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Assessing Cd, Pb Accumulation in the Tissues of *Chalcalburnus chalcoides* in Anzali Port

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Abstract: This study was performed in spring 2008 in order to determinate of Cd and Pb accumulation in gills, kidney, skin tissues and muscles of *Chalcalburnus chalcoides* in Anzali Port in the South west of Caspian Sea within 10 km² zone. The samples were collected from 12 stations, biometric measurements had been taken, thereafter, tissues were separated and digested chemically and then it were analyzed by Flame Atomic Absorption Spectrophotometer. Pearson correlation of metal's concentration in studied tissues with the weight and forked length of fish showed non significant relationship between weight and length ($p \geq 0.05$) and results showed that smaller fish have higher concentration of Cd and Pb than larger ones. Maximum concentrations of Cd and Pb were recorded in gills (0.94 and 8.33 ppm) while minimum concentrations were recorded in muscles (0.02 and 0.12 ppm). Results show that Cd and Pb were accumulated in descending order in gills>skin>kidney>muscles. The level of metals was compared with (WHO, 1993) standard and it was resulted that Cd in gills and Pb in all tissues were significant difference at ($p < 0.05$) levels.

Key words: Heavy metal, *Chalcalburnus chalcoides*, bioaccumulation, Anzali Port

INTRODUCTION

Technology improvement and increasing of population in cities and rural regions, cause pollution such as heavy metals form agriculture and industrial waste water and municipal sea wages into aquatic ecosystems. These pollutants like heavy metals after entering into aquatic environment cumulate in tissues and organs of aquatic organisms. The amount of absorption and assembling depends on ecological, physical, chemical and biological conditions and the kind of element and physiology of organisms (Jaffar *et al.*, 1998, 1999). Also sexuality, weight, age, feeding, habits and tissue race can be effective (Gil *et al.*, 1997; Fuhrer *et al.*, 1996). These metals after assembling in the body of aquatic organisms enter into food chain and extremely consumed by human. Reaction of these elements depends on the concentration, physical-chemical properties, chemical bonds and their solution that effects on the absorption, assembling, distribution in body and physiological effects of metals (Gharib, 2004b). Because of distractive and position trace elements such as Cd and Pb even in few amount (Agbozu *et al.*, 2007) is necessary to be controlled continuously.

One of the most important ways to control is to collect some fish as a bioindicator of metal pollutants in aquatic environments. In this case, different tissue of fish can be used to study the physiological effects of heavy metals (Obasohan, 2007). The concentration of heavy metals in organs of fish can be an introduction to precision of pollution level of water. Anzali Port in the South west of Caspian Sea is polluted by heavy metals because of ship traffic, oil tankers and entrance of municipal, industrial and agricultural sea wages by rivers and some from the sea. The aim of the present study is to evaluate Cd and Pb, in tissues of *Chalcalburnus chalcoides* in this port. In addition it has been tried to analysis tissues of this fish and measure the amount of Cd and Pb. In addition of

economic values, this fish plays an important role in people's food in Northern provinces of Iran, particularly Gilan Province.

MATERIALS AND METHODS

This study has been done of the spring 2008 in Anzali Port in the South west of Caspian Sea. The sampling stations are shown in Fig. 1.

The samples were collected from twelve stations for female of *Chalcalburnus chalcoides* in triplicate (*3 = 9 sample in each station). After measurements the tissues gills, kidney, skin and muscles were detached and washed. Then they were dried up at 60°C for 48 h and have been wet digested in nitric acid 65% and perchloric acid 75% (Nollet-Leo, 2004). Metal concentration survey by atomic absorption flame photometric model of Varian A110. In order to statistical calculates in descriptive analysis and illative research from statistic Z (normal distribution) various t-tests, ANOVA analysis in have been determined by SPSS soft ware.

