Recycling and ecotoxicity of flax/PLA composites: Influence of seawater aging

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ABSTRACT

Poly(lactic acid) is finding increasing applications as it is a bio-sourced compostable thermoplastic. Its properties can be improved by adding flax fibres, which are also compostable. The possibility to recycle the composite after service is attractive because it reduces waste in landfills, is a cost-effective measure to reduce the need for raw materials and extends the life span of composites. However, few reports are available to verify how recycling affects properties of flax-PLA composites. In addition, if these recycled materials are to be used in marine applications, it is important to investigate whether they will be adversely affected by seawater compared to virgin materials, as well as to investigate the toxicity of these materials for marine organisms. The goal of this work was to address these three points, i.e., to assess whether recycling and posterior environmental weathering (seawater exposure) changes the flax-PLA composite properties, and to assess these materials' toxicity for biota (a benthic copepod species, *Nitokra spinipes*). First, our results indicate that recycling up to four times hardly affects the composite properties. However, immersion in seawater at 40 °C for up to nine months reduces tensile strength of both virgin and recycled composite by up to 60%. Finally, the ecotoxicity evaluation indicated that neither leachates from PLA nor from Flax-PLA induce immobilization of *N. spinipes*, nor were any immobilization effects induced by leachates resulting from materials aged for two months in seawater. These results will contribute to assessing the sustainability of future bio-based polymer applications.

Introduction

Increasing concerns over end-of-life scenarios for plastics are driving research into novel material solutions. Plastic pollution of the oceans has become a major issue [1], and EU legislation such as the European Single-Use Plastics (SUP) Directive [2] is further encouraging the development of improved reusable and recyclable polymers. The objectives of this study, performed within the European Interreg project SeaBioComp [3], are: to provide alternatives to landfill, conserve resources and reduce plastic pollution by making recycling more accessible, and to reduce environmental impact through development of bio-sourced materials. One possibility is to reduce the use of thermoset polymers by replacing them with thermoplastics, allowing

multiple re-use cycles. Recycling of thermoplastics is now well established, with around 50% of polyethylene terephthalate (PET) bottles being recycled in the EU [4]. However, currently less than 10% of plastics are recycled worldwide [5].

PLA, poly(lactic acid), a thermoplastic polymer known to be compostable, was selected for study here. It is one of the most widely studied bio-sourced polymers since it was first synthesized in 1845. Recent papers provide an overview of its properties [6,7]. Following previous work [8–10], the Bio4Self and SeaBioComp projects further developed self-reinforced PLA (SRPLA) materials [11,12], integrating PLA fibres in a PLA matrix. This should lead to improved end-of-life recycling, as it removes the need for separating matrix and fibres, a key step in developing more efficient circularity [13]. A seawater aging study on these

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Fig. 1. Workflow of recycling flax reinforced PLA composites.

fibres (flax) into a PLA matrix polymer to improve its mechanical properties for use in marine applications. There has been extensive work on flax fibre reinforcements in recent years [17,18] and they are gradually being accepted in automotive applications [19], as a response to environmental legislation. Pilin et al. examined multiple recycling of flax/PLA by injection moulding, without adding virgin polymer, and showed that mechanical properties were retained for three recycling steps [20]. As the current project is aimed at marine applications, it is important to establish whether exposure to seawater affects the performance of these materials after recycling. Flax fibres are quite sensitive to water, so they need to be protected by a polymer matrix. Several authors have reported wet aging studies on PLA composites [21–23]. Le Duigou et al. [21] showed that the mechanical response of flax/PLA is strongly dependent on water temperature. In a study of PLLA reinforced by 30% by weight of flax fibres they showed that at temperatures close to the glass transition temperature (58 °C) degradation was very rapid, whereas at lower water temperatures (25 °C) the material was quite stable. Regazzi et al. [22] presented water diffusion and mechanical models for flax/PLA immersed at different temperatures and obtained good agreement with predictions of reversible mechanisms [22]. Gil-Castell et al. [23] aged SRPLA composites under different conditions (temperature, water, compost) and found that these could be degraded by composting in a similar way to unreinforced PLA.

While both recycling and seawater aging of PLA materials have been studied separately, little information is available in the published literature to assess quantitatively how recycling affects subsequent performance in a seawater environment. Beltran et al. [24] did examine the effects of recycling and seawater aging, but by applying different aging sequences before the recycling process. This is important information, in order to determine whether aged materials can be recycled or not, but results performed in the opposite order, showing how seawater aging affects recycled materials are not available. To fill this gap a study was initiated on seawater degradation, first of unreinforced PLA and then of flax fibre reinforced PLA materials which had undergone multiple recycling operations.

Following manufacturing processes most thermoplastics contain a variety of incorporated chemicals, including but not limited to small polymer units (i.e., oligomers and monomers), catalysts, solvents, and additives. The chemical compounds are associated to the polymer matrix by weak bonds, and can migrate, i.e. leach, to the surrounding marine environment [25–27]. Some of these leached chemicals such as Bisphenol A and phthalates have long been known to induce toxicity in marine organisms [28–30]. Hence, concerns have been raised on the ecological effects of leachates as aqueous solutions of various chemical compounds, on marine organisms [26,27,33,34]. There is an increasing body of evidence stating that the leachate from pure formula bio-based polymers does not induce acute effects on aquatic species such as *Daphnia magna* [43] and *Nitokra spinipes* [32], at a plastic-to-water ratio up to 100 g / L (reviewed in Curto et al. [44]). However, very few studies have focused on the effects of biocomposites, especially those exposed to a weathering process in the field. The determination of the ecotoxicological effects of leachates from biocomposites retrieved from the environment on marine organisms, compared to its pristine form, is a key element to assess the sustainability of future biocomposite applications and to support a transition process to improved plastic materials.

