CHAPTER 1

Compositional analysis of municipal solid waste (MSW) of Vadodara city and study of the fungal diversity

This chapter is dedicated to conducting a compositional analysis of the municipal solid waste (MSW) gathered from Vadodara city, along with an investigation into the fungal diversity present in dumpsites and landfill areas. To achieve this objective, a compositional analysis of the MSW scenario was carried out by visiting dumpsites and landfill locations to collect waste samples. The primary goal of the compositional analysis was to identify the non-biodegradable components within the MSW. The identified component will be selected for the further experimentation.

Furthermore, fungal strains were isolated from these sites using a combination of aero-microflora and soil-microflora techniques at each sampling spot. The isolated strains underwent thorough morphological and microscopic identification based on their distinguish characteristics. Subsequently, these isolated fungal strains were screened for their potential to degrade the selected non-biodegradable component of MSW, contributing to a comprehensive understanding of waste management and environmental sustainability.

COMPOSITIONAL ANALYSIS

METHODOLOGY

Study Area

Vadodara city is one of the well-known cities of Gujarat and talk about its Cultural happenings. The city has a total area of 108 sq. k. meters lies between 22°30′72″N and 73°18′12″E on the map of India with the population of approximately 13.23 lacs (male population- 9 lacs & female population- 8 lacs) (Census report 2011-19). The maximum and minimum temperature of the city throughout the year varies from 10 °C to 44.5 °C. Relative humidity of the city ranges from 45% to 90% and wind speed throughout the year ranges from 6 m/sec to 40 m/sec.

The waste management system of Vadodara has received limited attention compared to other cities. Daily collection of garbage accounts to much higher than 40 lac metric tonnes. While passing along the Vadodara-Bharuch national highway, the sight of a massive solid waste mountain at the Jambuva landfill area inspired us to explore potential solutions for this issue, leading me to select Vadodara city as the focus area for my study on municipal solid waste management. Importantly it was observed that the huge heap of accumulated MSW was solidified with the lower layers having only plastic bags popping out in the rest of degraded waste.

The present study has been carried out at different dump-sites of the city include a major landfill area and three temporary dumping sites. Four stations have been selected as study areas viz.; (i) Karelibaug for North zone, (ii) Waghodia for east zone, (iii) Atladara area for West & South zone, (iv) Jambuva area as a main landfill. A brief description of each station is given below:

(i) Karelibaug area- This station is located between latitude 22°32′ longitude 73°19′ in karelibaug area. Concrete surface has been constructed on the area of dumping site for north zone.

(ii) Waghodia area- This station is located between latitude 22°18′ longitude 73°13′ in waghodia area. Similarly, to Karelibaug dumpsite concrete surface has been constructed for east zone daily waste.

(iii) Atladara area- This station is located between latitude 22°15′ longitude 73°06′ near Mujhmahuda area. Internal road reaching to this site is not properly constructed hence even a short spell of rain could lead to slushy path.

(iv) Jambuva area- This landfill area of Vadodara city (Revenue Survey No. 346) is located between latitude 22°13′ longitude 73°12′ near Jambuva by-pass. Earlier Jambuva landfill had capacity of total 300 TPT waste and recently it is under maintenance for 750 TPT waste capacity. At this particular landfill VMC is mainly processing this waste in two ways; Biomethanation which produces compost & Biogas and plastic waste is being processed to produce Biodiesel.

Figure.1. Map showing the study area including three temporary dumpsites and a main landfill area (Source: Google maps)

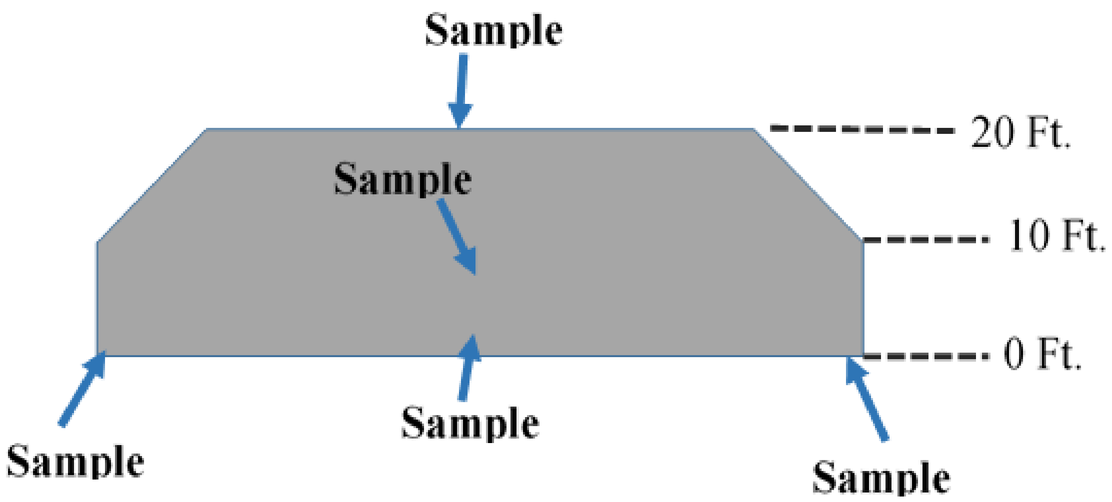

Figure.2. Sample collection methodology of Jambuva landfill area

Collection of the solid waste

For compositional analysis waste samples were collected from three corners (replicates) of temporary dumpsites. The replicates were collected as per the modified method of Central Public Health and Environmental Engineering Organization (CPHEEO) manual (2016). While Jambuva area is somewhat oval shaped and dumped waste has approximately 20 ft. in height. The Jambuva landfill was constructed in February, 2020 for MSW management. Hence approximately 2 kg solid waste samples were collected from three different depths of each side as shown in Figure.2.

