Effect of Pesticides on the Soil Microbial Activity

Saeeda Yousaf,1* Sardar Khan1 and Muhammad Tahseen Aslam2
1Department of Environmental Sciences, University of Peshawar, Peshawar 25120
2PCSIR National Physical Standard Laboratory, H-9, Islamabad

Abstract. -The purpose of this study was to evaluate the effect of commonly used pesticides such as Pendimethaline, Trifluralin, Glyphosphate, 2, 4-D, and MCPA (Chwastox) on microbial activities in soil. Two types of clean soils were amended with recommended level of pesticides and incubated in the laboratory at 35°C for 15 days. Microbial activities in the form of CO2 production were measured during incubation at 1, 2, 3, 4, 5, 7, 9, 11, and 15 days intervals. CO2 production was not affected substantially by any of the applied doses of pesticides. However, the total amount of CO2 produced during 15 days was suppressed by all pesticides, except MCPA (Chwastox). The effect of pesticides on microbial activities varied greatly with the type of pesticides used. The MCPA Chwastox did not exert any inhibitory effect on the respiratory rate of microbes, while other selected pesticides showed highly toxic effect on soil microbial activity.

Keywords: Pendimethaline, Trifluralin, Glyphosphate, 2, 4-D, MCPA (Chwastox), soil microbes

INTRODUCTION

Insecticides generally are the most hazardous to the environment, followed by fungicides and herbicides. This is a generalized statement, because certain herbicides are highly toxic and present a greater hazard to the environment than some insecticides. Thus, one has to be specific about which pesticide (including its dosages and methods of application) is being investigated in an ecological study. An estimated quantity of about 2.5 million tons of pesticides about US$ 16.3 billion is applied in world agriculture costing annually (Helsel, 1987). Despite the use of this huge amount of pesticides, besides several other controls methods, pests manage to destroy in the world 36% of all potential crops before harvest. There is now overwhelming evidence that some of these chemicals pose risk to humans and other life forms and unwanted side effects to the environment (Forget, 1993). No segment of the population is completely protected against exposure to pesticides and the potentially serious health effects, though a disproportionate burden are shouldered by the people of developing countries and by high risk groups in each country (WHO, 1990). The world-wide deaths and chronic diseases due to pesticide poisoning number about 1 million per year (EF, 1999).

The increased use of pesticides in agricultural soils causes the contamination of the soil with toxic chemicals (Muñoz-Leoz et al., 2013). When pesticides are applied, the possibilities exist that these chemicals may exert certain effects on non-target organisms, including soil microorganisms (Zhao et al., 2013). The microbes play an important role in the soil ecosystem (Khan et al., 2010), and their functions (Khan et al., 2007) are very crucial in nutrient cycling and decomposition (Lorenzo et al., 2001).

The study of pesticide effects on non-target populations is an accepted strategy to evaluate its associated potential environmental risks. Among non-target populations, soil microorganisms are extremely important, since they play an essential role in nutrient turnover (Aneja, 2004), maintaining generative capacity in agro-ecosystems (Bohlen, 2002). The processes of ecological succession are, among other factors, mediated by microorganisms and depend on a fine balance of their population dynamics (Kennedy, 1999). Under these circumstances, the impact inflicted on soil microbial populations caused by a specific pesticide is a potential indicator of the toxicity level of this product, and may represent a component of a broad study aiming to evaluate its potential impact on the environment (Kent, 2002).

Absorption of pesticides into the soil and their persistence varies with the composition, pH,
and temperature of the soil. Most organophosphate hydrocarbon can be degraded by plants, but organo-
chlorinated pesticides cannot, so they are taken up by them when present in soil (Gao et al., 2013; Gul 
and Khan, 2001). This study was aimed at evaluating the commonly used pesticides for their 
adverse effects on soil microbial activity.

**MATERIALS AND METHODS**

**Soil samples and preparation**

Laboratory incubation experiment was conducted to assess the effect of five different 
pesticides on microbial activity (respiration rate) in two different soils. Soil samples were collected 
from two sites (Pirsabak and KattiKhel) located nearCharsadda on Charsadda-Charsadda-Nowshahra Road.

