Chlorpyrifos Biodegradation Study in Egyptian Agricultural Soil

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ABSTRACT

Ten samples from various Egyptian governorates were collected from previously chlorpyrifos (CP) treated agricultural soils, to examine their ability to CP degradation under lab conditions. Each sample was divided into two parts; one of them was sterilized to be used as a control (SS), and the other was left without sterilization (nSS) to reply study hypothesizes. All samples (SS and nSS) were treated with CP at 10 ppm. CP residues were examined by GC analysis after time intervals. Results showed that completely CP disappearance was occurred within 14-28 days in nSS treatments, while in SS treatments ranged between 5-7% of initial concentration during the same period. Chlorpyrifos degradation was characterized using half-life values (DT50) and degradation rate constant (K) values. To confirm these results, soil samples that had a high capacity for CP degradation were treated again by CP at 20 ppm. Results showed that the CP disappearance was faster compared to first nSS treatments where CP was completely disappeared within 7-10 days. Also, the SS treatments showed negligible pesticide degradation. This high degradation ability can be attributed to the presence of microorganism's that have the capacity to degrade the tested pesticide. Our data confirm that the repeated application of the same pesticide for several years on the same soil piece may be led to the adaptation of microorganisms to this pesticide. Further study will be required to isolate and characterize a CP degrader strains that can be used after that in remediation of CP polluted sites.

Keywords: degradation; chlorpyrifos; soil; Egypt; accelerated degradation.

INTRODUCTION

Chlorpyrifos is a broad-spectrum insecticide which is considered one of the most frequently used chlorinated organophosphorus pesticide (Maya *et al*., 2011). It represents the highest consumed pesticide in Egypt (about 1280 ton annually according to 2011 Agricultural Pesticides Committee statistics, Ministry of Agriculture & Land Reclamation) (Eissa *et al*., 2014). Pesticide use in agriculture has contributed to significant environmental pollution and poses a major ecological concern globally (Guliy *et al*., 2003). Egypt like many other developing countries relies heavily on pesticides to boost crop yields and meet the demands of a growing population (Ibrahim and Shehata, 2022). Chlorpyrifos is one of the most widely used organophosphate insecticides, exhibits broad-spectrum effectiveness against economically important crop pests (Whitney, 1967). However, its usage has been linked to adverse effects on both insects and mammals, including humans (Maya *et al*., 2011). In Egypt, chlorpyrifos is extensively utilized, making it the most consumed pesticide in the country (Chishti *et al*., 2013). Consequently, chlorpyrifos residues have been detected in various ecological systems, impacting biogeochemical cycles and posing threats to human health (Xue *et al*., 2005). Microbial degradation plays a crucial role in the breakdown and detoxification of pesticides in soil and water (Surekha *et al*., 2008). The persistence of chlorpyrifos in soil varies depending on factors such as soil type, microorganisms, and climatic conditions (Singh *et al*., 2002). Previous studies have reported varying half-lives for chlorpyrifos, with tropical soils showing faster degradation rates under certain conditions (Chai *et al*., 2013). The half-life of chlorpyrifos varies from 10 to 120 days in the soil environment . This large variation in half-life has been attributed to different environmental factors, the most important of which are soil type, soil pH, climate conditions, organic carbon content and pesticide formulation (Singh and Allan 2006). Repeated application of a pesticide at the same site can often influence the activities of the soil microorganisms responsible for its biodegradation, and the phenomenon of enhanced or accelerated biodegradation is a common occurrence (Racke and Coats, 1990). However, limited research has focused on the persistence and degradation of chlorpyrifos in agricultural soils in Egypt. Therefore, this study aimed to investigate the degradation and enhanced degradation of chlorpyrifos in different agricultural soils collected from various sites in Egypt.

MATERIALS AND METHODS

Chemicals: -

Chlorpyrifos (CP, O,O diethyl- O-3,5,6-trichloro-2- pyridyl phosphorothioate) emulsifiable concentrate (Dorspan 48%) and standard analytical grade solution were obtained from Central Agriculture pesticides laboratory, Agriculture Research Center, Giza, Egypt.