Physical characterization of the solid waste

The collected samples were brought to the laboratory and segregated. MSW contains on an average between 30 to 50% compostable waste, about 4-6% recyclables and such constituents having high calorific value. Collected Solid waste samples were majorly categorized into Kitchen waste, recyclable waste (glass, packaging & paper), plastic waste and dry organic waste as described by Mehta and Pandey (2014) for its compositional analysis. In present study 10-year-old waste and daily waste samples were compared for determination of non-biodegradable components of MSW.

The VMC has proposed to clear up over 4 lac metric tonnes of garbage dumped at the disposal site, freeing 4.5 hectares of land which would be available for future garbage disposal (Times of India, 26th June, 2020). The waste would be segregated & products like refused- derived fuel (RDF), plastics & others could be made from the garbage.

RESULT AND DISCUSSION

Active disposal Sites

In India approximated 50-90% of the million tonnes of solid waste is collected and dumped into uncontrolled open landfill sites (Ayub and Khan, 2011). Personal communication at Vadodara Municipal Corporation (VMC) office revealed that Vadodara city has three temporary active dumping sites for different zones; (i) In Karelibaug area for North zone, (ii) for east zone it is located in Waghodia, (iii) Atladara area for West & South zone and from all the three dumpsites, wastes are being transported to the large landfill site which is located at Jambuva, Vadodara (Revenue Survey No. 346). At the three temporary sites dumper carrying the waste is transported and segregated into recyclable wste. Thereafter the remaining waste is transported to the main landfill at Jambuva. Earlier Jambuva landfill had capacity of total 300 TPT waste and recently it is under maintenance for 750 TPT waste capacity. At the main landfill VMC has undertaken processing this waste in two ways; Biomethanation which produces compost & Biogas and plastic waste is being processed to produce Biodiesel, presently both the processes seemed to be inactive during the visit. Jambuva landfill cell has 45000 sq.mt. area and it has 4 lac M.T. waste capacity. The area is covered with 1.5 mm thick H.D.P.E liner to protect landfill soil from dumped waste.

According to Central Pollution Control Board (CPCB), average collection coverage of solid waste ranges from 50%-90% and 94% of all collected waste is disposed of in an unacceptable manner. Hence it leads to contamination of groundwater and surface water through leachate (Kumar *et al.*, 2014). Vadodara city has similar scenario of waste management, as shown in Figure.3 (C) daily waste is simply given to cattle to be eaten which is completely objectionable manner of daily waste disposal. At Jambuva landfill area leachate collection system had been developed by using 100 mm thick perforated H.D.P.E. lateral pipe, but VMC doesn’t perform any further process for collected leachate. While collecting the leachate it seemed to be acidic in nature. The leachate is produced by the percolation of rainwater through the waste layers in a landfill. Therefore, it may show high concentrations of many compounds, in particular high concentrations of easily degradable organic components as volatile fatty acids. Particularly, landfill leachates may contain very high concentrations of dissolved organic matter, inorganic macro components and heavy metals (Kjeldsen et al., 2002). Many regulated landfills attempt to utilize landfill biogas, a renewable energy source, to generate electricity or heat, but no such practice observed in VMC waste management.

Collection and Physical Characterization of Solid waste

Near VUDA Bhawan (Karelibaug) dumping site segregation of recyclable waste is being conducted by rag-pickers. Further this segregated recyclable waste such as glass bulbs & bottles, milk bags, used plastic bottles (Cosmetics & Soap) (Figure.3.) are being supplied to scrap dealers. At Atladara and

Waghodia dumping sites no such segregation process is being conducted. The daily waste including all kind of wastes is being given to cattle to be eaten at Atladara dumpsite, while Gadheda market dumpsite is simply used as a transit for transferring daily waste to main landfill area. Physical characteristics of the solid waste collected and analyzed from the different dumping sited of Vadodara has been represented in Table.1-3.

Table.1. Physical composition of daily Solid waste from temporary dumping sites

Sr. No.	Nature of Waste	Percentage (%)		
		Site 1 (VUDA Bhawan)	Site 2 (Atladara)	Site 3 (Gadheda Market)
1	Kitchen waste (Rotten fruits & Vegetables, peels of vegetable & Fruits etc.)	53	67.7	59.5
2	Glass (Bottles/ Bulbs)	10.9	-	5
3	Packaging waste		-	-
	(i) Paper Boxes	6	4	-
	(ii) Food wrappers, Plastic bags of milk & other products	15.55	14.7	3.1
	(iii) Soap Bottles	3.3	1.5	-
4	Paper waste	3.6	1	7.6
6	Polyethylene Shopping bags	4.85	8	14
7	Disposable waste	2.25	2.05	3.15
8	Dried leaves & branches	-	-	1.2
9	Unclassified debris, soil	4.45	1.75	6.5

