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Changes in enzymes activities of soil samples exposed to electromagnetic radiations (EMR) from mobile phone

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This study aimed at investigating the changes in activities of dehydrogenase, catalase, alkaline phosphatase, acid phosphatase and alkaline protease of soil samples exposed to electromagnetic radiations (EMR) from mobile phone for the periods of 0, 30, 60 and 90 days. EMR-unexposed soil samples served as the source of control enzyme activities. The mean enzyme activities from the EMR-exposed soil were significantly ($p<0.05$) lower than those from the unexposed samples. The overall percentage changes in enzymes activities of the EMR-unexposed and exposed soil samples for dehydrogenase, catalase, alkaline phosphatase, acid phosphatase and alkaline protease relative to their respective starting values were 124.42 and -65.15%, 138.01 and -13.87%, 94.09 and 19.70%, 101.01 and -41.00%, and 162.55 and -21.71% respectively. The results show that EMR from mobile phones elicited significant negative impact on soil enzymes activities.

Key words: Electromagnetic radiations, mobile phones, enzymes.

INTRODUCTION

The use of mobile phones in recent years has raised and continued to raise tremendous safety questions. This is because users are exposed to electromagnetic radiations, the effects of which on the body depend on their frequencies, durations and powers.

The radiations generated by mobile cell phones are non-ionizing radiations (NIR). There are wide ranges of data documenting the ability of non-ionizing radiations (NIR) to affect living cells, including changes in the biochemical and molecular mechanisms of cells both *in vitro* and *in vivo* (Barnes, 1996). Changes also occur in cell metabolism and proliferation, inducing potentially damaging effects in various cell components ranging from the cytoplasmic membrane, where the distribution of proteins is modified (Bersani et al., 1997) to the cytoplasm itself and the nucleus, where the activities of intracellular enzymes and molecules regulating cell growth are altered (Hill, 1998).

Contrary to what the Telecommunication Industries proclaim, there are vast scientific, epidemiological and medical data that affirm that exposure to electromagnetic radiations from mobile phones and their towers, even at low levels, can have profound adverse effects on biological systems.

Experiments have revealed the biological effects that occur in activities of isolated enzymes, cell cultures and

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animals after exposures to low-intensity electromagnetic radiations. Mobile phone radiation exposure can cause cell damage via reactive oxygen species formation, and cell death (Oral et al., 2006; Sokolovic et al., 2008). Exposure of acetylcholinesterase, an important central nervous system enzyme, to mobile phone EMR altered the structural and biochemical characteristics of the enzyme, resulting to a significant change of its activities (Barteri et al., 2005). Three different cell types (rat hepatoma cells, egg cells of the Chinese hamster, human melanoma cells) were exposed for 1 h to a 450 MHz fields with a 16 Hz amplitude modulation and a power flux density of 10 W/m^2. The exposure raised ornithine decarboxylase (ODC) activity by a little more than 50%. The increased ODC activity remained fairly constant for several hours after the exposure. Similar fields with a 60 Hz and a 100 Hz modulation had no effects (Byus et al., 1988).

The mobile phones in their receiving and sending processes use (mostly) microwave frequency of 945 MHz (Bakr, 2004), emitting radiation that may be absorbed by various organs of the body depending on their location (Ozguner et al., 2005; Oktem et al., 2005). Mobile phones also emit low-frequency magnetic field pulse generated by battery currents in the phone that are too weak to produce non-thermal effects (Hyland, 2000). Recent studies have shown that the intense radiations from mobile phone towers adversely impact on every biological organism within 1 square kilometer (Bakr, 2004).

Mobile phone towers are especially dangerous because they emit electromagnetic radiations at a frequency range of 900 to 1900 MHz. Radiofrequency of this range is considered to be within high frequency range (HF-EMF) (Scehihr, 2006). However, the electromagnetic radiations emitted by mobile phones and base stations penetrate the living bodies to a distance that decreases with increasing frequency (Stewart, 2000).