The aim of this paper is to present new data to assist in the selection of more sustainable materials for marine applications. It will first present the influence of recycling on the mechanical behaviour of two materials, PLA and flax/PLA composite. The effects on structure and

SRPLA materials provided data on their degradation kinetics [12] and indicated a complete loss of mechanical properties within one year at 40 °C. Various studies on recycling of PLA are also available. For example, Rossi et al. [14] performed life cycle analysis (LCA) on end-of-life options for PLA packaging, including recycling, industrial composting and landfill. They concluded that for most impacts mechanical recycling is the most environmentally beneficial route. Maga et al. [15] also presented LCA results from studies on end-of-life options for PLA waste and showed environmental benefits for both mechanical and chemical recycling, by reducing virgin polymer consumption, leading to reduction in global warming and energy, and lower agricultural land occupation. Beltran et al. investigated the influence of additives such as chain extenders and concluded that they could improve the recyclability of PLA [16].

The main focus of the present study is the introduction of natural

Table 1
Unaged PLA matrix and flax/PLA composite properties. Mean (standard deviation).

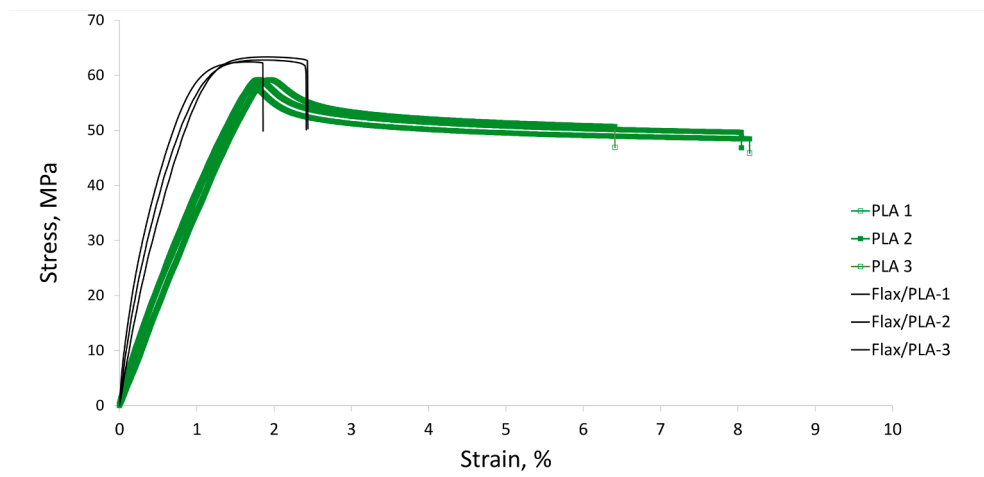| Materials	T_g (°C)	T_m (°C)	Density (g.cm^{-3})	E_t (GPa)	X_t (MPa)	E_f (GPa)	X_f (MPa)
PLA matrix	59 (±1)	Amorphous	1.259 (0.001)	3.81 (0.14)	57.1 (1.82)	3.74 (0.04)	109 (1.03)
Flax/PLA-V	63 (±1)	181 (±1)	1.311 (0.001)	6.82 (0.80)	58.4 (1.1)	6.19 (0.16)	109 (1.7)

Fig. 2. Examples of tensile stress-strain plots, for 3 unreinforced PLA and 3 virgin flax-reinforced PLA specimens.

Table 2
Influence of recycling on mechanical properties in tension and flexure. Mean (standard deviation).

Materials	E_t (GPa)	X_t (MPa)	E_f (GPa)	X_f (MPa)
PLA	3.81 (0.14)	57.1 (1.8)	3.74 (0.04)	109 (8)
Flax/PLA: V	6.82 (0.80)	58.4 (1.1)	6.19 (0.16)	109 (1.7)
R1	7.16 (1.61)	58.8 (1.9)	5.90 (0.06)	106 (1.5)
R2	6.62 (0.52)	57.6 (1.1)	5.89 (0.09)	108 (1.5)
3	6.51 (0.34)	61.4 (0.7)	5.75 (0.15)	108 (2.5)
R4	6.36 (0.11)	59.6 (1.4)	5.81 (0.07)	110 (1.8)

properties will be described. Then results from a seawater immersion aging study will be presented, on samples immersed for up to nine months at 40 °C. Finally, ecotoxicity results will be presented and discussed.

Materials & manufacture

The polymer used for the recycling study, PLA L130 (Total-Corbion), is a semi-crystalline PLA grade with a density of 1.24 g cm^{-3} and a melt flow index of 23 g / 10 min (at 210 °C, load 2.16 kg). The reinforcements are short flax fibres of 8 mm length from scutched tow supplied by Swerdloff. Before each step, the materials were dried at 80 °C in a Moretto DA427 - D2TS W dryer. Reference samples of pure L130 were made by injection moulding using an Arburg Allrounder 320 S 500 – 150 at 190 °C to make ISO 1A tensile bars. Flax reinforced PLA was made by compounding 10% flax (8 mm) as reinforcement and PLA L130 as matrix using a Leistritz ZSE 75HP Mic27 / GL-44D. ISO 1A tensile bars were injection moulded from this compound using the Arburg Allrounder 320 S 500 – 150 at 180 °C. Recycling of these materials has been done by first shredding the composite specimens to smaller pieces of around 1 cm^2 using a Herbold SML 22/30 S3-2 "C". These pieces were then compounded at 180 °C using the Leistritz ZSE 75HP Mic27 / GL-44D. ISO 1A tensile bars were made by injection moulding with the Arburg Allrounder 320 S 500 – 150 at 180 °C. This process has been repeated four times. The schematic diagram in Fig. 1 clarifies the manufacturing steps.

There were therefore six different injection moulded materials produced for seawater aging, which will be referred to subsequently as PLA, V, R1, R2, R3 and R4.

