ALTERATION IN ELECTROPHORETIC MUSCLE PROTEIN AND ESTERASE ISOZYME AS AN EFFECT OF SOME HEAVY METALS IN TWO SPECIES OF TILAPIA

EBTEHAG A. KAMEL

Central Laboratory for Aquaculture Research, Abbassa, ARC, Egypt

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Abstract

Two species of tilapia (*Sarotherodon galilaeus* and *Tilapia zillii*) were used as experimental fish and treated with two doses (2 and 4 ppm) of cadmium (Cd), copper (Cu) and zinc (Zn) heavy metals at 96 and 192 hrs as common environmental pollutions. The results of electrophoretic analysis of muscle protein and esterase isozyme in this investigation revealed that both *Sarotherodon galilaeus* and *Tilapia zillii* species were affected by the treatments with two doses (2 and 4 ppm) of each metal. Some bands (represent the gene expression) were susceptible to pollution and disappeared from the treated fish. Muscle protein in fish exposed to 4 or 2 ppm of used heavy metals showed variations in the intensities and numbers of bands. The degree of polymorphism was high in all treatments. The intensities of bands were decreased after treatments compared with control. The correlation coefficients in band numbers of *Sarotherodon galilaeus* between control and treated fish with 4 ppm were negative at 96 hrs in Cd, Cu & Zn and at 192 hrs in Cd & Zn, while positive correlation coefficient was recorded for fish treated with 4 ppm of Cu at 192 hrs.

Tilapia zillii showed a new band in treatment with 4 ppm of Cd at 96 hrs. The correlation coefficient was positive in fish treated with 4 ppm of used heavy metals and control.

Esterase isozyme showed high polymorphism in treated fish and some new bands appeared. There was decrease in band numbers in some treatments in *Tilapia zillii,* while some new bands were appeared in *Sarotherodon galilaeus.*

INTRODUCTION

Environmental pollution is a major problem increased in each part in the earth. There is several sources cause contamination for the aquatic ecosystem by heavy metals such as activity of industry, agriculture, municipal ... ect. Environmental pollution may affect genetic variation in a population inhabiting polluted sites (Frati *et al.*, 1992). Pollution affects levels of enzyme activity and quantitatively and qualitatively electrophoretic results (Poly, 1997).

Eboh *et al.* (2006) reported the heavy metals contaminants in five commercial fish species included tilapia, catfish, ilisha, bonga and mudskipper. The levels of environmental heavy metals in *Tilapia nilotica* were determined in different tissues to assess Nasser Lake water pollution with toxic metals by Rashed (2001 a & b). Atli *et al.* (2006) studied the effect of heavy metals in *Oreochromis niloticus* and explained

their effect on catalase activity as an enzyme in antioxidant defense system which protecting animal from oxidative. Bezerra *et al.* (2005) found that Cd, Zn and Cu inhibited alkaline protease activity in *Oreochromis niloticus*.

Elghobashy *et al.* (2005) showed the effect of some heavy metals on protein and isozyme electrophoresis in Nile tilapia (*Oreochromis niloticus)*. Effect of heavy metals on electrophoretic pattern of protein from *Oreochromis niloticus* in lakes Maryout and Nozha was studied by El-Demerdash and Elagamy (1999). Ibrahim (2004) pointed out the effect of Cu treatment on some electrophoretic protein bands of *Oreochromis niloticus*. The accumulation of zinc, cadmium and mercury were recorded by Cuvin-Aralar (1994) in two *Oreochromis niloticus* strains.

The responses of fish populations to environmental stresses may be genetically dependent and allozymic patterns are promising tools for the genetic monitoring of environmental stresses (Virgilio et *al.*, 2003).

Zinc metal caused indicated significant differences in allozymes of Minnow populations (*Gambusia affinis, Pimephales notatus*, and *Fundulus notatus*) from contaminated creek and this results support for using the genetic structure and variability as a bioindicator for heavy metal contamination (Roark and Brown, 1996).

Electrophoretic analysis of muscle protein and esterase isozyme were used in this study to show the effect of some heavy metals such as cadmium (Cd), copper (Cu) and zinc (Zn) as common environmental pollutions on two species of tilapia (*Sarotherodon galilaeus* and *Tilapia zillii)* which were used as experimental fish and treated with two doses (2 and 4 ppm) of these heavy metals.

