



Characterization of the bio-interaction and surface changes of paper cups treated with the fungus *Aspergillus terreus* using SEM and EDX

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Abstract

Background: The waste from disposable paper cups is an environmental problem because it contains materials and polymer layers that interfere greatly with the speed at which it can be broken down naturally. Bioremediation seeks to utilize metabolic activity of microorganism (fungi) and cause noticeable changes in waste materials composition and increase in waste environmental disintegration. Study **Objective:** The objective of this study was to assess the potential of biodeterioration by *Aspergillus terreus*, a facultative anaerobe, on a pure and active isolate of *Aspergillus terreus* (a soil isolate) on paper cup pieces. Materials and **Methods:** Fragments of paper cup of 0.2 g was added to Potato Dextrose broth (PDB) medium and was inoculated with the isolate *A. terreus*. The samples were incubated for 1 month to provide enough time for the growth of the fungus and its interaction with the paper. After incubation, the samples were analyzed by SEM and EDX, with elemental mapping, to evaluate morphological and elemental modifications on the surface of the material due to the fungal treatment. **Results:** Scanning electron microscopy (SEM) images showed extensive fungal growth on the material, including hyphae and spore-like structures. The surface also displayed changes in texture, irregularities, and fine cracks, suggesting that fungal activity affected the paper cup. Measurements of 50 surface features in the SEM images averaged 34.11 ± 13.82 nm. The median was 32.25 nm, and measurements ranged from 11.8 to 66.0 nm. A Shapiro–Wilk test found no evidence that the measurements differed from a normal distribution ($P = 0.220$). Energy-dispersive X-ray (EDX) analysis identified carbon and oxygen as the main elements. Carbon made up 62.77 wt% (70.03 at%), and oxygen accounted for 33.30 wt% (27.89 at%); small amounts of sodium and aluminum were also detected. Elemental maps showed that carbon and oxygen were distributed broadly and consistently across the treated surface. **Conclusion:** The results reveal that the fungus *Aspergillus terreus* can proliferate over the surface of paper cups and manifest morphological alterations on their surface, along with changing the elemental makeup of the material. These findings may encourage the inclusion of *A. terreus* in the fungal formulations for the investigation of biopulping and bioremediation of polymeric materials and paper waste. In addition, the work underscores that further comparative studies are needed to assess the real degree of decay and the efficiency of the fungus under different conditions.

Keyword : *Aspergillus terreus*; Bioremediation ;Biodeterioration; Paper Cup Waste; Scanning Electron Microscopy (SEM).

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INTRODUCTION

Paper and plastic wastes are major contributors to the environmental issue owing to the overuse of disposable products like paper cups [1]. Although paper cups are widely perceived to be more environmentally friendly than other plastic packaging products, they do consist of polymeric materials – which are used to make the cups resistant to water and other liquids – and these materials delay the natural decomposition of paper cups [2,3,4]. Biodegradation is a biotechnological process in the area of waste bioremediation that depends on the capacity of microorganisms including fungi to secrete enzymes and metabolic products capable of degrading complex organics [5,6]. The majority of fungi have an exceptional capacity to colonize organic

substrates and use them as a source of both energy and carbon, indicating that they are also key organisms for studies on bioremediation and environmental waste accumulation reduction [7]. *Aspergillus* genus is commonly found in soil and other environments. Some species of this genus have been also found to secrete several extracellular enzymes that may act in degradation of complex organic materials [8]. *Aspergillus terreus*, a soil-borne fungal species, plays an important role in environmental and microbiological studies owing to its metabolic versatility and ability to utilize wide array of nutrient sources as well as ability to grow under varied environmental conditions [9]. Bioremediation of polymeric substances depends on the interaction of microbes with the surface of the polymer, the degradation process is preceded by surface colonization, and the production of enzymes that can alter the chemical linkages and the components of the material, leading to changes in its elemental composition [10,11]. Therefore, analysing a material before and after fungal growth on it may offer some visual signs of the ability of the fungus to degrade/biotransform that substrate[12]. SEM is important in the study of morphological and surface changes of the materials; for example, the surface of paper cups can be visibly altered by fungal growth (cracking, erosion, pitting, or other types of surface alteration) [13]. Energy-Dispersive X-ray spectroscopy (EDX) can be used to observe the modification in the elemental composition and to detect the elements present in the sample prior to and after fungal treatment [14]. Since biological processes are crucial in reducing the build up of paper waste including polymeric materials, in this study we investigate the potential of the soil fungus *Aspergillus terreus* to degrade (that is alter) paper cups in which the fungus was grown in an aqueous nutrient medium. And it also attempts to characterize the changes of the material in morphology and element by SEM and EDX. The results of this work may provide a basis to evaluate the exploitability of native fungal species for the biodegradation of wastes and materials comprising polymeric substances.

MATERIALS AND METHODS

Fungal Isolation and Purification

Soil samples were taken to recover the fungus *Aspergillus terreus*. Fungal cultures were established and purified to obtain an active pure culture of *A. terreus*. The isolate was stored under appropriate conditions for later application in bioremediation.

Preparation of Paper Cup Samples

The fragments of the paper cups employed in the experiment were collected, and 0.2 g of the paper material was weighed out per each replicate. The sections were submerged in Potato Dextrose Broth (PDB) – a liquid culture medium – to establish the best conditions for the growth of the fungus and the subsequent exposure of the paper material to the fungus.