It has been reported that at 900-1900 MHz, EMR does not penetrate deeply into the body; instead, it is absorbed by the skin and the underlying tissues. The heat that is generated in the tissue is then channeled into blood circulatory system (Otto and von Muhlendahl, 2007). However, the same cannot be said for unicellular organisms or even enzymes where it is expected to penetrate deep into the nucleus where DNA is located.

The importance of microorganisms in the soil cannot be over emphasized. For example, the main role of mould and fungi in the soil is to breakdown the remains of plant materials using their appropriate extracellular enzymes such as pectinases, celluloses etc., and these are further broken down through the activities of bacterial enzymes. Through the activities of these microbial enzymes, carbon, nitrogen and other minerals are released to the soil for plants utilization. Therefore, such factors like temperature, pH, substrate concentrations etc that affect the activities of these enzymes cum their host microorganisms in soil, for instance, will negate the availability of these minerals and by extension soil fertility. Also, bacterial and fungal communities occupy overlapping niches in soil. Disturbing these communities, for instance, through the denaturation or reduction of their enzymes activities, may alter the balance existing between them. The resulting imbalance may affect the influence of bacteria and fungi on their niches, and consequently, to the functional ecosystem.

Duration of EMR exposure for instance, seems to be a major determinant of EMR effect on the activities of enzymes. The time of exposure and power density are correlated in a way that decrease in power density (PD) could be compensated by increase in duration of exposure.

MATERIALS AND METHODS

Sampling and sample preparation

Soil samples were collected from the surface to a depth of about 30 cm into thoroughly washed and air-dried plastic containers 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, in the month of August, 2013 and then homogenized (Kenwood, UK). Five kilograms (5 kg) of the homogenized soil sample were then weighed into each of two experimental tanks, measuring120 × 120 × 100 cm each.

Experimental set up

A commercially available Nokia 2700 mobile phone (90 0MHz band with modulated voice and Specific Absorption Rate of 0.927 W/kg) was used for the generation of EMR for this study. The mobile phone was sandwiched at a depth of 5cm in the 5 kg soil sample of experimental tank one. During exposure, the mobile phone was kept on talking mode for 4 h 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 150 m) from the exposed sample (with phone) and at the same atmospheric conditions.

Both the exposed and the unexposed soil samples were irrigated continuously to maintain 75% humidity and a room temperature of 28±2°C for periods of 0, 30, 60 and 90 days. The mobile phone was temporarily removed (2 min) from test experimental tank during every irrigation exercise. The exposed and unexposed sample setups were kept away from any instrument or machine to avoid interference from any form of unwanted EMR. At the end of each period, the soil enzyme activities were assayed.

Soil enzyme activities assay

Dehydrogenases (EC 1.1.1) activity

Soil dehydrogenase activity was assayed based on the estimation of the rate of reduction of triphenyltetrazolium chloride (TTC) to triphenylformazan (TPF) after incubation at 30°C for 24 h. Field-moist soil sample (5 g) was weighed into five test tubes and mixed with 5 ml of TTC solution. The tubes were sealed with rubber stoppers and incubated for 24 h at 30°C. After the incubation, 40 ml acetone was added to each tube, and the tubes were shaken thoroughly and further incubated at room temperature for 2 h in the dark (shaking the tubes at intervals). The soil suspension (15 ml) was then filtered and the optical density of the clear supernatant was measured colorimetrically against the blank at 546 nm (Thalmann, 1968).