MATERIALS AND METHODS

Fish samples of *Sarotherodon galilaeus* and *Tilapia zillii* species were collected from fish farm at Abbassa, Sharkia. The weight of fish samples were 50 ±10 gm. Fish were transferred to laboratory and acclimated condition for two weeks under control condition. Each ten fish were kept in glass aquarium (120 L). Experimental fish were randomly distributed into seven groups with three replicates for each species. Fish were fed at a rate of 3% of the biomass per day. Fish were treated by Cadmium {(Cd) $CdSO_4. 8H_2O$}, Copper {(Cu) $CuSO_4. 5H_2O$} and zinc {(Zn) $ZnSO_4. 7H_2O$} were used at doses of 2 and 4 ppm. Then, fish were kept under observation.

Samples preparation

Both 96 and 192 hours from treatment were used in this experiment to study the effect of those heavy metals. Random samples of 10 fish, which were apparently healthy, were collected from each treatment and stored at −30 ^{0}C. Muscles from each sample were dissected and kept in a freezer immediately until used for protein and

isozyme electrophoretic analysis. Samples from each treatment were ground with 1ml of protein extraction buffer (1x), (10%SDS, 10ml Glycerol, 1M Tris-HCl and 0.25M EDTA, pH 8.8) and left in a refrigerator over night. Then, samples were vortexes for 15 seconds and centrifuged at 10,000 rpm at 5°C for 15 min. Finally, the clear supernatants containing water-soluble proteins were used.

Protein and Isozyme analyses.

These analyses were carried out at Molecular Cytogenetics Lab, Faculty of Agriculture, Ain Shams University.

SDS-PAGE of muscle soluble protein and Isozyme electrophoresis.

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) was used as a powerful technique for protein analysis. Muscles soluble protein fractions were separated exclusively on a vertical slab (19.8 cm x 26.8 cm x 0.3 cm) using the gel electrophoretic apparatus (Manufactured by LABCONCO) according to the method of Laemmli (1970) as modified by Payne (1976).

Polyacrylamide standard gel (9 %) was used to analyze esterase isozyme according to the method of Vander Bank *et al.* (1989).

Gels statistical analysis

Gels were scanned using UVP-Video Documentation System and analyzed with Gel Works 1D Image Analysis Software supplied from the Manufacture and IBM Compatible Personal Computer 165-2072. The densitometric scanning of such band was based on its three dimensional characters. Each band was recognized by its length, width and intensity. Accordingly, relative amount of each band quantity could be measured and scored.

RESULTS AND DISCUSSION

The results of electrophoretic analysis of muscle protein and esterase used in this investigation revealed that both *Sarotherodon galilaeus* and *Tilapia zillii* species were affected by the treatments with two doses (2 and 4 ppm) of cadmium (Cd), copper (Cu) or zinc (Zn). Figures (1, 2) showed the qualitative and quantity effects of cadmium (Cd) , copper (Cu) and zinc (Zn) treatments.

Effect of heavy metal on muscle protein

1- ***Sarotherodon galilaeus***: fish exposed to heavy metals (Cd, Cu or Zn) at 4 or 2 ppm showed decrease in number of bands compared to the control. Some bands were susceptible to pollution and disappeared from treated fish. The lowest number of bands was recorded in Zn (2ppm) at 192 hrs (13 bands), while the highest number of bands was recorded in Cd (2ppm) at 96 hrs (21 bands) compared to the control which showed 23-24 bands (Table 1). Fish treated with Cu (4 ppm) demonstrated 14-15

bands at 96 hrs, while they were 15-16 bands at 192 hrs. Treated fish with 2 ppm (Cd) at 96 hrs revealed 15-21 bands and 18-20 bands for 4 ppm at 192 hrs. Both Zn and Cu (4 ppm at 192 hrs) treatments showed the same stressed effect and some bands were absence (15-16 bands).

