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IMPACT OF CYPERMETHRIN AND CHLORPYRIFOS ON MICROBIAL ACTIVITY AND BIOMASS IN A NIGERIAN SOIL

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ABSTRACT

The impacts of a synthetic pyrethroid—cypermethrin—and organophosphate insecticide—chlorpyrifos—on soil bacterial, fungal, and pseudomonad populations; basal and substrate-induced respiratory activities; and biomass-C in paddy rice and uncultivated soils were evaluated over a 30-day exposure period. The pesticides were applied at two rates: normal field concentration (NFC) and five times field concentration (X5FC). The growth on and mineralization of different concentrations of the pesticides by three *Pseudomonas* species were also assessed for 14 days. The effect on paddy rice root and shoot growth was evaluated. Results indicated that the dynamics of temporal changes in soil microbial populations were dependent on pesticide concentration and type. The suppressive effect on bacterial population lasted 14 days and over 30 days for fungal population in the X5FC pesticide-contaminated paddy rice soils. The suppressive effect was less than 7 days for both bacterial and fungal populations in the uncultivated soils contaminated at X5FC, though pseudomonad population was promoted almost immediately in the same soils. The mean shoot/root ratio of rice seedlings in the control soil (2.38 ± 0.16) was higher but not significantly different from those in the X5FC cypermethrin-treated (2.13 ± 0.10) and chlorpyrifos-treated soils (1.92 ± 0.15). Pesticide concentration aside from affecting microbial numbers in soil can also contribute to water pollution as a nonpoint source pollutant.

Keywords: Cypermethrin, chlorpyrifos, microbial biomass, substrate induced respiration, basal respiration.

INTRODUCTION

Developing countries like Nigeria, Ghana, Nepal, Ethiopia, and others have no standard or set procedures regarding the release of pesticides via point or nonpoint source into the soil or water bodies. Developed countries that have strict regulations on pesticides contamination still have cases of pesticides in water and soil at limits exceeding the set environmental standards (Stehle et al., 2012). The fate of pesticides used on farmlands has become a matter of concern in modern agricultural practice in the context of environment in general and ecotoxicology in particular (Perucci et al., 2000). The pesticides that are frequently used eventually reach the soil from the crop plants where activities of microbes such as feeding and reproduction are found to be maximum and could also be leached down in which case the groundwater is contaminated (Ayansina et al., 2003).

Chlorpyrifos is an organophosphate while cypermethrin is a pyrethroid pesticide (Han et al., 2017). The addition of organic matter increases the adsorption of pesticides and decreases their mobility in the soil (Briceño et al., 2007). Organic matter increases the activities of microorganisms in the soil. The increase in soil microbial load and activities tends to increase the number of organisms that will be available to degrade pesticides in the soil (Johnson et al., 1997). Autochthonous bioremediation involves sourcing the microorganisms from the contaminated site (Semrany et al., 2012). Results obtained from previous works on pesticide biodegradation has led to more work to isolate pesticide degrading microorganisms and techniques to make the process easier (Xiao et al., 2013; Perucci et al., 2000; Zhang et al., 2009). Various species and strains of *Pseudomonas* have been used in biodegradation of various environmental pollutants including hydrocarbons, gasoline, kerosene, diesel, jet fuel, and pesticides (Cycoń et al., 2017).

Anthropogenic activities such as land use and management may adversely impact the soil's capability to provide ecosystem services in which microbes are playing a key role. Because sampling is usually confined to the topsoil, little is known about effects of land use on ecosystem functioning down the soil profile. Land use exerts strong effects on soil microorganisms in the topsoil and microbial biomass and activity decrease with soil depth (van Leeuwen et al., 2017).

Microbial biomass acts as a soil ecological marker because of its active involvement in nutrient release and plays a major role in soil structure formation (Smith & Paul, 1990). Microbial biomass–mediated organic matter transformation has proved that it acted as source of nutrient elements in the soils having poor nutrients (Kang et al., 2012). The pesticides used in this study were chosen because they are popularly used by farmers locally and elsewhere. The aim of this study is to evaluate the effect of synthetic pesticides on soil microorganisms.

MATERIALS AND METHODS

The pesticides, cypermethrin and chlorpyrifos, and seeds of paddy rice (*Oryza sativa*) were obtained from a local agro-allied store at Ilorin, Nigeria. Culture media including nutrient agar, nutrient broth, Sabouraud dextrose agar, and Pseudomonad agar base with the **cetrimide fucidin cephaloridine** (CFC) supplements were manufactured by either Rapid Lab or Oxoid, UK. All chemicals used are of analytical grade (>95% purity).

The soil used in this study has been previously characterized with the following parameters: texture (sand = 89%, clay = 6%, silt = 5%), organic matter = 4.2%, organic carbon = 2.4%, total nitrogen = 0.54%, effective cation exchange capacity (ECEC) = 36.05 $Cmol^{-1}$, pH 9.35.

Field Potted Experiment and Sampling Regimes

The field potted experiment was carried out at the University of Ilorin Botanical Garden. Application was made and approval was received from the University Botanical Garden Committee for the piece of space used for the experiment. The soil used to plant the paddy rice was obtained from the garden and has been previously used to grow crops including paddy rice. Six pieces of large plastic pots (10 m^3) were filled with about 10 kg of soil and planted with paddy rice on the 16th of March 2015 at a rate of 20 seeds per pot for uniform spacing. Each pot was watered everyday with 2 L of water from River Oyun flowing through part of the Botanical Garden. Seeds were allowed to germinate and grow for two weeks before the application of the pesticides on the 1st of April 2015. The standard field application as recommended by the manufacturer (Jubaili Agrotec Ltd.) is 1 L of cypermethrin/chlorpyrifos to 400 L of water for a hectare of land. As the area of the pots used was 0.2 m^2, 0.3 mL of cypermethrin and 0.5 mL of chlorpyrifos each was diluted in 200 mL of water and applied to 10 kg of soil in the pots. Application of pesticide was made at two rates: normal field concentration (NFC; 1.5 mL/kg) and five times the field concentration (X5FC; 7.5 mL/kg) by direct spraying onto the surface of the soil. Duplicate pots were used for each treatment including the untreated control soil.