Table.1. represents the physical characteristics of the daily waste collected and dumped at the three different sites (Vuda Bhavan, Atladara & Gadheda market). As there are wastes collected from the residential areas it can be observed that the waste generated is the highest percentage followed by packaging wastes (food wrappers, plastic bags of milk & other products) at site 1 & 2. At site 3 following kitchen waste the polyethylene shopping bags are at higher percentage. The observation made at this specific site highlights the mindset of the residents in that area, particularly regarding the usage of plastic bags and the practice of disposing kitchen waste in such bags. This behavior can have significant implications for waste management and environmental sustainability. By identifying and understanding these patterns, it becomes possible to develop targeted strategies and interventions to address these specific issues.

Table.2. indicates that major amount of waste has either ability to be recycled or to be degraded naturally in the environment, and rest is causing damage to our ecosystems while ingested by mammals and also considered to be non-biodegradable. In this analysis highest percentage is of kitchen waste which is either eaten by cattle or degraded naturally in few months.

Figure.3. A-F: Temporary Dumping sites of Vadodara City
A-Karelibaug area; **B-** Waghodia area; **C-** Atladara area; **D-** Atladara dumpsite with ten-year old waste; **E & F-** Segregation of recyclable waste; **G-I-** Old dumpsite converted into garden at Atladara area; **J-L-** Jambuva Landfill area **(J & K-** Waste Disposal; **L**- Leachate separation)

Atladara dumping site has two locations in which one is for daily waste and other location has more than ten years old waste. Comparison of daily waste samples and ten-year old sample indicates that kitchen waste, paper & box waste get degraded by microorganisms in a period of few months as displayed in Table.2 & Figure.4. The analysis of waste samples collected over a ten-year period reveals important insights into the recycling patterns and composition of waste in Vadodara. The finding that soap bottles, milk packaging bags, and glass wastes are being recycled is encouraging, as it demonstrates a level of environmental consciousness and responsible waste management.

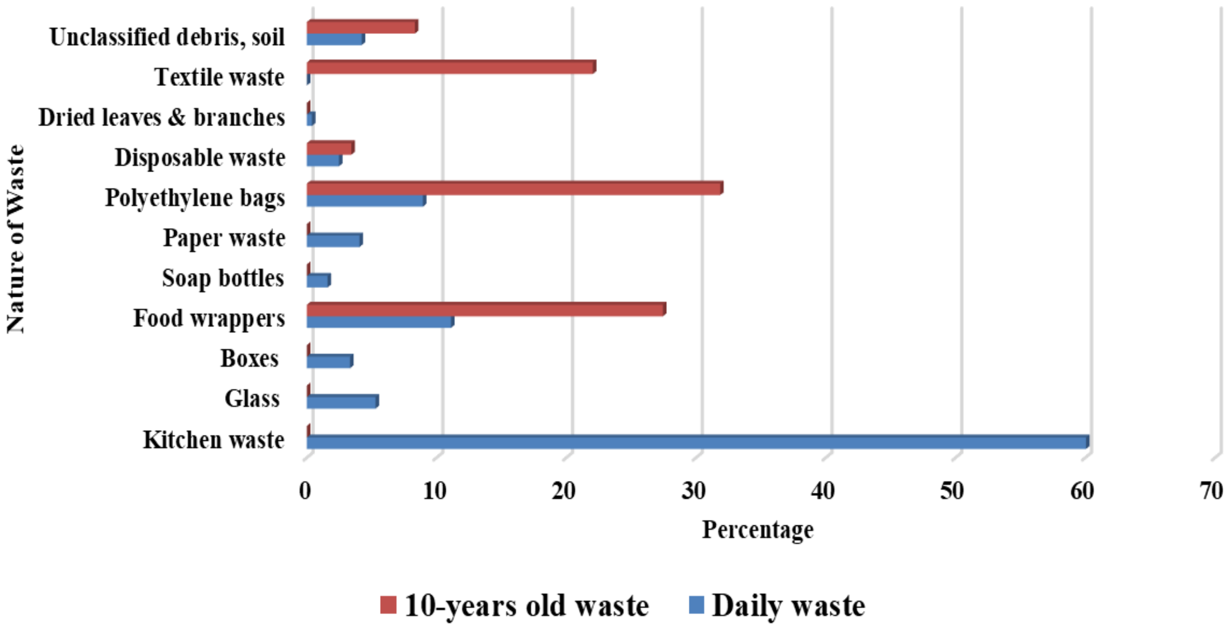

Figure.4. Comparison of daily waste (temporary dumping sites) and 10-years old waste samples (Atladara dumping sites)

Table.2. Physical composition of solid waste from Atladara dumpsite (Ten-year-old)

Sr. No.	Nature of waste	Percentage (%)
1	Polyethylene Bags	31.86
2	Packaging waste (Food Wrappers)	27.45
3	Coir	6.86
4	Textile waste (Leather, Fabric)	22
5	Disposable waste	3.43
6	Unclassified debris & Soil	8.33

Solid waste samples collected from Jambuva landfill area showed different composition in each depth. Solid waste sample from 0 ft. had highest content of soil & unclassified debris and lowest in 20 ft. waste sample that explains decomposition of degradable waste. Kitchen waste was only found in 20 ft. waste samples i.e., 24.44% of total waste sample. Textile waste was observed in all three depths samples. Plastic waste includes different kind of plastic products such as food wrappers, plastic bags of milk & other products, soap bottles & cosmetic packaging waste, disposable utensils and polyethylene bags.

