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Methodological Comparison of Field And Laboratory Sample Processing for Chlorophyll-A Measurement in Two Tropical Resevoir

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Abstract
Chlorophyll-a is widely used as an indicator of phytoplankton biomass and trophic condition. However, limited information is available on how sample volume and processing location affect chlorophyll-a measurements in tropical reservoirs. This study compared three sampling procedures in Cirata and Jatigede Reservoirs, West Java, Indonesia. The method compared a 1-L sample concentrated in the laboratory (1NF), a 1-L sample concentrated in the field using a 25-µm plankton net (1PN), and a 10-L sample concentrated in the field using the same net (10PN). Chlorophyll-a was extracted using 90% acetone and measured by UV–Vis spectrophotometry. In Cirata Reservoir, chlorophyll-a concentrations were 5.470, 3.333, and 1.228 µg/L for 1NF, 1PN, and 10PN, respectively. In Jatigede Reservoir, the corresponding values were 19.380, 24.967, and 4.370 µg/L. The 10PN treatment produced the lowest chlorophyll-a values in both reservoirs, while the response of the 1PN treatment differed between reservoirs. These findings indicate that sample volume and processing location may influence chlorophyll-a estimates. Further validation using a standard direct-filtration method and adequate replication is needed before one procedure can be recommended as the most accurate.

Keywords: Cirata reservoir, Jatigede Reservoir, Plankton net, Technique sampling, Trophic status.

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1. Introduction

Assessing the health and sustainability of aquatic ecosystems involves evaluation of trophic status, which reflects nutrient availability and productivity. Aquatic trophic status refers to the level of nutrient and biological productivity in a water body, classified from oligotrophic (low productivity) to eutrophic (high productivity). The trophic status of reservoirs with eutrophic levels is characterized by excess nutrients that cause algae blooms, decreased oxygen levels, and deteriorated water quality. The reservoirs that are already eutrophic in Indonesia are the Jatigede and the Cirata reservoirs (Herawati *et al*., 2022; Pratiwi *et al*., 2020; Rosadi *et al*., 2020). Both reservoirs serve multiple functions, including water supply, flood control, aquaculture, and

hydropower generation, making the assessment and monitoring of their trophic conditions important for sustainable reservoir management.

The input of organic matter into reservoir waters may affect water quality and the abundance of phytoplankton (Khan *et al*., 2021). Phytoplankton is photosynthetic microorganisms with chlorophyll pigments that absorb sunlight for photosynthesis (Khan *et al*., 2021; Zhang *et al*., 2021). Chlorophyll-a (chl-a) is widely used as an indicator of phytoplankton biomass and water quality and is an important parameter for assessing trophic status and eutrophication in aquatic ecosystems (Girdner *et al*., 2020; Hu *et al*., 2014; Rosadi *et al*., 2020). Therefore, reliable chl-a measurements are essential for monitoring changes in phytoplankton biomass, evaluating eutrophication, and supporting reservoir management.

However, the interpretation of chl-a measurements can be affected by spatial and temporal variability, environmental conditions, phytoplankton community composition, and sampling procedures (Girdner *et al*., 2020; Hu *et al*., 2014). In particular, the volume of water collected and the timing and location of sample concentration may influence chl-a recovery. Larger sample volumes may increase the amount of phytoplankton collected, whereas differences between immediate field concentration and subsequent laboratory concentration may affect sample handling and pigment recovery. The use of plankton nets for pre-concentration may also selectively retain phytoplankton according to cell size, potentially affecting the measured chl-a concentration.

Despite the importance of chl-a for trophic-status assessment, the effects of sample volume and field versus laboratory concentration procedures on chl-a measurements in tropical reservoirs remain insufficiently understood. Previous studies have largely focused on the use of chl-a as an indicator of water quality and trophic status, whereas less attention has been given to how alternative sampling and sample-concentration procedures may influence chl-a recovery. This methodological uncertainty is particularly relevant for tropical reservoirs, where differences in phytoplankton communities and environmental conditions may affect the performance of sampling procedures. Therefore, evaluating alternative sampling and concentration approaches is important to determine whether differences in sample volume and processing conditions can influence measured chl-a concentrations and their subsequent interpretation.

