

# Proximate and Heavy Metals Composition of Clam *Galatea Schwabi* (Clench, 1929) From the Lower Sanaga, Cameroon

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**Abstract** The clam *Galatea schwabi* is a bivalve found in the lower Sanaga, and exploited by local people for its meat and shell. This research aimed to determine the proximate and heavy metals (Cd, Pb and Hg) composition contained in fresh and smoked clam meat by standard analytical methods. Fresh clam meat was collected from the fishing area and smoked from Yakalak and Malimba Districts. The results showed a significant difference in the water, protein, lipid, and mineral content of smoked clams in Yakalak and Malimba District, except for the ash content which was similar in the different areas studied. The clam harvested in the lower Sanaga, regardless of its commercialized form is rich in proteins with  $27,31 \pm 0,27\%$  in fresh, for the smoked clams  $59,85 \pm 0,24\%$  in Yakalak,  $34,66 \pm 0,13\%$  in Malimba District, and minerals Ca, Mg and Na. The concentration of heavy metals Pb and Cd in fresh and smoked *G. schwabi* meat is above the WHO recommended tolerance limits, while Hg is below the same standard. The highest concentrations of heavy metals were observed in smoked clams from Malimba District. Although that *G. schwabi* clam constitutes an alternative source of nutrients for human and animal, the high concentration of heavy metals, show that the resource is subject to the effects of anthropic activity, and consuming it would be a risk for consumers.

**Keywords:** *Galatea schwabi*, proximate composition, heavy metals, Lower Sanaga

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## 1. Introduction

Bivalves such as oysters, clams, and mussels have been cultivated for centuries. They are a rich source for human food and an associated economic value for local communities [1]. Total aquaculture and fisheries production of bivalves was 17.7 million tons in 2020 [2], and clams occupy an important place among these bivalves. The analysis of the chemical composition of the shellfish meat has shown interesting nutritional values and the possibility of using this meat in preventive nutrition [3]. However, these animals that live buried in the mud feed by absorbing heavy metals who is a consequence of the consumption of contaminated food by the bivalves [4], or the organic matter and some inorganic materials from the nearby water source through filtration [5]. Bioaccumulation of these heavy metals is not directly or solely from the sediment, but from other sources such as suspended living

or dead particles and dissolved metals in the sediment [6]. The poisoning effects of heavy metals are due to their interference with the normal body biochemistry in the normal metabolic processes [7]. Three metal contaminants are most sought after in fishery products, namely lead, mercury and cadmium, because they have no role in life processes and are known to be toxic at relatively low doses to humans and organisms [8]. Human exposure to these metals after consumption is associated with health problems such as kidney and bone damage, lung cancer, effects on neurodevelopment and neurobehavior in fetuses, infants, and children, and elevated blood pressure in adults [9].

In Cameroon, the main areas where clams are found are located in the watersheds of the Sanaga and Wouri rivers [10,11]. Little research has been conducted on the exploitation of freshwater clam meat in Cameroon; and those exploited in the Sanaga River basin belong to a single species recently named *Galatea schwabi* by Kondakov et al. [12]. An estimated production of more

than 800 tons per year has been exploited along the Sanaga Delta for meat and shell by local fishermen in the Mouanko Sub-division [13]. The strong presence of the resource has led the populations to commercialize clams in various forms, notably fresh, smoked or cooked. Due to the perishable nature of clam, processing methods are used to stabilize the food from a sanitary point of view, increase shelf life, enhance the value of less noble or less sought after cuts, diversify supply, and preserve the product. Salting, cooking, drying, smoking and fermentation are the most frequency used processes [14]. The present study aims to evaluate not only the proximate composition, but also the bioaccumulation of heavy metals contained in fresh and smoked *Galatea schwabi* clam meat exploited by local people.

