

# Monitoring of Pesticide Residues in Lebanese Vegetables and Agricultural Soils and Their Impact on Soil Microbiological Properties

Mohamad H. Omeiri\*, Rony S. Khnayzer<sup>1</sup> and Hoda H. Yusef<sup>2</sup>

Department of Biological Sciences, Faculty of Science, Beirut Arab University, Debbieh, Lebanon

<sup>1</sup>Department of Natural Sciences, Lebanese American University, Chouran, Beirut 1102-2801

<sup>2</sup>Department of Botany and Microbiology, Faculty of Science, Alexandria University, Alexandria, Egypt

✉ Mohamad.omeiri@live.ca

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**Abstract:** The practice of pesticide application in agriculture done in Lebanon deviate from standard agricultural disciplines, raising awareness of their contamination of soil and vegetables as well as their repercussions on microbial communities. To this end, soil and vegetable samples from different pesticide-treated sites were quantified for chlorpyrifos (C) and methomyl (M) residues using Gas Chromatography-Mass Spectrometry. Moreover, clear soil specimens were treated with different concentrations of C, M, and CM assortment to assess their repercussion on the microbial population. High pesticide concentrations were recovered ranging between 31.13 and 53.74 mg/kg in eight agricultural fields, which abate gradually with time. After 20 days of pesticide implementation, their residues were above the maximum residual levels for lettuce, cabbage, tomato, and corn. Soil altered with pesticides showed fluctuation in its microbial community by suppressing some groups while favouring the propagation of tolerant species. Our data indicate the need for the requirement of strict laws to minimise pesticide usage and reduce their impact on soil quality, human health, and non-target organisms.

**Key words:** Chlorpyrifos, methomyl, agricultural soil, indigenous microflora, gas chromatography-mass spectrometry, colony-forming unit.

## Introduction

Anthropogenic activities have been on the rise causing the extensive release of contaminants into the environment. Pesticide contamination is among the factors implicated in environmental pollution worldwide despite their advantages in economic feasibility and high crop yield production. Concomitant to the inevitable use of pesticides, their residues yield significant risks to human health as well as wildlife due to their potential persistence in the food chain and the environment (Daisley et al., 2018).

One of the most important global forms of pesticides is organophosphates (Ops), which were introduced to agriculture in the 1960s during the Green Revolution. Among the OPs used is O, O-diethyl-O-3,5,6-trichloro-2-pyridyl commonly known as chlorpyrifos (C). Chlorpyrifos is partially metabolised in nature into 3,5,6-trichloro-2-pyridinol and chlorpyrifos oxon. It has been extensively used in agriculture for the protection and treatment of economically important plants against a broad spectrum of pests (Rathod & Garg, 2017). Chlorpyrifos typically works by inhibiting

\*Corresponding Author

brain monoacylglycerol lipase resulting in rodents' hypomobility. According to the WHO classification of pesticides, chlorpyrifos is classified as a moderately hazardous insecticide (class II). Upon unintentional exposure to high levels of C, poisoning may affect non-target species including humans leading to paralysis and coma, with thousands of deaths reported yearly (Rathod & Garg, 2017). Importantly, reports have revealed that this pesticide is persistent in the environment, with traces detected being in soils up to one year after its initial application (Chauhan et al., 2017).

Besides, carbamates are pesticides that contain OC=ON linkage. 5-Methyl-N-(methyl carbamyloxy) thioacetamide, commonly known as methomyl, belongs to the carbamate family that has been used as an insecticide and ovicide (Chen et al., 2015). The introduction of methomyl was in 1966 as a broad-spectrum insecticide. It triggers the inhibition of the essential enzyme acetylcholinesterase (Trachantong et al., 2017). Despite its efficacy in agricultural settings towards target organisms, the EPA (Environmental Protection Agency), WHO (World Health Organization), and ECC (European Chemical Classification) classify methomyl as a hazardous and very toxic insecticide to non-target organisms including humans (Kulkarni & Kaliwal, 2018).