RESULTS

The results of biometry measurements of fish samples are shown in Table 1. According to Table 2 and 3 maximum of Cd in gills (station 7) was 0.94 ppm and the minimum in muscles

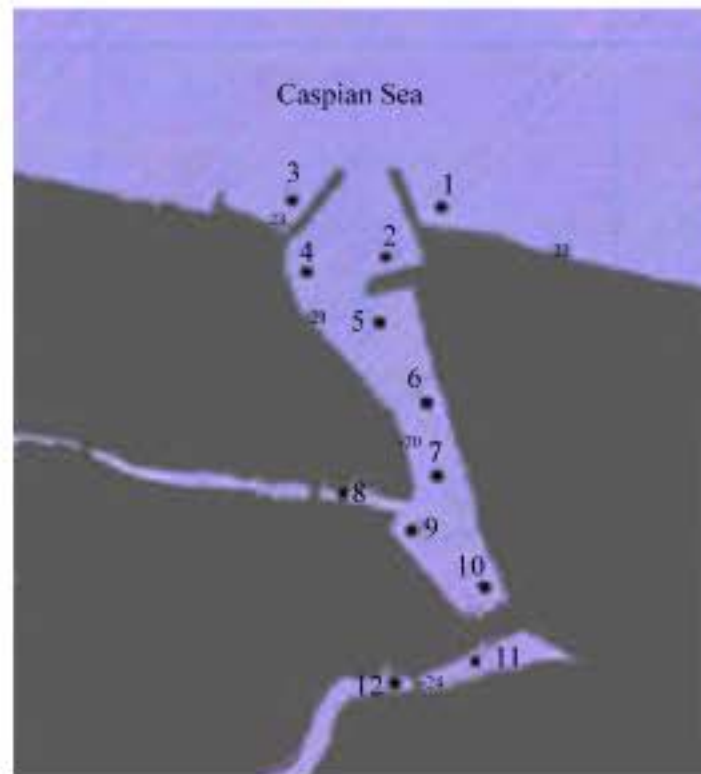


Fig. 1: The sampling stations of Anzali Port

Table 1: The average of weight and Forked length of *Chalcalburnus chalcoides* in Anzali Port

Station No.	Weight (g)	Forked length (cm)
1	29.15±0.70	13.9±0.3
2	57.70±2.82	17.9±1.3
3	56.45±1.58	17.0±1.2
4	61.40±5.43	17.4±0.8
5	63.22±4.47	18.7±1.4
6	67.23±4.35	18.6±1.3
7	43.14±2.44	16.2±1.1
8	51.71±1.32	17.9±0.9
9	79.79±1.41	19.4±0.7
10	37.96±4.12	15.1±1.7
11	47.39±1.70	16.3±1.3
12	54.78±3.60	18.6±0.4
Min	29.15	13.9
Max	79.79	19.4

Table 2: The average of Cd concentration (ppm) in the tissues of *Chalcalburnus chalcoides* in Anzali Port

Station No.	Muscles	Kidney	Liver	Gills
1	0.05±0.008	0.07±0.003	0.13±0.036	0.24±0.013
2	0.06±0.008	0.08±0.006	0.10±0.008	0.35±0.010
3	0.03±0.005	0.12±0.002	0.17±0.018	0.09±0.021
4	0.03±0.006	0.12±0.009	0.21±0.030	0.20±0.025
5	0.06±0.002	0.07±0.001	0.38±0.010	0.15±0.017
6	0.03±0.007	0.11±0.007	0.14±0.030	0.67±0.009
7	0.06±0.001	0.22±0.080	0.32±0.009	0.94±0.008
8	0.04±0.004	0.07±0.005	0.23±0.020	0.49±0.021
9	0.04±0.008	0.11±0.004	0.12±0.021	0.43±0.007
10	0.08±0.001	0.13±0.002	0.16±0.014	0.23±0.015
11	0.02±0.004	0.03±0.005	0.07±0.009	0.28±0.004
12	0.03±0.005	0.10±0.001	0.14±0.010	0.21±0.005
Min	0.02	0.03	0.07	0.09
Max	0.08	0.12	0.38	0.94