Fungal Treatment of Paper Cups

Some of the pure *Aspergillus terreus* culture was inoculated into a liquid medium with the paper cup pieces. The plates were left for 1 month so as to provide sufficient time for the growth of the fungus and an interaction with the paper, which makes it possible to evaluate whether the fungus has potential to modify the composition and structure of the material in the period.

Measuring Changes in the Paper Material

After the period of incubation, the pieces of the paper cup were taken out from the medium and were processed for the analysis in the laboratory. The specimens were dispatched to a specialized lab to study the alterations in the material due to fungal treatment.

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was used to investigate morphological and surface modifications on pieces of paper cups after treatment with fungi. The study analyzed changes in surface features including erosion, cracking, pitting and deterioration in material structure and related these with those of control untreated samples.

Energy-Dispersive X-ray Spectroscopy (EDX)

Energy-Dispersive X-ray Spectroscopy (EDX) analysis was performed to investigate the elemental composition of the samples and to check for possible modifications in the elemental composition of the paper substrate after treatment with fungi. The EDX results with the SEM images were evaluated to determine the degree of alteration in the surface and elemental characteristics of the substrate due to fungal growth.

RESULTS AND DISCUSSION

A pure, actively growing *Aspergillus terreus* isolate was cultured in potato dextrose broth with 0.2 g of paper-cup fragments for one month. Microscopy and elemental analyses were then conducted to provide complementary evidence of the fungus's interaction with the fragments. The terms below reflect the scope of each method: SEM documented fungal growth on the material and associated surface features, while EDX and elemental mapping assessed elemental composition and distribution. The results indicate potential for biodeterioration and bioremediation, although no untreated paper-cup EDX/SEM control was supplied and replicate mass-loss measurements, FTIR spectra, or mineralization data would be required for stronger evidence of complete polymer degradation.

Table 1. Experimental and analytical framework represented in the supplied dataset

Component	Recorded condition / evidence	Interpretive role
Biological agent	<i>Aspergillus terreus</i> isolated from soil; pure active isolate	Candidate fungal bioremediation agent
Substrate	Paper-cup piece, 0.2 g	Polymer-associated paper-cup waste
Medium and exposure	PDB; co-incubation for one month	Fungal-material interaction period
SEM	Images 1-11 at 5.00-330 kx	Colonization and surface morphology
EDX	20 kV; 10 nA; 5000x; 60 s; standardless PAP correction	Elemental composition
Elemental mapping	C, O, Na and Al maps plus combined overlay	Spatial distribution of detected elements

Microscopy is a first-line technique useful for showing attachment, colonization, and surface-associated alteration, and the experimental design ensures that the fungus remains in direct contact with the paper-cup material for a prolonged period. Gravimetric or chemical evidence is usually coupled with SEM because the latter mainly provides morphological information rather than direct evidence of chemical-bond cleavage. In comparable studies of fungal degradation, SEM has been used together with FTIR and gravimetric weight loss to support deterioration of polymeric materials [15,16]. The present dataset therefore represents a biologically relevant morphological and elemental level of evidence, which could be supplemented with chemical or mass-balance measurements for more convincing evidence of depolymerization.