Pesticides are used to safeguard plants from assorted pests. However, higher doses were used to overcome their resistance and adaptation (Parween et al., 2016). This eventually minimised the indigenous microflora that resides within the soil to enhance plant growth through nitrogen fixation, nutrient solubilisation, etc. (Gouda et al., 2018). Moreover, the pervasive use of these pesticides may cause toxicity to plants by affecting their growth and metabolism along with the persistence of pesticide residues in different plant parts (Sharma et al., 2016).

Chlorpyrifos and methomyl are widely used in Lebanon, aiming at protecting agricultural fields against potential pests as well as increasing the crop's yield. The land in Bekaa valley is considered very fertile in the region attracting large and various agricultural projects. In the current study, different Lebanese ecological sites were investigated for the potential contamination of pesticides in soil and plants, and the pesticide residues were quantified to project any resulting risk to health and the environment. Moreover, the impacts of these pesticides on the diversity of microorganisms in soil were examined. Sequentially, this will open the horizon for resistant microflora which can turn out to be the hot spots for pesticide degradation.

## Materials and Methods

### Chemicals and Reagents

Pure grade chlorpyrifos, methomyl, and other chemicals were acquired from Sigma-Aldrich (USA). Commercial pesticides were obtained from an insecticide supplier in Lebanon.

### Soil Samples

The soil samples used throughout the present study were collected from various locations within the Bekaa valley of Lebanon (Figure 1) by splitting the fields into sampling areas where each area was divided into systematic grids.

Four fields were chosen for chlorpyrifos and methomyl application. Each field was divided into 20 grids containing 20 cores (Figure 2). Soil samples (100 g) were taken using a clean soil auger from each core down to a depth of 6 inches. Upon mixing the soil samples, a composite sample was formed where 500 g of it was placed in the sample polyethylene bag provided. Subsurface soils were collected down to a depth of 24 inches. These samples were taken at different time intervals. The information on meteorology is listed in Table 1. Before analysis, all soil samples were stored at 4°C in a fridge. Pesticide extraction was performed as previously described by El-Saeid et al. (2010) and analysed using GC-MS.

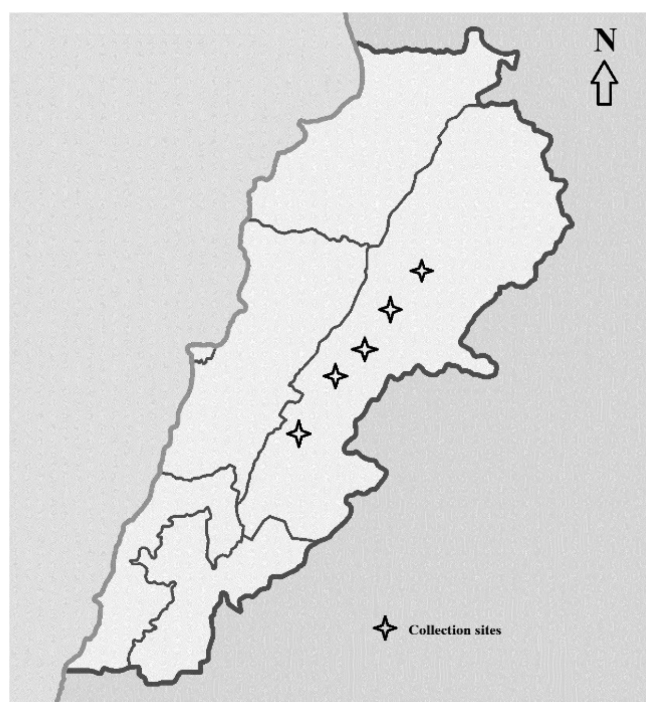
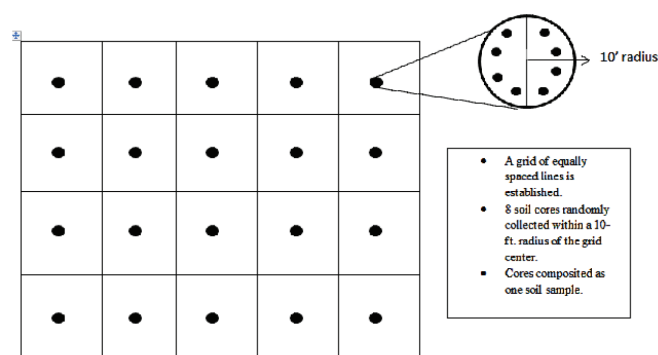


Figure 1: Location map of the study area.