Table 3: The average of Pb concentration (ppm) in the tissues of *Chalcalburnus chalcoides* in Anzali Port

Station No.	Muscles	Kidney	Liver	Gills
1	0.43±0.045	1.50±0.072	2.80±0.075	3.91±0.109
2	1.15±0.017	1.40±0.033	1.90±0.058	3.33±0.060
3	0.30 ±0.085	0.15±0.072	1.40±0.033	2.17±0.040
4	0.40±0.016	1.30±0.027	1.60±0.040	2.08±0.086
5	0.41±0.021	1.25±0.032	1.90±0.058	2.27±0.099
6	3.00±0.110	1.00±0.091	1.74±0.043	8.33±0.083
7	0.60±0.018	0.75±0.018	2.05±0.044	8.16±0.037
8	0.20±0.008	1.02±0.069	3.05±0.052	4.06±0.087
9	0.62±0.019	0.85±0.071	1.20±0.017	3.76±0.045
10	0.50 ±0.074	1.25±0.090	1.90±0.056	2.83±0.074
11	0.70±0.034	0.75±0.028	0.80±0.022	5.63±0.067
12	0.12±0.084	1.30±0.035	3.00±0.045	4.21±0.021
Min	0.12	0.15	0.80	2.08
Max	3.00	1.40	3.05	8.33

Table 4: Crosstabe of Cd level based on weight of *Chalcalburnus chalcoides* in Anzali Port

Range of weight		Range of Cd							Total
		<0.1 (ppm)	0.1-0.2 (ppm)	0.2-0.3 (ppm)	0.3-0.4 (ppm)	0.4-0.5 (ppm)	0.6-0.7 (ppm)	0.9-1.0 (ppm)	
1 (20-30 g)	% within weight	50	25	25	0	0	0	0	100
	% within Cd	10	7.1	14.3	0	0	0	0	8.3
2 (30-40 g)	% within weight	50	0	25	0	0	25	0	100
	% within Cd	10	0	14.3	0	0	100	0	8.3
3 (40-50 g)	% within weight	37.5	50	0	0	12.5	0	0	100
	% within Cd	15	28.6	0	0	50	0	0	16.7
4 (50-60 g)	% within weight	37.5	25	18.8	12.5	0	0	6.3	100
	% within Cd	30	28.6	42.9	66.7	0	0	100	33.3
5 (60-70 g)	% within weight	50	16.7	16.7	8.3	8.3	0	0	100
	% within Cd	30	14.3	18.6	33.3	50	0	0	25
6 (70-80 g)	% within weight	25	75	0	0	0	0	0	100
	% within Cd	5	21.4	0	0	0	0	0	8.3
Total	% within weight	41.7	29.2	14.6	6.3	4.2	2.1	2.1	100
	% within Cd	100	100	100	100	100	100	100	100

(station 11) was 0.02 ppm. Also maximum of Pb in gills (station 6) was 8.33 ppm and the minimum in muscles (station 12) was 0.12 ppm. Crosstabs Table 4 and 5 are based on Cd and Pb level in tissues based on weight that determine different absorption level in metal concentration in studied tissues

Table 5: Crosstabe of Pb level based on weight of *Chalcalburnus chalcoides* in Anzali Port

