

# Original Research Article

## TOTAL COLONY COUNTS OF SOME MICROORGANISMS IN SOIL SAMPLES EXPOSED TO ELECTROMAGNETIC RADIATIONS FROM MOBILE PHONES

### ABSTRACT

Effect of electromagnetic radiations (EMR) from mobile phones on some soil bacterial (*Bacillus*, *Micrococcus*, *Staphylococcus*, *Corynebacterium* and *Enterococcus*) and fungi (*Saccharomyces*, *Penicillium* and *Geotrichum*) strains were studied. At the study periods of 0, 30, 60, and 90 days, the total colony counts (CFU/g) and viability ratios of bacteria and fungi strains were determined on both the EMR unexposed and exposed soil samples. At the exposure periods of 30, 60, and 90 days, the mean total colony counts of all the organisms in the EMR exposed samples were statistically ( $p < 0.05$ ) lower than those of the EMR unexposed samples. The percentage changes in total colony counts of the EMR unexposed and exposed soil samples between 0 to 90 days for *Bacillus*, *Micrococcus*, *Staphylococcus*, *Corynebacterium*, *Enterococcus*, *Saccharomyces*, *Penicillium* and *Geotrichum* were 2.54% and -70.51%, 33.18% and -75.80%, 11.79% and -63.93%, 20.12% and -91.96%, 30.00% and -98.94%, 62.36% and -61.06%, 42.40% and -100%, and 50.46% and -78.48% respectively. These show that *Staphylococcus* and *Saccharomyces* strains being the most resistant, while *Enterococcus* and *Penicillium* were the most susceptible strains. The results generally indicate that EMR has negative impacts on both bacteria and fungi total colony counts.

**KEYWORDS:** Electromagnetic Radiations, Mobile Phone, Fungi, Bacteria.

### 1.0 Introduction

In the last few decades, the world has witnessed a remarkable increase in usage of wireless telecommunication network. Nigeria, like other nations of the world, with her overwhelming population, has warmly embraced the technology. This is evidenced by the number of users estimated at over fifty per cent (50%) of her one hundred and forty million populations (Zain, 2005). The increase in the number of users is not without an increase in the number of base stations spread across the nation, to receive and send communication signals.

The mobile phones in their receiving and sending processes use (mostly) microwave with frequency of 945MHz (Bakr, 2004), emitting radiations that may be absorbed by various organs of the body at various degrees depending on the place where they are located (Ozguner *et al.*, 2005; Oktem *et al.*, 2005).

Mobile towers are especially dangerous because they emit microwaves at a frequency range of 900 to 1900MHz. Recent studies have shown that the intense radioactivity from mobile phone towers adversely impact on every biological organism within 1 square kilometer (Bakr, 2004).

Mobile phones and base stations emit electromagnetic radiations that penetrate the living bodies to a distance that decreases with increasing frequency (Stewart, 2000), and poses threat to human and other flora and fauna in two (2) ways: direct (environmental) and indirect (health) hazard (Jain and Wullert, 2002).

Dreyfuss and Chipley, (1980) studied the effect of EMR at sub-lethal temperature on *Staphylococcus aureus* and suggested that the existence of a phenomenon different from thermal heating would have been responsible for altered activity of various metabolic enzymes. There have also been a lot of other reports favouring the existence of non-thermal effects (Carta and Desogus, 2010; Coptý *et al.*, 2006), but the studies refuting the possibility of non-thermal effects (Kozempel *et al.*, 2000; Shazman *et al.*, 2007; Vela and Wu, 1979) cannot be jettisoned. Studies on *E. coli* and *S. aureus* suggested that physical damage caused by microwaves led to alterations in membrane permeability and consequently influx of extracellular  $\text{Ca}^{2+}$ . An increase in cell permeability of up to 89.8% and 19.7% were obtained after MW treatment in *S. aureus* and *E. coli* respectively (Chen *et al.*, 2007). High frequency electromagnetic field of mobile phones affected the viability of *Staphylococcus aureus* but not its ability to produce biofilm (Boon *et al.*, 2000). At the exposure of three different bacterial species; *E. coli*, *Lectercia adecarbodxylata* and *Staphylococcus aureus* to EMR of 50Hz, the viability of *E. coli* was significantly reduced in comparison to the other two species, with *S. aureus* being the least affected (Fojt *et al.*, 2004). However; prolonged exposure to 50Hz Low Frequency-EMF has been shown to affect transposition activity in *E. coli* (Del Re *et al.*, 2003). Duration of EMR exposure therefore, seems to be a major determinant of EMR effect on microorganisms. The time of exposure and power density are correlated in a way that decrease in power density (PD) may be compensated by increase in duration of exposure.

