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STUDY OF BIOACCUMULATION OF METALS IN TISSUES OF *HIMANTOPUS HIMANTOPUS* (BLACK WINGED STILT) FROM COASTALAREAS OF KARACHI, PAKISTAN

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ABSTRACT

Levels of Cadmium (Cd) and Chromium (Cr) were measured in seawater samples and liver, pectoral muscles, kidney, and stomach of *Himantopus himantopus* (Black winged stilt) collected from Hawks Bay, Sands Pit, and Korangi Creek, Karachi, Pakistan. All samples were collected quarterly from 2006-2009 and analyzed using Atomic Absorption Spectrometer (Perkin Elmer. A. Analyst-700). Maximum levels of Cd were 0.46 mg/l during 3rd quarter at Hawks Bay and lowest amount of Cd was 0.0003 mg/l during 1st quarter at Korangi Creek. Highest and lowest concentration of Cr was 0.497 mg/l and 0.002 mg/l in 4th and 2nd quarter at Korangi creek and Hawks Bay, respectively. In body tissues, highest stage of Cd (49.250 ug/g) was detected in pectoral muscles during 3rd quarter at Hawks Bay and lowest Cd (0.001 ug/g) was noted in liver during 1st quarter at Hawks Bay. Highest levels of Cr (35.635 ug/g) was observed in kidney for the period of 3rd quarter at Korangi creek and lowest level of Cr (1.980 ug/g) was determined in pectoral muscles during 1st quarter at Sands Pit.

INTRODUCTION

The effluence of aquatic system is due not only to natural causes but all the anthropogenic activity such as discharges of domestic or industrial effluents, leaching and runoff of pesticides in agricultural lands are also accountable (Morley, 2010). Cadmium is recognized for its long half-life in biological systems (decades in humans and years in birds), and 0.1-1.0% of ingested Cd absorbs through the avian gastrointestinal tract to be circulated to kidney and liver (Thompson *et al*., 2007). In animals chromium can cause breathing tribulations, a lower ability to fight against disease, sterility and tumor development Chromium is not known to accumulate in the bodies of fish, but high concentrations of chromium, due to the disposal of metal products in surface waters, can damage the gills of fish that swim near the point of dumping and the seabirds take this chromium through food as they are at high trophic level in aquatic food chain. As seabirds are conspicuous animals they are appropriate choice to take part as sentinel organisms; sudden changes in their numbers offer an alarm that may show an unknown pollution or food supply problem. Seabirds are top consumers in marine food chains which offer opportunities to notice the toxicological effects of different elements on the marine ecosystem (Ferreira, 2011).

Karachi coastal areas (Hawks Bay, Sands Pit and Korangi Creek) get 90% of untreated industrial and domestic effluents of Karachi city through Lyari and Malir River and 2% of industries have the facilities to treat their effluents before releasing into the sea (Raza *et al*., 2012). As a result of these activities, level of pollution is increasing here. However, from the ecological point of view, these coastal areas are significant roosting, feeding, and nesting grounds for water fowl due to the rich mangrove growth. The present investigation includes the determination of metals (Cd, and Cr) in seawater and their bioaccumulation in liver, kidney, stomach, and pectoral muscles of *Himantopus himantopus* (Black winged stilt) collected from coastal areas (Hawks Bay, Sands Pit and Korangi Creek) of Karachi, Pakistan.

MATERIALS AND METHODS

Samples of Seawater and *Himantopus himantopus* (Black Winged Stilt) were collected quarterly from Hawks Bay, Sands Pit, and Korangi Creek to study bioaccumulation of metals. Three birds and three samples of sea water were collected in each quarter. The *Himantopus himantopus* (Black Winged Stilt) were shot with a 0.22 Chikosilvakia rifle and seawater samples were collected in untainted bottles.

Solvent extraction method: In the laboratory, seawater was filtered by using Whattman No.40 filter paper and acidified by using 0.1N 3ml nitric acid. The solvent extraction method of Kremling (1983) was used to digest water samples. An aliquot of 500 ml of acidified filtered water was collected in a beaker and 5 ml of citrate buffer was added to bring pH 4.5. The separatory funnel with 5 ml of Ammonium Pyrrolidine Dithiocarbamate (APDC) and 20 ml of Methyl Isobutyl Ketone (MIBK) was used to shake this water for 10-20 minutes. The lower aqueous layer was drained in a beaker and the upper organic layer was collected in a 100 ml flask. 10 ml MIBK was added in resulting aqueous layer and process was repeated. The last extract was evaporated to semi dryness and dissolved in 1 molar nitric acid and filtered. The resultant filtrate was stored for examination by Atomic Absorption Spectrometer (AAS).

