

NOTE

A simple method for the detection and the “a posteriori” correction of the interference of sulfide on phosphorus measurements*JOSEP M. GASOL¹ and ALEXANDER J. B. ZEHNDER²¹Institut de Ciències del Mar, CSIC, Passeig Joan de Borbó, s/n.
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SUMMARY: We report on the interference that hydrogen sulfide can generate on the measurement of phosphorus in seawater with the Murphy & Riley method. Free sulfide can be found in a variety of saline systems, from partially closed fjords to eutrophic estuaries, river saline wedges, saline lagoons, etc. in concentrations ranging from 0.02 up to 10 mM and, thus, may be present in water samples without the analysts suspicion. We tested the effects of sulfide concentrations ranging from 0.005 up to 0.7 mM on the measurement of phosphorus concentrations from 10 μgP to 1.5 $\text{mgP-PO}_4 \text{ l}^{-1}$. We characterized spectrophotometrically, the product and the dynamics of the interference and we provide a simple method to detect the interference “a posteriori” by computing the ratio between the absorbances at 350 and 690 nm. If sulfide has been measured in parallel, the absorbances, even those that have had high interference, can be corrected to values without the interference with the equations that we provide. We propose the routine use of this method whenever dealing with samples that could have some sulfide in solution.

Key words: Sulfide, phosphorus, interference in phosphorus measurement.

RESUMEN: UN MÉTODO SENCILLO PARA DETECTAR Y CORREGIR “A POSTERIORI” LAS INTERFERENCIAS DEL SULFHÍDRICO EN LAS MEDIDAS DE FOSFATO. – El trabajo muestra la interferencia que el ácido sulfhídrico puede causar en las mediciones de fósforo en agua de mar con el método de Murphy & Riley. Ácido sulfhídrico en forma libre puede encontrarse en diversos ecosistemas salinos: fiordos parcialmente cerrados, estuarios eutróficos, cuñas salinas en ríos, lagunas costeras, etc... en concentraciones que oscilan entre 0.02 y 10 mM. Puede, por tanto, encontrarse en muestras sin que el analista lo espere. Comprobamos los efectos de concentraciones de sulfhídrico entre 0.005 y 0.7 mM sobre las mediciones de concentraciones de fósforo entre 10 μgP y 1.5 $\text{mgP-PO}_4 \text{ l}^{-1}$. También caracterizamos espectrofotométricamente el producto y la dinámica de la interferencia y presentamos un método simple para detectar “a posteriori” la interferencia basado en el cálculo de la relación entre las absorbancias a 350 y 690 nm. Si el sulfhídrico fué medido en paralelo, la interferencia puede eliminarse de las absorbancias, incluso si fué muy importante mediante las ecuaciones que proporcionamos. Sugerimos el uso rutinario de este método siempre que deba trabajarse con muestras que pudieran tener sulfhídrico disuelto.

Palabras clave: Sulfhídrico, fósforo, interferencia en las mediciones de fósforo.

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The use of the molybdenum-blue method for the measurement of orthophosphate concentrations in water is widespread since the method was simplified in the late 50s and antimony was added to favor the development of color (Murphy and Riley, 1958; 1962). Despite the general use and simplicity of the method, researchers often report problems due to interferences from arsenate (Olsen, 1967; Chamberlain and Shapiro, 1973), silicates in acid media (Stainton *et al.*, 1977) and ferrous iron (Nürnberg, 1984). One of the molecules that can interfere with the analysis is hydrogen sulfide, which competes with phosphorus for antimony (De Jonge and Villerius, 1980; Nürnberg, 1984).

