Antimicrobial Activityof Copper and Copper Alloys against Bacteria Pathogens in Processed Canned Fish

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ABSTRACT

This research investigated the effects of copper and its alloys on bacterial pathogens in processed canned fish. The research was carried out between January 2024 and August, 2024. *Five(5) samples each of cannedFish were obtainedfrom asupermarket in Ogbomoso, Nigeria, and cultured for 24 hours by using Potato Dextrose Agar (PDA), MCconkey Agar (MCA) and Nutrient Agar (NA). The isolates culture was conducted by serial dilution and the bacteria isolates were biochemically characterized for identification. Fivehollowcylinderswerecastfromcopperanditsalloysatvaryingcompositionsof:100% Cu(A),90%Cu:10%Zn(B),80%Cu:20%Zn(C),70%Cu:30%Zn(D)and60%Cu:40% Zn(E).Theculturedsampleswereputineachofthecylindersandthemicrobialloadwere recorded for 6 hrs at 2 hrs interval. Scanning Electron Microscopy/Energy Dispersed Spectroscopy (SEM-EDS) wasusedtocapturesurfacestructuresofCopper and itsalloys with bacteria.* B*iochemical characterization of bacteria isolates from canned fish showed the presence of Salmonella Aureus, Vibrio Species, Staphylococcus Species and Listeria Species. The microbial colony reduction for fish at 0 hr, 2 hrs, 4 hrs, and 6 hrs for A, B, C, DandEwere150,100,50and0;150,120,70and10;150,130,76and12;150,138,78 and18;and150,142,86and20,respectively.The statistical analysis revealed significant variations in microbial growth inhibition, which were influenced by both the composition of the Cu-Zn mixture and the duration of exposure. For instance, at 100% Cu and 0% Zn, the colony count did not show a statistically significant reduction over time (F = 4.97, P = 0.067).Copperanditsalloyshavebeenconfirmed tobegoodantimicrobialagentincontrollingthegrowth ofbacteria.Therefore,theycanbe used as canning in food industries to control microbial growth and prolong shelf life.* Characterizationresultsonculturesampleonfish,deadlymicroorganismslike *Staphylococcusaureus*,issusceptibletogrowifcanningis defective.

Keywords: [canned Fish,isolates,copper alloy,microbial load}

1. INTRODUCTION

Canning of processed foods is an aspect of food preservation used to prevent wastage of raw food and to meet food demands in urban populated regions. It also helps to ease transportation of food from one place to another. However, food industries are confronted with food contamination challenges due to activities from microorganism especially bacteria. The spreading of deadly diseases through food pathogens is a major concern to numerous researchers as noted by Abushelaibi, (2005). Most of these pathogens emanate from production process, contaminated packaging materials and storage. In recent times, different techniques have been adopted by food processing industries to reduce the risk of pathogenic microorganisms. These include the use of low or high temperature techniques, irradiation, fermentation and packaging.

Lathan and Ramachandran (2013) confirmed fish to be primary sources of protein for human in many parts of the world, especially in most developed countries. Microbial growth massively affected one fourth of the world's food supplies and 30% of landed fish are lost through microbial infection. (Ghaly*et al*., 2010). Breeding fish in microbe rich environment is mostly invaded by pathogenic or opportunistic microorganisms (Zahang*et al.,*2008).

Takahashi *et al* (2003) noted *Morganellamorganii, Proteus Vulgaris, Photobateriumdamselae, and Raoultellaplanticola* as histamine forming bacteria from different type of fish. Lakshmanan*et al*.,(2002) identified dominion of aminobacteria such as, Alcaligenes*, flavobacterium, Acinetobacter, Shewanella, and Pseudomonas* during the storage of fish and shrimp. On risk based control of Biogenic Amines formation in fermented foods, EFSA (2011) revealed that, Biogenic amines are biologically active nitrogenous compounds of low molecular weight, mainly formed by the decarboxylation of amino acids.

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Biogenic amines are important due to the risk of food intoxication and to serve as chemical indicators of fish spoilage (Kim *et al.,* 2009). Various means had been used to prevent a biogenic amines formation; such as limiting microbial growth through irradiation, controlled atmosphere packaging, maintaining chilling and freezing, and/or the use of hydrostatic pressures (Suzzi and Gardini, 2003). Viscianoet *al.,*(2012) opined that, the control biogenic amines formation has mainly focused on controlling the growth of biogenic amines forming bacteria.