Phosphatases

The method used was based on the determination of p-nitro-phenol

released after the incubation of the soil samples with p -nitrophenyl phosphate (ρNP) for 1h at 37°C. Five grams (5 g) of the moist soil sample were placed in Erlenmeyer flasks (50 ml) and treated with 0.25 ml of toluene, 4 ml of buffer solutions (pH 6.5 for the assay of acid phosphatases and pH 11 for the assay of alkaline phosphatases), and 1 ml of p-nitrophenyl phosphate solutions made in the same buffer. After stoppering the flasks, the contents were mixed and incubated for 1 h at 37°C. After the incubation, 1 ml of $CaCl_2$ (0.5 M) and 4 ml of NaOH (0.5 M) were added. The contents were mixed and the soil suspension filtered through a Whatman filter paper grade 2. For the standard, 1ml of pNP solution was added after the addition of $CaCl_2$ (0.5 M) and 4 ml of NaOH (0.5 M) immediately before filtration of the soil suspension. Absorbance of the solutions were read at 400 nm (Eivazi and Tabatabai, 1977).

Proteases

Soil alkaline protease activity was assayed using Folin-Ciocalteu reagent based on the determination of amino acid released after the incubation of the soil samples with sodium caseinate for 30 min. The soil suspension was subjected to centrifugation (5,000 rpm for 10 min), and the supernatant was used for alkaline protease activities determination according to (Nigam and Ayyagari, 2007).1 ml of supernatant was mixed with 1 ml of casein (1%) [prepared in 0.1 M citrate buffer of pH 5 and 0.1 M glycine-NaOH buffer of pH 10] followed by incubation at 50°C for 30 min. Then 5 ml of 5% trichloroacetic acid (TCA) was added to precipitate the undigested protein. The solution was then centrifuged at 10,000 rpm for 10 min, and the supernatant subjected to estimation for the amount of amino acids released by Lowry's method (Lowry et al., 1951). International unit of protease was calculated as:IU= Net amount of amino acid released (µg) × dilution factor/ 181× 30, where 30=incubation period, 181=amount of protein present in the sample.

Catalase (EC1.11.1.6) activity

The method used was based on the volumetric determination of oxygen liberated after incubation of soil sample with hydrogen peroxide for 3 min at room temperature (Isamah et al., 2000; Cohen et al., 1970). Five grams (5 g) of moist soil samples were weighed into five test tubes, mixed in 20 ml of distilled water, and centrifuged at 3500 rpm for 10 min at 4°C. To the supernatant, 5ml of cold 6mM H_2O_2 was added and the reaction stopped after 3 min by adding 0.25 ml of 6 N H_2SO_4 rapidly with thorough mixing. Excess potassium permanganate (10 ml) was added, shaken gently and absorbance read at 480 nm for 30 s. The blank was prepared as the test but with the replacement of supernatant with distilled water.

A standard was prepared by mixing 10 ml of potassium permanganate with 5.5 ml of potassium phosphate buffer (pH 6.5) and 0.25 ml of 6N H_2SO_4 and the absorbance read at 480 nm. Enzyme activity was calculated using the following formula: $^{K}/_{0.00693}$.

Where the first-order rate constant $(K) = \text{Log}\left(\frac{S_o}{S_t}\right) \times \left(\frac{2.3}{t}\right)$.

Where, 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 (min).

Statistical analysis

All analyses were done in triplicate and the data generated were analyzed using ANOVA and Duncan's test with the aid of Statistical Package for Social Science (SPSS). Values for $p<0.05$ were considered statistically significant; data were presented as mean± standard deviation.

RESULTS AND DISCUSSION

The effects of non-ionizing radiation, emitted by mobile phones, in living cells have been widely reported, however, the mechanism of action has not been fully documented, though it is thought to be through the action of reactive oxygen species. Living tissues are 70-90% water by weight, non-ionizing radiation therefore, would be expected to induce cellular generation of free radicals by excitation of water molecules. The dividing line between radiations that excites electrons and radiation that forms ions is often assumed to be equal to the energy of ionization of water (1216 kJ/mol). Radiation that carries less energy can excite the water molecule and is therefore called non-ionizing radiation. Radiation that carries more energy than 1216 kJ/mol can remove an electron from a water molecule, and is called ionizing radiation (Daniel et al., 2008). The capability of non-ionizing radiation to induce cellular damage has been reported by Barnes (1996) and other scientists; whether these reported effects were as a result of free radical formation, thermal or athermal action, remained an interesting area for further investigation.

