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COMPARATIVE STUDY ON THE LEVELS OF HEAVY METALS IN THE BITTER LAKES AND LAKE TIMSAH, (SUEZ CANAL) IN RELATION TO THE REPRODUCTIVE CYCLE OF THE RABBIT FISH *SIGANUS RIVULATUS*.

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ABSTRACT

The concentrations of Cu, Zn, Pb, Cd, Fe, Mn, Cr, Ni and Co in muscles, liver and gonads of the marine rabbit fish *Siganus rivulatus* from the Bitter lakes and lake Timsah, (Suez Canal) were investigated seasonally, over the course of one year (November 2002 – August 2003). Muscles recorded the lowest concentrations of metals than other organs which ranged between 0.22 – 0.76 and 0.27 – 1.23, 1.95 – 3.67 and 3.01 – 5.08, 0.09 – 0.43 and 0.24 – 0.51, 0.04 – 0.16 and 0.11 – 0.37, 4.32 – 5.66 and 5.01 – 10.33, 0.12 – 0.29 and 0.26 – 1.35, 0.06 - 0.09 and 0.05 – 0.16, 0.02 – 0.39 and 0.03 – 0.51 and 0.05 – 0.24 and 0.06 – 0.31 µg/g wet weight for Cu, Zn, Pb, Cd, Fe, Mn, Cr, Ni and Co in Bitter lakes and lake Timsah, respectively. The highest concentrations of all studied metals were found in gonads. Variation of metals in fish muscles through different seasons indicated that the most studied metals exhibited their highest value during the hot seasons (spring & summer). Gonadosomatic index (GSI) and its relationship with metal accumulation was calculated for the studied fish. By using seasonal changes in the values of gonadosomatic index, it was found that their spawning season is during spring.

INTRODUCTION

Metals pollution has been identified in coastal regions of oceans and seas throughout the world. Knowledge of the concentration and distribution of heavy metals in seawater, sediments and biota can play a key role in detecting sources of pollution in aquatic systems. So, three measures of heavy metals levels, in marine habitats are usually available, namely: concentrations in water, sediment, and biota (Rainbow, 1995).

Heavy metals are elements having atomic weight between 63.546 and 200.590 (Kennish, 1992). Living organisms require trace amounts of some heavy metals, including cobalt, copper, iron, manganese, molybdenum, vanadium, strontium and zinc. Excessive levels of essential metals, however, can be detrimental to the organism. Non-essential heavy metals of particular concern to surface water systems are cadmium, chromium, mercury, lead, arsenic and antimony (Kennish, 1992). There are many biological and environmental factors affecting the accumulation of heavy metals such as water temperature, sexual state of the fish and metals concentration in the surrounding medium (Dallinger *et al.*, 1987; Nicoletto and Hendricks, 1988). Many studies were conducted to discuss the relationships between metals level and factors affecting their accumulation in the fish tissues (El-Moselhy, 1993, 1996 and 2000, Kock *et al.*, 1996; Lemus and Chung, 1999; Abd El-Azim 2002 ; EL-Moselhy & EL-Boray, 2004).

Siganus rivulatus is considered as the most important commercial fish in the Bitter lakes and lake Timsah. It is carnivorous fish, which feeds on bottom invertebrates (Fischer and Bianchi, 1984). Many studies were carried out on *Siganus rivulatus* that concerned with their biology, reproduction and metal accumulation (El-Mesery, 1993, EL-Moselhy, 1996 ; Abd El-Azim, 2002).

The present work aims to study the temporal variation of metals (Cu, Zn, Pb, Cd, Fe, Mn, Cr, Ni and Co) in the tissues of fish *Sigunus rivulatus* collected from the Bitter Lakes and lake Timsah. In addition, the relationships between accumulation of metals and maturity stage of fish gonads were investigated.

