Title
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Publication Date
2007-12-01

ENVIRONMENTAL FACTORS AFFECTING MICROBIAL MAT DISTRIBUTION IN MO'OREA, FRENCH POLYNESIA

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Abstract Communities of photosynthetic prokaryotes are found around the world in a huge variety of locations, one of which is hypersaline mudflats. The estuary in Tamae, Mo'orea has been mostly filled in by a golf course and severely cut the population of microbial mats. Microbial mats in the Tamae mudflats in Mo'orea, French Polynesia were studied. Eight different morphologies were described and mapped. Additionally, their microhabitats described based on salinity, temperature, number of crab holes, and ground water depth and all but temperature saw certain significant differences. Transects were run from one end to another, and using the same environmental parameters, changes over the entire mudflat were observed. There were no trends with temperature. All of the other parameters exhibited trends and seem to have an influence over mat distribution, however there is not concrete evidence to support the claim that these are the factors that limit these microbial mat distributions. This ecosystem is very complex and it is probable that many more factors affect their distribution. If this habitat is to be conserved it will need to be studied more.

Key words: Microbial mats, hypersaline,French Polynesia, Lyngbya, Cyanobacteria

INTRODUCTION

Microbial mats are communities of oxygenic photosynthetic prokaryotes, typically composed of cyanobacteria. Cyanobacteria are thought to be the first living organisms, dating back to the Precambrian, which produced a large amount of oxygen, thereby changing the Earth's atmosphere (Stewart, 1983). Microbial mats have been extensively studied around the world because their lithified layers form stromatolite which paleontologists use to study the early history of the earth and astrobiologists use as indicators of life on other planet (Lau C., 2005). They are also studied because of their important role in nutrient cycling, their extreme living conditions, and PCR analysis of their communities. They can be found almost anywhere, especially in extreme environments where they do not get out competed by other organisms (58 Renaut, Robin W. 1993). They have been studied in French Polynesia because of their unique thick mats termed "Kopara" which are approximately 20-50 cm (Trichet, 1967, Mao, C.L, 2001). Inter-tidal regions with high salinity and fluctuating water depths are typical regions mats inhabit (Hoffman L., 1999), and are important to intertidal mudflat production and function (Zedler 1980, Cohen et al. 1984, Cohen & Rosenberg 1989). Other studies in Mo'orea have been conducted in the Tamae estuary by students who have examined which blue-green algal species made up the mats and environmental parameters in diel cycles across the mats. They have since been covered by a golf course leaving my site as one of the last populations on the island. Microbial mats are known to be influenced by environmental parameters. They have been shown to have differing levels in tolerance to salinity, temperature, and desiccation(Al-Thukair,A.A. 2007, Wrenn et al., 1997, Margesin and Schinner, 2001 and Yakimov et al., 2004,), and "extreme conditions… are known to be major factors in biodiversity distribution…"(Frontier, 1985 and Atlas and Bartha, 1997). This study aimed to *i.* describe the mats *ii* map their distributions *iii.* describe each mat types' microhabitat *iv.* examine the environmental factors across the mudflat that could thereby influence mat distribution. I hypothesize that mats may exhibit different microhabitats and that certain parameters such as salinity and temperature may vary throughout the region and correspond to different mat type distributions.

METHODS

Study site

Mo'orea is a high volcanic island in the Society Island Archipelagos in the Pacific Ocean

The microbial mats studied are located on the northeast end of Mo'orea, French Polynesia at S 17° 28" and W 149°46" on the Tamae mudflats. This site was chosen for its unique microbial mats and its accessibility. The majority of the original mudflats along the estuary in Tamae were completely covered by a new golf course adjacent to the site. The study site is separated from the golf course by a corridor of hibiscus and palm trees. The region with microbial mats is approximately 120meters by 230 meters. Due to time restraints and human disturbance only a portion of the mudflats were studied. The study area surveyed was approximately 100 square meters with a lagoon to the North East and hibiscus trees to the South West. The high tide covers some of the mats while others stay fairly dry throughout the entire day unless it is rainy or very windy.

