IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 10, Issue 1 Ver. II (Jan. 2016), PP25-34
www.iosrjournals.org

Effects of Electromagnetic Radiations (EMR) on Some Soil Physicochemical Parameters, Catalase and Dehydrogenase Activities

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Abstract: *Effects of electromagnetic radiations (EMR) on some physicochemical properties (pH, moisture, organic matter, phosphate contents) of soil and activities of catalase and dehydrogenase were studied at exposure periods of 0, 30, 60, and 90 days. At the exposure periods of 30, 60, and 90 days, the mean values of organic matters from the EMR unexposed (control) samples were significantly ($p<0.05$) lower than the mean values from EMR-exposed samples. The mean values of moisture contents from the EMR-exposed samples at the exposure periods of 30, 60, and 90 days were significantly ($p<0.05$) lower than the mean values from the EMR-unexposed samples. At the exposure period of 30 days, the mean value (6.993±0.065 units) of pH from the EMR-exposed sample was significantly ($p<0.05$) lower than the mean value (7.353±0.049 units) from the EMR-unexposed samples. The overall percentage changes for the EMR-exposed and unexposed samples for catalase and dehydrogenase activities were -13.9%, 99.0% and -64.9%, 91.9% respectively. The findings from this study showed that EMR had negative impacts on the studied soil parameters in contrast to pH and phosphate concentration.*

Keywords: *Electromagnetic radiations, mobile phones, physicochemical, catalase, dehydrogenase.*

I. Introduction

Recently, our society has come to embrace the usefulness of telecommunication in all its facets. In the last one and half decade, countries of the world have witnessed remarkable surge in the use of the communication technology. In Nigeria for instance, it is estimated that over 50% of the populations are subscribers of mobile telecommunication services. The increase in the number of subscription is not without corresponding increases in the number of base stations erected at the nooks and crannies of the nation; sometimes on the roof tops and public places. Mobile phones; however, in their sending and reception of signals use mostly microwave with the frequency of 945MHz (1), while mobile towers emit microwaves at the frequency range of 900 to 1900MHz. The radio frequency emitted by the mobile phones and base stations penetrates bodies to a distance that decreases with increasing frequency (2). Recent studies have shown that the intense radioactivity from mobile phone towers adversely impact on every biological organisms within 1 square kilometer (1). The microwaves might travel for as far as 2 miles in hilly areas, and up to 45 miles where there are fewer obstructions and of course, they easily penetrate bricks, metals (3) and soil.

Electromagnetic radiation is at non-ionization radiation (NIR) level. Barnes (4) had reported the ability of non-ionizing radiation to induce cellular damage. Electromagnetic radiations (EMR) may exert its effects through thermal and/or athermal means. The thermal effects of EMR result from rise in temperature produced by the energy absorbed from oscillating electric fields. At the absorption of the electromagnetic radiations, ionic distortion is provoked, leading to heating (2). The thermal effect varies with the conventional heating effects. This is because unlike the conventional heating effect, thermal effect causes the rotation and dipolar polarization of molecules in an attempt to align the dipoles with the electromagnetic radiations (5). Athermal effect of EMR on the other hand, results from a direct stabilizing interaction of electric field with specific (polar) molecules in a reaction medium with no rise in temperature (6). The growing interest in athermal effect stems from the fact that thermal effects alone cannot fully explain the observed effects of EMR.

The aim of this study was to assess the effects of the radiations from mobile phones on biotic and abiotic components of soil.

Catalase

The enzyme catalase (hydrogen peroxidase oxidoreductase, EC1.11.1.6) has a detoxifying function in cell, catalyzing the following reaction:

H_2O_2 -----→ $H_2O + ½O_2$.

All aerobic and most of the facultative anaerobic bacteria exhibit catalase activity. Its estimation is based on the determination of released O_2 (7). Catalase activity is very stable in soil. It shows significant correlation with the content of organic carbon and decreases with soil depth (8). Storage of moist or air-dried soils at room temperature for 4 months had no effect on catalase activity, and good correlations have been found between catalase and dehydrogenase activities (7). These informed the choice of the enzyme for this study.

