



## Deliverable n°T2.4.3

Report on the biodegradation of the point-of-sale display components

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KAÏROS



**Interreg**   
EUROPEAN UNION  
France ( Channel  
Manche ) England

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## Partners

PP Leader : Kaïros

Partners involved : Portsmouth, UBS, Ecotechnilin

Deliverable N° and name :

- 2.4.3 Report on the biodegradation of the point-of-sale display components

## Content

### 1. Contexte de l'activité 2 – MT2

In this activity, Kaïros has developed new composite materials with a monolithic and sandwich structure using a non-woven preform of lightly oriented flax fibres. This was manufactured by Écotechnilin using the Vandecandelaère scutching process. These materials are intended for use in point-of-sale advertising. As a result, their surface finish must be smooth and free of visible defects to meet the aesthetic requirements of this application.

The environmental footprint of these new materials is reduced thanks to their high recyclability and compostability potential and the use of biosourced raw materials. Kaïros has to ensure that the materials meet the specifications imposed by the POP sector (machinability, aesthetic appearance, light weight, good mechanical strength) while at the same time checking that they can be recycled. These materials are produced using the thermocompression process, which ensures short manufacturing cycle times and low processing costs. Numerous tests, including mechanical strength tests in different environments, UV ageing tests and scratch resistance tests, are carried out to characterise the new material. The results obtained enable a detailed technical data sheet to be drawn up for the material, enabling it to be compared with conventional petro-sourced materials. The manufacture of composite sheets is also intended to produce a prototype of a typical POP product. The production of a piece of POS furniture will demonstrate the robustness of the material in this field of application.



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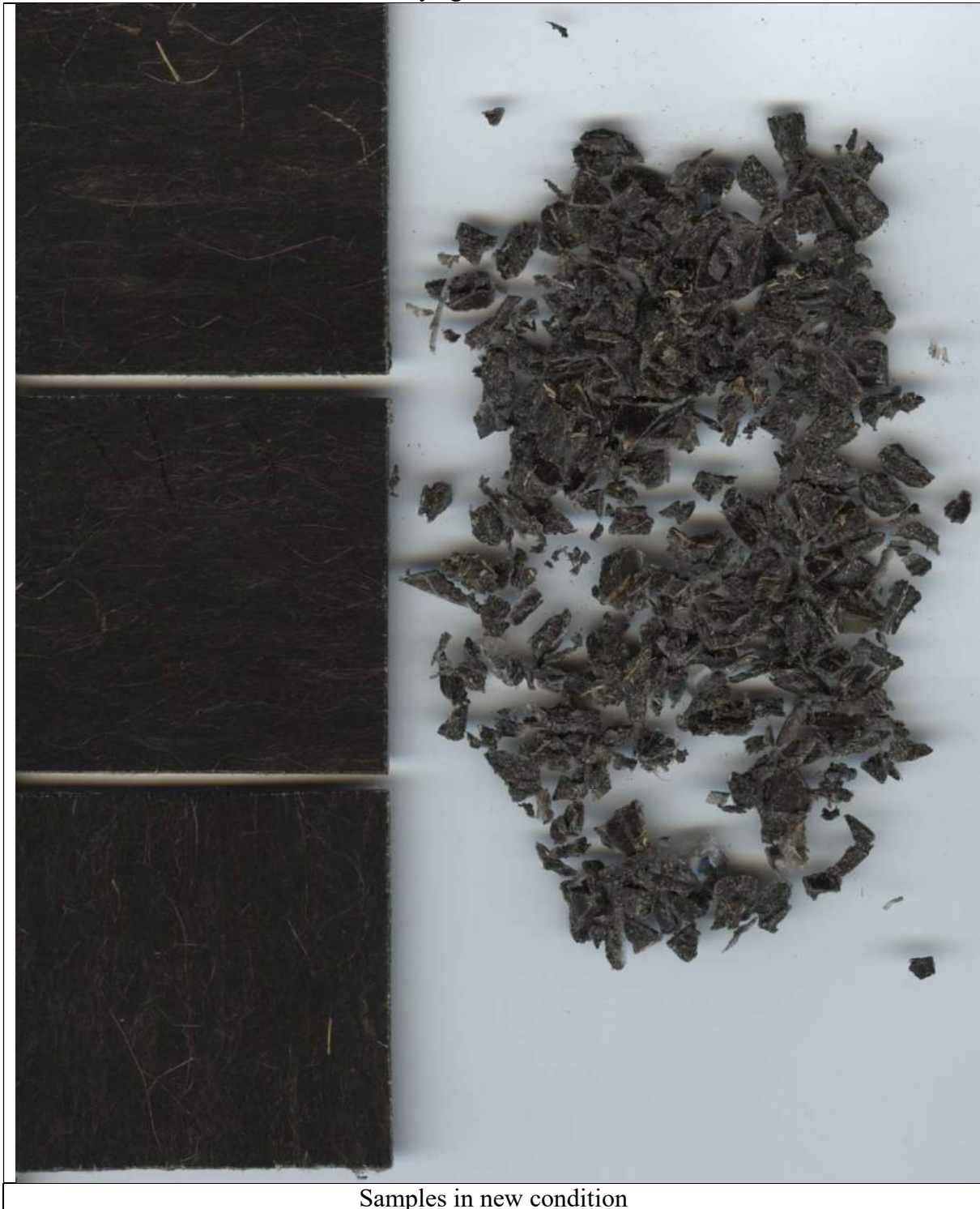
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## 2. Materials submitted to tests