The study aims evaluate alternative chlorophyll-a sampling and concentration methods by comparing different sample volumes and sample-processing locations in Cirata and Jatigede Reservoirs, Indonesia. The accurate measurements of chl-a are crucial to ensure reliable and useful information for aquatic management. Therefore, it is important to develop and apply targeted methods for chl-a measurement to enhance the understanding and effective management of aquatic ecosystems.

2. Methods

2.1 Study Area and Sampling Locations

The research was carried out in the Jatigede and Cirata Reservoir at West Java, Indonesia. Cirata and Jatigede are two major reservoirs in West Java, Indonesia, but they differ in their morphometric characteristics. Jatigede Reservoir located in Jatigede District, Sumedang Regency serving a dual purpose of irrigating agriculture and electrical hydropower. Jatigede Reservoir, located on the Cimanuk River, has a surface area of approximately 4,122 ha (41.22 km^2), a storage capacity of approximately 980 million m^3, a mean depth of approximately 23.8 m, and a maximum depth of approximately 95 m based on its design elevation range (Kementerian PU, 2016). Cirata Reservoir located in Purwakarta, Cianjur, and West Bandung regencies, West Java, is a multipurpose facility that functions as a hydropower plant, flood control, and irrigation agriculture. Cirata Reservoir is located along the Citarum River and has a maximum surface area of approximately 6,200 ha (62.0 km^2), a maximum storage volume of approximately 2,165 million

m³, a mean depth of 34.9 m, and a maximum reported depth of approximately 106 m (Purnamaningtyas and Tjahjo, 2008).

Sampling sites were determined using a purposive sampling method and selected to represent different areas of both reservoirs. The sampling locations were established in open-water areas adjacent to floating net cages (KJA), where water conditions could be assessed in relation to aquaculture activities. In the Jatigede Reservoir, sampling sites were located at coordinates S 06° 55' 02"–E 108° 05' 47" (JTG 2), S 06° 53' 53"–E 108° 07' 18" (JTG 3), and S 06° 51' 51"–E 108° 04' 30" (JTG 6). Meanwhile, sampling sites in the Cirata Reservoir were located at S 06° 43' 18.21"–E 107° 19' 44.34" (CRT N), S 06° 44' 28.04"–E 107° 17' 04.05" (CRT Q), S 06° 41' 34.31"–E 107° 20' 49.53" (CRT M), and S 06° 42' 31"–E 107° 20' 08" (CRT R). Figure 1 shows the sampling locations in the Jatigede and Cirata reservoirs.

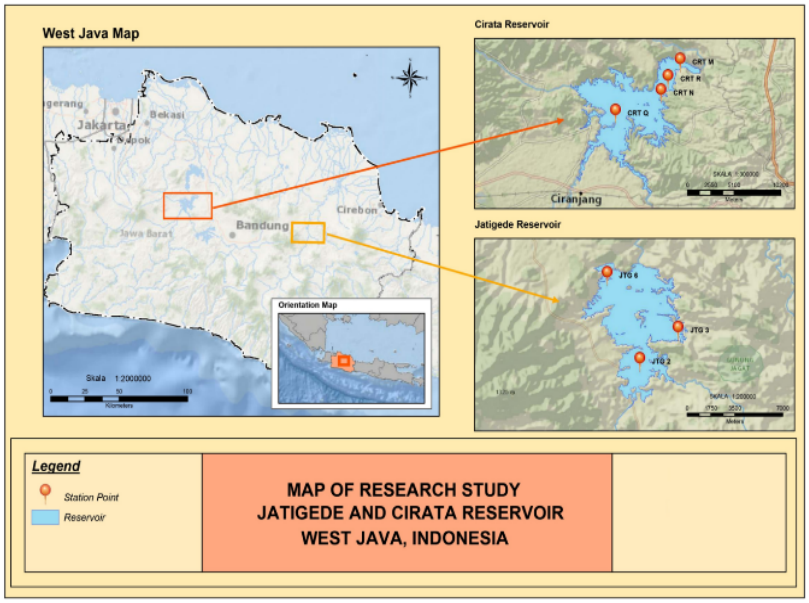


Figure 1. Research location sampling in Cirata and Jatigede Reservoir, West Java, Indonesia

2.2 Water Procedure

Water sampling was conducted in August 2023, during the dry season, to characterize water-quality conditions under relatively low-rainfall conditions. However, the results represent only the sampling period and may not reflect seasonal variations throughout the year. The physicochemical parameters used were temperature, pH, turbidity, transparency, total dissolved solid (TDS). In situ water temperature was measured using a Lutron series portable (DO-5510, Taiwan). pH and turbidity were measured using a Lutron series portable pH meter (Ph-207, Taiwan) and a portable turbidity meter (CD-4301, Taiwan). Transparency was measured using a Secchi disk. The total dissoloved solid (TDS) were separated by filtering the water through a 0.45 µm filter paper.