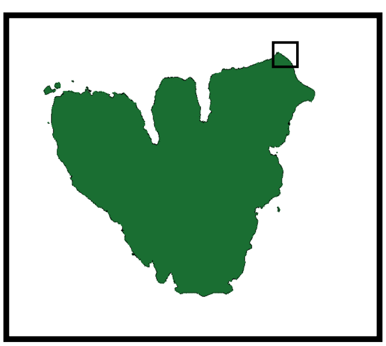

FIG. 1. Location of field site on the North East corner of Tamae.

FIG.2. Map of study site from Google Earth Pro.

Study Organism

Microbial mats are oxygenic photosynthetic prokaryotes which are typically comprised of cyanobacteria. Some of the blue-green algae in the community are *Lyngbya* and *Microcoleus.* They lay on top of the mud, either attached or unattached, and help to stabilize the surface.

Mat Types

Eight mat types were defined by different morphologies. Color and texture were used to determine the different types. For each mat type an approximately 10cm by 15cm by 10cm square piece was obtained with a trowel. They were placed in tupperware for protection and taken back to the lab. Next, they were then sliced into layers in which color, texture, size (cm), and adherence to the layer below it were cataloged.

Mapping

Eleven transects were run at 75°N, roughly perpendicular to the shore. Every ten meters mat type was recorded. I also recorded points where mat type changed. These points were then transferred to graph paper to make a rough map. Many of the mats are very patchy, all of which are not displayed on the map.

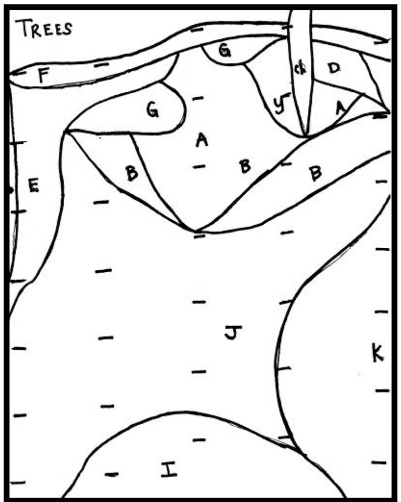

FIG. 3. Distribution Map, dashed lined indicate vertical transects.

Sampling

I laid five transects from the trees to the water approximately twenty meters apart at 75° North. Each transect was divided into six, fifteen meter sections resulting in 6 zones and 30 total points. Zone 1 was closest to the trees and zone 6 was generally closest to the water, although, the tide does not always flow over zone 6 first due to the direction of the water flow. Data was collected on 5 different days between October 31, 2007 and November 13, 2007. Each day 1 random point was sampled within each 15 meter section of each transect, 30 random points each day. This resulted in 25 points in each zone. First, mat type(s) present were recorded. Then, a Checkmate II was used to measure the surface temperature and subsurface temperature, approximately 5 centimeters beneath the surface of the mats. Additionally, temperature buttons were used to collect surface temperature every 10 minutes for 24 hours along each transect between November 6, 2007 and November 10, 2007. A hole was dug with a trowel to reach ground water and immediately the ground water depth was recorded. The holes were then left to fill with water until the water was significantly clear for salinity measurements. A refractometer was then used to measure salinity (ppt). The number of crab holes was counted in $1m^2$.

Statistics

JMP software was used to analyze the data. Oneway ANOVA's were used to analyze differences between zones for temperature, number of crab holes, salinity, and water depth. Means were compared using a Tukey- HSD analysis. The same was used to compare the mat types. Due to the unequal sample size of the mats sub-samples were taken randomly to obtain 9 points for each type, with the exception of types G and F, which had a lower sample size because of their restricted distributions. To analyze correspondence between ground water depth and; surface temperature, subsurface temperature, salinity, and crab holes a Bivariate test was run.

RESULTS

Mat Morphology

The different types of microbial mat were cataloged and recorded. After initial observations eight different morphologies were analyzed. *Lyngbya* was present in types A and G, and *Microcoleus* was present in G and F. Many of the mats exhibited a typical anoxic layer if the mats were well developed. Through observations I noticed a change in morphology after a long period with no rain or just after a storm. After a long period with no rain many of the mats had a white layer, presumably salt, and had cracks on their surfaces and had started to break apart. After rain algal blooms could be seen and then disappeared a couple of days later. The mats were generally darker in color and softer after the rain. The mats were much more easily broken apart from footsteps in after the rain. For a detailed description of each mat type see Appendix A.

Table 1. General description and location of each mat type.