Rhizospheric soil samples were taken from each planted pot at defined intervals (1, 2, 5, 9, 14, 21, and 30) over a period of 30 days' post-treatment with the pesticides. Samples (40–50 g) were collected using a clean trowel into aluminum foil, carefully wrapped and transported immediately to the laboratory for analysis. Composite samples of each treatment were used for the microbiological analyses including bacterial and fungal populations as well as microbial respiratory activities and biomass-C. Also, samples of the rice seedlings were uprooted carefully from each set of the pots to evaluate the effect on plant growth indices.

Lab Microcosm Experiment and Sampling Regimens

In another experiment, fresh uncontaminated soil (600 g), obtained from the same site, was divided into three: two were used as treatment samples and the third, as the untreated control soil. Pesticides were applied (at NFC and X5FC rates) by thoroughly mixing in stainless glass bowl following the one-step spiking procedure previously described in Reid et al. (1998). The control was mixed with an equal volume of water without any pesticide, and all three treatments were placed in 1000-mL glass jars covered with perforated aluminum foil to allow for exchange of air and stored at room temperature in a cool area in the laboratory. Subsamples were taken after 0, 1, 7, 14, and 21 days for analysis of bacterial, fungal, and Pseudomonad populations in the soils.

Measurement of Length of Root and Shoot of Rice Seedling

After the rice seedlings were cleaned with water and air-dried, the length of shoots and roots of the rice seedlings were measured. The average values of the data from seedlings collected from the triplicate pots were determined and recorded.

Enumeration of Microbial Populations in Soil Samples

The bacterial and fungal populations in the soil samples were determined using standard microbiological procedures (pour plate method). One gram of soil was introduced to 9 mL of distilled water in a test tube and vortexed for 30 seconds to dislodge the cells

from the soil particles. From the suspension, a series of dilutions (10^{-3} for fungi and 10^{-5} for bacteria) were made, and 1-mL aliquots were withdrawn and inoculated on nutrient agar (NA; Rapid Lab., UK) or Sabouraud dextrose agar (SDA; Oxoid, UK) for enumeration of bacteria and fungi, respectively. The NA plates were incubated at 37°C for 24 hours, and SDA plates, at 25°C for 96–120 hours before the enumeration. The Pseudomonad number was estimated on Pseudomonad agar base (Oxoid CM0559) with **cetrimide fucidin cephaloridine** (CFC) supplement. The preparation of the agar followed the manufacturer's instruction. Incubation was at 37°C for 24 hours.

Estimation of Soil Microbial Respiratory Activities and Biomass-Carbon

Weighed amounts (20 g) of the control or treated soil sample were placed into standard respiratory bottles and mixed with 2 mL of sterile mineral salts medium (MSM: 0.3 g of NaCl, 0.6 g of KNO_3, 0.25 g of KH_2PO_4, 0.75 g of K_2HPO_4, 0.15 g of $MgSO_4$, $7H_2O$ per litre) without glucose for the basal respiration assay while MSM containing glucose (2.5 mg mL^{-1}) in addition was used for the substrate-induced respiration (SIR) assay. Two milliliters of 1-M sodium hydroxide (NaOH) placed in EDTA bottles suspended in the respiratory bottles were used to trap the CO_2 evolved. Sodium hydroxide in the EDTA bottle was changed at intervals of 1, 2, and 3 days for basal respiration and 2, 4, 8, 12, 24, 48, and 72 hours for SIR setups. The trapped CO_2 was determined by titrimetric method using 1 M of HCl with phenolphthalein as indicator.

For the estimation of soil microbial biomass-carbon (MBC), the values of CO_2 evolved after 2 hours of glucose utilization was used with the formula postulated by Sparling and Williams (1986):

$$\text{Microbial biomass-C} = 40(X) + 0.37 \text{ (mg/g of soil)}$$

where X is the CO_2 evolved after 2 hours (mg/g of soil)

Isolation and Characterization of Pseudomonas Isolates

After 21 days of exposure to pesticides, distinct colonies on Pseudomonad agar inoculated with the X5FC soils were subcultured to obtain pure isolates. Three isolates were finally selected for further studies and tentatively identified based on the dissimilarities in their colonial and cellular morphologies and physiological and biochemical properties. The identities of the isolates are *Pseudomonas aeruginosa, Pseudomonas putida*, and *Pseudomonas desmolyticum*.

Growth, Harvest, and Quantification of Purified Cell Pellets

Portions of the pure culture of the isolates (24 hours old) were introduced into nutrient broth (100 mL) placed in 250-mL conical flasks and incubated on a rotary shaker (100 rpm) for 72 hours to allow the growth of cells till exponential phase under aerobic condition. Cell pellets were harvested by repeated centrifugation (Uniscope Laboratory Centrifuge, Model: SM 112) at 3500 rpm (1,986 g) for 10 minutes (for each run) until a clear supernatant was obtained. The supernatant broth was then carefully decanted and discarded. Sterile phosphate buffer solution (PBS: 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HP0_4, 0.24 g of KH_2PO_4 per liter of distilled water) was used to resuspend and wash the remnant of the broth from the cells. The purified cell pellets were finally resuspended in PBS and stored at 10°C until usage, but not more than 48 hours. Quantification of the inoculum size was done using the MacFarland's

suspensions (1% $BaCl_2$ and 1% H_2SO_4) on a spectrophotometer (Genesys 20 model) at 600-nm wavelength (0.5 MacFarland's was used). Where necessary, the suspensions of the individual isolates were appropriately diluted with PBS to give a final cell concentration of approximately 1.5×10^8 CFU/mL.

