



## Evaluation of Heavy Metals and Antimicrobial Efficacy of Hand Sanitizers Sold in Makurdi, Benue State-Nigeria

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**Abstract:** Hand sanitizer has become more popular than ever due to its effectiveness during the Corona Virus Disease 2019 (COVID-19) pandemic. Hand sanitizers kill germs on hands and other surfaces on contact, helping to slow the spread of transmissible diseases. This study evaluates heavy metals concentrations and the antimicrobial efficacy of four alcohol-based hand sanitizers used in Makurdi, Benue State. The assessment involved two locally-made hand sanitizers labeled as sample B and K, and two industrial-made hand sanitizers labeled as sample C and D. After digestion with concentrated nitric acid and perchloric acid, the concentrations of cadmium, nickel, mercury and lead were determined using Atomic Absorption Spectrophotometer (AAS). The concentration of cadmium and nickel were found in all the samples analyzed; Cd has  $0.0109 \pm 0.0001$ ,  $0.1235 \pm 0.0003$ ,  $0.1208 \pm 0.0002$ ,  $0.1136 \pm 0.0002$  mg/L for sample B, K, C and D respectively while Ni has  $0.011 \pm 0.000$ ,  $0.013 \pm 0.000$ ,  $0.070 \pm 0.000$ ,  $0.051 \pm 0.000$  mg/L for sample B, K, C and D respectively. Mercury and lead were not found in all the samples. It was observed that the concentrations of cadmium and nickel were slightly above the permissible limit by WHO, NESREA and EPA. The results also revealed that the zone of inhibition (antimicrobial efficacies) of hand sanitizers, B, K, C and D on *E. coli*, *S. aureus*, *P. aeruginosa* and *Candida* spp were ( $17.07 \pm 1.36$ ,  $12.20 \pm 1.3$ ,  $15.50 \pm 1.01$  and  $11.67 \pm 1.04$  mm), ( $11.30 \pm 0.89$ ,  $11.10 \pm 0.17$ ,  $11.06 \pm 0.40$  and  $6.67 \pm 0.29$  mm), ( $10.20 \pm 0.17$ ,  $11.30 \pm 0.30$ ,  $10.10 \pm 0.85$  and  $6.33 \pm 1.04$  mm) and ( $9.97 \pm 0.35$ ,  $10.40 \pm 0.17$ ,  $9.97 \pm 0.65$  and  $6.17 \pm 0.58$  mm) respectively. All samples had a bacteriostatic effect to all organisms but hand sanitizer B was generally observed to have higher effect on the entire test organism. Control was observed to be effective on all three bacterial strains while it was observed to be none effective on *Candida* spp. The study shows that the hand sanitizers analyzed contained some heavy metals (Cd and Ni) which are toxic to human body. Therefore, manufacturers must observe proper care while producing hand sanitizers.

**Keywords:** Hand sanitizer; Heavy metals; Anti microbial efficacy; Toxicity; AAS

## **Introduction**

The importance of sanitation cannot be over emphasized. Physical contact between people and objects is a key vehicle for the spread of pathogens (Kampf et al., 1999; Liu et al. 2010).

Hand hygiene is well known as one of the most significant of activities essential for the reduction of transmission of infectious diseases (Aiello et al., 2008; Hassan et al., 2012). Majority of early reports focused primarily on the role of hand washing as an infection control measure (Alvarado et al., 2009; Zapka et al., 2017; Pittet et al., 2006). This changed by the early 2000s, when the Centers for Disease Control and Prevention (CDC) issued a guideline authorizing that alcohol-based hand rub (ABHR) be regularly used for disinfecting hands (Baldissera et al., 2006; World Health Organization 2009). These ABHRs which are the most commonly used hand sanitizers are often composed of alcohol, ethanol, isopropanol or propanol (Chang et al., 2012; Pires et al., 2017). They have a recommended concentration range from 60-95 %. In addition to being useful in the absence of water, other advantages of the use of the hand sanitizers include, high antimicrobial activity in a shorter time, and the lack of requirement for drying of the hand (which could serve as another source of contamination).

The outbreak of corona virus in Wuhan China led to an increased awareness of the role of hand sanitizers in infection control (Garner & Favero, 1986; Centers for Disease Control and Prevention, 2002). and an upsurge of various brands of hand sanitizers into the Nigerian market (Pickering et al., 2010). Most of these products have made numerous claims, notably their ability to eliminate 99.9 % of microorganisms. A number of these claims have not been verified (Reynolds et al., 2006).

