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Accumulation and Loss of Chromium by Mussels (*M. galloprovincialis*)

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Chromium has been used by industry and sources to the environment include fossil fuel combustion, metal plating and refining, the leather industry etc. Although seawater concentrations of Cr range from 0.08 - 0.15 µg/L on surface water to deep water as hexavalent chromium (Cranston and Murray 1978) Langston (1990) had reported the chromium content as 0.088-0.55 µg/L while McNeely et. al. (1979) referenced that seawater may contain 0.00005 mg/L of chromium. It had also been reported by several researchers (Scoullos et al. 1992; Türkoglu et al. 1992) that chromium contents in many local seas exceeds this amount.

Chromium compounds are frequently encountered as environmental pollutants and have been known to produce toxic, mutagenic and carcinogenic effects in biological systems although Cr is an essential metal in glucose metabolism playing a cofactor role in insulin action and a role in peripheral activities of this hormone (Debetto 1988 a,b; Goyer 1991). Exposure of human populations to high levels of chromium (VI) results in increase incidence of lung cancer and it is clear that exposure to chromium (VI) results in DNA damage and may arise from both direct metal-mediated and indirect radical-medical pathways. However, both activation pathways may be important to the initiation and promotion of carcinogenesis by chromium(VI) compounds (Watterhahn and Stearn 1996). One of the most important properties of a toxic pollutant is its ability to accumulate in the tissues of organisms, Over a long period the pollutants present in the environment at very low levels may accumulate within the body of aquatic species by various mechanisms to the extent that they exert toxic effects. Therefore, it has great importance to know about the bioaccumulation potential of a pollutant (Parlak 1987). In this investigation, the accumulation and loss of chromium added to the medium as Cr(III) and Cr(VI) compounds were studied in mussel *Mytilus galloprovincialis*, Lamarck 1819. The total chromium content of mussels were measured without considering the changes in chemical state of both chromium compounds due to the reactions that may have occurred in seawater.

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MATERIALS AND METHODS

Accumulation of chromium added to the medium as Cr (III) and Cr (VI) compounds by mussels (*M. galloprovincialis*) have been examined. All individuals were measured as total length (6.44 +1 cm) and weight (9.2±1 g). After cleaning the shells the animals were placed in a container a week for acclimatisation aerated (2 L/h) continuously and fed with cultured phytoplankton of *Dunaliella sp,* and *Chlorella sp.*

The salinity of seawater was 38.5 ‰. The experiments were carried out under the controlled conditions as 14 ± 1 °C constant temperature and 12D-12L photoperiod.

For the uptake experiments, 50 individuals per experimental container (60x70x30cm) were placed in 42 L of filtered seawater and aerated continuously (2 L/h) maintained to fed on the same cultured phytoplankton. The Cr (III) and Cr (VI) were added to seawater as freshly prepared stock solutions using the salt of $KCrO_4$ and $CrCl_3$ to obtain 3 ppm concentrations. The exposure level was set as 3 ppm from the results of the pre-experiments carried on to estimate the sub-lethal concentration. Three of the experimental containers were prepared one for Cr (III), the other one for Cr (VI) and the third for control (blank). The fresh media was replaced daily. Uptake experiments were over in 13 days. The loss experiment was carried out using fresh and uncontaminated seawater, and was over in 11 days.

During the experiments three individuals were taken every other day and the soft parts of three mussels were done composite samples and divided three parts then prepared to analyse as duplicate. The 5 g wet weight subsamples were wet digested using $HNO_3 - HClO_4$ (5:1 v:v) and heated at 150°C until colourless solution were obtained then diluted with 0.1 N HCl up to the volume 50 ml (Bernhard 1976). Cr contents were determined using the atomic absorption spectrophotometer flame technique connected with NO_2-Acetylene mixture to obtain rich flame. The calibration was done with IAEA standard reference material gave a recovery of 85 %. The results were calculated as µg g^{-1} wet weight.

