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Articles and Statements

Bioaccumulation pattern of trace metals in commercially important crustaceans in Indian Sundarbans

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Abstract

We measured the concentrations of Pb and Cd, in muscle tissue of 3 commercially important crustacean species (*Penaeus monodon, Fenneropenaeus indicus* and *Metapenaeus brevicornis*) collected from the UNESCO declared world heritage site Indian Sundarbans. The Indian Sundarbans estuarine system is recognized as one of the most diversified and productive ecosystems in the world located at the confluence of Hooghly-Matla estuarine complex. Due to negative pressures like intense industrialization, urbanization and increase of anthropogenic activities in recent era, this ecosystem is getting contaminated with toxic heavy metals which vary with seasons. Significant variation of heavy metals in muscle tissue of 3 commercially important crustaceans collected from four different sampling stations (2 in central and 2 in western sector) of Indian Sundarbans were observed. Inductively coupled plasma – mass spectrometer was used to study the level of metals in tissues of selected species. The concentration of trace metals accumulated in all the muscle tissues of selected species followed the order Pb> Cd. The distribution of metals exhibited significant spatial variation and followed the order station 1 >station 2 > station 3 > station 4, which may be due to variable degree of contamination in different location ($p < 0.01$) indicating the adverse impact of industrialization and urbanization on the edible crustaceans community. When compared with the recommended value of World health Organization (WHO, 1989) in context to consumption of seafood, metal concentration in all the crustaceans showed higher value, the only exception was Sajnekhali (Station 4) for *Metapenaeus brevicornis*.

Keywords: lead, cadmium, crustaceans, inductively coupled plasma – mass spectrometer.

1. Introduction

Trace metals are introduced into the aquatic ecosystems in a number of ways. These chemicals accumulate in the tissues of aquatic organisms at concentrations many times higher than concentrations in water and may be biomagnified in the food chain to levels that cause physiological impairment at higher trophic levels and in human consumers (Raposo et al., 2009). Coastal zones can be considered as the geographic space of interaction between terrestrial and

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marine ecosystems that is of great importance for the survival of a large variety of plants, animals and marine species (Castro et al., 1999). Coastal pollution has been increasing significantly over the recent years and found expanding environmental problems in many developing countries. Urban and industrial activities in coastal areas introduce significant amount of trace metals into the marine environment, causing permanent disturbances in marine ecosystems, leading to environmental and ecological degradation and constitute a potential risk to a number of flora and fauna species, including humans, through food chains (Boran, Altınok, 2010).

There is an increasing concern regarding the roles and fates of trace metals in aquatic ecosystem of Indian Sundarbans. Much of this concern arises from the low level of available information on the concentration of these metals within the environment. Hence, it is very important to determine the concentrations of heavy metals in commercial fish and shrimps in order to evaluate the possible risk of human consumption (Cid et al., 2001).

The use of fin and shell fishes as bio-indicators of metal pollution of aquatic environments and suitability for human use from toxicological point has been documented (Amin et al., 2011). Apart from that, the sensitivity of crustaceans to heavy metals is well documented and for all these reasons, the importance of marine shrimp for environmental monitoring studies as bio-indicators of heavy metal pollution has been emphasized by several investigators (Yilmaz, Yilmaz, 2007).

The concentration of trace metals by seafood is a potential problem to man. The Indian Sundarban regions are no exception to this usual trend. The rapid industrialization and urbanization of the city of Kolkata, Howrah and the newly emerging Haldia port cum industrial complex in the maritime state of West Bengal has caused considerable ecological imbalance in the adjacent coastal zone (Mitra, 1998). The present paper aims to highlight the level of selective trace metals (Pb and Cd) in the muscle tissue of three commercially important species of shrimps, namely *Penaeus monodon, Fenneropenaeus indicus* and *Metapenaeus brevicornis* species selected for this study.

2. Materials and methods

Selection of the sampling stations

Two sampling zones were selected each in the western and central sectors of Gangetic delta at the apex of the Bay of Bengal. The western sector of the deltaic lobe receives the snowmelt water of mighty Himalayan glaciers after being regulated through several barrages on the way. The central sector on the other hand, is fully deprived from such supply due to heavy siltation and clogging of the Bidyadhari channel in the late 15th century (Chaudhuri, Choudhury, 1994). The western sector also receives wastes and effluents of complex nature from multifarious industries concentrated mainly in the upstream zone. On this background four sampling stations (two each in western and central sectors) were selected (Table 1) to analyze the concentrations of heavy metals in the muscles of common edible fishes collected during a rapid EIA study from 2nd November to 12th November, 2016 (Figure 1).

