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# BIOACCUMULATION OF METALS IN TISSUES OF KENTISH PLOVER (*CHARADRIUS ALEXANDRINUS*) IN COASTAL AREAS OF KARACHI, PAKISTAN

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## ABSTRACT

Concentration of Cadmium (Cd) and Chromium (Cr) were measured in seawater samples and body tissues (liver and kidney) of Kentish Plover (*Charadrius alexandrinus*) collected from Hawksbay, Sandspit, and Korangi Creek Coastal areas of Karachi. All samples were collected quarterly from 2006-2009 and examined using Atomic Absorption Spectrometer (Perkin Elmer. A. Analyst-700). In seawater, highest level of Cd (0.460 mg/l) was measured during 3$^{rd}$ quarter at Hawksbay and low quantity of Cd (0.003 mg/l) found during 1$^{st}$ quarter at Korangi Creek. Maximum and lowest concentration of Cr was 0.497 mg/l and 0.002 mg/l during 4$^{th}$ and 2$^{nd}$ quarter at Korangi creek and Hawks Bay, respectively. In the body tissues, peak stage of Cd (10.317 ug/g) was observed in liver during 2$^{nd}$ quarter at Sandspit and lowest Cd (0.857 ug/g) in kidney during 1$^{st}$ quarter at Korangi Creek. Maximum level of Cr (5.403 ug/g) was detected in kidney during 2$^{nd}$ quarter at Hawksbay and lowest level of Cr (0.743 ug/g) was found in Kidney in 2$^{nd}$ quarter at Sands pit.

**Keywords:** Kentish plover, metals, bioaccumulation, coastal areas.

## INTRODUCTION

Metals with high atomic numbers, high atomic weight and high densities are termed as heavy metals or trace metals. Heavy metals occur naturally in the world. Anthropogenic activities have led to metal toxicity effects in nature. Heavy metals like Cadmium, Arsenic, Manganese, Lead, Mercury, Zinc, Chromium and Silver accumulated in the tissues of living organisms and vegetation by absorption or ingestion of contaminated food, water, contaminated soil or absorption through skin. They entered our ecological system via fuel combustion, industrial wastages, agricultural waste and used batteries. Accumulation of these metal components in tissues or blood of living beings leads to serious health issues as well as high mortality rates. This also leads to biochemical disturbances, physiological malfunctioning and structural abnormalities in living beings.

Human interferences in nature and industrialization resulted in de-stability of ecological environment. All the anthropogenic activities near the wetlands resulted in contamination of water by those toxic heavy metals (Govind and Madhuri, 2014). Domestic waste material and agricultural products dumped into the water bodies increases the quantity of heavy metals in drinking water (Ghosh and Singh, 2005). Many researchers worked on heavy metals toxicity and effects of other toxic chemicals included petroleum, phenols and dyes on different living organisms (Gad and Saad, 2008; Jadhav *et al*., 2010). Different ecologies disturbed by toxicity of heavy metals include the fresh water ecosystem, terrestrial eco-zones, as well as marine biota. Directly or indirectly, metal toxicity adversely affects the biodiversity and living standards in particular habitat. The contamination of marine water through metals is very serious threat to biodiversity of the oceans. Industrial effluents contaminate the marine water at high extents. Toxicity in marine water ultimately leads to serious health effects in humans as they feed on fish and shell fish from marine environment (Emami *et al*., 2005). Ground water pollution by industrial effluents also causes contamination of marine water by runoff deposits while acid rain also results in pollution of marine environment with heavy metals.

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Biomonitoring acts as a specific tool to study the quantitative ratio of heavy metals. Many species of fish and aquatic birds have been used to analyze the excessive quantities of heavy metals in their bodies (Abdul Rashid *et al*., 2009). Since birds are present on the higher trophic level in any ecosystems, they considered as good choice to analyze high level of toxic components in aquatic ecosystem (Farriera, 2011). Toxic effects of different heavy metals have been discussed by researchers (Raza *et al*., 2016; Mehrotra *et al*., 2008; Sinka- Karimi *et al*., 2014). The persistent ability of Cadmium is so high that as it accumulates in the tissues, it remains there for several years and causes severe infectious problems in the human body. It may become carcinogenic leading to different types of cancers, respiratory tract infections, bone deficiencies and finally death (Govind and Madhuri, 2014). Very prolonged exposures of Cadmium results in abnormal liver tissues, thickening of blood arteries in liver, thinness of epithelial membrane, deformation of muscular membranes around gizzard and various kidney problems (Raza *et al*., 2016).