This work is designed to take a step look at the impact of EMR on microorganisms in the soil. It must be noted that soil flora and fauna are not exempted from the possible increasing impact of electromagnetic radiations from wireless networks, and as such, studies on microorganisms in their natural environment will provide insight into the overall effect of EMR on the soil.

## 2.0 Materials and Methods

### 2.1 Sample Collection

Soil samples were collected with the aid of thoroughly washed and air-dried plastic containers at a depth of 30cm from five different sites (at a distance of 2m from each other) on a five-year fallowed garden farm of Federal University of Technology, Owerri, Nigeria. They were collected in the month of August, 2013 and then homogenized (Kenwood, UK). Five kilograms (5 Kg) of the homogenized soil sample was then weighed into each of two experimental tanks, measuring 120×120×100 cm each.

### 2.2 Experimental Set up

A commercially available Nokia 2700 mobile phone (900MHz band with modulated voice and Specific Absorption Rate of 0.927W/Kg) was used for the generation of EMR for this study. The cell phone was sandwiched at a depth of 5cm in the 5kg soil sample of experimental tank one (Fig. 1A). During exposure, the cell phone was kept on talking mode for four hours per day, in vibration and the phone battery was also kept charged.

The unexposed sample (with no phone) was placed away (at a distance of 150m) from the exposed sample (with phone) at the same atmospheric conditions (Fig. 1B). Both the exposed and the unexposed soil samples were irrigated continuously to maintain 75% humidity and a room temperature of  $27 \pm 2^{\circ}\text{C}$  for periods of 0, 30, 60 and 90 days. The mobile phone was temporarily

removed (2minutes) from experimental tank one during every irrigation exercise. The exposed and unexposed sample set-ups were kept away from any instrument or machine to avoid interference from any form of unwanted EMR. At the end of each period, soil enzyme activities were assayed.

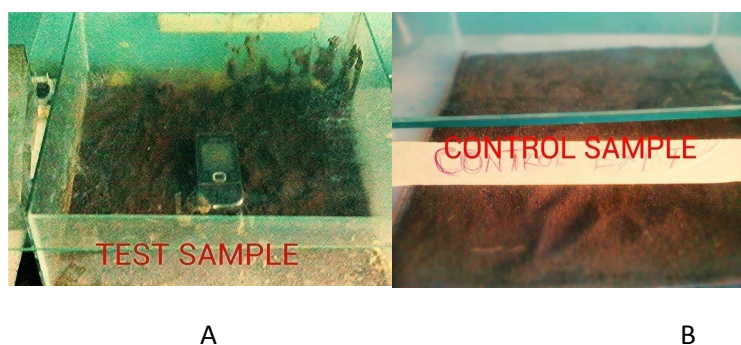


Figure 1: Experimental set-up of the Test (A) and Control (B) Samples

### 2.3 Preparation of Sample

Ten grams (10g) of the soil samples were dispersed into 90ml of sterile diluent to obtain  $10^{-1}$  dilution. Further dilutions were made until  $10^{-8}$  dilution was obtained (Beishir, 1987). Aliquot portions (0.1ml) of appropriate dilutions were inoculated onto surface dried nutrient and potato dextrose agar (PDA) respectively. The inocula were spread evenly on the media with a sterile hockey stick-like glass spreader. This was done to ensure even distribution of discrete and countable colonies. Nutrient agar plates were incubated at  $37^{\circ}\text{C}$ , while PDA plates were incubated at ambient temperature ( $28 \pm 0.2^{\circ}\text{C}$ ).

