

Assessment of Soil Dehydrogenase and Phosphatase Activities after Exposure to Certain Pesticides as Biomarkers for Pesticide Pollution

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ABSTRACT

Soil enzymes play a critical role in the biogeochemical cycles of essential nutrients and protect soil against the accumulation of harmful organic compounds. However, there is limited information on the effect of pesticides on enzyme activities in both old and newly reclaimed Egyptian soils. Laboratory studies were conducted to determine the effects of two nematicides (carbofuran and oxamyl) and two herbicides (ametryn and bentazone) on dehydrogenase and phosphatase activities in alluvial soil (clay soil) and calcareous soil (sandy soil) at concentrations of 25 and 100 µg/g soil over eight weeks. The results showed that dehydrogenase and phosphatase activities were generally enhanced in both clay and sandy soils treated with all tested pesticides. However, oxamyl had the lowest effect on dehydrogenase and phosphatase activities in both soil types compared to the other pesticides used in this study. Dehydrogenase and phosphatase activities in treated or untreated clay soil were higher than those in sandy soil. Additionally, the highest enhancement of dehydrogenase activity was observed at low concentrations of carbofuran and oxamyl, while a high concentration of ametryn and bentazone had a similar effect in both soil types. The highest stimulation of phosphatase activity was achieved at low pesticide concentration in clay soil, whereas high concentration was more effective in sandy soil. Based on the results obtained, it can be stated that the pesticide concentration, the soil type, and the incubation time are important factors affecting the pesticide-soil enzyme relationship. Accordingly, such a study can be used, which indicates the induction of such important enzymes in the soil, which can be used as an indicator to measure pesticide contamination in the soil within 60 days of exposure to pesticides.

Keywords: phosphatase, dehydrogenase, pesticide, soil enzymes, clay soil, sandy soil.

INTRODUCTION

Enzymes are proteins that catalyze specific biochemical reactions; they function to lower energy barriers and thus speed reactions which under normal conditions are extremely slow. Physico-chemical measurements indicate that enzyme catalyzed reactions

in soils have lower activation energies than non-enzyme catalyzed reactions and therefore have faster reaction rates. There are about 10,000 different enzymes in the known biochemical universe, but only several dozen are active in soils at any given time. Enzymes in soil are similar to enzymes in other systems in that their reaction rates are markedly dependent on pH, ionic strength, temperature, and the presence or absence of inhibitors (Van Elsas *et al.*, 2006; Gu *et al.*, 2019 and Kalyabina *et al.*, 2021).

Soil enzymes are produced from microorganisms, plants and animals. Microorganisms seem the main choice for supplying most soil enzyme activity because of their large biomass, high metabolic activity and the relatively larger amount of extracellular enzymes than plants or animals (Burns, 1982). Enzymes protect soil against the accumulation of harmful organic compounds by catalyzing degradation, polymerization, synthesis, coupling and incorporation into humic substances (Sumner, 2000b). Soil enzymes play a fundamental role in regulating the biogeochemical cycles of essential nutrients and the structure of the plant community and can therefore provide an early indication of change in soil functioning. This is caused by the fact that enzymes are involved in the processes that result in nutrient availability to plants, the synthesis of plant secondary compounds, and the turnover of soil organic matter (Wei *et al.*, 2024).

Pesticides are products that are widely used in agriculture to control harmful insects, weeds, plant pathogens, and other pests. Interactions between pesticides, soil organisms, and soil biochemical processes are affected by their physical-chemical properties, application methods and rates, as well as soil properties and environmental conditions (Tudi *et al.*, 2021). Among the herbicides currently used in agriculture in Egypt, the herbicides ametryn and bentazone stand out. Ametryn is a systemic type, selective against pre-emergent broadleaf and grass weeds used in the initial phase of cultivation of different crops. Its action starts with the initial phase of

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photosynthesis when chlorophyll formation is prevented (Sumekar *et al.*, 2023). Ametryn has specific characteristics for use in crops such as cotton, peanut, bean, and citrus crops. Given its different uses and risks, knowledge of its impact on soil activity becomes essential in assessing the impact of organic chemical compounds on the environment. Bentazone is commonly used all over the world to control grasses and broadleaf weeds in grain crops. Therefore, it has a chance to contaminate the soil causing negatively affect soil microorganisms, wildlife, terrestrial invertebrates, fish, birds, and mammals. Regarding nematicides, carbofuran is the most hazardous compound among carbamate pesticides to human health. Despite the prohibition of its application in developed countries, it is extensively used in developing and underdeveloped countries (Otieno *et al.*, 2010). Oxamyl is a member of a carbamate group, acutely hazardous. It is used to control pests of various crops and expand relevance in the fight against nematode communities predatory on roots. There is a lack of scientific data concerning soil microorganisms' response to a contaminated environment with this pesticide (Authority *et al.*, 2022).

Impact detection of pesticides on enzymes can provide an understanding of the changes in the functional capacity of the soil ecosystem and provide a method of assessing the risk of loss of sustainability in intensive agriculture and short-term economic benefits. It is believed that an understanding of their effects on enzymes is an important step to deciphering their consequences on soil quality and fertility. In most cases, agrochemicals used separately at their recommended field concentration induce slight changes in enzyme activity and key soil functioning (Sebai *et al.*, 2010). Pesticides have special effects on soil microorganisms that perform important biological activities in the soil, such as dehydrogenase and phosphatase enzyme production (Chia *et al.*, 2024). Assay of soil enzymes like amylase, cellulase, dehydrogenase, invertase, phosphatase, protease and urease indicate the importance of their role in complementing chemical and microbial analyses in the soil (Margesin *et al.*, 2000 and Sumner, 2000a). In order to analyze the toxicity of pesticides on soil microorganisms, dehydrogenase and phosphatase activities can be used as a measure of microbial population and their activities (Singh & Singh, 2005a and Nikolova *et al.*, 2023). Moreover, dehydrogenase and phosphatase activities are considered sensitive bioindicators of change occurring in soil ecosystems (Alkorta *et al.*, 2003). Despite this, there has been relatively little research on the impact of pesticides on enzymes compared to other areas of soil ecotoxicology (Margesin *et al.*, 2000).

Dehydrogenase is an intracellular enzyme that is released in relatively large amounts in comparison to

other enzymes due to damage of microbial cells and it has an exclusively microbial origin (Margesin *et al.*, 2000). Dehydrogenases catalyze oxidative activities in a cascade of events involving specific carriers, electrons are transferred from substrate to oxygen as the final acceptor (Gianfreda *et al.*, 2002). Dehydrogenase activity is a measure of the intensity of microbial metabolism and thus of microbial activity in soil (Gu *et al.*, 2019). Soil dehydrogenase activity could be affected or not-affected by pesticides (Trasar-Cepeda *et al.*, 2000). Dehydrogenase activity was increased with the treatment of tebupirimphos, permethrin, monocrotophos, cypermethrin, and fenvalerate (Madhuri & Rangaswamy, 2002 and Tu, 2008). While it was negatively affected by chlorothalonil (Singh *et al.*, 2002). The activity of this enzyme was not affected by chlorpyrifos, diazinon, fensulfothion, malathion, parathion, phorate, and terbufos (Tu, 1980). Phosphatases are extracellular enzymes that catalyze the hydrolysis of esters and anhydrides of phosphoric acid (Nannipieri *et al.*, 2011). They are produced by over 75% of soil microflora. Soil phosphatases play a major role in the mineralization of organic phosphorous substrates which is principally a microbial phenomenon (Nannipieri *et al.*, 2011). Phosphatase activity was stimulated with monocrotophos, cypermethrin, fenvalerate, dimethoate, and malathion (Hasan, 1999 and Subba Reddy *et al.*, 2011). While parathion, triazophos, permethrin and fonofos reduced the activity of phosphatase (Tu, 1980).

Consequently, it is very important to establish the influence of pesticides on the soil dehydrogenase and soil phosphatase activities. Therefore, our study aimed to evaluate the side-effect of herbicides ametryn and bentazone, and nematicides carbofuran and oxamyl on the activity of soil dehydrogenase and phosphatase, which are deemed as biological indicators of the changes in the soil microbiological composition. This work presents the results of the investigation of soil enzymes - dehydrogenase and phosphatase - in clay and sandy loam soil after treatment with the pesticides at the rates of 25 mg/kg (approximately equivalent to the recommended rate) and 100 mg/kg (as about 4 folds of the recommended rate).