De Jonge and Villerius (1980) suggested that the sometimes erratic values of phosphate concentration in some estuarine waters could be caused by sulfide interference. Sulfide is commonly found in many other environments: meromictic or saline lakes (e.g. Gorlenko *et al.*, 1983), coastal lagoons (e.g. Caumette *et al.*, 1983), deep-sea hydrothermal vents (e.g. Karl, 1982), eutrophic estuaries (e.g. Luther *et al.*, 1988) or partially land-locked fjords (e.g. Wassmann and Aadnesen, 1984), and it is present in the Black Sea below 200 m (e.g. Anderson and

Devol, 1987). It is also common in sludge and in water from wastewater treatment plants and can even be found in high concentrations in deep open ocean water in unventilated basins (e.g. Scranton *et al.*, 1987, Luther and Tsamakis, 1989). In Table 1 we compiled the maximum concentrations of sulfide found in some of these marine environments. These concentrations can be as high as 8 mM (~ 300 mg l⁻¹) and values around 0.5 mM are fairly common.

The measurement of sulfide concentrations is commonly not routine in oceanographic practice. Even though chemical oceanographers are well acquainted with the interference and the most common analytical solutions, marine biologists, which often do work on the phosphorus uptake dynamics of plankton living in microaerophilic environments, may not be well aware of that source of error in their measurements. In most cases, interference of sulfide on the measurement of phosphorus is deduced from the erratic behavior of replicates after the analysis has been completed. Since sulfide is volatile, the actual interference may depend on the amount of time that the sample has been left on the shelf before analysis. Possible aeration may lead to sulfide oxidation, thus contributing to random interferences as well.

TABLE 1. – Sulfide concentrations in some selected salt- or brackish- water environments

System	Reference	Maximal H ₂ S (mM)
Cariaco Trench	Atkinson and Richards, 1967	0.03
Cariaco Trench	Scranton <i>et al.</i> , 1987	0.05
Cariaco Trench	Zhang and Millero, 1993	0.06
Skan Bay and Tres Marias	Devol, 1987	0.20
Bannock basin, Mediterranean	Luther and Tsamakis, 1989	3.00
Black Sea	Atkinson and Richards, 1967	0.36
Black Sea	Sorokin, 1972	0.13
Black Sea	Karl, 1978	0.01
Black Sea	Millero, 1991	0.02
Gotlan basin, Baltic Sea	Gast and Gocke, 1988	0.04
Gotlan basin, Baltic Sea	Detmer <i>et al.</i> , 1993	0.07
Saanich Inlet, BC, CDN	Emerson <i>et al.</i> , 1979	0.04
Framvaren, N	Lidstrom, 1983	8.40
Vågsbøpollen fjord, N	Wassmann and Aadnesen, 1984	~0.64
Kviturdvikkollen fjord, N	Wassmann and Aadnesen, 1984	~0.46
Saelenvann estuary, N	Indrebø <i>et al.</i> , 1978	3.00
Chesapeake Bay, USA	Luther <i>et al.</i> , 1988	0.06
Pettaquamscutt Estuary, RI, USA	Sieburth and Donoghay, 1994	0.15
Emss estuary, NL	De Jonge and Villerius, 1980	5.00
Kalø Vig lagoon, DK	Ingvorsen and Jørgensen, 1979	1.00
Solar Lake, Israel	Jørgensen, <i>et al.</i> , 1979	1.00
Ace Lake Antarctica	Hand and Burton, 1981	~5.71
Lagune Ebrié, Cote d'Ivoire	Caumette <i>et al.</i> , 1983	10.67
Lake Kaiike, Japan	Matsuyama, 1977	0.53
Lake Nitinat, BC, CDN	Atkinson and Richards, 1967	0.30
Lake Mogilnoye, Barents Sea	Gorlenko <i>et al.</i> , 1978	4.69
Ebre River saline wedge, SP	Pedrós-Alió <i>et al.</i> , unpubl.	0.02
San Juan Is. marine snow	Shanks and Reeder, 1993	0.02

Here, we characterize the absorption spectrum of the sulfide-antimony compound that interferes with the assay, we show how we can detect the presence of interference in the phosphorus measurements and suggest a way for correcting it when the sample has already been analyzed.

