Determination of Heavy Metal Content in *Astacus leptodactylus caspicus* of Anzali Wetland, Iran†

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ABSTRACT : With attention to different human activities around Anzali wetland and introduction of different pollutants containing heavy metals into this water ecosystem and absorption and accumulation of heavy metals in shell and muscle of *Astacus leptodactylus* which is native benthose of Anzali wetland and enjoys food and economic importance; Sampling of waters and sediments from 18 stations and shell and muscle of *Astacus leptodactylus* from 5 stations had been conducted. After biometric evaluation of *Astacus leptodactylus*, the chemical analysis of the samples by using international and standard methods and then their heavy metals content was determined by Atomic Absorpotion Spectrophotometer (A.A.S), model P.U 9400 philips. Result of the analysis stated that: 1) The mean values of Cr, Zn, Pb, Ni, V in Anzali waters were 6.4, 184.5, 28.8, 28.9, 47.1 (μgr/lit) respectively, which comparing to the international standards, is not suitable for drinking, but it can be used for agricultural and aquacultural purposes. Even though the concentration of heavy metals in sediments were high, there are in range of acceptable limit for aquatics organisms; 2) Comparatively, the concentration of heavy metals in shell and muscle of *Astacus leptodactylus* in Anzali wetland (Cr, Zn, Pb, Ni, V in shell were 3.48, 109.3, 14.13, 7.17, 36.2 and in muscle were 2.98, 131.98, 3.12, 4.77, 9.05 ppm respectively) were more than what is determined for Aras dam (in shell were 1.6,224.3, 7.8, 7.6, 10.3 and in muscle were 1.4,60.2, 0.45, 2.7, 4.4 mg/kgr respectively); 3) For all samples, concentration of heavy metals in all *Astacus leptodactylus* in Anzali wetland and Aras dam, was at tolerance level for human consumption; 4) There was no association with significant difference between weight and size of *Astacus leptodactylus* with absorption and accumulation of heavy metals; but there was a significant association between heavy metals content in water and in sediments, and in sediments, shell and muscle of *Astacus leptodactylus* in Anzali wetland.

Key words : Anzali wetland, *Astacus leptodactylus*, Heavy metals, Pollution

INTRODUCTION

Anzali wetland is an Invaluable wetland and located in north of Iran or south west of caspian sea, which due to vast variety of her own plants and aquaties and incurred ecological, economical and social Impacts in the region enjoys great and special importance.

Conducting Industrial, Agricultural and urban activities around Anzali wetland and her nourishing rivers introduces big amount of pullotants such as heavy metalss in to the Wetland, who finally get through sediments as living place of valuable benthose like. *Astacus leptodactylus* Accumulation of different pollutants in water and sediments imposes a great danger to *Astacus leptodactylus* and cause deposition of heavy metals inside shell and muscle of this precious domestic benthose of Anzali Wetland. Considering nutritional, economical and exporting value (Thousands of USD annually), it seems necessary to us to conduct an study to determine heavy metals content inside shell and muscle of this bethose, followed by in water and sediments, which enable us to compare them with International standards and find appropriate solutions to save this rare water ecosystem.

MATERIALS AND METHODS

After initial studies on pollutants introduction in to Anzali Wetland and pinpointing the living place of *Astacus leptodactylus*, sampling of water sediments, *Astacas leptodactylus* in different sizes (Table1) of Anzali Wetland (on average 25 pcs in each station which finally terminates in to 6 pcs in small, medium and large sizes as samples were conducted from 18 and 5 stations respectively. Sampling from Aras depositing dam as control group were done too. Sampling of surface water to one meter depth, sediments of Wetland floor and *Astacus leptodactylus* were executed in Autumn by Nonsen bottle, van vien grab and special traps respectively. One liter of samples of each station decreased to 30 ml through mild heating after pushing through an No: 42 watman filter papers.