MATERIAL AND METHODS

1. Chemical and reagents

Technical grade of carbofuran (2,2-dimethyl-3H-1-benzofuran-7-yl) N-methylcarbamate) 95% a.i was obtained from FMC Corporation and Bayer. Oxamyl (N,N-dimethyl-2-methylcarbamoyloxyimino-2-(methylthio)acetamide) technical 95% a.i was obtained from DuPont, Agchem Access. Ametryn (N2-ethyl-N4-isopropyl-6-methylthio-1,3,5-triazine-2,4-diamine) technical 97% a.i. was obtained from Syngenta Crop

Protection. Bentazone (3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide) 98% was obtained from BASF Chemicals, Sharda Cropchem Limited. Chemical structure of tested pesticides is presented in Figure (1). Triphenyltetrazolium chloride (TTC), triphenylformazan (TPF), p-nitrophenyl sodium phosphate (PNSP) and p-nitrophenol (PNP) were obtained from Sigma Aldrich Co. (Spruce Street, Louis., MO, USA).

2. Apparatus and Instrumentation

A UV-Vis Spectrophotometer (Thermo Corporation, Nicolet, evolution 100, Germany), an Ultra Microplate Reader (Robonik, PVT. LTD), an Orbital shaker (Bibby Steril, LTD, UK), a Centrifuge (Model 90-1 UK), a water bath (LSB 0155, Korea), a Water Distillatory (DESA 0035, Eu), an Incubator (JSSI100T, Korea), a pH meter (Milwaukee, MARTN, Italy), and a Digital balance (ViBRA AJ-320E, 0.01-150 g, JAPAN).

3. Tested soil

The soil used in this investigation was gathered from the experimental farm of the faculty of Agricultural, Elmenofyia University, Egypt. The soil was taken from the 0-15 cm soil profile, air dried, and then ground to pass through a 2 mm sieve for removal of particles and non-decomposed plant residues. Throughout the investigations, the processed soil samples were kept in plastic bags at room temperature. A representative subsample was selected for the physicochemical examination. Table (1) shows the results for soil particle size distribution, total organic carbon, pH, and cation exchange capacity.

4. Soil Treatment

Soil samples was treated with two different concentrations (25 and 100 mg/kg) of the tested pesticides. The low concentration is equivalent to the recommended rate and the high concentration is equivalent to folds of the recommended rate. The soil sample (500 g on a dry weight basis) was placed in 2-L jar and mixed thoroughly with 100 and 75 ml of deionized water respectively for clay and sandy soils, the soil water content was 60% of water holding capacity. Samples of the test soil were kept together in the dark at a constant temperature $25\pm 2^{\circ}\text{C}$ in the incubator for one week before use. A known amount of solution containing ametryn, bentazone, carbofuran, and oxamyl individually was pipetted and thoroughly mixed with the preincubated soil samples, inside the glass bottle, before adding the water treatments. Both soil samples clay and sandy were spiked with 100 mg/kg and 25 mg/kg standard solution prepared in acetone to achieve the described concentrations. Acetone was allowed to evaporate for 1 hour and then the soil samples were mixed manually with a stainless-steel spatula. Bottles were then loosely covered by aluminum foil and kept a constant temperature set in the incubator. All samples were opened every 2 days to maintain aeration and moisture adjustment by replenishing the water amount lost, if any, after weighing the preincubated and incubated bottles. Upon experiment initiation, samples were collected at zero, 3, 7, 15, 30, 45, and 60 days after spiking with the tested pesticide. Three replicate samples were collected for the determination of enzyme activity in soil.

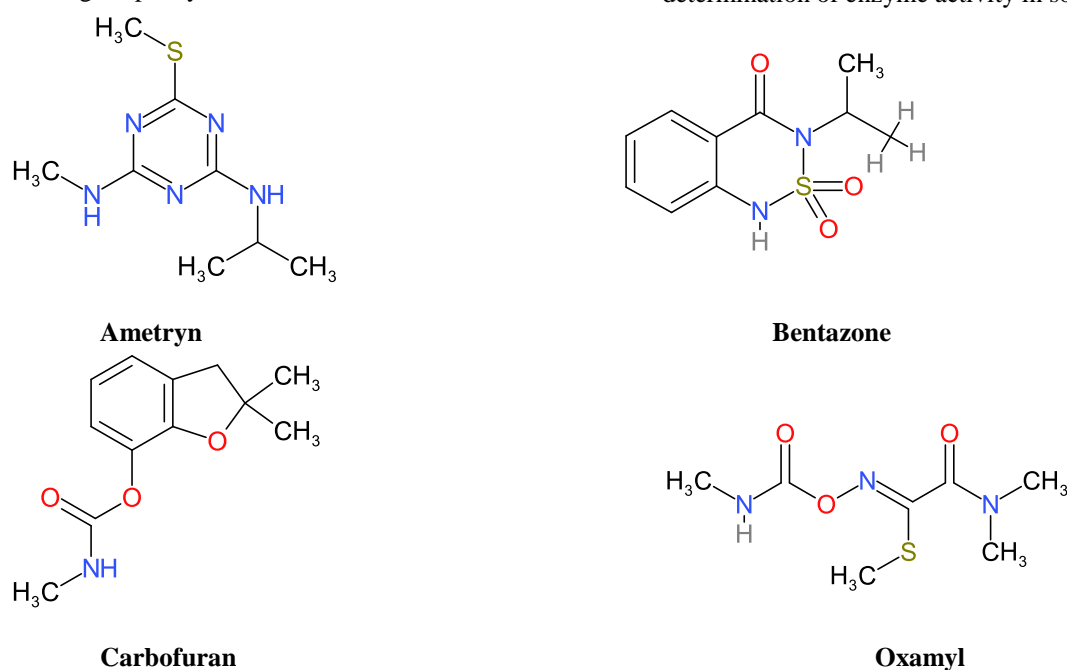
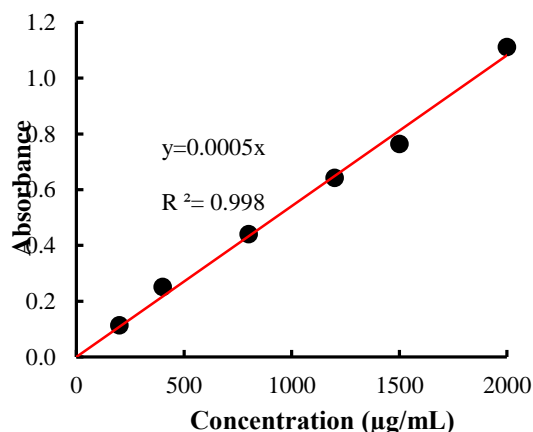


Figure 1. Chemical structure of tested pesticide

5. Determination of dehydrogenase activity

The dehydrogenase activity in the soil was measured using a colorimetric method. This involved reducing 2,3,5-triphenyltetrazolium chloride (TTC, colorless) to triphenylformazan (TPF, red color), which was then extracted using methanol and measured at 490 nm using a spectrophotometer (Thalman, 1968). For each sampling time, five grams of the treated soil sample were placed into a 10 mL capacity test tube, followed by the addition of 1 mL of 1% aqueous solution of TTC and 2 mL of distilled water. The tubes were then tightly covered with parafilm paper to create an anaerobic condition and incubated in the dark at 37°C for 24 hours. After the incubation, the TPF produced was extracted using four mL of methanol for each tube. The contents were shaken vigorously, stirred for one minute, and filtered through Whatman filter paper No. 1. This extraction was repeated three times, and the extracts were combined. The absorbance of TPF in the filtrate was determined at 490 nm using a spectrophotometer. A blank sample without the TTC solution was also prepared, and its methanol extract was used as a reference blank. The dehydrogenase activity was expressed based on the dry weight of soil in micrograms of TPF per gram of soil per 24 hours. A standard calibration curve for TPF was created in the range of 200-2000 mg/L, and the K value was computed to be 0.0005 (Figure 2).



6. Determination of soil phosphatase activity

The procedure involved placing a 1-gram soil sample (less than 2 mm) in a 50-ml Erlenmeyer flask. Then, 4 ml of modified universal buffer (MUB), 0.25 ml of toluene, and 1 ml of p-nitrophenyl disodium phosphate solution (made in the same buffer) were added and mixed by swirling for a few seconds (Tabatabai and Bremner, 1969). The flask was closed with a stopper and incubated at 37°C for 1 hour. After incubation, 1 mL of 0.5M CaCl₂ solution and 4 mL of 0.5M NaOH solution were added to the flask, which was then swirled and the soil suspension filtered through Whatman 42 filter paper. The intensity of the yellow color of the filtrate was measured using a spectrophotometer at a wavelength of 420 nm. For the control sample, the same procedure was followed, but 1 mL of the substrate solution was added after the addition of 1 mL 0.5M CaCl₂ and 4 mL of 0.5M NaOH solutions, immediately before filtration of the soil suspension. The p-nitrophenol content of the filtrate was calculated using a calibration graph (Figure 2) that plots standards containing 0, 10, 20, 30, 40, and 50 µg of p-nitrophenol. To prepare a standard curve, 1 mL of the standard p-nitrophenol solution was taken in a 100 mL volumetric flask and the volume was adjusted to 5 mL by the addition of distilled water. Then, 1 mL of 0.5M CaCl₂ solution and 4 mL of 0.5M NaOH solution were added to each flask and the resultant suspensions were filtered. The p-nitrophenol content of the filtrates was measured using a spectrophotometer at a wavelength of 420 nm.

