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STABILITY AND CATALYTIC EFFICIENCY OF THE ENZYMES IMMOBILIZED ON NATURAL AND SYNTHETIC ORGANO-CLAY COMPLEXES

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ABSTRACT

This investigation was conducted on soil samples collected from different fluvial and lacustrine depositional environments, irrigated for a long-time with either Nile water or wastewaters.

The main purpose of this study was to shed light upon the immobilization capability, kinetic behaviour and catalytic efficiency of phosphomonoesterase proteins immobilized on natural soil clay fractions and their synthetic organo-clay complexes. **The obtained results could be summarized as follows:**

- Immobilization and stabilization capability of acid-phosphatase protein on natural uncoated clay fractions and synthetic organo-clay complexes were higher than that of alkaline-phosphate protein, determined as bovine serum albumin (BSA).
- Immobilization capacity of the more hydrophobic cationic detergent-clay complexes for retention of phosphatase-proteins was greater than those of either less hydrophobic more hydrophilic humic acid-clay complexes or activated-coated clay fractions. The natural uncoated-clay fractions showed the highest binding capacity over all the other complexes.
- Immobilized-phosphatase-proteins on natural clay fractions and synthetic organo-clay complexes have been displayed low catalytic activities in comparison with their free-enzyme states.
- Specific enzyme protein activity (SEPA) in mg PNP released.(mg protein)$^{-1}$. hour^{-1} of free-acid-phosphatase and its affinity to react with its specific substrate (p-nitrophenyl phosphate) was markedly higher than those obtained of free-alkaline-phosphatase. Immobilized-phosphatase-proteins exhibited lower (SEPA) than the corresponding values of free-enzymes. Stabilized acid-phosphatase protein retained higher residual specific activity (RSEA%) than those of alkaline-phosphatase protein.
- Immobilized-phosphatase-proteins on the more hydrophobic cationic HDTMA-clay complexes exhibited greater (SEPA) values; retained highly (RSEA%) in comparison with either HA-clay complexes or coated- and natural clay fractions, which displayed the lowest values in spite of their highly immobilization capacity for retention of phosphatase-proteins.
- The K_m values in (mmoles L^{-1}) of immobilized-phosphatase-proteins were markedly increased, however, V_{max} values in [mg PNP released. (mg protein)$^{-1}$-hour^{-1}] were sharply decreased in comparison with their free-enzyme values. Little profound differences between K_m and V_{max} values of free- and immobilized-phosphatase-proteins on HDTMA-clay complexes could be discerned. However, on natural clay fractions, they displayed the highest K_m and the lowest V_{max} values.
- Alkaline-phosphatase protein was highly-sensitive and lowly- resistive for proteolysis, meanwhile, acid-phosphatase has a low-sensitivity and high-resistivity for proteolytic enzyme. Immobilized-phosphatase-proteins on HDTMA-clay complexes displayed more resistance and stabilization against proteolysis related to their free- enzymes, which lost almost their activities. Immobilization on natural

clay fractions showed the lowest stabilization and resistance towards proteolysis. No loss in the stabilization and activity of free- and immobilized-phosphatase-proteins were observed against the storage for 125 days.

** Soil specific enzyme activity in [mg PNP released. (kg soil)$^{-1}$.hour^{-1}) of acid-phosphatase was higher than that of alkaline-phosphatase, however, the specific protein concentration of alkaline-phosphatase was greater than of acid phosphatase. This means that acid-phosphatase have much more catalytic efficient in hydrolysis of phosphomonoesters in studied alluvial soils (low protein concentration and high specific activity) than does alkaline-phosphatase (high protein concentration but low specific activity).

Therefore, immobilized phosphomonoesterase on synthetic organo-clay complexes are strongly recommended to be used for reduction and detoxification of some xenobiotic substances which is considered to be a safe method for clean-up of the environment.

Keywords: Phosphomonoesterases, stabilization, immobilization, detoxification, xenobiotics, cationic detergent.

INTRODUCTION

Recently, the extensive use of xenobioxic substances has increased in spite of their potential environmental hazard (Madhun & Freed, 1990).

Dangerous accumulation levels, due to their persistence, are now present in terrestrial and aquatic environments (Bollag & Liu, 1990; Wolfe *et al.*, 1990 and Nannipieri & Bollag, 1991).

In fact, the use of enzymes capable of transforming xenobiotics and recalcitrant substances or their toxic derivatives into safer metabolites may represent a viable alternative *in situ* to reducing and detoxifying of environmental pollutants and considered to be means for the clean-up of the environment (Nannipieri *et al.*, 1990 and Gianfreda & Bollag, 1996).

With respect to their specificity, enzymes are classified into six groups: oxidoreductases; transferases; hydrolases; lyases; isomerases and ligases (Tabatabei, 1994 and Ruggiero *et al.*, 1996).

In general, enzymes act on a limited number of substrates that show similar structural features (substrate specificity), with the exception of hydrolytic enzymes, which react with a large number of substrates. Stereospecificity enables enzymes to recognize molecules that are sterically non-equivalent.

The major biological and non-biological reactions that contribute to transformations and detoxifications of various naturally occurring xenobiotics in the environment are hydrolysis; oxidation-reduction; generation of free radicals; condensation-polymerization and phototransformation (Bollag, 1992 and Ruggiero *et al.*, 1996).

Hydrolases such as phosphatases, hydrolyze numerous substances into less complex molecules, which usually lose their biospecificity and products with both a lower stability and a higher biodegradability, these enzymes have a very broad specificity and do not require cofactors or coenzymes (Schomburg & Salzmann, 1991 and Gianfreda & Bollag, 1994).

In spite of these numerous advantages many limitations still restrict the use of enzymes to degrade and detoxify xenobiotics in the environment. For example, free enzymes may exhibit a very short useful life and rapidly decomposed in an inhospitable soil environment. Enzymes may be inactivated through both non- biological and biological denaturation processes, such as adsorption on soil colloids, extreme acidity or alkalinity, or biodegradation by proteases (Dick & Tabatabai, 1987 and Tabatabai & Fu,1992).

Immobilization of enzymes on stable supports may circumvent the need for maintaining active biological systems; increase the stability of the enzymatic activity; provide a continuous or discontinuous, economical and safe method for decontamination of pollutant environments and protect enzymes against degradation and denaturation, usually it the cost of some loss of activity (Gianfreda & Bollag, 1994 ad Ladd *et al.,* 1996).

However, the successful use of immobilized enzymes for practical applications required certain prerequisites; (i) a simple immobilization method, (ii) and inexpensive and easily available support (iii) a high residual specific activity of the immobilized enzyme and (iv) a high stability of the immobilized enzyme for long-time storage and subsequent activity (Gianfreda & Bollag, 1994 and 1996).

A thorough understanding is still lacking on simple suitable methods of immobilization of enzymes and on the use of these immobilized enzymes in contaminated soil environments.