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Table 1. Mean of heavy metals in water samples (μg/ℓ) taken from different stations in Anzali wetland

No	Name of Station	Cr	Zn	Pb	Ni	V
1	Ghanadi	15	147	38	28	44
2	Pir bazar	13	130	40	24	43
3	Siah darvishan	4.5	99	21	14	38.4
4	Kolsar	5.7	96	21	10	36.6
5	Shijan	5.8	110	25	22	41.7
6	Pasikhan	4.5	100	24	19	39.3
7	Bagherkhaleh	2	150	36	12	36.6
8	Serkhancol	5	140	14	18	51
9	Siahkashim	6	170	27	32	54
10	Chafrod	7	290	27	32	50
11	Eest Wetland	9	140	36	37	54
12	West Wetland	10	190	49	52	56
13	Anzali roga	20	470	50	55	56.2
14	Nahang roga	4	150	18	24	52
15	Rase khaleh roga	2	170	12	15	51
16	Pir bazaar roga	6	350	23	21	54
17	Sosar roga	5	160	21	29	51
18	Canal	6	160	37	55	39.3
Min		2	96	12	10	36.6
Max		20	470	50	55	56.2
Mean		6.4	184.5	28.8	28.9	47.1
Standard deviation		4.17	109.36	11.16	14.08	7

Then they were filtered again after adding 1 ml Nitric Acid and poured inside polyethylen containers for injection in to Atomic Absorbtion system. Sediment samples were disiccated by electrical oven (60℃ for 24 hours) and filtered by No: 240 seive to seperate particles less than 63 micron. Separated samples, Them, were homogenized by acical mortar. Complete gestion of samples (one after the others) were done through placing one gram of each sample inside polyethylene container and adding flouridric (7ml), chloridric and Nitric Acids (5 ml) and using water bath. The volume of samples were increased to 30 ml by adding distilled water then (Roger and John 1994), to discover any possible fault, all above mentioned process were done inside an empty polyethylene container without sample as an control simultanously and then was injected in to Atomic Absorption system.

The results were reporied as nill. Heavy metal content in all samples were determined by Atomic Absorption system (A.A.S Pu 9400, Phillips) for three times (Van Loon 1980). All used containers were washed by diluted Nitric Acid and dried inside electrical oven. All sample analysis procedure were done by using Analytical AR Reagent. To ensure quality and accuracy of laboratory works, canadian standards i.e MESS-1 and BCSS-1 were applied and according to comparison which were made, The results were acceptable SPSS software statistical method of variance analysis and least signiticant Ditterence (LSD) test were applied for statistical analysis of samples and statistical signiticant Difference calculation ($p \leq 0/05$). Also, Kolmogorov- smirnov method and leven statistics test and regression method were conducted to determine the results and variances monotonicity and association and inter relations among data, respectively.

RESULTS

Analytical results of sediment and water and also shell and muscle samples of *Astacus leptodactylus* are shown on Tables 1 and 2 respectively. The highest and lowest levels of heavy metals in water samples are seen in station No. 13 (Anzali Roga) and stations No.4 and 7 (Kolsar and Bagher khaleh) with general average of 6.4, 184.5, 28.8, 28.9 and 47.1 microgram/liter for Cr, Zn, Pb, Ni, V respectively. The highest and lowest levels of heavy metals in sediment samples are observed in stations No. 13 and 16 (Anzali Roga and Pir Bazar Roga) and station No. 3 (Siah Darvishan) with general average of 85.6, 371.2, 59.04, 56.9, and 139.1 mg/kg for Cr, Zn, Pb, Ni, V respectively. the highest and lowest levels of heavy metals in *Astacus leptodactylus* shell are seen in stations No. 1 and 5 (ghanadi and sheijan) and station No. 7 (Aras dam) respectively. Also, the highest and lowest levels of heavy metals in *Astacus leptodactylus* muscle are observed in stations No. 2 and 5 (Pir Bazar river and sheijan) and stations No. 6 and 7 (Aras dam) respectively. The mean value of heavy metals in *Astacus leptodactylus* shell muscle of Anzali wetland and Aras Dam were shown in (Table 3). Generally speaking the level of heavy metals in shell is higher than muscle.