The following panels were tested for biodegradation:

- Monolithic plate 1.20 mm thick with 50.00 mm sides containing 81.90 % certified PLA, 17.80 % flax and 0.30 % dye.
- Monolithic plates 1.20 mm thick with 50.00 mm sides containing 81.90 % certified PLA, 17.80 % flax and 0.30 % dye ground to 8 mm.





### 3. Test for the detection of oestrogenic activity

#### 3.1. Trial method

##### Trace elements

Compounds	Results	Units	Maximum concentrations
Carbone		%	---
Mercure (Hg)	<0.05	mg/kg	0.50
Fluor (F)	<0.05	mg/kg	100.00
Arsenic (As)	<5.00	mg/kg	5.00
Plomb (Pb)	<5.00	mg/kg	50.00
Cadmium (Cd)	<0.40	mg/kg	0.50
Chrome (Cr)	<2.00	mg/kg	50.00
Cobalt (Co)	---	Mg/kg	38.00
Cuivre (Cu)	<3.00	mg/kg	50.00
Molybdène (Mo)	<1.00	mg/kg	1.00
Nickel (Ni)	<2.00	mg/kg	25.00
Selenium (Se)	<0.75	mg/kg	0.75
Zinc (Zn)	<5.00	mg/kg	150.00

**Complies with the requirements of the NF EN 14995**

#### IN VITRO TEST FOR THE DETECTION OF OESTROGENIC ACTIVITY - CELLULAR OEDT METHOD

##### 1. OBJECTIVE OF THE STUDY

The in vitro test for oestrogenic activity using the EMCDDA method enables endocrine disrupters with oestrogenic activity to be detected in a product using a cell test. If oestrogenic activity is found, a dose expressed as oestradiol equivalent is calculated according to the study model.

##### 2. TEST ELEMENTS

NAME	Kaïros monolithic slab, 1.20 mm thick and 50.00 mm square
OPERATOR	Prof. Le Tilly Véronique
PRESENTATION	Brown shavings (8mm shreds) and 5.0*5.0*1.2mm chips

##### 3. PRINCIPLE OF THE STUDY

The in vitro test for oestrogenic activity using the OEDT method is performed on living yeast cells genetically modified to produce the oestrogen receptor (ER) using spectrophotometric detection. These cells contain a reporter gene for oestrogen activity encoding the enzyme  $\beta$ -



galactosidase, the expression of which is controlled by activation of this receptor in the presence of an endocrine disruptor.

If an endocrine disruptor of the oestrogenic type binds to the oestrogen receptor, the latter becomes active and the reporter gene is expressed. This test can therefore be used to assess the level and nature of the associated biological risk.