## 2. Material and Methods

This study was conducted in the Mouanko Sub-division, Department of Sanaga Maritime, in the Littoral Region of Cameroon. The geographical coordinates of the study area

are between 3°14' & 3°50' North latitude, and 9°34' & 10°03' East longitude. The sites located as enclaves or peripheral to Douala-Edea national park. The surveyed in Yakalak District were made up of the villages Lolbethal and Nkangansok; and those of Malimba District were Moulongo, Bolounga, Malbengue and Maldjebou, and the fishing area downstream of the Sanaga River near the village of Bôngo following the coordinates 03°34'02.2" N, 009°42'20.8" E (Figure 1). The area is with Sanaga estuary closer to the giant mangrove forests of Mbiako [15]. River Sanaga is the longest of Cameroon river sweeping over 1/3 of country surface taking its rise from the Mandara Mountains of Bamenda Chain of Mountains. The study area has an equatorial-type climate with four seasons with Guinean variance: a long dry season (mid-November to mid-April), a short rainy season (mid-April to mid-June); a short dry season (mid-June to mid-August) and a long rainy season (mid-August to mid-November). The rainiest month is October with an average annual rainfall that varies between 3000-4000mm, and the least rainy is January. Moreover, yearly average temperature between 25 and 30°C [16].

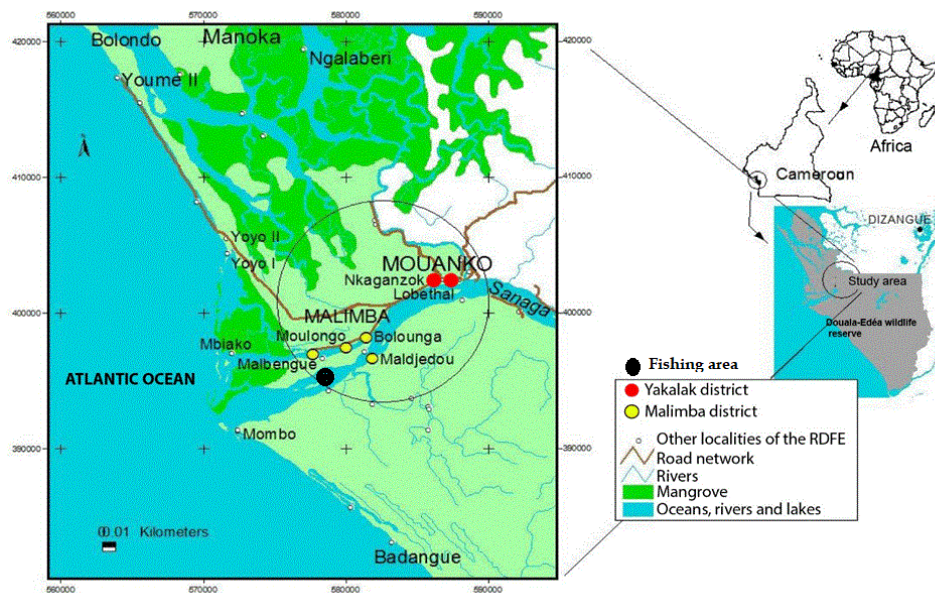


Figure 1. Map of the study area in the lower Sanaga



(A)

(B)

Figure 2. Samples collected from fresh (A) and smoked (B) clams

## 2.1. Sample Collection

Samples of *G. schwabi* clams were collected between February and May 2022 in the study area. Smoked clam samples were collected in Yakalak and Malimba District, the shell and fresh clams were collected in the fishing area. For smoking, the processors boil for 15 to 30 minutes fresh clams in cylindrical drums, then separated after cooling the flesh of the shell. The meat thus obtained is spread out on the racks for traditional heat smoking between 55 and 86°C for 12 to 72 hours with the “Banda” smoker. Woods from forests or mangroves are used for combustion [13]. To represent each District, 15 units were randomly sampled from the smokers the products treated for less than a week.

The fresh samples (Figure 2A) collected with shell were stored in a sterile cooler at a temperature near to 0°C, and the smoke (Figure 2B) were put into plastic bags that had been labeled according to each District from randomly selected processors before being transported to the laboratory for analysis. The choice of collection sites was based on the availability of the resource and the accessibility of the various smoking camps.

## 2.2. Proximate Analysis

### 2.2.1. Water Content

The water content of fresh and smoked *Galatea schwabi* clam meat was determined according to the method described by AOAC [17]. Samples were dried in an air oven at 105°C for about 10 hours until constant weights were obtained, then cooled in a desiccator before reweighed. The water content was taken to be the differential between fresh and dry weights.