DISCUSSION AND CONCLUSION

Analysis of water samples which were taken from 16 stations in Eastern, western, southern, central runways (out let brooks of Anzali wetland) (Table 1) showed the highest level of heavy metals in Anzali roga (Station No. 13) and levels of Cr, Pb, Zn, Ni, V as 20, 50, 470, 55, 56.2 μg/lit respectively. The lowest and general

Table 2. In mean value of heavy metals in sediments samples (mg/kg) taken from different station in Anzali wetland

No	Name of Station	Cr	Zn	Pb	Ni	V
1	Ghanadi	85	290	77	69	165.1
2	Pir bazar	81	289	75	66.6	163.5
3	Siah darvishan	54.6	219	28	62.4	87.6
4	Kolsar	71.7	258	30.3	67	102.9
5	Shijan	76.2	234	44.4	70.8	130
6	Pasikhan	72	216	36.3	58.2	170.1
7	Bagherkhaleh	64.8	288	40.2	61.8	159.3
8	Serkhancol	79	255	42	37.4	115.5
9	Siahkashim	70	390	55	37.8	142.2
10	Chafrod	48	255	48	53.4	138.5
11	Eest Wetland	81	405	65.7	71.7	154.2
12	West Wetland	67	270	80.7	64.5	123
13	Anzali roga	76.2	990	142.2	31.8	130.8
14	Nahang roga	82.1	267	64.5	39.6	136.5
15	Rase khaleh roga	60.3	420	79.2	33	160.1
16	Pir bazaar roga	303	600	53.4	96	162
17	Sosar roga	98.4	780	67.5	36.6	149.4
18	Canal	70.8	255	33.3	65.4	113.1
Min		48	216	28	31.8	87.6
Max		303	990	142.2	96	170.1
Mean		85.6	371.2	59.04	56.9	139.1
Standard deviation		55.48	212.41	27.13	17.32	23.73

average in descending order of heavy metals levels were seen in kolsar and siah darvishan stations and as Zn > V > Ni > Pb > Cr respectively (Table 1). Also, the highest and lowest mean values of Pb and Cr were observed in Eastern and central parts of wetland. considering to conducting different human and developmental activities and establishing various industries in Eastern side of wetland, the main source of contamination and increment of Pb and Cr in this area, is attributed to indirect introduction of Industrial effluents in to the rivers and finally eastern part of wetland.

The highest average of Cr, Ni, V in outlet brooks runways (stations No. 13 to 18) which could be attributed to runway effects and their high content of contaminates, oil contamination, vehicles and passing of commercial ships and ferries to Anzali wetland and their near areas (Nouroz 1993). On the other hand, considering the poor solubility of Cr and Pb (specially Cr) compare to other elements and high quantity of Zn in earth crust and it" s high solubility in water (Bowen 1979), we found the lowest and highest values of heavy metals for former and later respectively, comparing mean value of Cr, Pb, Zn, Ni, V in wetland water who and ISIRI standards revealed the suitability of wetland water for agricultural, aquacultural and Industrial but not for drinking purpose.