The quantity of oestrogen-type disruptors is determined by measuring  $\beta$ -galactosidase activity, which is correlated with the quantity of the receptor-disruptor complex (ER-PE) formed.

According to the recent classification established by the OECD (Organisation for Economic Co-operation and Development) working group on endocrine disruptors, the in vitro tests for transactivation of the oestrogen receptor (OECD TG 455) and binding between the oestrogen receptor and endocrine disruptors are classified as level 2 tests.

A recent publication has shown the consistency in terms of sensitivity to the detection of EPs between human cell lines, as used in the OECD TG 455 test, and our yeast model.

#### **4. CONDUCT OF THE STUDY**

##### **Study model : Cell test**

Activation measurements of the recombinant human oestrogen receptor expressed in *S. cerevisiae* (W303.1B) were reproduced independently in triplicate for each concentration tested. Three measurements were taken for each concentration.

In parallel, the oestrogenic activity curve was plotted as a function of oestradiol (E2) concentration.

Estrogenic activity is shown in Figures 1 and 2 as a histogram for each dilution tested.

The results in terms of oestrogenic activity are normalised according to the following formula:

$$\text{Activité œstrogénique relative} = (A_{\text{échantillon}} - A_{\text{min}}) / (A_{\text{max}} - A_{\text{min}})$$

$$A_{\text{min}} = A_{\text{solvant}}$$

$$A_{\text{max}} = A_{\text{E2max}}$$

In order to express oestrogenic activity values in oestradiol equivalents (contained in the culture medium), the calibration curve for oestrogenic activity as a function of oestradiol concentration (E2) is used (figure 3). If the oestrogenic activity value is too low, it is impossible to calculate an oestradiol equivalent. This means that the product has no oestrogenic activity, depending on the study model used.

If, on the other hand, the oestrogenic activity makes it possible to calculate an oestradiol equivalent, we calculate the circulating oestradiol equivalent in humans on the basis of our model. This model incorporates a precautionary principle, a notion of use (depending on the product tested) and a notion of threshold and therefore of potential risk..

##### **Study premise**

The product is absorbed entirely through the skin and diluted in the volume of blood circulating in the body (around 5L). The molecules present in the product are not metabolised by humans into other more or less toxic molecules.

##### **Notion of threshold - potential risk**



The quantities of circulating oestradiol E2 (expressed in g/5L) naturally found in 'humans' are given below:

- Menopausal women/Men: [ $5,4 \times 10^{-8}$  –  $2,7 \times 10^{-7}$ ]  
*équivalent à  $4.0 \times 10^{-11}$  –  $2.0 \times 10^{-10}$  mol/L*
- In non-menopausal women (excluding ovulation) : [ $1,4 \times 10^{-7}$  –  $8,2 \times 10^{-7}$ ]  
*équivalent à  $1.0 \times 10^{-10}$  –  $6.0 \times 10^{-10}$  mol/L*
- In women (ovulation) : [ $2,7 \times 10^{-6}$ ]  
*équivalent à  $2.0 \times 10^{-9}$  mol/L*

According to the study model, a value is considered critical when it is equal to or greater than half the average circulating oestradiol level in an ovulating woman ( $1.0 \times 10^{-9}$  mol/L).