Soil collecting: -

The soil samples were collected from different areas of Egypt having a history of chlorpyrifos usage in pest control activities for last 10-20 years as illustrated in (Table 1). Soil samples were collected from 0-15 cm depth from cultivated areas in sterile plastic bags and stored at 4ºC. It was air dried and sieved (2-mm mesh). Soil was mixed with 2 volumes of distilled water and pH was determined. The texture of soil was determined using hydrometer method as described by (Bouyoucos 1962). Organic matter content (OMC) and water holding capacity (WHC) of soil were determined by methods describes by (Ali *et al*., 2011). The physico-chemical properties of the collecting soils were shown in (Table 2).

Study the degradation of chlorpyrifos in soils:-

To maintain suitable moisture content for microbial activity throughout the experiment, the soil moisture content (MC) and Water Holding Capacity (WHC) were calculated. Each jar designated for soil samples (6 jars per soil type) contained 200 grams of dry soil. Three replicates were sterilized (SS) as control, and the other three replicates were non-sterilized (nSS). Chlorpyrifos solution was prepared in water, and uniformly added at a concentration of 10 ppm to each soil sample, whether sterilized or non-sterilized. The mixture was stirred to ensure homogeneity. The jars were incubated in a dark place under laboratory conditions. Soil moisture content was monitored throughout the experiment to maintain it at 65% of the soil's water-holding capacity (WHC). Soil samples were collected at specific time intervals (0, 1, 7, 14, 21 and 28 days). Soil samples were removed and air-dried, subjected to pesticide extraction the residual CP concentration was determined using gas chromatographic analysis (GC). The obtained CP concentration values were used to calculate the half-life (DT50) and daily destruction constant (K) for chlorpyrifos degradation. After complete dissipation of chlorpyrifos in the soil samples, soils showed the highest efficiency in chlorpyrifos degradation were treated again with the pesticide, but at a concentration of 20 ppm. Pesticide concentration was extracted and estimated as mentioned before.

Extraction of chlorpyrifos from soil:-

Soil samples were removed at different time intervals (0, 1, 7, 14, 21 and 28 days) and air dried then chlorpyrifos was extracted from the sample according to **(Akbar *et al*., 2014).** Average recovery of CP from soil ranged from 85 to 90 %.

Determination of chlorpyrifos:-

Gas chromatographic analyses were performed using an Agilent 6890N gas chromatograph (Agilent Technologies, USA), equipped with an electron capture detector (ECD). A fused silica capillary column (DB-1701, 30 m × 0.32 mm × 0.25 µm) was employed. The oven temperature was initially 80°C, held for 0.5 min, and raised to 230°C at 10°C/min and held for 10 min. The injector and detector were set at 230 and 300°C, respectively. Nitrogen was used as a carrier gas at a constant flow rate of 50 Ml/min.

RESULTS AND DISCUSSION

After the first soil treatment with 10 ppm chlorpyrifos (first enrichment), non-sterilized soil collected from B1, Ne1, I, S and F governorates exhibited the highest capacity to degrade chlorpyrifos within 14 days, while non-sterilized soil collected from Ne2, Ne3, B2 and M governorates degrade chlorpyrifos after 21 days. Soil collected from western part of Nubaria (Nw) achieved disappearance of pesticide after 28 days. The faster disappearance of chlorpyrifos from nSS collected from B1, Ne1, I, S and F may be attributed to factors such as pH levels (ranging from 6.93 to 8) and higher organic matter content (ranging from 0.28 to 0.53) in these samples (Table 2). pH and organic matter are known to significantly impact microbial activity and soil's ability to degrade chlorpyrifos. In comparison, the sterilized soils (SS) collected from forementioned governorates exhibited a pesticide disappearance rate ranging from 5 to 7% during the forementioned periods (Fig. 1). The DT50 half-life values and daily degradation constant (K) were calculated for all soil types and presented in Table (3). These values varied among the different governorates and soil types and are consistent with those obtained previously (Amin *et al*., 2021).

Repeated application of chlorpyrifos (20 ppm) (second enrichment) to non-sterilized soil collected from B1, Ne1, I, S, F and M governorates demonstrates the rapid disappearance of the pesticide from non-sterilized soil within 7-10 days (Fig. 2). In contrast, the sterilized soil in the forementioned governorates showed negligible chlorpyrifos disappearance during the same period.

