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# Microbe—Chloroacetanilide Herbicide Interaction across Soil Type

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## Abstract

**An investigation was carried out under laboratory conditions to study the persistence of butachlor applied at recommended dose (2 kg ai/ha) along with its impact on microbial activity as well as growth of colonial bacteria and fungi in alluvial (*Typic Haplaquent*), lateritic (*Typic Haplustalf*) and coastal (*Typic Haplaquept*) soils. Butachlor caused a significant increment in microbial activity following an initial diminution in between 10 to 22 days of incubation depending on the type of soil. The herbicide resulted in a significant shrinkage in bacterial community during later stages of incubation in lateritic and coastal soils in spite of a significant swelling on the 15th day in lateritic and alluvial soils. Fungal community significantly expanded at the initial stage in lateritic soil and during later stages in alluvial soil by the application of butachlor but shriveled during later stages in the lateritic soil, intermediate stage in coastal soil and initial stages in alluvial soil. Alluvial soil reared the highest population of colonial bacteria and exhibited highest microbial activity while coastal soil significantly pressed them down to the lowest. However, lateritic soil was the best niche for fungal community. Co-metabolism was the main phenomenon in butachlor metabolism particularly in coastal soil, though zymogenous microbes including bacteria and fungi also participated in both lateritic and alluvial soil at certain stages. The persistence of butachlor was the lowest in alluvial soil followed by lateritic and coastal soil, respectively. Among the soil types application of butachlor is safe in alluvial soil.**

## Keywords

**Alluvial Soil, Lateritic Soil, Coastal Soil, Bacteria, Fungi, Butachlor, Persistence**

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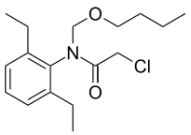
## 1. Introduction

Since man first began to cultivate crops, undesirable plants, called weeds, have been a problem causing reduction in agricultural production in several ways [1]. In India, as a whole, weeds cause an estimated 37% reduction of agricultural produce annually [2] and on global basis, a 10% reduction of crop yield [3]. Herbicides are used to kill or stunt weed infestation allowing crop plants to grow and gain a competitive advantage [4] and offer one of the most effective means to reduce labor costs on weed control. Herbicide usage in rice now accounts for about 30% of the total consumption of pesticides [5], and is expected to increase dramatically in India in future [6]. Butachlor (**Table 1**), available in granular as well as emulsifiable concentrate formulations, is a systemic pre-emergent herbicide controlling most of the annual grasses [7] [8]. Soil, the ultimate recipient of herbicides, comprises biotic and abiotic components. The abiotic components determine growth and activities of the biotic

**Table 1.** General characteristics of butachlor and test soils.

**A.** Properties of butachlor

Chemical structure




| | |
|---|---|
| Chemical class | Chloroacetanilide |
| Chemical name | [N-(butoxymethyl)-2-chloro-N-(2,6-diethylphenyl)acetamide] |
| Molecular formula | $C_{17}H_{26}ClNO_2$ |
| Molar mass | 311.85 g·mol$^{-1}$ |
| Solubility in water | 20 mg l$^{-1}$ (20˚C) |
| Vapour pressure | 1.8 × 10$^{-6}$ mm Hg (25˚C) |
| $LD_{50}$ | 1740 mg·kg$^{-1}$ (oral, rat) |

| **B.** Soil properties | Laterite | Soil type coastal | Alluvial |
|---|---|---|---|
| Water holding capacity (%) | 30.0 | 54.0 | 49.0 |
| pH (1:2.5) | 5.5 | 6.7 | 7.7 |
| Electrical conductivity (dsm$^{-1}$) | 0.06 | 1.55 | 0.48 |
| Cation exchange capacity [c mol (p$^+$) Kg$^{-1}$] | 9.1 | 4.7 | 16.9 |
| Total nitrogen (%) | 0.03 | 0.11 | 0.07 |
| Available nitrogen (%) | 0.0015 | 0.031 | 0.0196 |
| Available Phosphorus (ppm Olsen P) | 4.3 | 60.0 | 17.1 |
| Organic carbon (%) | 0.49 | 0.64 | 0.58 |
| C:N ratio | 17.5:1 | 5.7:1 | 8.5:1 |
| Mechanical analysis | | | |
| Sand (%) | 72.2 | 17.6 | 22.2 |
| Silt (%) | 8.4 | 35.8 | 35.4 |
| Clay (%) | 19.4 | 46.6 | 42.4 |
| Ca [c mol (p$^+$) kg$^{-1}$] | 2.5 | 8.2 | 10.4 |
| Mg [c mol (p$^+$) kg$^{-1}$] | 0.6 | 2.3 | 1.0 |
| Origin | Regional Research Farm, Jhargram, West Medinipur | No. 1 Farm, ICAR, Canning, 24 Pgs (S) | Univ. Research Farm, Mohanpur, Nadia |
| Soil taxonomy | *Typic Haplustalf* | *Typic Haplaquept* | *Typic Haplaquent* |

