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SEM-Based Surface Morphology and EDX Elemental Characterization of Plastic Plates Treated with *Penicillium notatum*

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Abstract

Plastic waste exhibits a significant environmental challenge as of its resistance to natural degradation, the study aimed to characterize outer layer morphological changes and elemental composition Related to with fungal treatment of plastic plates using imaging electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and basic mapping. A refined isolate of *Penicillium notatum*, collected from soil, was used for fungal exposure. SEM examination revealed surface-associated structures and morphological features; it may be associated using fungal interaction with the plastic material. Assess of 50 size measurements produced a mean of 34.96 ± 9.85 nm, with a median of 35.32 nm and a range of 16.68-56.95 nm. EDX assess detected carbon (56.40 wt%), oxygen (36.31 wt%), chlorine (7.29 wt%), with carbon and oxygen accounting for 92.71 wt% of the detected elemental composition. Elemental mapping showed a widespread distribution of carbon, whereas oxygen and chlorine exhibited comparatively less uniform distributions. These findings provide preliminary evidence of surface and elemental characteristics associated with fungal-treated plastic plates. For, because an untreated control, mass-loss measurements and polymer specific chemical analyses were unavailable, the observed findings cannot independently confirm polymer biodegradation or establish changes in the polymer's chemical structure, the studies incorporating appropriate controls and complementary analytical techniques are required to verify the biodegradation potential of *P. notatum*.

Keywords

Penicillium Notatum;
Electron Microscopy;
Energy-Dispersive X-Ray
Spectroscopy;
Elemental Mapping;
Plastic Waste.



Introduction

Plastic pollution represents a major environmental challenge since many synthetic polymers resist natural degradation, may persist in the environment to long periods. The rising accumulation of plastic waste has promoted the investigation of biological approaches to material deterioration, such as the potential role of fungi in changing plastic surfaces. Fungi are of particular interest due to they can produce extracellular enzymes and metabolites that may help to changes in the surface describes of certain polymeric materials below suitable environmental conditions [1,2]. Fungal influence with plastic surfaces may include the attachment of fungal structures, collection of biomass, and deposition of extracellular substances [3,4]. The interactions can be investigated through microscopic examination of surface morphology, the observation of surface related to structures or irregularities does not necessarily confirm the degradation of the basic polymer, microscopic findings should be explained carefully and supported by additional chemical and numerical analyses [5,6].

Scanning electron microscopy (SEM) is commonly used to examine the surface morphology of materials in different zooming, allowing the finding of localized structures, surface layers and morphological variations [7,8]. When combined with energy dispersive X-ray spectroscopy (EDX), SEM provides data bout the elemental essay of the examined surface. Elemental mapping unable to the visualization of the spatial distribution of found elements. EDX identifies elements instead than specific chemical bonds and cannot alone determine polymer identity or verify the cleavage of polymer chains [9,10]. Previous studies have inspected fungal interactions using plastic materials and stressed the importance of combining morphological noticing with complementary analytical techniques to assess potential biodegradation [11–13].

The approaches provide useful data about surface associated changes as emphasizing the need for extra evidence before concluding that biological degradation has took place [14,15]. The present study targeted to characterize the surface morphology of fungal healed plastic plates using SEM, assess the size distribution of selected surface connected structures, determine the elemental essay of the analyzed material using EDX, inspect the spatial allocation of carbon, oxygen, and chlorine via elemental mapping. The findings were

meant to provide initial evidence of fungal-material exchange and to assess the potential for top biodeterioration minus assuming that complete polymer biodegradation had been showed.

2. Materials and Methods

2.1 Fungal Isolate and Materials

A clean isolate of the fungus *Penicillium notatum* and gained from a soil sample, was used in this study to investigate its potential effects on the surface characteristics of plastic plates. Plastic plate samples were exposed to the fungal isolate under the experimental conditions established for the study. The fungal isolate was purified and maintained under appropriate laboratory conditions before use. After the treatment period, the samples were prepared for scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and elemental mapping. The isolate was specified as *Penicillium notatum* based on the detection records available for the experiment. The strain designation and molecular identification details were report where available.