Heavy metals are natural elements characterized by their high atomic mass and their high density. Although, typically occurring in lower concentration, they can be found all through the earth crust. Commonly, a density of at least  $5.5 \text{ g cm}^{-3}$  is used to define a heavy metal and differentiate it from other light metals. Some heavy metals like copper, selenium or zinc are essential trace elements with function indispensable for various biological processes also driving the entire human metabolism (Duffus, 2002). Zinc serves as a pivotal constituent of zinc finger enzymes (Duruibe et al., 2007). On the other hand many of them e.g mercury, cadmium, arsenic, chromium, thallium, lead and others, classically represent the “dark side of chemistry”; they exert toxic effects even at low concentration (Terfassa et al., 2014; Tamas et al., 2014; Florea & Busselberg, 2006).

## Materials and Methods

### Sample Collection and Preparation

Two industrially made and two locally made hand sanitizers were obtained in Makurdi, Benue State in August 2021. The samples were duly certified by Standard Organization of Nigeria (SON) and/or National Agency for Food and Drugs Administration and Control (NAFDAC). They were labeled C and D for industrial made and B and K for local made respectively and store at the room temperature in the laboratory prior to analysis.

### Sample Digestion

10 mL of each sample was measured in a measuring cylinder, 8 mL of concentrated nitric acid and 4 mL of perchloric acid were added to the conical flask containing the sample. The sample mixture was then placed on the hot plate and heated to boil. The flask was removed from the hot plate and cooled to room temperature. To the cooled sample, deionized water was added and the content was filtered in a 25 mL volumetric flask with No. 42 whatman filter paper. Subsequently, the solution was made up to volume using deionized water. Similarly, reagent blank was prepared by taking a mixture of the reagents (concentrated nitric acid and perchloric acid) and treating it in the same manner as the sample.

### Determination of Heavy Metals

Cadmium, nickel, mercury and lead were analysed after digestion with  $\text{HNO}_3$  and  $\text{HClO}_4$  using Atomic Absorption Spectrophotometer (AAS) with appropriate hollow cathode lamps. The whole procedure was done in triplicate.

### Microorganisms

The microorganisms used for the study were:

Bacteria: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*

Fungus: *Candida spp.*

These microorganisms were obtained from the Department of Biological Sciences, Benue State University, Makurdi.

### Preparation of Media

#### Nutrient Agar

Nutrient agar powder (T.M Media, 98 % pure) was collected and prepared according to the manufacturer's instruction in Microbiology Laboratory, Department of Biological Sciences, Benue State University, Makurdi. The nutrient agar (11.00 g) was weighed using an analytical weighing balance and dissolved in 500 cm<sup>3</sup> of distilled water and heated till the solution boiled and the heat, turned off. After cooling down, the nutrient agar solution was autoclaved for

about 15 min. The nutrient agar solution was dispensed into a sterile petri dish and allowed to set.

#### **Potato Dextrose Agar (PDA)**

Potato Dextrose Agar (T.M Media, 98 % pure) was collected and prepared according to the manufacturer's instruction in Microbiology laboratory, Department of Biological Sciences, Benue State University. The nutrient agar (19.50 g) was weighed using an analytical weighing balance and dissolved in 500 cm<sup>3</sup> of distilled water and heated till the solution boiled and the heat, turned off. After cooling down, the PDA solution was autoclaved for about 15 min. The PDA solution was dispensed into a sterile petri dish and allowed to set.

#### **Determination of Antimicrobial Activity Using Disk Diffusion Method**

##### **Antibacterial Testing**

The disk diffusion method was performed using nutrient agar. The test organism was transferred onto the petri dishes containing the molten nutrient agar. The inoculums were spread around the plate using a sterile wire loop. This was to give a uniform distribution of the test organism in the agar and allowed to set. Multiple disks were separately impregnated with both industrial made hand sanitizer and local made hand sanitizer. After making sure the disks were completely infused with the industrial made hand sanitizer and local made hand sanitizer respectively, it was aseptically transferred onto the plates containing *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The plates were incubated at 37 °C for 24 h. The diameter of the zone of inhibition was measured for both hand sanitizers.