		Range of Pb							Total
		0	1	2	3	4	5	8	
Range of weight		<1 (ppm)	1-2 (ppm)	2-3 (ppm)	3-4 (ppm)	4-5 (ppm)	5-6 (ppm)	8-9 (ppm)	
1 (20-30 g)	% within weight	75	0	0	0	0	25	0	100
	% within Pb	18.8	0	0	0	0	100	0	8.3
2 (30-40 g)	% within weight	25	50	0	0	0	0	25	100
	% within Pb	6.3	12.5	0	0	0	0	50	8.3
3 (40-50 g)	% within weight	25	50	0	25	0	0	0	100
	% within Pb	12.5	25	0	40	0	0	0	16.7
4 (50-60 g)	% within weight	31.3	31.3	25	6.3	0	0	6.3	100
	% within Pb	31.3	31.3	66.7	20	0	0	50	33.3
5 (60-70 g)	% within weight	33.3	25	8.3	16.7	16.7	0	0	100
	% within Pb	25	18.8	16.7	40	100	0	0	25
6 (70-80 g)	% within weight	25	50	25	0	0	0	0	100
	% within Pb	6.3	12.5	16.7	0	0	0	0	8.3
Total	% within weight	33.3	33.3	12.5	10.4	4.2	2.1	4.2	100
	% within Pb	100	100	100	100	100	100	100	100

Table 6: Crosstabe of Cd level in the tissues of *Chalcalburnus chalcoides* in Anzali Port

		Range of Cd							Total
		0	1	2	3	4	6	9	
Tissues		<0.1 (ppm)	0.1-0.2 (ppm)	0.2-0.3 (ppm)	0.3-0.4 (ppm)	0.4-0.5 (ppm)	0.6-0.7 (ppm)	0.9-1.0 (ppm)	
1 (Gills)	% within gills	8.3	16.7	33.3	8.3	16.7	8.3	8.3	100
	% within Cd	5	14.3	57.1	33.3	100	100	100	25
2 (Kidney)	% within kidney	50	41.7	8.3	0	0	0	0	100
	% within Cd	30	35.7	14.3	0	0	0	0	25
3 (Liver)	% within liver	8.3	58.3	16.7	16.7	0	0	0	100
	% within Cd	5	50	28.6	66.7	0	0	0	25
4 (Muscles)	% within muscle	100	0	0	0	0	0	0	100
	% within Cd	60	0	0	0	0	0	0	25
Total	% within tissues	41.7	29.2	14.6	6.3	4.2	2.1	2.1	100
	% within Cd	100	100	100	100	100	100	100	100

Table 7: Crosstabe of Pb level in the tissues of *Chalcalburnus chalcoides* in Anzali Port

		Range of Pb							Total
		0	1	2	3	4	5	8	
Tissues		<1 (ppm)	1-2 (ppm)	2-3 (ppm)	3-4 (ppm)	4-5 (ppm)	5-6 (ppm)	8-9 (ppm)	
1 (Gills)	% within gills	0	0	33.3	25	16.7	8.3	16.7	100
	% within Pb	0	0	66.7	60	100	100	100	25
2 (Kidney)	% within kidney	33.3	66.7	0	0	0	0	0	100
	% within Pb	25	50	0	0	0	0	0	25
3 (Liver)	% within liver	8.3	58.3	16.7	16.7	0	0	0	100
	% within Pb	6.3	43.8	33.3	40	0	0	0	25
4 (Muscles)	% within muscles	91.7	8.3	0	0	0	0	0	100
	% within Pb	68.8	6.3	0	0	0	0	0	25
Total	% within tissues	33.3	33.3	12.5	10.4	4.2	2.1	4.2	100
	% within Pb	100	100	100	100	100	100	100	100

based on weight. Crosstabs Table 6 and 7 are based on Cd and Pb level in different tissues. Error charts of Cd and Pb accumulation in the different weights and tissues are shown in Fig. 2 and 3. Scatter plot of Cd and Pb average in difference tissues are shown in Fig. 4.