The excess amount of Chromium in human body as well as in other living organisms causes several diseases including acute and chronic infections. The acute infections include hemorrhage in the gastrointestinal tract, destruction of red blood cells and kidney failure, while chronic effects include cancer in lungs. High levels of Chromium in aquatic species causes serious damage (Govind and Madhuri, 2014).

Our study was conducted to analyze the quantitative ratio of presence of Chromium and Cadmium in marine water and in the aquatic bird Kentish Plover (*Charadrius alexandrinus*) found in Hawksbay, Sandspit and Creek areas of Korangi. Most of the industrial and domestic waste from the Karachi city is dumped into water through outlets of small canals or rivers via Liyari river, Malir river and Hub river. Waste water treatment plants are not installed by most of the industries in Karachi that is why all the wastages from factories directly dump into sea. It is estimated that only 2% of industries follow the protocol before throwing waste water into the sea (Raza *et al*., 2012).

Karachi is the largest city of Pakistan with increasing population pressure along with intensive industrial developments. There are many different industries including textile, beverages, tobacco, paint, chemical, pharmaceutical, rubber, coal and petroleum that continuously discharge their waste into the seawater without treatment. About 90% of marine pollution is due to industrial wastages (Haq, 1976; Khan and Saleem, 1988).

## MATERIALS AND METHODS

Hawksbay, Sandspit and Korangi Creek were selected for our study and visited to collect water samples and to capture the birds. Water samples were collected in sterilized vessels.

### Solvent Extraction Method

Seawater samples were filtered by using a filter paper Whattman 40. Then samples were acidified by using 3ml of Nitric acid (0.1 N). Digestion of water samples performed by adopting the solvent extraction method according to Kremling (1983). In the next step, 500 ml of acidified filter sea water was poured in beaker and then 5ml of Citrate buffer was added into the beaker. The chemical prepared to have 4.5 pH. The separatory funnel with 5 ml of Ammonium Pyrrolidine Dithiocarbamate (APDC) and 20 ml of Methyl Isobutyl Ketone (MIBK) was used to shudder this water for 10-20 minutes. Lower layer, which was the aqueous layer, was drained into the beaker, while upper layer was collected in the flask which was the organic layer. The drained aqueous solution was added with 10ml of MIBK. Semi dried solution formed which was then dissolved in 1 molar Nitric acid and then filtered. The filtrates were analyzed by Atomic Absorption Spectrometer (Raza *et al*., 2012, 2013).

### Acid Digestion

This technique was used to analyze the metal composition in kidney and liver of *Charadrius alexandrinus* as described by Benton and J (1988). Desiccation of tissues was done by keeping that sample in heated oven at 50 degree Celsius. After drying, 0.5 grams of tissues were added with 2.5ml concentrated Nitric acid in a beaker. Beaker was placed on hotplate for 1 hour at 80 degree Celsius. Then it was allowed to cool down. 2.5 ml of concentrated Perchloric acid was added into the beaker and then placed on the hotplate for two to three hours at 200 degree Celsius. It was then allowed to cool down. To make a diluted mixture of 10 ml, distilled water was added to filtrate. Then it was placed in Atomic absorption spectrometer for further analysis of presence of heavy metals.

## RESULTS AND DISCUSSION

### Metals in Sea water

The present study shows high levels of Cd 0.460 mg/l, 0.068 mg/l, 0.018 mg/l in Hawksbay, Sandspit, and Korangi Creek, respectively. Whereas lower level of Cd was 0.02 mg/l, 0.03 mg/l, 0.04 mg/l in the same areas, respectively.