SEM evidence of fungal colonization and surface-associated structures

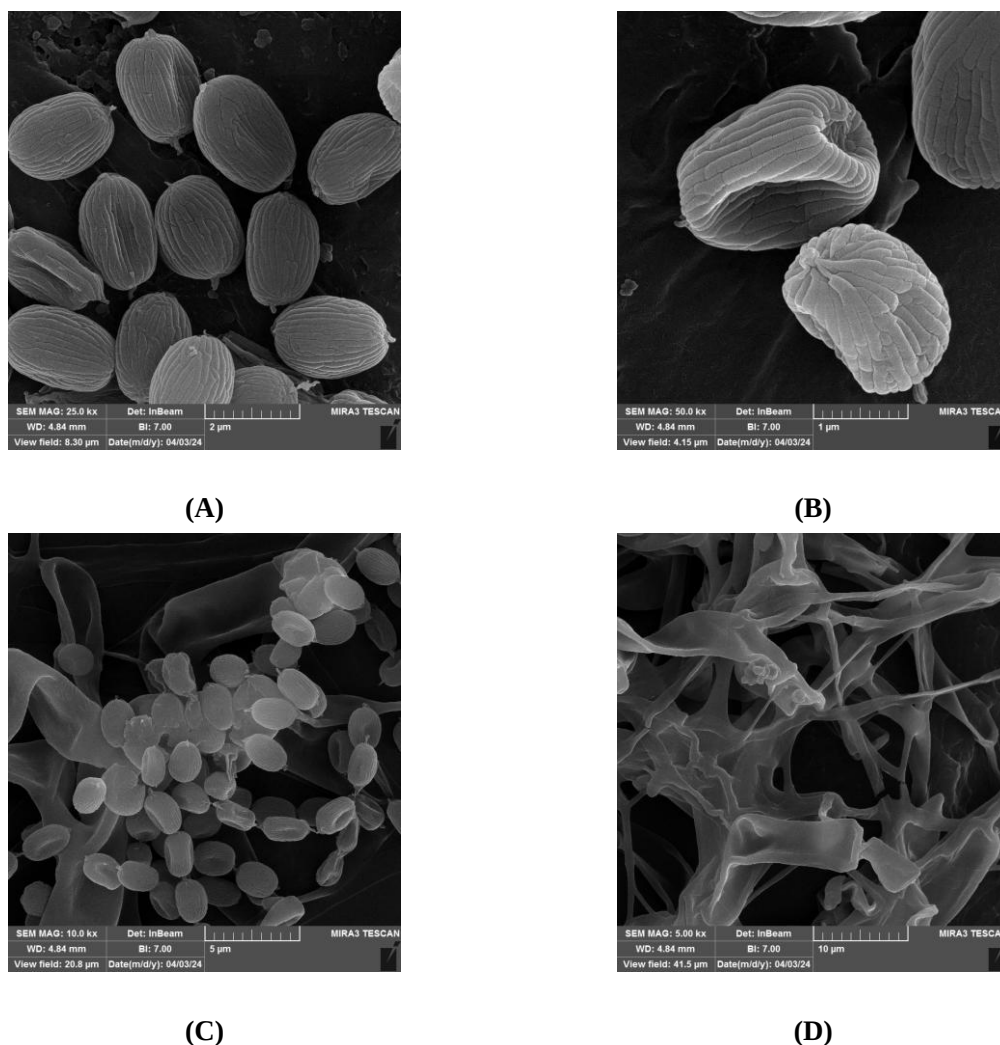
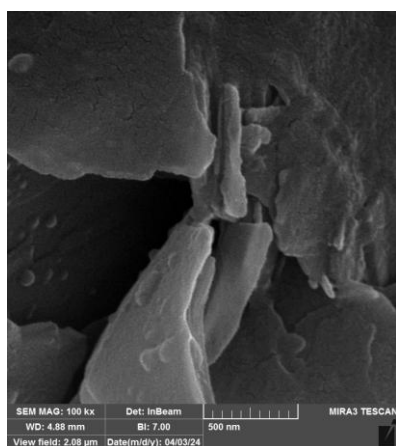
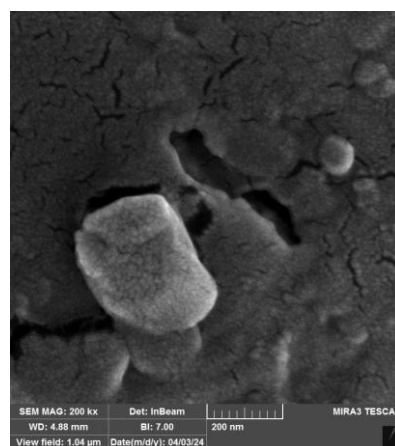


Figure 1. SEM micrographs 1-4 showing *A. terreus*-associated morphology across increasing fields of view. Images 1-3 show abundant oval to ellipsoidal fungal propagules with pronounced longitudinal surface ornamentation, whereas image 4 shows an interconnected filamentous network consistent with hyphal growth on/around the examined material

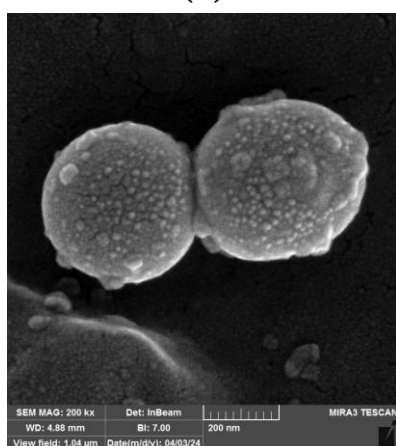
Images 1-4 are consistent with colonization by the inoculated *Aspergillus terreus* rather than a single particulate deposition. The repeated presence of oval-to-ellipsoidal bodies and the interconnected filamentous network suggest a persistent interface between the fungus and the substrate, an important precondition for localized extracellular enzymatic and oxidative activity. Fungal attack on plastic materials generally begins with surface attachment and hyphal colonization, followed by extracellular activity that can contribute to fragmentation and subsequent assimilation [17,18]. Similar surface colonization and SEM-visible alterations have been reported during *A. terreus* interaction with polymeric substrates, although confirmation of deterioration has relied on complementary gravimetric and spectroscopic evidence [16]. These findings therefore support establishment and close contact with the substrate, but they do not alone establish polymer-bond cleavage.



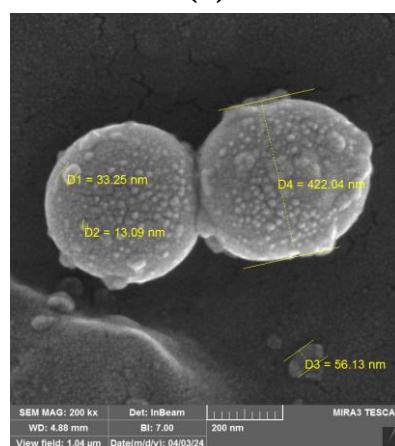
(A)



(B)



(C)



(D)

Figure 2. High-magnification SEM micrographs 5-8. Images 5-7 show localized surface discontinuities and rounded/irregular structures at submicrometre scale. Image 8 contains the instrument annotations D1 = 33.25 nm, D2 = 13.09 nm, D3 = 56.13 nm and D4 = 422.04 nm; these are retained exactly as supplied and are not treated as a population-size distribution

With higher magnification, the analyzed surface is heterogeneous, with fissure-type discontinuities, irregular edges, and discrete rounded structures. These features are compatible with localized surface modification and biological deposition at the fungus-material interface but do not specifically demonstrate chemical depolymerization. Comparable SEM-visible surface changes have been reported in polymers treated with *A. terreus* when independent chemical and gravimetric evidence was also available [16]. Distances D1-D4 in image 8 are not statistically interchangeable replicates because they are individual instrument measurements of different visible features. They were therefore not used to determine a mean particle size or to assess a normal distribution. This strict interpretation stops singulated image annotations from an isolated computational imaging endpoint and the SEM evidence remains morphologic.