Two additional slightly different PLA and PLA-Flax materials were prepared for the toxicity study: In this case the PLA matrix was produced from self-reinforced bi-component filaments with a core-sheath structure (at a ratio of 50 – 50). They were composed of high melt PLA (6202D, Natureworks, LLC: melting temperature = 165 °C) for the core region and low melt PLA (6302D, Natureworks, LLC: melting temperature = 130 °C) in the sheath region.

To produce the PLA-Flax specimens for the toxicity study these self-reinforced bi-component PLA filaments were mingled with flax fibres to
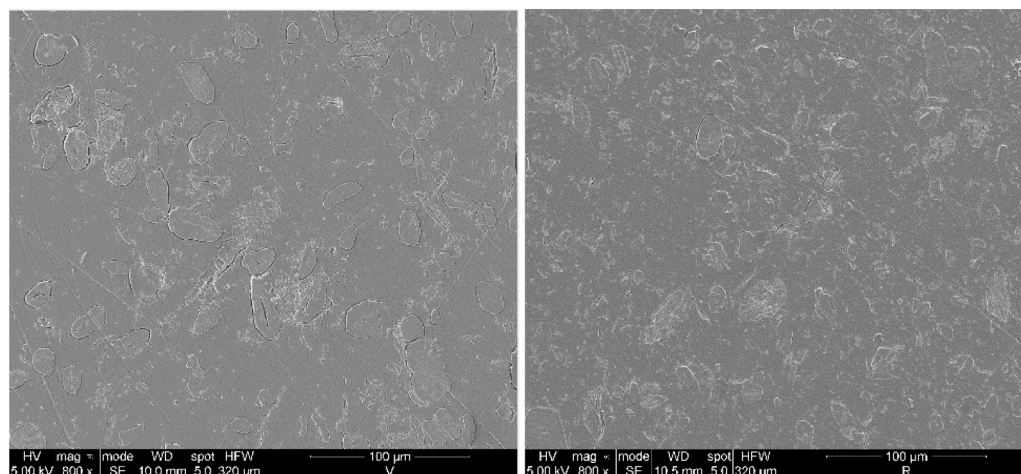

Fig. 3. SEM images, Virgin (left), and 4 times recycled (right) of unaged flax-PLA.
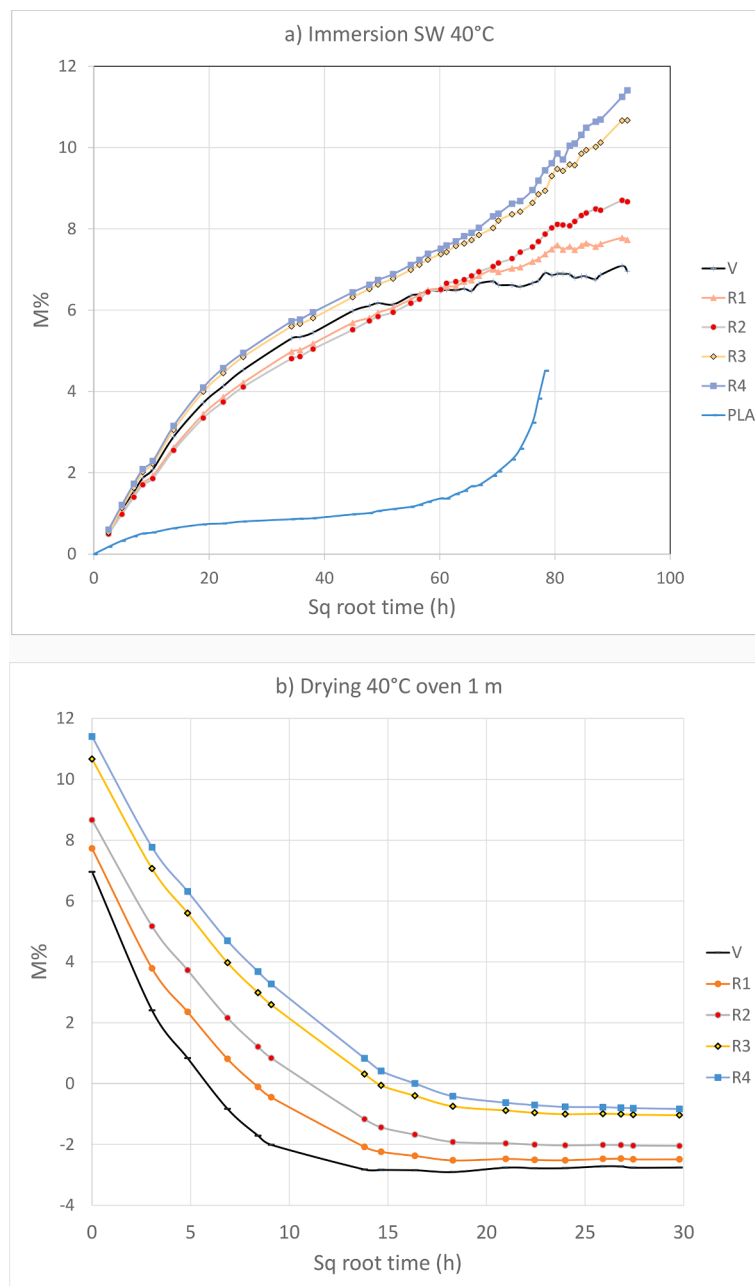
Fig. 4. Weight gain plots, all materials a) nine months 40 °C seawater immersion, b) Drying in air at 40 °C V: Virgin, R1 to R4: recycled 1 to 4 times. PLA: un-reinforced polymer.

obtain a non-woven mat by a needle-punch method. Then, a number of non-woven mats were stacked with several layers of low melt PLA film. This stack was placed in a mould and consolidated under heat (at 155 °C) and pressure (4 MPa) for 10 min by compression moulding to obtain composite plates. The final mass ratio of each constituent of the composite, i.e., flax fibres, self-reinforced bicomponent PLA filaments and low melt PLA film, were 28.7, 47.5 and 23.8%, respectively. The fibre content is therefore higher than for the recycled specimens.

