

Trace Metals in Selected Corals of Malaysia

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Abstract: The present study was carried out to determine the concentration of mercury, manganese, zinc and chromium in coral samples from shorelines of Tioman and Labuan Island, Teluk Sepangar and Tanjung Aru using neutron activation analysis (NAA). The coral heads of *Porites* sp. and *Favia* sp. were examined. The result indicated that the average chromium concentrations in *Porites* sp. from Tioman was $0.61 \pm 0.29 \mu\text{g g}^{-1}$, Teluk Sepangar $3.40 \pm 1.54 \mu\text{g g}^{-1}$, whilst Tanjung Aru and Labuan were below detection level ($< 0.01 \mu\text{g g}^{-1}$). Average chromium concentrations in *Favia* sp. from Tioman was $2.98 \pm 0.69 \mu\text{g g}^{-1}$, whilst Tanjung Aru and Labuan were below detection limit ($< 0.01 \mu\text{g g}^{-1}$). Average zinc concentrations in *Porites* sp. from Tioman was $0.84 \pm 0.18 \mu\text{g g}^{-1}$, Tanjung Aru ($26.02 \pm 8.57 \mu\text{g g}^{-1}$), Teluk Sepangar ($8.20 \pm 2.37 \mu\text{g g}^{-1}$) and Labuan ($0.16 \pm 0.13 \mu\text{g g}^{-1}$). Average zinc concentrations in *Favia* sp. from Tioman was $2.69 \pm 0.39 \mu\text{g g}^{-1}$, whilst Tanjung Aru and Labuan Island were below detection limit ($< 0.01 \mu\text{g g}^{-1}$). Concentrations of manganese in *Favia* and *Porites* sp. collected from waters off Tioman and Labuan islands were significantly different ($P < 0.05$) from each other and similar trends were observed for concentrations of mercury in *Favia* and *Porites* sp. from waters off Tioman Island. Concentrations of mercury in coral samples from Labuan were below the detection limit of $0.01 \mu\text{g g}^{-1}$.

Key words: Metal, coral, chromium, manganese, mercury, zinc, Malaysia, NAA

Introduction

The world of coral reefs is one of earth's most dynamic ecosystems and is on par with the uniqueness of the tropical rain forests. Coral reefs are filled with aquatic life and organisms and most of these areas have been converted into marine parks areas for conservation and sustainability of natural resources. The shorelines of Malaysia is part of a triangle comprising Malaysia, Philippines and Papua New Guinea and is regarded as the largest pool of marine life in the world. Later on, the study of coral included the study of patterns found on the coral surface and a study on taxonomy by Wood (1983) and Ditlev (1980). The growth of coral reefs, which is a benthos organism, has been used as an index to determine the environmental quality of the surrounding areas (Zainuddin and Yasin, 1992). Corals are sedentary organisms which have a slow growth rate and they experience all sorts of change in the environment which are reflected in their growth bands. The concentrations of metal in an aragonite (calcium carbonate) skeleton of a coral and sediment nearby, the coral can reflect the level of metals in the water (Howard and Brown, 1984; Shen and Boyle, 1988). The ability of corals to absorb metal allows studies that could be associated with pollution at a certain time period. Metals absorbed by corals will remain embedded forever because new growth will cover the old carbonate surface (St. John, 1974). According to Hanna and Muir (1990), corals from the Red Sea were found to be good bio-indicators for trace metals. Determination of heavy metals in corals is relatively quite new in Malaysia when compared to some countries like USA and Australia. Pollutants in coral studies were started in Sabah waters in 1994 (Mokhtar et al., 1994) and in waters of Peninsular Malaysia in 1996 (Mokhtar et al., 1997a, 1997b).

The objectives of this study were to measure content of the selected metals in coral samples collected from water of Tioman and Labuan Island, Tanjung Aru and Teluk Sepangar and to compare concentrations of same metals in different species of coral, at a particular location of sampling.

