Effect of pesticides on soil microorganisms

Mehjin A M AL-Ani [1], Rawaa M Hmoshi[2] , Ibtiha A Kanaan[2] and Abdullah A Thanoon[1]

[1]Department of Environmental Technology, College of Environmental Sciences and Technology, Mosul University
[2]Department of Environmental Sciences, College of Environmental Sciences and Technology, Mosul University

Email : mehjin2002@yahoo.com

Abstract. In light of the rapidly growing human population, extensive pesticides have been utilized to maximize crop production. This has become a major environmental concern. To assess the influence of commonly used pesticides on soil microorganisms counts and microbial activities in the form of CO_2 production, a factorial experiment was conducted. Herbicide (Glyset I.P.A, Glyphosate 48%) and insecticides Miraj (Alphacypermethrin 10%) and Malathion (50% WP) were separately added to the soil at 0, 50, 100 and 200 ppm and incubated in the laboratory at 30 °C. The counts of bacteria, fungi, actinomycetes and CO_2 production were examined weekly for 7 consecutive weeks. The results demonstrated that the addition of the three mentioned pesticides significantly decreased the microbial activities and counts of soil bacteria, fungi and actinomycetes. The observed effect was depended upon the type and amount of pesticide as well as the length of incubation period. The microbial activities and the number of bacteria, fungi, and actinomycetes were inversely proportional to the concentration of pesticides added to the soil. In most treatments, soil samples treated with 200ppm of Malathion demonstrated the lowest microbial activities and counts of bacteria, fungi and actinomycetes. This study suggests that the investigated pesticides negatively affect microbial counts and activity in soil, which confirms and reinforces previously reported environmental concerns. .

Key words: soil , pesticides, herbicide, insecticide, bacteria, fungi, actinomycetes

1. Introduction

Due to rapidly growing human population, extensive pesticides have been utilized to maximize crop production. The extensive consumption of pesticides in cultivated soils leads to the pollution of the soil with harmful materials (Muñoz- Leoz *et al.*, 2013). About 3 million tons of pesticides that costs about US$ 40 billion is utilized in world agriculture annually (Pan UK, 2003). About 99.9% from the applied pesticide not reached to target organisms and become as pesticide residues accumulation which pollute the soil environment and just 0.1% reached to target organisms(Carriger *et al.,* 2006; Pimental, 1995). Both pesticides residues accumulation and microorganisms activity usually present in the same reign, soil top layer (Harris and Sans, 1969; Alexander, 1961) . The impact of different pesticides on the growth of soil microorganisms and its activity are difficult to expect. Even if the pesticides used in low concentration they effect chemical and biological properties, biochemical activity and soil microorganisms (Cycon *et al.,* 2006; Singh *et al.,* 2008; Cycon *et al.,* 2010).

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Pesticides in the soil impact the non target and useful microorganisms (Singh and Prasad,1991; Bhuyan *et al.,* 1992) and their activities (Schuster and Schroder, 1990). Beneficial soil microorganisms play essential role in soil fertility and productivity such as organic matter biodegradation, nutrients recycling, humus formation, Soil structural stability, nitrogen fixation, plant growth promotion, disease biocontrol, and other biochemical transformation such as ammonification, nitrification phosphorus solubilizing (Prasad Reddy *et al.,* 1984; Husain *et al.,* 2003). The effect of pesticides on soil microorganisms and their activity depend upon the type of pesticides used , quantities and soil conditions (Subhani *et al.,* 2000).The objective of this study is to assess the influence of three usually used pesticides on soil microorganisms counts and microbial activities in the in the form of CO2 production.

2. Material and Methods

2.1. Soil sampling and analysis

The soil samples were taken from Alrashedia area 5 km north of Mosul city. The soil was taken from surface area (0-20) cm. To remove debris, the soil was sieved with 2 mm sieve. The physical and chemical characteristics of the soil were determined as following. Soil texture by hydrometer method, Soil Reaction (pH) by glass electrode method (1:2.5 soil water suspension), soluble salts by Electrical Conductivity, Organic material by rapid titration method (Black *et al.,* 1965), available phosphorus by Olsen's method (Olsen *et al*., 1954) Ca and Mg by Graham method (Graham *et al*.,1962) , Potassium and sodium by flame photometer, (Jackson, 1973). Some of soil physical and chemical properties were recorded in Table 1. .

