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Measurement Some of Immunological Parameters *Cyprinus Carpio* in the Polluted (Ahwaz) and Non-Polluted (Shoushtar) Area in Karun Riverian Iran

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Abstract
Karun Riverian Iranian important source of water which has a dramatic decline in water quality in the last 10 years because of the discharge of wastewater. Recently pollution level in this river has raised and it caused environmental hazards for aquatic organisms. Because the fish are biological indicators, In this study, immune factors of common carp *(Cyprinus carpio)* was evaluated in two area sAhvaz (pollution) and Shoushtar (non-polluted), in two seasons Autumn 2010 (low water) and spring 2011(high water). 62 samples of common carp with the average weight 245±24 g and total length of 23±8 cm were captured. After adaptation, blood samples were collected and immune factors, including lysozyme activity, total protein, albumin, and serum bactericidal activity was examined. Our results showed that total protein rate, lysozyme activity, and albumin in Ahwaz sample has significant difference ($P<0.05$) compared to Shoushtar sample. While, serum bactericidal activity showed that seasons had no significant impact on factors ($P>0.05$). Moreover there was no significant difference in male and female sex ($P>0.05$).
Keywords: Karun River, pollution, Biomarker, *Cyprinus carpio*, Immunological parameters

INTRODUCTION

Karun river is one of the important source of water in Iran, that it be affected by evacuation of pollution types [24]. This river be confronted the significant reduction of water quality during recent 10 years, and at now the pollution potential of this river has been high and higher and it has caused the environmental risks for aquatic creatures [4). About the negative effects of pollutant on aquatic we can mention some cases like genetic damage, the effect on reproduction, behaviour and immune system [17]. One of the most common ways of the study of pollution conditions is bio monitoring [1]. The development of biomarkers provided the possibility of using biological responses for evaluation of the effect of pollutants on aquatic environment in last 1980th [32]. The changes of aquatic ecosystems have recognized immediately and its destructive effects will vanish before it being uncontrollable, by bio monitoring [10].

Cyprinus carpio is one of the most important breeding fish of Khuzestan, Gilan and Mazandaran provinces of Iran that it is considered as a fish that it has economical and edible value in Karun River. Immune system is an important mechanism of physiological in animals for maintaining them against infections. Immunity can be nonspecific or acquired which acquired immunity includes immunity of humeral (producing immunoglobulin) and cellular [12]. In fish, humeral immunity responses evaluated more for examination of immune system and we commonly examine indexes that they are total protein, the power of destroying bacteria of serum, Lysozyme and IgM. Biomarkers are measurable properties in a biological system, which it changes due to disease or confronting of living creatures with chemical materials [15]. The national academy of biomarkers science has defined biological marker as created changes genobiologically in operation or structure or processes and cellular or biochemical, that these changes are in manner that they are measurable in sample or biological systems. Biomarkers have been effective and sensitive [30] and they react immediately than environmental changes which these immediate reactions can represent. There is much study that focuses on using fish species for evaluating biomarkers creations than environmental pollutant and as an index for healthy bio monitoring of aquatic ecosystem [2, 22]. All of environmental pollution have inflammatory and preventive effects on immune system of fish and their effect to some extent all of the immune system components [29]. There are some studies in which they have used from biomarkers of immune system (immunity and bloody factors) for examination of pollution effects, they were changes in bloody parameters of *Cyprinus carpio* fish after confronting with cadmium [31]. The pollution effects on bloody parameters of *Gobiusniger* that they were hunted from polluted an unpolluted regions [15], and also they show the examination of haematological parameters of silver carp blood in poisoning with threeclorofon (Naziffi, 2001). In a study that Rowan (2007), he used blood parameters of immune system in fish (*Ameiurus nebulosus*) as biomarker and he compared different stations with each other from a pollution level point. The achieved results showed there is on considerable in growth. Neutrophil levels in polluted areas were more than unpolluted areas. In a study which Sutheeshkumar *et al*, (2010) have did, this result achieved that the haematology parameters likes MCH, MCHC, HcT, Hb, RBC, WBC, MCV, valuable indicators and ability for monitoring of fish health. Also

