METAL BIOAVAILABILITY; TOXICITY IN SEDIMENTS AND ACCUMULATION IN FIREWORM *EURYTHOE COMPLANATA* (PALLAS, 1766) (POLYCHAETA: AMPHINOMIDAE) FROM BULEJI KARACHI, PAKISTAN

[1]MUHAMMAD UZAIR KHAN, [2]KANWAL NAZIM AND [3]MOINUDDIN AHMED

[1]Marine Reference Collection and Resource Centre, University of Karachi, Pakistan
[2]Sindh Coastal Forest Department
[3]Department of Botany, Federal Urdu University of Arts Sciences and Technology, Karachi, Pakistan
[1]Corresponding Author's e-mail: muzairjoji@gmail.com

خلاصہ

Eurythoe complanata (Pallas, 1766) ایک سمندری Polychaete دودہ (worm) ہے جسے بھاری دھاتی آلودگی کی نشاندہی کرنے والا بھی سمجھا جاتا ہے۔ حالیہ تحقیق کے دوران کراچی کے ساحلی علاقے Buleji سے دودہ اور مٹی (Sediments) کے نمونے low tide میں جنوری سے مارچ 2016ء کے دوران حاصل کئے گئے۔ بھاری دھاتوں Zn، Pb، Mn، Cu، Cd، Cr اور Ni کی مقدار Atomic Absorption Spectrophotometer کے ذریعے معلوم کی گئی۔ نتائج سے ظاہر ہوا کہ worms اور مٹی دونوں میں Zn کی سب سے زیادہ مقدار خشک وزن میں بالترتیب 14.86+1.64ug/g اور 36.16+3.04ug/g رہی جبکہ سب سے کم مقدار Ni کی 0.60+0.18ug/g اور 1.00+0.15ug/g رہی۔
worms میں بھاری دھاتوں کی مقدار ترتیب وار اس طرح رہی سب سے زیادہ مقدار Zn کی پھر Pb، Mn، Cu، Cr، Cd اور سب سے کم Ni اسی طرح سے مٹی کے نمونوں میں سب سے زیادہ مقدار Zn، اس سے کم Mn پھر Pb، Cu، Cr، Cd اور سب سے کم Ni کی تھی۔ مجموعی عناصر کی (BCF) Bio-concentration factor, کی مقدار 1 سے کم حاصل ہوئی۔ دودوں کے جسمانی وزن اور بھاری عناصر کے درمیان مثبت تعلق Zn، Pb، Cd، اور Ni میں ظاہر ہوا جبکہ Cr، Cu اور Mn کا منفی تعلق مشاہدے میں آیا۔

Abstract

Eurythoe complanata (Pallas, 1766) is a marine polychaete worm, which is considered as an indicator species of heavy metal pollution. During present investigation the worm and sediment samples were collected from the intertidal zone of the Buleji coast in Karachi, at low tide, from January to March 2016. Atomic Absorption Spectrophotometer was used to determine the concentrations of Zn, Pb, Mn, Cu, Cd, Cr, and Ni. The results showed that the mean concentrations of Zn was the highest 14.86 ± 1.64 in dry tissues of worms and 36.16 ± 3.04 $\mu g\ g^{-1}$ dry weight in sediments while Ni was the lowest 0.60 ± 0.18 (dry tissues of worms) and 1.00 ± 0.15 $\mu g\ g^{-1}$ were recorded in sediment. The trend of the metals concentration, in a decreasing order, was found to be Zn> Pb> Mn> Cu> Cr> Cd > Ni for the worm and Zn> Mn> Pb> Cu> Cr> Cd> Ni for the sediments samples. The bio-concentration factor (BCF) for all the metals studied were found to be <1. The correlation between body weight and the concentration of the heavy metals in the worm showed a positive relationship for Zn, Pb and Ni while for Cd, Cr, Cu and Mn negative relationship was observed.

Introduction

Eurythoe complanata (fire worm) belongs to the polychaete family, Amphinomidae. Bindra (1927) reported five species of *Eurythoe* from Manora and Oyster Islands, Karachi Pakistan. He described the taxonomic characteristics and keys for the identification of Genus and species. *E. complanata* are found in intertidal zones and in sandy beaches in tropical coastal areas mostly along the coast line (Barroso *et al*., 2010). According to Hartmann (1968) this species is commonly reported from tropical seas, also found in both sides of America. Fauchald (1977) stated that fire worms have circum-tropical diversity in the rocky intertidal and shallow sub tidal zones and associated with patches of sand, submerged rocks and among the coral reefs.