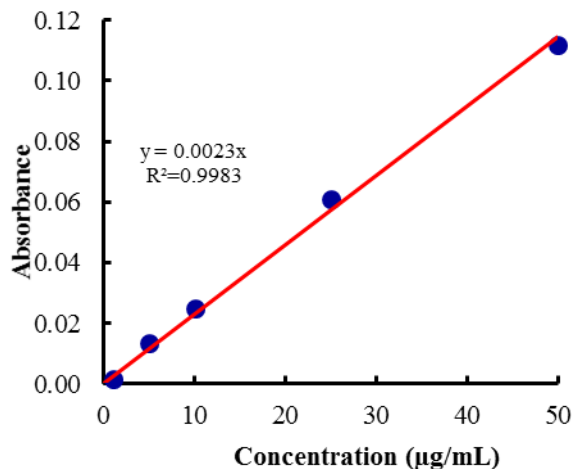


Figure 2. Standard curve of TPF (Left) and PNP (Right)

7. Statistical Analysis

The experimental data is shown as mean \pm standard error and statistical analysis was conducted using Minitab software. One-way analysis of variance (ANOVA) was used to analyze the dissipation and enzymatic activity data, and mean property values were separated ($p \leq 0.05$) using the Student Newman-Keuls (SNK) test.

RESULTS AND DISCUSSION

Effect of pesticides on dehydrogenase activity in soil

The side-effect of the tested pesticides was carried out depending on the effect on soil dehydrogenase activity which can be considered as an indicator of the biological activity and microbial population. The results of dehydrogenase activity in clay and sandy soil treated with carbofuran, oxamyl, ametryn and bentazone at two concentrations 25 and 100 $\mu\text{g/g}$ soil under the same conditions are shown in Table (1) and Figures (1-4).

Figure (3) showed that the dehydrogenase activity in clay soil treated with carbofuran increased at 3rd day followed by decreasing of about 25% and 50% with low and high tested concentrations, respectively. Then the enzyme activity increased up to 150% through the following 6 weeks (Figure 3A). Oxamyl reduced the activity of dehydrogenase after the first week, but it enhanced the activity within the second month (Figure 3B). In the case of herbicides ametryn and bentazone, dehydrogenase activity had the same pattern throughout the incubation for two months. The activity increased gradually until the 6th week in soil treated with ametryn, and it increased until 4th week in soil treated with bentazone (Figure 3C&D). In all treatments, after 60 days of treatment, the activity of dehydrogenase was

reached almost 100% like the control. Figure (4) exhibited that the dehydrogenase activity in sandy soil treated with carbofuran increased to about 200% at 3rd day, then it reduced to 100% after the first week of treatment. The enzyme activity enhanced to about 150% within the interval from 2nd-5th week, then it became equal to 100% on 60th day (Figure 4A). The activity of soil dehydrogenase was enhanced by 50% and 25% due to oxamyl at 25 and 50 $\mu\text{g/g}$ soil respectively throughout eight weeks (Figure 4B). In contrast to this, both herbicides ametryn and bentazone caused 25 and 50% increases in dehydrogenase activity in low and high concentration treatments respectively. At the end of two months, the activity reached 100+10% (Figure 4 C&D). The results indicated that both pesticide concentrations 25 and 100 $\mu\text{g/g}$ soil enhanced dehydrogenase activity expressed as % activity (as control activity 100%). It was observed that the low concentration of nematicides carbofuran and oxamyl increased the enzyme activity more than the high concentration whereas, the high concentration of herbicides increased the dehydrogenase activity more than the low concentration in either clay soil or sandy soil. In all treatments, after 60 days of treatment, the activity of dehydrogenase reached the normal level almost like the control. Data in Table (2) and Figure (5) indicated that the activity of dehydrogenase ($\mu\text{g TPF/g}$ soil) in clay soil was higher than that in sandy soil at all periods, whether the soil was treated or untreated. Also, dehydrogenase activities are always affected by all tested pesticides at concentrations of 25 and 100 $\mu\text{g/g}$ soil. In addition, the activities of dehydrogenase increased from the second to the eighth week in all treatments.

Table 1. Physical and chemical properties of soils

Parameters	Calcareous soil	Alluvial soil
Depth (cm)	0-15	0-15
pH (CaCl ₂)	7.93	7.82
Organic carbon content (%)	0.30	1.10
Organic matter content (%)	0.51	1.89
Electrical conductivity (ds/m)	29.53	1.91
Texture	Sandy clay	Clay loam

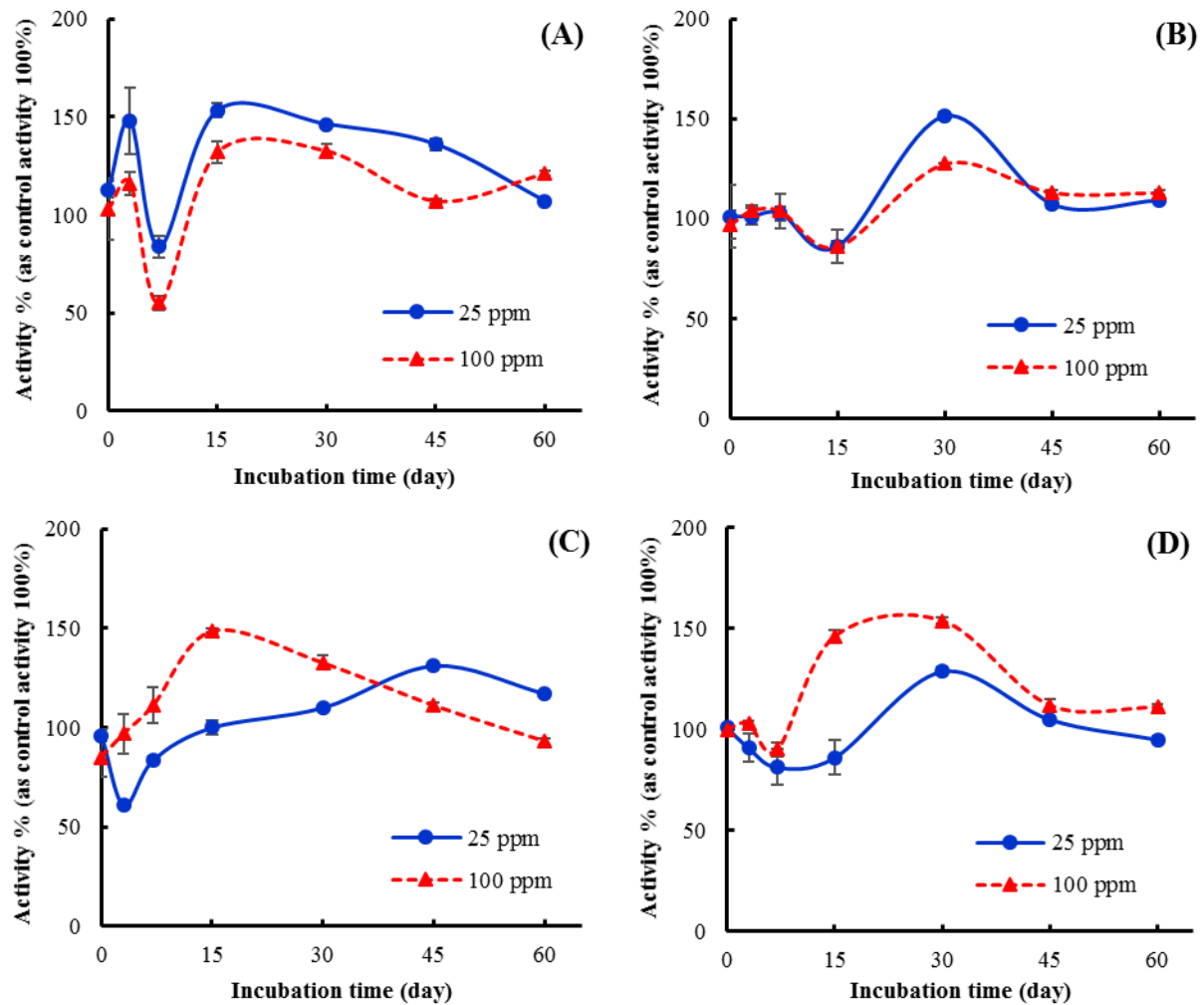


Figure 3. Effect of tested pesticides on dehydrogenase activity in clay soil over 60 days. The data are expressed in activity percentage (as control activity 100%) \pm SD. A: carbofuran B: oxamyl C: ametryn D: bentazone

