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Protein profile biomarker as bio indicator for aquatic pollution in Hila river, Iraq

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Abstract:

The freshwater species found in the Hilla River's water quality were assessed in the current study using a number of environmental biomarkers (molecular). Freshwater mussels (*Sesarma boulengeri*, *Unio tigridis*) and two kinds of fish (Tilapia zilli - Aspius vorax) were also collected throughout the six-month study period at three places along the Hilla River (from the Sinjar area to the north of the city to the Hashemia). When the level of statistical analysis was significant (p 0.05), there were significant links to the level of protein in the species of fish that were observed. The results of (physical and chemical parameters) were refers to that in three sites that more different, especially were in second site which was more pollutant. In addition to the moral distinctions between the locations, this also relates to the variance in water quality between them. As a result, the protein rate (expressed by the protein's Kda molecular weight) appeared. Several proteins were studied: Heat shock protein, 75 KDa as a response to stressors, and troponin, 180 KDa to 220 KDa, respectively, but in T. zilli, 28 KDa polypeptide as (rainbow trout by N-terminal), cortisol, 17 KDa, and Lysozyme, 17 KDa. Plasma membrane Ca ATPase 130 KDa and hemocynin 75 KDa are found in Sesarma boulengeri.Carbonic anyhydrase 28 KDa and apomyglobin 17 KDa in Uniotigridis. The purpose of this study was to ascertain the molecular weight of the proteins that appeared (protein profile, unknown proteins), for use in assessing the river's water quality.

Keywords: protein profile, biomarker, aquatic organism, water pollutant, Hilla river.

Introduction

Environmental stress may affects on the human health and cause imbalance of the ecosystem. Since ecosystems are complex systems, a number of different compensatory mechanisms operating at these levels. Using the environmental biomarker to assess ecosystem balance or organismal health is popular concept. These including biochemical, and histological biomarker [1, 2].

Characteristic protein expression patterns are observable in individual cells, communities of cells, organs, and whole organisms. Importantly, there are detectable differences in the patterns of protein expression in healthy versus compromised systems. Biomarkers can be used to measure the characteristic and dynamic pattern of protein expression that reflects the state of biological systems. [3, 4].

A biomarker that is dependable, reliable, and specific is needed for biomonitoring to determine the dangers to human health and numerous ecological challenges. Cellular and molecular biomarkers have a short response time to low levels of pollutants and some specificity for certain pollutant types. As a result, their reproducibility is enhanced, and their limitations are made clear [4].

In some study on aquatic species, adopting protein as an indicator of vital pollution and dependence on protein as biomarker. Biomarker where offered different concentrations of ammonium chloride and select the resulting protein type as a result of pressure exposure and this determines threat and the level of response to the contaminate aquatic organisms [5].

Nakisah *et al*. [6] conducted a study on gill, muscle and liver of fish *Acantho pagrusbutcheri*, they used Heat shock protein70, Only 11 fish per site are needed to detect significant intersite differences in muscle, but 14 and 21 fish per site, would be needed for gill and liver tissues. Field measurements of hsp70 should be supported by evidence of changes in other fish health indices.

in a study on zebra fish liver to see changes in the membrane protein profile after exposing those Fish to Methyl parathion , which are widely used for 24 hours using the different techniques , and found that nine proteins, seven of which were up-regulated and two of which were down-regulated, in membrane proteins. Quantitative real-time PCR was also used to examine the mRNA levels corresponding to these various membrane proteins. These findings may aid in the development of novel protein biomarkers for monitoring MP contamination levels in aquatic environments and beneficial insights into the hepatotoxic processes of MP. [7].

A study has demonstrated that the impact of several environmental stresses on blue crabs *Callinectes sapidus* and at the level of gene expression protein using the molecular weight as a bio indicator and done under conditions of 2-3 ppm for a period of five days. Using macro arrays, the expression of 10 partial genes cloned from the blue crab hepatopancereas was evaluated. It was found that Mn, SOD, hemocyanin, ribosomal S15, and L 23 expression were all considerably down-regulated (p 0.05). A down-regulation of hemocyanin transcription was also discovered by subtractive hybridization using RNA from normal and hypoxic hepatopancreatic tissue [8]. The aim of study to evaluated degree of damage in aquatic species through protein profile value in them.

Material and Methods

Description of the study area:

The first location, one of three on the Hilla River that were included in the study area, is located to the north of the city (Babylon Ancient site) This site is located approximately 7 kilometers from the city's center. Al-Farsi is located approximately 2 kilometers from the city center. Hashemia is located approximately 35 kilometers from the city center. The sites differ in terms of their composition and the variety of living things that can be found there Figure 1.