**Table 1: The information on meteorology during the sampling days**

| <i>Days of Sampling</i>   | <i>Day 0</i>    | <i>Day 1</i>            | <i>Day 4</i>            | <i>Day 10</i>   | <i>Day 20</i>   | <i>Day 40</i>         | <i>Day 70</i>  |
|---------------------------|-----------------|-------------------------|-------------------------|-----------------|-----------------|-----------------------|----------------|
| <i>Data</i>               | <i>10 April</i> | <i>11 April</i>         | <i>14 April</i>         | <i>20 April</i> | <i>30 April</i> | <i>20 May</i>         | <i>20 June</i> |
| <i>Season</i>             | <i>Spring</i>   |                         |                         |                 |                 |                       | <i>Summer</i>  |
| <i>Weather</i>            | <i>Cloudy</i>   | <i>Scattered clouds</i> | <i>Scattered clouds</i> | <i>Sunny</i>    | <i>Sunny</i>    | <i>Passing clouds</i> | <i>Sunny</i>   |
| Temperature (°C) high/low | 25/19           | 21/18                   | 19/16                   | 30/26           | 25/23           | 26/23                 | 38/32          |
| Humidity (%)              | 49              | 35                      | 56                      | 16              | 29              | 32                    | 21             |
| Wind (Km/h)               | 4               | 15                      | 12                      | 5               | 6               | 9                     | 6              |



**Figure 2: Systematic grid-square sampling pattern.** Schematic representation of sampling area upon dividing it into square grids from which soil cores were chosen (Midwest Laboratories, 2016).

### GC-MS Method and Analysis

The quantification of pesticides was done using GC-MS. All residual extracts were analysed using HP-5MS GC coupled to a mass selective detector. The GC columns used for chlorpyrifos and methomyl were HP-5MS and DB-WAX, respectively. One microliter of the extract was injected into the injection port using an auto-sampler. Helium was the carrier gas used for both columns. The initial flow was 2.0 ml/min with an average velocity of 53 cm/sec. The analysis time for chlorpyrifos and methomyl was 19 and 30 minutes, respectively, and the MS was operated in the full-scan mode. Quantification of pesticide residues was based on standard calibration curves showing the peak area with respect to concentration (ppm). The results were corrected for the percent recovery of pesticides and then averaged for at least three replicate samples.

### Chlorpyrifos and Methomyl Concentrations in Treated Vegetables

Lettuce and cabbage samples were taken randomly from a chlorpyrifos-treated field located in the Bekaa valley. In addition, corn and tomato were sampled from verified methomyl-treated fields. The dynamics of sampling

were 1, 10, and 20 days after application. Pesticide extraction was performed according to Lu et al. (2014). All experiments were carried out in triplicates.

### Effect of Insecticides Chlorpyrifos and Methomyl on Soil Microflora

Soil samples were obtained from the top layer (0-10cm) of the targeted fields. The samples were clear of any pesticide residues for the past few years. They were air-dried and stored at 4°C in sieved bags before use. A total of 50 g soil samples were added to sterile petri dishes with 5 mL of chlorpyrifos (C) or methomyl (M) and a mix of (CM) with specific concentrations. In parallel, the plate labeled as negative control contained pure water and the vehicle control contained acetone. Plates were incubated at 20°C for different time intervals. Total soil microbial quantifications were estimated based on the soil dilution plate technique. Following incubation, colonies were counted and the total number of microbial populations was recorded. All experiments were carried out in triplicates.

