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Bacterial responses to environmental herbicide pollutants (glyphosate and paraquat)

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ABSTRACT

The toxic effect of herbicides on non- target microorganisms may influence degradation of organic matter resulting in changes to nutrient cycling. In the present study, different strains of bacteria incubated in media containing different concentrations of glyphosate and paraquat were assessed over a period of two incubation terms. The deleterious impact of the herbicide was observed as glyphosate and paraquate treatments led to a reduction in the bacterial population. Analysis of the colony- forming unit (CFUs) showed a declining in microbial growth from 0 to 24 hours of incubation in all concentrations of glyphosate followed by a steady declining rate of the bacterial population after 48 h. The greatest bacterial population developed in media containing concentrations of glyphosate and paraquat was observed with strains S13.3, while strains S55 and S35 showed the lowest biomass production in response to all concentrations of glyphosate and paraquat. Based on the results obtained, strain S13.3 was determined to be resistant to the herbicides examined and may be useful for bioremediation of these compounds in soil.

Key words: Herbicides, Microbial populations, Microbial biomass, Glyphosate, Paraquat.

INTRODUCTION

Excessive use of herbicides in agriculture results in environmental contamination leading to accumulation of a large amount of chemical residues that may decrease the quality and productivity of soils. This can also impact ground water as well ascauses changesto the population dynamics of soil microorganisms. Microbes play important roles in soil ecosystems, where they have a major role in nutrient cycling and decomposition. In contrast, the accumulation of these toxic compounds in the food chain and drinking water creates a health hazard for the current and future generations (Chapalamadugu & Chaudry 1992; Margino *et al*. 2000; De - Lorenzo *et al*. 2001; Kolawole & Akinsoji 2011). Therefore, developing robust ways to biodegrade these chemicals in the environment is needed. Glyphosate and paraquat are two non-selective herbicides with a broad spectrum and rapid-action, and among the world's most widely used herbicides. Paraquat is immobilized on clay soil fractions shortly after application and is toxic because it diverts photosynthetic electron transport to oxygen to produce free radical that cause lipid peroxidation and membrane damage (Carp *et al.* 1985). Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, resulting in shikimate accumulation and reduced production of aromatic amino acids (Carp *et al*. 1985; Schonburnn *et al.* 2001; Yu *et al*. 2006). The use of microorganisms in reducing and detoxifying many herbicides, known as biological decontamination, is an

efficient method to reduce environmental and health impact of chemicals in the environment without producing toxic intermediates (Furukawa 2003; Mohammed 2009). The success of glyphosate and paraquat - degrading microorganisms in the soil depends on isolating bacteria with the ability to grow in the presence of these herbicides (Benslama & Boulahrouf 2013). If microorganisms are sensitive to particular herbicide, their application will interfere with vital metabolic activities of microbes, leading to disruption of the availability of nutrients in the soil (Oliveira & Pampulha 2006; Nautiyal 2006).

The effect of glyphosate on soil microbial processes has been an area of much research interest, although contrasting results have been also reported. Haney *et al.* (2000) asserted that usage of glyphosate resulted in an increase in soil microbial biomass.

In contrast, Busse *et al.* (2001) reported that glyphosate had no effects on soil microbial communities, while Weaver *et al*. (2007) reported that glyphosate had only small and transient impacts on the soil microbial community, even when applied at greater than field rates. The deleterious effect of the herbicides have been also reported by Adomako & Akyeampong (2016), as paraquat treatment resulted in reduction in the bacterial population in the soil. Similarly, Sebiomo *et al*. (2011) reported a significant response of soil microbial activity to herbicide treatment and increased adaptation of the microbial community to the stress caused by increase in concentration of the herbicides over weeks of treatment. Using biochemical, antibiotic and molecular 16S rRNA analyses, Sharifi *et al*. (2015) concluded that some bacteria isolated from some soils (Qom, Iran) could be applied for biodegradation of the herbicide. Regarding the increasing concern that herbicides not only affect the target organisms (weeds), but also the non - target soil microorganisms, the impacts of herbicide application should be taken into account as well.