E. complanata shows both sexual and asexual reproduction but the higher proportion of the population produce through asexual process by means of fragmentation and regeneration throughout the year (Kudenov, 1974). The ideal food source of *E. complanata* is carrion, but may feed on soft-bodied animals *i.e*. soft corals, sponges, ascidians and also consume some sea weeds (Kudenov, 1980). This is a nocturnal species inhabiting under rocks, inside calcareous algae and corals and burrowed in soft sediments during day time to avoid predation and desiccation, (Pardo and Amaral, 2006).

E. complanata is known as accumulator of heavy metal (Zn and Mn) in tissues (Amiard *et al*., 1987). Reish *et al* (1989) investigated the effects of Cd and DDT on *E. complanata* under experimental conditions. A toxicological experiment was conducted by Marcano *et al* (1997) based on coelomic lysozyme measurements, using Cu as a reference pollutant in the same species. Various studies have been conducted in temperate coastal areas regarding heavy metal accumulation in the tissues of various polychaete species in natural environment known as the accumulator of trace metals Mendez and Paez-Osuna (1998). However, in tropical and subtropical coastal areas especially in Pakistan coast, the information concerning these worms is limited. Bearing these considerations in mind, the present study was aimed to investigate trace metals contamination in sediments and tissues of *E. complanata*.

Materials and Methods

Buleji is a rocky ledge at a distance of 30 Km from Karachi (24° 8499’ N 66° 89418’ E). This is undisturbed coast compared with others. The estimated terrain elevation above sea level is 10 to 16 meter. A large number of rocky pools are present in the tidal zone. It also consists of sandy beach, which is a high energy zone with a wide variety of habitats which harbor a high diversity of marine organisms.

The complete specimens of *E. complanata* and sediments samples were randomly collected from Buleji intertidal zone, during January 2016 to March 2016. Specimens and sediments were rinsed initially with seawater at collection site to eliminate the adhered particles. Specimens were brought to the laboratory and placed in seawater at 4^{o}C up to 24 hours to relax the organisms for biometric measurement. Hundred individuals were randomly collected, eighty of them were divided into three groups according to their size and wet weight while twenty individuals representing all three classes were kept separately for individual worm analysis. All collected specimens were individually measured from the prostomium to the pigydium with a graduated ruler and weighed using a balance (±0.01g). Three sets of specimens from the collection site were selected according to their wet weight: (1) 32 individuals considering the 1- 2.56 g (wet wt) small size class; (2) 29 individuals of 2.71-3.89 g medium sized class; and (3) 19 individuals were pooled 4.1 – 5.41 g large sized class. The designed guidelines for animal ethics committee were followed for the use of animals in the laboratories abiding by the principles given by National Centre for the Replacement, Refinement and Reduction of Animals in Research (3 R's) and most importantly brought forward the concept of the fourth R, “rehabilitation” of used laboratory animals.

After biometric measurement all specimens were narcotized, dried at 60^{o}C (until dehydrated to achieve constant weight) and crushed. Suitable quantity (g) of crushed sample and dried sediments were taken and digested following El-Sayed *et al* (2011). The heavy metals Zn, Pb, Cd, Cr, Cu, Ni and Mn were determined using atomic absorption spectrophotometer model PG 990 (Lutterworth, U.K.).

In all size classes means ± SE were calculated. The regressions between body size and mean metal concentration were calculated following Paez-Osuna *et al* (1995), linear and exponential regression models (Microsoft Excel, 2010) were used to assess the relationship between individual worm weight correlated with the concentrations of metals.

Results and Discussion

Table 1 describes metal concentrations in sediments and tissues of *E. complanata*. In tissues samples Zn and Pb both were found to be the most abundant metals followed by Mn> Cu> Cd> Cr> Ni. Though, the values of Zn were within the range, enlisted in the same order of magnitude as estimated by Saiz-Salinas *et al* (1996) for several species of polychaetes. Similar results were found by Khan *et al* (2017) under the laboratory experiment in *E. complanata*.

