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Some Study on Heavy Metal and Bacterial infection Relationship in Some Cultured Marine Fish

Saad, T. T.

Poultry and Fish Disease Department, Faculty Of Veterinary Medicine, Alexandria University

ABSTRACT

This study was conducted for two main goals; Isolation and identification of the bacterial isolates affecting cultured marine fishes in Egypt as well as studying the relationship between heavy metal concentrations in water and fish tissues with the incidence of these bacterial diseases. In our study, A total number of (20) cage-cultured marine fishes (100 fish from Meagre and Grouper) were collected from Wadi-mariut region at Borg -El Arab city at Alexandria governorate . After culture on media and biochemical reactions, (90 isolates) from all naturally examined fish species were obtained as following: (50 isolates) from Meagre and (40 isolates) from Grouper. The incidence of bacterial isolates in the internal organs of all naturally examined fish species (Liver, Spleen, Heart and Kidney) has been studied and the results revealed that : The total number of bacterial isolates from liver was (100 isolates), (49 isolates) from heart, (62 isolates) from kidney and (39 isolates) from spleen and this means that the highest percentage of isolates were from Liver (about 40 %) from all bacterial isolates. The results revealed that : Heavy metal concentrations in water : Results showed an increase in all heavy metal concentrations above the permissible level in some cages and decrease in other cages (Due to the dilution factor) such as Copper, Cadmium, Lead, Mercury, Nickel and Iron while Zinc levels were lower than the permissible levels in all cages. Heavy metal concentrations in fish tissues: It is found that gills have the ability to accumulate heavy metals more than musculature. (a) In Meagre tissues: Zinc, Copper & Iron concentrations were lower in gills and musculature than permissible levels. On the other hand, Mercury and Lead were higher in gills than musculature as well as over the permissible levels. It worth to be noted that, Cadmium and Nickel concentrations were higher in gills than permissible levels and lower than their levels in musculature than the permissible levels. (b) In Grouper tissues: Copper and Zinc concentrations were lower in gills and musculature than permissible levels. Whereas, Iron and Cadmium concentrations were higher than permissible levels in gills as well as higher in their levels in musculature. While Mercury and Lead concentrations were higher in gills and musculature more than their permissible levels.

INTRODUCTION

Sadek (2000) cleared that Fish considered one of the main sources of the national income that Egypt depends on, stimulating local market economies, and important source of foreign exchange. Moreover, marine waters are the immediate alternative sources for water needed for mariculture and fortunately, Egypt has numerous marine resources of the Mediterranean and Red Seas.

Wedemeyer (1996) and Ellis (1999) mentioned that marine fishes represent the major investment choices for the national fishermen and also they are liable to variable number of environmental stressors, including chemical, natural and biological invaders. Such stressors are the main predisposing factors for the chronic immune suppression which ends for stimulating the bacterial invasion.

Bacterial diseases are responsible for heavy mortality in both wild and cultured fish and one of the most important factors of economic loss since the beginning of marine fish culture (Anderson and Conroy, 1970).

The prevalence of diseases and the number and types of bacterial pathogens have been well documented in several cultured and wild freshwater fish species, however, only a few bacteriological surveys on the prevalence of bacterial pathogens responsible for outbreaks in marine fishes. Therefore, we have to explore this field to know how we can protect the Egyptian Mari culture against these pathogens.

The present work aimed to study the following items

Monitoring of some heavy metal concentrations in both fish tissues and water of cages at Wadi-mariout in which fish reared in relation to the prevalence of bacterial diseases.

MATERIALS AND METHODS

A - MATERIAL

1. Fish

1.1. Naturally infected fishes

In our investigation, a total number of 200 cage-cultured marine fishes of different body weight range (50 g to 3 Kg) of four different species were collected; 100 of Grouper (*Epinephelus marginatus*) and 100 of Meagre (*Argyrosomus regius*) fishes were collected showing clinical signs from private fish farm at Wadi-Mariut region at west Alexandria governorate, Egypt.

1.2. Tools for determination of levels of heavy metals in both fish tissues and water samples

- Brown bottles for water samples and Polyethylene bags for fish samples.
- The samples were acidified by nitric acid.
- Ice box for transport of samples to the laboratory.
- Petri dishes, digestion flasks and ultra pure Conc. HNO_3 and H_2O_2 (1:1 v/v).
- Atomic absorption Spectrometer (Model Thermo Electron Corporation, S. Series AA Spectrometer with Gravities furnace, UK)

1.3. Disinfectants

70% ethyl alcohol solution: was used for disinfection of fish body surface before examination.

B- METHODS

1. Gross clinical examination

According to the method described by Amlacher (1970).

2. Postmortem (PM) examination

According to Conroy and Herman (1981).

3. Bacteriological examination

3.a. Sampling

The fish surfaces were swapped with 70 % ethyl alcohol for surface sterilization and then bacterial inocula were taken from liver, Kidney, spleen and heart under complete aseptic condition.

3.b. Bacterial culture

Smears from the above mentioned organs were cultured on tryptic soya broth (Difco, Detroit, MI, USA) supplemented with 3 % NaCl and incubated at 25 ^{0}C for 24 - 48 hrs then subcultured on Tryptic soya agar supplemented with 3% NaCl; TCBS (TCBS, Biolife, Milan, Italy) agar media supplemented with 3% NaCl; Blood agar media using 5% sheep RBCs supplemented with 3 % NaCl. The inoculated plates were incubated at 25 ^{0}C for 24 - 48 hrs.

3.c. Identification of bacterial isolates

According to the methods described by Austin and Austin (1999).

4. Serotyping of the bacterial isolates

4.a. Bacterial strains

All bacterial isolates were used as antigens in the agglutination tests. The strains were isolated from epizootics occurring in the Wadi-Mariut area at Alexandria governorate.

Stock cultures of the isolates were maintained by periodic transfer on tryptic soy agar (Oxoid Manual, 1982) slants with the appropriate salt concentration. For long term storage, cultures were kept at - 80 C^0 in medium with 15% glycerol.

4.b. Preparation of somatic "0" antigens

The bacteria were grown on brain-heart infusion agar (BHIA) (Difco) plates for 24-48 h at 25 C^0, harvested with phosphate-buffered saline (PBS), pH 7.2, and washed by centrifugation until clear. Cells from each isolate were re suspended in PBS (10% v/v) and then steamed at 100°C for one hour. Bacteria were again harvested by centrifugation and re suspended in PBS to obtain an adequate density (McFarland standard No. 3).

4.c. Slide agglutination tests

The tests were performed by mixing a drop of bacterial antigen suspension with a drop of undiluted or l/5 diluted antiserum. One antigen preparation was examined simultaneously with the different antisera using a multi well glass slide according to method described by ***(Toranzo et al., 1987).***

5. Spectrophotometeric method for detection the levels of heavy metals in water and fish tissues

5.1. Sampling

5.1.a. Water samples

Water samples were collected from different cages at Wadi-Mariuot area, where as the samples were taken during the period of collection of naturally infected fishes in dark brown bottles and the column sampler at a depth of half meter from the water surface. The samples were acidified by nitric acid and chilled on ice box for transport to the laboratory for heavy metals determination.

5.1.b. Fish sampling

At the same time, specimens of Megrea and Grooper were collected from the same areas. After dissection of fishes, parts of gills and dorsal musculature were carefully removed and prepared for metal analysis.

5.2. Determination of heavy metals levels

The method for analysis of the heavy metals in the water was carried out according to APHA (1995) and in the fish tissues according to Clesceri (1998) that was carried out using Atomic Absorption Spectrophotometery.

