IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 15, Issue 5 Ser. I (May 2021), PP 30-36
www.iosrjournals.org

Spectrofluorometric Procedure for Monitoring Formaldehyde In Aquaculture

Genilson Silva de Jesus, Monyque Palagano da Rocha, Herbert Lee Barbosa Veríssimo de Barros and Heberth Juliano Vieira

Federal University of Grande Dourados, Faculty of Exact Sciences and Technology, Rua João Rosa Góes, 1761 - Vila Progresso, Dourados, Mato Grosso do Sul, Brazil, CEP: 79825-070

Abstract:
Background*: Formaldehyde is an important chemical used in fish farming tanks for the control of parasites in fish. The main problem is that extensive use can lead to the dumping of the substance in the waters of rivers. In this context, it is extremely important to monitor this substance in the waters of fish-growing tanks. This work seeks to quantify the formaldehyde content in fish farm waters in the city of Dourados, Mato Grosso do Sul State, Brazil.*
Materials and Methods*: The emission spectra and excitation-emission matrix were obtained with spectrofluorometer (Cary Eclipse, Varian Inc.) equipped with a quartz bucket with four polished faces. In this work, the formaldehyde was reacted with Fluoral-P producing 3,5-diacetyl-1,4-dihydrolutidine. The fluorophore produced was monitored at 510 nm, after being excited at 410 nm.*
Results*: The proposed spectrofluorimetric procedure allowed the quantification of formaldehyde in water samples from fish-growing tanks with recovery ranging from 95 to 110%. It was found that the formaldehyde concentration found was not statistically different between the entry, tank and exit sampling points (ANOVA, $F_{calculated} < F_{crítical}$, 95% confidence). The formaldehyde concentration in the samples analyzed ranged from 2.2 to 5.2 µg/L.*
Conclusion: *The analytical procedure was used to quantify formaldehyde in water samples from fish growing tanks. The spectrofluorometric procedure is easy, simple, and accurate.*
Key Word*: Monitoring; Toxicity; Analytical procedure; Surface water; Carcinogenicity.*

Date of Submission: 30-04-2021 Date of Acceptance: 14-05-2021

I. Introduction

The increased demand for food is intrinsically linked to the significant increase in the world's population. Among the main sources of proteins consumed are those of animal origin, which have high nutritional and caloric value.

In this context, pisciculture emerges as one of the most healthy and viable food source alternatives for offerings in the coming years, as a result of the stagnation of fish production in the capture production[1]. It is possible to analyze this behavior when we observe the growth of each modality. In the period from 2015 to 2018 the capture modality obtained a stagnant production of about 220,000 tons (live weight), while aquaculture increased by 9.5 % in thesameperiod, reaching a productivity of about 520,000 tons[1].

Like other crops, pathogens and parasites impair the production of fingerlings promoting mortality causing economic damage[2]. There are several substances used in the elimination or control of these microorganisms, such as iodine, chlorine and benzalkonium chloride[3-4]. Formaldehyde has been used in the removal of parasites using the immersion of organisms[5], or added directly to the fishpond[3,4,6,7]. The immersion of the individual the solution can vary from 5 to 60 minutes depending on the applied concentration of formaldehyde[8]. After this exposure, the individual returns to the tank. Another form of treatment is the addition of formaldehyde directly to the tanks with the finalidade of parasitic control, in order to avoid the flowering of phytoplankton[4]. Both strategies can be applied to other chemicals in order to increase efficiency in asepsis, such as oxytetracycline and malachite green[9]. With this, fish farming tanks can be an emission point of chemical substances with high toxicity to river water, being of great importance the monitoring of these chemical species.

However, different studies have shown a concern about the increased use of formaldehyde[10]. Although few studies related to the effect of chronic exposure of formaldehyde the substance is considered possibly carcinogenic due to its mutagenic reaction with peptic bonds for protein formation[10,11].

Chromatographic procedures[12] are described for the quantification of formaldehyde in water samples using solid phase extraction[13,14,15], or liquid phase extraction as a sample preparation stage[14]. UETA et al. (2015)[16] proposed a procedure for the determination of formaldehyde in waters using capillarity extraction using

dynamic extraction and purge-and-trap methods. These procedures are generally expensive, time-consuming and require qualified analyst.