##### **Antifungal Testing**

The disk diffusion method was performed using potato dextrose agar. The test organism was transferred onto the petri dishes containing the molten potato dextrose agar. The inoculums were spread around the plate using a sterile wire loop. This was to give a uniform distribution of the test organism in the agar and allowed to set. Multiple disks were separately impregnated with both industrial made hand sanitizer and local made hand sanitizer. After making sure the disks were completed infused with the industrial made hand sanitizer and local made hand sanitizer respectively, it was aseptically transferred onto the plates containing *Candida spp*. The plates were incubated at 37 °C for 24 h. The diameter of the zone of inhibition was measured for both hand sanitizers.

#### **Results**

The results of the analysis (heavy metal concentrations and antimicrobial efficacy) are as presented in Table 1 and 2 respectively.

**Table 1: Heavy Metal Concentration in Some Selected Hand Sanitizers Sold in Makurdi, Benue State, Nigeria**

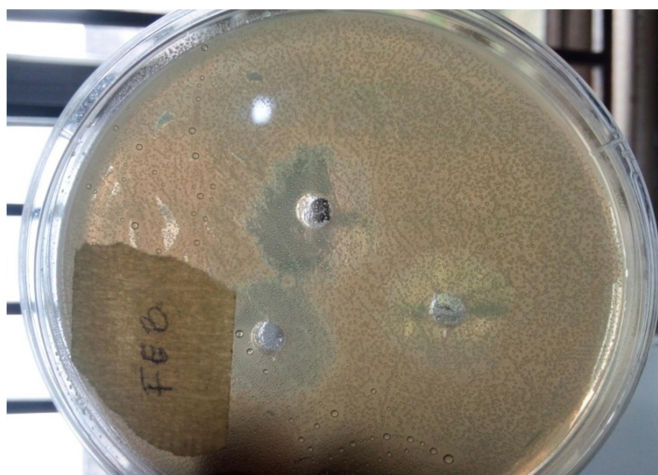
Sample	Cadmium (mg/L)	Nickel (mg/L)	Mercury (mg/L)	Lead(mg/L)
B	0.0109±0.0001	0.011±0.000	ND	ND
K	0.1235±0.0003	0.013±0.000	ND	ND
C	0.1208±0.0002	0.070±0.000	ND	ND
D	0.1136±0.0002	0.051±0.000	ND	ND
WHO	0.003	0.01	0.01	0.1
NESREA	0.01	0.05	0.01	-
EPA	0.005	0.02	0.002	-

- Values are Mean ± SD in triplicates ND=Not Detected

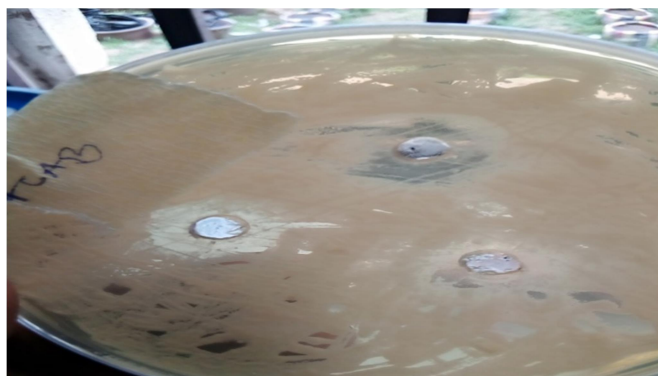
**Table 2: Zone of Inhibition of hand sanitizers in some strains of bacteria and fungus**

Hand sanitizers	Zones of inhibition (mm)				
	P. Aeruginosa	S. aureus	E. Coli	Candida Spp	FLSD (P<0.05)
B	15.50±1.01 <sup>b</sup>	12.20±1.31 <sup>ab</sup>	17.07±1.36 <sup>b</sup>	11.67±1.04 <sup>ab</sup>	0.945
K	11.06±0.40	11.10±0.17	11.30±0.89	6.67±0.29	1.000
D	9.97±0.65	10.40±0.17	9.97±0.35	6.17±0.58	4.233
C	10.10±0.85	11.30±0.30	10.20±0.17	6.33±1.04	3.767
CON.	5.55±0.39	5.10±0.17	5.40±0.35	0.00±0.00	NS

- Values are Mean ± SD in triplicate
- NS =Not Significant
- FLSD= Facial Least Significance Difference



**Figure 1:** A sample of the sensitivity disc, incubated after 24 hour at 37 °C showing the zone of inhibition against bacteria.