### 2.4 Determination of Microbial Total Colony Counts

A total colony count was determined manually by dividing the plates at the reverse side and counting visible colonies; the total colony count was then expressed as colony forming units per grams (CFU/g) according to Harrigan and McCance (1990).

Microorganisms isolated from the soil samples were characterized on the basis of colonial, microscopic and few biochemical tests as described by Cheesbrough (2000); Harrigan and McCance (1990).

The traditional method of microscopic examination of bacteria and yeast in the laboratory is the Grams' staining method. The staining method employed in this study was according Gardner and Provine (1975); Cheesbrough (2000). At the end of the process, bacteria that retained the violet-Iodine complex after washing in ethanol were classified as Gram positive, while those that lost this complex were classified as Gram negative.

The method applied for the microscope study of fungi was according Harrigan and McCance (1990). A large drop of mounting fluid (10%KOH) was placed on a clean grease-free glass slide. A bit of the specimen was carefully laid out in this small lake and a cover slip placed. The slide was gently warmed to remove air bubbles and to enhance the action of the staining fluid. The

preparation was allowed to stand for 10-15 minutes to remove air bubbles. By gently pressing down the cover slip or blotting the excess mounting fluid or both rendered the organisms easier to see. It was then examined with the high dry objective (40x) microscope.

Some biochemical reactions: carbohydrate fermentation, catalase and oxidase tests were carried out as described by Cheesbrough (2000), to identify species that could not be distinguished by their cultural and micro characteristics.

## 2.5 Viability Ratios of the Isolated Microbes

The viability ratios of the organisms were calculated by dividing the number of viable cells after exposure ( $cfu_i$ ) with the number of viable cells of control ( $cfu_o$ ) i.e. Viability ratio =  $\frac{cfu_i}{cfu_o}$

## 2.6 Statistical Analysis

All analyses were done in triplicate and the data generated were analyzed using One-way ANOVA and Duncan's test with the aid of Statistical Package for Social Science (SPSS). Values for  $P < 0.05$  were considered statistically significant.

## 3 Results and Discussion

Radiations generated by mobile phones are non-ionizing (NIR). There is a wide range of data documenting the capability of non-ionizing radiation to affect living cells, including causing changes in the biochemical and molecular mechanisms of cells both *in vitro* and *in vivo* (Barnes, 1996). Cell metabolism, cell proliferation, components of cytoplasmic membrane (Bersani *et al.*, 1997; Chen *et al.*, 2007), cytoplasm and nucleus, are also affected following exposure to EMR (Hill, 1998). Mobile phone radiations exposure can also cause cell damage and death by reactive oxygen species formation (Oral *et al.*, 2006; Sokolovic *et al.*, 2008). Furthermore, the ideas of resonant absorption and resonant interactions have been proposed as an explanation for the marked sensitivity of living systems to EMR (Cosic, 1997). In the present study, the effect of electromagnetic radiations from mobile phone on total colony counts (CFU/g) of soil *Bacillus*, *Micrococcus*, *Staphylococcus*, *Corynebacterium*, *Enterococcus*, *Saccharomyces*, *Geotrichum* and *Penicillium* stains, for the exposure periods of 0, 30, 60 and 90 days were examined. The results generally indicated that the total colony counts and viability ratios of the bacteria and fungi strains for the EMR exposed samples decreased with increase in exposure periods from zero (0) to ninety (90) days compared to the EMR unexposed samples. Though, bacterial species have been reported to differ in their susceptibility to EMR inactivation (Najdovski *et al.*, 1991), the results however, were consistent with the findings of Komarova *et al.*, (2008); Strasak *et al.*, (2002); Fojt *et al.*, (2007), that an increase in magnetic strength caused mortality in *E.coli* with the organism losing its ability to form colonies and consequently grew at a slower rate than the control. This observation have be attributed to non-thermal (athermal) effect, which has been suggested to have an important role in the suppression of microorganisms in suspension (Jeng *et al.*, 1987).The mechanism of action has, however, not been reported.

