Open Journal of Applied Sciences, 2025, 15(10), 3380-3400
https://www.scirp.org/journal/ojapps
ISSN Online: 2165-3925
ISSN Print: 2165-3917

Conception of an Optimized Sequential Extraction Protocol for Studying Phosphorus Mobility in the Sediments from a Tropical Lagoon Estuary

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How to cite this paper: Abbas, H.C., Yao, M.K., Konan, A.F.K. and Drida Bi, J.-C.B. (2025) Conception of an Optimized Sequential Extraction Protocol for Studying Phosphorus Mobility in the Sediments from a Tropical Lagoon Estuary. *Open Journal of Applied Sciences*, **15**, 3380-3400.
https://doi.org/10.4236/ojapps.2025.1510218

Received: September 18, 2025
Accepted: October 28, 2025
Published: October 31, 2025

Abstract

This study aimed to develop an optimized and adapted sequential phosphorus extraction protocol for the superficial sediments from the area II of the Ébrié system. To that end, four existing protocols (Bonzongo, Hieltjes and Lijklema, Paludan and Jensen, and Azzouz) were assessed. The adapted protocol was designed based on the one that exhibited the highest seasonal recovery rates and allowed for the analysis of a greater number of phosphorus fractions. Protocol optimization was achieved by adjusting the extractant volumes from 10 to 50 mL to minimize interferences with colorimetric orthophosphate analysis. Sediment samples were collected over one year, from January to December 2024, and the mean seasonal concentrations of total phosphorus and its various fractions were measured in accordance with ISO 6878 standard. Only the Bonzongo and the Paludan and Jensen protocols showed recovery rates exceeding 80% during the rainy and cold seasons. The Paludan and Jensen protocol, offering the ability to analyze a larger number of phosphorus fractions, was therefore selected as the foundation for the optimized protocol. This new protocol achieved seasonal phosphorus recovery rates ranging from 87.5% ± 6.3% to 114.7% ± 15.5%, demonstrating high precision and notable efficiency for studying phosphorus mobility in these sandy sediments. This study highlighted the necessity of developing a sequential extraction protocol specifically tailored to the sediments from a given study area to assess the mobility of phosphorus.

Keywords

Atlantic Ocean, Eutrophication, Ébrié System, Phosphorus, Sequential Phosphorus Extraction Protocol

1. Introduction

Phosphorus is an essential element for aquatic organisms, as it's involved in numerous physiological processes. While its excessive presence in terrestrial ecosystems benefits plant growth, the same isn't true for surface waters. In fact, phosphorus is one of the key nutrients implicated in eutrophication. This ecological scourge manifests as a massive proliferation of aquatic vegetation of all kinds, leading to an ecological imbalance in these environments. This is why phosphorus, along with carbon, is one of the most studied nutrients in aquatic ecosystems [1] [2]. Sedimentary phosphorus, whether in its inorganic or organic form, plays an absolutely critical role in the eutrophication process of aquatic ecosystems. The inorganic phosphorus is recognized as the main driver of sedimentary release (or internal flux). Conversely, the study of the organic phosphorus is indispensable not only for assessing the available nutrient stock but also for evaluating the potential for delayed sediment release. Consequently, understanding these dynamics is fundamental for the modeling and sustainable management of aquatic ecosystems facing the challenges of eutrophication [3]-[5].

Understanding phosphorus mobility in sediments has led to various approaches, including phosphorus speciation. In this regard, the first historical approach was phosphorus speciation using the Chang and Jackson protocol [6]. It is a classic and pioneering method for the sequential speciation of inorganic phosphorus in soils, which was subsequently widely adapted for the study of sediments. Its primary objective is to fractionate phosphorus into different chemical forms, typically bound to specific metallic cations, to better understand their origin, mobility, and bioavailability. However, while this method is essential, it has revealed limitations over time, including:

- lack of extraction specificity: for example, ammonium fluoride can extract both Al-P and Fe-P [7];
- re-adsorption of the extracted fraction onto other sediment phases during subsequent steps, which can skew the results [7] [8];
- the absence of a step for organic phosphorus in the original version, which constitutes a limitation for a comprehensive study of the phosphorus cycle [7] [9].

For these reasons, the Chang and Jackson method has undergone numerous modifications and improvements aimed at increasing its specificity and applicability to sediments, particularly for lacustrine and marine sediments whose composition can differ from that of terrestrial soils [7]. These include the sequential extraction protocols of Bonzongo [9], Hieltjes and Lijklema [10], Paludan and Jensen [11], and Azzouz [12], which were used in this study. Unlike certain protocols, such as SEDEX [13] and Modified SEDEX [14], which are specifically designed for iron-rich sediments, these protocols are suitable for all sediment types. The comparative analysis of phosphorus sequential extraction protocols revealed that while the majority of methods allow for the estimation of organic phosphorus, each protocol offers a unique analytical perspective. The combined use of the Hieltjes and Lijklema protocol [10] and its derivative, the Bonzongo protocol [9], is essential for evaluating the influence of both the sediment's nature and the extractants on the quantification of different phosphorus forms. Furthermore, the Paludan and Jensen protocol [11] is distinguished by quantifying the highest number of phosphorus fractions, particularly the specifically bound to humic acids. Lastly, the Azzouz protocol [12] highlights the critical influence of extractant volumes on the concentration of the fractions obtained. Consequently, the combined approach utilizing these four protocols is indispensable for ensuring a complete and nuanced assessment of the objectives of this study.

The Lagoon area II, the largest natural estuary of the Ébrié system [15], has long been landlocked due to the silting of the Grand-Bassam inlet since the early 1990s. During this period, studies by Bamba *et al.* [16] and Keumean *et al.* [17] highlighted the hypereutrophication of its waters. To facilitate the flushing of pollutants carried by Comoé River (notably invasive aquatic vegetation) into Atlantic Ocean, and to support regional economic development through the establishment of a fishing port, this channel was permanently reopened in 2022. This reopening has led to a significant marine intrusion into this lagoon estuary, consequently altering its hydrochemical functioning. Drida and Yao [18] have highlighted this situation, noting an alteration of the physical and chemical characteristics of the superficial sediments, as well as a change in their metal pollution. The reopening of the inlet could also impact phosphorus mobility in these sediments, thus influencing the trophic level of the water in this lagoon ecosystem. However, data on phosphorus mobility in the superficial sediments from this lagoon area are nonexistent, both for the period of the inlet's closure and for its reopening. Thus, understanding phosphorus mobility in these sediments constitutes a significant scientific challenge, given the rich biodiversity of this ecosystem and its major socioeconomic and ecotourism roles. It is within this context that the present study was conducted. The main objective of this study is to develop an optimized and adapted sequential phosphorus extraction protocol for these sediments in order to better assess the seasonal mobility of this nutrient. To achieve this, the secondary objectives were to determine the seasonal phosphorus recovery rates using four existing protocols: those of Bonzongo [9], Hieltjes and Lijklema [10], Paludan and Jensen [11], and Azzouz [12]. This approach aims to design an adapted protocol by considering all the phosphorus forms measured by these methods, while optimizing its efficiency by adjusting the extractant volumes.