Evaluation of Tolerance and Growth of Isolates to Increasing Concentration of Pesticides

The assay was designed to evaluate the effect of pesticide on the ability of the isolates to utilize and grow on simple carbon substrates like glucose. Solutions consisting of 49-mL MSM with glucose (3 mM) initially spiked with increasing concentrations of the pesticides (0, 0.3, 0.5, and 1.0 ppm) in 100-mL flasks were spiked with 1-mL cell suspensions (1.0×10^4 CFU/mL) of the individual isolates and incubated on an orbital shaker (100 rpm) for 14 days. The glucose–MSM solution was composed to adequately provide the essential nutrients and trace elements that can limit C-glucose mineralization. The bacterial cell concentrations were quantified by the plate count method. After 0, 1, 7, and 14 days of incubation, 1 mL of the culture was withdraw and serially diluted, and 0.1-mL aliquots from appropriate diluents were taken and inoculated on Pseudomonad agar plates. Following incubation at 37°C for 24 hours, the distinct colonies were enumerated and recorded.

Biodegradation of Pesticides in Amended Soils by Pseudomonad Isolates

The assay was designed to monitor mineralization in control soil and soils freshly amended with the pesticides at NFC and X5FC rates in a soil slurry system. Weighed amounts of soil (10 ± 0.2 g) was placed in 250-mL respiratory bottles containing 20 mL of MSM with glucose (1.2 mg g^{-1}) and incubated on an orbital shaker (25 ± 2°C; 100 rpm) for 14 days. Two milliliters of 1-M NaOH placed in EDTA bottles suspended in the respiratory bottles were used to trap the CO_2 evolved. NaOH in the bottles was replaced after 1, 3, 5, 7, 10, and 14 days. The trapped CO_2 was determined by titrimetric method using 1 M of HCl with phenolphthalein as indicator.

RESULTS AND DISCUSSION

In this study, effects of the organophosphates cypermethrin and chlorpyrifos, applied at the recommended field concentration (NFC) and five times the field concentration (X5FC), on soil microbial ecology and functioning were evaluated.

Effect of Pesticide Concentration on Plant Growth Indices

The rice seedling growth indices (root and shoot) in the control and pesticide-contaminated soils are shown in Table 1. One-way ANOVA indicated no significant difference in the ratios of shoot to root. This implies that neither cypermethrin nor chlorpyrifos, even at a rate of X5FC, had significant effect on the plant growth. This result is in disagreement with the work of Adetitun et al. (2016), who reported that plant growth parameters were negatively affected as the concentration of spent engine oil increased. This may be due to the fact that pesticides applied to farmlands have been tested for their toxicity on plants before commercial production. Also, the pesticides are meant to enhance plant growth. Hence, they can't be toxic to plants but only to pests.

Effect of Pesticide Concentration on Soil Microbial Populations

Figures 1 and 2 present the changes in the bacterial and fungal populations,

Table 1. Effect of Pesticide Concentrations on Paddy Rice Root and Shoot Growth

Days Post-treatment	Control			Cypermethrin						Chlorpyrifos					
				NFC			X5NF			NFC			X5NF		
	Root (cm)	Shoot (cm)	Ratio	Root (cm)	Shoot (cm)	Ratio	Root (cm)	Shoot (cm)	Ratio (s/r)	Root (cm)	Shoot (cm)	Ratio	Root (cm)	Shoot (cm)	Ratio
0	8.1	29.9	3.2	–	–	–	–	–	–	–	–	–	–	–	–
2	10.0	28.8	2.9	15.6	33.5	2.1	16.1	32.6	2.0	16.5	34.5	2.1	11.1	28.2	2.5
5	21.1	45.0	2.1	17.1	36.4	2.1	15.1	33.9	2.2	18.4	41.8	2.3	20.2	36.9	1.8
9	18.3	33.3	1.8	20.6	32.6	1.6	21.3	36.9	1.7	25.3	37.2	1.5	21.9	30.1	1.4
14	13.6	32.0	2.4	21.9	37.9	1.7	22.7	53.1	2.3	22.2	43.2	1.9	22.5	45.2	2.0
21	23.0	58.2	2.5	23.3	49.1	2.1	23.7	54.2	2.3	26.3	54.4	2.1	22.4	44.6	1.9
30	30.2	78.3	2.6	23.9	55.0	2.3	25.6	59.5	2.3	25.4	55.4	2.2	31.0	57.5	1.9
Mean			2.38			1.98			2.13			2.02			1.92
SEM			0.16			0.11			0.10			0.12			0.15

Key: NFC = normal field concentration. X5FC = five times field concentration.

respectively, in pesticide-contaminated paddy rice soils over a 30-day post-treatment. Results indicated that pesticide concentration affected microbial numbers and was dependent on the pesticide type. In particular, there were significant reductions in bacterial populations in the first 14 days (Fig. 1). However, after 21 days post-treatment, bacterial populations have either recovered or even increased significantly in cypermethrin-contaminated paddy rice soils. This was dependent on the pesticide concentration. The negative impact on the fungal population extended over 30 days, particularly in the X5FC cypermethrin-contaminated paddy rice soil, although, in the NFC chlorpyrifos-contaminated paddy rice soil, fungal populations recovered and increased after 21 days post-treatment (Fig. 2).