Orthophosphate was measured according to the standard Murphy and Riley (1958; 1962) method. We used a final Sb concentration of 50 μM . Ammonium molybdate and potassium antimonyl-tartrate react in acid medium with the orthophosphate to give a compound that, when reduced with ascorbic acid, can be quantified by its absorption peak at 885 nm (Standard Methods, 1985) in a spectrophotometer. The compound, however, has another absorption peak at 690 nm which is approx. 70% of the peak at 885 nm. Most of the results reported here correspond to the measurement of the absorbance at 690 nm, but the method proposed can equally be established with the absorbance at 885 nm. All dilutions were made with deio-

nized water and all glassware had been acid-rinsed. Hydrogen sulfide was measured by the methylene-blue method of Pachmayr (1960) as described in Trüper and Schlegel (1964). Total phosphorus was measured as orthophosphate after digestion in the presence of acid (0.6 N H_2SO_4) and persulphate ($\text{K}_2\text{S}_2\text{O}_8$) after heating for an hour. After the heating, the sample was either diluted with deionized water or neutralized with 1 N NaOH.

Orthophosphate was added from a stock solution of K_2HPO_4 in deionized water and preserved under refrigeration with 1 ml chloroform per L^{-1} of solution. Organic phosphorus was added from a stock solution of adenosin-diphosphate (Sigma). The hydrogen sulfide solution was made from $\text{Na}_2\text{S}\cdot 7\text{-}9\text{H}_2\text{O}$ and Na_2CO_3 (analytical grade) in pre-boiled oxygen-free water. The solution was dispensed in small tubes that had been autoclaved. When sulfide had to be used, a tube was neutralized with 1 N HCl to reach a final S^{2-} concentration of 70 mM.

