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Microbiological Quality, Proximate Composition and Heavy Metal Contamination of Pond-raised Catfish (*Clarias gariepinus*)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Catfish (*Clarias gariepinus*) is a commonly consumed fish in Nigeria. Its high demand necessitated fish farming in ponds which is now the current trend for fish production in Nigeria. Thus, the safety and quality of pond raised catfish has to be ascertained. The nutritional content, microbiological quality and heavy metal contamination of twenty (20) pond-raised catfish purchased from four different markets in Lokoja, Nigeria was determined using standard laboratory methods. The total bacterial count ranged between 1.5 x 10^3 - 3.9 x 10^3 cfu/g while the total fungal count ranged between 1.0 x 10^3 - 2.2 x10^3 cfu/g from the different sampling locations. The fungi from the pond raised fish samples were identified as *Rhizopus stolonifer, Aspergillus niger*, *Mucor sp*., *Penicillum sp*. and *Fusarium sp*. The bacterial isolates were identified as *Escherichia coli, Micrococcus sp., Salmonella sp., Staphylococcus aureus*, *Proteus sp., Bacillus sp.,* and *Pseudomonas sp*. Iron (Fe), copper (Cu), nickel (Ni), zinc (Zn) were present in all the fishes examined at concentrations not exceeding the approved permissible limits in food although lead (Pb), and cadmium (Cd) were below detection limits in all the fish samples. Pond raised catfish is a rich source of protein and fat.

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The incidence of some likely enteric and toxigenic organisms in the fish calls for a systematic approach in ensuring safety of the consumers. The consumption of the fish does not pose a likely source of heavy metal accumulation however the source of zinc in the fish should be identified as they can bio-accumulate quickly and pose a serious health risk to man.

Keywords: Catfish (Clarias gariepinus); food safety; microbes; heavy metal pollution; nutrients.

1. INTRODUCTION

Fish is a protein-rich food consumed by a large percentage of the populace because of its availability and palatability [1]. It is safer, healthier and an excellent source of protein as compared to other protein sources like beef, chevon and mutton due to its amino acid composition and protein digestibility. Fish is also one of the main sources of protein in the developing countries [2]. In Nigeria, fish is eaten fresh, fried or smoked and it forms part of a cherished delicacy that cuts across socio-economic, age, religion and educational barriers [3]. The African catfish (*Clarias gariepinus)* is a dominant fresh water fish with length varying between 1.4 - 2 m long and weighs between 8 - 59 kg. Its body colouration varies from olive green, to brown and black with the flanks often uniform grey to olive- yellow with a back that is a dark slate or greenish brown. Catfish can be found in their natural aquatic habitat like rivers, dams, weirs, lakes, damps, swamps, muddy waters, floodplains and other water bodies. In Nigeria, owing to the demand for the supply of catfish, they are also raised in man-made ponds. Ponds are built over clay-rich soil and filled with ground water. The production of pond-raised catfish begins with fertilized eggs under controlled aquaculture conditions and are harvested all year round. After hatching in tanks and initial growth, sac fry is transferred to a nursery pond. At four to six inches, the fingerlings are transferred again to raised-embankment, rectangular grow out ponds having surface areas of 10 - 20 acres. Feed consists of plant protein pellets made from soybeans, corn, wheat, and supplements [4].

The pollution of the aquatic habitat including fish ponds caused by heavy metals is of major concern due to their persistent and accumulative nature [5]. Heavy metals are toxic or poisonous even at low concentrations. Fishes are inhabitants of the aquatic environment that cannot escape from the detrimental influence of these pollutants. Fishes living in polluted water may accumulate higher amount of toxic heavy metals through their food chain. Fishes reared in ponds and lakes with artificial feed sometimes contain heavy metals because of the environmental contamination and contamination during processing [6]. The metal once absorbed is transported via blood to the muscles, bone, liver, kidney, gills and tissues and can become accumulated [7]. Metal accumulation in fish tissues poses a threat to human beings [8]. Almost all heavy metals cause health hazard to consumers and once ingested, numerous health problems arise [9]. Heavy metals having penetrated into humans through food chains cause various disturbances or serious diseases [10]. Most cause renal disease, some cause problems in the stomach, damage the central nervous system, retard growth in children and cause cancer [5].

