A SULFIDE DETECTION TEST FOR FIELD USE[1]

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Hydrogen sulfide in marshes and streams can be continuously formed from the decomposition of organic material or by its introduction from artesian wells. Sulfide may be directly toxic to fish or it can act as a scavenger for dissolved oxygen, and therefore stress or kill the organisms indirectly by lowering dissolved oxygen levels. For mosquito control agencies, the problem seems particularly important in salt marsh impoundments, where management practices in altered salt marsh habitats may contribute to the sudden release of hydrogen sulfide from sediments possibly causing the death of fish and aquatic invertebrates.

The toxicity of hydrogen sulfide for fish and invertebrates has been studied extensively by Smith et al. (1976). However, none of the detection methods described in this report (Broderius et al. 1976) or those listed by the American Public Health Association (1985), are practical as a field test. Field, rather than lab testing is essential because of the instability of hydrogen sulfide solutions. A field test would be useful as an early warning device for possible corrective measures when sudden sulfide loads occur in managed sensitive habitats. Among several qualitative and quantitative methods for sulfide determination, the methylene blue method, studied in detail by Cline (1969) can be easily adjusted for use in the field.

Procedure. Dilute hydrochloric acid (50%). Mix 0.5 liter hydrochloric acid (36–38%) with an equal volume of water. Dissolve 1 gm N,N-dimethyl-p-phenylenediamine sulfate in 0.5 liter dilute hydrochloric acid. Dissolve 1.5 gm ferric chloride ($FeCl_3 \cdot 6H_2O$) in 0.5 liter dilute hydrochloric acid. These two solutions, stored in a refrigerator, are stable indefinitely. The sulfide reagent consists of an equal mixture of both solutions which is stable for many months. The reaction is carried out in a 16 x 100 mm culture tube (or any other size tube) with a mark at the 5 ml level. Add 0.5 ml of the sulfide reagent to each tube. Add test sample to the 5 ml mark. A transient pink color appears first both in the presence or absence of sulfide. In the presence of sulfide, the pink color is replaced in about 1 minute by a blue reaction product (methylene blue) proportional to the amount of sulfide. With a large excess of sulfide, the color development may be delayed 5 or more minutes or will not develop at all. For that reason, it is advisable when sulfide in the sample is suspected (usually by smell) and no color appears, to dilute the sample 10× with water and react again.

Sensitivity and precision. The smallest amount detectable with this method is 0.05–0.1 mg/liter. The blue color is stable for at least 6 months and therefore, can be measured quantitatively in a spectrophotometer, at a later time. Carried out as described, the optical density is 0.17 per microgram sulfide at 650 nm. The relationship between color density and concentration is linear only to about 5 micrograms (optical density = 0.85). It is therefore recommended, if color density appears to be high and will be measured quantitatively, to react simultaneously a tenfold dilution of the sample. The reaction is highly specific for sulfide and not affected by salinity (sea water) or sediments scooped up with bottom samples.

Instability of sulfide solutions. To illustrate the instability of dilute hydrogen sulfide solutions and therefore the usefulness of carrying out the reaction in the field, I used sewage and artesian water. In raw domestic sewage at the entry point to the treatment plant, 15 mg/liter sulfide was present. In a full stoppered bottle, it increased to 40 mg/liter in 3 days. When refrigerated, the sulfide increased only slightly but when the same bottle was warmed to room temperature, it increased again to 40 mg/liter. Apparently under anaerobic conditions, sulfur reducing bacteria continued to produce hydrogen sulfide in raw sewage. However, when sewage was maintained in a beaker, in contact with air, sulfide was reduced overnight to 1–3 mg/liter, somewhat dependent on the height of the liquid level.

When a sample of artesian water, with 5 mg/liter sulfide at the well point was kept in an open beaker, only 1 mg was left after 6 hours, and similar losses occurred in a stoppered bottle overnight, even when refrigerated. With lower sulfide levels, the problem of transporting a water sample for analysis is proportionally aggravated. Artesian water (with 5 mg/liter sulfide) could be tested directly, but raw sewage did not develop the blue color unless diluted.

Field application. In general, surface water of the Indian River Lagoon and associated salt

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marshes was negative, but sulfide was usually present near the bottom and in the bottom mud. However, we found sulfide in the surface water of the perimeter ditch of the North Deerfield Impoundment, located in north eastern Indian River County, presumably originating from a submerged artesian well. The level fell from 2 mg to 0.2 mg per liter in about 100 meters, and was the same at the surface and near the bottom (40 to 60 cm below the surface). Further downstream, the surface water became negative whereas the bottom water still contained some sulfide. This 20 ha impoundment which contains a perimeter ditch around the dike interior is not flooded by pumping. However, it is intertidal through a breach in the dike. Salinities vary greatly and commonly range from 1 ppt near the artesian well to near lagoonal estuarine conditions (25–30 ppt) at the dike breach. Several fish including *Gambusia affinis* (mosquito fish) and *Centropomus undecimalis* (snook) were observed in the area of maximum sulfide concentration.

Smith et al. (1976) conducted acute and chronic toxicity studies with seven fish species in the laboratory and concluded that the maximum safe sulfide level varied between 0.0004 and 0.01 mg per liter, depending on species. These tolerance levels are several orders of magnitude below the levels found in our salt marsh study area. These discrepancies suggest that further studies are needed to establish whether fish kills in managed impoundments are due to sulfide.

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