Table.3. Physical composition of Municipal Solid waste from Jambuva landfill Area

Sr. No.	Nature of waste	Percentage (%)		
		0 Ft.	**10 Ft.**	**20 Ft.**
1	Rubber waste	0	30.47	0
2	Kitchen waste (Rotten fruits & Vegetables, dried vegetable skins)/ coconut outer layer	0	0	24.44
3	Glass (Bottles/ Bulbs)	0	6.59	0
4	Fabric/ Leather	11.94	21.39	6.03
5	Paper Boxes	1.16	1.49	6.15
6	Food wrappers, Plastic bags of milk & other products	**9.04**	**6.71**	**6.84**
7	Soap Bottles/ cosmetic tubes	**0.80**	**0**	**0**
8	Polystyrene Packaging waste	0.68	0	4.04
9	Polyethylene Shopping bags	**10.19**	**12.56**	**16.23**
10	Disposable waste	**4.47**	**2.48**	**2.05**
11	Tooth brush/pen/ comb/CDs/ bottle cap/ toys	**1.36**	**0.87**	**0.31**
12	Dried leaves & branches	2.17	40.29	19.15
13	Unclassified debris, soil/ rocks	58.14	26.74	15.48

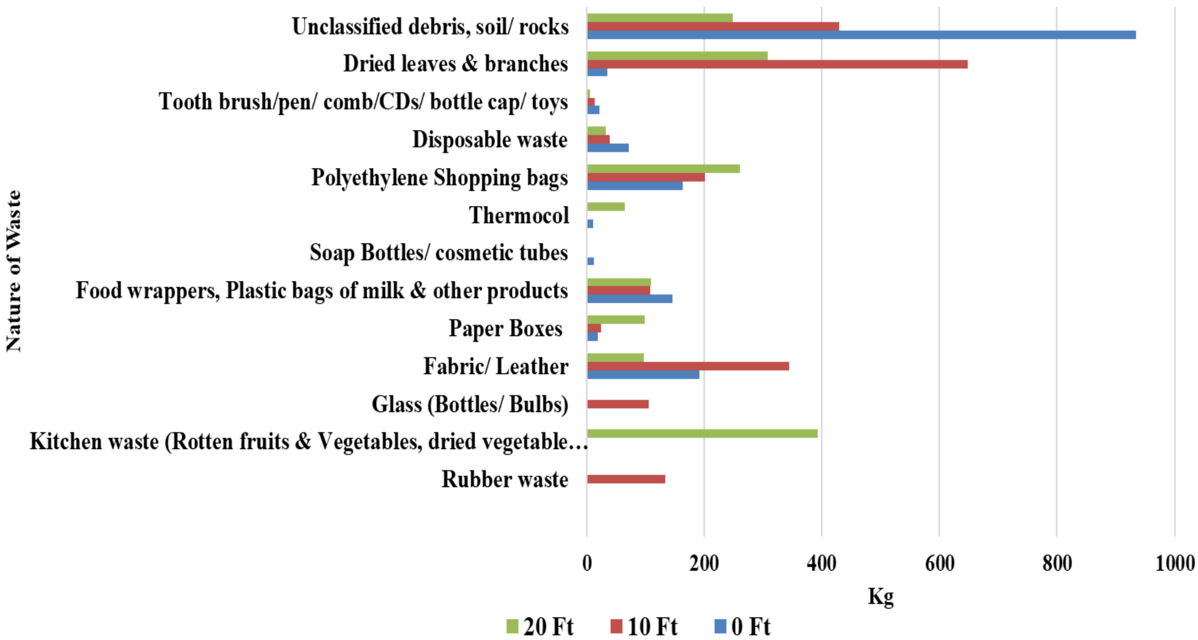

Figure.5. Comparison of Jambuva landfill waste samples from three different depths

In this analysis it reveals that approximately 25% of plastic waste is present in municipal solid waste. Among these all-plastic waste products highest percentage was of polyethylene bags. (Table.3 & Figure.4.) No significant difference was noted in the composition of waste when two different seasonal waste sample were compared. As per review article given by Sharholy *et al.*, 2008 MSW from metro cities of India contain about 3.9% of plastic waste while in this study both the seasons MSW contains approximately 26% of plastic waste materials which are causing damage to our ecosystems. Plastic materials widely used and contributing to plastic waste are polyethylene shopping bags and plastic water bottles. Visually also very clearly it could be seen that polyethylene bags remained persistent in all the lower levels (10-year-old).

As a result, the decision was made to choose polyethylene material as the non-degradable component for the subsequent fungal degradation experiments.

FUNGAL DIVERSITY IN MUNICIPAL SOLID WASTE

According to the previous analysis of municipal solid waste, polyethylene material was identified as the primary cause of plastic pollution, as it was the major non-degradable component found in 10-year-old waste samples. In light of this finding, the fungal microbiome of all three temporary dumping sites and the landfill area was studied to identify potential strains capable of degrading non-biodegradable components, such as polyethylene material.