Soil of Pirsabak site was silty clay loam, calcareous, non-saline up to more than 3ft deep and was 
classified as coarse loamy mixed hyperthemsic-typicustic. The sample was taken from the 
depth of 90 cm. Its pH was 9.3, had 0.81% organic matter and 0.47% organic carbon. Soil of KattiKhel 
site possesses the same characteristic as Pirsabak soil except that this soil was saline and was 
classified as coarse loamy mixed hyperthemic-cacrichaplaquepts. The soil samples were taken 
from the depth of 90 cm. Its pH was 9.9, had 1.26% of organic matter and 0.73% of organic carbon.

After collection, soil samples were broken down gently by hand. Stones and visible plant roots 
liters were removed and sieved (2 mm). Field moist soil samples were used in the incubation. Triplicate 
soil samples from each soil series were analyzed for key soil properties.

**Treatment of soil samples with pesticides**

Following pesticides, fungicides and insecticides were used in this study. Pendimethalin EC
active ingredients (a.i.) 33% W/V Trifluralin EC
a.i. 46.3% the herbicide Glyphosate 41EC i.v. 41% 
W/W, and insecticide. MCPA 30EC a.i. 44.25% of Chwastox; and 2, 4-Dichlorophenoxy Acetate (2, 4-
D) a.i. were 92%.

**Measurement of microbial activity in soil**

Microbial activities were measured in the form of CO$_2$ evolved during incubation by the 
method of Andreson et al. (1980). In this method, 50 g soil often samples as taken in a 500 ml conical 
flask. The samples were amended with appropriate pesticides in solution form and thoroughly mixed. 
Four (2 for Pirsabak and 2 for KattiKhel) flasks were kept amended with pesticides to act as control 
treatment. Each treatment was arranged in duplicate, 
thus a total of 26 flasks were arranged.

A 10 ml of 0.3M NaOH solutions was taken 
in a glass vial and suspended carefully in each flask 
with the help of a string. The flasks were sealed 
properly with rubber bungs to avoid any gaseous 
exchange between the flasks and outside 
atmosphere. A blank, in duplicate, was also run to 
account for the amount of CO$_2$ already present in the 
flask’s atmosphere. The flasks were placed in the 
incubator at 35°C and taken out at 1, 2, 3, 4, 5, 7, 9, 11 
and 15 days of incubation. At termination of an 
experiment the vial was carefully taken out of flask 
and the NaOH solution was transferred to clean 
250ml flask. For next incubation fresh NaOH 
solution was suspended in the same flask and returned 
to the incubator. The procedure was repeated at the end of each previous incubation period. After adding 10ml of 1M BaCl$_2$ solution and 
few drops of phenolphathalein, to the recovered 
NaOH solution, it was titrated against 0.1M HCl 
solutions until the pink color disappeared.

During the reaction one mole of CO$_2$ 
neutralizes two moles of NaOH. The amount of CO$_2$ 
produced was calibrated as g/g of moist soil/h.

**RESULTS AND DISCUSSION**

The effect of pesticides was measured on 
microbial activity in terms of CO$_2$ production in the 
pesticides amended and un-amended soil samples. 
The results obtained are presented below.

