

cc of filtrate, a profitable response from phosphorus fertilization will be secured if other limiting factors are also supplied. Soils which contain from .008 to .013 milligrams of phosphorus in 25 cc of filtrate are in an intermediate zone where response from fertilization is usually slight. The soils which were studied in this experiment and contained more than .13 milligrams of phosphorus in a 25 cc aliquot did not respond to phosphorus fertilizers applied to ordinary field crops.

Some soils which are particularly deficient in easily soluble phosphorus may have a high absorptive capacity for that element when it is applied to the soil, and under such conditions crops may not respond appreciably to phosphorus fertilization. When a soil filtrate contains less than .002 milligrams of phosphorus in 25 cc, it may be desirable under certain soil conditions to determine the ability of those soils to fix large amounts of phosphorus. This can be done by leaching a soil with a solution containing a known amount of mono-potassium or mono-calcium phosphate, followed by a second leaching with 200 cc of tenth normal acetic acid. The amount of absorption can be determined by difference after subtracting the easily soluble phosphorus removed by a similar quantity of acid passing through an untreated sample of the same soil.

Tenth normal acetic acid will dissolve considerable amounts of freshly precipitated iron, aluminum and manganese phosphate; consequently in certain soils which have some calcium phosphate present in them which is available to the plant roots which come in contact with it, very little phosphorus may be extracted by distilled water, carbonated water or sodium acetate solutions, buffered to pH 4, 5 and 6, because of the absorption of the dissolved phosphorus by the soil. Under such conditions it is necessary to use a solution which is more acid than a saturated aqueous solution of carbon dioxide to keep the dissolved phosphorus from being absorbed by the soil particles. Since acetic acid will separate soils which contain all their phosphorus in the form of iron or aluminum phosphate from soils which contain varying amounts of calcium phosphate, and also has a high buffer capacity which tends to keep the reaction of the extracting solution more nearly constant for different soils, it is more desirable than other solvents which have been recommended.

The rate of percolation of a solution through a soil will depend to a considerable extent upon the texture and structure of the soil particles. In these experiments 200 cc of acetic acid percolated through the average soil in two to five hours. In case of coarse sandy soils the time required for the solvent to pass through the soil may frequently be less than thirty minutes. In case of clay soils sometimes the

rate of percolation is very slow. In some instances this can be hastened by mixing sand with the soil, which may reduce the total amount of phosphorus extracted. In other instances mixing the soil with large amounts of sand will not increase the rate of percolation. If the determinations are started in the evening, in most cases the solvent will pass through the soil before morning. In case of calcareous soils in which considerable amounts of occluded phosphate occurs, the method can not be recommended. When small amounts of calcium carbonate exist in the soil, the result obtained by this method agree very well with crop yields secured from the application of phosphate fertilizers applied to the soil.

It is quite possible that plant methods might be used to good advantage on soils which are in the intermediate zone as determined by this test. In case of calcareous soils and other soils which do not absorb large quantities of phosphorus after it is brought into solution by the extracting medium, a less acid solvent could be used. This will reduce the error which may occur due to the solution of the protective coating surrounding occluded phosphates which are frequently found in calcareous soils.

When crops are planted on soils containing a large amount of organic phosphorus which is readily mineralized by bacterial action, frequently the response from phosphorus fertilization is slight, even though only small amounts of phosphorus are removed by dilute acid solutions. Under such conditions extraction of the soil with a solution containing one per cent. of sodium or potassium carbonate may be used to detect a phosphorus deficiency.

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CIRCULATION AND AERATION APPARATUS FOR AQUATIC MEDIA

WITH the growing recognition of the value of fishes and fish eggs as laboratory material for physiological and embryological study¹ the problem of adequately aerating the incubating eggs becomes important. The common method used in state fish hatcheries demands a continuous flow of fresh water from sources native to the eggs used. This is obviously impossible in the average laboratory, and experience has indicated that the mortality caused by chemical content, together with a predilection toward fungi, when flowing city water is used, is too high to make the method practical. The following easily constructed apparatus overcomes these difficulties.

The container is a quart milk bottle (see Fig. 1). Paraffin is melted in the bottom of the bottle to form a layer about $\frac{3}{4}$ " in depth. While this is cooling two

¹Floyd J. Brinley, "Eggs of Fresh-Water Fishes Suitable for Physiological Research," *SCIENCE*, 74: 295-296, 1931.