### 2.2.2. Ash Content

The ash content was determined by the procedure described by the AOAC [17]. In fact, dried samples obtained in the process of water content determination were heated in the muffle furnace at 550°C for several hours. After incineration and cooling in a desiccator, the percentage of ash was calculated by subtracting the weight of ash from the initial weight.

### 2.2.3. Mineral Content

The presence of the mineral content Ca, Na and Mg of the ash was determined by using air-acetylene flame atomic absorption spectrophotometer, Perkin-Elmer HGA 700 brand Model analyst according to the method described by the AOAC [17].

### 2.2.4. Crude Protein Content

Crude protein content of smoked and fresh clam was analyzed by the Kjeldahl's method [18]. In fact, the samples went through the three digestion, distillation and titration using a conversion factor of 6.25 to convert total nitrogen to crude protein. The percentage of protein in the samples was calculated thereafter.

### 2.2.5. Lipids Content

Lipids content was determined by weighing 10g of each sample wrapped in a filter paper in a Soxhlet apparatus

using hexane. This was done each for 6 hours. After this, the extracted materials left after the solvent had evaporated were weighed, and the lipids content was calculated according to the procedure described by [17].

## 2.3. Heavy Metals Analysis

Heavy Metals Analysis were determined by weighing 1g of oven-dried samples and made into fine powder for digestion. The samples were digested with concentrated nitric and hydrochloric acid in the ratio 3:1, and the mixture was placed on a water bath until the color changes. The resulting solution was cooled, filtered into a 100ml standard flask and made to mark with distilled water [19]. The heavy metals (mercury, cadmium and lead) were determined using flame furnace Atomic Absorption Spectrometry, Perkin Elmer HGA 700 brand. Each sample set has its own blank, while correction or adjustment was made through reference to blank. For mercury (Hg), the assay was carried out by measuring the absorbance with a UV visible spectrophotometer at 420nm of each of the standards on the calibration curve and of the samples. In the course of the experiment, glassware was washed with acid and distilled water twice. All reagents were of analytical grade. Certified reference material was checked to ensure of the analytical procedure.

## 2.4. Statistical Analysis

The obtained data were entered into an Excel sheet, coded, and then exported to StatView v5.0 software for statistical analyses. Qualitative data were presented in tables and graphs. Pearson's Chi-square test of independence and Fisher's exact probability were used to make comparisons between variables (bivariate statistics). Data were tested for compliance with the Cochran rule before statistical testing. Associations meeting the Cochran rule were tested by Pearson's Chi-square test of independence and, where appropriate, Fisher's exact probability test. The threshold for statistical significance was set at p-value < 0.05.

## 3. Results

The percentage proximate composition of the fresh and smoked meat of the *G. schwabi* clam collected in the fishing area and the Malimba and Yakalak District revealed the content of water, ash, protein, lipids and minerals as presented in Figure 3, Figure 4 and Table 1.

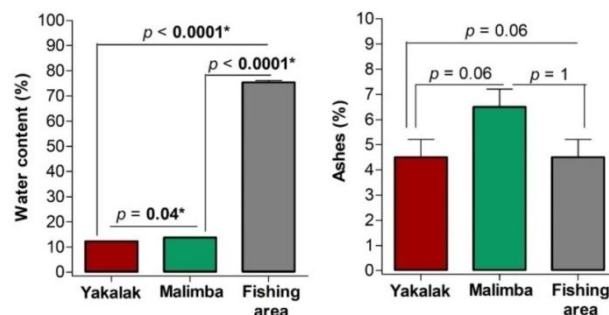


Figure 3. Water and ash content of *Galatea schwabi* clam meat in the different area



The values obtained of water and ash content expressed as percentage of dry matter of fresh flesh of *G. schwabi* was 75.23% and 6.50% respectively. It was also observed that there was a statistically significant difference ( $p < 0.05$ ) between the water content of smoked clams from the two Districts. The values obtained were  $13.84 \pm 0.80\%$  in Yakalak District, and  $15.68 \pm 4.20\%$  for Malimba District. As for ash content, no significant difference was observed with values obtained of  $4.50 \pm 0.71\%$  and  $6.50 \pm 0.70\%$  respectively.

