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MICROBIOLOGICAL, PROXIMATE AND HEAVY METAL CONCENTRATION IN *PENAEUS* SP. (SHRIMP) AND *CALLLINECTES* SP. (CRAB) FROM CREEKS IN NIGER DELTA NIGERIA

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ABSTRACT

Microbial quality of *Penaeus* sp. and *Callinectes* sp. from Itu and Oron creeks in Niger Delta was investigated. The total bacteria population of samples varied from 1.60×10^7 – 9.70×10^8 cfu/g and 1.03×10^7 – 8.90×10^8 cfu/g for *Penaeus* sp. and *Callinectes* sp. from Itu and Oron creeks respectively. The results show that the samples contain unacceptable levels of bacteria with *Penaeus* sp. from Itu creek contaning up to 9.70×10^8 cfu/g. The coliform levels were generally high ranged from 6.20×10^7 – 9.40×10^7 cfu/g and 5.30×10^7– $8.40 \times \times 10^7$ cfu/g for the samples. The Vibro count varied from 6.90×10^7 – 8.20×10^8 cfu/g and 5.20 – 5.90×10^7 cfu/g for for the samples. The fungi count ranged from 3.10 – 3.70×10^7 cfu/g and 3.20 – 4.00 $\times 10^7$ cfu/g. The bacteria isolates were *E. coli, Streptococus* sp. *Serratia marcesens, Shigella* sp, *Bacllus* sp, *Vibrio* sp. The fungi isolates were *Aspergillus niger, Aspergillus terreus* and *Apergillus flavus.* The total microbial counts obtained from this work were found to be higher than the specified standard limits ($1x10^5$cfu/g) for bacteria and fungi and $1.x10^2$ cfu/g for coliforms) by ICMSF and USFDA. Proximate determination shows that samples from Oron creek were nutritionally richer than that from Itu creek. Percentage protein content for the two creeks ranged from 29.85^d to 54.13^a % in which *Penaeus* sp. from Oron creek significantly had the highest. Callinectes sp. from Oron creek had the highest Ca (0.94^a mg/kg), P (0.74^a mg/kg) and K (0.21^a mg/kg) content. The concentration of metal ranges of Fe, Mg, Zn, Pb, Cd, Mn and Cu in the samples were 17.2^d – 22.7^a mg/kg, 130 – 281^a mg/kg, 24.4^d – 94.2^a mg/kg, 0.28^d– 0.84^a mg/kg, 0.02^d – 0.15^a mg/kg, 24.2^d – 88.6^a mg/kg and 3.4^d – 7.4^a mg/kg respectively. *Penaeus* sp. from Oron creek had the highest concentration of metals except for Pb and Cd. It was observed that different metals were present in the samples at different levels but majority were found to be within the standard limits prescribed by EU and FAO. Crustacean from the creeks in Niger Delta though nutritionally rich harbor pathogenic microorganism and heavy metals which can pose a serious health hazard to consumers as they are not totally safe for human consumption.

Key words: Microbial, *Penaeus* sp., *Callinectes* sp., Metals, Proximate

INTRODUCTION

Crustacean constitutes an important food component for a large section of the world population, more so in developing countries where shellfish forms a cheap source of protein [1]. Among the range of shellfish, shrimps and crabs are the most important thereby accounting for more than 70% of the total earnings of marine export products [2]. Shellfish are nutritionally balanced, an excellent source of proteins, a good source of minerals and some vitamins and are also low in fats and cholesterol [3]. Crabs and shrimps have been used by riverine dwellers in Niger Delta as food condiments and are recommended for pregnant women and protein malnourished cases. They are also known to reduce the risk of coronary heart disease. They are used in weight control and other disorders including cancer [4, 1 and 5].

A survey of the microbiological quality of some shellfish has shown shellfish to harbor pathogenic organisms [6, 7].These pathogenic organisms have been implicated in the outbreak of food-borne diseases in many parts of the world. These illnesses include typhoid fever, hepatitis and similar disorders of the digestive system [8 and 9].