Pathogenic bacteria in fish could also be checked by using antibiotics (Lewbart, 2001). However, studies have shown antibiotic resistant disease from *A.hydrophilla* and *A. Sobria*isolated from tilapia hybrid resisted oxtetracycline, erythromycin and sulfadiazine, mostly used in treatments prevention of pathogenic diseases in fish.(Wang *et al*, 2003).

In food processing plants, microorganisms can attach to solid surface from various nutrients, minerals, and organic matter to form micro colonies or biofilms on food contact equipment surfaces. To eliminate such pathogenic microorganisms, there is a need to introduce antimicrobial agents into processing units or production lines, industrial storage, canned packaging, or conveyance systems. Copper possesses antimicrobial characteristics and has been proven to have strong antimicrobial effects against food-borne pathogens such as *Salmonella typhimurium*as a constituent in animal feed (Beal *et al*., 2003) and *Listeria monocytogenes* on food contact surfaces (Abushelaibi, 2005).The research of Fowler *et al*., (2019) confirmed efficacy of copper alloys to have an-timicrobial activity on pathogens. Various studies proofed plastic and stainless steels having poor antimicrobial agents, virus survives for up to 48 to 72 hrs respectively (Hutasoit. *et al.,* (2020).

Canned fish is susceptible to the growth of microorganisms if proper measures are not put in place at processing stages to the packaging. Bacterial inactivation on copper surfaces is a viable approach for preventing food-borne bacteria contamination on food contact surfaces. Elgundi*et al*., (2011) reported that copper is effective in the rapid mortality of bacteria on contact. This inactivation is enhanced by ambient relative humidity, minimal media, and high corrosion rates. Various laboratory studies have shown that surfaces containing at least 55 percent to 70 percent of copper inhibited many infectious seeded microorganisms including *Staphylococcus aureus*, *E. coli*, *Enterococcus faecalis*, *S. enterica*, *Campylobacter jejuni*, *S.bacillus*, and fungi like influenza A-viruses and HIV Prado *et al*., (2012). The release of copper ions from a copper surface is very efficient for the bacteria-killing process because, the killing rate is faster, especially when intimate contact occurred without an inert polymer grid on the copper surface. However, when those bacteria cells were exposed to copper ions in the presence of 4 mM $CuSO_4$, they died within 100 minutes (Matthews, *et al*., 2013). These findings suggest that bacteria's direct contact with the copper surface causes severe cell membrane damage, making the cell more vulnerable to the released copper ions.Shazadi, *et al*, (2018) expressed the development in performance of nano particles ($CuNP_S$) as having a substantially larger surface – area – to volume ratio increased in Toxicity compared to metal, with projected optical properties, these make them a desirable replacement. Stokes *et al,* (2019) buttressed differences in the physiological state of the microbial population with obvious effects of their susceptibility to antimicrobials.

Antimicrobial Food packaging helps in extending shelf- life and also safe for food consumption and risk of contact with pathogens (Appendini and Hotchkiss,2002). Canning is a food preservation method in which food contents are processed and sealed in a hermetic container (such as metal, glass jar, plastic cans, thermos table, plastic, or a multilayered flexible pouch). Canning is also known as Appertization from the name of its first inventor, Nicolas Appert (Featherstone, 2015).Mehtar*et al.* (2008) tested the antimicrobial activity of copperand copper alloys against nosocomial pathogens and mycobacterium tuberculosis, the research confirmed high mortality of bacterial within few hours.

The aim of this research is to determine the effectiveness of the use of copper and copper alloys coated materials as useful antimicrobial agents in canned fish production. The targets to achieve the aim are to

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i. cast copper and copper alloy cylinders using five material compositions;

ii. perform microbial test on the selected sample of canned fish using serial dilution method and to characterize the bacteria isolates from the selected sample;

iii. evaluate the antimicrobial activities of the copper and copper alloys on culture bacteria from fish, as compared to the performance of stainless steel that are used as control.

2. MATERIAL AND METHODS

3.1 Materials

The materials selected for this research was canned fish.Canned fish is widely consumed daily both by elderly and the young. Cases have been reported about spoilt canned fish when opened for consumption due to growth of bacterial. Health challenges that these spoilt foods caused serve as motivation for the choices of this material for investigation and to delve into scientific approach to inhibit the growth of microorganisms.