2. Materials and Methods

2.1. Presentation of the Study Area

The lagoon area II is located at the far eastern part of the the Ébrié system (**Figure**

1), specifically between 3˚40' and 3˚50' West longitudes and 5˚20' and 5˚21' North latitudes. Its mean length from East to West is 17.143 km, while its mean width is 5.714 km [19]. This lagoon ecosystem covers an area of 87 km^2 [16]. The hydrology of this lagoon area is primarily influenced by Comoé River, Mé River, and Atlantic Ocean. The influence of the latter on the zone has increased considerably with the reopening of the Grand-Bassam inlet. Its continental watershed is mainly that of Comoé River, which drains the entire Côte d'Ivoire from Northeast to South over an area of 78,000 km^2 [20], given that Mé River watershed covers only 4300 km^2 [21]. Mé River contributes to this lagoon site, as do the Aghien and Potou lagoons. The altitude of the Ébrié system in general, and the lagoon area II in particular, relative to Atlantic Ocean, favors permanent inputs of water and sediments from Comoé River towards Atlantic Ocean [18]. This has long been the cause of the silting and subsequent closure of the Grand-Bassam inlet [16].

The strong marine intrusion, caused by the reopening of the Grand-Bassam inlet, is likely to alter its mode of functioning, particularly its hydrochemistry [18]. Such a situation has already been observed by Albaret and Ecoutin [22], who noted a modification of the floristic composition and the mortality of certain macrophytes, such as *Eichhornia crassipes*, during the penultimate reopening of this pass in 1987.

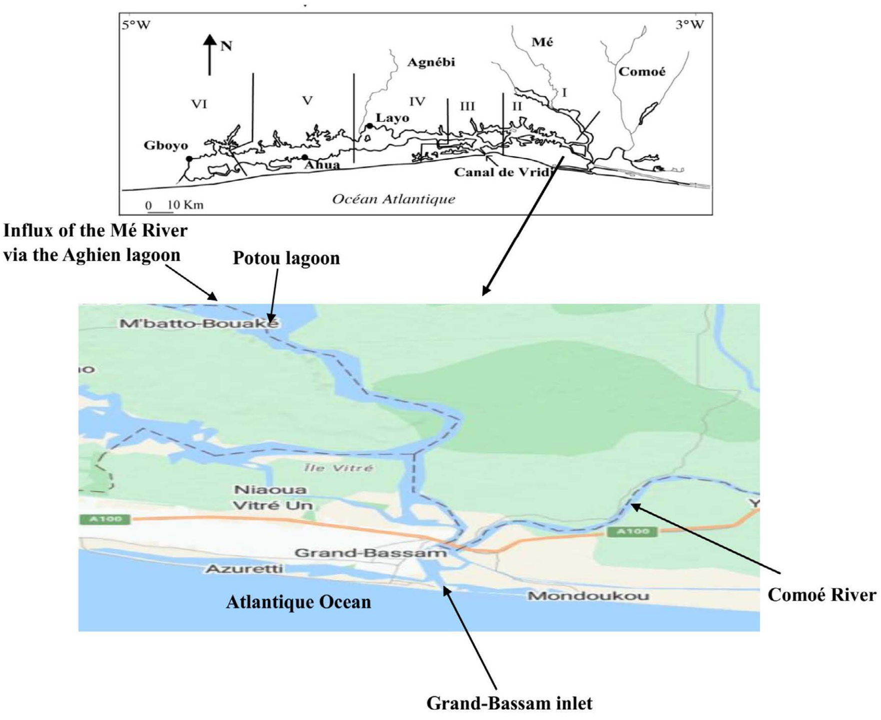


Figure 1. The lagoon area II of the Ébrié lagoon system [18].

Considering the hydrological regime of Mé River, which depends on the subequatorial climate in the South, and that of Comoé River, which is a function of the subequatorial climate in the South, the humid tropical climate in the Center, and the dry savanna tropical climate in the North [23], on the one hand, and the hydrological seasons of Atlantic Ocean off Abidjan on the other, Drida and Yao [18] defined the hydrological seasons of this lagoon ecosystem as follows: Hot Season (HS), from December to April; Rainy Season (RS), from May to July; Cold Season (SF), from August to September and; Flood Season (FS), from October to November. The continental watershed is dominated by the intensive practice of agriculture with chemical inputs, industrial and mining activities, as well as the practice of illegal gold mining [19].

Drida and Yao [18] observed that, just after the reopening of the inlet, the surface sediments were dominated by very coarse, coarse, and medium sands, originating mainly from Comoé River. A similar finding was reported by Adopo *et al.* [15] and Irié *et al.* [24] before the pass was reopened, with the latter noting that these sands were deposited in a fluviatile, beach-type environment. Their mineralogy is diverse, including the presence of monazites, kaolinites, magnetites, ilmenites, and feldspars, as reported by Irié *et al.* [24].

2.2. Sample Collection and Processing

This study spanned a full year, from January to December 2024. Samples were collected from five sampling sites. These sites are identical to those used by Mahi *et al.* [19] just prior to the reopening of the inlet, as well as those employed by Drida and Yao [18] just after this reopening (**Figure 2**).