Aquatic organisms, in general, accumulate contaminants from the environment and; therefore, have been extensively used in marine pollution monitoring programmes [10, 11]. Heavy metals discharged into the marine environment can damage both marine species diversity and ecosystem, due to toxicity and accumulative nature of the metals. The consequence of heavy metal pollution can be hazardous to man, based on the level of consumption of shellfish in riverine area, which is relatively high. There is need to check microbial and chemical contaminants in foods from the aquatic environment in order to understand their hazard levels and thus creating awareness to the public on health risks in consuming raw or undercooked and under -processed shellfish.

This study was undertaken to enumerate the microbial quantity as well as proximate, mineral composition and heavy metal concentration in edible portion of *Peanaeus* sp. and *Callinectes* sp. from two creeks in Niger Delta Nigeria in order to evaluate their hazard level in relation to the maximum residual limit for human consumption.

METHODOLOGY

Collection of Samples

A total of one hundred and twenty samples each of shrimp and crab (*Peanaeus* sp. and *Callinectes* sp respectively) used in this study were freshly harvested mechanically from Itu and Oron Creeks in Oron and Itu local government area of Akwa Ibom state, Eastern Nigeria both which are famous shellfish producing areas in Niger Delta, Nigeria. The sample was collected in triplicate. It was placed in ice and brought to the laboratory.

Microbiological analysis

The samples were washed and the edible portion of the meat removed, homogenized and about 10g taken for microbiological analysis. Standard pour plates were prepared from 10 – fold dilutions into nutrient agar medium for total heterotrophic bacteria counts, MacConkey agar was used for total Coliform counts, Salmonella/Shigella agar for total Salmonella/Shigella counts, Thiosulphate citrate bile salt sucrose agar for total Vibrio counts and Sabouraud dextrose agar for total fungal counts. The bacterial plates were incubated at 37^{o}C for 24-48 hours, while fungal plates were incubated at room temperature (28 ± 2^{o}C) for 3-5 days. Colonies were selected randomly and were characterized using morphological and biochemical test such as gram stain, spore stain, motility, catalase, oxidize, coagulase, indole, MR-VP and Urease and sugar fermentation tests. Bacterial isolates were identified with reference to Cowan and Steel's Manual for the identification of Medical Bacteria [12] and Bergey's Manual of Determinative Bacteriology [13]. Fungal isolates were identified based on their morphological and cultural characteristics as recommended by Sampson *et al.*,[14].

Proximate and mineral analysis

Proximate composition was determined according to the method of A.O.A.C. [15]. This includes determination of Ash content, Crude protein, Dry matter, Moisture content and Crude fiber. The mineral contents were also determined using Jenway Digital flame photometer (PFP 7 Model).

Metal concentration analysis

The edible portions of the meat from the samples were removed, homogenized and about 2.5g taken for analysis. Ten milliliters of nitric acid - perchloric acid (10:4) mixture were added to the sample, covered and left overnight at room temperature. The samples were digested, allowed to cool to room temperature, filtered using glass wool and made up to 50ml. The filtered samples were analyzed in triplicate, using Buck 2000 Atomic Absorption spectrophotometer (AAS) as per standard conditions (Table 1). The blanks and calibration standard solutions were also analyzed in the same way as for the samples. All the chemical analyses were carried out in duplicate.

RESULTS

The levels of microbial load in the shrimps and crabs from the two creeks in Niger Delta are shown in Table 2. The total bacteria population of samples varied from 8.50 x 10^7 – 9.70 x 10^8 cfu/g and 1.60 x 10^7 – 1.45 x 10^8 cfu/g for *Penaeus* sp. from Itu and Oron creeks, 7.00 x 10^7 – 8.90 x 10^8 cfu/g and 1.03 x 10^7 - 1.10 x10^8cfu/g for *Callinectes* sp. from Itu and Oron creeks.

Penaeus sp.from Itu creek therefore had the highest level of bacterial contamination. The coliform count ranged from 6.20 – 6.80 x 10^7 cfu/g and 8.90 – 9.40 x 10^7 cfu/g for *Penaeus* sp. from Itu and Oron creeks, 5.30 – 6.40 x 10^7 cfu/g and 7.30 – 8.40 x x 10^7 cfu/g for *Callinectes* sp. from Itu and Oron creeks.

