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Physiological effect of two heavy metals on esterase isozyme of digestive gland of garden flatworm *Bipalium kewense* (Moseley, 1878), 1ST record in Egypt

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Abstract: *Taxonomically, electrophoresis of isozymes might possibly contribute a better tool for taxonomy within the genera and species. However, physiological studies on the Egyptian flatworms are insufficient. Generally, esterase isozymes are found in some organs of flatworms, including digestive gland. Thereby, the present work included electrophoretic feature of esterase isozymes in the garden worm Bipalium kewense, 1st record in Egypt, as well as studying the effects of two heavy metals, zinc and copper, on esterase isozymes of digestive gland of this species. After the electrophoretic run, the Bipalium-esterase had been classified into three groups; the fastest arylesterase, aliesterase, and the slowest cholinesterase. The present results revealed that the most sensitive components to Zn and Cu, for 72 hours, were aliesterase and cholinesterase whereas arylesterase group showed the highest susceptibility to both metals.*

Keywords: *Turbellaria, Bipalliidae, Bipalium SPP., electrophoresis, Esterase isozymes, Zinc, Copper.*

I. INTRODUCTION

Planarians are terrestrial invertebrates which have great economic importance. Shovel-headed garden worm, *Bipalium kewense*, is a free-living terrestrial species. It was first discovered in Kew Gardens in England in 1878. Its body length is up to 30 cm and body width 3 mm[1]. It considers carnivore flatworm as natural enemy of invasive land flatworms ([2],[3]).

Electrophoresis gave some information about their variation in tolerance to heavy metals. Taxonomically, esterase isozymes were useful in separating some species ([4],[5]). In this regard, Avise (1975) [6] suggested that the electrophoretic mobility of isozymes provides indirect information about DNA, where their raw data are in the form of band that considered as "allozymes". In this respect, Augustinson (1961) [7] classified esterase isozymes into three groups; arylesterase, aliesterase and cholinesterase. The fastest group is arylesterase that is dominant followed in the speed by aliesterase one and the slowest category is cholinesterase. Abd-Allah (1990) [8] studied similarity index and genetic distance of esterase in the desert snail *Eremma* sp. Subsequently, Radwan *et al.* (1992) [9] treated *B. kewense* with five carbamate compounds and revealed that these compounds inhibited cholinesterase and aliesterase activities. In the next year, Stewart and Blackshaw (1993) [10] revealed the genetic variation in 12 populations of the planarian *Artioposthia triangulata* and confirmed that there was no relationship between statistical genetic distances for these populations Riutort, *et al.* (1992) [11] referred to the enzyme polymorphism and r-RNA sequences to measure genetic distances between species of family Planariidae and the relationships between genera and subgenera of family Dugesiidae. Then, Carranza *et al* (1998) [12] analyzed classification of families, from which Terricola, by molecular data from complete sequences of 18S r-DNA and 18S r-RNA. Subsequently, Guecheva *et al.* (2001) [13] referred that the planarians are suitable organisms for, in vivo, detection of copper geno-toxicity that can be used to assess both acute and chronic exposure to Cu, for 7 days, in aquatic ecosystems. Generally, physiological studies on the Egyptian flatworms are insufficient. Thereby, the present work included electrophoretic feature of esterase isozymes in a garden worm *Bipalium kewense* and studying the effects of two heavy metals, zinc and copper, on esterase isozymes of its digestive gland.

II. MATERIALS AND METHODS

II.1. Sampling:

Living *Bipalium kewense*-worms (Moseley, 1878 [14]) had been manually collected from the Botanical garden in the College of Education, Ain Shams University, Cairo. They are often found beneath fallen logs, leaf litter, stones and flowerpots.

II.2. Experimental studies:

The flatworms *B. kewense* were brought in small cans in the laboratory [each 10 flatworms in a glass box of 60 x 40 x 30 cm dimensions] with a mud substrate. The cans had been kept at the laboratory conditions (25±2 °c and 60-70% R.H.) for laboratory acclimation. Stock solutions of zinc and copper metals, as zinc acetate and copper acetate, had been used for treatment. Experimental worms were divided into three groups; untreated or control specimens, treated specimens with zinc acetate (concentrations 30, 55, 75 and 90 mg/L^{-1}) and treated specimens with copper acetate (the same concentrations of zinc acetate).

