Evaluation of the efficacy and optimization of indigenous microbial isolate for herbicides (Paraquat Dichloride, Glyphosate, and Glyphosate isopropylamine) degradation

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https://doi.org/10.53858/bnas01022430

ABSTRACT

The presence of herbicides in soil is a serious problem for the environment. Studies on degradation of Herbicide (Paraquate dichloride (PD), Rake out (RO) and Gobara (GB)) by bacteria and fungi species isolated from soil environment in Keffi Metropolis Nigeria were carried out. A total of twenty (20) soil samples were collected. The bacteria and fungi were isolated from the soil and identified using standard microbiological methods. The herbicides utilization was determined using Atomic Adsorption Spectrometer. The effect of temperature on utilization of the herbicides by *Enterobacter asburiae* at 26^{O}C ranges from 1.23±0.11 mg/ml for PD, 1.14±0.29 mg/ml for RO and 0.53±0.86mg/ml for GB, *Pseudomonas aeruginosa* utilization ranges from 1.45±0.17 mg/ml for PD, 1.17±0.35 mg/ml, for RO 1.12± 0.82mg/ml for GB. *Aspergillus flavus* ranges from 2.12±0.19 mg/ml for PD, 2.00±0.03 mg/ml for RO and 2.02±0.57 mg/ml for GB, *Fusarium redolens* were 2.19±0.26 mg/ml for PD, 2.15±0.08 mg/ml for RO and 1.92±0.16 mg/ml for GB. Effect of incubation time on microbial herbicides degradations: for *E. asburiae* on PD it ranges from day 1 with 0.24±0.37 mg/ml to day 20 with 2.06±0.11 mg/ml. for *P. aeruginosa* on PD ranges from day 1 with 0.38±0.08 mg/ml to day 20 with 2.39±1.45 mg/ml. The Utilization of herbicides by *A. flavus* on PD ranges from day 1 with 0.10±0.01 mg/ml to day 20 with 2.29±0.12 mg/ml. for *F. redolens* in PD, it ranges from day 1 with 0.27±0.08 mg/ml to day 20 with 2.57±0.27mg/ml. The process of degradation of herbicide has become very attractive as it allows for removal of herbicide over a relatively broad range of pH and temperature.

Keywords: *Herbicide;* Paraquate dichloride, Rake out; Gobara; bacteria; fungi

Received: 21 June 2021, Revised: 09 August 2021, Published: 20 August 2021

Citation: Ibrahim, Z.M., Makut M.d., Omale, A.A., Abubakar, M.U. (2021). Evaluation of the efficacy and optimization of indigenous microbial isolate for herbicides (Paraquat Dichloride, Glyphosate, and Glyphosate isopropylamine) degradation*, BIOMED Natural and Applied Science,1(2):24-30,* https://doi.org/10.53858/bnas01022430

1.0 Introduction

During the last decades, the scientific community, including government and non-government organizations have increased their interest in detecting and controlling the environmental agents responsible for damages to the human health and sustainability of the ecosystems [1]. This interest has been intensified by the frightening increase in the reports of the anthropogenic action on the environment responsible for damages to the ozone layer, accidental release of wastes and radioactive gases, as well as contamination by pesticides used in agriculture. However, the growth of the human population and of the activities associated with agriculture, industrialization, and urbanization have contributed to the depredation of biodiversity and genetic variability, resulting in the compromise of several species, including man [2,3]

Before the introduction of selective herbicides as an agricultural practice, the removal of weeds was accomplished manually in an extremely laborious form. Thus, the farmers sought other forms to control weeds, such as integrating other weed control practices such as crop rotation, tillage, and fallow systems [4]. The introduction of selective herbicides in the late '40s and the constant production of new herbicides in the following decades gave farmers a new tool in the control of weeds [4]. Therefore, the

process of modernization of agriculture introduced, in the '60s, the use of new biological varieties considered more productive, but dependent on chemical fertilizers and intensive use of pesticides, in order to increase productivity [5].

The use of these chemical agents resulted in the increase of productivity, but, on the other hand, brought adverse consequences, since many are harmful substances for man and the environment [6]. The world practice of using agrochemicals for long periods, often indiscriminate and abusive, has raised concerns among the public authorities and experts of public health and sustainability of natural resources [7].Many agrochemicals are very toxic substances whose absorption in man is almost exclusively oral and can also occur by inhalation or dermally. As a consequence of human exposure to pesticides, a series of disturbances can be observed, such as gastric, neurological, and muscular [8].

