Determination of Nitrate in Waters from Fish Ponds

Leaflet 99/December 1980

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THE PHENOLDISULFONIC ACID METHOD has been widely used for determining nitrate-nitrogen in water. In this procedure, water is evaporated and nitrate in the residue is treated with phenoldisulfonic acid to form a colorless nitro derivative. In alkaline solution, the nitro derivative is transformed to a yellow-colored compound. The intensity of the yellow color is proportional to the concentration of nitrate, permitting colorimetry. This procedure is time-consuming because of the evaporation step and because a number of substances interfere with the analysis.

Recently, another nitrate procedure has gained popularity. Nitrate is reduced quantitatively to nitrite when a sample is passed over cadmium filings that have been treated with copper sulfate. The nitrite is reacted with sulfanilamide and N-(1-naphthyl)-ethylenediamine to form a highly-colored azo dye so its concentration may be measured colorimetrically. A correction must be made for nitrite initially present in the sample.

The present study was initiated to compare the precision and accuracy of the two methods of determining nitrate in waters from fish ponds.

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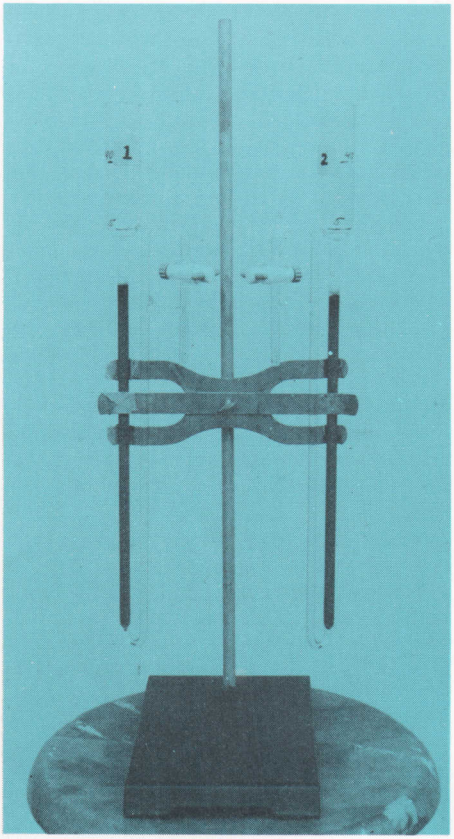

FIG. 1. Cadmium reduction column packed with cadmium filings.

METHODS

The phenoldisulfonic acid method was conducted according to standard procedure[1]. The cadmium reduction method followed the suggestions of Strickland and Parsons[2]. However, there were two variations from their suggestions. Instead of constructing the reduction columns from simple

[1]BOYD, C. E. 1979. Water Quality in Warmwater Fish Ponds. Auburn Univ. (Ala.) Agr. Exp. Sta. 359 p.

[2]STRICKLAND, J. D. AND T. R. PARSONS. 1972. A Practical Handbook of Seawater Analysis. Bull. 167. Fish. Res. Bd. Canada. 311 p.

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materials, pre-fabricated glass columns, figure 1, were purchased from the Robert Anderson Glass Company, Old Turnpike Road, Fitzwilliams, New Hampshire. Cadmium filings were prepared according to Strickland and Parsons, but before transferring the cadmium filings to the column, the filings were washed several times with distilled water to remove excess colloidal copper. Following reduction of nitrate to nitrite in the column, nitrite was determined by diazotization[1].

Water samples were obtained from ponds on the Fisheries Research Unit, Auburn University Agricultural Experiment Station, Auburn University, Alabama. These samples initially contained 0.2 mg/liter or less of nitrate-nitrogen, so small amounts of sodium nitrate were added to some samples to increase nitrate-nitrogen concentrations. Before analysis, all samples were filtered through glass fiber filters (Gelman Type A-E) to remove particulate matter.

Precision estimates were based on seven replicate analyses of each of six samples[3]. Percentage recovery was used to estimate accuracy[3]. After determining nitrate initally present, 28 samples were spiked with either 0.05 mg/liter nitrate-nitrogen (cadmium reduction method) or 0.3 mg/liter nitrate-nitrogen (phenoldisulfonic acid method) and nitrate-nitrogen was again measured. The percentage recovery was estimated by the following equation[4]:

$$\text{Percent recovery} = \frac{F}{1 + S} \times 100;$$

F = the final concentration of nitrate-nitrogen; I = the initial concentration of nitrate-nitrogen; S = the concentration of nitrate-nitrogen added. The closer to 100 percent the recovery, the greater the accuracy. Finally, nitrate-nitrogen was determined in 25 samples by both methods and the results were subjected to regression analysis.

RESULTS AND DISCUSSION

The cadmium reduction method permits determination of lower concentrations of nitrate-nitrogen than are possible with the phenoldisulfonic acid method, table 1. Nitrate-nitrogen concentrations in fish ponds are often below 0.1 mg/liter[1], so the cadmium reduction procedure has a distinct advantage over the phenoldisulfonic acid method.