2.2 Scanning Electron Microscopy (SEM)

Surface morphology was examined using scanning electron microscopy at magnifications ranging from approximately 5,000× to 330,000×. The available SEM acquisition records included ten imaging fields with different magnifications and pixel sizes, together with a separate elemental-mapping field acquired at approximately 10,000×. The micrographs were assessed to characterize the shape, aggregation and distribution of surface associated structures. Particular attention was provided to oval to irregular structures, clustered deposits, and their regional relationship with the basic substrate. The morphological observations were analyzed descriptively to detect features possibly associated with fungal material interaction.

2.3 Measurement of Surface-Associated Structures

A total of 50 size measurements obtained from the supplied SEM data were included in the analysis. The measurements were used to calculate the mean, standard deviation (SD), median, minimum, maximum, and coefficient of variation (CV). A histogram with a layered normal curve was used to

visualize the distribution of the measured values. The calculation was interpreted as dimensions of the selected surface-associated structures rather than as definitive measurements of fungal cells or polymer particles, because their features could not be established from the present data alone.

2.4 Energy-Dispersive X-ray Spectroscopy (EDX)

Elemental composition was defined using the obtainable quantitative EDX spectrum. The reported acquisition conditions were an accelerating voltage of 20.0 kV, a beam latest of 10.000 nA, a nominal zoom of 5,000×, and a live acquisition time of 60 seconds. Quantification was performed using unstandardized PAP correction and based on the supplied analytical report. The detected elements were carbon, oxygen, and chlorine. Their relative abundances were reported as atomic percentages (at%) and weight percentages (wt%). The net signal intensities were also recorded to support the descriptive interpretation of the spectrum.

2.5 Elemental Mapping

Elemental mapping was used to investigate the spatial distribution of carbon, oxygen, and chlorine within the selected SEM field. The elemental maps were examined qualitatively to describe the distribution of the detected elements across the

substrate and surface-associated structures. The mapping results were interpreted alongside the SEM morphology and quantitative EDX data. Elemental distribution was not considered sufficient evidence of polymer-chain degradation or chemical bond cleavage. The available images included the reference SEM micrograph, individual maps for C Ka, O Ka, and Cl Ka, a combined elemental map, and an overlay of the combined map on the reference micrograph.

RESULTS AND DISCUSSION

A data set of SEM acquisition metadata, mapped SEM, quantitative size measurements and EDX spectroscopy and elemental mapping of plastic-plate associated material that has been exposed to a fungal treatment. The files do not determine species of fungus or type of polymer. Evidence is interpreted based on the level of data, morphology and size describing surface associated structures and EDX and elemental mapping describing composition and spatial distribution. Although no gravimetric data for mass loss or polymer specific spectroscopy is available, the results suggest fungal interaction and potential ability to biodegrade, but not necessarily the polymer.

3.1. SEM acquisition range and mapped-surface morphology

Table 1. SEM acquisition metadata supplied for the plastic-plate-associated material.

SEM field	Magnification	Pixel size (nm/pixel)
1	9,996.5×	27.041
2	25,005×	10.810
3	5,003.3×	54.027
4	330,000×	0.819
5	200,000×	1.352
6	200,000×	1.352
7	200,000×	1.352
8	100,000×	2.703
9	50,000×	5.406
10	100,010×	2.703
Mapping field	10,000×	27.031

Acquisition can take place at 5,000 $\times$ all the way to a 330,000 $\times$ scale - from wide surface fields all the way down to nanoscale level of detail. This is an ideal range for the study of association of fungi with surfaces and small localized structures. The 10 HDR files provide data of the acquisition conditions of the single SEM fields and the visual field of the

direct morphological interpretation is displayed in the rendered micrograph (which is included in the mapping report). The multiscale design is thus an asset for the data, but the possibilities for comparisons based on image morphologies are limited to the images rendered in the reports.

