Accumulation pattern of heavy metals in marine organisms collected from a coal burning power plant area of Malacca Strait

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ABSTRACT: Nowadays, the marine environment is becoming vulnerable because of anthropogenic pollutants such as heavy metals carried by small particles of fly ash generated by coal burning power plants. Toxic heavy metals such as Cd, Cu, Zn, Pb, and Cr were estimated in three types of marine organisms, i.e., Arius maculatus, Penaeus merguiensis, and Anadara granosa, collected from a coal burning power plant area of Malaysia. An independent-sample t-test was conducted to compare the metal concentration in analysed species where significant differences were observed between A. maculatus, P. merguiensis, and A. granosa in the case of Cd, Cu, and Cr concentrations. Moreover, there were statistically significant difference between A. maculatus and A. granosa, as well as between P. merguiensis and A. granosa, in the case of Zn concentration. Similarly, A. maculatus and P. merguiensis, and A. maculatus and P. merguiensis demonstrated significant differences in the case of Pb concentration. Nevertheless, most of the species examined during this study exhibited concentrations that were lower than the permitted guideline. The hierarchical cluster analysis revealed two groups of analysed species where the first group included fish (A. maculatus) and shrimp (P. merguiensis), whilst the second group consisted of cockles (A. granosa). The calculated values of biota-sediment accumulation factor were 0.79, 0.22, 0.59, 0.07, and 0.06 for A. maculatus; 0.62, 2.31, 0.64, 0.05, and 0,04 for P. merguiensis; 5.10, 0.66, 0.79, 0.05, and 0.10 for P. merguiensis in the case of Cd, Cu, Zn, Pb, and Cr, respectively. The patterns of biota sediment accumulation factor (BSAF) were Cd > Zn > Cu > Pb > Cr for A. maculatus, Cu > Zn > Cd > Pb > Cr for P. merguiensis, and Cd > Zn > Cu > Cr > Pb for A. granosa. However, the BSAFs revealed a higher accumulation ability of heavy metals in A. granosa. Therefore, this species can be used as a bio-indicator of marine pollution.

KEYWORDS: BSAF, cadmium, copper, zinc, lead, chromium

INTRODUCTION

Increasing trends of human population and coastal development contribute to the increase in anthropogenic pollution load, which has become a major threat to marine and aquatic habitats. Large amounts of heavy metals can be deposited in the aquatic system near the coal burning power plant area, as the smallest particles of fly ash are enriched with heavy metals¹ and this holds ecological importance because of their toxicity, persistence, and bio-accumulation. As a result, there has been a growing interest in determining the heavy metal levels in marine environment and attention has been drawn to the measurement of contamination levels in public food supplies, particularly fish^{2–4}. Elevated amounts of metal concentrations in the southern areas and near power stations indicate the impact of coal-fired power stations on the lake. Aquatic organisms inhabiting sites polluted by coal ash are at risk because they accumulate extremely high concentrations of teratogenic trace elements, such as Cd, Cu, and Se, in their tissues. Previous studies suggested that coal combustion byproducts can disrupt the biology of amphibians⁵.

Additionally, heavy metal concentrations in aquatic organisms along with bio-concentration have been extensively studied in various places around the world^{6–12}. Attention has been given to the organism ability to reflect the environmental levels of trace metal contaminants in marine and estuarine ecosystems¹³. Since marine organisms accumulate contaminants from the environment, they have been used in marine pollution monitoring programmes. Heavy metal studies in aquatic biota indicate that

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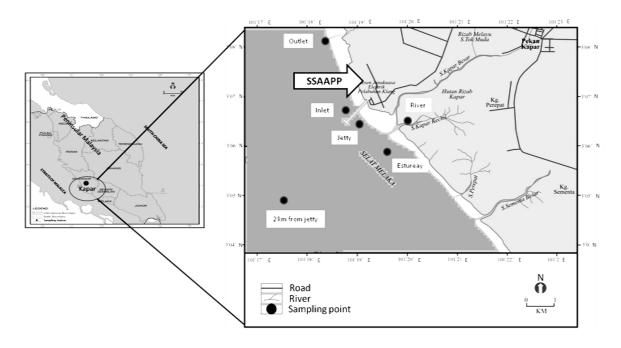


Fig. 1 Sampling locations at Kapar coastal area near the Sultan Salahuddin Abdul Aziz power plant.

heavy metals in aquatic organisms could be a more reliable water-quality indicator than chemical analysis of water column and sediment^{7,14}. Bivalve molluscs, which are sedimentary, widespread, and have a long life span, have been well established as a bio-indicator for monitoring the concentration of heavy metals in many areas of the world. Similarly, fish is a good bioindicator because it is easy to be obtained in a large quantity, has a potential to accumulate metals, has a long lifespan, has an optimum size for analysis, and is easy to be sampled¹⁵. Studies regarding the heavy metal accumulation in crustaceans are scant.