The DT50 values and daily degradation constant (K) for chlorpyrifos degradation during the second enrichment are presented in Table (4). These values are in consistent with those obtained previously (Fang *et al*., 2008) in which repeated pesticide treatments can enhance the soil ability to degrade the pesticide. The results highlight the increased capacity of the soil to degrade chlorpyrifos due to repeated applications, which can lead to a decrease in its effectiveness against target pests.

CONCLUSION

All the obtained results indicate, without any doubt, that the repeated application of the same pesticide for several years on the same soil piece may be led to the adaptation of microorganisms to this pesticide. Further study will be required to isolate and characterize a CP degrader strain that can be used after that in remediation of CP polluted sites.

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Table 1: Location of soil samples collecting sites with name of pesticides applied on crops.

S. No.	Soil Collection Site	Symbols	Previous Pesticides treatments	Duration and method of application
1	Beheira Governorate	B1	Chlorpyrifos, Malathion, Lambda-Cyhalothrin	Spray more than twenty years
2	Beheira Governorate	B2	Chlorpyrifos,Malathion, Lambda-Cyhalothrin Lambda-Cyhalothrin	Spray for ten years
3	Nobaria east city	Ne1	Chlorpyrifos,Lambda-Cyhalothrin, Fenamiphos Lambda-Cyhalothrin	Spray and injection more than twenty years
4	Nobaria east city	Ne2	Chlorpyrifos,Lambda-Cyhalothrin, Fenamiphos	Spray and injection more than ten years
5	Nobaria east city	Ne3	Chlorpyrifos,Lambda-Cyhalothrin, Fenamiphos	Spray and injection more than ten years
6	Ismailia Governorate	I	Chlorpyrifos,Lambda-Cyhalothrin, Azoxystrobin	Spray more than fifteen years
7	Nobaria west city	Nw	Chlorpyrifos,Lambda-Cyhalothrin, Imidacloprid	Spray for ten years
8	Sohag Governorate	S	Chlorpyrifos,Lambda-Cyhalothrin, Imidacloprid	Spray and injection more than fifteen years
9	El-Fayoum Governorate	F	Chlorpyrifos,Lambda-Cyhalothrin, Imidacloprid and Vydate	Spray and injection more than ten years
10	Matruh Gavernorate	M	Chlorpyrifos, Lambda-Cyhalothrin, Imidacloprid	Spray for ten years

Table 2: Physico-chemical characteristics of the soils used in the study.

Soil N.	Soil sample site	Sand	Silt	Clay	pH	Ec	%MC	%WHC	%OM
1	B1	20.77	26.08	53.15	7.30	1.85	8	45	0.53
2	B2	27.16	20.70	49.14	8.93	1.65	5	33	0.29
3	Ne1	65.66	14.19	20.66	7.13	1.70	6	18	0.50
4	Ne2	71.13	15.77	13.10	6.80	1.97	1	17	0.25
5	Ne3	63.16	21.16	15.68	6.54	1.37	7	19	0.26
6	I	14.17	67.33	53.36	8.00	1.51	4	27	0.30
7	Nw	63.19	24.64	12.17	6.00	1.61	2	22	0.17
8	S	10.13	37.68	52.19	7.50	1.16	4	31	0.44
9	F	28.16	44.19	27.65	6.93	1.75	4	28	0.35
10	M	75.16	7.02	20.82	7.94	2.43	1	19	0.28

Table 3: Kinetic studies of chlorpyrifos degradation in different soils treatment with initial CP concentration of 10mg/ kg soil.

Soil N.	Soil sample site	Treatment	DT_{50} (day)*	K (day^{-1})**	R^2
1	B1	SS	60.06	0.0041	0.9826
		nSS	3.13	0.3252	0.947
2	B2	SS	27.84	0.0249	0.9556
		nSS	5.03	0.2289	0.9062
3	Ne1	SS	28.17	0.0246	0.8699
		nSS	2.13	0.3970	0.9428
4	Ne2	SS	23.50	0.0295	0.8182
		nSS	6.03	0.2710	0.8722
5	Ne3	SS	22.58	0.0307	0.9205
		nSS	6.00	0.2630	0.8841
6	I	SS	24.32	0.0285	0.7461
		nSS	4.15	0.2111	0.9757
7	Nw	SS	49.87	0.0023	0.6667
		nSS	7.04	0.1018	0.963
8	S	SS	29.00	0.0239	0.6897
		nSS	3.99	0.2090	0.9413
9	F	SS	28.41	0.0244	0.9593
		nSS	4.21	0.1734	0.9785
10	M	SS	25.11	0.0276	0.6572
		nSS	5.13	0.2100	0.9494