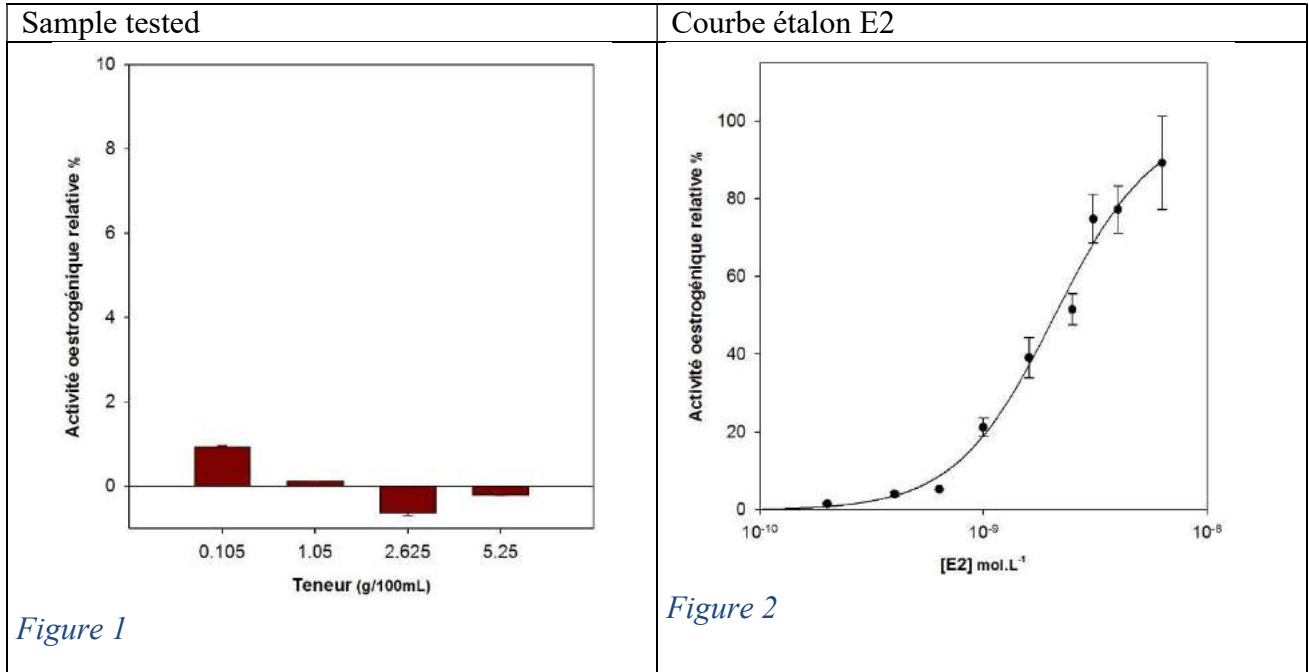
### Cell test protocol

1. Preparation of the pre-culture in a selective medium: the pre-culture, prepared from a glycerol-coated stock of co-transformed *S. cerevisiae* yeasts, is incubated at 30°C with agitation for 14h.
2. Preparation of the culture in enriched medium: 150mL of culture medium is adjusted with pre-culture medium so that the absorbance measured at 600nm is 0.1. The culture was then incubated at 30°C with agitation for 8 h.
3. Induction of oestrogen receptor expression: as soon as absorbance at 600nm is 0.4 - 0.6. The expression of the oestrogen receptor (hER $\alpha$ ) is induced (revealed) by adding galactose to the culture medium.
4. Preparation of the test sample: 20g were incubated in 200mL of acetone at 30°C for 22h with stirring. The solution was filtered and then concentrated by evaporation of the solvent at 30°C. The resulting solution was highly viscous, greyish brown in colour and had a final volume of 1.5mL. Measurements were carried out on the test sample undiluted and diluted in acetone.
5. Stimulation of the culture (4mL) by adding 40 $\mu$ L of pure oestradiol solution (E2) in order to obtain the reference curve (incubation at 30°C with agitation for 6h)
6. Stimulation of the culture (4mL) by adding 40 $\mu$ L of the sample to be tested, more or less diluted, (incubation at 30°C with shaking for 6h).
7. Measurement of transcriptional activity.



### 3.2. Results of the trial

The results of the activity measurements used to assess the biological risk associated with oestrogenic activity are shown below.



**Negative result:** Under the experimental conditions, the product "Kairos Monolithic Plates crushed 8mm/plates 5\*5\*1.2" does not have oestrogenic activity.