3.1.1 Sample Collection

Selected canned fish were purchased from Ogbomoso, one of the cities in the South West of Nigeria, located on latitude 8.1227^{o}N and Longitude 4.2436^{o}E. All samples were taken to the laboratory immediately after purchase. Five canned fish were used as samples.

3.1.2 Laboratory Materials

The following laboratory materials were used for carrying out the microbial test on the selected samples. Disposable Petri dishes, Conical flask, Autoclave, Sensitive Analytical weighing balance, Spatula, PDA (Potato Dextrose Agar), Nutrient Agar (NA), Mconkey, CA agar, ethanol, incubator, distilled water, paper tape, measuring cylinder and slant bottle.Laboratory experiments wereconducted in the Department of Mechanical Engineering, LadokeAkintola University of Technology, Ogbomoso, Nigeria and Department of Education Technology, Faculty of Vocational, Innovation and Engineering Education, Emmanuel Alayande University of Education, Oyo, Oyo State, Nigeria,

3.2 Casting of Antimicrobial cylindrical can

The casting of copper and zinc cylinders were done in percentage composition as stated in the objectives.

3.2.1 Samples of Cast Copper Cylinder and the Alloys composition

Casting was done at the *Federal Institute of Industrial Research, Oshodi (FIIRO),* Lagos, Nigeria. The compositions were made up of 100%Cu 0%Zn ; 90%Cu 10%Zn ; 80%Cu 20%Zn ; 70%Cu 30%Zn ; 60%Cu 40%Zn. The cast composition relative to weight measures are:

First composition (100wt% Cu : 0wt% Zinc – 200 grams of copper : 0 gram of zinc)
Second composition (90wt% Cu : 10wt% Zinc -180 gram of copper : 20 gram of zinc)
Third composition (80%wt Cu : 20wt% Zinc -160 gram of copper : 40 gram of zinc)
Fourth composition (70wt% Cu : 30wt% Zinc -140 gram of copper : 60 gram of zinc)
Fifth composition (60wt% Cu : 40wt% Zinc - 120 gram of copper : 80 gram of zinc

Copper and its alloys were chosen to test antimicrobial activity on pathogens due towide information from the literatures. Lacquering or chemical painting can be performedon cast copper and its alloys to remove toxicity.

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3.2.4 Experimental Control Test

Stainless steel cylindrical cup which was known to have poor anti-microbial effects was used as control.

3.3 Preparation of Potato dextrose agar, Nutrient agar and MaCconkey agar

Dextrose Agar (PDA), Nutrient agar (NA) and MaCconkey agar (MCA) were prepared according to the manufacturer's specification that is, 39 g of PDA, 28 g of NA and 51.1 g of MCA in 1000 ml of distilled water each. Bacterial contamination was inhibited by aseptically adding 2 g of tetracycline to 1000 mls of the sterile medium prior to pouring into sterile petri dishes for fungi plates while fungi contamination was inhibited by aseptically adding 2 ml of nystatin to 1000 mls of the sterile medium prior to pouring into sterile petri dishes for bacterial plates. The media was prepared, mixed thoroughly and sterilized by autoclaving (Plate 3.) at 121°C for 15 min.

A gram of each sample was suspended in 9 ml of double distilled water to make microbial suspensions (10^{-1} to 10^{-5}). Dilution of 10^{-3}, 10^{-4} and 10^{-5} were used to isolate both fungi and bacterial. One millilitre (1 ml) of microbial suspension of each concentration was then added to sterile Petri dishes (triplicate of each dilution) containing 15 ml of sterile Potato Dextrose Agar for fungi isolation and Nutrient Agar for bacterial isolation.

After the plates have been solidified, the petri dishes for fungi was incubated at 30^{o}C for 3 days while bacterial petri dishes was incubated at 37^{o}C for 24 hours.

