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Evaluation of Material Biodegradability in Real Conditions–Development of a Burial Test and an Analysis Methodology Based on Numerical Vision

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This work validated a burial protocol for *in situ* testing and presents a robust, repeatable and time-saving technique to measure degraded areas in the sample, i.e. an image analysis method. 1440 specimens of degraded samples have been compiled in a data base. To this end, twenty samples presenting different levels of biodegradability (i.e. PHBV/HV, PLA, PCL, PCL-Starch, paper, PE, PE-Starch) were buried at 4 different locations and then disinterred at 4, 6, 9, 12, 18, and 24-month intervals. The biodegradation levels of these samples were determined by computing weight and area loss. Weight loss was measured after careful cleaning, whereas area loss was quantified using image analysis. Image analysis gives reliable information on visual pollution while only requiring a rudimentary and thus quicker cleaning of the samples.

KEY WORDS: Soil burial test; polymer biodegradation: weight loss; human estimation; image analysis; in situ testing.

INTRODUCTION

Polymer materials have considerably improved our every day life. The use of these materials continues to grow: for instance, the annual French consumption is 5 million tons. Their application range is very large: packaging, in building, consumer goods, in agriculture, etc. This, of course, means an increase of plastic waste quantity and poses the problem of how to process and eliminate these waste materials [1, 2].

Recent legislation on packaging wastes [3] has reenforced the need to find new ways of processing waste materials. The biodegrading of wastes is one alternative, hence new materials must be designed which offer better biodegradability [4, 5, 6]. When mixed with organic waste or buried in soil, these materials would be totally eliminated by microorganism activity. The most promising market sectors for these products would be packaging; for domestic use (i.e., films and bags) and in agriculture (e.g. mulching and small tunnels). However, developing and selling biodegradable materials implies a very precise measurement of the biodegradability of these products.

Up to now, information on the fate of such products in the environment was very limited. No official methodology or norms have been applied to the study of plastic material biodegradation in soil. Some laboratory experiments have been carried out using soil samples [7, 8, 9, 10, 11]. Other studies, especially in Japan, looked at exposure of samples in real conditions [12, 13, 14, 15]. The method developed during the Japanese studies is the most appropriate when assessing the fate of a material in natural conditions however it is specific to the environment studied. For a given material, samples should be exposed in different biotopes to give a wide range of biodegradation kinetics.

Various methods allow us to estimate film sample

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biodegradation, when put in a solid matrix (soil, compost); these deal with appearance modification, mechanical property changes [16, 17], molecular weight alteration [12] and weight loss [18, 19]. Weight loss evaluation is one of the most accurate and reliable means of estimating the extent of degradation in buried films [20, 21]. However, the weight loss method is less sensitive when soil burial lasts for 3 months or more, because the removal of adhering fungal mycelium and soil particles becomes problematic after long burial periods [7, 18, 22]. Moreover, this method does not estimate the phenomenon of visual pollution caused by a certain material in the environment.

This study examines the degradation kinetics of various materials in real conditions. A protocol has been put forward and then validated for burial of the samples in soil. A new methodology based on image analysis has been developed and validated to accurately measure levels of material biodegradability.

MATERIALS AND METHODS

Material Preparation

Soil burial tests were carried out on twenty films including most so-called "biodegradable" films available in 1994 (see Table I). High density polyethylene (HDPE) was chosen for its "non-degradability" as a negative reference.

5 × 20 cm^2 samples were referenced and then dried at 50°C until a constant weight was obtained. Film samples were inserted ~6 by 6- between two 0,5 × 0,5 cm^2 polyethylene net meshes (Occitania Agri, France). These sample sets were held in frames which made the burial and extraction processes easy.

Soil Burial and Removal

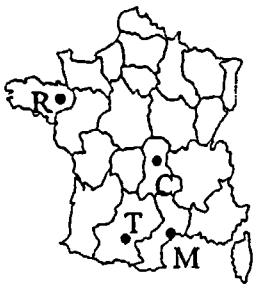
The experiments were carried out in 6 testing periods over 2 years. The experimental field (around 30 m^2) was partitioned into 6 blocks which corresponded to sample removal after 4, 6, 10, 14, 18, and 24 months (Fig. 1a). Three replicates of each sample were randomly located in each block to minimize the effect of soil heterogeneity. Thus, 360 (20 samples × 3 replicate × 6 testing periods) samples were buried at each location. These tests were carried out at four sites in France, where shuttered cropping is intensive and providing a wide variety of soil and climate conditions (Table II). For all sites, 1440 (= 4 × 360) samples have been cut, then dried at 50°C until a constant weight was obtained, weighed, set

Table I. Test Films Used in This Study and Percentage of Weight Loss Obtained on Materials and Four Burial Sites After 2 Years of Exposure

