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[illegible] for the Detection of Airborne Microbic Risks

[illegible]
[illegible]
[illegible]

[illegible] Bevölkerungsschutz, Bonn-Bad Godesberg
Issue No 2, pp 85-96

[illegible] Bevölkerungsschutz, Bonn-Bad Godesberg, Bundesrepublik Deutschland
Bundesamt für Zivilschutz, Bonn
London, Martin Kitsen

[illegible]

A field examination preliminary study of methods for determining microorganisms in the air and the usability of these methods in field studies concerning the natural content of microorganisms and other particles in air. [illegible]

[illegible] have included air sample collection and the counting of particles 1-5 µm in size with Casella and Anderson samplers, detection of nucleic acid containing particles in air with acridine orange staining and analysis of the composition of air particles with electron microprobe. The viability of collected microorganisms was recorded with conventional culture technique. The field experiments were carried out during one year with sampling about once a month at three different localities for sea, land and city air.

Individual viable microorganisms (recorded as full grown colonies) were detected without difficulty in the sample air by the culture technique. In spite of the good linearity of the acridine orange method in tests with bacteria aerosols, no [illegible] correlation between the fluorescence values and the results from the culture technique was obtained during the field tests concerning background conditions. The electron microprobe has been found to make possible studies of air particles with disturbing influence on the acridine orange method.

Introduction

There appears to be general agreement about the need for detecting with the least possible delay the presence of airborne biological weapons. The advantage to the defense of such an ability is obvious. The existence of reliable methods of detection is also believed to be likely to reduce the chances for B-weapons being used at all. The problem has been discussed for example at several Pugwash conferences and since 1965 has been followed up in international committee work. "BC (Försvarets Forskningsanstalt - Defence Research Institute) The Issues about BC weapons" (Tammelin, Larsson, Sorbo, Svensson and Persson 1964) provides an easily accessible comprehension of the potentialities of the B weapons. A critical survey has also recently been published in Science Journal (Clarke, 1966).

The concept detection in the present paper means measures resulting in a determination that abnormal contents of microorganisms occur in the air that is being continuously examined. The concept does not presuppose an identification of the organisms, which is not excluded, however. The detection is intended to lead to "early warning" for the taking of protective measures within less than an hour from the time of the sampling.

The possibility of variation of B weapons, both with regard to effects and tactical application, combined with the circumstance that the effective amounts can be as small as some ten bacteria or virus particles with a mass of about 10^{-12} g or less poses exceptional demands on the methods and instruments that can be used for detection. A process must be carried out by means of particularly advanced and costly apparatus, which as far as Sweden is concerned exists in only a few specimens. These circumstances have led to orientation of the work at FOA toward methods where special prerequisites existed within the country.

The goal of the experiments that are reported in the following is necessarily limited.

The studies should in the first place concern the detection of individual microorganisms within the size range 1-5 μ thus excluding viruses and rickettsias ($< 1\mu$) and larger bacteria aggregates (75μ). Particles between these size limits get into the lung alveoles and are thereby most effective for the spreading of virulent agents.

Methods for size-discriminating collection of particles in air should be developed and established on the basis of existing commercial apparatus.

[illegible] methods of detection of viable cells to substances specific [illegible] which, first of all, methods which we should be [illegible] of separating microorganisms from cells and particles. [illegible] possible to [illegible] individually with [illegible] becoming basis [illegible].

With the methods [illegible] limited field tests should be carried out in a few localities in different climates and at different seasons of the year in order to determine on the one hand, and possibly, about the [illegible] with respect to the sampling methods used, and on the other hand to provide [illegible] information about the occurrence of microorganisms in the air.

[illegible]

Choice of methods: As regards particle collecting apparatus, two commercial particle collecting devices have long been used at FOA, Casella cascade impactor (May 1949) and the Andersen sampler (Andersen 1958). Both are particle size discriminating. The former concentrates the fractions on a narrow band; the latter distributes them at a plurality of points on the collecting surface, as further described below.

The development work concerning these devices was for the purpose of calibration, individually and against each other, and the production of an easily portable unit combined with generator and air pump for the field tests.

For detection there was need for a method with fundamental development possibilities as regards specificity, speed and [illegible] ability. The method developed at the Institute for medical cell research at Karolinska Institutet, to analyze the nucleic acid contents in individual cells by means of acridine orange [illegible] probably be regarded as meeting these demands (Rigler [illegible]). Theoretically the method permits the detection of as little as 10^{-15} g nucleic acid, which corresponds approximately to the nucleic acid content in 1-1/10 bacteria. The method is described below.

The development was for the purpose of determining its [illegible] activity to bacteria, possible disturbing substances and background fluorescence in field tests.

It was natural to use tested culture technique as an independent detection and control method. The Andersen sampler is intended to permit particle collection directly on culture plates. Due to the long time for growing the colonies, the method is not regarded as suitable for further development to be used for early warning.