3.3.1 Isolation of Bacterial and Fungi for Characterizations

Isolation of bacteria from the selected samples were done in the laboratory by preparing media for the growth of bacteria colony. MaCconkey Agar (MCA) and Nurient Agar (NA) were used.From the selected samples, the bacteria were grown by culturing through serial dilution plate method. The microorganisms of fungi and bacterial were isolated by serial dilution plate method. (Plate 3a) shows laboratory set up for serial dilution method that was used for the isolation of microorganisms while (Plate3b) shows the material for preparation of agar media. The bacteria isolates were done by making use of 24 hours cultures that are gram-stain for cell morphological differentiation. Identification of the detected bacteria was done by characterization at Nigeria Stored Product and Research Institute, Ilorin.

3.3.2 Characterization of Bacteria and Fungi Isolates

Cellular characteristics of the bacterial isolates was carried out by using parameters such as size, elevation, pigment, surface, opacity, edge and shape. Cellular characteristics of the isolates, such as, gram's staining, motility test, spore staining, capsule staining, catalase test, oxidase test, methyl red test, indole test, starch hydrolysis test, citrate utilization test, sugar fermentation test, and oxygen relationsship test and these were determined using the procedures employed by Olutiola*et al*. (1991).

3.3.3 Laboratory Identification of Fungi

3.3.3.1 Identification of yeast

Different colony types were purified by transferring into sterile potato dextrose plate. Each pure colonial isolates (hg) was inoculated into a sterile potato dextrose agar starts and stored in refrigerator.

3.3.3.2 Colonial Morphology

The vegetative cells of the pure culture of the yeast isolates streaked on sterile potato agar plate. The plates were incubated at 30^{0}C for 48 hours. The shapes, colour, edge and the growth elevation were examined.

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3.3.3.3 Cellular Morphology

Smear of the pure cultures of the yeast isolates were prepared and stained with lactophenol-in-cotton blue and allowed to act for 30 sec and then washed off with distilled water. The slides were blot dry in between fold of filter paper and observed at oil immersion X40 objective lenses. Starch hydrolysis test, Citrate utilization test, Sugar fermentation test and Oxygen relationship test were carried out to describe the shape, structure, form and size of cells.

3.3.4 Microbial colony reduction

The colony of bacteria formed from culture samples of fish were dispensed into each of the cast copper and copper alloy. The colony of bacteria formed from each isolate was properly sealed and stored in the refrigerator at $4^{o}C$ with consistent power supply for 24 hours to prevent further growth of bacteria. Each cultured sample from fish was sealed in cylinder of pure copper and alloys were poured and counted on the plates at 2 hours interval for 6 hrs. Stainless steel was used as control of the experiment.

3. RESULTS AND DISCUSSION

4.1 Cast Copper and Copper alloy cylinders

The results of the cast showed that copper cylinder is reddish brown in colour at composition 100% Cu: 0% Zn. As percentage of copper reduces the cylinder changes from reddish brown to yellowish gold. At Cu 60%; Zn 40, copper alloy cylinder change fully to yellowish gold (Plate 1 a –e). In casting it was discovered that the cylinder made from Cu 50%: Zn 50% is brittle. The cast composition was then limited to Cu 60% and Zn 40% during casting.

4.2 Characterization of Bacteria and Fungi Isolates

Table 1 shows the results of biochemical characteristics of bacterial isolates from cannned fish.From the four isolates: A5, A6, A7 and A8, the characteristics of the bacteria are displayed in the respective column. The Bacterium detected from isolate A5 is *Salmonella Aureus*, *Vibrio Species* from A6, *Staphyloccus Species* from A7 and *Listeria Species* from A8.These bacteria pose great risk to human health if control measures are not adopted during food packaging. Table 2 shows the results of Characterisation of fungi Isolates for canned fish**.** From the three isolates; F4, F5, and F6, the characteristics of the fungi were displayed in the respective column.

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a

b

c

d

e

f

Plate 1 a : Raw cast before machining
b: Cu 100% and Zn 0% cylinder after machining
c: Cu 90%: Zn 10% cylinder after machining
d: Cu 80% and Zn 20% cylinder after machining
e: Cu70% and Zn 30% cylinder after machining
f: Cu 60% and Zn40% cylinder after machining

Plate 2: Stainless steel for control experiment

The fungi detected from isolate F4 is Alternaria species, PenicilliumSpecies from F5 and*Cladosporium species* from F6. These fungi pose great risk to human health if control measures are not adopted during food packaging.