The objectives of the present study were:(1) to shed light upon the behaviour and catalytic efficiency of the free and immobilized phosphomonoesterases on natural clay fractions and their synthetic organo-clay complexes as well as their kinetic properties and stabilization (ii) to determine the specific activity of these hydrolytic enzymes in studied soils and (iii) to relate enzymes activity to their specific enzyme protein concentrations.

MATERIALS AND METHODS

Locations description and soil sampling:

A representative composite surface samples of arable soils were collected form different locations at Kafr El-Sheikh and El-Gharbia Governorates. The selected locations represent different fluvial and lacustrine depositional environments in the northern Delta, irrigated for a long-time with Nile water and different sources of treated and untreated wastewaters.

The homogenized field moist soil samples passed through a 2-mm metal sieve, were subdivided into two main groups. The first one was immediately maintained in deep freezer and then thawed just prior to analysis for determination of soil enzymes activity. The second group was air dried, gently crushed and again sieved < 2 mm for the physical-chemical analysis and for isolation of the clay fraction < 2 µ. Soil characterizations were determined using the classical methods as described by Page *et al.* (1982); Klute (1986); Carter (1993) and Rowell (1996) as listed in Table (1).

Isolation of clay fraction < 2 μ:

Soil carbonates and organic matter were removed from 2-mm portion of air-dried soils using 1 *M* HCl and H_2O_2 30% treatments with heating to about 90°C until organic matter was destroyed and to remove excess H_2O_2. Iron oxides were removed with dithionite-citrate-biocarbonate with stirring at 80°C as reported by El-Kammah (1990) and Sheldrick & Wang (1993).

Table (1): Selected physical-chemical characterizations of the studied soils.

Alluvial soils depth section (0-30 cm)	pH 1: 2.5	EC dSm^{-1} soil paste	Ionic strength mmoles L^{-1}	$CaCO_3$ %	CEC Cmoles kg^{-1} soil	Bulk density Mg m^{-3}	Clay %	Organic-C %
Kafr Dokhmeas (fluvial soil)	8.04	4.16	68.5	3.85	50.7	1.38	56.2	1.67
Messier (fluvial soil)	8.26	5.34	81.3	3.24	46.6	1.26	50.7	2.53
Mean I	**8.18**	**4.75**	**74.9**	**3.55**	**48.7**	**1.32**	**53.4**	**2.10**
Mutobis Lacustrine soil)	7.97	2.78	46.7	8.73	42.3	1.34	48.9	1.85
El-Khashaa (Lacustrine soil)	8.13	5.69	78.9	12.56	39.8	1.20	43.6	2.06
Mean II	**8.05**	**4.24**	**62.8**	**10.65**	**41.1**	**1.27**	**46.3**	**1.96**
Grand mean	**8.12**	**4.49**	**68.9**	**7.09**	**44.9**	**1.29**	**49.8**	**2.03**

Notes, Each value is a mean of 2 replicates.

Soluble salts were removed by stirring, washing with distilled water and filtrating. The suspensions were dispersed with sodium hexametaphosphate as dispersing agent and the clay fraction < 2 μ was collected by sedimentation technique as described by El-Kammah (1979 and 1990).

The natural clay fractions were converted to Ca-monoionic clays by intensive shaking with 1 *N* $CaCl_2$.

The clays were dried at 60°C for 48 hours, ground to pass through 0.25 mm screen.

Total surface area of clay fractions was determined spectrophotometrically at wavelength 510 nm as m^2/g by the adsorption method using ortho-phenanthroline monohydrate [C_{12} H_8N_2. H_2O, mol. wt. 198.2, Sigma P9375) as described by Lawrie (1961). Standard curve of orthophenanthroline equation was as follows:

$$E = 0.003225\ C_{\mu moles\ L}\text{-}1 \text{ bei } 510 \text{ nm}$$

Cation exchange capacity (cmoles kg^{-1} soil) of the studied soils and clay fractions was determined using sodium acetate pH 8.2 as saturation solution and ammonium acetate as repeller solution according to the method reported by Page *et al.* (1982). Standard curve of sodium equation was as follows:

$$R = 0.008095\ C_{mg\ Na/100\ ml}$$

Homogenized matured co-composed cotton stalks, which minerally augmented was decalcified with 0.1 *N* HCl for 24 h to remove carbonate, exchangeable bases and inorganic materials.

Humic acids were extracted, purified and dialysed as described by El-Kammah (1990) and Stevenson (1994). Humic substances were extracted by shaking the composted materials with 0.1 *M* $Na_4P_2O_7$. 10 H_2O/0.1 *M* NaOH for 24 hours under N_2. The supernatant was isolated by decantation and centrifugation. The filtrate was fractionated by acidification with conc. H_2SO_4 until pH 2 to precipitate the gel humic acid.

Ash-free of acidified, precipitated humic acid was obtained by treated the redissolved humic acid with diluted acidic solution containing hydrofluric acid (0.3 *N* HF + 0.1 *N* HCl). The coagulated gel humic acid (ash-free) was washed free of salts by centrifugation, dialysed extensively against distilled water in semi-permeable membrane (cellophane bags) for one week. Dialysis solutions were continuously agitated and changed twice daily until pH 6 and the Cl^- and SO_4^{2-} tests were negative ($AgNO_3$ and $BaCl_2$ solutions). The non-dialysable isolated, purified ash-free humic acid (H-form) was dried at 45°C for 24 hours and pulverized to pass through 0.10 mm- screen.

Organo-clay complexes:

The synthetic organo-clay complexes were prepared as described in details by El-Kammah (1990). Briefly, the suspension contained 250 mg (excess, 5 mg/ml) ash-free humic acid (dialysed H-form) mixed with 250 mg (5 mg/ml) clay fraction < 2 µ (monoionic Ca-form) and 50 ml 0.05 *M* NaOH.

The suspensions were intensively shaken for 24 hours at 25°C on end-over-end shaker to reach the equilibrium conditions. The precipitated organo-clay complexes were isolated by centrifugation at 5000 rpm for 20 min, purified by washing and filtration several times, firstly with 0.05 *M* NaOH to remove the excess of humic acid, then with distilled water until the light color disappearance in the washing solution and finally with MUB-buffers (pH 4.5 or pH 10.4). The washed organo-clay complexes were frozen, freeze-dried, then stored until used for enzymes immobilization processes.