#### 4. Disintegration test and preparation for ecotoxicity tests for monolithic sheets 1.20 mm thick and 5 cm square

N° de l'essai	Quantité initiale de biodéchets (kg)	Quantité initiale de matériau d'essai (g)	Durée totale de compostage (jours)	Quantité de matériau > 10 mm (g) et en (%)	Fraction entre 2 mm et 10 mm (g) et en (%)	Fraction inférieure à 2mm (g) et en (%)
Essai 1	10.00	100.20	84.00	3.27 3.26	0.00 0.00	96.93 96.74
Essai 2	10.00	100.40	84.00	18.15 18.08	0.00 0.00	82.25 81.92
<b>Moyenne</b>	<b>10.00</b>	<b>100.30</b>	<b>84.00</b>	<b>10.71</b> <b>10.70</b>	<b>0.00</b> <b>0.00</b>	<b>89.59</b> <b>89.32</b>

The disintegration test carried out using 5\*5\*1.2 chips in the composting unit is within the limits of compliance with the requirements of NF EN 14995. However, the use of 8mm shreds at 10 times the normal dilution dose did not reveal the slightest residue after the 12-week composting period (see photo "preparation for ecotoxicity tests").

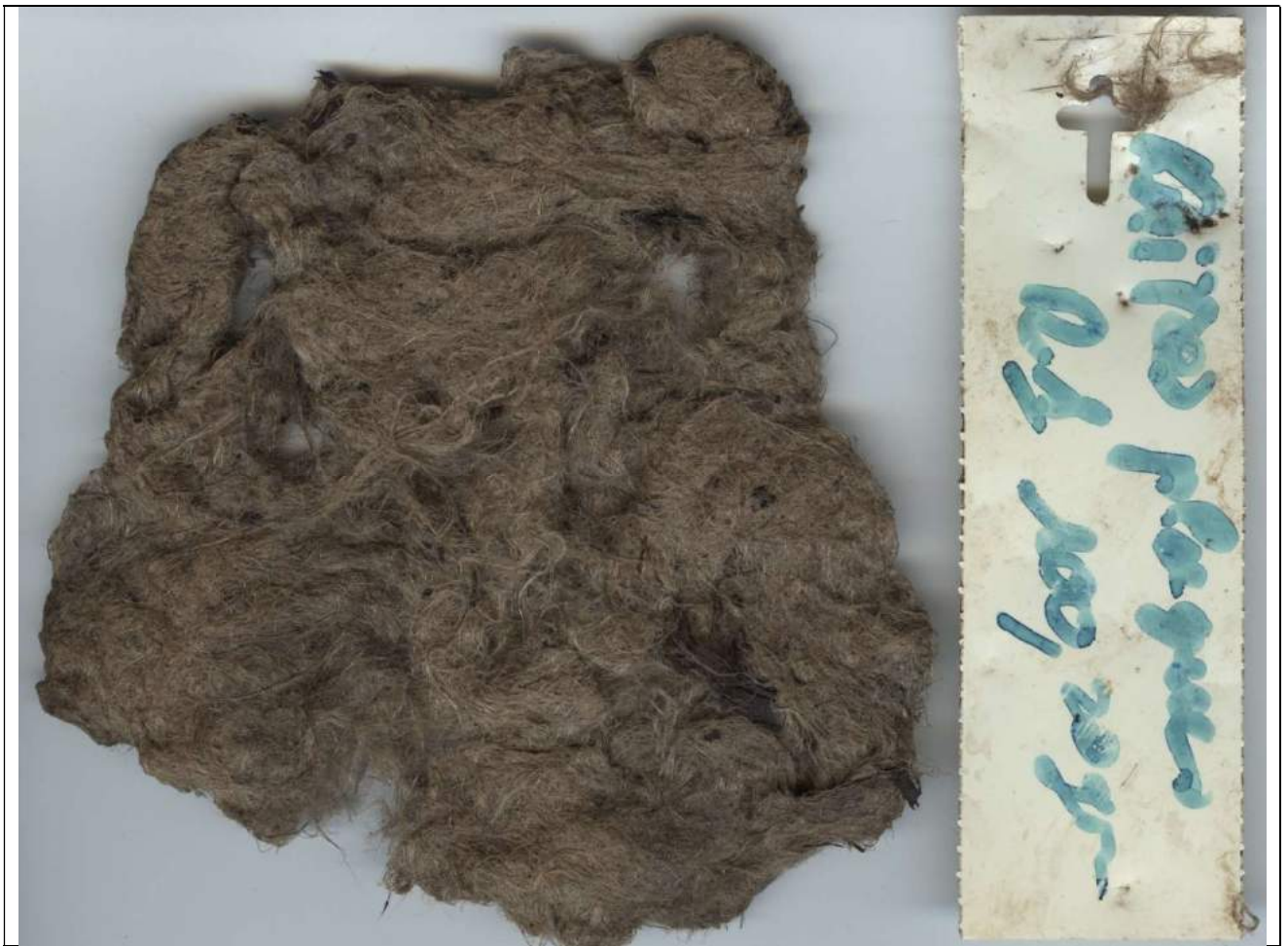
The product in its shredded state fully complies with the requirements of NF EN 14995..