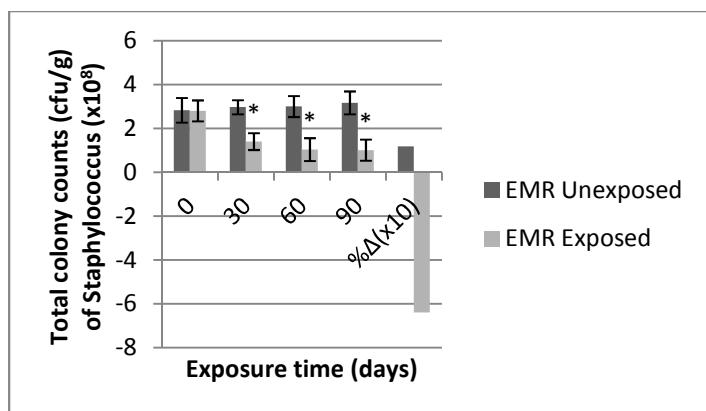


Fig.2: *Staphylococcus* strain total colony counts (CFU/g) of electromagnetic radiations (EMR) exposed and unexposed soil samples. %Δ, overall percentage change in *Staphylococcus* strain total colony counts. \* Values are significant ( $P<0.05$ ) in comparison with their respective EMR unexposed control.

*Staphylococcus* is a genus of gram-positive bacteria. *Staphylococci* are facultative anaerobes that are found worldwide and are small components of soil microbial flora. They form clusters, produce catalase and have appropriate cell wall structure (including peptidoglycan type and teichoic acid presence) (Harris and Foster, 2002). Figure 2 presents the *Staphylococcus* strain total colony counts (CFU/g) of electromagnetic radiation (EMR) exposed and unexposed soil samples. It shows significant ( $P<0.05$ ) reduction in *Staphylococcus* strain total colony counts (CFU/g) of EMR exposed samples at the exposure periods of 30, 60 and 90 days with the overall percentage changes of -63.9% and 11.8% for the EMR exposed and unexposed samples respectively, indicating the negative impact of the treatment on the organism. This finding agrees with Boon *et al.*, (2000); Mohd-Zain *et al.*, (2012), who reported loss of viability of the organism when exposed to electromagnetic radiation for more than 30 minutes.

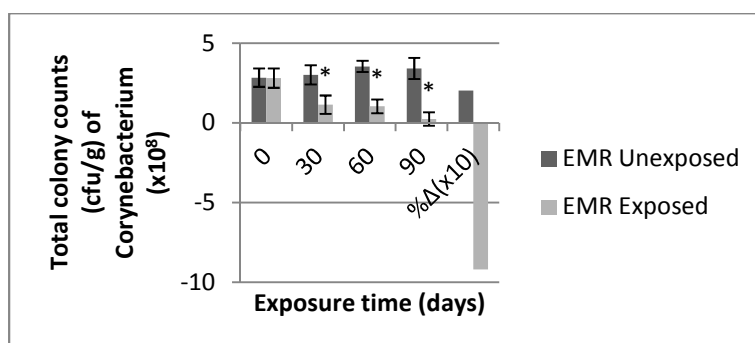
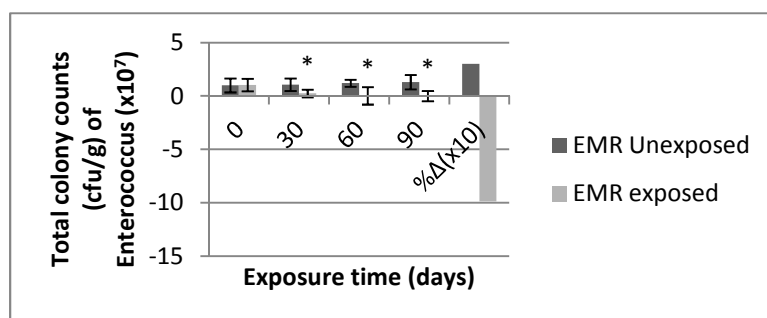


Fig. 3: *Corynebacterium* strain total colony counts (CFU/g) of Electromagnetic radiations (EMR) exposed and unexposed soil samples. %Δ, overall percentage change in *Corynebacterium* strain total colony counts. \* Values are significant ( $P<0.05$ ) in comparison with their respective EMR unexposed control.