The analytical methods that employ molecular spectroscopy are more economically accessible, are simple, do not require complex sample treatment and allow low detection limit. These analytical procedures are adequate for monitoring polluting chemical species. Analytical procedures have been described for the determination of formaldehyde in samples and waters employing 3,4-diaminoanisole[17], 4-amino-3-penten-2-one[18] as spectrofluorimetric reagents. A flow injection analysis (FIA) procedure with spectrofluorimetric detection was proposed for determination and formaldehyde using acetoacetanilide and ammonia as a reagent for fluorophore formation. The procedure was applied in several environmental matrices[19].

In this work, we describe the use of a bench-top spectrofluorimetric procedure for the determination of formaldehyde in psiculture waters using Fluoral-P as a fluorophore reagent.

Experimental Part

instrumentation

The emission spectra were obtained in a spectrofluorometer (Cary Eclipse, Varian Inc.) equipped with a quartz bucket with four polished faces, optical path of 10 mm and internal volume of 3.5 ml. The equipment has a pulsed xenon lamp and a photomultiplier tube as a detector, coupled to an angle of 90°C of the source and detection cell.

Reagents and Solutions

The solutions were prepared with deionized water obtained by the Gehaka purification system, model OS10LXE (18.3 MΩ×cm at 25° C).

The reagents to glacial acetic acid, ammonium acetate, acetylacetone and formaldehyde were analytical in grade and used without additional purification. The Fluoral-P reagent solution was prepared by adding 300 μL of glacial acetic acid, 15.43 g of ammonium acetate and 200 μL of acetylacetone, in a 100 ml volumetric flask, completing the volume with deionized water[18]. The Fluoral-P reagent solution was stable for 30 days when stored at 4°C.

Preparation of fish water samples

The water samples of pisciculture were collected in six different days, in two aquaculture crops from different localities. The containers for storing the samples were previously cleaned with cationic surfactant, rinsed with tap water shortly after deionized water then washed with methanol. They were then packed in the greenhouse for 24 h to 200°C. The samples collected were filtered using a PVDF syringe filter (0.45 μm) and conditioned to a refrigerator at 2°C.

Spectrofluorometric procedure for the determination of formaldehyde in fish water samples

The procedure for the determination of formaldehyde was performed by adding 400 μL of sample solution containing formaldehyde and 4.00 ml of Fluoral-P solution in a 5.0 ml volumetric flask, completing the volume with desionized water. In this study, the emission intensity was obtained at 510 nm, while the solution was excited at 410 nm, after 90 min of reaction. The standard solutions containing formaldehyde to obtain the analytical curve were obtained by adequately dilution of the formaldehyde stock solution in 5.0 ml volumetric flask. The final concentrations of the solutions used to obtain the analytical curve ranged from 2.4 μg/l to 30 μg/l. Emission spectra of the solutions between 250 and 750 nm were obtained, with excitation wavelength of 410 nm. The emission intensities of the standard solutions at 510 nm were used to construct the analytical curve. Formaldehyde concentrations were obtained by interpolation, using the analytical curve obtained on the same day.

II. Results and Discussions

Preliminary studies

The spectrofluorometric method employed in this procedure was based on the reaction of formaldehyde with 2,4-pentanodione and concentrated ammonium acetate. The fluorophore 3,5-diacetyl-2,6-dihydrolutidine obtained exhibits fluorescence at 510 nm, when excited at 410 nm[20].

Initially, the spectroscopic characterization of the product of the formaldehyde reaction using Fluoral-P was performed. In this study, the excitation-emission matrix of a standard formaldehyde solution 20 μg/l was obtained. The excitation-emission matrix, presented in Figure 1, presents different emission spectra of the analyzed solution, obtained sequentially at different excitation wavelengths. The result of the overlap of this emission spectra was the contour plot, in which the signal modulated by the equipment indicates to both the radiation range absorbed by the solution $\lambda_{(excitation),}$ as well as the radiation range emitted by the solution $\lambda_{(emission).}$ The Figure 1 shows two excitation radiation ranges obtained of formaldehyde solution 20 μg/l (330 to

375 nm) and another that presents higher radiation absorption ranging from (360 to 450 nm). The wavelength range with the highest radiation emission is between 480 and 570 nm.