EMR is also believed to exert its biological effects through: non-thermal action, thermal action, and/or a combination of the two. EMR emitted by mobile phone is at non-thermal power density level, so far, no common ground exist on non-thermal exposure levels of EMR in literatures. Thermal effect results from the conversion of the EMR energy into heat energy in the living systems. Polar molecules in cells exist in the form of water, DNA and proteins, and these molecules respond to electromagnetic radiations by rotating. This rotation creates an angular momentum which results in friction with neighboring molecules, thereby developing a linear momentum (vibrational energy) (Saifuddin et al., 2009), through this means, radiation energy is converted into thermal energy.

It can therefore be said that the effect resulting from vibrational energy is ther-mal effect which occurs in a biosystem due to penetration of electromagnetic radiation into biological materials and subsequently heating up the intra- and extra- cellular fluids by transfer of vibrational energy (Tahir et al., 2009). It must be noted that EMR thermal effects is different from conventional heating effect. This is because dipolar polarization and rotation of molecules in an attempt to align the dipoles with applied electromgnetic field produces effects which cannot be achieved by conventional heating (Zelentsova et al., 2006).

The non-thermal effect of EMR has been a matter of debate in scientific community. A non-thermal effect has been suggested to result from a direct stabilizing interaction of electric field with specific (polar) molecules in reaction medium with no rise in temperature (Herrero et al., 2008). The interest in non-thermal effect is predicated on the established fact that thermal effects alone

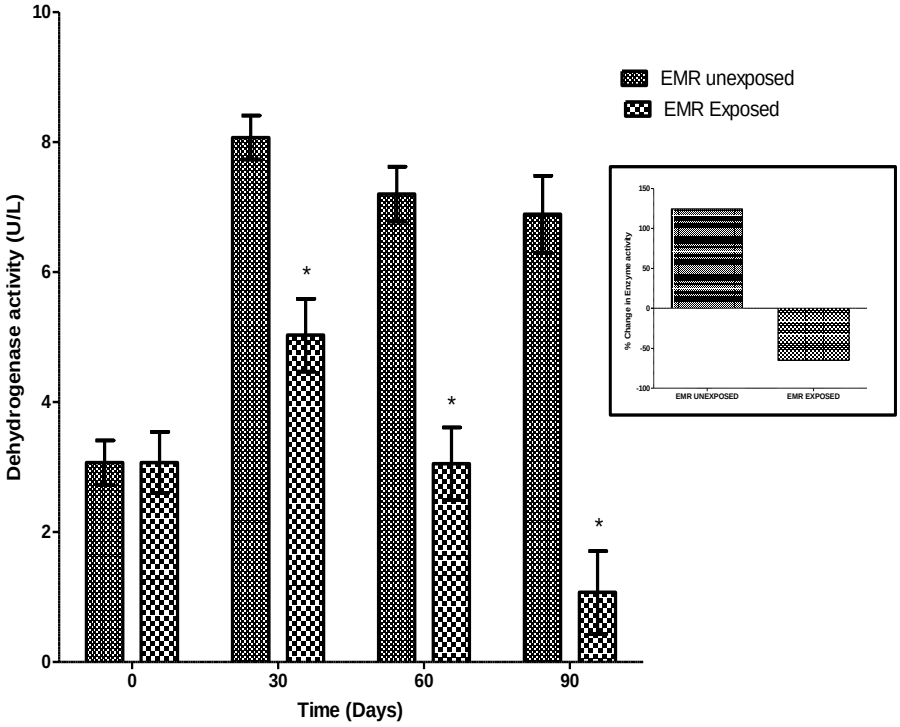

Figure 1. Dehydrogenase activity (U/L) of Electromagnetic radiation (EMR) exposed and unexposed soil samples. %Δ, *Values are significant ($p < 0.05$) in comparison with their respective EMR unexposed control. Insert chart; overall percentage change in dehydrogenase activities of EMR unexposed and exposed soil samples.

