

EVALUATION OF HEAVY METALS CONTAMINATION IN MANGROVE SEDIMENTS AND THEIR ALLIED FIDDLER CRAB SPECIES (*AUSTRUCALINDENSIS* (ALCOCK, 1900) FROM HAWKS BAY, KARACHI, PAKISTAN

Noor Us Saher* and Asmat Saleem Siddiqui

Centre of Excellence in Marine Biology, University of Karachi, Karachi, Pakistan

*Corresponding author: noorusaher@yahoo.com

ABSTRACT

The aim of the study was to determine the heavy metals (Fe, Cu, Zn, Co, Cr, Ni, Pb and Cd) in mangrove sediments and their associated fiddler crab (*Austruca sindensis* (Alcock, 1900) from the mangroves of Hawks Bay, Karachi, Pakistan. According to the sediment quality guideline, sediment was contaminated by Ni, Cu, Cr, Pb and Cd and assessed infrequent or frequent adverse effect of these metals on benthic fauna. The mean accumulation pattern of the heavy metals was observed as Pb > Cu > Zn > Fe > Ni > Cr > Co > Cd in the fiddler crabs. All metals concentrations were detected significantly greater in the male crab as compared to female crabs, except Cr. Fiddler crabs were noticed the highest bioaccumulation of Pb, which was observed two to three times higher concentrations than those in the sediment. Sediment biota accumulation factor (SBAF) also revealed the active accumulation of Pb in both genders, whereas Cu, Cd, and Zn showed active accumulation only in male crabs. The results indicated that *A. sindensis* may be employed in pollution monitoring programs, especially Pb contamination, along the coastal areas of Pakistan.

Keywords: Bioaccumulation, fiddler crab, heavy metals, sediment contamination, mangroves, Hawks Bay.

INTRODUCTION

Heavy metal contamination in coastal environments due to anthropogenic activities and their consequences as bioaccumulation and toxicity in ecosystems, has become a global issue. The coastal regions especially, mangroves are the natural and productive environment, constitute a unique fauna and flora and a number of important fisheries linked with them, especially shrimp and crabs (Dubinski *et al.*, 1986; Saifullah *et al.*, 2002). Mangrove sediments act as a chelating matrix for trace metals, reducing the mobilization of these chemicals to the mangrove plant tissues by trapping in the typical mangrove root system, therefore, availability of contaminants extend to the resident biota (Silva *et al.*, 1990; Struve and Falconer, 2001; Pinheiro *et al.*, 2012). The heavy metals can be accumulated in tissues of aquatic animals and this bioaccumulation shows a large variety for both metals and species, therefore heavy metals measured in tissues of these organisms can reflect past exposures (Rainbow, 1993; Canli and Atli, 2003; Beltrame *et al.*, 2010).

Crustaceans are known to accumulate metals from the surrounding environment through assimilation or absorption from food, water and/or sediment (Bryan, 1971; Na and Park, 2012). Crabs are a significant component of benthic fauna and considered as ecosystem engineers because of their dynamic role as a borrower in mangrove and estuarine environment. Fiddler crabs (genus *Uca* = *Austruca* by recent classifications) belong to the family Ocypodidae, are ubiquitous among the crab species, they associated with wide-ranging habitats throughout tropical and subtropical regions of the world (Saher and Qureshi, 2012). Despite their importance, only a few studies have been reported on the bioaccumulation of heavy metals in fiddler crabs (Ismail *et al.*, 1991; Blasco *et al.*, 1999; Gbaruko and Friday, 2007). *Austruca sindensis* (Alcock, 1900) is commonly known as Indus fiddler crab, and they have relatively restricted distribution in the northern Arabian Sea and the Northern and Northwestern coasts of the Persian Gulf. They are small, highly social and prefer habitat in higher intertidal areas or even in sheltered shores far from the sea (Crane, 1975; Saher and Qureshi, 2012; Shih *et al.*, 2015).

This study evaluates the heavy metals concentrations in *Austruca sindensis* from the coastal habitat of Pakistan. The objectives of this study were: (i) to determine the eight heavy metal (Fe, Cu, Zn, Co, Cr, Ni, Pb, and Cd) concentrations in mangrove sediments and their inhabitant fiddler crab (*A. sindensis*) from Hawks bay (ii) to evaluate gender effect on metal accumulation in fiddler crab; and (iii) to compare metal levels in sediment and crabs.