			
Refus >10mm	Fraction > 2mm et < 10mm	Refus >10mm	Fraction > 2mm et < 10mm
Control compost after 12 weeks of composting		Fraction > 2mm et < 10mm Fraction > 2mm and < 10mm Preparation for ecotoxicity tests Control compost with "Kairos monolithic slabs 1.20 mm thick crushed to 8 mm" 10% mass after 12 weeks of composting..	
			
Refus >10mm	Fraction > 2mm et < 10mm	Refus >10mm	Fraction > 2mm et < 10mm
Disintegration Mix compost with " <b>Kairos monolithic slabs 1.20 mm thick</b> " at 1% mass after 12 weeks of composting. Repetition 1		Disintegration Compost mixed with "Kairos monolithic slabs 1.20 mm thick" at 1% mass after 12 weeks of composting. Repetition 2	



**Scanning of residues obtained from 5\*5\*1.2**



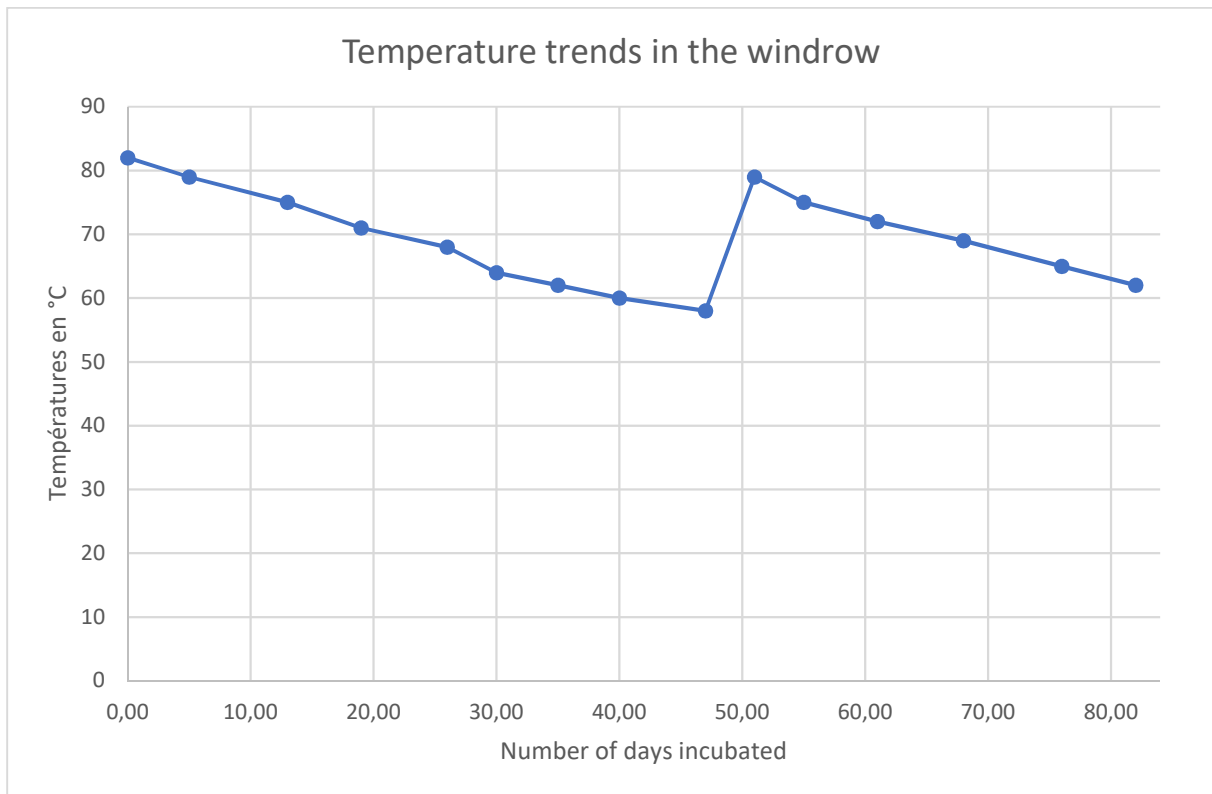
Repetition 1 – Filamentous residue obtained - 3.27 grams for 100.20 initial grams