cannot explain the manner in which the EMR affects biological systems. As a result of pausity of information on the exact mechanism involved in *'nonthermal* EMR effects' (*athermal* effects), their existence has been a subject of controversy. The influence of EMR on the soil enzymes may also result from its effect on the proteinaceous nature of the enzymes that changes the secondary and tertiary protein structure of enzymes and thus, denaturation of enzymes. These changes in enzyme activities may be correlated with the probability of electroporation (Reina et al., 1998) and reported irreversible cell membrane break down of microbial hosts.

The concept of resonant absorption and resonant interactions has been suggested as another possible explanations for the marked sensitivities of living systems to EMR (Cosic, 1997). In the present study, changes in enzymes activities of soil samples exposed to electromagnetic radiations (EMR) from mobile phone were assessed.

Figure 1 shows the dehydrogenase (DHG) activity of the electromagnetic radiation (EMR) of exposed and unexposed soil samples. It indicates significant ($p < 0.05$) reductions in DHG activity of EMR exposed soil samples at exposure periods of 30, 60 and 90 days, giving an overall percentage change in DHG activity between 0 to 90 days of 124.42% and -65.15% for the EMR unexposed and exposed soil samples respectively. Dehydrogenases are used as an indicator of overall soil microbial activity because they occur intracellularly in all living microbial cells. They are tightly linked with microbial redox processes (Moeskops et al., 2010). Most importantly, dehydrogenases do not accumulate extracellular in the soil. They play vital roles in the biological oxidation of soil organic matters by transfer of hydrogen from organic substrates to inorganic acceptors (Zhang et al., 2010). DHG activity, therefore, reflects metabolic ability of the soil and it is usually proportional to the biomass of the microorganisms in soil (Salazar et al., 2011). Stress on microbial biomass is reflected on dehydrogenase activities.

Our results show that between 0 to 30 days of EMR exposure to the soil samples, the activities of catalase were enhanced (Figure 2). This finding is consistent with the induction of catalase and peroxidase activities following microwave pretreatment of wheat seedlings (Chen et al., 2008). Generally, the catalase activity of the EMR exposed soil samples decreased significantly ($p<0.05$) with increase in duration of exposure, and in comparison with their respective EMR unexposed controls. The overall percentage change in catalase activities between 0 to 90 days of EMR exposed soil sample was -13.87% as compared to 138.01% from the EMR unexposed soil samples, thus indicating the negative impact of the treatment on the enzyme activities. Catalase, also known as hydrogen peroxidase oxido-reductase is an