Table 1: Mean ± SE of metal concentration in sediments and tissues (µg.g^{-1}dry wt.) of *E. complanata* collected from Buleji.

Metals / Samples	Zn µg.g^{-1}dry wt.	Pb µg.g^{-1}dry wt.	Cd µg.g^{-1}dry wt.	Cr µg.g^{-1}dry wt.	Cu µg.g^{-1}dry wt.	Ni µg.g^{-1}dry wt.	Mn µg.g^{-1}dry wt.
Sediments	36.16±3.04	17±4.40	2.2±0.25	5.7±0.83	8.16±1.15	1±0.15	17.7±2.98
Tissues	14.86±1.64	11.12±1.18	2.11±0.38	1.05±0.21	3.67±1.26	0.60±0.18	6.4±1.01

The higher mean concentrations of Zn, Mn and Pb ($p < 0.05$) and a lower level of Ni ($p < 0.05$) in sediments showed difference compared to other metals. The higher concentrations of metals may be due to the influence of untreated domestic and industrial effluents of Lyari River which have been reported by various researchers (Jilani 2014; Imtiaz-Uddin *et al*., 2014). Similar concentration of Cd was accumulated in *E. complanata* tissues as it was found in the sediments, where no evident differences were observed between sediments and tissues. According to Phillips (1980) and Rainbow (1990) higher accumulation of metals in tissues may depend on the metal, season, size of animal, metabolic rate and feeding habitat.

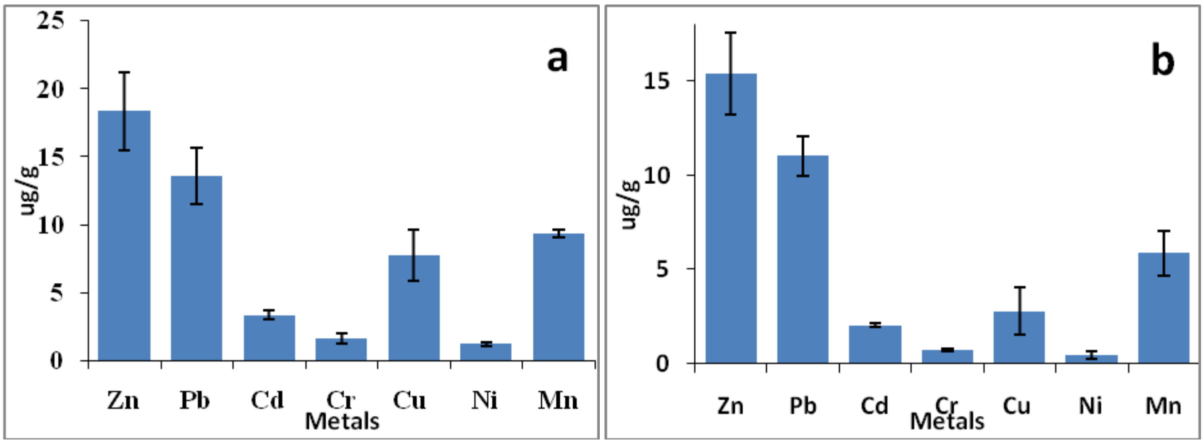
Coefficient of variation (CV%) describes the amount of variability relative to the mean. The highest percentages of Cu 96.07%, 76.34% and 41.48% were recorded in large, medium and small organisms respectively, while the lowest (Mn 5.47%), (Cd 7.39%) and (Ni 11.11%) were estimated in small, medium and large respectively among groups and metals. In general, the variations of the different cases were relatively higher (>41.48%) than small-sized class, where 76.34% and 96.07% of Cu was calculated in medium and large-sized class respectively (Table 2). Although, the large and medium sized worm has greater value of coefficient of variation but small sized worms has much more variability to this mean. It may be due to the reason that in *E. complanata*, the higher proportion of the population reproduced asexually by fragmentation and through regeneration process (Kundenov, 1974). So, small sized worms might be older in age but smaller in size containing metals. This may be the reason that the small worms (asexually reproduced) already accumulated metals, while more metals are accumulated for the growth of regenerating part than the other members of the same size class.