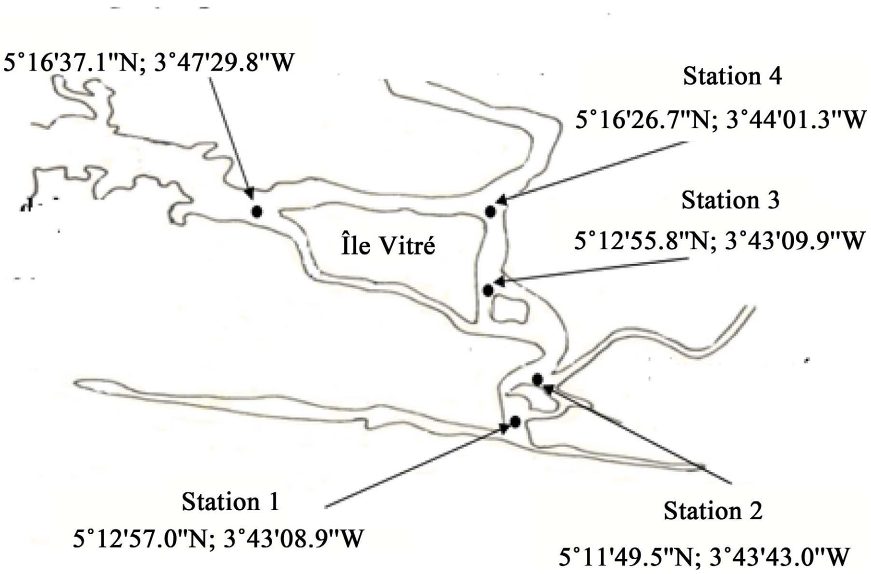


Figure 2. Geographic locations of the sampling sites used during the study period, the same as those used by Drida and Yao [18] and Mahi *et al.* [19].

Samples were collected from the study sites using a Van-Veen grab, taking the top 0 - 5 cm of sediment in strict compliance with the ISO 5667-19 standard [25]. Upon collection, the samples were immediately transferred to polyethylene bottles, as recommended by the ISO 5667-15 standard [26]. Monthly collections were conducted, yielding a total of 60 sediment samples over the entire study period.

Samples were processed in the laboratory by first removing coarse materials such as plant debris, rubble, and shells. They were then homogenized and oven-dried in accordance with the ISO 17892-1 A1 standard [27] before being ground to a particle diameter less than 0.63 µm. After processing, the samples were hermetically sealed in polyethylene bottles and stored in a moisture-free environment, as recommended by the ISO 5667-15 standard [25].

2.3. Total Phosphorus Analysis

Total phosphorus concentrations in the various samples were determined by the acid persulfate digestion method, in accordance with the ISO 6878 standard [28]. All experiments were performed in triplicate (n = 3).

2.4. Determination of Different Phosphorus Fractions in Sediment Samples

The various phosphorus fractions were determined using the sequential extraction protocols of Bonzongo [9], Hieltjes and Lijklema [10], Paludan and Jensen [11], and Azzouz [12]. The detailed implementation of these protocols is presented in **Figure 3**. The concentrations of the different phosphorus fractions were obtained by the ammonium molybdate spectrometric method, according to the ISO 6878 standard [28]. All experiments were also performed in triplicate (n = 3).

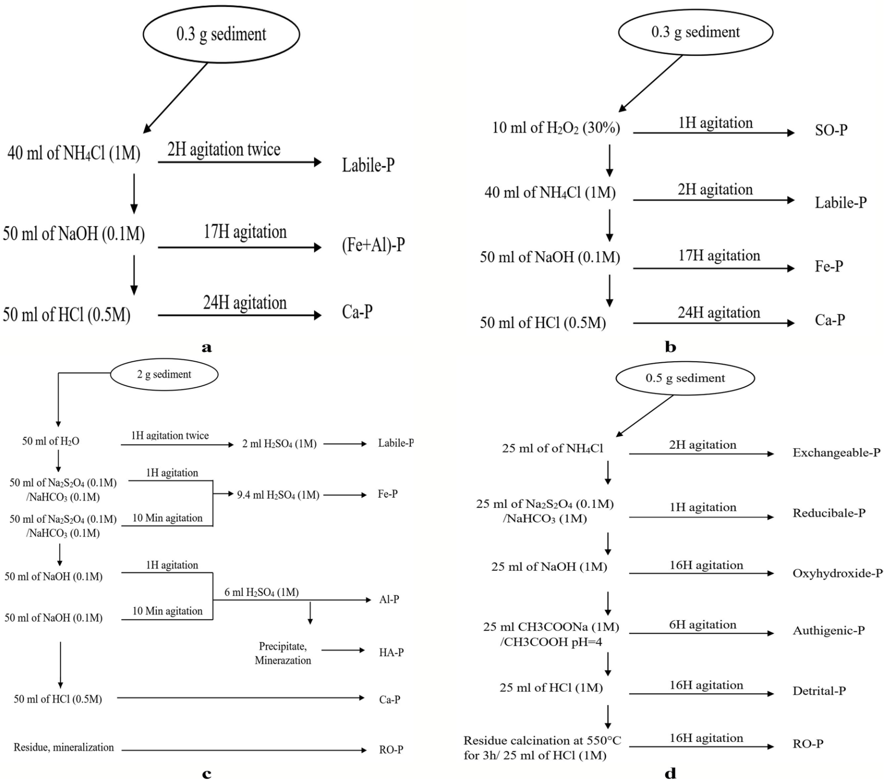


Figure 3. Sequential extraction protocols of Hieltjes and Lijklema (a) [10], Bonzongo [9] (b), Paludan et Jensen [11] (c), and Azzouz [12].

Based on the results obtained, the seasonal phosphorus recovery rates (R) in these substrates were evaluated using the following relationship:

$$\mathrm{R}\left(\%\right)=\frac{\sum \text{Phosphorus fraction concnetration}}{\text{Total phosphorus concentration}}\times 100$$

A protocol demonstrates satisfactory efficiency and precision in a given season when its recovery rates fall within the 80% to 120% range [29].

2.5. Conditions for Developing an Optimized Sequential Phosphorus Extraction Protocol for These Sediments

The development of a sequential phosphorus extraction protocol adapted to these sediments will be based on the structure of the protocol, among the four studied, that exhibits the highest seasonal recovery rates. If several protocols yield similar results, the one capable of determining the greatest number of phosphorus fractions will be selected. Subsequently, distinct phosphorus fractions measured by the other three protocols will be integrated into the chosen methodology, while carefully assessing the impact of the extractants on the subsequent analytical steps to avoid any interference.