Table 1. Some soil physical and chemical properties

Characteristics	**pH**	**Ec (µS/cm).**	**Organic matter (%)**	**Available Phosphor us (mg\L)**	**Ca (meq/L)**	**Mg (meq/L)**
Results	7.8	99	1.08	26	98	15
Characteristics	K (meq /L)	SO_4 (meq/L)	Clay (%)	Silt (%)	Sand7 (%)	Soil Texture
Results	122	25.8	14.77	53.08	32.58	Silt Loam

2.2. Soil sample preparation and Experimental design

200 gm of sieved soil was placed in 250 ml flask. The treatments involved of three pesticides . Herbicide (Glyset I.P.A, Glyphosate 48%) and two insecticides Miraj (Alphacypermethrin 10%) and Malathion (50% WP). pesticides were applied at 0, 50, 100. 200 ppm levels. The pesticides pollution include twelve treatments in a completely randomized design replicated three times . All components were gently mixed with the soil. The moisture content of soil was got to 60% water holding capacity. Distilled water was added to maintain them 60% of WHC. For measuring CO_2 production, 10 ml of 2M sodium hydroxide solutions was placed in a glass tube and put the tube gently on soil surface in each flask. The flasks were closed well with rubber stoppers to avoid any gaseous exchange between the flasks and outside atmosphere. A blank, in three replicate, was also done to account for the quantity of CO_2 already present in the flask's atmosphere. The flasks were incubated at a temperature of 30C. Soil sampling was conducted weekly intervals for 7 weeks .

2.3. Measurement of microbial activity

activities of microorganisms were determinded in the form of carbon dioxide production according to Anderson *et al.,* 1982. the glass tube was gently got out of flask weekly and the sodium hydroxide solution was transmitted to clean flask. For following incubation fresh sodium hydroxide solution was put in clean glass tube and placed in the same flask and it is gave back to the incubator. The process was reiterate at the finish of each former incubation period. After addition 10ml of 1M barium chloride solution and drops of phenolphthalein, to the recovered sodium hydroxide solution and titrated against 1 M hydrochloric acid solution till the pink color is gone. During the reaction one mole of carbon dioxide equalize two moles of sodium hydroxide. The quantity of released CO_2 was adjusted as mg CO_2/100g soil

$$2NaOH + CO_2 \longrightarrow Na_2CO3$$
$$Na_2CO_3 + BaCl_2 \longrightarrow 2NaCl + BaCO_3$$

2.4. Microbial analysis

In assessing microbial population, standard plate count methods were used to prepare nutrient agar (NA) for assessment of the bacteria population , potato dextrose agar (PDA) for assessment of fungi, Starch casein nitrate (SCN) agar - for assessment of actinomycetes, . One gram each of the soil samples were measured into the test tube containing 9 ml sterile distilled water and serially diluted to dilution factor 10^5 and 1 ml of the proper dilutions was pipette into sterile plate with appropriate medium which were incubated at 30˚C. All plated were incubated inverted wise. Microbial counted were done at 48 hours for NA and 72 hours for PDA and 6 days for(SCN) (Stanley,2015; Adesina and Adelasoye, 2014)

2.5. Statistical analysis

ANOVA was carried out. The means were compared using Least Significant Difference LSD test at $p < 0.05$ after, ANOVA.

3. Results and Discussion

3.1. Effect of pesticides on bacteria count

Table 2 shows the addition of pesticides decreased the count of bacteria in all pesticides types and at all incubation periods. In the first week of incubation period, the addition of Glyset (Glyphosate 48%) 50ppm, 100ppm and 200ppm decreased the count of bacteria by 4%, 11% and 13% respectively. While at 7^{th} week of incubation, the reduction in bacteria count was 6%, 9% and 9% respectively. This depression was significant just at 100 ppm and 200 ppm. This results are consistent with (Newman *et al.,* 2016; Aralujo *et al.,*2003) who concluded that the presence of glyphosate decreased the number of bacteria, microbial biomass and acidobacteria population. They believed the reduction in the bacteria population for a long time could weaken some biogeochemical reactions accomplished by these microorganisms. (Grossbard and Atkinson, 1985) reported that the toxic effects of pesticides as result of inhibition of amino acid synthesis via the shikimic acid pathway. Conversely, other studies have demonstrated significant increase in bacteria count after glyphosate treatments (Partoazar *et al.,* 2011; Wardle and Parkinson,1990). .

Our results in table 2 also show that the addition of Miraj ((Alphacypermethrin 10%) insecticide decreased the population of bacteria. In the first week of incubation period, the addition of Miraj

50ppm, 100ppm and 200ppm decreased the count of bacteria by 18%, 24% and 32% respectively. While at 7th week of incubation, the reduction in bacteria count was 9%, 17% and 45% respectively. Similar results were observed by (Goswami *et al.,* 2013; Wesley *et al.,* 2017) who reported that the decrease in the soil microbial count and biomass can be associated with the toxic effect of Cypermethrin on soil microorganisms. The presence of Cypermethrin and thiamethoxam inhibited the metabolic process and significantly decreased ammonifying, nitrifying and denitrifying bacteria compared to the untreated sample (Nicoleta *et al.,* 2015).