N°	Film based on:	Grade	Thickness (μm)	Weight loss (%)				Biodegradability (1)
				Toulouse	Rennes	Clermont	Montpellier	
A	Polyhydrobutyrate	same	65	96	100	99	94	***
B	hydroxyvalerate [PHBV]		150	77	93	74	45	**
C	Polycaprolactone [PCL]	I	465	94	100	100	100	***
D	with starch	II	27	100	96	100	98	***
E		II	55	61	53	44	32	**
F	Starch with	same	35	99	80	78	72	**
G	biodegradable additives		116	52	37	40	32	**
H	Poly(lacticacid) [PLA]	III	120	62	31	22	88	**
I		IV	354	0,4	0,0	0,5	17	**
J	Polycaprolactone [PCL]	--	47	98	100	100	100	***
K	Cellophane	—	24	100	100	100	100	***
L	Cellophane + 2 nitrocellulose sides	—	25	79	85	95	82	**
M	Cellophane + 2 PVDC sides	--	36	84	75	90	74	**
N	PLA - 50g/m^2	same	369	50	100	100	85	**
O	PLA - 100g/m^2		379	23	100	100	42	**
P	Protein	—	45	100	100	100	100	***
Q	PE + Starch + additives	V	130	0,1	0,1	0,1	0,0	*
R	PE + Starch + oxidant additives	VI	51	0,1	-0,2	0,0	-0,1	*
S	Paper	—	365	100	100	100	100	***
T	Polyethylene [PE]	—	11	0,0	0,7	-0,5	-0,2	*

(1) *: non biodegradable, **: intermediary biodegradable, ***: biodegradable.

Table II. Description of Experimental Sites



	Site	Climate	Soil	Average temperature after 2 years (°C)	Average temperature after 2 years (mm)
R	INRA Rennes	Oceanic	Silty organic	11.2	102
M	Cemagref Montpellier	Mediterranean	Silty sandy	14.5	98
C	Cemagref Varennes	Continental	Sandy	10.6	99
T	INRA Auzeville	Continental	Silty sandy clay	14.0	99

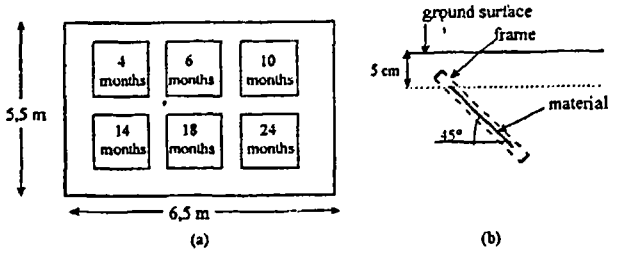


Fig. 1. Burial of test specimens: (a) map of the experimental field with 6 blocks, (b) the material exposure.

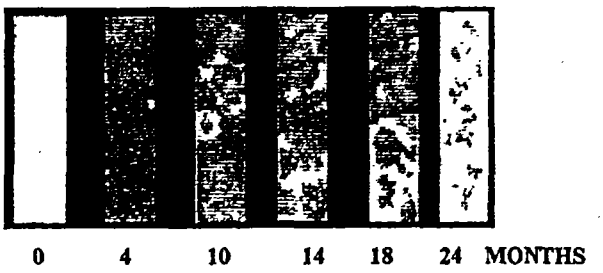


Fig. 2. Original material "B" (0 month) and extraction after 4, 10, 14, 18 and 24 months of exposure in Rennes soil.

in a frame and buried. The ground of each parcel was dug beforehand to a 30-cm depth. Large lumps, plant waste and other debris were removed. Each sample was buried with a 45° angle to avoid water stagnation (Fig. 1b) and covered with 5 cm of sieved earth to prevent superficial drying. During the exposure period, burial sites were regularly weeded.

After a given degradation time, samples were carefully removed to avoid damage. Then, they were introduced into individual referenced envelopes respecting the depth gradient. Upon retrieval, samples were gently brushed to remove adhering soil and mycelium fragments, while preserving the original shape. After image analysis, samples were rinsed in deionized water and dried at 50°C to determine the weight loss.

Image Analyses

Although human vision is probably the most useful sense when assessing quality, it is subjective and can lead to errors. Therefore, in numerous industries, image analysis is replacing human vision to automatically control aspect defects, shape and color, etc. [23]. As human vision is used to characterize samples after degradation [7, 24], we suggest replacing this subjective assessment with image analysis.

When the degraded film sample is set against a background, the more advanced the degradation is, the more the background area is visible in the sample holes (Fig. 2). If the background color is sufficiently different from the studied material, it is possible to distinguish two parts in the image. A black background was suitable for about 90% of the films. For the ten percent remaining, a white background was used.