This work aims to evaluate the effect of the commonly - used herbicides glyphosate and paraquat on bacterial populations.

MATERIALS AND METHODS

Herbicide treatment

The herbicide treatments consisted of paraquat (Gramoxone) and glyphosate (Roundup). So that, 4 different concentrations (0, 10, 100 and 1000 mg kg^{-1}) of paraquat and glyphosate were applied in this study.

Bacteria inoculation

At first, all bacterial strains encoded as S1, S3, S4, S6, S8.3, S13.3, S35, S55 and S05 were prepared for inoculation into test tubes. These bacteria were previously obtained from herbicide degradation tanks in Brazil.

Enumeration of microbial population

Enumeration of the microbial populations was done using specific media. X medium was used for the enumeration of total bacteria by the pour plate method. Incubation was done at 30 ^{o}C, for 24 – 48 h, as sampling times. All bacterial strains were streaked on Luria-Bertani (LB) plates and incubated at 30°C. About 10 µl of wet cells of bacteria from the 30°C - incubated LB plates was transferred into separated small flask cultures of R- media containing NH_4NO_3 and glucose and then kept in shaker for 24 hours at 30°C. Tolerance experiments were designed to examine the influence of 0, 10, 100 and 1000 ppm of each herbicide on growth. Medium containing no herbicides served as a control. The experiment was performed using triplicate samples. The cell pellet was prepared to approximately optical density (OD) 600 nm =1.0 using PBS to dilute the cell pellets. About 10 µl of $1x10^9$ CFU were added to each test tube with 1 ml of (phosphate buffered saline) PBS or PBS with herbicide. The drop plate method, conducted under sterile condition, was used for enumeration of the colonies. The sterile plates were prepared with 180 µl of PBS. Enumeration of bacterial colonies was achieved using the colony counter after 24 h and 48 h.

Statistical analysis

Data were generated from bacterial enumeration and expressed in line graphs and subjected to analysis of variance (ANOVA) to compare the means of the different sampling times.

RESULTS AND DISCUSSION

The effect of herbicide treatments on microbial population was determined based on the inhibition rate of the growth of bacterial colonies in each treatment medium. Analysis of the CFUs showed a declining in microbial growth from 0 to 24 hours of incubation in all concentrations of glyphosate followed by a steady declining rate of the bacterial population after 48 hours. However, interestingly, an increasing trend with a very gentle slope at all levels was observed with strains S4 and S6, after 48 hours of culture (Figs. 1 - 2).

This was because of recovery of microbial populations after initial inhibition either due to microbial adaptation to these herbicides or due to their degradation.

This phenotype may also be due to the microbial multiplication on increased supply of nutrients available in the form of microorganisms killed by the herbicides (Latha & Gopal 2010; Vandana *et al*. 2012, Baboo *et al*. 2013). The gradual increase in microbial counts may also be attributed to the herbicide acting as a nutrient source and their ability to temporarily mineralize and use herbicides as an energy source (Wardle & Rahman 1992; Baboo *et al*. 2013).