Microbes do extremely well in thriving on herbicide compounds in the soil by utilizing them as a supply of nutrients and energy. Many herbicides serve as good carbon and/or nitrogen sources for soil microorganisms [9]. Evidence for their remarkable range of degradative abilities can be seen in the recycling rather than the accumulation of vast quantities of biological materials that have been produced throughout the history of life on earth [10]. Microbial degraders work in natural environments, and some alterations are imperative to encourage the organisms to degrade the herbicide at a faster rate in a limited time frame. Hence, to achieve successful bioremediation of herbicide contamination, it necessitates the construction of a unique niche for the desired microbes, so they can be productively exploited [11]. Biostimulation plays a role here, by the facile addition of substrates or nutrients to the microbial habitat strictly on a "need only" basis consequently invigorating the biodegradation of target herbicide compound [12]. The aim of the present study is to evaluate the potential and optimum condition for microbial degradation of herbicide contaminated soil obtained from different farm locations around Nasarawa State University, Keffi, Nasarawa State, Nigeria

3.0 Materials and Methods

3.1 Chemical and Reagents

The chemicals and reagents that were used in the study include; Ethanol, Rakeout Glyphosate (Touchdown, Sygenta Crop Protection AG Basle, Swizerland), Paraquat dichloride (Pentashi Anhui Zhongshan Chemical Industry Co Ltd, Address: Xiangyu Town Chemical Industry Park Dongzhi county, Anhui Province China). Gobara (Touchdown, Sygenta Crop Protection AG Basle, Swizerland). Lactophenol, Cotton Blue, Crystal Violet, Potassium Iodide, Iodine Crystal, Safranin, Indole Reagent, Methyl Red, Oxidase Reagent, Hydrogen Peroxide (H_2O_2), Sodium Chloride (NaCl), Sucrose, Sodium Nitrate, Magnesium Sulphate, Calcium Chloride, and Ferrous Sulphate,

3.2 Sample Collection

Soil samples for this study were collected from four (4) different farm locations in Nasarawa State University, Keffi, Nasarawa State, Nigeria between longitude 8.55^0 and latitude 7^0N and above the sea level of 630m. Top soil of (5-10 cm depth) was collected using a sterile spatula and transferred into sterile polythene bags which were transported to the laboratory for further analysis [13]

3.3 Isolation and identification of bacteria and fungi

The isolation of the bacteria and fungi isolates were carried out as described in our previous study [14]**.** The pure cultures of the bacteria isolates were identified based on their cultural, morphological and Biochemical test as described by Damales and Eleftherohorinos [15], while the fungi isolates identification was carried out as described by Makut and Ekeleme [16].

3.4 Determining the effects of temperature on microbial biodegradation of herbicides

Experiment was carried out at two different temperatures (26^{o}C and 37^{o}C) in order to determine the effect of temperature on biodegradation of the RO, PD, and GB herbicides by bacteria and fungi. The

https://doi.org/10.53858/bnas01022430

isolates were Inoculated in Czapek Dox broth medium containing different herbicides of 50 ppm (NaNO3 3.0 g, K2HPO4 1.0g, KCl 0.5g, MgSO4 0.1 g, FeSO4.2H2O 0.1g, Sucrose 30g) were incubated for 15days [17].

3.5 Determining the effects of pH on microbial biodegradation of herbicides

In order to determine the effect of pH on biodegradation of RO, PD, and GB herbicides by the bacteria and fungi. The isolates were Inoculated in Czapek Dox broth medium containing different herbicides of 50 ppm (NaNO3 3.0 g, K2HPO4 1.0g, KCl 0.5g, MgSO4 0.1 g, FeSO4.2H2O 0.1g, Sucrose 30g) the pH were adjusted to pH 4.5 and pH8.5 and were incubated for 15days [17].

3.6 Determining the effects of incubation time on microbial biodegradation of herbicides

Effect of Days on biodegradation of on biodegradation of RO, PD, and GB herbicides by the bacteria and fungi isolates was carried out as described previously [17]. The isolates were Inoculated in Czapek Dox broth medium containing different herbicides of 50 ppm (NaNO3 3.0 g, K2HPO4 1.0g, KCl 0.5g, MgSO4 0.1 g, FeSO4.2H2O 0.1g, Sucrose 30g) and were incubated for different days ranging from 1-12 days.

