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Mineralizing effectiveness of *Pleurotus ostreatus* and *Pleurotus djamor* in the treatment of lignocellulosic waste from cigarette butts, Cusco (Peru)

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Abstract*– Considering the environmental pollution caused by cigarette butts, due to their toxic content which hinders their proper recycling, this study applied a mineralizing and detoxifying treatment using saprobic macromycetes—Pleurotus ostreatus and Pleurotus dejamor—as regenerative treatment systems for the mineralization of toxic substances, particularly through the enzymatic degradation of cellulose and lignin fibers via cellulase activity. Fungal growth occurred under controlled conditions of humidity, temperature, and light exposure. The degradation of lignocellulosic fibers was monitored using SEM analysis at 45 and 75 days, while EDS analysis was used to evaluate the mineralization of key elements. Weekly evaluations (every three days) over a four-month period confirmed the progressive growth of the mycelium on the cigarette butt substrate. The results showed increased mineralization of Cl and S at 45 days, and of Ti, Si, and Al at 75 days. Controlled environmental parameters were essential for basidiomycete development, with carbon availability being a key growth factor. The resulting substrate is characterized by being partially degraded, demineralized, and containing fewer toxic substances; therefore, it can be reused as raw material, contributing to circular economy strategies, improving waste valorization, and ultimately achieving zero environmental impact.

Keywords*-- Pleurotus ostreatus, Pleurotus djamor, biotechnology, lignocellulosic waste, cigarette butts.

I. Introduction

Anthropological pollutionhas generated various environmental challenges, with cigarette butts being one of the most prevalent and persistent contaminants in natural ecosystems [1]. These residues contribute significantly to biodiversity loss and ecosystem degradation [2], as they contain a range of toxic substances, including arsenic (As), nicotine ($C_{10}H_{14}N_2$), cadmium (Cd), and toluene ($C_6H_5CH_3$) [3,4]. These compounds release toxins upon contact with water, particularly heavy metals such as arsenic—which pose threats to aquatic fauna and human health [5]. Managing cigarette waste remains a global challenge [6], and in Peru, their presence in public spaces and water bodies further threatens biodiversity and environmental integrity [7,8].

One promising bioremediation approach is mycoremediation, which utilizes fungi to degrade or immobilize contaminants [9]. Several fungal species have demonstrated the capacity to absorb heavy metals and degrade synthetic polymers, such as cellulose acetate—a primary component of cigarette filters [10]. These fungi may also assimilate the toxic substances in cigarette waste and store them within their mycelial structures.

Cellulose is one of the main components of plant biomass, it is composed of polymeric glucose chains. Other components are hemicellulosic fibers, form sugar polymers such as xylose ($C_5H_{10}O_5$) and mannose [11,12]. The hydrolysis of cellulose and hemicellulose depends on their molecular architecture, monomeric composition, and inter-unit bonding [13–15]. In nature, this decomposition is catalyzed by cellulases and other enzymatic complexes [16], which are found in actinomycetes, bacteria, filamentous fungi, and macrofungi [17,18].

Macrofungi, in particular, play essential ecological roles in trophic networks and biogeochemical cycles, facilitating the distribution and transformation of organic and inorganic compounds; so, they are considered key species for environmental remediation strategies [19,20].

The objective of this study was to evaluate the effectiveness of *Pleurotus ostreatus* and *Pleurotus djamor* in the mineralization of lignocellulosic residues derived from cigarette butts and the detoxification of associated toxic compounds, using a protocol tailored to the biological characteristics of these basidiomycetes [21,22]. Furthermore, this study aimed to determine the optimal environmental conditions—relative humidity (RH), temperature (T), and photoperiod (HL)—for fungal growth and assess the degradation of cellulose and hemicellulose fibers in the treated waste.

II. Materials and Methods

A. Materials

The study was conducted in the district of San Jerónimo, located in the southern province of Cusco, Peru. A total of

2,500 cigarette butts were used as a lignocellulosic substrate to evaluate their mineralization and detoxification using *Pleurotus ostreatus* and *Pleurotus djamor*. The experiment was structured in two treatment phases, conducted at 45 and 75 days, during which the cigarette filters remained in contact with actively growing fungal mycelia.