Basic material analysis of [illegible] collected particles in background tests would hardly have been possible before with methods then available. Some years ago instruments were developed, however, which combine the magnifying ability of the electron microscope with a recording of the characteristic x-ray that occurs by electron radiation of the elements. Both occurrence and content of an element in extremely small amounts can be established (Kiessling 1960). At the time of the experiments only two instruments were present in Sweden. The most suitable of the two for the purpose was the one at the Institute for Structure Research, Chalmers Technical Institute, which was made available with personnel by the head of the institute, Professor Cyrill Brosset.

The purpose of the field tests was to give experience concerning the [illegible] of the method, [illegible] with regard to the background conditions in the air and give information about the natural microbial flora.

Particle collection. Casella cascade impactor -- The apparatus (May 1945) consists of four parts joined together (Fig. 1), each having a slot through which the air passes and a removable collecting surface disposed crosswise to the slot (Fig. 2). The slots become successively smaller, which leads to increased velocity of the air that is sucked through. Depending on the velocity of the air stream, particles with decreasing mass at each step are hurled against the collecting surface, where they get stuck in an adhesive coating on the surface.

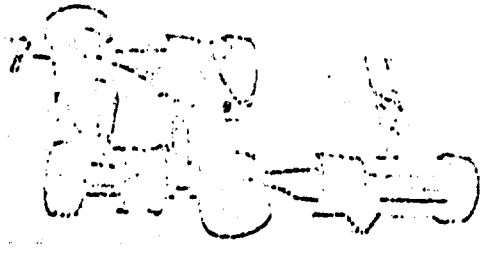


Fig. 1. Casella cascade impactor with the step sequence used in the experiments

Fig. 2. Diagrammatic sketch of Casella cascade impactor. The air passes in the direction of the arrows. The particle fractions are collected on the adhesive coated glass plates (2)

Each step is numbered by the manufacturer from 1 to 4, corresponding to the catching of particles within size intervals provisionally indicated for the respective steps (Fig. 3). For more exact measurements each step must be calibrated separately and in combination. The calibration is carried out by measuring the particle distribution on the collecting surfaces under the microscope with a measuring ocular.

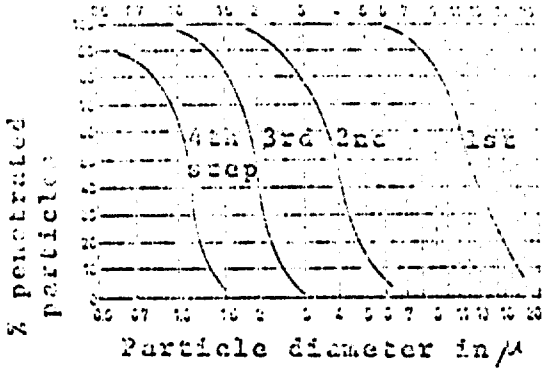


Fig. 3. Collecting effectiveness in the four steps of the cascade impactor (Proc. May 1945)

With the sucking through velocity of 1,050 liters per hour normally used, and the step combination 1,2,2,3 and 4 the particle distribution obtained in step 3 was 0.9 - 5μ over a deposition surface of 2.0 x 15.7 mm^2. The deposition surface consisted of a cover glass (26 x 23 x 0.3 mm) mounted in a brass plate especially manufactured for these experiments. Object glass and cover glass were easily broken if they were inserted directly

in the steps. In steps for particle sizes without interest, only brass plates were inserted. All collecting surfaces were covered with a sterile adhesive composed of 1 g gelatin, 25 g glycerol and 175 g water. The cover glasses were washed with alcohol prior to coating.

The particles were distributed in a characteristic manner when they were caught on the adhesive coated glass (Fig. 4). Air particles did not appear to fasten reproducibly to clean uncoated glass. An average of 75% of the number of particles that fastened to coated surfaces adhered to polished brass. Material collected in step 3 (1-5/4) of the Casella impactor was studied by the acridine orange method.

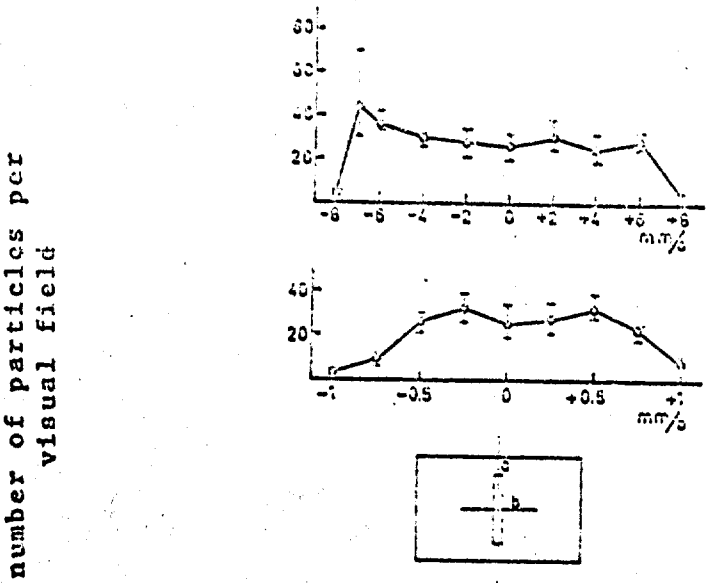


Fig 4. Particle count for dust collected on the adhesive coated step 3 of the Casella impactor. The sketch at the bottom indicates at which section of the preparation the count took place. Two preparations were counted; the longitudinal section (a) 3 times and the transverse section (b) twice for each preparation. The greatest deviations from the indicated average values are marked.