A monochrome CCD (Charge Coupled Device) camera captured the image of the sample fragments which were illuminated by 4 halogen lamps (Fig. 3). After a 8 bit A/D conversion, the numerical image was converted into a matrix made up of 512 × 512 elements (or pixels). Each pixel had a luminance ranging from 0 (when black) to 255 (when white). Then, a numerical analysis was carried out by a piece of software specially written by *Cemagref*. The region of interest was the polygon surrounding the sample. This program took into account the occurrence of sample shrinking. In that case, the operator had the opportunity to define a smaller window around the sample edges. Once this area was defined, its luminance histogram was com-

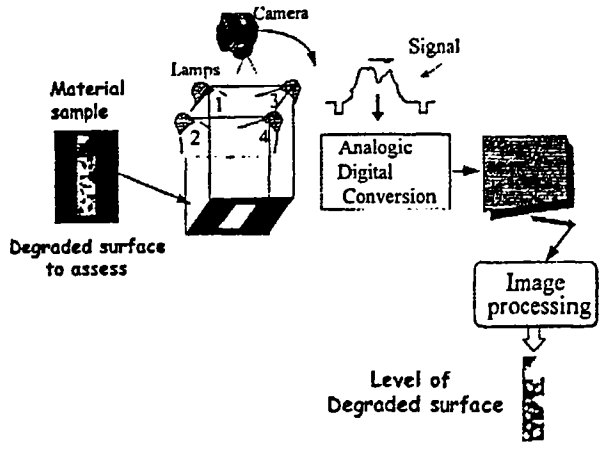


Fig. 3. Principle of image acquisition.

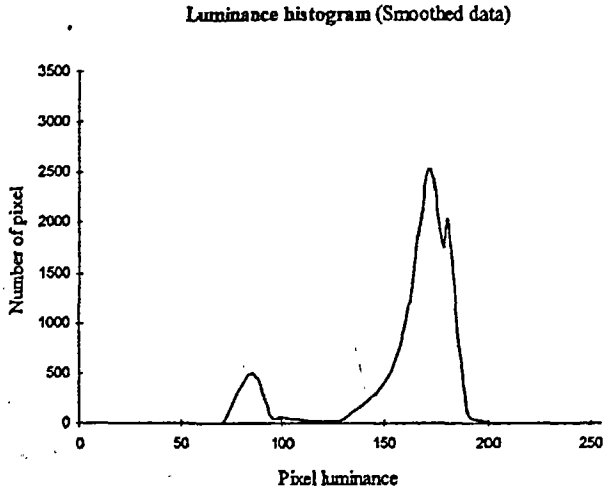


Fig. 4. Example of luminance histogram.

puted (Fig. 4). On the x axis, the gray levels of the pixels ranging over 256 levels; the y axis corresponds to the number of pixels having the x gray level. This histogram was generally bimodal, i.e. had 2 peaks, representing two different populations; the first population (or mode)—the darkest pixels—corresponds to the background i.e. the degraded part; the second mode—the brightest pixels—corresponds to the original material. In general, in the luminance histogram, the limit between these two parts was not clearly defined, the modes partially overlap.

Image processing allowed us to determine this threshold in order to compute the ratio of the dark over total surface pixels. This threshold value varied from one image to another, because of differences in sample colors, global luminance of the scene or lighting variations. To set up an automated process, an adaptive thresholding procedure was developed. At the beginning of the degradation process, when degraded area was null, the first mode was missing. Consequently, this procedure was based upon the second mode only, i.e., the one corresponding to the material. The degraded area was computed as the number of pixels, whose gray levels were lower than the threshold. This corresponds to the integral value of the luminance histogram from zero to the threshold.

- For each sample and each condition (i.e., a given location and a given duration), three replicates were analyzed. They were averaged to give the final "visual pollution" index.

Robustness Assessment

Before testing, the robustness of this method was assessed. A reference sample presenting a 15% degradation level (histogram shown in Fig. 4), was submitted to various lighting conditions: one and then two lamps out of a set of four were turned off. The degraded area was computed in each case to assess the effect of luminance variability on the results.

Image Analysis and Human Perception

Several authors [7, 24, 25] have conducted visual assessment of degradation. The results obtained show that human assessment can be naturally biased. Thus, the degraded surface measured by image analysis was compared to human visual estimation. Twenty people were asked to estimate the degraded surface of ten material samples by giving a mark between 0 (not degraded) and 5 (totally degraded). For each sample, the marks given by the twenty people were then averaged.

Weight Loss

After image analysis, samples were washed with deionized water and dried at 50°C until a constant weight is obtained. Each sample was weighed before and after degradation using a Mettler AE 163 digital weighing scales (0,1 mg precision). Weight loss was calculated using the following relationship: $W_L = (M_I - M_T)/M_I) \times 100$ (1) where M_I and M_T are the initial weight and the weight at time t, respectively.