components, which, in turn, regulates the physical, chemical and biological properties of soil. The living organisms exhibit different types of inter-relationships leading to establishment of a biological equilibrium in soil. Among different internal and external factors affecting the equilibrium, soil pH, and level of organic matter and electrical conductivity of soil have drawn maximum attention [9]. Generally neutral to slightly alkaline soil reaction support higher growth of bacteria [10], while acidic soils harbor more fungal propagules [11]. Level of organic matter in soil also determines the growth as well as activities of microorganisms [12]. Soil salinity on the contrary exerts diminutive impact on the preponderance of microbes in soil [12]. Soil micro flora exists normally in a state of starvation due to low energy substrate supply [13] which can be changed temporarily into their active growth and metabolism on availability of energy and nutrients from applied herbicides [14]. As a consequence, the herbicides are degraded in soils by biotic agencies. Though herbicides effectively control weeds, they also cause qualitative and quantitative alterations in the soil microbial populations and their enzyme activities [15]-[17], resulting in decline in crop productivity [18]. So herbicides are aptly considered as "two-edged sword" [19] and viewed as a serious threat to global environment [20]. They kill species of bacteria, fungi and protozoa combating pathogenic microorganisms and thus upset the balance between pathogens and beneficial organisms [21]. Biochemical transformation is the principal avenue of pesticide metabolism in addition to physical and chemical transformations [22] wherein micro-organisms are mainly involved [23] [24]. Among them, bacteria and fungi exhibit maximum capability of degrading pesticides [25]. Some of them utilize herbicide as energy and nutrient sources [26] while others degrade pesticide without exploiting them as energy and nutrient sources [22] by a process called co-metabolism [27]. Reports on negative effect of butachlor on the growth and activities of microbes are also available [28]. However, Information regarding fate and behavior of butachlor under varying soil conditions affecting growth and activities of micro-organism is scanty. An attempt was thus made to study the persistence of butachlor at the recommended dose in alluvial, lateritic and coastal soils along with its impact on microbial activity as well as growth of colonial bacteria and fungi.

## 2. Materials and Methods

### 2.1. Chemicals

Butachlor of analytical grade (97.7%) was obtained from Sigma-Aldrich (Germany). All solvents and chemicals were of A. R. grade.

### 2.2. Soil Collection and Analysis

The investigation was based on three simultaneous experiments in the laboratory of Department of Agricultural Chemistry and Soil Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India and the experiments were conducted with three soils from three different geographical origins possessing a wide variation in physicochemical properties (**Table 1**). Surface soil samples (0 - 10 cm), collected from the monoculture (*kharif* rice) cultivated fields of all three different locations, were kept in a green house at 25˚C ± 5˚C at 60% - 80% of maximum water holding capacity. Shortly before the study, soils were air-dried, ground and passed through a 80 mesh sieve, thoroughly homogenized and analyzed for pH (1:2.5 soil-water ratio) [29]; organic carbon by 1 N $K_2Cr_2O_7$ solution method [30]; total nitrogen by modified Kjeldahl method [29] and available $P_2O_5$ by extraction with alkaline $NaHCO_3$ (pH 8.5) method [31]. Mechanical analysis for determining the texture of the soil was done by Hydrometer method [32] [33]. Carbon-di-oxide evolved per 100 gm soil following the method of Pramer and Schimidt [34] by absorbing the evolved carbon-di-oxide in NaOH solution and back titrating with hydrochloric acid using Barium chloride and phenolphthalein as indicator.

### 2.3. Experiments

#### 2.3.1. Experiment 1

A set comprising three earthen pots were filled with 2 kg for each of the three soil types left as such to be considered as control. Another set of similar pots were thoroughly blended with butachlor at the rate of 2 kg a.i./ha-separately for each soil type in a tumbling mixture (30 ± 2 rpm) for 1 h. The moisture content of the soils was maintained at 50% of the water holding capacity and checked gravimetrically every week throughout the experimental period. The pots were covered with black polyethylene sheet to avoid photo degradation of the herbicide and to minimize moisture loss. The pots were then arranged in a Completely Randomized Design with three

replications of each of the two treatments viz. control and treated, and were incubated at 30˚C ± 1˚C for 60 days.

#### 2.3.2. Experiment 2

In order to study the influence of butachlor on microbial activity across soil type another experiment was conducted in one litter conical flasks containing 100 g soil with the same treatments (with or without butachlor at 2.0 kg a.i./ha), replications (3), soil types (alluvial, lateritic and coastal) and environmental conditions (moisture 50% of water holding capacity and incubation temperature 30˚C ± 1˚C). Microbial activity was determined at periodic intervals in terms of mg of carbon-di-oxide evolved per 100 gm soil following the method of Pramer and Schimidt [36] by absorbing the evolved carbon-di-oxide in NaOH solution and back titrating with hydrochloric acid using Barium chloride and phenolopthalein as indicator.

#### 2.3.3. Experiment 3

Third experiment was carried out in 50 ml conical flask containing 10 gm of soil with the same treatments (with or without butachlor at 2.0 kg a.i./ha), replications (3), soil types (alluvial, lateritic and coastal) and environmental conditions (moisture: 50% of water holding capacity, incubation temperature 30˚C ± 1˚C and incubation period 60 d) to determine the dissipation pattern of butachlor in each soil type in absence of microorganisms. For this one batch of soil sample was sterilized by autoclaving at 15 psi and 121˚C [35]. The experimental set-up for sterilized soils was performed aseptically.