To optimize this protocol, the volumes of the extractants will be adjusted from 10 to 50 mL. The selection of this range of extractant volumes is not arbitrary; rather, it results from a careful balance between chemical efficiency and analytical precision. Extractant volume primary role is to ensure thermodynamic equilibrium by maintaining a sufficient reagent concentration to shift the equilibrium toward the dissolution of sedimentary phosphorus. This volume is directly related to the solid-to-liquid ratio (S/L), a critical parameter that influences kinetics. An optimal volume ensures efficient contact between the solid and liquid phases. Furthermore, a sufficient volume is crucial for preserving the extractant's buffering capacity, thereby maintaining a constant pH or ionic strength. This stability enables the selective solubilization of phosphorus (particularly that bound to iron and aluminium oxides). Ultimately, this specific range represents an indispensable analytical compromise: it maximizes the extraction yield while ensuring the final phosphorus concentration in the solution remains high enough for accurate measurement using standard laboratory techniques (UV/Vis) [12].

The optimal volume will be the one that yields the highest concentration for the specific phosphorus form under consideration. In the case where a single extractant is used to determine the concentrations of two phosphorus forms, the best volume will be the one that simultaneously provides the highest concentrations for both forms. If two volumes of a single extractant lead to maximum concentrations for a phosphorus form that are not statistically different, the smaller volume will be retained in the interest of reagent economy.

A protocol demonstrates satisfactory efficiency and precision in a given season, therefore on all the study period, when its recovery rates fall within the 80% to 120% range [29].

2.6. Statistical Analysis of Results

In addition to standard univariate statistical techniques (namely the mean (m), standard deviation (s), coefficient of variation (VC), minimum value (Min), and maximum value (Max)), a one-way ANOVA was used. The one-way ANOVA aimed to determine if there was a statistically significant difference between the various seasonal phosphorus recovery rates obtained with the four protocols. This statistical test was also employed during protocol optimization to determine if the concentrations of the different phosphorus forms obtained with various volumes of an extractant were statistically different. When such a difference was significant, three post-hoc tests (Fisher's LSD, Tukey's HSD, and Dunnett's test) were implemented to identify distinct seasonal means and homogeneous subgroups that did not differ from one another. The results of the one-way ANOVA and post-hoc tests were considered statistically significant at $p < 0.05$.

3. Results and Discussion

3.1. Results

3.1.1. Mean Seasonal Total Phosphorus Concentration

The concentration of total phosphorus in these sediments ranged from 849.9 ± 109.9 (RS) to 1199.9 ± 104.7 (HS) (**Figure 4**). A one-way ANOVA revealed a significant overall difference between the seasonal total phosphorus concentrations in these substrates ($p < 0.05$). Subsequent post-hoc tests showed that only the total phosphorus concentration in the HS was statistically different from that in the RS ($p < 0.05$). Thus, the seasonal total phosphorus concentrations in these sediments, over the study period, followed the following increasing order:

$$\text{Ptot (HS)} > \text{Ptot (RS)} = \text{Ptot (CS)} = \text{Ptot (FS)}.$$

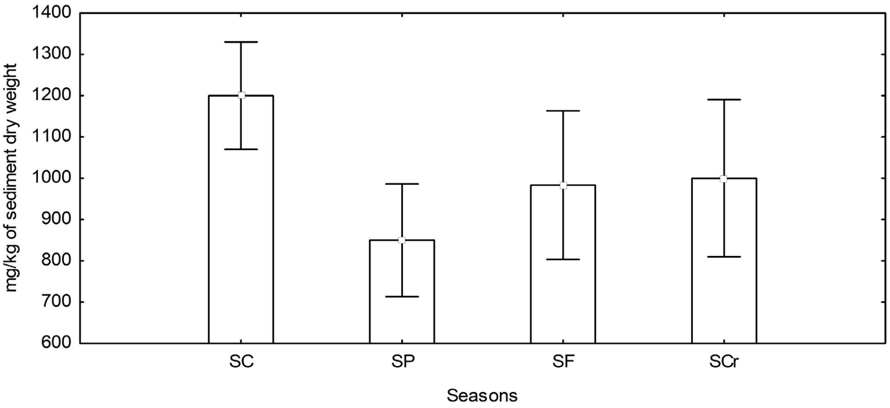


Figure 4. Seasonal evolution of total phosphorus concentration in surface sediments of the area II of the Ébrié system over the study period.

3.1.2. Seasonal Phosphorus Recovery Rates of the Different Protocols

The seasonal phosphorus recovery rates in these substrates, obtained with the Paludan and Jensen protocol [11], were found to be the highest, except during the RS, where the highest rate was reportedly determined using the Bonzongo protocol [9]. The Azzouz protocol [12], in contrast, showed the lowest seasonal recovery rates (**Figure 5**).

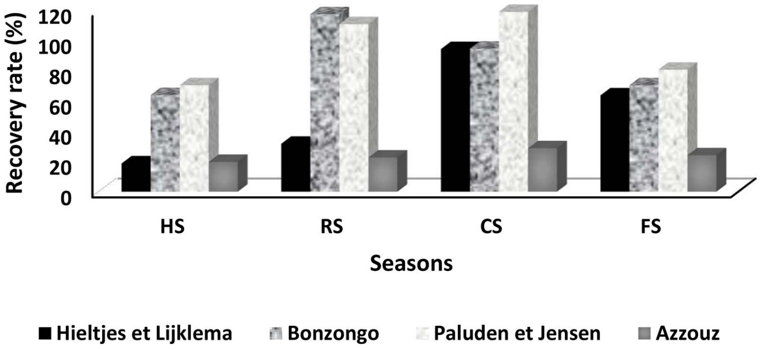


Figure 5. Mean seasonal phosphorus recovery rates in the superficial sediments of the study area, obtained with the four sequential extraction protocols used in this study.

A one-way ANOVA revealed overall significant differences between the mean seasonal phosphorus recovery rates of these protocols. Post-hoc tests demonstrated that only the phosphorus recovery rates determined with the Bonzongo protocol [9] were not statistically different from those obtained with the Paludan and Jensen protocol [11]. Consequently, in virtually every season, the phosphorus recovery rates followed the following order of importance:

TR (Bonzongo) = TR (Paludan et Jensen) > TR (Hieltjes et Lijklema) > TR (Azzouz)

Considering the efficiency requirement for a sequential extraction protocol, which necessitates a recovery rate between 80% and 120%, it is clear that only the protocols of Bonzongo [9] and Paludan and Jensen [11] are suitable for studying phosphorus mobility in these substrates during the RS and CS, while they are unsuitable during the HS and FS.

Given the advantages offered by the Paludan and Jensen protocol [11] compared to that of Bonzongo [9], particularly its ability to determine a greater number of phosphorus fractions, it is more appropriate to develop an optimal extraction protocol for these sediments based on this methodology.