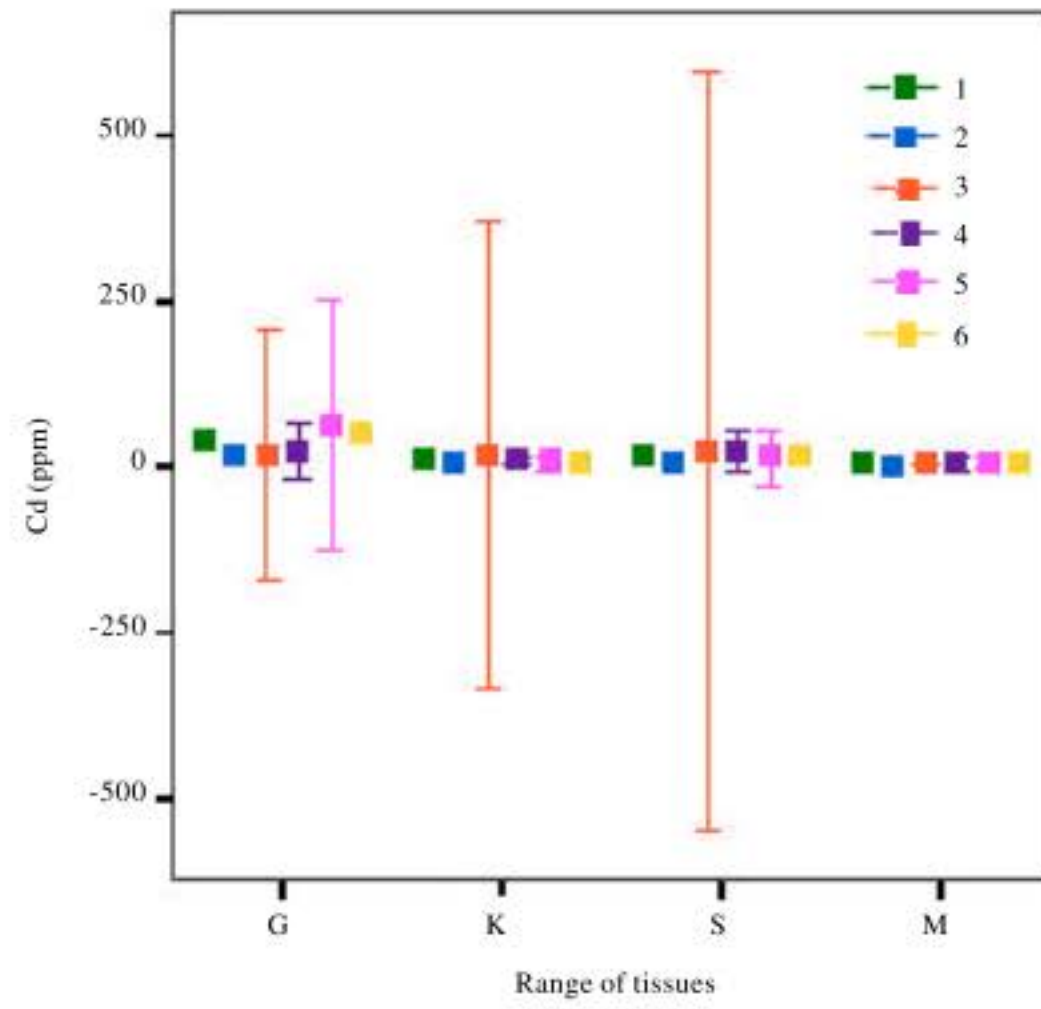


Fig. 2: Error chart of Cd concentration in the different weights and tissues of *Chalcalburnus chalcoides* in Anzali Port