Highest value of Cr was 0.007 mg/l, 0.034 mg/l, and 0.497 mg/l in Hawksbay, Sandspit, and Korangi Creek, respectively; minimum level of Cr was found in Hawksbay (0.002 mg/l), Sandspit (0.024 mg/l), and Korangi Creek (0.315 mg/l) ( Figs. 1-3).

Fatoki and Mathabatha (2001) found amounts 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. The results from Fatoki and Mathabatha (2001) shows higher values as compared to the results found in current study due to the higher contamination of heavy metals recorded in water from East London harbor and Port Elizabeth.

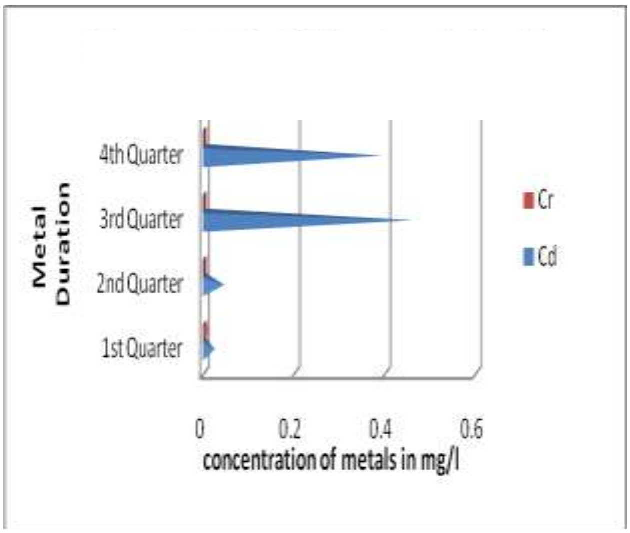


Fig. 1. Concentration (mg/l) of Cd and Cr in water samples at Hawksbay.

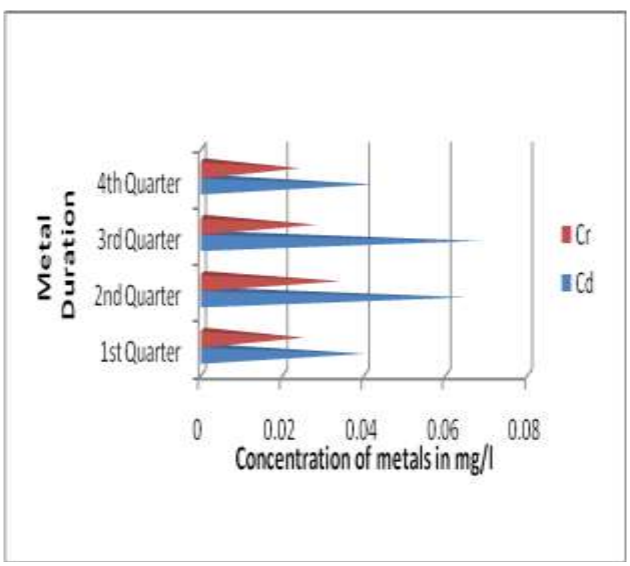


Fig. 2. Concentration (mg/l) of Cd and Cr in water samples at Sandspit.

Saleem (2002) measured contaminations of Cd (0.485 mg/l) in Karachi harbor, half of its concentration was reported in Korangi Creek and 0.063 mg/l was found in Buleji. These results are in line with present study for Cd. Another study (Qari and Siddiqui, 2004) found 0.02-0.11 mg/l Cd in Paradise point, Karachi. This amount of Cd is also line with the present study at Sands Pit and Hawks Bay samples. Industrial waste is considered to be the main source of Cd in this marine environment. 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).