Environmental parameters were monitored using calibrated thermo-hygrometers within aseptic cultivation chambers, designed to prevent contamination and promote optimal mycelial development.

B. Methods

B.1. Environmental monitoring

To monitor environmental parameters—temperature (T), relative humidity (RH), and photoperiod (HL)—thermo-hygrometers were used, calibrated with a resolution of ±1% RH and ±0.1°C, along with an external IP67-rated thermistor. Humidity levels ranged from 20% to 90%, and light exposure was controlled according to the research monitor's criteria.

Custom-designed cultivation chambers were prepared to accommodate fungal growth on cigarette butt substrates, allowing systematic evaluation of *P. ostreatus* and *P. djamor* under controlled conditions.

B.2. Substrate Inoculation and Incubation

The experiment followed a systematic procedure. Workspaces and cultivation chambers were first sterilized. Each container was prepared with 60 g of cigarette butts and inoculated with fungal mycelium in a 2:1 substrate-to-mycelium ratio. The cigarette butts were manually hydrated with distilled water to reach a relative humidity of 60–80%. All containers and materials were sterilized using autoclave prior to use. After inoculation, the containers were sealed, labeled, and transferred to a sterile incubation chamber. Environmental parameters were monitored every three days. Substrates were incubated at a minimum temperature of 15 °C for either 45 or 75 days to allow the formation of fungal primordia and fruiting bodies under optimal conditions.

B.3. Analytical Methods

Chemical and structural changes in the cigarette but substrates were evaluated at the end of each treatment phase using high-resolution Scanning Electron Microscopy (SEM, Hitachi SU8230) and Energy-Dispersive X-ray Spectroscopy (EDS). SEM imaging allowed for the observation of surface morphology and degradation of cellulose and hemicellulose fibers. EDS provided elemental analysis to detect chemical mineralization or detoxification, quantifying changes in the presence of key elements (e.g., Mg, Al, Si, K, P, S, Cl, Ca) that are indicative of toxic contamination or successful bioremediation.

III. Results and Discussions

Table I summarizes the chemical elements identified in untreated cigarette butt samples. SEM images of the untreated filters, shown in Fig. 1, revealed heavily worn cellulose fibers with visible residues of magnesium (Mg), aluminum (Al), silicon (Si), phosphorus (P), sulfur (S), chlorine (Cl), and potassium (K). These elements were present either in free form or chemically bonded within the fiber matrix, reflecting the inherent toxicity of cigarette waste.

TABLE I
EDS analysis of cellulose acetate fiber from untreated cigarette butts

Element	At. No	Netto	Mass (%)
Carbon	6	10139	48.81
Oxygen	8	10695	27.08
Magnesium	12	8883	4.25
Aluminum	13	1057	0.46
Silicon	14	357	0.27
Phosphorus	15	4001	1.24
Sulfur	16	5787	1.73
Chlorine	17	12169	3.86
Potassium	19	34066	12.30
		Sum	100.00

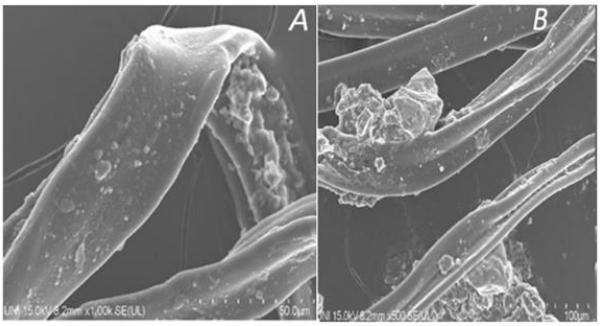

Fig. 1 Cigarette butt fibers without detoxification and mineralization treatment.
Note: (A) 50 µm-15 kV (B) 100 µm-15 Kv

C. Phase I: 45-Day Biodegradation

A continuous 45-day monitoring was conducted to track relative humidity (RH), temperature (T), and light exposure (LE), while cigarette butt substrates—classified as highly toxic solid waste—were inoculated with mycelia of *Pleurotus ostreatus* and *Pleurotus djamor*. Average environmental conditions recorded were 22.17 °C and 52.46% RH for Sample 1 (*P. ostreatus*), and 19.05 °C and 53% RH for Sample 2 (*P. djamor*). Both fungal strains achieved 100% colonization, demonstrating their adaptation and biodegradative capacity for cellulose and lignin.