they explained that biochemical parameters of blood serum including glucose, total protein, cholesterol and urea can use due to monitoring any change in water quality. In Iran, up to now, there are a few field examinations of pollutants effects on biomarkers of immune system and performed researches had been lab on greatly. We have studied in this article the changes of immunity factors of *Cyprinus carpio* under effect of Karun River and seasonal changes. The fundamental aim of this research is bio monitoring of aquatic environment with study of immune factors of *Cyprinuscarpio* at two different areas of Karun River. Also it will be indicator of that whether we can attribute the portable changes in immune factors at different areas and seasons, to present pollutants or can't attribute. In this study, also we will compare the immune factors at both Ahvaz (including high pollution) and Shushtar (the region without any pollution), in autumn season (draughtiness) and spring (watery season).

MATERIALS AND METHODS

Fish hunting performed in this experiment, in two different areas of Karun River. The first, Ahvaz contains all of kinds of pollutions and many of industrial, house-made and agricultural wastes enter to this area of karun. Shushtar is the second areas of sampling that it has a distance of 90 kilometres with Ahvaz, the first area (figure 1). This area have had a little pollution, with respect to established studies [5, 8, 24] and also the recorded statistics and information related to laboratory of quality determining of Khuzestan waters, this area have been a region with very low pollution. Therefore it considers as evidence region.

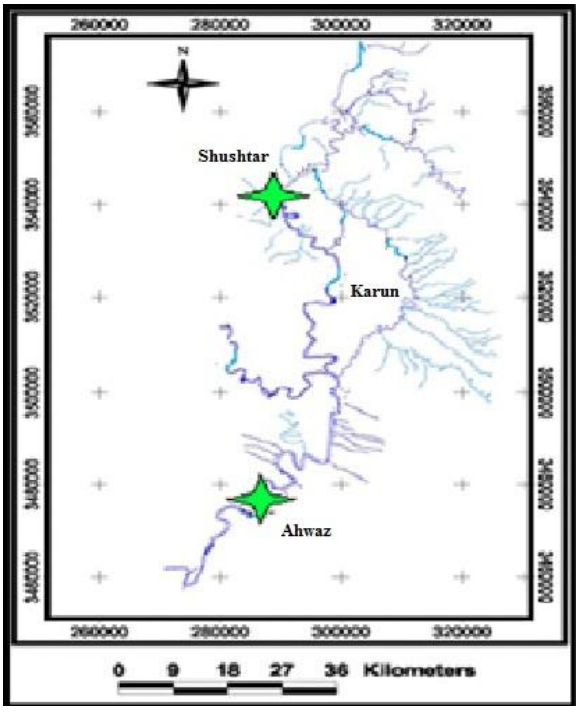

Figure 1. Sampling Stations

Sampling

In this study, we investigated 62 *Cyprinus carpio* with weight average 245gr and total length of 23cm, from two areas of Ahvaz and Shushtar that they hunted by net with spring of 8cm. The sampling performed during both autumn 2010 (the drought season of karun) and spring 2011 (the watery season of karun). Fish transported to the laboratory of Chamran veterinary medicine college of Ahvaz, after hunting in these two areas by air pump, where they transferred to fibreglass tanks and the bleeding operation performed after adaptation (about 24 hours). At first, fish have been anaesthetized by clove flower powder [27] and they biometry. The blood samples were collected from the area of tail vein. The blood samples transferred to micro tube contain heparin. Then, samples centrifuged. Their blood serum separated and factors examined. For measuring of Lysozyme activity of serum, it be used from the method of Agars Liz plate that it has recommended by Roed *et al*, (1993) and ability of killing bacteria of serum samples from the recommended method by Kajita *et al*, (1990). The total protein of serum calculated according to this method [18] and also with use of standard kit of protein estimating related to Biochemistry Company. This method is named modi biuret. The Albumin measurement by the method of BCG (bromocresol green method) and also with use of recognitive kit related to Biochemistry Company [7].

