INVESTIGATIONS ON THE MICROBIOLOGY OF MARINE AIR

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All the viable microorganisms in the air have been transferred to the atmosphere either from the land or the sea. A comparison of the micro-flora of marine air with that of normal sea water and of land areas should indicate the major source of organisms in the atmosphere over the ocean.

There have been few attempts to determine the origin of the bacteria occurring in marine air. Fischer (1886) assumed all the bacteria in the air came from the land, and concluded that under normal conditions the ocean air is nearly germ free 120 miles from shore. ZoBell and Mathews (1936) found both marine and land bacteria in the air over the ocean, and distinguished between them on the basis of growth on different types of media. The investigations of Levin (1899), Gazert (1902, 1903), Hesse (1913), Pirie (1913), and others were mainly concerned in showing the presence or absence of bacteria in the air over certain marine locations and have little direct bearing on this paper.

EXPERIMENTAL

The field investigations were carried out on board the research vessel *E. W. Scripps*, in an area off the Pacific Coast ranging from Cedros Island to Monterey, and from the mainland to 400 miles at sea. The observations were made during a series of cruises in 1938 and 1939, and so the stations occupied were widely separated in both location and time. A board frame constructed to hold nine Petri dishes, was attached to the crosstree of the mainmast sixty-five feet above the deck. The section of the frame holding the dishes projected out about three feet from the crosstree, and was clear of all the rigging. The board was so oriented that when the boat hove to on location the wind blew across it into the rigging, thus avoiding contamination from the ship.

According to ZoBell and Feltham (1933) most marine bacteria cannot grow in fresh-water medium while most terrigenous forms fail to multiply

* Contribution from the Scripps Institution of Oceanography, New Series No. 77. Assistance in the preparation of these materials was furnished by the personnel of the Works Progress Administration Official Project No. 665-07-3-141.

TABLE I: Number of Bacteria and Molds Developing on Plates Exposed to Marine Air

Station	*Date and location*	*Nearest land in nautical miles*	*Wind direction and force (Beaufort scale)*	*Minutes exposed*	*Sea-water medium*		*Tap-water medium*	
					Bacteria	*Molds*	*Bacteria*	*Molds*
FPS 229	9/20/38	0.5	calm	15	51	59	23	61
	121° 47.9′ W.				79	51	28	98
	36° 48.2′ N.				36	95	17	47
					89	58	15	34
FPS 258	9/30/38	15	NW. 2	10	5	44	3	44
	120° 2.5′ W.				3	65	1	64
	34° 14′ N.				3	60	3	71
					3	41	2	87
FPS 187	8/3/38	110	NW. 2	60	2	4	2	5
	119° 37′ W.				0	4	0	5
	32° 23′ N.				0	3	2	5
					8	2	2	2
EWS 21	12/13/38	110	ESE. 3	5	79	1	1	3
	120° 12′ W.				16	3	0	12
	32° 33.5′ N.				23	5	3	6
					7	5	0	4
F 78A	7/4/39	360	NW. 2	15	75	21	96	1
	120° 10′ W.				19	9	14	4
	26° 18′ N.				210	15	37	51

in sea-water medium. Since both types of organisms were expected, part of the nutrient medium used (0.3% peptone, 0.2% proteose-peptone, 0.2% beef extract, 0.0025% $FeCl_3 \cdot 6H_2O$, 1.5% agar) was made up with fresh-water and part with sea water. 10 to 15 cc. of medium were poured into Petri dishes and allowed to harden. The dishes were then stored in sterile cans until exposed. When the boat stopped on a station, four dishes containing sea-water medium and four containing tap-water medium were placed in the frame on the mast. The covers were removed and the plates exposed to the air for a period varying between five minutes and two and a half hours. A ninth plate was placed in the frame without removing its lid. It served as a control to indicate if contaminants were picked up during transport, manipulation, and storage. The direction and force of the wind during exposure was recorded as was the longitude and latitude of the station. After removal from the mast, the plates were incubated until returning to the laboratory where the colonies that developed were enumerated. The number of bacteria and molds were counted separately. The yeast were included with the bacteria since they could not always be distinguished by their colony characteristics alone. Their abundance was later estimated on the basis of a microscopic examination of a representative number of colonies.