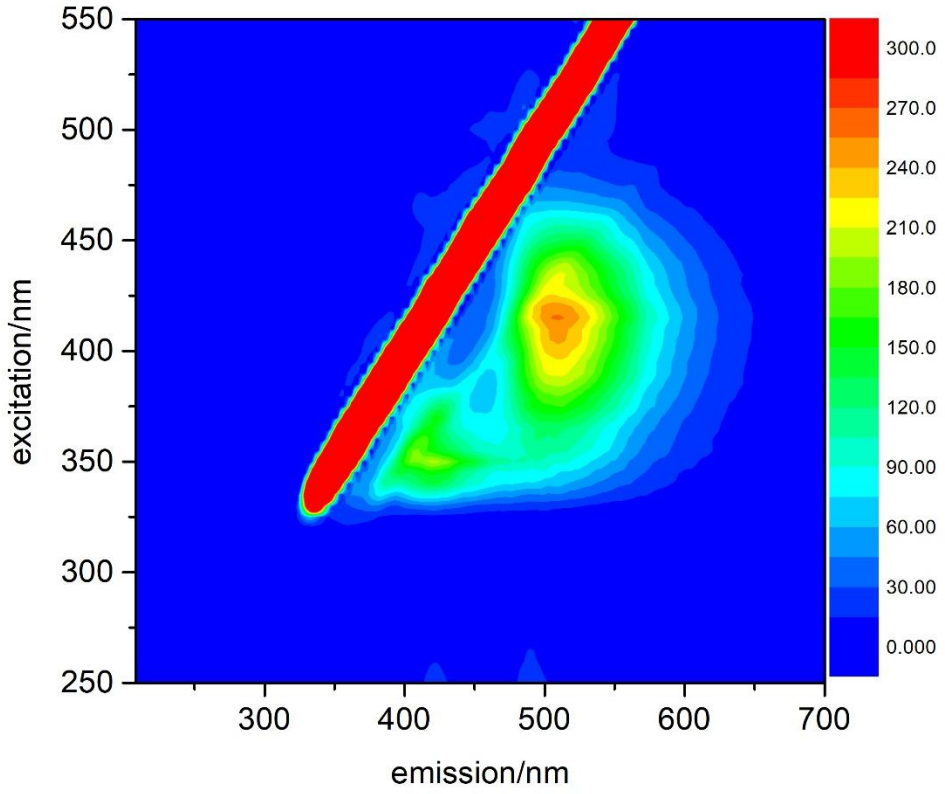

Figure 1: Excitation emission matrix of a standard formaldehyde solution 20 µg/l reacted with Fluoral-P. Excitation slit = 10 nm; emission slit =10 nm. PMT voltage= 700 V.

Fluorophore stability

The stability of the fluorophore produced by the reaction between formaldehyde and Fluoral-P was evaluated monitoring the solution containing the mixture between Fluoral-P and formaldehyde 3.6 µg/l and for 90 min. The behavior of the fluorophore formed was showed in the Figure 2. In this study, a constant increase in radiation emission intensity at 510 nm up to 80 min reaction. After 90 min of reaction, a constant radiation emission with intensity variation of less than 1.5% was achieved. This result is in accordance with that found by COMPTON and PURDY (1990)[20].

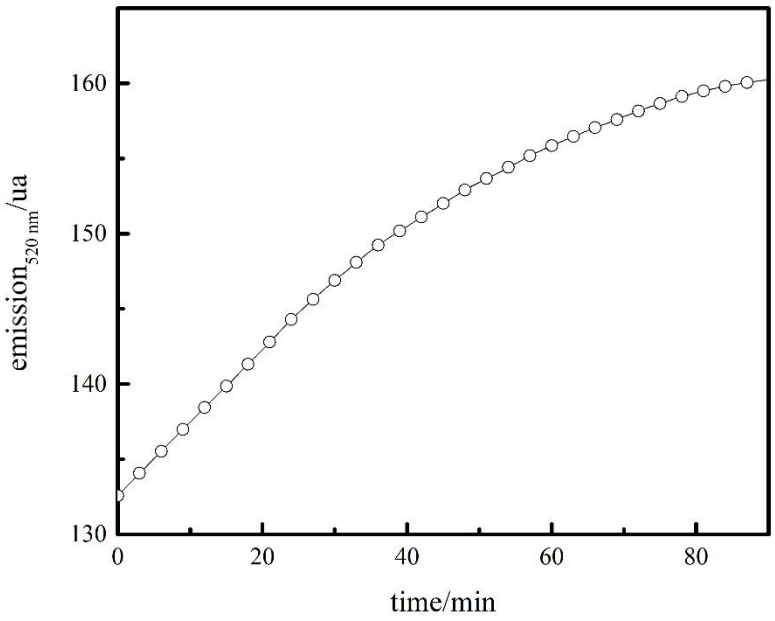

Figure 2: Evaluation of fluorophore stability formed between formaldehyde 3.6 µg/l and Fluoral-P reagent.