Mat	Color	Texture	Zones
F	Grey/Brown	Silty	1
G	Brown/Red	Smooth	1
Y	Green	Filmy	1
A	Green/Brown	Thick/Rubbery	1,2

Ch	Dark Brown	Grainy/ Smooth	1,2,3
E	Grey/Brown/Blue	Grainy/ Smooth	1,2,3,4,5
J	Brown	Grainy/muddy	1,2,3,4,5,6
K	Grey/Brown	Sandy	3,4,5,6

Mapping

The rough distribution of mats was mapped. There is a wider range of mats found in the first and second zone, than in the others. (Species diversity decreases with every zone getting closer to the water with a chi² value of <0.001)?

Temperature

There was no significant difference found between the different mat types for temperature ($p<0.1754$). When comparing across the six different zones I found no correlation with temperature ($p< 0.7570$). Using the data from the temperature buttons a high of 52°C and a low of 22°C was measured and an average change from day to night was measured at 24.5°C over the course of 4 sunny days. I found a significant correlation between surface and subsurface temperature to groundwater depth, both with a p-value less than 0.0001. As ground water depth increased so did temperatures.

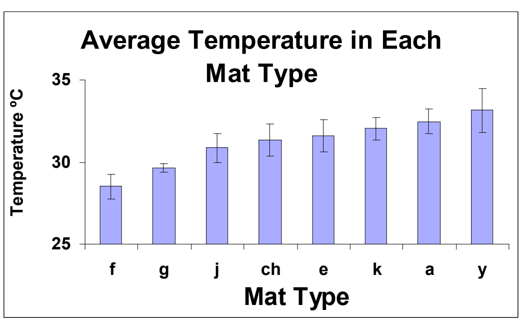

FIG. 4. Average temperature in each mat type (p-value =0.1754).

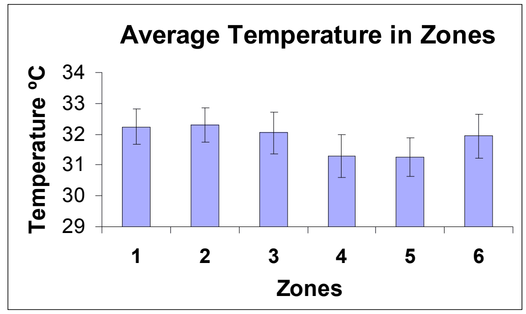

FIG. 5. Average surface temperature for each zone (p-value = 0.7570).

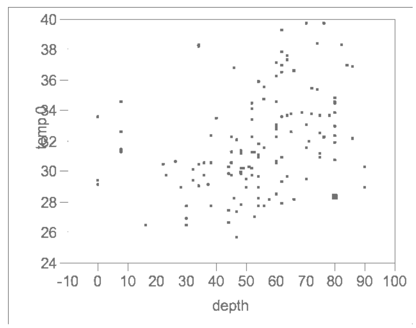

FIG. 6. Surface temperature compared to ground water depth (p-value=0.0001)

FIG. 7. Surface Temperature over the course of four days.

Salinity

Type "F" exhibits a significantly lower salinity (p-value=0.0025) than all of the other mats except for type G. Zone 1 showed a significantly lower salinity than zones 4 and 6 with a p-value of 0.007. There was no correlation between salinity and the other parameters and crab hole or temperature or the different days.

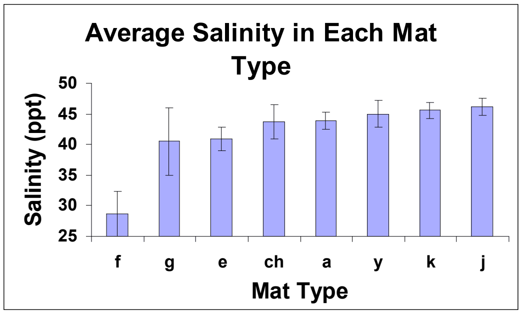

FIG. 8. Average salinity for each mat type (p-value=0.0025)