Figure 4 presents the protein and lipid contents in fresh and smoked *G. schwabi* clam meat from the fishing and smoking area in each District. The protein content found on fresh clam meat was  $27.31 \pm 0.27\%$ , and lipid  $9.50 \pm 0.71\%$ . However, after smoking, protein and lipids increased significantly, and there was a significant difference ( $p < 0.05$ ) between smoked clams from the two Districts. The smoked clams collected in Yakalak District had the highest protein ( $59.85 \pm 0.24\%$ ) and lipid ( $9.50 \pm 0.71\%$ ) contents. Those in Malimba District were  $34.66 \pm 0.13\%$  and  $9.50 \pm 0.71\%$  respectively. However, no significant difference ( $p > 0.05$ ) was observed between the lipid content of the fresh clam and that smoked in Malimba District.

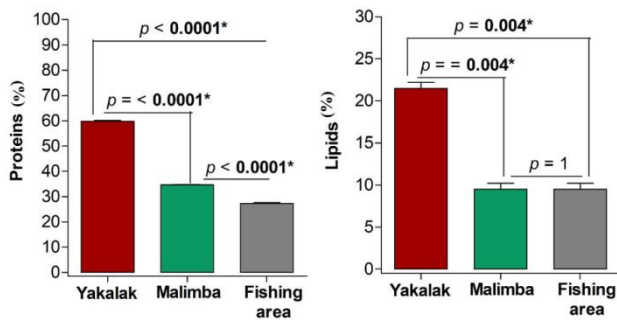


Figure 4. Proteins and lipids contents of *Galatea schwabi* clam meat in the different area

The analysis carried out the determination of the mineral content of clam meat from the different collection areas. The values obtained in Na, Mg and Ca expressed as a percentage of dry matter are presented in Table 1. The recorded Ca contents showed the highest values in fresh

and smoked clam meat, and an increase in Mg content after smoking. In return, the Na content varied significantly between the areas studied. There was a significant difference ( $p < 0.05$ ) between the different minerals sought and the sampling area. With respect to these minerals Na, Mg and Ca, the clams smoked in Yakalak District showed better results than those in Malimba District.

Table 1. Mean ( $\pm$ SD) concentration of minerals in *Galatea schwabi* clam meat in the different area

Districts	Concentration of minerals (mg/100g)		
	Na	Mg	Ca
Yakalak <sup>1</sup>	124,82 ± 0,26	58,51 ± 0,27	656,50 ± 0,71
Malimba <sup>2</sup>	16,78 ± 0,18	87,54 ± 0,09	352,50 ± 0,71
Fishing area <sup>3</sup>	20,98 ± 0,11	19,47 ± 0,04	384,50 ± 0,71
<i>p</i> -value (1 vs 2)	< 0,0001*	< 0,0001*	< 0,0001*
<i>p</i> -value (1 vs 3)	< 0,0001*	< 0,0001*	< 0,0001*
<i>p</i> -value (2 vs 3)	0,0002*	< 0,0001*	< 0,0001*

Duncan's ANOVA and post hoc tests were used to make comparisons of each area with numbers 1, 2 and 3.

\*Statistically significant at  $p$ -value < 0.05

The concentration of heavy metals determined on smoked and fresh flesh of *Galatea schwabi* collected from the smoking and fishing areas are recorded in Figure 5.

The level of mercury (Hg) obtained for each sample (smoked and fresh) is lower than the value indicated by the usual WHO standard, which is  $0.50 \mu\text{g/g}$ . No significant difference ( $p > 0.05$ ) was observed between the different collection areas. Therefore, lead (Pb) and cadmium (Cd) levels increased after smoking from  $11.35 \mu\text{g/g}$  to  $12.65 \mu\text{g/g}$  in Yakalak District and  $15.00 \mu\text{g/g}$  in Malimba. Cadmium levels increased from  $6.80 \mu\text{g/g}$  to  $9.05 \mu\text{g/g}$  in Yakalak District and  $10.95 \mu\text{g/g}$  in Malimba District. These results obtained are higher than the same standard, which is  $1.50 \mu\text{g/g}$  and  $1.00 \mu\text{g/g}$  respectively on a fresh weight basis. For smoked products, no significant difference ( $p > 0.05$ ) was obtained between the heavy metals Cd and Pb from the two Districts. However, there was a significant difference ( $p < 0.05$ ) in Cd concentration between smoked meat from Malimba District and that from the fishing area.