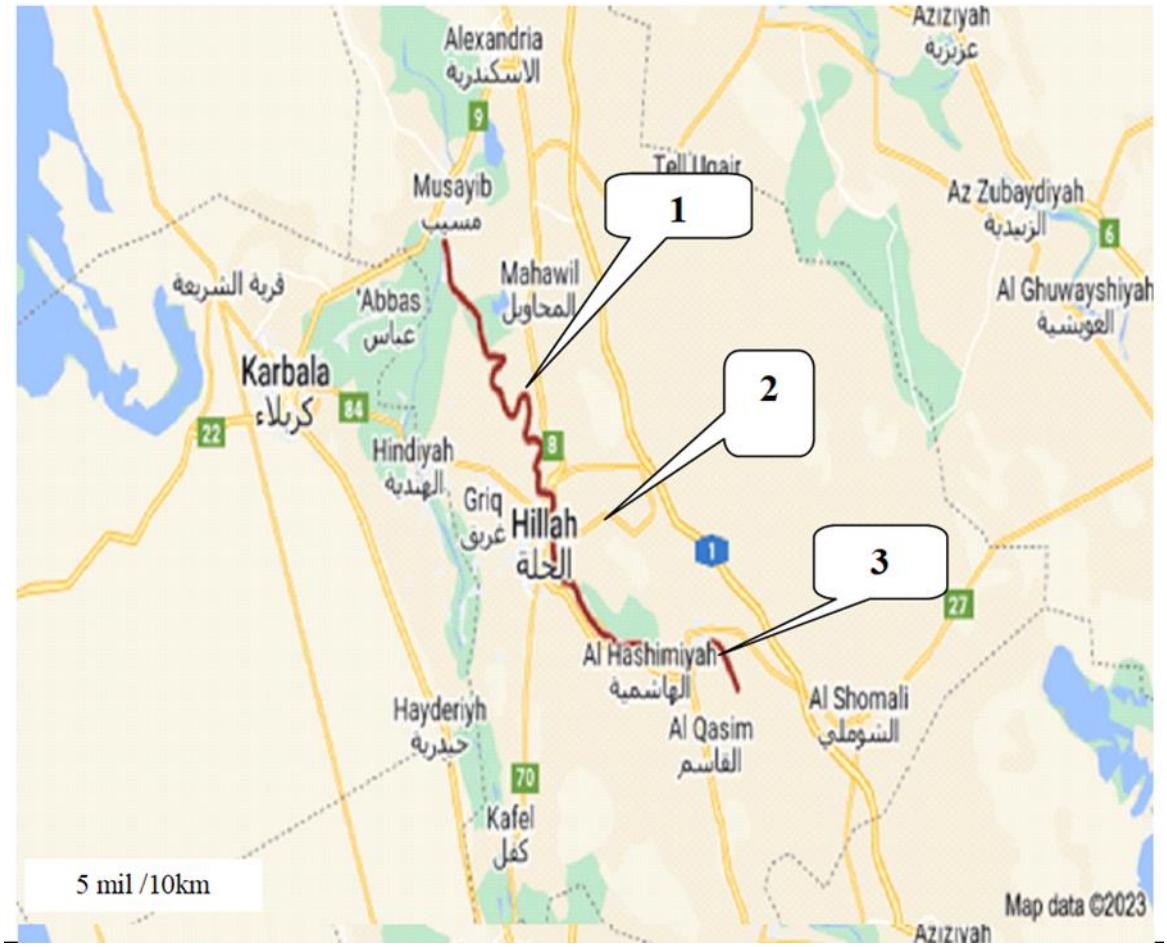

Fig. 1: Map of the Al Hilla - River (site 1,site 2,site 3)

A samples of fish, were collected from the study sites by small nets to maintain the vitality of the work while protein profile was extracted from the samples in laboratories (Fish - liver) and freeze for conducting tests.