Table (2) showed the disappearance and absence frequency of bands which ranged from completely (100%) to partial (67 to 33%). Three bands were disappeared from total bands scored in fish samples treated with 4 and 2 ppm of Cd at 96 hrs while five bands in 2ppm of Cd at 192 hrs. The correlation coefficient values between band numbers in control and treated fish with 4 ppm were negative at 96 hrs in Cd, Cu, &Zn and at 192 hrs in Cd & Zn, while positive correlation coefficient value was recorded for fish treated with 4 ppm of Cu at 192 hrs and control (Table 3). The interaction between 4 & 2 ppm of heavy metals showed negative correlation coefficient values in Cd at 192 hrs and Zn at 96 hrs. While they were positive at Cd, Cu at 96 hrs and Cu, Zn at 192 hrs. Zero correlation coefficient value was recorded only in 2 ppm of Zn at 96 hrs and control fish.

2-***Tilapia zillii***: The control samples demonstrated 24-25 bands as shown in figure (2) and table (1). Fish treated with 4 ppm of Cd revealed 18-24 bands at 192 hrs, while they were 20-23 at 96 hrs. Zinc treatments showed very close effect for both concentrations at 96 hrs which were 18-21 bands for 4 ppm and 19-21 bands for 2 ppm. The exposure to 4 ppm Cu had low effect at 96 and 192 hrs which were obvious by number of bands 23-24 at 192 hrs and 21-23 at 96 hrs.

Table (2) showed that band No.26 was very sensitive to environmental pollution and disappeared completely from all treated *Tilapia zillii* at different concentration and time except for 2 ppm of Cd at 192 hrs. On other hand band No.17 was recorded in 4ppm of Cd at 96 hrs which represent new genotype. From table (2) it was clear that 4 ppm of Cu and Cd at 192 hrs showed similar effect and three bands were disappeared completely from treated fish.

The correlation coefficient values were positive in fish treated with 4 ppm of Cd, Cu and Zn at both 96 and 192 hrs and control (Table 3). Negative correlation coefficient values were recorded for the interaction between 2 & 4 ppm and 4 ppm & control in fish treated with Cu at 96 hrs and Zn at 192 hrs while, they were positive for all other treatments. Zero correlation coefficient value was recorded only in 2 ppm Cd at 192 hrs and control fish.

From the previous results, it was noticed that all used heavy metals in this study were affected muscle protein in tested fish. The number of bands was varied compared to control. Polymorphism was recorded in most treated samples. Some bands were susceptible to that environmental pollution and disappeared. This effect

maybe due to the DNA mutation and represented the changed in genes products, other process (physiological & biological) were related to the changes in gene products as effect of heavy metals. The results of this study were agreed with Rizkalla *et al.* (2006) who study the individual effect of Cd, Zn, Cu or in combination in carp fish. The results revealed the variation in relative mobility, intensity and number of bands. Sharf-Eldeen and Abdel-Hamid (2002) mentioned that expose the fish to copper metal induced a disappearance of some protein fractions and changed the relative electrophoretic mobilities that indicated genetic mutation. New band was recorded in this study which could find the proper environment to express its effect as genetic environment interaction. Elghobashy *et al.* (2005) reported that electrophoretic protein in *O. niloticus* fish showed variations in numbers of phenotypic bands, relative electrophoretic mobilities, optical densities and molecular weights. The variations between and within each site were clear. Some individuals showed sensitive genotype which represented by loss of some phenotypic banding patterns and pointed out that some genotypes were expressed as a result of pollution. Ibrahim (2004) reported that some protein bands were missing when *O. niloticus* exposed to different concentrations of copper and lead. While others appeared as new bands.

In this study some bands were susceptible to heavy metals and disappeared while others were more resistance. Farag (2001) recorded that some protein bands were missed while new bands were appeared in *O. niloticus* collected from polluted area in Altal Alkaber and Shader Azam where high concentration of lead, copper, zinc and iron.

El-Demerdash and Elagamy (1999) reported marked difference in electrophoretic patterns of protein in *O. niloticus* from Maryout Lake which where contained higher concentrations of cadmium than Nozha . Rashed *et al.* (1992) mentioned that changes in muscle protein electrophoresis of fish species after exposure to organophosphorus pesticide might be a result of pesticide stress and some unexpressed genes might have come to be expressed to contradict such effect. Manna and Mukherjee (1986) reported some variations in numbers, densities, and mobilities of protein bands as effect Malathion of organophosphate insecticide on tilapia. Manna and Sadhukhan (1992) noticed marked variations in protein bands in the functional aspect of genetic materials for the treatments of malathion, mercuric chloride and sodium arsenic for *O. mossambicus.* This results would very likely imply that these chemicals had affected the DNA which impaired genetic function and lead to the variation in bands.