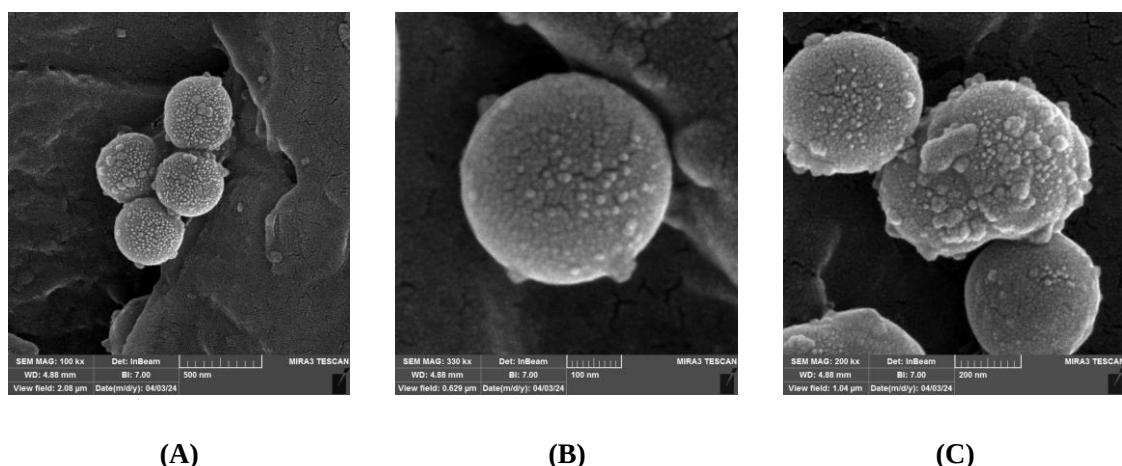


Figure 3. SEM micrographs 9-11 showing clustered rounded structures and nanoscale surface texture at 100-330 kx. Scale bars and original microscope annotations are preserved

Images 9-11 extend the morphological sequence from micro to nanometre scale showing at higher magnifications roundish clustered features and a heterogeneous surface roughness. These results are in agreement with patchy biological deposition and surface related modification, although they are not indicative of a distinctive signature of polymer-chain scission. Comparable surface alterations were also reported detected during fungal degradation of plastic materials while more compelling proof of degradation is usually backed up by supplementary chemical, gravimetric, respiratory, or mineralization evidence[16,19,20]. Since a matched untreated SEM control was not presented, Figure 3 should be interpreted as a representation of ongoing colonization and surface alteration as opposed to a quantitative measure of degradation rate or total mineralization.

SEM size distribution

The analysis included 50 measurements taken from SEM images. The structures averaged 34.11 nm in size (SD = 13.82 nm), with a median of 32.25 nm and values ranging from 11.8 to 66.0 nm. A 40.51% coefficient of variation points to considerable variation among the measured structures. The Shapiro–Wilk test found no statistically significant difference from a normal distribution ($W = 0.969$, $p = 0.220$). A histogram overlaid with a normal curve is therefore appropriate for presenting the data descriptively.

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

N	Mean (nm)	SD (nm)	Median (nm)	Min (nm)	Max (nm)	CV (%)	Shapiro-Wilk P
50	34.11	13.82	32.25	11.8	66.0	40.51	0.220

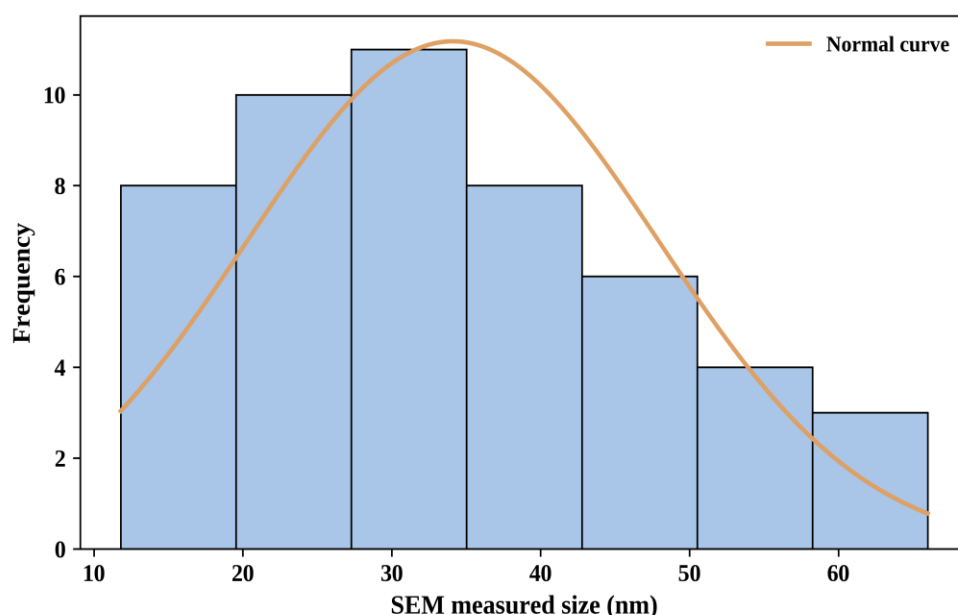


Figure 4. Histogram of the 50 provided SEM size measurements with a fitted normal density. The sizes measured range from 11.8 to 66.0 nm and, according to the Shapiro-Wilk test ($P = 0.220$), they do not significantly deviate from normality.