(SS) sterilized soil; (nSS) non-sterilized soil. (*) Disappearance time calculated by T1/2 = ln2/k. (**)Linear equation between ln(Ct/C0) of chemical data and time yielded regression equation and regression coefficient (R2)

Table 4: (Second Enrichment) Kinetic parameters of Chlorpyrifos following repeated application in different soil treatments with initial CP concentration of 20mg/ kg soil:

Soil sample site	Treatment	DT50 (day)*	K (day-1)**	R^2
B1	SS	16.74	0.0414	0.9275
	nSS	2.37	0.5076	0.9686
Ne1	SS	16.05	0.0435	0.9745
	nSS	1.23	0.5619	0.9735
I	SS	34.8	0.0199	0.9634
	nSS	2.98	0.4101	0.8782
S	SS	17.60	0.0395	0.9494
	nSS	2.40	0.4939	0.9033
F	SS	20.10	0.0345	0.9702
	nSS	2.90	0.4008	0.9425
M	SS	14.70	0.0473	0.9070
	nSS	3.09	0.3905	0.9435

(SS) sterilized soil; (nSS) non-sterilized soil. (*) Disappearance time calculated by T1/2 = ln2/k (**) Linear equation between ln(Ct/C0) of chemical data and time yielded regression equation and regression coefficient (R^2).