The internal tissues of all healthy animals, is considered sterile in live, healthy fish, but becomes contaminated from numerous sources during harvest, slaughter, dressing, processing and packaging. The pond environment is recognized as a source for bacteria, especially *Salmonella* and *Vibrio* [11]. Poor water quality, farm run-off, feeds, non-sanitary processing conditions, and poor distribution, handling and preparation practices of pond-raised catfish can be part of the contamination pathway [11]. Processing facilities can harbour *Salmonella* and *Listeria monocytogenes* and workers can carry *Staphylococcus aureus*, Hepatitis A virus and Noroviruses [12]. *Salmonella* has a history of contamination of aquaculture products (International Commission on Microbiological Specifications for Foods [13]. *Salmonella* is the second most common violation found in imported fishery and seafood products. It has been reported that *Salmonella* levels in ponds are enhanced by high stocking densities and warm water temperatures [14].

The nutritional, heavy metal composition and microbiological quality and safety of pond-raised catfish in Lokoja have not been established. The aim of this study is to determine the safety of pond-raised catfish to its consumers as the fish is

now a most sought-after food used in preparing several delicacies in Lokoja.

2. MATERIALS AND METHODS

2.1 Collection of Pond-raised Catfish Samples

A total of 20 fresh pond-raised catfish samples were collected from four (4) different markets (Old, New, Lokongoma and Ganaja) in Lokoja**,** Kogi State, Nigeria in between 7.00 am and 8.00 am. The samples were collected into separate sterile coolers and transported immediately to the laboratory for analysis. They were refrigerated at 4°C for subsequent analysis.

2.2 Isolation, Identification and Enumeration of Bacteria and Fungi

The method of Ibrahim et al. [15] was employed. The head, gill, skin and intestine of the pond-raised catfish were macerated in a stomacher machine (Stomacher 400® circulator by John Morris scientific) using a stomacher bag. One gram (1 g) of the stomached fish sample was weighed and dispensed into 9 mL of distilled water. This was shaken vigorously for 10 min and allowed to stand for 20 min, after which a tenfold serial dilution was carried out in duplicate and viable aerobic bacterial counts were enumerated in standard plate count agar after incubation at 37°C for 48 h. The aliquot (0.1 mL) of the tenfold dilution was used to inoculate nutrient agar and potato dextrose agar for the isolation and enumeration of total bacteria and fungi respectively. Distinct colonies of the bacterial isolates were streaked on agar slants. The agar slants were incubated at 37°C for 24 h.

The bacterial isolates were identified using their cultural characteristics and biochemical tests such as Gram staining, catalase test, coagulase test, sugar fermentation test, starch hydrolysis test and citrate utilization test employing the method of Fawole and Oso [16] and methyl red test [17]. The fungal isolates were identified by macroscopic examination through observing the colonial morphology like diameter, pigmentation of hyphae, texture, shape and surface appearance and elevation. Microscopic examination was carried out after staining with lactophenol dye [18]. The morphological and biochemical characteristics of the fungal isolates were compared with those of known taxa.

2.3 Heavy Metal Analysis

The method according to Ogwok et al. [19] was employed. The fish samples (10 g) were pre-dried in a muffle furnace at 150°C for 1 h to allow carbonization. The temperature was then increased to 250°C and 550°C after 1 h and 2 h respectively in order to minimize volatization of lead. The muffle furnace was put off after a day and opened when the temperature had dropped to 250°C. The ash was digested in 5 mL of 10% nitric acid with warming to ensure total dissolution and the mixture filtered through an acid washed filter paper into a 25 mL volumetric flask ashed in a furnace at 550°C. The samples were digested in a flask with 20 mL of trioxonitrate (V) acid. The solution was then diluted to the mark of the volumetric flask with distilled water. The heavy metals were determined at wavelengths 283.3nm, 324.8 nm and 248.3 nm for Pb, Cu and Fe respectively. Heavy metals were analysed using the atomic absorption spectrophotometer (AAS) – AA-6800 Series (SHIMADZU) model.