With this, the parameters set for the spectrofluorimetric determination of formaldehyde using Fluoral-P are presented in Table 1.

Table 1: Parameters used for the determination of formaldehyde using the spectrofluorimetric procedure.

λ excitation	410 nm
λ emission	510 nm
excitation/emission slits	10/10 nm
Detector voltage	700 V
Reaction time	90 min

Calibration curve

The Figure 3 shows the emission spectra of the standard formaldehyde solutions obtained for the construction of the calibration curve. The calibration curve was constructed with the emission intensity values at 510 nm of the emission spectra obtained and presented in the Figure 4.

The calibration curve of the spectrofluorimetric procedure proposed for determination of formaldehyde in pisciculture waters presented a linear regression between emission intensity and the formaldehyde concentration range between 2.4 µg/l and 30 µg/l. The calibration curve obtained, presented in Figure 4, can be represented by regression equation I=4.0 (±0.06) × [formaldehyde] – 2.27 (±0.89); r = 0.9998, where I is emission intensity and [formaldehyde] is the formaldehyde concentration in µg/l. The limit of detection (LD) and the limit of quantification (LQ) of the procedure were estimated using the standard deviation of the linear coefficient (s_b) of the regression equation of the analytical curve[21]. The obtained detection and quantification limits were 2.0 µg/l (3× s b) and 3.5 µg/l(10× sb), respectively. The precision in the determining a standard solution of formaldehyde 5 µg/l was less than 2.0% (n=10).

The residual graph can provide important information for the validation of the regression model obtained from the analytical curve of the proposed spectrofluorimetric procedure[22]. The residuals obtained from the regression equation of the analytical curve are randomly dispersed along the horizontal axis,shown in Figure 4 (right). This behavior indicates that the proposed procedure for the determination and formaldehyde does not present systematic error.

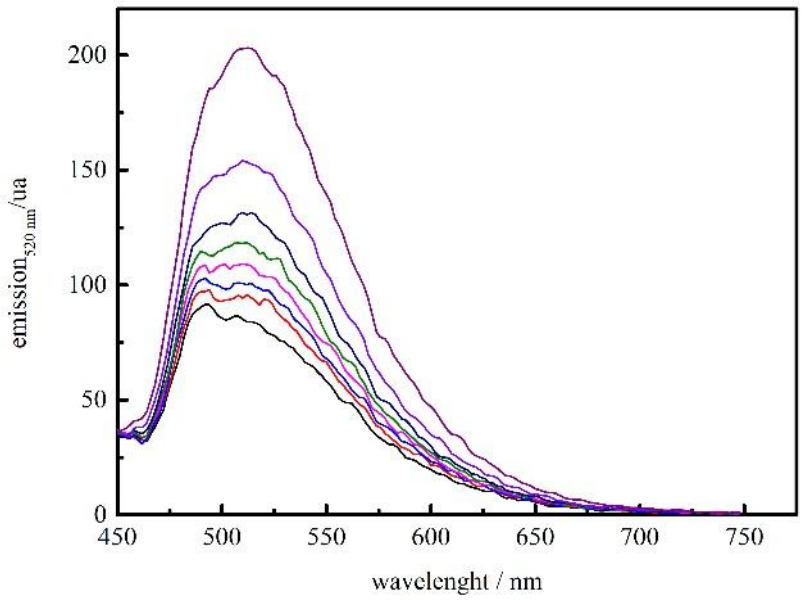

Figure 3: Emission spectra of standard formaldehyde solutions at concentrations (µg/l). (a) white; (b) 2.40 (c) 4.80; (d) 7.21; (e) 9.61; (f) 12.0; (g) 18.0 and (h) 30.0. The conditions of obtaining are described in Table 1.

