stress, preventing oxidative damage (Giulia et al., 2012). In general, a slight inhibition of the catalase (CAT) activity was found in all the treatments with APW presence compared with the control treatment in both cases, with and without earthworms (Fig. 1). In presence of the earthworms, only the treatments with PS showed statically lower values than the control treatment, showing the rest of AWP treatments LDPE and PET values statistically similar or slightly lower than the control treatment. A potential reason for the observed fall at these low levels could be due to the ability of some plastic additives to act as CAT inhibitors. Additives such as hydroxylamine and metallocenes are widely used as UV and light stabilizers in the film manufacturing process, while resorcinol is an efficient gas barrier of several polymers (Rodríguez-Seijo et al., 2018). These compounds show affinity for binding catalase enzyme site and leads the enzyme to permanent inactivation (Pritchard, 1998; Rodríguez-Seijo et al., 2018). Thus, it seems to the presence of APW in the proportion tested (1.25% f. w.) lead to a partial inhibition in CAT production, but not induce a clear detoxifying response in *E. fetida*. In the biofilm samples, all APW treatments led to a sharp reduction in the CAT activity in relation to the substrate, with a mean decrease of 85% (Fig. 1).

Concerning to the carboxylesterase (CbE) activity, in general, the different AWP treatments seemed to induce an increase in the values of the CbE activity, especially in absence of *E. fetida,* with the values closer to the control treatment with the LDPE + LLDPE presence. The presence of LDPE, LLDPE, PS and PET during assay led to a significate increase in CbE activity compared with the control treatment without plastic with a mean increase of 39.7%. When the control treatment without AWP is compared with the presence and absence of earthworms, an increase in

