Open Access Research Journal of
Life Sciences
Journals home page: https://oarjpublication/journals/oarjls/
ISSN: 2783-025X (Online)

OARJ OPEN ACCESS RESEARCH JOURNALS

(RESEARCH ARTICLE)

Biochemical Composition and Microorganisms Associated with *Senilia senilis* (Linnaeus, 1758) in Mangrove Swamps of Iko Estuary, Southeast, Nigeria

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Open Access Research Journal of Life Sciences, 2022, 03(01), 127–139

Publication history: Received on 14 January 2022; revised on 09 March 2022; accepted on 11 March 2022

Article DOI: https://doi.org/10.53022/oarjls.2022.3.1.0028

Abstract

The bloody cockle (*Senilia senilis*), has been harvested by humans over thousands of years and can therefore be a good candidate for assessing past variations of key environmental parameters. Proximate and Microbes associated with *S. senilis* were evaluated as indices for food safety and biomarker of pollution. Standard microbiological techniques and standard methods of AOAC were employed. Results showed that *Bacillus subtilis, Micrococcus species, Proteus species, Klebsiella species, Staphylococcus aureus, Vibrio cholerea, Vibrio parahaemolyticus, Enterobacter sp, Escherichia coli, Bacillus cereus,* and *Chromatium species* were the probable bacteria while *Rhizopus stolonifer*, *Aspergillus niger, Penicillium species, Candida tropicalis, Fusarium species* and *Aspergillus flavus* were the probable fungi isolated from the sample. The mean crude protein, moisture, carbohydrate, ash, lipid and crude fibre contents of the soft tissues were 55.86±2.80, 45.01±1.73, 28.59±2.88, 5.96±0.35, 6.61±0.81 and 4.52±0.19% respectively, while the energy or caloric value was 397.65±11.97 Kcal. The mean Total Heterotrophic Bacterial Count, Total Vibrio Count, Total Coliform Count, Total Salmonella Shigella, Total Staphylococcus Count and Total Fungal Count in fresh sample were $1.72\pm0.85x10^5$, 7.62±1.11x10, 3.33±1.73x10, $2.40\pm0.14x10^2$, $2.08\pm0.21x10^1$ and $3.33\pm0.68x10^4$ cfu g^{-1} respectively and concentrated mostly in the gut. Although, *S. senilis* is highly nutritious, the high microbial load make its consumption life threatening. Proper monitoring and surveillance should therefore be adopted by Government and non-governmental agencies to check pollution of the aquatic environments and proper processing should be adopted before consumption for good public health.

Keywords: Ash; Marine; Nutrient; Protein; Seafood; Shellfish

1. Introduction

The Niger Delta region of Niger is very rich in biodiversity, especially bivalve molluscs assemblage [1]. The intertidal mudflat of Iko estuary provides excellent habitat and spawning ground for commercially important shellfishes species such as Bloody cockle (*Senilia senilis*), Knife clam (*Tagelus adansonii*), Donax clam (*Donax rugosus*) and Mangrove oyster (*Crassosstrea gasar*). The West Africa bloody cockle *S. (=Anadara) senilis* is one of the 220 species of bivalves in the family arcidae. The species is so called because of the red liquid when seeing or smelling blood cockle. This red liquid is similar to human blood. The red corpuscle carries haemoglobin which helps it to survive in low level oxygen environment. They inhabits West African estuaries and lagoons and occur naturally from Senegal to Angola in the West African coastal region. *Senilia senilis* lives in habitats with high temperature all the year round, the annual variation being only 5 °C (ranges of 26.7-31.6 °C). The salinity range tolerated by the species is between 7.5 and 27.5%. The maximum depth at which *S. senilis* lives is 5 m. Larvae settle preferentially in water less than 3 m deep. These observation suggest that the distribution of *S. senilis* is influenced by water depth [2]. However, the region is constantly receiving pollutants from different sources such as oxygen demanding organic waste mostly from industrial, municipal/urban and domestic activities; pathogens, plant nutrients, certain organic chemicals, minerals and trace

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metal and radioactive minerals and compounds [3]. According to Solomon *et al*., [4], streams, rivers and lagoons of the region are highly polluted and poses great ecological threat to the ichthyo-faunal assemblage and human consuming them. Many infectious outbreaks such as vibriosis, shigellosis, cholera, salmonellosis and hepatitis A have been linked with the consumption of clam [5 – 7]. The bloody cockle *S. senilis*, has been harvested by humans over thousands of years and can therefore be a good candidate for assessing past variations of key environmental parameters such as temperature, primary production, and Saharan dust advection within West African coastal ecosystems. They are well known seafood sources of animal protein, and are commonly sold in most fish markets, especially those located close to coastal areas. Food composition described in terms of classes of substances is generally referred to as proximate composition. Information on the gross chemical or proximate composition of marine invertebrates including shellfishes contributes remarkably to our understanding of the species. Analysis of proximate composition such as protein, lipids and carbohydrate as well as other essential minerals has great importance since they are major constituents of living matter [8, 9]. Measurements of such biochemical composition is necessary to ensure that they meet the requirements of food regulations and commercial specifications. Evaluation of the microbial load is also important for safety assurance of this important species of bivalve. There is paucity of information on the proximate composition and microbes associated with *S. senilis* from this study area. This study therefore seeks to present information on the biochemical profile and microbes associated with *S. senilis* from this area.