Test methods and conditions

Physico-chemical testing

The microstructure of the six different materials for seawater aging, both initially and after recycling and aging, was investigated using five techniques: Differential Scanning Calorimetry (DSC), density

measurements (gas pycnometer), scanning electron microscopy, X-ray tomography and Gel Permeation Chromatography (GPC). The DSC analyses were run with a ramp at 10 °C/minute on TAI 25 Discovery™ equipment. They provided glass transition (Tg) and melt temperatures (Tm). Density values were obtained using an Accupyc™ 1340 helium pycnometer from Micromeritics.

Scanning Electron Microscopy (SEM) and X-ray Tomography were used to examine the internal structures of injected specimens. For the former observations were made on Au-Pd coated polished sections in a FEI Quanta™ 200 microscope. For the latter Nikon (Xtec) XTH225 X-ray micro-computed tomography (μ-CT) equipment was used.

GPC-SEC (Size Exclusion Chromatography) was performed by PeakExpert (Tours, France) on dried samples of unaged and seawater aged specimens of PLA and Flax/PLA in order to quantify molecular weight changes after ageing. The solvent was chloroform at 30 °C, Agilent 5 μm columns were used with polystyrene calibration.

Fig. 5. PLA matrix tensile specimen pieces after eight months' immersion in seawater at 40 °C.

Table 3
Values of molecular weight, Mn, (kg/mol) from GPC.

Material	Unaged	9 months SW
PLA	78.5	2.0
V	66.8	48.7
R4	73.4	41.7

Mechanical testing

Mechanical tests were performed in two test laboratories; first, to examine the influence of recycling tensile and flexural tests were performed on unaged specimens. Rectangular specimens with the cross-sections of 10×4 mm^2 were subjected to tensile tests using a universal testing machine (Instron 1185). The gage length was 100 mm and the crosshead speed was set to 1 mm/min. For each case, at least 10 tensile specimens were tested and the average and standard deviation values were recorded.

Three-point bending tests were performed on rectangular specimens with the dimensions of $80 \times 10 \times 4$ mm^3 using the same testing machine (Instron 1185). The loading pins had a radius of 5 mm, whereas the support pins had a radius of 2 mm. The cross-head speed was set to 2 mm / min. For each case, at least 10 flexural specimens were tested and the average and standard deviation values were recorded.

Second, in order to quantify the influence of seawater aging tensile tests were performed on an Instron™ 5966 frame with a 10 kN load cell and an Instron™ optical extensometer. Tests were performed at a loading rate of 2 mm / minute. Aged specimens were kept in water until testing and 3 or 4 specimens were tested for each aged condition.

Seawater aging

Tensile test specimens were aged in natural, continuously renewed seawater maintained at 40 °C for up to nine months. Seawater is pumped from the Brest Estuary to temperature-controlled tanks. The only treatment is a sedimentation to remove particles. The tank temperature is measured and recorded every 10 min and was within 40 °C $\pm$ 1 °C for over 99% of the aging period. This temperature was chosen based on previous PLA results, as degradation at lower temperatures is very slow [12]. Witness specimens, one of each material, were periodically removed and weighed on a Sartorius balance (to the nearest mg) to provide weight gain versus immersion time data. After immersion for 9 months the six witness specimens were dried in an oven at 40 °C until constant weight, to provide desorption curves.

Ecotoxicity assessment

The strain of *Nitokra spinipes* was isolated from sediments in the Tvaren Bay, Baltic Sea in 1975 and has been in continuous culture ever since in darkness at 22 $\pm$ 1 °C [35]. According to protocol ISO 14,669 [36], the culture medium was prepared in diluted natural seawater (source: Oostende port, Belgium; salinity: 7 PSU) and filtered through a 0.2 μm sterile filter. The *N. spinipes* cultures were maintained in darkness under static-renewal conditions and fed weekly with a commercial salmon feed (Astra-Ewos, Södertälje, Sweden).

Leachates of pristine and seawater-aged biocomposites were generated in diluted natural seawater (surface salinity: 7 PSU) [36]. Prior to the leachate preparation, PLA and Flax-PLA flakes ($2 \times 4 \times 0.3$ cm) were immersed for two months in temperature-controlled tanks at IFREMER (France), supplied with natural seawater pumped from the Brest Estuary (France). Seawater was continuously renewed, and the tanks used in this step were maintained at 25 $\pm$ 2 °C. Pristine and seawater-aged biocomposites were then incubated at 22 $\pm$ 1 °C in the dark until dry, and stored in light-sealed containers until further use. Thermal extractions following a non-target screening were performed using Gas chromatography–mass spectrometry (GC-MS) for both pristine and seawater-aged biocomposites to profile the chemical components of tested items and assess chemical shifts of the aging process (for further information please see supplementary information, S1). To extract leachates from tested items, we followed the Capolupo et al. [31] methodology, where 26 g plastic flakes were added at a solid-to-liquid ratio of 80 g / L in 320 mL water media in a pre-sterilized 500 mL Erlenmeyer flask wrapped in aluminum foil. To monitor the background variance of water physico-chemical parameters (pH, and salinity), triplicated water blanks (not containing plastics) were incubated alongside the samples. Both samples and blanks were incubated on an orbital shaker with a speed of 80 rpm, at room temperature (22 $\pm$ 1 °C) for 21 days in the dark. Upon sampling, the leachates were filtered through a 0.2 μm cellulose filter, to an end volume of 320 mL per treatment.