Atomic Absorption Spectrophotometer (Model Thermo Electron Corporation, S. Series AA Spectrometer with Gravities furnace, UK,) instrument was used to detect the heavy metals. The concentrations of heavy metals were expressed as mg/l for water and µg/g dry wt. for fish tissues. Fish specimens were digested according to AOAC (1996).

6. Statistical analysis

The data of bacteriological examinations and heavy metal concentrations were statistically analyzed using Chi-square and ANOVA tests according to (SAS, 1987). After that the results presented in the form of figures according to Harvard graphics 4 computer programs.

RESULTS

1. Results of naturally collected fishes

1.1.a. Clinical signs of naturally examined Meagre

Some naturally examined Meagre showing erythema and reddening of the mouth with opaqueness on the eye (Fig. 1); hemorrhagic caudal and anal fins (Fig. 2); erosions and hemorrhages at caudal fin and reddening around the anal opening (Fig. 3); while others show ulceration at the caudal peduncle with hemorrhagic tail fin (Fig. 4).

1.1.b. PM lesions of naturally examined Meagre

Some naturally examined Meagre showing hemorrhagic ascetic fluid (arrow) and congestion of gills (Fig. 5) while other fish showing whitish nodules in liver (arrow) (Fig. 6).

Fig. (1): A naturally examined Meagre showing erythema and reddening of the mouth with opaqueness on the eye.

Fig. (2): A naturally examined Meagre showing hemorrhagic caudal fin in all fish and also at anal fins.

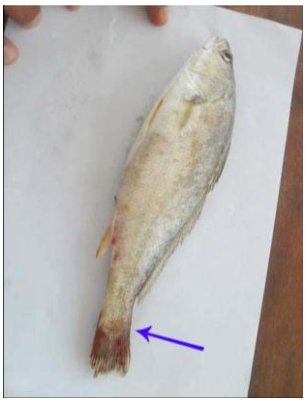

Fig. (3) : A naturally examined Meagre showing erosions and hemorrhages at caudal fin and reddening a round the anal opening.

Fig. (4): A naturally examined Meagre showing ulceration at the caudal peduncle with hemorrhagic tail fin.

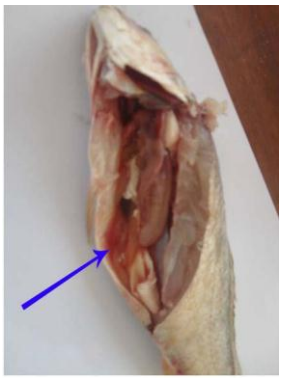

Fig. (5): A naturally examined Meagre showing hemorrhagic ascetic fluid (arrow) and congestion of gills.

Fig. (6): A naturally examined Meagre showing paleness color of the liver (arrow) and serous ascetic fluid in other fish.

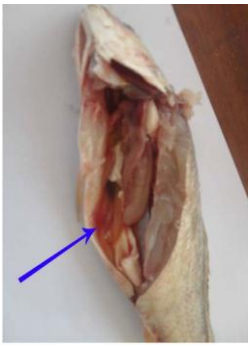

Fig. (7): A naturally examined Grouper showing congestion at liver and at the intestine.

Fig. (8): A naturally examined Grouper showing severe congestion at gills and liver (arrow) in comparison with other fish.

2. Clinical signs of naturally examined grouper

A naturally examined Grouper showing opaqueness, hemorrhages and bulging of the eye (exophthalmia) (arrows) (Fig. 9) as well as there was ulceration in between eyes of the examined fish (Fig. 12).

2.1. PM lesions of naturally examined grouper

Naturally examined Groupers showing congestion of the gills (Fig. 10); congestion in spleen and intestine at (Fig. 11).

Fig. (9): A naturally examined Grouper showing opaqueness, hemorrhages and bulging of the eye (exophthalmia) (arrows).

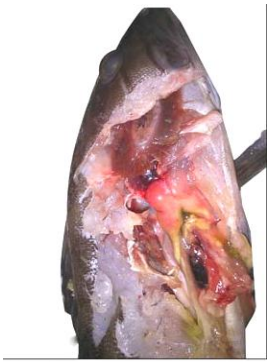

Fig. (10): A naturally examined Grouper showing congestion in the gills and in the internal body organs.

Fig. (11): A naturally examined Grouper showing ulceration in between the eyes and on the body surface .

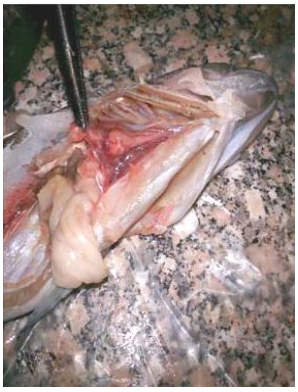

Fig. (12) : A naturally examined Grouper showing congestion in internal organs.

3. Prevalence of bacterial serotypes among naturally infected Meagre

According to Table (1) and Fig. (6); the highly prevalent bacterial serotype in naturally infected Meagre was *V. alginolyticus* (10 %), *T. maritimum* was (8 %) followed by *V. harveyi* that was (6 %).

4. Prevalence of bacterial serotypes among naturally infected Grouper

According to Table (2) and Fig. (7); the highly prevalent bacterial serotype in naturally infected Grouper was *V. alginolyticus*, *Vagococcus salmoninarum*, *Ps. aeruginosa* and *Ps. anguilliseptica* (7.5 % for each serotype).

6. Total prevalence of bacterial serotypes retrieved from the internal organs of all naturally infected marine fishes

The total prevalence of bacterial serotypes retrieved from the internal organs of all naturally infected marine fishes was illustrated in Table (4) and Fig. (9) Which showed that the high incidence of bacterial serotypes was in Liver (40 %), followed by the kidney (24.80 %), (19.60 %) in the heart and the lowest incidence occur in Spleen (15.60 %).

Table (1) : Prevalence of different bacterial isolates in Meagre.

Bacterial isolates	Number of isolates	Prevalence (%)
Ph. damselae subsp. Piscicida	2	4
Ph. damselae subsp. damselae	2	4
Vagococcus salmoninarum	0	0
Strept. parauberis	1	2
Strept. agalactiae	1	2
Strept. iniae	3	6
Lactococcus garvieae	1	2
Lactococcus piscium	1	2
Tenacibaculum maritimum	4	8
V. anguillarum serotype 01	2	4
V. alginolyticus	5	10
V. vulnificus biotype 1	1	2
V. viscosus	0	0
V. harveyi	3	6
V. vulnificus biotype 2	1	2
V. ordalii	4	8
V. anguillarum serotype 023	2	4
Ps. Aeruginosa	0	0
Ps. Pleoglossicida	3	6
Ps. Fluorescens	2	4
Ps. Chlororaphis	1	2
Ps. Anguilliseptica	0	0
Total number of isolates	**50 isolates**	

Table (2) : Prevalence of different bacterial isolates in Grouper.