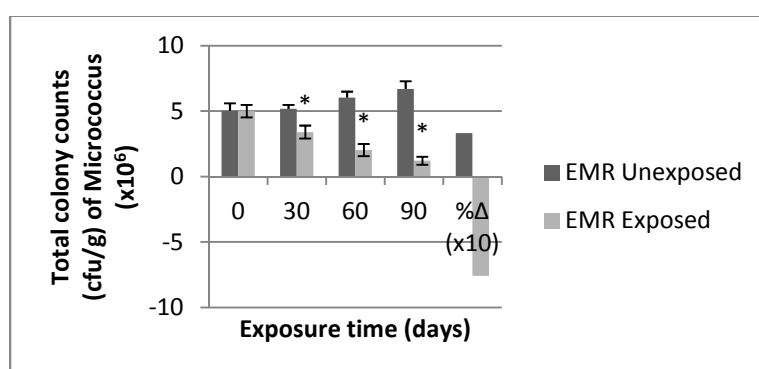
*Corynebacterium* is a genus of Gram-positive, rod-shaped bacteria. They are widely distributed in nature and are mostly innocuous. They are catalase positive, none spore-forming, none motile, rod-shaped bacteria that are straight or slightly curved. They occur commonly in nature in the soil, water, plants and food products. Figure 3 presents the *Corynebacterium* strain total colony counts (CFU/g) of electromagnetic radiation (EMR) exposed and unexposed soil samples with

179 overall percentage changes of -92.0% and 20.10% in the total colony counts of the organism for  
 180 the EMR exposed and unexposed samples respectively. It shows significant ( $P<0.05$ ) decline in  
 181 the number of viable cells at those exposure periods.



182  
 183 Fig. 4: *Enterococcus* strain total colony counts (CFU/g) of Electromagnetic radiations  
 184 (EMR) exposed and unexposed soil samples. %Δ, overall percentage change in  
 185 *Enterococcus* strain total colony counts. \* Values are significant ( $P<0.05$ ) in comparison  
 186 with their respective EMR unexposed control.

187 Figure 4 shows the *Enterococcus* strain total colony counts of electromagnetic radiation (EMR)  
 188 exposed and unexposed soil samples. It indicates significant ( $P<0.05$ ) decreases in total colony  
 189 counts of EMR exposed samples at the exposure periods of 30, 60 and 90 days. The overall  
 190 percentage changes in the total colony counts of the organisms for EMR unexposed and exposed  
 191 samples were of 30.0% and -98.94% respectively, indicating the negative impact of the  
 192 radiations on the exposed organisms. Enterobacteriaceae are rod-shaped, typically 1-5μm in  
 193 length and are Gram-negative stains. They are facultative anaerobes that ferment sugars to  
 194 produce lactic acid and various other end products. They are spore-forming and generally lack  
 195 cytochrome C oxidase, although there are exceptions such as *Plesiomonas shigelloides* (Harris  
 196 and Foster, 2002).



197  
 198 Fig. 5: *Micrococcus* strain total colony counts (CFU/g) of Electromagnetic radiations (EMR)  
 199 exposed and unexposed soil samples. %Δ, overall percentage change in *Micrococcus* strain  
 200 total colony counts. \* Values are significant ( $P<0.05$ ) in comparison with their respective  
 201 EMR unexposed control.

202 *Micrococcus* is a genus of bacteria in the micrococcaceae family. It occurs in a wide range of  
 203 environments, including water, dust, and soil. They are Gram-positive, spherical cells ranging  
 204 from 0.5 to 3 μm in diameter and typically appear in tetrads. They are catalase positive, oxidase  
 205 positive, indole negative and citrate negative (Daniel *et al.*, 2008). They can grow well in

environments with little water or high salt concentrations. Though not a spore-former, micrococcus cells can survive for an extended period of time (Greenblat *et al.*, 2004). Figure 5 shows the *Micrococcus* strain total colony counts of electromagnetic radiation (EMR) exposed and unexposed soil samples. It reveals significant ( $P<0.05$ ) decreases in total colony counts of the EMR exposed samples, with overall percentage changes of 33.2% and -75.8% in total colony counts of the organisms for EMR unexposed and exposed samples respectively. This demonstrates the negative impact of the radiations on the organism.