MATERIALS AND METHODS

Site description

Pakistan shoreline covers approximately 990 km north margin of the Arabian Sea. The Karachi coastline is situated in the Sindh province that comprises about 167 km long ocean front (Saher and Siddiqui, 2016). Karachi is

one of the most populated mega city and main hub of industrial and commercial activities along the coastline of Pakistan. Therefore, it is threatened by pollution through various points and non-point sources. Lyari and Malir Rivers are the leading sources of pollution in Karachi coastal areas, are contaminated by various links of unprocessed metropolitan and industrial wastes, and at the end drain into the beaches of Karachi into the Arabian Sea (Rizvi *et al.*, 1988; Saher and Siddiqui, 2016). The current study site positioned at 24° 50' 51" N and 66° 54' 8" E, it is located near Hawks Bay, Karachi (Fig. 1). The site is sheltered by the dense mangrove vegetation (*Avicenna marina*).

Sampling procedure

To monitor the heavy metal contamination in mangrove sediment and the fiddler crabs from Hawks Bay backwater mangrove area, Karachi. The sampling was conducted during Jan. 2012 during low tide. Fiddler crabs were randomly collected by excavating their burrows and gathered in plastic bottles. The sediment cores (N = 3, approx. 20 cm deep) was also collected through PVC core from the site. All samples kept in the icebox and brought to the laboratory for analysis.

Laboratory analysis

The each sediment core thoroughly mixed and homogenized. A sub-sample from it was taken (200 g) and dried at 75 °C for 24 h. Dried sediment samples (100 g) were ground and homogenized, then screened with US standard sieves to separated gravel (G), sand (S) and mud (M) fractions. One gram of sediment (mud) was mixed with 10 mL mixtures of HNO₃ and HCl (1:3) and digested at 90 °C for an hour on a hot plate and then allowed to cool at room temperature. The sample was filtered and diluted to 50 mL with distilled water (Saher and Siddiqui, 2016). The crabs were washed with plenty of distill water to remove the debris than crabs were pooled according to gender and make a composite sample as an individual crab sample was not sufficient for analyses after desiccation. The both samples were oven dried at the constant weight, then grounded and homogenized. Dry crab samples (1.0 g) were mixed with 5 mL of HCl and 2 mL of HNO₃ and heated to near dryness, then added 10 mL of H₂O₂ and allowed the sample cool at room temperature. Samples were filtered and diluted to 50 mL using distilled water (Leung and Furness, 1999). Sediment and crab samples were analyzed for the eight heavy metals (Fe, Cu, Zn, Co, Ni, Cr, Pb, and Cd) through Atomic Absorption Spectrometer A Analyst 700 (Perkin Elmer USA).

RESULTS AND DISCUSSION

Heavy metals contamination in mangrove sediment

The eight heavy metals (Fe, Cu, Zn, Co, Cr, Ni, Pb, and Cd) concentrations in mangrove sediments ($\mu\text{g g}^{-1}$ dry weight) collected from Hawks Bay mangrove area is presented in Fig. 2. The mean concentrations of metals, Fe, Cu, Zn, Co, Ni, Cr, Pb and Cd were 1009.9, 36.33, 71.86, 11.52, 50.89, 121.6, 44.08 and 1.39 $\mu\text{g g}^{-1}$ respectively in mangrove sediment. According to sediment quality guidelines, Ni concentration in mangrove sediment was exceeded in their effective range medium (ERM) limits, indicated the frequent adverse effects of Ni contamination on benthic fauna. The concentrations of Cu, Cr, and Cd in sediment were exceeding their effective range low (ERL) limits, suggested that adverse effects of these metals were occasionally observed on allied fauna. The Pb concentration in sediment was close to their ERL values indicated the rare adverse effects of Pb, while Zn concentration was below their ERL values suggested that no or rare adverse effect of Zn was observed on sedimentary fauna. Numerical sediment quality guideline (SQG) is a valuable index used for the evaluation of contamination and toxicity of metals in sediment. It has been developed using a variety of approaches, typically involving statistical comparisons of chemical concentrations and measures of adverse biological effects upon exposure to sediments (Long and MacDonald, 1998; Saher and Siddiqui, 2016).