Esterase Isozyme

Three distinct zones were recorded in *Tilapia zillii* (A,B,C) while four were recorded in *Sarotherodon galilaeus* (A,B,C,D) as shown in figure (3). The variations

were higher in the band intensities and there were polymorphisms in band numbers in all treatments (Figure 3 and Table 4)

Sarotherodon galilaeus

After the treatments the intensities of bands were decreased and some bands starting to disappeared (Figure 3 and Table 4). Most of effects were recorded in C and D zones. Two new bands were recorded in 4 ppm of Zn at 96 hrs (band No. 3 and 4) with heavy intensities. There was an increase in band numbers in some samples of the treatments with 4 ppm of Cu at 96 and 192 hrs, while decrease in one band was recorded in 4ppm of Cd at 96 hrs and 2 ppm of Cd at 192 hrs. There was equal effect of 4 ppm of Cd at 96 hrs and 4 ppm of Cu at 96 and 192 hrs. However, polymorphism was high in all treatments at different time of exposure.

Tilapia zillii

This species showed high variations in band intensities in all treatments. Also, there was decrease in number of bands in all treatments. Zn (2 & 4 ppm) and Cu (2 ppm) at 192 hrs showed the same number of bands (3-4 bands). The lowest number of bands was recorded in 2 ppm of Zn at 96 hrs.

Esterase isozyme showed high polymorphism in treated fish and some new bands were appeared. There was decrease in band numbers in some treatments in *Tilapia zillii,* while some bands increased in *Sarotherodon galilaeus.* These results were agreed with Bezerra *et al.* (2005) who reported that protease activity in *Oreochromis niloticus* was strongly inhibited by Cd followed by Cu and Zn heavy metals. Elghobashy *et al. (*2005) noticed variations in isozyme band numbers, optical density were related to sources of pollution. Roark and Brown (1996) noticed that allozyme was sensitive to zinc for Gpi and Pgdh loci, while it was not sensitive to lead. There were significant differences between creeks of the allozymes GPi in *F. notatus* and *P. notatus* species, Mdh in *G. affinis* and Pgdh in *F. notatus.* The proportion of heterozygous genotypes of *P. notatus* and *G. affinis* species were higher in contaminated creek fish. Almeida *et al.* (2002) noticed increase in LDH of red muscle, decreased in white muscle and changes in SOD in muscles of Nile tilapia (*Oreochromis niloticus*) which exposed to cadmium. Gillespie and Guttman (1988 & 1993) recorded that the genetic structure of allozyme in fish populations is sensitive to changes in water quality. Allele and genotype frequencies in fish populations from the field were varied according to exposure to contaminants. Certain allozyme genotypes of fish were more sensitive to toxic effects of contaminants than other genotypes. Long term exposure to contaminants by aquatic populations may decrease genetic diversity by selecting against sensitive to allozyme genotype and remaining population is therefore more vulnerable to extinction due to reduced ability to adapt to further environmental stress.

Significant variability in genotypic and allelic frequencies was detected in six gene loci of *H. diversicolor*. Patterns differentiation, which could be related to the contamination levels, were found at loci of LDH, PGI and SDH Virgilio *et al.* 2003).

Mishra and Shukla (2003) reported that endosulfan treatments reduced significantly the activity and specific activity of cMDH and mMDH but had no effect on total cytoplasm and mitochondrial protein contents. The PAGE showed the presence of one predominant specific LDH in liver as well as in muscles. This indicates that inhibitory effect of endosulfan on MDH and LDH skeletal muscle of fresh water catfish. Schlueter *et al.* (1995) recorded that there were relationships between allozyme and toxicity. The difference survivorship of individual with sensitive and resistant alleles was at MDH-2* and IDHP-I* loci. Survivors of copper exposure were genetically selected for copper resistance, resulting in the significant reduction in frequencies of several sensitive genotypes in the population.