C.1. Cellulose and Lignin Detoxification – Phase I

SEM images confirmed the detoxification of cellulose and hemicellulose fibers in degraded cigarette butts exposed to both fungal species. Images captured under high voltage (15.0 kV) at magnifications of 50 and 100 µm highlighted the structural breakdown of fibrous components, reinforcing the biodegradation potential of *P. ostreatus* and *P. djamor*.

C.2. Pleurotus ostreatus Treatment – Phase I

Energy-dispersive X-ray spectroscopy (EDS) of regions treated with *P. ostreatus* revealed that oxygen constituted 97.21% of the atomic percentage, with a relative error of ±11.13%. Other detected elements included K (1.01%), Ti (0.99%), S (0.36%), and P (0.43%), as shown in Table II and Fig. 2. This reflects a significant increase in oxygen and the complete disappearance of elements such as Al, Si, Mn, Mg, and Cl. In a second analysis, oxygen levels remained high (96.67% ±11.2%), with Ti at 0.94%, K at 1.41%, P at 0.38%, S at 0.29% and 0.93%, and Al at 0.31%.

These results confirm enhanced mineralization and detoxification due to fungal catalase activity, leading to the stabilization and detoxification of previously hazardous elements.

TABLE II
EDS ANALYSIS OF CELLULOSE ACETATE FIBER FROM CIGARETTE BUTTS MINERALIZED BY *PLEUROTUS OSTREATUS* - SPECTRUM 1 AND 2 (PHASE I)

Spectrum 1				
Element	**At. No**	**Netto**	**Mass (%)**	**Atom (%)**
Carbon	6	149940	0	0
Oxygen	8	32894	93.29	97.21
Potassium	19	1376	2.37	1.01
Titanium	22	888	2.85	0.99
Sulfur	16	512	0.7	0.36
Phosphorus	15	579	0.79	0.43
		Sum		100
Spectrum 2				
Element	**At. No**	**Netto**	**Mass (%)**	**Atom (%)**
Carbon	6	120045	0	0
Oxygen	8	29303	91.27	96.67
Titanium	22	862	3.69	0.94
Potassium	19	1760	3.79	1.51
Phosphorus	15	423	0.7	0.38
Sulfur	16	358	0.55	0.29
Aluminum	13	246	0.5	0.31
		Sum		100

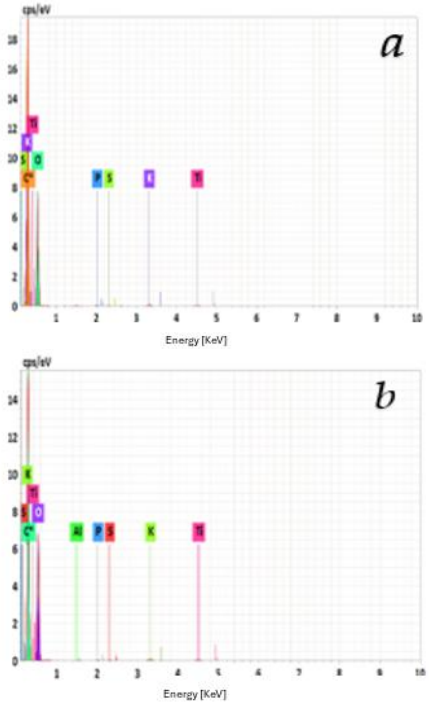

Fig. 2 Mineral spectrum in cellulose acetate fiber in Phase I - *Pleurotus ostreatus*
Note: (a) Spectrum 1 of minerals on cellulose acetate fiber at 20 µm – 15kV at 45 days (b) Spectrum 2 of minerals on cellulose acetate fiber at 20 µm – 15kV at 45 days

C.3. Pleurotus djamor Treatment – Phase I

EDS spectra from samples treated with *P. djamor* showed oxygen at 67.42% ±12.50%, alongside Si (9.53%), Al (1.56%), P (1.54%), S (2.10%), Cl (2.21%), K (10.74%), and Ca (4.89%), as shown in Table III and Fig. 3. The increased oxygen content and elimination of Mg and Mn indicate active detoxification.