The Salmonellae / Shigella count ranged from 5.20 x 10^7 -5.50 x 10^8 cfu/g and 6.90 x 10^7 – 7.20 x 10^8 cfu/g for *Penaeus* sp. from Itu and Oron creeks, 7.00 – 7.50 x 10^7 cfu/g and 6.20 – 6.80 x 10^7 cfu/g for *Callinectes* sp. from Itu and Oron creeks.

The Vibro count varied from 6.90 x 10^7 – 7.20 x 10^8 and 8.00 x 10^7 – 8.20 x 10^8 cfu/g for *Penaeus* sp. from Itu and Oron creeks, 5.20 – 5.30 x 10^7 and 5.60 – 5.90 x 10^7 for *Callinectes* sp. from Itu and Oron creeks.

The fungi count ranged from 3.10 – 3.40 x 10^7 cfu/g and 3.50 – 3.70 x 10^7 cfu/g for *Penaeus* sp. from Itu and Oron creeks, 3.20 – 3.50 x 10^7 cfu/g and 3.70 – 4.00 x 10^7 cfu/g for *Callinectes* sp. from Itu and Oron creeks.

Morphological and biochemical characteristic of the bacteria and fungi isolated from the samples from the two creeks are shown in Tables 3 and 4.

The organisms isolated from the samples were, *Escherichia coli, Salmonella* sp., *vibrio* sp., *shigella* sp., *Serratia marcesens*, *Bacillus* sp., and *Streptococcus* sp. for bacteria and *Aspergillus flavus, Aspergillus niger,* and *Aspergillus terreus* for fungi (Table 5).

The percentage proximate and mineral composition of the samples is shown in Table 6. The crude protein ranged from 29.85 ^{d} – 54.13^{a} % in which the highest was obtained from Oron creek. *Penaeus* sp. and *Callinectes* sp. from Oron creek has the highest Na, Ca, K and P content.

The concentration of different metals detected in the edible portion of the samples from the two creeks is shown in Table 7. The heavy metal concentrations are found to be significantly higher ($P<0.05$) in *Peaneus* sp. from Oron creek except for Pb and Cd than samples from Itu creek. *Peaneus* sp. from the two creeks had significantly high concentration of metals.

The zinc content in the samples ranged from 24.4^{d} – 94.2^{a} mg/kg in shrimps and in crabs from the two creeks. The highest concentration was detected in shrimp from Oron creek.

Lead was detected in all samples but the highest concentration (0.84^{a} mg/kg) was found in *Callinectes* sp. (crab) from Itu Creek.

Cadmium was also detected virtually in all the samples. The lowest concentration was detected in shrimp from Oron creek that contained 0.02^{d}mg/kg and the highest was from Crab (0.15^{a}mg/kg) from Itu creek.

The concentration of copper ranged from 3.4^{d} - 7.49^{a} mg/kg in which the highest was found in Peaneus sp. from Oron creek. Manganese was also detected in all the samples and the concentration ranged from 24.2^{d} - 88.6^{a} mg/kg, the highest being detected from Peaneus sp. from Oron creek.

DISCUSSION

The total microbial counts obtained from this work were found to be higher than the specified standard limits ($1x10^5$cfu/g) for bacteria and fungi and $1.x10^2$ cfu/g for coliforms) by ICMSF [16] and USFDA [17]. This high level of microbial loads could be as a result of human activities in the rivers where the shellfish are harvested. These activities include; bathing, washing of clothes or other materials, disposal of faecal matters and sewage discharge by municipal authorities and independent oufits.

All these organisms isolated have health implications on man. The presence of *E.coli* in the *Penaeus* sp. and *Callinectes* sp. is an indication of secondary contamination as *E.coli* are known to be associated with gastrointestinal tracts of warm-blooded animals and are known to be present in the environment as a natural flora. This secondary contamination may be as a result of sewage contamination *Penaeus* sp. and *Callinectes* sp. harvesting areas. E. coli is the causative agent of diarrhea, dysentery, hemolytic uremic syndrome, bladder and kidney infection, septicemia, pneumonia and meningitis [18].