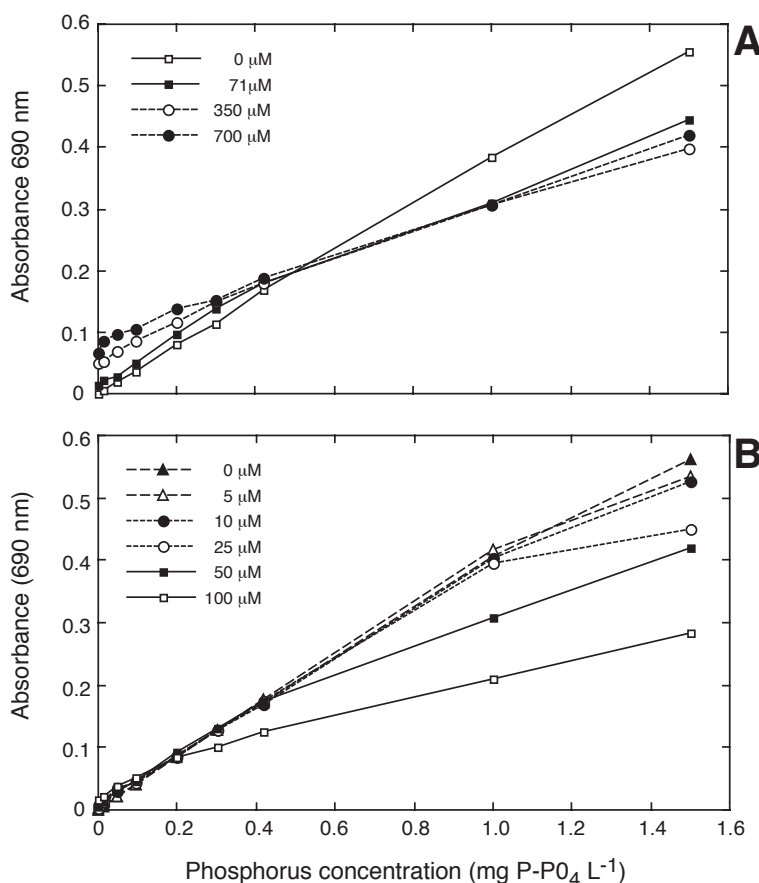


FIG. 1. – Interference of sulfide in the measurement of the absorbance at 690 nm. in a 1-cm path cell of different series of phosphorus concentrations after having reacted with the molybdate-antimonil-ascorbic reagent. A) High sulfide concentrations (0 to 0.7 mM). B) Lower sulfide concentrations (0 to 0.1 mM). Legend in the figure. The amount of Sb in the reagent used for the run in A was higher than in the reagent used in the run B.

Multiple regression was performed using the multiple general linear hypothesis module of Systat (Wilkinson, 1989). To compensate for inherent bias in log-transformed equations, we provide correction factors as $CF = \text{antilog } 1.1513 \times (SEE)^2$, SEE being the standard error of the estimate (square root of the residual mean square). Predictions converted to arithmetic scale must be multiplied by CF.

The interference of sulfide on phosphorus measurements has been studied by De Jonge and Villerius (1980) and Nürnberg (1984). The interference is in fact double. On the one hand S competes with P for Sb and, thus, higher concentrations of sulfide produce lower absorbances at high concentrations of phosphate (Fig. 1). It is at high P concentrations that Sb may be limiting and S and P compete for the available Sb. As pointed out by De Jonge and Villerius (1980) this problem can be solved by just incrementing the concentration of Sb in the reagent. On the other hand a yellow-brownish compound that increases the absorbance is produced at lower concentrations of P (Fig. 1A). The formation of this product is only slightly dependent on the P concentration, and is mainly dependent on the amount of sulfide present (Fig. 2, and Figure 3A). The interference of S on P measurements is thus double: competition for the Sb, and development of this yellowish-brown product. In the measurements of total phosphorus only the first interference is present and the yellowish-brownish colored product did not develop, probably because the treatment (boiling in presence of acid and persulphate) affected the chemical reactivity of the product.

The reaction between Sb and S and P is not simple. De Jonge and Villerius (1980) have identified several different compounds that are produced at different concentrations of Sb and S. We observed a regular increase of absorbance at all wavelengths when adding different concentrations of S even without any P added (Fig. 3A). Concentrations of sulfide as low as 0.06 mM already superseded the absorbance of the blue color generated by $0.3 \text{ mgP-PO}_4 \text{ l}^{-1}$ at all wavelengths (including both 690 and 885 nm, Fig. 3B). The effect of the sulfide interference was much lower when high concentrations ($1.5 \text{ mP-PO}_4 \text{ l}^{-1}$) of P had been added (Fig. 3B). However, both the absorbance at 690 nm and that at 885 nm were modified by the presence of 0.35 mM of sulfide. When we extended these observations to wavelengths lower than 500 nm, we detected a peak of absorbance of the product Sb-S at $\sim 350 \text{ nm}$ (Figure 4). The peak at 350 nm, in fact, consisted of a portion of the absorbance generated by the P reaction product and a portion generated by the Sb-S product itself (Fig. 4B).

Because it appeared that the absorbance at a given wavelength could be described as the addition of the absorbance due to the compound Sb-S (that has a peak at 350 nm) plus the absorbance of the Sb-P compound (which has peaks at 690 and 885 nm), we set up an experiment to determine whether the ratio between the absorbances at 350 nm and 690 (or 885) nm could be used as an index of sulfide interference in the phosphorus measurement.