The particles are generally small, measuring in the lower-to-middle nanometre range, though their sizes vary considerably. The coefficient of variation is 40.51%. This heterogeneity is consistent with the variable rounded and irregular nanoscale features seen in the high-magnification SEM images. The non-significant Shapiro-Wilk result indicates that a normal-curve overlay can be used as a descriptive visualization, but it does not imply that every measured structure belongs to a single biological population. The quantitative size-distribution information therefore complements, rather than substitutes for, the qualitative SEM evidence of colonization and surface-associated alteration.

EDX elemental composition

Table 3. Quantitative EDX composition of the analyzed paper-cup-associated field

Element	Line	Intensity	Weight %	Atomic %	ZAF	Peak/Background
C	K α	277.0	62.77	70.03	0.4998	7849.09
O	K α	41.9	33.30	27.89	0.1450	1091.10
Na	K α	4.4	1.45	0.84	0.3643	72.82
Al	K α	12.7	2.48	1.23	0.6272	86.98
Total			100.00	100.00		

Carbon and oxygen were dominant in the EDX field and together accounted for 96.07 wt%, with carbon representing 62.77 wt% and oxygen 33.30 wt%. Sodium and aluminium were only minor constituents. The corresponding atomic composition, 70.03% carbon and 27.89% oxygen, is consistent with a carbon-rich matrix containing oxygen. This profile can be associated with an organic paper- or polymer-related substrate and may also include contributions from fungal biomass. EDX cannot determine polymer type or chemical bonding, however. The prevalence of carbon and oxygen is thus to be expected as a context to the element for the studied surface and not a result of direct cleavage of certain polymer bonds. To demonstrate chemical conversion, molecular spectroscopy or direct assessments of mineralization are necessary [19,20].

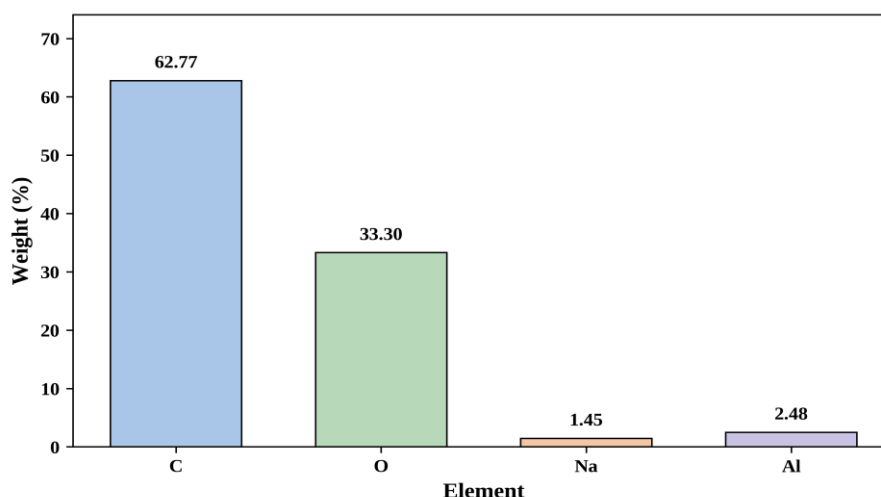


Figure 5. EDX weight-percentage profile of C, O, Na and Al

The weight-percentage distribution is clearly dominated by carbon and oxygen, with sodium and aluminium as minor constituents. This is compatible with a carbon- and oxygen-rich paper-cup-associated surface, but EDX alone cannot differentiate among cellulose, polymer coating, fungal biomass, oxidation products, or the source of the minor elements. Disposable paper cups commonly use cellulose-rich paper with a hydrophobic polymer lining, so a carbon- and oxygen-rich elemental profile is plausible without identifying a specific polymer from EDX alone [21]. The graph should therefore be considered a compositional profile of the analyzed field. Replicate measurements from both treated and untreated samples, together with molecular spectroscopy, would be necessary to determine whether fungal exposure systematically shifts the carbon-to-oxygen balance [18].