Water samples were collected using the grab sampling method at each sampling site. The samples were collected directly from the water surface at each sampling point. Three replicate samples were collected at each sampling point. Following collection, the water samples were transported to the laboratory for water quality analysis. The samples were stored in sample bottles and kept in a cool box during transportation. Upon arrival at the laboratory, the samples were stored at approximately 4°C until analysis.

Three chlorophyll-a sampling treatments were used to evaluate the effect of sample volume and the timing of concentration on chlorophyll-a recovery: (1) 1-L laboratory concentration (1NF), (2) 1-L field concentration (1PN), and (3) 10-L field concentration (10PN). For the 1NF treatment, a 1-L water sample was collected in the field, transported to the laboratory under cooled conditions, and subsequently concentrated using a 25-µm mesh plankton net. For the 1PN treatment, a 1-L water sample was concentrated immediately in the field using the same 25-µm mesh plankton net. Thus, the 1NF and 1PN treatments used the same sample volume and mesh size but differed in the

timing and location of the concentration step. The 10PN treatment used a 10-L water sample that was concentrated immediately in the field using the same plankton net, allowing the effect of a larger sample volume to be evaluated under field-concentration conditions. In all treatments, the concentrated sample (approximately 15–20 mL) retained by the plankton net was filtered through a Whatman GF/C filter paper. The filter paper used to filter the sample water was then placed in a glass bottle containing 15 ml of 90% acetone solution for the extraction process. The sample bottle was stored at 4ºC for 12-14 hours. After storage, sample was placed in a 10 ml centrifuge tube and rotated in a centrifuge at 3500 rpm for 10 minutes to clear the solution and supernatant. The sample was then poured into a cuvette and absorbed on a UV-Vis spectrophotometer at wavelengths of 630, 647, 664 and 750 nm. Chl-a concentration using formula: [SN1.1]

$$Chlorophyll\ (\frac{mg}{l}) = \frac{Ca\ \times v}{V\ \times d}$$

Where Ca is filtered chl-a, v is aseton volume 90% (Liter), d is diameter of cuvet (cm); V is water sample (Liter). T-test analysis was used to see the variation of chl-a among different stations (Parson *et al.,* 1984).

2.3 Data Analysis

Statistical analyses were conducted to evaluate differences in physicochemical water quality between reservoirs and differences in chl-a concentrations among sampling treatments. An independent-samples t-test was used to compare physicochemical water-quality parameters between Cirata and Jatigede reservoirs. For chl-a, paired-samples t-tests were used to compare the three sampling treatments (1-L laboratory concentration [1NF], 1-L field concentration [1PN], and 10-L field concentration [10PN]) within each reservoir. The paired analysis was used because the three treatments were applied to samples collected from the same sampling points. The mean and standard deviation (SD) for each parameter were calculated from all replicate data across sampling points within each reservoir, with a total of three replicates per point. Statistical significance was assessed at $p < 0.05$.