For each site, the average percentage of weight loss,

given as a function of time, was the average of the three replicates.

Weight Loss and Image Analysis Correlation

For each measurement technique, errors may occur during the sample extraction and cleaning phases (called "sampling error" or S.E.) or during the measurement process (called "measurement error" or M.E.). These two types of errors (SE and ME) give an overall error which appears as a standard deviation in repeatability tests (SD). Thus, $SD^2 = SE^2 + ME^2$ (2).

For each technique, the overall error can be evaluated by the lack of repeatability of the triplicate outputs.

Let us define $CV_i = s/m$ s: standard deviation for a triplicate and m: average for a triplicate.
Thus, for the 20 samples:

$$SD = \sqrt{\frac{\sum_{i=1}^{n} CV_i^2}{n}} \quad (3)$$ where n: number of triplicate

For each technique, M.E. was easy to evaluate: the whole measurement procedure (after extraction and cleaning) was repeated 20 times on the same sample. The standard deviation gives the measurement error, M.E.

The sampling error (S.E.) can not be experimentally evaluated. However, SE can be calculated from the two other values (Eq. 2). These various errors (SD, SD and ME) were compared for the two measurement methods (weight loss and image analysis).

RESULTS AND DISCUSSION

Optical Changes with Time

During the exposure in natural conditions several phenomena could be observed on the sample. Color changed in association to a microbial growth and/or morphological alterations (mechanical properties, etc.); small stains evolved to form holes and sometimes led to the partial or total disappearance of the sample; thinning of the film sample.

Weight Loss

Firstly, we were interested in the weight loss percentage obtained after the two years of exposure in the four sites. As shown in Table I, three types of degradation can be distinguished: 1) total degradation 2) partial degradation and 3) zero degradation.

The first class represents materials that, after a 2-year burial period, have completely disappeared. In this class, K, S, J, P, A, C and D materials are to be found. The second class corresponds to materials with an intermediary level of biodegradability. The percentage of degradation is linked to material composition. Sample H and I (based on PLA with different additives) show different levels of degradation due to the difference between additives, whereas C and D based on PCL with starch (two grades) show a total degradation. The degradation percentage is also linked to material thickness. For instance, results obtained on pairs of samples show a significant difference depending on thickness. The thinner the sample, the higher the degradation level. Material "I" has been placed in this class because it started to degrade in the site of Montpellier. Finally, three materials (Q, R and T) did not present any signs of degradation, after a 2-year burial period and were placed in the last class. The polyethylene and the material "R" have even been increased in mass.

Table I also showed that the general behavior of each material was generally the same whatever the location, i.e., "totally degradable" materials in Clermont were not "intermediary degradable" in Rennes. There was no correlation between the weight loss and the locations that would show that one biotope was on the whole more adequate for degradation. However intermediary materials, containing PCL and/or starch seemed more likely to degrade in Rennes, Toulouse or Clermont than Montpellier. This was due either to different ecotypes or to climatic conditions, Montpellier having a Mediterranean rain regime. Alternating periods of heavy rainfall and long dry seasons are more frequent in Montpellier and can affect abiotic degradation processes. These materials degraded completely in Clermont and Rennes, and only partially in Toulouse and Montpellier.

Results obtained on certain materials: "T" and "Q" and in particular "N" and "O" materials may cast doubt on the validity of the weight loss method. The mass of these materials actually increased in some cases, due to high adherence level of soil particles and mycelium fragment. For instance, during the fifth exposure period in Clermont, sample "N" mass (Fig. 5) increased by 160%.

The main difficulties posed by this method is the cleaning process either the sample is thoroughly cleaned and some sample material fragments can be lost or the sample is less intensively cleaned and some soil particles

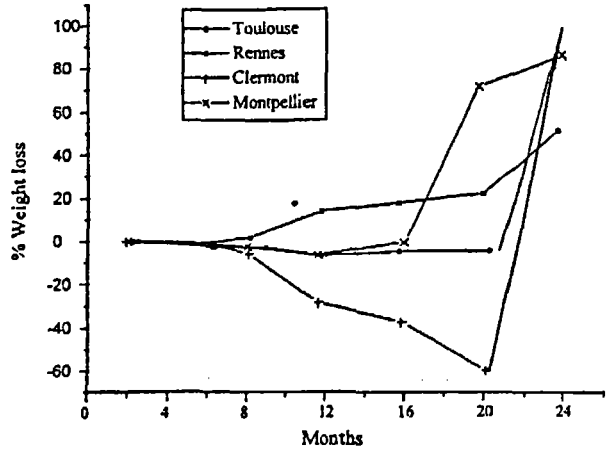


Fig. 5. Percentage of weight loss of the material "N" for four burial sites (this curve should be always positive).