2. Material and Methods

2.1 Description of the Sampling Site

Eastern Obolo L.G.A. of Akwa Ibom State, Nigeria lies between longitude 7^{0}35’ E and latitude 4^{0}30’ N (Fig. 1). It has many tributaries and creeks that drain into the Atlantic Ocean and the Bight of Bonny. The climate of the area is tropical with distinct rainy (April to October) and dry season (October to May) with a high annual rainfall averaging about 250 mm. The area is characterized by an extensive mangrove swamp with inter-tidal flats influenced by the semi-diurnal tidal regime of estuary [10]. The Obolo (Andoni) are bounded by the Reo-real estuary in the west and the Cross-River estuary in the East. On the North and South are the Ogoni, Ibibio and Atlantic Ocean [11].

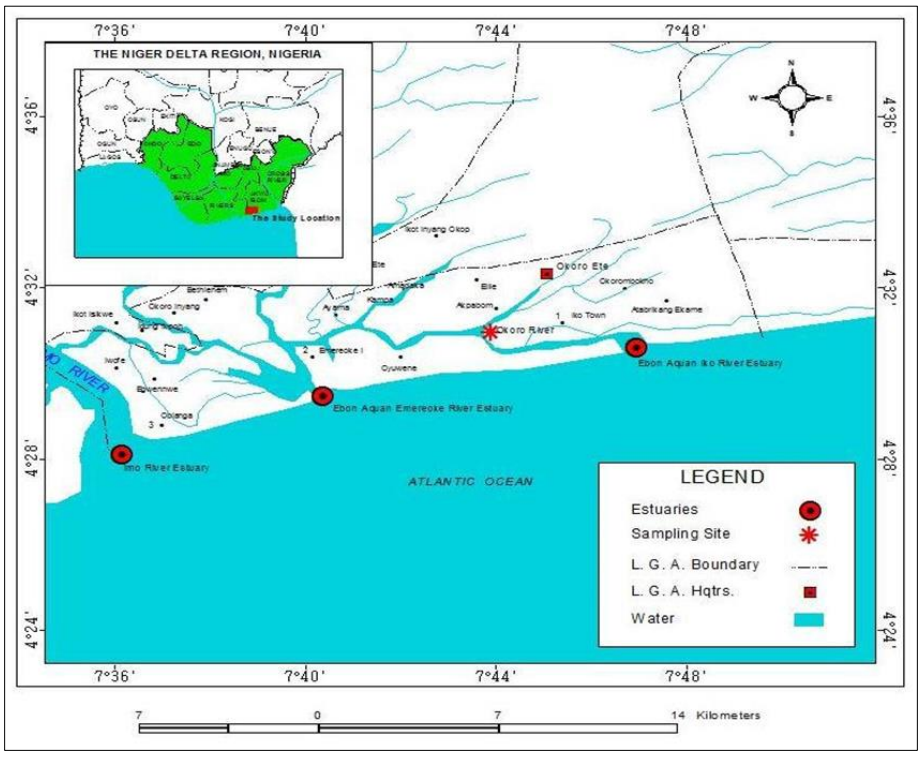

Figure 1 Niger Delta Region of Nigeria Showing the Sampling Sites

2.2 Sample Collection and Preparation

West African bloody cockles were collected monthly (January – December 2019) from women fisher folks on the Iko Town River landings areas. All samples were collected in sterile polythene bags, transported to the laboratory within an hour of collection, and held at 4 °C until tested. The shellfish shells were scrubbed and aseptically shucked into sterile polythene bags, diluted, and macerated (Colworth 400 Stomacher) as required for each proximate and microbiological analyses.

2.3 Laboratory Analyses

Samples of *S. senilis* were first cleansed with a brush and water to remove all materials adhering to the shells and allowed to air dry. Care was taken to ensure that all the fresh clams sample analyzed were healthy-looking in appearance and with no signs of diseases infections noted. Soft tissues of *S. senilis* were extracted from their shells using a sterile stainless-steel knife as recommended in previous studies [12 – 14]. Soft tissues of *S. senilis* were dried by thinly spreading them in a hot-box oven at a temperature of 105 °C for 48 hours [15, 14], until they were sufficiently dried to be ground. Porcelain mortar and pestle were used to grind sampling units of each class size to fine powdery form and then screened through a sieve of 2 mm mesh size [16] for analyses of proximate composition.

2.3.1. Proximate Composition

Proximate analysis was carried out in triplicate according to procedures described by Yolanda [17]. Ash content was determined by ashing samples in an electric muffle furnace at 500 °C for 3 hours. Moisture content was determined by heating 1g oven-dried finely ground sample in a hot box oven at 105 °C to a constant dry weight. Protein content was determined by the Kjeldahl method in which the total nitrogen content of the sample was first estimated from which crude protein content was estimated by multiplying by the conventional factor of 6.25 (i.e. %N x 6.25). Lipid was estimated by the batch solvent extraction technique in which lipid was extracted using diethyl ether as solvent and the lipid content of sample determined by evaporating the solvent [15]. The carbohydrate content was estimated as the Nitrogen-Free-Extract (NFE) by subtracting the sum of the weights of protein, fiber, and ash from the total dry matter of sample.