Bacterial isolates	Number of isolates	Prevalence (%)
Ph. damselae subsp. Piscicida	2	5
Ph. damselae subsp. Damselae	2	5
Vagococcus salmoninarum	3	7.5
Strept. Parauberis	2	5
Strept. Agalactiae	**2**	**5**
Strept. Iniae	1	2.5
Lactococcus garvieae	2	5
Lactococcus piscium	2	5
Tenacibaculum maritimum	2	5
V. anguillarum serotype 01	1	2.5
V. alginolyticus	3	7.5
V. vulnificus biotype 1	2	5
V. viscosus	**1**	**2.5**
V. harveyi	2	5
V. vulnificus biotype 2	2	5
V. ordalii	1	2.5
V. anguillarum serotype 023	1	2.5
Ps. aeruginosa	**3**	**7.5**
Ps. pleoglossicida	0	0
Ps. fluorescens	1	2.5
Ps. chlororaphis	2	5
Ps. anguilliseptica	3	7.5
Total number of isolates	**40 isolates**	

Table (3): The prevalence of bacterial isolates in different organs of naturally infected marine fishes:

Fish / Organ	Meagre		Grouper	
	No.	**%**	**No.**	**%**
Liver	20	8	15	6
Heart	4	1.6	5	2
Kidney	16	6.4	16	6.4
Spleen	10	4	4	1.6
Total	50	20	40	16

5. The prevalence of bacterial isolates in the internal organs of the naturally infected marine fishes

The incidence of bacterial isolates retrieved from internal organs in each fish species of naturally infected marine fishes was illustrated in Table (3). and Fig. (8).

7. Results of heavy metal concentrations in both water and fish tissues

7.1. Copper (Cu) levels

Copper (Cu) level was higher in Cage (IV) and Cage (III) (0.68±0.001) and (0.513±0.001) than permissible level (0.2) while Copper level was lower than permissible level in Cage (II and Cage (I) respectively.

7.2. Zinc (Zn) levels

Zinc (Zn) levels were lower in all cages than the permissible level where as Zinc levels were 0.464±0.001, 0.050±0.001, 0.026±0.001 and 0.016±0.001 in cage IV, III, II and I respectively and they have no toxic effects.

7.3. Cadmium (Cd) levels

Cadmium levels were higher in all water samples from all cages over the permissible level so that it considered toxic for

Table (4): The total prevalence of bacterial serotypes retrieved from the internal organs of all naturally infected marine fishes.

Strains \ Organs	Liver		Heart		Kidney		Spleen	
	No.	%	No.	%	No.	%	No.	%
Ph. damselae subsp. piscicida	4	1.6	2	0.8	5	2	2	0.8
Ph. damselae subsp. Damselae	2	0.8	4	1.6	4	1.6	2	0.8
Vago. Salmoninarum	4	1.6	2	0.8	1	0.4	1	0.4
Strept. Parauberis	5	2	2	0.8	2	0.8	1	0.4
Strept. Agalactiae	2	0.8	2	0.8	2	0.8	2	0.8
Strept. Iniae	3	1.2	1	0.4	2	0.8	3	1.2
Lactococcus garvieae	6	2.4	2	0.8	2	0.8	2	0.8
Lactococcus piscium	2	0.8	2	0.8	2	0.8	2	0.8
Tenacibaculum maritimum	9	3.6	6	2.4	4	1.6	2	0.8
V. anguillarum serotype 01	2	1.2	1	0.4	3	1.2	3	1.2
V. alginolyticus	17	6.8	12	4.8	4	1.6	2	0.8
V. vulnificus biotype 1	2	0.8	1	0.4	3	1.2	1	0.4
V. viscosus	2	0.8	1	0.4	2	0.8	1	0.4
V. harveyi	6	2.4	2	0.8	5	2	2	0.8
V. vulnificus biotype 2	3	1.2	1	0.4	2	0.8	2	0.8
V. ordalii	5	2	1	0.4	3	1.2	1	0.4
V. anguillarum serotype 023	8	3.2	1	0.4	3	1.2	1	0.4
Ps. Aeruginosa	3	1.2	2	0.8	2	0.8	2	0.8
Ps. Pleoglossicida	4	1.6	1	0.4	3	1.2	2	0.8
Ps. Fluorescens	4	1.6	1	0.4	3	1.2	2	0.8
Ps. Chlororaphis	3	1.2	1	0.4	2	0.8	1	1.2
Ps. Anguilliseptica	4	1.6	1	0.4	3	1.2	2	0.8
Total number of isolates	**100**	**40 %**	**49**	**19.60 %**	**62**	**24.80 %**	**39**	**15.6 %**

all fishes. Cadmium levels follow this order: Cage IV > Cage III > Cage I > Cage II over the permissible level.

7.4. Lead (Pb) levels

Lead levels were higher in Cage (III) and (II) over the permissible level while in Cage (I) and Cage (IV); Pb levels were lower than the permissible levels.

7.5. Mercury (Hg) levels

Mercury levels were higher in Cage I, II and III than the permissible levels. Moreover, Mercury levels follow the following order: Cage III > Cage II > Cage I > Cage IV over the permissible level.

7.6. Iron (Fe) levels

Iron levels were higher in all water samples from all cages over the permissible level. Moreover, Iron levels follow the following order: Cage III > Cage IV > Cage I > Cage II.

Table (5): Heavy metal concentrations (mg/L) in water samples from different cages in comparison with the permissible levels :

Element	Cage (I)	Cage (II)	Cage (III)	Cage (IV)	Permissible level
Copper (Cu)	E 0.011±0.001	D 0.035±0.001	B 0.513±0.001	A 0.68±0.001	C 0.2 *
Zinc (Zn)	E 0.016±0.001	D 0.026±0.001	C 0.050±0.001	B 0.464±0.001	A 2.0 *
Cadmium (Cd)	C 0.007±0.001	D 0.005±0.001	B 0.009±0.001	A 0.044±0.001	E 0.004 **
Lead (Pb)	D 0.028±0.001	B 0.065±0.001	A 0.099±0.001	E 0.012±0.001	C 0.050 **
Mercury (Hg)	C 0.002±0.001	B 0.003±0.001	A 0.004±0.001	D 0.001±0.001	D 0.001 **
Iron (Fe)	C 0.570±0.001	D 0.425±0.001	A 1.420±0.001	B 0.720±0.001	E 1.00 ***
Nickel (Ni)	D 0.002±0.001	A 0.005±0.001	B 0.004±0.001	C 0.003±0.001	E 0.001 **

Means within the same row of different litters are significantly different at (P < 0.01) .

7.7. Nickel (Ni) levels

Nickel levels were higher in all water samples from all cages over the permissible level. Moreover, Nickel levels follow the following order: Cage II > Cage III > Cage IV > Cage I.

8. Heavy metal concentrations (µg/g dry weight) in gills and musculature of Meagre from Wadi-Mariut :

According to Table (6) and Fig. (11); generally, all heavy metal concentrations were higher in Gills more than musculature in both Meagre and Grouper.

In comparison with the permissible levels (PL) of heavy metals; Zinc (Zn), Copper (Cu) and Iron (Fe) levels were lower in gills and musculature samples than the PL While Mercury (Hg) and Lead (Pb) levels were higher in Gill samples over the musculature samples over the PL and have the following order; Gills > Musculature > PL. On the other hand; Cadmium (Cd) and Nickel (Ni) levels were higher in Gill samples over the PL and their levels in musculature samples are the lowest one and they have this order; Gills > PL > Musculature.

9. Heavy metal concentrations (µg/g dry weight) in gills and musculature of Grouper from Wadi-Mariut

According to Table (7) and Fig. (12); In comparison with the permissible levels (PL) of heavy metals; Zinc (Zn) and Copper (Cu) levels were lower in gills and musculature samples than the PL While Iron (Fe) and Cadmium (Cd) levels were higher in Gill samples over the PL and their levels in musculature samples are the lowest one and they have this order; Gills > PL > Musculature.

On the other hand; Mercury (Hg), Lead (Pb) and Nickel (Ni) levels were higher in Gills samples over musculature samples over the permissible levels and follow this order; Gills > Musculature > PL.