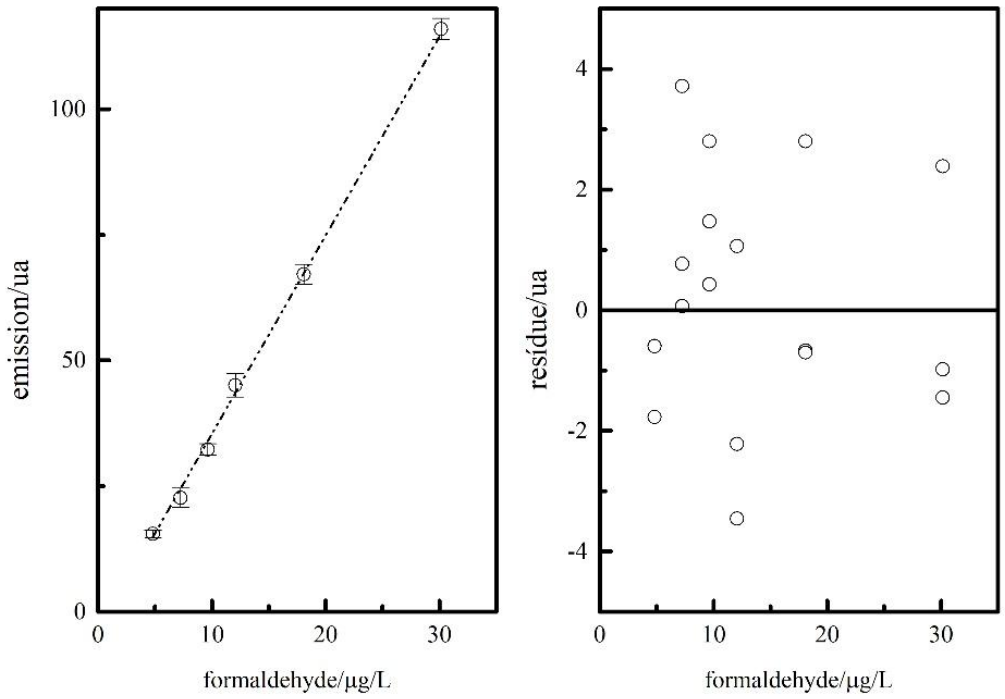

Figure 4: Analytical curve for determination and formaldehyde using the proposed spectrofluorimetric procedure (left); Residual graph obtained from the analytical curve (right).

The precision of the calibration curve of the spectrofluorimetric procedure for the determination of formaldehyde was evaluated. The procedure showed an excellent intra-day and inter-day precision ranged from 1.2 to 8.0% (CV%), indicating a robustness of the proposed spectrofluorimetric procedure.

Formaldehyde recovery in fish water samples

After optimized the spectrofluorimetric procedure for determination and formaldehyde in samples and psiculture waters, the effect of the sample matrix on quantification of formaldehyde in samples of psiculture water was evaluated. In this study, aliquots of standard formaldehyde solution were added to the water samples and the amount of formaldehyde added was determined using the proposed procedure. The concentrations added were 3.0, 5.0 and 20 μg/l. The formaldehyde recoveries in the evaluated samples were from 95 to 110% (n=3), indicating no interference of the sample matrix in the determination of formaldehyde in the evaluated samples.

The interference caused by acetaldehyde in the determination of formaldehyde was evaluated in the study by ANDRADE et al. (1996)[18]. The authors were founded that acetaldehyde does not cause interference at a concentration 1000 times higher than the formaldehyde concentration. SUGAYA et al. (2001)[23] determined aldehydes in waters finding formaldehyde at a concentration of up to 59 μg/l, while the concentration of acetaldehyde found was up to 260 μg/l. In the work of KIM et al. (2011)[24], the concentration of formaldehyde in water samples found ranged from 2.7 to 117 μg/l, while the concentration of acetaldehyde determined was up to 11.9 μg/l. Thus, it is evident that the concentration of acetaldehyde generally found in water samples is not an important interfering factor in the determination of formaldehyde in waters in analytical procedures that employs Fluoral-P as a derivatizing reagent.

The recoveries obtained in our study were higher than those obtained by COTSARIS and NICHOLSON (1993) in the work in which was employed 2,4-diffenilhydrazine as derivatizing reagent and quantification using HPLC[13]. The recoveries obtained in this study were higher than those obtained by GIROUSI et al. (1997), in which it used 3,4-diaminoanisole as a derivatization reagent[17]. About the detection limit, the work of JONES et al. (1999)[15] the proposed HPLC procedure presented a higher detection limit than the LD obtained in this study

Determination of formaldehyde in fish water samples

The spectrofluorimetric procedure using Fluoral-P was applied in the determination of residual formaldehyde in samples of water from pisciculture collected in two localities. In each fish farm, the sampling was carried out on 3 different breeding tanks (source, tank and effluent), collected on different days. The results of formaldehyde concentrations found in the samples collected are shown in Figure 5.