Table 1. Selected sampling stations with coordinates and salient features

Stations	Latitude	Longitude	Anthropogenic activities
Sagar island (Station 1)	21° 39' 04" N	88° 01' 47" E	Situated at the confluence of the River Hooghly and the Bay of Bengal on the western sector of Indian Sundarbans. It is noted for pilgrims in 'mahakumbh mela' every year and unplanned tourism.
Kakdwip (Station 2)	21°52′06″ N	88°11′12″E	Located in the western sector of Indian Sundarbans. Noted as a fish landing station; high anthropogenic pressure is observed on it as a result of unplanned tourism, transportation through passenger vessel jetties, extensive repairing and conditioning of boats and fishing vessels.

Gosaba (Station 3)	22° 15' 45" N	88° 39' 46" E	Located in the Matla Riverine stretch in the central sector of Indian Sundarbans. Noted for unorganized fish landing and shrimp culture.
Sajnekhali (Station 4)	22°05'13.4" N	88 ° 46'10.8" E	Located in the central sector of Indian Sundarbans. Noted for its wilderness and mangrove diversity; selected as our control zone.

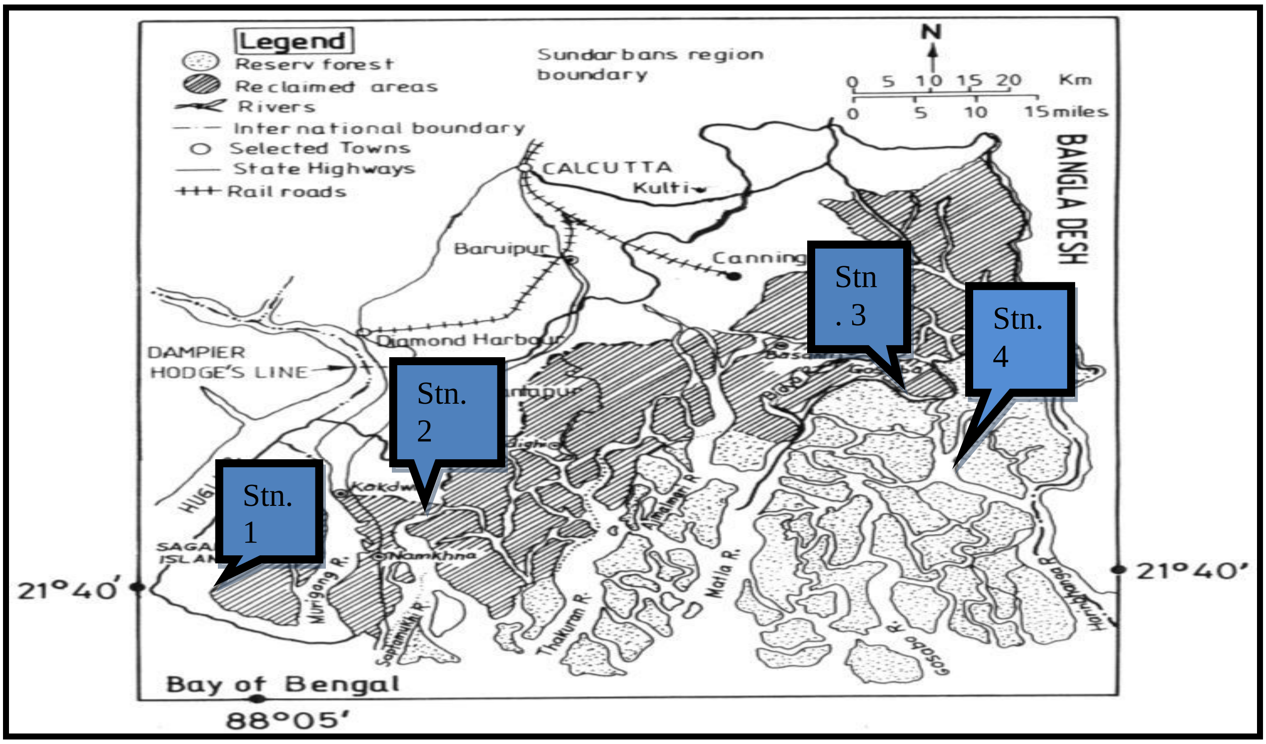

Fig. 1. Map of study area in Indian Sundarbans estuarine region

Collections of specimens

Three species of crustaceans, namely *Penaeus monodon, Fenneropenaeus indicus and Metapenaeus brevicornis* were collected during high tide condition from the selected stations were collected during low tide condition from the selected stations (Table 1). The collected samples were stored in a container, preserved in crushed ice and brought to the laboratory for further analysis. Similar sized specimens of each species were sorted out for analyzing the metal level in the muscle.

Analysis of heavy metals in muscle tissue

Inductively coupled plasma – mass spectrometry (ICP-MS) is now - a - day accepted as a fast, reliable means of multi-elemental analysis for a wide variety of sample types (Date and Gray, 1988). A Perkin-Elmer Sciex ELAN 5000 ICP mass spectrometer was used for the present analysis. A standard torch for this instrument was used with an outer argon gas flow rate of 15 L/min and an intermediate gas flow of 0.9 L/min. The applied power was 1.0 kW. The ion settings were standard settings recommended, when a conventional nebulizer/spray is used with a liquid sample uptake rate of 1.0 mL/min. A Moulinex Super Crousty microwave oven of 2450 MHz frequency magnetron and 1100 W maximum power Polytetrafluoroethylene (PTFE) reactor of 115 ml volume, 1 cm wall thickness with hermetic screw caps, were used for the digestion of the muscle samples of the shellfish. All reagents used were of high purity available and of analytical reagent grade. High purity water was obtained with a Barnstead Nanopure II water-purification system. All glasswares were soaked in 10% (v/v) nitric acid for 24 h and washed with deionised water prior to use.