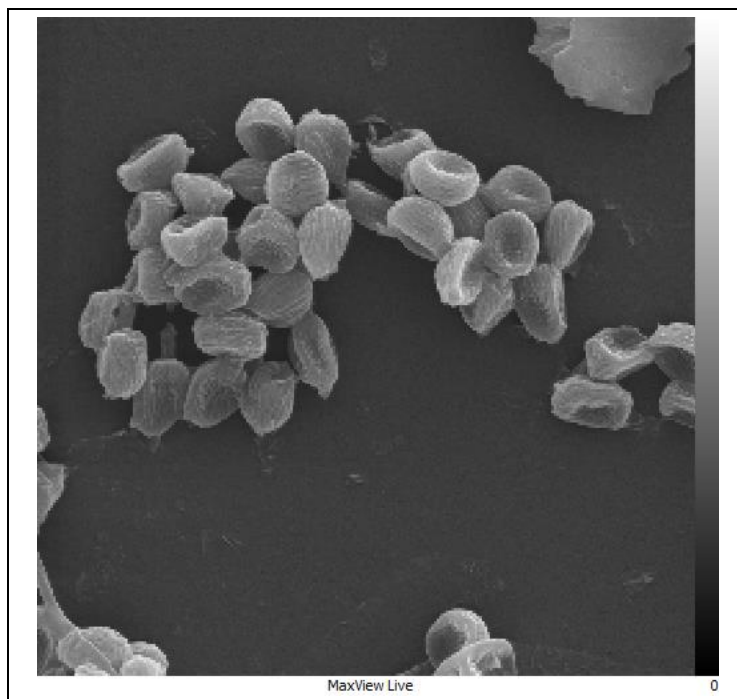


Figure 1. SEM-associated reference micrograph from the elemental-mapping field at approximately 10,000 $\times$.

The mapped field features biological structures in oval to irregular shapes which are clustered together on a relatively flat substrate. They are grouped together in a not necessarily regular particulate layer and are possibly fungal structures attached to the substrate. Surface attachment is an early and important stage in the interaction between fungi and plastics as it exposes the

fungal biomass and extracellular products to the plastic. Typically, such features (polymer-chain degradation) require chemical or gravimetric measurements before they can be interpreted as fungal colonization, though a few studies have relied on SEM to record surface changes, cracks, and grooves as evidence of fungal colonization [16,17,18].

3.2. SEM size distribution

Table 2. Descriptive statistics and normality assessment of the supplied SEM size measurements.

Statistic	Value
N	50
Mean (nm)	34.96
SD (nm)	9.85
Median (nm)	35.32
Minimum (nm)	16.68
Maximum (nm)	56.95
CV (%)	28.18
Shapiro-Wilk W	0.975
Shapiro-Wilk P	0.378

The mean size of the 50 measurements was 34.96 nm while the median was 35.32 nm, which were close. The range was 16.68–56.95 nm with CV of 28.18%, which was considered moderate. Shapiro-Wilk test showed no significance ($W = 0.975$, $P = 0.378$) which means that the measurements were

not significantly different from normal. These statistics argue for displaying the data in a histogram with a normal curve, but also in a way that allows for the fact that the SEM-visible structure can be quite heterogeneous in surface population.