The samples were taken and treated in the same way with the other samples to estimate the distribution of Cr in several tissues and organs (such as; foot, gonad, mantle, muscle, gill, hepatopancreas and byssus) at the termination of uptake experiments.

The total Cr content was estimated from the absorbance values and non-linear estimation using least square methods were applied for calculation of accumulation and loss curve as well as biological half life ($t_{½}$) values. According to this method accumulation curve function were estimated $Y=A\ (1-e^{-bt})$ where Y is Cr concentration, A is constant (intercept), b is slope and t is the time (Nakamura et al 1982; Ueda et al. 1982). Loss curve function was estimated as

Table 1. Accumulation and loss of Cr by mussels, *M. galloprovincialis.* n=3 (the mean of three analyses of composite samples)

	Day	Cr (VI) group				Cr(III) group			
		*E.cons ppm	%	* P.value ppm	%	*E.cons ppm	%	*P.value ppm	%
Uptake	0	0.300	1.89	0.00	0.00	0.30	3.07	0.00	0.00
	1	5.10	32.07	2.48	19.21	3.00	30.70	1.34	15.05
	3	5.83	36.48	6.20	48.02	3.43	35.14	3.58	40.41
	5	8.70	54.72	8.73	80.79	4.90	50.20	5.27	59.48
	7	10.24	64.41	10.43	80.85	6.97	71.41	6.56	74.04
	11	9.11	57.30	12.38	95.89	7.26	74.38	8.29	93.57
	13	15.89	100.0	12.91	100.0	9.76	100.0	8.86	100.0
Loss	0	15.89	100.0	15.06	100.0	9.76	100.0	7.35	100.0
	1	13.90	87.47	14.50	96.28	5.08	52.05	6.89	93.74
	3	13.00	81.81	13.44	89.18	4.50	46.11	6.06	82.45
	5	12.60	79.30	12.45	82.67	4.91	50.30	5.33	72.52
	8	10.70	67.34	11.11	73.77	5.82	59.63	4.39	59.73
	11	10.40	65.45	9.90	65.80	3.67	37.60	3.62	49.25

*E.Cons: Experimental Concentrations *P.value. Predicted values

$Y = Ae^{-bt}$ (Scholz 1980) and biological half life ($t_{½}$) was calculated with the formula $t_{½} = 1n\ 0.5/b$. The statistical analyses of differences between accumulations and loss pattern of Cr (III) and Cr (VI) compounds were checked by one-way ANOVA test.

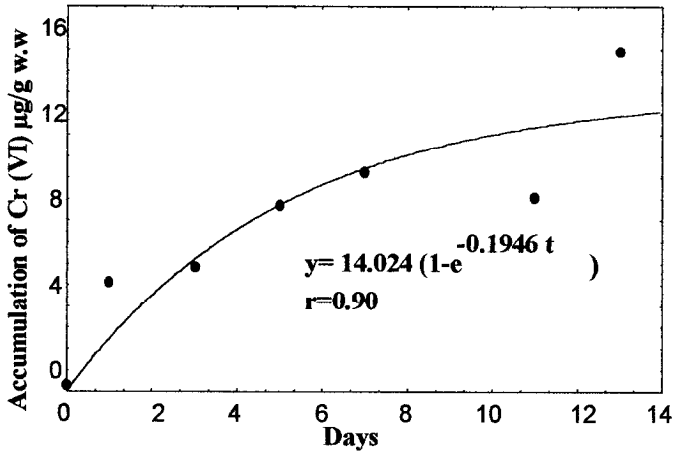
RESULTS AND DISCUSSION

In this investigation the accumulation and loss of chromium compounds as Cr (III) and Cr (VI) have been studied. Cr was accumulated up to 15.9 µg Cr g^{-1} wet weight during 13-day from the medium containing Cr (VI) compound while the content was 0.3 µg Cr g^{-1} at the beginning of the experiment, The concentration of Cr from Cr (VI) compound in total tissue of mussel was decreased up to 10.4 µg Cr g^{-1} during 11-day loss experiment. Biological half-life of Cr (VI) compound was estimated as 18.2 days from these results (Table 1, Figure 1).