Table (6) : Heavy metal concentrations (µg/g dry weight) in gills and musculature of Meagre from Wadi-Mariut

	Gills	Musculature	PL *
	Mean± S. E.	Mean± S. E.	
Zinc (Zn)	B 21.69±0.24	C 13.58±0.09	A 60.0
Copper (Cu)	B 0.55±0.0034	C 0.26±0.0026	A 3.0
Iron (Fe)	B 38.15±0.89	C 20.52±0.32	A 50.0
Nickel (Ni)	A 1.39±0.11	C 0.36±0.15	B 0.50
Mercury (Hg)	A 1.66±0.0086	B 0.73±0.0024	C 0.50
Cadmium (Cd)	A 0.35±0.0098	C 0.16±0.0078	B 0.20
Lead (Pb)	A 3.97±0.016	B 1.30±0.018	C 0.20

*** Permissible levels (PL) according to *WHO (1989)*.**
Means within the same row of different litters are significantly different at (P < 0.01)

Table (7) : Heavy metal concentrations (µg/g dry weight) in gills and musculature of Grouper from Wadi-Mariout :

Fish tissue / Metals	Gills	Musculature	PL *
	Mean± S. E.	Mean± S. E.	
Zinc (Zn)	B 58.15±2.04	C 36.68±1.02	A 60.0
Copper (Cu)	B 0.63±0.0038	C 0.32±0.005	A 3.0
Iron (Fe)	A 59.82±1.08	C 37.79±0.71	B 50.0
Nickel (Ni)	A 1.59±0.29	B 0.51±0.12	C 0.50
Mercury (Hg)	A 1.70±0.89	B 0.73±0.13	C 0.50
Cadmium (Cd)	A 0.59±0.75	C 0.18±0.18	B 0.20
Lead (Pb)	A 4.33±0.059	B 1.34±0.021	C 0.20

*** Permissible levels (PL) according to *WHO (1989)* .**
Means within the same row of different litters are significantly different at (P < 0.01).

DISCUSSION

In the present work, we spot light on the clinical picture and PM lesions of the most predominant bacterial pathogens affecting some marine fishes native to Wadi-mariut region, Borg El-Arab city, Alexandria governorate. Moreover, isolation and identification of these bacterial infections by both biochemical traditional methods serological , studying the relationship between heavy metal concentrations in water and in fish tissues with the prevalence of bacterial diseases.

Concerning the clinical signs and Postmortem (PM) lesions of some naturally examined marine fishes; the clinical picture and gross lesions of Vibriosis (Listonellosis) in naturally infected some marine fishes; our results were in concordance to that obtained by *Actis et al. (1999)*; where they stated that fish affected by *Listonella anguillarum* (*L. anguillarum*) showed typical signs of a generalized septicemia with hemorrhage on the base of fins, exophthalmia and corneal opacity. Also, *Austin and Austin (2007)* who reported that the typical external clinical signs of *V. anguillarum* infection include red spots on the ventral and lateral areas of the fish and swollen and dark skin lesions that can ulcerate and bleed. The eyes were also infected, resulting in opacity at first, and later in ulceration and exophthalmia.

On the other hand; *V. harveyi* have several virulence factors such as the ability to attach and form biofilms *(Karunasagar et al., 1994)*; Capacity to bind iron *(Owens et al., 1996)*; Lipopolysaccharide *(Montero and Austin, 1999)*; extra-cellular products (cysteine protease, phospholipase, haemolysin) (*Soto-Rodriguez et al., 2003)* and Bacteriocin-like substance *(Prasad et al., 2005)*.

In concern to the results of clinical signs and PM lesions of Tenacibaculosis in naturally infected fishes; our results were agreed with that obtained by *Salati et al. (2005)* where they recorded that *T. maritimum* caused a disease characterized by fin erosion and necrotic ulcers of skin and muscle in Meagre *(Dicentrarchus labrax*), gilthead Grouper *(Sparus aurata)*.

These results may be attributed to the virulence factors of *T. maritimum* as the synergistic interaction of the toxins and enzymes present in the extra-cellular products (ECP) which have high proteolytic activity with an ability to degrade gelatin, amylase, casein and nucleases as well as a positive cytotoxic activity (*Baxa et al., 1988)*; Adhesion (*Burchard et al., (1990)*; Hemagglutinating activity (*Pazos, 1997)*; the lipopolysaccharides (LPS) of cell wall *(Vinagradov et al., 2003)*; Capsular structure *(Avendaño-Herrera, 2005*) and iron uptake mechanisms *(Avendaño-Herrera et al., 2005c)*.

These results may be attributed to phosphoglucomutase enzyme as the virulence factor for S. iniae *which inter-converts glucose-6-phosphate and glucose-1-phosphate which play important role in the production of* S. iniae *polysaccharide capsules* (Buchanan et al., 2005).

In regards to the biochemical characterization of *S. iniae* isolates in this study, the results of biochemical tests were in complete agreement with those obtained by *Roberts (2001)* and (*Whitman, 2004*).

In regard to the culture and morphological characteristics of Vibrio species noticed in this study, the results were in concordance with those reported by (*Balebona et al., 1998b*) and (*Megahed, 2000).* In regards to the biochemical characterization of Vibrio isolates in this study, the results of biochemical tests were in complete agreement with those obtained by (*Roberts, 2001)* and *(Zorrilla et al., 2003a).*

In regards to the biochemical characterization of Photobacterium isolates in this study, the results of biochemical tests were in complete agreement with those obtained by

(*Hawke et al., 1987*); (*Hawke, 1996*) and (*Roberts, 2001).*

In regards to Serotyping of bacterial isolates; our results revealed that (78 %) fishes were found to be infected with Gram negative bacteria and only (22%) fishes were infected with Gram positive bacteria and *V. alginolyticus* was the most prevalent bacterial isolate (14%) from the percentages of all isolates. These results were in agreement with that obtained by (*Zorrilla et al., 2003b)* where they stated that most of the bacterial isolates were Gram-negative (93.19%) and 69.90% of these isolates were identified as species of Vibrio as well as *V. alginolyticus* being the most frequent Vibrio species (21.35%). Similarly, *Austin and Austin (1993) and Toranzo et al. (1993)* also illustrated that in marine fish, the most prevalent infectious pathologies affecting farmed gilt-head Grouper in the sea water of Spain were of bacterial origin, Gram negative bacteria being the most frequently isolated more than 93%. This high incidence of Vibrio and *V. alginolyticus* was also determined in a previous survey carried out in the same area by (*Balebona et al., 1998b).*

V. alginolyticus was not dominant in samples obtained from Common dentex (*D. dentex, L.*) (*Company et al., 1999*), although its detection was noteworthy. Moreover, *Toranzo et al. (1993)* did not detect this species in the microflora associated with healthy and diseased turbots from farms in northwestern Spain. However, other authors (*Angulo et al., 1993)* and *(Blanch et al., 1997*) have isolated *V. alginolyticus* from the intake water at turbot farms and from turbot larvae during a period of 40 days in the north of Spain.

In concern to the prevalence of bacterial isolates in naturally infected Meagre; Our results were not in concordance with that obtained by *(Nedoluha and Westhoff, 1997)* where they suggested that the most predominant bacterial pathogens randomly isolated from diseased striped bass, *Morone saxatilis L.* and hybrid striped bass, *Morone chrysops L.* and *Morone saxatilus L.* collected from different marine aquaculture systems were accounted for Aeromonas species (19%), Pseudomonas species (6%) and on the other hand, *Staphylococcus aureus* and Vibrio species were isolated from all systems but in low numbers.