3.7 Quantification of the herbicide degradation by the bacteria and fungi isolates

The quantification of RO, PD, and GB herbicides degradation was carried out using a method described previously [17]. After incubation for days the broth media was centrifuged at 1000rpm for 2mins. The residues of the herbicide were analyzed by UV- visible Spectrophotometer**.**

3.0 Results

3.1 Microbial utilization of herbicides at various temperature

The effect of temperature on the utilization of herbicides (PD, RO, and GB) by fungal and bacterial species isolated from farmlands is shown in figure 1. The effect of temperature on utilization of herbicide, by *Enterobacter asburiae* at 26^{O}C ranges from 1.23±0.11 mg/ml for PD, 1.14±0.29 mg/ml for RO and 0.53±0.86 mg/ml for GB, *Pseudomonas aeruginosa* utilization ranges from 1.45±0.17 mg/ml for PD, 1.17±0.35 mg/ml, for RO and 1.12±0.82 mg/ml for GB. *Aspergillus flavus* ranges from 2.12±0.19 mg/ml for PD, 2.00±0.03 mg/ml for RO and 2.02±0.57 mg/ml for GB, *Fusarium redolens* were 2.19±0.26 mg/ml for PD, 2.15±0.08 mg/ml for RO and1.92±0.16 mg/ml for GB

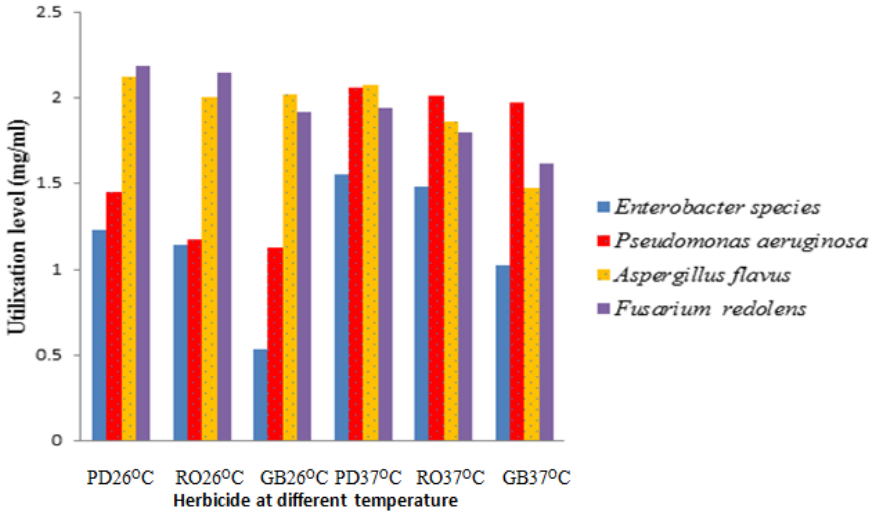


Figure 1: Effect of Temperature on Utilization of Herbicides by Fungal and Bacterial Species. PD= Paraquat Dichloride; RO= Rake out (Glyphosate)t; GB= Gobara (Glyphosate isopropylamine)

3.2 Microbial utilization of herbicides at various pH

The effect of pH on the rate of utilization of herbicides (PD, RO, and GB) by fungal and bacterial species isolated from farm lands is given in figure 2. pH 4.5 and 8.5 were used to determine the utilization of herbicides capacity of *E. asburiae, P. aeruginosa*, *A. flavus, F. redolens* which show that at pH of 4.5 the range for *E. asburiae* degradation was 1.38, 1.74, and 2.06 mg/ml at pH 4.5 and 0.82, 0.56 and 0.69 mg/ml at pH 4.5 for PD, RO and GB respectively, while for *P. aeruginosa* herbicide degradation were 1.19, 1.27 and 1.99 mg/ml at pH 4.5, and 0.79, 0.97 and 0.71 mg/ml at pH 8.5 for PD, RO and GB respectively. The degradation by *A. flavus* were 1.04, 1.61 and 1.59 mg/ml at pH 4.5, and 1.00, 0.62 and 0.87 mg/ml at pH 8.5 for PD, RO and GB respectively, while for *F. redolens* the degradation rate were 2.08, 2.15, and 2.03 mg/ml at pH 4.5, and 1.10, 1.00 and 0.92 mg/ml at pH 8.5 for PD, RO and GB respectively