2.4 Determination of Moisture Content

The method of AOAC [20] was employed. The powdered sample (2 g) was weighed (W_1) into pre-weighed crucible (W_0) and placed into a hot drying oven at 105°C for 3 h. The crucible was removed, cooled in a desiccator and weighed. The process of drying, cooling and weighing were repeated until a constant weight (W_2) was obtained. The weight loss due to moisture was obtained thus;

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{W_1 - W_0} \times 100$$

Where: W_0= weight of the empty crucible (g) W_1= weight of the powder sample + empty crucible (g) W_2= weight of dried sample + empty crucible.

2.5 Determination of Ash Content

The method of AOAC [20] was used. The dried powdered samples (2 g) was weighed (W_1) into a pre-weighed empty crucible (W_0) and placed into a lenton muffle furnace at 550°C for 5 h. The ash was cooled in a desiccator and weighed (W_2). The weight of the ash was determined by the difference the dry powdered leaves sample, pre-weighed and the ash in the crucible. Percentage ash was obtained by equation;

$$\text{Ash (\%)} = \frac{W_2 - W_0}{W_1 - W_0} \times 100$$

Where: W_0 = weight of empty crucible (g), W_1 = Weight of crucible + powdered sample (g), W_2 = weight of crucible + ash sample (g).

2.6 Determination of Crude Fibre Content

Percentage of crude fibre was determined by the method of AOAC [20] in which 2 g of ground sample was weighed (W_o) into a 1dm^3 conical flask. Water (100 cm^3) and 20% H_2S0_4 (20 cm^3) were mixed and boiled gently for 30 min. The content was filtered through a Whatman No.1 filter paper. The residue was scrapped back into the flask with a spatula. Water (100 cm^3) and 20 cm^3 of 10% NaOH were added and allowed to boil gently for 30 min. The content was filtered and the residue was washed thoroughly with hot distilled water, and then rinsed once with 10% HCl and twice with ethanol and finally three times with petroleum ether. It was allowed to dry and scrapped into the crucible and dried overnight at 105°C in a hot air oven. It was then removed and cooled in a desiccator. The sample was weighed (W_1) and ashed at 550°C for 90 min in a lenton muffle furnace. It was finally cooled in a desiccator and weighed at again (W_1). The percentage crude fibre was calculated using this equation:

$$\text{Crude fibre (\%)} = \frac{W_1 - W_2}{W_0} \times 100$$

Where: W_0 = weight of sample, W_1 = weight of dried sample, W_2 = weight of ash sample.

2.7 Determination of Crude Protein

The crude protein of the sample was determined using the micro Kjedahl method described by AOAC [20]. The sample (0.5 g) was mixed with 10 mL concentrated sulphuric acid in the micro Kjedahl digestion flask. A tablet of selenium catalyst was added. The flask was heated on the digestion block in a fume cupboard at 100°C for 4 h until the solution became clear (the digest). The flask was removed from the block and allowed to cool. The digest was diluted to 100 mL in a volumetric flask and used for the analysis. The 10 mL of the digest was mixed with equal volume of 45% NaOH solution in a Kjedahl distillation apparatus. The mixture was distilled into 10 mL of 40% boric acid containing 3 drops of mixed indicator (bromocresol or methyl red). A total of 50 mL of the distillate was collected and titrated against 0.02 EDTA until a colour change from green to a deep red end point was observed.

The crude protein was calculated using the formula:

$$\text{Crude protein (\%)} = \%\ N_2 \times 6.60$$

The nitrogen content of the sample is given by the formula below:

$$N_2\,(\%) = \frac{100}{W} \times \frac{N \times 14}{100} \times \frac{V_t \times T.B}{V_a}$$

Where:

W = Weight of sample (0.5 g)
N = Normality of titrant
V_t = Total digest volume (100 mL)
Va = Volume of digest analysed (10 mL)
T = Sample titre value
B = Blank titre value

2.8 Determination of Carbohydrate

The method of AOAC [20] was adopted. The total carbohydrate content in the samples was obtained by calculation using the percentage dry method. That is by subtracting the % sum of other food nutrients from 100%. This is done by using the equation below:

CHO (%) =100% - (% crude protein + % crude lipids + % crude fiber + % ash + % moisture).