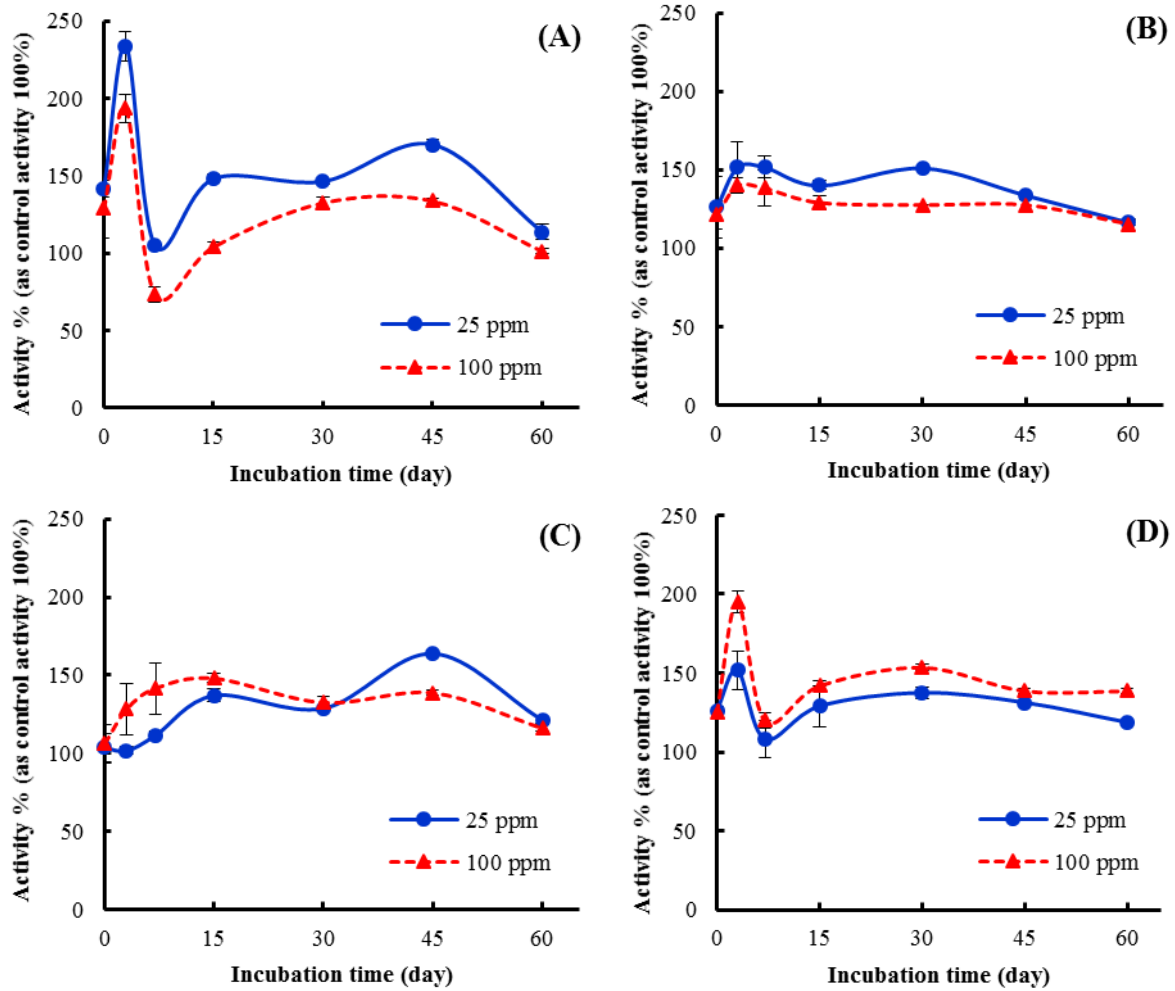


Figure 4. Effect of tested pesticides on dehydrogenase activity in sandy soil over 60 days. The data are expressed in activity percentage (as control activity 100%) \pm SD. A: carbofuran B: oxamyl C: ametryn D: bentazone

Table 2. Dehydrogenase activity in clay and sandy soil treated with different pesticides over 60 days

Treatment	Conc. (ppm)	Dehydrogenase activity ($\mu\text{g TPF/g soil}$)						
		Incubation time (day)						
		0	3	7	15	30	45	60
Control	Clay	200 \pm 5.66	200 \pm 16.97	160 \pm 16.97	200 \pm 11.31	160 \pm 5.66	198 \pm 19.80	200 \pm 16.97
	Sandy	160 \pm 16.97	120 \pm 16.97	136 \pm 16.97	198 \pm 14.14	160 \pm 5.66	158 \pm 19.80	160 \pm 16.97
Concentration (25 ppm)								
Carbofuran	Clay	226 \pm 8.49	296 \pm 33.94	134 \pm 8.49	307 \pm 7.54	234 \pm 2.83	272 \pm 5.66	214 \pm 2.83
	Sandy	226 \pm 8.49	280 \pm 11.31	126 \pm 2.83	296 \pm 5.66	234 \pm 2.83	272 \pm 5.66	182 \pm 8.49
Oxamyl	Clay	202 \pm 31.11	202 \pm 8.49	164 \pm 5.66	172 \pm 16.97	242 \pm 2.83	214 \pm 2.83	218 \pm 2.83
	Sandy	202 \pm 31.11	182 \pm 19.80	182 \pm 8.49	280 \pm 5.66	242 \pm 2.83	214 \pm 2.83	186 \pm 2.83
Ametryn	Clay	192 \pm 5.66	122 \pm 2.83	134 \pm 2.83	200 \pm 7.54	176 \pm 0.00	262 \pm 2.83	234 \pm 2.83
	Sandy	166 \pm 14.14	122 \pm 2.83	134 \pm 2.83	274 \pm 8.49	206 \pm 2.83	262 \pm 2.83	194 \pm 2.83
Bentazone	Clay	202 \pm 2.83	182 \pm 14.14	130 \pm 14.14	172 \pm 16.97	206 \pm 2.83	210 \pm 2.83	190 \pm 2.83
	Sandy	202 \pm 2.83	182 \pm 14.14	130 \pm 14.14	258 \pm 25.46	220 \pm 5.66	210 \pm 2.83	190 \pm 2.83
Concentration (100 ppm)								
Carbofuran	Clay	206 \pm 31.11	232 \pm 11.31	88 \pm 5.66	264 \pm 11.31	212 \pm 5.66	214 \pm 2.83	242 \pm 2.83
	Sandy	206 \pm 31.11	232 \pm 11.31	88 \pm 5.66	208 \pm 5.66	212 \pm 5.66	214 \pm 2.83	162 \pm 2.83
Oxamyl	Clay	194 \pm 14.14	208 \pm 5.66	166 \pm 14.14	172 \pm 5.66	204 \pm 0.00	226 \pm 2.83	226 \pm 2.83
	Sandy	194 \pm 14.14	168 \pm 5.66	166 \pm 14.14	258 \pm 8.49	204 \pm 0.00	204 \pm 5.66	184 \pm 5.66
Ametryn	Clay	170 \pm 19.80	194 \pm 19.80	178 \pm 14.14	297 \pm 1.89	212 \pm 5.66	222 \pm 2.83	186 \pm 2.83
	Sandy	170 \pm 19.80	154 \pm 19.80	170 \pm 19.80	296 \pm 5.66	212 \pm 5.66	222 \pm 2.83	186 \pm 2.83
Bentazone	Clay	200 \pm 5.66	206 \pm 2.83	144 \pm 5.66	292 \pm 5.66	246 \pm 2.83	224 \pm 5.66	222 \pm 2.83
	Sandy	200 \pm 5.66	234 \pm 8.49	144 \pm 5.66	284 \pm 5.66	246 \pm 2.83	222 \pm 2.83	222 \pm 2.83