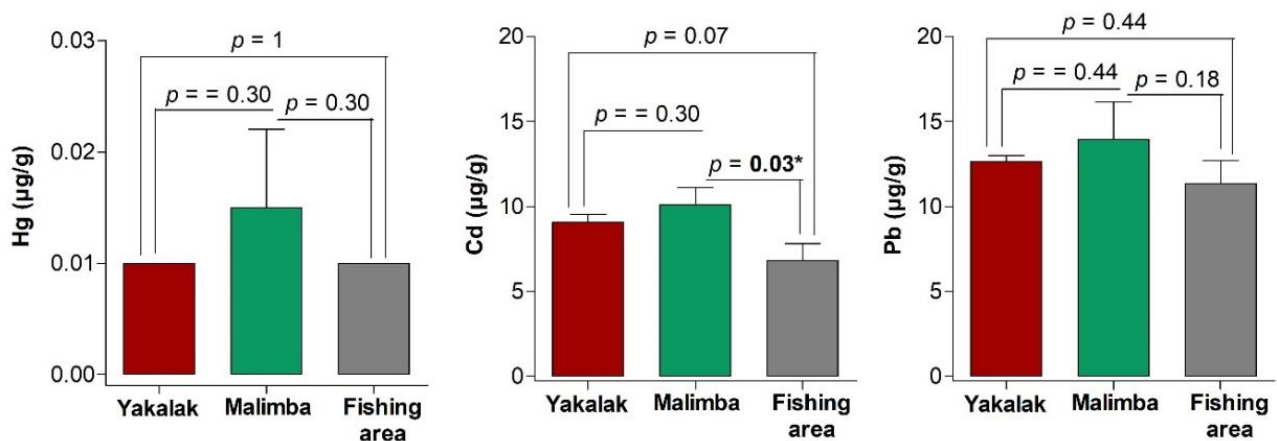


Figure 5. Concentration of heavy metals in smoked and fresh clam meat of *Galatea schwabi* in the different ar

## 4. Discussion

The nutritional attributes of animal-source foods are evaluated on the basis of their nutrient composition (proteins, lipids, carbohydrates, vitamins, minerals) and their ability to cover humans nutritional needs [14]. The proximate composition of the clam can increase or decrease due to the state in which it is found (processed or fresh) and also tells us about the ecosystemic situation of the environment. The results on the water, lipids and proteins content of smoked clam meat collected in Malimba and Yakalak District indicated significant differences, except for the ash and heavy metal content.

Water content is one of the main factors limiting the growth of microbial cells and regulating the level of food spoilage. It is a primary factor responsible for safeguarding the food matrix, stabilizing the food supply, budding of various types of shelf-stable products, stability of foods, and assessment of microbial species present in foods [20]. Water content also influences other chemical compounds in the food. The results of water, lipid and protein content obtained on fresh *G. schwabi* flesh are comparable to those of other authors. An important caveat to bivalve production is the health benefit of low fat, protein meat, and mineral content [1]. The concentrations of these different nutrients vary from species to species. Plana et al. [21] obtained 78% water, 25 to 27 mg/g lipid and 86 to 92% proteins on the flesh of young clams *Ruditapes philipinarum*. Ogidi et al. [5] obtained similar values on the flesh of *Mercenaria mercenaria* collected in the community of Ekowé in Nigeria. The different values were obtained by Bityutskaya et al. [3] in the fresh meat of *Anadara kagoshimensis*, *Donax trunculus* and *Chamelea gallina* clams collected from the Sea of Azov in Russia. This difference could be explained by the fact that some aquatic organisms such as bivalves show significant seasonal variations in chemical composition. They can also vary from one species and from one individual to another depending on age, diet, sex, area and sampling period. It has also been shown that water content may depend on the portion collected. The work of Chouba et al. [22] reported a mean value of 28% water content in the visceral mass of *Venerupis decussata* clams. The part of the sample taken would also be a significant factor influencing the water content of the product analyzed. The latter also showed that protein contents ranging from 48% to 58%, and total lipid contents ranging from 3 to 5% on clam meat vary according to the collection areas. This difference could be attributed on the one hand to the method used for the determination of proteins and the extraction of clam oils. On the other hand, the increase in protein and lipid content is observed in situations of dehydration of the product. The significant decrease in water content during the treatments would have led to an increase in the protein and lipid content of the product. These phenomena of dehydration who depends on the conditions of the smoking method, concentrate the dry matter and those of nutrients contents [23].