Table I shows the field data from five typical stations. In most cases the counts on all plates of the same medium exposed at a given station were of the same order of magnitude, showing the microorganisms were usually uniformly distributed over a small area. At station F 78A, however, dishes exposed simultaneously at a distance not greater than a foot apart gave results as divergent as 210 and 19 bacteria and 51 and 1 mold per plate. This lack of uniformity introduces difficulties in interpreting the data obtained. The large and consistent differences between the number of bacterial colonies developing on the sea-water and tap-water media at station FPS 229 leads one to conclude that most of the bacteria in the air at that location originated from the ocean. A comparison of the average values at station F 78A of 101 bacteria per sea-water plate and 49 per tap-water plate would give the same conclusion. On the other hand examination of the results on individual plates reveals that the variation between counts on the same medium is greater than the difference between those on the two types of media. Instead of postulating a majority of marine bacteria one could ascribe the higher average of the sea-water plates to a fortuitous distribution of microorganisms in the air.

Table II gives the average colony counts calculated on an hour exposure time obtained at twenty-five different locations. The stations are arranged in order of increasing distance from land. The wind directions are given in relation to the position of the nearest shore, blowing over the ocean to the land during an onshore breeze and the reverse during an offshore wind.

TABLE II

AVERAGE NUMBER OF MICROORGANISMS DEVELOPING ON PLATES PER HOUR EXPOSURE

Station	*Nearest land in nautical miles*	*Sea-water medium*		*Tap-water medium*		*Wind direction*
		Bacteria	*Molds*	*Bacteria*	*Molds*	
FPS 228	0.5	4	36	7	55	onshore
FPS 229	0.5	255	263	83	240	calm
FPS 235	3.5	20	25	12	34	onshore
FPS 202	5.0	8	5	2	6	offshore
FPS 237	6.0	24	132	23	441	offshore
FPS 246	6.0	17	30	19	54	calm
FPS 242	7.0	23	424	12	730	offshore
FPS 243	9.0	6	5	2	42	offshore
FPS 258	13	14	489	14	400	onshore
EWS 18A	68	0	9	0	6	offshore
EWS 9A	75	18	39	3	48	onshore
EWS 9	91	17	42	9	50	onshore
EWS 6	93	36	72	9	57	onshore
FPS 190	105	2	12	5	8	calm
FPS 180	107	11	3	66	4	calm
FPS 187	110	3	3	2	4	onshore
EWS 21	110	375	42	12	75	offshore
EWS 22	125	3	78	8	40	offshore
EWS 24	165	broken	broken	60	90	offshore
FPS 182	215	6	9	3	39	onshore
FPS 185	230	2	18	3	12	onshore
FPS 185A	230	0	9	3	10	onshore
FPS 186	240	1	23	3	22	onshore
F 78A	360	404	60	196	75	onshore
F 77	380	13	0	4	3	onshore

The air was not completely free of microorganisms at any station. On several plates there was no development at all but others in the same series showed either bacteria or molds or both. Even four hundred miles out at sea there was an abundant micro-flora, and the largest bacterial population was found at F 78A, 360 miles from shore. The large variability of results from station to station is to be expected as we are not dealing with a single air mass; certain regularities in the distribution, however, become apparent on further analysis of the data.

At fourteen stations the average number of bacterial colonies developing on sea-water medium was greater than the number on tap-water medium, while the opposite was true at eight stations. Only at five stations was the variation between plates of different media greater than that between plates of the same medium. In three of these the sea-water count was greater, in two the tap-water. This would indicate that either the number of marine and land bacteria were about the same or else that the organisms in the air could develop on both types of media. In order to further clarify this point 168 colonies were picked directly from the original sea-water plates and transferred to tap-water slants while 96 colonies were taken from tap-water plates to sea-water slants. 130, or 77 per cent of the former, and 69, or 72 per cent of the latter, developed. Accepting as marine bacteria those that do not grow on tap-water media, then less than 25 per cent of the viable bacteria in the marine air originated from the sea.