A second spectrum revealed oxygen at 80.32% ±11.65%, with K (6.83%), Ca (6.81%), Cl (1.65%), S (1.24%), P (1.27%), Si (0.92%), and Al (0.95%). Again, a marked reduction of Ca and absence of Mg and Mn were observed, consistent with catalytic detoxification by *P. djamor*, resulting in the chemical stabilization of residual elements.

TABLE III.
EDS ANALYSIS OF CELLULOSE ACETATE FIBER FROM CIGARETTE BUTTS MINERALIZED BY *PLEUROTUS DJAMOR* - SPECTRUM 1 AND 2 (PHASE I)

Spectrum 1				
Element	**At. No**	**Netto**	**Mass (%)**	**Atom (%)**
Carbon	6	86454	0	0
Oxygen	8	25514	17.91	49.08
Silicon	14	32163	4.44	12.18
Aluminum	13	4167	0.7	1.92
Phosphorus	15	6257	0.79	2.17
Sulfur	16	9551	1.12	3.06
Chlorine	17	10557	1.3	3.57
Potassium	19	49866	6.97	19.11
Calcium	20	19367	3.25	8.92
			Sum	100
Spectrum 2				
Element	**At. No**	**Netto**	**Mass (%)**	**Atom (%)**
Carbon	6	171302	0	0
Oxygen	8	58974	28.65	80.32
Potassium	19	47397	5.95	6.83
Calcium	20	40358	6.09	6.81
Chlorine	17	13159	1.31	1.65
Sulfur	16	9801	0.89	1.24
Phosphorus	15	9244	0.87	1.27
Silicon	14	5793	0.57	0.92
Aluminum	13	4931	0.57	0.95
			Sum	100

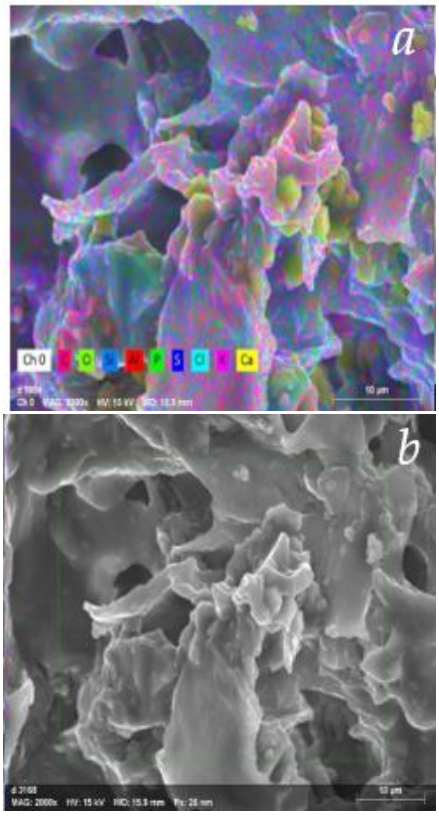

Fig. 3. Mineral spectrum in cellulose acetate fiber in Phase I - *Pleurotus djamor*

Note: (a) Spectrum 1 of minerals on cellulose acetate fiber at 10 µm – 15kV at 45 days (b) Spectrum 2 of minerals on cellulose acetate fiber at 10 µm – 15kV at 45 days

D. Phase II: 75-Day Biodegradation

D.1. Cellulose and Lignin Detoxification – Phase II

During the 75-day Phase II, relative humidity (RH), temperature (T), and light exposure (LE) were continuously monitored in substrates composed of cigarette butts and treated with mycelia of *Pleurotus ostreatus* and *Pleurotus djamor*. The average environmental conditions were 23 °C and 54.25% RH for Sample 1 (*P. ostreatus*) and 19.15 °C and 55% RH for Sample 2 (*P. djamor*). Both fungal species achieved full colonization of the substrate, confirming their optimal adaptation and robust biodegradative capabilities for cellulose and lignin.

D.2. Pleurotus ostreatus Treatment – Phase II

EDS analysis of a region treated with *P. ostreatus* revealed oxygen at 81.08% atomic concentration (±11.39% relative error), along with Mg (2.68%), P (3.88%), S (1.24%), Cl (1.42%), K (5.66%), and Ca (4.04%), as shown in Table IV and Fig. 4. A notable increase in oxygen content and the elimination of Al, Si, and Mn were observed, alongside a significant reduction in Ca content. These results indicate

effective elemental mineralization and detoxification catalyzed by *P. ostreatus* enzymes, leading to the stabilization and neutralization of toxic compounds.