Andersen sampler -- The apparatus (Andersen 1958) consists of six aluminum dishes placed in series, each with 400 holes of successively diminishing size (Fig. 5). Inthe intervals between the dishes collecting surfaces can be placed. When air

[illegible] through the apparatus the diminishing hole size, as [illegible] the droplets impacted, result in increased air velocity, whereby particles of even smaller mass are hurled against the collecting surfaces where they become attached (Fig. 6).

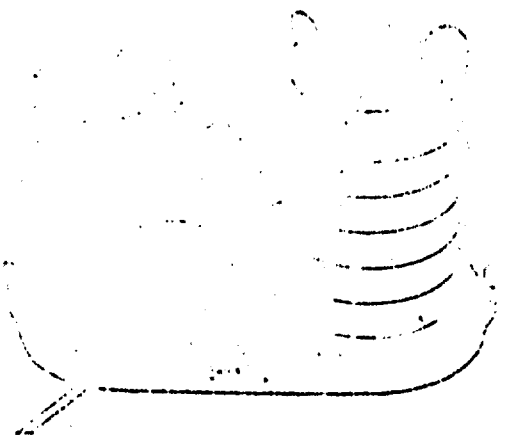


Fig. 5. Andersen sampler. Unit with sampler on the right and pump on the left in picture

air sample

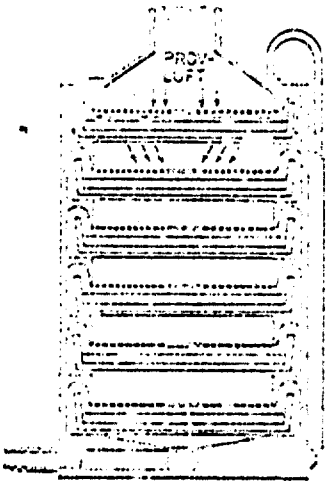


Fig. 6. Diagrammatic sketch of Andersen sampler. The air is taken in and passes through the apparatus in the direction of the arrows. For each step (detachable) the pore size in the step diminishes. Between the step insert dishes are placed with culture medium which also serves as collecting surface.

The collecting surfaces consist of petri dishes with a suitable medium for growing the collected microorganisms. Different from the Casella impactor, the Andersen sampler can therefore record viable microorganisms distributed by different sizes on the six collecting surfaces. The sampling time ought to be chosen so that the number of viable organisms does not approach 400 in any petri dish. The result will then be uncertain because of greater probability that two organisms will fasten under the same hole. The [illegible] observation only one grown colony can as a rule be recorded under each hole in the sampler.

In the commercial model (manufactured by [illegible] Consulting Service, USA) the apparatus is provided with a pump that gives a definite air speed. For data from the Andersen sampler and Casella impactor to be comparable, the recommended air velocity for the former is reduced from 1600 liter per hour to 1000 liter per hour. Furthermore, the step sequence in the Andersen sampler is changed from the normal to the sequence 1,2,3,5,6,4. Then a particle distribution of [illegible] to 6.8μ will be obtained in step 5.

At equal running times in the same localities the Casella impactor gave ca 30% lower particle number than the Andersen sampler, however. The difference applies mostly to small particles in the size range of 0.8 - 1μ. This deviation must be accepted, however, as none of the other combinations gave better results.

The calibration was carried out on adhesive covered object glass lying in recesses in aluminum insets suitable for petri dishes (Fig. 7).

Fig. 7. Various types of inset dishes used in Andersen sampler. Left, dish with recessed metal plate for adhesive coated object glass (for microscope study of collected particles); center, same with recesses for copper plate (for electron microprobe measurements) and adhesive covered cover glass (for comparative microscope studies); right, petri dish for growing bacteria and fungi.

[illegible] was used for [illegible] the collecting [illegible] petri dishes and [illegible] glass dishes. [illegible] minutes. Cultivation was also carried out on the plates [illegible] the steps with [illegible] [illegible] of minutes from 1-5 μ respectively. An example of the appearance of the plates is presented in Fig. 6.