Heavy metal concentrations in allied fiddler crab (*Astruca sindensis*)

The mean heavy metals concentrations (Fe, Cu, Zn, Co, Cr, Ni, Pb and Cd) in fiddler crab (*A. sindensis*) is shown in Fig. 3. The gender effects on heavy metal accumulation were scrutinized by one-way ANOVA analysis followed by Tukey's comparison, significant differences were accepted at $p = 0.05$. Mean accumulation pattern of the eight heavy metals in fiddler crabs was observed as $\text{Pb} > \text{Cu} > \text{Zn} > \text{Fe} > \text{Ni} > \text{Cr} > \text{Co} > \text{Cd}$. Lead presented the highest concentration in crabs. The significant variations were observed in Pb concentrations between the genders, moreover significantly lower concentrations were detected in females ($74.37 \mu\text{g g}^{-1}$) and higher in male ($113.89 \mu\text{g g}^{-1}$). Pb is a toxic and non-essential element for crabs, therefore it could not regulate through metabolic processes, only accumulate and tends to be detoxified by metallothioneins or phosphate granules and stored permanently in tissues (Rainbow, 1997a; MacFarlane *et al.*, 2000).

Copper exhibited the second highest concentration in the fiddler crab among the metals (Fig. 3). The significant dissimilarities were noticed in Cu concentrations between the genders, furthermore significantly lower concentrations were observed in female ($19.0 \mu\text{g g}^{-1}$) and higher in male ($97.35 \mu\text{g g}^{-1}$). Cu is a micronutrient for aquatic life, but it is converted into toxic at higher concentration. It is essential for crustaceans and is part of the respiratory pigment hemocyanin (Young, 1972; Pinheiro *et al.*, 2012). During Ecdysis, water absorption decreases the concentration of this metal and it is consumed during the course of the post-molt in the synthesis of hemocyanin and also for exoskeleton hardening (Engel and Brouwer, 1991; Pinheiro *et al.*, 2012). Therefore the excessive concentration of Cu indicated the regularity of metabolic activities. Zinc displayed the third highest concentration in the fiddler crab among the studied metals (Fig. 3). The significant discrepancies were identified between the gender for Zn concentration, and it fluctuated from 1.99 to $76.65 \mu\text{g g}^{-1}$ in female and male respectively. Zn is used as an active center for metallo enzymes and activators of other enzyme systems, i.e. carbonic anhydrase (Rainbow, 1997b; MacFarlane *et al.*, 2000).

The significant inter-sexual difference was observed in Fe concentrations and it was significantly lower concentrations were observed in female than the male, ranged from $4.51 \mu\text{g g}^{-1}$ in female to $61.62 \mu\text{g g}^{-1}$ in male (Fig. 3). Fe is an essential element for the physiological needs of decapod crustaceans. Nickel and Chromium concentrations were also apparent significant variations between the genders (Fig. 3). Nickel showed significantly lower concentrations in female ($19.34 \mu\text{g g}^{-1}$) and higher in male ($32.49 \mu\text{g g}^{-1}$). Whereas, Cr showed significantly higher concentrations in female ($7.52 \mu\text{g g}^{-1}$) than male ($7.08 \mu\text{g g}^{-1}$). Edible crustacean's tissues rarely contain Cr above from 0.3 mg/kg (Eisler, 1981). The highest value (0.6 mg Cr/kg fresh weight) reported in the muscle of rock crab (*Cancer irroratus*) was from specimens collected near an ocean dump site receiving large quantities of metals. Moreover, tissues of digestive glands and in gills of rock crab also contained the highest Cr residues (Greig *et al.*, 1977; Eisler, 1986). Cobalt extended from $3.34 \mu\text{g g}^{-1}$ in female to $10.18 \mu\text{g g}^{-1}$ in male, moreover, it showed significant differences between the genders (Fig. 3). Cadmium fluctuated from 0.90 to $2.18 \mu\text{g g}^{-1}$ in female and male, respectively, and the significant intersexual variations in Cd accumulation were noticed between the genders (Fig. 3). The Cd has no recognized biological function, but produces potential toxicities in organisms. The Cd is non-essential for crabs, and enters cells through transporters for divalent metals such as Calcium (Rainbow and Black, 2005; Pinheiro *et al.*, 2012).