## Results and Discussion

### Chromatographic Analysis

Chlorpyrifos and methomyl peaks appeared at retention time ( $t_R$ ) 11.05 and 11.28 min on HP-5MS and DB-WAX columns, respectively. The total analysis times were 19 and 30 minutes for chlorpyrifos and methomyl, respectively. Methods were optimised for optimum resolution which facilitated the quantification and identification of the insecticides.

### Method Validation

Quantification of pesticide residues was accomplished using standard curves for both chlorpyrifos and methomyl. Recovery assays of both pesticides were determined using three replicates. The average chlorpyrifos and methomyl recoveries in the soil were 99.3±26.1% and 99.8±7.6%, respectively. Chlorpyrifos

average recoveries for lettuce and cabbage were  $98.4 \pm 23.6\%$  and  $99.4 \pm 9.6\%$ , respectively. Moreover, the average methomyl recoveries for tomato and corn were  $90.2 \pm 9.4\%$  and  $93.4 \pm 5.2\%$ , respectively. The limit of detection (LOD) and the limit of quantification (LOQ) were determined from the chromatogram of the standard solutions and calculated using equations 1 and 2 (Barberis et al., 2019). For both pesticides, the LOD for the GC analysis was 1 ppm and the LOQ was 3 ppm. In all curves, the quantitative analysis of both pesticides was achieved using a linear function ( $R^2 \geq 0.98$ ). The peaks of both pesticides were well resolved with no interferences from the matrix. The data obtained from the recovery studies showed that the two pesticides in both soil and vegetables are near quantitative which is consistent with the findings of Lang et al. (2005) who showed that the extraction process of pesticides using the Soxhlet method exhibited 80.7%-96.1% recoveries with STD ranges 6.1%-15%.

$$\text{LOD} = 3 \times (\text{S/N}) \quad (1)$$

$$\text{LOQ} = 10 \times (\text{S/N}) \quad (2)$$

### Quantification of Pesticide Residues in Soil Samples

The identification of pesticides was performed according to the retention times obtained from the pure analytical standards and NIST library identification. According to the results illustrated in Figures 3 and 4, the concentrations of both chlorpyrifos and methomyl applied to the soil ranged from 33 to 47.1 mg/kg and 31.13 to 53.74 mg/kg, respectively. These pesticides were persistent in soil which was reflected by their leaching into lower layers in the ground. The sorption of chlorpyrifos by the top 24 inches of soil was assessed and was found to increase at lower levels and decrease at the upper soil levels (0-6 inches). Vis-a-vis the concentration of chlorpyrifos in soil, methomyl insecticide was lower in the soil's lower levels. The