The analyses were carried out on composite samples of 20 specimens of each species having uniform size. This is a measure to reduce possible variations in metal concentrations due to size and age. 20 mg composite muscle samples from 10 individuals of each species of shell fishes were weighed and successively treated with 4 ml aqua regia, 1.5 mL HF and 3 ml H2O2 in a hermetically sealed PIFE reactor, inside a microwave oven, at power levels between 330-550 W, for 12 min to obtain a clear solution. After digestion, 4 ml H2BO3 was added and kept in a hot water bath for 10 min, diluted with distilled water to make up the volume to 50 ml. Taking distilled water in place of muscle samples and following all the treatment steps described above the blank process was prepared. The final volume was made up to 50 ml. Finally, the samples and process blank solutions were analysed by ICP-MS. All analyses were done in triplicate and the results were expressed with standard deviation.

Statistical analysis

Analysis of variance (ANOVA) was performed to assess whether heavy metal concentrations varied significantly between sites. Possibilities less than 0.01 ($p < 0.01$) were considered statistically significant. All statistical calculations were performed with SPSS 14.0 for Windows.

3. Result

The accuracy and precision of our results were checked by analyzing standard reference material (SRM, DORM-2). The results indicated good agreement between the certified and the analytical values (Table 2). Number of sample, scientific name, common name and feeding habits of each sample are summarized in Table 3.

Table 2. Concentrations of metals found in Standard Reference Material DORM-2 (dogfish muscle) from the National Research Council, Canada (all data as means ± standard errors, in ppm dry wt.)

Value	Pb	Cd
Certified	0.065	0.043
SE	0.009	0.005
Observed*	0.060	0.040
SE	0.006	0.006
Recovery (%)	92.3	93.0

Table 3. Number of samples and its common name and feeding habit of studied shrimps

Species	Common name	Feeding habit	Number
Penaeus monodon	Black tiger shrimp	Crustaceans, fishes, molluscs and polychaetes	10
Fenneropenaeus indicus	Indian white shrimp	Diatoms, copepoda, ostracods, amphipods, small crustaceans, molluscan larvae, polychaetes and detritus	10
Metapenaeus brevicornis	Yellow shrimp	Vegetable matter, small crustacea, echiurid setae, large crustacea, remains of fishes, polychaeta and Sand grains	10