In the lab microcosm experiment, the effect of pesticide concentration followed similar trend of an initial reduction in microbial populations, although the extent of the impact was not as prolonged as in the contaminated paddy rice soils (Fi. 3). In the unvegetated pesticide-contaminated soils, bacterial and fungal numbers were significantly reduced in the first day after pesticide application, as compared to the control soil (Fig. 3: 1A, 1B, 2A,

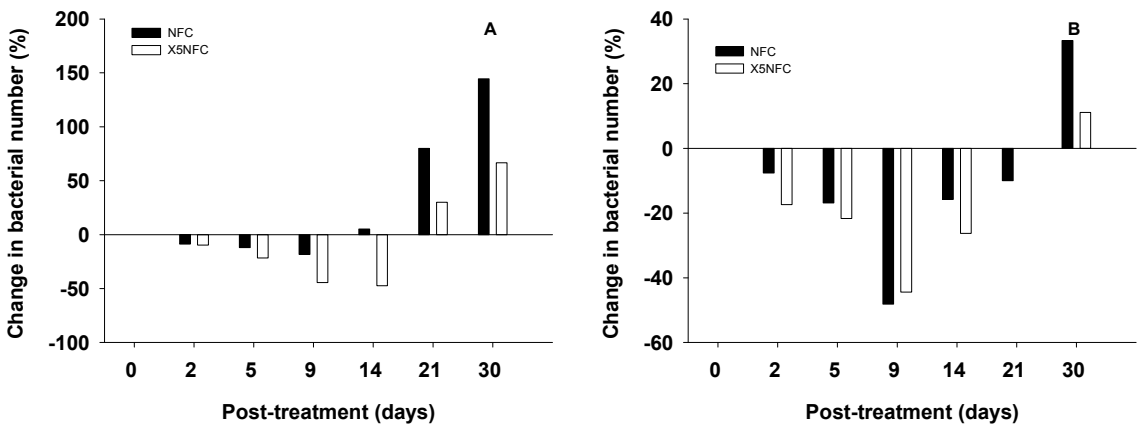

Figure 1. Change in total bacterial population in paddy rice soils treated with (A) cypermethrin and (B) chlorpyrifos over 30 days post-treatment as compared to the control soil.

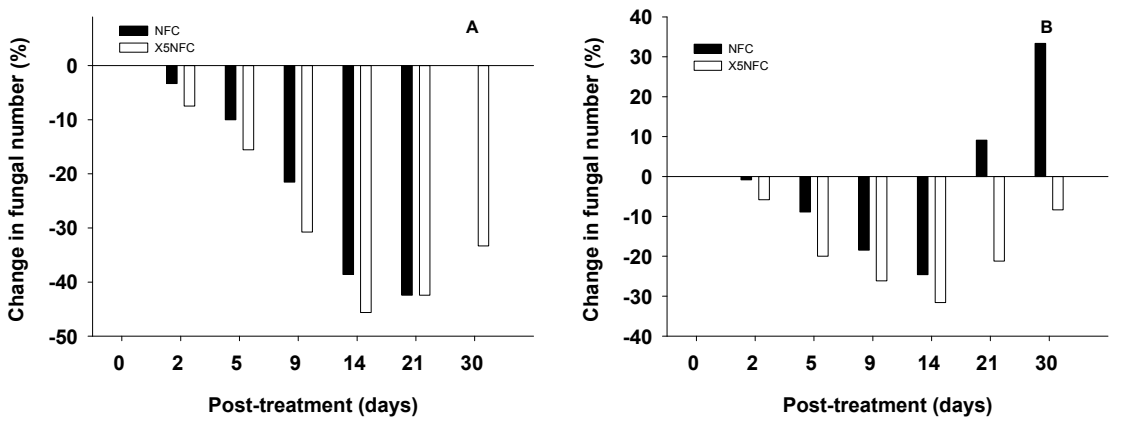

Figure 2. Change in fungal population in paddy rice soils treated with (A) cypermethrin and (B) chlorpyrifos over 30 days post-treatment as compared to the control soil.