As there is very little information regarding heavy metal behaviour around the coal burning power plant areas, particularly with respect to accumulation in marine organisms, this study aims to determine the concentrations of toxic heavy metals (Cd, Cu, Zn, Pb, and Cr) in the edible portion of fish, crustaceans, and molluscs, collected from the local fish market of Kapar coastal area of Malaysia, which is adjacent to a coal burning power station.

MATERIALS AND METHODS

Samplings were carried out around the Sultan Salahuddin Abdul Aziz power station, which is located at the western coast line of west Malaysia, at the Malacca Strait. It is the largest power station in Malaysia with a generating capacity of 2420 MW contributing about 23% of the country energy demand. Sediment samples were collected from six stations along the coastal area (Fig. 1) and organism samples were purchased from the local fish market of the village Tok Muda in August 2008, December 2008, and February 2009. A total of 15 samples for each organism were collected during every sampling period and the catch locations were verified with the fishermen.

The organism and sediment samples were transported to the laboratory and preserved in a freezer for further analysis. The organism samples were dissected to obtain the edible parts (muscle). The organisms and sediment samples were oven dried at 60 °C. After drying, the sediment sample was ground using mortars (Gelman No. 4012). Then, the sediment samples in the form of powder were sieved using a 200 µm diameter sized sieve (Retsch). Three replicates of organisms and sediment samples were analysed to measure heavy metals such as Cu, Cd, Zn, Pb, and Cr. All the glasswares used for analysis were acid washed to avoid possible contamination. About 0.3-0.5 g of dried samples for each replica were weighted in a beaker using electronic scales. The samples were then digested with a mixture of 30 ml nitric acid (HNO₃, GR, 65%, Merck) and 5 ml of concentrated perchloric acid (HClO₄, GF, 70%, Merck). After that, 10 ml of concentrated hydrochloric acid (HCl, GR, 37%, Merck) was added to the samples and heated until dry. After cooling

Elements	SRM	Analysis					Certified	Mean re-
		Rep-1	Rep-2	Rep-3	Rep-4	Mean	value	covery (%)
Cd	IAEA 407	0.15	0.15	0.18	0.14	0.16 ± 0.02	0.19	82.34
	NIST 1646a	0.10	0.11	0.17	0.12	0.12 ± 0.03	0.15	83.03
Cu	IAEA 407	2.91	2.82	3.06	2.88	2.92 ± 0.10	3.28	88.89
	NIST 1646a	7.34	7.27	8.56	7.54	7.68 ± 0.60	10.01	76.69
Zn	IAEA 407	54.35	59.48	57.83	50.48	55.5 ± 4.0	67.10	82.77
	NIST 1646a	34.24	35.43	57.21	37.95	41 ± 11	48.90	84.27
Pb	IAEA 407	0.11	0.11	0.11	0.09	0.10 ± 0.01	0.12	85.75
	NIST 1646a	8.82	8.67	11.11	8.85	9.4 ± 1.2	11.70	80.02
Cr	IAEA 407	0.67	0.62	0.58	0.50	0.59 ± 0.07	0.73	81.29
	NIST 1646a	33.31	33.86	33.65	33.29	33.53 ± 0.27	40.90	81.98

Table 1 Analytical result for the reference materials IAEA 407 (fish) and NIST 1646a (estuarine sediment) along with the certified value for each metal ($\mu g/g$ dry weight of fish).