Acute copepod tests were performed in two independent batches (i.e. pristine and aged biocomposites) according to ISO 14,669 [36]. Considering the complexity in chemical compositions of the leachate, nominal dilution series (%) instead of absolute concentrations (mg / L) were used. In brief, three to four-week-old copepods were exposed to different concentrations (22, 36, 60, and 100%) of leachates of each biocomposite in complete darkness and without food for 96 h. For each concentration treatment, 20 organisms were randomly selected regardless of gender (except egg-carrying females) and separated into four replicates of five organisms. They were exposed in 2.5 mL of test medium spiked with the respective test substance in sterilized 10 mL glass vials. During the test, organisms were permanently kept at 22 $\pm$ 1 °C. After 96 h, immobility was defined as no swimming and no appendage movement during 10 s as described in ISO 14,669 [36].

All statistical analyses were performed in R v4.1.2 [37]. Data concerning the count of immobile copepods were analyzed per leachate using a non-parametric test, because the ANOVA assumptions of normality were not met after data transformation ($p < 0.05$, Shapiro–Wilk test). Therefore, a Kruskal-Wallis rank sum test was performed to check if the count of immobile copepods was dependent on dilution levels (%) of leachates (CI, confidence Interval = 95%). The Dunn's multiple comparison test (p values adjusted with the Bonferroni
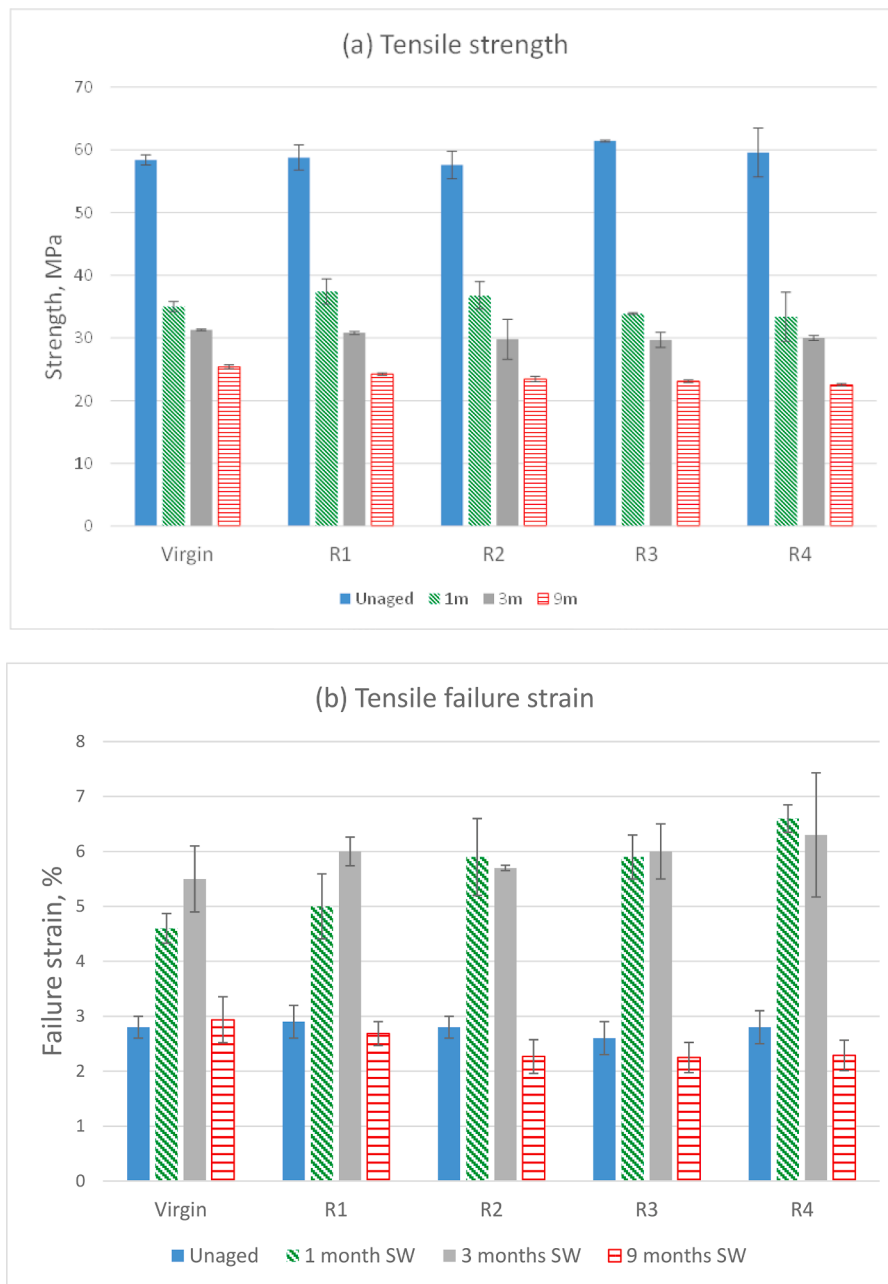
Fig. 6. Influence of aging on tensile stress-strain response. a) maximum stress, b) strain to fail Error bars show standard deviations.

method) using the FSA package [38] was applied when there were significant differences among treatments.

Results and discussion

Characterization of initial material state

The PLA and virgin Flax/PLA were characterised and their tensile and flexural properties are given in Table 1. The addition of quite a small amount of fibres (10%) results in a significant change in mechanical behaviour, Fig. 2. The presence of these short flax fibres increases stiffness and reduces failure strain but does not modify strength significantly.

Influence of recycling

Recycling involves both mechanical size reduction operations, which

will affect the fibre orientation and fibre length distribution, and thermal forming cycles which will affect the matrix microstructure. The former will be revealed by mechanical property changes while the latter can be investigated by thermal analyses (calorimetry). A third parameter is the creation of defects during recycling, which can also affect mechanical properties, and this can be examined by microscopy and tomography. Table 2 provides a summary of tensile and flexure properties.