2.3.2. Estimation of Caloric Value (Energy)

The caloric value of the sample was obtained by multiplying the value of the crude protein, lipid and carbohydrate by 4, 9, 4 Kcal respectively and taking the sum of the product.

2.4 Preparation for Microbiological Analysis

The samples were all deshelled with a sterile surgical blade. All utensils were washed and dried. Nutrient agar was weighed to a beaker and covered with foil paper. The quantity measured depends on the given dosage. The formula used for the measurement is given as

$$2x \div x/2 \qquad (1)$$

Where x is the given dosage in gram.

Though any agar (nutrients that help the parameter of the media to grow) can make all the microorganisms available to grow, however the suitable nutrient agar for each media will identify its specific parameters by causing the parameters to show its distinguished colour. The agar was diluted by adding sterile water to it, all ingredients were sterilized. Agar such as Potato Dextrose Agar (PDA), MacConkey Agar (MCA), Monnitol Salt Agar (MSA), were sterilized using pressure from the autoclave. However, Eosine Ethylene Blue Agar (EMBA), Salmonella Shigella Agar (SSA), Theosulphate Citrate Bile-salt Agar (TCBA) were sterilized by heating in a hot plate so that the ingredients do not appear potent.

2.5 Serial Dilution of Homogenate

One ml of the supernatant was drawn into the syringe for further dilutions. The dilutions of the media known as serial dilution helps to reduce the amount (in terms of abundance) of cells of the different parameters so that it will not be over crowded when view from the microscope. When diluted, the cells will be scanty while each individual parameter cells are still identified. Serial dilution can be carried out into many powers as power 1, 2, 3, 4, 5 etc. This involves drawing the supernatant into a syringe and injecting it into a sterile water in test tube. After the first dilution which is power 1, 1 ml of power 1 is drawn into another sterile water in another test tube that is power 2. After diluting another sterile water presents in another test tube i.e. power 3 and so on. Power 3 was used for the test. After preparing the

sterile dilution of power 3, one ml was drawn into a petri dish. The media was prepared in triplicate. Twelve petri dish were used. One ml of power 3 was put into 1 petri dish and all petri dishes were filled with the media and covered accordingly. The sterile ingredient which was stirred concomitantly with the preparation of media was allowed to cool. The ingredient which identifies a colony of cells of a particular parameter was mixed with the media in the petri dish and allowed to solidify. Each active ingredient was capable of identifying the distinguish column of microorganisms of the media.

2.5.1. Estimation of Microbial Load of Samples

Standard microbiological techniques described by Harrigan and McCance [18] were employed for the analyses.

2.5.2. Determination of Heterotrophic Bacteria

The total heterotrophic bacterial and fungal count in the sample were determined by the pour plate and spread plate method (this method employs putting the serial diluted sample into a petri dish and then putting the nutrient agar to it) using Nutrient agar (NA) and Saboraud Dextrose Agar (SDA) as the analytical media respectively. The plates were incubated at 28 °C for 24 hours and 5-7 days for bacterial and fungal densities respectively.

2.5.3. Densities of Population Indicator Bacteria

Using the same pour plate and spread method, the coliform count, fecal coliform (*Escherichia coli) Salmonella* and *Shigella, Vibrio, Staphylococcus aureus and Pseudomonas* were estimated using MacConkey agar (MA), Eosine Methylene Blue Agar (EMBA), Salmonella Shigella Agar (SSA), Thiosulphate Citrate Bile-salt Sucrose agar (TCBS), Mannitol Salt Agar (MSA), Pseudomonas Isolation agar (centrimide agar) as analytical media respectively [19] Incubation of Culture Plates and Counting of Microbial Colonies. The bacterial and fungal plates were incubated for 24 hours at 28 °C. Microbial colonies that emerged on the incubated plate were counted with the aid of Quebec colony counter and recorded as colony forming unit per gram of the sample (cfu g^{-1}).

2.5.4. Purification and Maintenance of Microbial Isolates

Representative colonies from culture plates were picked for characterization. These colonies were repeatedly sub-cultured into freshly prepared nutrient agar plate by streaking methods and incubated for growth at 28 °C for 24 hours before transferring them to agar slant [20]. The pure isolates of bacteria were maintained on agar slant as stock and preserved in the refrigerator for further use.

2.6 Characterization and Identification of Microbial Isolates

Bacterial isolates were characterized and identified presumptively based on their morphological, cultural and physiological characteristics, confirmatory identification was based on biochemical characteristics. The following biochemical tests were carried out: Gram staining, motility, coagulase, catalase, spore staining, crease, and citrate.