Salmonella, one of the most important food-borne pathogens is indication of sewage contamination and appear to be associated with number a of non-human hosts for example, reptiles [19]. It has been reported to survive and persist in the aquatic environment. *Salmonella* has been detected in periwinkles from different creeks [7], in the gut of tilapia and crab [20; 21] and causes new-born meningitis and infantile diarrhea. The presence of *Streptoccus* sp. is also implicated in humans infections like pharyngities, scarlet fever and pneumonia. *Shigella* sp. and *Salmonella* sp. are causative agents of illnesses like shigellosis and salmonellosis in human who are the only reservoir of these organisms [22]. *Bacillus* sp. causes a toxin mediated diseases rather than infections such as diarrhea and emetic illness characterized by nausea and vomiting [23].

Serratia marcensens an *enteriobacteria,* has been implicated in human pulmonary and urinary infection [24]. *Vibrio* sp., a natural habitants of sea water, has been reported by Cash *et al.*,[25] that an oral dose of 10^4 + 10^8 *Vibrio cholerae* organisms can routinely induce cholera infection in humans. *A. niger, A. flavus* and *A. terreus* have also been implicated in causing mycetoma in human [24]. *Aspergullus flavus* is involved in allergic aspergillosis (pulmonary aspergillosis) and also produces aflatoxin that is highly carcinogenic [26].

Proximate analysis has shown sample from Oron creek to be nutritionally richer than samples from Itu. This may be attributed to the fact that Oron creek has brackish water, which is often known to be nutritionally richer than fresh water found in Itu creek. The moisture content was found to be higher in *Penaeus* sp. from Itu creek. Since Itu has fresh water, the shellfish from this water tends to absorb water from the external environment into their cells which are of higher concentration in order to balance the osmotic pressure between the cell and the surrounding water. The fat,

fiber and moisture contents in samples from Itu creeks are constituents in shellfish, which provide an energy source to the consumers.

The presence of mineral elements like sodium, calcium, potassium and phosphorus found in brackish water which are major constituents of protein, nucleic acid, co-factors and other cell components, when absorbed into the cells. Shellfish has been reported to serve as a source of protein and mineral elements [27], which helps in the repair of worn-out tissue and body building. The mineral elements analyzed in this work are essential minerals required by humans. The sodium and calcium are essential elements found in human bones phosphorus plays a major role in glucose metabolism and also essential element of the DNA molecule. Iron and Magnesium are essential trace elements; they play a major role in the metabolic processes that take place in human system and regulation of blood. Magnesium may function as a co-factor to some enzymatic activities. Iron is a major component of the hemoglobin found in human blood.

Zinc is an essential element for animals and humans, the recommended daily allowance is 10mg/day in growing children and 15mg/day for adults [28]. It has a protective effect against the toxication of both cadmiun and lead [29 and 30]. A deficiency of zinc is marked by retarded growth, loss of taste and hypogonadism, leading to decrease fertility. Zinc toxicity is rare, but, at concentrations in water up to 40mg/kg, may induce toxicity, characterized by symptoms of irritability, muscular stiffness and pain, loss of appetite, and nausea [28].

Lead was detected in all samples; however, all the *Peaneus* sp. from the two different creeks contained lead below 0.5mg/kg [31] and 1.0 mg/kg [32] and Crab from the two different creeks contained lead, the concentration ($0,62^{d}$ – 0.84^{a}mg/kg) of which is above the standard limit of EC [31] and not above that of FAO [32]. Lead causes renal failure and liver damage in humans [33, 34].

The concentration of cadmium in these shellfishes was below standard limit of EC [31] (0.5mg/kg) and 2.0 mg/kg of FAO [35]. Humans are exposed to cadmium through food and the average daily intake for adults has been estimated to be approximately 50mg [29]. The standard threshold for acute cadmium toxicity would appear to be a total ingestion of 3 -15mg. Severe symptoms have been reported to occur with ingestions of 10 - 326mg, while ingestions exceeding 350mg can result in shock and acute renal failure [36].

The concentrations of copper in the samples were much below the limit of 10mg/kg [32], Copper is an essential part of several enzymes and it is necessary for the synthesis of hemoglobin. Shellfish is the richest sources of copper especially oysters and Crustaceans [37]. Underwood [37] reported that deficiencies of copper in infants can lead to anemia and hypoproteinemia and no deficiency of copper in adult has been reported.

Manganese was also detected in all the samples, the highest being detected in *Peaneus* sp. from Oron creek. Manganese deficiencies can lead to severe skeletal and reproductive abnormalities in mammals. It is widely distributed throughout the body with little variation and does not accumulate with age. Total daily intake varies from 2.5 to 7.0mg [38].