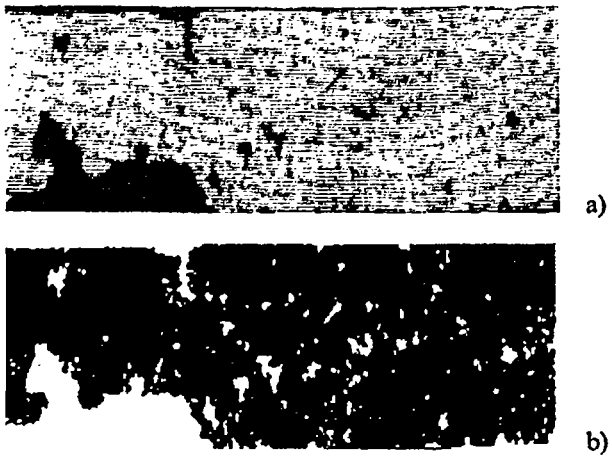


Fig. 6. Original (a) and processed image (b) of material "A" sample (extraction after 6 months of exposure in Toulouse soil).

can remain attached to it. The inaccuracies of the residual material mass measurement, already pointed out by Lee *et al.* and Goheen *et al.* [7, 26] are mainly caused by these experimental difficulties.

In conclusion, weight loss measurement is effective in revealing differences in biodegradation depending on the materials used or the sites concerned. However, this method is subject to criticism, as the sample requires a thorough but not overzealous cleaing to be sure that no material is lost or no foreign matter is added. Image analysis should cope with this obstacle.

IMAGE ANALYSIS

Robustness Assessment

The original image and the image after processing of the "reference" material "A" are given in Fig. 6. The percentage of degraded surface is 15%. Table III shows the degraded areas computed by the software when one (first column) or two (second column) lamps were turned off. Even in these severe conditions, the maximum difference between the various predictions of degraded surface was only 1%. Thus, the software developed at *Cemagref* was considered to be very robust.

Comparison with Human Assessment

For a number of samples (10), the percentage of degraded surface was compared to that estimated through human assessment. Fig. 7 shows a plot of the degraded surface percentage calculated by numerical

Table III. Computed Degraded Surface Obtained When One or Two Halogen Lamps are Turned Off

N° of lights turned off	Computed degraded surface (%)	Lights turned off	Computed degraded surface (%)
None	15.4	1&2	14.6
1	15.3	1&3	14.5
2	14.9	1&4	14.5
3	15.6	2&3	14.9
4	15.7	2&4	15.1
		3&4	14.9

vision against the average human mark. A good correlation is obtained even if the human panel underestimated the lower levels of degradation. This was due to a well known psycho-visual phenomenon. This experiment confirms the need of an objective system to accurately measure the degraded surface.

Image Analysis

Image analysis was carried out before the weight loss measurement. The cleaning process is faster, less time consuming and less tiring. Moreover, weight loss measurement can overestimate (loss of material during extraction and cleaning) or underestimate (accumulation of soil particles or microorganisms) the level of degradation. Image analysis can only overestimate the part of degraded material by loss of material during extraction. For instance, evolution curves of "N" and "O" materials show that the degraded surface gradually increases with time (Fig. 8) whereas weight increases!

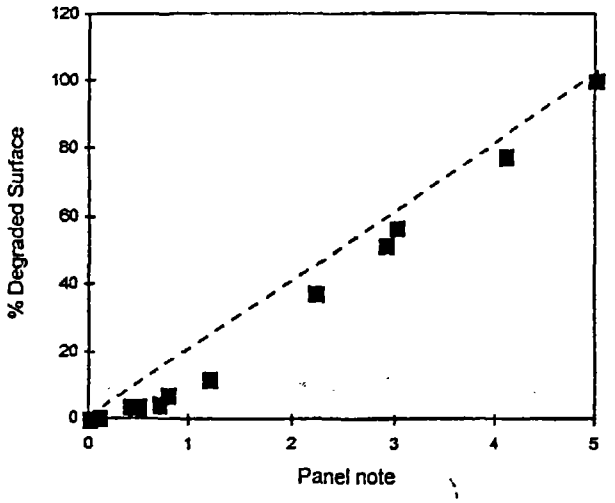


Fig. 7. Relationship between the degraded surface percentage and the note obtain by the panel of human estimation.

Another advantage of image analysis is the mapping of microbial action. Indeed, if sample orientation is carefully recorded during recovery (for instance "top"–"bottom" indicated) and if the sample is not too fragmented, it is possible to study the influence of depth on microbial attack. This is impossible with weighing. No obvious relationship has been established between degradation, depth influence and site (Fig. 9).