In water ecosystems, the floor sediments are considered as depositing and most vulnerable part. The descending order of heavy metals deposition in Anzali wetland sediments is as follows Zn > V > Cr > Pb > Ni (Table 2). Intensity of agricultural, urban and industrial activities in Eastern part of wetlands and further sedimentation of suspending particles, made the sediments of eastern, or wetland outlet brooks (Stations No. 13 to 18) as most contaminant parts. The highest and lowest levels of Pb and Zn were found in sediments of station No. 13 (Anzali raga) and coliding points of southern rivers respectively, Although the highest level of Pb and Zn were found in sediments of Anzali roga but they showed the lowest level of Ni. Also, maximum and minimum level of V and Zn were found in this station respectively this shows that there is no association among heavy metal levels and also variety in sources large amount of Cr, Zn, Pb and V were found in siah kashim region (station No. 9), an environment protected area, which showed only little difference compare to heavy metal content in sediments of it" s Northern part (central part). But the level of heavy metals in runway floor sediments, specially in Anzali roga and Pir bazaar roga, stands for highest amount, which we found the main reasons as various human activities on that area and interactions between wetland outlet circulations and shore waves and water circulation of Caspian sea and therefore heavy metal accumulation in sediments of this area Although comparing of floor sediments of Anzali wetland and available standards for appropriate sediments for benthose living revealed increment of certain elements above acceptable values but fortunately it dose not reach to lethal and regarding unacceptable level regarding to benthose living (Table 4).

In the mean time, water and sediment sample analysis results in different stations or parts of Anzali wetland, revealed an proper association and direct relation between these two phases (Table 5), In most areas, Increment of heavy metals in water is bind with increase of heavy metals in sediments.

Results of *Astacus leptodactylus* muscle and shell analysis in various size and weight, for Anzali wetland and Aras dam could be seen in (Table 3). The results shows that size and weight impose no effect on level of Cr, Zn, Pb, Ni and V in shells and muscles. Comparison of heavy metal accumulation and absorption in Anzali wetland and Aras dam *Astacus leptodactylus* shells and muscles indicates the highest and lowest heavy metals level respectively.

Table 3. In mean value of heavy metals in shell and muscle of *Astacus leptodactylus* (mg/kg dry weight) in Anzali wetland and Aras dam

No of station	Place of sampling	Cr		Zn		Pb		Ni		V	
		Shell	Muscle	Shell	Muscle	Shell	Muscle	Shell	Muscle	Shell	Muscle
1	Ganari river	3.5	2.3	126	135	13.2	4.3	8.1	3.1	35.1	6.13
2	Pir bazar river	3.5	2.9	99	161.3	16	5.2	6.7	4.1	35	11.6
3	Siah darvishan river	3.3	1.7	101	124	13.7	2.5	6.6	3.14	38.2	11.36
4	Kolsar river	3.35	3.3	112.5	114.3	13.75	4.2	6.75	4.2	36.7	5
5	Shijan river	4.28	4.7	108	125.3	14	4.4	7.74	9.3	38.6	11.16
6	Aras dam (Male)	2	1.9	249	77	10.18	0.7	8.5	2.56	11.46	2.16
7	Aras dam (female)	1.26	0.9	199.5	43.3	4.8	0.2	6.9	2.8	9.16	6.63
Min		1.26	0.9	99	43.3	4.8	0.2	6.6	2.56	9.16	2.16
Max		4.28	4.7	249	161.3	16	5.2	8.5	9.3	38.6	11.6
Total mean		3.02	2.53	147.28	111.46	9.91	3.07	7.33	4.17	29.17	7.07
Anzali Wetland mean		3.48	2.98	109.3	131.98	14.13	3.12	7.17	4.77	36.2	9.05
Anzali Wetland SD		0.479	1.137	10.78	17.95	1.08	1.81	0.69	2.85	1.68	3.21

Table 4. Comparison of water, sediment, shell and muscle Analysis results of *Astacus teptodactylus* in Anzali wetland with existed standards

Elemelt	Standard of drinking water		LC 50 for Aquatics life	Current research on wetland water	Sediment standard				Current research on wetland sediment
	Max of optimum	Max of acceptable			Earth crust	Ocean	Dangerous limit	Acceptable limit	
Cr	0.05	0	2~20	6.4	100	90	110	26	85.6
Zn	15	5	1~10	184.5	70	165	820	120	371.6
Pb	0.1	0	1~40	28.8	12.5	80	250	31	59.04
Ni	0.5	-	-	28	75	225	75	16	56.8
V	-	-	-	47	135	120	-	-	139