The significantly lower water content of smoked clams is caused by smoking techniques that would be applied in the same way in both Districts. Smoking is the preferred method of shore dwellers since it extends the shelf life of large quantities of harvested clams. According to Rifna

et al. [20] the use of heat, natural drying, forced drying, freezing, crystallization and osmotic concentrations are the basic techniques applied to reduce the water content of food. Trisyani et al. [24] obtained lower water content in dried clam meat of *Solen sp.* in Indonesia. The results obtained are also comparable to similar studies conducted on smoked fish [25,26,27]. But, the smoking time of fish products is not long enough to reduce moisture to the recommended level of 10% or less [28]. The moisture contents obtained in the studied District are higher than this standard, and this would be explained on the one hand by the different treatments applied such as de-roasting and sun drying which are two treatments that favor the reduction of water following the coagulation of proteins before the actual smoking. According to Assogba et al. [29] water losses during cooking are often related to the denaturation of muscle proteins after the death of the animal. On the other hand, storage conditions could have an influence on the dehydration of the smoked product. In the case of this study, the smoked products are spread out on shelves in the kitchens that continue to dry the clams thanks to the heat produced by the wood fire. This measure is taken by the local residents to avoid the appearance of insects and mold on the smoked products during storage. The water content would therefore correlate with the ash content of the products. The ash content of *G. schwabi* is between 4.50 and 6.50%, which would indicate that the clam would contain many minerals regardless of its form (fresh or smoked). The loss of water in the food would obviously lead to an increase in ash content. Trisyani et al. [24] obtained 12.28% on fresh meat, and 3.99% on dried meat of *Solen sp.* These values are still high compared to those of Bityutskaya et al. [3] who obtained ash contents below 2.00%.

Minerals are inorganic nutrients, which generally require small amounts ranging from less than 1 to 2500 mg per day depending on the mineral [30]. They are known to play an important role in maintaining various biochemical activities in the body. Analysis of Na, Mg and Ca minerals from *G. schwabi* from the lower Sanaga, showed that regardless of its form (fresh or smoked) or the area sampled, calcium has the highest values than the others. This could be explained by the fact that *G. schwabi* clam is a bivalve that lives buried on the sediment, and filters organic or inorganic particles from detritus suspended in the water to feed. Calcium plays an important role in human and animal nutrition, as it contributes to the normal development and maintenance of bones and teeth, the clotting of the blood, the nervous irritability of the blood, the normal action of the heart, muscle activity and the activation of enzymes [5]. Furthermore, the roles of potassium, magnesium and calcium have been demonstrated in the prevention and treatment of hypertension [31]. Our results are comparable to the work done on clam meat from the Sea of Azov [3] and those of Yelengwe Ndjamou et al [32] done on smoking and fresh clam meat from the lower Sanaga. Similar observations were obtained on the meat of the clam *Venerupis decussata* from different regions of the Tunisian coastline [22] and *Mercenaria mercenaria* from the Ekowe community in Nigeria [5]. The low Na content of *G. schwabi* smoked in Malimba District awakens that it is an excellent food for people with heart conditions such

as high blood pressure. During the pre-treatment process, there would be a significant leakage of soluble mineral salts into the cooking liquid during the detoxification. Although the smoking process is applied in the same way in both Districts, the boiling time during the de-broiling phase would be the element that marks the difference between the values obtained. The cooking processes carried out under certain temperature and pressure conditions can lead either to the production of substances harmful to humans or to the loss of certain important nutrients.