The degradation of the mechanical properties of flax/PLA composites with recycling cycle is negligible (Table 2). The tensile and flexural moduli were slightly decreased by 6 – 7% as the composites were subjected to recycling. Conversely, the tensile and flexural strengths were almost identical up to the fourth recycling cycles (Table 2).

These results are in line with earlier work by Le Duigou et al., who worked on similar materials [20]. In that work PLLA reinforced with two fibre contents, 20% and 30% by weight of 4 mm long flax fibres, was recycled 6 times. Tensile strengths for both series were retained for 3 cycles before dropping. Also, tensile modulus was not affected by
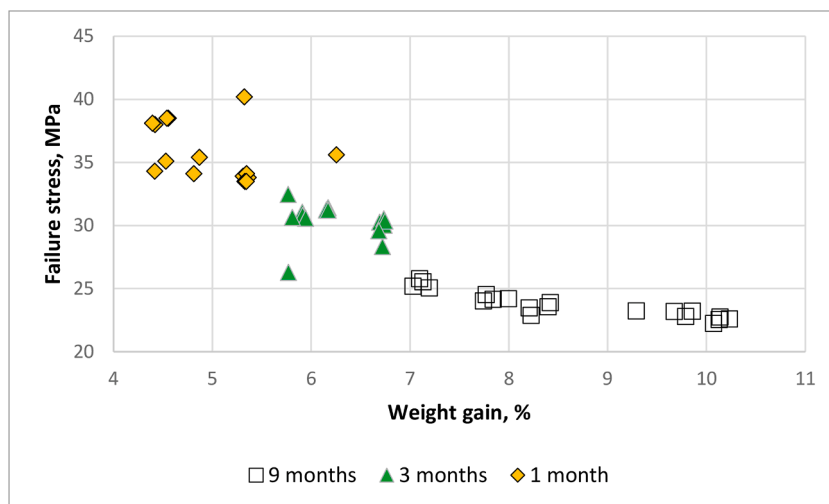
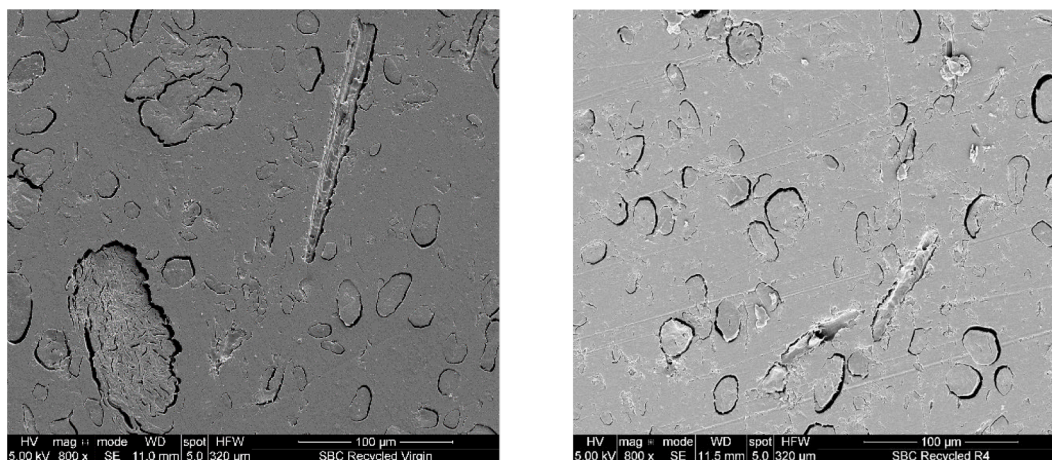**Fig. 7.** Strength versus weight gain for each tested flax-PLA specimen.

Fig. 8. SEM images of nine month aged specimens. Left: Virgin, Right: recycled four times.

Table 4

Influence of seawater aging on transition temperatures and densities.

Material	Tg (°C)		Tm (°C)		Density (g.cm^{-3})	
	Unaged	9 m SW	Unaged	9 m SW	Unaged	9 m SW
V	63.3	63.5	180.5	174.9	1.311	1.328
R1	62.0	63.3	177	174.3	1.315	1.303
R2	61.4	61.8	178.5	175.5	1.314	1.303
R3	61.1	61.6	175	173.7	1.315	1.285
R4	61.4	62.3	175	174.9	1.315	1.296

recycling.

Examination of specimens by tomography revealed very few features and no voids, so no images are shown here. Polished sections cut transverse to the tensile specimen loading direction provided more information. Both materials appear compact with no sign of porosity, which confirms the conclusion from tomography. The main difference between virgin and recycled specimens is the more homogeneous appearance of the latter. This is presumably due to larger fibres and heterogeneous matter being progressively broken down into smaller elements, Fig. 3.

Influence of seawater aging on properties

Immersion in natural seawater

The weights of the five flax/PLA materials over a nine-month immersion period all show large increases, Fig. 4a.

PLA alone in seawater at 40 °C shows a slowly increasing weight increase between 1 and 2% for the first six months, followed by a rapid increase to over 4%. At that point, after around eight months in seawater, the specimen was completely brittle and it broke into four pieces during weighing, Fig. 5. A similar degradation rate was noted in a previous PLA study under these aging conditions [12].