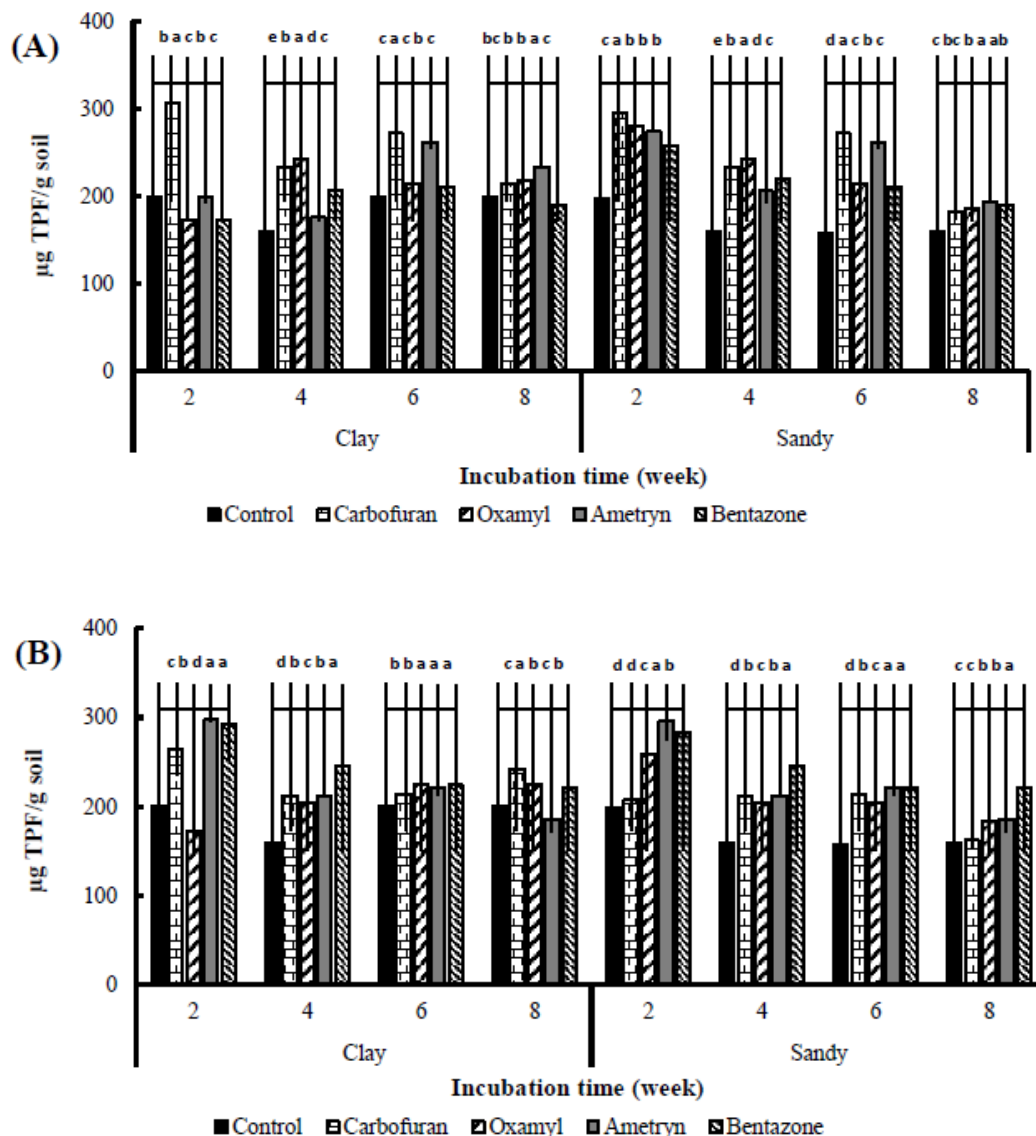


Figure 5. Comparison among dehydrogenase activities in clay and sandy soil (as µg TPF/g soil) treated by different pesticides over two months. A: 25 µg/g soil, B: 100 µg/g soil. The columns in each time interval followed by different letters indicate significant difference between treatments at P ≤ 0.05

The statistical analysis indicated that the dehydrogenase activities increased significantly in treated clay and treated sandy soil compared to untreated soil. In the case of 25 µg/g soil treatment (Figure 5A), clay and sandy soil treated with carbofuran had the highest set of soil dehydrogenase activities after the second and sixth week of treatment when compared to other pesticides treatments, while soils treated with oxamyl and ametryn had the highest dehydrogenase activity after the fourth week and eighth week of treatment, respectively. Whereas in the case of 100 µg/g soil treatment (Figure 5B), clay soil treated with

carbofuran had the highest set of soil dehydrogenase activities after the 8th week of treatment, while clay soil treated with oxamyl had the highest set of dehydrogenase activity after the 6th week. Both clay and sandy soils treated with ametryn had the highest dehydrogenase activity after the 2nd and 6th week, while clay and sandy soils treated with bentazone had the highest set of dehydrogenase activity after the 4th and 6th week. One month after treatment, the treatments of clay and sandy soil could be arranged based on the dehydrogenase activity in order as follows; at low concentration, oxamyl > carbofuran > bentazone >

ametryn > control and at high concentration, bentazone > carbofuran = ametryn > oxamyl > control. It was observed that after two weeks of incubation, the herbicides ametryn and bentazone at 100 µg/g soil while the nematicides carbofuran and oxamyl at 25 µg/g soil, were the highest compounds enhancement of dehydrogenase activity in clay and sandy soil. Moreover, in the treatment of 100 µg/g soil, the herbicides ametryn and bentazone were the highest enhancement pesticides on dehydrogenase activity. Whereas in the treatment of 25 µg/g soil, the nematicide carbofuran was the highest stimulation pesticide on dehydrogenase activity in clay and sandy soil (Figure 5).

In literature, various effects of different pesticides on several soil enzymes are reported (Quilchano & Marañón, 2002; Gianfreda *et al.*, 2005 and Deborah *et al.*, 2013). In particular, the effects on soil dehydrogenase activity (Sebiomo *et al.*, 2010; Nare *et al.*, 2014; Arora *et al.*, 2019 and Singh & Singh, 2023). Dehydrogenase activity is only present in viable cells, and it is also an indirect indicator of soil microbial biomass (Kucharski *et al.*, 2009). Dehydrogenase activity is a sensitive bioindicator of the microbial activity response to pesticide inputs.

The obtained results indicated that soils treated with oxamyl had the lowest dehydrogenase activity, while soils treated with carbofuran had the highest dehydrogenase activity when compared to other pesticides used in this study. Application of the tested pesticides led to a significant increase in dehydrogenase activity irrespective of doses. At the initial stage with the application of pesticide dehydrogenase activity gradually decreased up to 7-15 days, after that the activity slowly increased. This result is in agreement with that obtained from other studies (Dzantor & Felsot, 1991b and Sebiomo *et al.*, 2010). They indicated that this effect might be due to the degradation of the tested compounds and the increase in microbial populations with the capability of utilizing the pesticide formulation as a carbon source. The difference in the dehydrogenase activity in the two soils may be ascribed to the difference in the decomposition rates of the tested pesticides or their transformation to less toxic by-products in both soils. In general, the dehydrogenase enzyme activity was significantly increased in all treatments compared with the control at all-time intervals. In this study, dehydrogenase activity increased from the second to the sixth week of treatment.

The increased activity of dehydrogenase during the periods of incubation in soil treatments with carbofuran, oxamyl, ametryn and bentazone in this study, suggests increased microbial activities during this period. Similar observations were reported Yao *et al.* (2006); Singh &

Kumar (2008) and Nare *et al.* (2014) who indicated that dehydrogenase activities were stimulated by pesticides including endosulfan, deltamethrin and profenofos. Also, Yao *et al.* (2006) showed that after 14 days of application of acetamiprid at rates from 0.5 to 50 mg/kg soil dehydrogenase activity was stimulated (Yao *et al.*, 2006). In the other hand some studies showed dehydrogenase activities were inhibited by insecticides such as chlorpyrifos and quinalphos (Pandey & Singh, 2004; Singh & Singh, 2005b; Tejada *et al.*, 2011 and Singh & Singh, 2023), diazinon (Cycoń *et al.*, 2006 and Cycoń *et al.*, 2010), and inhibited by herbicide glyphosate (Milošević and Govedarica, 2002), atrazine (Dzantor and Felsot, 1991a), 2,4-D (Arora *et al.*, 2019). Also, it was reported that after application of captan the dehydrogenase activity decreased at a heavier rate (Chen *et al.*, 2001). No effects on soil dehydrogenase activity were detected (Nakamura *et al.*, 1990). Furthermore, it was reported that insecticides and herbicides can be classified into two groups, one group with positive effects and another group with negative effects (Riah *et al.*, 2014).