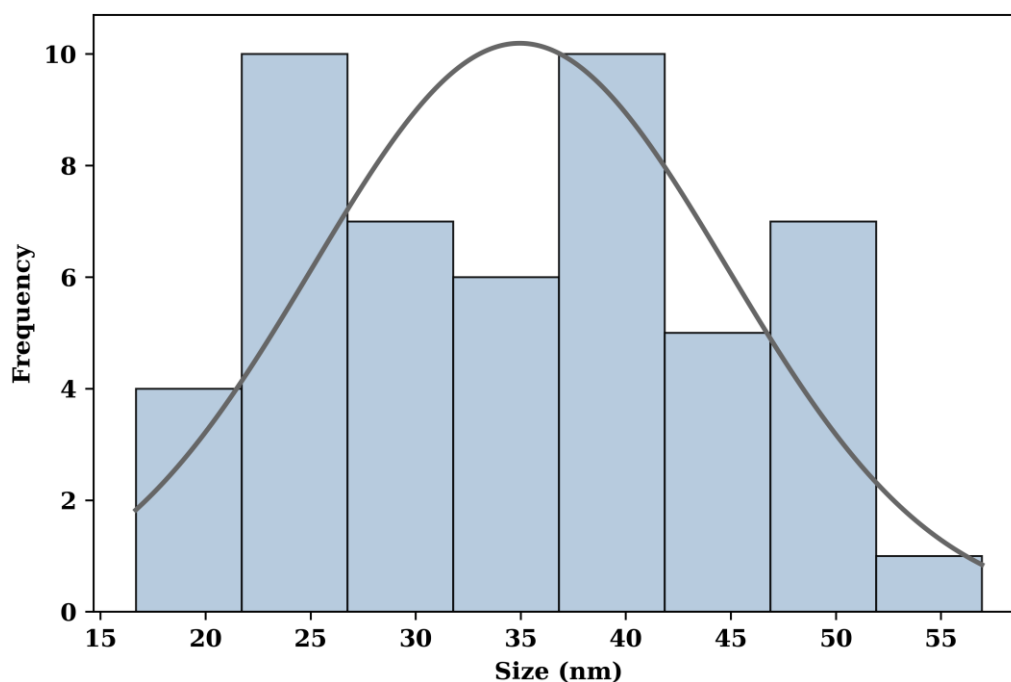


Figure 2. Distribution of 50 supplied SEM size measurements with a superimposed normal curve.

Data are not clumped in a narrow range (bin) and the histogram is centered around the mid 30 nm range. This is similar to the coefficient of variation and is a degree of variation that might be detected in the size of the structures analyzed. The Shapiro-Wilk result was not significant; thus, the normal-curve overlay can be used as a descriptive tool. It must not, however, be considered as proof that all measured objects are from one population, biological or material; the size analysis is

complementary to the microscopy and to the morphological interpretation.

3.3. EDX elemental composition

The quantitative EDX spectrum was collected at 20.0 kV, 10.000 nA, nominal magnification of 5,000× and live time of 60 s. The report was corrected using a standardless PAP correction. Carbon, oxygen and chlorine were quantified from the spectrum provided.

Table 3. Quantitative EDX composition of the analyzed plastic-plate-associated field.

Element	Line	Intensity	Weight %	Atomic %	ZAF	Pk/Bg
C	Kα	868.0	56.40	65.48	0.2871	5424.46
O	Kα	281.1	36.31	31.65	0.1468	1406.93
Cl	Kα	285.4	7.29	2.87	0.8441	381.41

The field is enriched in carbon and oxygen, the combined amount of which is up to 92.71 wt% of the analyzed field. Carbon is the largest component at 56.40 wt% and 65.48 at%, followed by oxygen at 36.31 wt% and 31.65 at%. Chlorine is definitely detected at lower levels (7.29 wt % and 2.87 at %). The presence of an organic plastic-associated surface and a possibly biological deposit of material is indicated by the carbon-oxygen dominance and

chlorine is indicative of a chlorine-bearing material within the analyzed area. EDX is not able to distinguish between the chlorine that is part of the base polymer, additive, coating or surface residue and cannot give information about chemical bond cleavage. In addition, complementary molecular characterisations would be required to check for polymer identity and chemical transformation