Acid digestion: Acid digestion of kidney, liver, stomach, and pectoral muscles was completed according to Benton and J, Jr. (1988). All tissues were dehydrated in a heating oven at 50°C to a constant weight. 0.5 g dried tissue was sited in a beaker with 2.5 ml concentrated nitric acid and permitted to stand overnight. It was then placed on a hot plate at 80°C for 1 hour in a fuming chamber, and cooled. An aliquot of 2.5 ml concentrated Perchloric acid was added, and then beaker was covered and allowed to digest on hot plate at 180- 200°C for 2-3 hour. The cover was removed and the mixture was heated at 80°C and then beaker was detached from hot plate and cooled to room temperature. Distilled water was added to make up the volume to 10 ml. The seawater and body tissues samples were analyzed by Atomic Absorption Spectrometer (Perkin Elmer. A. Analyst-700).

RESULTS AND DISCUSSION

Seawater samples: The present study shows highest levels of Cd 0.460 mg/l, 0.068 mg/l, 0.018 mg/l in Hawks Bay, Sands Pit, and Korangi Creek, respectively (Figs. 1-3). Whereas lower level of Cd was 0.022 mg/l, 0.04 mg/l, 0.003 mg/l in the same areas, respectively. Fatoki and Mathabatha (2001) determined ranges of Cd between 0.2-72.0 mg/l at East London harbor and 0.3-4.0 mg/l at sites in Port Elizabeth. These results are higher than present study. Saleem., (2002) observed concentration of Cd 0.485 mg/l in Karachi harbor, half of its concentration was reported in Karachi creek and 0.063 mg/l was found in Buleji. These results are in line with present study for Cd. Qari and Siddiqui (2004) found 0.02-0.11 mg/l Cd in Paradise point, Karachi, Pakistan. This result is in line with the present study at Sands Pit and Hawks Bay samples as industrial waste is considered to be the main source of Cd in the marine environment.

Highest value of Cr was noted in 0.007 mg/l, 0.034 mg/l, and 0.497 mg/l in Hawks Bay, Sands Pit, and Korangi Creek, respectively in present study. Minimum level of Cr was found in Hawks Bay (0.002 mg/l), Sandspit (0.024 mg/l), and Korangi creek (0.315 mg/l) (Figs. 1-3). High concentration of Cr is due to untreated tannery waste which is being dumped in the Korangi Creek via Malir River (Raza *et al*., 2012). Saleem (2002) determined concentration of Cr ranges from 2.61-2.13 mg/l in Karachi Harbor and Gizri creek. These results are higher than present study, possibly due to less impact of pollutants at present study areas. Khan *et al* (2003) reported 0.21-0.54 mg/l Cr in Gharo Creek, Pakistan. This result was high than Hawks bay samples in the present study but almost in the line with Korangi Creek samples. Qari and Siddiqui (2004) reported Cr 0.06-1.33 mg/l in seawater of Paradise Point, Karachi. This result is almost in the line with Korangi Creek samples in the present study. Now in Karachi, three treatment plants (located in Sher Shah, Mehmood Abad, and Mari Poor) are working and only 10% industrial waste is treated. However, 90% industrial effluent is directly dumped into sea without treatment (Raza *et al*., 2012). Ismail *et al*., (2006) examined Cr 0.068 mg/l in Korangi creek; 0.062 mg/l in Karachi harbor; and 0.291 mg/l in Sands Pit. However in the present study, levels of Cr are high in Korangi creek samples. Marcovecchio aftepossir *et al*., (2010) reported Cr concentration and distribution within Bahia Balance estuary, Argentina. The results showed that heavy metal pollution was localized in areas close to both industrial effluents discharge system and urban sewage out fall discharge. In present study, it is determined that levels of Cd and Cr are high due to different anthropogenic activities. Zhao *et al*., (2013) assessed 450 and 2510 mg/l of Cd, and Cr in the seawater samples, respectively from Deer Island, Liaoning Province, China during 2010–2011. These results are much high as compare to present study, probably due to less impact of environmental pollutants.