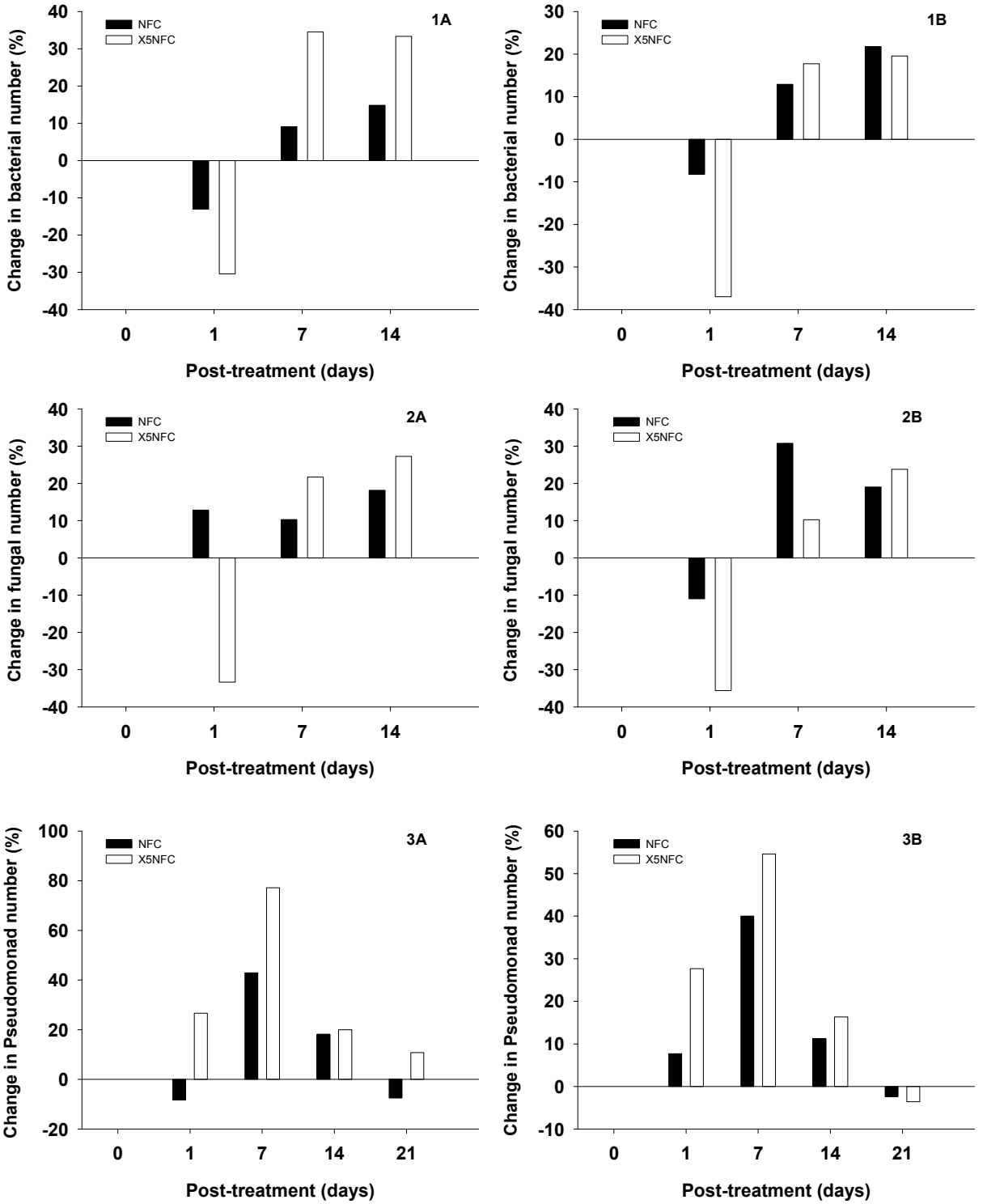

Figure 3. Change in bacterial, fungal, and *Pseudomonas* populations in uncultivated soils treated with (A) cypermethrin and (B) chlorpyrifos over 21 days post-treatment as compared to the control soil.

and 2B). However, as from 7 days afterwards, the microbial populations have regained and increased in the contaminated soils. The early recovery of microbial population from the initial impact of pesticide application in the lab microcosm experiment may be due to the difference in pesticide application methods and storage effect among other factors. Further studies comparing the effect of pesticide concentration on microbial populations in soils with different crops may provide a clearer understanding of the influence of plant type on the changes in the rhizospheric microbial ecology.

Importantly, this study revealed that the effect of pesticide on soil microbial ecology is selective on certain microbial groups. While there were, in most instances, significant reductions in the overall microbial populations, the Pseudomonad numbers actually increased almost immediately and remained significantly higher until after 14 days in the pesticide-contaminated soils, as compared to the control soil (Fig. 3: 3A and 3B). However, the pesticide concentration affected the extent of increase in Pseudomonad numbers in the contaminated soils. In the work of Adetitun et al. (2013) and Ekpo and Ekpo (2006), it was noted that the preliminary response of a natural microbial population in contact with hydrocarbon is most often a decrease in the microbial biomass followed by a rise in the number of biodegraders. This can be seen as an initial adjustment, a sort of lag phase before actual degradation begins exponentially. Zhang et al. (2009) reported an increase in bacterial population after the application of cypermethrin pesticide on a pepper plant phyllosphere. This increase is similar to that reported in this work.

Effect of Pesticide Concentration on Soil Microbial Activity and Biomass-C

Basal respiration is acknowledged to measure the decomposition of organic C within soils by capable and active microorganisms (Dawson et al., 2007). SIR can determine the utilization of specific substrates by certain communities within the general microbial population (Anderson & Domsch, 1978).

Changes in basal respiration and SIR in the contaminated paddy rice soils are presented in Figures 4 and 5, respectively. Both cypermethrin and chlorpyrifos have similar effect, which was dependent on their concentration, on basal respiration (Fig. 4). In both NFC and X5FC cypermethrin-contaminated soils, basal microbial respiratory activity increased initially but decreased progressively after 14 days till the end of the experiment (Fig. 4A). Meanwhile, basal microbial respiratory activity was higher in the NFC chlorpyrifos-contaminated paddy rice soil for over 21 days but did not last beyond 5 days in the X5FC chlorpyrifos-contaminated soil, compared to the control soil (Fig. 4B). Juxtaposing these findings with the observed decrease in microbial populations within this period suggests an immediate stress condition following pesticide contamination of the paddy rice soil.

Noteworthy, changes in SIR followed a dissimilar trend to the basal respiration in the pesticide-contaminated soils (Fig. 5). In the cypermethrin-contaminated soils, a short period of initial decrease in SIR was followed by increase in SIR throughout the rest days of the experiment; the extent was dependent on its concentration, with greater SIR recorded in the NFC cypermethrin-contaminated soil (Fig. 5A). Equally, increased SIR was observed in both chlorpyrifos-contaminated soils, as compared to the control soil, throughout the 30 days post-treatment. The increase in SIR peaked after 14 and 21 days in the NFC and X5FC chlorpyrifos-contaminated soils, respectively (Fig. 5B).