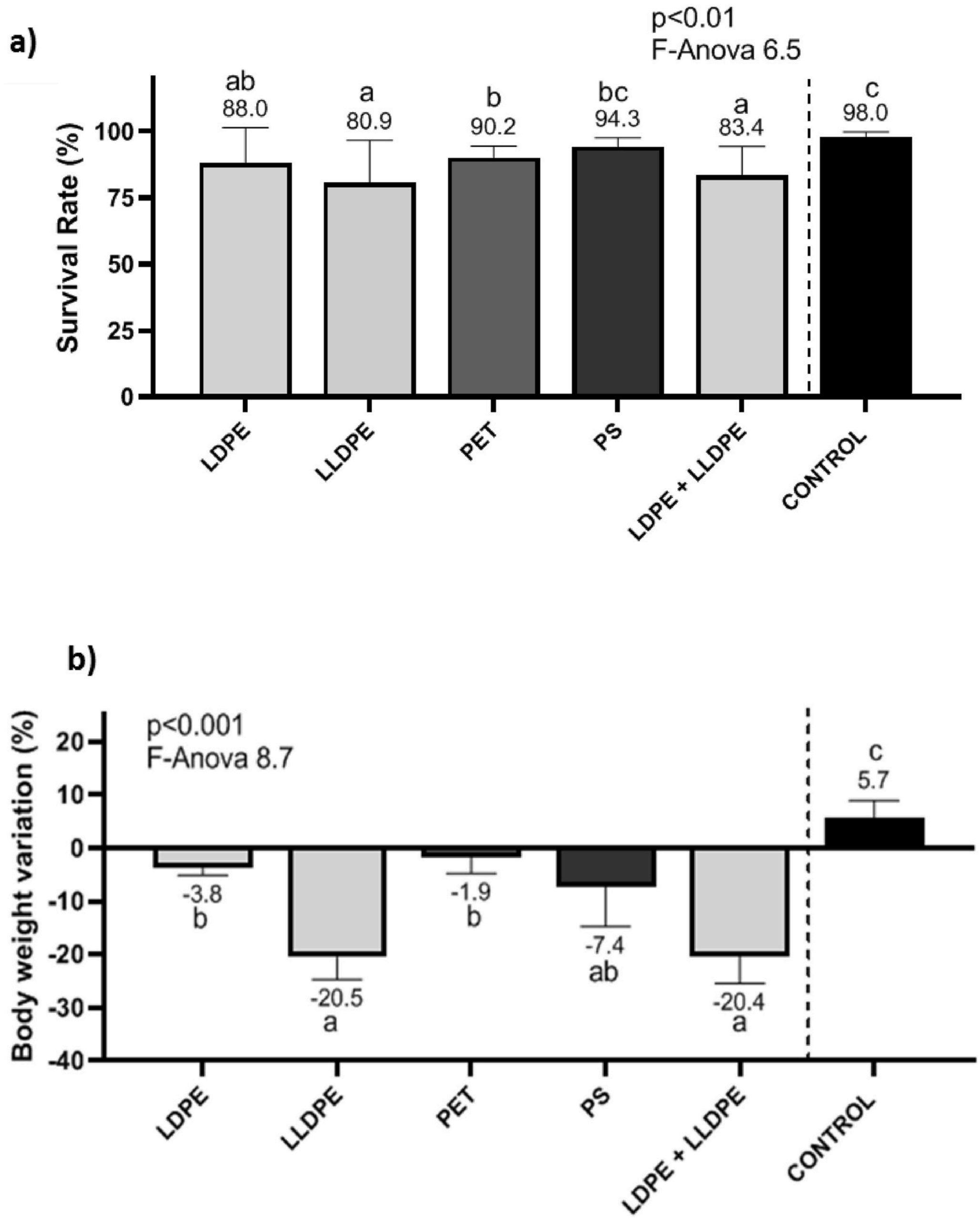

Fig. 2. a) Survival and b) body weight variation in *Eisenia fetida*. Different letters indicate significant statistical differences ($P < 0.05$).

CbE activity is observed, probably due to this esterase is mainly secreted in the intestinal lumen of the earthworms themselves (Sánchez-Hernandez et al., 2009) and therefore, earthworms casting may act as source of stable and active CbE activity (Sánchez-Hernández et al., 2015). This increase in presence of plastic materials could be due to the occurrence of cellular damages, which may produce changes in energy consumption to counteract the effects imposed by oxidative stress or other mechanisms (Rodríguez-Seijo et al., 2018). In the biofilm samples, all samples obtained final values close to the control treatment without earthworms, not being affected for earthworms and/or APW presence, independently of the type of plastic material. The strong inhibition observed could indicate a bioscavenging role of this esterase in response to close contact with APW material. In soils have been described a mechanism of passive protection of biofilm against plastic particles, which are dependent on the physical-chemical properties of the biofilm matrix (Jing et al., 2014). Unfortunately, no data are available on the effects of MPs or film debris (mesoplastics) in organic waste biofilm formation.

The DHE activity is directly related with biological oxidation of organic matter. The type of AWP seemed not to induce a significant effect on the DHE activity in the environment with *E. fetida*, observing the same values as for the control treatment (Fig. 1). However, without earthworms, a slight increase was observed for all the AWP treatments, especially for LDPE, PET and PS. These results seem to indicate that the digestive system of earthworms was able to break down the organic matter in the feedstock with APW, increasing the particle surface-to-volume ratio, and thereby the maintenance the number and activity of the microorganisms. Moreover, the higher DHE activity found may be due to a lower stabilization reached in these samples. This is corroborated by the higher WSC content remained in samples without earthworm presence. In contrast with the results observed in CAT and CbE, the DHE activity remained very high in the biofilm samples. The trend of DHE in APW biofilm was similar to that observed for the substrate without earthworms.