The worms had been kept in the treated solutions and their media were daily renewed. Each experimental exposure was performed 3 times for both zinc and copper metals. Then, the mortality of the experimental worms (treated and untreated groups) was daily recorded. Dose mortality rate obtained from bioassay tests was plotted using log-probit graph (Swaroop, 1966 [15]). Finally, surviving specimens had been used for experimental electrophoretic studies, after 72 hours of exposure to heavy metal-solutions.

II.3 Electrophoretic Preparation:

II.3.1 Preparation of samples:

The healthy flatworms *B. kewense* had been used in the present work, since the physiological state of the flatworms is very important for the electrophoretic technique, as recommended by Davis (1978) [16].

II.3.2 Electrophoretic technique:

II.3.2.1 Agarose-gel preparation:

Agarose-gel electrophoresis method had been used for separation of the esterase isozymes.

II.3.2.2 Electrophoretic run:

Horizontal electrophoretic apparatus of the Central Laboratory, College of Education, Ain Shams University, had been used for application of the electrophoretic run.

II.3.2.3 Staining of samples:

After the electrophoretic run, the bands in the gel on the glass-plates that carries esterase isozymes, displayed darkish-brown stain using 1-naphthyl acetate compound and fast blue RR stain**.**

II.3.2.4 Scanning:

The yielded results of esterase isozymes were applied to HELENA SCANNER (FRANCE) to reveal scanning of esterase zymograms "electro-phoregrams". Moreover, the scanner directly determined the relative intensity, according to Augustinson (1961) [7] and Wilkinson (1970) [17].

III. RESULTS AND DISCUSSION

It is evident that the cumulative mortality rate of exposed *B. kewense* to Zn and Cu, after 72 hours of exposure, increased with the increase of concentrations, as displaying in table (1). From table (1), LC_{50} of both Zn and Cu was 75 mg/L^{-1}. Thus, the behavioral responses of the exposed flatworms to LC_{50} of the two metals Zn and Cu were nearly similar. Esterase isozymes are known to be found in digestive gland of *B. kewense*. It is known that polypeptides, or protein, are considered the building units of esterase isozymes. Electrophoresis utilizes an electrical field in order to separate electrically charged molecules. Davis (1978) [16] identified isozymes as hydrolyze carboxylic esters. In this concern, esterase isozymes migrate through the electric field at different rates according to their charge, size, molecular shape and weight. Thus, electrophoresis of isozymes gave some information about their variation in tolerance to heavy metals.

The present electrophoregram-results revealed that the esterase isozymes of digestive gland in untreated *B. kewense* are separated in five bands; included the fastest arylesterase followed by aliesterase and the slowest cholinesterase (Figs. 1-6). The present results agree with Augustinson (1961) [7] and Abdel-Haleem (1999) [18] who revealed that esterase isozymes composed of the same present three components. The present tested Zn and Cu-metals had qualitative and quantitative effect on the esterase-bands of *B. kewense*. From the present results, Zn-metal has higher effect on esterase isozymes of *B. kewense* than Cu-element, especially after 75 mg/L^{-1}-concentration. In this respect, the most sensitive components of esterase to 55 and 75mg/L^{-1}Zn and Cu were aliesterase and cholinesterase, which are frequently absent, whereas arylesterase portion revealed the highest susceptibility to 55 and 75mg/L^{-1}Zn and Cu (Figs. 8-9 for Zn & 10-11 for Cu).

The present resulted electrophoregrams of untreated and treated worms had been statistically analyzed the similarity index (I). Tables (2&3) showed the effects of Cu and Zn on esterase isozymes between untreated "control" and treated worm and showed that the statistical similarity index (I) between control, treated worms with both 75mg/L^{-1}Zn and 55 and 75mg/L^{-1}Zn is similar (50±0.98); whereas (I)-data between both control and 75mg/L^{-1}Cu (44.44±0.98) is lower than those between 75mg/L^{-1}Cu and 55 and 75mg/L^{-1}Cu (50±0.98). The present data of similarity index (I) are agree with those of Abd-Allah (1990) [8] on the desert snail *Eremina desertorum*.