In this study, the concentrations of Pb in the crustacean muscle were found to range from 4.86 ppm dry wt. (in Sajnekhali) to 9.22 ppm dry wt. (in Sagar island) in case of *Penaeus monodon*. In case of *Fenneropennaeus indicus,* it ranged from 5.81 ppm dry wt. (in Sajnekhali) to 12.8 ppm dry wt. (in Sagar island) whereas, in case of *Metapennaeus brevicornis,* it ranged from 1.02 ppm dry wt. (in Sajnekhali) to 5.87 ppm dry wt. (in Sagar island) (Figure 2).

The concentrations of Pb in the crustacean muscle were found to range from 0.56 ppm dry wt. (in Sajnekhali) to 1.89 ppm dry wt. (in Sagar island) in case of *Penaeus monodon*. In case of *Fenneropennaeus indicus,* it ranged from 0.72 ppm dry wt. (in Sajnekhali) to 3.84 ppm dry wt. (in Sagar island) whereas, in case of *Metapennaeus brevicornis,* it ranged from 0.21 ppm dry wt. (in Sajnekhali) to 2.17 ppm dry wt. (in Sagar island) (Figure 3).

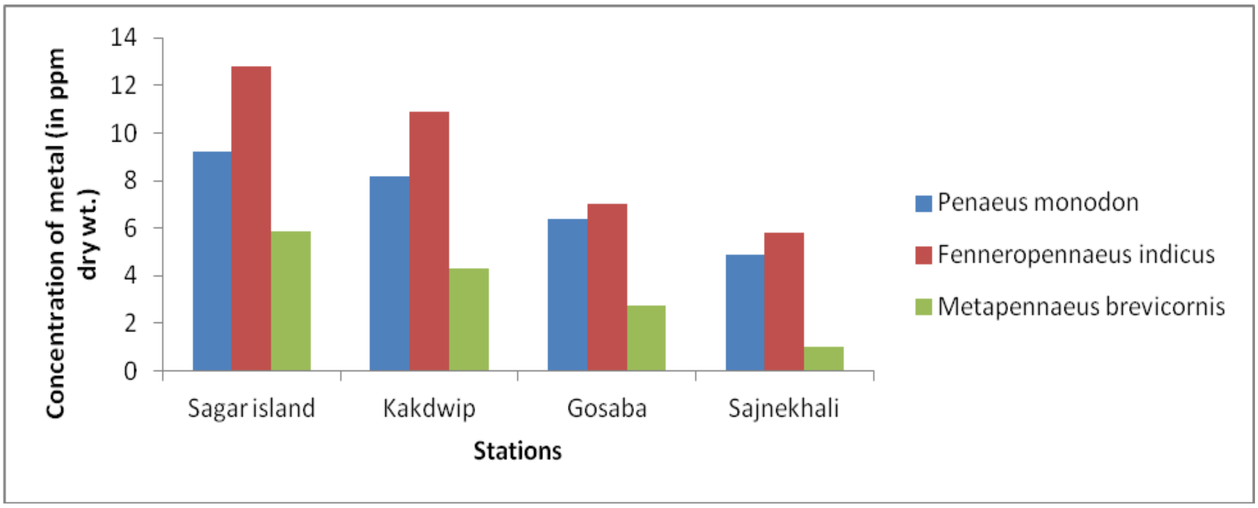

Fig. 2. Concentration of Pb in crustacean muscle in the selected stations for the study period