### 2.4. Sample Collection and Analysis

Soil sample was drawn from the respective pots periodically ($5^{th}$, $10^{th}$, $15^{th}$, $30^{th}$, $45^{th}$ and $60^{th}$ days after incubation) from the replicated pots of each treatment. The moist soil samples were analyzed to count the colony forming units (CFU) of total bacteria and fungi following serial dilution and pour plate technique [36] in asparagine-mannitol agar [37] and dextrose-rose bengal agar [38], respectively. Soils (sterile and non-sterile) were also analyzed for the presence of butachlor by extracting the soils following the procedure described by Debnath *et al.*, [39]. For residue analysis, soil samples (sterile and non-sterile) were also collected on "0" day (1 h) in addition to the periods stated above. Herbicide residues were analyzed by GLC [Hewlett Packard (USA), model 5890A] coupled with a $Ni^{63}$—electron capture detector, a glass column ($6^{/}$ × 2 mm) packed with 3% OV-101 on 80 - 100 mesh chromosorb-W (HP), and 3329A integrator. During analysis following gas chromatographic parameters were maintained: injection temperature, 250˚C; oven temperature, 200˚C; detector temperature, 250˚C; carrier gas, nitrogen having 65 mL/min flow rate. The residue data were processed to calculate the half-life ($T_{1/2}$) following the method of Hoskins [40].

### 2.5. Statistical Analysis

The results were evaluated by analysis of variance (ANOVA), and the statistical significance (P = 0.05) of difference between means within factors (herbicide, soil type and incubation time) was computed using Completely Randomized Design following the method of Gomez and Gomez [41].

## 3. Results and Discussion

### 3.1. Butachlor Dissipation

Sorption and degradation are two fundamental processes which govern predicting the environmental fate and behavior of organic contaminants in soil, which are affected by many factors like interaction with microorganisms, chemical and soil constituents [42]. A study on butachlor dissipation was carried out in soil in presence and absence of biological activity under laboratory condition. The dissipation and thus persistence of butachlor varied across soil types and prevailing conditions (sterilized and non-sterilized). After 60 days of incubation, 45.9% - 49.5% and 83.9% - 90.5% attenuation of butachlor residues were noticed in three soils under sterilized and non-sterilized condition, respectively (**Table 2**). When log residues in all cases were plotted against time, a linear relationship ($r^2$, 0.85 - 0.98) was observed suggesting a first order reaction in the dissipation behavior of the herbicide (**Figure 1**), which conforms earlier findings [43] [44]. The half-life ($T_{1/2}$) values were calculated by means of log 2/K, where K represents to the slope of the straight line regression. Under non-sterilized condition

**Table 2.** Dissipation pattern of butachlor in sterilized and non-sterilized soil.

| Days after application | Sterilized | | Non-sterilized | | % loss due to biotic forces |
|---|---|---|---|---|---|
| | Residues* (ppm) | % loss due to abiotic forces | Residues* (ppm) | % total loss | |
| | | | ***Laterite soil*** | | |
| 0 | 0.961 ± 0.009 | - | 0.965 ± 0.015 | - | 0 |
| 5 | 0.839 ± 0.006 | 12.64 | 0.719 ± 0.019 | 25.49 | 12.85 |
| 10 | 0.748 ± 0.008 | 22.16 | 0.576 ± 0.016 | 40.31 | 18.15 |
| 15 | 0.639 ± 0.009 | 33.51 | 0.411 ± 0.011 | 57.41 | 23.9 |
| 30 | 0.621 ± 0.004 | 35.38 | 0.292 ± 0.01 | 69.74 | 34.36 |
| 45 | 0.547 ± 0.007 | 43.08 | 0.208 ± 0.008 | 78.44 | 35.36 |
| 60 | 0.509 ± 0.009 | 47.03 | 0.118 ± 0.018 | 87.77 | 40.74 |
| Regression equation | Y = 1.91 − 0.004X | | Y = 1.89 − 0.014X | | |
| $r^2$ | 0.867 | | 0.975 | | |
| $RL_{50}$ (days) | 75.25 | | 21.50 | | |
| | | | ***Coastal soil*** | | |
| 0 | 0.953 ± 0.018 | - | 0.956 ± 0.021 | - | 0 |
| 5 | 0.84 ± 0.100 | 11.86 | 0.735 ± 0.100 | 23.12 | 11.26 |
| 10 | 0.746 ± 0.020 | 21.72 | 0.586 ± 0.010 | 38.7 | 16.98 |
| 15 | 0.648 ± 0.020 | 32 | 0.442 ± 0.020 | 53.76 | 21.76 |
| 30 | 0.584 ± 0.040 | 38.71 | 0.316 ± 0.016 | 66.94 | 28.23 |
| 45 | 0.563 ± 0.020 | 40.92 | 0.23 ± 0.030 | 75.94 | 35.02 |
| 60 | 0.516 ± 0.016 | 45.85 | 0.154 ± 0.020 | 83.89 | 38.04 |
| Regression equation | Y = 1.89 − 0.003X | | Y = 1.89 − 0.012X | | |
| $r^2$ | 0.850 | | 0.971 | | |
| $RL_{50}$ (days) | 100.33 | | 25.08 | | |
| | | | ***Alluvial soil*** | | |
| 0 | 0.98 ± 0.080 | - | 0.982 ± 0.080 | - | 0 |
| 5 | 0.844 ± 0.020 | 13.88 | 0.698 ± 0.012 | 28.92 | 15.04 |
| 10 | 0.736 ± 0.020 | 24.9 | 0.546 ± 0.020 | 44.39 | 19.49 |
| 15 | 0.626 ± 0.020 | 31.12 | 0.354 ± 0.020 | 63.95 | 32.83 |
| 30 | 0.591 ± 0.009 | 39.69 | 0.252 ± 0.022 | 74.33 | 34.64 |
| 45 | 0.535 ± 0.015 | 45.41 | 0.172 ± 0.012 | 82.48 | 37.07 |
| 60 | 0.493 ± 0.007 | 49.49 | 0.093 ± 0.007 | 90.52 | 41.03 |
| Regression equation | Y = 1.91 − 0.004X | | Y = 1.87 − 0.015X | | |
| $r^2$ | 0.851 | | 0.967 | | |
| $RL_{50}$ (days) | 75.25 | | 20.06 | | |