Weight Loss/Image Analysis Correlation

For "A" and "B" materials, weight loss and degraded surface indexes have been plotted as a function of time (Fig. 10). The two indexes do not evolve in the same way. These indexes evolve in parallel for "A" material, whereas, for "B" material, the degraded surface develops much more slowly than weigh loss. These different evolution kinetics can be revealed by plotting weight loss against the degraded surface (Fig. 11). The thicker the sample, the less linear the relationship. This is particularly noticeable at the beginning of the experiment, where the thicker material presents a very sharp slope. Thick materials first degrade in their thickness, leading to a sharp weight decrease without modification of the original surface appearance. When thickness continues to decrease, holes appear. Therefore, the degraded surface area increases and simultaneous the weight loss does the same [27, 28, 29]. Consequently, image analysis is a satisfactory technique for estimating visual pollution. It reveals the physical endurance of a material even if this sample has lost much weight. For instance, for material "B" weight loss is estimated to be 80% while surface loss is only 20%!

Finally, the comparison of the reproducibility obtained by the two techniques shows that degraded surface measurement is the most accurate. The standard deviation (SD) of the repeatability test (Eq. 3) is better for the image analysis method: 0.26 as compared to 0.43 with the weight loss measurement. The measurement error is 0.004 for image analysis and about 0 for weight. Thus, the sampling error is SE # 0.26 for the image analysis method and SE # 0.43 for weight loss measurement. As already mentioned, weight loss method is very sensitive to the sampling and cleaning processes. Even if the measurement error is slightly higher with image analysis, the latter is globally more robust. More-

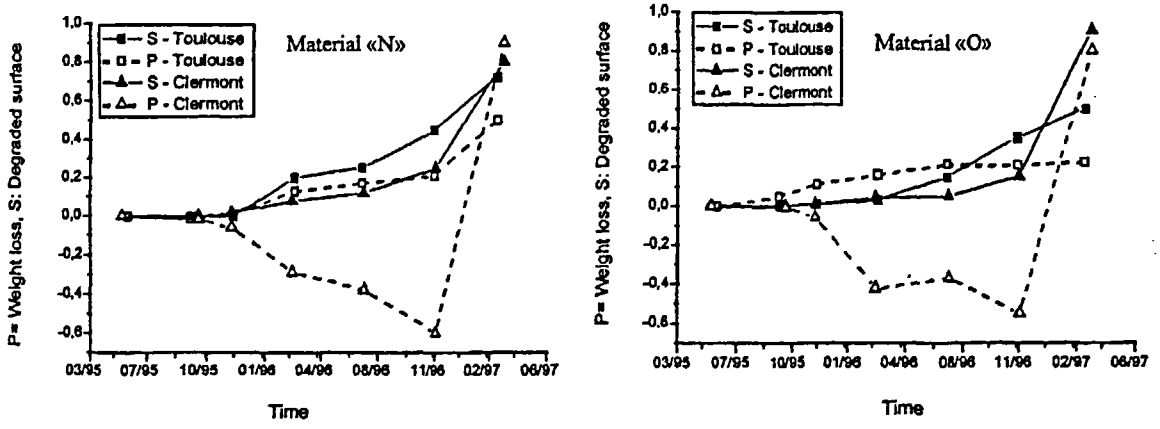


Fig. 8. Degradation of materials "N" and "O": evaluation of visual pollution (degraded surface – S) and the mass pollution (weight loss – P) for Toulouse and Clermont sites.