The comparative view of heavy metals concentrations in *A. sindensis* with other three fiddler crab species collected from different coastal and estuarine environment of the world is shown in Table 1. The concentrations of Cu, Co, Ni, Cr and Pb were observed higher in *A. sindensis* as compared to other three species of fiddler crabs. These metal concentrations were perceived higher than those reported for *A. tangeri* collected from River Guadalquivir Estuary, Spain after the accident of Aznalcóllar (Blasco *et al.*, 1999). The concentrations of Zn in *A. sindensis* were observed similar to the values found in *A. tangeri*, whereas Fe and Cd concentrations in *A. sindensis* were observed lower than stated for *A. tangeri* and *A. annulipes*, respectively (Table 1). The comparison reveals the high metal accumulation in *Austruca sindensis*, it may be indicative of the high tendency of metals accumulation in this species, especially in male crabs due to their one enlarge chela as metals storing organ, or due to taken of complete animal with their exoskeleton which also have the high affinity towards the metals or the bioavailability of metals in the environment also influential in bioaccumulation processes in an organisms.

Sediment-Biota Accumulation Factor (SBAF)

To evaluate the active accumulation of heavy metals in fiddler crabs, sediment biota accumulation factor (SBAF = $\text{Metals}_{\text{Biota}} / \text{Metals}_{\text{Sediment}}$) was calculated (Fig. 4). The SBAF values greater than one (>1.0) indicate the active accumulation of heavy metals and while, SBAF <1.0 showed non active bioaccumulation. Average SBAF in crabs follow the order $\text{Pb} > \text{Cu} > \text{Cd} > \text{Co} > \text{Ni} > \text{Zn} > \text{Cr} > \text{Fe}$. The SBAF values showed significant differences ($p < 0.001$) between the genders for all studied metals. Moreover, the values for all metals were observed significantly higher in male than the female, except Cr. SBAF values for all metals were detected <1.0 in females, indicates the no active bioaccumulation of heavy metals, except Pb. Whereas, SBAFs for Pb, Cu and Cd were observed greater than 1.0 in male, indicates the active bioaccumulation of these metals. Active accumulation of Cu in crabs is not an unusual phenomenon, Cu is an essential metal for decapod crustaceans and regulates the metabolic activities, but at a higher level, it also accumulated in tissues (Bryan, 1971; Rainbow, 1985). Conversely, Pb and Cd are not essential metals, but they are toxic at low concentrations. Non-essential metals are not regulated and accumulation can occur at all concentrations (Brouwer and Lee, 2007; Rainbow, 1985). According to Bondgaard and Bjerregaard (2005), the molting of the crab *Carcinus maenas* did not reduce Cd levels, which may also explain the high SBAF for toxic metals in the fiddler crab.



Fig. 1. Map showing the study site Hawks Bay, Karachi, Pakistan (Google Map).

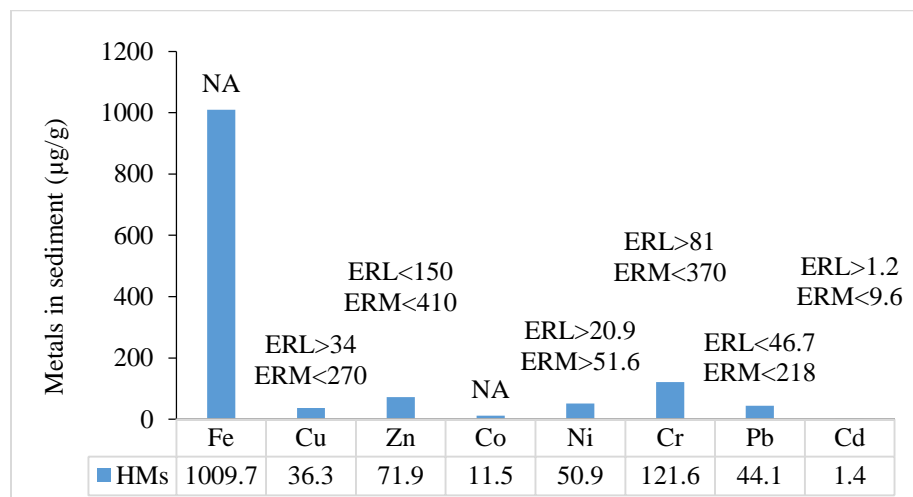


Fig. 2. Mean heavy metals concentrations ($\mu\text{g g}^{-1}$) in sediment from Hawks Bay, Karachi. ERL and ERM guidelines for heavy metals and their comparison with metal levels in Hawks Bay sediment ('<' and '>' sign shows the above and below limits of corresponding metals in sediment with their respective guidelines; NA shows unavailability of guideline value).