**Rate of CO$_2$ evolution**

Rate of CO$_2$ evolution in the pesticides 
amended and un-amended (control) soil samples 
was measured from day 1 to day 15 of incubation 
period (Table I). The results showed that no 
considerable differences in the rate of CO$_2$ evolution 
were observed between the pesticides amended and 
control soils on day 1 to day 15 of incubation period.
Table I.- Rate of CO₂ evolution (mg CO₂ kg⁻¹ soil hr⁻¹) in the pesticides amended and un-amended soil samples during different incubation periods.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 9</th>
<th>Day 11</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pirsabak Soil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1 (Control)</td>
<td>858</td>
<td>784</td>
<td>784</td>
<td>708</td>
<td>616</td>
<td>321</td>
<td>384</td>
<td>215</td>
<td>54</td>
</tr>
<tr>
<td>T2</td>
<td>858</td>
<td>773</td>
<td>756</td>
<td>642</td>
<td>641</td>
<td>328</td>
<td>211</td>
<td>113</td>
<td>47</td>
</tr>
<tr>
<td>T3</td>
<td>858</td>
<td>793</td>
<td>600</td>
<td>659</td>
<td>639</td>
<td>420</td>
<td>186</td>
<td>170</td>
<td>76</td>
</tr>
<tr>
<td>T4</td>
<td>858</td>
<td>825</td>
<td>702</td>
<td>681</td>
<td>642</td>
<td>349</td>
<td>222</td>
<td>207</td>
<td>158</td>
</tr>
<tr>
<td>T5</td>
<td>858</td>
<td>783</td>
<td>670</td>
<td>687</td>
<td>678</td>
<td>399</td>
<td>185</td>
<td>186</td>
<td>93</td>
</tr>
<tr>
<td>T6</td>
<td>858</td>
<td>609</td>
<td>601</td>
<td>669</td>
<td>658</td>
<td>427</td>
<td>215</td>
<td>143</td>
<td>115</td>
</tr>
<tr>
<td><strong>KattiKhel soil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T7</td>
<td>858</td>
<td>858</td>
<td>858</td>
<td>709</td>
<td>641</td>
<td>335</td>
<td>235</td>
<td>158</td>
<td>90</td>
</tr>
<tr>
<td>T8</td>
<td>858</td>
<td>858</td>
<td>858</td>
<td>713</td>
<td>682</td>
<td>181</td>
<td>191</td>
<td>148</td>
<td>102</td>
</tr>
<tr>
<td>T9</td>
<td>858</td>
<td>858</td>
<td>858</td>
<td>725</td>
<td>641</td>
<td>431</td>
<td>241</td>
<td>144</td>
<td>104</td>
</tr>
<tr>
<td>T10</td>
<td>858</td>
<td>858</td>
<td>858</td>
<td>743</td>
<td>698</td>
<td>327</td>
<td>256</td>
<td>129</td>
<td>97</td>
</tr>
<tr>
<td>T11</td>
<td>858</td>
<td>858</td>
<td>858</td>
<td>743</td>
<td>653</td>
<td>387</td>
<td>228</td>
<td>196</td>
<td>108</td>
</tr>
<tr>
<td>T12 (Control)</td>
<td>858</td>
<td>858</td>
<td>858</td>
<td>729</td>
<td>629</td>
<td>415</td>
<td>140</td>
<td>194</td>
<td>194</td>
</tr>
</tbody>
</table>

Pirsabak soil: T1, Soil only (control); T2, Soil+Trifluralin at 1000ml/ha; T3, Soil+Pendimethalin at 1000ml/ha; T4, Soil+MCPA (Chwastox) at 500ml/ha; T5, Soil+2,4-D at 750g/ha; T6, Soil+Glyphosate at 1900 ml/ha. KattiKhel soil: T7, Soil+Trifluralin at 1000ml/ha; T8, Soil+Pendimethalin at 1000ml/ha; T9, Soil+MCPA (Chwastox) at 500ml/ha; T10, Soil + 2,4-D at 750g/ha T11, Soil+Glyphosate at 1900 ml/ha; T12, Soil only (control).

On first day of incubation, there was no difference in CO₂ production between pesticide amended and un-amended soils and the amount of CO₂ produced was 858mg CO₂ kg⁻¹ soil hr⁻¹. Similarly, no difference was observed between soils of Pirsabak and KattiKhel series. On second day of incubation, the CO₂ production was slightly reduced from 609 to 825mg CO₂ kg⁻¹ soil hr⁻¹ in all treatments of Pirsabak soil compared to day 1 (Table I). In the KattiKhel series the CO₂ production remained the same on the second day of incubation in all pesticides amended and un-amended samples.

On third day of incubation, the overall production of CO₂ in Pirsabak soil was further reduced and ranged from 600 to 756mg CO₂ kg⁻¹ soil hr⁻¹ in the pesticide amended treatments compared to control (784mg CO₂ kg⁻¹ soil hr⁻¹). Like the first two days, on third day of incubation the KattiKhel soil treatments were not shown any change in the amount of CO₂ production (858mg CO₂ kg⁻¹ soil hr⁻¹) in both amended and un-amended soils. The pattern of CO₂ production in all the treatments on the fourth day of incubation was almost similar to that of the third day of incubation in the Pirsabak series. In case of KattiKhel soil, the CO₂ production in the pesticides amended treatments showed a decrease and ranged from 709 to 743mg CO₂ kg⁻¹ soil hr⁻¹ as compared 729mg CO₂ kg⁻¹ soil hr⁻¹ control soil. However, the CO₂ production was remained the same in both series of soils on the fifth day as observed on the fourth day of incubation. There were only minor differences in CO₂ production between similar treatments on both days of incubation.