Repetition 2 – Filamentous residues and a few slightly disintegrated platelets - 18.15 grams for 100.40 grams initial weight



## Validation elements



## Average composition of Humipro (initial mixed compost, of the same nature as the incubation compost) :

- NPK: 2; 1; 3 (on dry product)
- 62.3 % dry matter
- Fertilising units in organic form
- OM: 74 % on dry matter or 46.1 % on raw product

## Incubation compost:

Burial of test bags (disintegration (1% mass) and ecotoxicity (10% mass)) and controls in a fermentation windrow at a depth of 80 cm. The ventilated windrow is made up of a mixture of 70 t of agri-food wastewater treatment plant sludge and 60 t of green waste.

## Analysis of the compost obtained

CARACTERISATION DE LA VALEUR AGRONOMIQUE				Résultats exprimés sur		Critères NF U 44-051		Observations et paramètres calculés
DETERMINATIONS		Symboles	Unités	sec	brut	Seuil de la norme	Conformité à la norme	
Matière sèche (NF EN 12880)	MS	%			<b>65,4</b>	>= 30	Conforme	C organique : 314 g.kg-1 de sac 205 g.kg-1 de brut N organique : 1,46 % brut Rapport C/Norg : 13,8 Rapport C/Nr : 14,1 <i>Conforme</i> (Seuil de la norme > 8)* (N-NO <sub>3</sub> +N-NH <sub>4</sub> +N <sub>uréique</sub> ) / NT : Inf à 3,6 (%) <i>Conforme</i> (Seuil de la norme < 3,3%)* *Excepté pour les Amendements Organiques avec engrais L'expression des résultats en % est équivalente à l'expression en kg/Quintal. Pour convertir ces résultats en g/kg ou kg/T, il vous suffit de les multiplier par 10.
Humidité (NF EN 12880)	H	%			<b>34,6</b>			
pH (M.L. selon NF EN 15953)				7,7				
Conductivité (M.L. selon NF EN 12176)	CE	mS.cm-1		<b>0,77</b>				
COMPOSITION DU PRODUIT								
Perte au feu de la M.S. (NF EN 12879)	MO	%	62,8					
Perte au feu de la M.S. (NF EN 12879)	MO	%		<b>41,1</b>		>= 25	Conforme	
Matières minérales (NF EN 12879)	MM	%	37,2	<b>24,3</b>				
Azote Kjeldahl (NF EN 13342)	NTK	%	2,23	<b>1,46</b>				
Azote global (NTK+N-NO <sub>x</sub> )	NT	%	2,23	<b>1,46</b>		< 3	Conforme	
Rapport MO/N organique				<b>27,6</b>				
Azote ammoniacal	N-NH <sub>4</sub>	%	inf à 0,001	<b>inf à 0,001</b>		La norme s'applique par défaut sur le brut		
Azote nitrique	N-NO <sub>3</sub>	mg.kg-1	inf à 15,3	<b>inf à 10,00</b>				
Azote uréique (M.L.-spectrophotométrie)	N <sub>uréique</sub>	%	< 0,03	<b>&lt; 0,02</b>				
Phosphore	P <sub>2</sub> O <sub>5</sub>	%	1,25	<b>0,82</b>			< 3	Conforme
Potassium	K <sub>2</sub> O	%	2,80	<b>1,83</b>		< 3	Conforme	
Magnésium	MgO	%	0,60	<b>0,39</b>				
Calcium	CaO	%	3,75	<b>2,45</b>				
Sodium	Na <sub>2</sub> O	%	0,04	<b>0,03</b>				
Total Nr + P <sub>2</sub> O <sub>5</sub> + K <sub>2</sub> O					<b>4,11</b>		< 7	Conforme
Chlorure	Cl	g.kg-1						
Soufre	SO <sub>3</sub>	%	0,65	<b>0,43</b>				
Aluminium	Al	%						
Fer	Fe	mg.kg-1			Valeurs limites		<p>Norme NF U 44-051 Teneurs relatives, en % de la valeur limite</p>	
Manganèse	Mn	mg.kg-1			120			
Chrome	Cr	mg.kg-1	<b>45,9</b>		300			
Cuivre	Cu	mg.kg-1	<b>105</b>		60			
Nickel	Ni	mg.kg-1	<b>27,2</b>		600			
Zinc	Zn	mg.kg-1	<b>119</b>		18			
Arsenic	As	mg.kg-1	<b>4,1</b>		3			
Cadmium	Cd	mg.kg-1	<b>0,19</b>		180			
Plomb	Pb	mg.kg-1	<b>12,5</b>		2			
Mercuré (M.L. AUREA17-AME-IT-011)	Hg	mg.kg-1	<b>inf à 0,10</b>		12			
Sélénium	Se	mg.kg-1	<b>inf à 0,5</b>					
Molybdène	Mo	mg.kg-1						
Bore	B	mg.kg-1						
Cobalt	Co	mg.kg-1						