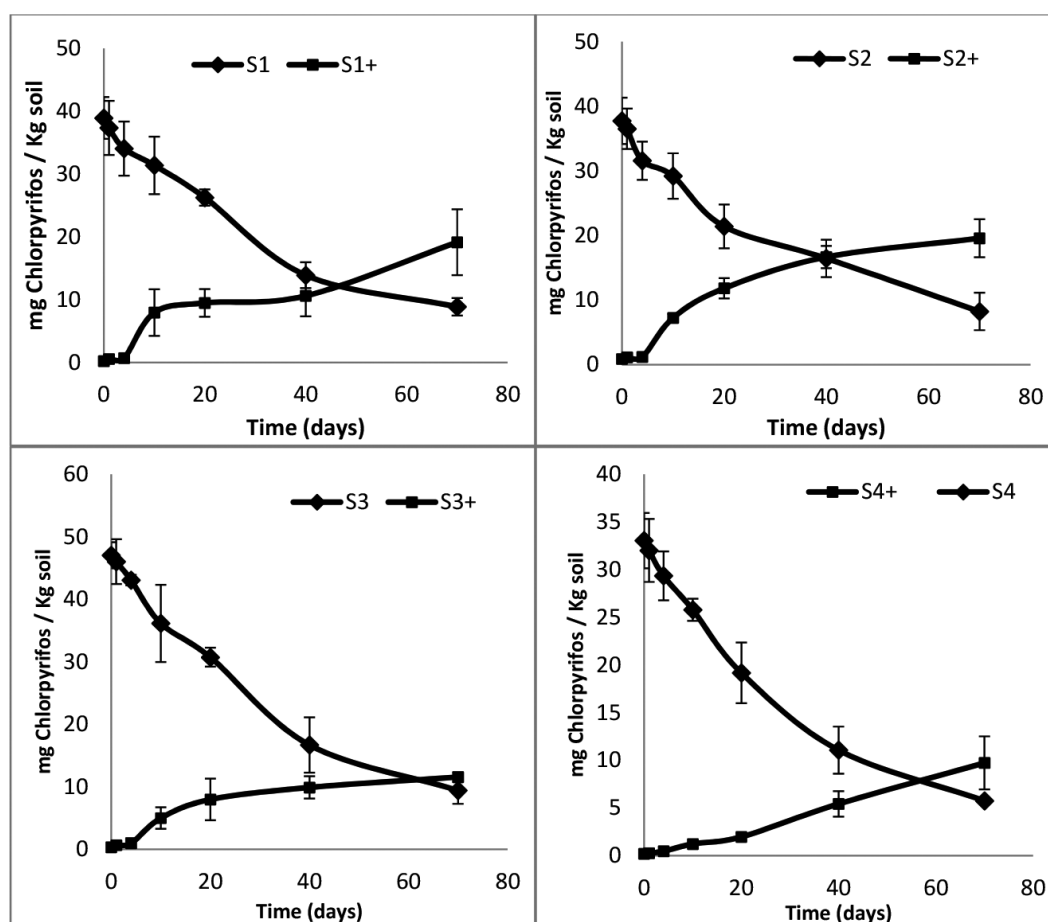
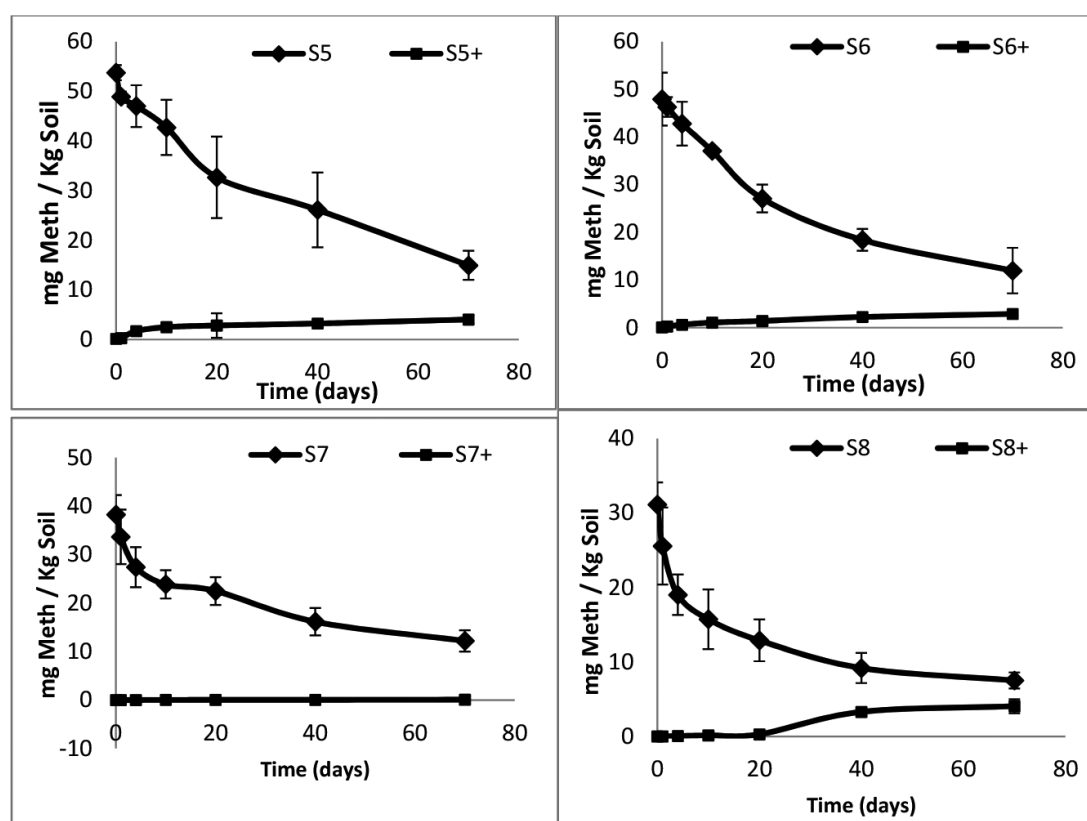