3. Results and Discussion

3.1 Water Quality Measurement

The physicochemical characteristics of Cirata and Jatigede Reservoirs are presented in Tables 1 and 2. Water temperature ranged from 26 to 30°C in Cirata Reservoir and from 27 to 30°C in Jatigede Reservoir. Both reservoirs had the same mean temperature of 28.25°C, although temperature variation was slightly higher in Cirata Reservoir. The water in both reservoirs was slightly alkaline. The mean pH was 7.86 ± 0.55 in Cirata Reservoir and 8.07 ± 0.16 in Jatigede Reservoir. Jatigede Reservoir therefore showed a slightly higher and more stable pH than Cirata Reservoir. Turbidity ranged from 4.20 to 14.35 NTU in Cirata Reservoir, with a mean of 9.71 ± 4.18 NTU. In Jatigede Reservoir, turbidity ranged from 9.80 to 14.70 NTU, with a higher mean value of 13.53 ± 3.31 NTU. Water transparency ranged from 0.16 to 1.45 m in Cirata Reservoir and from 0.88 to 0.95 m in Jatigede Reservoir. Mean transparency was 0.76 ± 0.53 m in Cirata and 0.90 ± 0.04 m in Jatigede. clearer water, while Cirata Reservoir showed greater spatial variation in transparency. Total dissolved solids ranged from 148 to 168 mg/L in Jatigede Reservoir, with a mean of 156 ± 10.58 mg/L. The TDS values in both reservoirs were reported to be below the reference value of 1,000 mg/L.

Table 1. Physicochemical water quality in Cirata Reservoir, Indonesia

Parameters	Units	Cirata Resevoir			Std*
		Min	Max	Mean±STD	
Temperature	°C	26	30	28.25±1.71	Dev 3
pH		7.14	8.31	7.86±0.55	6 - 9
Turbidity	NTU	4.2	14.35	9.71±4.18	
Transparency	m	0.16	1.45	0.765±0.53	4
TDS	mg/L	110	150	122.50±18.92	1000

Standard water quality based PP No. 22 Tahun 2021

Table 2. Physicochemical water quality in Jatigede Reservoir, Indonesia

Parameters	Units	Jatigede Resevoir			Std*
		Min	Max	Mean±STD	
Temperature	°C	27	30	28.25±1.26	Dev 3
pH		7.97	8.25	8.07±0.16	6 - 9
Turbidity	NTU	9.8	14.7	13.53±3.31	
Transparency	m	0.88	0.95	0.90±0.04	4
TDS*	mg/L	148	168	156±10.58	1000

Standard water quality based PP No. 22 Tahun 2021. Significant level *, p< 0.05

The slightly alkaline conditions in both reservoirs might be influenced by the surrounding limestone geology and photosynthesis process. Both reservoirs showed slightly alkaline water conditions, which may be influenced by the geological characteristics of their respective catchments and photosynthesis process. They receive inputs from many rivers and various anthropogenic activities, such as floating net cages, agriculture, domestic waste, and industrial waste. These waste inputs can affect water quality parameters in the reservoirs, including turbidity, transparency, and TDS.

Measuring chl-a levels can provide crucial information for determining the trophic status of a lake. The classification of trophic status in Cirata and Jatigede reservoir was classified as eutrophic status which has been established through the prevalence of phytoplankton communities and elavated concentrations of chlorophyll-a, nitrogen, and phosporus (Herawati *et al*., 2022; Pratiwi *et al*., 2020). Based on result, chl-a concentration in Cirata Reservoir using the 1NF, 1PN, and 10PN variation methods showed consecutive values of 5.470 µg/L, 3.333 µg /L, and 1.228 µg /L, respectively (Figure 2). The observed decrease in chl-a from the 1PN and 10PN variations over 1NF was 39.06 % and 77.53% higher, respectively. On the other hand, the difference between 1PN and 10PN showed only a 63.16% decreasing in chl-a concentration. The t-test results showed that the non-filtration and filtration techniques weren't significantly different. Meanwhile, there was a significant between water sampling techniques between 1 PN and 10PN.

The results of chl-a concentration in the Jatigede Reservoir with the 1NF, 1PN, 10PN methods showed 19.380 µg/L, 24.967 µg/L, and 4.370 µg/L, respectively (Figure 2). The observed increase in chl-a from the 1PN variations over 1NF was 22.38% higher. On the other hand, the difference between 1PN and 10PN showed 82.49% decrease in chl-a concentration. There was a highly significant difference between water sampling techniques between 1NF and 10PN or 1PN, but no significant difference between using 1 liter filtration in laboratory and field.