On day 7 of incubation, the CO₂ production in Pirsabak soil was slightly increased and ranged from 328 to 427mg CO₂ kg⁻¹ soil hr⁻¹ in the pesticide amended soil as compared to 321 mg CO₂ kg⁻¹ soil hr⁻¹ in the unamended soil. Except for MCPA (Chwastox) amendment, the rate of CO₂ production was reduced from 181 to 431mg CO₂ kg⁻¹ soil hr⁻¹ compared to 415mg CO₂ kg⁻¹ soil hr⁻¹ in the control KattiKhel soil (Table I). On 9th and 11th day of incubation, the pattern of CO₂ production in all the treatments was similar to each other. The least reduction in the CO₂ production occurred in the pesticides amended soils of Pirsabak.

On 15th day of incubation, the overall production of CO₂ evolution in all the treatments was reduced. In the Pirsabak series, the rate of CO₂
Table II. Cumulative CO$_2$ production (mg CO$_2$ kg$^{-1}$ Soil) in the pesticides amended and un-amended soil samples during different incubation periods.

<table>
<thead>
<tr>
<th>Treatments*</th>
<th>Day 1 (mg CO$_2$ kg$^{-1}$)</th>
<th>Day 2 (mg CO$_2$ kg$^{-1}$)</th>
<th>Day 3 (mg CO$_2$ kg$^{-1}$)</th>
<th>Day 4 (mg CO$_2$ kg$^{-1}$)</th>
<th>Day 5 (mg CO$_2$ kg$^{-1}$)</th>
<th>Day 7 (mg CO$_2$ kg$^{-1}$)</th>
<th>Day 9 (mg CO$_2$ kg$^{-1}$)</th>
<th>Day 11 (mg CO$_2$ kg$^{-1}$)</th>
<th>Day 15 (mg CO$_2$ kg$^{-1}$)</th>
<th>Mean (mg CO$_2$ kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pirsabak Soil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1 (Control)</td>
<td>858</td>
<td>1642</td>
<td>2426</td>
<td>3134</td>
<td>3750</td>
<td>4071</td>
<td>4455</td>
<td>4070</td>
<td>4724</td>
<td>3237</td>
</tr>
<tr>
<td>T2</td>
<td>858</td>
<td>1631</td>
<td>2387</td>
<td>3029</td>
<td>3676</td>
<td>4004</td>
<td>4215</td>
<td>4328</td>
<td>4375</td>
<td>3167</td>
</tr>
<tr>
<td>T3</td>
<td>858</td>
<td>1651</td>
<td>2251</td>
<td>2910</td>
<td>3545</td>
<td>3969</td>
<td>4155</td>
<td>4325</td>
<td>4401</td>
<td>3118</td>
</tr>
<tr>
<td>T4</td>
<td>858</td>
<td>1683</td>
<td>2385</td>
<td>3066</td>
<td>3708</td>
<td>4057</td>
<td>4279</td>
<td>4486</td>
<td>4644</td>
<td>3241</td>
</tr>
<tr>
<td>T5</td>
<td>858</td>
<td>1641</td>
<td>2311</td>
<td>2998</td>
<td>3676</td>
<td>4015</td>
<td>4200</td>
<td>4386</td>
<td>4479</td>
<td>3174</td>
</tr>
<tr>
<td>T6</td>
<td>858</td>
<td>1467</td>
<td>2068</td>
<td>2737</td>
<td>3395</td>
<td>3622</td>
<td>4037</td>
<td>4180</td>
<td>4295</td>
<td>2962</td>
</tr>
<tr>
<td><strong>KattiKhel Soil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T7</td>
<td>858</td>
<td>1716</td>
<td>2547</td>
<td>3283</td>
<td>3924</td>
<td>4259</td>
<td>4494</td>
<td>4652</td>
<td>4742</td>
<td>3386</td>
</tr>
<tr>
<td>T8</td>
<td>858</td>
<td>1716</td>
<td>2547</td>
<td>3299</td>
<td>3969</td>
<td>4140</td>
<td>4331</td>
<td>4479</td>
<td>4581</td>
<td>3324</td>
</tr>
<tr>
<td>T9</td>
<td>858</td>
<td>1716</td>
<td>2547</td>
<td>3317</td>
<td>3940</td>
<td>4371</td>
<td>4612</td>
<td>4756</td>
<td>4860</td>
<td>3442</td>
</tr>
<tr>
<td>T10</td>
<td>858</td>
<td>1716</td>
<td>2547</td>
<td>3317</td>
<td>4015</td>
<td>4342</td>
<td>4598</td>
<td>4727</td>
<td>4824</td>
<td>3438</td>
</tr>
<tr>
<td>T11</td>
<td>858</td>
<td>1716</td>
<td>2547</td>
<td>3392</td>
<td>3970</td>
<td>4357</td>
<td>4595</td>
<td>4791</td>
<td>4899</td>
<td>3518</td>
</tr>
<tr>
<td>T12 (Control)</td>
<td>858</td>
<td>1716</td>
<td>2547</td>
<td>3303</td>
<td>3932</td>
<td>4347</td>
<td>4487</td>
<td>4681</td>
<td>4256</td>
<td>3347</td>
</tr>
</tbody>
</table>