Dehydrogenase

Soil dehydrogenases (EC1.1.1.) are the major representatives of the oxidoreductase enzymes class. Among all enzymes in the soil, dehydrogenase is one of the most important, and has been useful as an indicator of overall soil microbial activity (9) because they are tightly linked with microbial oxidoreduction processes. They occur intracellularly in all living microbial cells (10), strongly increase under anaerobic conditions and do not accumulate extracellularly in the soil. Dehydrogenases play a significant role in the biological oxidation of soil organic matter (SOM) by transferring hydrogen from organic substrates to either nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH) (11; 12). This also explains the choice of the enzyme for this study, as it will offer insight into the relationship between the enzyme, soil organic matters and the impact of the EMR on the relationship.

Soil Moisture

Water availability strongly affects soil microbial activity, community, composition (13), and consequently on soil enzymes activities. As soils dry, the water potential increases, and as well microbial activity as intracellular enzyme activity slows down (13). In the case of wet soils, increased moisture could bring into soil solution soluble organic matter, which could cause increase of bacterial population number (11).

Organic Matter Content

Soil organic matter (SOM) is used to describe the organic constituents in the soil (tissues from dead plants and animal products produced as these decompose and the soil microbial biomass). Organic carbon refers to the carbon occurring in the soil in SOM. SOM plays an important role in soil biological (provision of substrate and nutrients for microbes), chemical (buffering and pH changes) and physical (stabilization of soil structure) properties. These properties, along with soil organic carbon (SOC), nitrogen and phosphorus, are considered critical indicators for the health and quality of the soil (14).

Soil organic matter (SOM) has important effects not only on soil enzymes activities but first of all on microbial activities. Soil OM has been considered as an indicator of soil quality (similarly like DHA), because of its character of nutrient sink and source that can enhance soil physical and chemical properties, and also promote biological activity (9). It is evident that soil enzyme activity is strongly connected with soil OM content. The higher OM level can provide enough substrate to support higher microbial biomass, hence higher enzyme production (10). Kumar *et al,* (15) indicated that DHA displayed the close and positive correlation with OM content of four forests stands (two at low and two at higher attitudes).

pH

Enzyme activities tend to increase with soil pH. According to (16), the weakening of enzymatic activity in soil with the increase of soil acidity is the effect of destroying ions and hydrogen bonds in enzyme active centre. It is often assumed that pH may affect soil enzyme level in three different ways (17)

a. By changing the ionic form of the active sites of the enzymes, which consequently affect the enzyme activity and hence the reaction rate.
b. By altering the three-dimensional shape of enzyme, and
c. By affecting the affinity of the substrate for the enzyme.

Therefore, the pH factor is seen to be the best predictor of DHA in the soil environment (18).

Phosphate

In natural systems like soil and water, phosphorus will exist as phosphate (PO_4^-). Phosphorus (P), next to nitrogen, is often the most limiting nutrient for crop and forage production. The primary role of Phosphorus in plants is to store and transfer energy produced by photosynthesis for use in growth and reproductive processes.

Adequate P levels promote root and shoot growth, stimulate tillering, and hasten maturity. Thus, P-deficiency stunts vegetative growth and grain yield. Soil phosphorus is relatively stable in soil, and leaches very little compared to nitrogen. The release of inorganic phosphate from organic phosphates, called mineralization, is a result of microbial breakdown of organic compounds, and this role of microorganisms is influenced by soil temperature, soil aeration (oxygen levels), salinity (salt content/electrical conductivity) and soil moisture. The process is most rapid when soils are warm, moist, well aerated and well drained, and much slower on saturated wet soils.

Soils with inherent pH values between 6 and 7.5 are ideal for P-availability, while pH values below 5.5 and between 7.5 and 8.5 limits P-availability to plants due to fixation by aluminum, iron, or calcium often associated with soil parent materials (19). Phosphorus availability is controlled by three primary factors: soil pH, amount of organic matter, and proper placement of phosphorus; and the potential for P-loss is especially related to erosion and runoff.

II. Materials And Methods

Sampling and Sample Preparation

Samples were collected through the depth of 0-30cm into thoroughly washed and air-dried plastic containers with airtight capped covers, from five different sites (2m from one another) on about five-years-fallowed Garden of the Federal University of Technology, Owerri, Imo State, Nigeria on the month of September, 2013. The soil samples were homogenized (Kenwood, UK) and 5 kg weighed into two experiment tanks measuring 12x12x10cm each.