3.1.3. Development and Optimization of a Sequential Extraction Protocol

1) Protocol Development

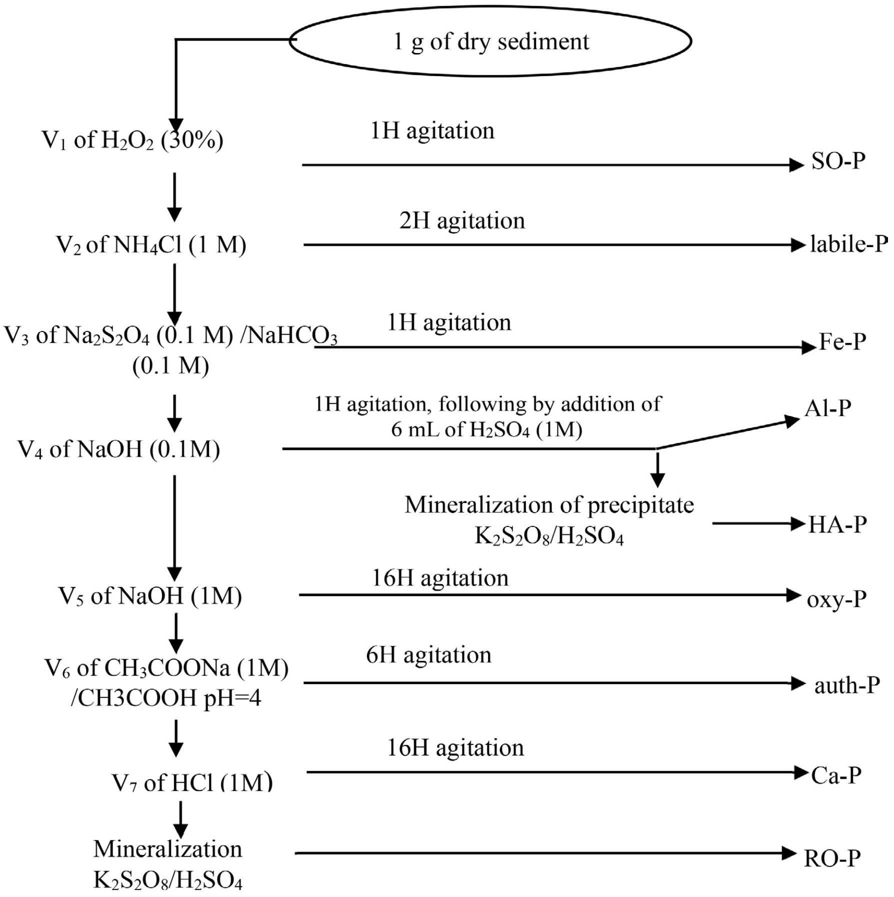
A new protocol was devised to integrate nearly all the phosphorus fractions proposed by the four methods used in this study. The flowchart for this protocol is presented in **Figure 6**.

2) Protocol Optimization

a) Selection of Optimal Extractant Volumes

Table 1 presents the mean concentrations of the different phosphorus fractions obtained using volumes ranging from 10 to 50 mL.

The mean concentration of SO-P in these sediments was observed to increase progressively with increasing the volume of 30% H_2O_2. A one-way ANOVA



SO-P: soluble organic phosphorus; labile-P: labile phosphorus; Fe-P: iron bound to phosphorus; Al-P: Aluminium bound to aluminium; HA-P: humic acid bound to phosphorus; oxy-P: metal (oxy)hydroxides bound to phosphorus; auth-P: authigenic phosphorus; Ca-P: detrital phosphorus; RO-P: residual organic phosphorus.

Figure 6. Sequential extraction protocol implemented for studying phosphorus mobility in the superficial sediments from the study area.

Table 1. Mean concentrations of the different phosphorus fractions considered in the developed protocol, at various volumes of the extracting agents (n = 3).

Phosphorus fraction	Extractant	Statistical parameters	Volume (mL)				
			10	20	30	40	50
SO-P	H_2O_2 (30%) (V_1)	m ± s	5.3 ± 3.9	13.7 ± 15.1	11.2 ± 12.4	15.5 ± 12.8	32.5 ± 27.2
		VC (%)	73.3	109.7	110.5	82.7	83.6
		Min-Max	0.7 - 11.9	1.3 - 46.6	1.3 - 35.9	2.7 - 42.7	3.3 - 96.7
labile-P	NH_4Cl (1M) (V_2)	m ± s	6.3 ± 3.8	19.1 ± 14.1	28.9 ± 14.4	52.7 ± 29.8	64.3 ± 31.8
		VC (%)	59.3	73.5	60.2	56.5	49.5
		Min-Max	1.9 - 13.3	3.9 - 50.7	7.9 - 53.3	18.7 - 106.7	19.9 - 116.7
Fe-P	$Na_2S_2O_4$ (0.1 M) /$NaHCO_3$ (0.1 M) (V_3)	m ± s	93.3 ± 17.1	198.4 ± 24.1	194.7 ± 29.8	350.7 ± 48.1	415.8 ± 81.6
		VC (%)	18.2	12.1	15.3	13.7	19.6
		Min-Max	73.3 - 121.3	170.7 - 246.7	158.7 - 246.7	285.2 - 421.3	299.9 - 559.9

Continued

Al-P	NaOH (0.1 M) (V_4) following by addition of 6 mL de H_2SO_4 (1 M) (V_4)	m ± s	5.2 ± 1.9	11.6 ± 4.4	19.7 ± 16.8	105.3 ± 152.7	34.2 ± 14.4
		VC (%)	36.9	37.4	85.3	144.9	42.1
		Min-Max	1.9 - 8.7	3.9 - 21.3	1.3 - 47.9	5.3 - 373.3	16.7 - 59.9
HA-P		m ± s	16.5 ± 7.2	138.7 ± 64.9	158.9 ± 12.9	315.3 ± 39.8	359.9 ± 32.1
		VC (%)	43.8	46.8	8.1	12.6	8.9
		Min-Max	7.3 - 33.3	30.7 - 214.7	131.9 - 179.9	263.9 - 378.7	309.9 - 416.7
oxy-P	NaOH (1M) (V_5)	m ± s	3.9 ± 1.4	20.2 ± 23.9	3.9 ± 2.4	6.7 ± 3.5	8.3 ± 3.2
		VC (%)	35.9	118.3	61.9	52.7	37.8
		Min-Max	1.9 - 6.7	2.7 - 79.9	1.3 - 10.7	2.7 - 15.9	3.3 - 16.6
auth-P	CH_3COONa/ CH_3COOH (pH = 4) (V_6)	m ± s	25.7 ± 8.1	39.9 ± 11.6	31.1 ± 7.1	44.7 ± 18.7	53.3 ± 25.4
		VC (%)	31.7	28.9	22.7	41.9	47.6
		Min-Max	11.3 - 35.9	19.9 - 53.3	15.9 - 42.7	13.3 - 85.3	6.7 - 93.3
Ca-P	HCl (1 M) (V_7)	m ± s	2.3 ± 1.1	4.8 ± 2.3	3.6 ± 1.8	6.7 ± 3.5	7.3 ± 2.6
		VC (%)	46.8	47.9	48.5	52.7	34.9
		Min-Max	0.7 - 4.7	1.3 - 7.9	1.3 - 7.9	2.7 - 15.6	3.3 - 13.3