METHODOLOGY

Isolation and identification of microbial strains

Microbial strains were isolated by performing aero-microflora and soil-microflora techniques for each spot. Soil and leachate samples were collected in sterile zip-lock pouches from each selected study area. The soil samples were serially diluted by standard methods for the isolation of microbial strains (Geldreich et al., 1972). 1 gm of soil sample was drowned and mixed with 9 mL of sterile water. The samples were then serially diluted from 10^{-4} to 10^{-6} with sterile water and spread plated on Potato dextrose Agar (PDA) & Malt extract Agar (MEA) for isolation of fungal species and Nutrient Agar (NA) & Luria Bertani Agar (LBA) for isolation of bacterial species, further petri-plates were kept for incubation at room temperature (35°± C).

Obtained microbial strains were sub-cultured for observation of morphological and microscopic characteristics.

(i) Bacterial identification- Colonies showed different morphological characteristics such as size, shape, color, elevation and margin were identified from different plates streaked with diluted samples. Gram staining technique was followed for observation of microscopic characteristics (Mac Faddin, 1980). Species showing purple stain were categorized into Gram positive while species with pink stain were categorized into Gram negative bacteria.

(ii) Fungal identification- For morphological characteristics of fungal isolates, they were stained with lactophenol cotton blue reagent and examined under a brightfield and phase-contrast microscope, standard manual was followed for identification (Nagamani et al., 2006). Isolates were identified based on morphological characteristics such as growth pattern, hyphal characters, color of colonies on the medium, surface texture, aerial mycelium, mechanism of spore production and characteristics of the spores.

RESULT AND DISCUSSION

Isolation and identification of microbial strains

A collective number of nine bacterial and seven fungal strains were isolated from soil and leachate sample collected from MSW dumping sites. Microbial strains were stained and identified on the basis of morphological and microscopic characteristics.

Bacterial strains:

Gram staining technique for bacterial identification revealed five strains were stained pink hence noted as gram negative (-ve) strains and remaining four strains noted as gram positive (+ve) strains. Further morphological characters such as colony color & size, surface, shape, margin and elevation were observed and is represented in Table.4. Distinguished colony characters of each bacterial strains are mentioned below:

Table.4. Colony characteristics of bacterial isolates

Strain No.	Size	Color	Surface	Shape	Margin	Elevation	Gram Stain
1	Small (1mm)	Reddish brown	Granular	Irregular	Undulate	flat	-ve
2	Small (0.5-1mm)	Creamish pink	Smooth	Irregular	Undulate	Raised	-ve
3	Small (1mm)	Light green	Slimy & smooth	Circular	Entire	Convex	+ve
4	Small (1-1.5mm)	Dull cream	Granular	Irregular	Undulate	Flat	+ve
5	Small (1-2mm)	Light brown	Rough	Irregular	Undulate	Flat	-ve
6	Medium (2-3mm)	Dirty brown	Slimy	Irregular	Entire	Pulvinate	-ve
7	Small (1mm)	Yellow	Smooth	Circular	Entire	Raised	+ve
8	Large (4mm)	Cream	Rough	Irregular	Lobate	Flat	+ve
9	Small (0.5mm)	Blue to green	Slimy & smooth	Irregular	Enitre	Raised	-ve

Nine bacterial strains could be identified as to whether to +ve or –ve strain, further identification required study at molecular level which was time consuming and expensive. On the other hand, fungal species are generally more effective at degrading polyethylene material due to its capability of secreting enzymes for plastic degradation compared to bacterial strains, as supported views expressed by Muhonja et al. (2018) and Anastasi et al. (2013). Since fungal strains have not been extensively investigated for their polyethylene degradation capabilities, the decision was made to exclude bacterial strains from further experimentation.