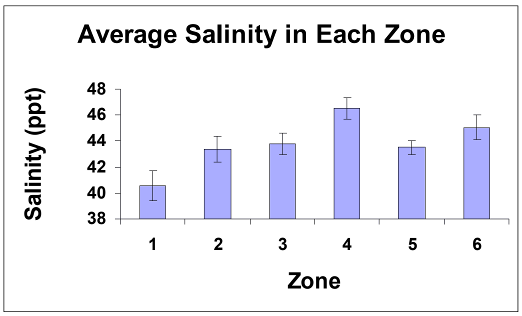

FIG.9. Average salinity in each zone (p-value= 0.007)

Crab Holes

The number of crab holes found in $1m^2$ in the different mat types was significantly different with a p-value of 0.0001. Type "J" was found with the highest average number of crab holes and G, Y; A had the lowest numbers respectively. Although there are not clear trends in they are significantly different. Zones 1 and 2 had a significantly ($p<0.002$) lower number of crab holes than zone 5, which showed the highest average.

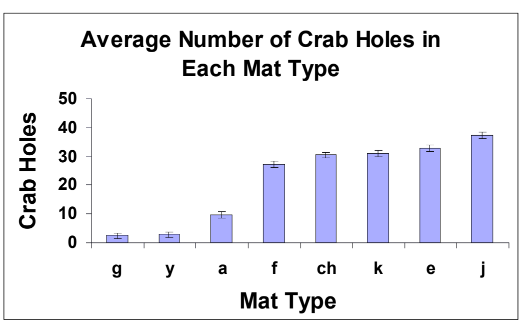

FIG.10. Average number of crab holes found in $1m^2$ (p-value=0.0001). Difference is seen only between type F and others.

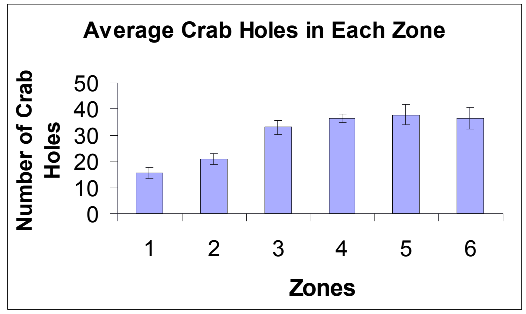

FIG.11. Average number of crab holes found in $1m^2$ in association with each zone (p-value =0.002)

Ground Water Depth

Zone 6 had significantly lower ground water depths than zone 2 (<0.0194). I found no significant correlation between crab hole numbers and water depth ($P<0.1584$). There was also no correlation between salinity and ground water depth ($P<0.1482$).

Table 2. Average number of crab holes, ground water depth, surface temperature °C, subsurface temperature C°, and salinity for each mat type

	Crab Holes	Depth	Surface T	Sub-surface T	Salinity
A	10	28.5	32	29	43.8
Ch	30	29.8	31.3	29.5	43.8
E	33	30.2	31.6	29.1	40.9
F	27	26	28.5	27.7	28.7
G	3	29.5	29.7	29	40.5
J	37	24.6	30.9	28	46.1
K	31	37	32	29.8	45.6
Y	3	29.5	33.1	29.3	45

DISCUSSION

Mat Morphology

Mat types were distinguished by morphologies on the top and therefore it is possible that some of them are composed of similar communities, PCR analysis is needed to determine whether or not there are real differences between all of the mat types. The changes in mat morphology correlating to weather are typical responses to drying, "desiccation and dehydration are possibly the most cited causes of mat destruction or death" (Renaut W. 1993). The mats seemed to come back to life after rain and obtain similar morphologies to before the drying period, although some differences were noticed, such as a larger distribution of type Y. A typical anoxic layer was seen in places where the mats were well developed (Villbrandt,M. 1991).

Mapping

Mapping was done by hand and is less accurate than using a GPS unit. The distribution of the mats probably changes over the different seasons. Long term aerial photographs and mapping could see a more detailed view of how the mats change.

Temperature

For the different mat types and zones we saw no difference between temperatures. Although the tide water does cover some of the mats for a period of time which could lower temperature, they are not significantly lower; the entire region exhibits very hot temperatures, which are similar to other findings in intertidal regions which displayed extremes of 55°C (Hoffman, 1996). They have very high heat tolerance and have a huge variation in temperature, for the four days the temperature buttons were out we saw an average difference of 24.5°C from day to night. Temperature increased with ground water depth most likely because water has a cooling effect. Because the temperature regime is so extreme it is likely that they are all adapted to very high and variable temperatures, therefore it would not affect their distributions. A common feature of microbial mat communities is
their ability to rapidly acclimate to changing environmental conditions (Joye B. 1993 ;). Therefore, the mat microhabitats, in reference to temperature, are not significantly different and there is no significant trend across the mats.
A desiccation experiment could show the limits of the mats. From these observations it is probable that the mats would display an extremely high tolerance to heat and possibly come back to more typical states after returning to "normal" conditions.