Figure 6 presents the change in microbial biomass-C in the pesticide-contaminated paddy rice soils, as compared to the control soil. Changes in microbial biomass-C were

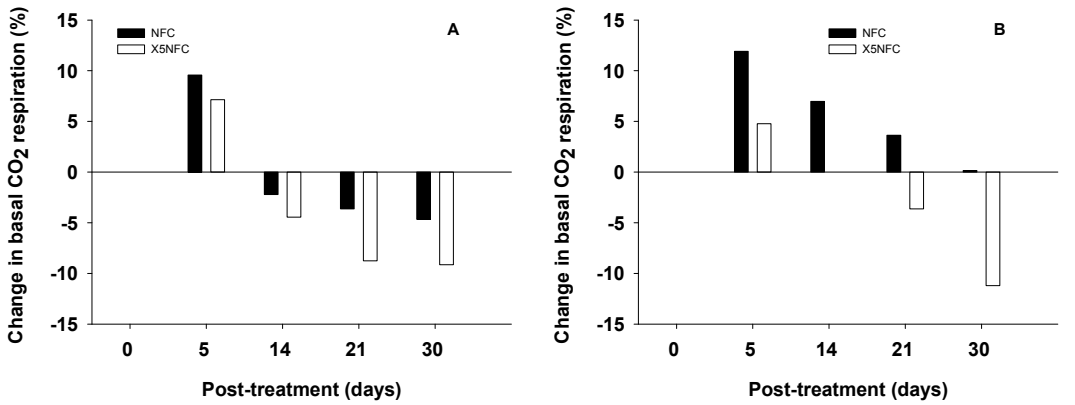

Figure 4. Change in basal respiration in paddy rice soils treated with (A) cypermethrin and (B) chlorpyrifos over 30 days post-treatment as compared to the control soil.

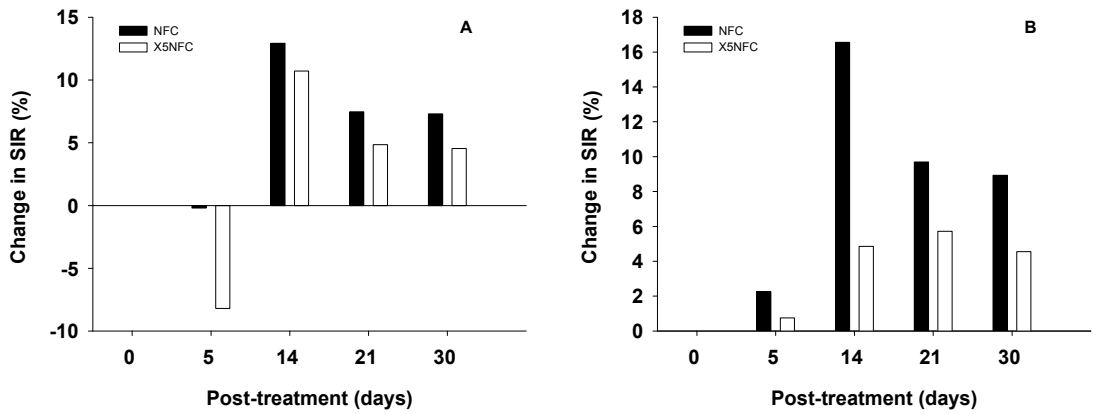

Figure 5. Change in substrate induced respiration (SIR) in paddy rice soils treated with (A) cypermethrin and (B) chlorpyrifos over 30 days post-treatment as compared to the control soil.

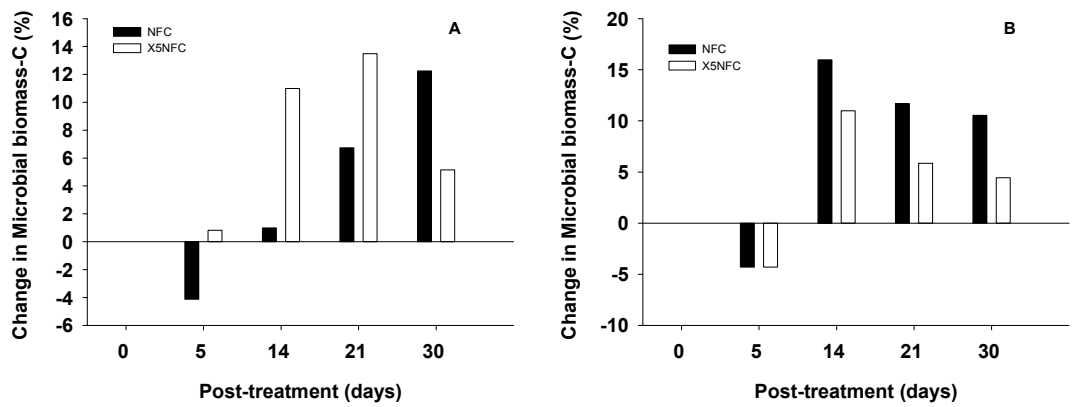

Figure 6. Change in microbial biomass C in paddy rice soils treated with (A) cypermethrin and (B) chlorpyrifos over 30 days post-treatment as compared to the control soil.

similar in both cypermethrin- and chlorpyrifos-contaminated soils; though while cypermethrin had greater effect in the NFC contaminated soil, the effect of chlorpyrifos was greater in the X5FC contaminated soil (Fig. 6). Biomass-C initially decreased slightly and then increased progressively; it peaked on day 30 in the NFC and day 21 in the X5FC cypermethrin-contaminated soils (Fig. 6A).

The initial decrease in soil microbial biomass may be due to the toxic effect of cypermethrin and chlorpyrifos on aspects of the soil microflora, which probably cannot tolerate the effect of these pesticides. The decrease was followed by an increase in the total number of bacteria count at 21 days after treatment till the day 30. Nevertheless, when compared with the untreated soil, which decreased till day 30, cypermethrin and chlorpyrifos had no adverse effect on the soil flora at various treatment levels over a period of time.