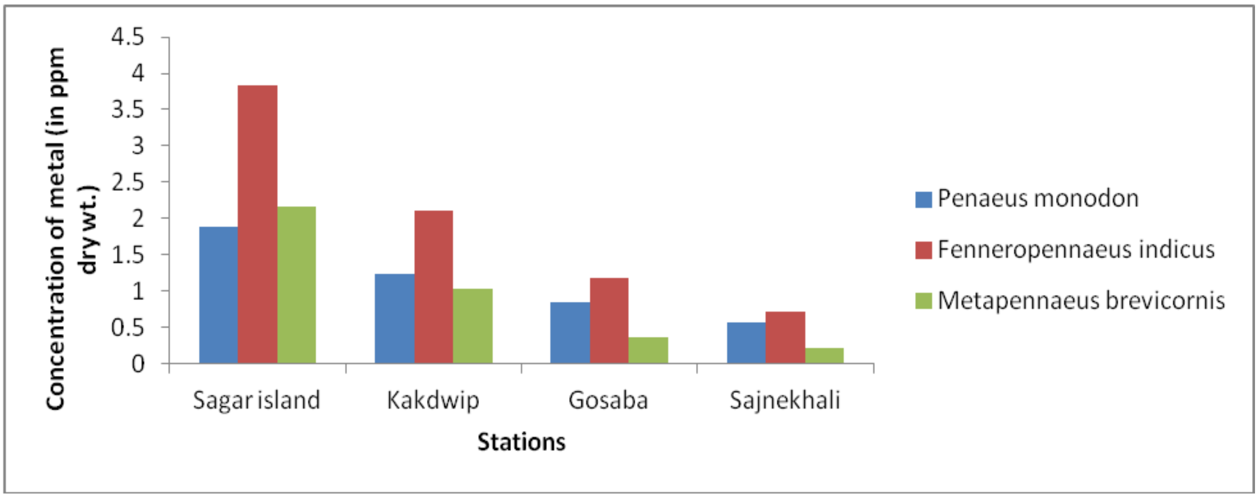

Fig. 3. Concentration of Cd in crustacean muscle in the selected stations for the study period

4. Discussion

Trace element concentrations varied markedly among species. These variations are presumably due to individual samples being of different size categories, from different ecological niches and from different trophic levels. Possibly, species also have variable metabolic requirements for specific trace element (Soegianto et al., 2008). The western part of the Gangetic delta is connected to Himalayan glacier through Bhagirathi River. Researchers pointed out that the glaciers in the Himalayan range are melting at the rate of 23 m/yr (Hasnain, 2002). This along with Farraka discharge has resulted in gradual freshening of the system, which has role in elevation of dissolved metal level in the system by way lowering of pH. The presence of chain of factories and industries along the bank of Hooghly estuary is another major cause of increased metal level in the aquatic phase of Hooghly estuary that have been reflected in the shellfish muscles of stations 1 and 2.

Pb and Cd are non-essential element for most of the living organisms. Pb is a neurotoxin that causes behavioral deficits in aquatic organisms and decreases in survival, growth rates and metabolism (Burger et al., 2002). There is often little accumulation of Pb in marine and freshwater species. Consequently lead is not a threat to fisheries resources except at extreme pollution (Clark, 1997). The most toxic of the heavy metals is Pb, which finds its way in coastal waters through the discharge of industrial waste waters, such as from painting, dyeing, battery manufacturing units and oil refineries *etc*. Antifouling paints used to prevent growth of marine organisms at the bottom of the boats and trawlers also contain lead as an important component. These paints are designed to constantly leach toxic metals into the water to kill organisms that may attach to bottom of the boats, which ultimately is transported to the sediment and aquatic compartments. When compared with the recommended value of World health Organization (WHO, 1989) in context to consumption of seafood (0.5 ppm for Pb), the concentrations in all the shrimp species in all the stations were found much higher than the prescribed limit. Cadmium is regarded as a priority pollutant because of its toxicity to organisms in the aquatic environment (Saddiq, 1992). When excess Cd is absorbed through sea food it tends to accumulate in the liver and in the kidneys of the human body (Mol et al., 2010). The main sources of Cd in the present geographical location are electroplating and an important metal with many industrial applications. In addition to this, Cd is a by-product of Zn and Pb mining and smelting (Mitra, 1998). Very small amount of Cd was recorded in the shrimp muscle from stations 4 that is located almost in industry-free zone surrounded by mangrove vegetation. For Cd, the WHO permissible limit is 0.5 ppm dry wt. for seafood (WHO, 1989). In all the stations and for the entire selected crustacean species, the concentration of Cd were found higher than the prescribed limit; the only exception was Sajnekhali (Station 4) for *Metapenaeus brevicornis*.

The findings of other studies are summarized in Table 4, and are compared with the concentrations reported in this study and elsewhere in the world. In the present study, the pattern of trace metal concentration of five shrimp species were found as order Pb> Cd. Comparing the present data with guidelines and limits (Table 5), it can be seen that most of metal concentrations found in the tissues of aquatic animals proved to be below (excluding Pb) the tolerance levels for human consumption.