On the contrary, our results were disagreed with that obtained by *(Marzouk et al., 2009)* where they stated that the main cause of mortality of red grouper, Meagre, Grouper was Photobacterium species (*Pasteurella damselae)* as a primary cause during this period. Similarly, *Soliman et al. (2011)* demonstrated that the most prevalent isolates that may lead to mortalities of adult grouper were *Pasteurella piscicida* (64%).

These differences may be attributed to the differences in fish species, Seasons, water quality, locality and types of aquaculture.

In concern with the results of the incidence of bacterial isolates retrieved from the internal organs of naturally infected Grouper were not in agreement with that obtained by (*Zorrilla et al., 2003b)* where they stated that the bacterial isolates from Grouper were isolated from spleen (49.51%), liver (29.12%), kidney (11.65%) and other origins such as ulcers and exophthalmic eyes. Similarly, our results were not in concordance with that recorded by (*El-Gendy, 2007)* and *(Moustafa et al., 2010*) where they mentioned that the highest prevalence of *Ps. fluorescence* was recorded in Kidneys (40.32 %), Liver (27.41 %), spleen (20.96 %) and only (11.29 %) from the gills. In the same manner, they recorded that the highest incidence of *V. anguillarum* was (37.03 %) from spleen, liver (33.33%), Kidney (22.22 %) and (7.40 %) from the gills. Moreover, they recorded that the highest prevalence of *P. piscicida* was (39.06 %) from spleen, Kidney (28.12 %), Liver (21.87 %) and only (10.93 %) from the gills.

The pollution of the aquatic environment with heavy metals becomes a serious health concern during recent years. Lead, mercury and cadmium are the most dangerous metals causing serious health hazards in humans (*Abd El-Hady,*

2007). Heavy metals have the tendency to accumulate in various organs of marine organisms, especially fish, which in turn may enter into the human metabolism through consumption causing serious health hazards (*Puel et al., 1987*).

In regards to the heavy metal concentration in water; our results revealed that all heavy metal concentrations were higher in all cages over the international permissible limits. Moreover, heavy metal concentration in cage (III) and Cage (IV) were higher than that of cage (I) and Cage (II) and this may be attributed to the dilution factor whereas the heavy metal concentrations in near cages (III and IV) were higher than which far away in the water of Wadi-Mariut.

In regards to heavy metal concentrations in fish tissues; our results showed that the metal concentrations in fish tissues (musculature and gills) of cultured Meagre and Grouper were closely associated with metal content of water in Wadi-mariut and this may be attributed to the fact that if an environment receives foreign pollutants (e.g. heavy metals), the organisms living in it could take up the pollutants from the water or/and food and concentrate it in their bodies (*Ravera, 2001*); (*Shakweer, 1998*) and (*Eiman and Zamzam, 1996)*.

Our results were in agreement with many authors who have reported that gills have a high tendency to accumulate heavy metals (*Wong et al., 2001)* and *(Coetzee et al., 2002*). Also, *Dural et al. (2006)* investigated the bioaccumulation of the heavy metals (Fe, Zn, Cd) in the liver, gill, gonad and muscle tissues of *Dicentrarchus labrax L.*, *Mugil cephalus* and *Sparus aurata L.* and they found that the heavy metal levels were generally higher in the liver and gill than the gonad than muscle tissues in the three species.

In concern heavy metals in musculature; our results showed that musculature retained the lowest concentration of the measured metals. This finding was in agreement with the observations of many authors who have shown that fish musculature has a low tendency to accumulate the heavy metals to which they are exposed (*Blasco et al., 1998); (Canlı et al., 1998); (Ibrahim et al., 1999); (Canlı and Atlı, 2003); (Karadede et al., 2004)* and *(Yılmaz, 2005*). The concentration of heavy metals in the edible part (musculature) of Meagre and Grouper at Wadi-mariut may pose health risk to the consumer, as the concentration of Mercury (Hg) and Lead (Pb) exceed those of the international permissible limits according to (*WHO, 1989*).

In regards to Iron (Fe) levels; iron was the most abundant metal in the studied tissues of the fishes; whereas Fe concentrations were higher in tissues of Grouper more than that of Meagre and (59.82 and 37.79 µg / g dry weight in gills and musculature respectively). These results agreed with that obtained in a study carried out by (*Türkmen et al., 2010)* where they stated that the average levels of Fe in Gilthead Grouper and Meagre were higher in gill tissue and reported as 38.9 and 28.9 mg kg-1, respectively.

The concentration of Fe in the water of the four cages of Wadi-mariut as well as in tissues was higher than the permissible level (0.3 mg/L) recommended by the (*Egyptian Organization for Standardization, 1993)* which could be attributed to Fe liberation from Sediments as sulphides (*Abo El-Ella et al., 2005*).

In concern to Lead (Pb) levels; the principle source of Lead in the marine environment appear to be the exhaust of vehicles run with Leaded fuels that reaches the Sea water by the way of rain and wind blow dust (*Castro and Huber, 1997*).

The average levels of Pb in Grouper and Meagre samples were found above the maximum acceptable limit for human consumption. The Pb concentrations were higher than the permissible level in water

(*WHO, 1984*) as well as in tissues of Meagre and Grouper stated by *WHO (1989).*

In regard to Zinc (Zn) levels; among the fish species analyzed in this study, Zn levels were lower than the permissible limits; our findings for gilthead Grouper and Meagre were lower than the findings of (*Çelik and Oehlenschläger, 2005)* and (*Olowu et al., 2010)* where they found higher Zn levels than permissible levels of Meagre and Grouper.

In concern Copper (Cu) levels; Cu levels presented in this study are in agreement with values reported by many researchers (*Mol et al., 2010*) and (*Arafa and Ali, 2008*) whereas their findings regarding the levels of Cu in Gilthead Grouper and Meagre have showed that Cu levels in fish samples is quite below the maximum acceptable limit, similar to those in our study.

In regard to Nickel (Ni) levels; results of Ni levels in water as well as in tissues of Meagre and Grouper were higher than the permissible levels and these findings were agreed with the findings of (*Abida et al., 2009*) where they reported the highest levels of Ni were recorded in the gills and muscles in *Labeo rohita.*

Concerning Mercury (Hg) levels; methyl mercury which is formed in aquatic sediments through the bacterial methylation of organic mercury, it is toxic chemical compound of mercury and in fact, nearly all of the mercury in fish musculature occur as methyl mercury (*Joiris et al., 1999*). Methyl mercury affects the kidneys and central nervous system, particularly during development as it crosses the blood brain barrier (*Clarkson, 2002*).

In regards to Cadmium (Cd) levels; our finding regarding Cd concentration in a study carried out by (*Türkmen et al., 2010),* where they stated that the levels of Cd in gilthead Grouper and Meagre were determined as 0.30 and 0.10 mg kg-1, respectively. These results are above the maximum acceptable limit similar to those in our study. In contrary, our results were lower than the findings obtained by (*Enkaleda et al., 2010).*

Generally, Heavy metals are chemical stressors and the development of disease will reflect interactions between the host, the disease causing situation and stressors (*Austin and Austin, 1993*) and this may be attributed to suppression of immune system and immune response which provide opportunities for entering of many pathogens, but till now the effect of heavy metals on the immune system and immune response is not fully understood (*Storelli et al., 2002*).