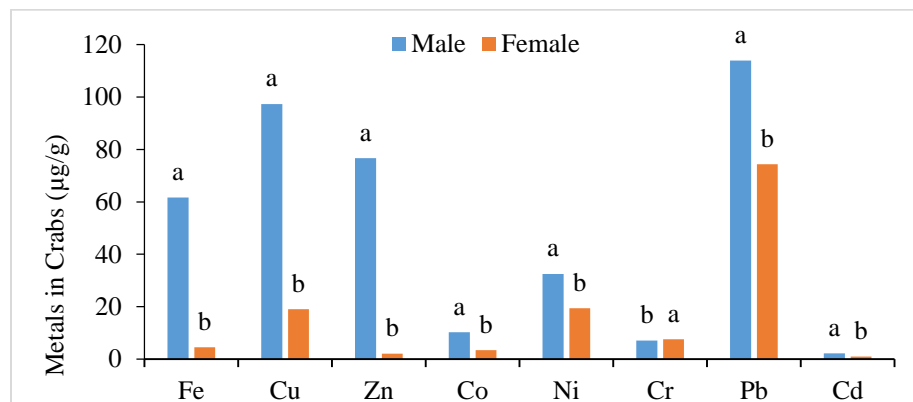


Fig. 3. Mean heavy metals concentrations ($\mu\text{g g}^{-1}$) in both genders of fiddler crab from Hawks Bay, Karachi (One-way ANOVA: Different superscripts shows significant differences in mean concentrations of metals between male and female fiddler crabs at $p < 0.001$).

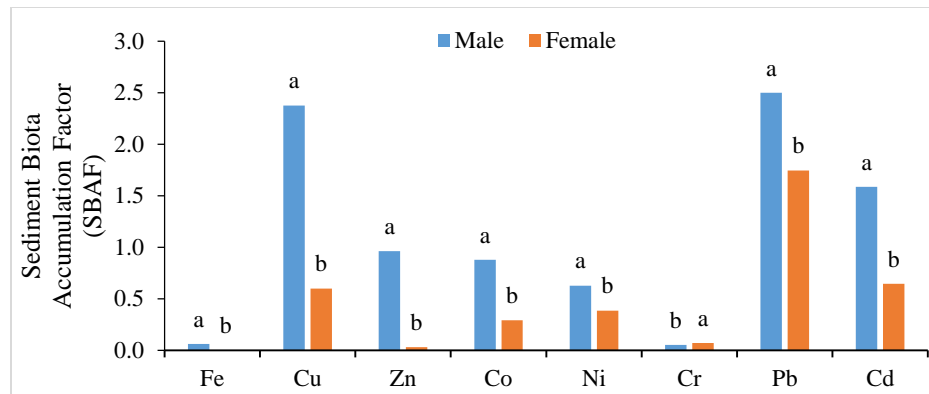


Fig. 4. Sediment Biota Accumulation Factor (SBAF) for heavy metals in both genders of fiddler crab from Hawks Bay, Karachi (One-way ANOVA: Different superscripts shows significant differences in mean SBAF values between male and female fiddler crabs at $p < 0.001$).

Table 1. The comparison of heavy metals concentrations ($\mu\text{g g}^{-1}$ dry weight) in fiddler crab species reported from different coastal and estuarine environments of the world.

Crab species	<i>A. sindensis</i>	<i>A. pugilator</i>	<i>A. tangeri</i> *	<i>A. annulipes</i> *
Fe	3.4 – 63.6	–	37.5 – 421	–
Cu	18.9 – 97.5	2.24 – 2.32	29.8 – 53.7	7.91 – 23.9
Zn	2.0 – 76.9	7.00 – 7.08	14.6 – 81.9	10.2 – 23.8
Co	2.56 – 11.06	–	0.08 – 0.25	–
Ni	17.48 – 33.57	–	0.31 – 0.89	–
Cr	7.02 – 7.60	0.54 – 0.60	–	–
Pb	71.26 – 115.4	1.05 – 1.09	0.00 – 0.76	3.04 – 11.6
Cd	0.88 – 2.24	–	0.02–0.28	1.47 – 3.24
Reference	This study	Gbaruko and Friday (2007)	Blasco <i>et al.</i> (1999)	Ismail <i>et al.</i> (1991)
Location	Hawks Bay, Pakistan	Niger Delta, Nigeria	River Guadalquivir Estuary, Spain	Northern Peninsular Malaysia

* indicates metal content in this species as $\mu\text{g g}^{-1}$ wet weight.