Me trial and Method

Protein profile

By using the method described by Garfin, Electrophoresis of polyacrylamide gel with sodium dodecyl sulfate (SDS-PAGE), was used to evaluate the protein composition of mollusks [9]. The following are the solutions needed for this procedure, and adding 140 ml of deionized water were used to make the stock solution (30% acrylamide), which was then stored in a refrigerator at 4°C after being made. The resolving gel buffer solution is made up of 100 ml of deionized water and 12.11 g of Tris-base (1M Tris-HCl pH 8.8). (pH: 8.8). Keeping: R T , Then we attended The buffer solution for the stacking gel was made by dissolving 6.055 g of Tris-base in 100 ml of deionized water (0.5M Tris - HCl pH 6.8). (PH: 6.8) Preservation: Room Temperature. Then it was taken 50 g of sodium dodecyl sulfate (SDS) were dissolved in 350 ml of deionized water, and the amount was then increased to 500 ml by adding more deionized water. Stock sample buffer for 2X Laemmli samples: The process involved combining the following: Bromphenol blue (2.8 ml), D.W. (1.2 ml), 1M Tris-HCl pH 6.8 (1 mg),10% SDS (four milliliters), glycerol, and two milliliters] , Then we add Ammonium per sulfate (APS) 10% is made by mixing 1 ml of deionized water with 100 mg of APS , When employed in preparation gel, TEMED (N, N, N, N-tetramethylenediamine) is added to the final volume . Electrophoresis buffer 10 X: [Tris-base (0.025 M), Glycine (0.192 M), and SDS, (0.1% w/v)] and finished to 100ml Deionized D.W. staining stopper (Coomassie brilliant blue R-250) [Glacial acetic acid, Methanol, 45 ml of deionized water, and 0.25 g of Coomassie brilliant blue R-250 (10ml).
Glacial acetic acid (100ml), methanol, and deionized water (700ml)] (200ml). Then come step Preparation for resolving gel 10%. [30% Acrylamide (1.665 ml), 1 M Tris-HCL (1.875 ml), 10% SDS (50 l), 1.39 ml of deionized water, 2.5 l each of

10% APS and TEMED, and 1.39 ml The entire volume must not exceed 5ml. preparing gel for stacking. [30% Acrylamide (0.47), 0.5 M Tris-HCL (pH 6.8) (1.25), 10% SDS (25 l), Deionized D.W. (1.38), 10% APS (25 l), and TEMED (2.5 l) should be added together to make a total volume of 3 ml. loading sample, 2-6-5-3-1 The identical amount of stock sample buffer and standard protein solution were combined with ten microliters of samples, and each sample was then incubated at 95–100 Co for five minutes

Standard proteins (Protein Ladder).

Eneaid Company in the United States offers three different protein standards, ranging in molecular weight from 10 to 180 KDa. For monitoring protein separation during SDS-polyacrylamide gel electrophoresis, this ladder was created.

Electrophoresis:

A vertical electrophoresis chamber (CS-Cleaver scientific Ltd., U.K.) filled with 1X electrophoresis buffer was used to position the gel after casting. Each gel well received twenty microliters of samples. Electrophoresis was accomplished at 2 mA/gel at 40 volt for the initial moment, lasting for 30 minutes, and then at 20 mA/gel at 200 volt for the resolving stage, lasting for 1-2 hours [10]

once electrophoresis has finished. Gel was placed in a staining buffer for two hours while being shaken at room temperature. A de-staining buffer was then added.

The relative mobility (Rm) was attained based on the migration distance of samples and standard proteins from the starting point to the center of the protein band. From the beginning point to the dye band's center, the migration distance of bromophenol blue was measured. Some measurements of relative mobility (Rm) were made.

$$\textbf{(Relative Mobility) R.m.} = \frac{\text{Mobility of protein}}{\text{Mobility of bromophenol blue}}$$

Physical and Chemical Analysis

Temperture

Was measured the temperature of the water in sample collection sites using a device (Multi 350 i).

pH Value

was measured using a device (Multi 350i)(WTW -wissenschaftich) in the field directly and solutions used to calibrate the device organization.

Electrical conductivity :

electrical conductivity Was determined by using a device (Multi 350 i), Expressed the product in units of (µs/cm).

Total Dissolved Soild :

Was measured the (TDS) of the water in sample collection sites using a device (Multi 350 i). Expressed the product in units of (mg /L).

Total Soild Suspended :

Was measured the (TSS) of the water samples filtering(100) from sample on the filter paper (0.45) µ known Weight (B) and then drying the paper in a temperature oven (103-105) Cº for (24) hour and then was weighing (A) (APHA, 2003): . Expressed the product in units of (mg /L) , T.S.S.(mg/L) = (A-B) × 103 Of sample Volum .

Dissolved Oxygen (DO) :

Was measured of the water in sample collection sites using a device (Multi 350 i), Expressed the product in units of (mg/l). **Biology oxygen demand (BOD)** :

That method was used to (winkler) (APHA, 2003) and expressed in units of output in mg / l.