Concerning the relationship between Fe toxicity and the incidence of fish bacterial diseases; our results illustrated that in excess iron concentration in water than the permissible levels can accelerate of bacterial growth as Gram negative bacteria as Vibrio species as well as some Gram positive bacteria as Streptococcus species and Staphylococcus species and this is considered as an excellent bacterial growth stimulant and this may be attributed to many factors as following; its properties as an excellent oxygen transporter, iron tends to stimulate the growth of common bacteria (*Kutsky, 1982*); the acquisition of iron by pathogens in vivo as it was shown that availability of iron in vivo is restricted by chelation to host transferrin and lactoferrin, and that injection of iron salts enhanced virulence of many pathogens in various animal models (*Bullen, 1981*); Concerning the relationship between Cu toxicity and the incidence of fish bacterial diseases; copper concentrations in water were higher than the permissible levels and can increase the infection with Vibriosis especially *V. Anguillarum* infection and similar results obtained by (*Rodsaether et al., 1977*) where they described the debilitating effects of copper, in terms of concentration and time of exposure with regard to increasing susceptibility to Vibriosis (e.g. *Vibrio anguillarum*).

These results of Cu may be attributed to the fact that the exposure to copper toxicity

resulted in coagulation of the mucus layer of the gills, which inhibited oxygen transport and caused respiratory stress or reduced the number of lymphocytes and granulocytes in the blood, leading to reduced phagocytosis (*Mushiake et al., 1985*).

Further studies; *Omima Aboud (2010)* stated that lead, mercury and cadmium have inhibitory effect on phagocytic activity of fish macrophages and manifested by low levels of antibodies and high mortality rates in fish exposed to these metals than in the control fish after experimental infection by *Ps. Fluorescence*.

REFERENCES

Abd-El-Hady, B. A. (2007): Compare the Effect of Polluted and River Nile Irrigation Water on Contents of Heavy Metals of Some Soils and Plants. Research Journal of Agriculture and Biological Sciences, 3(4): 287 - 294.

Abida, B.; Harikrishna, S. and Irfanulla, K. (2009): Analysis of heavy metals in water, sediments and fish samples of Madivala Lakes of Bangalore, Karnataka. International Journal of Chemical Technology Research, 1(2): 245 - 249.

Abo El-Ella, S. M.; Hosny, M. M. and Bakry, M. F. (2005): Utilizing fish and aquatic weeds infestation as bioindicators for water pollution in Lake Nubia, Sudan. Egyptian Journal of Aquatic Biology and Fisheries, (9): 63 - 84.

Actis, L. A.; Tolmasky, M. E. and Crosa, J. H. (1999): Vibriosis. In: Woo, P.T.K., Bruno, D.W. (Eds.), Fish Diseases and Disorders, vol. 3. CAB Intern., Publ., United Kingdom, pp. 523– 558.

Amlacher, E. (1970): Textbook of fish diseases, T.E.S. Publication, NewJersey. USA, P. 117-135.

Anderson, J. I. and Conroy, D. A. (1970): Vibrio disease in marine fishes. In: S. F. Snieszko (Editor), a symposium on Diseases of Fishes and Shellfishes. Am. Fish. Sot. Spec. Publ., (5): 266 - 272.

Angulo, L.; Lo´pez, J. E. and Vicente, J. A. (1993): Distribucio´n del ge´nero Vibrio en el agua de entrada de dos piscifactorias de rodaballo (*Scophthalmus maximus*). In: Cervin˜o, A., Landi´n, A., de Coo, A., Guerra, A., Torre, M. (Eds.), Proceedings of the IV National Congress of Aquaculture, Xunta de Galicia, Vilanova de Arousa (Spain), pp. 611 – 616.

AOAC (Associatian of Official Analytical Chemists) (1996): "Official Methods for Minerals in Plants. Method 975.03. B.b." In: Official Methods of Analysis of AOAC International, 16th ed., V (1), Arlingoton (Va.): AOAC International Section 3, pp 3-4.

APHA (American Public Health Association) (1995): Standard Methods for the Examination of Water and Wastewater. 19th Edn., Washington DC.

Arafa, M. M. and Ali, A. T. (2008): Effect of some heavy metals pollution in Lake Mariout on *Oreochromis niloticus* fish in Egypt. J. Comp. path. & Clinic. Path., Vol. (21) No. (3): 191 – 201.

Austin, B. and Austin, D. A. (1993): Bacterial Fish Pathogens: Disease of Famed and wild Fish, 2nd ed. Chichester: Ellis Horwood.

Austin, B. and Austin, D. A. (1999): Bacterial fish pathogens: disease of farmed and wild fish, 3rd edn (revised). Springer-Praxis series in aquaculture and fisheries. Springer-Verlag, London.

Austin, B. and Austin, D. A. (2007): Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish, 4th edn. Springer-Praxis Publishing, New York-Chichester.

Avendaño-Herrera, R. (2005): Avances en el conocimiento del patógeno de peces *Tenacibaculum maritimum*: implicaciones en el diagnóstico y prevención de la enfermedad. PhD thesis, Universidad de Santiago de Compostela.

Avendaño-Herrera, R.; Toranzo, A. E.; Romalde, J. L.; Lemos, M. L. and Magariños, B. (2005c): Iron uptake mechanisms in the fish pathogen *Tenacibaculum maritimum*. Appl. Environ. Microbiol., (71): 6947 – 6953.

Balebona, M. C.; Andreu, M. J.; Bordas, M. A.; Zorrilla, I.; Morin˜igo, M. A. and Borrego, J. J. (1998a): Pathogenicity of *Vibrio alginolyticus* for

cultured gilthead Seabream (*Sparus aurata L.)*. Appl. Environ. Microbiol., (64): 4269 – 4275.

Balebona, M. C.; Zorilla, M. I.; Morinigo, M. A. and Borrego, J. J. (1998b): Survey of bacterial pathologies affecting farmed gilthead Seabream (*Sparus aurata L.)* in south-western Spain from 1990 to 1996. Aquaculture, (166): 19 – 35.

Baxa, D. V.; Kawai, K. and Kusuda, R. (1988): In vitro and in vivo activities of *Flexibacter maritimus* toxins. Rep. Usa Mar. Biol. Inst. Kochi Univ., (10): 1 – 8.

Blanch, A. R.; Alsina, M.; Simon, M. and Jofre, J. (1997): Determination of bacteria associated with reared Turbot (*Scophthalmus maximus*) larvae. J. Appl. Microbiol., (82): 729 - 734.

Blasco, J.; Rubio, J. A.; Forja, J.; Gomezparra, A. and Establier, R. (1998): Heavy metals in some fishes of the Mugilidae family from salt ponds of Cadiz Bay, Spain. Ecotoxicology and Environmental Restoration, (1): 71 - 77.

Buchanan, J. T.; Stannard, J. A.; Lauth, X.; Ostland, V. E.; Powell, H. C.; Westerman, M. E. and Nizet, V. (2005): S. *iniae* phosphoglucomutase is a virulence factor and a target for vaccine development. Infection and Immunity, (73): 6935 - 6944.

Bullen, J. J. (1981): The significance of iron in infection. Review of Infectious Diseases, (3): 1127 − 1137.

Burchard, R. P.; Rittschof, D. and Bonaventura, J. (1990): Adhesion and motility of gliding bacteria on substrata with different surface free energies. Appl. Environ. Microbiol., (56): 2529 – 2534.

Canlı, M. and Atlı, G. (2003): The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, and Zn) levels and the size of six Mediterranean fish species. Environmental Pollution, (121): 129 -136.