The results in table 2 show that the presence of malathion insecticide decreased bacteria number. In the first week of incubation period, the addition of malathion 50ppm, 100ppm, and 200ppm decreased the number of bacteria 40%,42% and 59% respectively. While at 7th week of incubation period, the reduction was 32%, 38% and 41% respectively. These results agree with (Haleem *et al.,* 2013; Gonzalez- Lopez *et al.,*1993) who concluded the presence of malathion significantly decreased the population of bacteria.

Table 2. Effect of pesticides on Bactria count x 10^5

	Incubation periods / weeks						
Treatments	**1**	**2**	**3**	**4**	**5**	**6**	**7**
(Unamended Control)	73.22	70.2	66.11	65.22	62.44	59.65	57.44
Glyset 50 ppm	70.12	66.11	63.48	61.22	59.11	58.05	54.10
Glyset 100 ppm	65.18	58.45	59.58	55.14	55.33	54.25	52.11
Glyset 200 ppm	59.62	55.20	54.10	56.67	56.55	55.59	52.33
Miraj 50 ppm	60.11	55.12	50.22	55.34	53.55	55.11	52.22
Miraj 100 ppm	55.39	52.00	50.22	51.32	44.59	46.91	47.39
Miraj 200 ppm	50.11	48.29	46.11	47.15	40.48	45.49	31.40
Malathion 50 ppm	44.22	40.59	38.66	36.88	36.26	40.16	38.84
Malathion 100 ppm	42.44	40.38	39.36	35.65	34.22	36.40	35.64
Malathion 200 ppm	30.11	38.22	32.48	31.11	30.32	32.87	33.84
LSD	6.48	7.22	6.51	5.57	4.80	4.40	4.51

Table 3 shows that the presence of glyset herbicide decreased fungi count in all glyset concentration. However, the reduction in fungi count was just significant at 200ppm. The addition of glyset 200ppm, decreased the fungi count by 20% and 13% at first week of incubation period and 7th week respectively. Tanney and Hutchison, 2010 reported that the addition of glyphosate depressed the growth of 21 from 22 fungal species.

Out results in table 3 show that the presence of Miraj insecticide reduced the fungi count at all concentrations and period of incubations. The addition of Miraj at 50ppm, 100ppm and 200ppm, decreased the fungi count in the first week of incubation 60%, 61% and 63% respectively. While the reduction at 7th week was 48%, 50% and 62% respectively. Goswami *et al.,* 2013 concluded that cypermethrin application had toxic effects on soil microorganisms.

Table 3 shows the addition of malathion decreased the fungi counts at all concentrations and periods. During the first week of incubation, the addition of malathion at 50ppm,100ppm and 200ppm decreased fungal population 56 %, 62% and 66% respectively. While during the 7th week, the reduction was 58%, 64% and 65% respectively. Similarly, studies have shown that the presence of malathion insecticide decreased fungal population (Walia *et al.,* 2018; Smith, *et al.,* 2000) . Our results show that the most adverse effect was seen in soil treated with malathion specially at 200ppm.

Table 3. Effect of pesticides on fungi count x 10^3

	Incubation periods/ weeks						
Treatments	1	2	3	4	5	6	7
(Unamended Control)	23.11	22.32	23.68	20.11	18.19	19.68	18.88
Glyset 50 ppm	22.19	20.68	22.67	18.62	17.28	18.89	17.74
Glyset 100 ppm	21.11	19.66	22.22	17.89	16.00	18.49	17.37
Glyset 200 ppm	18.51	17.76	19.88	16.22	15.45	17.30	16.38
Miraj 50 ppm	9.20	10.33	9.42	9.38	11.12	11.34	9.88
Miraj 100 ppm	9.06	10.20	9.11	8.78	10.11	9.33	9.48
Miraj 200 ppm	8.66	7.22	6,88	7.11	7.38	7.22	7.11
Malathion 50 ppm	10.11	11.12	10.88	9.39	10.11	8.88	7.98
Malathion100ppm	8.88	7.48	7.66	8.11	7.22	7.48	6.88
Malathion200ppm	7.88	5.98	5.56	6.11	6.38	6.12	6.55
LSD	3.48	2.81	3.21	2.88	2.52	2.22	1.86

The effect of pesticides on actinomycetes population was shown in table 4. The presence of glyset herbicide inhibited actinomycetes population. During the first week of incubation, the addition of glyset 50ppm, 100ppm and 200ppm declined actinomycetes population by 5%, 7% and 22% respectively. However, during the 7th week, the reduction was 7%, 9% and 10% respectively. Our results show that the reduction of actinomycetes population as a result of glyset addition was significant just at 200ppm.