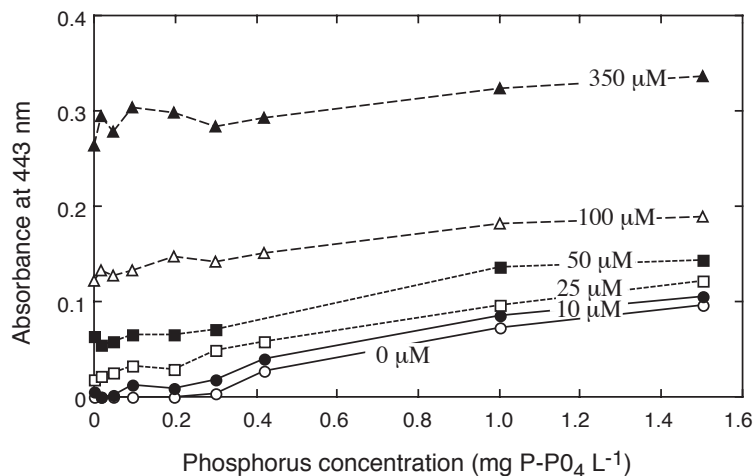


FIG. 2. – Absorbance at 443 nm in a 1-cm path cell of different combinations of phosphorus and sulfide concentrations. 0 μM sulfide (m), 10 μM (j), 25 μM (q), 50 μM (n), 100 μM (Δ) and 350 μM (s).

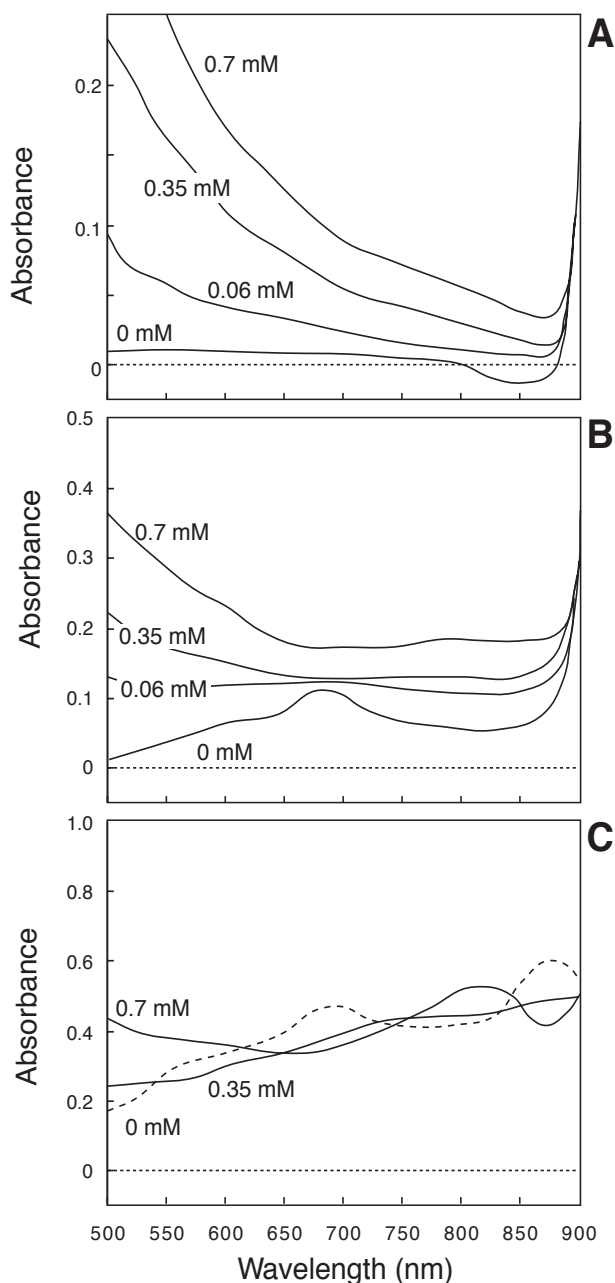


FIG. 3. – Absorption spectra of the combined sulfide and phosphorus product of reaction with antimony. A) No phosphorus added. B) 0.3 mgP-PO₄ l⁻¹ added. C) 1.5 mgP-PO₄ l⁻¹ added.