*Mean value of three replications.

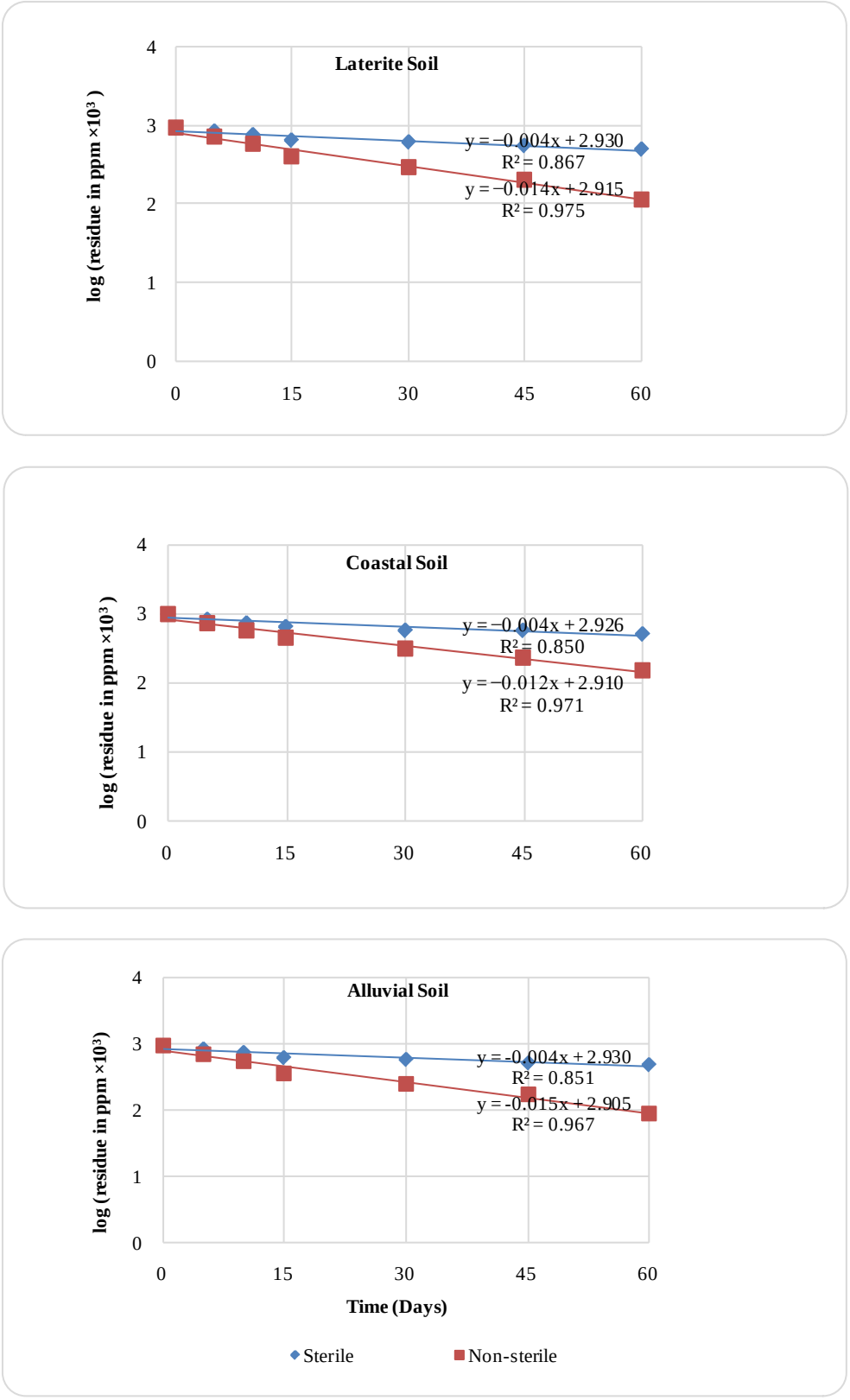



**Figure 1.** Linear plot for first order reaction of butachlor dissipation in sterile and non-sterile laterite, coastal and alluvial soils.