Table 1. The effect of heavy metals on band numbers of tilapia species

Species	Time	96 hrs				192hrs				Control	
	heavy	4ppm		2ppm		4ppm		2ppm			
	metal	max	min	max	min	max	Min	max	min	max	min
S. galilaeus	Cd	20	19	21	15	19	18	20	18	24	23
	Cu	15	14	16	15	16	15	16	14		
	Zn	19	15	17	15	16	15	18	13		
T. zillii	Cd	23	20	23	18	24	18	24	22	25	24
	Cu	23	21	22	18	24	23	20	19		
	Zn	21	18	21	19	19	18	22	19		

Table 2 . The band numbers, its disappearance, absence and their percentage of muscle protein.

Species	Heavy metals	Time	Rate of band Disappearance		
			100%	67%	33%
Sarotherodon galilaeus	Cd 4ppm	96 hrs	2,16, 20	5,15, 24	23
	Cd 2ppm		2, 20, 22	5, 14, 15, 16	1,17, 18, 23, 24
	Cu 4ppm		1, 2, 6, 9, 16, 20, 22, 23, 24	3, 5	7
	Cu 2ppm		1, 2, 5, 8, 15, 20, 22, 23, 24	******	3
	Zn 4ppm		20, 22, 23, 24	1, 2, 5, 16, 19	6, 15
	Zn 2ppm		4, 16, 22, 23, 24	2, 15, 20	1, 3, 5, 7, 19
	Cd 4ppm	192 hrs	2, 5, 15, 16, 20, 22	23	*****
	Cd 2ppm		2, 5, 15, 22, 23	20	16
	Cu 4ppm		1, 2, 5, 8, 16, 20, 22, 23, 24	15	******
	Cu 2ppm		1, 2, 8, 15, 20, 22, 23, 24	2, 5	14, 16, 17, 18
	Zn 4ppm		1, 2, 8, 16, 20, 22, 23, 24	5, 15	6
	Zn 2ppm		2, 5, 16, 22, 23, 24	1, 8, 14, 15, 20	6
Tilapia zillii	Cd 4ppm	96 hrs	16, 24, 26,	15, 21, 22	2, 3
	Cd 2ppm		*****	3, 15, 20, 21, 22, 24, 26	5, 6, 16
	Cu 4ppm		21, 24, 26	3, 5, 8, 15	1, 2, 6, 16, 18, 20, 22
	Cu 2ppm		26	14, 22	1, 3, 16, 24
	Zn 4ppm		8, 15, 22, 24, 26	3, 5, 16	21
	Zn 2ppm		14, 15, 22, 24, 26	6	1, 5, 8, 21
	Cd 4ppm	192 hrs	21, 22, 24, 26	15	8, 23
	Cd 2ppm		21, 24, 26	8, 15	5, 6, 16, 18, 20, 22
	Cu 4ppm		22, 26	5	24
	Cu 2ppm		8, 14, 15, 22, 24, 26	******	3, 6, 16, 27
	Zn 4ppm		1, 5, 15, 24, 26	14, 16, 18, 19, 22	3
	Zn 2ppm		14, 15, 24, 26	3, 19	1, 5, 16

Table 3 . The correlation coefficient between band numbers in treatments and control.

Species	Time	96 hrs			192hrs		
S. gallilaeus	Heavy metals	2, 4 ppm	4 ppm	2 ppm	2, 4 ppm	4 ppm	2 ppm
	Cd	0.1890	-1.0000	-0.1890	-0.8660	-0.5000	0.8660
	Cu	0.5000	-0.5000	-1.0000	0.5000	1.0000	0.5000
	Zn	-0.2402	-0.9707	0.0000	0.9177	-0.5000	-0.8030
T. zillii	Cd	0.8660	0.9449	0.9820	0.5000	0.8660	0.0000
	Cu	-1.0000	0.5000	-0.5000	0.5000	0.5000	1.0000
	Zn	0.9820	0.9449	0.8660	-0.9449	0.5000	-0.1890

Table 4. Showed the variation in isozyme band numbers at different treatment and control.