In a second spectrum of the same sample, oxygen was present at 73.70% ±12.55%, with P (5.13%), S (1.93%), Cl (3.00%), K (10.63%), Ca (3.06%), Mg (1.95%), and Al (0.60%). Again, oxygen content increased while Si and Mn were fully eliminated and Ca content reduced, reinforcing the consistency of detoxification induced by *P. ostreatus.*

TABLE IV

EDS ANALYSIS OF CELLULOSE ACETATE FIBER FROM CIGARETTE BUTTS MINERALIZED BY *PLEUROTUS OSTREATUS* -SPECTRUM 1 AND 2 (PHASE II)

Spectrum 1				
Element	**At. No**	**Netto**	**Mass (%)**	**Atom (%)**
Oxygen	8	82926	29.07	81.08
Carbon	6	200034	0	0
Magnesium	12	13686	1.46	2.68
Phosphorus	15	34923	2.69	3.88
Sulfur	16	11846	0.89	1.24
Chlorine	17	13289	1.13	1.42
Potassium	19	43587	4.96	5.66
Calcium	20	26063	3.63	4.04
			Sum	100
Spectrum 2				
Element	**At. No**	**Netto**	**Mass (%)**	**Atom (%)**
Carbon	6	63552	0	0
Oxygen	8	24572	17.63	73.7
Phosphorus	15	16866	2.38	5.13
Sulfur	16	7124	0.92	1.93
Chlorine	17	11599	1.59	3
Potassium	19	37639	6.21	10.63
Calcium	20	8958	1.84	3.06
Magnesium	12	2982	0.71	1.95
Aluminum	13	1279	0.24	0.6
		Sum		100

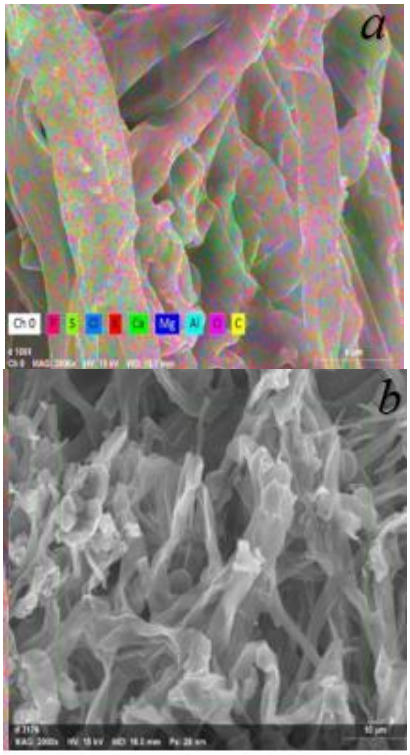

Fig. 4 Mineral spectrum in cellulose acetate fiber in Phase II - *Pleurotus ostreatus*

Note: (a) Spectrum 1 of minerals on cellulose acetate fiber at 10 µm – 15kV at 75 days (b) Spectrum 2 of minerals on cellulose acetate fiber at 10 µm – 15kV at 75 days

D.3. Pleurotus djamor Treatment – Phase II

Analysis of a region treated with the red strain, *P. djamor*, revealed oxygen at 42.88% ±16.21%, with elevated concentrations of K (22.97%), Ca (17.54%), Cl (3.85%), S (3.51%), P (4.79%), Si (1.36%), and Al (3.10%), as shown in Table V and Fig. 5. A slight decrease in oxygen content and Ca levels was observed, while Mn was entirely eliminated. This supports the detoxifying role of *P. djamor* through its catalase activity.

A second spectrum showed oxygen at 67.01% ±11%, accompanied by Mg (0.80%), Al (2.79%), Si (26.08%), P (0.42%), S (0.13%), K (1.49%), Ca (0.32%), and Fe (0.95%). In this case, a modest increase in oxygen and the disappearance of Cl and Mn were observed, alongside a marked reduction in Ca levels, confirming the elemental stabilization and detoxification capacity of *P. djamor*.