SO-P: soluble organic phosphorus; labile-P: labile phosphorus; Fe-P: iron bound to phosphorus; Al-P: Aluminium bound to aluminium; HA-P: humic acid bound to phosphorus; oxy-P: metal (oxy)hydroxides bound to phosphorus; auth-P: authigenic phosphorus; Ca-P: detrital phosphorus; RO-P: residual organic phosphorus.

revealed statistically significant overall differences among these concentrations as a function of the volume ($p < 0.05$). Post-hoc tests showed that only the mean SO-P concentration obtained with a 50 mL volume was statistically different from those obtained with the other volumes ($p < 0.05$). Consequently, the 50 mL volume of 30% H_2O_2, which yielded the greatest POS concentration in these substrates, is considered optimal for this study.

The mean concentration of labile-P also increased progressively with the increasing volume of 1 M NH_4Cl. A one-way ANOVA highlighted overall significant differences among the mean P-labile concentrations ($p < 0.05$). Post-hoc tests revealed that only the labile-P concentrations obtained with 40 mL and 50 mL were not statistically different ($p < 0.05$). Therefore, in the interest of both efficiency and reagent economy, it is advisable to retain the 40 mL volume for the extraction of this phosphorus fraction from these substrates.

Just as with the mean concentrations of SO-P and labile-P, the concentration of Fe-P increased progressively with the increasing volume of the combined 0.1 M $Na_2S_2O_4$/0.1 M $NaHCO_3$ reagent. A one-way ANOVA highlighted overall significant differences among the mean Fe-P concentrations obtained with the different volumes ($p < 0.05$). Post-hoc tests revealed that only the Fe-P concentrations obtained with the 20 mL and 30 mL volumes were not statistically distinct ($p > 0.05$). Furthermore, the highest Fe-P concentrations in these sediments were obtained with 50 mL. Consequently, this volume was retained for the extraction of this phosphorus fraction in the remainder of the study.

The mean concentration of Al-P increased progressively with the 0.1 M NaOH volume from 10 to 40 mL, then slightly decreased at a volume of 50 mL. Conversely, the mean concentration of HA-P increased consistently with the increasing volume of this extractant. A one-way ANOVA revealed overall significant differences between the mean concentrations of these two phosphorus forms obtained with the different volumes of 0.1 M NaOH ($p < 0.05$). Post-hoc tests revealed the following:

- the Al-P concentrations obtained with 20 mL and 30 mL were not statistically distinct ($p > 0.05$);
- only the HA-P concentrations obtained with 40 mL were statistically distinct from those obtained with the other volumes ($p < 0.05$);

The difference between the mean Al-P concentrations obtained with 40 mL and 50 mL of 0.1 M NaOH is less significant than with the other volumes (10, 20, and 30 mL). To simultaneously obtain high concentrations for both Al-P and HA-P in these substrates, it is therefore judicious to select a 40 mL volume.

The highest mean concentration of oxy-P was observed at a 20 mL volume of 1 M NaOH, while the lowest was observed with 10 mL and 30 mL of this extracting agent. A one-way ANOVA highlighted significant overall differences among the oxy-P concentrations obtained with these volumes ($p < 0.05$). Post-hoc tests revealed that only the oxy-P concentrations obtained with the 20 mL volume of 1 M NaOH were statistically different from those obtained with all other volumes (10, 30, 40, and 50 mL) ($p < 0.05$). Consequently, the 20 mL volume is deemed optimal for the extraction of this phosphorus fraction in this context.

The highest mean concentrations of auth-P were observed with 20, 40, and 50 mL volumes of the CH_3COONa/CH_3COOH buffer solution (pH = 4). The lowest was noted with a 10 mL volume. A one-way ANOVA highlighted overall significant differences among the mean auth-P concentrations obtained with these different volumes ($p < 0.05$). Post-hoc tests revealed that:

- the concentrations obtained with 20, 40, and 50 mL volumes were statistically different from those obtained with 10 mL ($p < 0.05$);
- the concentrations obtained with 30 mL were different from those obtained with 40 mL ($p < 0.05$);
- there was no statistically significant difference between the mean P-auth concentrations determined with 20, 40, and 50 mL volumes ($p > 0.05$).

Given these observations and in the interest of reagent economy, it is more judicious to select the 20 mL volume for the extraction of this fraction.