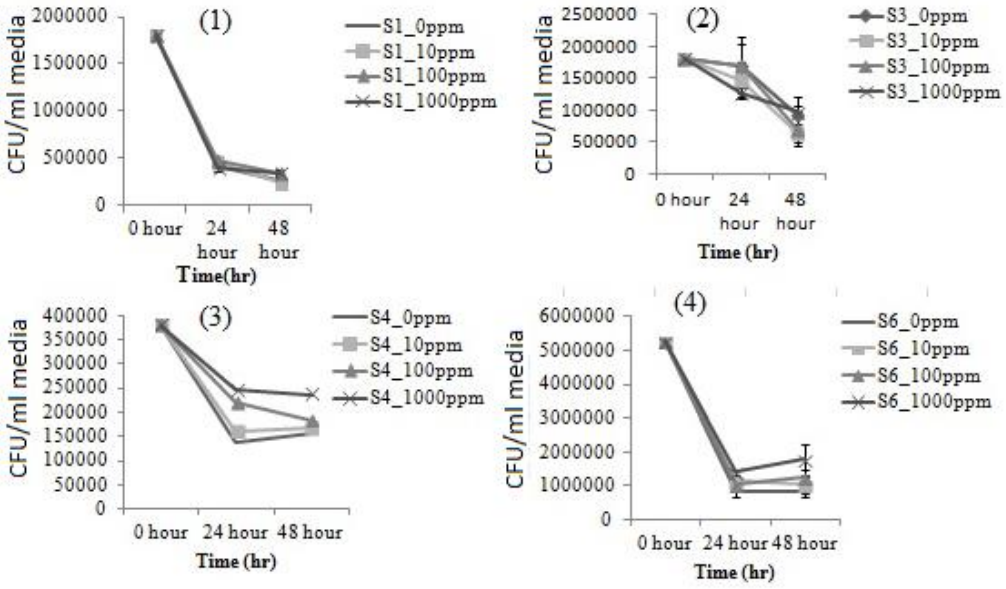

Fig. 1. Effect of herbicide glyphosate on isolated bacteria (S1, S3, S4 and S6) population. Error bars indicate standard error (n = 3). Colonies enumerated on the media (CFU ml^{-1}) after their incubation at 0, 24 and 48 hours.

Growth inhibition showed an increasing trend with increased herbicide concentrations, and microbial population showed different degrees of sensitivity to the herbicide compounds (glyphosate or paraquat) at different sampling times. Herbicides may alter the quality and quantity of microbial populations, through the direct toxicity of applied herbicides to the susceptible microbial species (Matsurnara & Boush 1971). The decrease in colony development of bacterial strains in response to the herbicides, relative to non-herbicide treatment (control), is shown in Figs. 1 - 4. The greatest population with 1000 ppm glyphosate was recorded with strain S13.3, whereas the lowest population was observed with S55 and S35. These two strains were the most sensitive

to glyphosate (Figs. 1 - 2). The sensitivity of specific bacteria in response to increasing concentrations of glyphosate in strain S13.3 is also evident in Fig. 5.

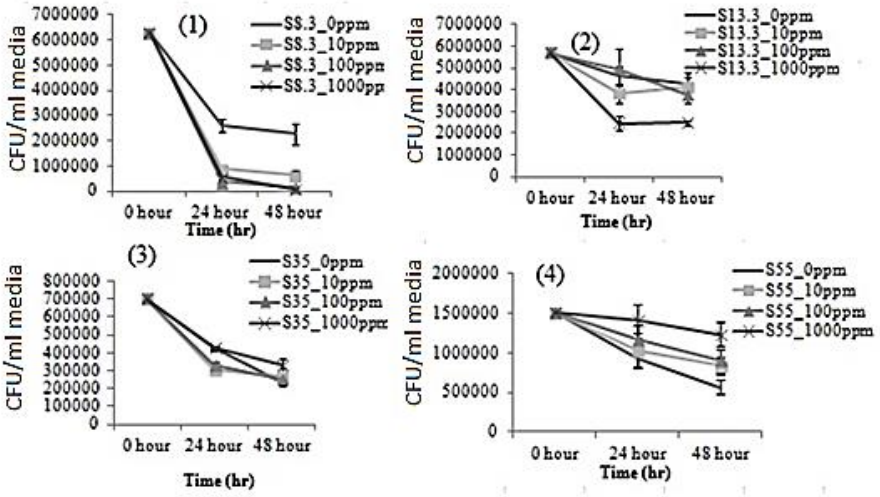

Fig. 2. Effect of herbicide glyphosate on isolated bacteria (S8.3, S13.3, S35 and S55) population. Error bars indicate standard error (n = 3). Colonies enumerated on the media (CFU ml^{-1}) after their incubation at 0, 24 and 48 hours.