Cationic detergent-clay complexes:

Synthetic clays saturated with cationic hexadecyltrimethyl ammonium ($HDTMA^+$) prepared as described by Boyd & Mortland (1985a and b). Briefly, clay fractions < 2 µ were redispersed in distilled water and collected by sedimentation technique several times. The suspensions were treated with hexadecyltrimethyl-ammonium bromide (Sigma H6269, $C_{16}H_{33}$ N $[CH_3]_3$ Br; mol. wt. 364.5) at concentrations several times the cation exchange capacities of the clay fractions in order to replace the $HDTMA^+$ cations on the exchange sites of clay fractions. The suspensions were continuously shaken for 24 hours at 25°C on end-over-end shaker to reach the equilibrium states. After shaking, the HDTMA-clay complexes were separated by centrifugation at 5000 rpm for 20 min, and purified by washing and filtrating with distilled water to remove the excess organic salt, then with MUB-buffers pH 4.5 and 10.4.

The washed HDTMA-clay complexes were frozen, freeze-dried, then stored until used for enzymes immobilization processes.

Purified reference enzymes:

Reference proteins of phosphomonoesterases, purified from plant and microbial sources were purchased from Sigma Chemical Company. Acid-phosphatase (orthophosphoric-monoester phosphohydrolase [acid optimum]; EC 3.1.3.2.) from wheat germ, type 1; product number P3627 preweighed lyophilized (freeze-dry) powder. Alkaline-phosphatase (orthophosphoric monoester phosphohydrolase [alkaline optimum]; EC 3.1.3.1.) from bovine (calf) intestine type 1: product number P3877.

Reference enzyme protein contents of these commercial purified acid- and alkaline-phosphatases were determined by the method described by Lowry *et al.* (1951) as bovine serum albumin (BSA).

Enzymes immobilization:

Phosphomonoesterases were covalent-immobilized on natural soil clay fractions < 2 µ as reported by Gianfreda & Bollag (1994) which described by Ruggiero *et al.* (1989); Sarker *et al.* (1989). Briefley, 200 mg of clay fractions were incubated at 45°C with 5 ml of 0.5 *M* HNO_3 for 2 hours with shaking, centrifuged at 12000 x g and washed with distilled water until the supernatants reached a neutral pH. Five milliliters of a 2% 3-aminopropyltrimethoxy Silane in pure acetone were added to the activated clay fractions and the suspensions were shaken overnight at 45°C. After centrifugation, the activated pellets were treated with 5 ml of 5% glutaraldehyde solution in 0.1 *M* sodium phosphate buffer (pH 7), evacuated in a vacuum desiccator to remove trapped air bubbles, and incubated for one hour at room temp. After several washings with deionized water and phosphate buffer, the pellets were incubated with 2 ml of 0.1 *M* phosphate buffer (pH 7) containing the suitable amounts (6 mg as BSA) of either acid- or alkaline-phosphatases. The enzyme-clay mixtures were shaken gently at 4°C for 24 hours. The immobilized enzyme complexes were recovered by centrifugation; washed several times with buffer solution until no enzymatic activity was detected in the washings.

The centrifugates were checked for enzyme proteins by NaOH alkalization and until disappearance of the red-violet color using the Biuret reaction (Metzner, 1982).

Clay-enzyme complexes were stored at 4°C as suspensions in 0.1 *M* phosphate buffer, pH 7.0

For comparison, control was prepared as 2 ml of 0.1 *M* phosphate buffer pH 7 containing 6 mg acid- and alkaline-phosphatases without pellets referred to as "free enzyme" and treated in the same fashion.

Reference proteins assay were performed on the supernatants and washing buffers, as well as on the free enzymes according to the method described by Lowry *et al.* (1951) using bovine serum albumin (BSA) as mentioned after.

The amounts (milligrams) of enzyme proteins immobilized were evaluated by the differences between milligrams of enzymes protein as BSA initially added and that recovered in the supernatants and washing buffers.

The immobilization of phosphomonesterases on organo-clay complexes were performed by incubating 200 mg materials with 2 ml of 0.1 *M* phosphate buffer pH 7 containing the suitable amounts (6 mg as BSA) of the

enzymes and treated in the same fashion as mentioned before for activated and coated clay minerals.

Protein micro-determination:

Total proteins content of the purified commercially preparations acid and alkaline-phosphatases obtained from Sigma company were colorimetrically microdetermined at wavelength 540 nm with special reference protein of bovine serum albumin (BSA) as protein standard solution according to the method described by Lowry *et al.* (1951). Details about the reagent preparations were described by Metzner (1982). BSA was purchased via Sigma Chemical Company (product No. P5304). Each vial contains 20 g/dL in 0.85% NaCl inluding 0.1% sodium azide as preservative.

BSA standard curve was prepared using BSA working solution (1 mg BSA/ml) and the standard NaCl solution 0.85% (Sigma 430 AG-4) as diluent. Standard curve of BSA equation was as follows:

$$E = 0.0085178\ C_{\mu g\ BSA\ ml}^{-1} \text{ at wavelength 540 nm}$$

Soil enzymes assay:

The enzyme activities were assayed at their optimal pH and temp. in oven dry equivalent weight of field-moist soils < 2 mm by the methods described by Tabatabai (1994); Nannipieri (1995) and Nannipieri *et al.*(1996).

Assay of phsophomonoesteraes activity based on colorimetric determination of ρ-nitrophenol released by phosphatase activity when the soil samples were incubated at 37°C for one hour with tolune and buffered 50 m*M* disodium ρ-nitrophenyl phosphate hexahydrate [$C_6H_4NO_6PNa_2$. $6H_2O$, mol. wt. 371.1, Sigma 104-0] as a substrate. The employed buffer solution was the modified universal buffer (MUB) pH 6.5 for assay of acid-phosphatase or pH 11 for assay of alkaline-phosphatase. The ρ-nitrophenl (PNP) released was extracted by filtration and the yellow color intensity was spectrophotometrically measured at wavelength 420 nm.

The concentration of PNP was calculated by reference to a calibration graph. Standard curve of PNP equation was as follows:

$$E = 0.00075\ C_{\mu gPNP/5ml} \text{ at wavelength 420 nm.}$$

The phosphomonosterases activity was recorded as µg PNP released. kg^{-1} soil. $hour^{-1}$.

Specific enzymes activity:

The specific activity [mg PNP released. (mg protein)$^{-1}$.h^{-1}] of phosphomonoesterases was determined by the methods described by Klose e& Tabatabai (2002). Activity of free and immobilized acid-phosphatase was assayed by incubating 1-ml modified universal buffer (MUB) pH 4.8 containing 1 mg of each free or immobilized reference enzyme protein (115 µg of specific acid-phoshatase protein); 3-ml MUB pH 4.8 and 1 ml of buffered 50 m*M* ρ-nitrophenyl phosphate hexahydrate (PNPP) disodium salt (6.96 mg PNP) as chromogenic ester in total volume 5 ml at 37°C for 30 min. with hand occasionally shaking.

The specific activity of free and immobilized alkaline-phosphatase was assayed by incubating a 1-ml MUB pH 10.4 containing 1 mg of each free or immobilized reference enzyme protein (812 µg of specific alkaline-

phosphatase protein); 3-ml of MUB pH 10.4 and 1-ml of buffered 50 m*M* PNPP (6.96 mg PNP) in total volume 5 ml at 37°C for 30 min. with hand occasionally shaking.