Results and Discussion:

The physicochemical parameters

Changes during this study that recorded in the study sites were varied and diverse, which included physical and chemical characteristics and that affected implied on some traits of the species under study follows water temperature aged between(12- 22 Cº) in the first site and(13-22 Cº)In a second site, (11-25 Cº)in the third site ,the results indicated the presence of significant differences($P< 0.05$),The highest value and the lowest value recorded in the third site was in the month October (2014) and Month November (2014), respectively and found significant positive correlation between temperatures. (PH) water in three sites was ranged between the values of(12- 8.70) in the first site (8.10- 11) in the second

site (6.70 – 12.3) in the third site .Electrical conductivity Values ranged between highest value .(1255µs/cm) in third site during march 2015 and with lowest value (1042 µs/cm) in third site also during October 2014.

The values of salinity is variation between the three sites were at (p< 0.05) has recorded the highest value (6%) in the first site during the October (2014) and the lowest (0.3%) but in the second site during the December (2014) did not study refers to, the values of (Total dissolved solid) in this study showed that significant changes at the level (P < 0.05) and ranged between(1082- 1237mg/g) in the first site(1092-1243mg/g) and second site and (1040-1254mg/g)in the third site, with the highest values in the third site through February 2015 and the lowest during November 2014While total suspended solids values ranged between study sites. (20.2-29.1 mg/g) in the first site(22.36 – 50.20 mg/g)in the second site (24.20 – 34.2 mg/g) and in the third site .The results of the study also indicated that there are significant differences at (p <0.05) values in dissolved oxygen in study sites where so the values ranged(1-6.80 mg/g) in the first site(1.50- 6.70 mg/g)in the second site and (24.2 – 34.2 mg/g)in the third site ,but in the first site .And ranged values(biological oxygen) in the results of the study to the existence of moral changes(p< 0.05) inorganic dissolved oxygen to water the three sites where values ranged between values (0.1 – 2.30 mg/g) in the first site (0.1 – 2.5 mg/g)in the second site(0.01- 3.5 mg/g)in the third site has registered the highest value (3.5mg/g) on the site third through October(2014) and the minimum value(0.01mg/g) through march(2015),. All previously mentioned values and significance differences were clarified in Table 1 and Figures (1-2).

Table 1 : physicochemical parameters in study sites Rang (first line) Mean±SD(second line) of some during study period

Sites	Site 1	Site 2	Site 3
Parameters			
W. Tem. . C°	**(12-22.8) (16.9667± 4.04063)**	**(13- 22) (17.3333 +- 3.82971)**	**(11-25) (17.1667 +- 4.833391)**
pH	**(8.70- 12.00) (9.6667 +- 1.20941)**	**(8.10-11.0) (10.3333+ - 1.14833)**	**(6.70-12.30) (9.3833 +- 1.87554)**
E.C(µc/cm)	**(1088-1236.0) (1.1937+ - 61.28513)**	**(1088-1236.0) (1.1927 +- 59.46315)**	**(1042-.1255) (1.1820 +- 58.92)**
Salinity ppt	**(0.50- 6.00) (1.5667+- 2.18785)**	**(0.50 – 0.60) (0.5500+- 0.05477)**	**(0.30- 6.00) (0.5167 +-0.050)**
TDS(mg/l)	**728.9-828.1 (1.1952 +- 64.16048)**	**728.9-828.1 (1.1957+- 60.06219)**	**(698.1- 840.8) (1.1855+-59.54829)**
TSS(mg/g)	**(20.2 – 29.1) (23.7550 +- 3.00009)**	**(22.63 – 50.20) (35.4717 +-4.4790)**	**(24.20-34.20) (29.1783+ - 3.99740)**
DO(mg/l)	**(1.5 -6.80) (5.6267 + - 2.27338)**	**(1.50- 6.70) (5.7167 + -2.07115)**	**(6.00- 9.00) (7.3833 +- 0.97245)**
BOD_5(mg/l)	**(0.1-2.30) (0.8967+-0.90778)**	**(0.1-2.5) (1.3000 +-0.98184)**	**(0.01- 3.5) (1.1850 +- 1.42582)**