Table 4. A comparison of heavy metals concentrations (ppm in dry weight) in crustaceans collected from different parts of the world

Species	Location	Pb (in ppm dry wt.)	Cd (in ppm dry wt.)	References
Fenneropennaeus indicus	Egypt	0.03-0.14	0.04-1.47	Ahdy *et al.*, 2007
Metapenaeus brevicornis	Thane basin, India	0.01-0.02	0.1-0.4	Krishnamurti and Nair, 1998
Penaeus monodon	Bangladesh	0.8-1.3	0.2-0.4	Hossain and Khan, 2001
Fenneropennaeus indicus	Thane basin, India	0.02-0.09	0.04-0.1	Krishnamurti and Nair, 1998
Penaeus monodon	Gulf of Fonseca	0.035-0.5	0.002-0.03	Carbonell *et al.* 1998
Penaeus monodon	Malaysia	BDL	0.002	Ismail et al., 1995
Metapenaeus brevicornis	Uram coast, India	0.4457-2.6529	0.001-0.231	Meshram et al., 2014
Penaeus monodon	Malad area, India	0.213	0.211	Zodape, 2013
Metapenaeus brevicornis	Malad area, India	BDL	0.110	Zodape, 2013

Penaeus monodon	Indian Sundarbans	4.86-9.22	0.56-1.89	In this study
Fenneropennaeus indicus	Indian Sundarbans	5.81-12.8	0.72-3.84	
Metapenaeus brevicornis	Indian Sundarbans	1.02-5.87	0.21-2.17	

Table 5. Maximum permitted concentration of metals in crustacean (*Penaeus monodon, Fenneropennaeus indicus* and *Metapenaeus brevicornis)* as per WHO, 1989

Trace metal	Prescribed limit (in ppm dry wt.)
Pb	0.5
Cd	0.5

Significant variations were observed ($p < 0.01$) in the trace metal concentrations between species. This may be due to variations in potentiality of species towards bioaccumulation. Variations of trace metal concentration between stations may be the effect of dynamic nature of physic-chemical variables in the study area (Table 6). Among the three crustacean species studied the trace metal accumulation followed the general order as: *Fenneropennaeus indicus* > *Penaeus monodon* > *Metapenaeus brevicornis* and among the stations selected for this study the order of metal accumulation was: Station 1 > Station 2 > Station 3 > Station 4.

Table 6. ANOVA results showing spatio-temporal variations between crustacean species and stations

Factors	Variables	F_{cal}	F_{crit}
Muscle Pb	Between species	52.39881	5.143253
	Between stations	27.83714	4.757063
Muscle Cd	Between species	70.18088	5.143253
	Between stations	16.0561	4.757063

5. Conclusion

The shrimp species *Penaeus monodon*, *Penaeus indicus* and *Metapennaeus brevicornis* are commonly available in the mangrove dominated Indian Sundarbans region, at the apex of Bay of Bengal. The knowledge of heavy metal concentrations in crustaceans are very important with respect to nature management, human consumption of these species and to determine the most useful biomonitor species and the most polluted area. Information on the distribution pattern of toxic heavy metal pollutants in aquatic environment becomes important so as to know the accumulation of such pollutants in the organisms and final transfer to man through sea foods. The International official regulatory agencies have set limits for heavy metal concentrations above which the fish is considered unsuitable for human consumption. However in the Indian sub-continent there is no safety level of heavy metals in fish and shrimp tissues. The present zone of investigation situated in and around Indian Sundarbans, a world Heritage Site, demands regular monitoring of metal status for effective management and conservation of this famous mangrove gene pool. The present study is important not only from the human health point of view, but it also presents a comparative account of heavy metals in edible shellfishes from two different sectors of Gangetic delta that are physico-chemically different. The high concentrations of heavy metals in commercially important crustaceans sampled from Nayachar island (station 1) is a cause of concern, and requires regular monitoring of water quality around the point sources present opposite to the western bank of the island, in combination with the fact that shrimp consumption is the main source of heavy metal intake in people not occupationally exposed, amplifies the need for preventive measures to safeguard public health.

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