Body tissues: Cd is the most toxic element mercury (Hg) for the marine life. It is accumulated in the body of the marine organisms due to its regulatory ability, as recorded by Pentreath (1976) and Olafson (1977). Stoneburner *et al* (1980) reported 3790 ug/g Cd in liver, 23500 ug/g Cd in kidney, 1110 µg/g Cd in muscles and 2830 ug/g Cd in stomach of sooty terns from Dry Tortugas (Florida). These results are much higher than the present findings, possibly due to influence of pollution in Florida. Norheim (1987) analysed levels and interactions of heavy metals in seabirds from Svalbard and the Antarctic from Spitbergen. Cd was 0.4-9.4 ug/g in liver and 4.1-58 ug/g in kidney of Glaucous gull (*Larus hyperboreus*) while 6.1-32 ug/g Cd was in kidney of Fulmar (*Fular glacialis*). These results are in the line with the present study at Sands Pit and low levels were found in Hawks Bay and Korangi Creek, due to similar amount of intake of pollutants through food chain by marine birds (Fig. 4, 6, and 8). Warren and Wallace, (1990) calculated concentration of Cr in blue winged teal from South plains Texas. Cr was 0.20 ug/g in liver and 0.22 ug/g in muscles. In present findings, levels of Cr are comparatively elevated in liver and pectoral muscles of *Himantopus himantopus* (Black winged stilt) at Hawks Bay, Sands Pit, and Korangi creek (Figs. 5,7,and 9).

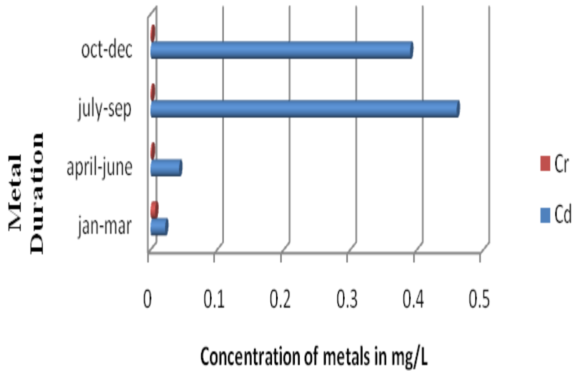

Fig. 1. Concentration of **Cd** and **Cr** in water samples (in mg/L) at Hawks bay.

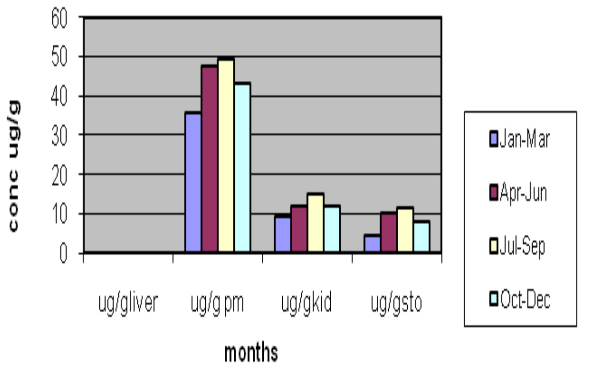

Fig. 4. Concentration of **Cd** in tissues of Black Wing Stilt at Hawks bay.

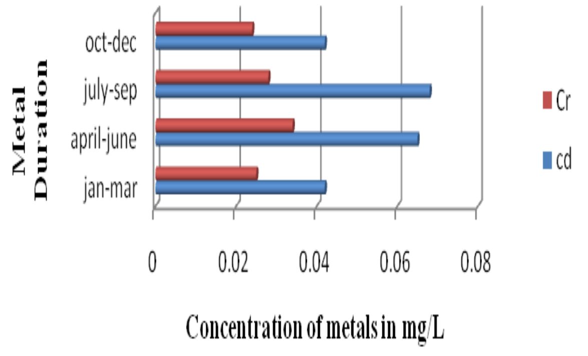

Fig. 2. Concentration of **Cd** and **Cr** in water samples (in mg/L) at Sands pit.

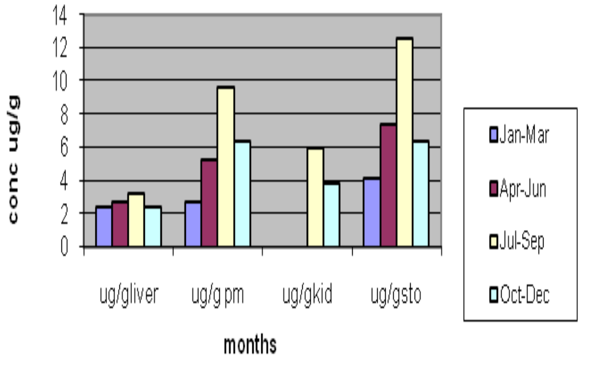

Fig. 5. Concentration of **Cr** in tissues ofBlack Wing Stilt at Hawks bay.