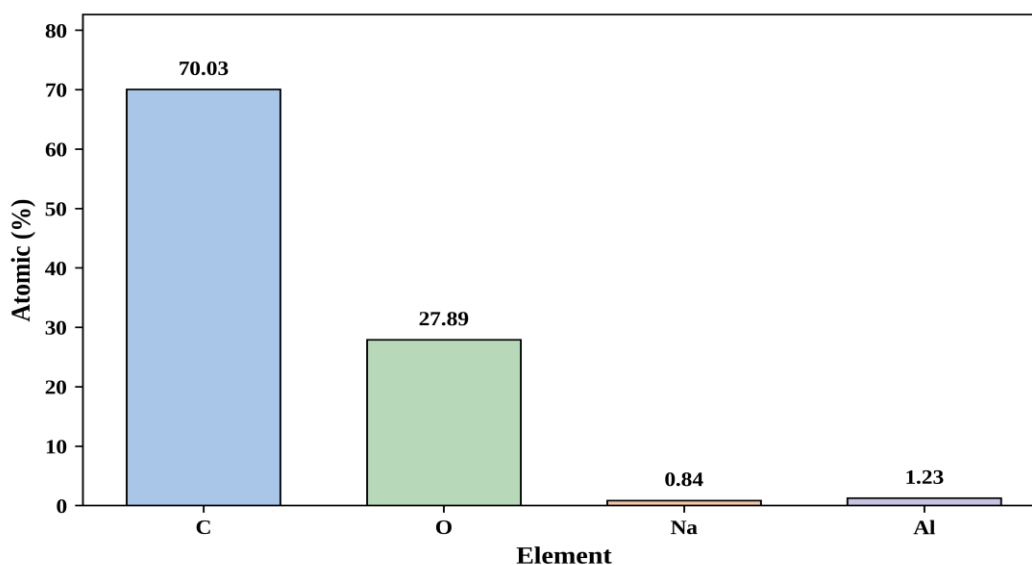


Figure 6. EDX atomic-percentage profile of C, O, Na and Al

The atomic percentages reflect the same compositional hierarchy, with carbon above oxygen and both far above aluminium and sodium. Agreement between the atomic-percentage and weight-percentage rankings strengthens confidence that the analyzed field is predominantly carbon- and oxygen-rich. This is compatible with the organic paper and lining matrix expected in disposable

paper cups[21], but it does not discriminate among cellulose, polyethylene or other copolymers, fungal biomass, or oxidation products. Complementary molecular techniques such as FTIR or Raman spectroscopy would therefore be needed before assigning a specific polymer identity or chemical transformation [18,19].

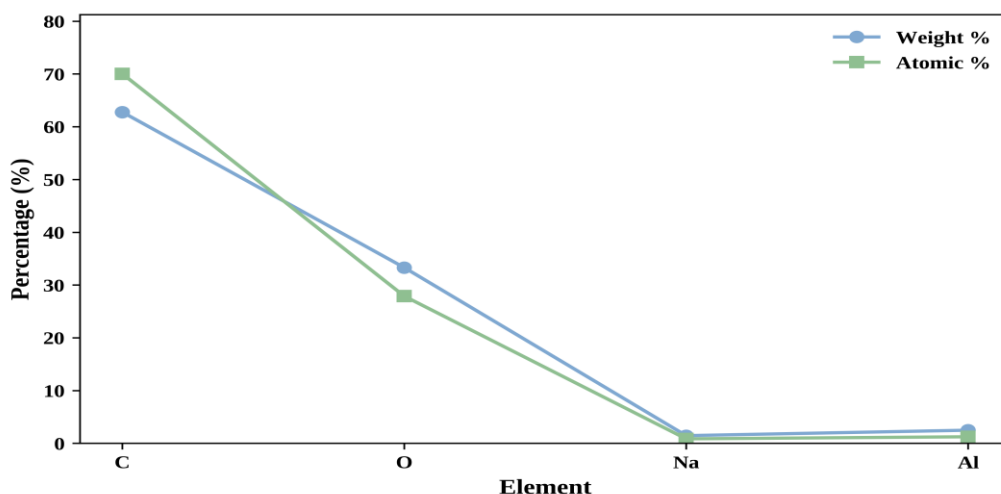


Figure 7. Comparative elemental composition profile based on EDX weight and atomic percentages

The comparative profile shows that carbon remains the predominant element under both weight- and atomic-percentage reporting, while oxygen remains the second major element. This concordance serves as an internal compositional check and is relevant to the paper-cup bioremediation setting because disposable paper cups commonly contain a cellulose-rich paper component together with a hydrophobic polymer lining [21]. The present EDX result should not, however, be used to identify a specific polymer because EDX is not molecularly specific. Thus, the comparison serves to verify the uniformity in elemental composition across the two modes of reporting, and not to its utility in identifying polymers or as a method of direct evidence of bond scission.

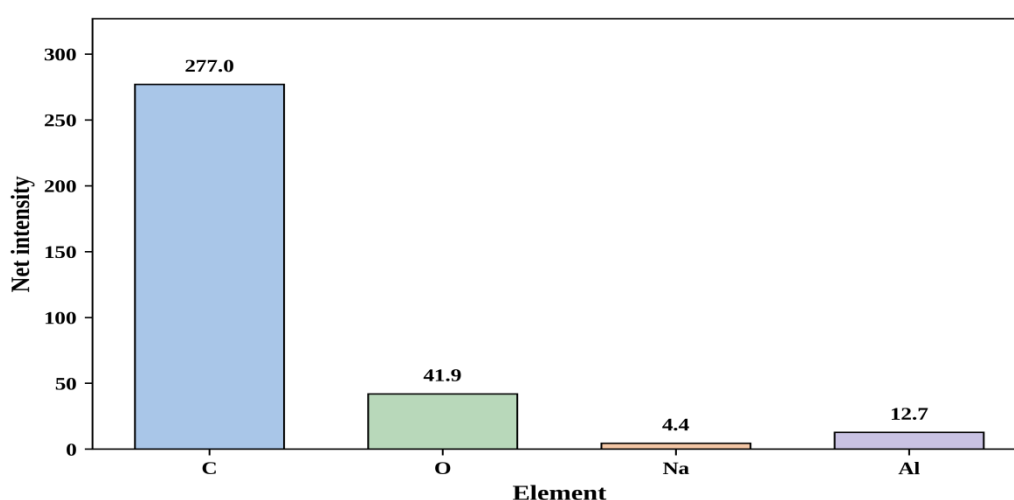


Figure 8. Net EDX signal intensity for the detected elements

Carbon produces a much stronger signal than oxygen, aluminium, or sodium, matching the ranking in the reported composition. This consistency offers a basic check on the elemental profile

shown in the spectrum. However, the graph should be treated as a signal profile, not as a quantitative comparison: only one quantitative EDX analysis was available. Without replicate spectra and a comparable untreated control, it is not possible to determine whether treatment increased or reduced the levels of any elements.