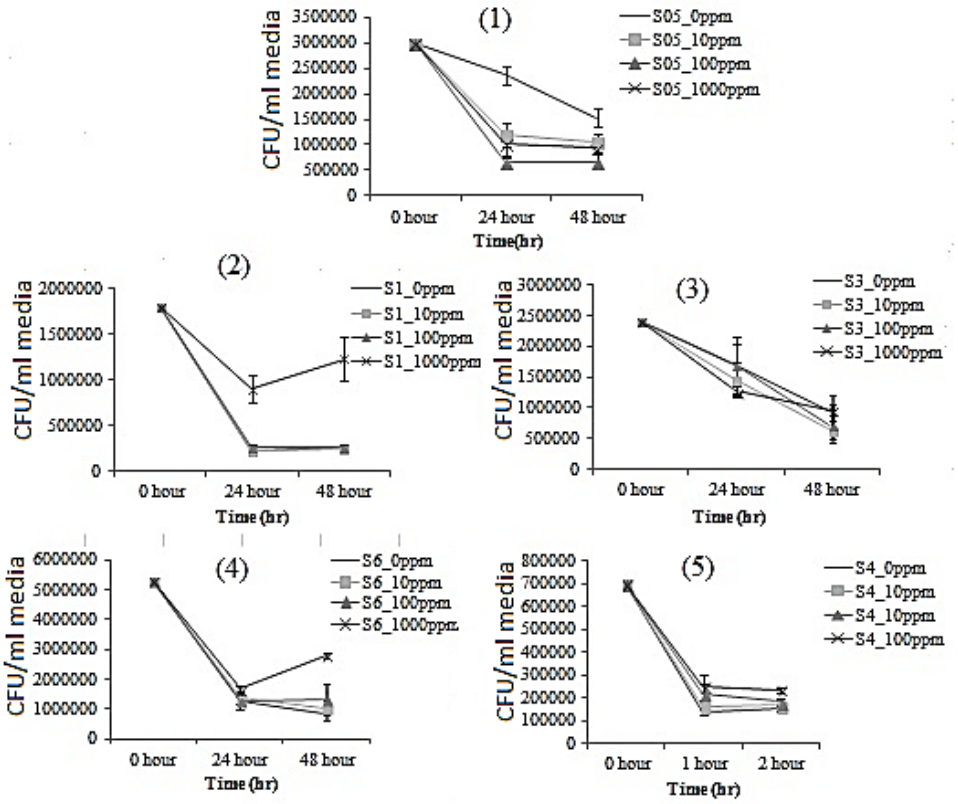

Fig. 3. Effect of herbicide paraquat on isolated bacteria (S05, S1, S3, S6 and S4) population. Error bars indicate standard error (n = 3). Colonies enumerated on the media (CFU ml^{-1}) after their incubation at 0, 24 and 48 hours.

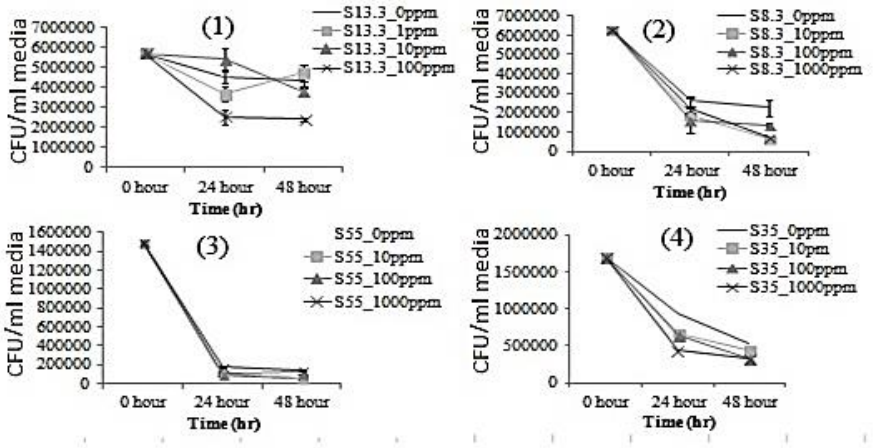

Fig. 4. Effect of herbicide paraquat on isolated bacteria (S13.3, S8.3, S55 and S35) population. Error bars indicate standard error (n = 3). Colonies enumerated on the media (CFU ml^{-1}) after their incubation at 0, 24 and 48 hours.