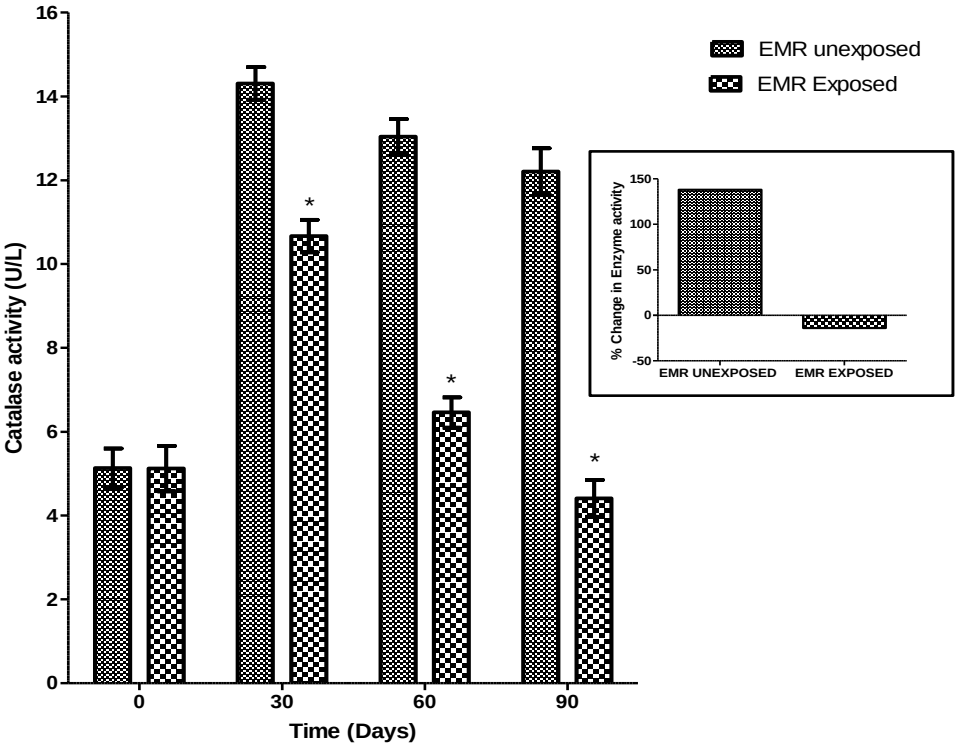

Figure 2. Catalase activity (U/L) of Electromagnetic radiation (EMR) exposed and unexposed soil samples. %Δ, *Values are significant ($p < 0.05$) in comparison with their respective EMR unexposed control. Insert chart; overall percentage change in catalase activities of EMR unexposed and exposed soil samples.

enzyme that has a detoxifying function in cells, catalyzing the reaction: H_2O_2 ------→ H_2O + ½O_2. All aerobic and most facultative anaerobic bacteria, but not obligate anaerobic bacteria, exhibit catalase activity. Catalase activity has been shown to be very stable in soil and significantly correlates with the content of organic carbon and decreases with soil depth (Ladd, 1978). However, no relation has been detected between catalase activity and soil biomass. Furthermore, storage of moist or air-dried soils at room temperature for up to a period 4 months had not been found to have effect on catalase activity.

Figures 3 and 4 show the effect of EMR exposure on alkaline phosphatase (ALP) and acid phosphatase (ACP) activities of soil samples respectively. ALP and ACP are both phosphatases, which are known to catalyse the hydrolysis of organic phosphomonoester to inorganic phosphorus. Based on their optimum pH, the enzymes are classified as acid, neutral and alkaline phosphatases. Acid (pH optimum: 4 – 6.5) and alkaline phosphatase (pH optimum: 9-10) have been found in soils (Speir and Ross, 1978). Acid phosphatase is predominant in acidic soils, while alkaline phosphatase prevails more in alkaline soils (34). Both ALP and ACP activities were significantly ($p<0.05$) lower in the soil samples exposed to the EMR. The observed reductions in the phosphatase activities decreased with increase in duration of exposure giving an overall percentage activity decrease of -19.70 and -41.00% in comparison with the increased activity observed in the unexposed soil samples (94.09% and 101.01%) for ALP and ACP respectively. Generally, ACP recorded minimal activities than ALP in both the EMR exposed and unexposed samples. This is because the soil samples were basic with an average pH value of 9.40.

Alkaline proteases are degradative enzymes that catalyze the partial or the total hydrolysis of proteins. These enzymes are mainly produced by bacteria and fungi. Microbial proteases are predominantly extracellular and can be secreted in the fermentation medium by several species of bacteria, bacillus and fungi for example *notatum* (Ellaiah et al., 2002; Raju et al., 1994; Haq et al., 2006). In this study, EMR inhibited alkaline protease activities by -21.71% and this is consistent with the report of Dholiya et al. (2012). However, between 0-30 days, the activities of the protease were enhanced in the EMR-exposed samples. This agrees with the findings of Afzal and Mansoor (2012) who reported increased activities of proteases following EMR exposure to radicles for 72 h. While the alkaline protease activity remained high in the EMR unexposed samples, that of the exposed samples decreased significantly ($p<0.05$) with increased duration of exposure. Thus, the overall percentage changes