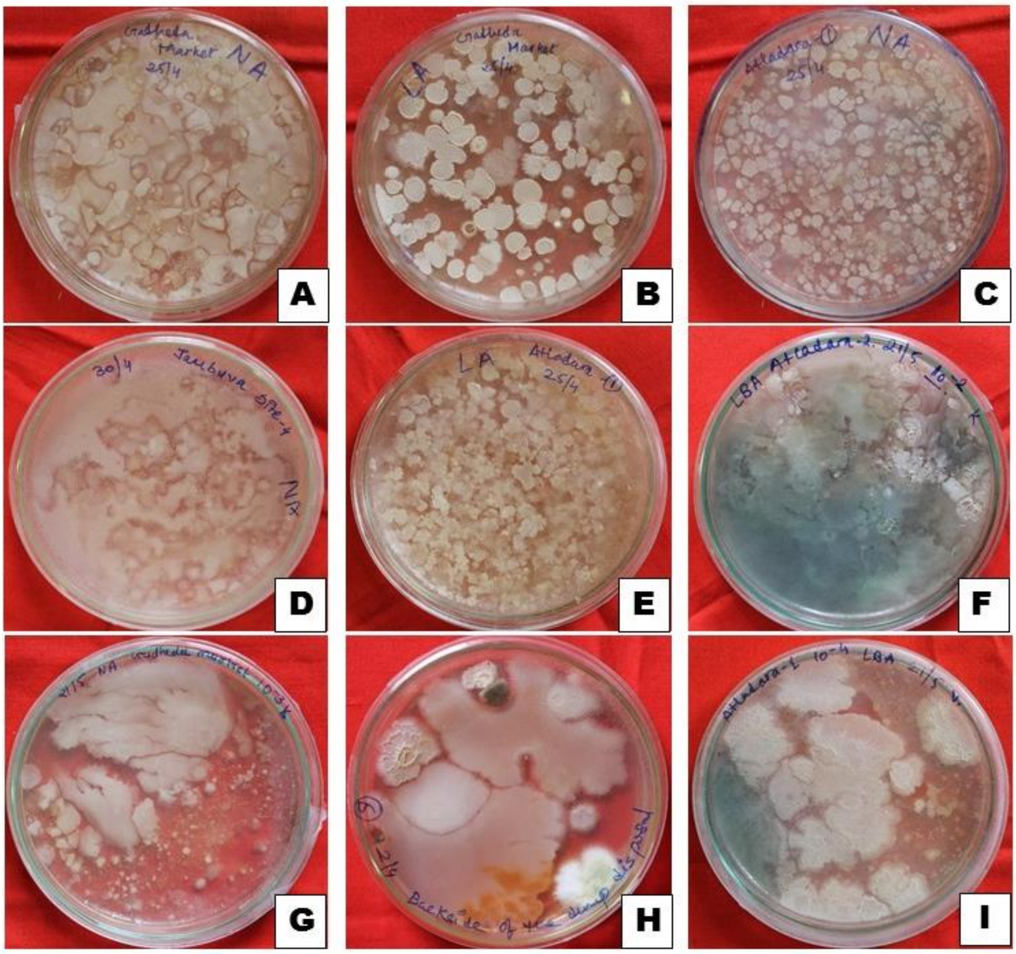

Figure.6. Bacterial isolates in the mixed culture showing distinguished morphological characteristics:

A. Strain 1 (Gram -ve), **B.** Strain 2 (Gram -ve), **C.** Strain 3 (Gram +ve), **D.** Strain 4 (Gram +ve), **E.** Strain 5 (Gram -ve), **F.** Strain 6 (Gram -ve), **G.** Strain 7 (Gram +ve), **H.** Strain 8 (Gram +ve), **I.** Strain 9 (Gram -ve)

Fungal strains:

Figure.7 & 8 describes the characteristic features of the colony and microscopic characteristics respectively which has been described in Table.5. Fungal strains were isolated from soil and leachate samples collected from MSW dumpsites. The strains were designated serially with SA1 to SA19 and then based on colony characteristics & microscopic features tentatively identified. After screening and identifying the potential strains, only strains confirmed with their potentiality to degrade polyethylene were authenticated further for the molecular identification.

Three different species of *Aspergillus sp.* were identified on the basis of its fast-growing fungal colonies with blue-green, green, brown and black color (Figure.8-A, B, C, D, G) and the notable structure of phialides (Nagmani et al., 2016).

A species of *Trichoderma* was isolated from soil and aero-mycoflora method and later identified with its effuse colonies and conidiophores with lateral branches. Hyaline aerial hyphae, stolons, pigmented rhizoids and differentiation into stolons & nodes with rhizoids were the main observed characteristics of *Rhizopus* species. Uniseriate phialide condition is discussed when phialides are in one series while phialides in two series are called as biseriate. Predominately biseriate phialides are reported to be present in *A. flavus.* Carbon black-colored conidial heads and conidiophores up to 3mm in length were clearly seen in *A. niger* (Figure.8-A). Distinguished morphological and microscopic characteristics of each isolate have been described in Table.5.