The flax reinforced specimens show much higher weight gains, as flax fibres can absorb over 20% moisture by weight [40,41]. It is interesting to compare the recycled specimen weights to the virgin material, which stabilizes around 7%; the R1 and R2 specimens stabilize around 8% while specimens R3 and R4 are continuing to gain weight around 10%. There is thus a small difference in diffusion behaviour depending on the number of recycling steps. The fact that the virgin material weight gain stabilizes suggests that fibres have saturated and that the matrix of that material is not yet degraded. The recycled materials R3 and R4, with the same amount of fibres, do not stabilize and show an accelerating weight gain similar to the unreinforced PLA, suggesting that the matrix degradation has started. This is confirmed by the desorption plots, Fig. 4b, which indicate weight loss for all samples
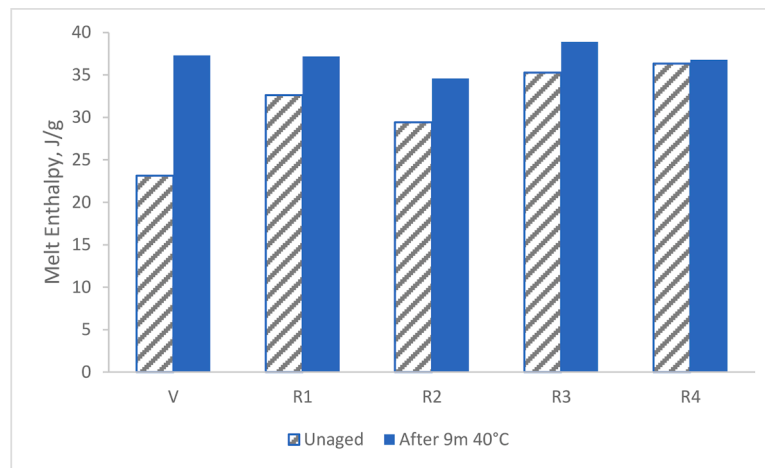
Fig. 9. Melt enthalpies of flax-PLA unaged and after nine months in seawater (variability ± 2 J/g).
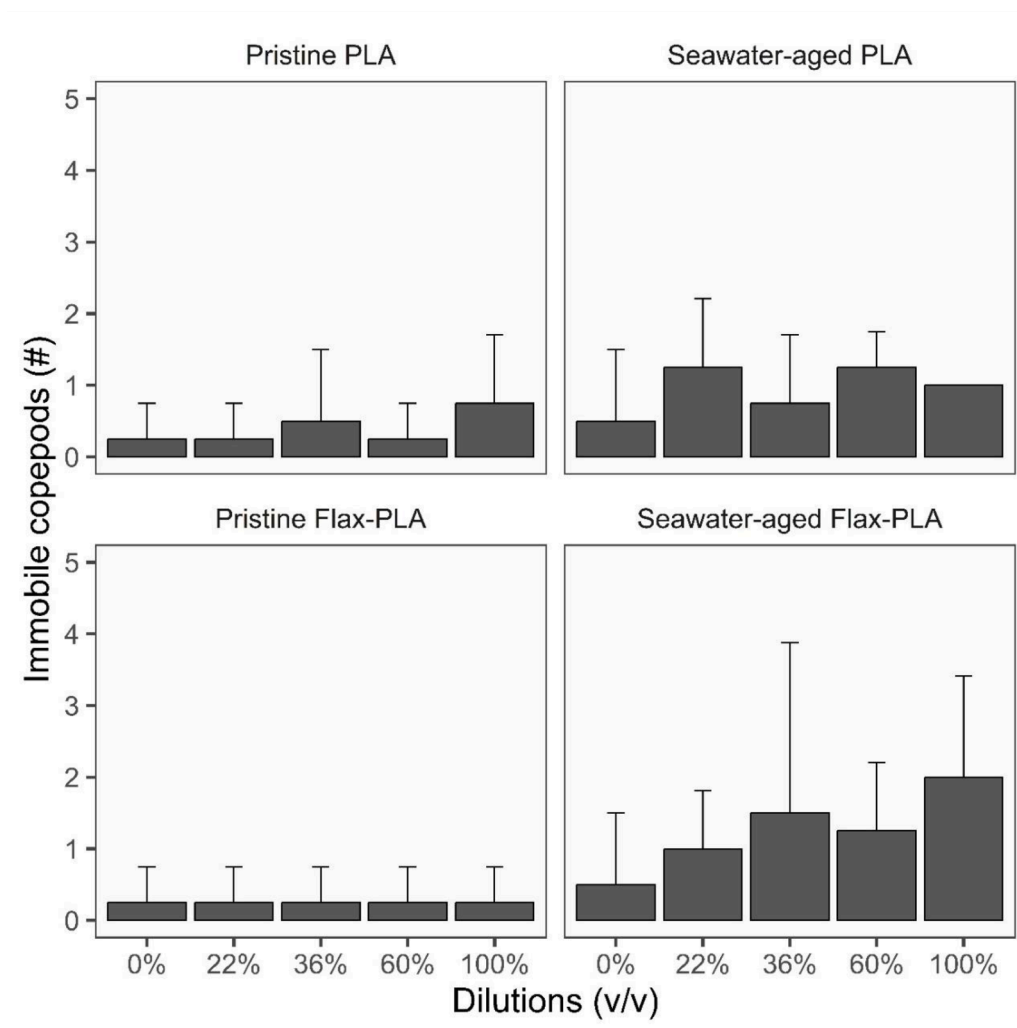

Fig. 10. Bar chart of immobilization of N. spinipes, with the mean count of immobile copepods (#, $n = 4$) after 96 h with corresponding standard deviation (SD) versus the dilutions (%) of leachates from A) pristine PLA; B) seawater-aged PLA; C) pristine flax-PLA; D) seawater-aged Flax-PLA. The initial number of copepods was 5 # in all vials.

with respect to their initial dry weights.

Additional information was obtained from GPC analysis of selected specimens, Table 3.

These results indicate that four recycling steps have not significantly affected the molecular weight compared to that of the unreinforced polymer. They also show that, in contrast to the very severe degradation of the unreinforced matrix after 9 months' immersion, the matrix of the flax reinforced composite is only partially hydrolysed by this aging

condition.

Tensile tests after aging

Tensile tests were performed on wet aged specimens after one, three- and nine-months' immersion in natural seawater. Fig. 6 shows the results compared to the unaged values.