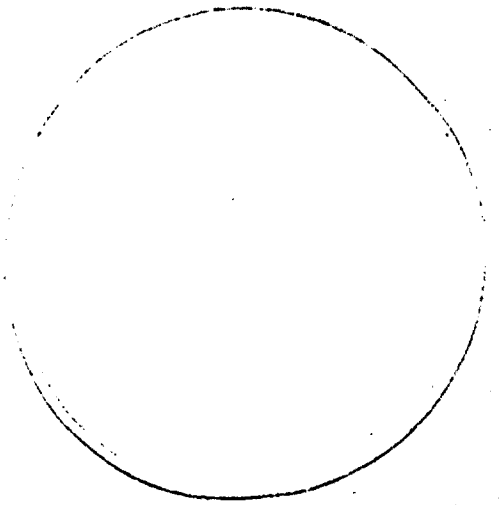
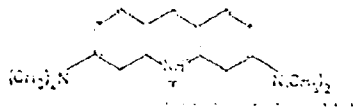


Fig. 6. Exposed culture plate of the Andersen sampler after cultivation of collected microorganisms. The smaller, smooth colonies derive from bacteria; the larger, dull colonies consist of fungi.

The samples to be analyzed in the electron microprobe were collected during 15-30 minutes on electropolished copper plates. The plates, which were one inch in diameter and 3 mm thick were placed in a recess in an aluminum inset for a petri dish and were introduced instead of the agar layer in step 5 (1-5μ). In the same aluminum inset there was also a recess for an adhesive covered half object glass on which collections for other analytical purposes could be made (Fig. 7).

Detection methods: The acridine orange method -- Acridine orange (AO) as a basic dye with strong fluorescence and marked metachromic characteristics. Its bonds with nucleic acids can [illegible] be regarded as being well clarified. (Rigler 1966) Essentially [illegible] is an ion bond between the basic dye and the phosphate groups in the nucleic acid, but a certain intercalation of the AO molecules in double chain nucleic acid might also occur. In high concentrations (small distance between the molecules) AO



shows a strong metachromic effect [illegible] fluorescence is changed to [illegible] to separate single from [illegible] strand nucleic acid (as a rule similar to RNA) [illegible] molecules AO per unit of length than single strand [illegible] (as a rule similar to RNA), schematically illustrated in Fig. 9. The effect of this will be that the AO-DNA complex shows green fluorescence (maximal at 530 nm), while the AO-RNA complex fluoresces in a red color with maximum at 670 nm (Fig. 10).

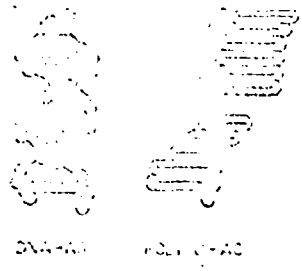


Fig. 9. Diagram of the bond of acridine orange to double strand (DNA-AO) and single strand (RNA, polyuracil, poly U-AO) nucleic acid respectively. The closer bond of acridine orange to poly-uracil (or RNA) causes the metachromic change from green to red fluorescence. From Rigler 1966.

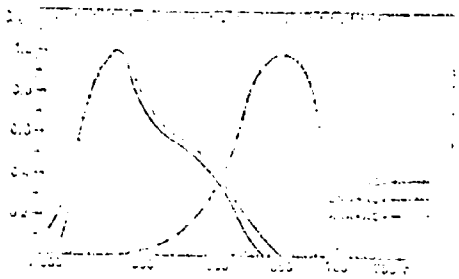


Fig. 10. [illegible] double stranded nucleic acid and [illegible] orange [illegible] compared with 4.5 nm for single stranded nucleic acid. From Rigler 1966.

The band of AO to nucleic acids has been used by [illegible] (1966) to identify and measure the amount of nucleic acid in individual cells. As a rule the dyeing is [illegible] place at pH 4.1 to prevent binding of the basic dye to the carboxyl groups of the proteins.

The sample glasses were fixed 5 min in vapor from 37% formaldehyde, then 2-24 hours in ethanol/acetone 50:50. After treatment in pyridine (5 min), pyridine: acetic acid anhydride 3/2 parts by volume (15 min) and ethanol from 100 to 30% (total 20 min), the glasses were transferred to double distilled water (3 min). After 5 min in citric acid-phosphate buffer, pH 4.1 they were dyed 15 min with 10 10^{-4}% in the same buffer. They were mounted in buffer under cover glass. For other details see (Rigler 1966). No experiments to shorten the process timewise have yet been made.

For the spectrum studies and routine fluorescence measurements of collected air samples, a fluorescence microspectrograph was used, which has recently been developed at the Institution for Medical Cell Research and Genetics (Caspersson, Lomakka and Rigler 1966). This is provided with double monochromators and quartz optical system throughout, which gives the opportunity to register both excitation and emission spectra as fluorescence intensity in ultraviolet and visible light from small particles. Theoretically there is no lower limit for the size of particles that can be measured (the emitted light from a particle can be measured even if its size falls below the power of resolution of the microscope). In practice the sensitivity is limited by noise from the photomultiplier that is used, and Rigler has

calculated that the limit for AO-dyed DNA lies at ca. 5 x 10^{-13} moles DNA-PO_4, corresponding to ~ 10 T$_2$-phage particles (or 1/10 E. coli). With instruments designed with a view to maximal sensitivity, this limit could probably be brought down considerably.