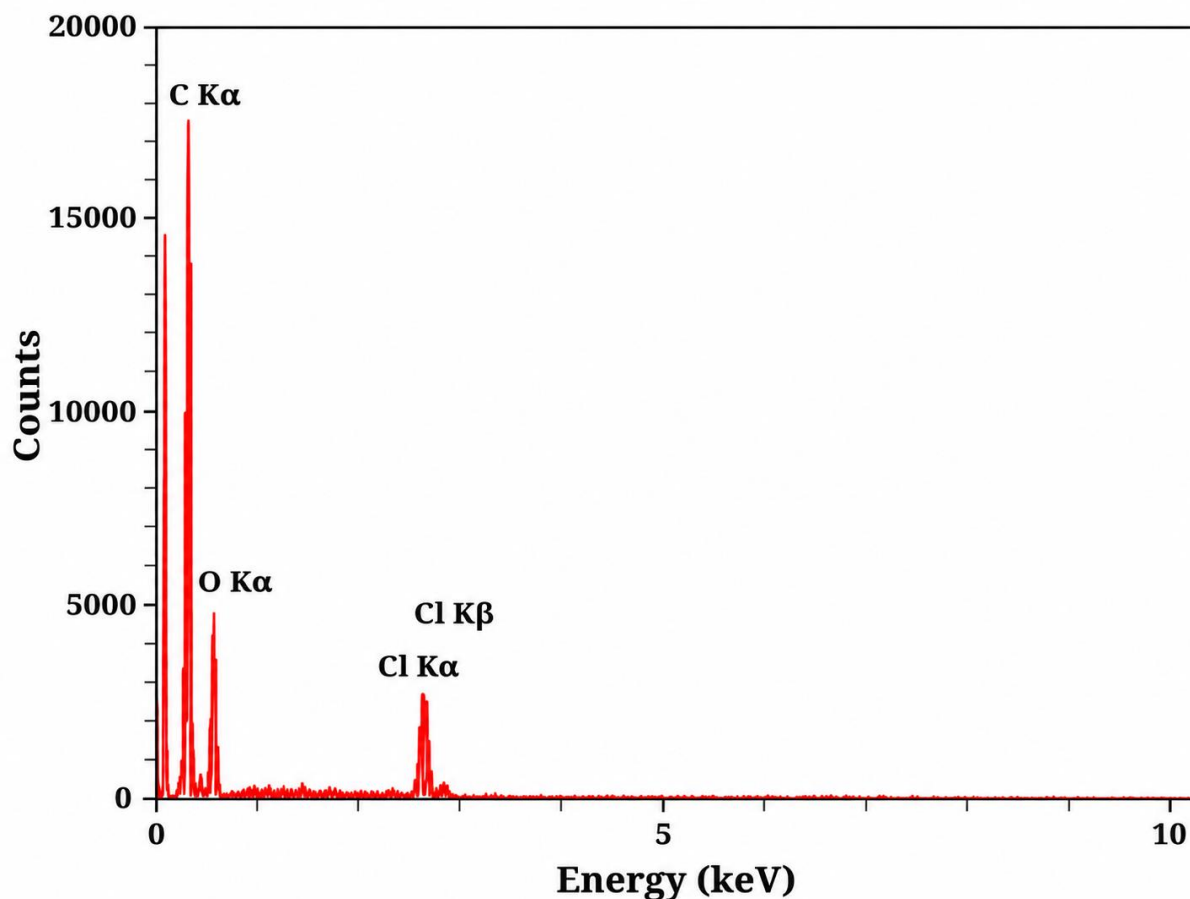


Figure 3. Original EDX spectrum showing the dominant C Kα, O Kα, and Cl Kα/β signals.

The spectrum also visually represents the quantitative ranking. The largest peak is the carbon peak, followed by the lower energy oxygen peak, and a higher energy chlorine peak. The EDX result is internally consistent as the peak pattern of the

spectrum is consistent with the quantitative table. Only one quantitative spectrum, however, is included and this should not be used as a measure of enrichment or depletion after treatment.

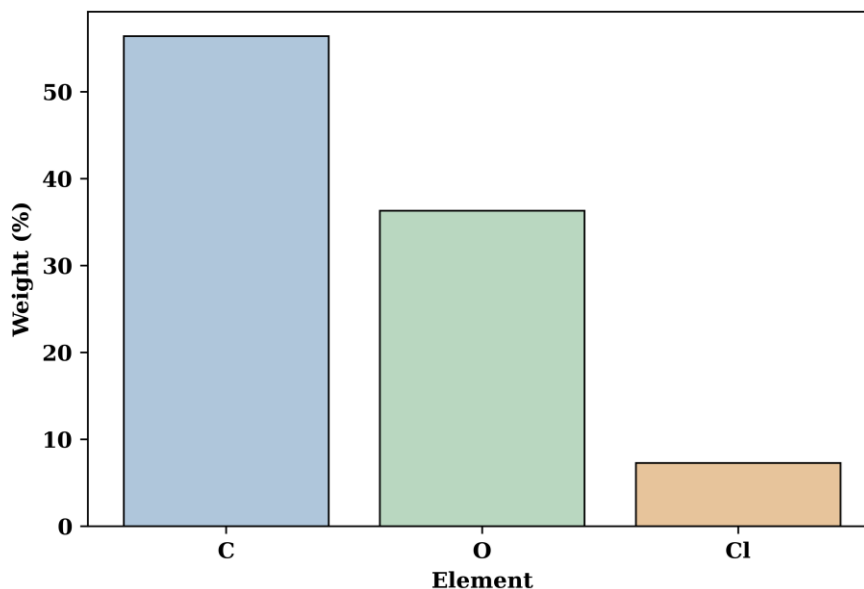


Figure 4. EDX weight-percentage profile of carbon, oxygen, and chlorine.