Canli, M.; Ay, O. and Kalay, M. (1998): Levels of heavy metals (Cd, Pb, Cu and Ni) in tissues of Cyprinus carpio, Barbus capito and Chondrostoma regium from the Seyhan river. Turkish Journal of Zoology, 3(22), pp 149 - 157.

Castro, P. and Huber, M. E. (1997): Marine biology. 2nd edition, Book (ISBN 0697243605).

Çelik, U. and Oehlenschläger, J. (2005): Zinc and copper content in marine fish samples collected from the eastern Mediterranean Sea. Eur. Food Res. Technol., (220): 37- 41.

Clarkson, T. W. (2002)*:* The three modern faces of mercury. Environ. Monit. Assess. (121): 431 – 438.

Clesceri, L. S. (1998): Standard methods for the examination of water and waste water. In Collection and Preservation of Samples and Metals (eds Arnold, E., Greenbergy and Eaton, A. D.), APHA, AWWA, WEF, Washington, DC.

Coetzee, L.; du Preez, H. H. and van Vuren, J. H. (2002): Metal concentrations in Clarias gariepinus and Labeo umbratus from the Olifant and Klein Olifants River, Mpumalanga, South Africa: Zinc, copper, manganese, lead, chromium, nickel, aluminium and iron. Water SA, (28): 433 - 448.

Company, R.; Bobadilla, A. S.; Pujalte, M. J.; Garay, E.; Pellitero, P. A. and Saanchez, J. P. (1999): Bacterial and parasitic pathogens in cultured Common dentex (*Dentex dentex L*.) J. Fish Dis., (22): 299 - 309.

Conroy, D. A. and Herman, L. R. (1981): Text book of fish diseases. T.F.H. publ., West Sylvania.

Dural, M.; Lugal, M. Z.; Ksu, G.; Akif, A. and Derici, B. (2006): Bioaccumulation of some heavy metals in different tissues of *Dicentrarchus labrax L.*, 1758, *Sparus aurata L.*, 1758 and *Mugil cephalus L.*, 1758 from the Camlik lagoon of the eastern coast of the Mediterranean (Turkey). Environmental Monitoring and Assessment, (118): 65 – 74.

Egyptian Organization for Standardization (1993): Egyptian standard, maximum levels for heavy metal concentrations in food. ES 2360-1993, UDC: 546.19: 815, Egypt.

Eiman, M. and H. Zamzam, (1996): Effect of selenium-mercury interaction on Clarias lazera fish. Proceeding of the 3rd Congress of Toxicology in the Developing Countries, Cairo, Egypt, 19, pp: 379 - 392.

El-Gendy, M. Y. (2007): Epizootiologyical studies on some bacterial infections in marine fishes. Thesis, M.V.Sc., Fish Disease and Management, Fac. Vet. Med. Cairo Univ.

Ellis, A. E. (1999): Immunity to bacteria in fish. Fish & Shellfish Immunol., (9): 291 – 308.

Enkaleda, O.; Luljeta, D.; Jetmira, A.; Muhamet, Z.; Lmer, H.; Doriana, B. and Fatgzim, L. (2010): Heavy metals in fish for public consumption and consumer protection. Natura Montenegrina, Podgorica, 9(3): 843 – 851.

Hawke, J. P. (1996): Importance of a siderophore in the pathogenesis and virulence of *Photobacterium damsela subsp. piscicida* in hybrid-Striped bass (Morone saxatilis x Morone chrysops). Ph.D. Dissertation. Louisiana State University and Agricultural and Mechanical College, Baton Rouge, LA.

Hawke, J. P.; S. M. Plakas, R. V. Minton, R. M. Mcphearson, T. G. Snider and A. M. Guarino (1987): Fish pasteurellosis of cultured Striped bass (Morone saxatilis) in coastal Alabama. Aquaculture, (65): 193 - 204.

Ibrahim, A. M.; Bahnasawy, M. H.; Mansy, S. E. and El-Fayomy, R. I. (1999): Heavy metal accumulation in water, sediment and some fishes in Lake Manzala, Egypt. J. Egypt. Ger. Soc. Zool., (29): 43 - 58.

Joiris, C. R.; Holsbeek, L. and N. L. Moaatermi (1999): Total methylmercury in Sardines, Sardinella aurita and Sardina pilchardus from Tunisia. Mar. Pollut. (38): 188 – 192.

Karadede, H.; Oymak, S. A. and Ünlü, E. (2004): Heavy metals in mullet, Liza abu, and catfish, Silurus triostegus, from the Atatürk Dam Lake (Euphrates), Turkey. Environment International, (30): 183 - 188.

Karunasagar, I.; Pai, R.; Malathi, G. and Karunasagar, I. (1994): Mass mortality of *Penaeus monodon* larvae due to antibiotic resistant Vibrio harveyi infection. Aquaculture, (128): 203 - 209.

Kutsky, R. (1982): Handbook of Vitamins, Minerals and Hormones, 2nd ed., Van Nostrand Reinhold.

Marzouk, M. S.; Hanna, M. I. and Amany M. Kenawy (2009): Monitoring the Cause of Mortality in Some Marine Fishes in Matrouh Governorate, Egypt during the summer 2008. American-Eurasian J. Agric. & Environ. Sci., 5 (2): 148 - 158.

Megahed, A. A. (2000): Studies on some Gram negative bacteria of fish. Thesis, M.V.Sc., Microbiology, Fac. Vet. Suez Canal Univ.

Mol, S.; Özden, Ö. and Oymak, S. A. (2010): Trace metal contents in fish species from Atatürk Dam Lake (Euphrates, Turkey). Turkish Journal of Fisheries and Aquatic Sciences, (10): 209 - 213.

Montero, A. B. and Austin, B. (1999): Characterization of extracellular products from an isolate of *Vibrio harveyi* recovered from diseased post-larval *Penaeus vannamei* (Bonne). J. Fish Dis., (22): 377 – 386.

Moustafa, M.; Laila, A. M.; M. A. Mahmoud, W. S. Soliman and M. Y. El-gendy (2010): Bacterial Infections Affecting Marine Fishes in Egypt. Journal of American Science, (6): 603 – 612.

Mushiake, K.; Nakai, T. and Muroga, K. (1985): Lowered phagocytosis in the blood of eels exposed to copper. Fish Pathology, (20): 49 - 53.

Nedoluha, P. C. and Westhoff, D. (1997): Microbiology of Striped bass grown in three aquaculture systems. Food Microbiol., (14): 255 - 264.

Olowu, R. A.; Yejuyo, O.; Adejoroia, A.; Adewuyi, G. O.; Osundiya, M. O.; Onwordi, C. T.; Yusuf, A. and Owolabi, M. S. (2010): Determination of heavy metals in crabs and prawn in Ojo rivers Lagos, Nigeria. Journal of Chemistry. 7 (2): 526 – 530.

Omima, A. S. A. Aboud (2010): Impact of pollution with lead, mercury and cadmium on the immune response of Oreochromis niloticus. J. New York Science, 3(9): 12 – 16.

Owens, L.; Austin, D. A. and Austin, B. (1996): Effect of siderophore production in Vibrio harveyi isolates. Dis. Aquat. Org., (27): 157–160.

Oxoid Manual (1982): Oxoid manual 5th Ed. Published by Oxoid Limited Hampshire, England.

Pazos, F. (1997): Flexibacter maritimus: estudio fenotípico, inmunológico y molecular. PhD thesis, Universidad Santiago de Compostela.