Our results indicated that the activity of dehydrogenase in clay soil was higher than that in sandy soil at all periods, whether the soil was treated or untreated. The adverse impact of pesticides on the soil environment is dependent primarily on the dose applied, environmental persistence, frequency of use, the physicochemical properties of soil, pH, temperature, moisture content, and sorption capacity (Kucharski *et al.*, 2009). Low concentration of nematicides carbofuran and oxamyl increased the enzyme activity more than the high concentration whereas, the high concentration of herbicides increased the dehydrogenase activity more than the low concentration in either clay soil or sandy soil. The response of dehydrogenase activity to herbicides with high concentration was explained by Sebiomo *et al.* (2010) who reported that microbial activity increased as an adaptation to the stress caused by an increase in concentration of the herbicides over weeks of treatment, demonstrating a potential capacity for adaptation of the microorganisms in soils when large amounts of herbicides are added. In all treatments, after 60 days of treatment, the activity of dehydrogenase reached the normal level almost like the control. Also, the effect of herbicides on dehydrogenase activity was observed over the entire experimental period and decreased at a very slow rate (Kucharski *et al.*, 2009).

Effect of pesticides on phosphatase activity in soil

The influence of four pesticides (carbofuran, oxamyl, ametryn, and bentazone) on phosphatase activities in both soil types of clay and sandy was investigated, the obtained results are presented in Figures (6-8) and Table (3). Phosphatase activity in clay

soil is illustrated in Figure (6). The results indicated that all the pesticides except oxamyl showed an enhancement effect toward the phosphatase activity until the end of the experiment. The highest enhancement was detected on the third day of treatment. Oxamyl showed an inhibitory effect on the enzyme activity from 15th day until 45th day of treatment, while carbofuran stimulated the enzyme activity to 130 and 140% within the time period from 15th day to 45th day of high and low concentration treatment, respectively. Also, ametryn and bentazone increased the phosphatase activity to more than 200% within the first week of treatment then the enzyme activity gradually decreased until the 45th day. In all pesticide treatments, the phosphatase activity started to come back to normal after the 45th day of treatment. The enhancement of phosphatase activity in clay soil treated with all pesticides at 25 µg/g soil is higher than that in soil treated with 100 µg/g soil. Figure (7) shows the effect of the tested pesticides on phosphatase activity in sandy soil. All treatments stimulated the enzyme activity to about 225% within the first week of treatment, then the enzyme activity was gradually reduced to come back to normal on the 60th day of treatment. It was observed that

in sandy soil treated with all pesticides, both concentrations of pesticides (25 and 100 µg/g soil) increased the phosphatase activity, but the high concentration increased the activity more than the low concentration.

The data presented in Table (3) showed the effect of tested pesticides on phosphatase activity in clay and sandy soil. The enzyme activity in clay soil was more than that in sandy soil treated with all pesticides as well as untreated soil. The phosphatase activity in clay soil was higher by 2-folds at 0 and 3rd day, and it was higher by 5-folds after two weeks and two months of treatment. The data of phosphatase activity in clay and sandy soil treated with all pesticides at 25 and 100 µg/g soil were exhibited in Figure (8). The statistical analysis showed that the highest phosphatase activity was obtained after six and eight weeks in clay soil while it was obtained after two weeks in sandy soil. After two weeks of treatment, the herbicides ametryn and bentazone (at 25 and 100 µg/g soil) significantly stimulated the enzyme activity more than the nematicides carbofuran and oxamyl in clay soil.

Table 3. Phosphatase activity in clay and sandy soil treated with different pesticides over 60 days

Treatment	Conc. (ppm)	Phosphatase activity (µg PNP/g soil)						
		Incubation time (day)						
		0	3	7	15	30	45	60
Control	Clay	65 ± 6.15	65 ± 3.07	43 ± 4.61	61 ± 2.46	65 ± 3.07	217 ± 6.15	250 ± 3.07
	Sandy	26 ± 6.76	33 ± 3.07	43 ± 3.07	130 ± 9.22	54 ± 6.15	78 ± 1.54	74 ± 2.46
Concentration (25 ppm)								
Carbofuran	Clay	76 ± 12.30	133 ± 12.30	59 ± 3.07	74 ± 6.15	91 ± 6.15	246 ± 6.15	289 ± 9.22
	Sandy	26 ± 3.07	74 ± 3.07	77 ± 7.69	213 ± 3.07	74 ± 6.15	95 ± 7.69	79 ± 0.15
Oxamyl	Clay	56 ± 13.53	187 ± 3.07	79 ± 4.61	65 ± 2.46	50 ± 6.15	164 ± 7.69	257 ± 15.68
	Sandy	30 ± 3.07	75 ± 1.54	101 ± 4.61	213 ± 1.69	83 ± 6.46	111 ± 3.07	79 ± 1.08
Ametryn	Clay	58 ± 1.54	173 ± 10.76	75 ± 4.46	122 ± 0.61	77 ± 7.69	228 ± 6.15	268 ± 4.61
	Sandy	20 ± 4.00	52 ± 3.69	86 ± 6.92	159 ± 9.22	65 ± 0.92	104 ± 2.77	85 ± 1.54
Bentazone	Clay	57 ± 3.07	122 ± 21.52	88 ± 10.76	117 ± 18.45	80 ± 6.15	240 ± 1.54	270 ± 9.22
	Sandy	28 ± 2.77	64 ± 1.54	67 ± 5.84	176 ± 6.15	62 ± 1.54	89 ± 3.07	88 ± 0.46
Concentration (100 ppm)								
Carbofuran	Clay	46 ± 15.22	129 ± 29.21	59 ± 12.30	70 ± 11.07	83 ± 9.22	205 ± 13.83	241 ± 3.07
	Sandy	24 ± 1.38	50 ± 6.15	80 ± 6.15	270 ± 6.15	84 ± 0.31	103 ± 4.61	84 ± 0.31
Oxamyl	Clay	36 ± 0.77	153 ± 16.91	74 ± 12.30	55 ± 3.69	48 ± 2.46	161 ± 12.30	235 ± 3.07
	Sandy	27 ± 1.23	68 ± 4.61	92 ± 4.61	258 ± 7.69	91 ± 6.15	120 ± 4.00	81 ± 1.08
Ametryn	Clay	49 ± 13.83	169 ± 15.53	55 ± 7.69	99 ± 2.46	72 ± 12.30	170 ± 3.07	246 ± 2.00
	Sandy	26 ± 3.07	47 ± 4.61	98 ± 1.84	192 ± 10.76	68 ± 0.31	121 ± 5.53	109 ± 6.15
Bentazone	Clay	49 ± 4.30	58 ± 4.61	72 ± 6.15	109 ± 1.84	77 ± 4.61	182 ± 7.69	205 ± 13.83
	Sandy	26 ± 1.08	54 ± 9.22	84 ± 4.61	209 ± 3.07	82 ± 1.38	116 ± 7.69	93 ± 9.22