Table 2. Comparison among three matrices i.e. sediments, male and female fiddler crabs from Hawks Bay, Karachi.

Inference	Metals	Matrices			One-way ANOVA	
		Sediment	Male	Female	F-value	p-value
I, $S > M > F$	Fe	1009.7 ^a	61.62 ^b	4.508 ^c	201448.79	<0.001
	Ni	50.898 ^a	32.495 ^b	19.34 ^c	240.25	<0.001
II, $M > S > F$	Cu	36.33 ^b	97.353 ^a	19.001 ^c	571.46	<0.001
	Cd	1.39 ^b	2.183 ^a	0.905 ^c	746.18	<0.001
III, $S \approx M > F$	Zn	71.86 ^a	76.65 ^a	1.991 ^b	207.53	<0.001
	Co	11.522 ^a	10.186 ^a	3.346 ^b	119.80	<0.001
IV, $S > M \approx F$	Cr	121.6 ^a	7.077 ^b	7.52 ^b	112.12	<0.001
V, $M > F > S$	Pb	44.08 ^c	113.89 ^a	74.37 ^b	489.93	<0.001

Note: S, Sediments; M, Male; and F, Female crab.

Comparison of heavy metals in sediments and crabs

The heavy metals comparison was scrutinized by one-way ANOVA analysis followed by Tukey's comparison in three matrices i.e. sediment, male and female fiddler crabs are shown in Table 2. Five conditions were observed among the eight heavy metals concentrations in different medium analyzed in this study. The first condition in which sediment has highest metal loads than male and female crab ($S > M > F$) was examined for Fe and Ni. Iron is the most abundant element in the earth's crust as well as act as micronutrients for biota. For decapod crustaceans it

is also as essential metals for metabolic activities, no information about Fe toxicity was reported in crabs. While, Ni concentrations in sediment were exceeding their both ERL and ERM guidelines, and Hawks Bay sediment showed frequent adverse effects of Ni on sediment-associated fauna. Low levels of Ni in fiddler crabs may indicate their tolerance level, do not accumulate the certain metal even if present abundant in the environment, conversely it also indicates the low bioavailability of Ni to organisms. The second condition presented, the highest metal load in male than sediment and female ($M > S > F$). It was observed for Cu and Cd and they were observed two times greater concentrations in male than those within the sediment.

The third condition showed that there were no significant differences between metal concentrations in sediment and male, but significantly lower concentration noticed in the female crab ($S \approx M > F$). It was observed for Zn and Co, reveals that robust metal accumulation can be responsible for gender specific activities. In the case of *Astruca* especially, male crabs have one enlarged chela which has 10 times greater muscles than their body and may be responsible for high metal accumulation. The fourth condition was observed for Cr, in which sediment has 12 times greater metal load than crabs, but relatively similar metal concentrations were detected in male and female crab ($S > M \approx F$). This condition may also indicate the high tolerance ability towards the metal accumulation in crabs, those metals which already present in high amount in the sediments. The last condition was observed for Pb, in which male crab has highest metal loads than female crab and sediment ($M > F > S$). The results of the present study clearly indicated that Pb bioconcentration occurred in both genders of the fiddler crab. The concentrations in female and male were two to three times greater, respectively, than those within the sediments. The high variability in different metal accumulation in *A. sindensis* may indicate their sensitivity towards the metals contamination.

The current study revealed that this species may act as biomonitor species for lead contamination, furthermore the intersexual variations in metal accumulation indicate that both genders may be included in monitoring programs. The results also suggested that evaluation of spatial and temporal variations in metal bioaccumulation should be studied in future monitoring to justify the role of this crab species as potential bioindicator of metal contamination in the marine and estuarine environment.

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