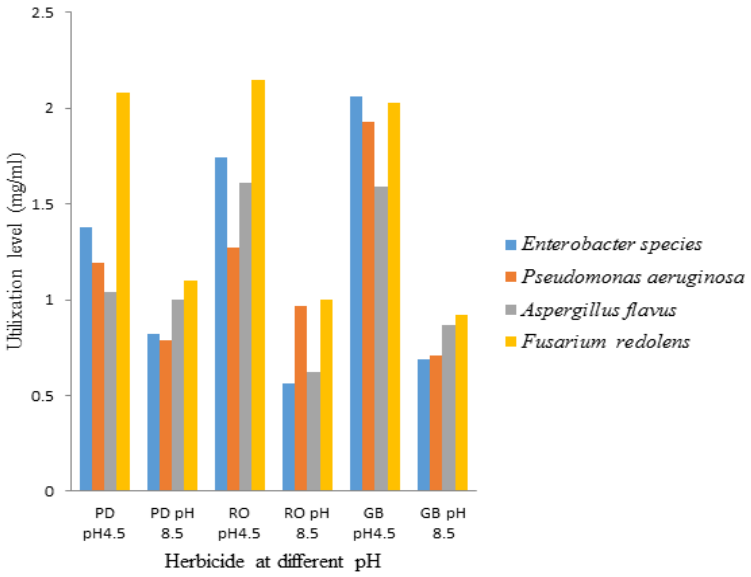


Figure 2: Effect of pH on the utilization of herbicides by Bacteria and fungal isolates.PD= Paraquat Dichloride; RO= Rake out (Glyphosate)t; GB= Gobara (Glyphosate isopropylamine)

3.3 Effect of Time on microbial Utilization of Herbicides by Bacterial species

The Utilization of Herbicides by bacteria over a period of 20 days was taken at intervals of 1, 4, 8, 12, 16, and 20 days respectively. Effect of time on microbial Utilization of Herbicides: for *Enterobacter asburiae* it ranges from on PD day 1 with 0.24±0.37 mg/ml to day 20 with 2.06±0.11 mg/ml (Table 1). for *Pseudomonas aeruginosa* on PD ranges from day 1 with 0.38±0.08 mg/ml to day 20 with 2.39±1.45 mg/ml (Table 2). The Utilization of Herbicides by *Aspergillus flavus* on PD ranges from day 1 with 0.10±0.01 mg/ml to day 20 with 2.29±0.12 mg/ml (Table 3). for *Fusarium redolens* in PD it ranges from day 1 with 0.27±0.08 mg/ml to day 20 with 2.57±0.27 mg/ml (Table 4).

Table 1: Effect of Time on Herbicides Utilization by *Enterobacter asburiae*

Time (Days)	PD mg/ml	RO mg/ml	GD mg/ml
Day 1	0.24±0.37	0.39±0.64	0.19±0.99
Day 4	0.88±0.61	0.73±0.02	0.63±0.14
Day 8	1.00±0.47	1.00±0.90	0.98±0.66
Day 12	1.22±0.86	1.12±0.97	1.01±0.13
Day 16	1.53±0.79	1.32±0.12	1.15±0.93
Day 20	2.06±0.11	1.92±0.98	1.77±0.10

PD= Paraquate Dichloride, RO= Rake out (Glyphosate),
GB= Gobara (Glyphosate Isopropylamine)

https://doi.org/10.53858/bnas01022430

Table 2: Effect of Time on Herbicide Utilization by *Pseudomonas aeruginosa*

Time (Days)	PD mg/ml	RO mg/ml	GD mg/ml
Day 1	0.38±0.08	0.22±0.01	0.23±0.06
Day 4	0.66±0.15	0.57±0.07	0.42±0.01
Day 8	0.97±0.68	0.90±0.65	0.89±0.22
Day 12	1.06±0.07	1.00±0.47	1.06±0.17
Day 16	1.94±5.89	1.88±0.75	1.79±0.16
Day 20	2.39±1.45	2.01±0.06	1.92±0.67

PD= Paraquate Dichloride, RO= Rake out (Glyphosate),
GB= Gobara (Glyphosate Isopropylamine)