Table. 1 shows the results of characterization fungi isolates for canned fish. From the three isolates; F4, F5, F6, the characterization of the bacteria were displayed in the respective column. The fungi detected from isolate F4 is *Alternaria species, Penicillium species from* F5 and *Cladosporium Species* F6. These fungi pose great risk to human health if control measures were not adopted during food packaging.

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Table 1: Biochemical Characteristic of Bacteria Isolates from Fish

Laboratory Code	A5	A6	A7	A8
Cultural characteristics of isolate	Flat, Smooth, Spherical, opaque, creamy	Flat, Smooth, Long rods, Rhizoid, Creamy, Opaque	Raised, Smooth, Spherical, Entire creamy translucent	Flat, Rough, Short Reds, Entire Yellowish opaque
Gram	+	+	+	+
Motility	-	+	+	+
Catalase	+	+	-	-
Starch hydrolysis	+	+	-	+
Spore	-	+	-	-
Glucose	+	-	+	+
Lactose	+	+	+	+
Sucrose	+	-	+	+
Fructose	-	+	+	+
Galactose	+	+	+	+
Maltose	-	+	+	+
Probable Identity of Isolates	*Salmonella Aureus*	*Vibrio Species*	*Staphylococcus Species*	*Listeria Species*

Table 2: Characterization of fungi Isolates from canned Fish

Laboratory Code	**Colony Morphology**	**Cell Shape**	**Ascospore**	**Pseudomycellum**	**Isolate Identity**
F4	Cream Coloured Smooth edge Surfaced and Raised	Oval	+Ve	-Ve	*Alternaria species*
F5	White mold to grey in colour Black colour underneath	Mold	-Ve	+Ve	*Penicillium Species*
F6	Cream coloured smooth edge surfaced and raised	Ellipsoidal	+Ve	-Ve	*Cladosporium Species*

The fungi detected from isolate F1 is*CurvulariaLunata*, *Mycelia Sterilia* from F2 and *Aspergillusspp* from F3. These fungi pose great risk to human health if control measures are not adopted during food packaging.

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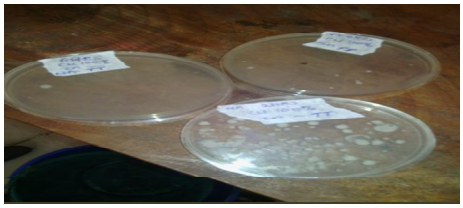
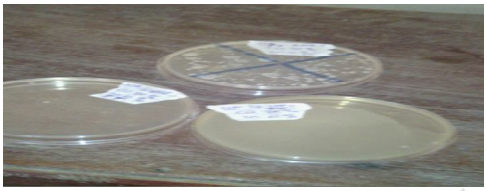


a



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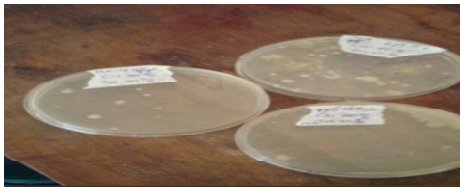

c

d

e

Plate 3a: Colony counts of Cu 100%: Zn 0% (fish)
b: Colony counts of Cu 90% : Zn 10% (fish)
c: Colony counts of Cu 80 : Zn 20% (fish)
d: Colony counts of Cu 70% : Zn 30% (fish)
e: Colony counts of Cu 60%: Zn 40% (fish)

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It can be observed generally that the composition with higher percentage of copper killed faster than the one with reduced percentage of copper. However, all the five composition displayed good antimicrobial property. Therefore any of the composition can be used as antimicrobial agent depending on availability of FUNDS because copper is more expensive than zinc. And also the time required for performance of antimicrobial works may determine the applicable one. Figure 1 shows the colony reduction graphs when colony counts were plotted against time (hours) in canned fish.

4.3.3Reason for mortality of bacteria

The movement of living bacterium was observed under microscope of high magnification of 1000X. And these bacteria also move in colonies and sometimes stayed as single entity. Bacteria can also be categorized as living being due to ability to move and therefore required oxygen to survive as other animals. When inoculated on copper and alloys of copper surfaces, it was observed that mortality rate increases until none was found. Copper (Cu) has affinity for oxygen and makes and formed CuO_2.