MATERIALS AND METHODS

Siganus rivulatus fish were collected monthly from Bitter Lakes and lake Timsah during November 2002- August 2003 (Fig. 1). All samples were weighed, and measured and dissected where their gonads, were examined morphologically to determine the maturity stages. Gonadosomatic index (GSI) (the percentage of gonad weight to the gutted weight of fish) was determined seasonally and calculated as :

$$GSI = \text{gonad weight} / \text{gutted weight} \times 100$$

The preparation of samples to determine concentration of heavy metals was carried out according to FAO (1976). Fish organs (muscles, liver and gonads) were separated and a known weight was digested by conc. HNO_3 in Teflon digestion vessels. Wet digested samples were diluted with deionized distilled water and analyzed, using flame atomic absorption spectrophotometer (Perkin Elmer Model A analyst 100). The obtained data were expressed as µg/g wet weight. The data were statistically analyzed, using computer program: statistica, version 6. The precision of analytical method was checked by replicate measurements for the studied metals in marine fish. The obtained results showed precision of 4.3-11.8 % for all studied metals.

RESULTS AND DISCUSSION

1- Metals concentration in different fish organs:

Mean concentrations of the studied heavy metals in different organs of the fish *S. rivulatus* are presented in Table (1). According to the distribution of metals in the fish organs, muscles showed the lowest values for all studied metals which ranged between 0.22 – 0.76 and 0.27 – 1.23, 1.95 – 3.67 and 3.01 – 5.08, 0.09 – 0.43 and 0.24 – 0.51, 0.04 – 0.16 and 0.11 – 0.37, 4.32 – 5.66 and 5.01 – 10.33, 0.12 – 0.29 and 0.26 – 1.35, 0.06 - 0.09 and 0.05 – 0.16, 0.02 – 0.39 and 0.03 – 0.51 and 0.05 – 0.24 and 0.06 – 0.31 µg/g wet weight for Cu, Zn, Pb, Cd, Fe, Mn, Cr, Ni and Co in Bitter lakes and lake Timsah, respectively. The internal organs particularly gonads exhibited the highest values for all metals.

Data of the present study indicated that, heavy metals concentrations in *Siganus rivulatus* tissues were relatively high in Lake Timsah compared with that of Bitter lakes. This may be attributed to the heavy pollution that Lake Timsah suffered especially from sewage and agricultural effluent; and consequently had high concentrations of heavy metals. Hamed (2005) reported that heavy metals concentrations of sea water in Bitter lakes were lower than those found in Lake Timsah. EL-Moselhy (2006) studied the level of mercury in the organisms from Bitter lakes and Lake Timsah; and recorded that Lake Timsah organisms have high concentration of Hg than those in the Bitter lakes.

The present results demonstrated that there is an organ-metal-specific accumulation. This pattern is in agreement with the findings of Fujise *et al.* (1988), EL-Moselhy (1999, 2000), Abd El-Azem (2002) and EL-Moselhy & EL-Boray (2004). Therefore, muscles were selected to discuss the seasonal variation of metals in *Siganus rivulatus*.

Spring and summer showed higher values of the most studied metals in fish muscles obtained from Lake Timsah and Bitter lakes samples, while the lowest values were recorded in autumn (Table 1).

A number of factors may contribute single or combined with seasonal changes of metals accumulation in fish, as sexual differences, water temperature and ambient concentration of metals (Dallinger *et al.*, 1987; Nicoletto and Hendricks, 1988). Many studies have shown that toxicity and accumulation of metals in fish were associated with an increase in temperature (Hodson and Sprague, 1975; Roach and Maly 1979; McGeachy and Dixon, 1989, El-Moselhy, 1993; Koch *et al.*, 1996; Lemus and Chung, 1999). The mechanisms by which temperature modifies accumulation of metals are not yet fully understood. Activity of fish may increase by increasing the water temperature that can lead to increased gill ventilation rates owing to higher oxygen demand for metabolic requirement and / or decreasing oxygen concentration in water (Heath, 1987; Niimi, 1987; Douben, 1990). Data of the present study coincide with the previous studies that reported increased of metals concentration in fish tissues with increasing of temperature.

Table (2) showed the correlation matrix between metals in different organs of *Siganus rivulatus* collected from Bitter Lakes and Lake Timsah. It is obvious that there are very highly significant *correlations between Pd and Mn (r=0.99)*, Cr and Ni and Co (r=0.98 and 0.88, respectively) and between Zn and Cr, Ni and Co (r = 0.85, 0.87 and 0.84, respectively) in muscles of *Siganus rivulatus* from Bitter Lakes. Moreover, similar highly significant correlations were found between Cr, Zn, Pb, Cd, Fe, Mn and Co except Cr and Ni in in all pairs of metals in liver of *Siganus rivulatus* collected from Bitter Lakes.