rate of attenuation was highest in alluvial soil ($T_{1/2}$, 20.1 d) followed by lateritic soil ($T_{1/2}$, 21.5 d) and coastal ($T_{1/2}$, 25.1 d) soils. However, under sterilized soil the trend was as follows: alluvial soil ($T_{1/2}$, 75.3 d) = lateritic soil ($T_{1/2}$, 75.3 d) < coastal soil ($T_{1/2}$, 100.3 d). The half-life found in the present investigation under non-sterilized condition is quite comparable with the half-life values found earlier ($T_{1/2}$, 16 - 19 d, [45]) under field study, using application rate similar to our present study (2 kg ai/ha). However, the observed minor difference might be due to the differences in experimental conditions in two studies. Opposite to our experiment they conducted the experiment in rice grown soil under field condition with and without organic manure treatment, which attributed to difference in physiological metabolic activities in soil. The biochemical activity is also encouraged by root exudates [46]. However, quality and quantity of root exudates depend on plant types and microorganisms also have a choice on root exudates utilization [47]. In a separate experiment half-life value of butachlor in the rhizosphere soil containing three different plants ranged from 5 - 10 d [28], which also reflects the same explanation. Faster degradation of butachlor in alluvial than that of coastal soil, observed in the present study, was also previously reported [48]. The low persistence of the chemical in alluvial soil was earlier demonstrated due to the most congenial environmental factors particularly pH [44], favoring microbial degradation process through enhanced activities of micro-organisms [49]. On the other hand, it was highly persistent in coastal soil due to higher salt content [50] adversely affecting growth and activities of micro-organisms as evidenced from the least microbial activity across soil type. Commendable dissipation was observed in sterile soil. This indicates the involvement of abiotic forces (sorption and chemical factors) other than photo degradation as the soils were kept under dark during the entire experimental period. In non-sterilized soil herbicide dissipation was higher than that in sterilized soil, confirming the possible microbial role in addition to abiotic factors. It is also interesting to note that irrespective of soil types and days of incubation there was a general trend of more dissipation of the chemical due to abiotic forces (49.5% loss after 60 d) than biotic forces (41.0% loss after 60 d) (**Table 2**). Sorption plays a vital and influencing role on organic contaminants fate and behavior in soil because the process exerts tremendous impact over the bioavailability of the chemical for degradation [51]. Adsorption of butachlor in soil is well established phenomenon and well documented [52]-[54].

### 3.2. Effect of Butachlor on Microbial Activity across Soil Types

Substantiating the results of Min *et al.*, [15] and Barman *et al.*, [55] butachlor effectuated significant detrimental influence on carbon di-oxide production, the virtue of microbial activity, from the beginning up to 10, 13 and 22 d of incubation in coastal, lateritic and alluvial soil, respectively (**Table 3**). The initial detrimental influence reflected the lag phase or adaptive phase of microbial community for synthesizing required enzymes [56] to degrade the herbicide with the formation of new strains through mutation [57] and then multiply their population to the required level not only for the detoxication [26] but also the utilization of either herbicide [58], the dead susceptible biotic agents [59] or both as energy and nutrient sources. Consequently, supporting the reports of Min *et al.*, ([15] there was a significant boost in microbial activity from 13$^{th}$, 16$^{th}$ and 28$^{th}$ day of incubation in coastal, lateritic and alluvial soil, respectively, persisting thereafter throughout. Among the three soil types, significant higher amount of carbon di-oxide was released from alluvial soil followed by lateritic and coastal soil, respectively. The lowest microbial activity in coastal soil was due to the hypertonic soil environment [60] while

**Table 3.** Effect of butachlor on the evolution of $CO_2$ (mg 100 $g^{-1}$ dry soil) across soil type (values are mean of 5 replications) Con = Control, Tre = Butachlor treated at 2 kg a.i. per ha.