Table 2: Coefficient of variation (CV%) for trace metals in tissues of *E. complanata*.

Groups	Zn	Pb	Cd	Cr	Cu	Ni	Mn
Small	27.19	26.08	18.36	16.66	41.48	16.43	5.47
Medium	32.54	32.03	7.39	19.92	76.34	75.25	34.27
Large	16.75	30.14	40.52	83.39	96.07	11.11	43.77

Fig. 1 shows the variation in heavy metal concentrations in three size classes (small, medium and large) of *E. complanata* on the bases of wet weight. The level of Zn was maximum (18.4 ± 2.88 µg g^{1}dry wt) recorded in small-sized group with mean dry weight 437.5 ± 25.98 mg while the minimum level (10.83 ± 1.04 µg/g) was found in large sized having mean dry weight 1158.42 ± 23.82 mg). The level of lead (13.63 ± 2.05 µg/g) was the second highest metal estimated in small-sized group while, the minimum concentration of Pb (8.7 ± 1.51 µg/g) was found in the large-sized group. The range of Pb accumulation (5.9-17.3 µg g^{1}) in fire worm was within the range of values stated by Carral *et al* (1995). The highest concentration of Pb (13.63 ± 2.05 µg g^{1}) was estimated in the small-sized class (Fig 1a) and the minimum (8.7 ± 1.51) in the large-sized class (Fig 1c).

Similarly, Mn (9.36 ± 0.29 µg/g), Cu (7.8 ± 1.86 µg/g), Cd (3.4 ± 0.36 µg/g), Cr (1.83 ± 0.17 µg/g) and Ni (1.26 ± 0.12 µg/g) were found higher in small-sized group however, the minimum concentration of Mn (3.46 ± 0.87 µg/g), Cu (0.43 ± 0.24 µg/g), Cd (0.86 ± 0.2 µg/g), Cr (0.56 ± 0.27 µg/g) and Ni (0.09 ± 0 µg/g) were observed in large-sized group. The concentrations of Mn, Ni, Cu and Cr in different groups of *E. complanata* were compared with the estimated values of other researchers in different polychaetes species. Bryan *et al* (1987) reported that, in other polychaete species *N. diversicolor* requires Zn for the development of its jaws and also enhance body size during growth period. Present study also depicts that the accumulation of Zn, Pb, Ni and Cd in small worms were more efficient than large worm of *E. complanata*. This may be the reason that the worms which are being considered as small might be a fragment of parent worm due to the process of regeneration through asexual reproduction (Kudenov, 1974).