2.1 Experimental Set Up

A commercially available GSM mobile phone (900MHZ band) was used for the generation of EMR for the study. The mobile phone was sandwiched in the five kilogram soil sample of the test experiment tank. During exposure, mobile phone was kept on talking mode, in vibration and the phone battery was also kept charged.

The control sample (with no mobile phone) was placed away from the radiation in the same atmospheric conditions.

Unexposed and exposed soil samples were irrigated continuously to maintain 75% humidity, at room temperature of 28 ± 2^0C for a period of one, two and three months. The samples (control and test) were placed away from any instrument or machine to avoid interference from any form of unwanted EMR. At the end of each period, the soil samples were subjected to physicochemical and biochemical studies.

2.2 Physico-chemical Parameters Assays

Moisture.

The analysis of the moisture contents of the samples was as described by (20). Briefly; a porcelain crucible was placed in an oven at a temperature of 105^0C and allowed to stand for 2 hours. It was allowed to cool at room temperature in desiccators. The empty crucible was weighed (empty crucible weight = x). Ten grams (10g) of sample were weighed in the crucible and weighed again (sample + crucible weight = Y). The crucible was placed for 12 hours in the oven at 105^0C. The setup was allowed to cool at room temperature in a desiccator and weighed again (Z). Moisture content of the samples was calculated thus:

M (Moisture content) % = $\frac{(Y-Z)\times\ 100\%}{(Z-X)}$

MCF (moisture correction factor = $\frac{100+M\%}{100}$

pH

The pH of the soil was measured potentiometrically in a 1:2 soil-water suspensions as described by (21). Briefly; twenty five grams (25g) air-dried, 1 mm sieved soil sample was weighed into a 100 ml flask, and 50 ml distilled water added and agitated for one hour. The pH meter was calibrated using pH buffer, after which the pH of the suspension was measured.

Phosphate

Phosphate was determined by the method described by (22). Phosphate in the soil samples were extracted with sodium bicarbonate solution of pH 8.5 in an acid ammonium fluoride solution. Phosphate in the soil sample was determined colorimetrically with ammonium molybdate as the colouring reagent. Briefly; five grams (5g) of soil samples were weighed into 250 ml shaking bottles, 100 ml of sodium bicarbonate extractant added and agitated for 30mins. The setup was filtered through Whatman filter paper.

Five milliliter (5ml) of the standard series or blank sample was pipette to a test tube and 5ml of the mixed reagent was added. The solution was shaken and stood for one hour for the blue colour development, subsequently; the concentration of the solution was measured at 720nm, with spectrophotometer. The concentration of phosphate in the sample was determined using calibrated spectrophotometer with series of known concentrations and calculated thus,

$$\text{Available P (mg/kg)} = \frac{(s-B) \times D \times \left[100 + \left\{w - \left(\frac{w}{mcf}\right)\right\}\right] \times mcf}{W_{(g)}}$$

where:
S = P concentration in sample (mg/L) read by spectrophotometer
B = P concentration in blank (mg/L) read by spectrophotometer
D = Dilution factor (standard 1 for undiluted samples)
W = Weight of sample
mcf =Moisture correction factor
100 = volume of extractant

Organic Carbon

The organic carbon in the sample was oxidized with potassium dichromate and sulphoric acid according to the method of (14). Briefly, one gram (1g) of the soil sample was weighed into a 500ml conical flask (Borosil/corning) and then, ten milliliter (10ml) of 1N $K_2Cr_2O_7$ and 20ml of conc.H_2SO_4 were added. The flask was swirled carefully and allowed to stand for 30 minutes, 200ml distilled water and 10ml H_3PO_4 were added slowly. One milliliter (1ml) of diphenylamine indicator was added and then titrated against 0.5N ferrous ammonium sulphate solution until green colour appeared, indicating the end point. A blank was run simultaneously and organic carbon was calculated thus:

$$\textit{Organic carbon}(\%) = \frac{\mathbf{10(B-S) \times 0.39 \times mcf}}{\mathbf{B \times W}}$$

where,
B = ml of ferrous ammonium sulphate solution used for blank
S = ml of ferrous ammonium sulphate solution used for sample
mcf = moisture correction factor
W = sample weight (g).
0.39 = conversion factor (including a correction factor for a supposed 70% oxidation of organic carbon).
According to (23), the easily oxidizable organic carbon can be converted to total organic carbon by multiplication with 1.30 correction factor (or by dividing with 0.77). The total organic carbon was then converted to organic matter by the following equation:

$$\textbf{\% organic matter} = \frac{\%\textit{total carbon} \times 1.729}{0.58}$$