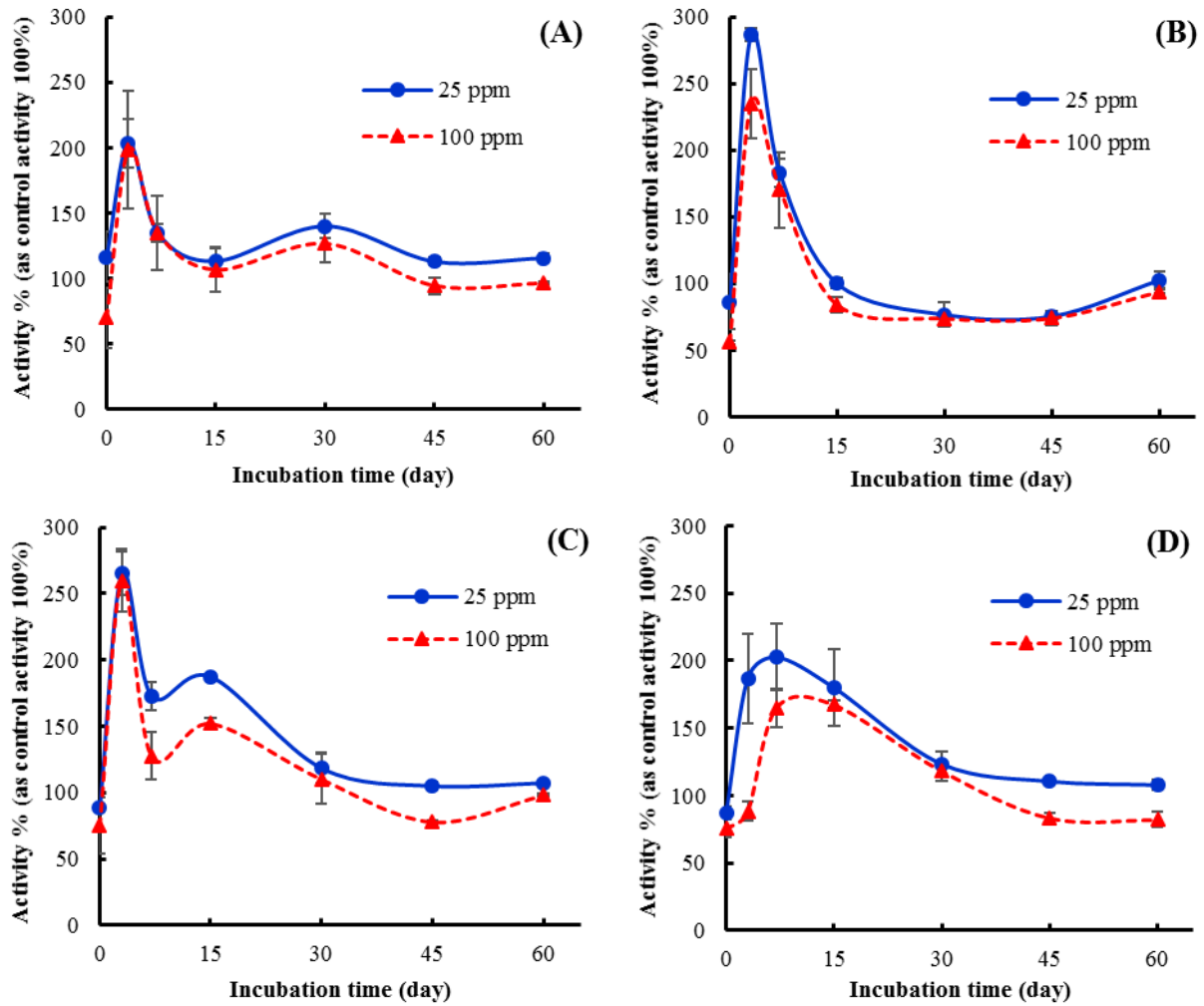


Figure 6. Effect of tested pesticides on phosphatase activity in clay soil over 60 days. The data are expressed in activity percentage (as control activity 100%) ± SD. A: carbofuran B: oxamyl C: ametryn D: bentazone

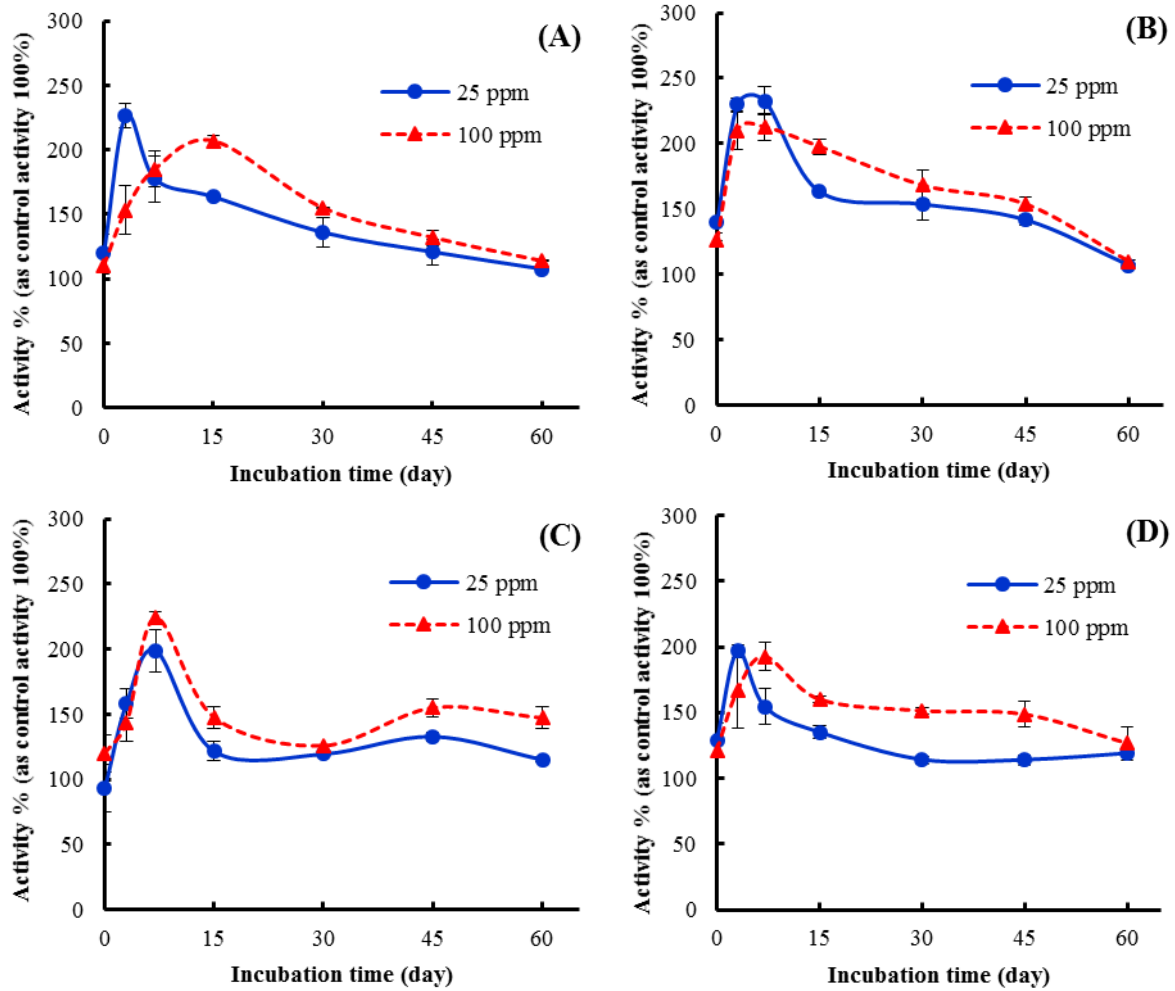


Figure 7. Effect of tested pesticides on phosphatase activity in sandy soil over 60 days. The data are expressed in activity percentage (as control activity 100%) ± SD. A: carbofuran B: oxamyl C: ametryn D: bentazone