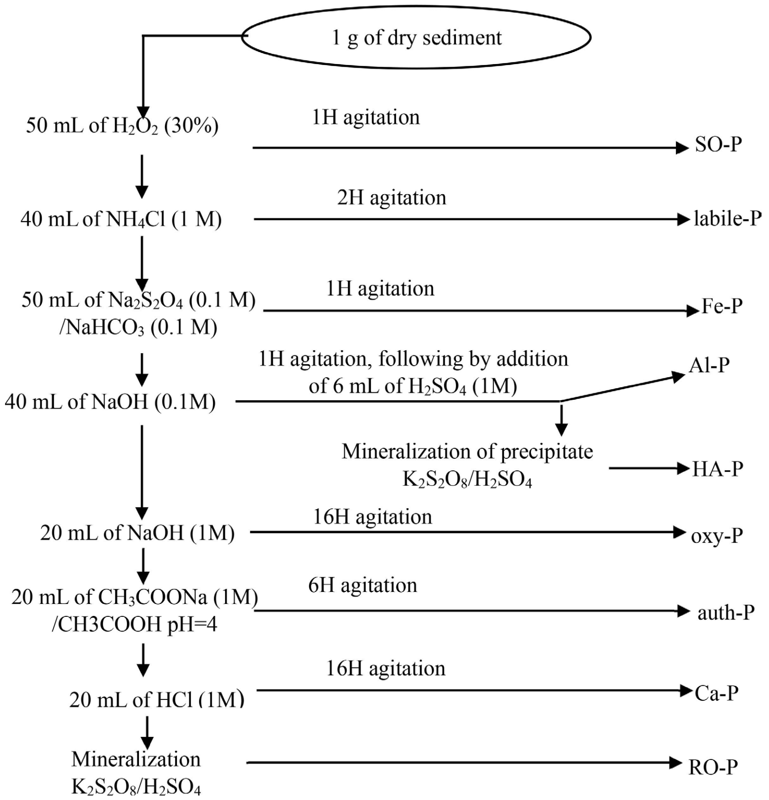
The mean concentrations of Ca-P progressively increased with the 1 M HCl volume from 10 to 20 mL, and then from 30 to 50 mL. A one-way ANOVA revealed overall significant differences among the mean concentrations of Ca-P obtained with the different volumes of this acid. Post-hoc tests showed no statistically significant difference between:

- the mean P-det concentration obtained with a 10 mL volume and that determined with a 30 mL volume;

- the mean P-det concentration determined with a 20 mL volume and those obtained with 40 and 50 mL volumes;
- the mean P-auth concentration (likely P-det) obtained with a 40 mL volume and that determined with a 50 mL volume.

From these observations, it's appropriate to select 20 mL as the optimal volume of this extracting agent for this phosphorus fraction in these sediments. This choice balances the need for high extraction efficiency with reagent economy.

The optimized sequential extraction scheme for the different phosphorus fractions considered in this study is presented in Figure 7.



SO-P: soluble organic phosphorus; labile-P: labile phosphorus; Fe-P: iron bound to phosphorus; Al-P: Aluminium bound to aluminium; HA-P: humic acid bound to phosphorus; oxy-P: metal (oxy)hydroxides bound to phosphorus; auth-P: authigenic phosphorus; Ca-P: detrital phosphorus; RO-P: residual organic phosphorus.

Figure 7. Sequential extraction protocol established for the optimal study of phosphorus mobility in the superficial sediments of the study area.

b) Protocol Efficiency

The seasonal phosphorus recovery rates in the surface sediments of the study area ranged from 85.7% in the HS to 114.7% in the RS, with low intra-seasonal variations. All these rates were found to be higher than those obtained with the Paludan and Jensen protocol [11], with the exception of the CS rates (Table 2).

These seasonal rates, all of which fall between 80% and 120%, confirm the efficiency of this method for studying phosphorus mobility in these substrates. Furthermore, this protocol enables the assessment of SO-P, auth-P, and oxy-P concentrations, in addition to those accounted for by the Paludan and Jensen protocol [11]. As a result, it ensures high efficiency for the study of phosphorus mobility, regardless of the season.

Table 2. Statistical parameters for the mean seasonal total phosphorus concentration and phosphorus recovery rate in the surface sediments of the study area, using the developed protocol (n = 3).

Sequential extraction protocol	Statistical parameters	Seasons				Annual
		HS	RS	CS	FS	
Paludan et Jensen (1995) [9]	m ± s	70.4 ± 11.5	110.8 ± 13.8	118.9 ± 29.7	80.7 ± 32.5	95.2 ± 30.1
	VC (%)	16.4	12.5	24.9	40.3	31.6
	Min-Max	57.9 - 82.2	91.3 - 123.2	78.3 - 144.4	33.5 - 113.5	33.5 - 144.4
Implemented protocol in this study	m ± s	85.7 ± 6.3	114.7 ± 15.5	110.7 ± 15.1	102.5 ± 15.8	103.4 ± 16.9
	VC (%)	7.4	13.5	15.4	15.4	16.4
	Min-Max	79.2 - 95.6	89.9 - 128.3	91.9 - 125.4	84,1 - 125.5	79.2 - 128.3

3.2. Discussion

Total phosphorus concentrations in the superficial sediments of this estuary were exceptionally high throughout the study period, surpassing values documented in comparable environments, such as the Al-Shaibah and Al-Budhia lagoons (Red Sea, Saudi Arabia) [30], the northern Adriatic Sea [31], and the Shamei Lagoon [32]. This enrichment is attributed to the combined influence of the sandy sediment texture, the estuary's hydrology, and its overall sedimentological processes [33]-[35]. This is driven by the presence of detrital phosphorus associated with weathering-resistant primary minerals, coupled with the significant accumulation of organic matter in the sedimentary environments [33] [35]. The strong intrusion of Atlantic oceanic waters during the HS drives the relatively high phosphorus content observed in these substrates. The elevated salinity and ionic composition of the oceanic input, coupled with increased oxygen levels, catalyze phosphorus-trapping processes (flocculation and precipitation) that sequester phosphorus originating from the Comoé River. This leads to the accumulation of phosphorus-rich, fine-grained material within the sandy matrix [29] [35] [36]. These stabilizing phenomena are significantly reduced during high-precipitation seasons (from RS to FS) due to inhibited oceanic influence by Comoé River. Consequently, the high total phosphorus concentration measured during the HS was statistically greater than those observed during the other three seasons.

Analysis of the seasonal phosphorus recovery rates demonstrates that sequential extraction protocols utilizing both a strong oxidant and large reagent volumes (the protocols of Bonzongo [9] and Paludan and Jensen [11]) achieved significantly higher rates. These results are in sharp contrast to those from protocols that use no oxidizing agent (the Hieltjes and Lijklema protocol [10]) and those that employ low volumes of extraction reagents (the Azzouz protocol [12]). The Hieltjes and Lijklema protocol [10] has known limitations for phosphorus speciation, especially in complex mineral matrices like rock fragments. These issues stem from reagent inadequacy [37] [38]. The Bonzongo protocol [9], developed as a modification of the Hieltjes and Lijklema protocol [10], overcomes these challenges in geological matrices through the critical preliminary step of using concentrated H_2O_2 (30% - 35%). This powerful oxidizing agent targets organic matter and sulphides (major immobilizers of phosphorus), catalyzing the degradation of organic bonds and releasing phosphorus as soluble phosphate ions [7] [39] [40]. By eliminating organic matrix interferences and improving access to occluded phosphorus, H_2O_2 serves as an essential pretreatment, significantly enhancing the efficiency and precision of subsequent extraction steps [9]. This mechanistic improvement ultimately justifies the significantly higher seasonal phosphorus recovery rates observed with the Bonzongo protocol [9] compared to the Hieltjes and Lijklema protocol [10] in this study. The Paludan and Jensen protocol [11] notably improves speciation by providing access to the RO-P. This is achieved using $Na_2S_2O_8$, a stronger oxidizing agent than H_2O_2 used in the Bonzongo protocol [9]. Due to its comprehensive methodology, the Paludan and Jensen protocol yielded seasonal total phosphorus recovery rates in the studied sediments that were statistically identical to those obtained with the Bonzongo protocol [9]. In contrast, the Azzouz protocol [10] exhibited low seasonal P recovery rates due primarily to two factors: insufficient extractant volumes and the inadequacy of its digestion method for these complex sediments [41]-[44].