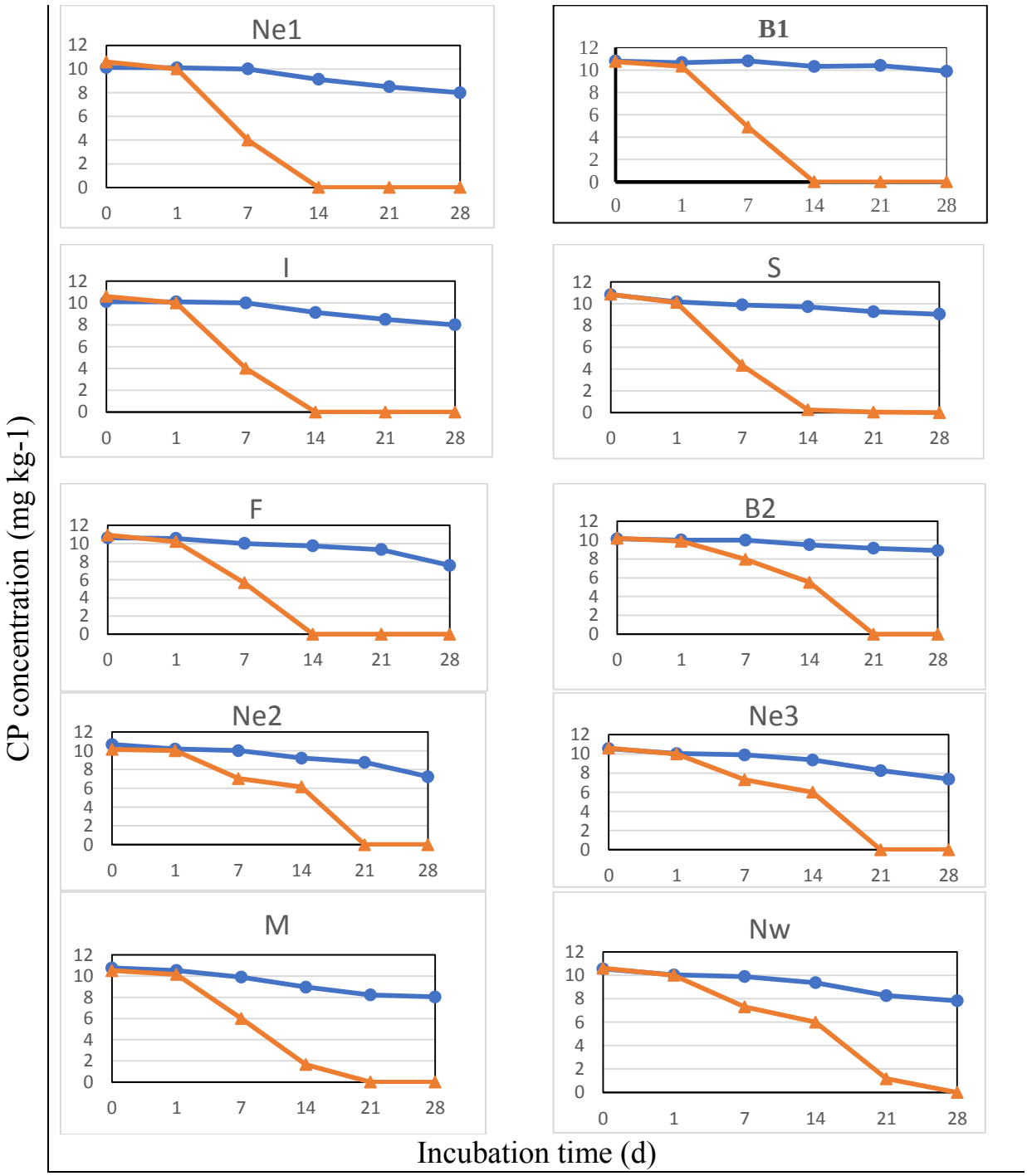

Figure 1 (First Enrichment): Degradation dynamics of chlorpyrifos in different soil treatments with initial CP concentration of 10mg kg^{-1} soil. Symbols: (●) (SS) sterilized soil, (▲) (nSS) non-sterilized soil. Ne1,Ne2, Ne3 (Nobaria district east), B1, B2 (Beheira), I (Ismailia), S (Sohag), F (Fayoum), M (Matroh) and Nw (Nobaria district weast)