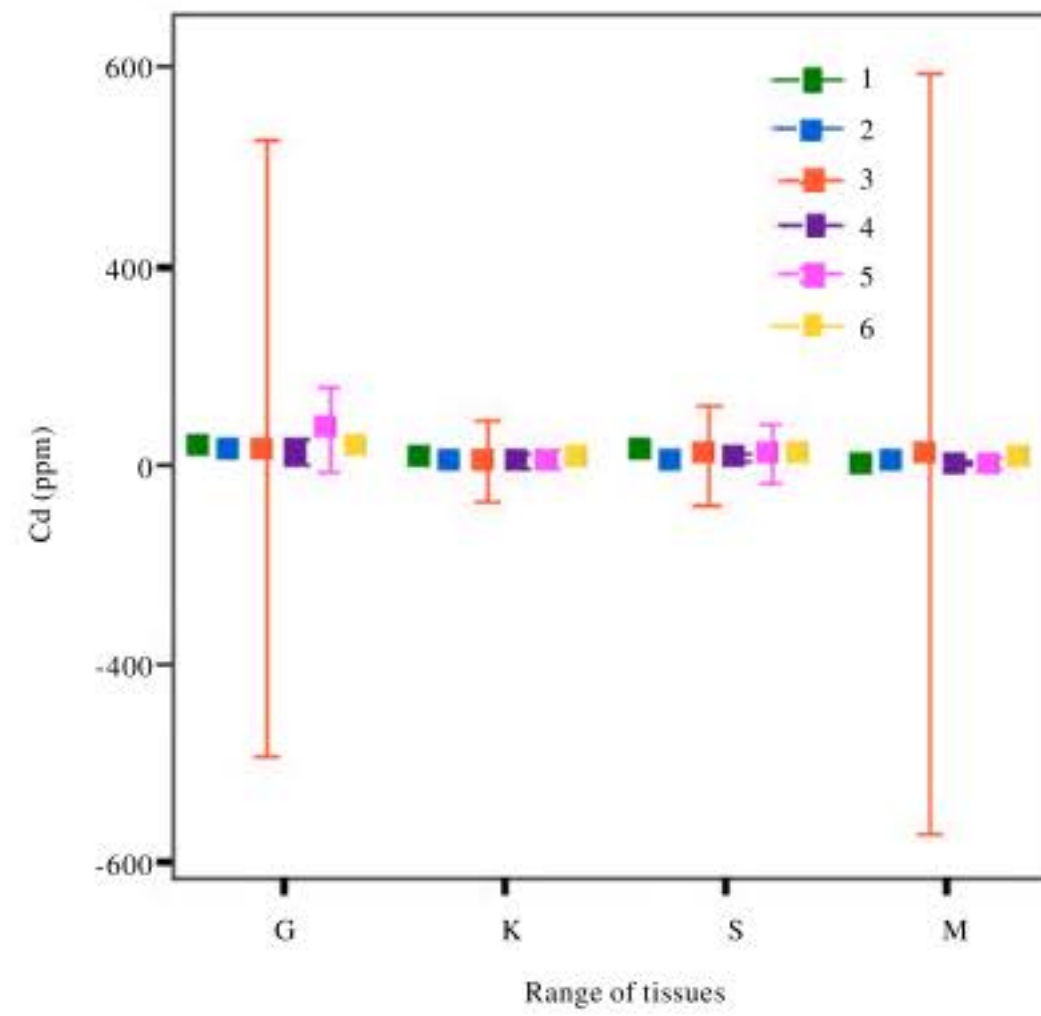


Fig. 3: Error chart of Pb concentration in the different weights and tissues of *Chalcalburnus chalcoides* in Anzali Port