The relative abundance of carbon and oxygen with respect to chlorine is displayed in the weight-percentage profile. This elemental pattern is helpful for describing the post-treatment surface, but should not be used directly as a polymer

assignment. Mostly morphological and elemental changes are correlated with FTIR and/or mineralization or mass loss measurements in studies dealing with the interaction between fungi and plastics [18,19].

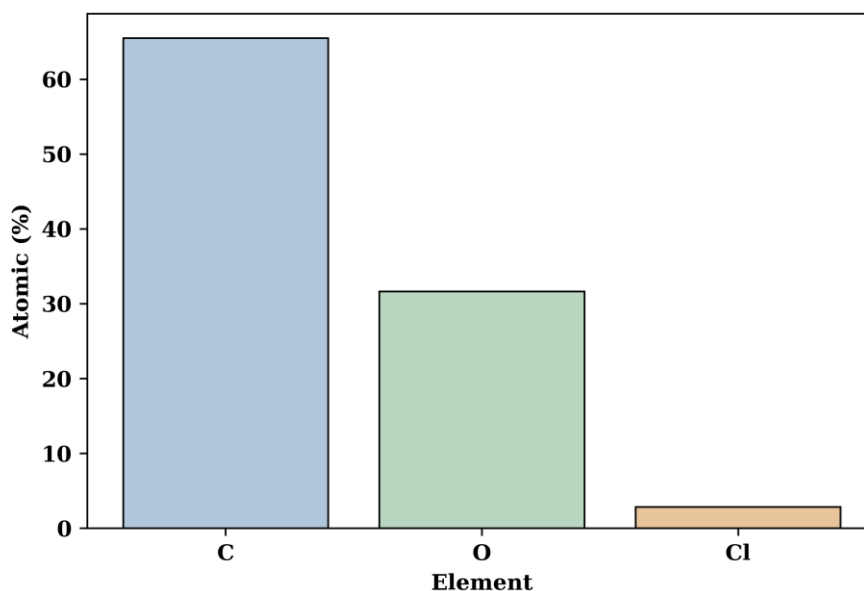


Figure 5. EDX atomic-percentage profile of carbon, oxygen, and chlorine.

Order of atomic percentage is the same as that of weight percentage, the positions of the elements being carbon predominant, oxygen subdominant and chlorine minor. The agreement between the different reporting bases highlights the general elemental ranking of the analyzed field. The various

weight and Atomic percentages are expected due to the calculations based on different properties of the elements detected. The data thus generated can therefore best be used for a description of composition, rather than as a direct measure of biodegradation.