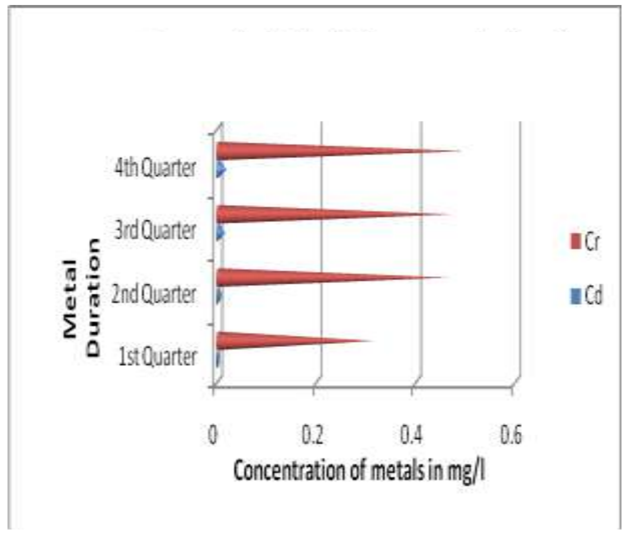


Fig. 3. Concentration (mg/l) of Cd and Cr in water samples at Korangi Creek.

Saleem (2002) determined concentration of Cr ranged 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, this result was higher than Hawks bay samples in the present study but almost in the line with Korangi Creek samples. Earlier study by Qari and Siddiqui (2004) reported Cr 0.06-1.33 mg/l in sea water of Paradise Point, Karachi. Result of this study is almost in line with Korangi Creek samples from our present study. In Karachi, there are three treatment plants located in Sher Shah, Mehmood Abad and Mari Poor where 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) found Cr 0.068 mg/l in Korangi Creek, 0.062 mg/l in Karachi harbor and 0.291 mg/l in Sandspit. However, in the present study, levels of Cr are high in Korangi Creek samples because of the great influence of industrial pollutants at that location. Qari and Siddiqui (2008) recorded concentrations of Cr (0.05-1.04

mg/l) and Cd (0.03-0.2 mg/l) in the surface and tide pools seawater of Nathia gali coast of Karachi.

Marcovecchio *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 the present study, it is determined that levels of Cd and Cr are high due to different anthropogenic activities. Another study by Joksimovic and Stankovic (2012) determined the concentration and accumulation of Cd 2500, 6200, 1900 and 7000 mg/l in sea water at four locations on the Montenegrin coastline, Sveta Stasija, Herceg Novi, Zanjice, and Bar, respectively. In the present study, levels of Cd were very low. It could be due to less pollutants in the study area as compare to Montenegrin coastline. A recent study 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. Their results are much higher than our study, possibly due to less impact of environmental pollutants. In 2013, Daniszewski calculated the annual average concentration of Chromium to be 2.67 mg/l in 2008 and 2.64 mg/l in 2009. These results are much high as compare to our work. Sarinas *et al*. (2014) found average dissolved chromium of .545 mg/l at Villa Beach, Iloilo City. This result is in range with our study. In the Iranian coast along the Oman Sea, 150 mg/l Cd and 201 mg/l Cr were found in the Gulf Chabahar (Bazzi, 2014). It shows great influence of marine pollutants on the Iranian coast along the Oman Sea as compare to present study. Recently Srichandan *et al*. (2016) studied the distribution of trace metals in surface seawater of the Bay of Bengal, off Rushikulya estuary, East Coast of India. The rank order distribution of trace metals in terms of their average concentration in seawater was reported as Fe > Ni > Mn > Pb > As > Zn > Cr > V > Se > Cd. In our study Cr distribution was found to be higher than Cd. Mary *et al*. (2017) assessed heavy metals in coastal water of Kalapet area, Pondicherry State, India. The metal concentration in seawater was recorded as 680 mg/l Cd and Cr at 910 mg/l. These results are much higher compared to the present investigation. It shows huge accumulation in coastal water of Kalapet area compared to Karachi Coast. Mortuza and Al-Misned (2017) studied environmental contamination and assessed heavy metals Cr (1.36 mg/l) and Cd (0.17 mg/l) in water of Red Sea Coast of Jizan, Saudi Arabia. These both values were higher than the recommended maximum concentration of Cr for drinking water (0.1 mg/l) set by WHO (1993). In the present report, levels of Cr were low but still higher than the recommended maximum concentration of Cr for drinking water (0.1 mg/l) set by WHO, 1993.

**Metals in Kentish Plover Body Tissues**

Kentish Plover (Fig. 4) one of the smallest plovers with long legs. They breed on most continents. Due to several anthropogenic activities in the coastal areas their population is decreasing and their current status is of threatened species (BirdLife International, 2016).