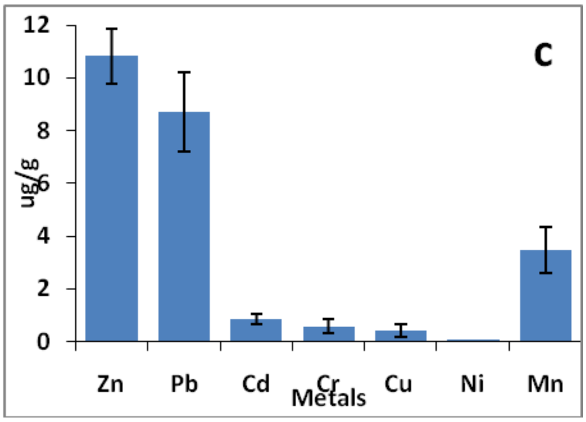
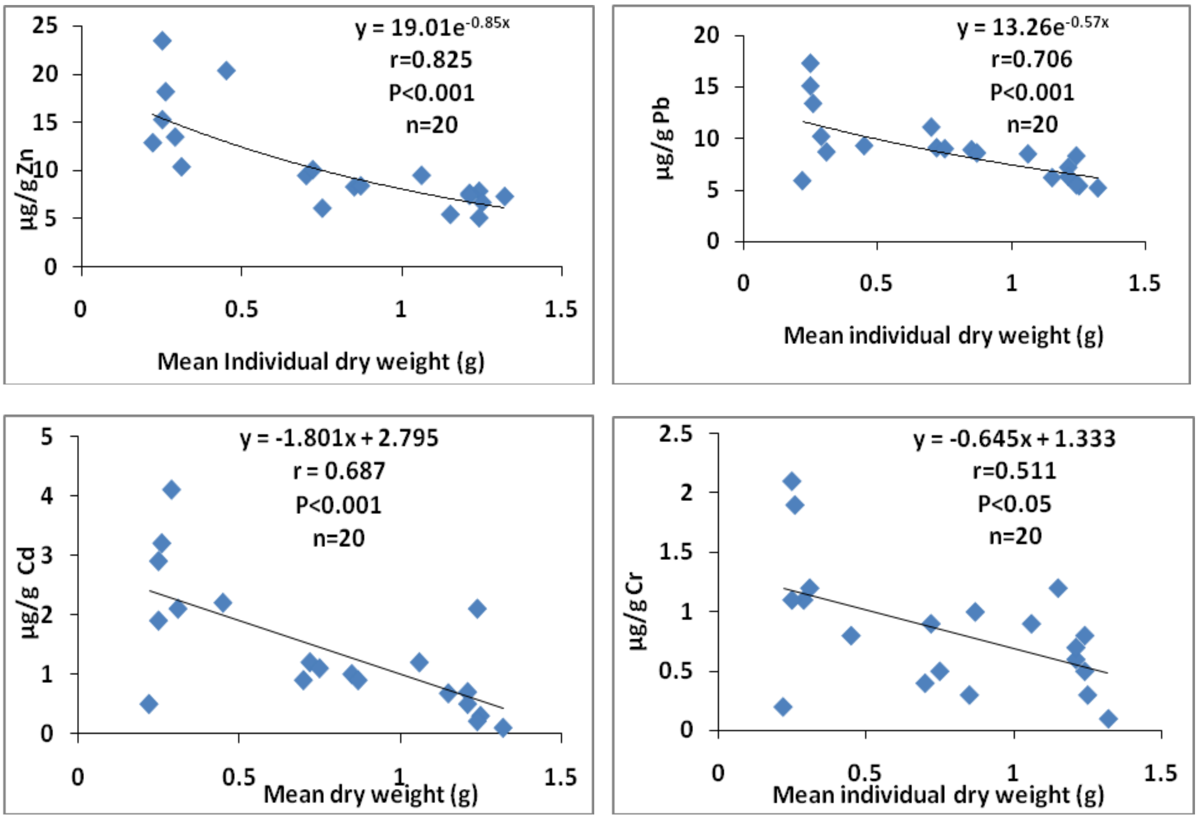


Fig. 1. Variation in trace metal concentrations in three classes of *E. complanata*on on the bases of wet weight. (a) mean ± SE of 32 individuals ranging 01-2.56 g wet wt; (b) mean ± SE of 29 individuals ranging 2.71-3.89 g wet wt; (c) mean ± SE of 19 individual up to 4.1-5.41g wet wt.

Fig. 2 shows regression between dry weight and accumulation of metal in twenty individuals of *E. complanata*. Four metals (Cd, Cr, Cu and Mn) displayed linear regression while three metals (Zn, Pb and Ni) showed exponential regression, however all metals showed significant relationship between dry weight and metal accumulation. The negative linear regression may be verified by the further study of lifecycle (asexual reproduction occurs in *E. complanata*) and the other environmental factors Rainbow (1990) such as habitat and mode of feedings *e.t.c.* Because *E. complanata* is also reproduced by body fragments and during this process the nutrient desire and the growth rate also varies as compared to normally growing worms.

Howard and Brown (1983) described a seasonal influence on Cu concentrations and Zn levels, described that accumulation of Zn and Cu increase during the growth periods of this species. Finally, it may be concluded that *E. complanata* is a bio-indicator species of metal pollutant, more probably for Pb and Cu as also investigated by (Mendez and Paez-Osuna, 1998). It was concluded that small worms group has tendency to accumulate more metals than the large group which have propensity to regulate metals at a stage rather than accumulation. However, it may be strongly suggested that surveys and experiments should be done in field as well as in laboratory to monitor this species.