On the other hand, in gonads, highly significant correlations were recorded between Zn, Cd, Fe, Mn, Co and Cu, Cd, Fe, Mn, Cr, Co and Zn, Fe, Mn, Cr, Co and Cd and Mn, Cr, Co and Fe. The correlation matrix between studied metals in different organs of *Siganus rivulatus* collected from Lake Timsah are presented in Table (2). Cu and Cr (r = -0.95), Zn and Ni (r = -0.98), Cd and Fe (r = -0.95), Mn and Ni (r = -0.97) showed insignificant correlations with each other in muscles of *Siganus rivulatus* collected from Lake Timsah. Meanwhile, in liver of *Siganus rivulatus* from Lake Timsah, very highly significant correlation between all pairs of metal studied except Cu and Pb were reported, but, in Gonads, there are highly positive correlation between all pairs of studied metals except Cu.

This mean that, the factors which are responsible for the presence of these metals in seawater are similar, whether coming from land activities or naturally as background level. Similar highly significant correlations were recorded between Fe, Mn, Zn and Cu in different marine environments (Beltagy *et al.*, 1983).

Abd El-Azim (2002) reported that heavy metals concentration in muscles of *Siganus rivulatus* collected from El-Defresoir (Suez canal) were Cu : 0.582, Zn : 2.43, Pb : 1.382, Cd : 0.095, Fe : 5.48, Mn : 0.3, Cr : 0.15, Ni : 0.66 and Co : 0.07$\mu g.g^{-1}$. Data of the present study are comparable with that of Abd el-Azim (2002), where levels of the studied metals in the muscle tissues are below WHO limits (FAO, 1992) which were Cu = 30, Zn = 1000, Pb = 2.0 and Cd = 2.00 $\mu g.g^{-1}$, while the maximum concentrations of Cu, Zn, Pb and Cd in the internal organs (liver and gonads) were higher than those of WHO limits.

2- Relationship between gonadosomatic index (GSI) and metals accumulation:

In the present study, macroscopical examination was carried out for *S. rivulatus* and showed the following maturity stages: 1- dormant, 2- prespawning, 3- spawning and 4- post spawning.

1- **Dormant stage :**Testes are thin, flaccid, grayish and sometimes with white areas. Ovaries are thin, flaccid, congested and sometimes contain large opaque-yellow residual eggs. This stage was reported in autumn.

2- **Prespawning:** According to their morphology males and females were easily distinguished by the naked eye. Testes are thickened, opaque, filling two thirds of the body cavity, with creamy white colour and smooth texture. Ovaries are more rounded, translucent, thickened and large eggs extruded by pressure on abdominal wall. This stage was found in winter.

3- **Spawning:** Testes fill the body cavity, pure white, smooth texture and milt extruded by pressure on abdominal wall. Ovaries show somewhat shrinkage due to discharge of a considerable amount of eggs. This stage was obtained in spring.

4- **Post spawning:** Testes are thin, flaccid, grayish and sometimes with white areas (residual sperms). Ovaries are thin, flaccid, congested and sometimes contain large opaque-yellow residual eggs. This stage was reported in summer.