The MUB buffers and the reagents were prepared as described by Tabatabai (1994). The specific enzymes activities were calculated as described before, using the standard curve equation and recovered as mg PNP released. (mg protein)$^{-1}$.hour^{-1}.

Kinetic studies:

For the Michalelis-Menten studies, acid- and alkaline-phosphatase activities were assayed at pH 4.5 and 10.4 respectively, at 25°C and different substrate concentrations in MUB buffer. Concentrations of p-nitrophenyl phosphate hexahydrate (PNPP) ranged from 0.5 to 15 m*M* (stock solution 50 m*M*) in total volume 5 ml containing 1-ml solution included 1 mg free or immobilized reference enzymes proteins.

Enzymes stabilization:

The storage stability of free and immobilized enzymes was evaluated by periodically determined the activities of the MUB-buffered enzymes at 4°C for 125 days.

Stability to proteolytic enzymes:

Resistance of free and immobilized enzymes to decomposition by proteolytic enzyme (Protease; type IV bacterial, purified from *Streptomyces caespitosus*, Sigma P0384) was also evaluated.

After addition 1 ml 50 m*M* PNPP to 2-ml MUB buffers (pH 4.8 and 10.4) and 1 ml buffered protease containing 5 mg enzyme, the preparations were held of 20°C for 30 min., then added 1 ml buffered solution containing 1 mg free or immobilized acid- and alkaline-phosphatases. The mixtures were shaken at 37°C for 30 min. and analyzed the activity of phosphatases.

RESULTS AND DISCUSSION

Immobilization capacity of the enzymes:

Data listed in Tables (2.1 and 2.2) revealed that the various coated and uncoated clay fractions as well as the synthetic organo-clay complexes showed different immobilization capabilities for the reference purified acid and alkaline-phosphatase-proteins.

Generally, synthetic organo-clay complexes displayed markedly higher binding capacities than coated clay fractions. The mean values of immobilized enzymes on synthetic organo-clay complexes were 5.095 and 3.408 mg for acid- and alkaline-phosphatases proteins, determined as bovine serum albumin (BSA). These values referred to as 84.8% and 56.8% (w/w) of the initial reference enzyme proteins added to the immobilization mixtures. Meanwhile, the immobilized amounts on coated clay fractions were 2.725 and 2.15 mg acid and alkaline proteins referred to as 45.4% and 35.83% (w/w).

The immobilization capability of acid and alkaline enzyme proteins could be arranged in the following descending order as follows:

Organo-clay complexes > H.A-clay complexes > coated clay fractions

Immobilization capacity of the more hydrophobic cationic detergent-clay complexes (HDTMA-clay complexes) was higher than those of the less hydrophobic more hydrophilic humic acid clay complexes (HA-clay complexes), since as much as 89.7% and 60.20% by weight of the added initial reference enzyme proteins of acid- and alkaline-phosphatases to immobilization mixtures, can be bound to these complexes before appreciable enzymes remain in solutions. Humic acid-clay complexes, immobilized-phosphatase-proteins relatively lower than those obtained by HDTMA-clay-complexes. The natural uncoated-clay fractions displayed obviously the highest binding capacities for retention phosphatases proteins in comparison with synthetic organo-clay complexes and natural uncoated clay fractions.

Table (2.1): Immobilization of acid-phosphatase protein (EC 3.1.3.2.) on natural clay fractions and synthetic organo-clay complexes.

Clay fractions and synthetic organo-clay complexes	Immobilized acid phosphatase		Specific enzyme protein activity (SEPA %)	Loss of specific activity %	Residual specific activity (RSEA %)
	mg protein as (BSA)	% (w/w)			
Free acid-phosphatase protein					
	-	-	15.07	-	-
Immobilized acid-phosphatase protein					
Clay fraction 1 Kafr Dokhmeas	5.56	92.8%	6.32	58.06	41.94
Clay fraction 2 Mutobis	5.22	87.0%	5.24	65.23	34.77
Mean I	**5.39**	**89.8%**	**5.78**	**61.65**	**38.85**
Coated clay fraction 1	3.08	51.3%	8.61	42.87	57.13
Coated clay fraction 2	2.37	39.5%	6.98	53.68	46.32
Mean II	**2.725**	**45.4%**	**7.20**	**48.27**	**51.73**
HDTMA-clay complexes 1	5.62	93.6%	14.863	1.59	98.41
HDTMA-clay complexes 2	5.14	85.8%	12.39	17.78	82.22
Mean III	**5.38**	**89.7%**	**13.61**	**9.69**	**90.31**
HA-clay complexes 1	4.95	82.3%	12.26	18.65	81.35
HA-clay complexes 2	4.66	77.6%	11.52	23.58	76.42
Mean IV	**4.81**	**79.9%**	**11.89**	**21.11**	**78.89**
Mean of organo-clay complexes III + IV	**5.095**	**84.8%**	**12.75**	**15.40**	**84.60**
Grand mean	**4.57**	**76.2%**	**9.77**	**35.18**	**64.31**

Notes:

1. **Bovine serum albumin (BSA-protein).**
2. **Reference enzyme protein of acid-phosphatase was 96.55 (determined as BSA protein).**
3. **Specific enzyme protein of acid-phosphatase was 11.5% (as determined by Klose & Tabatabai (2002).**
4. **Specific enzyme protein activity (SEPA %) in mg PNP released. (mg protein)$^{-1}$. hour^{-1}**
5. **Residual specific enzyme protein activity (RSEA %).**

Commonly, the dominant mechanisms of immobilization, stabilization and protection of enzymes proteins have been concluded for organic and inorganic colloids by Body & Mortland (1985a, b and 1990); Sarkar *et al.* (1989); Tabatabai &Fu (1992) and Ladd *et al.* (1996).

These mechanisms include adsorption; microencapsulation; cross-linking; copolymerization; entrapment; ion-exchange; adsorption and cross linking; covalent attachment; H-bonding; ionic bonding and lipophilic reactions. Although it is possible that more one, and even all these processes are involved in binding enzymes.

The differences between these immobilization capacities may be due to the king and nature predominant bonding mechanisms between catalytic functioning sites of the enzymes and organic portions in organo-clay complexes matrices.

Table (2.2): Immobilization of alkaline-phosphatase protein (EC 3.1.3.1.) on natural clay fractions and synthetic organo-clay complexes.