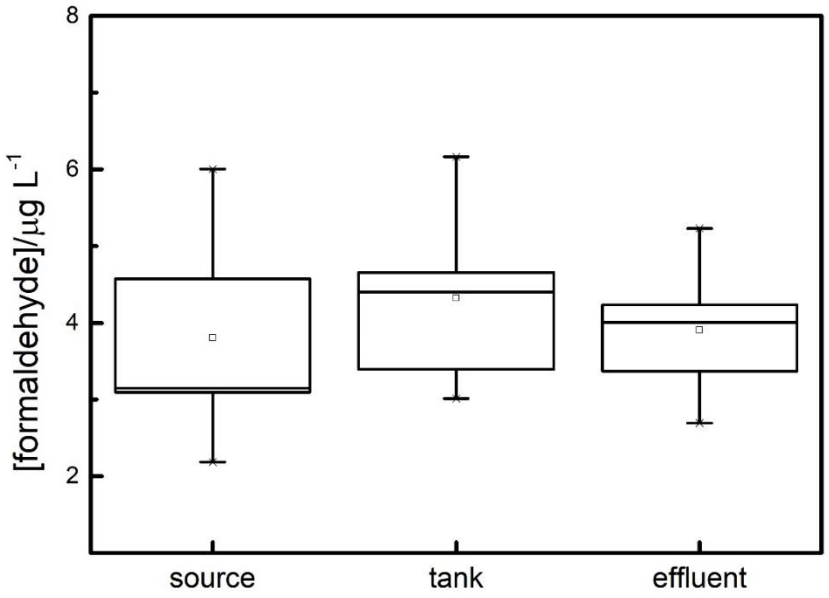

Figure 5. Box-plot with formaldehyde concentrations determined in water samples of fish farm with proposed spectrofluorimetric procedure.

It can be verified that the spectrofluorometric procedures allowed the quantification of formaldehyde in the samples of fish-growing waters in the analyzed samples. The procedure allowed the determination of formaldehyde in 30 samples per hour. The formaldehyde concentrations found were higher than the limit of quantification, but no statistical difference was observed between the samples when the analysis of variance was

performed single factor (Fcalculated=0.24) at the confidence level of 95% (Fcritical [1;12; α=0.05]=4.47). The low formaldehyde concentration found in the analyzed samples is probably due to the period in which the tanks were left without formaldehyde treatment. Another possibility is the use of other chemicals in the management of these tanks.

III. Conclusion

The procedure developed presented be simple, sensitive, and low cost, thus can be applied in monitoring of formaldehyde in fish farm. The procedure is safe for the analyst and requires only filtration as sample preparation. The procedure presents accuracy and adequate detection limit for monitoring the formaldehyde concentration in samples of pisciculture waters.