Elemental mapping

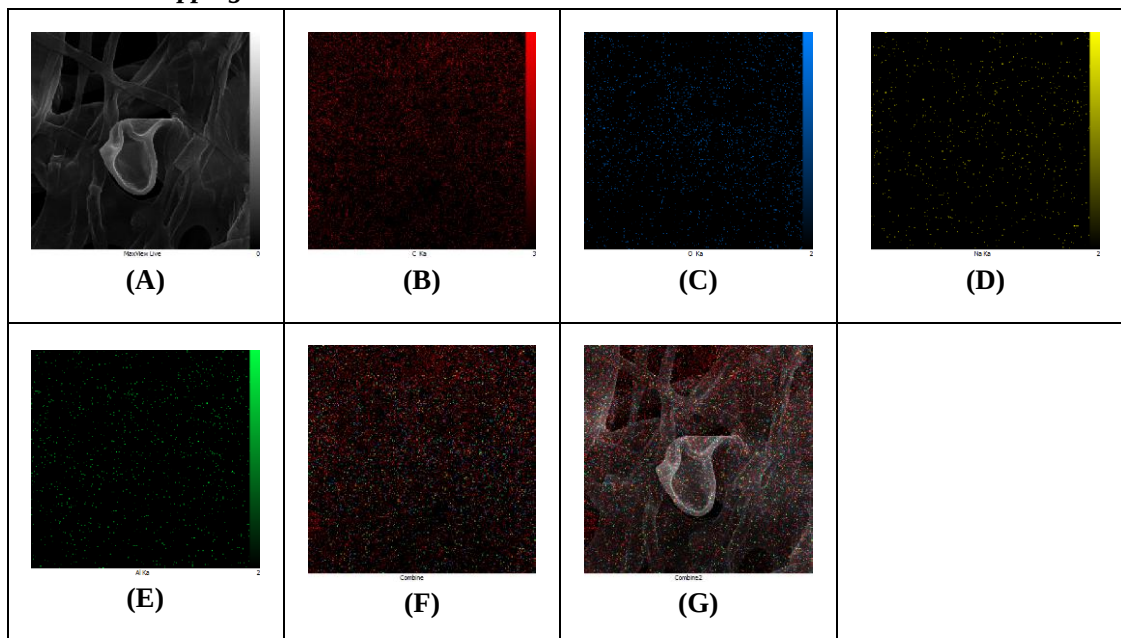


Figure 9. SEM-associated elemental mapping: (A) reference morphology, (B) C K α , (C) O K α , (D) Na K α , (E) Al K α , (F) combined elemental map, and (G) combined map overlaid on the SEM field

Carbon is widely distributed throughout the mapped field, while oxygen is also broadly present but visually less intense. Signals for sodium and aluminium are sparse and dispersed. The combined overlay shows that the strong carbon and oxygen signals occur over the same general surface structure seen in the reference micrograph, suggesting that the carbon-oxygen signature is distributed across the surface rather than localized to a single point. Elemental mapping can therefore be used to show spatial heterogeneity and co-localization but cannot identify functional groups, polymer type, or cleavage of chemical bonds. Comparable fungal-plastic studies require additional chemical or mass-balance evidence before a mechanism of polymer degradation can be established [16,19,21,22]. The mapping therefore supports intimate association between the fungus and material and a heterogeneous carbon- and oxygen-rich surface after incubation, rather than a stand-alone chemical mechanism of degradation.

Across SEM morphology, quantitative size distribution, EDX composition, and elemental mapping, a consistent pattern of persistent fungal contact, heterogeneous nanoscale surface-associated features, and a carbon- and oxygen-dominated analyzed field is observed. This convergence is compatible with *Aspergillus terreus*-associated biodeterioration and bioremediation potential. Surface colonization and morphological modification are important components of the evidence for fungal interaction with polymeric materials, but more convincing degradation claims require additional chemical, gravimetric, respiratory, or mineralization data [16,17,20,23]. The present data should therefore not be interpreted as absolute verification of complete polymer degradation without a matched untreated control, replicate mass-loss measurements, polymer-specific spectroscopy, or mineralization data.

CONCLUSION

The SEM, quantitative size-distribution, EDX, and elemental-mapping data clearly illustrate interaction between *Aspergillus terreus* and paper-cup-associated material after one month of incubation in potato dextrose broth. Fungal propagules and dense hyphal networks can be found along the full length of the SEM series and in conjunction with surface discontinuities and irregular morphology at multiple nanoscale steps. In total, 50 SEM observations were provided for analysis, which resulted in an average of 34.11 ± 13.82 nm and a median value of 32.25 nm, ranging from 11.8 to 66.0 nm. The distribution did not significantly deviate from normal (Shapiro-Wilk $P=0.220$). EDX analysis showed carbon and oxygen as the dominant elements, while elemental mapping revealed extensive carbon and oxygen signals with sparse sodium and aluminium signals. These complementary observations support persistent fungal colonization and surface-associated biodeterioration consistent with bioremediation potential. They do not establish complete polymer degradation or define its chemical mechanism in the absence of a matched untreated control, replicate mass-loss data, polymer-specific spectroscopy, or mineralization measurements.