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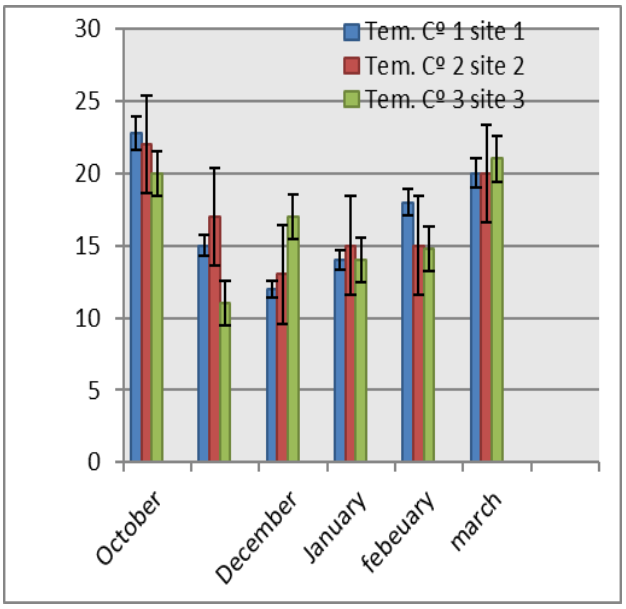

Fig. A: value of Tem. in 3 sites

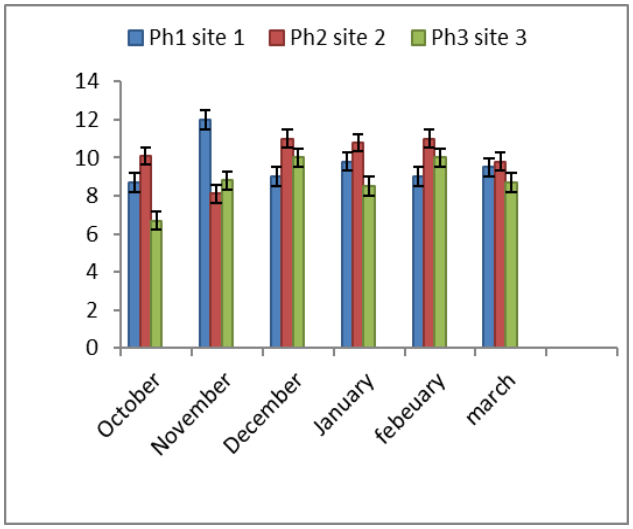

Fig. B :value of PH in 3 sites

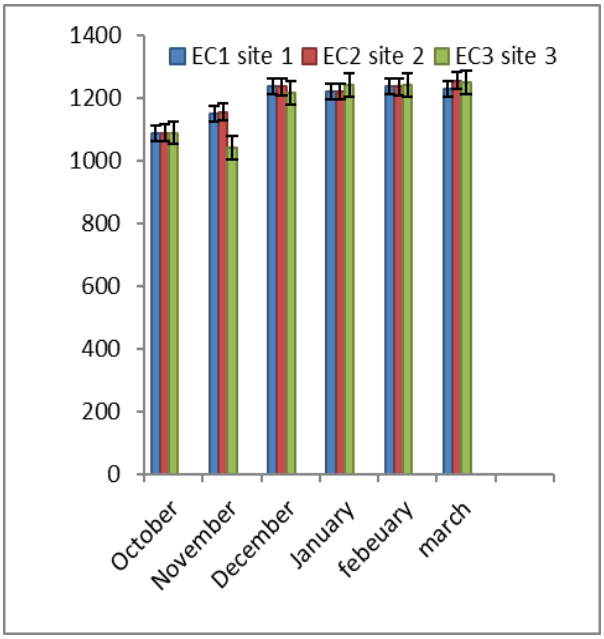

Fig. C: value of EC.in 3 sites

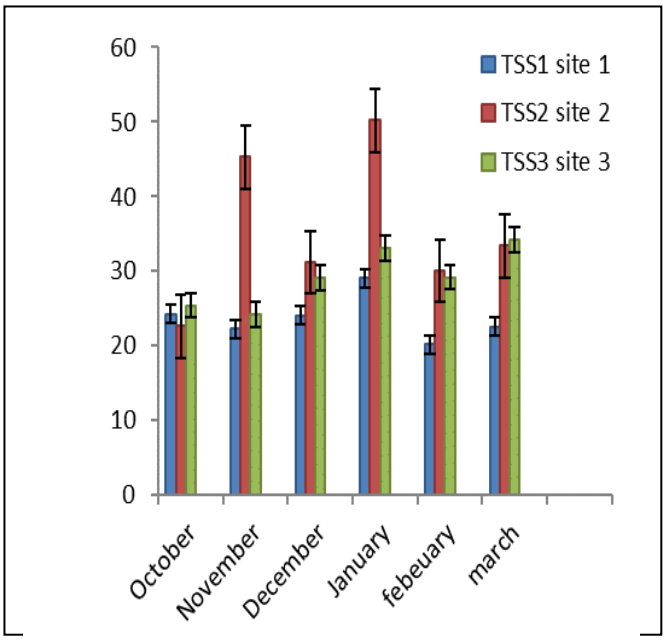

Fig. D: value of TSS.in 3 sites

Figure 1 : **A**: value of Temperature , **B**: value of PH, **C**: value of EC ,**D** value of TSS.: in 3 sites