Materials and Methods

The coral samples of *Porites* and *Favia* sp., two of the more commonly found species in Malaysian waters were collected between February and June of 1999 from the waters of Tioman island, situated east of the shoreline of Johor, Peninsular Malaysia at $002^{\circ} 55' \text{N}$ and $104^{\circ} 07' \text{E}$; Labuan Island, one of Malaysia's marine parks and situated at coordinates $005^{\circ} 14' \text{N}$ and $115^{\circ} 11' \text{E}$; Tanjung Aru situated at $5^{\circ} 55' \text{N}$, $116^{\circ} 03' \text{E}$ and Teluk Sepangar situated at $6^{\circ} 04' \text{N}$ and $116^{\circ} 05' \text{E}$. Tanjung Aru and Teluk Sepangar is respectively 6 and 14 km away from Kota Kinabalu by road. Teluk Sepangar waters is plied by ships and tankers. Tanjung Aru is a tourist beach area. Labuan Island has a population of 60,000 people and a port known as Victoria Port. Two abandoned oil ridges are located about 3 Km away.

Sampling: Small coral heads measuring about 20 cm in diameter were sampled by Scuba divers by dislocating them off their substrates. Identification of coral was based on its calice morphology. The coral heads were put into sample nets and carried up to the surface where they were kept in cooled boxes during transportation to the laboratories.

Sample preparation: All apparatus were cleaned and then soaked in 10% HNO_3 for overnight. They were rinsed with distilled water, then dried in the oven at 120°C for 1 h to dry. Coral samples were soaked in a mixture of sodium hypochlorous solution and distilled water with a ratio of 1:4 (Guzman and Jimenez, 1992) to remove gelatine as well as organisms or organic materials from the coral surface. After soaking for 24 h, samples were dried in the oven at a temperature of 105°C for 48 h. Dried coral samples were sliced vertically into slabs with a thickness of about 1 cm. Cutting was carried out by using a rock saw at the Geological Laboratory of UKM. Sliced samples of coral were then cleaned using the method of Shen and Boyle (1988). Annual bands on the sample were traced and sketched under fluorescent lighting. Bands were assumed to represent one year (Barnes and Lough, 1992). Every band was separated by using a chisel, followed by secondary

cleaning and then pulverised. Cleaning of the coral was done using a modified method of Shen and Bolye (1988). This cleaning process involved pre and secondary cleaning stages. Pre cleaning removed contaminants from the sample surface. Samples were cleaned twice using distilled water followed by 0.15 N HNO₃ twice. During secondary cleaning every band was separated using chisel, hammer and then crushed into smaller pieces of 5 mm using a pounder. Samples were then washed with distilled water for 10 min, 0.5 N HNO₃ for 3 min, washed again with a mixture of oxidizing agent, 30% H₂O₂ and 0.2 N NaOH (1:1) on a water bath for 5 min and cleaned with 0.15 N HNO₃ for 3 min until the colour of the sample was bleached before being dried in the oven at 105°C for 24 h. Secondary washing was done to remove surface contaminants before grinding. Dried coral samples were brought to MINT for pulverization. The method used to determine the amount of heavy metals in these two species was neutron activation analysis (NAA). Heavy metals analyzed were chromium (Cr), manganese (Mn), mercury (Hg) and zinc (Zn).

Neutron activation analysis (NAA): Crushed homogenized sample and 0.2 g of reference material were weighed and placed in polythene containers. Reference material for mercury, manganese, chromium and zinc were prepared and introduced into a polythene container, then dried in an oven at 50°C for 24 h. Dried coral samples and reference material in polythene containers were sealed tightly. Samples were placed in a reactor TRIGA MK II with a power of 750 kW and radiated with thermal neutron flux of 4X10¹² neutron cm⁻²s⁻¹ for 6 h. After radiation, samples were removed from the reactor and left to cool for 3 weeks. A high resolution gamma spectrometer was used to measure radionuclide activity. Counting took 3000 to 3600 s after three weeks of cooling off. Concentration of metals were determined from these measurements. The gamma lines for quantitative determination of the elements using NAA comparative to standard techniques were as follows: 279 keV of ²⁰³Hg, 1811 keV of ⁵⁶Mn, 1115 keV of ⁶⁵Zn and 320 keV of ⁵¹Cr. Correction of 279 keV gamma line of ²⁰³Hg is necessary in samples containing selenium due to contribution from 279 keV gamma line of ⁷⁵Se. The gamma spectra consisting of the photo peaks of interest were analyzed using an *uS AMPO* programme purchased from Canberra, Australia.