Fig. 4. Kentish Plover *Charadrius alexandrinus***.**

Levels of Cd and Cr in the liver and kidney of *Charadrius alexandrinus* are presented in Figures 5-10. Present study shows highest level of Cd (10.317 ug/g) in liver of Kentish plover at Sandspit and lowest Cd (0.857 ug/g) was noted in kidney at Korangi Creek. Maximum and lowest levels of Cr (5.403 ug/g and 0.743 ug/g) was detected in kidney during 2$^{nd}$ quarter at Hawksbay and Sandspit, respectively.

Pentreath (1976) and Olafson (1977) reported that marine organisms accumulate Cd due to its regulatory ability. Stoneburner *et al.* (1980) recorded 3790 ug/g Cd in liver, 23500 ug/g Cd in kidney of sooty terns from Dry Tortugas, Florida. Their results are much higher than the present findings, possibly due to influence of other pollution factors in Florida. Norheim (1987) analyzed 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 *Larus hyperboreus* (Glaucous gull), while 6.1-32 ug/g Cd was in kidney of *Fular glacialis* (Fulmar). Their results are in line with the present study at Sandspit and low levels were found in Hawksbay and Korangi Creek, due to similar amount of intake of pollutants through food chain by marine birds. Warren and Wallace (1990) calculated concentration of Cr in Blue winged teal from South plains Texas, Cr was 0.20 ug/g in liver. In our findings, levels of Cr are comparatively elevated in liver of Kentish Plover at Hawksbay and Sandspit.

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 line with present study at Hawksbay and Sandspit but at Korangi Creek, low levels of Cr were recorded in liver samples. Low levels of Cd in the present study may be due to monthly oscillation of metal ions and also these marine birds are at their higher trophic level.

More than 200 tannery units are functioning in Karachi and the waste material Cr, Cd, Pb, Mn, and Mg is directly discarded in to the marine water (Raza *et al*., 2012). Szarek *et al*. (2001) studied Cd levels in young coots originating from industrial and agricultural regions of North middle Poland. The results showed that in coots from industrial areas, levels of Cd were decreased due to better system of controlling pollutants production. In the present findings, Cd was 10.317 ug/g in liver of *Charadrius alexandrinus* (Kentish Plover) at Sandspit. This area is close to Lyari industrial area, thus pressure of metals is comparatively high. Szymezyk and Zalewski (2003) reported Cd contents in liver of *Anas platyrhychos* (mallards) and other hunting fowl species in Warmia and Mazury in 1999-2000.

In the present work, concentration of these metals was near the ground, perhaps due to less influence of marine pollutants on marine life. Augusta *et al*. (2005) measured Cd of 4.8 ug/g in liver and 40.7 ug/g in kidney of *Larus crassirostris* (Black tailed gull) collected from Rishiri Island, Japan. In the present findings, levels of Cd are high in liver at Sandspit but lowest in kidney.

Borga *et al*. (2006) studied regional and species specific bioaccumulation of major and trace elements in Arctic seabirds. These levels were diverse among species. Kojadinovic *et al*. (2007) studied trace elements in three marine birds *Pterodrome basani* (Barau's petrel), *Puffin iherminieri bailloni* (Andubon's shearwater) and *Phaethon lepturus* (White tailed trophic bird) 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 and White tailed trophic bird, respectively. Their results are quiet high compared to our work, possibly due to great influence of aquatic pollution in Reunion Island.

Kim and Koo (2007) observed Cd of 13.4 ug/g and 1.41 ug/g in liver of *Nycticorax nycticorax* (Black crown night Heron) and *Ardea cinerea* (Grey Heron), respectively from Pyeongtaek, Korea. These results are in line with present study, possibly due to having the same environmental pollution status. Farreira (2010) studied samples of liver and kidney of *Egretta caerulea* (Little blue Heron) collected from Sepetiba Bay, Riode Janeiro, Brazil to analyze Cadmium and Chromium. Cd in amounts of 6.32 ug/g and 6.57 ug/g and and Cr at 2.80 ug/g and 4.16 ug/g were detected in liver and kidney, respectively. These results are in line with present work. Jakimska *et al*. (2011) reported 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 birds feed on is also essential is also essential in investigating the levels of pollutants in their soft tissues.