TABLE III

RELATION BETWEEN NUMBER OF MICROORGANISMS AND DISTANCE FROM LAND

Distance from land in nautical miles	*Average number of colonies per hour exposure*			
	Sea-water medium		*Tap-water medium*	
	Bacteria	*Molds*	*Bacteria*	*Molds*
0–10	45	115	20	200
10–150	48	79	13	69
150–400	71	20	39	36

The number of molds developing on tap-water was greater at 16 stations and less at 8 than those developing on sea-water medium. In only two cases was the variation between media greater than the variation between plates of the same medium. 100 transfers were made from sea-water plates to tap-water slants. 97 of these cultures developed, and if anything, grew better on tap-water medium than on sea-water medium. The relative rate of growth on the two media was estimated by measuring the diameters of three hundred colonies developing on plates exposed at stations FPS 185 and FPS 185A. After the same period of growth an average colony diameter of 5.8 mm. on the tap-water medium and 2.3 mm. on the sea-water medium was found. These results strongly indicate the terrigenous origin of all the molds.

If most of the organisms in the ocean air originated from land sources we might expect to find a regular decrease in their numbers as we go farther away from shore provided we remain in the same air mass. Since our observations were separated by long intervals of distance and time the data does not represent a homogeneous air mass, but even so it was hoped that combining all results would bring out certain trends.

Table III was obtained by averaging all the results between given distance intervals from land. These intervals were arbitrarily chosen so as to include approximately equal amounts of data in each division.

The decrease in number of molds with distance from shore is quite apparent confirming our conclusion as to their land origin. The same relation does not appear to exist for the bacteria. This lack of a regular decrease might be explained in several ways. An examination of Table II shows three stations whose bacterial count on sea-water medium is much higher than the rest. Because of the small amount of data these high counts dominate the averages and may mask any relation that does exist. If we assume that these three results represent abnormal conditions in the atmosphere and omit them from our calculations, we obtain 15 as the average

TABLE IV

RELATION BETWEEN NUMBER OF MICROORGANISMS AND WIND DIRECTION

Wind direction	*Average number of colonies per hour exposure*				*Ratio of sea-water to tap-water counts*	
	Sea-water medium		*Tap-water medium*			
	Bacteria	*Molds*	*Bacteria*	*Molds*	*Bacteria*	*Molds*
offshore	63	99	17	204	3.70	0.49
onshore	41	63	21	63	1.95	1.00
calm	71	77	43	77	1.65	1.00

number of colonies developing per plate per hour exposure between 0–10 miles at sea, 11 between 10–150 miles, and 4 between 150–400 miles, showing a decrease with distance from shore.

On the other hand we can assume a significant number of organisms are carried into the atmosphere from the surface ocean water. This number would depend on the population of the water and the amount of agitation and would have no relation to the distance from land except in the narrow tidal zone where breaking waves send large amounts of spray into the air. The counts being the sum of a variable number of bacteria originating from the ocean and a constantly decreasing amount from land, would not be a function of the distance from shore. For reasons discussed below it is believed that the number of viable marine bacteria in the air is small so this latter explanation is only of minor significance.

The life history of an air mass should influence the number and type of organisms it contains. The number of bacteria in sea water is usually less than 500 per cc. (ZoBell and Feltham, 1934; Benecke, 1933) which is quite small compared to what is found in soil, dust, and other terrigenous surfaces.

Thus air traveling over the ocean for a long distance should contain fewer organisms and have a higher proportion of marine bacteria than air coming from the land. ZoBell and Mathews (1936) found a much higher ratio of marine to land bacteria during onshore winds than during offshore winds. They were working in the tidal zone where due to the large amount of spray in the air, a change in wind direction would have an almost immediate effect on the types of organisms found. It must be pointed out, however, that the wind direction at a given location does not necessarily indicate the life history of the air mass.

Table IV, which averages the results of all stations having similar wind directions, bears this out. Although the total number of organisms found during offshore winds was somewhat higher than during onshore winds, the ratio of sea-water to tap-water bacterial counts was also higher during offshore winds.

The morphological, biochemical and physiological properties of 100 cultures picked at random from sea- and tap-water plates were determined. 32 of this group were yeast, 30 cocci, 15 gram-negative rods and 23 gram-positive spore-forming rods. Fifteen different species listed below were identified by comparison with the characteristics given by Bergey (1939).