*For details of treatment, see footnote of Table I.

Production ranged from 47 to 158 mg CO$_2$ kg$^{-1}$ soil hr$^{-1}$ in the pesticide amended soil, while CO$_2$ production was 54 mg CO$_2$ kg$^{-1}$ soil hr$^{-1}$ in control soil. The rate of CO$_2$ evolution in the KattiKhel series ranged from 97 to 108 mg CO$_2$ kg$^{-1}$ soil hr$^{-1}$ in the pesticides amended soil, which is slightly lower than that of control soil (194 mg CO$_2$ kg$^{-1}$ soil hr$^{-1}$).

The CO$_2$ production decreased in all treatments at the end of incubation compared to that of day 1. Based on these findings, no appreciable effect was observed on the rate of CO$_2$ production after application of selected pesticides.

### Cumulative CO$_2$ production

The effect of pesticides on cumulative CO$_2$ production was measured in two series of the soil samples during different incubation periods. Amount of CO$_2$ produced during 15 days of incubation varied greatly with the pesticides treatments. The amount of cumulative CO$_2$ in the MCPA (Chwastox) was 3241 mg kg$^{-1}$ of soil which was the highest amount of CO$_2$ produced in the Pirsabak series (Table II). The lowest CO$_2$ (2962 mg kg$^{-1}$) was produced after the Glyphosate treatment, followed by 3174 mg kg$^{-1}$ after 2, 4-D treatment, 3167 mg kg$^{-1}$ in Trifluralin treatment, 3118 mg in the Pendiimethaluln treatment. Similarly, the cumulative CO$_2$ production was 3442 mg kg$^{-1}$ in the MCPA (Chwastox) treated soil of the KattiKhel series, while the the lowest amount (3324 mg kg$^{-1}$) of CO$_2$ was produced in the Pendimethalin treatment, followed by Trifluralin (3386 mg kg$^{-1}$), 2,4-D (3438 mg kg$^{-1}$) and Glyphosate (3438 mg kg$^{-1}$) treatments. These results indicated that all the pesticides (except MCPA (Chwastox)) could be toxic to soil microbes in both soils (Pirsabak and KattiKhel). It is evident from literature that some pesticides were highly toxic to soil microorganisms and inhibit their biochemical activities, while others might be less toxic and could be easily degraded by microorganisms (Gao et al., 2013; Muñoz-Leoz et al., 2013).

### CONCLUSIONS

In this study we have evaluated five commonly used pesticides (Pendiimethalin, Trifluralin, MCPA (Chwastox), Glyphosate, and 2, 4-D) for their effects on microbial activities in agriculture soils from District Charsadda. It has been concluded that in both series of soils (Pirsabak and KattiKhel), all the pesticides used were highly toxic to soil microbes, as evidenced by the suppression of CO$_2$ produced.

### REFERENCES

Bohlen, P.J. Edwards, C.A. Zhang, Q.; Parmelee,


FORGET, G., 1993. Balancing the need for pesticides with the risk to human health. Impact of pesticide use on health in developing countries. IDRC, Ottawa.2.


(Received 6 August 2012, revised 25 July 2013)