| Soil type | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 10 | 13 | 16 | 19 | 22 | 25 | 28 | 35 | 42 | 49 | 56 | 63 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Laterite | Con | 8.06 | 16.02 | 23.70 | 36.13 | 47.53 | 58.54 | 69.32 | 80.91 | 93.58 | 106.25 | 119.81 | 133.90 | 148.36 | 163.19 | 178.86 | 195.49 | 213.45 | 232.68 | 254.13 |
| | Tre | 5.56 | 12.82 | 20.15 | 32.25 | 44.10 | 55.53 | 66.59 | 79.37 | 92.75 | 106.67 | 121.10 | 136.10 | 151.40 | 166.85 | 182.95 | 200.45 | 219.35 | 240.35 | 264.85 |
| Coastal | Con | 9.10 | 17.15 | 25.47 | 34.13 | 42.11 | 49.34 | 55.35 | 61.64 | 69.07 | 77.27 | 86.37 | 96.48 | 107.40 | 108.63 | 124.25 | 141.35 | 160.18 | 182.57 | 207.80 |
| | Tre | 6.06 | 13.22 | 21.42 | 31.08 | 40.04 | 47.82 | 53.92 | 61.27 | 69.40 | 78.40 | 88.90 | 100.15 | 112.03 | 124.78 | 142.35 | 163.00 | 185.05 | 211.51 | 241.26 |
| Alluvial | Con | 17.00 | 33.13 | 48.41 | 61.94 | 73.14 | 84.04 | 95.57 | 109.16 | 124.44 | 144.03 | 168.52 | 195.41 | 221.36 | 244.27 | 266.12 | 286.70 | 305.70 | 322.80 | 339.55 |
| | Tre | 13.06 | 25.64 | 36.88 | 47.17 | 57.12 | 69.02 | 81.34 | 96.37 | 105.15 | 137.69 | 165.32 | 195.17 | 221.24 | 244.76 | 267.88 | 290.21 | 309.31 | 327.26 | 344.09 |
| CD (P = 0.05) | | 0.003 | 0.009 | 0.005 | 0.003 | 0.003 | 0.001 | 0.003 | 0.001 | 0.005 | 0.009 | 0.017 | 0.014 | 0.015 | 0.016 | 0.018 | 0.014 | 0.019 | 0.049 | 0.036 |

the highest microbial activity in alluvial soil [61] was due to the most favorable environmental condition particularly the pH [44]. Consequently during a period of 63 d, the cumulative release of carbon di-oxide from alluvial soil was 33.6% higher than that of lateritic soil which, in turn was 22.3% higher than that of coastal soil.

### 3.3. Effect of Butachlor on Colonial Microbes across Soil Types

Butachlor brought about differential influence on colonial micro-organisms at different stages of incubation which, in turn, varied in different soil types. The herbicide caused an initial non-significant influence on bacterial population in each soil type—10 d for lateritic as well as alluvial soils and 45 d for coastal soil (**Figure 2**). For lateritic and alluvial soil the non-significant influence reflected stationary phase. Whereas, significant stimulation of bacteria by application of butachlor on the 15$^{th}$ day of incubation in spite of significant detrimental influence on microbial activity during initial 13 and 22 d of incubation in lateritic and alluvial soil, respectively, ensured that the bacterial flora derived their energy and nutrients from herbicide for their growth and multiplication [62]. Consequently the population of bacteria significantly increased in lateritic and alluvial soil by 22.7 and 12.4%, respectively over their respective control on the 15$^{th}$ day. The stimulation was more pronounced in lateritic soil. That was due to the additive effect of energy and nutrient from the susceptible dead cells as evidenced from significant enhancement in microbial activity on the 16$^{th}$ day of incubation. Then there was another stationery phase persisting up to 30$^{th}$ day and 60$^{th}$ day for lateritic and alluvial soil, respectively. Butachlor at the later stages in lateritic soil, however, induced significant detrimental influence on bacterial population manifesting the activation process through which a putative herbicide was converted to toxic molecules [26]. As a result, there was a progressive activation of butachlor resulting in significant gradual reduction in colonial bacteria from 16.3% on the 45$^{th}$ day to 30.0% on the 60$^{th}$ day of incubation as compared to that of untreated control in