Concentrations of mercury, manganese, chromium and zinc in a sample were determined from the following equation:

$$C_x = (A_x + C_s) / A_s \text{ ----- Equation (1)}$$

Where, C_x is the Concentration of mercury, manganese, chromium or zinc in a sample (µg g⁻¹), A_x is the Radionuclide activity of mercury, manganese, chromium or zinc in a sample, A_s is the radionuclide activity in mercury, manganese, chromium or zinc in a reference material and C_s is the concentration of mercury, manganese, chromium or zinc in a reference material (µg g⁻¹) quality assurance of data was carried out using standard reference material (SRM) of Soil-7 (IAEA) and Lake Sediment SL-1 (IAEA). No coral SRMS were obtainable. According to Yam (1999), Soil-7 was the most suitable and commonly used reference material in coral analysis, even though it is a lake sediment sample. Analysis of variance (ANOVA) were carried out on results of this study to determine if there is a significant difference between two values. T-tests were carried out to determine significant differences between two values only at a time. ANOVA and t-test tested at 95% accuracy level using Microsoft software of office Excel were used on data of reference material.

Results and Discussion

NAA analysis were also performed on reference materials Soil-7 and SL-1. Neutron activation analyses of this study had been able to detect concentrations of Zn, Cr, Mn and mercury with good degree of accuracy and precision. This was based on Z-scores

which showed values of less than 2 for both the reference materials of Soil-7 and SL-1 (Table 1). This shows that the data obtained in this study are accurate and precise.

The yearly bands on each coral head samples were sketched under fluorescence lighting. Each sample of coral was marked with numericals where number one represents the outermost (youngest) band.

The concentrations of Hg, Mn, Cr and Zn in coral samples from water of Tanjung Aru were determined (Table 3).

Correction for selenium disturbance: In NAA analysis technique, measurement of peak strength is used to determine the content of metals in the sample. The peak strength for mercury (Hg) is 279 keV with a half life of 46.9 days and for selenium (Se) a peak strength of 265 keV with a half life of 75 days. In some samples, disturbance occurred where the selenium peak overlapped with the mercury peak. Corrections were made on samples of this study so as to get accurate results for mercury (Glascock, 1985). According to Glascock (1985) the overflow of selenium at the 265 keV energy peak is 58.6% and at the 279keV peak 24.7%. This means, the energy peak of 279 keV is the result of the existence both mercury and selenium.

Concentration of chromium, zinc, manganese and mercury in coral samples: Each yearly band is marked with a number that is considered to represent a certain period of time. An assumption is made that coral species takes up metals at one time into each band.

Tioman Island: In this study, the highest reading for zinc was 3.04 µg g⁻¹ and was found in the third band of *Favia* sp. The lowest reading was 0.62 µg g⁻¹ in the second band of *Porites* sp. (Table 2). The highest Cr reading was 3.88 µg g⁻¹ in the fifth band of *Favia* sp. and the lowest reading of 0.32 µg g⁻¹ in the fourth band of *Porites* sp. There were significant differences in terms of zinc and chromium concentrations between bands in *Favia* sp., but no significant differences (P<0.05) between bands in *Porites* sp. These might be caused by different diet of corals, which consisted zooplanktons, organic material and particles (Lasker, 1980), skeletal structure of each species (Howard and Brown, 1984), shape of colony morphology and polyps, eating habits and physiological mechanisms (St. John, 1974). T-test results did not show significant differences between chromium and zinc in *Porites* and *Favia* sp. And then mean concentration of manganese from Tioman Island was 1.41 and 0.53 µg g⁻¹, respectively. According to ANOVA, concentrations of manganese in each band were significantly different from each other in both samples of *Favia* and *Porites* sp. This shows that the amount of manganese absorbed by corals are affected by the surroundings (Howard and

Table 1: Concentrations of mercury, manganese, zinc and chromium in the reference materials and comparison with the certified values

