

made for salinity and other interfering substances if calibration curves are used because these interfering factors with one exception, that of arsenic, are taken care of in the preparation of the curves. Arsenic, if present as arsenate, and phosphorus are determined together in the Denigès method, so it is necessary to evaluate the arsenate by suitable means if true phosphorus values are to be obtained.

Farber and Youngberg² have shown that with the Denigès³ method, copper, iron and sulfates do not interfere with the color production in phosphorus determinations if appropriate amounts of reagents are used. Cooper⁴ has recognized that there is an interfering effect of the salts present in sea water. Brujewicz⁵ gives tables of correction for various salinities, showing that the latter have an effect on color production, and he evaluates a corresponding factor. Kalle,⁶ on the other hand, maintains that in the true sense of the word there is no salt error, but that the effect obtained is due rather to the copper present in the sea water. Using the same instrument as Kalle, Robinson and Wirth⁷ found a decided decrease in color intensity due to the presence of salts. Our results show that there is decided salt effect from redistilled water to a salinity of 5 o/oo, but that this effect increases only a small amount from this point to 30 o/oo salinity. Also, in agreement with Farber and Youngberg, copper and iron in the magnitudes tested have no effect on color production.

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A METHOD OF FREEING FUNGI FROM BACTERIAL CONTAMINATION¹

IN work with fungi it is often necessary to obtain cultures which are entirely free of bacterial contamination. This problem presents particular difficulties with such groups as the aquatic Phycomycetes, in which the whole mycelium is immersed in water or culture medium. Several methods have been described to secure bacteria-free material of such forms, but many of them rely on complicated apparatus, difficult technique or long and time-consuming procedure. The following method, requiring a minimum of effort and

² J. E. Farber and G. E. Youngberg, *Ind. Eng. Chem., Anal. Ed.*, 4: 107, 1932.

³ G. Denigès, *Compt. rend.*, 171: 802, 1920.

⁴ L. H. N. Cooper, *Jour. Mar. Biol. Assoc.*, n. s., 18: 677, 1933.

⁵ S. W. Brujewicz and V. S. Krasnova, *Bull. State Ocean. Inst. U. S. S. E.*, 14: 24, 1933.

⁶ K. Kalle, *Ann. d. Hydrographie*, 63: 65, 1935.

⁷ R. J. Robinson and H. E. Wirth, *Ind. Eng. Chem., Anal. Ed.*, 7: 147, 1935.

¹ John R. Raper, *Jour. Elisha Mitchell Sci. Soc.*, 52: 274-289, 1936.

time, has been used successfully to free a number of water molds from their bacterial contaminants.

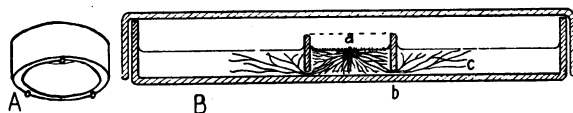


FIG. 1

To one end of a Van Teighem ring are fused three small glass beads, one third to one half mm in diameter, as shown in A of the accompanying diagram. The ring is placed in a Petri dish with the beaded surface resting on the bottom of the dish. Into the Petri dish enough nutrient agar (2 per cent. agar plus some suitable nutrient) is poured to bring its surface well up on the sides of the ring as in B. After the agar has solidified, a bit of inoculum is transferred to the area within the ring (a). As growth takes place some of the hyphae extend down into the agar, grow under the ring (b) and into the agar lying beyond it; the contaminating bacteria, however, do not follow the hyphae but are retained at the surface of the semi-solid within the ring. Cubes of agar containing numerous hyphal tips from this outlying portion of the mycelium (c) are therefore bacteria free; from these it is simple to start fresh cultures perfectly free from contamination.

This method has been used so far only for the purpose of freeing water molds from bacteria, but it should prove equally effective in securing pure cultures of any fungus which does not form an aerial mycelium. It is described here in the hope that it may be of use to other students working with such forms.

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