Elemelt	Mammals		Sea fishes	Maximum of acceptable level in benthose consumed by human	Current research in muscle	Current research in shell
	Muscle	Shell				
Cr	< 0.002~0.84	0.1~33	0.03~2	-	13	3.6
Zn	240	75~170	9~80	40	10~15	109.3
Pb	0.2~3.3	3.6~30	0.001~15	1	0.075~0.015	14.14
Ni	1.2	<0.7	0.01~4	0.5	1.2	7.18
V	0.002~0.02	0.003~0.03	0.3	-	-	36.7

Accumulation and absorption of all heavy metals in *Astacus lepodactylus* shell is higher than muscle except for Zn which is in contrast physiological condition or the benthose trend or need to absorb and accumulate Zn in it" s muscle and over limit accumulation of Zn comparing with other elements in water and sediments of under covered areas and it" s appropriate solubility, all could explain the matter. The same results were taken in analysis of Anzali wetland fish viscera in 1993 (Poorang, N) and Mexican

Table 5. Comparison of heavy metals levels (ppm) in *Astacus leptodactylus* with other research results

Place of research	Type of research	Cr	Zn	Pb	Ni	V	Reference
Cooks River	Oyster	0 (0~0)	1294 (830~1615)	1.46 (1.1~2.1)	-	-	CSAHS Public Health Unit Technical Report PO Box 437. 1997.
Mexico	Rock Oyster	1.8	798	-	4.1	-	Martin et al. 1998-99
Massachusetts	Crayfish		0.48~1.1	0.28~0.89			Mierzykowski et al. 2000
Honeyhouse Creek of Swan Lake National Wildlife Refuge Klombia, Misoury	Turtel Liver	1.0	-	< 0.5	1.6	-	Colette, S. 1993.
From Mazaltan, Sinaloa, Mexico	Rock Oyster (*Crassostrea iridescens*)	1.6	3.7±0.5	670±100	-	-	Martin, G., Monica, A.J., Isidor. J. (1998-99)
River Thames Tennesse	*Orconectes australis* *Combarus tenebrosus*	0.8~0.9	-	-	-	-	Dickson et al. 1979
Grove & plow shop Wetland	Crayfish	0.5~1.2	-	0.28~0.89	-	-	Mierzykowski et al. 2000
Anzali Wetland	Muscle	3	132	4	4.7	9.05	Riahi et al. 2001
Anzali Wetland	Shell	3.6	109.3	14.14	7.18	36.7	

beaches oysters in 1998-9 (Martin, C) and Massachusetts bivalves in 2000 (Mierzxkowski).

The analysis of Anzali wetland *Astacus leptodactylus* samples and the level of elements accumulation and absorption in their shells and Muscles showed descending pattela in sampling stations (Nourishing rivers in to wetland) from east to south and south west parts. Also, the highest heavy metals accumulation and absorption was found in *Astacus leptodactylus* samples taken from eastern part of Anzali wetland possibly due to various urban, Industrial and Agricultural activities in this area.

The same results were achieved by other researchers (Blevins and Pancorbo 1986, Anonymous 1993, Creswell 1993, Karimpor and Hoseinpour 1998, Martin *et al*. 1998, Mierzykowski and Carr 2000), *Astacus leptodactylus* growth (weight & length) showed almost no difference compare to 2000 report (Karimpour and Hoseinpour 1998) but it is obvious that Aras dam Astacus leptodactylus are bigger and more heavier then their counterparts in Anzali wetland.

Also as already has been confirmed (Martin *et al*. 1998) there is no association between weight and length of *Actacus leptodactylus* and accumulation and absorption of heavy metals in their shells and muscles. Comparison of heavy metals content in *Astacus leptodactylus* muscle with FDA standards (FDA-2000) indicates that the heavy metals content in *Astacus leptodactylus* in under covered areas is acceptable for daily human consumption (Table 4).

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