Species	heavy metal	96 hrs	192 hrs	control
S. gallilaeus	CD 4ppm	5-6	6-7	6
	CD 2ppm	6	5-6	
	CU 4ppm	6-7	6-7	
	CU 2ppm	7-8	6	
	ZN 4ppm	8-9	5-7	
	ZN 2ppm	7-9	5-8	
T. zillii	CD 4ppm	3-6	4-5	5
	CD 2ppm	4-6	3-6	
	CU 4ppm	4	4-5	
	CU 2ppm	3-4	5-6	
	ZN 4ppm	4-5	3-4	
	ZN 2ppm	2-4	3-4	

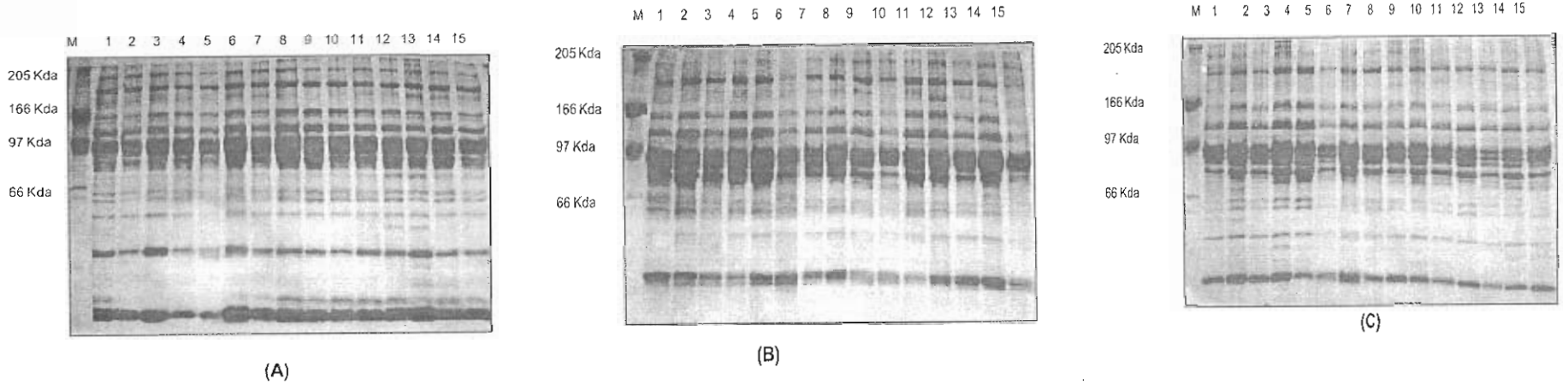

Fig.1. The effect of cadmium (Cd), copper (Cu) and zinc (Zn) treatments in the intensities and band numbers on muscle protein of *Sarotherodon galilaeus*

A: cadmium		B: copper		C: zinc	
1-3	Cd 4ppm at 96hrs	1-3	Cu 4ppm at 96hrs	1-3	Zn 4ppm at 96hrs
4-6	Cd 2ppm at 96hrs	4-6	Cu 2ppm at 96hrs	4-6	Zn 2ppm at 96hrs
7-9	Cd 4ppm at 192hrs	7-9	Cu 4ppm at 192hrs	7-9	Zn 4ppm at 192hrs
10-11	Cd 2ppm at 192hrs	10-11	Cu 2ppm at 192hrs	10-11	Zn 2ppm at 192hrs
12- 15	Control	12- 15	Control	12- 15	Control