Metals	Soil -7	SL-1
Mercury		
Certified concentration (µg g ⁻¹)	0.04 ± 0.00	0.13 ± 0.00
Concentration from this study (µg g ⁻¹)	0.05 ± 0.03	0.49 ± 0.35
Z-score	0.30	0.22
Manganese		
Certified concentration (µg g ⁻¹)	631 ± 20	3460 ± 160
Concentration this study (µg g ⁻¹)	683 ± 64	3288 ± 87
Z-score	0.70	0.90
Zinc		
Certified concentration (µg g ⁻¹)	104 ± 5	223 ± 10
Concentration this study (µg g ⁻¹)	105 ± 4	220 ± 11
Z-score	0.16	0.22
Chromium		
Certified concentration (µg g ⁻¹)	60 ± 11	104 ± 9
Concentration this study (µg g ⁻¹)	69 ± 2	111 ± 9
Z-score	0.80	0.56

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Table 2: Concentrations of mercury, manganese, chromium and in the coral samples from marine waters off Tioman Island

Metals	Species	Bands	Average ($\mu\text{g g}^{-1}$)	Range ($\mu\text{g g}^{-1}$)
Mercury	<i>Porites</i>	5	0.02±0.01	0.01-0.03
		4	0.03±0.02	0.01-0.04
		3	0.15±0.09	0.06-0.24
		2	0.06±0.01	0.05-0.07
		1	0.08±0.02	0.06-0.10
	<i>Favia</i>	5	0.18±0.11	0.14-0.24
		4	0.28±0.11	0.06-0.08
		3	0.04±0.01	0.03-0.05
		2	2.36±0.10	0.06-0.08
		1	2.23±0.21	0.14-0.24
Manganese	<i>Porites</i>	5	0.46±0.01	0.45-0.47
		4	0.88±0.16	0.72-1.04
		3	0.53±0.05	0.48-0.58
		2	0.13±0.01	0.12-0.14
		1	0.65±0.12	0.53-0.77
	<i>Favia</i>	5	0.74±0.07	0.67-0.81
		4	1.69±0.05	1.64-1.74
		3	1.52±0.07	1.45-1.59
		2	1.41±0.08	1.33-1.49
		1	1.71±0.23	1.48-1.94
Chromium	<i>Porites</i>	5	1.06±0.28	0.86-1.26
		4	0.32±0.10	0.25-0.39
		3	0.46±0.15	0.35-0.56
		2	0.51±0.04	0.48-0.53
		1	0.69±0.23	0.53-0.85
	<i>Favia</i>	5	3.88±0.06	3.83-3.92
		4	3.11±0.11	3.19-3.23
		3	3.31±0.04	3.26-3.36
		2	2.36±0.10	2.29-2.43
		1	2.23±0.21	2.08-2.37
Zinc	<i>Porites</i>	5	0.85±0.18	0.72-0.97
		4	1.05±0.16	0.96-1.20
		3	0.96±0.18	0.77-1.03
		2	0.62±0.05	0.58-0.68
		1	0.71±0.12	0.62-0.79
	<i>Favia</i>	5	2.86±0.01	2.85-2.87
		4	2.86±0.15	2.75-2.96
		3	3.04±0.08	2.98-3.10
		2	2.66±0.07	2.61-2.71
		1	2.04±0.11	1.96-2.12

Detection limit for NAA method is 0.005 $\mu\text{g g}^{-1}$
 Band 1: The outermost band (most recent)

Brown, 1984). Relatively, the average concentrations of manganese in *Favia* sp. were higher than those in *Porites* sp. T-tests showed significant differences ($P < 0.05$) between *Favia* sp. and *Porites* sp. Average concentrations of mercury in coral samples from Tioman Island were 0.15 and 0.07 $\mu\text{g g}^{-1}$ for *Favia* and *Porites* sp., respectively.

Tanjung Aru, Kota Kinabalu: Concentrations of zinc in *Favia* sp. and concentrations of chromium in *Porites* and *Favia* sp. were below the detection limit of 0.01 $\mu\text{g g}^{-1}$ (Table 3). Concentrations of chromium in coastal waters of Sabah were below detection limit (Anonymous, 1998). According to Hanna and Muir (1990), concentration of chromium in coral was also low because the composition of the coral skeletal and tissues were correlated linearly with changes in sea water concentration which includes the contents of heavy metals. Concentrations of zinc in the different bands ranged from 15.64-45.64 $\mu\text{g g}^{-1}$. Concentrations of zinc at Tanjung Aru were caused by leaching from barren developed earth and wastes from industrial activities around Kota Kinabalu. The fourth band which was apparently equivalent to the year 1996, had the highest concentration of zinc, followed by a decline in this value in the third band of 1997. Coral samples at Tanjung Aru had an average manganese concentration of 5.07±0.56 $\mu\text{g g}^{-1}$ in *Favia* sp. and 2.73±0.15 $\mu\text{g g}^{-1}$ in *Porites* sp. Concentrations of manganese in corals were hard to determine because the level of manganese can change drastically even within a short distance (Brown and Holley, 1982).