The variation of GSI is generally used to indicate the maturity stages, and spawning season of the fish. It is observed that GSI values of males and females were low during autumn season. Index values began to increase after winter season and reached maximum values in spring season. They then began to decrease towards summer season. When the GSI reaches its maximum value, this gives a perfect indication about the time of spawning (EL-Maghraby *et al.*, 1974). GSI of *S. rivulatus* showed that the spawning season in the two regions (Bitter lakes and Timsah lake) takes place in spring season, where their values are 0.4 and 1.1 % for Bitter lakes and Lake Timsah, respectively. Compared to other studies carried out along the southern Mediterranean and Red Sea regarding the reproductive biology of the rabbit fish, some differences appear in the present study. Indeed, among the earlier studies Ben-Tuvia (1986), reported that the larvae and juveniles of *S. rivulatus* from the eastern Mediterranean are found between July to November and mature females appear during summer months. Amin (1985 a) reported that *S. rivulatus* from the Jeddah region of the Red Sea, reached sexual maturity earlier than those from the eastern Mediterranean. Moreover, Amin, (1985 b) using the temporal changes in GSI values, from different samples collected from Red Sea, showed that gonadal maturation occurs over a three month period (January – March), with the spawning period continuing for about seven months (March – September). So, the present study coincides with the latter results and also with EL-Mesiry, (1993), who studied this fish from Red Sea. This results may be related to the fact that the water temperature in Red Sea is considerably higher than that of the Mediterranean. In addition, there are some contradictions between the present results and those mentioned by some authors including Hussein (1986), Akşiray (1987), Golani (1990) and Avşar (2000) who reported that reproduction of rabbitfish takes place during summer months.

Figures 2 and 3 showed the variation of metals content in the fish gonads according to changes in level of GSI, which mainly represent the maturity stages. In fish from Bitter lakes, Mn, Cr and Ni exhibited their highest level in the dormant stage, while other metals appeared in the spawning and post spawning stages. For fish from Lake Timsah, most studied metals gave their highest level in the dormant stage. In contrast,, the lowest concentration of metals except Cu, were found in spawning and post spawning stages (Fig. 3). The concentration of metals in male fish fluctuated during the reproductive cycle for all studied organs. Lamb, (1983) and Heath, (1987) reported that a reproductive cycle does not appear to be the cause of the observed variation of metals concentration in the tissues.

Metallothionein (MT) plays an important role in metal regulation in fish. Its main functions are detoxification of non-essential metals, like Cd and Hg and maintenance of the homeostasis as a function of the reproductive status of Zn and Cu (Roesijadi, 1992). The concentration of hepatic MT in fish fluctuates as a function of reproductive status for Zn and Cu, since these elements are essential cofactors of many enzymes involved in the synthesis of DNA, RNA and proteins (Olsson *et al.*, 1987) and Overnell *et al.*, 1987). In the present study, Cu and Zn in gonads of *Siganus rivulatus* recorded the highest concentration. This means that such metals play an important role in gonadal development.

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Table 1: Seasonal variation of metals concentration (mean and standard error) in muscle, liver and gonad of *Siganus rivulatus* from Bitter lakes and Lake Timsah during 2002-2003.