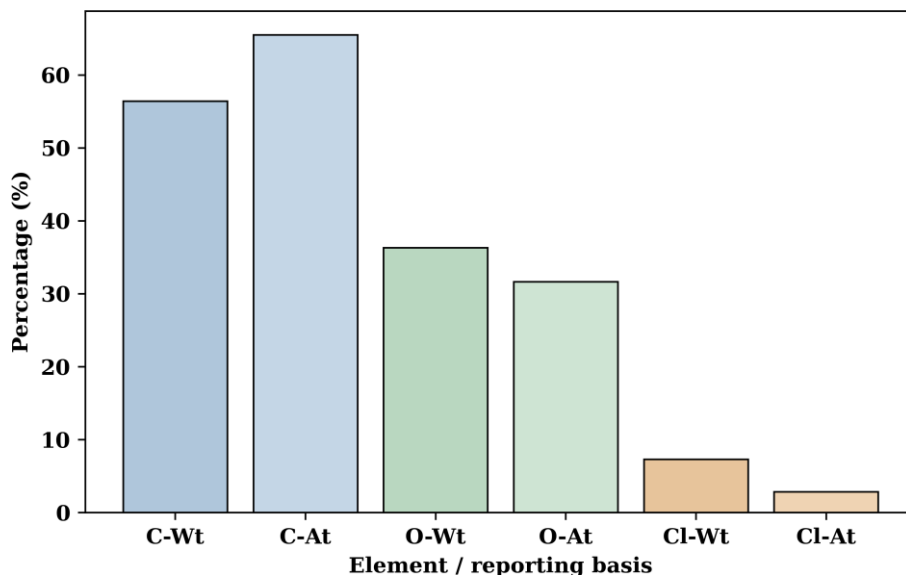


Figure 6. Comparative EDX composition profile using weight- and atomic-percentage values.

The paired profile means the relative order of any three of the elements remains the same regardless of whether they are expressed as mass or as atoms. Carbon is still the main component, and chlorine is still a minor component, in both forms. This uniformity is helpful in analysis, but through this

comparison, the source of each element is not illustrated. In particular, the chlorine signal should be reported as a feature on the surface to be analyzed and not as a plastic type, unless accompanied by polymer-specific spectroscopy.

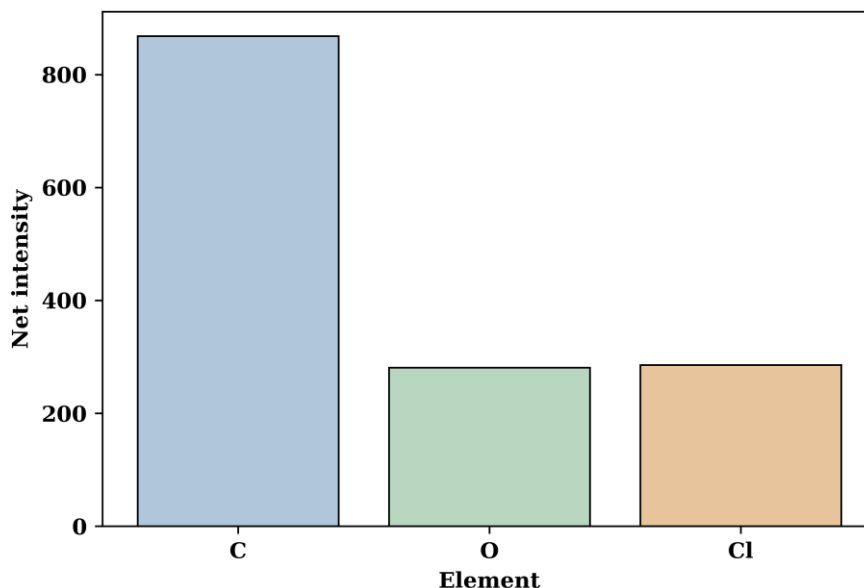


Figure 7. Net EDX signal intensity of the quantified elements.

Again, the net-intensity graph exhibits the prominent carbon response and the less prominent oxygen and chlorine responses. Although the raw net intensities of oxygen and chlorine are similar, the weight and atomic fractions are not similar as

quantification with EDX does not rely on peak height alone, but also incorporates element-specific corrections. The graph is thus a valuable visual aid to the spectrum and it is not a quantitative, independent, comparison or an inferential test.