3. RESULTS

3.1 Microbiological Quality of Pond-raised Catfish (*Clarias gariepinus*)

A total of twenty-two (22) bacterial species were isolated comprising of *Escherichia coli* (1), *Salmonella* sp. (4), *Proteus* sp. (3), *Staphylococcus* sp. (5), *Bacillus* sp. (4), *Pseudomonas* sp. (2) and *Micrococcus* sp. (3) were isolated from the pond-raised catfish from fish farms in Lokoja as shown in Table 1. Five (5) fungal species were identified consisting of *Fusarium* sp., *Rhizopus* sp., *Penicillium* sp., *Aspergillus* sp. and *Mucor* sp. Table 2.

The head and skin of the catfish had the highest total bacterial count (3.9×10^3 and 3.8 x 10^3 cfu/g respectively). It was observed that the head, gills, intestine and skin of all the catfishes purchased from Lokongoma market had the highest total bacterial count as shown in Table 3. The skin of the catfish had the highest mean total fungal count (2.2×10^3 cfu/g) as shown in Table 4.

3.2 Proximate Composition of Pond-raised Catfish (*Clarias gariepinus*)

The moisture, protein, fat, ash, crude fibre and carbohydrate content of pond-raised catfish examined ranged from 72.61 - 78.18%, 8.23 – 9.96%, 14.75 – 32.26%, 4.05 – 4.51%, 0.03-0.24% and 7.27 – 17.27% respectively as shown in Table 5.

3.3 Heavy Metal Contamination of Pond-raised Catfish (*Clarias gariepinus*)

Heavy metals such as iron, copper, nickel and zinc were detected in all the pond raised catfish although lead and cadmium were below detectable limit in all the fish samples collected from different locations as shown in Table 6.

4. DISCUSSION

Catfish is consumed by a large proportion of the populace who consume it because of its availability, flavour and palatability while others do so because of its nutritional value. It is therefore imperative that pond raised catfish is of standard quality so as to ensure the safety of the citizenry. The incidence of pathogenic bacteria such as *Escherichia coli* could be as a result of the pollution of the ponds with faecal matter from the manure applied to the fishes or from the fish themselves [21] inadequate water change in the ponds from time to time, unhygienic habits of food handlers and fish farmers. *Salmonella* sp. *Bacillus* sp. and *Staphylococcus* sp. occurred most in the fish samples. In a similar study, these organisms were reported in fish samples [22]. Some of the identified organisms such as *Salmonella* sp. and *Staphylococcus* sp. cause food borne diseases such as salmonellosis and staphylococcal food poisoning and their incidence are of public health significance [23]. The presence of *Aspergillus* sp. in the fish samples portends high health risk especially if the strain produces mycotoxins.

Bacterial counts on the skin and head of catfish samples were higher. The high occurrence of bacteria in these parts of the fish may be attributed to their constant contact with the water body [5] and this agrees with the study by Ajani et al. [24]. The high fungal load on the skin of the fish could be due to the high moisture content of the fish which encourages fungal growth. The high microbial load reported in this study may have been due to high level of contaminants in the market where these catfish were being sold and obtained. Also, the high microbial load on the skin and head may be as a result of the fish being kept in dirty buckets and baskets before sampling [25]. It may also be attributed to the unhygienic practices of the fish handlers. The bacterial count of the catfish samples studied falls within the maximum recommended bacterial count for good quality fish product, i.e. 5 x 105 cfu/g [13, 9].

The study revealed that catfish is a rich source of fat, moisture and protein. The oil from catfish could be a rich source of omega -3- fatty acid which has a lot of health benefits such as decreasing the risk of heart disease and stroke while also helping reduce symptoms of depression, hypertension, attention deficit hyperactivity disorder (ADHD), joint pain, arthritis and chronic skin ailments like eczema. Fish oil intake has also been associated with aiding the body in weight loss, fertility, pregnancy and increased energy [26]. The fish is a good source of protein and will be of immense nutritional benefit to malnourished children and adults.

The presence of these heavy metals; iron, copper, nickel and zinc may contribute to the dietary intake required once they do not exceed the recommended dietary allowance.