	Immobilized acid phosphatase		Specific enzyme protein activity (SEPA %)	Loss of specific activity %	Residual specific activity (RSEA %)
	mg protein as (BSA)	% (w/w)			
Free alkaline-phosphatase protein					
	-	-	2.808	-	-
Immobilized alkaline-phosphatase protein					
Clay fraction 1 Kafr Dokhmeas	4.75	79.16	1.05	62.61	37.39
Clay fraction 2 Mutobis	3.15	52.50	0.66	76.50	23.50
Mean I	**3.95**	**65.83**	**0.855**	**69.55**	**30.45**
Coated clay fraction 1	2.32	38.67	1.45	48.36	51.64
Coated clay fraction 2	1.98	33.00	1.19	57.63	42.37
Mean II	**2.15**	**35.83**	**1.32**	**53.00**	**47.00**
HDTMA-clay complexes 1	4.15	69.17	2.60	7.40	92.60
HDTMA-clay complexes 2	3.07	51.20	2.34	16.67	83.33
Mean III	**3.61**	**60.20**	**2.47**	**12.04**	**87.96**
HA-clay complexes 1	3.36	56.00	2.20	21.65	78.35
HA-clay complexes 2	3.05	50.80	1.99	29.11	70.89
Mean IV	**3.205**	**53.40**	**2.095**	**25.38**	**74.62**
Mean of organo-clay complexes III + IV	**3.408**	**56.80**	**2.283**	**18.71**	**81.29**
Grand mean	**3.169**	**52.82**	**1.685**	**47.09**	**52.91**

Notes:

1. **Reference enzyme protein of alkaline-phosphatase was 90.53% (determined as (BSA-protein).**
2. **Specific enzyme protein of alkaline-phosphatase was 81.5% as determined by Klose and Tabatabai (2002).**
3. **Speici enzyme protein activity (SEPA %) in mg PNP released. (mg protein)$^{-1}$. hour^{-1}.**
4. **Residual specific enzyme protein activity (RSEA %).**

It could be said that, reference enzyme phosphatase-proteins may be immobilized on organo-clay complexes by hydrophobic bonding mechanism as reported by Body and Mortland (1985a, b); Ladd *et al.* (1996) and Ruggiero *et al.* (1996).

Binding capacities of reference acid and alkaline enzyme proteins immobilized on uncoated clay fraction (1) isolated from Kafr Dokhmeas fluvial soils were greater than those obtained on clay fraction (2) isolated from Mutobis lacustrine soils. These differences between immobilization capacities

may be due their chemical and physical properties. Clay fractions (1) and (2) have specific surface areas (472.8 and 416.2 m^2/g); CEC (65.4 and 53.3 cmoles kg^{-1} soil) and surface change densities (0.145×10^{-2} and 0.138×10^{-2} meq/m^2), respectively. It could be said that phosphatase-proteins binding on monoionic Ca-clay fractions is principally by hydrogen bonding between its oxygen or hydroxyl groups and appropriate functional groups of phosphatase molecules as reported by Ladd *et al.* (1996).

Immobilization affinity of reference acid-phosphatase protein on clay fractions and organo-clay complexes generally was higher than that of reference alkaline-phosphatase protein under the same conditions.

The analysis of the purified reference acid- and alkaline-phosphatase-proteins determined by the method of Lowry *et al.* (1951) showed that they contained 96.5% and 90.53% proteins as bovine serum albumin (BSA). Because the purified reference proteins contained proteins other than those of the phosphatases, the percentages of the specific acid- and alkaline-phosphatase-proteins have been determined by Klose & Tabatabai (2002) using sodium dodecyl polyacrylamide gel electorphoresis (SDS-PAGE) as 11.5% and 81.2% for the same types of the studied acid alkaline-phosphatases, respectively.

Specific enzyme protein activity:

Data illustrated in Tables (2.1 and 2.2) showed also that, specific enzyme protein activity (SEPA) in mg PNP. $(mg\ protein)^{-1}\ hour^{-1}$ of free acid-phosphatase (15.07) was markedly higher than those obtained for free alkaline-phosphatase (2.808) at the same conditions. This means that, the affinity of acid-phosphatase protein to react with their specific substrate (p-nitrophenyl phosphate) was higher than of alkaline-phosphatase, protein.

Obtained results showed that acid- and alkaline-phosphatase proteins immobilized on different natural clay fractions and synthetic organic-clay complexes displayed specific activity (SEPA) lower than that of the free enzymes i.e. great differences between free and immobilized enzymes can be discerned.

In this respect, the results are in agreement of those reported in other studies. It has been demonstrated that reaction of enzymes with clays or organic polymers usually resulted in decrease in specific enzyme activities; but may stabilize the bound enzymes proteins against degradation by proteinases and/or denaturing agents (Burns, 1982 and 1986; Boyd & Morland, 1985a, b and 1990; Sarkar ***et al.*** 1989; Nannipieri *et al.,* 1995 and Ruggiero *et al.,* 1996).

These differences in activities may have depended on the relation of binding sites to active sites on the enzyme molecules.

The reduced activity is generally interpreted in terms similar to those for enzymes immobilized on other solid supports that; steric and diffusional restrictions; direct involvement of the active site in binding to the support and modified conformation of the immobilized enzymes by electrostatic and hydrophobic forces (Goldstein, 1976 and Ruggiero *et al.,* 1996). Such reactions may (1) enhance the rigidity of the enzyme molecules, thereby stabilizing them against denaturation by heating or drying, (2) render the

active sites of enzymes less accessible to the substrate and make them inaccessible to attack by fee proteinases and (3) if the bound enzyme is itself a proteinase prevent destruction by autogestion (Tabatabai & Fu, 1992).

Ruggiero *et al.* (1989) and Sarkar *et al.* (1989) immobilized various enzymes on different clay minerals by using 3-aminopropyl triethoxysilane plus glutaraldehyde to ensure that the enzymes remained far enough from the clay surface to retain most of their original activity. Dick & Tabatabai (1987) reported that enzyme molecules less tightly bound on exterior surface (secondary surface adsorption) may retain the activity of free enzymes as was the case with phosphatase-clay complexes.

The obtained specific activities of acid- and alkaline-phosphatase-proteins immobilized on the studied materials could be arranged in the following descending order as follows:

Cationic HDTMA-clus complexes > HA-clay complexes > Coated clay fractions > uncoated clay fractions.

Residual specific activity:

Residual specific activities of the immobilized-phosphatase-proteins were calculated as percentages of the specific activities of their free enzymes.

As shown in Tables (2.1 and 2.2) immobilized acid-phosphatase protein retained higher residual specific activity (64.82%) than those obtained by alkaline-phosphatase protein (52.91%).

Data revealed also generally that, phosphatase-proteins immobilized on synthetic organo-clay complexes retained more residual activities (84.6% and 81.29%) and lost less enzyme activities (15.4 and 18.71%) for acid- and alkaline-phosphatase-proteins, respectively.