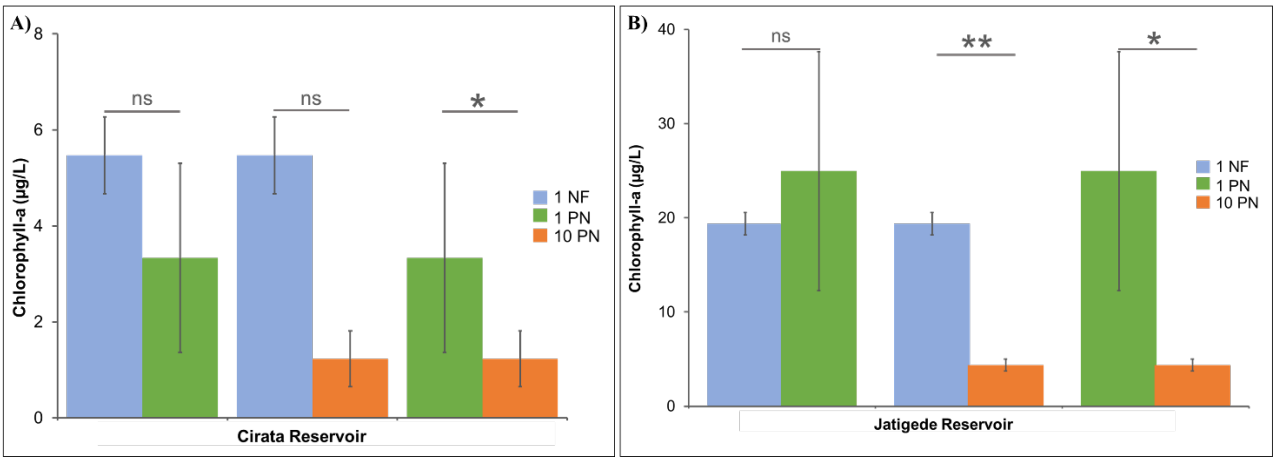


Figure 2. T-test and comparison of non-filter and filter chl-a collection methods with plankton net in Cirata Resevoir (A) and Jatigede Resevoir (B). Significance level: ns, $p>0.1$; # $p<0.1$; * $p<0.05$; **$p<0.01$. Abbreviations are defined as follow 1NF = filter 1 L in lab; 1PN = filter 1L in field; 10PN = filter 10L in field

Chl-a measurements in the Cirata Reservoir indicated that all methods water samples were classified as mesotrophic (1 to 15 µg/L). In contrast, the Jatigede Reservoir was classified as eutrophic, with chl-a concentrations exceeding 20 µg/L, except for the 10PN treatment. These findings indicate that Cirata Reservoir exhibited low chl-a concentrations during the dry season, likely due to the extensive coverage of water hyacinth on the water surface. This is consistent with the findings of Ariyani *et al* (2019), who reported relatively low chl-*a* concentrations during the dry season despite high nutrient availability, particularly in areas covered by water hyacinth. The higher Chl-a values at Jatigede suggest a greater phytoplankton biomass and potentially higher trophic status compared to Cirata, which may reflect differences in nutrient loading, water residence time, or land use in the surrounding catchment areas.

The chlorophyll-a concentrations showed different responses to the sampling treatments between the two reservoirs. Chl-a concentrations were consistently higher at Jatigede Reservoir than at Cirata Reservoir across all treatments. At the 1 NF treatment, Jatigede recorded approximately 19.380 µg/L compared to 5.470 µg/L at Cirata, while at the 1PN treatment, Jatigede yielded ~24.967 µg/L versus ~3.333 µg/L at Cirata. Both reservoirs exhibited a similar pattern, with the 10PN treatment producing the lowest chl-a concentrations. These findings suggest that increasing the sample volume does not necessarily improve chl-a estimation, particularly in eutrophic ecosystems. In contrast, the 1PN treatment showed different trends between the two reservoirs, with chl-a concentrations increasing in Jatigede Reservoir but decreasing in Cirata Reservoir. However, the differences among sampling techniques and between reservoirs were not statistically significant.The chl-a obtained from filtration with a water volume of 10 liters exhibits the lowest concentration compared to other treatments. This allows for a less accurate determination of the total chl-a concentration in the water system being studied. This indicates that using a larger sample volume does not always result in a significant increase in chl-a measurement results.