The efficiency of the Bonzongo [9] and Paludan and Jensen [11] protocols for studying phosphorus mobility in the sediments of this estuary varies seasonally. Their inadequacy during certain periods is explained by both the sediment mineralogy and the volumes of extractants used. Although these sediments predominantly originate from Comoé River, their mineralogical composition changes seasonally, as the river's watershed is sedimentary in the south and rests on a geological basement from the center to the north [18] [20]. The results obtained with these two protocols indicate that the sediments of this estuary contain more easily extractable phosphorus forms during the CS and RS, compared to the other two seasons. Furthermore, the excess volume of extractants used during sequential phosphorus extractions may have skewed the concentrations due to their interference with the reagents employed for orthophosphate determination via colorimetry, particularly with sediments sampled during the HS and FS. These interferences have been widely documented, showing that extractant residues can compromise analytical accuracy, especially for samples with high mineral phosphorus content [45].

These observations highlight that phosphorus sequential extraction in these sediments is intrinsically impacted by their sandy nature, the estuary's hydrology, and its sedimentological processes. These factors determine how phosphorus is stored, remobilized, and, consequently, the quantity recovered in each operational fraction (labile, Fe/Al oxide-bound, organic, and residual) [33]-[35] [46]. The water regime controls the sedimentary phosphorus stock through two mechanisms. First, external inputs (meteorological runoff, Comoé River during the FS, and Mé River via the Potou and Me lagoons during the RS) introduce an increased quantity of suspended matter rich in organic phosphorus and primary mineral phosphorus. These inputs are likely to increase sedimentary phosphorus because the material is freshly deposited and less chemically transformed [37] [46]. Second, the estuary's shallow depth and water residence time control oxygenation [46]. During seasons of high meteorological inputs (notably in the FS), poor water ventilation leads to significant organic matter degradation, resulting in anoxic conditions at the water-sediment interface. This anoxia triggers the reduction of iron (Fe(III) to Fe(II)), which dissolves oxide matrices and releases the Fe-bound to phosphorus (Fe-P) [5] [33] [36], thereby significantly influencing the measured speciation profile. Furthermore, during the HS, strong oceanic water intrusion can increase water salinity, promoting phosphorus desorption via ionic competition and potentially affecting the most labile fractions [35]. The sandy nature of these sediments translates into a low overall phosphorus adsorption capacity. In this sediment type, phosphorus is not trapped by the surface of the grains themselves but is strongly bound to Fe/Al oxide coatings or to fine organic matter associated with the sand [36] [47]. Consequently, any sequential extraction performed on these sediments must effectively target these coatings (notably via NaOH or H_2O_2), as the phosphorus is not homogeneously distributed [39] [40]. Furthermore, both the quantity and nature of the organic matter directly influence the organic phosphorus fraction [33] [35]. A sedimentology that favours the accumulation of autochthonous organic matter leads to a more labile phosphorus organic fraction compared to phosphorus organic originating from more refractory allochthonous organic matter [34]. This process significantly impacts the measured speciation profile.

The suitability of the implemented protocol for studying phosphorus mobility in these substrates, regardless of the season, is based on two major methodological adaptations. On the one hand, the use of a H_2O_2 pretreatment to oxidize organic matter increased the precision and efficiency of the subsequent extraction methods. On the other hand, adapting the volumes of the extractants was essential to minimize interference from their residues with the reagents used for orthophosphate determination via colorimetry. Despite the robustness of this approach, the low phosphorus recovery rate observed in the sediments of the HS, compared to that of other seasons, illustrates a high degree of crystallization of this nutrient during this period. This contrasts with the presence of Atlantic oceanic waters in this estuary, which normally promotes phosphorus trapping on the sediments through flocculation and precipitation [33] [36]. The high crystallization of these substrates considerably limits phosphorus extraction, highlighting its low mobility and geochemically stable nature [37] [38] during this season. The identification of multiple organic phosphorus fractions is essential for assessing the lagoon's potential for delayed P release. The mobility and bioavailability of organic phosphorus typically decrease in the order: SO-P > HA-P > RO-P [4]. By quantifying these specific fractions, this protocol allows researchers to distinguish the immediately threatening phosphorus (SO-P) from the stabilized stock (RO-P), thereby providing a more precise picture of the sediment's actual trophic potential.

4. Conclusion

This study highlighted the need to develop a sequential extraction protocol adapted for studying phosphorus mobility in the soils and sediments of a specific environment, which is justified by the unique nature of these substrates. The parameters considered enabled the design of a sequential phosphorus extraction protocol, providing information on several of its forms. The use of this optimized sequential extraction protocol provides Ébrié Lagoon managers with a chemical and temporal roadmap for nutrient management (quantification and targeting of the internal flux, seasonal adaptation of strategies, and improved ecological modeling). Thus, it transforms a simple static measurement into a dynamic management tool, enabling the Ébrié Lagoon authorities to shift from a reactive approach (treating the symptoms) to a proactive and targeted strategy (preventing the underlying mechanisms) against eutrophication.

Author contributions

H. C. Abbas: Data curation; Formal analysis; Investigation; Software; Roles/Writing—original draft.
M.K. Yao: Project administration; Validation; Supervision; Visualization; Resources; Roles/Writing—original draft; Writing—review & editing; Resources.
K.F.A. Konan: Methodology; Roles/Writing—original draft; Writing—review & editing; Resources.
J-C. B. Drida Bi: Methodology; Roles/Writing—original draft; Writing—review & editing; Resources.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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