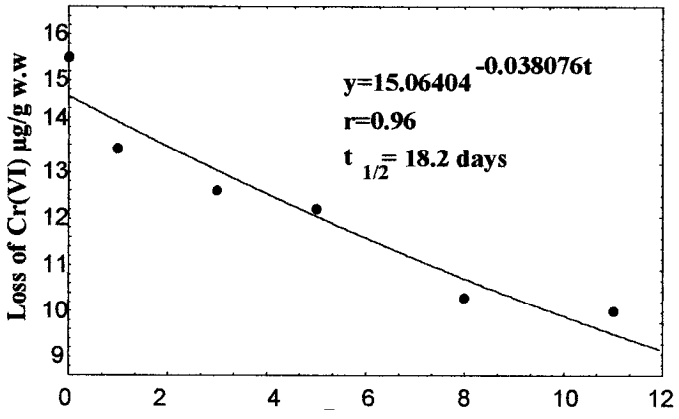
Distribution of Cr (VI) compound in tissues and various organs of mussels had different patterns when compared with controls. The descending order was gill< muscle< gonad< hepatopancreas< foot < byssus (Figure 3, Table 2).

Cr (III) accumulation was increased from 0.3 to 9.76 µg Cr g^{-1}. During the loss experiment, the body concentration of chromium decreased from 9.76 to 3.67 µg Cr g^{-1} in 11 days, Biological half-life of Cr (III) compound was calculated as 10.7 days (Table 1, Figure 2). Distribution of Cr (III) compounds in various tissues and organs were shown in descending order as mantle< muscle< foot< gonad< gills< hepatopancreas< byssus (Figure 3, Table 2).

From these results it can be concluded that accumulation of Cr (VI) were higher than that of Cr (III). The rate of loss of both chromium groups show that Cr (VI)



(A)



(B)

Figure 1. Accumulation (A) and loss (B) of Cr by mussel, *M. galloprovincialis* in Cr (VI) group.

need longer time ($t_{½}$= 18.2 days) to be cleared from the body then Cr (III) ($t_{½}$= 10.7 days).

The one-way ANOVA test had shown that there was not statistically significant difference between the accumulations of both group as Cr (III) and Cr (VI). However, the differences of loss of both groups were found statistically significant ($p<0.0002$)

Also, the distribution of Cr from Cr (VI) and Cr (III) groups had different pattern. Higher concentrations found in byssus, foot and hepatopancreas in the mussels kept in the medium containing Cr (VI) compound while byssus, hepatopancreas and gills had high Cr concentration from Cr (III) compound.