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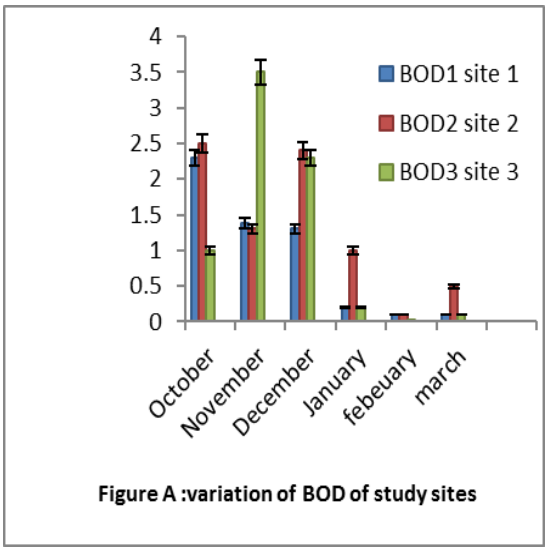

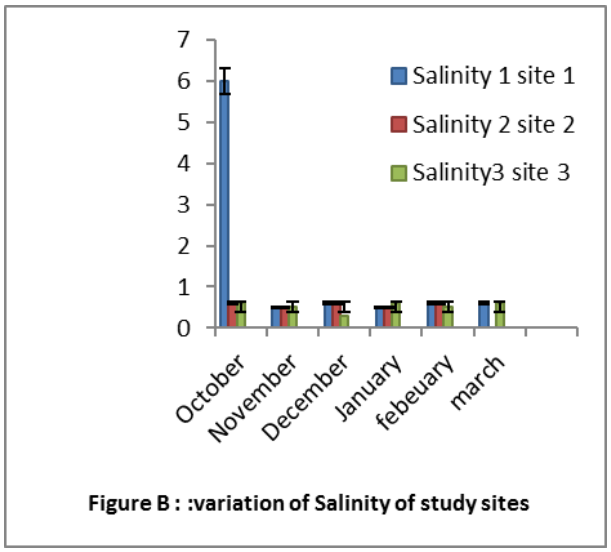

Fig. A: value BOD.in 3 sites **Fig. B: value of salinty.in 3 sites**

Figure 2 : **A**: value of BOD , **B**: value of salinty.: in 3 sites

The figure (3 , 4) showed that proteins have been identified in the species under study by comparing it with protein ladder which represents the molecular weights of proteins represented by(Kda) . There are several proteins were identified in studied T. zilli , A. vorax ,Sesarmaboulengeri ,Uniotigridis: Vitellogenin like protein 180 KDa to 220KDa , and Heat shock protein , 75 KDa as response to stresses , and Troponin 35 KDa, but in T. zilli : 28 KDa poly peptid as (rainbow trout by N-terminal), cortisol 17KDa, wihle in A.vorax Lysozyme 17 KDa .Sesarmaboulengeri, Uniotigridis : Plasma membrane Ca ATPase 130 KDa, Haemocynin 75KDa .In Uniotigridis: Carbonic anyhydrase 28KDa ,Apomyglobin 17 KDa .

Within ten minutes of micro-bial exposure to chemical pollutants in the following order: xylene, toluene, benzene, and TCE, protein yield and radioisotope incorporation were decreased. Different polypeptide profiles, radioactive polypeptide banding patterns, and radioisotope incorporation rates were formed as a result of the freshwater microbial community's adaptation to chemical contaminants before radioisotope incorporation [11].

In this study determine the molecular weights of unknown proteins, construct a standard curve by plotting the molecular weights of known protein markers on the y-axis against the migration distances of those markers on the x-axis. Representing the molecular weights on a logarithmic scale results in a linear.

through results (physical and chemical) in 3 sites that more different were in second site , for this reason ,the results of protein profile were more damage in spices in this site .