TABLE 1. COMPARISON OF ABSORBANCE READINGS FOR NITRATE-NITROGEN CONCENTRATIONS USING THE CADMIUM REDUCTION METHOD AND THE PHENOLDISULFONIC ACID TECHNIQUE

	Absorbance in 1-cm colorimeter cell*	
Nitrate-nitrogen (mg/liter)	Cadmium reduction	Phenoldisulfonic acid
0.01	0.036	0.004
0.05	0.167	0.022
0.10	0.328	0.046
0.15	0.502	0.071
0.20	0.658	0.092

*Suitable accuracy for routine purposes can be achieved if absorbance values fall between 0.025 and 0.700. For rigorous work, absorbance values should be between 0.100 and 0.450.

Precision estimates, table 2, suggest that both methods of nitrate analysis are relatively precise. However, the precision of both methods tends to decrease at low nitrate-nitrogen concentrations. The percentage recovery of nitrate-nitrogen was significantly greater ($t = 5.14$; $P<0.001$) for the cadmium reduction method than for the phenoldisulfonic acid method, table 3, suggesting that the cadmium reduction method is more accurate. Percentage recovery for the phenoldisulfonic acid method was even poorer (58.3 percent) than indicated in table 3 for eight samples initially containing less than 0.15 mg/liter nitrate-nitrogen. However, even when these samples were removed, the percentage recovery for the remaining 20 samples was only 91.6 percent. Percentage recovery by the cadmium reduction method was not affected by the initial nitrate-nitrogen concentration.

Correlations between concentrations determined in the same samples by the two methods were not significant ($r^2 = 0.17$; $P>0.05$) for 13 samples containing low concentrations of nitrate-nitrogen. Since accuracy

[3]UNITED STATES ENVIRONMENTAL PROTECTION AGENCY. 1972. Handbook For Analytical Quality Control in Water and Wastewater Laboratories. Analytical Quality Control Laboratory, Cincinnati, Ohio. 97 p.

[4]BOYD, C. E. 1979. Determination of Total Ammonia Nitrogen and Chemical Oxygen Demand in Fish Culture Systems. Trans. Amer. Fish. Soc. 108: pp. 314-319.

TABLE 2. PRECISION ESTIMATES FOR NITRATE-NITROGEN DETERMINATIONS MADE BY THE CADMIUM REDUCTION METHOD AND BY THE PHENOLDISULFONIC ACID TECHNIQUE. SAMPLES WERE ANALYZED SEVEN TIMES

	Sample[1]					
	A	B	C	D	E	F
Cadmium reduction:						
Mean (mg/liter)	0.001	0.008	0.114	0.198	0.662	1.14
Standard deviation (mg/liter)	0.0004	0.0008	0.0036	0.0040	0.0042	0.010
Coefficient of variation (pct.)	25.0	10.0	3.2	2.0	0.6	0.9
Phenoldisulfonic acid:						
Mean (mg/liter)	0.03	0.15	0.21	0.61	0.81	2.93
Standard deviation (mg/liter)	0.004	0.01	0.011	0.034	0.05	0.063
Coefficient of variation (pct.)	13.3	6.7	5.2	5.6	6.2	2.2

[1]Sample A for cadmium reduction was not the same sample as Sample A for phenoldisulfonic acid, etc.

TABLE 3. SPIKE-RECOVERY ESTIMATES OF ACCURACY FOR NITRATE-NITROGEN DETERMINATIONS MADE BY THE CADMIUM REDUCTION METHOD AND THE PHENOLDISULFONIC ACID TECHNIQUE (TWENTY-EIGHT SPIKE-RECOVERY TESTS WERE MADE WITH EACH PROCEDURE)

	Cadmium reduction	Phenoldisulfonic acid
	Pct.	*Pct.*
Mean	99.6	82.1
Standard deviation	2.96	17.8
Coefficient of variation	3.0	21.7

was superior for the cadmium reduction method, table 3, data obtained by the phenoldisulfonic acid method were assumed to be erroneous. There was a significant correlation ($P < 0.01$) between nitrate-nitrogen concentrations determined on the same samples for higher nitrate-nitrogen levels, figure 2. However, the cadmium reduction method gave consistently greater values than the other method.

CONCLUSIONS

Because of its greater sensitivity and accuracy, the cadmium reduction method is superior to the phenoldisulfonic acid technique for measuring nitrate-nitrogen in waters from fish ponds. The cadmium reduction columns are initially difficult to prepare, but once columns are prepared, the cadmium reduction method is no more complicated to perform than is the phenoldisulfonic acid procedure.

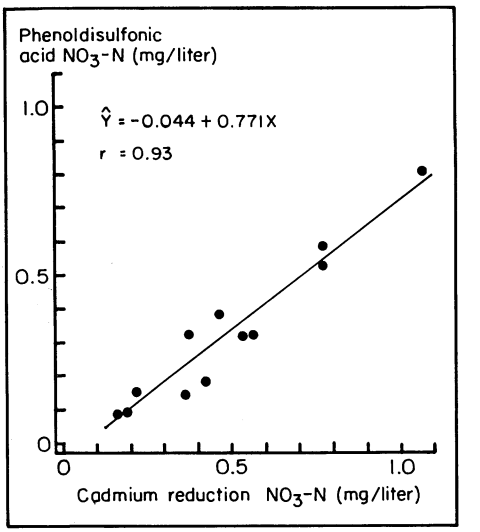

FIG. 2. Relationship between nitrate-nitrogen concentrations measured on the same samples by the cadmium reduction method and the phenoldisulfonic acid method.