TABLE V
EDS ANALYSIS OF CELLULOSE ACETATE FIBER FROM CIGARETTE BUTTS MINERALIZED BY *PLEUROTUS DJAMOR* - SPECTRUM 1 AND 2 (PHASE II)

Spectrum 1				
Element	**At. No**	**Netto**	**Mass (%)**	**Atom (%)**
Carbon	6	116214	0	0
Oxygen	8	4152	4.22	42.88
Potassium	19	42900	5.53	22.97
Calcium	20	26471	4.33	17.54
Chlorine	17	7821	0.84	3.85
Sulfur	16	6737	0.69	3.51
Phosphorus	15	8009	0.91	4.79
Silicon	14	1814	0.24	1.36
Aluminum	13	3047	0.51	3.1
		Sum		100
Spectrum 2				
Element	**At. No**	**Netto**	**Mass (%)**	**Atom (%)**
Carbon	6	67632	0	0
Oxygen	8	159246	36.94	67.01
Magnesium	12	8147	0.67	0.8
Aluminum	13	36278	2.59	2.79
Silicon	14	378869	25.24	26.08
Phosphorus	15	6085	0.45	0.42
Sulfur	16	1794	0.15	0.13
Potassium	19	18737	2.01	1.49
Calcium	20	3470	0.45	0.32
Iron	26	4834	1.83	0.95
		Sum		100

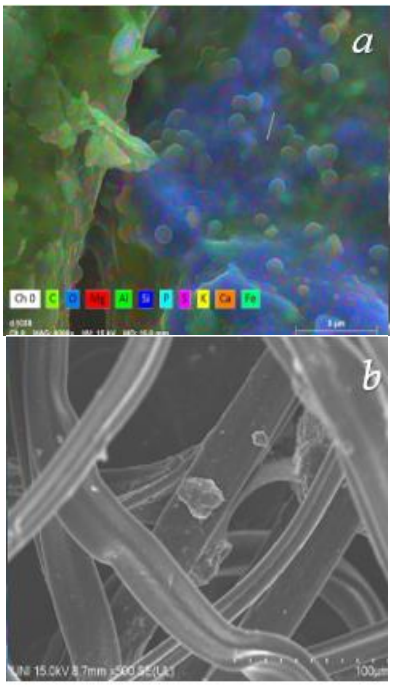

Fig. 5. Mineral spectrum in cellulose acetate fiber in Phase I - *Pleurotus djamor*

Note: (a) Spectrum 1 of minerals on cellulose acetate fiber at 5 µm – 15kV at 75 days (b) Spectrum 2 of minerals on cellulose acetate fiber at 100 µm – 15kV at 45 days

Finally, Tukey's test indicated the formation of a homogeneous group in terms of the effectiveness of *P. ostreatus* and *P. djamor* in reducing sodium concentrations in cigarette butt waste. All treatments showed high levels of mineralization, *P. djamor* at 45 days, and the most effective in reducing iron content was *P. ostreatus* at 75 days, as shown in Table VI.

TABLE VI
TUKEY'S TEST FOR HOMOGENEITY OF THE AVERAGE PERCENTAGE OF MINERALIZATION IN THE PRESENCE OF MINERALS

Strains	**N**	**Average**	**Group**
PD 45	2	1.895	A
PO 45	2	1.770	A
PO 75	2	0.6100	A

IV. CONCLUSIONS

The mineralizing efficacy of *Pleurotus ostreatus* and *Pleurotus djamor* in the treatment of lignocellulosic waste from cigarette butts was confirmed, demonstrating their ability to biodegrade cellulose and lignin. SEM imaging revealed pronounced mineralizing, detoxifying, cleansing, and catalytic

activity of fungal catalases from both basidiomycetes across the two evaluation phases—at 45 and 75 days of biological activity.

Specific environmental conditions of relative humidity (RH), temperature (T), and light exposure (LE) were established to support optimal fungal growth and biodegradation, with systematic environmental monitoring every three days during both phases.

Detoxification of cellulose and hemicellulose fibers in degraded cigarette butt residues was verified through SEM and EDS analyses, identifying both fungi as active mineralizing agents. According to the number of mineralized compounds observed throughout the process, *Pleurotus ostreatus* proved to be more effective in the mineralization and detoxification of cellulose acetate, followed by Pleurotus djamor.

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