metals	Seasons	Muscle		Liver		Gonad	
		Bitter lakes	Lake Timsah	Bitter lakes	Lake Timsah	Bitter lakes	Lake Timsah
Cu	Autumn	0.22 ± 0.26	0.27 ± 0.82	9.40 ± 2.57	9.71 ± 1.78	53.20 ± 6.42	61.60 ± 57.46
	Winter	--	0.36 ± 0.13	--	10.57 ± 3.28	--	84.25 ± 13.61
	Spring	0.76 ± 0.31	1.23 ± 2.06	4.30 ± 1.40	5.51 ± 2.49	74.60 ± 22.14	51.14 ± 26.03
	Summer	0.31 ± 0.21	0.84 ± 0.39	8.22 ± 1.98	9.80 ± 2.94	50.52 ± 35.24	--
Zn	Autumn	1.95 ± 1.84	3.01 ± 1.60	34.15 ± 6.42	43.90 ± 7.40	22.20 ± 11.30	26.59 ± 13.44
	Winter	--	3.48 ± 1.44	--	61.50 ± 8.46	--	40.46 ± 16.90
	Spring	3.67 ± 1.22	3.89 ± 1.46	50.20 ± 13.90	54.09 ± 5.98	44.99 ± 21.30	51.20 ± 28.44
	Summer	2.21 ± 0.57	5.08 ± 1.52	41.30 ± 0.89	51.30 ± 4.09	39.23 ± 15.56	--
Pb	Autumn	0.09 ± 0.60	0.24 ± 0.13	0.35 ± 0.15	1.67 ± 0.58	8.20 ± 5.89	15.90 ± 12.75
	Winter	--	0.25 ± 0.16	--	2.58 ± 0.81	--	14.70 ± 4.13
	Spring	0.43 ± 0.35	0.51 ± 0.23	0.76 ± 0.31	1.20 ± 0.04	12.44 ± 9.98	14.52 ± 12.15
	Summer	0.37 ± 0.03	0.49 ± 0.07	0.79 ± 0.08	1.19 ± 0.79	13.13 ± 5.57	--
Cd	Autumn	0.04 ± 0.05	0.11 ± 0.17	1.72 ± 0.55	1.89 ± 0.85	2.64 ± 1.63	3.72 ± 1.55
	Winter	--	0.37 ± 0.58	--	1.44 ± 0.69	--	3.75 ± 1.54
	Spring	0.16 ± 0.07	0.32 ± 0.12	0.15 ± 0.09	1.49 ± 0.03	4.35 ± 7.46	4.96 ± 1.45
	Summer	0.14 ± 0.03	0.22 ± 0.11	0.94 ± 0.06	1.45 ± 0.27	2.75 ± 2.63	--
Fe	Autumn	4.63 ± 2.00	5.01 ± 2.70	71.55 ± 54.14	109.9 ± 17.88	113.64 ± 10.50	17.88 ± 15.27
	Winter	--	10.33 ± 3.39	--	110.14±61.01	--	200.6 ± 14.46
	Spring	4.32 ± 0.82	6.75 ± 3.81	44.02 ± 36.10	56.42 ± 35.20	98.66 ± 16.14	111.7 ± 14.45
	Summer	5.66 ± 2.22	7.19 ± 2.72	84.40 ± 8.05	87.10 ± 37.1	230.3 ± 23.7	--
Mn	Autumn	0.12 ± 0.27	0.31 ± 0.34	9.90 ± 2.65	10.10 ± 3.21	24.21 ± 5.99	24.99 ± 12.41
	Winter	--	0.85 ± 0.09	--	12.59 ± 4.43	--	32.11 ± 9.42
	Spring	0.29 ± 0.07	1.35 ± 0.63	6.92 ± 0.87	7.84 ± 1.02	17.30 ± 7.91	22.70 ± 16.70
	Summer	0.14 ± 0.08	0.26 ± 0.08	6.55 ± 7.19	8.04 ± 2.20	19.58 ± 4.28	--
Cr	Autumn	0.06 ± 0.05	0.05 ± 0.10	2.82 ± 2.07	2.86 ± 4.92	3.21 ± 2.14	4.32 ± 2.27
	Winter	--	0.14 ± 0.05	--	4.61 ± 2.55	--	5.83 ± 2.64
	Spring	0.07 ± 0.11	0.08 ± 0.05	1.20 ± 0.35	1.60 ± 0.45	1.88 ± 1.31	2.86 ± 1.62
	Summer	0.09 ± 0.09	0.16 ± 0.06	1.70 ± 0.74	2.50 ± 0.82	1.95 ± 0.44	--
Ni	Autumn	0.02 ± 0.01	0.03 ± 0.05	1.60 ± 0.12	2.05 ± 1.72	14.70 ± 6.64	18.40 ± 6.67
	Winter	--	0.06 ± 0.01	--	3.64 ± 1.33	--	15.52 ± 0.72
	Spring	0.17 ± 0.08	0.18 ± 0.07	3.84 ± 1.57	4.70 ± 2.16	4.84 ± 0.98	6.02 ± 1.44
	Summer	0.39 ± 0.06	0.51 ± 0.14	1.07 ± 0.30	1.25 ± 0.41	12.70 ± 1.57	--
Co	Autumn	0.05 ± 0.05	0.06 ± 0.02	1.03 ± 0.94	1.70 ± 1.07	2.15 ± 1.54	4.81 ± 2.88
	Winter	--	0.07 ± 0.02	--	7.17 ± 1.36	--	6.65 ± 3.71
	Spring	0.09 ± 0.02	0.11 ± 0.06	0.79 ± 0.16	0.98 ± 0.26	4.86 ± 3.62	7.60 ± 2.90
	Summer	0.24 ± 0.17	0.31 ± 0.07	0.90 ± 0.47	0.91 ± 0.53	3.89 ± 1.67	--

Table 2: Correlation between the different measured metals in muscles of *Siguns rivulatus* collected from Bitter lakes and lake Timsah during 2002-2003.