Prasad, A. S.; Morris, P. C.; Hansen, R.; Meaden, P. G. and Austin, B. (2005): A novel bacteriocin-like substance (BLIS) from a pathogenic strain of *Vibrio harveyi*. Microbiology, (151): 3051 –3058.

Puel, D.; Zsuerger, N. and Breittmayer, J. P. (1987): Statistical assessment of a sampling pattern for evaluation of changes in Hg and Zn concentration in Patella coerulea. Bull. Environ. Contam. Toxicol., (38): 700 –706.

Ravera, O. (2001): Monitoring of the aquatic environment by species accumulator of pollutants: a review. J. Limnol., (60): 63 - 78.

Roberts, R. J. (2001): Fish Pathology, 3rd edn. W. B. Saunders, Philadelphia, PA.

Rodsaether, M. C.; Okfsen, J.; Raa, J.; klyhre, K. and Steen, J. B. (1977): Copper as an initiating factor of vibriosis (*Vibrio anguillarum*) in eel (*Anguilla anguilla*). Journal of Fish Biology, 17 - 21.

Sadek, S. (2000): Seabream culture in Egypt; status, constraints and potential. Fish Physiol. Bioch., (22): 171 – 178.

Salati, F.; Cubadda, C.; Viale, I. and Kusuda, R. (2005): Immune response of Seabass, Dicentrarchus labrax L. to Tenacibaculum maritimum antigens. Fish Sci., (71): 563 – 567.

SAS (1987) : Statistical analysis system. User's Guide Statistics. SAS Institute Cary, North Carolina.

Shakweer, L. M. (1998): Concentration levels of some trace metals in *Oreochromis niloticus* at highly and less polluted areas of Mariut Lake. J. Egypt. Ger. Soc. Zool., (25): 125 - 144.

Soliman, W. S.; Samira, S. Rezika; Al-Garib, S.; El-Waer, O. and Eldaghayes, I. (2011): Study on Grouper fish mortality phenomenon at the east costal Libyan area of the Mediterranean Sea with reference to bacteriological and parasitological examinations. New York Science Journal, (4): 6 - 14.

Soto-Rodriguez, S. A.; Roque, A.; Lizarraga-Partida, M. L.; Guerra-Flores, A. L. and Gomez-Gil, B. (2003): Virulence of luminous vibrios to Artemia franciscana nauplii. Dis. Aquat. Org., (53): 231 – 240.

Storelli, M.; Giacominelli-Stuffler, R. and Marcotrigiano, G. (2002): Mercury accumulation and speciation in muscle tissue of different species of sharks from Mediterranean Sea, Italy. Bull. Environ. Contam. Toxicol. (68): 201 – 210.

Toranzo, A. E. and Barja, J. L. (1993): Virulence factors of bacteria pathogenic for cold water fish. Annu. Rev. Fish Dis., (3): 5 – 36.

Toranzo, A. E.; Santos, Y.; Lemos, M. L.; Ledo, A. and Bolinches, J. (1987): Homology of Vibrio anguillarum strains causing epizootics in turbot, salmon and trout reared on the Atlantic coast of Spain. Aquaculture, (67): 41 - 52.

Türkmen, A.; Türkmen, M.; Tepe, Y. and Çekiç, M. (2010): Metals in tissues of fish from Yelkoma Lagoon, northeastern Mediterranean. Environmental Monitoring and Assessment, (168): 223 - 230.

Vinagradov, F. Q.; Jian, J. C. and Wu, Z. H. (2003): Characterization of extracellular proteases from *Vibrio alginolyticus* isolated from maricultured fish. Acta. Hydrobiologica. Sinica. (30): 553 - 558 (in Chinese with English abstract).

Wedemeyer, G. A. (1996): Physiology of Fish in intensive culture Systems. Chapman & Hall, New York. 232 pp.

Whitman, K. A. (2004): Finfish and Shellfish Bacteriology Manual; Techniques and Procedures. ISBN 0-8138-1952-0 – Iowa State Press.

WHO, World Health Organization (1984): Guidelines for drinking water quality. Geneva No. 111.

WHO, World Health Organization (1989): Guidelines for the safe use of wastewater and excreta in agriculture and aquaculture. Measures for public health protection. London School of Hygiene and Tropical Medicine, UK. ISBN 92 4 152248 9.

Wong, C. K.; Wong, P. P. K. and Chu, L. M. (2001): Heavy metal concentrations in marine fishes collected from fish culture sites in Hong Kong. Arch. Environ. Contam. Toxicol., (40): 60 - 69.

Yılmaz, A. B. (2005): Comparison of heavy metal levels of grey mullet (*Mugil cephalus*) and Seabream (*Sparus aurata*) caught in İskenderun Bay (Turkey). Turk. J. Vet. Anim. Sci., (29): 257 - 262.

Zorrilla, I.; Arijo, S.; Chabrillon, M.; Diaz, P.; Martínez-Manzanares, E.; Balebona, M. C. and Moriñigo, M. A. (2003a): Vibrio species isolated from diseased farmed Sole, *Solea senegelansis* (Kaup), and evaluation of the potential virulence role of their extracellular products. Journal of fish diseases, (26): 103-108.

Zorrilla, M.; Chabrillon, A. S.; Rosales, P. D.; Manzanares, E. M.; Balebona, M. C. and Morinigo, M. A. (2003b): Bacteria recovered from diseased cultured gilthead Seabream, *Sparus aurata L.* in southwestern Spain. Aquac., (218): 11 – 20.

بعض الدراسات على المعادن الثقيلة وعلاقتها بالإصابات البكتيرية فى بعض أسماك المياه المالحة المستزرعة

طلعـــت طلعـــت سعـــد

تم إجراء هذه الدراسة من أجل عزل والتعرف على أنواع البكتيريا التى تصيب أسماك المياه المالحة فى مصر ومقارنة تواجدها بنسب المعادن الثقيلة فى الماء والأنسجة الخاصة بالأسماك .

فى هذه الدراسة تم العمل على عدد (20) قفص من أقفاص المياه المالحة حيث تم استخدام عدد (100) سمكة من أسماك الوقار و (100) من أسماك المياجرى تم تجميعها من وادى مريوط ومنطقة برج العرب بمحافظة الإسكندرية .

تمت الزراعة على الأوساط المختلفة للبكتيريا وتم عزل (90) عترة من الأسماك المصابة (50) من أسماك المياجرى و (40) من أسماك الوقار نسب البكتيريا فى الأعضاء الداخلية تم عزلها غالبا من الكبد والطحال والقلب والكلى حيث كان (100) عترة من الكبد و (49) من القلب و (62) من الكلى و (39) من الطحال .

أما بالنسبة لنتائج المعادن الثقيلة فى الماء فتم تسجيلها بنسب مختلفة على حسب درجة تخفيف الماء كالآتى :

(النحاس , الكادميوم , الرصاص , الزئبق , النيكل , الحديد , الخارصين) نسب المعادن الثقيلة فى الأنسجة كانت على النحو التالى : فى أسماك المياجرى الزنك , النحاس , الحديد كانت بتركيزات أقل فى الخياشيم عن العضلات فى حين أن النسب الخارصين والرصاص فكانت فى الخياشيم أعلى من العضلات بالإضافة إلى نسب الكادميوم والنيكل كانت إلى حد ما بنسب متساوية .

أما بالنسبة للوقار فكان النحاس والخارصين فكانت بنسب أقل فى الخياشيم عن العضلات على العكس من ذلك كان الحديد والكادميوم بينما الرصاص والخارصين كانت إلى حد ما بنسب متساوية .