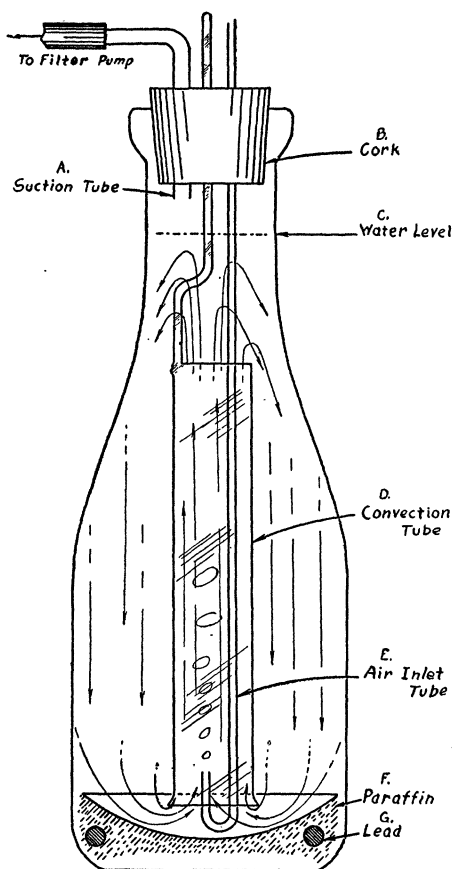


FIG. 1

10 gram pieces of lead are embedded near the lower margin of the paraffin to overcome its tendency to float, in the absence of a perfect seal at the periphery. When cooled it is made cup-shaped with a flame provided by connecting a glass tube, drawn to a tip, to a gas jet. By repeatedly melting the center of the paraffin, and slowly revolving the inclined bottle to allow the melted portion to build up on the sides, the desired cup is obtained. When hard, this can be fire-polished smooth by lightly passing the flame over the surface. The well, thus formed, serves to direct the eggs under the mouth of the convection tube.

The convection tube (D) is made from a 1" test-tube 6" long, from the bottom of which 1" is cut. A $\frac{1}{8}$ " glass rod 6" long with a $\frac{1}{2}$ " double bend about $\frac{3}{4}$ " from one end is fused by its short section to the cut end of the convection tube, so that when the long section is inserted in the cork the line of suspension

is vertical and along the axis of the convection tube. The working distance of this tube is about $\frac{1}{2}$ " above the paraffin, varying with the size of the eggs and the amount of circulation desired. The air inlet tube (E) is $\frac{1}{8}$ " glass tubing 14" long with a $\frac{3}{8}$ " U bend $\frac{1}{2}$ " from one end. This is suspended inside the convection tube so that the air jet is in the center. The suction tube (A) is $\frac{3}{4}$ " glass tubing 5" long with a right angle bend in the middle. The cork (B) is drilled in the center to accommodate the convection tube rod, and $\frac{1}{4}$ " along a diameter on each side of the center to accommodate the inlet and suction tubes. The apparatus is set up as shown in the figure (all unions being air-tight) and attached to a filter pump.

When in operation the suction, created by the jet of air passing through the convection tube from the air inlet, will circulate the eggs up through this tube, discharging them at the top; they will then be carried to the bottom of the bottle by gravity and the current, where they will again be drawn up into the convection tube (circulation indicated on figure). The entire medium is filled with moving eggs, which are constantly suspended in the highly oxygenated water. The volume of air passed through, and the relationship of the inlet tube to the bottom of the convection tube, determine the velocity of the current. Dr. Floyd J. Brinley, of this department, has found the apparatus highly successful for the incubation of eggs of the wall-eyed pike (*Stizostedion vitreum*) with respect to percentage and speed of hatching. About 20,000 eggs can be handled in each bottle.

Since the apparatus permits control of temperature, aeration and circulation, thus maintaining a uniform medium under ordinary laboratory conditions, it lends itself readily to many problems involving effects of temperature, chemical solutions, gases, etc., on aquatic organisms. It may be useful in chemical problems utilizing solutions and precipitates. Modifications of the idea also suggest themselves for many problems; for example, the efficiency of the apparatus described by J. Henry Walker, Jr., in *SCIENCE* for June 26, 1931 (method for oxygenating an aquarium), can be increased by the addition of the convection tube about the air intake. The current created carries air bubbles down into the medium, permitting greater diffusion.

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SPECIAL ARTICLES

THE VAGINAL SMEAR METHOD OF DETERMINING VITAMIN A¹

IN the quantitative determination of vitamin A,²

¹ From the Laboratory of Agricultural Chemistry, University of Wisconsin, Madison, Wisconsin.

three different biological methods have been used. These are what may be called the growth method, the ophthalmic method, and the vaginal smear method.

² In this paper "vitamin A" refers to all substances which show vitamin A activity.