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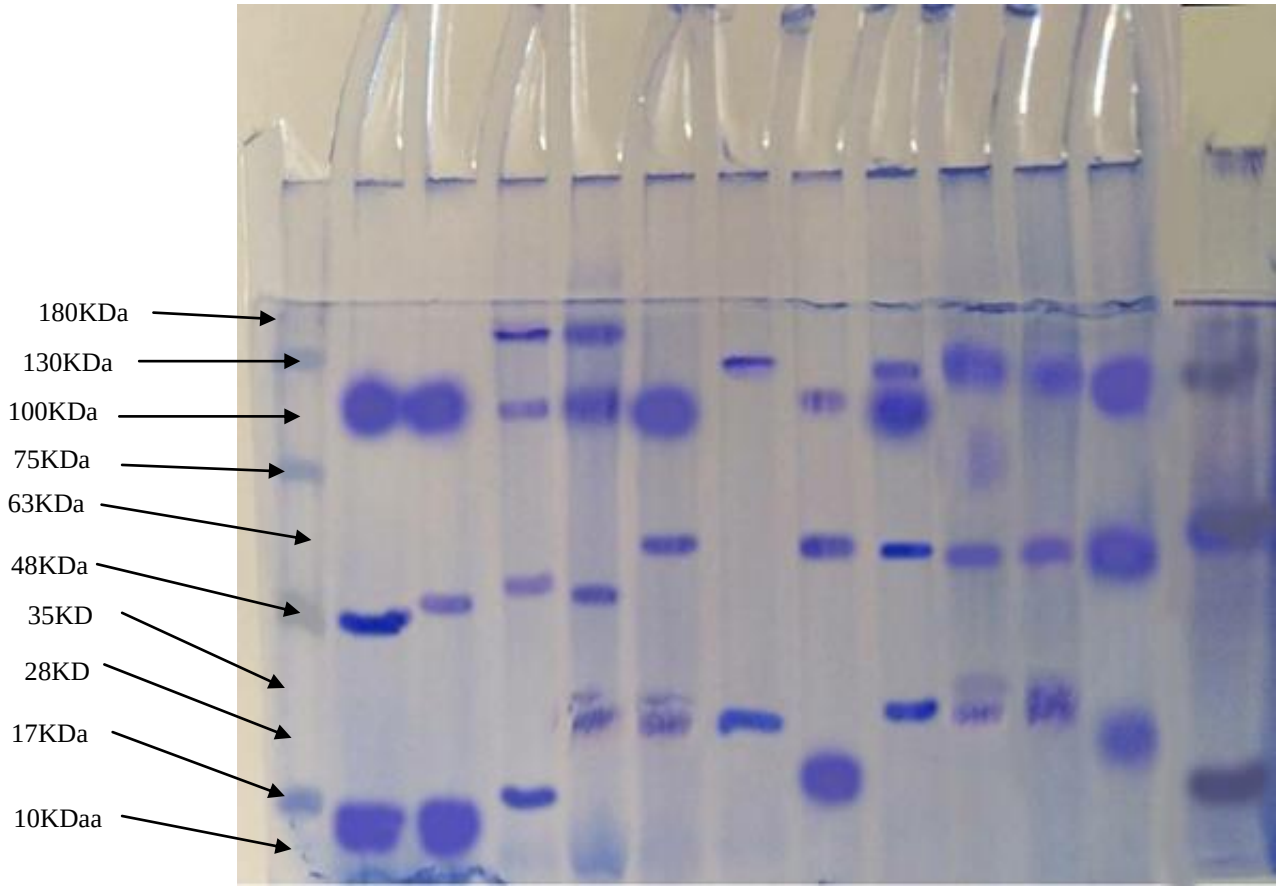

Fig. 3 : Ladder protein(1) p. profile of (U. tigridis (2-3-4), S. boulengeri(5-6-7) T. zilli(8-9-10) , A.vorax(12-13-14) according to SDS- PAGE