Figure 3: Concentration of Chlorpyrifos (mg/kg soil) in different sites of the Bekaa valley. (Samples from S1, S2, S3 and S4 were 0-6 inches deep. Samples from S1+, S2+, S3+ and S4+ were 6-24 inches deep). Vertical bars represent the standard deviation of the means of the three replicates.





**Figure 4: Concentration of Methomyl (mg/kg soil) in different sites of the Bekaa valley. (Samples from S5, S6, S7 and S8 are 0-6 inches deep. Samples from S5+, S6+, S7+ and S8+ are 6-24 inches deep). Vertical bars represent the standard deviation of the means of three replicates.**

dissipation of chlorpyrifos and methomyl in the agricultural sites within 70 days ranged from 77.2% to 82.6% and 68.1% to 75.8%, respectively. This agrees with Chapman and Harris (1980) who reported that 50% of chlorpyrifos remained in soil after 8 weeks of application and 9% remained after 1 year. Hwang et al. (2015) also reported that chlorpyrifos presence in the soil can exceed 90 days post-treatment. Many studies reported the contamination of different water resources in Lebanon with chlorpyrifos and methomyl (Aisha et al., 2017; Jabali et al., 2020). However, the rate of pesticide degradation in soil was previously found to be affected by pesticide nature, application rate, environmental conditions, etc. (Fantke & Juraske, 2013). Our results showed an increase in pesticide concentrations in the lower levels of the soil surface which might indicate the potential migration of these pesticides down the soil profile. The leaching of these pesticides under study may be due to the continuous and excessive watering of the plants within the soil which might facilitate its movement to subsoil levels.

### Chlorpyrifos and Methomyl Concentrations in Treated Vegetables

Trace amounts of insecticide residues may be found in crops after harvest that might originate from direct absorption of the pesticide by plant leaves and/or roots, which can move through the plant to other parts. The mean residue levels of chlorpyrifos and methomyl detected in different plants at different time intervals are presented in Tables 2 and 3. The initial chlorpyrifos residues in cabbage and lettuce after 1 hour from its application were  $5.9 \pm 0.3$  and  $4.9 \pm 0.2$  mg/kg, respectively. Such residues showed a 39-46% decrease after 20 days of application. Similarly, methomyl residues recorded  $4.9 \pm 0.4$  and  $4.67 \pm 0.15$  mg/kg in tomato and corn, respectively. These values decreased by 43-48% following 20 days of treatment. According to the Commission of the European Union (2016, 2018), the MRLs of chlorpyrifos and methomyl in lettuce, cabbage, tomato, and corn was 0.01 mg/kg, which was far below our values. Similar to our study, Elgueta et al. (2020) found that the chlorpyrifos and methomyl residues in lettuce and tomato in Chile were

**Table 2: Chlorpyrifos residues in cabbage and lettuce plants after treatment**

| <i>Days after treatment</i> | <i>Cabbage</i>             |                    |              | <i>Lettuce</i>             |                    |              |
|-----------------------------|----------------------------|--------------------|--------------|----------------------------|--------------------|--------------|
|                             | <i>Residue*±SD [mg/kg]</i> | <i>% persisted</i> | <i>% RSD</i> | <i>Residue*±SD [mg/kg]</i> | <i>% persisted</i> | <i>% RSD</i> |
| Initial                     | 5.93±0.25                  | 0                  | 4.2          | 4.90±0.17                  | 0                  | 3.5          |
| 10                          | 4.37±0.21                  | 73.7               | 4.8          | 3.87±0.21                  | 79                 | 5.4          |
| 20                          | 3.23±0.15                  | 54.5               | 4.7          | 3.0±0.10                   | 61.2               | 3.3          |
| MRL                         | 0.01 mg/kg                 |                    |              | 0.01 mg/kg                 |                    |              |

\*Average of three replicates, SD-standard deviation of three replicates, RSD-relative standard deviation, MRL-maximum residue limit.