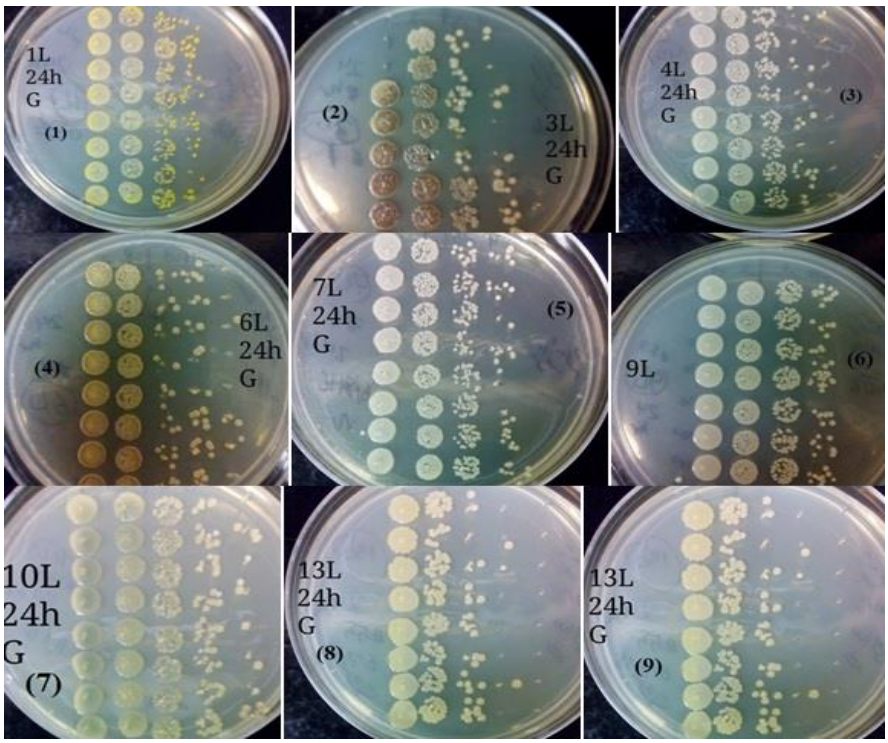

Fig. 5. Observation on the accumulation of cell aggregates of different isolated bacteria in culture media containing different concentrations of glyphsate. Each row from left to right represents the colonies obtained from 0, 10, 100 and 1000 ppm glyphosate, respectively. 1, 2, 3, 4, 5, 6, 7, 8 and 9 indicated strains S05, S01, S03, S04, S06, S8.3, S13.3, S35 and S55, respectively.

Increasing in paraquat concentration had less impact on all bacteria, which was in agreement with that of other researchers who found that paraquat inhibited fungal counts in soil (Sahid *et al.* 1992). The highest bacterial population development in the media containing the

varying concentrations of paraquat was observed on S13.3, while strains S55 and S35 showed the lowest biomass production in response to all concentrations of paraquat (Figs. 3 - 4). As shown in Fig. 6, the highest number of colonies, in terms of paraquat concentration, can be seen in the case of S13.3, while the lowest number is related to strains S55 and S35.

The data presented in Figs. 1 – 4 also revealed that paraquat caused higher inhibition of bacterial populations in comparison with glyphosate. The growth inhibition of microbial colonies caused by the herbicide paraquat was similar to those recorded for other bacteria which increased with the enhanced application rates of paraquat (Adomako & Akyeampng 2016).

The diversity of the results, which differed with herbicides type and concentration along with bacteria strains, illustrates the complexity of investigations on this very important biotic activity.

The reaction of microorganisms, in general, did not show a concentration dependency on applied herbicide doses. Generally, all doses of glyphosate and paraquat failed to impact on the examined microorganisms, as judged by CFUs, except for strain S8.3 in terms of glyphosate and strains S1 and S5 in terms of glyphosate, where more difference was between control and other concentrations (Figs. 1 - 4).