According to FAO [27] and Olaifa et al. [5], the acceptable limit of copper in fish samples is 30 mg/kg. The pond raised catfish samples that were examined in this study had less than the acceptable limit. Iron is an essential element in the human diet. Iron forms part of hemoglobin which allows oxygen to be carried from the lungs to the tissues [5]. Demirezen and Uruc [28] reported a permissible limit of iron in food to be in the range of 30 – 150 mg/kg. High concentrations of iron can cause tissue damage as a result of the formation of free radicals. The iron content is within the permissible limit range and thus does not present any health risk to consumers.

The metal with the overall highest concentration in the fish was Zn. The maximum zinc level permitted for fish is 50 mg/kg according to Food Codex [29]. The Zn concentration of the pond raised catfish was within the permissible limits however, if the source of the introduction of zinc is not controlled, they can reach a dangerous concentration that can affect consumer's health. Lead and cadmium were below detectable limits however their permissible limit according to WHO/FAO is 1 mg/kg and 0.2 mg/kg respectively

Table 1. Identification and occurrence of bacterial isolates in catfish samples

Isolates	Microscopic and biochemical characteristics	Number of isolates	% Occurrence in catfish samples
***Bacillus* sp.**	G +ve rods, positive reaction to catalase, methyl red and starch hydrolysis. Negative reaction to coagulase, citrate and indole test. Fermentation of sucrose and galactose with gas production. fermentation of glucose, fructose and lactose with no gas production.	4	18.2
***Micrococcus* sp.**	G +ve cocci, positive reaction to catalase, methyl red and indole tests. Negative reaction to coagulase, citrate and starch hydrolysis tests. Fermentation of glucose, sucrose, fructose and galactose with gas production. Fermentation of lactose with no gas production.	3	13.6
***Salmonella* sp.**	G -ve rods, positive reaction to catalase, methyl red, starch hydrolysis and indole tests. Negative reaction to coagulase and citrate tests. Fermentation of glucose and fructose with gas production. No fermentation of sucrose, lactose and galactose	4	18.2
***Proteus* sp.**	G -ve rods, positive reaction to catalase, coagulase and indole tests. Negative reaction to citrate, methyl red and starch hydrolysis tests. Fermentation of glucose and sucrose with gas production. Acid production only from fructose and galactose. No fermentation of lactose	3	13.6
Escherichia coli	G -ve rods, positive reaction to catalase, methyl red and indole tests. Negative reaction to citrate, coagulase and starch hydrolysis tests. Fermentation of sucrose and lactose with gas production. Acid production only from fructose and glucose. No fermentation of galactose	1	4.5
***Pseudomonas* sp.**	G -ve rods, positive reaction to methyl red and starch hydrolysis tests. Negative reaction to citrate, indole, catalase and coagulase tests. Acid production only from glucose. No fermentation of galactose, glucose, sucrose and lactose,	2	9.1
Staphylococcus aureus	G +ve rods, positive reaction to catalase, citrate, methyl red and indole tests. Negative reaction to coagulase and starch hydrolysis tests. Fermentation of sucrose, fructose, glucose and lactose with only acid production. No fermentation of galactose	5	22.7

Table 2. Morphological and Microscopic Characteristics of Fungal Isolates

Colonial morphology	Microscopic characteristics	Probable isolates
Cotton white to brown, rapid growth and is asepted	Spores with dark pigment	*Rhizopus* sp.
White on PDA, rapid growth from white to black due to conidophores, septate hyphae	Conidophore with conidium of dark pigment	*Aspergillus* sp.
White at first, changes to cream yellow, septate and fast growing with spores	Sporangia with a slimy texture; spores with dark pigment	*Mucor* sp.
Colonies are usually fast growing, in shades of green, sometimes white, mostly consisting of a dense felt of conidophores	Many-branched condiospores sprout on the mycelia, bearing individually constricted conidiospores	*Penicillum* sp.
Dark in colour, thick-walled, smooth or wrinkled with chlamydospores	Chlamydophore with dark pigments of chlamydospore	*Fusarium* sp.