The present scores of the statistical relative intensity % of the present two concentrations of Zn "55 and 75mg/L^{-1}Zn" revealed the fragmentation of the second widest band to three bands and finally the three bands of control increased in the six bands; whereas relative intensity % of the two concentrations of Cu "55 and 75mg/L^{-1}Cu" revealed lighting and narrowing the first control-band (tables 4&5). The present scores of the relative intensity% are also agree with the results of Abd-Allah (1990) [8] on the snail *E. desertorum*.

Radwan *et al.* (1992) [9] treated *B. kewense* with five carbamate compounds and revealed that these compounds inhibited cholinesterase and aliesterase activities. The present results reinforced results of Radwan *et al.* (1992) [9], where the present results of both cholinesterase and aliesterase are highly sensitive to Cu and Zn-metals. Guecheva *et al.* (2001) [13] stated that the planarians are suitable organisms, in vivo, for detection of copper geno-toxicity that can be used to assess both acute and chronic exposure to Cu for 7 days.

Table (1): Percentage of cumulative mortality of ***Bipalium kewense*** after 72 hours exposure to different concentrations of Zn and Cu

Concentrations Mg/L^{-1}	Cumulative Mortality % Mean (%) ± SD	
	Zn	Cu
Control	0 ± 0	0 ± 0
30	20 ± 0.817	10 ± 0.943
55	35 ± 1.414	30 ± 1.886
75	50 ± 2.828	50 ± 2.828
90	60 ± 2.357	65 ± 3.771

(Table 2): Similarity index (I) between esterase isozyme of six of controls (untreated) ***Bipalium kewense*** Moseley (mean %± SE).

Controls	Controls				
	1	2	3	4	5
5	75.00 ± 0.99	57.14 ± 0.98	57.14 ± 0.98	62.50 ± 0.98	-
4	33.33 ± 0.97	80.00 ± 0.99	100.00 ± 0.99	-	-
3	40.00 ± 0.98	50.00 ± 0.98	-	-	-
2	80.00 ± 0.99	-	-	-	-
1	-	-	-	-	-

Table (3): *Similarity index (I) between esterase isozyme of control and treated Zn and Cu of* ***Bipalium kewense*** Moseley (mear % ± SE).

	Control	Zn	
		Concentration 1	Concentration 2
Concentration - 2	50.00±0.98	50.00±0.98	-
Concentration - 1	0.00±0.00	-	-
Control - Zn	-	-	-

	Control	Cu	
		Concentration 1	Concentration 2
Concentration - 2	44.44±0.98	50.00±0.98	-
Concentration - 1	0.00±0.00	-	-
Control - Zn	-	-	-

Concentration 1 = 75 mg / L^{-1}
Concentration 2 = 55 mg / L^{-1}

(Table 4): Relative intensity (%) for untreated samples of ***Bipalium kewense*** Moseley.