Immobilized acid and alkaline-phosphatase-proteins on clay fractions saturated with more hydrophobic (long chain alkyl groups) HDTMA-cations (HDTMA-clay complexes) were absolutely retained the highest residual specific activities (90.31% and 87.96%) and the lowest values of lost-activities (9.69% and 12.04%). The clay fractions saturated with humic acid (HA-clay complexes) were relatively retained less residual activities (78.89% and 74.62%) and loss relatively higher activities (21.11% and 25.38%) for acid- and alkaline-phosphatase proteins.

This suggested that, depending on the nature of the enzyme, the active sites may be free and highly available to substrate. The model provided here of HDTMA-clay complexes and phosphatases has some similar and dissimilar features when compared with data obtained by others on soil phosphomonoesterases.

Phosphatase-proteins immobilized on uncoated and coated clay fractions retained the lowest residual activity levels and lost the highest enzymes activities. This means that the active sites may be relatively blocked or obscured by the immobilization process and these active sites may, indeed by closely related to binding sites. The hydrophobic bonding process would seem to be a likely one in soils where natural organic matter, both free and complexes with clays, might provide zones or areas of hydrophobicity which

interact with similar areas on enzymes (Boyd & Mortland, 1985a, b and 1990; Gianfreda & Bollag, 1994).

Kinetic parameters:

In general, the reaction catalyzed by an immobilized enzymes obeys the hyperbolic Kinetics of Michaelis-menten.

The graphically lineweaver-Burk straight line relations were plotted, from which the kinetic parameters were calculated by determining the intercepts and the slops. Table (3) showed the V_{max} values [maximum velocity in mg PNP released (mg protein)$^{-1}$. hour^{-1}] and K_m values [Michaelis constant in (mmoles L^{-1}), which is the substrate concentration at V = 0.5 V_{max}]. As reviewed in literature the K_m value of an free enzyme is constant only under a specific set of conditions i.e. it remained virtually unchanged (Ruggiero *et al.*, 1996).

The K_m values of acid- and alkaline-phosphatases immobilized on clay fractions as well as organo-clay complexes were markedly increased in comparison with K_m of their free enzymes.

Grand mean values of K_m were 1.756 and 2.753 (39% increase) for free and immobilized acid-phosphatase, while its values were 0.735 and 1.614 (119.25% increase) for free and immobilized alkaline-phosphatase. Acid- and alkaline-phosphatases mmobilzied on HDTMA-clay complexes displayed the lowest K_m values.

The K_m values were 1.98 (13% increase) and 0.956 (30% increase) mmoles L^{-1} for immobilized acid and alkaline enzymes on HDTMA-clay complexes, while the corresponding values were 2.22 (26% increase) and 1.35 (83% incase) mmoles L^{-1} on HA-clay complexes.

Table (3): Michaelic kinetic parameters of the free and immobilized phosphomonoesterases on natural clay fractions and synthetic organo-clay complexes.

Clay fractions and synthetic organo-clay complexes	Acid-phosphatase (EC 3.1.3.2.)		Alkaline-phosphatase (EC. 3.1.3.1.)	
	V_{max} mg PNP (mg protein^{-1}) hour^{-1}	K_m mmoles L^{-1}	V_{max} mg PNP (mg protein^{-1}) hour^{-1}	K_m mmoles L^{-1}
	Free enzymes (control)			
	16.5	1.756	3.15	0.735
	Immobilized enzymes			
Clay fraction 1 Kafr Dokhmeas	7.11 (57%)	3.36 (91%)	1.43 (55%)	2.05 (179%)
Clay fraction 2 Mutobis	6.75 (59%)	3.95 (125%)	1.14 (64%)	2.29 (212%)
Mean I	6.93 (58%)	3.65 (108%)	1.28 (59%)	2.17 (195%)
Coated clay fraction 1	8.25 (50%)	3.03 (73%)	1.93 (39%)	1.85 (152%)
Coated clay fraction 2	6.77 (59%)	3.29 (87%)	1.56 (50%)	2.11 (186%)
Mean II	7.51 (54%)	3.16 (80%)	1.75 (44%)	1.98 (169%)
HA-clay complexes 1	13.86 (16%)	2.07 (18%)	2.44 (22%)	1.27 (73%)
HA-clay complexes 2	12.85 (22%)	2.36 (34%)	2.16 (31%)	1.42 (93%)
Mean III	13.40 (19%)	2.22 (26%)	2.30 (20%)	1.35 (83%)
HDTMA-clay complexes (Mean IV)	15.35 (10%)	1.98 (13%)	2.58 (18%)	0.956 (30%)
Grand mean value	10.79 (35.25%)	2.753 (39%)	1.978 (36.75%)	1.614 (119.25%)

V_{max} Values expressed as percentages of the specific activity of free enzymes

K_m Values expressed as percentages of Michaelis constants of free enzymes

However, the immobilization of these enzymes on uncoated and coated clay fractions showed the highest K_m values.

In comparison, the K_m values of immobilized acid- and alkaline-phosphatases on HDTMA-clay complexes (1.98 and 0.956 moles L^{-1}) were about as well as K_m of their free enzymes (1.756 and 0.735), where little differences between free and immobilized enzymes can be discerned.

The hydrophobic binding mechanism has apparently provided an environment for the enzyme which does not greatly affect the catalytic functioning sites of the enzyme. The obtained v_{max} values of immobilized acid and alkaline-phosphatases were relatively decreased in comparison with their free enzymes. Little differences were obtained between V_{max} values of free acid and alkaline-phosphatases and their corresponding values immobilized on HDTMA-clay complexes.

These findings can be explained on the basis of conformational, steric, partitioning and diffusional effects of the sorbed acid and alkaline-phosphatases as explained by Goldstein (1976) and Ruggiero *et al.* (1996).

Properties of immobilized enzymes:

- **Stability to proteolytic enzymes:**

Data listed in Table (4) showed the resistance of free and immobilized acid- and alkaline-phosphatase-proteins to proteolysis with proteolytic enzymes (protease). Results demonstrated generally that after exposure the free and immobilized-phosphatase-proteins to protease (5 gm/5 ml) in immobilization mixture, their residual specific activities were sharply decreased. Grand mean values of residual specific activity of free enzymes were 8.43% (91.6% lost of activity) and 4.34% (95.66% lost of activity) for acid and alkaline-phosphatases. This means that alkaline-phosphatase protein is highly-sensitive and lowly-resistive for proteolysis, meanwhile, acid-phosphatase protein has low-sensitivity and high resistivity for proteolytic enzyme protease.

Table (4): Stabilization of free and immobilized acid- and alkaline-phosphatases as affected by proteolytic enzyme protease.