Salinity

When looking at salinity in each of the mat types "F'' is the only type that shows a significant difference from the rest (with the exception of type "G"), this is most likely due to the fact that it is uniformly the furthest from the tide water and closest to the influx of fresh water, and "G" is generally the close to "F". Except for "F" with an average of 28.7 ppt, their average salinities are in the low to mid 40's which is typical of intertidal mats (40 Al-Thukair,A.A. 2007;). Many of the mats are clustered in the same region and do have a lower average than type "J" which is found consistently closer to the water, however this is not a statistically significant difference. Even though some variation is seen, their microhabitats are not significantly different in regard to salinity, except for type "F". It is possible that some mats, like type "F", are restricted to their locations based on salinity, but experimental data would be needed to support these findings.

In the zones we did see a difference when comparing zone 1 to zone 1 and zone 1 zone 6. Salinity slowly increases when getting closer to the water, but only at the peaks is there a significant difference. The tide water does not come in over the site in a uniform pattern, this could account for zone 4 having higher average salinity than zone 5, however this difference is not significant. Although a trend is seen across the mudflat it is not conclusive evidence that salinity drives spatial differentiation.

Crab Holes

Although crab hole numbers are significantly different it is difficult to analyze the relationship between the mats and the crabs. The highest number is found in type J and the least in types G, Y and A, which are typically found in zones 1 and 2. These 2 zones have significantly lower crab holes compared to zone 5 which might be preferred by the crabs because it gets covered by tide water, but not as often or as long as zone 6. Crabs may not like to eat the mats in zones one and two as much (Walter etal., 1973; De Deckker, 1987), but this does not seem to correlate with observations. It might also be harder for the crabs to dig their holes in zones one and two because some mats associated with that area have less porous soil under them, like mat G, which also exhibits the lowest number of crab holes. It is also possible that because there are fewer crabs in zones 1 and 2 a wider variety of mats can establish themselves, possibly because they have lower tolerances for bioturbation. It is possible that they get too fragmented to stay established in the regions with higher crab densities (Fenchel T., 1998). Type "J", found with the highest amount of crabs is a lot patchier in its distribution; many times the bottom of the soil covers the mat because of the crab activity, although this does not necessarily prevent mat growth, which would allow type "J" to persist in a patchy distribution (McNamara, 1992). Therefore, although there are certain microhabitat differences it is unclear what drives the differences in crab hole numbers and if this influences the distribution of the mats. It seems like there would be a difference between crab hole number and depth, but no trend was observed. It is hard to conclude what influences crab hole distribution and thereby hard to say if it affects mat distribution. Although trends were seen it cannot be said if the crabs are necessarily preventing some of them from spreading to regions with higher bioturbation and/or herbivory.

Ground Water Depth

Zone 6 has a shallower water table because it is closer to the influx of tide water, while zone 2 was the furthest away from the tide water but not as close to the incoming fresh water as zone 1 and thus exhibits the deepest water table. Zones 6, 5, and 4 were typically covered by tide water for varying amounts of time. It seems like there would be a difference between crab hole number and water table depth, but no trend was observed. It is possible that trends would be seen if water was allowed to settle for a longer period of time.

Conclusion

Although trends were seen and certain factors are certainly correlated, the results do not indicate that these parameters dictate mat distributions. This ecosystem is much more complicated, there are clearly other unmeasured factors at play including nutrient availability, long term storm patterns, maximum and minimum microhabitat descriptions, desiccation patterns, and seasonal variability. This makes conservation of this area much more complicated than it appears. Although the parameters studied influence the mats, more experimental and long term data is needed to see the limits of the different types to test if these factors force their distributions.

ACKNOWLEDGMENTS

Thanks to all of the professors Jere Lipps, George Roderick, Carole Hickman, Rosmary Gillespie, James Bartolome and GSI's: Joel Abraham, Erica Spotswood Andrea Swei. Additionally to Richard Moe and Brent Mishler for help identifying some of the mats. Thanks to the entire class for making it the best class ever! And a special thanks to my muddy buddies!