2.6.1. Gram's Reaction

The gram staining was done according to the method described by Bergey and Holts [21]. It differentiates bacteria into gram-positive and gram-negative organisms. A heat fixed smear from 18-24 hours old culture was prepared on a clean slide and stained with crystal violet solution for 60 seconds. It was washed off with distilled water and flooded with Lugol's iodine for 60 seconds, after which it was rinsed off with distilled water. The iodine served as a mordant. Seventy percent alcohol was then used to decolorize and washed off immediately (1-2 seconds) with water safranin, a counterstain was applied and allowed for 30 seconds, rinsed with water, air dried and the slide observed under oil immersion objective lens (x 100). Gram-negative bacteria were decolorized by alcohol and hence took up the colour of the counter stain appearing pinkish while Gram-positive bacteria appeared purple or violet because they retained the colour of the primary dye which was crystal violet.

2.6.2. Coagulase Test

This test was carried out to detect the ability of organism to agglutinate human blood plasma by producing the protein called coagulase. Coagulase activity is used to distinguish between pathogenic and non-pathogenic organisms. The test organisms were placed on a clean slide and a drop of a loopful saline was added to it and mixed properly. A drop of plasma was then added and mixed gently. Visible clumping reaction by the test organism after 5-10 seconds indicated positive reaction while no clumping/agglutination reaction showed negative result.

2.6.3. Catalase Test

Most bacteria that utilize oxygen (aerobes and facultative anaerobes) in their respiration produce hydrogen peroxide which is toxic to their own enzyme systems. Their survival is due to the production of catalase which converts hydrogen peroxide to water and oxygen. This test was performed to determine the ability of the isolates to breakdown hydrogen peroxide with the production of oxygen and water (O_2 and H_2O). Precisely 3% hydrogen peroxide was used. The test was carried out by bringing the test isolates in contact with the 3% hydrogen peroxide (H_2O_2). The production of effervescence gas bubbling immediately indicated a positive reaction, while the non-production of effervescence, indicated a catalase negative reaction.

2.6.4. Indole Test

This test was performed to determine the ability of an organism to breakdown the amino acid tryptophan with the production of indole. Recently, tryptone is preferred since it contains more tryptophan. Precisely 3 milligrams of sterile tryptone water was put in each test tube and the test organisms were inoculated in the tubes. It was incubated for 48 hours at 30 ℃ after 48 hours, 0.5 ml of Kovac's reagent was added into each of the test tubes and mixed properly, then it was observed after 10 minute for the formation of a red ring at the top layer of the tubes which indicated a positive result while the absence of red ring showed a negative result.

2.6.5. Urease Test

This test was based on the ability of the test isolates to produce urease enzyme which split urea to produce ammonia and carbon dioxide and water. Urea agar was employed. The urease test was used to distinguish members of proteus from others or lactose fermenting enteric bacteria. Test tubes containing urea agar on slants was inoculated with the test organisms and incubated at 30 ℃ for 24 hours. The culture was observed for change in colour from yellow to pink which indicated a positive result.

2.6.6. Citrate Utilization Test

This test identifies members of the family Enterobacteriaceae and was performed to detect the ability of an organism to utilize citrate as its sole source of carbon and energy. The prepared agar was sterilized by autoclaving for 15 minutes at 121 ℃. The agar was cooled and poured into plates and allowed to set. The test organisms were inoculated into the plate and incubated at 37 ℃ for 48 hours. A positive result was indicated by a change in coloration from green to blue.

2.6.7. mr-vp Test

This distinguishes between organisms which produce and maintain a high acidity from those producing an initially lower acidity which reverts towards neutrality. Voges proskauer test is used to determine the formation of acetyl carbinol (acetone) from dextrose. This is oxidized by the reagent to diacetyle which produces a red colour with guanidine.

2.6.8. Motility Test

This test was performed to differentiate between the mobile and non-motile bacterial species. The motility of isolates was detected by cultivation in semi-solid nutrient agar. Semi Solid nutrient agar in test tube were inoculated with the test organisms using the inoculating needle by stabbing method. The needle was used to pick the organisms after which they were stabbed into the agar and incubated for 24 hours at 30 ℃, then observed for growth. Occurrence of growth along the line of stabbing indicated a positive result, which means organism is motile.

2.6.9. Spore Staining Test

Certain bacteria, particularly members of the genera *Bacillus and Clostridium* produce endospores. Spores are resistant structures capable of surviving for long periods in an unfavourable environment and then give rise to new bacterial cells. This test was carried out to test the presence of spores in the test isolates. The bacterial smears were prepared as that used for Gram staining technique. The slides were heat-fixed by passing it several times over the flame. Thereafter, the slides were placed over the boiling water flooded with malachite green stain and left for 10 minutes. Washed with slow-running water, the slides were then flooded with safranin for 20 seconds, washed under tap water and then blot dried. The slides were viewed under oil immersion objective. The vegetative portion of the bacterium stained pink while the spore stained green.

2.6.10. Starch Hydrolysis

In this reaction, carbon compounds serve as substrate for biochemical activities which involve the production of specific enzymes that degrade the compound. The test organisms were inoculated on starch agar and incubated at 37°C for 24 hours. The surface was flooded with Lugol's iodine. Isolates which have the amylase enzyme hydrolyzed the starch which gave a positive result, but the blue-black coloration was a negative result.