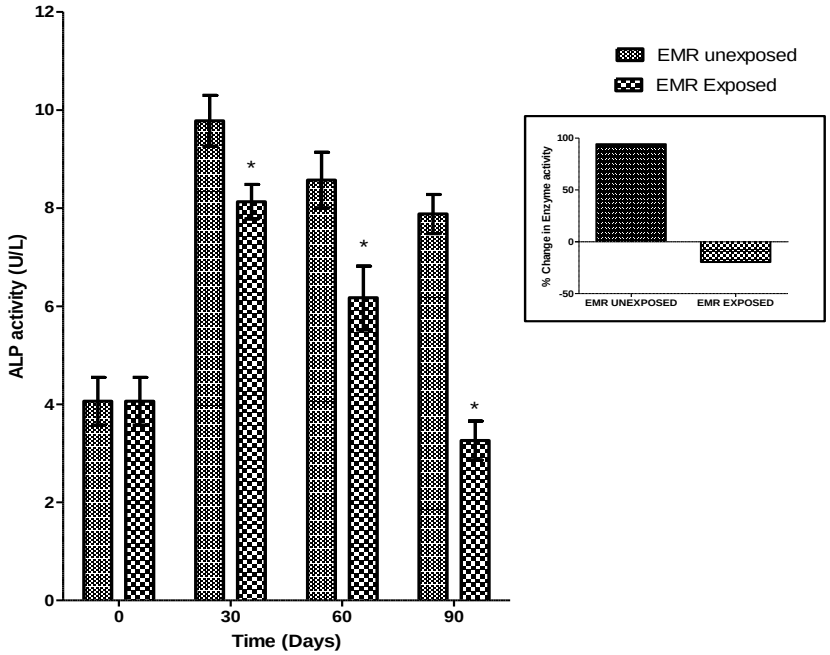

Figure 3. Alkaline phosphatase (ALP) activity (U/L) of Electromagnetic radiation (EMR) exposed and unexposed soil samples. %Δ, *Values are significant ($p < 0.05$) in comparison with their respective EMR unexposed control. Insert chart; overall percentage change in ALP activities of EMR unexposed and exposed soil samples.

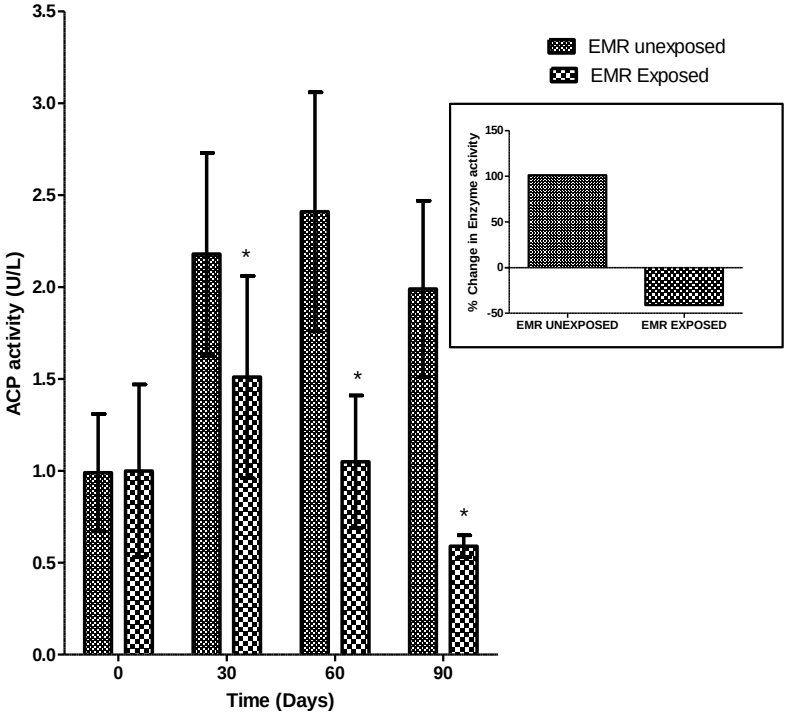

Figure 4. Acid phosphatase (ACP) activity (U/L) of Electromagnetic radiation (EMR) exposed and unexposed soil samples. *Values are significant ($p < 0.05$) in comparison with their respective EMR unexposed control. Insert chart; overall percentage change in ACP activities of EMR unexposed and exposed soil samples.