Mansouri *et al*. (2012) determined the levels of Cr in kidney of Western reef heron (*Egretta gularis*) and Siberian gull (*Larus heuglini*) were (0.96 and 2.32 μg/g), respectively, whereas in liver they were (1.05 and 2.75 μg/g), respectively. The samples were collected from November to December 2010 throughout the Hara Biosphere Reserve. In the present study, levels of Cr are high in kidney and liver at Korangi Creek, Sandspit and Hawksbay. Untreated wastes from the industries and sewage enhances the assembly of metals and is picked up by the marine organisms which through the food chain ends up in marine birds as they are at upper trophic stage in a marine environment.

Sinka-Karimi *et al*. (2014) determined Cd and Cr in tissues of *Anas platyrhynchos* (Mallard) and did risk assessment of food consumption in the southeastern Caspian Sea. Liver and kidney showed the highest bioaccumulation of metals. In the present study, we found high levels of Cd (10.31ug/g) in liver at Sands Pit and Cr (5.40 ug/g) in kidney at Korangi Creek. another study Sinka-Karimi *et al*. (2015) reported mean cadmium concentrations in livers (1.63 ± 0.66 ug/g) of *Aythya ferina* (Pochards) collected from southeastern Caspian Sea in the winter of 2012. Recently, Plessl *et al*. (2017) analyzed samples of liver for Cr (0.000001 ug/g) and Cd (0.0000002 ug/g) in the Mallard *Anas platyrhynchos* collected from Eastern Austria. In present investigation, highest level of Cd (10.317 ug/g) and Cr (4.017 ug/g) was reported in liver of *Charadrius alexandrinus* (Kentish Plover) at Sandspit and Hawksbay, respectively possibly due to great influence of metal accumulation in these coastal areas.

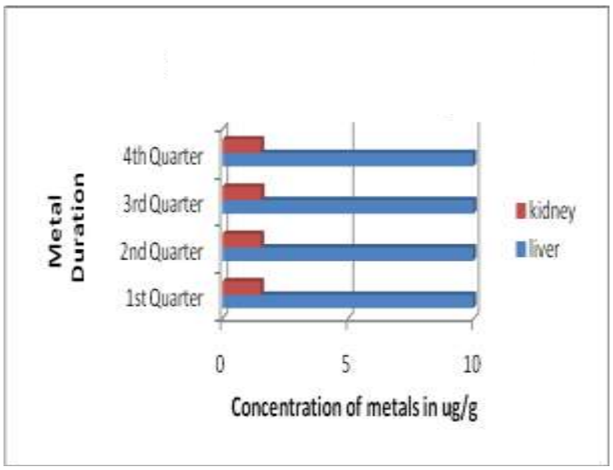


Fig. 5. Concentration of Cadmium in body tissues of Kentish Plover at Hawksbay.

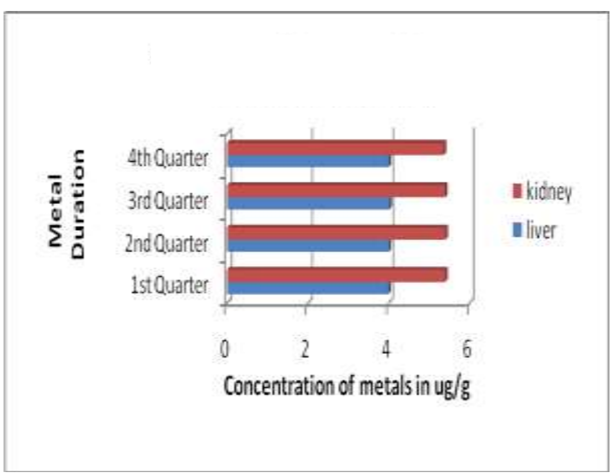


Fig. 6. Concentration of Chromium in body tissues of Kentish Plover at Hawksbay.