2.3 Assay of Enzyme Activities

Dehydrogenase (1.1.1) Activities

The method of (24) was used and is based on the estimation of the triphenyltetrazolium chloride (TTC) reduction rate to triphenyl formazan (TPF) in the soil samples after the incubation at 30^0C for 24h.
Five grams (5g) of field-moist soil samples were weighed into test tubes and mixed with 5ml of TTC solution. The tubes were sealed with rubber stoppers and incubated for 24h at 30^0C. After the incubation, 40 ml acetone was added to each tube, and the tubes were agitated thoroughly and further incubated at room temperature for 2h in the dark (agitating the tubes at intervals). Fifteen milliliter (15ml) of the soil suspension (red coloured) was then filtered and the optical density of the clear supernatant was measured against the blank at 546nm. TTC and TPF are sensitive to light, therefore; all the procedures were performed under diffused light.

Catalase (1.11.1.6) Activity

Five grams (5g) of soil samples were mixed in 20ml of distilled water balanced and centrifuged at 3500g for 10 minutes at 4^0C (25). Five millilitres (5mls) of cold 6mM H_2O_2 was added to the supernatant. The reaction was stopped after 3mins by adding 0.25ml of 6N H_2SO_4 rapidly and then agitated. Excess potassium permanganate (10mls) was added, agitated gently. A standard was prepared by mixing 10ml of potassium permanganate with 5.5mls of potassium phosphate buffers (pH 6.5) and 0.25ml of 6N H_2SO_4, and Absorbance was read at 480nm (26). Catalase activity was calculated thus:

$K/_{0.00693}$. where the first-order rate constant (K) = Log $\left(\frac{S_o}{S_t}\right) \times \left(\frac{2.3}{t}\right)$.
S_0 (Initial substrate concentration) = Absorbance of standard minus Absorbance of blank,
S_t (final substrate concentration) = Absorbance of standard minus Absorbance of sample,
t = reaction time.

2.4 Statistical Analysis

All readings from the study were obtained, at least in triplicate and the data generated were analyzed using Analysis of Variance (ANOVA) and Duncan's test with the aid of Statistical Package for Social Sciences (SPSS). Values for $p<0.05$ were considered as statistically significant; data were presented as mean±standard deviation.

III. Results

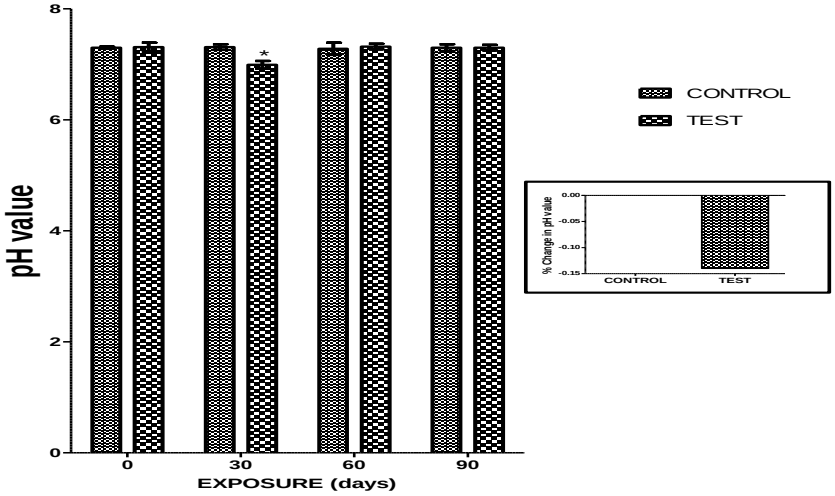

Fig.1: pH of electromagnetic radiation (EMR) exposed and unexposed soil samples. Insert plot; %Δ, overall percentage change in pH of EMR unexposed and exposed soil samples.
* Values are significant ($p<0.05$) vis-à-vis their respective EMR unexposed control.