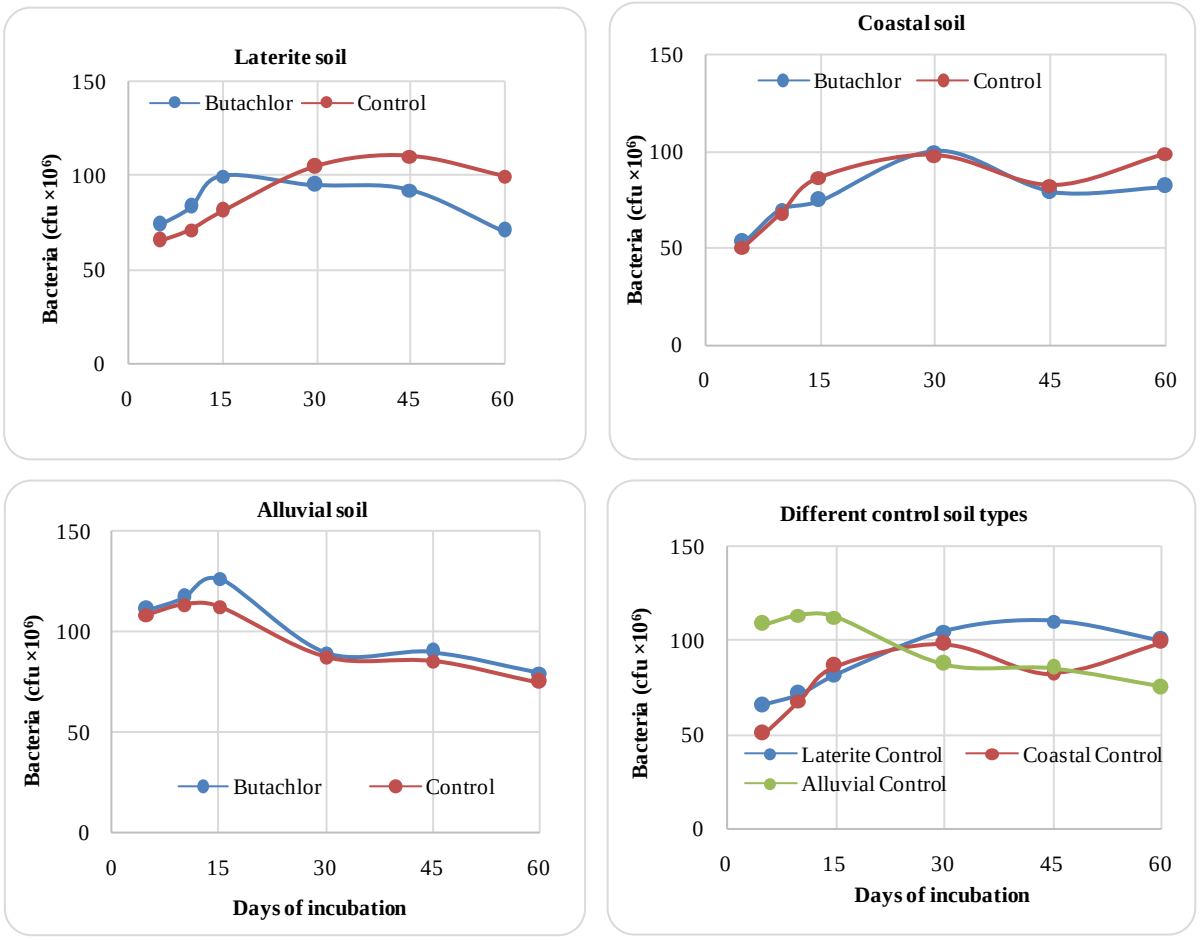
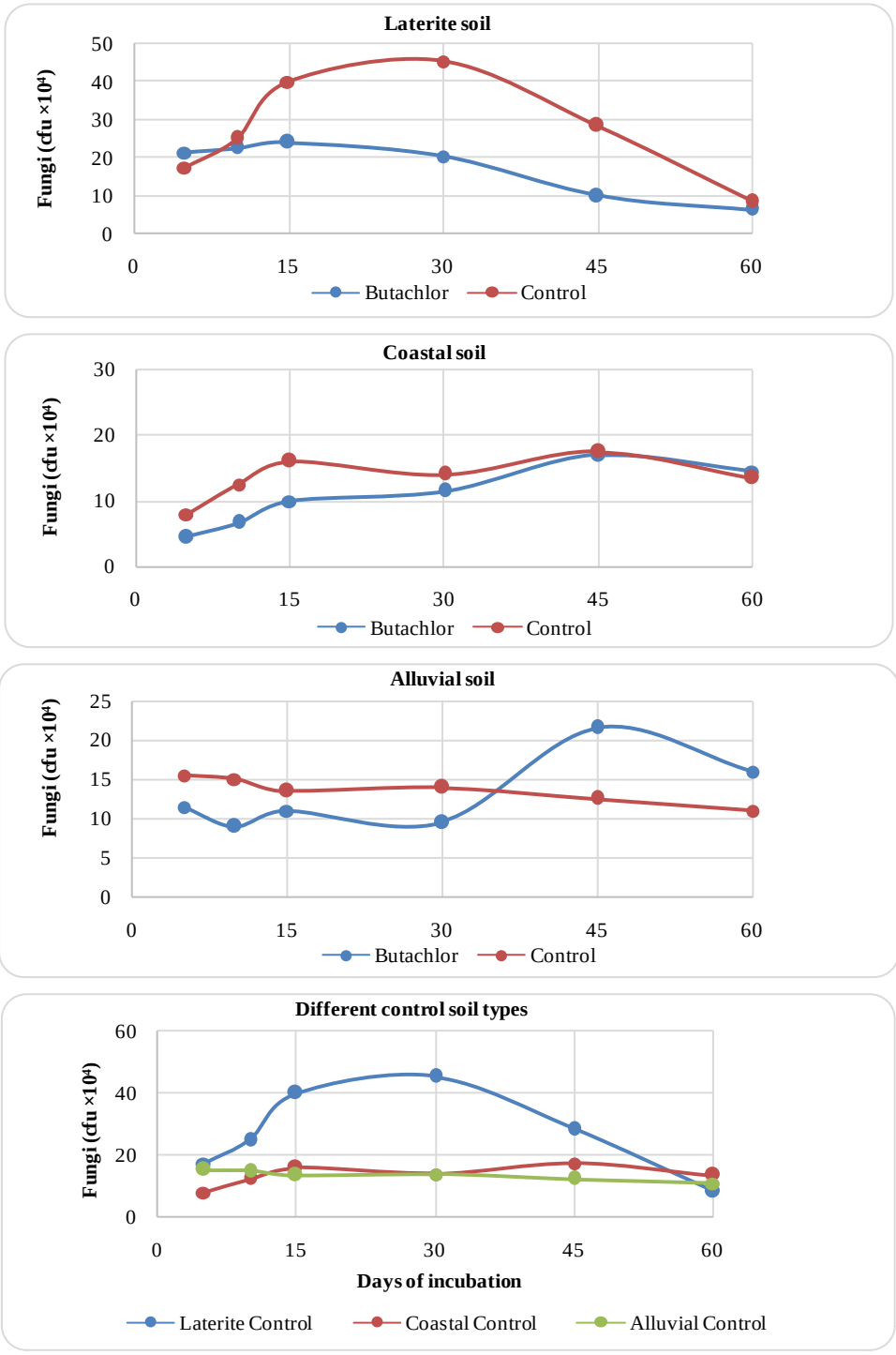