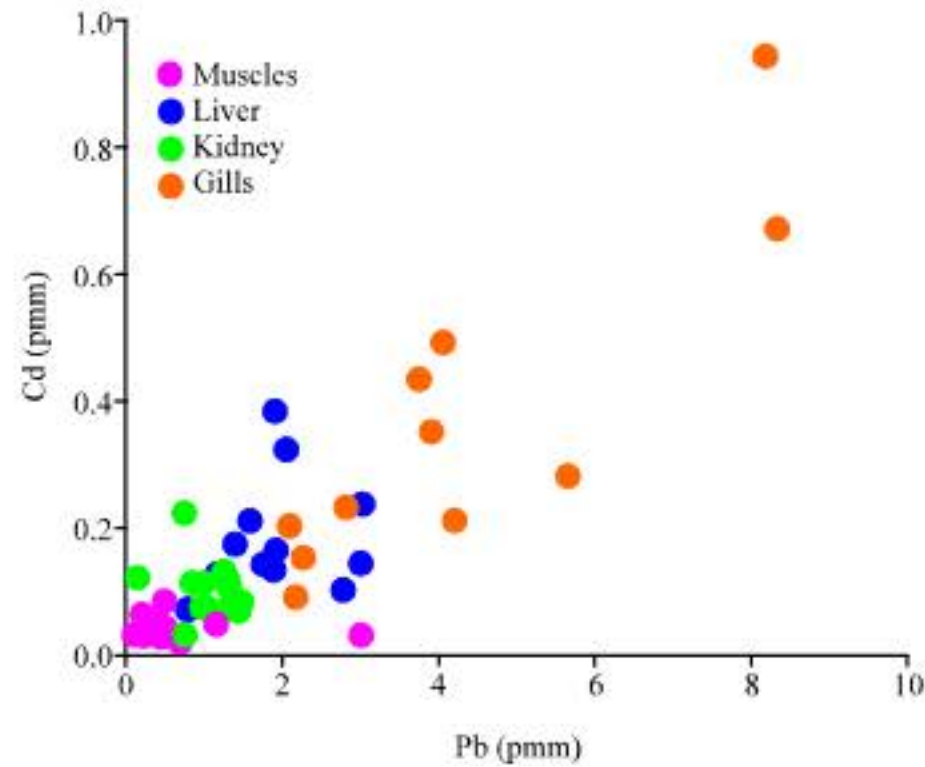


Fig. 4: Scatter plot of Cd and Pb average in the tissues of *Chalcalburnus chalcoides* in Anzali Port

DISCUSSION AND CONCLUSION

Results in Table 2 and 3 show that maximum concentration of Cd and Pb in gills were found in stations 6 and 7. This could be attributed to traffic of ships, particularly commercial oil ships. The lowest value of Cd and Pb found in stations 11 and 12 where the distance from Anzali Port and port activity is not too much. Pearson correlation of metal's concentration in studied tissues with the weight and forked length of fish showed non significant relationship between weight and length ($p \geq 0.05$). To consider the results of Table 4, maximum Cd (0.8 to 0.9 ppm) were found in 25% of fish in ranged from 20 to 30 g and 6.3% of them in ranged from 40 to 50 g. Also the value 0.7 to 0.8 ppm of this metal is in fish with range of 10 to 20 g. Based on Table 5, maximum concentration of Pb was 9 to 10 ppm in fish with range of 40 to 50 g and fish with range of 20 to 30 g, had concentration of 6 to 7 ppm and it is assembled is in fish with weight range of 40 to 60 g. At the end, results show that smaller fish have higher concentration of Cd and Pb than larger ones. Table 6 and 7 show that accumulation of Cd and Pb is different in various tissues and ranged as follows: gills > skin > kidney > muscles. In other reports about heavy metals accumulation in different species of fish have driven that muscles has the less amount of metals. Also gills have the most concentration of heavy metals (Munn *et al.*, 1995; Obasohan, 2007). Table 8 shows the quality of correlation of Cd and Pb in studied tissues. According to the reports between two measured metals using Pearson correlation $r = 0.870$ in gills. It could be attributed to the same source of entering solvent of Cd and Pb salts. According to Fig. 2 maximum standard deviation of Cd accumulation was found in fish ranged between 30-40 g in weight. Also according to Fig. 3 maximum standard deviation of Pb accumulation was recorded between 30-40 g of its weight of muscles. The pearson correlation of gills and muscles according to Pb concentration is $r = 0.917$ and about of gills and kidney according to Cd concentration is $r = 0.577$. It means that absorption of solvent salts of Cd and Pb by gills is more than concentration of Pb in muscles and Cd in kidney. On the other hand the amount of (r) shows that influence of gills absorption and other agents. Comparison to quantity of metals in different tissues is done by one way (ANOVA). Results show in all tissues there is significant difference ($p > 0.05$), except gills and skin for Cd then kidney and muscles for Pb. Comparison the concentration of Cd and Pb in various tissues by attention to ranking the weight and sampling distance, did not show meaningful difference in ANOVA experiment alone ($p \geq 0.05$) while comparison of them in various tissues in limited weight and different sampling distances in reaction with each other was studied with multi way analysis (GLM) and