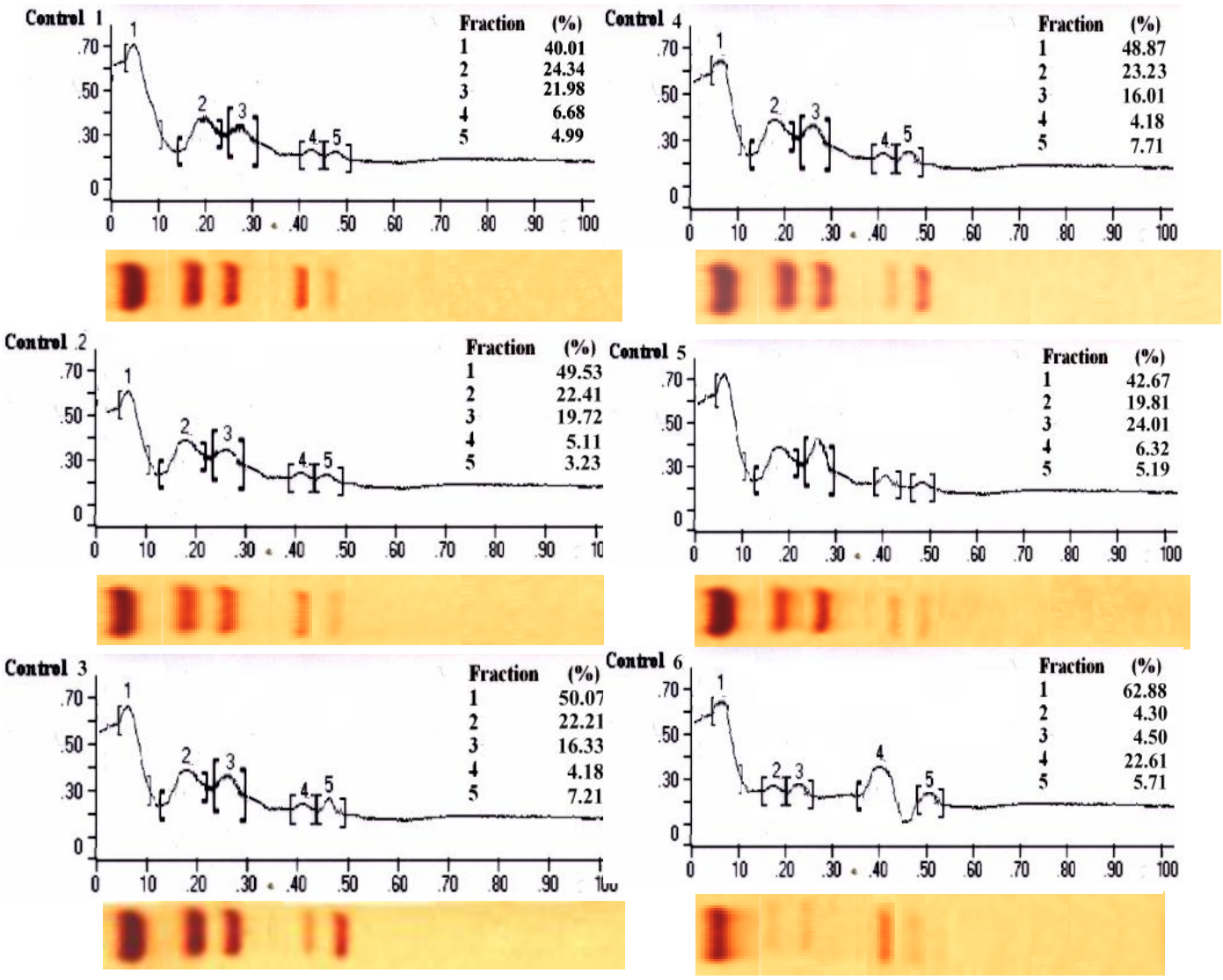
Samples / Bands	Controls				
	C1	C2	C3	C4	C5
b_1	70.787	61.539	80.214	64.725	54.506
b_2	72.514	88.462	59.786	63.315	72.838
b_3	66.699	55.565	67.765	81.960	42.981
b_4	55.786	84.244	70.707	61.339	69.682
b_5	-	-	-	-	-

Table (5): Relative intensity (%) for treated samples of ***Bipalium kewense*** Moseley by Zn and Cu.