Inertes selon NF U44-164

Humidité : 44,00 %

Poids sec : 555,8 g

## MASSES D'ÉLÉMENTS SECS (en g)

Mailles (en mm)	Cailloux Calcaire	Verre	Métaux	Plastiques durs, textile	Films, PSE	Pourcentage du poids sec
> à 5 ronde	19,94	0,00	0,00	0,02	0,00	3,59 %
De 2 à 5 ronde	10,46	0,00	0,16	0,10	--	1,93 %
< 2 ronde	23,57	--	--	--	--	4,24 %

## INERTES (en % du poids sec)

Désignation	Cailloux Calcaire	Verre	Métaux	Plastiques durs, textile	Films, PSE	INERTES TOTAUX
Inertes >5 mm	3,59	0,00	0,00	0,00	0,00	3,59 %
Inertes totaux	9,71	0,00	0,03	0,02	0,00	9,76 %

## CONFORMITÉ AUX NORMES NF U 44-051 (2006) ET NF U 44-095/A1 (2008)

En % du poids sec	Verre, et métaux > 2 mm	Plastiques durs, textile > 5 mm	Légers > 5 mm	Lourds > 5 mm	INERTES TOTAUX
Votre produit	0,03	0,00	0,00	3,59	9,76 %
Seuils	2,00	0,80	0,30	-	-

The ecotoxicity report is currently being drafted by the laboratory and will be delivered at the end of July.

## 5. Conclusion

This composite material for point-of-sale advertising, developed as part of the FLOWER project, was subjected to oestrogenic detection, biodegradation and ecotoxicity tests in accordance with standard NF EN 14995. The tests were carried out using two samples: firstly 5x5x1.2 cm sheets and secondly 8 mm shredded material.

Under the experimental conditions, both samples showed no oestrogenic activity. In terms of biodegradation, the 5x5x1.2 cm wafers did not meet the requirements of the standard. On the other hand, the 8 mm ground product fully complies with the requirements of standard NF EN 14995. The ecotoxicity report is currently being drafted by the laboratory.