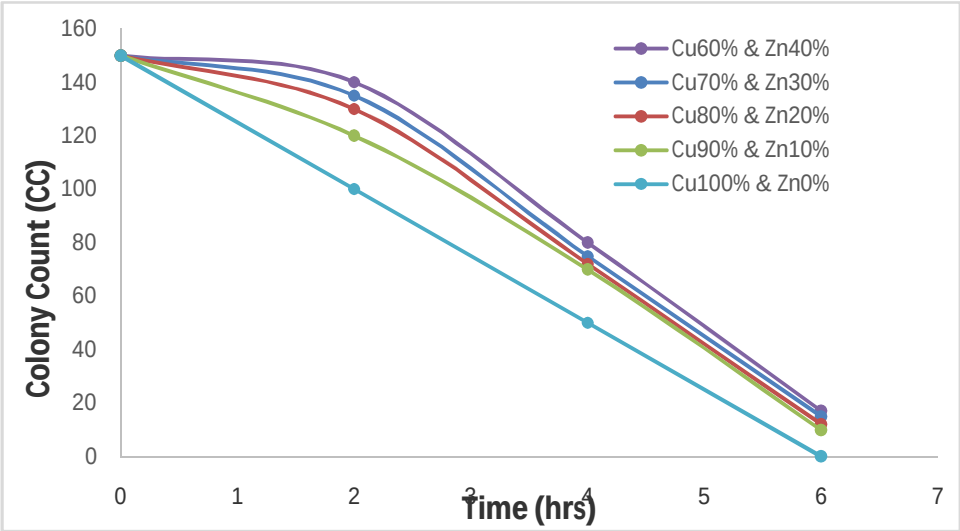

Figure 1: Colony Reduction Graph (Fish)

The oxygen extracted from bacteria colony led to mortality of microorganisms. When Copper comes in contact with living microorganisms, itextracts off the oxygen which usually leads to the death of microorganisms within few hours. Due to lack of oxygen, it resulted to mortality of bacteria.

4.3.4 Statistical Analysis

The antimicrobial activity of Cu-Zn mixtures at varying compositions was evaluated based on colony count reduction over different time intervals (2, 4, and 6 hours). The analysis revealed significant variations in microbial growth inhibition, which were influenced by both the composition of the Cu-Zn mixture and the duration of exposure. For instance, at 100% Cu and 0% Zn, the colony count did not show a statistically significant reduction over time ($F = 4.97$, $P = 0.067$). This result suggests that while copper alone possesses antimicrobial properties, its effectiveness may increase over the tested time intervals. Copper ions are known to disrupt microbial cell walls and metabolic processes, but the absence of Zn in the mixture may limit the potential synergistic effects that could enhance antimicrobial activity (Godoy-Gallardo *et al*., 2021).

5.1 Conclusion

From the results obtained, the following conclusions can be drawn

i. From the casting of copper and zinc composition at varying percentages, the colour of each composition differs from one another. 100% Cu 0% Zn displayed reddish brown.As zinc percentage increases in the compositions, faint yellowish colour appeared and it became more pronounced till it changed to yellowish gold at composition Cu 60% Zn 40%. Further addition of zinc beyond 40% made the composition brittle and porous which was unsuitable for this research.

ii. From characterization results on culture sample on fish, deadly microorganisms like *Staphylococcus aureus*, is susceptible to grow if canning is

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defective. From microbial colony reduction experiments with copper and zinc, all the above microorganisms were effectively killed within hours. It was observed that all five compositions displayed antimicrobial property with Cu 100% Zn 0% at fastest rate. Stainless steel used as control displayed no antimicrobial property, the organisms remain viable after 48 hours. And this confirmed the results of other researchers that stainless steel has poor antimicrobial property as stated in the literatures.

iii. Apart from food industries; antimicrobial activity of copper can be tested in the hospital bed, door handles in public houses, hospital trays, hospital equipment, Toxicity can be removed by coating with composite of acrylic lacquer polimer and TiO_2nanoparticles. (Sina et al. 2023).

5.2.Contributions to knowledge

i. the antimicrobial effects of copper and its alloys surfaces on fish were established.

ii. the mortality rate of bacteria pathogen depend on the percentage of copper

iii. the research revealed the clear evidence that copper and its alloys killed microorganisms .

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Disclaimer (Artificial intelligence)

Option 1:
Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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