The introductory measurements to [illegible] that these at the time of final [illegible] None of the bacteria strains [illegible] fluorescence). The continuous measurements thereafter took place at only one wavelength, 530 nm. The [illegible] intensity from ca 50 visual fields along a line across the 1 mm wide strip of particles from the Casella impactor was used as a relative measure of the amount of green (530 nm) fluorescing material in the sample. No attempts were made to identify and measure the fluorescence from individual bacteria in routine samples.

An apparatus for semi-automatic measurement of the fluorescence from collected particles was designed to gather experience for a possible future automation of the method. The stage was provided with a device that permitted moving the preparation with constant speed past the measuring objective. The signals from the photomultiplier that read the fluorescence were fed via an amplifier to a recording device that recorded the distribution of fluorescence along the scanned line. The fluorescence along this line was at the same time integrated automatically. Thus the integral gave a relative measure of the amount of green fluorescing material in the sample. In this case a slit-shaped surface (10 x 80 μm) was used as measuring field.

Cultivation technique. The microbiological cultivation of colonies from the collected microorganisms in petri dishes took place completely according to conventional technique.

The nutrient agar plates were usually incubated 5 days at room temperature, sometimes for control an additional day at 32° C. The number of grown colonies was then counted, whereby bacteria were separated from fungi. See also Fig. 5.

The results were recorded so that the numbers of colonies from steps 1, 2 and 3 were brought together, while the numbers from steps 5 and 6 respectively were recorded separately. The samples from step 4 (the last) were not counted. In this way a division theoretically by particle size was obtained:

step 1-3	particles $> 6\mu$
step 5	$0.8\text{-}6\mu$
step 6	$< 0.8\mu$

of [illegible] analysis. The method is much too complicated to describe in this article. Those who are interested are referred on the one hand to [illegible] and on the other hand to the original literature ([illegible], Castaing 1951). For the actual experiments only a [illegible] of the resources of the instruments had to be made use of [illegible] could [illegible] register the [illegible] particles [illegible] could therefore [illegible] but the emitted x-ray radiation could not [illegible]. The measurements were carried out by [illegible] Institute [illegible] the direction of [illegible].

As the samples were collected on the [illegible] polished copper plates, whose size was adapted to the electron microprobe apparatus, they were shaded by a 100 [illegible] carbon layer to avoid electrostatic charges under the electron beam. Such charges result in the material moving. The shading was done at Meteorological Institute, Stockholm University or the State Bacteriological Laboratory.

One or more suitable areas for study were localized on the plate under the dissection microscope and were marked so they could be sought out in the electron microscope.

The electron beam sweep first reproduced the sample surface with the collected particles on an oscilloscope whose screen was photographed with a polaroid camera. In the same way the excited x-ray radiation was recorded for one element at a time. Recording of 4-6 elements in the particles on a sample surface could be carried out routinely in about one hour.

By comparing the photographs of the total number of particles (the electron pictures) with the x-ray pictures from the same area, a rough estimate could be obtained of the composition of the particles with reference to the examined material. See Fig. 11.

No quantitative determination was carried out. Due to the waste of time with the measurements only a few elements could be included in the study. In order to obtain indications of the occurrence of mineral particles in the samples silicon, aluminum and calcium were included. Chlorine was regarded as an indication of the occurrence of salt crystals in the air over the sea. Particles which, as mentioned above, were recorded in the electron picture but not formed again in any of the element recordings, could be regarded as showing the presence of organic particles.