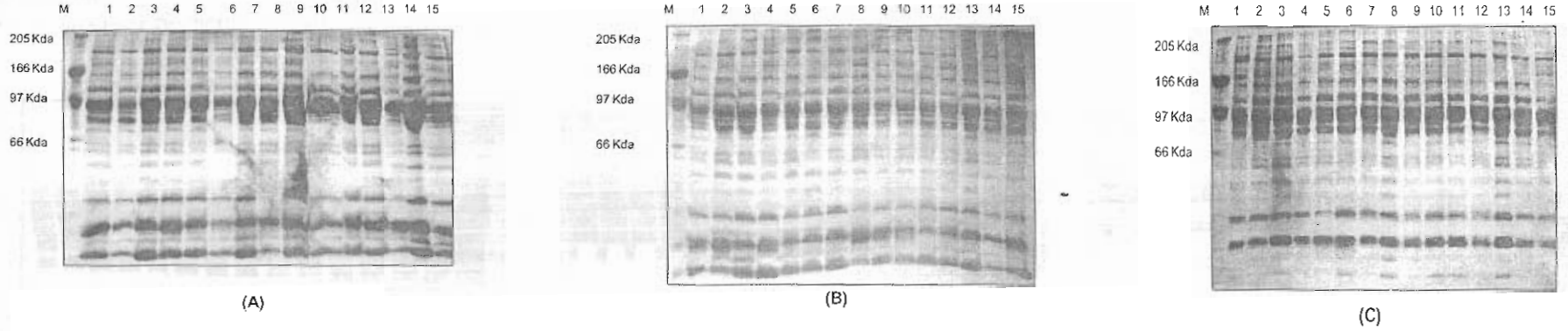

Fig. 2. The effect of cadmium (Cd), copper (Cu) and zinc (Zn) treatments in the intensities and band numbers on muscle protein of *Tilapia zillii*

A: cadmium		B: copper		C: zinc	
1-3	Cd 4ppm at 96hrs	1-3	Cu 4ppm at 96hrs	1-3	Zn 4ppm at 96hrs
4-6	Cd 2ppm at 96hrs	4-6	Cu 2ppm at 96hrs	4-6	Zn 2ppm at 96hrs
7-9	Cd 4ppm at 192hrs	7-9	Cu 4ppm at 192hrs	7-9	Zn 4ppm at 192hrs
10-11	Cd 2ppm at 192hrs	10-11	Cu 2ppm at 192hrs	10-11	Zn 2ppm at 192hrs
12- 15	Control	12- 15	Control	12- 15	Control