Enzymes conditions	Acid-phosphatase activity mg PNP released (mg protein)$^{-1}$. hour^{-1}				Alkaline-phosphatase activity mg PNP released (mg protein)$^{-1}$. hour^{-1}			
	Protease 5 mg/5 ml		Loss of activities %	Residual specific activity (RSEA%)	Protease 5 mg/5 ml		Loss of activities %	Residual specific activity (RSEA%)
	Without	With			Without	With		
Free enzymes	15.07	1.27	91.6%	8.43%	2.808	0.122	95.66%	4.34%
Immobilized enzymes on:								
Clay fractions	5.78	1.02	82.85	17.65	0.855	0.126	85.25	14.75
Coated clay fractions	7.80	1.98	74.62	25.38	1.32	0.275	79.11	20.89
HDTMA-clay complexes	13.61	8.54	37.26	62.74	2.47	0.985	60.12	39.88
HA-clay complexes	11.89	4.12	65.35	34.65	2.095	0.539	74.28	25.72
Grand mean	9.77	3.92	64.90	35.10	1.685	0.481	74.69	25.31

Each figure is a mean of 6 determination (3 replicates x 2 fractions).

Grand mean values of residual specific activity of immobilized enzyme proteins 35.10% (64.9% lost) and 25.31% (74.69% lost) were at least (4.16- and 5.83-fold) greater than that the values of free acid and alkaline-phosphatase-proteins respectively.

Immobilized acid and alkaline-phosphatase proteins on the more hydrophobic HDMTA-clay complexes retained the highest residual specific activity values 62.74% (37.26% lost) and 39.82% (60.12% lost). However, on H.A.-clay complexes retained less residual specific activity values 34.65% (65.35% lost) and 25.75% (74.28% lost) respectively.

Enzymes immobilized on uncoated and coated clay fractions retained the lowest residual activity values and lost the highest activity. It could be concluded that, acid and alkaline-phosphatase-proteins immobilized on cationic HDMTA-clay complexes were resulted in more resistance and stabilization to proteolysis. This results suggested the existence of protective mechanisms associated with organo-clay complexes that shield the enzymes against proteolytic activity without hindering the diffusion of substrate molecules in the active sites. Sarkar (1986); Nannipieri *et al.* (1988), Ladd *et al.* (1996) and Ruggiero *et al.* (1996) reported that phosphatases are immobilized on organic-mineral complexes in such a manner that pores on the organic portion of the three-dimensional network of clay-organic matter-enzymes complexes surrounding the enzymes permit the passage of small molecules of substrate and product, but not those of large molecules such as proteolytic enzymes.

This structure confers a measure of stabilization on the enzyme while allowing retention of enzyme activity as elucidated before.

- **Storage stabilization:**

Analytical results concerning the stabilization of free and immobilized acid- and alkaline-phosphatase-proteins against the storage revealed that, no loss of the initial activities of the free and immobilized-phosphatase-proteins and retained almost 100% of their initial activities. Little differences between free and immobilized alkaline-phosphatases can be discerned over the experimental period elongated 125 days.

Phosphomonoesterases activity in soils:

Data given in Table (5) showed that, specific enzyme activity of acid-phosphatase in studied alluvial soils were generally higher than that obtained of alkaline-phosphatase. Grand mean values of specific activities of acid- and alkaline-phosphatases were 413 and 263.5 mg PNP released (kg soil^{-1}). hour^{-1}. Commonly, fluvial soils (Kafr Dokhmeas and Messier soils) had higher specific activities 480 and 292 than those obtained in lacustrine soils (Mutobis and El-Khashaa soils) which were 346 and 235 mg PNP released. (kg soil)$^{-1}$. hour^{-1} for acid- and alkaline-phosphatases.

Differences between enzyme specific activities in the two different depositional environmental soils and in between themselves may be due to their physicochemical properties as shown in Table (1).

Table (5): Specific activity of acid- and alkaline-phosphatases and their specific enzyme protein concentrations in studied alluvial soils.

Alluvial soils depth section (0-30 cm)	Specific activity mg PNP_1 released (kg soil)$^{-1}$. hour^{-1}		Specific protein concentrations mg protein (kg soil)$^{-1}$	
	Acid (EC 3.1.3.2.)	Alkaline (EC 3.1.3.1.)	Acid (EC 3.1.3.2.)	Alkaline (EC 3.1.3.1.)
Kafr Dokhmeas Fluvial soils	396	264	26.28	94.01
Messier Fluvial soils	564	320	37.42	113.96
Mean I	**480**	**292**	**31.85**	**103.98**
Mutobis Locustrine soils	294	203	19.51	72.29
El-Khashaa Lacustrine soils	398	267	26.40	95.08
Mean II	**346**	**235**	**22.96**	**83.69**
Grand mean	**413**	**263.5**	**27.40**	**93.84**

Each value is a mean of 3 replicates

Enzyme protein concentrations were calculated as mg protein. (kg soil)$^{-1}$ for acid- and alkaline-phosphatases in studied alluvial soils in order to prove whether there is a relationship between the activity of any enzyme and its specific protein concentration in soils. These calculations are based on the specific activities of the purified reference enzymes (i.e. activity values per mg protein) and the activity values obtained in the soils.

The acid-phosphatase protein concentrations ranged from 19.51 to 37.42 (avg. = 27.40) mg protein (kg soil)$^{-1}$. The corresponding values of alkaline-phosphatase protein concentrations ranged from 72.29 to 113.96 (avg. 93.84).

Generally, alkaline-phosphatase of specific protein concentrations were higher than that of acid-phosphatase protein concentrations in studied alluvial soils.

On the other hand, fluvial soils contained specific phosphomono-esterase proteins more than lacustrine soils. This means that alkaline-phopshatase protein is predominant in fluvial alluvial soils than that in lacustrine soils. These means that alkaline-phosphatase protein is predominant in fluvial alluvial soils than that in lacustrine soils. These results support those reported by Juma and Tabatabai (1978), Nannipieri (1995) and Klose & Tabatabai (2002) showing that acid-phosphatase is predominant in acid soils and that alkaline-phosphatase is dominant in alkaline soils.

The activity values of the phosphomonoesterases corresponded well with the protein concentrations of these enzymes in soils. Comparison of the alkaline-phosphatase activity values relative to their estimated protein concentrations with those obtained for acid-phosphatase in soils. Data tabulated in Table (5) suggested that acid- phosphatase was much more efficient in hydrolysis of phosphomonoesters in soils (low protein concentration and high specific activity) than that of alkaline-phosphatase (high protein concentration and low specific activity) in alluvial soils.

It could be concluded that, enzyme protein concentrations revealed that the catalytic efficiency varies among soil enzymes, with acid-phosphatase showing a greater catalytic efficiency than does alkaline-phosphatase in studied alluvial soils.

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ثبات وفاعلية الإنزيمات التنشيطية المحملة على معقدات الطين الطبيعية والصناعية

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أجريت هذه الدراسة على عينات سطحية مختارة لأراضى رسوبية تمثل بيئات ترسيبية نهرية وبحيرية فى منطقة شمال الدلتا ، تروى بمياه نهر النيل ومياه صرف مختلطة (زراعى ــ صحى ــ صناعى) من مصرف الغربية الرئيسى (كوتشنر).