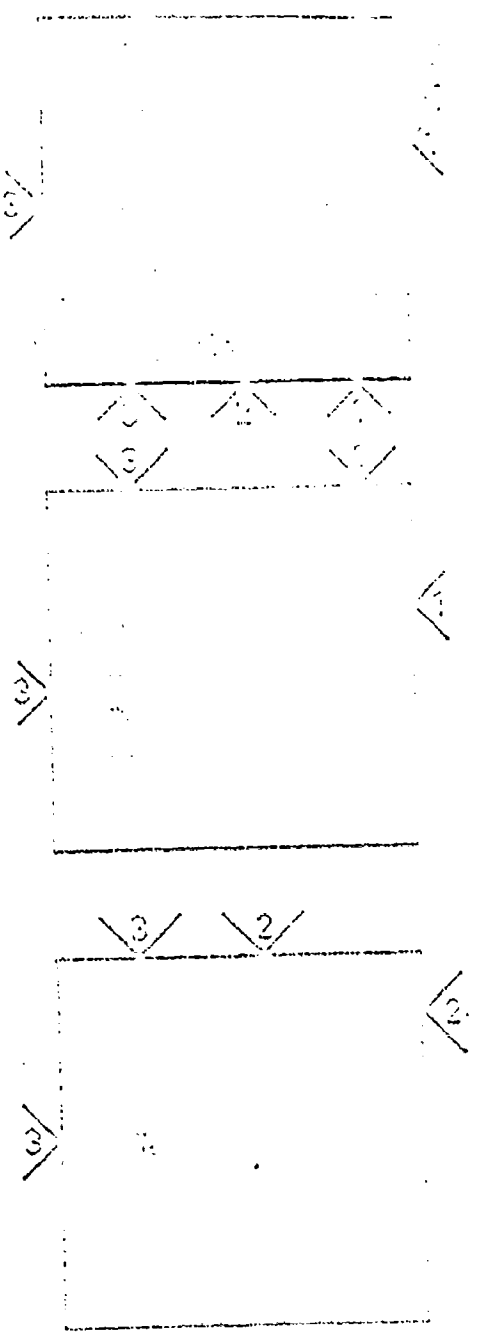


Fig. 11. Example of element detection with electron microprobe in particles collected on copper plate mounted in an Andersen sampler. Polaroid camera pictures of the oscilloscope of the instrument (see text). Top, electron picture of the sample surface, in the middle, silicon and at the bottom aluminum radiation from exactly the same area. The numbered arrows show examples of identifiable particles on the electron picture, which particles contain either silicon or aluminum or both elements.

[illegible]

[illegible] as possible.

1. [illegible] air -- the [illegible] 5 m above the ground.

2. [illegible] air -- the Järva [illegible], ca 2 m above ground.

3. [illegible] air -- the Lock in Stockholm, ca 2 m above the ground.

To have the influence of different seasons included the samplings were extended over a calendar year starting in March 1965 and ending in January 1966. The sampling frequency had to be limited to once a month.

The sampling units consisted of a Casella impinger and an Andersen sampler with appurtenant air pumps. The air intakes for the respective samplers were placed in the immediate vicinity of each other, directed into the wind. The portable gasoline driven generator for the pump operation was placed ca 50 m downwind from the air intakes of the sampling apparatus, so the exhaust gases would not disturb the sampling.

Sampling occasions were chosen when stable weather was forecast for a couple of days in succession. Samples were taken at Lambarfors on one day and at the Lock and Järvafältet the next day. Occasionally one more day was required.

At each locality 2 samples were taken in each of the samplers with a collecting time of 15 minutes (the Lock), 25 and 30 minutes (Järva) and 30 minutes (Lambarfors). The collected samples were quickly transferred to closed special containers. The samples for the electron probe were collected after the exposure

samples for 15-30 minutes. At each [illegible] wind velocity, barometric pressure and [illegible] as date and time of the day were noted. [illegible] reported in this paper.

Results

The acridine orange [illegible] cence measured in relative units to [illegible] of bacteria that were [illegible] coated cover glass in the [illegible] bacteria were sprayed into a [illegible] air in the air intake of the Casella [illegible] applied even if the glasses had been [illegible] collection with a uniform amount of [illegible] changed with different types of dust [illegible].

It was found that different types of dust behaved on the one hand fluoresced differently after staining with acridine orange and on the other hand also had different autofluorescence, i.e., fluorescence independent of the acridine orange staining. Both types of fluorescence were studied in a number of [illegible] dusts.

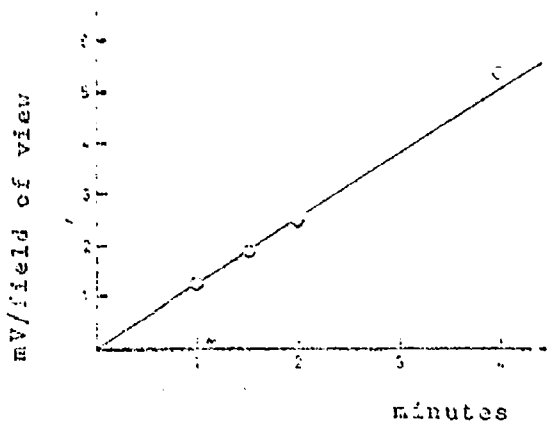


Fig. 12. Diagram of the average fluorescence intensity per field of view, from preparations collected in the Casella impactor. An increased bacteria density (longer exposure of the glass in the impactor) gives a proportional increase of the fluorescence.

[illegible]	[illegible]	[illegible] orange
[illegible]		
[illegible]	+	+
[illegible]	++	++
[illegible]	++	++
[illegible]	++	+
[illegible]		
acid	++	+
neutral	++	++
basic	++	++
[illegible]	+	+
[illegible]	+	+
[illegible]	+	+

It appears, [illegible] that autofluorescence [illegible] plant parts of various kinds. These [illegible] acridine orange staining, like all the other dyes [illegible] studied. It was found above all that cellulose fluoresces strongly after acridine orange staining.

In an attempt to reduce the influence of autofluorescence from unspecified particles in collected air samples, some filters were divided in two, one half stained with AO, the other examined unstained. The process was technically difficult, therefore only a small number of attempts were made. These showed nevertheless that unspecific autofluorescence contributed with only one half of the fluorescence that was measured at 550 nm after AO staining.

Preliminary experiments have been made to automat the fluorescence measurements described above. The results shows that the measurements can be made considerably more rapidly than hitherto.

Electron microscope analyses. The method was found suited for determining the presence of elements looked for in individual collected dust particles. (Fig. 11) The external circumstances with the apparatus located elsewhere one were the reasons why it could be used only for a few field experiments.