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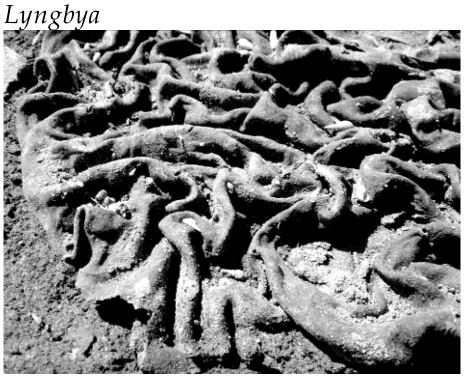
Appendix A

Mat Type A

Layer	Color	Texture	Size	Adherence
1	Green/Brown	rubbery	0.05	pulls off
2	Green	fuzzy	0.05	scrape off
3	Brown/Black	fuzzy	0.05	scrape off
4	Brown	sticky	>1	scrape off
5	Brown	grainy	>1	scrape off
6	Red/brown	grainy	1	falls off

This Mat appears in patches typically on top of type J. It can be dark brown and wrinkly, but when it gets very dry it turns white and breaks apart. A portion of the blue-green algae is *Lyngbya*



Mat Type Ch

Layer	Color	Texture	Size	Adherence
1	Dark brown, very few specks	Smooth/ Grainy	0.1	scrape off
2	Black	Smooth	1	scrape off
3	Grey, lots of white specks	Grainy	1	scrape off

This type was found in the channels and was always more wet than the surrounding areas unless it had just rained. When it got very dry this kind had still not cracked

Mat Type E

Layer	Color	Texture	Size	Adherence
1	Grey /Brown/Blue,	Grainy	0.1	scrape off
2	Red	Sticky	Patchy	scrape off
3	Dark Grey	Grainy	2	falls off
4	Light Grey	grainy	>1	falls off

Mat Type F

Layer	Color	Texture	Size	Adherence
1	grey	grainy	0.1	scrape off
2	black	sticky	0.1	scrape off
3	dark grey	sticky	1.5	falls off
4	light grey	grainy	>2	falls off

This mat was found only under the trees. A portion of the blue-green algae is probably *Lyngbya* and *Microcoleus*

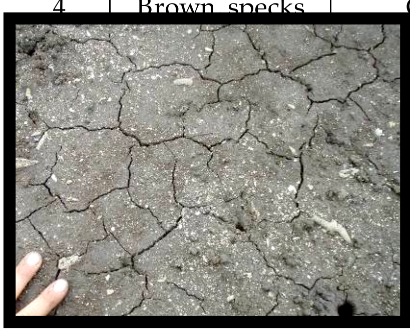
Mat Type G

Layer	Color	Texture	Size	Adherence
1	Brown/Red	smooth	0.2	scrape off
2	Black	smooth	0.3	scrape off
3	Light Grey	grainy	0.4	falls off
4	Red/Grey	grainy	0.3	scrape off
5	Brown	sticky	0.2	.
6	Light Grey	grainy	>1	falls off

This mat is very smooth and does not have any grains on the surface, unlike most of the other types. A portion of the blue-green algae is probably *Microcoleus* and *Lyngbya*.

Mat Type J

Layer	Color	Texture	Size	Adherence
1	Brown	Grainy	0.2	pulls off
2	Dark Brown, Green	Smooth/Rubbery	0.1	scrape off
3	Green	Fuzzy	0.1	Pulls off
3	Brown	Smooth	0.5	pulls off
4	Brown, specks	Grainy	>1	falls off



Very Dry
Very Wet

Mat Type K

Layer	Color	Texture	Size	Adherence
1	Grey/Light Brown, white specks	sandy	0.3	falls off
2	Dark grey/Black	smooth	<1	scrape off
3	grey/brown, white specs	sandy	>1	falls off

The second layer was not always present.

Mat Type Y

Layer	Color	Texture	Size	Adherence
1	Green	filmy	<0.1	scrape off
2	Brown/Light brown	smooth	<0.1	scrape off
3	Black	smooth	1	scrape off
4	Grey, white specks	grainy	2	falls off

This was very thin and its distribution got wider after rain fall.