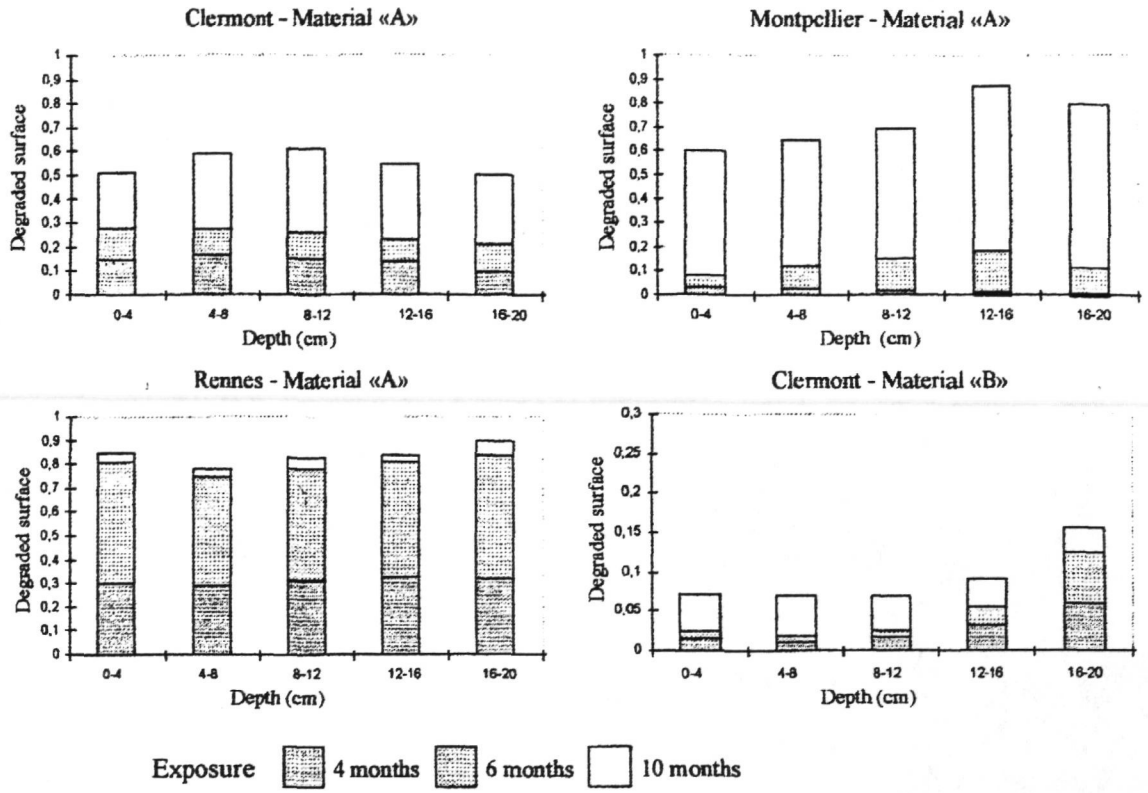


Fig. 9. Degraded area percentage measured at various levels of the same sample buried at 45°; the results are given for material "A" on three locations: Clermont, Montpellier and Rennes and for material "B" at Clermont-Ferrand.

over the cleaning is much easier and faster with image analysis.

Krupp *et al.* [30] asserted that the weight loss method offered proof of "non biodegradability" if its result was negative. Indeed, the polyethylene showed "zero biodegradability" because the weight loss and the degraded surface were negative. On the other hand, a negative result on only weight loss did not inevitably imply zero degradation. "N" and "O" materials accumulate soil particles, but image analysis confirmed that the samples degraded (presence of holes). Image analysis can quantify hole appearance even if the material accumulates weight.

CONCLUSION

Real-life experiments are laborious and expensive. However they are necessary to estimate the *in situ* biodegradability of polymer materials.

The following conclusions can be drawn from our work. A protocol for burying samples in various biotopes is proposed and validated: 1440 samples have been buried on 4 sites. To measure the *in situ* biodegradability, the classic loss weight method was used. Moreover, a new method based on image analysis has been developed. This last technique has been validated with regard to robustness, repeatability and reliability. This method offers three different advantages: a better reproducibility as compared to the classic weight loss method; it allows a faster and less tedious cleaning process and accurately calculates visual pollution. Moreover, it can provide the biodegradability gradient in relation to depth. The variability of the degradation kinetics depending on the site and the thickness of materials has been shown using the biodegradation data bank (20 materials, four sites).

Further works will deal with making the relationship between laboratory and *in situ* experimental results. The aim is to predict *in situ* biodegradability using laboratory results and site characteristics (climate and soil).