All of the statistical analysis fulfilled with SPSS 16, All first data examined in viewpoint of being normal and it applied the data was analyse by ANNOVA.

RESULTS AND DISCUSSION

The Effect of Area on Immune Factors

The results of the study of immune factors average showed there is a significant difference between Ahvaz and Shushtar, in Lysozyme activity ($p<0.01$). This way in factor of Shushtar shows a considerable increasing than Ahvaz. The results of variance analysis showed the significant difference in total protein and albumin between two regions ($p<0.01$). While the ability of serum bacterial killing showed significant difference between two regions ($p>0.05$) (table 1).

The Effect of Season on Immune Factors

The received results from the study of the average of immune factors in two season of sampling showed that the value of these factors in autumn and spring have not any significant difference ($P>.05$), (table 2).

Reciprocal Results of Season and Region on Immune Factors

The received results from study of reciprocal effects of season (autumn and spring) and region (Ahvaz and Shushtar) on immune factors of *Cyprinus carpio* showed that the value of these factors in Ahvaz and Shushtar in autumn and spring season have not any significant ($p>.05$) with each other (table 3).

Table 1. The main effects of regions (Ahvaz and Shushtar) on immune factors of *Cyprinus carpio* in the Karun River

immune factors	Area	Number fish	mean ± S.D.	Data Analysis		
				Df	F	Sig
Lysozyme activity(µ/l)	Ahvaz	32	241.88±22.658	1	11.909	0.001**
	Shoushtar	30	267.57±33.759			
Total protein(mg/l)	Ahvaz	32	5.436±2.263	1	20.078	0.000**
	Shoushtar	30	3.37±0.852			
Albumin(mg/l)	Ahvaz	32	0.551±0.361	1	16.984	0.000**
	Shoushtar	30	0.883±0.227			
serum bactericidal activity	Ahvaz	32	255.41±65.212	1	0.051	0.822 ns
	Shoushtar	30	251.7±41.471			

*P <0.05%, **P <0.01% and ns is not a significant difference between two regions.

Table 2. The effect of seasons on immune factors of *Cyprinus carpio* in the Karun River.

immune factors	Season	Number fish	mean ± S.D.	Data Analysis		
				Df	F	Sig
Lysozyme activity(µ/l)	Autumn	27	254.63±23.439	1	0.531	0.469 ns
	Spring	35	254.06±36.339			
Total protein(mg/l)	Autumn	27	4.717±2.455	1	0.026	0.872 ns
	Spring	35	4.219±1.584			
Albumin(mg/l)	Autumn	27	0.701±0.278	1	0.31	0.58 ns
	Spring	35	0.72±0.392			
serum bactericidal activity	Autumn	27	255.07±66.709	1	0.017	0.896 ns
	Spring	35	252.49±44.097			

*P <0.05%, **P <0.01% and ns is not a significant difference between two regions.

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Table 3. The reciprocal effects of season (autumn and spring) and region (Ahvaz and Shushtar) on immune factors of *Cyprinus carpio* in the Karun River

immune factors	**area**	**season**	**Number fish**	**mean ± S.D.**	**Data Analysis**		
					Df	**F**	**Sig**
Lysozyme activity(µ/l)	Ahvaz	autumn	17	246.941±23.644	1	0.493	0.458ns
		spring	15	236.133±20.77			
	Shoushtar	autumn	10	267.7±17.081			
		spring	20	267.5±40.016			
Total protein(mg/l)	Ahvaz	autumn	17	5.53±2.761	1	0.076	0.783ns
		spring	15	5.328±1.612			
	Shoushtar	autumn	10	3.334±0.693			
		spring	20	3.387±0.938			
Albumin(mg/l)	Ahvaz	autumn	17	0.602±0.268	1	0.648	0.424ns
		spring	15	0.493±0.447			
	Shoushtar	autumn	10	0.87±0.216			
		spring	20	0.89±0.238			
serum bactericidal activity	Ahvaz	autumn	17	256.235±79.079	1	0.0	0.991ns
		spring	15	254.466±47.621			
	Shoushtar	autumn	10	253.1±1.613			
		spring	20	251.1±42.464			

*P <0.05, **P <0.01 and ns is not a significant difference between two regions.