The report of Tu (1992) on eight herbicides applied to a loamy sand at 10 mg kg^{-1} soil to determine if these materials caused any serious effect on microbial activities related to soil fertility showed that soil respiration increased significantly after 96-hour incubation with atrazine. The study also reported that the herbicidal treatments at the tested levels were not harmful to soil microbial activities, which are important to soil fertility. Another report by Haney and Senseman (2000), who worked on glyphosate soils, showed that glyphosate significantly stimulated soil microbial activity as estimated by C and N mineralization but soil microbial biomass was not affected. An increase in the mineralization rate of C occurred from the first day following the introduction of glyphosate and persisted up to 14 days. These workers showed that the pesticide appeared to be directly and rapidly degraded by microbes, even at high application rates, without adversely affecting microbial activity. The stimulation of microbial activities by these chemicals agrees with our findings, and it's logical because feeding is an active process naturally. The increase in basal respiration rate with increase in concentration was also similar to that in our findings. This is also logical as higher concentration leads to higher activities and, hence, increase in respiration and its rate too.

Effect of Pesticide Concentration on Pseudomonas Tolerance and Growth

Bacteria belonging to the family Pseudomonadaceae are known to tolerate and possess the ability to degrade organic contaminants like the organophosphates. As observed earlier while the general microbial population decreased, at least in the first two weeks, Pseudomonad population actually increased within the period. The effect of increasing concentration of the pesticides on the tolerance and glucose utilization capacity of three Pseudomonad species isolated in X5FC contaminated soils 21 days post-treatment was evaluated in aqueous phase (Fig. 7). The isolates apparently exhibited different patterns of tolerance. As compared to the control without pesticide, the isolates showed pesticide concentration dependent decreases in growth after an initial short-span tolerance to cypermethrin (Fig. 7: 1A, 1B and 1C). However, the growth pattern of the isolates indicated that they tolerated and possibly utilized and grew on chlorpyrifos (Fig. 7: 1A, 1B, and 1C). It is not confirmed whether the degradation of these pesticides were assimilatory or dissimilatory.

Pseudomonas desmolyticum NCIM 2112 was reported in the degradation of chlorpyrifos into nontoxic metabolites such as 2-pyridinol and thiophosphate by Rokade and Mali (2013). These workers observed that the degradation was influenced by other carbon sources. Akbar and Sultan (2016) reported the isolation of *Achromobacter xylosoxidans*

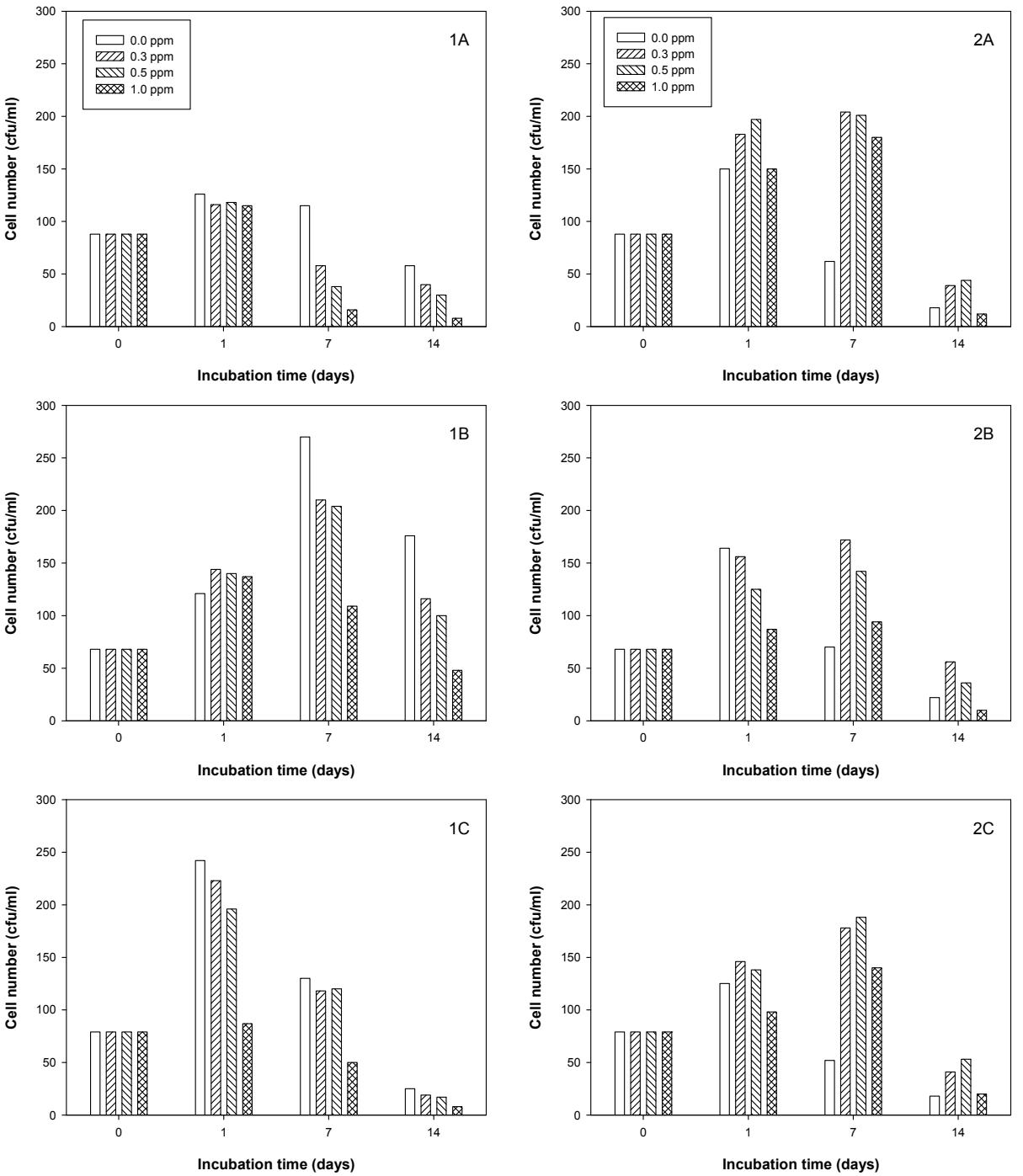

Figure 7. Tolerance and growth profile of *Pseudomonas* isolates A, B, and C to increasing concentration of (1) cypermethrin and (2) chlorpyrifos in aqueous phase.