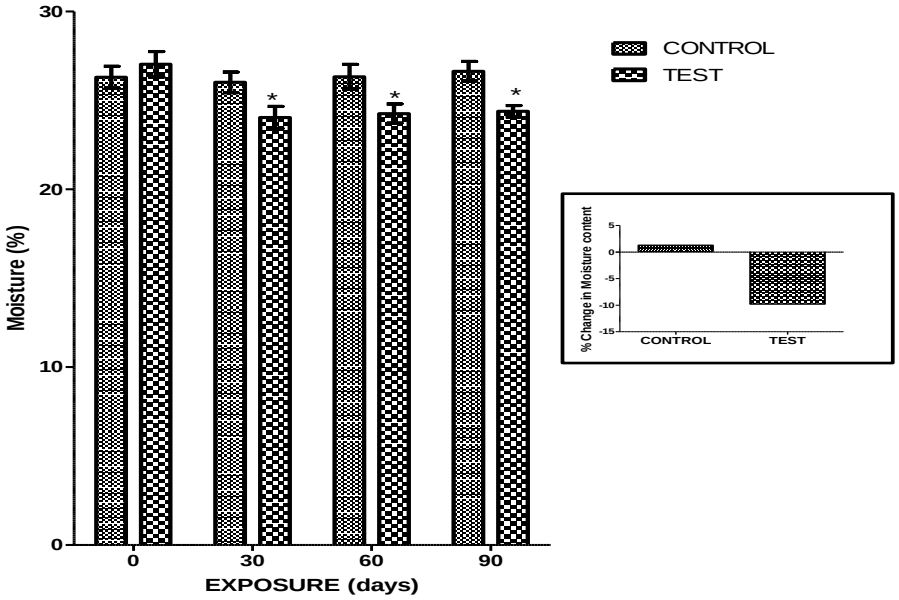

Fig.2: Moisture of electromagnetic radiation (EMR) exposed and unexposed soil samples. Insert graph; %Δ, overall percentage change in moisture of EMR unexposed and exposed soil samples.
* Values are significant ($p<0.05$) vis-à-vis their respective EMR unexposed control.

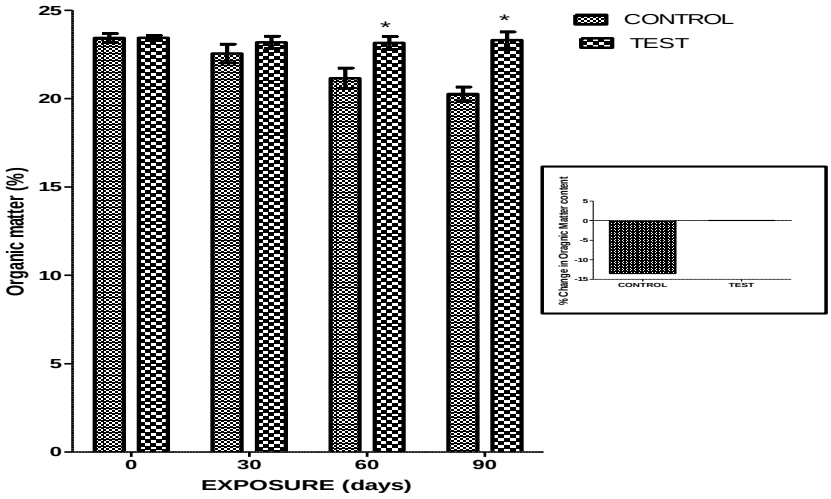

Fig.3: Organic matters (%) of electromagnetic radiation (EMR) exposed and unexposed soil samples. Insert plot; %Δ, overall percentage change in organic matters of EMR unexposed and exposed soil samples.
* Values are significant ($p<0.05$) compared to their respective EMR unexposed control.