2.6.11. Haemolysis test

This test provides information on haemolytic enzyme, a bacterium possesses by inoculating the test organisms on blood agar. Haemolysis was determined by streaking the test organism on a blood agar plate and incubating for 18-24 hours at 37 °C after which haemolytic activity was observed (alpha, beta or gamma haemolysis). Beta-haemolysis is indicated by zones around the line of streak. Discoloured or darkened medium demonstrates alpha-haemolysis while no discernible change in the colour of the medium shows gamma haemolysis.

2.7 Statistical analysis

All findings for the most-probable number (MPN) for Total Heterotrophic Bacterial count (THBC), Total Vibrio Count (TVC), Total Salmonella Shigella (TSS), Total Coliform Count (TCC) Total Staphylococcal Count (TSC) and Total Fungal Count (TFC), were analyzed using analysis of variance (ANOVA). Where significant difference existed ($p<0.05$), *post hoc* test was used to separate the mean using Duncan Multiple Range Test (DMRT). IBM SPSS statistical version 20 was used.

3. Results

3.1 Proximate Composition

Proximate Composition of bloody cockle is shown in table 1. The moisture content of the mollusc ranged from 3.75 to 4.50%, showing significant ($P<0.05$) variation in their moisture content and was higher in large class. The ash content ranged from 2.43 to 5.96% showing significant difference ($P<0.05$) among the three classes of cockle. The fibre content ranged from 2.11 to 4.52% and was significantly higher in the large sized cockle. The protein content of the molluscs ranged from 39.89 to 55.81%. The large class of *S. senilis* showed significantly higher ($P<0.05$) protein content. Lipid content ranged from 3.76 to 6.61% and was significantly higher ($P<0.05$) in large class. Carbohydrate content ranged from 18.28 to 28.59% and was also significantly higher ($P<0.05$) in the large class of cockle.

Table 1 Proximate Composition of African bloody cockle in Mangrove Swamps of Iko Estuary, Southeast, Nigeria

Parameters (%)	Mean	Standard Deviation
Moisture	45.01	1.73
Ash	5.96	0.35
Fibre	4.52	0.19
Protein	55.81	2.80
Lipid	6.61	0.18
CHO	28.59	2.88
Caloric value (Kcal)	401.07	10.35

CHO = Carbohydrate

3.2 Caloric value (energy)

Caloric value ranged from 397.65 to 401.07 kcal and there was no significant difference ($P>0.05$) in the caloric values of the different classes of molluscs.

3.3 Isolation, Characterization and Identification of Bacterial Isolates from *Senilia Senilis*

The type of Bacterial shapes, odour, pigment, elevation, colour, consistency optical characteristic, Gram reaction catalyst, coagulase, motility, starch hydrolase, citrate, urease, mr vp, spore formation, H_2S, Hae, Glucose, maltose, lactose, fructose, sucrose, galactose identified were used to isolate the present of *B. substilis, Micrococcus sp, Proteus sp, Klebsiella*

sp, Staphylococcus aureus, V. cholerea, Enterobacter sp, Shigella Spp., Bacillus aureus and *Chromatium Spp.* as most probable organisms found in *S. senilis*. This is shown in Table 2.

Table 2 Cultural, Morphological, Structural and Biochemical Characteristic of Bacterial isolate from *Senilis senilis* caught in Mangrove Swamps of Iko Estuary, Southeast, Nigeria

Bacteria shape	**Rod**	**Cocci in pair**	**Rod**	**Rod**	**Cocci in cluster**	**Cocci**	**Rod**	**Rod**	**Rod**	**Rod**	**Rod**
Gram reaction	+	+	+	-	+	-	-	-	-	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	-
Coagulase	-	-	-	-	+	-	-	-	-	-	-
Motility	+	-	+	+	-	+	-	-	+	+	+
Starch hydrolase	+	+	+	-	-	+	+	+	-	+	+
Citrate	+	+	+	+	-	-	+	-	-	-	+
Urease	-	+	+	+	-	-	-	-	-	-	-
MR	-	+	+	+	-	-	-	-	+	-	-
VP	+	-	-	-	+	+	+	-	-	+	+
Form spore	+	-	-	+	-	-	+	-	-	-	+
H_2S	+	-	-	+	-	-	-	+	-	+	+
Haemolysis	-	-	-	-	β	-	-	-	-	-	-
Glucose	AG	-	AG	AG	A	A	A	AG	A	AG	AG
Maltose	A	A	A	AG	A	A	A	AG	AG	A	AG
Lactose	-	+-	AG	AG	-	-	AG	AG	-	AG	+
Fructose	A	A	AG	-	AG	A	A	AG	-	A	-
Sucrose	-	-	AG	AG	A	AG	-	AG	-	-	A
Galactose	A	A	AG	AG	A	A	A	AG	AG	-	AG
Mannitol	-	-	-	-	AG	-	-	-	-	AG	-
Probable organism	*Bacillus subtilis*	*Micrococcus sp*	*Proteus sp.*	*Klebsiella sp.*	*Staphylococcus aureus*	*Vibrio cholera*	*Vibrio parahaemolyticus*	*Enterobacter sp.*	*Escherichia coli*	*Bacillus cereus*	*Chromatium sp.*

Key: +=positive reaction, - = negative reaction, A=Acid only, AG=Acid and Gas, β=beta haemolysis

3.4 Isolation, characterization and identification of fungal isolates from *Senilia senilis*

The type of fungal pigmentation, soma, special vegetative structure, asexual spore, special reproductive structure, conidial head, vesicles shape and nature of hyphae identified were used to isolate the presents of *Aspergillus terreus, Rhizopus stolonifer*, *Aspergillus flavus*, *Aspergillus fumigates, Mucor* and *Candida tropicalis* as probable organisms found in *S. senilis*. This is show in Table 3.