**Figure 2:** A sample of the sensitivity disc, incubated after 24 hour at 37 °C showing the zone of inhibition against fungi.

### Discussion

The concentration of cadmium (Table 1) was  $0.0109 \pm 0.0001$  mg/L in sample B,  $0.1235 \pm 0.0003$  mg/L in sample K,  $0.1208 \pm 0.0002$  mg/L in sample C and  $0.1136 \pm 0.0002$  mg/L in sample D. It was observed that the concentration of cadmium in sample K was slightly higher compared to other samples. The concentrations of cadmium in all the hand sanitizers analyzed were slightly above the permissible limit of WHO, NESREA and EPA as shown in the table 1.

The concentration of nickel was  $0.011 \pm 0.000$  mg/L,  $0.013 \pm 0.000$  mg/L,  $0.070 \pm 0.000$  mg/L and  $0.051 \pm 0.000$  mg/L in sample B, K, C and D respectively as shown in Table 1. It was observed that the concentrations of nickel were generally below the permissible limit of NESREA (0.05

mg/L) but slightly higher than those of WHO (0.01 mg/L) and EPA (0.02 mg/L). Mercury and lead were not found in all the test samples exhibiting their safe levels.

From Table 2, the efficacy of different hand sanitizers used in Makurdi on some selected strains of bacteria and fungus was presented. It was observed that hand sanitizer B showed higher effect on *E. coli* ( $17.07 \pm 1.36$  mm) than the other test organisms. This effect on *E. coli* was significantly higher than the effect on *S. aureus* ( $12.20 \pm 1.31$  mm) ( $p=0.038$ ); and *Candida spp* ( $11.67 \pm 1.04$  mm) ( $p=0.018$ ) respectively. However, no significant difference was observed between *E. coli* than *S. aureus* and *P. aeruginosa* ( $15.50 \pm 1.01$  mm) ( $p=0.425$ ). The effect of hand sanitizer K was also observed to be slightly higher on *E. coli* than *S. aureus* and *P. aeruginosa* but with no significant differences between them ( $p > 0.05$ ). On the other hand, a highly significant difference was observed when its effect on the three bacterial strains was compared to its effect on *Candida spp* ( $p < 0.05$ ) with the effect observed to be higher on the bacterial strains.

The effect of the sanitizer D was also significantly higher on the bacterial strains compared to the *Candida spp* ( $6.17 \pm 0.58$ ) ( $p=0.000$ ). Its effect was however slightly higher on *S. aureus* ( $10.40 \pm 0.17$  mm) compared to *E. coli* ( $9.97 \pm 0.35$  mm) and *P. aeruginosa* ( $9.97 \pm 0.65$  mm) with no significant difference between them ( $p > 0.05$ ). In the same manner, sanitizer C showed a slightly higher effect on *S. aureus* ( $11.30 \pm 0.30$  mm) than *E. coli* ( $10.20 \pm 0.17$  mm) and *P. aeruginosa* ( $10.10 \pm 0.85$  mm) with no significant difference between them ( $p > 0.05$ ). Its effect on the three bacterial strains was however significantly higher than on the *Candida spp* ( $6.33 \pm 1.04$  mm) ( $p=0.000$ ).

The control was observed to have effect only on the bacterial strains with no significant difference between them ( $p > 0.05$ ). Sample B was generally observed to have higher effect on the entire test organism compared to all other samples used in the study (Table 2). This may be due to the type of alcohol formulations used, since alcohol serves as the main ingredient in bacteriostatic effect (Aiello et al., 2008; Alvarado et al., 2009; Shoge et al., 2021). Ethanol and isopropyl alcohol formulations are popularly used worldwide. Methanol or ethylene glycols are quite poisoning, the metabolites of isopropyl alcohol are considerably less toxic, and treatment is largely supportive (Tamas et al., 2014).

## Conclusion

All the tested samples of hand sanitizers showed a satisfactory bacterial reduction to *E. Coli*. The findings of this study indicate that all the hand sanitizer samples had a bacteriostatic effect to *Staphylococcus aureus*, *Peudomonasaeruginosa*, *Escherichia coli* and *Candida spp*.

The study also showed that some of the hand sanitizers, contain heavy metals which are toxic to the human body, therefore, manufacturers must carefully select gelling agents and other additives during production to safeguard human health.

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