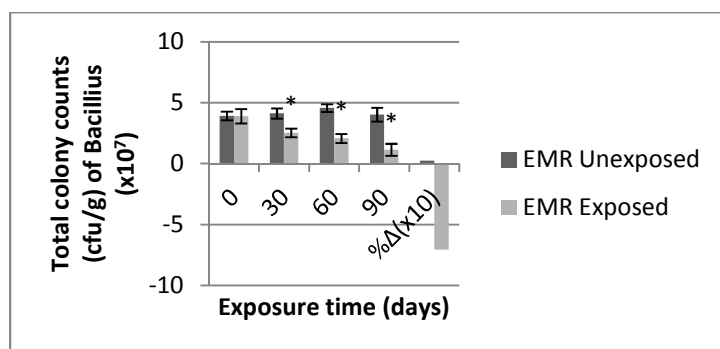


Fig. 6: *Bacillus* strain total colony counts (CFU/g) of Electromagnetic radiations (EMR) exposed and unexposed soil samples. %Δ, overall percentage change in *Bacillus* strain total colony counts. \* Values are significant ( $P<0.05$ ) in comparison with their respective EMR unexposed control.

*Bacillus* is an extremely diverse group of bacteria that include the causative agent of anthrax (*Bacillus anthracis*). They are rod-shaped, gram-positive sporulating aerobes, or facultative anaerobes (Harris and Foster, 2002). Figure 6 shows the *Bacillus* strain total colony counts of electromagnetic radiation (EMR) exposed and unexposed soil samples. It reveals significant ( $P<0.05$ ) decrease in total colony counts of EMR exposed samples at the exposure periods of 30, 60 and 90 days. It also presents the overall percentage changes of 2.50% and -70.50% in total colony counts of the organisms for EMR unexposed and exposed samples respectively. The finding is consistent with the report of Otludil *et al.*, (2004) that reported negative impact of the radiations on the bacillus bacterial strain.

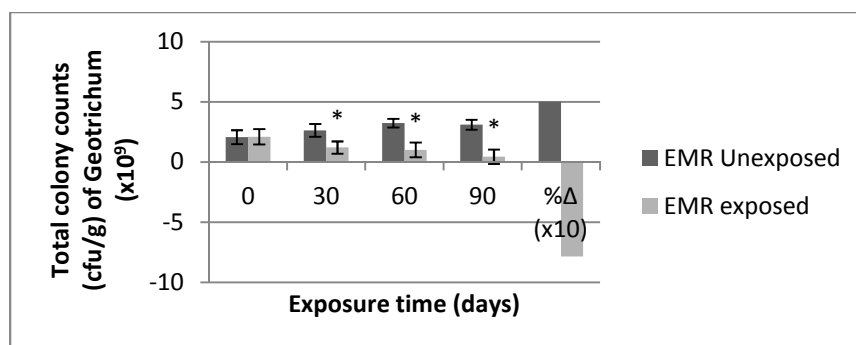


Fig.7: *Geotrichum* strain total colony counts (CFU/g) of Electromagnetic radiations (EMR) exposed and unexposed soil samples. %Δ, overall percentage change in *Geotrichum* strain total colony counts. \* Values are significant ( $p<0.05$ ) in comparison with their respective EMR unexposed control.

*Geotrichum* is a yeast found worldwide in soil, water, air, and sewage, as well as in plants, cereals, and dairy products (Bouakline *et al.*, 2000). *Geotrichum* strains produce rapidly growing, white, dry, powdery to cottony colonies, resembling "ground glass." When disturbed on the surface, the colony becomes yeast-like or slimy. The optimal growth temperature is 25°C. Figure 7 shows the *Geotrichum* strain total colony counts of electromagnetic radiation (EMR) exposed and unexposed soil samples. It indicates significant ( $P<0.05$ ) decrease in total colony counts of EMR exposed samples at the exposure periods of 30, 60 and 90 days, with overall percentage changes of 50.24% and -78.57% in total colony counts of the organisms for EMR unexposed and exposed samples respectively, revealing the negative impact of radiation on the organism.