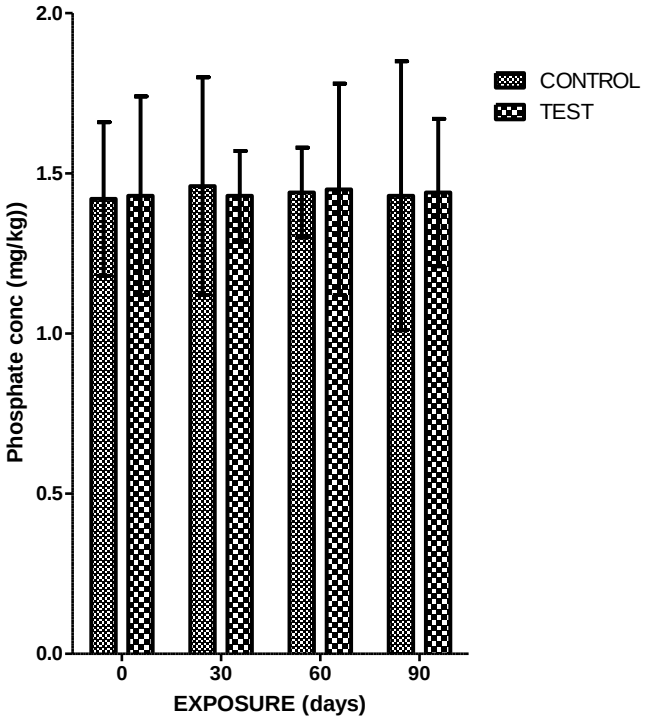

Fig.4: Phosphate (mg/kg) of electromagnetic radiation (EMR) exposed and unexposed soil samples. Insert graph; %Δ, overall percentage change in phosphate of EMR unexposed and exposed soil samples.
* Values are significant ($p<0.05$) vis-à-vis their respective EMR unexposed control.

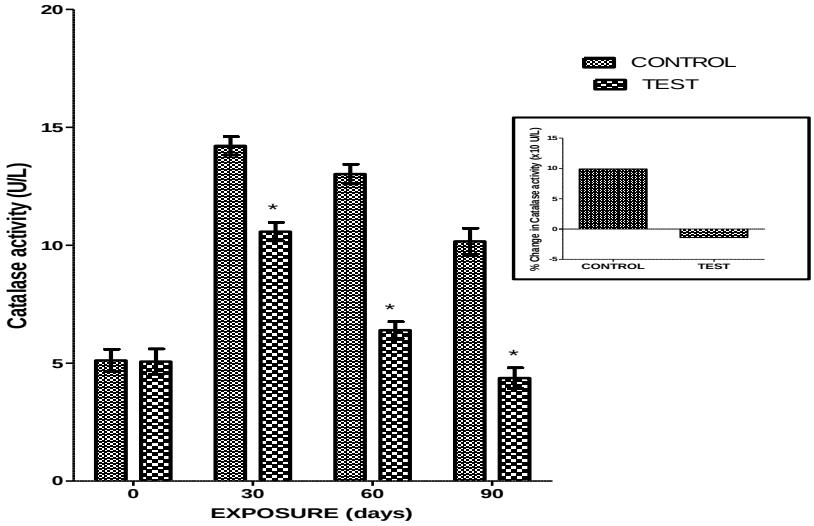

Fig.5: Catalase activities (U/L) of electromagnetic radiation (EMR) exposed and unexposed soil samples. Insert graph; %Δ, overall percentage change in catalase of EMR unexposed and exposed soil samples.
* Values are significant ($p<0.05$) vis-à-vis their respective EMR unexposed control.