الهدف من هذه الدراسة هو إلقاء الضوء على السلوك ، والقدرة التنشيطية والتحليلية ، والحركية لإنزيمات الفوسفاتيز الحامضية والقاعدية الحرة والمقيدة على كل من معادن الطين الطبيعية ومعقداتها العضوية الصناعية ، وتقدير القدرة التحميلية لهذه المعقدات لتقييد وربط هذه الإنزيمات بصورة تجعلها تحتفظ بأكبر قدر من النشاط الإنزيمى بالإضافة إلى تقدير النشاط والتركيز النوعى لبروتين هذه الإنزيمات بالأراضى الرسوبية المدروسة لمعرفة كفاءتها التحليلية تحت هذه الظروف الترسيبية المختلفة.

ويمكن تلخيص النتائج المتحصل عليها فى الأتى:

- القدرة التحميلية (Immobilization capability) لبروتين إنزيم الفوسفاتيز الحامضى للتقييد والارتباط بسطوح معادن الطين الطبيعية ومعقداتها العضوية الصناعية عموما كانت أعلى من تلك المتحصل عليها لبروتين إنزيم الفوسفاتيز القاعدى ، مقدرة فى صورة بروتين (البيومين مصل الأبقار) Bovine Serum Albumin (BSA).
- إدمصاص (تحميل) المواد العضوية بميكانيكيات مختلفة على سطوح معقدات الطين العضوية الصناعية أدى إلى انخفاض نسبى فى قدرة وكفاءة هذه السطوح على تحميل ، وتقييد ، وارتباط إنزيمات الفوسفاتيز الحامضية والقاعدية مقارنة بسطوح معادن الطين الطبيعية مفردة بدون ادمصاص مواد عضوية عليها.

- معقدات معادن الطين الطبيعية مع الكاتيونات العضوية للمواد المنشطة للسطوح (Cationic HDTMA detergent-clay complexes) الأكثر كرها للماء "More hydrophobic" أظهرت سعة إدمصاصيه أعلى لتقييد وارتباط إنزيمات الفوسفاتيز الحامضية والقاعدية على سطوحها مقارنة بمعقدات الطين الطبيعية مع الأحماض العضوية الدبالية Humic acid-clay complexes الأكثر حبا للماء "More hydrophilic" ، وأيضا أعلى من معقدات معادن الطين الطبيعية المنشطة بالأحماض المعدنية والمغطاة بالمواد العضويةactivated-coated clay fractions ، بالاضافة الى أن قدرتها التحميلية تكاد تقترب من القدرة التحميلية لمعادن الطين الطبيعية بمفردها ، تلك التى أظهرت أعلى القيم لتحميل وربط هذه الإنزيمات على سطوحها.
- ارتباط معادن الطين الطبيعية بالمواد العضوية وتكوين المعقدات العضوية الصناعية أدى أيضا إلى انخفاض نسبى فى الكفاءة التحليلية والنشاط الإنزيمى النوعى (SEPA) لبروتين إنزيمات الفوسفاتيز الحامضية والقاعدية المحملة والمقيدة على سطوح هذه المعقدات (بالمليجرام بارانيتروفينول منطلقة لكل مليجرام بروتين إنزيمى لكل ساعة) مقارنة بتلك المتحصل عليها لإنزيمات الفوسفاتيز فى صورتها الحرة التى أظهرت قيم SEPA أعلى ، وكانت قيم النشاط الإنزيمى النوعى لبروتين إنزيم الفوسفاتيز الحامضية الحرة والمقيدة أعلى من نظيرتها لبروتين إنزيم الفوسفاتيز القاعدى الحرة والمقيدة. وأن هذه القيم تقترب من قيم إنزيمات الفوسفاتيز الحامضية والقاعدية فى حالتها الحرة.
- قيم K_m (بالمليمول/لتر) ، V_{max} (بالمليجرام بارانيتروفينول منطلقة لكل مليجرام بروتين إنزيمى لكل ساعة) لإنزيمات الفوسفاتيز الحامضية الحرة أعلى من نظيرتها لإنزيمات الفوسفاتيز القاعدية الحرة ، وأوضحت النتائج زيادة فى قيم K_m وانخفاض فى قيم V_{max} لإنزيمات الفوسفات الحامضية والقاعدية المقيدة على معادن الطين ومعقداتها العضوية مقارنة بالإنزيمات فى صورتها الحرة ، وقد أظهرت معقدات الطين مع الكاتيونات العضوية أقل زيادة فى قيم K_m وأقل انخفاضا فى قيم V_{max} وهذه القيم تقترب من قيم الإنزيمات فى صورتها الحرة.
- أظهرت النتائج انخفاضا حادا فى النشاط الإنزيمى النوعى لبروتين إنزيمات الفوسفاتيز الحامضية والقاعدية الحرة المعرضة لتأثير نشاط إنزيمات التحلل البروتينى ، وكان بروتين إنزيم الفوسفاتيز القاعدى أكثر حساسية وأقل مقاومة من بروتين إنزيم الفوسفاتيز الحامضى الأقل حساسية والأكثر مقاومة. ومن ناحية أخرى أظهرت إنزيمات الفوسفاتيز الحامضية والقاعدية المقيدة على سطوح معادن الطين الطبيعية ومعقداتها العضوية مقاومة عالية وأكثر ثباتا للتحلل الإنزيمى البروتينى مقارنة بالإنزيمات فى حالتها الحرة. وأوضحت النتائج أن تخزين إنزيمات الفوسفاتيز الحرة والمقيدة لمدة ١٢٥ يوم لم يؤدى إلى فقد فى نشاط الإنزيمات النوعى.
- نشاط إنزيم الفوسفاتيز الحامضى فى الأراضى الرسوبية المدروسة أعلى من نشاط إنزيم الفوسفاتيز القاعدى فى حين أن تركيز البروتين الإنزيمى لإنزيمات الفوسفاتيز القاعدية أعلى من نظيره للفوسفاتيز الحامضية وهذا يوضح أن الفاعلية التنشيطة (Catalytic efficient) لإنزيم الفوسفاتيز الحامضية أعلى من إنزيم الفوسفاتيز القاعدى فى الأراضى الرسوبية المدروسة.

بناء على ضوء النتائج المتحصل عليها فإن الأهمية التطبيقية لهذه الدراسة تنطوى على إمكانية استخدام إنزيمات الفوسفاتيز المحملة على معقدات معادن الطين الطبيعية مع منشطات السطوح العضوية فى إزالة سمية الملوثات العضوية كوسيلة لتخلص البيئة من هذه الملوثات ، لما تتمتع به هذه الإنزيمات المقيدة من كفاءة تحليلية ونشاط إنزيمى نوعى عالى يكاد يقترب من نشاط هذه الإنزيمات فى الصورة الحرة.