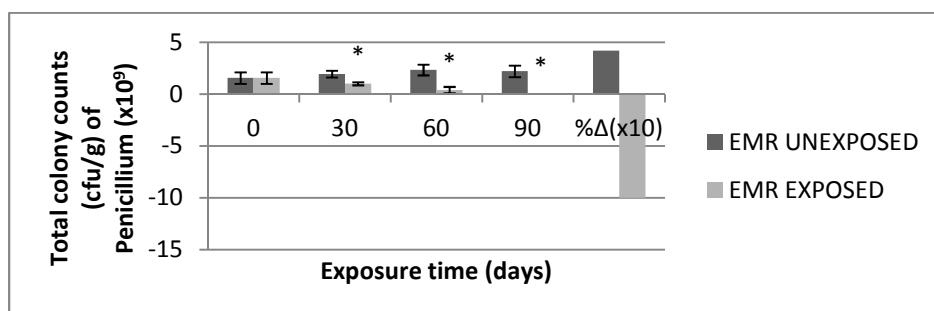


Fig.8: *Penicillium* strain total colony counts (CFU/g) of Electromagnetic radiations (EMR) exposed and unexposed soil samples. %Δ, overall percentage change in *Penicillium* strain total colony counts. \*Values are significant ( $P<0.05$ ) in comparison with their respective EMR unexposed control.

*Penicillium* is a large and difficult genus encountered almost everywhere, and usually the most abundant genus of fungi in soils. There are approximately 200 species of this filamentous fungus, many of which give off a musty odor. Species of *Penicillium* are recognized by their dense brush-like spore-bearing structures called penicillin. Figure 8 shows the *Penicillium* strain total colony counts of electromagnetic radiation (EMR) exposed and unexposed soil samples. It shows significant ( $P<0.05$ ) decrease in total colony counts of EMR exposed samples at the exposure periods of 30, 60 and 90 days. The overall percentage changes in total colony counts of the organisms are 41.94% and -100% for EMR unexposed and exposed samples respectively. At 90 days of exposure, no growth was recorded in the EMR exposed samples. This shows the extremely deleterious effects of EMR on *Penicillium* strain.

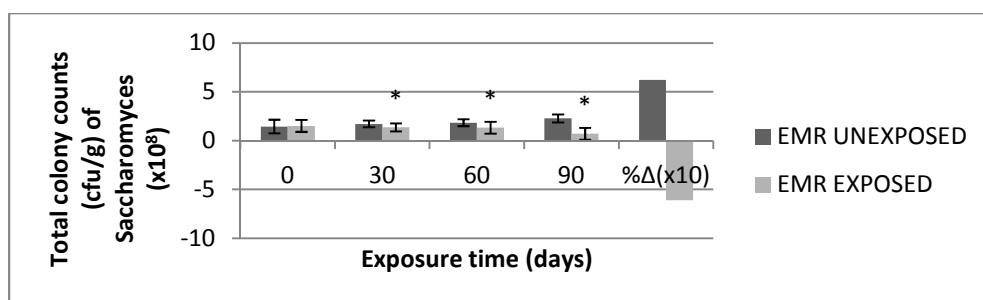


Fig. 9: *Saccharomyces* strain total colony counts (CFU/g) of Electromagnetic radiations (EMR) exposed and unexposed soil samples. %Δ, overall percentage change in *Saccharomyces* strain total colony counts. \* Values are significant ( $P<0.05$ ) in comparison with their respective EMR unexposed control.

*Saccharomyces* is yeast, which can exist either as single-celled organisms or as pseudomycelia. The cells are reproduced by multilateral budding. They can be differentiated based on growth characteristics and physiological traits. *Saccharomyces* strains are frequently isolated from the stools and throats of normally healthy individuals. *S. cerevisiae* for instance, is a normal inhabitant of soils and is widespread in nature. These traits enhance the organism's ability for long term survival (LaVeck, 1991). Since soil is a natural habitat for *S. cerevisiae*, it would be expected to survive well in soil. Figure 9 shows the *Saccharomyces* strain total colony counts of electromagnetic radiation (EMR) exposed and unexposed soil samples. It reveals significant ( $P<0.05$ ) decrease in total colony counts of EMR exposed samples at the exposure periods of 30, 60 and 90 days, with the overall percentage changes being 62.14 % and -61.11% for EMR unexposed and exposed samples respectively. This is consistent with the findings of Grundler *et al.*, (1977), who reported decrease in population of *Saccharomyces cerevisiae* following microwave irradiation.