STATISTICAL ANALYSIS

SEM size measurements, with a total sample size of 50, were summarized using the mean, standard deviation, median, range, and coefficient of variation. Distributional normality was evaluated using the Shapiro-Wilk test and yielded $W = 0.969$ and $P = 0.220$. Because $P > 0.05$, a histogram with a superimposed normal curve was used for descriptive visualization. The EDX dataset consisted of a single quantitative elemental spectrum and was therefore summarized descriptively using weight percentage, atomic percentage, net intensity, and elemental profiles without inferential testing. A matched untreated quantitative dataset was not available, so no inferential comparison between treated and untreated material was performed. Descriptive and normality procedures were specified for IBM SPSS Statistics version 32.0, while publication graphics were formatted according to GraphPad Prism version 11.1.0 conventions[24,25,26,27].

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توصيف التفاعل الحيوي والتغيرات السطحية في الأكواب الورقية المُعالجة بفطر *Aspergillus terreus* باستخدام تقنيتي SEM و EDX

الملخص

خلفية الدراسة: تُمثل نفايات الأكواب الورقية ذات الاستخدام الواحد مشكلة بيئية؛ لاحتوائها على مواد وطبقات بوليمرية تُعيق بشكل كبير سرعة تحليلها الطبيعي. ويهدف المعالجة الحيوية (Bioremediation) إلى الاستفادة من النشاط الأيضي للكائنات الدقيقة (مثل الفطريات) لإحداث تغييرات ملموسة في تركيب النفايات، مما يساهم في تسريع تفككها بيئياً. هدف الدراسة: هدفت هذه الدراسة إلى تقييم قدرة فطر *Aspergillus terreus* -وهو كائن اختياري اللاهوائية (facultative anaerobe)- تم عزله من التربة- على إحداث تدهور حيوي في قطع من الأكواب الورقية. المواد والطرق: وُضعت قطع من الأكواب الورقية (بوزن 0.2 جرام) في وسط غذائي من مرق "البطاطس والدكستروز" (Potato Dextrose Broth - PDB)، ثم لُفحت بعزلة فطر *Aspergillus terreus*. خُصنت العينات لمدة شهر كامل لإتاحة الوقت الكافي لنمو الفطر وتفاعله مع الورق. وبعد فترة التحضين، خُصنت العينات للتحليل باستخدام المجهر الإلكتروني الماسح (SEM) وتقنية تشتت الأشعة السينية (EDX)، مع إجراء مسح للعناصر (elemental mapping)، وذلك لتقييم التغيرات المورفولوجية والعنصرية التي طرأت على سطح المادة نتيجة المعالجة الفطرية. النتائج: أظهرت صور المجهر الإلكتروني الماسح (SEM) نمواً فطرياً كثيفاً على المادة، تضمن خيوطاً فطرية (hyphae) وهياكل تشبه الأبواغ. كما أظهر السطح تغيرات في ملمسه، مع ظهور نتوءات وشقوق دقيقة، مما يشير إلى أن النشاط الفطري قد أثر على الأكواب الورقية. بلغ متوسط قياسات 50 سمة سطحية رُصدت في صور SEM نحو 34.11 ± 13.82 نانومتر، حيث كانت القيمة الوسيطة 32.25 نانومتر، وتراوحت القياسات بين 11.8 و 66.0 نانومتر. وأظهر اختبار "شابيرو-ويلك" (Shapiro-Wilk test) عدم وجود أدلة على اختلاف القياسات عن التوزيع الطبيعي ($P = 0.220$). أما تحليل تشتت الأشعة السينية (EDX)، فقد كشف أن الكربون والأكسجين هما العنصران الأساسيان المكونان للمادة. شكّل الكربون نسبة 62.77% من الوزن (70.03% من عدد الذرات)، بينما شكّل الأكسجين نسبة 33.30% من الوزن (27.89% من عدد الذرات)؛ كما تم الكشف عن كميات ضئيلة من الصوديوم والألمنيوم. وأظهرت خرائط العناصر توزيعاً واسعاً ومتسقاً للكربون والأكسجين عبر السطح المُعالج. الخلاصة: تكشف النتائج أن فطر "أسبرجيلوس تيريوس" (*Aspergillus terreus*) يمكنه النمو على أسطح الأكواب الورقية، مسبباً تغيرات مورفولوجية (شكلية) على تلك الأسطح، بالإضافة إلى تغيير التركيب العنصري للمادة. قد تشجع هذه النتائج على إدراج فطر "*A. terreus*" ضمن التركيبات الفطرية المستخدمة في دراسات المعالجة الحيوية (bioremediation) وعمليات اللب الحيوي (biopulping) للمواد البوليمرية ونفايات الأكواب الورقية. علاوة على ذلك، يؤكد هذا العمل على الحاجة إلى إجراء دراسات مقارنة إضافية لتقييم مدى التحلل الفعلي وكفاءة هذا الفطر في ظل ظروف مختلفة.

الكلمات المفتاحية: أسبرجيلوس تيريوس (*Aspergillus terreus*) ؛ المعالجة الحيوية؛ التحلل الحيوي؛ نفايات الأكواب الورقية؛ المجهر الإلكتروني الماسح (SEM).