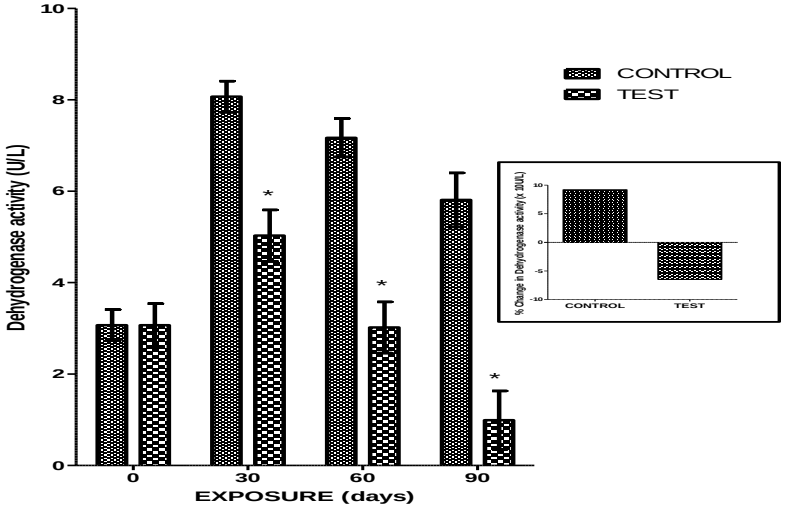

Fig.6: Dehydrogenase activities (U/L) of electromagnetic radiation (EMR) exposed and unexposed soil samples. Insert graph; %Δ, overall percentage change in dehydrogenase of EMR unexposed and exposed soil samples.
* Values are significant ($p<0.05$) compared to their respective EMR unexposed control.

IV. Discussion

Within the last few decades, the world population has adopted the use of mobile phones in a horrendous way. Increasing use of these electromagnetic devices has become a trend as well as a need in a large section of the society. Mobile phones transmit electromagnetic waves of very low intensity and there has been a lot of discussion on possible adverse effects of these radiations on the living and non-living components of the environment. It has been established that the radiations generated by mobile phones are non-ionizing radiations (NIR). Available data have shown the ability of non-ionizing radiation to produce such effects as change in the biochemical and molecular mechanisms of cells both *in vitro* and *in vivo* (4), inducing potentially damaging effects in all cell components including cytoplasm and nucleus; altering cell metabolism and cell proliferation (27; 28). Electromagnetic radiation exerts its effects through: thermal action, non-thermal actions, and/or combination of the duo. Mobile phones emit EMR at non-thermal power density levels. Thermal effects result from the conversion of the EMR energy into heat energy. Polar molecules (proteins, DNA, etc) respond to EMR by rotation. Angular momentum created from this rotation results in friction with neighboring molecules, thus developing vibration energy (29). Through this means, radiation energy is converted into thermal energy. The effect of vibration energy is therefore, thermal effect which happens in biosystems because of the absorption of

EMR into biological materials, which subsequently heat up the intra- and extra-cellular fluids by transfer of vibration energy (30).

The effect of EMR on enzymes may as well results from the denaturation of the proteins consequent to the thermal effect of the radiation thus, causing changes in the secondary and tertiary protein structure. The mechanism of this change on the enzyme may be through electroporation (31). The effect of EMR can also be accounted for by the concept of resonance absorption and resonance interaction (32).

The mean values of pH from the control and test soils revealed that the soil samples were slightly basic. The means 7.31±0.03, 7.32±0.05, and 7.30±0.05 of pH from the test samples for the exposure periods of 0, 60, and 90 days were not significantly ($p<0.05$) different from the mean values 7.30±0.02, 7.28±0.11, and 7.30±0.06 from control samples for the same periods respectively. So far, no evidence exist that EMR can affect pH directly, however, it is well-established that changes in environmental conditions can induce bacterial cells to alter the pH of their medium (33). This explains the significant ($p<0.05$) difference observed for the mean value 6.99±0.07 from the test sample compare to the mean value 7.31±0.05 from the control at the exposure period of 30 days.

One of the applications of microwave (at the same frequency as EMR) is based primarily on its thermal effects includes moisture removal, and this explained the trend of the results on moisture content where the mean values 24.04±0.62, 24.26±0.54, 24.39±0.32 from the test samples for the exposure period of 30, 60, and 90 days were significantly ($p<0.05$) lower than the mean values 26.02±0.58, 26.33±0.69, 26.64±0.55 from the controls for the same periods respectively.

Soil organic matter is derived from residual plant and animal materials decomposed by microorganisms under the influence of temperature, moisture and optimal soil conditions. Most soil microbes have the enzymes needed for the decomposition. Dehydrogenases particularly, play a significant role in the biological oxidation of soil organic matter (OM) by transferring hydrogen from organic substrates to inorganic acceptors (12). However, any factor that affects the activities of soil microbial dehydrogenases can as well affect the degradation of soil organic matter. This therefore explains the mean values 22.55±0.53%, 21.15±0.57%, 20.26±0.39% from the control for exposure periods of 30, 60, and 90 days which were significantly ($p<0.05$) lower than the relatively unaffected mean values 23.18±0.35 %, 23.15±0.36 % and 23.46±0.46% from the test soil sample. The treatment therefore, had no direct effect on the organic matter content of the test soil, instead; it further revealed the presence of stress factor on the enzymes required to metabolize the soil organic matters in the sample.