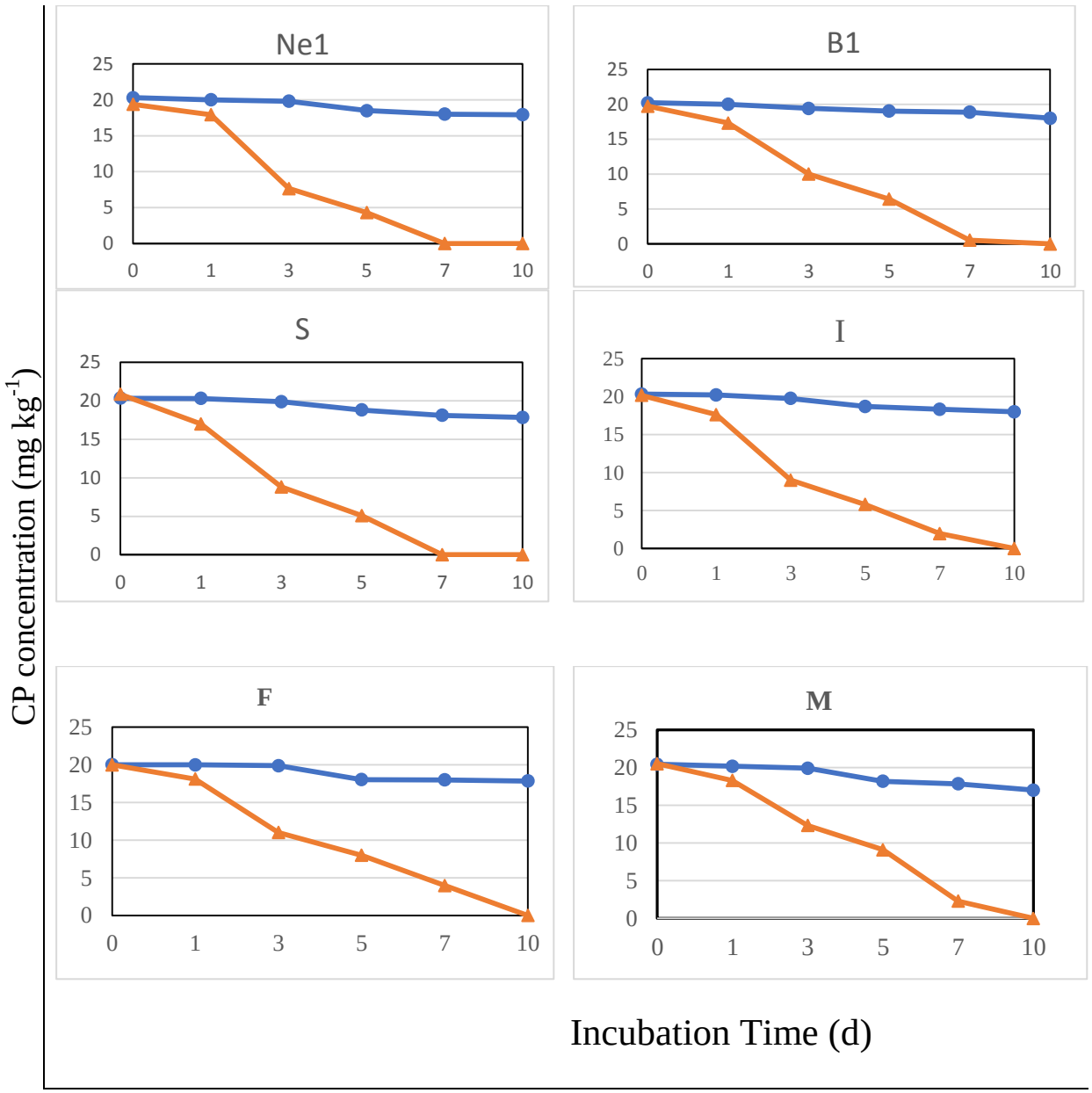

Figure 2 (Second Enrichment): Degradation dynamics of chlorpyrifos following repeated application in different soil treatments with initial CP concentration of 20mg/ kg soil. (●) (SS) sterilized soil, (▲) (nSS) non-sterilized soil. Ne1 (Nobaria district east), B1 (Beheira), I (Ismailia), S (Sohag), F (Fayoum) and M (Matroh)

دراسة التحطم الحيوي للكلوربيريفوس في التربة الزراعية المصرية

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الملخص العربي

تم جمع التربة المصرية التي سبق معالجتها بالكلوربيريفوس من الأراضي الزراعية في المحافظات المختلفة لفحص قدرت تلك التربة على تحلل المبيدات. تم تقسيم التربة التي تم تجميعها إلى قسمين: تربة معقمة (SS) (المجموعة الضابطة) ,تربة غير معقمة(nSS) . تم معاملة عينات التربة بتركيز 10جزء في المليون من الكلوربيريفوس ثم تم متابعة اختفاء المبيد بعد فترات زمنية محددة (0؛ 1؛ 7؛ 14؛ 21؛ 28يوم). أوضحت النتائج ان المبيد اختفى تماما من التربة الغير معقمة خلال فترة تراوحت من 14 الى 28 يوم؛ بينما اختفى المبيد من التربة المعقمة بنسبة من 5 الى 7% فقط خلال نفس الفترة. تم توصيف تحطم الكلوربيريفوس باستخدام حساب فترة نصف العمر (DT50) او ثابت التحطم اليومي (K). عينات التربة التي أظهرت كفاءة اعلى في تحطيم المبيد في نهاية فترة التجربة الاولى تم تكرارمعاملتها بتركيز اخر اعلى من الكلوربيريفوس (20 جزء في المليون). أوضحت النتائج انه في التربة الغير معقمة (nSS) كان اختفاء المبيد اسرع وذلك خلال من 7 الى 10 أيام في حين أظهرت التربة المعقمة قدرة ضعيفة جدا على تحطيم المبيد. يمكن أن تعزى هذه القدرة العالية على التحلل إلى وجود الكائنات الحية الدقيقة التي لديها القدرة على تحلل المبيد الذي تم اختباره. تؤكد بياناتنا أن الاستخدام المتكرر لنفس المبيد لعدة سنوات على نفس قطعة التربة قد يؤدي إلى تكيف الكائنات الحية الدقيقة مع هذا المبيد. ستكون هناك حاجة إلى مزيد من الدراسة لعزل وتمييز سلالات قادرة على استخدام الكلوربيريفوس كمصدر للكربون والتي يمكن استخدامها بعد ذلك في علاج المواقع الملوثة بالكلوربيريفوس.

الكلمات الاسترشادية: التحطم، الكلوربيريفوس، التربة، مصر، التدهور المتسارع.