Table 3 Cultural, Morphological, Structural and Biochemical Characteristic of Fungal isolates from Senilia senilis from caught in Mangrove Swamps of Iko Estuary, Southeast, Nigeria

Pigmentation	Type of Soma	Nature of hyphae	Special vegetable structure	Asexual spore	Special reproductive structure	Conidial head	Vesicles shape	Probable organism
White becoming greyish	Filamentous	Coenocytic	Stolon rhizoids	Ovoid sporangiospores	Tall sporangiophores in group, black brown sporangia	-	-	*Rhizopus stolonifer*
Black colony	Filamentous	Septate	Foot cell	Globose conidia	Smooth wall erects conidiophores	Globose	Globose	*Aspergillus niger*
Dark green colony	Filamentous	Septate	Broom like appearance	Globose conidia produce in long columnar	Erect conidiophores terminating in whorls of phialides	-	-	*Penicillum sp*
Creamy white colony	Pseudo-hyphae	Septate	Apothelium	Blastoconidia	Conidia	Radiate	Globose	*Candida tropicalis*
pink	Filamentous	Septate	-	Micro conidia	Short branch conidiophores	-	-	*Fusarium* sp
Yellow	Filamentous	Septate	Foot cell	Globose conidia	Phialides born directly on the vesicle sclerotia	Radiate	Sub-globose	*Aspergillus flavus*

3.5 Microbial load of *Senilia senilis*

Microbial loads of the sample are shown in Table 4. Mean THBC of 1.72 $\times 10^5$ cfu g^{-1} with the least seen in shell and the highest in the gut. Mean TVC was 7.62 $x10^{-1}$ cfu g^{-1} with the least seen shell and the highest in gut. Mean TCC was 3.33 $\times 10^1$ cfu g^{-1} with the least in clam flesh and the highest in gut. Mean TSS of 2.40 $\times 10^2$ cfu g^{-1} was recorded and followed the same pattern as others. Mean TSC was 5.62 x 10^1 cfu g^{-1} with no staphylococcal count on the shell. Mean TFC was 3.33×10^4 cfu g^{-1} with least in shell and highest in clam homogenized flesh. Total heterotrophic Bacteria Counts (THBC) in clam gut was significantly higher ($p<0.05$) compared to flesh and shell. Total Vibrio Count (TVC), Total Coliforms Count (TCC), Total Salmonella Shigella (TSS) and Total Staphylococcal Count (TSC) portrayed a similar trend, being significantly higher ($p<0.05$) in the gut than flesh and shell. The reverse was the case of Total Fungal Count (TFC) which was higher in the flesh than the gut.

Table 4 Microbial load (cfu g^{-1}) of *Senilia senilis* samples caught in Mangrove Swamps of Iko Estuary, Southeast, Nigeria

Anatomical sites	THBC ($x10^5$)	TVC ($x10^1$)	TCC ($x10^1$)	TSS ($x10^2$)	TSC ($x10^1$)	TFC ($x10^4$)
Clam gut	1.92 ± 2.11^c	8.28 ± 2.33^c	3.58 ± 1.52^c	3.02 ± 2.12	4.53 ± 2.64^a	3.30 ± 1.58^b
Clam flesh	1.64 ± 1.55^a	7.82 ± 2.67^b	2.70 ± 1.62^a	2.10 ± 1.91	6.71 ± 3.12^c	4.34 ± 1.42^c
Clam shell	1.61 ± 1.72^a	6.75 ± 1.83^a	3.71 ± 1.01^d	2.09 ± 2.50^a	-	2.35 ± 1.21^a
MEAN±SD	1.72 ± 0.85^b	7.62 ± 1.11^b	3.33 ± 1.73^b	2.40 ± 0.14^c	5.62 ± 0.96^a	3.33 ± 0.68^b

Mean±sd in the same column with different alphabets are significantly different ($p<0.05$). Values are mean ± SD. THBC = Total Heterotrophic Bacterial count, TFC = Total fungal count TVC= Total Vibrio Count TCC = Total Coliform Count, TSS = Total *Salmonella shigella,* TSC = Total Staphylococcal Count.