Samples from animal stables, where air particles of predominantly organic origin could be expected were found, as anticipated, to contain particles visible in the electron microscope which largely did not give any energy radiation

corresponding to the examine elements. It is probable that [illegible] consisted of the materials hydrogen, carbon, oxygen, [illegible], which are not detectable by the apparatus.

Field experiments. The aerial orange fluorescing particles caught in the Casella impactor showed no direct link with the number of viable microorganisms counted on the same time in the Anderson sampler (see Fig. 13). The fluorescence intensity after sucking through an air volume of [illegible] measuring values of between 0.5 and [illegible]. A series of samples taken successively on the same day (one hour) showed fairly constant values. A certain correlation was, however, found in the results from [illegible], the highest values in the summer. The high fluorescence values [illegible] and January were recorded from samples taken in [illegible], when a quantity of small fluorescent droplets of the snow [illegible] were observed.

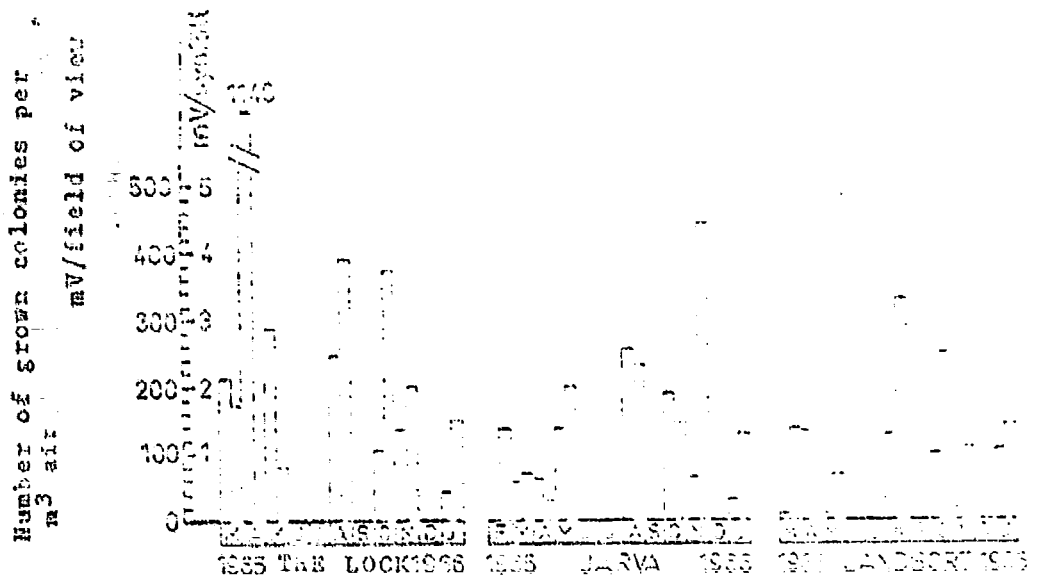


Fig. 13. Result of field experiments designed to characterize collected particles in the size range 1-5 μ on the one hand as viable microorganisms (number of grown colonies/m^3 sample air, unfilled columns) on the other hand as the fluorescence intensity after orange staining (mV/field of view). On the abscissa the year and month of sampling for each sampling location (The Lock, Jarva, Landsort)

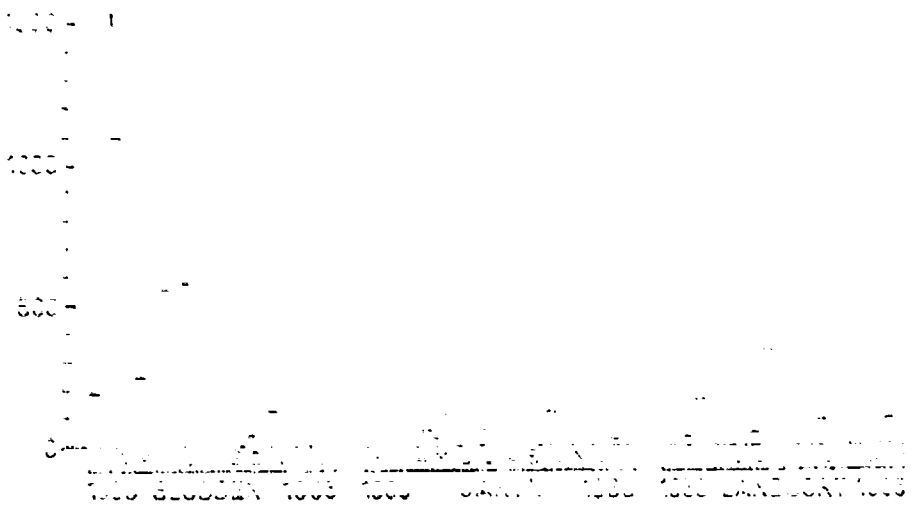


Fig. 24. [illegible] of field capacity and living fine root [illegible] at the end of October, respectively, in a number of paired columns per m² surface of [illegible] within the pine [illegible] in three sampling locations (the beech, [illegible], homogenate at various times of the year). Unfilled columns: biomass (g) per m² of air. Solid columns: [illegible] per m² of air.