CONCLUSION

The present study revealed that shrimps and crabs from creeks in Niger Delta Nigeria contain unacceptable levels of microorganisms (as laid down by ICMSF [16] and USFDA [17]). Though nutritionally richer, the heavy metals analyzed from this work provide information about the concentration of metals in crustaceans, which can be related to the industrial activity and other human activities taking place in these two areas (Itu and Oron). Majority of the metal level remain within their permissible safe levels for human consumption as laid down recently by EC [31] and FAO [32] except in few cases.

The study on the whole evidenced the microbial, nutritional and metal status of shrimps and crabs from Niger Delta. However, it did bring out the probable hazards associated with their consumption.

Table1: Working conditions for the analysis of trace elements by atomic absorption spectrophotometer

Metals	Wavelength(nm)	Silt width(mm)	Lamp current (mA)	Fuel and oxidant	Support	Sensitivity (mg/l)
Fe	248.3	1.0	5	Acetylene	Air	300
Mg	285.2	1.0	5	Acetylene	Air	15
Zn	213.8	1.0	5	Acetylene	Air	40
Pb	283.3	1.0	10	Acetylene	Air	500
Cd	228.8	0.5	5	Acetylene	Air	40
Mn	279.4	0.2	5	Acetylene	Air	150
Cu	324.7	0.5	4	Acetylene	Air	200

Table 2: Total count (cfu/g) of the microbial groups in *Peneaus* sp. and *Callinectes* sp. from Niger Delta creeks

Source	sample	Total bacteria count x10^7	Total *Coliform* count x10^7	Total *Salmonellea/Shigella count* x10^7	Total *Vibro* count x10^7	Total fungi count x10^7
Itu creek	Shrimps	9.7x10^8	6.80	5.5 x10^8	7.2 x10^8	3.40
	Crabs	8.90	6.40	7.50	5.30	3.50
Oron creek	Shrimps	1.45x10^8	9.40	7.20	8.20	3.70
	crabs	1.03x10^8	8.40	6.80	6.60	4.20
Itu creek	Shrimps	8.50	6.20	5.20	6.90	3.10
	Crabs	7.00	5.30	7.00	5.20	3.20
Oron creek	Shrimps	1.60	8.90	6.90	8.00	3.50
	crabs	1.10	7.30	6.20	5.90	4.00

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Table 3: Morphological and Biochemical characteristics of bacteria isolated from *Peaneus sp. and Callinectes sp.* from Niger Delta creeks

Isolate	Colonial and cell morphology	Gram reaction	catalase	oxidase	Methyl red	VP	Indole	citrate	motility	Spore	Urease	Coagulase	Hs_2	Glu	Lac	Gal	Suc	Manitol	Probable organism
1	Creamy yellow,circular convex and opaque	-Rod	+	+	+	-	+	-	+	-	-	-	-	A	A	-	A	A	*Eschericia coli*
2	Yellow and smooth	-Rods	+	-	+	-	+	+	+	-	-	-	-	A G	A	A	-	A	*Salmonella sp.*
3	Orange and smooth cocci in cluster and single	-Curve Rods	+	-	-	+	+	+	+	-	-	-	-	A	A	A	A	A	*Vibrio sp*
4	Large cream with rhizoid-like edge	-Rod	+	-	+	-	-	-	-	-	-	-	+	A	A	A	-	A	*Shigella sp.*
5	White irregular flat and tranluscent	-Rods	+	-	-	+	-	+	+	-	-	-	+	A	A	A	A	A	*Serratia marcessens*
6	Yellow colour, cocci in cluster and chain	+Rodi	+	-	-	+	+	+	-	+	-	-	-	A	-	-	A G	A G	*Bacillus sp.*
7	Milky white colours entire edge,	+Cocci	-	-	+	-	+	+	-	O	-	-	-	A	A	-	A	A	*Streptococcus sp.*

+-positive, - - negative, A- acid, AG- acid and gas

Table 4: Morphological characteristic of Fungi isolated from *Peaneus* sp. and *Callinectes* sp. from Niger Delta creeks

Isolates	1	2	3	4
Colony description	Dense felt yellow dreen colony	Black colony	Brownish colony becoming darker with ages	White becoming grayish brown
Morphological characteristic	Filamentous,radiate conidia head	Conidia terminated in vesicles, conidia in chains.	Filamentous, foot cell, short conidiophores	filamentous Stolon, rhizoids Ovoid sporangiospores
Nature of hyphae	septate	septate	septate	coenocytic
Probable identity	*Aspergillus flavus*	*Aspergillus niger*	*Aspergillus terreus*	*Rhizopus* sp.