Table 4 shows actinomycetes population was decreased as a result of Miraj insecticide addition. During the first week of incubation, the depression in actinomycetes population was 54%, 56% and 60% as a result of addition of miraj insecticide 50ppm, 100ppm and 200ppm respectively. During the 7th week, the depression was 63%, 64% and 69% respectively.

Actionomycetes population was depressed in the soil treated with malathion(table 4). During the first week of incubation period, the addition of malathion 50ppm, 100ppm and 200ppm decreased actinomycetes population by 34%, 36% and 40% respectively. While during the 7th week, the reduction in actinomycetes population was 37%, 42% and 50% respectively. Our results show that the most adverse effect was seen in soil treated with malathion specially at 200ppm.These results are coordinated with several studies (Walia *et al.,* 2018; Haleem, *et al.,* 2013) who reported that the population of actinomycetes decreased by malathion treatment.

Table 4. Effect of pesticides on Actenomycetes count x 10^3

	Incubation periods/ weeks						
Treatments	1	2	3	4	5	6	7
(Unamended Control)	140.21	140.14	137.12	121.44	124.22	120.44	121.66
Glyset 50 ppm	133.42	133.00	130.15	112.53	117.8	114.32	113.55
Glyset 100 ppm	130.88	131.22	124.67	115.64	116.6	112.12	111.22
Glyset 200 ppm	109.22	107.22	123.30	100.43	105.55	109.2	109.52
Miraj 50 ppm	65.12	61.42	59.44	60.22	46.22	45.44	45.22
Miraj 100 ppm	61.46	58.22	57.22	55.11	45.44	43.22	44.12
Miraj 200 ppm	55.42	52.42	52.42	50.66	40.32	40.66	38.18
Malathion 50 ppm	92.22	88.68	93.44	85.56	80.22	78.22	77.22
Malathion 100 ppm	90.42	85.22	88.22	80.34	76.11	72.44	70.42
Malathion 200 ppm	83.44	75.11	79.14	72.44	69.12	66.48	60.44
LSD	12.22	15.22	17.12	14.22	11.22	11.52	10.82

3.2. Microbial activity

The pesticides treatment utilized in the current study caused adverse impact on the microbial activity in the form of CO_2 production (table 5). There were significant decreases in CO_2 production and these decreases significant in all pesticides types and concentrations used except Glyset 50 ppm, 100 ppm and miraj 50 ppm. The addition of 200ppm glyset decreased the CO_2 production during first and 7th week of incubation by 18% and 26% respectively. During the first week, the addition of Miraj insecticide 100ppm and 200ppm, decreased CO_2 production by 25%, 29% respectively. While the reduction during the 7^{th} week was 32% and 36% respectively. The most adverse effect was seen with soil treated with malathion insecticide. During the first week of incubation, the addition of malathion 50ppm, 100ppm and 200ppm depressed CO_2 production by 31%, 37% and 43% respectively. While the depression in CO_2 production during 7th week was 42%, 45% and 52% respectively. The same results were shown by Goswami *et al.,* 2013 who reported that the application of cypermethrin insecticide on soil at high concentration leads to poisonous impact on soil biomass, respiration and FDHA activity. Yousaf *et al.,* 2013 concluded that the pesticides were very poisonous to soil microbes, as showed by the decrease of CO2 produced.

Table 5. Effect of pesticides on CO2 evolution (mg CO2 100g soil) during different incubation periods

	Incubation periods/ weeks						
Treatments	1	2	3	4	5	6	7
(Unamended Control)	140	122	111	118	116	120	117
Glyset 50 ppm	131	115	106	119	110	114	109
Glyset 100 ppm	127	110	119	115	107	112	110
Glyset 200 ppm	115	88	85	89	91	92	87
Miraj 50 ppm	126	120	122	106	110	111	109
Miraj 100 ppm	105	102	96	98	88	84	80
Miraj 200 ppm	100	100	90	88	80	76	75
Malathion 50 ppm	96	88	80	76	72	70	68
Malathion 100 ppm	88	84	78	74	70	64	64
Malathion 200 ppm	80	66	60	64	62	60	56
LSD	15.2	17.1	13.5	14.4	13.2	11.2	10.4

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