(JCp4) and *Ochrobactrum* sp. exhibiting chlorpyrifos-degradation potential from pesticide contaminated agricultural fields. These organisms degraded 84.4% and 78.6% of the initial concentration of chlorpyrifos (100 mg L^{-1}) within a period of only 10 days. Pankaj et al. (2016) reported the ability of *Bacillus* sp. to degrade cypermethrin. These workers isolated *Bacillus* sp. from pesticide-contaminated soil of a rice field in India. They discovered that *Bacillus* sp. degraded the compound up to 81.6 % within 15 days.

Effect of Pesticide Concentration on Biodegradation by Pseudomonad Isolates

Figure 8 represents the effect of pesticide concentration on mineralization in soil. There was no significant difference in the initial rate and extent of mineralization in the cypermethrin-contaminated soils, as compared to the control soil (Fig. 8A). However, while the initial rate was not different, the later rates and overall extents of mineralization were appreciably higher in the chlorpyrifos-contaminated soils than in the control soil (Fig. 8B). This observation collocates with the results of the ability of certain Pseudomonad species to grow well in the presence of the chlorpyrifos.

Biodegradation was influenced by the levels of hydrogen peroxide and nitrogen along with microbial community, temperature, and pH changes (Javaid et al., 2016). According to Cycon et al. (2009), biodegradation of the organophosphorus insecticide diazinon was achieved using *Pseudomonas* sp. and *Serratia* sp. **The use of the genera** *Pseudomonas* in biodegradation of pollutants has been over flogged in literature. Many species of *Pseudomonas* can carry out nitrate respiration. They are chemoorganoheterotrophic and nutritionally highly adaptable. This property makes *Pseudomonas* species to be able to utilize many carbon compounds. Some strains can grow chemolithautotrophically (Singleton, 2004).

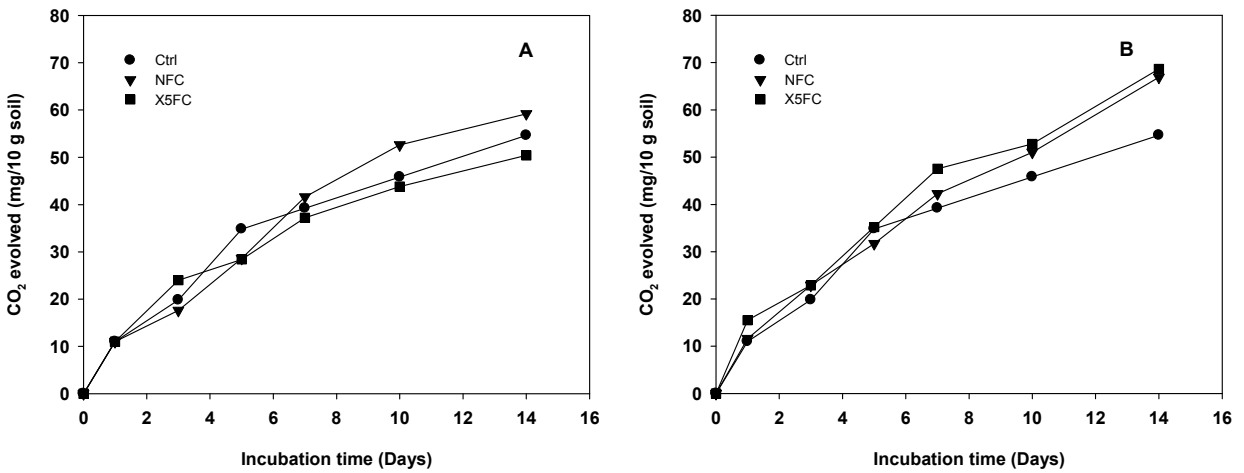

Figure 8. Effect of pesticide concentration on mineralisation in soils treated with (A) cypermethrin and (B) chlorpyrifos.

CONCLUSION

It is noteworthy that the pesticides used in this work did not have any direct toxic nor adverse effect on the planted rice. This is a good development that should be improved upon by makers of the pesticides to ensure continuous harmlessness of the chemicals to plants. Though pesticides at the rates applied may not affect plant growth significantly, they affected microbial populations, respiratory activities, and biomass-C. With the increase in microbial biomass and especially in the *Pseudomonas* group together with the measured biodegradation, there is great hope that pesticide-contaminated soil and water can be remedied.

This remediation can be sustained if the pesticide pollution is not intermittent. Like water, soil and any other ecological habitat have the innate ability to self-purify. This self-purification will however be affected if the pollutant is added continuously. There is urgent need for regulation and enforcement of such regulations on the application of pesticides in Nigeria. Also, manufacturers of these pesticides can work on ensuring that the pesticides do not constitute a source of pollution to water and soil. The same mechanism used, which made the pesticides nontoxic to plants, can be explored as it relates to the receiving soil, water, micro-flora, and micro-fauna in them.

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