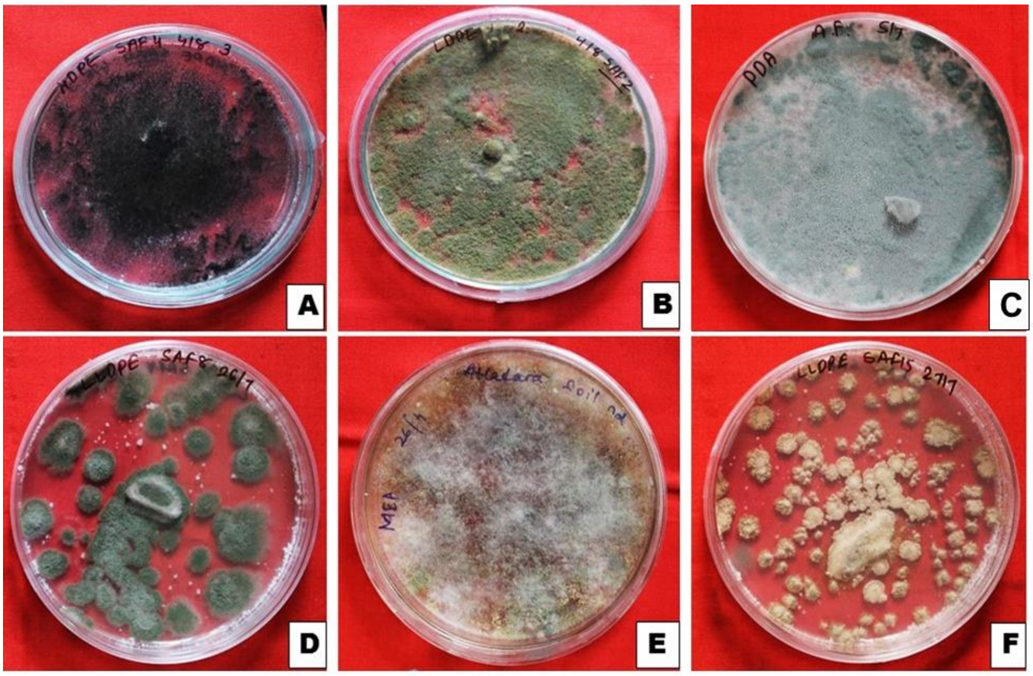

Figure.7. Fugal isolates showing distinguished morphological characteristics:
A. *Aspergillus niger*; **B.** *Aspergillus flavus*; **C.** *Aspergillus fumigatus*; **D.** *Trichoderma* sp.; **E.** *Rhizopus* sp.; **F.** *Aspergillus oryzae*

The compositional analysis experiment revealed the non-degradable component of municipal solid waste (MSW) responsible for the plastic menace. Although kitchen waste was expected to be the predominant component in the daily waste samples, it surprisingly appeared to be easily compostable. However, upon examining 10-year-old samples, it was found that polyethylene bags were present in the largest amount.

The nineteen fungal strains designated as SA1 to SA19 were identified as given in Table.6. Eight fungal strains isolated from dumping sites of Vadodara, along with four procured from National Fungal Culture Collection of India (NFCCI), Pune and other seven fungal strains readily available in the laboratory at The Maharaja Sayajirao University of Baroda (MSU), Vadodara (Total 19) were screened with HDPE, LDPE and LLDPE powders to identify its potentiality to degrade plastic components. Table.6 describes the source of the fungal strain and numbers assigned to them for conducting the experiments. Therefore, total number of nineteen fungal strains were further screened with polyethylene materials in the next experiment.

Table.5. Distinguished characteristics of fungal isolates

Sr. No.	Strain	Fungal Species	Morphological characteristics	Microscopic Characteristics	Source of isolation
1	SA4	*Aspergillus niger* Tiegh.	Moderately growing black colored colonies; reverse colorless to pale yellow	Large & black conidial heads; non-septate conidiophores; globose conidia	Soil, Air & Leachate
2	SA2	*Aspergillus flavus* Link.	Rapidly growing light green colored colonies; reverse colorless to pale tallow brown	Yellow conidial heads; conidiophores arising separately from the substratum; biseriate phialides; globose conidia	Soil, Air & Leachate
3	SA3	*Aspergillus fumigatus* Fresen.	Rapidly growing dull blue-green colonies; reverse colorless	Septate conidiophores, gradually enlarging into a flask shaped vesicle; uniseriate phialides; globose conidia	Soil, Air & Leachate
4	SA5	*Aspergillus oryzae* (Ahlb.)	Rapidly spreading light brown colonies; at first white, later becoming light brown; reverse light yellow	Radiate conidial heads; up to 5mm long conidiophores; globose to subglobose conidia	Soil & Air
5	SA8	*Trichoderma* sp.	Effuse colonies with olive to dark green color, reverse yellow	Irregularly branched conidiophores; Septate conidia	Soil & Air
6	SA12	*Rhizopus* sp.	Very fast-growing grey, dark brown colonies; aerially growing filaments or stolons	Pigmented rhizoids; unbranched sporangiophores	Soil & Air
7	SA15	*Aspergillus oryzae* (Ahlb.)	Moderately spreading light brown colonies; at first white, later becoming brown; reverse dark yellow to brown	Radiate conidial heads; up to 5mm long conidiophores; globose to subglobose conidia	Leachate

Table.6. List of nineteen fungal strains screened with Polyethylene

Sr.No	Fungal species		Source
1.	*Aspergillus tubingensis* R. Mosseray	**SA1**	NFCCI
2.	*Aspergillus flavus* Link	**SA2**	Dumpsite
3.	*Aspergillus fumigatus* Fresenius	**SA3**	Dumpsite
4.	*Aspergillus niger* van Tieghem	**SA4**	Dumpsite
5.	*Aspergillus oryzae* (Ahlburg) E. Cohn	**SA5**	Dumpsite
6.	*Trichoderma reesei* Simmons	**SA6**	MSU
7.	*Trichoderma viride* Pers.	**SA7**	MSU
8.	*Trichoderma* sp.	**SA8**	Dumpsite
9.	*Penicillium oxalicum* Currie, J.N.; Thom, C.	**SA9**	NFCCI
10.	*Penicillium chrysogenum* Thom	**SA10**	NFCCI
11.	*Pennicillium citrinum* Thom, C.	**SA11**	Dumpsite
12.	*Rhizopus* sp.	**SA12**	Dumpsite
13.	*Pestalotiopsis* sp.	**SA13**	NFCCI
14.	*Curvularia* sp.	**SA14**	MSU
15.	*Aspergillus oryzae* (Ahlburg) E. Cohn	**SA15**	Dumpsite
16.	*Lentinus sajor-caju* (Fr.) Fr.	**SA16**	MSU
17.	*Fusarium solani* Link	**SA17**	MSU
18.	*Phanerochaete chrysosporium* P. Karst	**SA18**	MSU
19.	*Flavodon* sp.	**SA19**	MSU