Bacillus subtilis
Bacillus flavus
Bacillus megatherium
Bacillus mycoides
Bacillus tumescens
Bacillus cohaerens
Bacillus laterosporus
Flavobacterium aquatilis
Achromobacter liquifaciens
Staphylococcus aureus
Staphylococcus albus
Staphylococcus citreus
Micrococcus flavus
Micrococcus candidus
Sarcina flava

This group shows a surprising resemblance to those found by Proctor (1934, 1935) and Proctor and Parker (1938) in the upper atmosphere. The following sentences quoted directly from Proctor's paper (1934) apply equally well to the entire group of cultures studied in this investigation. "Every species identified was found to be one which is commonly found in the soil, water, or air . . . It is interesting to note that no pathogens were found. Neither *Escherichia coli*, or similar intestinal types were found . . . None of these cultures have the ability to ferment common sugars with the formation of gas."

Another group of 120 colonies from stations F 77 and F 78A were stained and their morphology determined. There were two yeasts, 12 cocci, and 32 gram-negative rods. The remainder were gram-positive rods, most of which showed spores.

One hundred typical mold colonies were isolated and given to Dr. O. A.

Plunkett, and Mr. Kilpatrick, of the Department of Botany, University of California at Los Angeles, for identification. Most of them proved to be forms commonly found in the soil and none showed any characteristics which would indicate a marine origin. The following is a list of the genera found together with the number of times each genus occurred.

Actinomycete	2	Macrosporium	2
Alternaria	6	Pennicillium	18
Aspergillus	2	Penozythia	1
Cantenularia	1	Spicaria or Paecilomyces	1
Cephalosporium	7	Sporotrichium	7
Cladosporium	7	Stemphylium	3
Helminthosporium	1	Trichoderma	1
Hormodendron	15		

DISCUSSION

The micro-floras of sea water and marine sediments have certain characteristics which distinguish them from the populations of other natural environments. Fischer (1894) in the first comprehensive survey of marine bacteria remarked on the scarcity of molds in ocean waters. He found yeast in several samples but not in great abundance. Cocci and members of the genus *Bacillus* were almost entirely absent. Gazert (1912) and Hesse (1913) also found an almost complete lack of cocci and spore-forming organisms. ZoBell and Feltham (1934) found that about 90 per cent of the marine bacteria were gram-negative and less than 5 per cent were cocci.

This is quite different from what is found in marine air. Molds represented over 50 per cent of the population even on a medium not especially adapted to their needs. Yeasts constituted 15 per cent, cocci 19 per cent, gram-negative rods 21 per cent, and gram-positive rods 45 per cent of the remaining organisms.

A comparison of these two types of populations shows that land forms predominate in the air over the area studied. The great abundance of spore-forming organisms, both bacteria and molds, might be explained on the basis of their greater ability to survive adverse conditions. The presence of large numbers of these bacteria 400 miles from shore reveals the possibility of long distant transport of microorganisms through the atmosphere. The qualitative differences between the ocean and air micro-flora also shows that most of the organisms settling from the air into the ocean do not develop.

Although this work has been presented from a biological viewpoint there are unexplored possibilities of solving problems in other fields. At the present time there does not seem to be any property of an air mass which can serve to identify its source and history. It may be that an intensive study of the types and sources of organisms in the air may serve as a tool in tracing the life history of air masses and in understanding the mixing processes they undergo (see the following paper by Jacobs).

SUMMARY

The microorganisms in the air over an area of the Pacific Ocean were studied by exposing nutrient media to the atmosphere. The average numbers of bacteria and molds developing on sea-water and tap-water medium were determined at 25 different stations.

The air was not completely free of organisms at any station. The numbers found varied greatly in different plates at a given station, and from station to station, but there appeared to be a general decrease in numbers with distance from shore.

Molds were the predominant group of organisms and spore-forming bacteria were next. Fifteen species of bacteria and the same number of mold genera were identified. The differences between the ocean and the air micro-flora indicates the terrestrial origin of most of the organisms.

The possible use of these investigations in solving meteorological problems is mentioned.

Acknowledgements

I wish to thank Dr. O. A. Plunkett and Mr. Kilpatrick of the Dept. of Botany, University of California at Los Angeles for undertaking to identify the mold cultures. I am also indebted to Mrs. C. Feltham for technical assistance and to Dr. C. E. ZoBell for advice and encouragement in the carrying out of this investigation.

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