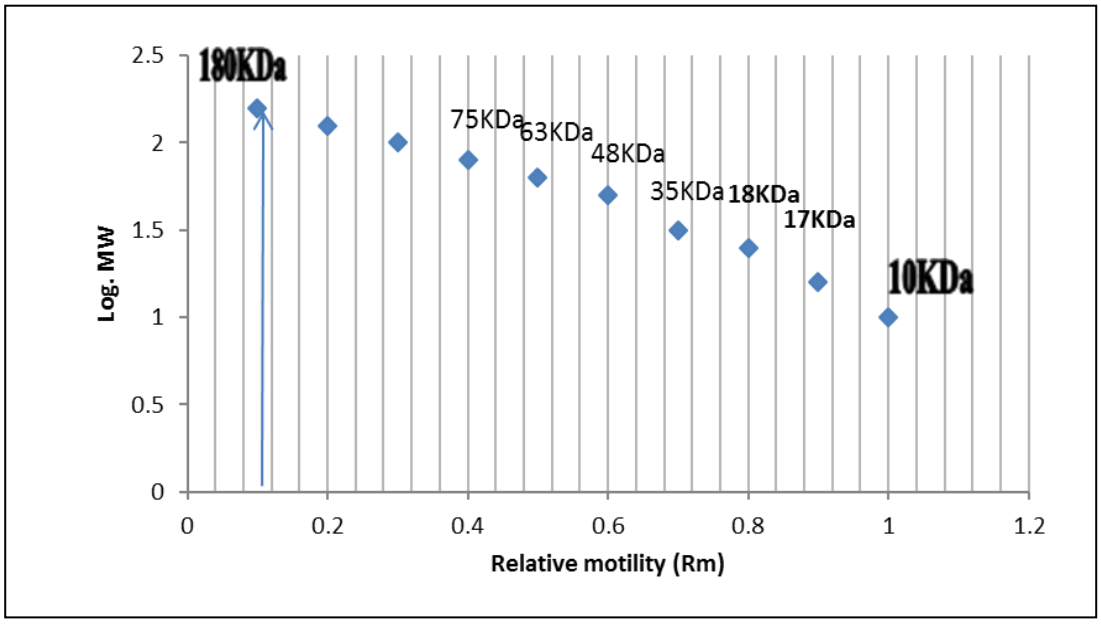

Fig. 4 : Standard curve for detection of Selected unknown proteins from Standard Protein Molecular weights.

More pollutants, whether heavy metals or organic compounds that are transformed or degraded by aquatic organisms, enter the environment due to the quick development of industry and agriculture [12].

Induction of stress protein synthesis by contaminant is reported to be highly tissue – specific in aquatic animal, among the tissue analysed (gills- skeletal –muscle-hepatopancreas) [13].

The results showed (SDS-page) for detection the types of proteins,such as Vitellogenin :like protien, heat shock protien as response to surrounding (vairty of xenobiotic)This protein back in the four types withinit molecular weight [14]. :Heat shock protein HSP and the level were correlated with the occurrence of DNA damage and auseful biomarker for monitoring of environmental pollution [15]. It finds in two species., Its reasons that lead to the production of these proteins increasing in hypoxic condition are one of major factors responsible for declines in estuarine habitate quality [16, 17].

The difficulty of determining the molecular and cellular functions of the many protein products encoded by prokaryotic and eukaryotic genomes has recently been addressed by breakthroughs in genomic and proteomic technologies. Particularly, chemical methods for proteome analysis have been developed, allowing for global protein activity profiling. Here, we highlight these chemical proteomic techniques and their use to identify and characterize enzyme activity linked to illness. [18]

Fish species have drawn a lot of attention in research examining biological and biochemical reactions to environmental contaminants for a variety of reasons. Following certain practical guidelines, monitoring species should be chosen from an exposed community based on their link to the assessment endpoint. Both requirements for evaluating the quality of aquatic environments are satisfied for many fish species. Fish may be found almost everywhere in the aquatic environment, and because they transport energy from lower to higher trophic levels, they play a significant ecological role in the aquatic food webs. So, there may be significant ecological value in studying fish behavior, toxicant uptake, and reaction. The majority of the generic biomarker requirements (see the introduction to this chapter) seem to apply equally to several fish biomarkers. Yet, there may be significant differences across fish species in terms of both the fundamental physiological characteristics and the susceptibility of some biomarkers to environmental pollution. Fish are typically regarded as the most practical creatures for monitoring pollution in aquatic environments, despite their limitations, such as a relatively high level of movement. [19, 20, 21].

Conclusions

High correlation between water quality and variation of environmental biomarker (protein profile) of some aquatic organism in Al- Hilla river. It is possible and necessary to consider the results of the study as real and supportive indicators for monitoring pollution in Shatt Al-Hillah. Environmental treatments must be taken in the 2site becauae was more pollution from another sites, order the results in this site for protein profile of organism .

Author Contributions Statement :

Shaimaa Satae M. Ali : working steps of the expremental and results analysis

Ayad M.J. Al-mamoori: Revision and correction (scientific and linguistic) .

Declaration of competing interest

The author confirms that there was no competing interest with others.

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