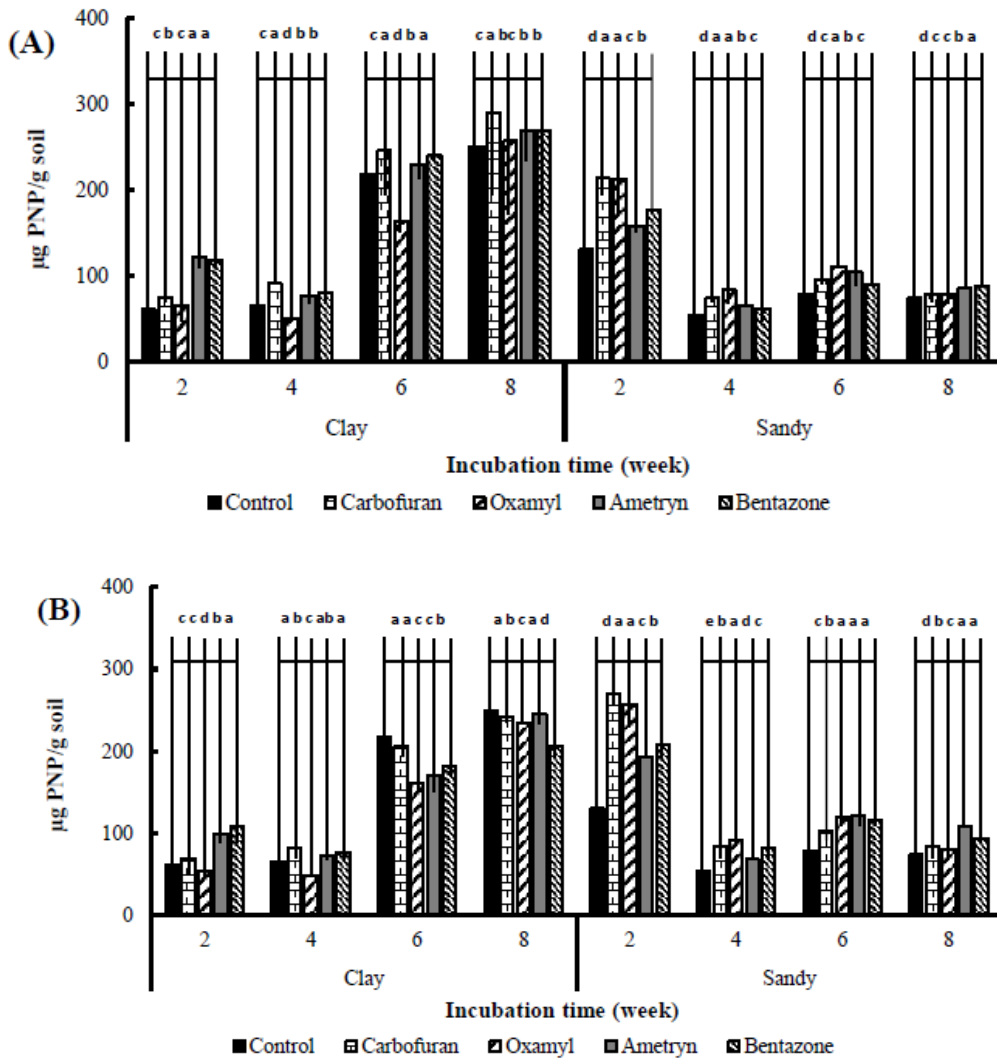


Figure 8. Comparison among phosphatase activities in clay and sandy soil (as µg PNP/g soil) treated by different pesticides over two months. A: 25 µg/g soil, B: 100 µg/g soil. The columns in each time interval followed by different letters indicate significant difference between treatments at P ≤ 0.05

While the nematicides carbofuran and oxamyl significantly stimulated the enzyme activity more than the herbicides ametryn and bentazone in sandy soil. In contrast, after eight weeks of treatment, the herbicides ametryn and bentazone significantly stimulated the phosphatase activity more than the nematicides carbofuran and oxamyl in sandy soil. In addition, after four and six weeks of treatment by both concentrations, oxamyl was the lowest enhancement pesticide for phosphatase activity in clay soil while it was the highest enhancement pesticide in sandy soil. After six weeks of treatment, the highest enhancement pesticide for phosphatase activity was carbofuran in clay soil,

whereas it was the lowest enhancement pesticide in sandy soil.

In soil, the phosphatase enzyme is believed to play a critical role in phosphorus cycles and key roles in the soil system (Kumar and Singh, 2023). The obtained results indicated that in clay soil and sandy soil that were treated with all pesticides, both concentrations of pesticides 25 µg/g soil (recommended rate) and 100 µg/g soil (4-folds of the recommended rate) increased the phosphatase activity. In the case of clay soil, the low concentration increased the activity more than the high concentration. In the case of sandy soil, the high concentration increased the activity more than the low concentration. This result demonstrated the importance

of pesticide concentration. It was reported that the effect of glyphosate on the soil phosphatase activity depended on the herbicide dosage (Płatkowski & Telesiński, 2015 and Płatkowski & Telesiński, 2016). It was revealed that carbosulfan and chlorpyrifos enhance phosphatase activity in soil when administered at field application rates (Swetha *et al.*, 2021). Also, the activity of phosphatase was enhanced in the presence of butachlor (Xia *et al.*, 2011), monocrotophos and chlorpyrifos (Srinivasulu *et al.*, 2012), endosulfan (Surya Kalyani *et al.*, 2010).

All the tested pesticides enhanced phosphatase activity in clay and sandy soil, except oxamyl. These results are in agreement with previous studies that indicated the effect of pesticides on soil phosphatase gave conflicting results: inhibition (Sannino & Gianfreda, 2001 and Yu *et al.*, 2011), and stimulation (Nakatani *et al.*, 2014 and Cherni *et al.*, 2015). The effect of pesticides on phosphatase is higher in sandy soil than in clay soil (Tejada, 2009 and Płatkowski & Telesiński, 2015). The stimulatory effect of the tested pesticides on phosphatase may be attributed to the effect of the pesticides on the microorganisms. Oxamyl slightly inhibited the activity of phosphatase in clay soil and sandy soil. This result may be attributed to the soil enzyme activity correlated with microorganisms. Microbial degradation of herbicides in soil is a function of three key variables: the ability of the microorganisms to degrade the pesticides, the quantity of these microorganisms in the soil, and the activity of the soil microbial enzyme system (Sannino and Gianfreda, 2001). The results clearly indicated that the phosphatase activity in treated or untreated clay soil was more than that in sandy soil at all periods. Soil texture influences enzyme activity, clay soil has greater ability to store organic matter that promotes microbial communities, and clay forms clay-enzyme complexes (Maphuhla and Oyedeji, 2024).

CONCLUSION

All the pesticides, except oxamyl, significantly enhanced dehydrogenase and phosphatase activities compared to the control at all-time intervals. Oxamyl showed the lowest dehydrogenase and phosphatase activities in both clay and sandy soils compared to the other pesticides. The low concentration of nematicides carbofuran and oxamyl, increased dehydrogenase activity more than the high concentration. In contrast, high concentrations of herbicides ametryn and bentazone increased enzyme activity more than low concentrations in both clay and sandy soils. In clay soil, low concentrations of pesticides increased phosphatase activity more than high concentrations. Conversely, in sandy soil, high concentrations of pesticides increased phosphatase activity more than low concentrations. The

activity of both soil enzymes, dehydrogenase and phosphatase, was higher in treated or untreated clay soil than in sandy soil at all periods. In all pesticide treatments, dehydrogenase and phosphatase activities began to return to normal after the 60th day of treatment. Further studies on the effects of pesticide residues and their metabolites on soil enzyme activities are recommended. Accordingly, This study may open new channels for research on estimating the level of these enzymes in the soil in different sectors of Egyptian lands, especially in natural habitats that were not exposed to pesticides. Accordingly, they can be used as scientific indicators to measure the degree of pesticide pollution during 60 days of exposure to pesticides.

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الملخص العربي

تأثير بعض المبيدات الحشرية على نشاط إنزيمي الديهيدروجينيز والفوسفاتيز في التربة

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المستخدمة في هذه الدراسة. كان نشاط إنزيم الهيدروجين والفوسفات في التربة الطينية المعالجة أو غير المعالجة أعلى من نشاطه في التربة الرملية. بالإضافة إلى ذلك، لوحظ أعلى تحفيز لنشاط ديهيدروجينيز عند التركيزات المنخفضة من الكاربوفوران والأوكساميل، في حين كان للتركيزات العالية من الأميترين والبننازون تأثير مماثل في كلا نوعي التربة. تم تحقيق أعلى تحفيز لنشاط الفوسفاتيز عند التركيز المنخفض من المبيد في التربة الطينية، بينما كان التركيز العالي أكثر فعالية في التربة الرملية. بناءً على النتائج التي تم الحصول عليها والتي تؤكد أن تركيز المبيد ونوع التربة ومدة الحضانة من العوامل المهمة التي تؤثر على العلاقة بين إنزيمات التربة والمبيدات. وبناء عليه يمكن استخدام مثل هذه الدراسة التي تشير إلى حدوث حث لمثل هذه الإنزيمات الهامة في التربة والتي يمكن أن تستخدم كمؤشر قياس التلوث بالمبيدات في التربة خلال ٦٠ يوم من التعرض للمبيدات.

تلعب إنزيمات التربة دورًا حاسمًا في الدورات البيوجيوكيميائية للعناصر الغذائية الأساسية وتحمي التربة من تراكم المركبات العضوية الضارة. ومع ذلك، هناك معلومات محدودة عن تأثير المبيدات الحشرية على أنشطة الإنزيمات في كل من التربة المصرية القديمة والمستصلحة حديثًا. أجريت دراسات مخبرية لتحديد آثار مبيدين للديدان الخيطية (كاربوفوران وأوكساميل) ومبيدين للأعشاب (أمترين وبننازون) على نشاط إنزيمي الديهيدروجينيز والفوسفاتيز في التربة الغرينية (التربة الطينية) والتربة الجيرية (التربة الرملية) بتركيزات ٢٥ و ١٠٠ ميكروغرام/ جرام من التربة خلال ثمانية أسابيع. أظهرت النتائج أن نشاط إنزيمي الديهيدروجينيز والفوسفاتيز يتعزز بشكل عام في كل من الترب الطينية والرملية المعاملة بجميع المبيدات المختبرة. ومع ذلك، كان للأوكساميل أقل تأثير على نشاط إنزيم الهيدروجين والفوسفات في كلا نوعي التربة مقارنة بالمبيدات الأخرى