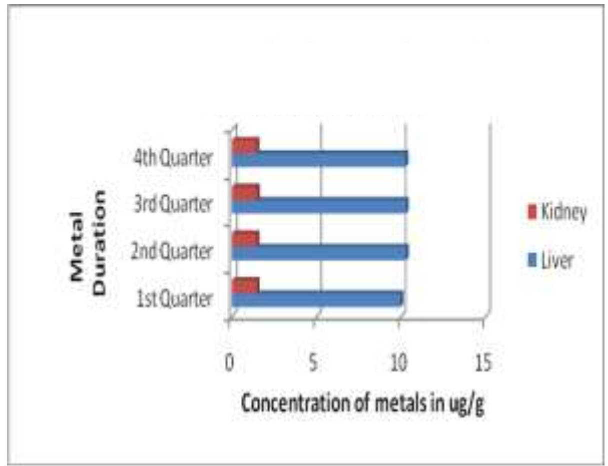


Fig. 7. Concentration of Cadmium in body tissues of Kentish Plover at Sandspit.

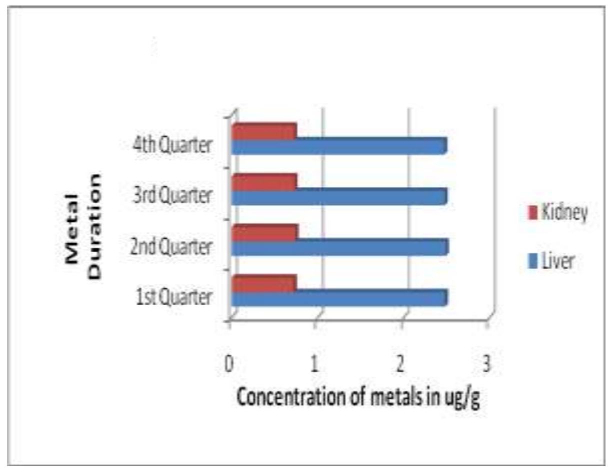


Fig. 8. Concentration of Chromium in body tissues of Kentish Plover at Sandspit.

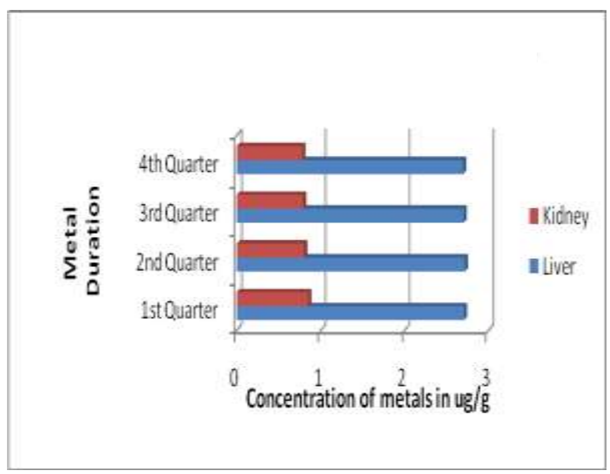


Fig. 9. Concentration of Cadmium in body tissues of Kentish Plover at Korangi Creek.

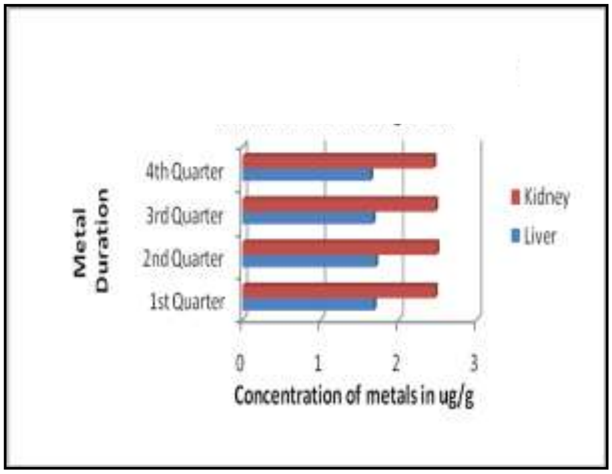


Fig. 10. Concentration of Chromium in body tissues of Kentish Plover at Korangi Creek.

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