**Table 3: Methomyl residues in tomato and corn plants after treatment**

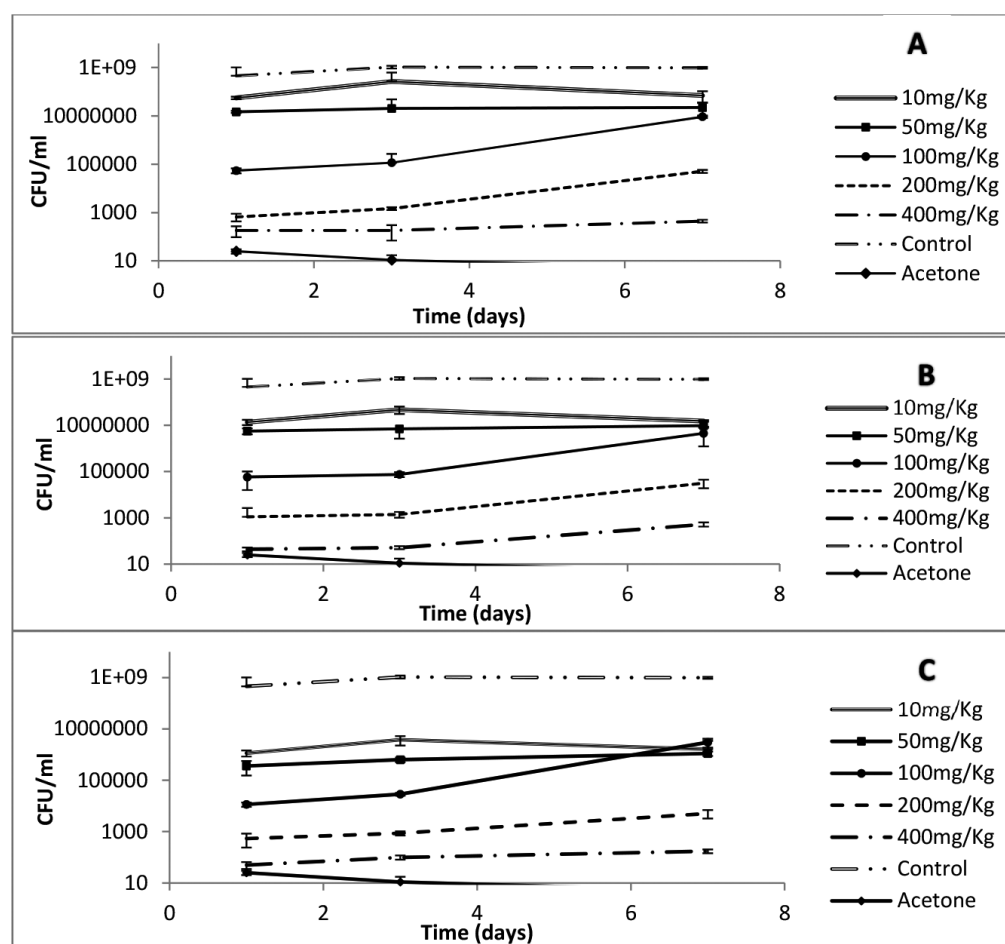
| <i>Days after treatment</i> | <i>Tomatoes</i>            |                    |             | <i>Corn</i>                |                    |             |
|-----------------------------|----------------------------|--------------------|-------------|----------------------------|--------------------|-------------|
|                             | <i>Residue*±SD [mg/kg]</i> | <i>% persisted</i> | <i>%RSD</i> | <i>Residue*±SD [mg/kg]</i> | <i>% persisted</i> | <i>%RSD</i> |
| Initial                     | 4.90±0.40                  | 0.0                | 8.2         | 4.67±0.15                  | 0.0                | 3.3         |
| 10                          | 3.77±0.25                  | 76.9               | 6.7         | 3.70±0.20                  | 79.3               | 5.4         |
| 20                          | 2.80±0.10                  | 57.1               | 3.6         | 2.43±0.06                  | 52.1               | 2.4         |
| MRL                         | 0.01 mg/kg                 |                    |             | 0.01 mg/kg                 |                    |             |