Different results were also observed in the extensive work of Zain *et al*. (2013) where the herbicide treatments significantly inhibited the development of microbial populations in the soil, and the degree of inhibition closely related to the rates of their concentrations and varied with the types of herbicide.

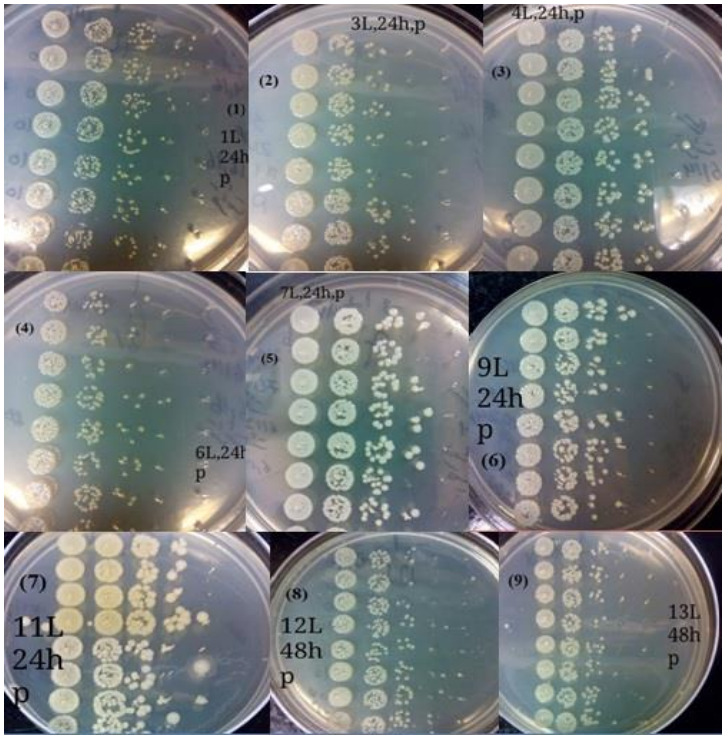

Fig. 6. Observation for the accumulation of cell aggregates of different isolated bacteria in culture media containing different concentrations of paraquat. Each row from left to right represents the colonies obtained from 0, 10, 100 and 1000 ppm paraquat, respectively. 1, 2, 3, 4, 5, 6, 7, 8 and 9 indicated strains S05, S01, S03, S04, S06, S8.3, S13.3, S35 and S55, respectively.

CONCLUSION

Before determining which microorganisms are responsible for glyphosate and paraquat biodegradation, a search for strains that are resistant to these herbicides are needed. According to the present study, strain S13.3

was considered to be resistant to both herbicides and may be useful in biodegrading these herbicides in soil.

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پاسخ باکتریایی به علف‌کش‌های آلاینده زیست محیطی (پاراکوات و گلایفوزیت)

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چکیده

علف‌کش‌ها می‌توانند مواد آلی را به عنوان عامل غیر هدف تحت تاثیر قرار دهند و سبب تغییر در چرخه عناصر غذایی شوند. در مطالعه حاضر، سویه‌های مختلف باکتریایی در محیط حاوی غلظت‌های مختلف گلایفوزیت و پاراکوات در طول دو دوره انکوباسیون ارزیابی شدند. نتایج نشان داد که اثر مخرب علف‌کش‌های گلایفوزیت و پاراکوات سبب کاهش جمعیت باکتری‌ها شد. بیشترین جمعیت باکتریایی در محیط کشت حاوی غلظت‌های گلایفوزیت و پاراکوات با سویه های S13.3 مشاهده شد، در حالی که سویه‌های S55 و S35 کمترین تولید زیست‌توده را در تمامی غلظت‌های گلایفوزیت و پاراکوات نشان دادند. بر اساس نتایج این تحقیق، سویه S13.3 به مقاومت در برابر علف‌کش‌های آزمایش شده در این تحقیق مقاوم بوده و می‌تواند برای زیست‌پالایی این ترکیبات در خاک مفید باشد.

*مولف مسئول