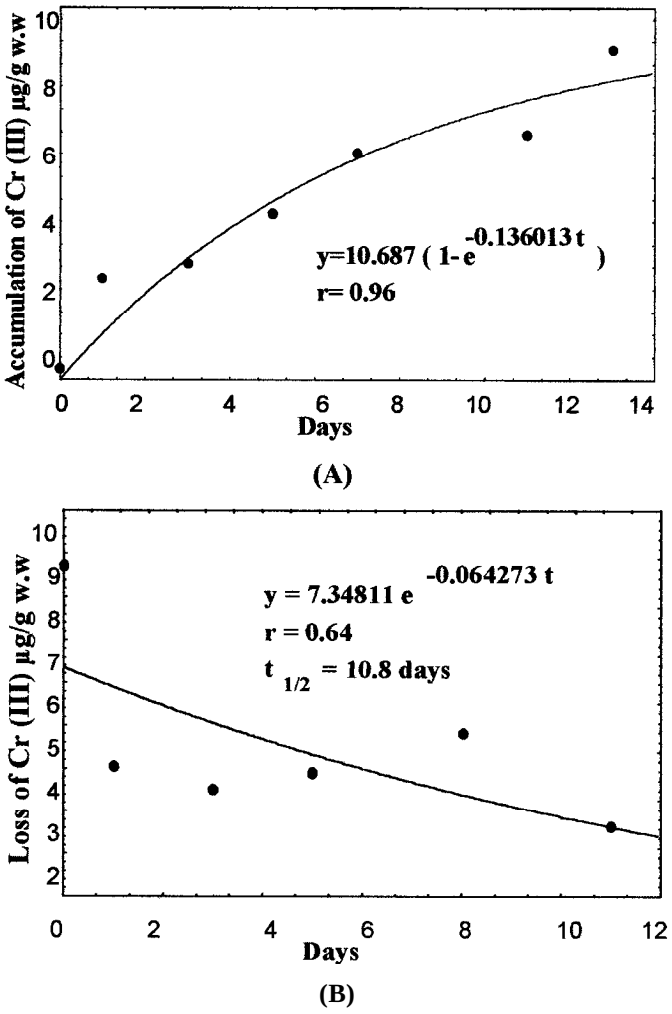


Figure 2. Accumulation (A) and loss (B) of Cr by mussel, *M. galloprovincialis* in Cr (III) group.

concentration. Andreatis and Papadopoulou (1982) had also reported that chromium were accumulated in higher amounts in hepatopancreas and gills for mollusc *Meretrix chitane.* Ikuta (1986) had studied the correlation between ratios of metal concentrations in byssuses to these in soft body parts and metal concentrations in soft bodies of bivalves and reported that three-quarters of the whole body burdens were found on manganese and zinc in byssuses.

According to these results, it is necessary to continue the studies about the effects of chemical speciation of Cr on mussels using biochemical and histopathological methods to illuminate the difference in their accumulation, loss and distribution in tissues.

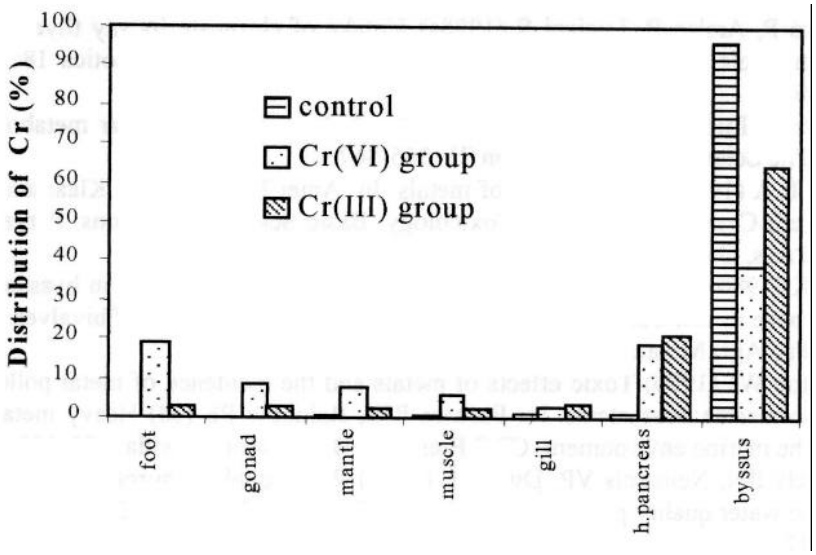


Figure 3. Distribution of Cr in tissues of mussel, *M. galloprovincialis*

Table 2. The distribution of total Cr in the tissues of mussels, *M. galloprovincialis* (μg/g wet weight).

Tissues	Control		Cr(VI) group		Cr(III) group	
	Cons. ppm	%	Cons. ppm	%	Cons. ppm	%
Foot	n.d	n.d	79.74	18.99	8.77	2.91
Gonad	n.d	n.d	35.58	8.50	8.79	2.95
Mantle	0.20	0.41	33.42	7.98	6.59	2.19
Muscle	0.30	0.62	26.19	6.25	7.04	2.34
Gill	0.58	1.19	12.93	3.08	10.68	3.55
Hepatopancreas	0.62	1.28	79.74	18.99	64.32	21.58
Byssus	46.66	96.48	162.26	38.77	194.40	64.67

n.d: not detected (lower than detection limit, 0.005 μg/L)

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