DISCUSSION

The immunity is an important mechanism of physiological in animals, in order to protecting against infections and setting internal environmental of body [12]. In recent years, in ecotoxicology studies, there be used from wild and hunter species for examination of immunological effects of pollutants, that we can say the main and final aim of these studies had been the using of fish species for monitoring of aquatic ecosystem [22]. Our results showed that Lysozyme, total protein and albumin have a significant difference in Ahvaz than Shushtar.

The comparison of these results with previous studies that mostly they are established in laboratory, revealed this topic that such changes in immune factors, greatly related to the presence of metal pollutant in Karun and at Ahvaz. Therefore, if there be specific, the type of changes that various pollutants on biomarkers of immune system, then for determining the type of pollutants which change aquatic environment, there is not any great necessity to measure pollutions value in water ecosystems. in our study, the results more indicates the presence of metal pollutants in Karun and certainly at Ahvaz region, in a way that measuring of pollutants amount of Karun in established research indicate high degree of heavy metals (chromium, cadmium, lead, etc) in this area [4, 13, 19]. The essence of Lysozyme changes that make by heavy metals and environmental pollutants was complex and it shows different results (increasing/decreasing), that it depends on factors like pollutant, species and etc. Consequently, these differences cause we cannot interpret results comfortably [3]. Our results, indicated the decreasing of Lysozyme amount in Ahvaz region than Shushtar region, that the Lysozyme decrease cause the sensitivity of fish go up against disease. Also decreasing of WBC can change the amount of Lysozyme [9]. According to Dye and Donaldson statements (year), confronting with heavy metals in fish increase cortisol level and this increasing to decrease WBC specially lymphocytes. Because Lysozyme has leukocyte resource, we can conclude that decrease in WBC amount can also decrease the Lysozyme level [25].

Plasma proteins synthesis by liver and apart of plasma proteins excrete from body, in many of disease and physiological disorders [26]. Using from total protein, the albumin of fish blood serum can apply as an indicator for evaluation of pollutants stress responses. As it is clear from following results, the total amount of protein has a significant increase in Ahvaz than shushtar ($p<0.01$), while albumin didn't show a significant reduction, which this increasing in protein amount is due to beginning. Protein synthesis in liver [33] These results correspond with findings of researchers who they have used from these indexes for evaluation of pollution effects. As an example, the confrontation of *Cyprinus carpio* with heavy metals like lead, mercury, nickel and copper in primary 12 hours either in pulling concentration or in concentration under pulling shows the increasing in total protein amount and decreasing albumin amount. The decrease of albumin level in polluted environment with metal pollutants is due to providing of energy requirements. S albumin transfers material from apart of fish body to other parts. They have dietetic, protective and transitional performance [11].

However, applying from these biomarkers still have not completed for evaluation of ecological risks. The reason of this topic can be to effect of immune system by wide range of stressors. Temperature is one of the most important parameters that it can consider as stressor. Because fish cannot regulate their body temperature, therefore they have affected of environment temperature. But commonly, it be proved that higher temperature (about 5-10°c higher than the temperature of available normal conditions) can increase immune responses [6; 20]. In this study, the water temperature in both Ahvaz and Shushtar, during autumn and spring seasons, was optimum temperature for *Cyprinus carpio* consequently, temperature factor can't represent as stressor in this study. The measurement and analysis of immune biomarkers that they be used in this study, they provide two series of valuable information for us. Firstly, they show how a fish can react than the presence of collection of changes in qualitative parameters of water that it is their live environment. Secondly, many studies are necessary, until they protect water ecosystems against destroyer pollutants related to Environment, with use of biological methods.

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