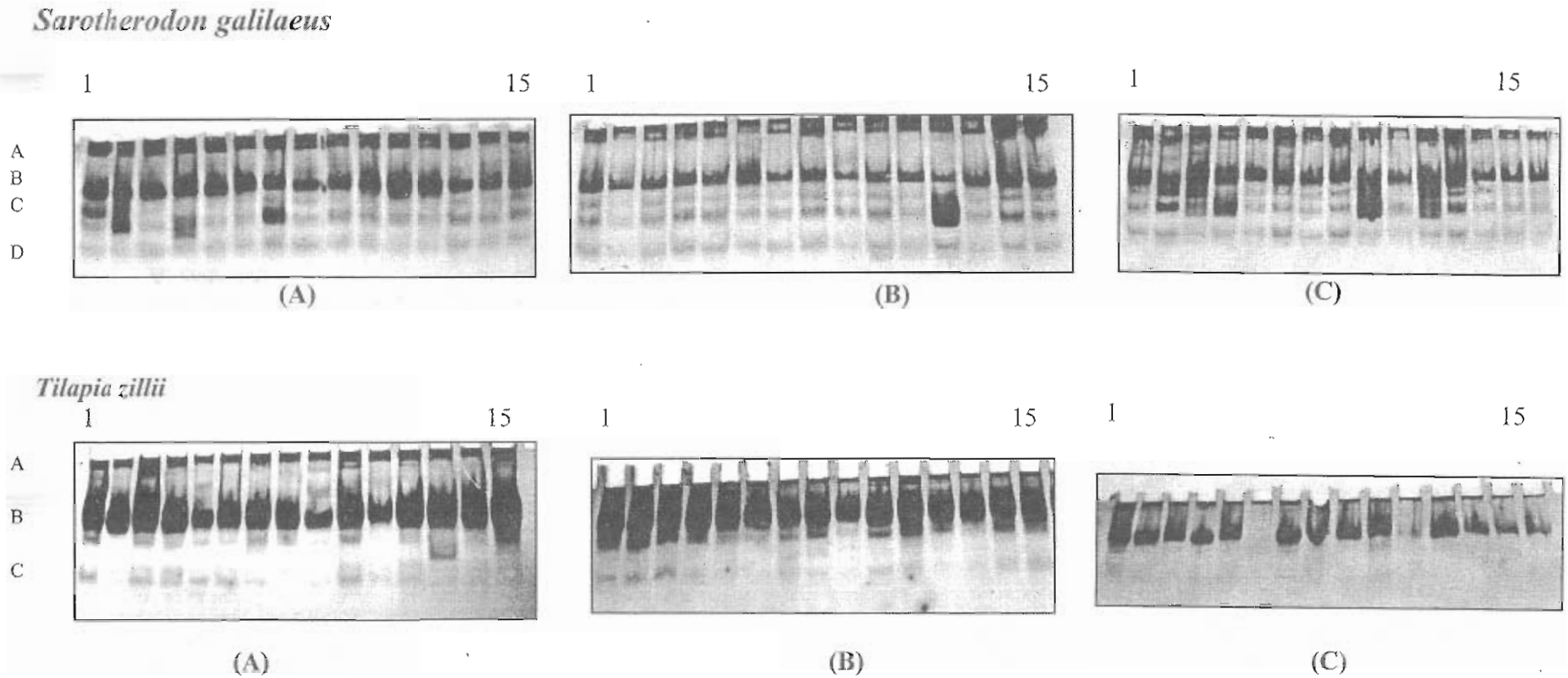

Fig. 3.The effect of cadmium (Cd), copper (Cu) and zinc (Zn) treatments on esterase isozyme of *Sarotherodon galilaeus* and *Tilapia zillii* species

A: cadmium		B: copper		C: zinc	
1-3	Cd 4ppm at 96hrs	1-3	Cu 4ppm at 96hrs	1-3	Zn 4ppm at 96hrs
4-6	Cd 2ppm at 96hrs	4-6	Cu 2ppm at 96hrs	4-6	Zn 2ppm at 96hrs
7-9	Cd 4ppm at 192hrs	7-9	Cu 4ppm at 192hrs	7-9	Zn 4ppm at 192hrs
10-11	Cd 2ppm at 192hrs	10-11	Cu 2ppm at 192hrs	10-11	Zn 2ppm at 192hrs
12- 15	Control	12- 15	Control	12- 15	Control

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التغيرات في التفريد الكهربى للبروتين العضلات ومتشابه انزيم الاستريز كتأثير لبعض العناصر الثقيلة في صنفين من البلطى

ابتهاج عبد الرازق كامل

قسم الوراثة –المعمل المركزي لبحوث الثروة السمكية بالعباسة

أجريت هذه التجربة علي صنفين من اسماك البلطي هما البلطي الجاليلي والبلطي الزيللي وتم معاملتهما بتركيزين هما ٤ و ٢ جزء في المليون من الكادميوم و النحاس و الزنك كملوثات بيئية.
وقد أظهرت نتائج تحليل التفريد الكهربي لبروتين العضلات ان كلا من البلطي الجاليلي و الزيللي قد تأثرا بكلا المعاملتين.
وقد تأثر التعبير الجيني للأسماك نتيجة التلوث البيئي الناتج عن التعرض لدرجات مختلفة من المعاملة بالمعادن الثقيلة حيث أن بعض الحزم كانت ذات حساسية للتلوث و قد اختفت كليا (١٠٠٪) او جزئيا (٣٠٪) من بروتين عضلات الأسماك والتي تعرضت إلى ٢ و ٤ جزء في المليون وقد أوضحت اختلاف في كثافة و أعداد الحزم.
وكان تعدد الأشكال فى بروتين العضلات كثيرا في كل المعاملات و قد قلت كثافة الحزم بعد المعاملة بالمقارنة بالأسماك غير المعاملة. وقد كان معامل الارتباط سالبا بين أسماك البلطى الجاليلي الغير المعامل والمعامل فى تركيز ٤ جزء فى المليون فى ٩٦ ساعة للكادميوم و النحاس والزنك و ١٩٢ ساعة للكادميوم و الزنك بينما كان معامل الارتباط موجبا لتركيز ٤ جزء فى المليون للنحاس فى ١٩٢ ساعة.
اما بروتين العضلات في البلطي الزيللي فقد أوضح حزمه جديده في المعاملة ٤ جزء في المليون للكادميوم في ٩٦ ساعة و قد كان معامل الارتباط موجبا في الأسماك المعاملة و غير المعاملة.

في حين أن مشابه انزيم الاستريز كان عاليا في الأسماك المعاملة و قد ظهرت بعض الحزم الجديدة وقد كان هناك انخفاض في عدد الحزم في بعض المعاملات في اسماك البلطي الزيللي بينما كان هناك زيادة في عدد الحزم في البلطي الجاليلي.