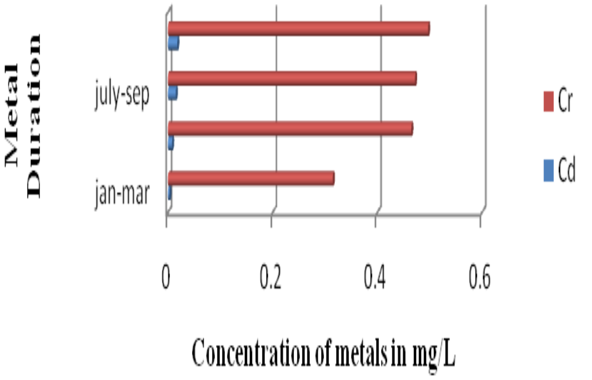

Fig. 3. Concentration of **Cd** and **Cr** in water samples (in mg/L) at Korangi Creek.

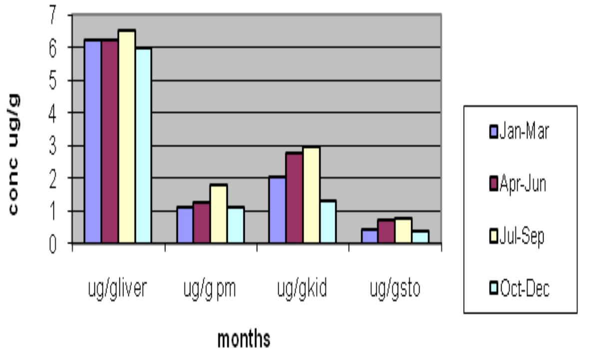

Fig. 6. Concentration of **Cd** in tissues of black wing silt at Sand pit.

Elliott *et al*., (1992) assessed heavy metal concentration in Atlantic Canadian seabirds. Concentration of Cr (2.68 ug/g), and Cd (32 ug/g) were recorded in liver of Leach's storm. These results for Cr are in the line with present result at Hawks Bay and Sands Pit **(Fig.5 and 7)** but at Korangi Creek, high levels of Cr were recorded in liver samples **(Fig. 9)**. Whereas levels of Cd are low in the present study, may be due to monthly oscillation of metal ions and their intake by birds as marine birds are at their higher trophic level. More than 200 tannery units are functioning in Karachi and the waste material of these tanneries is directly discarded in to the sea. These tanneries mostly discharge Cd, Pb, Mn, and Mg in to the sea (Raza *et al*., 2012). Szefer *et al*., (1993) examined major essential elements in seals, Penguins, and other representative fauna of Antarctic. Distinct inter tissue differences in the metal concentration were determined. Szarek*et al*., (2001) studied Cd levels in young coots originating from industrial and agricultural regions of North middle Poland. The results demonstrate that in coots from industrial areas, levels of Cd were decreased due to better system of controlling pollutants production. In the present findings, among all organs, Cd was 0.030 ug/g in liver of *Himantopus himantopus* (Black winged stilt) in Hawks Bay **(Fig.4)**. This area is close to Lyari industrial area, Karachi, Pakistan. So here pressure of metals is comparatively high. Szymczyk and Zalewski (2003) reported Cd contents in liver and muscles of mallards (*Anas platyrhychos*) and other hunting fowl species in Warmia and Mazury in 1999-2000.

In the present work, concentration of these metals was near to the ground, perhaps due to less influence of marine pollutants on marine life. Agusta *et al*., (2005) studied Cd 4.8 ug/g in liver and 40.7 ug/g in kidney and 0.393 ug/g in muscles of black tailed gull (*Larus crassirostris*) collected from Rishiri Island, Japan. In the present findings, levels of Cd are high in muscles but in kidney, concentration of Cd is lowest. The present result show levels of Cd in liver at Hawks Bay and Korangi creek **(**Figs. 4 and 8). Borga *et al*., (2006) studied regional and species specific bioaccumulation of major and trace elements in Arctic seabirds. These levels were diverse among species. The present study confirms these findings.