Maintaining soil pH between 6 and 7 will generally result in the most efficient use of phosphate. Organic matters contain soluble phosphate, organic phosphate, and inorganic phosphate compounds that are quite available.

When phosphate in organic matters comes in contact with the soil, various reactions are initiated that make the phosphate less soluble and less available. The rates and products of these reactions are dependent on such soil conditions as pH, moisture content, temperature, and the minerals already present in the soil. The mechanisms for the changes in phosphate are complex and involve a variety of compounds. In alkaline soils (soil pH greater than 7), Ca is the dominant cation that reacts with phosphate. Sequences of reactions in alkaline soils lead to the formation of dibasic calcium phosphate dihydrate, octocalcium phosphate, and hydroxyapatite. The formation of each product results in a decrease in solubility and availability of phosphate. In acidic soils (especially with soil pH less than 5.5) Al is the dominant ion that will react with phosphate. In these soils the first products formed would be amorphous Al and Fe phosphates, as well as some Ca phosphates. The amorphous Al and Fe phosphates gradually change into compounds that resemble crystalline variscite (an Al phosphate) and strengite (Fe phosphate). Each of these reactions will result in very insoluble compounds of phosphate that are generally not available. Reactions that reduce P availability occur in all ranges of soil pH but can be very pronounced in alkaline soils ($pH > 7.3$) and in acidic soils ($pH < 5.5$) (19).

The means values 1.43±0.14mg/kg, 1.45±0.33mg/kg, 1.44±0.23mg/kg of phosphate from the test samples for the exposure periods of 30, 60, and 90 days were not statistically ($p<0.05$) different from the mean values 1.46±0.34mg/kg, 1.44±0.14mg/kg, 1.43±0.42mg/kg from the control samples respectively.

The influence of temperature (which is usually associated with the microwave component of EMR) on the equilibrium concentration of phosphate in the soil solution in literature appears to be those of (34). He reported reversible increase of 1 to 2% per centigrade in the phosphate concentration with increasing temperature in the range of 0 to 30°C. This is inconsistent with our findings as no significant effect of the radiation was observed on the concentration of the soil phosphate in EMR exposed samples vis-à-vis the unexposed samples. This may be due to the absence of supplying source of P or absence of the enabling environment for continuing mineralization of the soil samples.

Our results showed increased activities of catalase between 0 to 30 days exposure of the soil sample to electromagnetic radiations (fig.5). This observation is consistent with the induction of catalase and peroxidase activities following microwave pretreatment of wheat seedling (35). Beyond this period, the activities of

catalase in the EMR exposed soil decreased significantly ($p<0.05$) with increasing duration of exposure and in comparison with the respective EMR unexposed controls. The overall percentage change in catalase activities between 0 to 90 days of EMR exposure was -13.9 % vis-à-vis the 99% of the EMR unexposed soil samples, thus revealing the negative impact of the treatment.

The activity of dehydrogenases was enhanced between 0 to 30 days of EMR exposure and decreased progressively at subsequent exposure periods of 60 and 90 days. Fig.6 demonstrated the activity of dehydrogenase in the EMR exposed and unexposed soil samples. It showed significant ($p<0.05$) decrease in the EMR exposed soil samples at the exposure periods of 30, 60, and 90 days, with overall percentage change of -64.9%; 91.9% for EMR exposed and unexposed soil samples respectively.

V. Conclusion

The work assessed the Effect of Electromagnetic Radiations (EMR) on Some Soil Physicochemical Parameters, Catalase and Dehydrogenase Activities at the exposure periods of 0, 30, 60 and 90 days. And from the findings, it can be concluded that sub-chronic exposure of the soil samples to electromagnetic radiations (EMR) from mobile phone had negative impact on the studied soil parameters but for soil pH and phosphate concentration. The findings have therefore paved the way for further studies on soil samples near telecommunication masks.

Acknowledgement

The authors appreciate the technical staffs of the Departments of Biochemistry and Physics, Federal University of Technology, Owerri, for their technical assistances in the course of the research.

Competing Interest: The authors hereby declare that no conflicting interests exist.

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