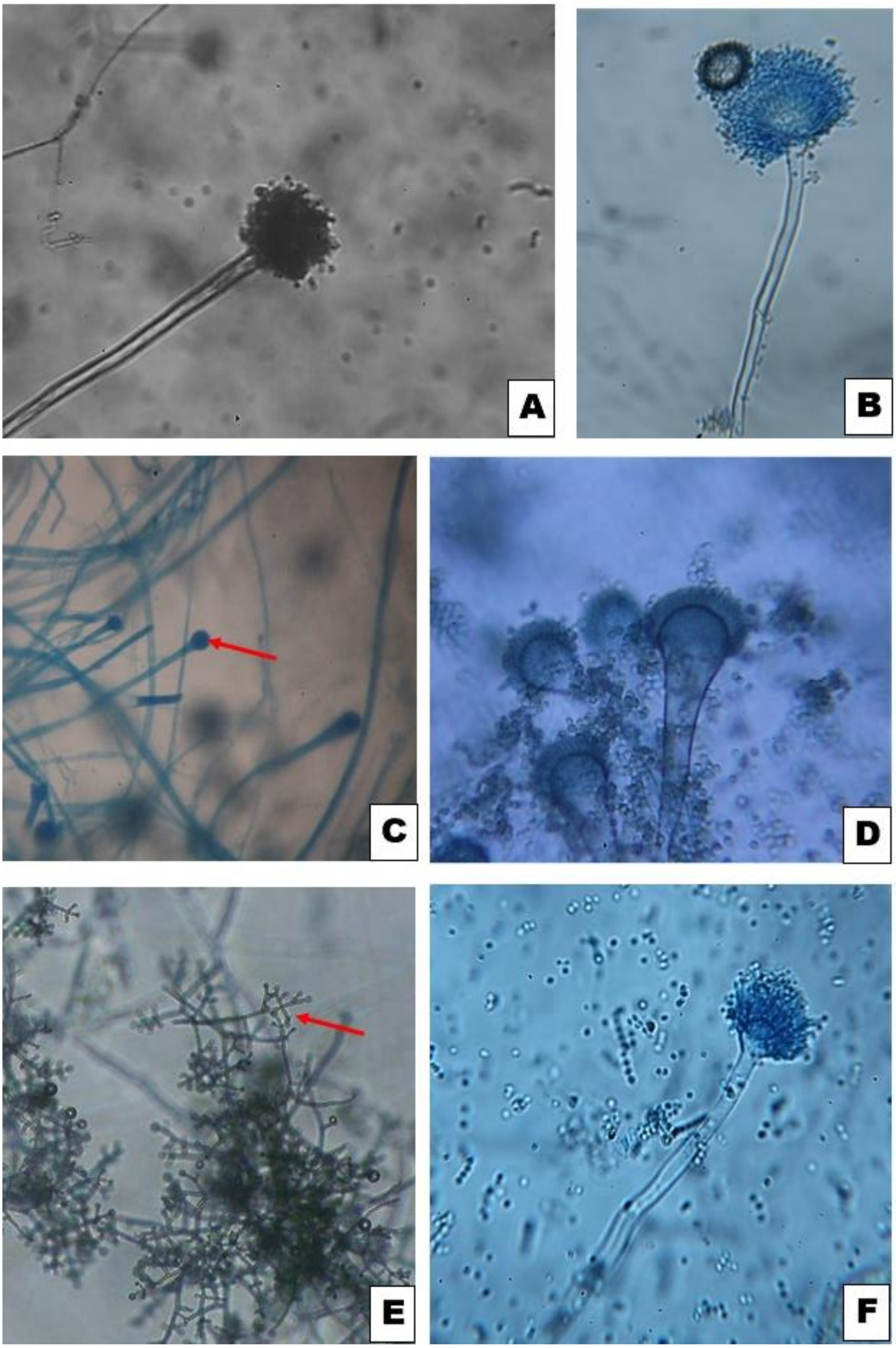

Figure.8. Fugal isolates showing distinguished microscopic characteristics:
A- *Aspergillus niger*; **B-** *Aspergillus fumigatus*; **C-** *Aspergillus flavus*; **D-** *Rhizopus* sp.; **E-** *Trichoderma* sp.; **F-** *Aspergillus oryzae* STR2

Key observations of the study

The municipal solid waste scenario in the Vadodara study was determined by visiting three temporary dumping sites and the Jambuva landfill area. The fungal microbiome of these sites was studied.

- The compositional analysis of MSW collected from the dumpsites revealed that polyethylene bags were the most responsible component for plastic pollution, as they were found in higher percentages even in 10-year-old waste samples.
- The polyethylene material was chosen for degradation experiments.
- A total of seven fungal strains were isolated from the dumping sites. Among them, five strains belonged to *Aspergillus* species, morphologically identified as *A. niger*, *A. flavus*, and *A. fumigatus*, along with two strains of *A. oryzae*. Additionally, *Trichoderma* sp. and *Rhizopus* sp. were also isolated and morphologically identified.