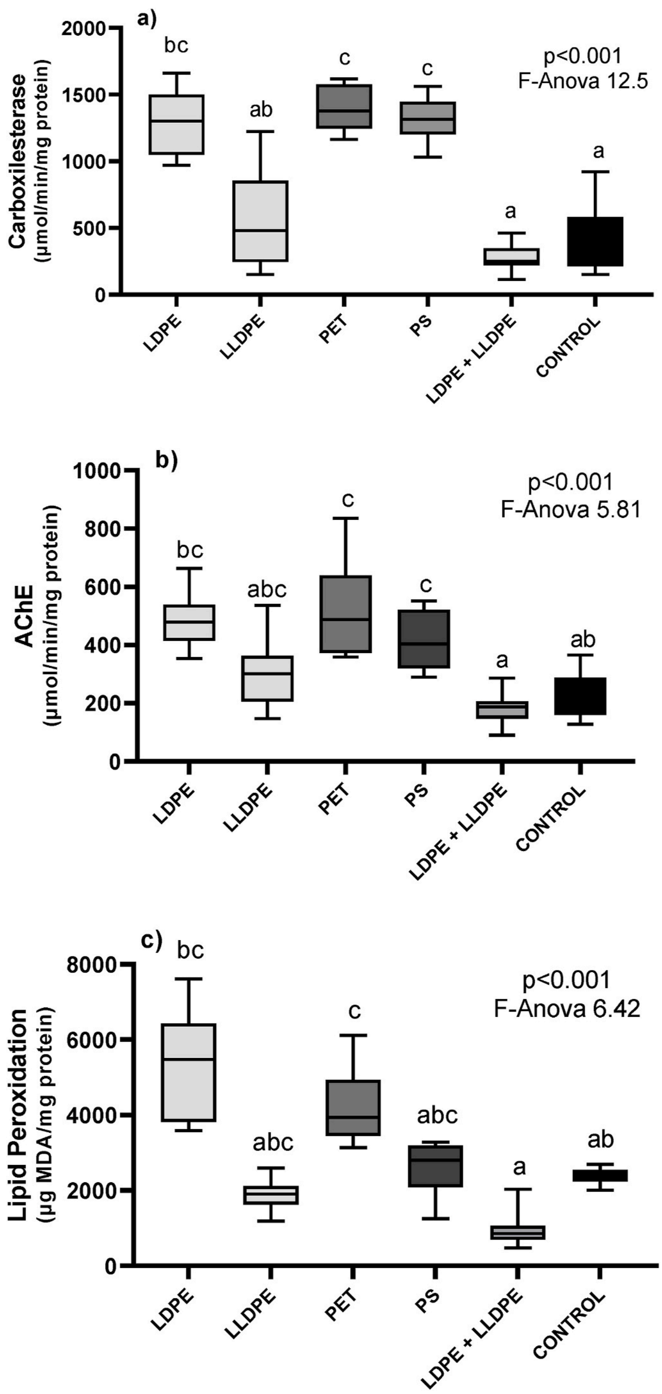

Fig. 3. Response of biomarkers: (a) carboxylesterase, (b) acetylcholinesterase (AChE) and (c) lipid peroxidation determined in *Eisenia fetida* tissue. Different letters in the box indicate significant statistical differences ($P < 0.05$).

3.3. *Effects of AWP presence on Eisenia fetida: survival, body weight and response of biomarkers*

The presence of APW in the feedstock seemed to induce a decrease in the *E. fetida* survival compared with the control treatment (Fig. 2). The treatments with LLDPE and the mixture LDPE + LLDPE produced the lowest value of *E. fetida* survival at the end of the exposure assay. In the PS, PET and LDPE treatments, statistical effects were detected, but with a decrease of the survival rate of less than 10% compared to the control treatment. This affectation observed in epigeic earthworms when they are exposed to APW material under vermicomposting conditions has been described as a set of biotic factors, which affects various physiological processes, such as respiration rate, reproduction rate, feeding rate and burrowing activity (Yadav et al., 2011). Concerning to weight variation, *E. fetida* exhibited a higher susceptibility to negative morphological effects by exposure to LLDPE and/or LDPE + LLDPE plastic treatments, obtaining similar values of negative weight variation. In the rest of treatments (PET, PS and LDPE), a similar trend was observed, although with minor differences compared with the control treatment. The substrate corresponding to the control treatment was able to maintain a higher density of earthworms with less mortality, also founding an enhancement of the body weight of *E. fetida* compared with the results observed in the substrate with plastic. This demonstrates that the nutrient capacity of the substrate material was not a limiting factor for *E. fetida* development, in the experimental bioassay. Thus, these results can indicate that the loss of weight in the treatments with the plastic presence could be induced by stress in the earthworm physiological activity.

Concerning the biomarkers studied in the *E. fetida* tissue, two different stress responses were observed in the *E. fetida* tissue by exposure to APW materials (Fig. 3). The treatments with the APWs including LLDPE in the backbone polymer formation (LLDPE and LLDPE + LDPE) were the only treatments that showed the mean value of CbE activity close to the control treatment. On the other hand, the highest CbE mean values were observed in the treatments where *E. fetida* was exposed to PS and PET. Lackmann et al. (2022) studied the CbE as a biotransformation enzyme involved in the xenobiotic metabolism, but reported that overall change in *E. fetida* was not significantly affected by the microplastic (Polystyrene-HBCD) exposure in soil for 28 days. Thus, the results obtained seem to suggest a different molecular mechanism underlying the earthworm response to the different APW tested. Chen et al. (2020) also studied the effect of different types of microplastic and reported a varying mode of action depending on the plastic type, but also the shape, size and potential influence of additives. Our results might indicate that the chemical nature of the plastic polymer is also determinant in the pathway followed by this type of esterase.