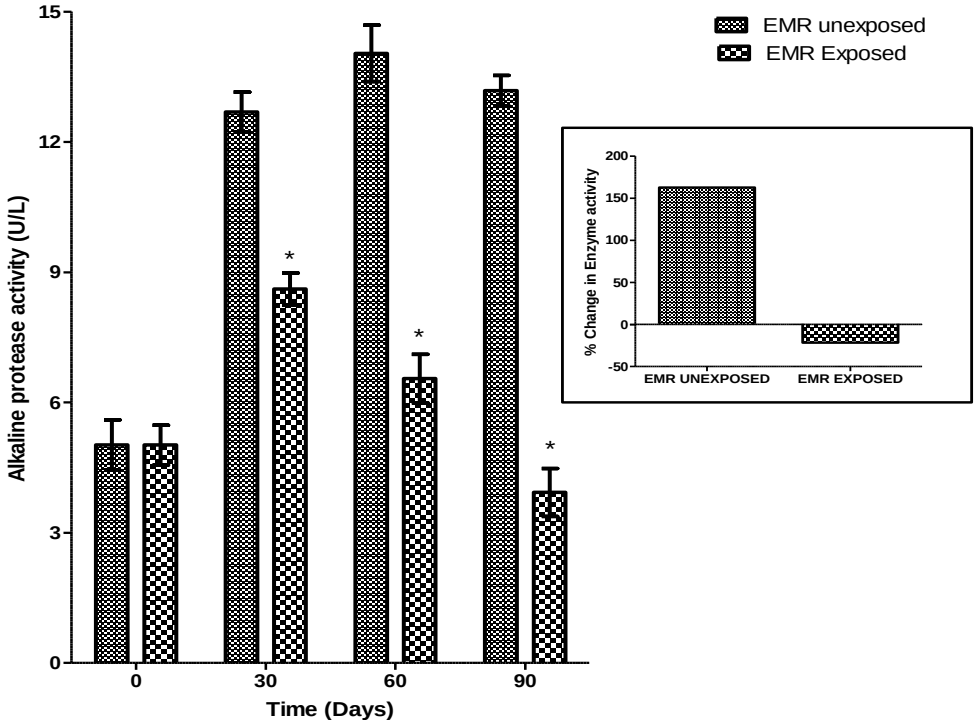

Figure 5. Alkaline protease activity (U/L) of Electromagnetic radiation (EMR) exposed and unexposed soil samples. *Values are significant ($p < 0.05$) in comparison with their respective EMR unexposed control. Insert chart; overall percentage change in alkaline protease activities of EMR unexposed and exposed soil samples.

in activities of the enzymes between 0 to 90 days were found to be -21.71 and 162.55% for the exposed and unexposed samples respectively (Figure 5).

Conclusion

This study indicated that EMR had significant negative impacts on soil enzyme activities at the exposure periods of 30, 60, and 90 days, with dehydrogenase being the most affected and catalase the least affected vis-a-vis their corresponding enzymes activities in the unexposed soil samples. It can be suggested that the EMR effects might be the result of micro-thermal heating that was importantly different from conventional heating.

Based on the observed effects, it is therefore concluded that chronic ex-posure to electromagnetic radiations from mobile phones can inhibit enzyme activities in soil. The findings have further paved ways for studies on the impact of EMR on soil and its biomass around telecommunication masks.

Conflict of interests

The authors do hereby declare that no conflict of interest exist.

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