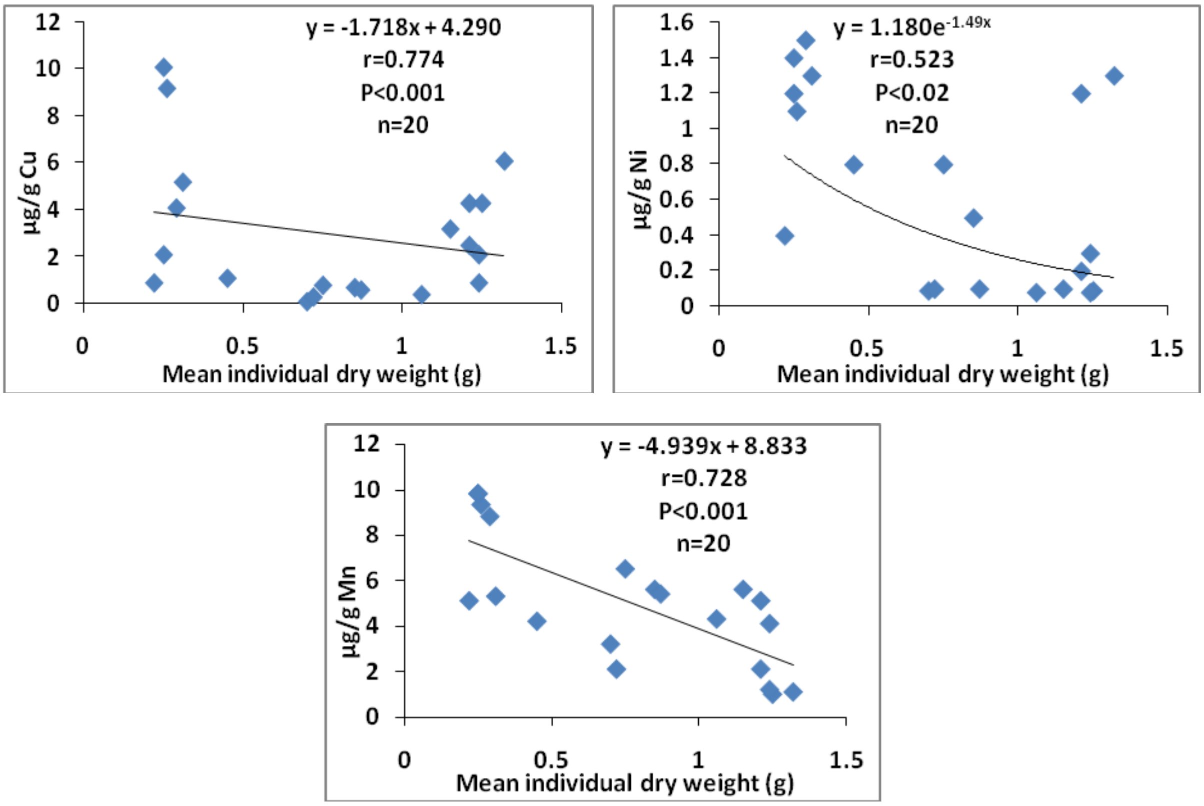


Fig. 2. Regression between individuals dry weight and metal concentrations of *E. complanata*.

Acknowledgement

We are grateful to Professor Dr. Javed Mustaquim of the Centre of Excellence in Marine Biology, University of Karachi, for identifying the worms.

References

Amiard, J. C., Amiard-Triquet, C., Berthet, B. and Metayer, C. (1987). Comparative study of the patterns of bioaccumulation of essential(Cu, Zn) and non-essential (Cd, Pb) trace metals in various estuarine and coastal organisms. *Journal of Experimental Marine Biology and Ecology* 106: 73-89.

Barroso, R., Klautau, M., Sole-Cava, A. M. and Paiva, P. C. (2010). *Eurythoe complanata* (polychaeta: Amphinomidae), the ‘cosmopolitan’ fire worm, consist of at least three cryptic species. *Marine Biology* 157: 69-80.

Bindra, S. S. (1927). Fauna of Karachi. 1- A study of the genus *Eurythoe* (Family Amphinomidae). *Memoirs of the Department of Zoology, Punjab University*, Vol. 1: 1-18.

Bryan, G. W., Gibbs, P. E., Hummerstone, L. G. and Burt, G. R. (1987). Copper, zinc, and organotin as long-term factors governing the distribution of organisms in the Fal estuary in southwest England. *Estuaries* 10: 208-219.