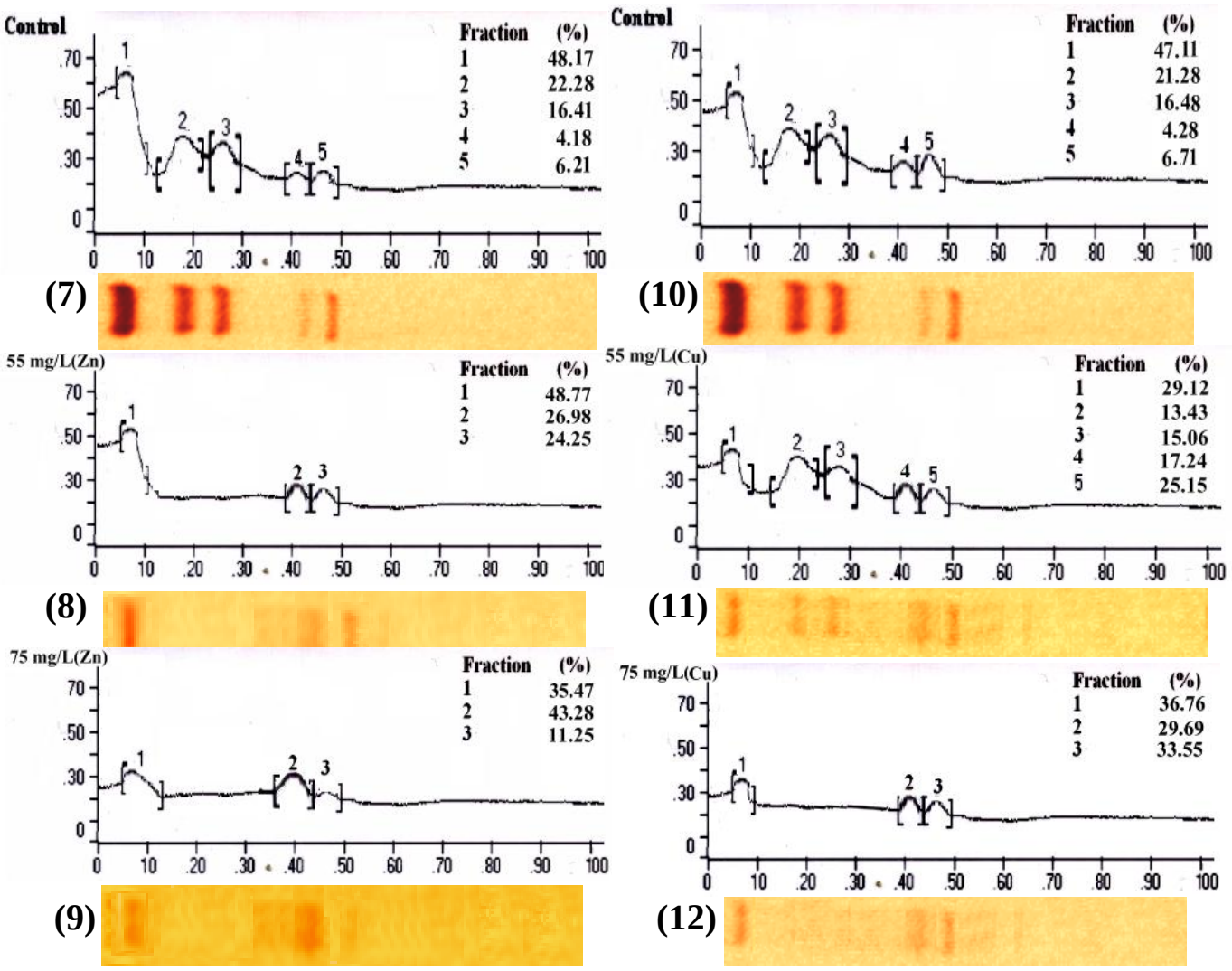
Samples / Bands	Control	Zn	
		Concentration 1	Concentration 2
b_1	87.91	51.12	59.94
b_2	72.09	48.88	40.05
b_3	83.60	39.03	47.46
b_4	79.94	-	-
b_5	48.88	-	-

Samples / Bands	Cu		
	Control	Concentration 1	Concentration 2
b_1	87.82	60.22	60.43
b_2	83.59	57.20	37.46
b_3	78.57	59.03	43.03
b_4	88.55	-	58.57
b_5	85.82	-	47.82

Concentration 1 = 75 mg / L^{-1}
Concentration 2 = 55 mg / L^{-1}



Figs. 1-6. Electrophoregrams of esterase isozymes obtained from digestive gland of the six control "untreated" *B. kewense* worm.



Figs. 7-12. Electrophoregrams of esterase isozymes obtained from digestive gland of the treated *B.kewense* with Zn (Figs. 7-9) and Cu (Figs. 10-12).

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