Table 3: Effect of Time on Herbicide Utilization by *Fusarium redolens*

Time (Days)	PD mg/ml	RO mg/ml	GD mg/ml
Day 1	0.27±0.08	0.18±0.05	0.20±0.01
Day 4	0.62±0.18	0.49±0.02	0.52±0.01
Day 8	1.17±0.01	1.26±0.09	1.17±0.03
Day 12	1.73±0.18	1.84±0.28	1.56±0.00
Day 16	2.10±0.79	2.00±0.59	2.04±0.10
Day 20	2.57±0.27	2.33±0.38	2.15±0.20

PD= Paraquate Dichloride, RO= Rake out (Glyphosate),
GB= Gobara (Glyphosate Isopropylamine)

Table 4: Effect of Time on Herbicide Utilization by *Aspergillus flavus*

Time (Days)	PD mg/ml	RO mg/ml	GD mg/ml
Day 1	0.10±0.01	0.16±0.02	0.19±0.08
Day 4	0.51±0.37	0.42±0.47	0.58±0.47
Day 8	1.05±0.64	1.18±0.69	1.16±0.57
Day 12	1.73±1.34	1.57±0.30	1.62±0.60
Day 16	2.00±2.10	2.04±0.03	2.09±0.65
Day 20	2.29±0.12	2.30±0.91	2.61±0.44

PD= Paraquate Dichloride, RO= Rake out (Glyphosate),
GB= Gobara (Glyphosate Isopropylamine)

4.0 Discussion

The utilization of Herbicide content of medium by bacteria and fungi isolates such as *Enterobacter asburiae, Pseudomonas aeruginosa*, *Aspergillus flavus, Fusarium redolens* as observed in this study was totally in agreement with the studies earlier described by Olukanni *et al*., [18] and Samarth *et al*. [19].

This study also shows that the rate of utilization of Herbicide by bacteria and fungi observed in this study is similar to the study earlier reported by Olukanni *et al*., [18] the reduction in the concentration of Herbicide content in the medium by *Pseudomonas aeruginosa* than *Enterobacter asburiae* and *Fusarium redolens* than *Aspergillus flavus* was not surprising although studies have reported that some bacteria have greater absorption capacity due to the thick layer of peptidoglycan [20,21].

Studies that show the high absorption capacity of Herbicide by *Pseudomonas aeruginosa* than other species of bacteria and *Fusarium redolens* fungi isolates from contaminated soil by herbicide appear to be new to the best of our knowledge although the mechanism of removal or biosorption of Herbicide content by bacteria was not evaluated in this study.

https://doi.org/10.53858/bnas01022430

The effect of temperature on the removal or reduction of herbicide concentration shows that the reduction rate was higher at 37°C than at 26°C by bacteria and 26°C than 37°C by fungi for degradation of herbicide concentration such as Paraquat Dichloride; Rake out (Glyphosate) and Gobara (Glyphosate Isopropylamine) this suggest that temperature is a necessary factor affecting degradation or removal of herbicide by bacteria and fungi tested in this study.

The findings on the effect of temperature on degradation of herbicide by microorganism tested were observed not to be in agreement with studies done by Goyal et al. [22]. From this study, we also observed that utilization of herbicide by species of bacteria and fungi was higher at pH of 4.5 than at pH 8.5 and this finding is in agreement with the study earlier reported by Silva *et al*., [2] and it also suggest that as the pH of the solution increases the utilization rate of herbicide and this shows that pH and ionic strength may be very important factors in the herbicide biosorption or utilization capacity of bacteria and fungi species [23]

5.0 Conclusion

From this study bacteria and fungi species ability to remove or utilize herbicide has been shown to be efficient for herbicide removal from solution. The process of degradation of herbicide has become very attractive as it allows for removal of herbicide over a relatively broad range of pH and temperature. Many researchers have studied the removal of herbicide by different microorganisms which provides enough arguments for the use of microbial species for herbicide removal from solutions. Consequently, through further research and studies, microbial removal of herbicide would be the most conventional, environmentally friendly means of removal of herbicide not only from the soil but from all forms that are contaminated by herbicide.

Conflict of Interest: The author declared no conflict of interest exist

Author's contributions: Author ZHM, design and conducted the study, and wrote the original draft of the manuscript. Authors AAO and MUA participated in the data collections and analysis. Author MMD supervised the work, reviewed the draft and edited the manuscript. All authors read and approved the final version of the manuscript

Funding: This work received no external funding

Acknowledgements: Our appreciation goes to Dr. D. Ishaleku, Prof. Y. B. Ngwai, and all the lecturers of the Microbiology Department, Nasarawa State University, Nigeria

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https://doi.org/10.53858/bnas01022430