Table 3: Concentrations of chromium and zinc in the coral samples from water of Tanjung Aru

Metals	Species	Bands	Average ($\mu\text{g g}^{-1}$)	Range ($\mu\text{g g}^{-1}$)
Zinc	<i>Porites</i> sp.	9	25.11±0.17	24.99-25.23
		8	30.83±0.38	30.56-31.10
		7	15.64±1.49	14.58-16.70
		6	19.32±0.50	18.97-19.68
		5	22.34±1.15	21.53-23.15
		4	45.64±0.93	44.98-46.30
		3	22.51±0.65	22.05-22.96
		2	26.13±0.94	25.46-26.74
		1	26.64±1.07	27.89-29.40
			Average	26.0
	<i>Favia</i> sp.	9	< LD	< LD
		8	< LD	< LD
		7	< LD	< LD
		6	< LD	< LD
		5	< LD	< LD
Chromium	<i>Porites</i> sp.	9	< LD	< LD
		8	< LD	< LD
		7	< LD	< LD
		6	< LD	< LD
		5	< LD	< LD
	<i>Favia</i> sp.	9	< LD	< LD
		8	< LD	< LD
		7	< LD	< LD
		6	< LD	< LD
		5	< LD	< LD

< LD: Limit of detection (LD) for the NAA in this study was 0.01 $\mu\text{g g}^{-1}$
 < LD : Below the limit of detection
 Band 1: The outermost (most recent) band

Table 4: Concentrations of zinc and chromium in the coral samples from Teluk Sepangar

Species	Metals	Bands	Average ($\mu\text{g g}^{-1}$)	Range ($\mu\text{g g}^{-1}$)
<i>Porites</i> sp.	Zinc	4	6.40±0.23	6.24-6.56
		3	6.40±0.11	6.32-6.48
		2	8.62±10.16	8.51-8.73
		1	11.40±0.11	11.32-11.47
			Average	8.20±2.37
	Chromium	4	5.31±0.25	5.13-5.49
		3	2.73±0.16	2.62-2.85
		2	3.83±0.13	3.74-3.92
		1	1.73±0.17	1.61-1.85
		Average	3.40±1.54	

Only *Porites* sp. was sampled from Teluk Sepangar
 Band 1: The outermost (most recent) band

Teluk Sepangar: Only *Porites* sp. was sampled from water of Teluk Sepangar. The concentration of zinc was relatively high compared to chromium (Table 4). Teluk Sepangar is situated near the traveling route of large ships and the concentrations of zinc might be contributed by oil discharge from these and from runoffs of nearby on-shore industrial area.

Labuan Island: Concentrations of zinc in *Porites* sp. reached 1.32 $\mu\text{g g}^{-1}$ in the fourth band and recorded the lowest value of 0.97

Table 5: Concentrations of metals in the coral samples from water of Labuan Island

Metals	Species	Band	Average ($\mu\text{g g}^{-1}$)	Range ($\mu\text{g g}^{-1}$)	
Zinc	<i>Porites</i>	5	1.22±0.17	1.10-1.34	
		4	1.32±0.02	1.31-1.34	
		3	1.17±0.03	1.11-1.19	
		2	0.97±0.09	0.91-1.01	
		1	1.10±0.05	1.07-1.13	
			Average 1.16±0.13		
	<i>Favia</i>	5	< LD	< LD	
		4	< LD	< LD	
		3	< LD	< LD	
		2	< LD	< LD	
		1	< LD	< LD	
	Chromium	<i>Porites</i>	5	< LD	< LD
			4	< LD	< LD
			3	< LD	< LD
2			< LD	< LD	
1			< LD	< LD	
<i>Favia</i>		5	< LD	< LD	
		4	< LD	< LD	
		3	< LD	< LD	
		2	< LD	< LD	
		1	< LD	< LD	
			Average < LD		
Manganese		<i>Porites</i>	6	9.47±0.29	9.18-9.76
			5	9.33±0.11	9.22-9.44
			4	11.95±0.30	11.65-12.25
	3		6.53±0.13	6.40-6.66	
	2		5.06±0.05	5.01-5.11	
	<i>Favia</i>	5	7.05±0.38	6.67-7.43	
		4	6.97±0.13	6.84-7.10	
		3	4.48±0.18	4.30-4.66	
		2	4.14±0.12	4.02-4.26	
		1	3.35±0.18	3.37-3.73	
	Mercury	<i>Porites</i>	5	< LD	< LD
			4	< LD	< LD
			3	< LD	< LD
			2	< LD	< LD
1			< LD	< LD	
<i>Favis</i>		5	< LD	< LD	
		4	< LD	< LD	
		3	< LD	< LD	
		2	< LD	< LD	
		1	< LD	< LD	