4. Discussion

The results obtained from this study showed that *S. senilis* has relatively low moisture content. The range of values for moisture (37.61±0.98-45.05±1.73) obtained from this study show that they are lower than that of oyster, clam, rough and smooth periwinkle and whelk which recorded 73.37, 73.72, 84.80, 80.22 and 60.97 percent respectively [22]. However, these values are higher than 3.29±0.45 - 4.65±0.33% reported by Umesi *et al*., [23] for *S. senilis* from the Andoni River. These variations in moisture content of these molluscs could be due to the effect of environment as reported by Osibona *et al.*, [24]. Ash content was about 60% higher in large class clam than in lower class and about 40% higher that of the medium class clam. The range of 2.43 to 5.96% for ash reported in this study is similar to that of Umesi *et al*., [23] which ranged from a minimum in small-sized clams (2.32 ± 0.16%) to a maximum in large-sized clams (3.48 ± 0.28%). However, these were lower than values reported by Kiin-kabari *et al.*, [22] for other shellfishes which ranged from 6.85% for clam to 14.02% for whelk. High ash content of molluscs is an indication that they are rich in micronutrients (minerals). Crude fibre content was lower than that reported by Umesi *et al*., [23]. However, other works had not reported any fibre content of molluscs therefore this result is subject to more research. The protein content of these mollusc are far higher than 9.97% to 13.96% recorded for other molluscs [22] and 28 – 39% recorded by Umesi *et al*., [23]. The result had shown that molluscs constitute a rich source of protein. According to Das [25] molluscan shellfishes are of high biological value. Protein is the major structural component of cells and is responsible for the building and repair of body tissues. Lipid content ranged from 3.76% to 6.61% for different classes of the mollusc. This lipid contents were high compared to other species of molluscs. Judith and Jenny [26] indicated that consumption of molluscs in large proportion reduced the risk of hypercholesterolemia which is capable of causing cardiovascular disease, due to its high omega-3 fatty acid content. Carbohydrate content ranged from 18.28- 28.59%. This is higher than 0.92%, 0.72%, 0.55%, 0.26%, and 0.93% for oyster, clam, rough periwinkle, smooth periwinkle and whelk respectively reported by [22], 4. 33 – 14.22% by Umesi *et al*., [23] for *S. senilis* and 7.66% carbohydrate reported earlier by Obande *et al.,* [27] for the Fresh water snail (*Pila ampullacea*) from River Benue. High carbohydrate content indicates that high consumption of molluscs can be supplemented with low energy-rich foods to balance the energy-protein intake requirement. This is further supported with high caloric values recorded for these two species of molluscs. Meanwhile, clear variance in carbohydrate, protein, lipid, and fiber contents were noticed in soft tissues of *S. senilis* in which mean values generally increased from a minimum in small-sized clams to peak values in large-sized individuals. The carbohydrate and lipid contents of clams recorded in this study were thrice greater than those of small-sized clams. It is, therefore, unarguable that large-sized clams will provide better nutrients compared to small-sized and medium-sized clams. Size does not seem to affect moisture and lipid content of *S. senilis* especially when comparing medium-sized and large-sized class. The mineral content in the ash provide measures of quality of foods. While ash content measures the total amount of minerals present within a sample, mineral content provides a measure of the amount of specific inorganic components present within a sample. Carbohydrate and lipid also contribute to good food quality. These findings therefore show that consumption of this mollusc can meet the recommended daily dietary allowance for protein and other nutrients of an adult male and female.

Senilia senilis caught in Iko region of the Niger Delta estuaries accumulate and concentrate microbial pathogens and those that are GRAS (Generally Regarded as Safe) present in the water. In all, eleven (11) bacterial species and six (6) fungal species were isolated. *Bacillus subtilis* isolated from the sample was an indication that not only pathogenic microbes but useful ones were also isolated. For instance the isolation of *B. subtilis* was an indication that commercially useful bacteria (GRAS) was present in the samples. According to Martinez [28], *B. subtilis* is important in biotechnology for enzyme production. In food industry, it is used in the production of animal feed additives, sweeteners, fermented products and flavour enhancers. The production of household detergent, vitamins and antibiotics is also accomplished using *B. subtilis.* Above all, it is used in the development of vaccines and in the development of sporicides. *Micrococcus species* is very useful in cheese production. It helps in the ripening cheeses made from farm animals' milk; halotolerant *M. Species* are major component of micro flora of some cheeses and help in desired body, texture and flavour of soft cheese. Although not considered a pathogen, *M. Species* has generally been regarded as opportunistic pathogen since some strains such as *M. luteus* has been implicated as the causative agent of septic arthritis, recurrent bacteraemia etc. and pneumonia in patient with acute leukaemia [29]. Some of the isolates may have been derived from external sources during handling and as such, the clams become transient carriers of such microbes. For instance, isolation of *Proteus* Spp. may mean that the sample was not prepared hygienically so that it entered from the environment and when consumed by humans it can cause osteomyelitis [30]. However, in this study most isolates from the clam gut and homogenized flesh may be accounted for mainly by the filter feeding effect of the clam [31]. *Klebsiella* Spp can causes diseases and spoilage therefore its isolation from *S. senilis* mean that if eaten raw or not well processed it can cause UTIs, wound and respiratory infection as well as serious pneumonia in humans [32]. *Staphylocccus aureus* is pathogenic and has been isolated in the sample. This means that consumption of this sample by human can result in the type of food poisoning known as SFP (staphylococcal food poisoning) and late on-set infection in neonates [33]. *Vibrio cholera* and *Vibrio parahaemolyticus* were also isolated from *S. senilis* and according to Ababouch *et al*, [34] they both cause