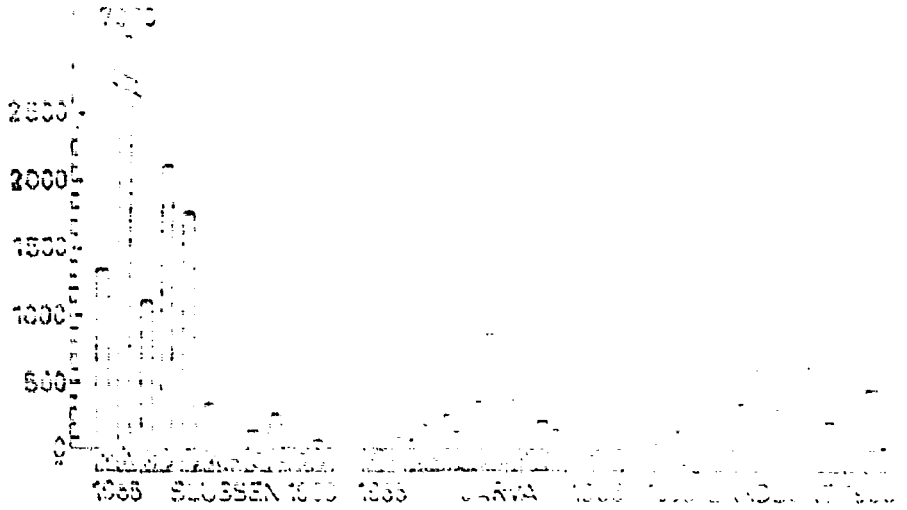


Fig. 15. Total number of viable [illegible] collected in Anderson [illegible] colonies per m³ of sample air, at the three sampling localities The Lock, Järva and Landsort. Unfilled columns: bacteria per m³ of air. Solid columns: fungi per m³ of air

The result of the culture tests from the field experiments appears in Fig. 13-15. First it must be stated that human personnel probably played an important role in the sampling. The bacterial count in the layer next to the ground was not noted, but the influence of the weather factors could be observed at Landsort and The Lock. Thus at Landsort a lower bacterial count was met with a sea breeze than with a land breeze. At The Lock the high bacteria counts during April and June were obtained with low air humidity, high temperature and weak wind.

The total number of recorded viable [illegible] per m³ did not exceed 1000 in any locality (Fig. 15) and the highest values for all three localities were measured during July and August. The measured number of bacteria in land and sea air was low, with some exceptions not exceeding 50 bacteria per m³.

Samples for electron microprobe analysis could only be taken in the field samplings in November 1967 and January, 1968. Rain had a disruptive effect on the values from Landsort and Järva in the former case, wherefore these were not included. The results are recorded in Table XI.

or incubation temperatures might have produced values different from those given here.

The good correlation between acridine orange fluorescence and bacteria count in the model experiments with test aerosols did not prevail in the field experiments. The principal reason for this appears to be the relatively low natural occurrence of microorganisms (bacteria background) in the air samples and an unexpectedly high content in the samples of method disturbing substances with fluorescence in the measured wave length and adjacent ones. The disturbing particles probably consist of silicon compounds and organic material (for example, material containing cellulose). It must furthermore be pointed out that the acridine orange method theoretically can detect all microorganisms collected in the Casella impactor, while only the viable organisms are recorded after collection in the Andersen sampler.

It was characteristic that the fluorescence measuring values were low in comparison with those from the test bacteria aerosols and that they had relatively limited distribution when measurements from the same day were compared. It is therefore quite conceivable that a massive appearance of bacteria in the sampling air (e.g. originating from a nearby source of distribution) could produce significant evidence of the presence of bacteria. No field experiments have yet been made in this direction.

Another possibility for increasing the sensitivity of the acridine orange method appears to be simultaneous measurement on several wavelengths other than green or red, whereby greater opportunities would be obtained for discriminating the fluorescence that is obtained, especially from nucleic acids. As unspecific particles as a rule show green or blue fluorescence after staining with AO (max 450-530 nm) further increased specificity can be gained by denaturing double strand nucleic acid to single strand. This changes the fluorescence color from green to red. Further advantages could be gained if such measurements could be carried out only on particles of a certain size and be increased in number. Hereby the automation that already has been worked out could be tried and some form of size discrimination of fluorescent particles could be introduced.

In this connection it should be pointed out that fluorescent antibody technique, for which similar apparatus can be used as for the acridine orange method, offers a practicable method for detection and in addition for exact typing of microorganisms.

The electron microprobe involves a practicable method for analysis of the chemical composition of individual particles. In recent times considerably faster instruments have been developed than that used in our investigation.

Certain constant differences for the various collecting places have been obtained. Especially the low natural bacteria content in the samples from field and sea air should be pointed out. The latter sampling location also showed the lowest level of unspecified fluorescent particles.

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