Acetylcholinesterase (AChE) is a biomarker of neuro-toxicity, which degrades acetylcholine to remove the neurotoxic effects of pollutants in many species (Zhang et al., 2020). The presence of APW materials produced an interaction of CbE and AChE of *E. fetida* in a similar way. AChE activity in *E. fetida* exposed to LDPE, LLDPE, PET, PS, significantly increase compared to the control, showing also the highest values with the exposure to PS and PET as it has been previously reported for CbE activity (Fig. 3). However, the individuals of *E. fetida* in LDPE + LLDPE and LDPE treatments showed similar AChE mean values than individuals in the control treatment. Previous studies have also shown an increase of AChE when *E. fetida* has been exposed to plastic materials, which could be due to the presence of plastic produces a stimulation of the neurotoxicity response in *E. fetida* to have a specific regulatory effect on neurotoxins (Chen et al., 2020; Zhong et al., 2021; Zhang et al., 2020). Thus, Chen et al. (2020) found an increase in AChE in *E. fetida* exposed for 21 days and 28 days at 1.0–1.5 g/kg of LDPE in soil. Zhong et al. (2021) also reported increases in AChE in a study about the effect of microplastics in sludge on the vermicomposting process.

Concerning to lipid peroxidation (Fig. 3), the presence of LDPE and PET induced the highest values of lipid peroxidation, compared to the control treatment without APW exposure. Recent studies have demonstrated that microplastics with a wide variety of chemical composition can cause in terrestrial organism such as ciliates, collembolans and earthworms skin damage, tissue lacerations, immunity disturbing and neurotoxicity with subsequent increase in lipid peroxidation activity (Sarker et al., 2020; Wang et al., 2020). Some studies have even shown that exposure to MPs (HDPE, PP and LDPE) with size less than 300 µm by E. *fetida* (Chen et al., 2020; Jiang et al., 2020) led to the occurrence of clear inflammatory processes, between the gut epithelium and the chloragogeneous tissue, sometimes with the development of fibrosis and congestion (Rodríguez-Seijo., 2018).

Table 2
Effect of the different factors (AWP presence, particle size and UV pre-treatment) on the main physico-chemical and chemical characteristics of the vermicompost obtained.

	pH	EC (dS m^{-1})	OM (%)	TOC (%)	TN (%)	P (%)	K (%)	WSC (%)	C_{FA} (%)	C_{HA} (%)
AWP presence										
Presence of AWP	7.6 b	4.2 a	54.5 b	26.8 a	2.3 a	0.7 a	1.2 a	8.6 a	2.5 a	3.3 a
Absence of AWP	7.5 a	5.1 b	52.0 a	25.3 a	2.4 b	0.8 a	1.2 a	8.2 a	2.9 b	3.8 a
F-Anova	*	***	***	*ns*	*	*ns*	*ns*	*ns*	*	*ns*
Particle size										
Film debris (mesoplastic)	7.6 a	4.0 a	54.1 a	24.5 a	2.4 a	0.7 a	1.0 a	8.3 a	2.4 a	3.1 a
Microplastic	7.7 b	4.4 b	54.8 a	29.2 b	2.3 a	0.7 a	1.3 b	9.0 b	2.5 a	3.5 b
F-Anova	*	***	*ns*	***	*ns*	*ns*	***	*	*ns*	*
Pre-treatment										
No pre-treatment	7.6 a	4.2 a	54.5 a	25.9 a	2.3 a	0.7 a	1.1 a	8.5 a	2.5 a	3.2 a
UVC – 250h	7.8 b	4.5 b	54.8 a	31.2 b	2.4 a	0.8 a	1.4 b	9.3 a	2.6 a	4.0 b
F-Anova	*	*	*ns*	***	*ns*	*ns*	***	*ns*	*ns*	**

EC: Electrical conductivity. TOC: Total organic carbon. OM: Total organic matter. WSC: water-soluble carbon, C_{FA}: fulvic acid-like C; C_{HA}: humic acid-like C. n.s.: not significant $P > 0.05$; *, **, ***: significant at $P \leq 0.05$, 0.01 and 0.001, respectively. Average values in a column followed by the same letter are not significantly different at $P < 0.05$ (Tukey-b post-hoc test).