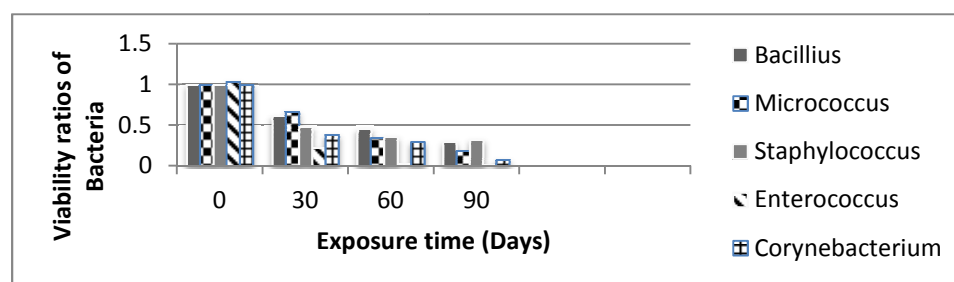


Fig. 10: Viability ratios of bacteria strains

Figure 10 shows the viability ratios of the bacteria isolates. It reveals that *Staphylococcus* and *bacillus* strains offered more resistance at the end of 90 days of exposure to the radiation than the other bacteria strains, with a decreasing order of resistance of: *Staphylococcus*>*Bacillus*>*Micrococcus*>*Corynebacterium*>*Enterococcus*.

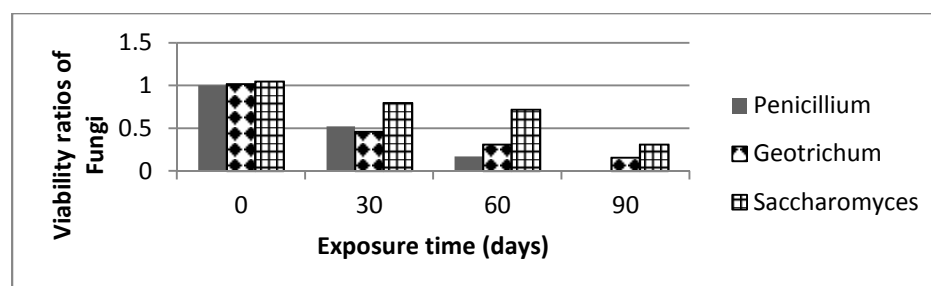


Fig.11: Viability ratios of Fungi strains.

Figure 11 shows the viability ratios of the fungi isolates. It indicates that *Saccharomyces* strain was more resistant to the radiation at the end of 90 days of exposure than the other fungi strains. The decreasing order of resistance is *Saccharomyces*>*Geotrichum*>*Penicillium*, with *Penicillium* completely losing its viability (100%) at 90 days of exposure.

293     **4           CONCLUSION**

294     The work examined the impact of electromagnetic radiation from mobile phones on total colony  
 295     counts of soil *Baccillus*, *Micrococcus*, *Staphylococcus*, *Corynebacterium*, *Enterococcus* bacteria  
 296     stains and *Saccharomyces*, *Penicillium*, *Geotrichum* fungi strains at exposure periods of 0, 30, 60  
 297     and 90days. Results indicate that EMR has negative impact on the total colony counts of the  
 298     studied microorganisms. However, the susceptibility of the organisms to the treatment varied,  
 299     with *Staphylococcus* and *Saccharomyces* strains being the most resistant, while *Enterococcus*  
 300     and *Penicillium* were the most susceptible strains. Based on the observed effects, it is concluded  
 301     that EMR from mobile phone has tremendous negative impact on the studied microorganisms.

303     **Competing Interests.** The Authors do hereby declare that no competing interests exist.

306                                     **References**

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