Organ	Metal	Cu	Zn	Pb	Cd	Fe	Mn	Cr	Ni	Co
Bitter Lakes	Cu	1.00								
	Zn	0.60	1.00							
	Pb	0.57	-0.06	1.00						
	Cd	-0.55	-0.21	0.14	1.00					
	Fe	-0.36	0.19	0.05	0.91	1.00				
	Mn	0.58	0.06	0.99*	0.22	0.18	1.00			
	Cr	0.76	0.85	-0.06	-0.69	-0.35	-0.02	1.00		
	Ni	0.63	0.87	-0.26	-0.64	-0.28	-0.20	0.98*	1.00	
	Co	0.39	0.84	-0.50	-0.53	-0.15	-0.43	0.88	0.96*	1.00
Lake Timsah	Cu	1.00								
	Zn	-0.39	1.00							
	Pb	0.62	0.48	1.00						
	Cd	0.81	0.22	1.00*	1.00					
	Fe	-0.60	-0.50	-1.00	-0.95	1.00				
	Mn	-0.03	-0.91	-0.80	-0.61	0.80	1.00			
	Cr	-0.95	0.66	-0.30	-0.59	0.30	-0.28	1.00		
	Ni	-0.19	0.98	0.60	0.41	-0.70	-0.97	0.49	1.00	
	Co	-0.34	1.00*	0.50	0.27	-0.60	-0.93	0.61	0.99	1.00