Carral, E., Puente, X., Villares, R. and Carballeira. (1995). Background heavy metal levels in estuarine sediments and organisms in Galicia (northwest Spain) as determined by modal analysis. *The Science of the Total Environment*. 172: 175-188.

El-Sayed, A., El-Ayyat, M. S., El-Sayeed, N. and Khater, Z. Z. K. (2011). Assessment of heavy metal in water, sediments and fish tissues from Sharkia province, Egypt. *Egypt. Journal of Aquatic Biology and fish*. 15: 125-144.

Fauchald, K. (1977). Polychaetes from intertidal areas in Panama with a review of previous shallow-water records. *Smithsonian Contributions to Zoology* 221: 1-81.

Hartmann, O. (1968). *Atlas of the Errantiate Polychaetous Annelids from California*. Allan Hancock Foundation, University of Southern California, Los Angeles.

Howard, L. S. and Brown, B. E. (1983). Natural variations in tissue concentration of copper, zinc and iron in the polychaete *Nereis diversicolor*. *Marine Biology* 78: 87-97.

Imtiaz-Uddin, S. M., Mumtaz, M. and Ahmed, T. (2014). Physico-chemical analysis and heavy metal concentration in textile effluents in Karachi Region of Pakistan. *Global Journal of Environmental Science and Technology*. Vol. 2(5): 071-074.

Jilani, S. (2014). Status of metal population in the Rive and coastal areas of Karachi. Middle-East. *Journal of Science and Research*. 22: 1288-1293.

Khan, M. U., Moinuddin, A. and Kanwal, N. 2017. Heavy metal accumulation in fire worm *Eurythoe complanata* (pallas, 1766) under the influence of industrial waste. *FUUAST J. BIOL*., 7(1): 9-12.

Kudenov, J. D. (1980). *Annelida: Polychaeta*. In: Brusca, R.C. (Ed.), Common Intertidal Invertebrates of the Gulf of California. The University of California Press, Tucson. 77-123.

Kudenov, J. D. (1974). *The reproductive biology of Eurythoe complanata (PALLAS, 1766) Polychaeta Amphinomidae*. Thesis Ph.D, Department of Biological Sciences, University of Arizona.

Marcano, L., Nusetti, O., Rodriguez-Grau, J., Briceño, J. and Vilas, J. (1997). Coelomic fluid lysozyme activity induction in the polychaete *Eurythoe complanata* as a biomarker of heavy metal toxicity. *Bulletin of Environmental Contamination and Toxicology*. 59: 22-28.

Mendez, N. and Paez-Osna, F. (1998). Trace metals in two populations of the fire worm Eurythoe complanata from Mazatlan Bay: effect of body size on concentrations. *Environmental Pollution.* 102: 279-285.

Paez-Osuna, F., Perez-Gonzalez, R., Izaguirre-Fierro, G., Zazueta-Padilla, H. M. and Flores-Campaña, L. M. (1995). Trace metal concentrations and their distribution in the lobster *Panulirus inflatus* (Bouvier, 1895) from the Mexican Pacific coast. *Environmental Pollution*. 90: 163-170.

Pardo, E. and Amaral, A. (2006). Foraging and mobility in three species of Aciculata (Annelida: Polychaete). *Brazilian Journal of Biology*. 66(4): 1065-1072.

Phillips, D. J. H. (1980). Quantitative Aquatic Biological Indicators. *Applied Science, London*.

Rainbow, P. S. (1990). Heavy metal levels in marine invertebrates. In: Furness, R.W., Rainbow, P.S. (Eds.), *Heavy Metals in the Marine Environment*. CRC Press, Boca Raton. 67-80.

Reish, J. D., Asato, S. L. and Lemay, J. A. (1989). The effect of cadmium and DDT on the survival and regeneration in the amphinomid polychaete *Eurythoe complanata*. VII *Simposio International de Biología Mavina*, 1989, La Paz, Mexico. 107–111.

Saiz-Salinas, J. I., Ruiz, J. M. and Frances-Zubillaga, G. (1996). Heavy metal levels in intertidal sediments and biota from Bidasoa Estuary. *Marine Pollution Bulletin*. 32: 69-71.