[1]. ORGANIZATION, A. State of the World Fisheries and Aquaculture 2014. [S.l.]: Food & Agriculture Org, 2020.
[2]. LEAL, J. F. et al. Use of formalin in intensive aquaculture: properties, application and effects on fish and water quality. Reviews in Aquaculture, v. 10, p. 281–295, Jun. 2016.
[3]. CENGIZLER, İ.; AYTAÇ, N.; ALDIK, R.U. H.Y. Biocide use and its potential risks in aquaculture in Turkey. Eastern Anatolian Journal of Science, v. 3, p. 1–5, 2017.
[4]. TONGUTHAI, K. The use of chemicals in aquaculture in Thailand. Use of Chemicals in Aquaculture in Asia: Proceedings of the Meeting on the Use of Chemicals in Aquaculture in Asia 20-22 May 1996, Tigbauan, Iloilo, Philippines. [S.l.]: [s.n.]. 2000. p. 207–220.
[5]. DA SILVA, A. L. et al. Use of sodium chloride, formalin and the association of these products in the control of ectoparasites in Tilapia larvae (Oreochromis niloticus). Bulletin of the Fisheries Institute, v. 35, p. 597-608, 2018.
[6]. FRANCIS-FLOYD, R. Use of formalin to control fish parasites. [S.l.]: University of Florida Cooperative Extension Service, Institute of Food and ..., 1996.
[7]. MACEDO, C.F.; SIPAÚBA-TAVARES, L. H. Eutrophication and water quality in fish farming: consequences and recommendations. Bulletin of the Institute of Fisheries, v. 36, p. 149–163, 2018.
[8]. PAHOR-FILHO, E.; MIRANDA-FILHO, K.C.; JR, J. P. Parasitology of juvenile mullet (Mugil liza) and effect of formaldehyde on parasites and host. Aquaculture, v. 354, p. 111–116, 2012.
[9]. LUVIZOTTO-SANTOS, R. et al. The use of pesticides in fish farms and fishing grounds located in the Mogi-Guaçu river basin. Bulletin of the Institute of Fisheries, v. 35, p. 343–358, 2018.
[10]. ORGANIZATION, W. H.; Others, i'M SORRY. IARC monographs on the evaluation of carcinogenic risks to humans. Volume 88: Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol. IARC monographs on the evaluation of carcinogenic risks to humans. Volume 88: Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol. , 2004.
[11]. HOQUE, M. S. et al. Evaluation of artificially contaminated fish with formaldehyde under laboratory conditions and exposure assessment in freshwater fish in Southern Bangladesh. Chemosphere, v. 195, p. 702–712, 2018.
[12]. WHITTLE, P. J.; RENNIE, P. J. Determination of formaldehyde in river water by high-performance liquid chromatography. Analyst, v. 113, p. 665–666, 1988.
[13]. COTSARIS, E.; NICHOLSON, B.C. Low level determination of formaldehyde in water by high-performance liquid chromatography. Analyst, v. 118, p. 265–268, 1993.
[14]. VELIKONJA, Š. et al. Comparison of gas chromatographic and spectrophotometric techniques for the determination of formaldehyde in water. Journal of Chromatography A, v. 704, p. 449–454, Jun. 1995.
[15]. JONES, S.B. et al. Determination of Submicromolar Concentrations of Formaldehyde by Liquid Chromatography. Analytical Chemistry, v. 71, p. 4030-4033, 1999. Available at: <https://doi.org/10.1021/ac990266s>.
[16]. UETA, I. et al. Determination of formaldehyde in aqueous samples with a miniaturized extraction capillary coupled to high-performance liquid chromatography. Analytical Sciences, v. 31, p. 99–103, 2015.
[17]. GIROUSI, S. T. et al. Fluorometric determination of formaldehyde. Fresenius' journal of analytical chemistry,v. 358, p. 667–668, 1997.
[18]. DE ANDRADE, J.B. et al. Spectrofluorimetric determination of formaldehyde in liquid samples. American laboratory (Fairfield),v. 28, p. 56–58, 1996.
[19]. LI, Q.; OSHIMA, M.; MOTOMIZU, S. Flow-injection spectrofluorometric determination of trace amounts of formaldehyde in water after derivatization with acetoacetanilide. Talanta, v. 72, p. 1675–1680, 2007.
[20]. COMPTON, B.J.; PURDY, W.C. The mechanism of the reaction of the Nash and the Sawicki aldehyde reagent. Canadian Journal of Chemistry, v. 58, p. 2207–2211, 1980.
[21]. MILLER, J. N.; MILLER, J.C. Statistics and Chemometrics for Analytical Chemistry. [S.l.]: Pearson/Prentice Hall, 2005. ISBN ISBN: 9780131291928LCCN: 2004060168. Available at: <https://books.google.com.br/books?id=Efx77dxOC3sC>.
[22]. LOCO, J. V. et al. Linearity of calibration curves: use and misuse of the correlation coefficient. Accreditation and Quality Assurance, v. 7, p. 281–285, Jul. 2002.
[23]. SUGAYA, N. et al. Analysis of Aldehydes in Water by Head Space-GC/MS. Journal of Health Science,v. 47, p. 21–27, 2001.
[24]. KIM, H.-J.; SHIN, H.-S. Simple and automatic determination of aldehydes and acetone in water by headspace solid-phase microextraction and gas chromatography-mass spectrometry. Journal of Separation Science, v. 34, p. 693–699, Feb. 2011.

Genilson Silva de Jesus, et. al. "Spectrofluorometric Procedure for Monitoring Formaldehyde In Aquaculture." *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 14(5), (2021): pp 30-36.