We ran several series of analysis with different combinations of phosphorus (ranging from 0 to 1 mgP-PO₄ l⁻¹) and sulfide (ranging from 0 to 350 μM), we recorded the absorbances at the different wavelengths and we computed the ratio between the absorbances at 690 nm and at 350 nm. We found that the ratio of absorbances (Ratio = A₆₉₀:A₃₅₀) was very strongly related (P < 0.00005) to the concentrations of P and sulfide in the following way:

$$\text{Log Ratio}(A_{690}:A_{350}) = 0.14 (\pm 0.06) - 0.49 (\pm 0.02) \text{LS} + 0.22 (\pm 0.02) \quad (1)$$

with N=52, r²= 0.92, SEE= 0.13 and CF= 1.05 being LS= log sulfide concentration (mM) and LP= log phosphorus concentration (μgP-PO₄ l⁻¹).

The ratio (A₆₉₀:A₃₅₀) was found to have values above 1 (and mostly around 4) when the interference of sulfide was low, that is, when there was no sulfide in the sample; and values below 1 when the interference was more severe (Fig. 5A). Figure 5A can be used “a posteriori” to detect and interpret the interference of sulfide on a given P measurement, because the ratio A₆₉₀:A₃₅₀ is directly related to the concentration of sulfide in the sample with independence of the phosphorus concentration.

To estimate the concentration of P from the concentrations of sulfide and the ratio of absorbances, Equation #1 cannot be used directly. The following equation, which is obtained from the same data set, can be used instead:

$$\text{LP} = 0.11 (\pm 0.23) + 1.58 (\pm 0.16) \text{LS} + 3.26 (\pm 0.28) \text{Log Ratio}(A_{690}:A_{350}) \quad (2)$$

with N=52, r²= 0.73, SEE= 0.51 and CF= 1.98

Because samples without interference had been used to build the regression equation, the equation will predict correct P concentrations if sulfide is not present. However, and because of the collinearity between LS and LRatio, this second equation predicts with lower accuracy than does equation #1. These equations discriminate the contribution of different solvents to the global absorbance of the mixture. They work, thus, in a way equivalent to that used in computing the concentration of a given pigment from the absorbances of a mixture of pigments in a solvent.

To solve the interference by sulfide in the phosphorus measurements, De Jonge and Villerius (1980) and Nürnberg (1984) have suggested several techniques. The first one is the increase in the concentration of Sb in the reagent to avoid the interference by competition at high phosphorus values. However, this method only solves one of the two interference problems, and it leaves untouched the production of the yellowish compound that absorbs at 433. Acidification of the sample is sometimes suggested to be a way to volatilize and consequently eliminate the dissolved sulfide from the sample. In natural samples, however, this method, together with the method where hydrogen peroxide is added may result in the hydrolysis of low molecular weight P-

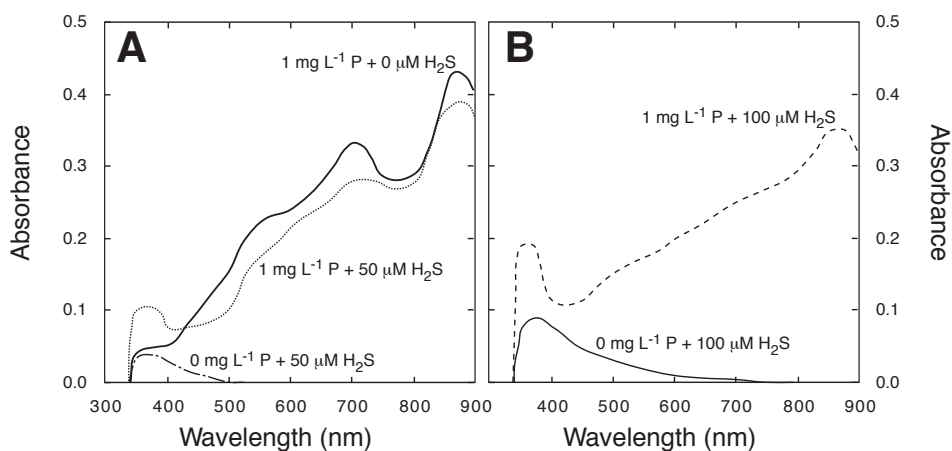


FIG. 4. – Combined absorption spectra of different phosphorus and sulfide concentrations.