Table 3. Total bacterial count of pond-raised catfish from Lokoja markets

Fish samples from different markets	Gills	Skin	Intestine	Head
Old	2.0×10^3 cfu/g	3.3×10^3 cfu/g	2.2×10^3 cfu/g	3.5×10^3 cfu/g
New	1.6×10^3 cfu/g	2.4×10^3 cfu/g	1.5×10^3 cfu/g	3.2×10^3 cfu/g
Lokongoma	3.0×10^3 cfu/g	3.8×10^3 cfu/g	3.1×10^3 cfu/g	3.9×10^3 cfu/g
Ganaja	2.0×10^3 cfu/g	3.8×10^3 cfu/g	2.5×10^3 cfu/g	3.6×10^3 cfu/g

Table 4. Total fungal count of pond-raised catfish from Lokoja markets

Fish samples from different markets	Gills	Skin	Intestine	Head
Old	1.2×10^3 cfu/g	2.0×10^3 cfu/g	1.5×10^3 cfu/g	1.3×10^3 cfu/g
New	1.5×10^3 cfu/g	1.8×10^3 cfu/g	1.4×10^3 cfu/g	1.5×10^3 cfu/g
Lokongoma	1.1×10^3 cfu/g	1.7×10^3 cfu/g	1.0×10^3 cfu/g	1.5×10^3 cfu/g
Ganaja	1.7×10^3 cfu/g	2.2×10^3 cfu/g	1.5×10^3 cfu/g	1.7×10^3 cfu/g

Table 5. Nutritional composition of pond-raised catfish

Fish samples from different markets	Moisture content (%)	Protein (%)	Fat (%)	Ash (%)	Crude fibre (%)	Carbohydrate (%)
Old	73.92 ± 0.1	9.96 ± 0.1	27.01 ± 0.19	4.05 ± 0.1	0.07 ± 0.12	14.94 ± 0.2
New	72.61 ± 0.53	8.23 ± 0.1	32.26 ± 0.86	4.17 ± 0.27	0.05 ± 0.18	17.27 ± 0.2
Lokongoma	75.13 ± 0.72	8.50 ± 0.2	25.68 ± 0.23	4.37 ± 0.32	0.24 ± 0.62	13.68 ± 0.33
Ganaja	78.18 ± 0.1	9.83 ± 0.16	14.75 ± 0.31	4.51 ± 0.29	0.03 ± 0.2	7.27 ± 0.15

Table 6. Heavy metal composition of pond-raised catfish

Fish samples from different markets	Fe (mg/g)	Pb (mg/g)	Cu (mg/g)	Zn (mg/g)	Ni (mg/g)	Cd (mg/g)
Old	0.12±0.08	BDL	0.018±0.011	0.19±0.13	0.0055±0.0035	BDL
New	0.12±0.08	BDL	0.011±0.006	0.37±0.25	0.0067 ±0.0037	BDL
Lokongoma	0.23±0.16	BDL	0.012±0.007	0.32±0.22	0.0042±0.0022	BDL
Ganaja	0.16±0.11	BDL	0.027±0.017	BDL	0.0065±0.0045	BDL

Key: BDL: Below detection limits

[30]. The presence of these two metals in food is not tolerated because they can be toxic even in trace amounts [31]. The WHO permissible limit for Nickel in foods is 0.2 mg/kg [32]. The fish samples examined in this study had Ni concentrations far below the permissible limit and thus the consumption of the fish cannot cause cancer or toxigenicity. Overall, the pond-raised catfish examined in this study are not toxic and do not represent any health risk.

5. CONCLUSION

The study indicated the presence of bacteria and fungi such as *Salmonella* sp., *Staphylococcus aureus*, *E. coli* and *Aspergillus* sp. that may be pathogenic and toxigenic in pond-raised catfish.

Catfish is a rich source of moisture, protein and fat hence its consumption should be encouraged in individuals requiring meals containing protein and good fat such as omega-3-fatty acid.

The pond raised catfish did not contain heavy metals in high concentrations and were within the acceptable limits set by standard organizations like WHO and thus do not present any health risk to the consumer. The presence of zinc in the catfish samples examined was in trace amounts however, since they can bio-accumulate quickly in human tissues, fish farmers and feed producers should take measures to ensure the concentration of the metal is insignificant or not present at all in fish feeds or fish pond water.

Pond-raised catfish should be properly processed before consumption so to eliminate pathogenic, spore forming bacteria and likely toxin producing fungi that may be present.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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