The increases in failure strain with immersion time suggest that for one- and three-month durations in water plasticization is the main mechanism. After nine months the failure strain drops to unaged values. When measured strength is plotted versus the weight gain of each sample before testing, Fig. 7, there is some variability but a general decreasing trend is observed. The values appear to stabilize at the highest weight gains.

Scanning electron microscopy on polished sections was again used to examine the material microstructure. Fig. 8 shows images of the materials aged for 9 months in seawater, taken at the same magnification as the images of the unaged specimens in Fig. 3. Comparison between Figs. 3 and 8 clearly shows that the fibre/matrix interfaces in the seawater aged specimens have been degraded. Flax fibres swell in water and retract during drying, resulting in interfacial stresses.

The physical properties before and after immersion for 9 months are summarized in Table 4. There are no large differences between the thermal properties of the five materials after aging but the recycled materials show higher apparent crystallinity than the unaged specimens, Fig. 9. As the number of fibres in each small DSC capsule may vary, only the measured enthalpies are plotted on the figure but the difference for the virgin samples are significant. Assuming 10% by weight of fibres and a 100% crystallinity enthalpy value of 93 J/g [42], this would correspond to an increase in matrix crystallinity from 19% initially to 36% after nine months in water at 40 °C. The severe degradation of the matrix alone (Fig. 5 and Table 3) after this immersion period indicates that the amorphous regions of the composite matrix are sensitive to hydrolysis under these conditions. A higher degree of crystallinity after recycling will therefore be beneficial. After aging for nine months the melt enthalpies of all materials are similar. The composite density is only slightly higher than that of the unreinforced PLA, due to the small difference between densities of the matrix (1.26) and flax fibres (around 1.45) [39]. Densities of aged samples tend to drop with recycling. Given the large amounts of water in the specimens after nine months this may be caused by volume increase due to swelling.

The DSC transition temperatures, Tg and Tm, and the density for each material before aging and after 9 months' immersion are given in Table 4 (three samples of each, temperature variation ±1 °C, density variability ±0.001 g/cm^3).

Ecotoxicity

Neither leachates from PLA nor from Flax-PLA (up to 100% v / v) induced immobilization of *Nitokra spinipes* (Fig. 9), suggesting that leachates from PLA and Flax-PLA had no acute effects on the tested organisms. The harpacticoid copepod, *N. spinipes*, was exposed to the leachates of pristine and seawater-aged biocomposites in two independent batches, and the immobile copepods were counted for the first ninety-six hours (Fig. 10). In the batch of pristine biocomposites, the average counts of immobile copepods in the control, pristine PLA exposures and pristine Flax-PLA exposures are 0.3 ± 0.4, 0.4 ± 0.7 and 0.3 ± 0.4 #. Neither leachates of pristine PLA nor leachates of pristine flax-PLA had an effect on the number of immobile copepods ($p > 0.05$, Kruskal-Wallis). Similarly, both seawater-aged PLA and seawater-aged Flax-PLA did not induce immobilization of *N. spinipes* ($p > 0.05$, Kruskal-Wallis). After 96 h exposure, 0.5 ± 1, 1.1 ± 0.7 and 1.4 ± 1.4 # immobile *N. spinipes* were found in the control, seawater-aged PLA exposures and seawater-aged Flax-PLA exposures respectively.

These results for PLA leachates are in line with the results of Bejgarn et al. [32], who observed that the leachates from commercial PLA did

not cause the mortality of *N. spinipes* even at a higher plastic-to-water ratio (100 g / L). At a similar plastic-to-water ratio (100 g / L), Gewert et al. [45] confirmed that leachates from polystyrene PS, Polypropylene and polyethylene terephthalate had no lethal effects on *N. spinipes* except for polyvinyl chloride (gloves) with a high content of additives. It should be noted that the chemical profiles of both PLA and flax-PLA (Table S1) are relatively pure compared to commercial plastics reported by Bejgarn et al. [32] and Gewert et al. [45]; this is expected as the biocomposites in the present study are still test specimens and not final products. Future studies are recommended to assess both potential acute and chronic effects of the leachate of final biocomposite products recycled from the real environment on aquatic species.

Although the leachates from seawater-aged biocomposites had no acute effects in *N. spinipes* (Fig. 9), we observed qualitative changes of the volatile organic components (VOCs) profiles in both seawater-aged PLA and flax-PLA (Table S1). For example, lactic acid was observed in seawater-aged flax-PLA but not in pristine Flax-PLA (Table S1), suggesting the occurrence of hydrolysis of Flax-PLA during the two-month seawater aging [46,47]. For PLA, lactic acid was observed in both pristine and seawater-aged samples, however, we cannot conclude on either the formation of lactic acid or the hydrolysis of PLA since no internal-standards were included during the thermal extraction. Future studies are recommended to verify the hydrolysis of biocomposites by quantifying the specific compounds selected according to the non-target screening with internal standards [31,44].

Conclusions

Addition of 10% by weight of short flax fibres to PLA is shown to increase the material stiffness but not affect strength. Recycling the composite up to 4 times does not change tensile or flexural strength nor stiffness. Seawater aging of these materials results in large weight gains, up to 10% after nine months, and up to 60% reduction in strength. However, there is no difference in seawater aging behaviour between virgin and recycled flax/PLA. Both show significant fibre/matrix interface degradation after aging and matrix hydrolysis. The latter is slower when flax fibres are present. Neither leachates from pristine PLA and flax-PLA, nor from materials aged for two months in seawater, induce immobilization of *N. spinipes*. We anticipate that these results will contribute to assessing the sustainability of future bio-based polymer applications and to supporting a transition process to more sustainable plastic materials.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which might be considered as potential competing interests: The author Elke Demeyer is employed by Centexbel, the technical centre for the Belgian textile industry, headquartered in Kortrijk, Belgium. The other authors report no conflict of interest.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcomc.2023.100379.

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