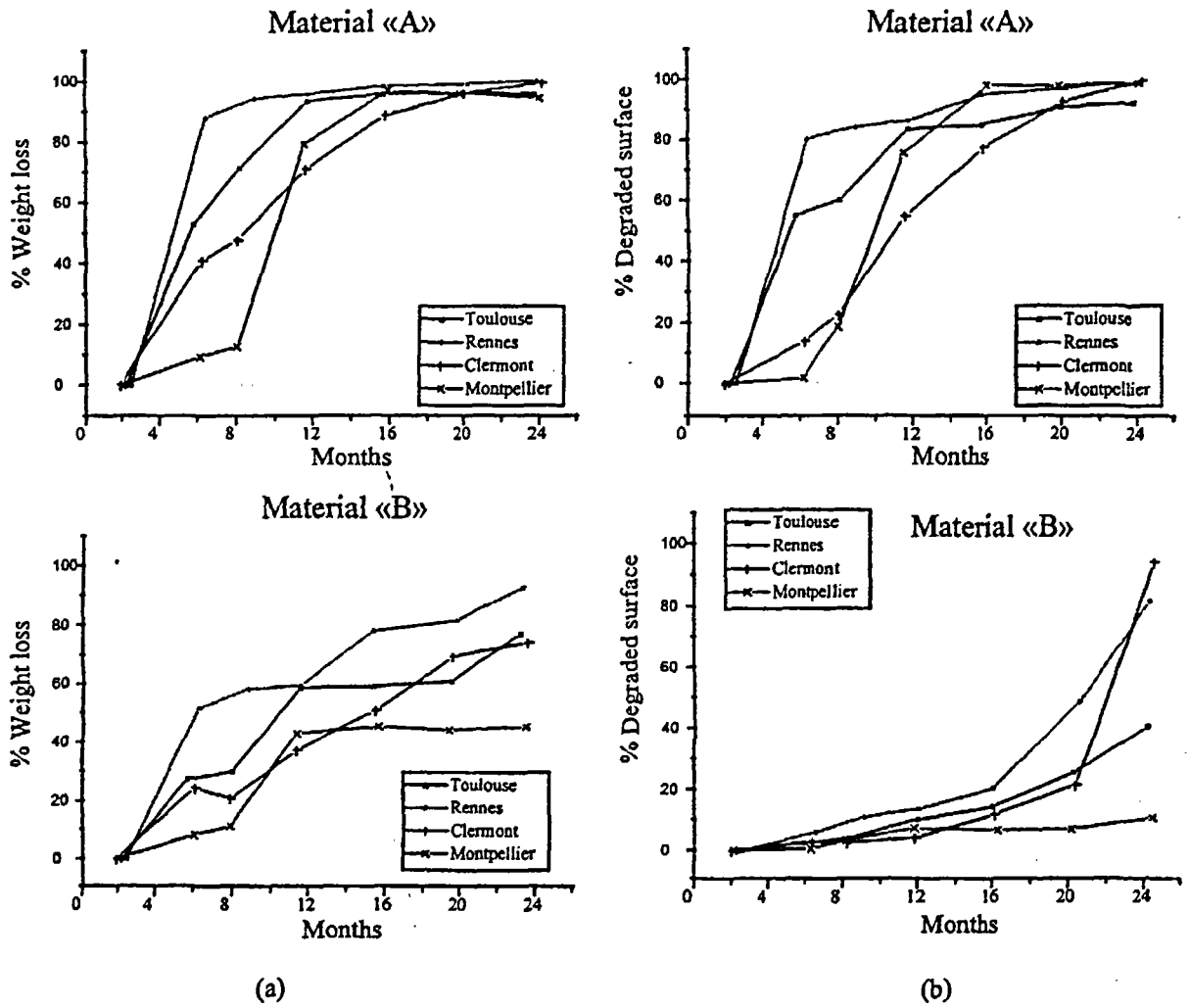


Fig. 10. Degradation of PHBV based materials of two thickness: e = 55 μm (A) and e = 150 μm (B) ((a) weight loss and (b) degraded surface).

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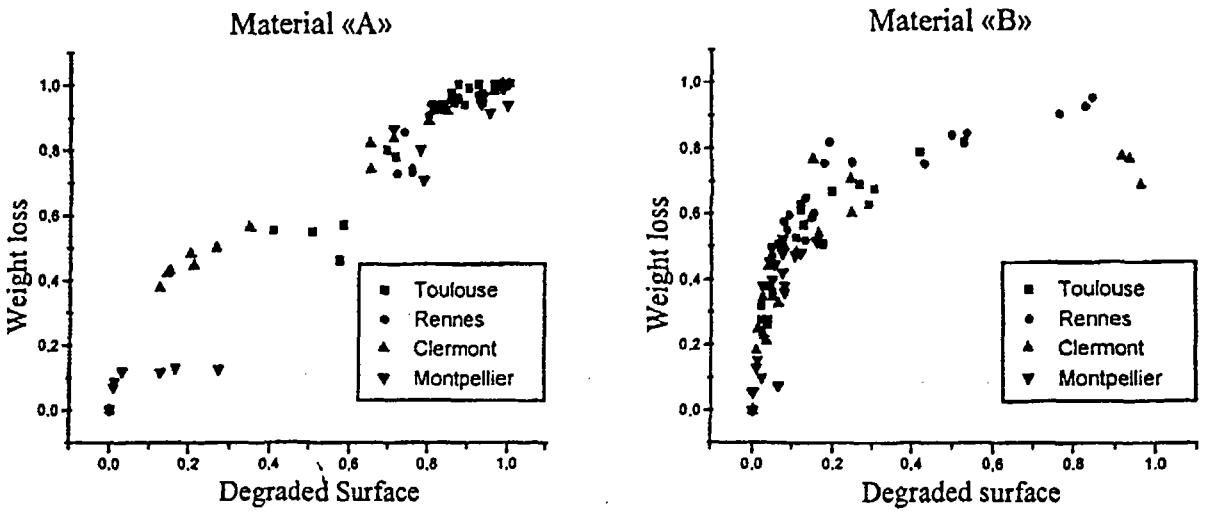

Fig. 11. Comparison between the degradation of "A" and "B" materials with different thickness - A: e 55 μm and B: e = 150 μm.

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