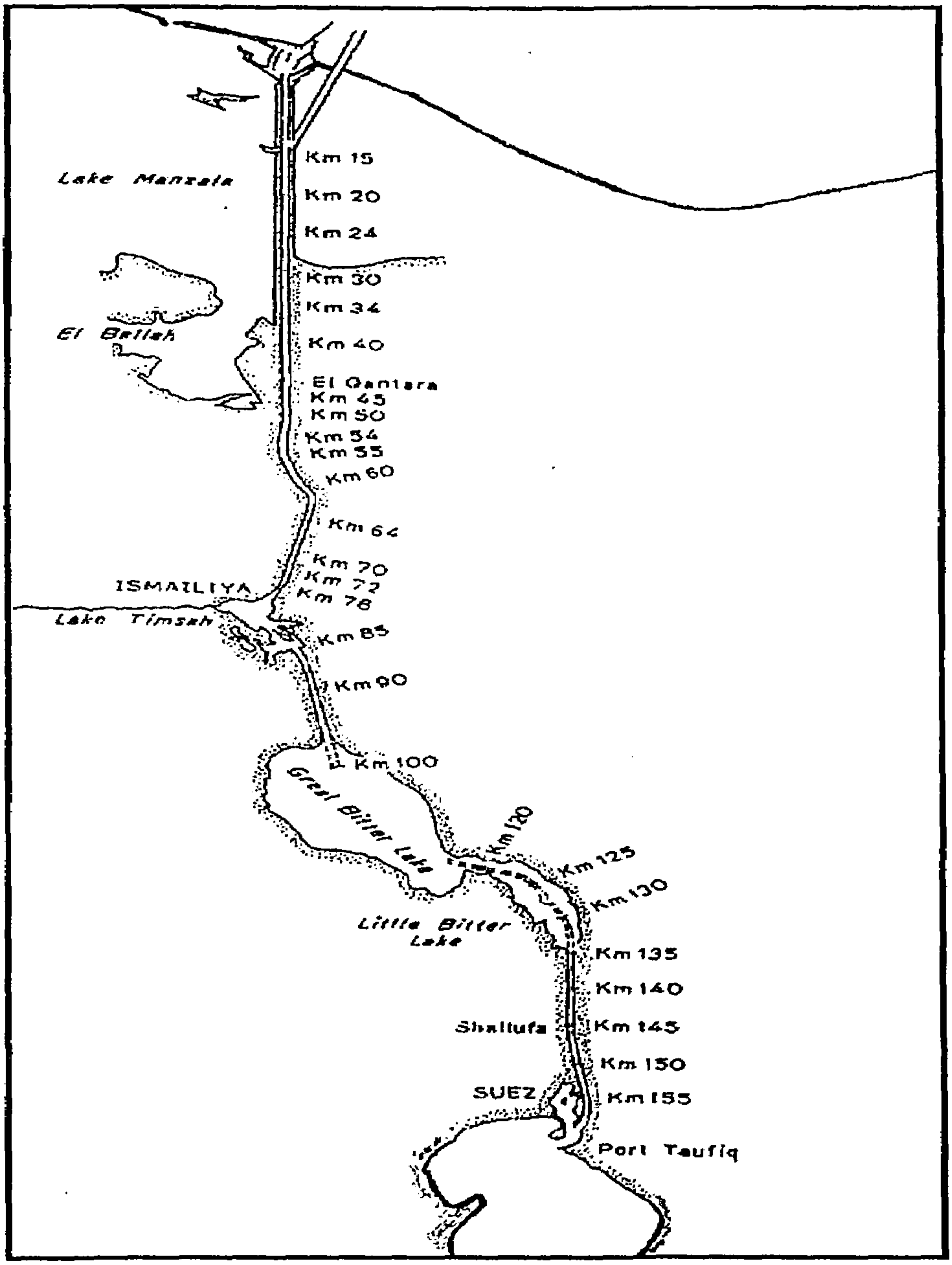

Fig. (1): Map of Suez Canal demonstrating the Bitter lakes and lake Timsah.

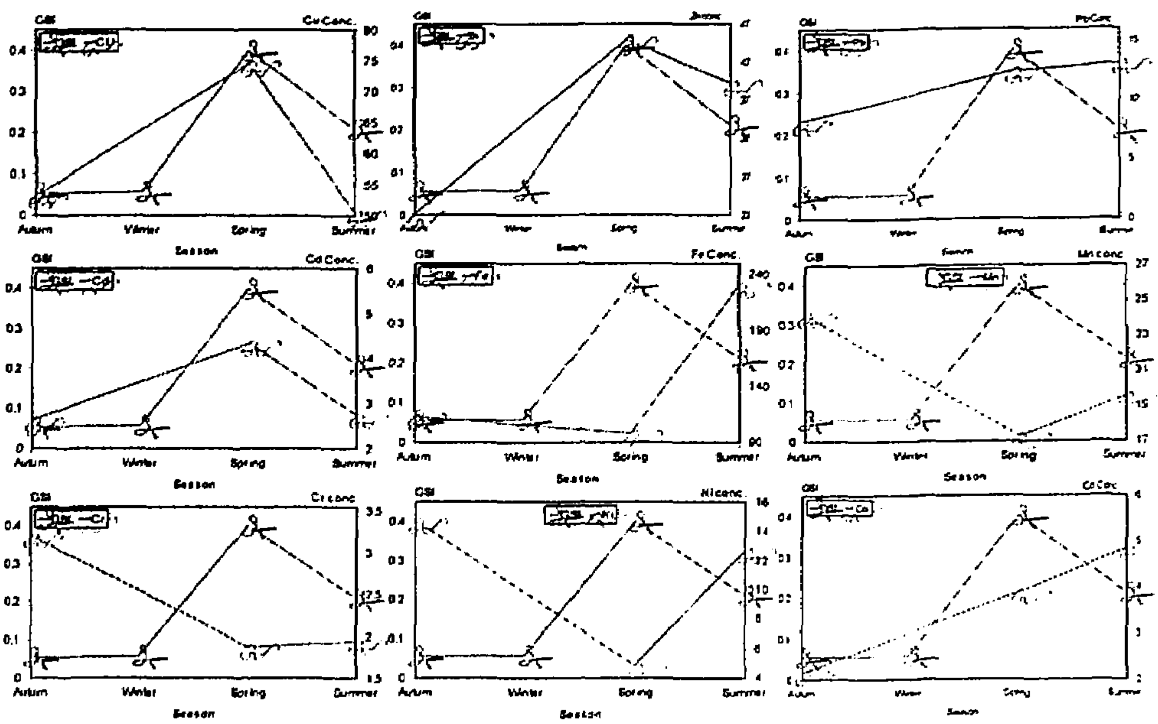

Figure 2: Relationship between metals concentration (µg/g) and GSI in gonad of fish, *Sigauns rivulatus,* in Bitter lakes.