Table 8: Pearson correlation in the tissues of *Chalcalburnus chalcooides* in Anzali Port

		Cd				Pb			
		Gills	Liver	Kidney	Muscles	Gills	Liver	Kidney	Muscles
Cd	r	1.00							
Gills	p	0.00							
Cd	r	0.209	1.00						
Liver	p	0.514	0.00						
Cd	r	0.577	0.401	1.00					
Kidney	p	0.049	0.196	0.00					
Cd	r	-0.195	0.189	-0.117	1.00				
Muscles	p	0.544	0.555	0.718	0.00				
Pb	r	0.870	-0.040	0.346	-0.420	1.00			
Gills	p	0.000	0.903	0.270	0.174	0.00			
Pb	r	0.126	0.181	0.046	0.414	-0.007	1.00		
Liver	p	0.697	0.573	0.888	0.181	0.984	0.00		
Pb	r	-0.131	-0.020	-0.251	0.498	-0.172	0.501	1.00	
Kidney	p	0.685	0.952	0.431	0.100	0.592	0.097	0.00	
Pb	r	0.416	-0.211	0.014	-0.220	0.617	-0.251	0.008	1.00
Muscles	p	0.178	0.511	0.965	0.493	0.032	0.431	0.981	0.00

showed significant difference ($p < 0.05$). Also comparison between the concentration of Pb and Cd in GLM test shows significant difference ($p < 0.05$) about Pb, regarding to rank the tissues and weight, while did not show any meaningful difference ($p \geq 0.05$) in Cd. Concentration factors of Cd in tissues were analyzed and three factors determined. First factor was the concentration of Cd in gills and Pb in muscles and gills, the second factor was Cd concentration in muscles and Pb in skin and kidney, the third factor was Cd concentration in skin and kidney. The first one shows that gills have the most important role in Cd and Pb concentration and muscles in Pb. The second and third factors show the less important role of muscles, skin and kidney in Cd and Pb concentration. Comparison of Cd and Pb in tissues was done with one sample t-test by standard (WHO, 1993). The results of Cd show meaningful difference ($p < 0.05$) in gills while in skin did not show ($p \geq 0.05$). The amount of metals in kidney and muscles is less than standard level. Also the results of mentioned test about Pb pollution in studied tissues showed meaningful difference ($p < 0.05$) with standard (WHO, 1993). Traffic of commercial ships and port activities, entering the civic and agricultural waste waters, cause the pollution (Rashed, 2001). In this case north- west to south- west streams in Caspian Sea, cause entering the mineral and organic pollutants from Azarbaijan Beach to this zone. Average received amount of Cd and Pb in Iranian women is 0.027 and 0.145, in men is 0.046 and 0.128 ppm (Gharib *et al.*, 2003). Also average received amount of Cd and Pb in people of Northern provinces is 0.127 and 0.236 ppm (Gharib, 2004b). Considering in the above amounts and position of fish in feeding of people in northern provinces specially Gilan, it is necessary to control pollution level in Anzali Port Because by consuming the studied fish more often, that may cause increasing the accumulation of metals in human body. This matter may cause some disease by receiving metals more than maximum acceptable weekly amount of Cd and Pb, in adults which in 0.007 and 0.025 ppm (ENHIS, 2007).

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