**Figure 2.** Effect of butachlor application on bacterial population in three soils at different periods of incubation.

lateritic soil. Butachlor also brought about significant reduction in colonial bacteria by 17.2% as compared to the untreated control in the coastal soil on the 60th day of incubation. Sustaining the reports of Min *et al*., [63] fungal propagules were significantly increased on the 5th day of incubation by the application of butachlor over that of untreated control in lateritic soil illustrating the utilization of the chemical as nutrient and energy sources by the chemo heterotrophs (**Figure 3**). Then there was manifestation of the stationery phase on the 10th day before the




Anova (P value at 0.05): Treatment (Tr) 1.25, Days (D) 1.20, Interaction (Tr x D) 3.81

**Figure 3.** Effect of butachlor application on fungal population in three soils at different periods of incubation.

influence of activated compounds. Thereafter, there was a significant progressive devastating impact of activated herbicide molecules resulting in sharp decline in fungal propagules from 40% on the 15th day to 64% on the 45th day of incubation. But on the 60th day there was again detoxification process as evidenced from the non-significant influence in the lateritic soil. In contrast, butachlor resulted in the stationary phase of fungal propagules on the 5th day in coastal soil before induction of activated compounds. Then activated compounds of butachlor resulted in significant reduction in fungal propagules from 46.4% on the 10th day to 37.5% on the 15th day revealing a significant gradual declining impact of activated compounds of butachlor and/or butachlor as such, which were detoxified thereafter from 30th to 60th day of incubation as evidenced from their non-significant influence. This conformed earlier finding [62]. Whereas, butachlor resulted in significant reduction in fungal propagules in alluvial soil as compared to untreated control on the 5th day of incubation. The impact was more severe as evidenced from the 40% reduction in fungal propagules on the 10th day. Following a mysterious non-significant influence on the 15th day the activated butachlor molecules again resulted in a significant fall in fungal propagules by 32.1% as compared to untreated control on the 30th day. Thereafter, butachlor resulted in a significant enhancement in fungal propagules during later stages of incubation in alluvial soil, commemorating the findings of Yang *et al.*, [28]. The extent of increment, however, gradually decreased from 75% on the 45th day to 45.5% on the 60th day as compared to untreated control in alluvial soil. So far as soil types are concerned, alluvial soil, owing to most favorable environmental conditions particularly the pH, was the best niche for the maximum proliferation of colonial bacteria followed by lateritic and coastal soils, respectively. On the other hand, lateritic soil due to favorable acidic pH harbored the highest fungal propagules followed by alluvial and coastal soil, respectively. Coastal soil reared the least colonial bacteria and fungi due to higher osmotic soil environment. The results substantiate the findings of [14]. Butachlor was subjected to co-metabolism by commensal microbes during initial 10 days of incubation and 19.5% residues were dissipated as evidenced from non-significant influence of herbicide on colonial microbes in alluvial soil (**Figure 2** & **Figure 3**, **Table 2**). Then the significant increment in colonial bacteria on the 15th day pointed out that 13.3% butachlor residues were utilized by zymogenous [64] bacteria as energy and nutrient sources besides commensal microorganisms from the 10th to 15th day of incubation. Again there was 1.8% of residual loss in butachlor through co-metabolism from the 15th to 30th days. But during later stages, 6.4% of butachlor residues were utilized by colonial fungi in alluvial soil along with co-metabolic micro-organisms. On the other hand, co-metabolism was the only phenomenon prevailing in coastal soil and 38.0% of butachlor residues were co-metabolized in coastal soil by commensal microbes (**Figure 2** & **Figure 3**, **Table 2**). However, along with co-metabolic microbes, colonial fungi were also the responsible agents during initial 5 d in lateritic soil and 12.9% of butachlor residues were utilized by them as nutrients and energy sources as evidenced from significant increment in fungal propagules. Then there was co-metabolism of 5.3% of butachlor residues from 5 to 10 d of incubation. But again from the 10th to 15th days of incubation about 5.7% of butachlor residues were metabolized by zymogenous bacteria together with co-metabolic ones as evidenced from their significant stimulation. Thereafter, from 30th to 60th day about 10.4% of butachlor was co-metabolized in soil.

## 4. Conclusion

Albeit considering the importance and significance of abiotic forces in dissipating butachlor in soil, it is understood that highest microbial activity and maximum enlargement of microbial community resulted in the least persistence of butachlor in alluvial soil. On the other hand, the herbicide induced highest persistence in coastal soil due to inimical influence on the growth and the activity of micro-organisms. So among the three soil types alluvial soil furnishes its suitability for butachlor application.

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