compounds. Such hydrolysis could lead to an overestimation of the concentration of orthophosphate (Stainton, 1980). Degassing the sample (e.g. by bubbling N_2 gas through) is probably the best available solution to remove all sulfide before the analysis. We used the ratio $A_{690}:A_{350}$ to ascertain the effect of aeration and acid addition to several series of P concentration to some of which we had added $100 \mu M$ sulfide. The ratio had values above 1 when no sulfide had been added (Fig. 5B), shifted to values below 1 when the sulfide was added and recovered values above 1 after 15 min N_2 bubbling and acid (1:100 of 6N H_2SO_4) addition.

Thus, we suggest the routine incorporation of the measurement of the absorbance at 350 nm when sampling areas where sulfide could be in solution. In case sulfide has been simultaneously measured, equation (1) can be used to recover the correct phosphorus concentration. If sulfide has not been measured, but one part of the sample has been saved for later measurements, looking at the index would indicate the need for repeating the measurement after the proper measures to get rid of the sulfide have been taken. A final use of the index would indicate whether the correction had been successful.

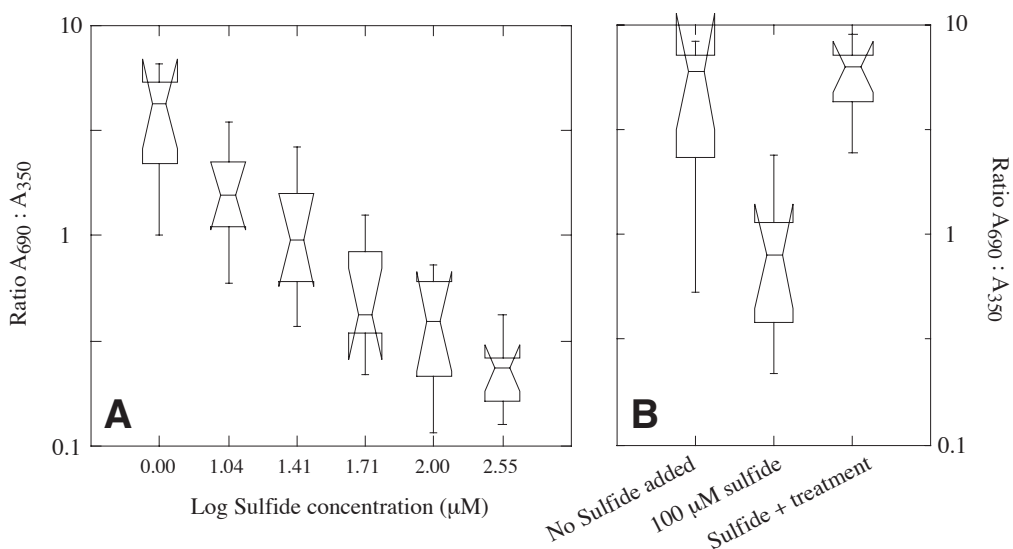


FIG. 5. – A) Values of the ratio $A_{690}:A_{350}$ for different sulfide concentrations. Each box-and-whisker plot (see Wilkinson 1989) correspond to several series of phosphorus concentrations ranging from 0 to $1 \text{ mgP-PO}_4 \text{ l}^{-1}$ (10 samples per series) and having a fixed sulfide concentration. B) Values of the same ratio for other series of phosphorus concentrations, without sulfide additions, with the addition of $100 \mu M$ of sulfide, or with the addition of sulfide plus treatment with nitrogen bubbling and acidification.

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