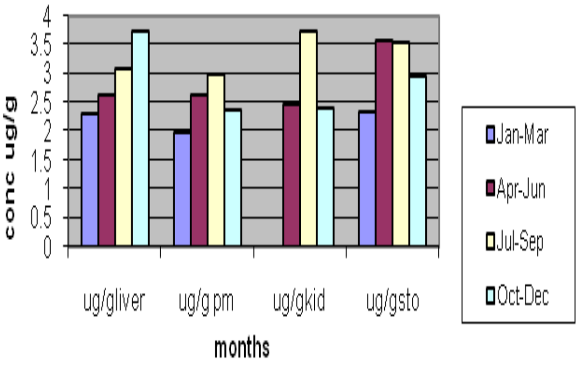

Fig. 7. Concentration of **Cr** in tissues of Black Wing Stilt at Sand pit.

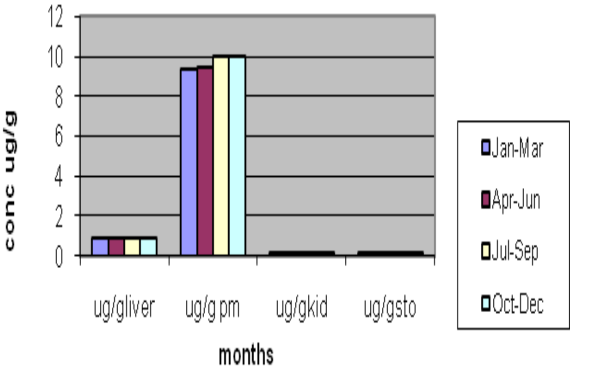

Fig. 8. Concentration of **Cd** in tissues of Black Wing Stilt at Korangi Creek.

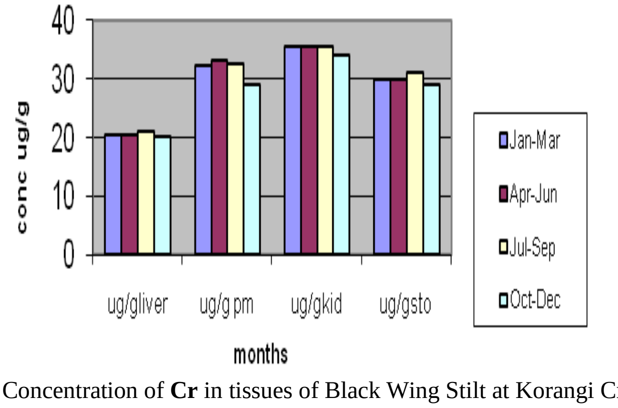

Fig. 9. Concentration of **Cr** in tissues of Black Wing Stilt at Korangi Creek.

Kojadinovic *et al*., (2007) studied trace elements in three marine birds Barau's petrel (*Pterodrome basani*), Andubon's shearwater (*Puffin iherminieri bailloni*), and white tailed trophic bird (*Phaethon lepturus*) breeding on Reunion Island (Western Indian Ocean). A highest value of Cd was 145 ug/g, 147 ug/g, and 117 ug/g in kidney of Barau's petrel, Andubon's shearwater, white tailed trophic bird respectively. These results are very high than present study, possibly due to great influence of aquatic pollution in Reunion Island. Kim and Koo., (2007) observed Cd 13.4 ug/g ,1.41 ug/g in black crown night heron (*Nycticorax nycticorax*), and grey heron (*Ardea cinerea*) respectively from Pyeongtaek, Korea. These results are in line with present study, possibly due to having the same environmental pollution status. Jakimska *et al*., (2011) investigated that the animals most exposed are those at the top of the trophic pyramid and metal levels in their tissues are the highest. The type of food of birds is also essential to investigate the levels of pollutants in their soft tissues. Mansouri *et al*., (2012) determined the levels of chromium in kidney of Western Reef heron (*Egretta gularis*) and Siberian gull (*Larus heuglini*) were (0.96, 2.32 μg/g), respectively, whereas in liver they were (1.05, 2.75 μg/g), respectively. The samples were collected from November to December 2010 throughout the Hara Biosphere Reserve. In present study, levels of Cr are high in liver at Korangi creek **(Fig.9)** but are in line with the results at Sands Pit and Hawks Bay (Fig. 5 and 7). However, in kidney, Cr is high at Korangi creek as compare to Sands Pit and Hawks Bay. Actually unprocessed waste from the industries and sewage enhances the gathering of metals and is picked up by the marine organisms which through the food chain end up with marine birds as they are at upper trophic stage in a marine environment.

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