Three metallic contaminants, toxic to humans, notably lead (Pb), cadmium (Cd) and mercury (Hg), are considered from the point of view of their pathway in the environment, in particular their access to the consumer through food of marine origin [8]. Although in low concentrations, they accumulate in the flesh of bivalves [33]. The mercury value of clam reported here (less than  $0.02\mu\text{g/g}$ ) was lower than what reported by Fauziah et al. [34] who reported an average value of  $0.35\mu\text{g/g}$  for meat clam *Batissa violacea* from the Teuom river in Indonesia. In fact, mercury is introduced into the environment through cosmetic products as well as manufacturing processes like making of sodium hydroxide. It is toxic and has no known function in human biochemistry and physiology. Inorganic forms of mercury cause spontaneous abortion, congenital malformation, gingivitis, stomatitis, neurological disorders [7]. The level of Pb and Cd obtained in the flesh of *G. schwabi* in the Yakalak District varied according to the sampling area and the type of product analyzed (fresh or smoked). The results observed are comparable to those on clams [4,5,6,32], and on processed fish meat and crab [23,35]. However, the work done by Chouba et al. [22] in Tunisia does not corroborate with the results obtained in the present study. They reported low concentrations of Pb and Cd in the visceral mass of the clam *Venerupis decussata* depending on the sampling stations. Meteigner et Pierriere-Rumebe [36] obtained similar results when assessing the chemical quality of areas producing burrowing shellfish in the Arcachon basin. Assessment of the toxicity of these two heavy metals (Cd and Pb) in Asian clam *Corbicula fluminea* was done by Ai Yin Sow et al. [37]. The high concentration of Pb and Cd in the clam *G. schwabi* from the lower Sanaga is not surprising and could be explained on the one hand by the mode of nutrition and the state of the living environment of the clam, and on the other hand by the method used for the treatment (smoking). Regarding its mode of nutrition and its living environment, many works have shown that clams feed by filtration accumulating different particles, pollutants and microorganisms and their mode of contamination in water [36,38]. In accordance with what has been observed, the clams of the Lower Sanaga are mollusks living buried in the sand. The surrounding environment would therefore be a factor that could promote the increase of heavy metals Cd and Pb in clam meat through water pollution due to anthropogenic activities. Water pollutants that are either physical or chemical have various origins among which: phytosanitary products, nitrates and phosphates (eutrophication), Polychlorinated Biphenyls (PCBs), by hydrocarbons (oil spill), by bacteria (fecal coliforms), by acid mine drainage, by heavy metals (cadmium, lead, etc.) [39]. It should be noted that industrial development

located upstream of the clam fishing area (metal company, hydroelectric dams), and the discharge of their effluents into the water, could lead to an increase in the level of these two heavy metals in fresh and smoked clam meat in Mouanko. According to Clemens and Feng [40], Cd, Pb, and Hg are all highly toxic to both plants and humans in their ionic forms. These elements can damage different cellular structures and a variety of tissues and organs. Low-dose exposure far below the thresholds for acute toxicity can cause disease because of long-term bioaccumulation in the human body.

## 5. Conclusion

In conclusion, analysis of the proximate composition of *G. schwabi* meat clam of the Lower Sanaga, indicated the presence of nutrients protein, lipid, mineral, and could serve as a source of nutrients for the maintenance of healthy body. The heavy metal content of fresh and smoked clam was analyzed to ensure that it was safe for consumption. The concentration of Pb and Cd is high compared to the limit proposed by the WHO standard. However, the heavy metals analyzed raise questions among local residents about the pollution of the ecological environment of the *G. schwabi* clam in the Lower Sanaga. The presence of Cd, Hg and Pb in the fresh and smoked tissues of clam implies that human activities that release these heavy metals into the environment should be put in check.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that there are no conflicts of interest relating to this article.

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## Authors' Contributions

This research work, design and analysis were carried out by all the authors. The manuscript was put together by AOA. The final version was read and approved by

all authors. All authors read and approved the final manuscript.

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