< LD Limit of detection (LD) for the NAA in this study was $0.01 \mu\text{g g}^{-1}$

< LD : Below the limit of detection

Band 1: The outermost (most recent) band

$\mu\text{g g}^{-1}$ in the second band (Table 5). Labuan Island has of a large port know as Victoria Port, one of the busiestport in East Malaysia. Concentrations of zinc might be due to the zinc content found in oil, brought in by currents and waves around this island. Concentration of zinc in *Favia* sp. and chromium in *Porites* and *Favia* sp. was below detection limit.

Concentration of mercury in corals from waters of Labuan Island and Tanjung Aru: No readings was obtained from both sampling locations in Labuan Island and Tanjung Aru waters and it was expected that the intake of mercury by sample would also be minimal. This is in line with the Environmental Quality Data which shows that the concentration of manganese in the marine water of Sabah was extremely low.

Comparison of zinc concentrations between locations: Similar trends for all four locations and all four yearly bands of the coral were obtained. Tanjung Aru recorded the highest concentration zinc followed by Teluk Sepagar, Labuan and Tioman Island, in that decreasing order. Tanjung Aru and Teluk Sepangar are situated at the shorelines of city of Kota Kinabalu where pollutants originated from areas of factories and development projects. Tioman Island had the lowest concentration of zinc in all four growth bands, due to the fact that it is one of the 21 islands declared as a marine

park. Environmental protection laws at marine parks is much more stringent compared to other places.

Comparison of manganese concentrations in *Favia* sp. from three locations: Average concentrations of manganese in *Favia* sp. obtained from Labuan Island, Tanjung Aru and Tioman Island were 5.24 ± 0.21 , 5.07 ± 0.56 and $1.41 \pm 0.10 \mu\text{g g}^{-1}$, respectively. Relatively, mean concentration of manganese in *Favia* sp. from Labuan Island was the highest of all three locations. T-test analysis showed that the concentration of manganese in all three location varied significantly. This might be contributed to higher anthropogenic activities on-going at the shorelines of Labuan Island in comparison to Tanjung Aru and Tioman Island.

Comparison of manganese concentrations in *Porites* sp. from the sampling locations: Average concentrations of manganese in *Porites* sp. obtained from Labuan Island, Tanjung Aru and Tioman Island were 7.52 ± 0.17 , 2.73 ± 0.15 and $0.53 \pm 0.07 \mu\text{g g}^{-1}$, respectively. Relatively, average concentrations of manganese in *Porites* sp. from Labuan Island also has the highest readings compared to the other two study locations. T-test analysis showed significant difference in term of manganese concentration in *Porites* sp. samples in all three study locations. Generally, the content of manganese analyzed in both *Favia* and *Porites* sp. from Labuan Island showed the highest level in comparison with the other three sampling location. This might be because Labuan Island is a town with anthropogenic activities which is relatively high compared to the other two sampling locations.

It is concluded that concentrations of Cr, Hg, Mn and Zn concentrations in selected Malaysian coral samples using neutron activation analysis (NAA). The concentrations of these metals in coral were believed to be representative of non-polluted ranges. Levels of concentration of the metals studied here were found to be relatively highest in water of Labuan believed to be due to anthropogenic activities near Labuan shipyards and ports. This study showed that *Porites* sp. is suitable for use as metal pollution historical recorders in Malaysian waters.

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