gastrointestinal disease. It can hereby be deduced that eating of raw or unprocessed *S. senilis* from this study area can result in outbreak of cholera *V. parahaemolyticus* infection. According to Powel *et al*. [35] *V. parahaemolyticus* has been responsible for several recent seafood-associated outbreaks and therefore considered an emerging bacterial pathogen in Europe. The isolation of *Enterobacter* Spp. from the sample is an indication that consumption of unprocessed clam from this study area can result in enterobacter infections. Such infection include but not restricted to UTIs, bacteraemia, endocarditis, osteomyelitis etc. *Escherichia coli* isolation is an indicator for pathogenic organisms. The isolation of *E. coli* from *S. senilis* in this study area is an indication of fecal contamination by man and his livestock. This is of public health significance as these organisms have generally been agent of gastroenteritis in humans [36 - 38]. For instance ***E. coli*** have been reported to be responsible for foodborne illnesses and deaths [39] through poor processing and handling of foods or farm animals. *Bacillus cereus* is known to cause cutaneous disease. Its isolation from this sample is an indication that eating of unprocessed clam from this water body may result in skin infection as well as pneumonia, meningitis and necrotizing fasciitis [40]. *Chromatium* Spp was also isolated from the sample. It is not pathogenic, rather it is used in photosynthetic sulfur oxidation which is an indication that it entered the clam body via the water body. The six (6) species of pathogenic fungi isolated from *S. senilis* in this study area include: *Rhizopus stolonifer*, *Aspergilus niger*, *Penicillum Spp*, *Candida tropicalis, Fusarium Spp* and *Aspergilus flavus*. This shows that consumption of unprocessed clam from this study area can result in mucormycosis, rot, food spoilage, candidiasis, opportunistic mycosis and production of aflatoxins which can be of serious health implication to human and his livestock. According to Zhai, *et al*. [41] *C. tropicalis* can cause fatal digestive system disease and septicemia. *Aspergillus* Spp has been associated with outbreaks of seafood diseases known as nosocomial aspergillosis which is a serious threat for severely immune-compromised patients [42].

The level of mean THBC reported in this study was higher than the range reported by Udoh *et al*. [43] for *G. paradoxa* in Cross River Estuary and Volta Lake while TCC fell within the range [43]. Total heterotrophic bacteria and coliform may be directly influenced by many anthropogenic activities and rainfall. Run-off from rain might carry raw sewage from the surrounding villages and leachate from waste sites in the catchment area into the freshwater; the clams being filter feeders are able to accumulate the isolates in their tissues to level twice that in the surrounding waters [44]. This is in agreement with Antai [46] who reported that the high microbial load in the sample is a clear indication that clam serves as a medium through which microbes multiplied rapidly. Udoh, *et al*. [43] added that these species of microbes are demonstrating strong resistance to novel. Generally, microbial load was higher in the gut than other parts confirming the fact that clam should be degutted before processing for consumption. *Senilia senilis* are protein rich food and therefore serves as a suitable substrate in supporting growth of different types of bacteria and fungi; the microbial growth in these fresh seafood will encourage food spoilage and seafood poisoning. On the other hand estuaries or rivers are constantly polluted with faecal matter from riverine dwellers. This findings agrees with the work of [47] that shellfish and coastal waters contaminated by human pathogens could be sources of shellfish-borne or water-borne outbreaks. In fact, shellfish can accumulate and concentrate microbial pathogens present in waters by their filter-feeding activities. The risk of disease from these agents varies by pathogen, dose, host and characteristics of the seafood matrix. The extent to which a microbial hazard is likely to be present in seafood and give rise to a public health and safety risk depends on numerous factors, including the biology of the particular seafood species, its growth environment and the specific activities along its production and processing supply chain [48]. This gives relevant information regarding the food safety and sanitary conditions of *S. senilis* and the coastal water [49]. The results obtained from this study also showed that *S. senilis* appears in the river between the months of February – January.

5. Conclusion

The findings from this work showed that bloody cockles contain considerable amount of protein and other nutrients. High values of moisture, ash, fibre, protein, lipid and carbohydrate were recorded in African bloody cockle. With increased consumption of bloody cockle, the serious problem of macronutrient deficiency can be addressed. However, the bio-accessibility of these nutrients and the digestibility of the mollusc's protein should be investigated. These findings also showed that *S. senilis* contains considerable number of pathogenic microorganisms which are major sources of water borne diseases and death. From the foregoing, it has been established that this aquatic ecosystem is under threats of pollution and so clams as well as other aquatic food organisms must be processed properly before consumption. Considering the importance of shellfishes as primary protein source, proper monitoring and surveillance should be adopted by Government to check pollution of the aquatic environments.

Compliance with ethical standards

Acknowledgments

The authors acknowledge the support of Prof. Okon M. Udoidiong of the Department of Fisheries and Aquatic Environmental Management, University of Uyo, Uyo, for species identification. The labouratory Technologists, Mr Bassey Akpan is also acknowledged for his assistance analyses carried out during this project.

Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

No funding was obtained during this project. This was an M. Sc. Project and funding assistance was offered by the project student and the supervisor.

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