Table 5: Microorganism isolated from *Peaneus* sp. and *Callinectes* sp. from Niger Delta creeks

Isolates	Itu creek		Oron creek	
	Peaneus sp.	*Callinectes sp.*	*Peaneus sp.*	*Callinectes sp.*
E.coli	+	+	+	+
Salmonella sp.	+	+	-	+
Shigella sp.	-	-	+	+
Serratia marcessens	-	+	+	+
Sreptococcus sp.	_	+	+	+
Enterobacter sp	+	+	+	+
Bacillus sp.	+	+	+	+
Vibrio sp.	+	+	+	_
Aspergillus flavus	_	+	-	+
Aspergillus niger	+	+	+	+
Aspergillus terreus	_	+	-	+
Aspergillus niger	-	+	+	+
Rhizopus sp.	-	+	+	+

+ - present, - - absent

Table 6: Proximate composition (%) and mineral contents (mg/kg) of *Peneaus sp.* and *Callinectes sp.* from Niger Delta

Source	sample	Crude protein	Crude fat	Crude fiber	Ash content	Dry matter	Moisture content	Na	K	Ca	P
Itu	A_1	49.85 ^{b}	3.29 ^{d}	1.23 ^{d}	14.12 ^{d}	89.86 ^{d}	10.14 ^{a}	0.37 ^{b}	0.13 ^{d}	0.84^{c}	0.36^{b}
	B_1	29.85 ^{d}	6.25 ^{a}	1.75 ^{b}	26.21 ^{b}	91.87 ^{b}	8.13 ^{c}	0.31 ^{c}	0.18 ^{b}	0.88^{b}	0.65^{d}
Oron	A_2	54.13 ^{a}	3.47 ^{c}	11.15 ^{a}	17.26 ^{c}	91.39 ^{c}	8.61 ^{b}	0.43 ^{a}	0.16 ^{c}	0.92 ^{a}	0.41^{c}
	B_2	31.47 ^{c}	5.89 ^{b}	1.68 ^{c}	28.29 ^{a}	92.68 ^{a}	7.32 ^{d}	0.33 ^{c}	0.21 ^{a}	0.94 ^{a}	0.74^{a}

a,b,c = Values with superscripts within each row are significantly different ($P<0.05$) by
Duncan's Multiple Range Test A_1 = *Peaneus* sp., B_1 = *Callinectes* sp. from Itu creek, $A_{2=}$ *Peaneus* sp. and $B_{2=}$ *Callinectes* sp. from Oron creek

Table 7: Concentration of heavy metals (mg/kg) in *Peaneus* sp. and *Callinectes* sp. from Niger Delta creeks

Source	Sample	Fe	Mg	Zn	Pb	Cd	Mn	Cu
Itu	A_1	22.7[b]	276[b]	91.6[b]	0.28[d]	0.04[c]	85.2[b]	6.8[b]
	B_1	17.2[d]	130[d]	24.4[d]	0.84[a]	0.15[a]	24.2[d]	3.4[d]
Oron	A_2	24.2[a]	281[a]	94.2[a]	0.33[c]	0.02[d]	88.6[a]	7.4[a]
	B_2	18.6[c]	137[c]	26.2[c]	0.62[b]	0.11[b]	26.5[c]	4.7[c]

a,b,c = Values with superscripts within each row are significantly different ($P<0.05$) by
A_1 = *Peaneus* sp., B_1 = *Callinectes* sp. from Itu creek, $A_{2=}$ *Peaneus* sp. and $B_{2=}$ *Callinectes* sp. from Oron creek

ABREVIATION

UNEP - Unite Nations Environmental Programme

ICMSF- International Commission of Microbiological Specification for Food

NAS – NRC - National Academy of Sciences - National Research Council.

FNB - Food and Nutrition Board.

FAO - Food and Agricultural Organization

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