\*Average of three replicates, SD-standard deviation of three replicates, RSD-relative standard deviation, MRL-maximum residue limit.

above the MRLs. Islam et al. (2019) also reported the presence of chlorpyrifos in Dhaka cabbage samples with concentrations exceeding the MRLs. In Lebanon, Nasreddine et al. (2016) found that chlorpyrifos is frequently detected in 37.1% of semi-rural diets. Chlorpyrifos is typically absorbed from the roots of the plant which is then transported to its upper parts since it is a systemic insecticide (Hwang et al., 2018). In our study, although the number of insecticides in plants was high, there was a significant decrease in the residue levels from day 1 till day 20 as anticipated in comparison with the previous study (Katna et al., 2018). Besides, the variation in dissipation percentages of these pesticides between different plants may be due to the surface area and surface properties of the plant leaves (Jie et al., 2021). Moreover, pesticide residues present in the food samples will have a negative influence on normal health and physiological process. Fu et al. (2019) reported that long-term accumulation of low doses of chlorpyrifos (0.11 mg/kg) had synergistic effects inducing cell apoptosis and oxidative stress leading to tissue damage and subcellular lesions.

### Effect of Pesticides on Soil Microflora

Soil microbial flora plays an important role in nutrient and biogeochemical cycles which are depicted by the nitrification and denitrification processes (Patil et al., 2020). These, in turn, are advantageous to the physiological development of most plants and crops (Jha and Kumar, 2021). According to Araujo et al. (2003), insecticide application will result in its long-time persistence in soil which will negatively impact its soil microflora by either inhibiting or killing some specific groups of microorganisms. Besides, Meena et al. (2020) recorded that chlorpyrifos is considered the most destructive insecticide of the soil bacterial flora. Our results showed that microbial population decreased on the 1<sup>st</sup> day following pesticide application with the major drop occurring among those treated with 200 and 400 mg/kg (Figure 5). On the 7<sup>th</sup> day following application, a slight increase in the microbial count was noticed in soil samples with 10, 50, and 400 mg/kg concentrations of C, M, and CM in addition to the negative control. However, a significant increase in the soil microbial colony forming units was observed



**Figure 5: Bacterial colony forming units (CFU) quantified from soil treated with different pesticides at different concentrations and both negative (water) and vehicle (acetone) controls. (A) Chlorpyrifos (C) treatment; (B) methomyl (M) treatment; (C) chlorpyrifos+methomyl (CM) treatment. Vertical bars represent the standard errors of the means of three replicate.**

for concentrations 100 and 200 mg/kg of C, M, and CM. Johnsen et al. (2001) reported that such changes in microbial communities and their diversity may arise from the fact that such pesticides may be considered toxic to some microbial groups and on the other hand, favours propagation of some tolerant species. These tolerant species might be able to utilize these pesticides as an energy and nutrient source. In that perspective, the isolation of such microbial flora from pesticide-contaminated soil may be beneficial in the remediation of massive polluted agricultural sites.

### Conclusion

Our study focused on variable soil and plant samples collected from one of the largest agricultural fields in Lebanon. The irrational use of pesticides comes at high costs to the environment and human health. The data

obtained from this investigation lays the foundation for public health studies as well as risk assessments due to exposure to high levels of pesticides in Lebanon. Although our study is focused on specific pesticides used in a target agricultural area in Lebanon, our results likely indicate excessive use of pesticides by farmers which resulted in their presence and persistence in vegetables and soil in high amounts. Pesticide application should be frequently monitored by local authorities to enforce government regulation and control pesticide uses.

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