The use of a plankton net for sample pre-concentration may influence chlorophyll-a measurements because the 25-µm mesh can allow smaller phytoplankton, including some nanoplankton, to pass through the net net and potentially contribute to an underestimation of chlorophyll-a. Consequently, the measured chl-a concentration may not fully represent the total phytoplankton biomass present in the original water sample. The effect of the concentration method on chlorophyll-a recovery varied between reservoirs. In Cirata Reservoir, the 1NF method produced the highest chlorophyll-a concentration, whereas in Jatigede Reservoir, the highest concentration was observed using the 1PN method. This variation indicates that chl-a recovery

was not consistently determined by the concentration method alone. However, differences in phytoplankton community composition and cell-size distribution between reservoirs may also influence the extent of biomass retained by the net. Therefore, the observed differences among the concentration methods should be interpreted with consideration of both the methodological effect of the 25-µm mesh and the characteristics of the phytoplankton communities in each reservoir.

pH can indicate potential changes in chl-a concentrations, understanding these complexities is crucial for interpreting their relationship in lake ecosystems accurately (Kärcher *et al*., 2020). The relatively high pH in both reservoirs may be influenced by the limestone geology, which contributes to the carbonate buffering capacity of the water. In Cirata Reservoir, the surrounding Citatah Karst is composed of limestone dominated by $CaCO_3$, and previous studies have suggested that limestone dissolution and the subsequent transport of carbonate materials through runoff may contribute to increased pH in the reservoir (Wiranegara *et al*., 2023). In the Jatigede area, the Cinambo Formation includes calcareous lithologies, such as calcareous mudstone and sandstone, indicating the presence of carbonate-bearing geological materials (Rizal and Gumilar, 2019). However, geological conditions alone may not fully explain the observed pH, as biological processes, particularly phytoplankton photosynthesis, which removes dissolved CO_2 from the water and shifts the carbonate equilibrium toward higher pH. That reasons may also contribute to pH variation in reservoir waters. Therefore, the slightly alkaline conditions observed in both reservoirs likely reflect the interaction of geological and biological processes rather than a single controlling factor.

The correlation between water transparency and chl-a concentration is a key indicator for assessing water quality and overall ecosystem health in lakes and reservoirs. In this study, transparency in both Cirata and Jatigede reservoirs was relatively low, which is consistent with eutrophic conditions where high concentrations of phytoplankton and suspended particles reduce light penetration through the water column (Golubkov & Golubkov, 2024). This was further reflected by the Secchi disk depth recordings, which generally remained below 2 meters in both reservoirs.

The implications of this study for trophic-status assessment are important because chl-a is a widely used indicator of phytoplankton biomass and trophic condition in lakes and reservoirs. However, the differences in chl-a concentrations among the sampling treatments suggest that the choice of sampling and concentration procedures can influence the interpretation of trophic status. Therefore, differences in chl-a concentrations obtained using the 1NF, 1PN, and 10PN treatments could potentially lead to different trophic-status classifications, particularly when measured concentrations are close to classification thresholds. The inconsistent response among treatments between Cirata and Jatigede further indicates that trophic-status assessment should not assume a uniform effect of a given concentration procedure across different reservoir systems. Therefore, chl-a based trophic-status assessments should consider the sampling method and sample volume and, where possible, be supported by complementary indicators such as total phosphorus and water transparency. This approach may reduce the risk of misclassifying reservoir trophic status based solely on chlorophyll-a measurements (Carlson, 1977).

A limitation of this study is that the size distribution of phytoplankton passing through the 25-µm mesh was not directly quantified. Therefore, the contribution of phytoplankton size to the observed differences in chlorophyll-a recovery could not be directly determined. Future studies should consider direct filtration or finer-mesh concentration methods and assess phytoplankton size fractions to better quantify the effect of sampling procedures on chl-a recovery.

4. Conclusion

This study showed that sample volume and the location of sample concentration influenced the measured chlorophyll-a concentrations in Cirata and Jatigede Reservoirs. The 10PN treatment consistently produced the lowest values, while the 1PN treatment showed different responses between the two reservoirs. Concentrating a 1-L sample in the field using a 25-µm mesh plankton net may offer practical advantages, but its accuracy could not be confirmed in this study. Further validation using a standard direct-filtration method, adequate replication, and seasonal sampling is needed before one procedure can be recommended for routine chlorophyll-a monitoring.

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6. Conflict of Interest Declaration

The authors declare that there is no conflict of interest regarding the publication of this article. The Authors confirmed that the paper was free of plagiarism.

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C : **C**onceptualization
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