Thus, the plastic with less effect on the response of the biomarkers studied in the *E. fetida* tissue was the mixture of LDPE + LLDPE in all the cases, with values statistically similar to those obtained in the control treatment without plastic. This fact could be related to the production of these plastics (LDPE + LLDPE), since the joint extrusion process of both generates a new polymer. This mixture would have had a different crystallinity than the individual plastics, which could change the possible release of toxic substances that would alter enzymatic activity, such as those of CbE and AChE, as well as lipid peroxidation.

3.4. *Effects of the plastic materials on vermicompost properties and on E. fetida tissue biomarkers: AWP presence, particle size and UV pre-treatment*

All the plastic factors studied (presence, particle size and pre-treatment) had a significant effect on the physico-chemical parameters (pH and electrical conductivity) in all the scenarios studied (with and without earthworms) (Table 2). However, the effect on the rest of chemical parameters was different depending on the factor considered, with a more similar behaviour in case of the particle size and the UV-pre-treatment, both affecting the contents of total organic C, K and humic acid-like C. This similarity could be explained due to the changes produced at physical and/or chemical level, which can induce changes in the polymeric structure of the AWP materials, becoming the bulk polymer more available for biological attack (Shah et al., 2008; Urbanek et al., 2021).

Slight differences were found for the enzyme activities considered depending on the AWP particle size (film debris or microplastic-MP) and the application or not to the AWP materials of the UV pre-treatment (Fig. S1). For both factors, the only enzyme activities affected were CbE and DHE, not observing any significant effect on the catalase activity. Moreover, film debris seems to induce a lower inhibition in CbE and DHE activity than MP. Likewise, the pre-treatment of the plastic materials with UV-C during 250 h produced a lower activity in these enzymes. The lower enzyme activities observed for the AWP with smaller size (MP) or pre-treated with UV could be related with a lower biological activity oxidation of the organic matter content in the substrate due to the potential release of toxic compounds derived from the plastic materials when they are physically and/or chemically modified. Thus, the significant differences found in the enzymatic activities related to the size and/or composition changes can be an evidence of the presence of derived compounds from plastic material that can induce adverse effects. On the other hand, the particle size or the UV pre-treatment seem not to have a significant effect on the biomarkers studied (carboxylesterase, AChE and lipid peroxidation) in the *E. fetida* tissue (Fig. S2). Several studies have reported that the effects of MPs on terrestrial organism is closely related with different physico-chemical properties of polymer such as shape, size, types and additives (Chen et al., 2020; Lambert et al., 2017). However, the results obtained in this study are not in accordance with that reported in previous studies with earthworms in soil.

4. Conclusions

The presence of agricultural plastic waste-AWP (microplastic and film debris) influenced the final characteristics of the vermicomposts obtained, concretely organic matter and NPK contents, especially in the presence of PET and PS. The exposure to the AWP materials produced in *E. fetida* a higher susceptibility to negative morphological effects, observing the lowest value of survival and the highest loss of weight at the end of the exposure assay, especially with the treatments with LLDPE and LDPE + LLDPE. This fact was also reflected in the signs of oxidative stress and neurotoxicity observed in *E. fetida* related with APW exposure, especially with MP or AWP pre-treated with UV, which seemed to trigger a biochemical response that was not observed in the biomarkers response. However, more research is necessary to better understand the mechanism involved in the detoxification response system of *E. fetida*.

Credit author statement

Z.E. Blesa Marco (Methodology) (Investigation) (Validation) (Writing - original draft); J.A. Sáez: (Methodology) (Investigation) (Validation) (Writing - original draft); A.M. Pedraza Torres (Methodology) (Investigation) (Validation); E. Martínez Sabater (Methodology) (Investigation) (Validation); L. Orden (Methodology) (Investigation) (Validation); F.J. Andreu-Rodríguez: (Supervision) (Investigation) (Methodology) (Validation); M.A. Bustamante: (Investigation) (Data curation) (Writing -review & editing); F.C. Marhuenda-Egea (Investigation) (Data curation) (Writing -review & editing); M.J. López: (Conceptualization), (Supervision), (Writing -review & editing), (Project administration) (Funding acquisition); F. Suárez-Estrella (Methodology) (Investigation) (Validation); R. Moral: (Conceptualization), (Supervision), (Writing -review & editing), (Project administration) (Funding acquisition) (Resources).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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