3.4. Elemental mapping

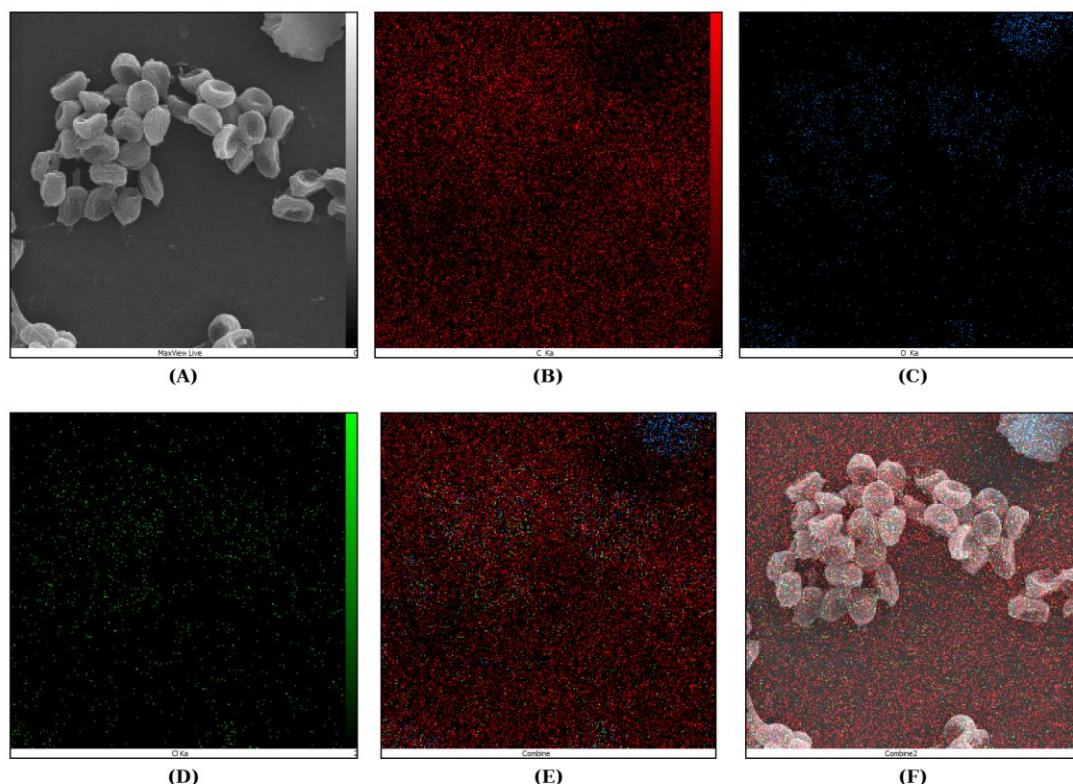


Figure 8. SEM-associated elemental mapping: (A) reference morphology, (B) C Ka, (C) O Ka, (D) Cl Ka, (E) combined elemental map, and (F) combined map overlaid on the reference field.

Carbon is ubiquitous in the mapped area, whereas oxygen and chlorine are not as intense and are more localized in distribution. The three detected elements are not localized at one point, but are spread throughout the same analyzed field, as the combined map indicates. The overlay also checks elemental signals which are present on the smoother background and also on the clustered structures. This spatial information can be correlated with the EDX spectrum indicating where the elements were detected, but is not an indication of bond breaking and/or functional groups. Currently, surface colonization, morphology, spectroscopy, mineralization and mass-balance measurements are used as complements and not as alternatives [19,20].

CONCLUSION

Data from microscopy, size-distribution, EDX and elemental-mapping are consistent and provide a description of the interaction of the fungi with the material within the plastic plates. The mapped area of the SEM field shows that there are clustered biological structures in close proximity to the surface, and 50 measurements given, resulted in a

mean size of 34.96 ± 9.85 nm with a median of 35.32 nm and range of 16.68-56.95 nm. There was no significant deviation from normality in the size data. EDX showed the presence of a carbon and oxygen rich field with a small but distinct chlorine component and the elemental maps indicated that the C, O and Cl signals were ubiquitous throughout the area mapped. All these results taken together suggest the surface association and biodeterioration potential in the conditions studied. They can't be used alone to diagnose the plastic polymer or total chemical degradation. Untreated matched control, replicate mass loss measurements and polymer specific techniques such as FTIR or Raman spectroscopy would greatly enhance a future degradation claim.

STATISTICAL ANALYSIS

The mean, standard deviation, median, minimum, maximum and coefficient of variation of the 50 measurements in the SEM size data set were calculated to summarise the data set. The Shapiro-Wilk test was used for assessment of distributional normality and the results were: $W = 0.975$ and $P = 0.378$. P -values > 0.05 means that descriptive

visualization of the data is given by a histogram of the data with the normal curve overlaid. The EDX data has been descriptively analyzed based on weight percentage, atomic percentage, net intensity and elemental mapping and obtained one quantitative spectrum. An inferential comparison of treated and untreated material was not conducted as an untreated quantitative data set was not provided.

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