the sample, 2.5 ml of HNO₃ was added into the samples. A total of 20 ml of de-ionized distilled water was added into the beaker containing the sample and filtered through a filter paper (Whatman, GF/C; diameter 47 mm; pore size 0.45 µm). Subsequently, the filtered solutions were added with de-ionized distilled water up until 70 ml to make it into 0.5 M HNO₃. Determination of heavy metals was carried out using the inductively coupled plasma mass spectrometry (Perkin Elmer-Elan 9000). In order to confirm the validation of the method, SRM-407 provided by the International Atomic Energy Agency, was used for fish samples and SRM-1646a for estuarine sediment supplied by the National Institute of Standards and Technology was used for sediment analysis. The analytical results for the investigated heavy metals in the reference material were within or near the certified values (Table 1). Therefore, the recoveries of all the metals were satisfactory. Independent samples t-test was performed using the analytical software SPSS to compare the accumulation pattern of heavy metal in marine organisms. The following equation was used to calculate the biota-sediment accumulation factor¹⁶:

$$BSAF = C_{org} (dry weight) / C_{sed} (dry weight),$$

where $C_{\rm org}$ is the concentration of heavy metal in organism and $C_{\rm sed}$ is the concentration of heavy metal in sediment.

RESULTS AND DISCUSSION

The concentrations of analysed toxic metals in marine organisms during different sampling periods are presented in Fig. 2. An independent *t*-test was conducted to compare the concentration of cadmium in *Arius maculatus* and *Penaeus merguiensis* where a significant difference was observed (t(88) = 2.01,

p = 0.04). Similarly, significant differences were discovered in the case of A. maculatus and Anadara granosa (t(45) = 11.38, p = 0.00), as well as P. merguiensis and A. granosa (t(46) = 11.80, p =0.00). However, A. granosa $(1.48 \pm 0.55 \,\mu\text{g/g} \,\text{dry wt})$ had the highest concentration of cadmium, followed by A. maculatus ($0.23 \pm 0.09 \,\mu\text{g/g}$ dry wt) and P. merguiensis (0.18 \pm 0.11 µg/g dry wt). Phillips¹⁷ also reported a higher amount of cadmium in molluscs. None of the species analysed in this study were found to contain cadmium concentration above the proposed permitted concentration and the values were within the range of other reported values^{12, 17–43} (Table 2). A high concentration of cadmium causes several health problems to humans. Cadmium and its compound along with mercury and some other dangerous metals are included in the blacklist. Ingestion of cadmium produces shock acute renal failure when the amount exceeds 350 mg⁴⁴.

Mean copper concentration was highest in P. merguiensis (21.6 \pm 7.8 µg/g dry wt), followed by A. granosa (6.1 \pm 4.2 µg/g dry wt) and A. maculatus $(2.01 \pm 0.57 \text{ }\mu\text{g/g} \text{ dry wt})$. There were significant differences between A. maculatus and P. merguiensis (t(44.35) = 12.51, p = 0.00), A. maculatus and A. granosa (t(46.49) = 6.88, p = 0.00), and P. merguiensis and A. granosa (t(56.23) = 9.27), p = 0.00). Barwick and Maher⁴⁵ observed comparatively lower copper concentration in fish than the crustaceans and molluscs which support the present study. Copper concentrations measured in organisms are compared with the reported values of other places and the guideline (Table 2). The calculated values are within the range of previous studies and lower than the guidelines. The higher copper concentration found in P. merguiensis and A. granosa probably reflects active accumulation of copper by these species

Species	Standard/Place	Cd	Cu	Zn	Pb	Cr	Reference
Fish	USEPA limits	2	120	120^{*}	4	8	Refs. 18, 19 [*]
	WHO	1	30	100	2	50	Ref. 20
	Food standard of Malaysia	1	30	100	2		Ref. 21
	Caspian Sea	0.0032	1.65	20.656	0.0144	0.35	Ref. 22
	China	0.01 - 0.04	0.06-0.16	2.39-4.49			Ref. 23
	Bahrain	0.03			0.13		Ref. 24
	Yugoslovakia	0.01 - 0.84			0.02 - 1.7		Ref. 25
	Barent Sea	< 0.01	0.6	5.6-7.8	< 0.1		Ref. 26
	Mumbai, India	0.02	0.31	8.36	0.08	0.78	Ref. 27
	Afyon, Turkey	0.01	0.33-0.6	6.73–10.74	0.02		Ref. 28
	China	0.004-0.021	0.228-1.89	16-130	0.177-0.289		Ref. 29
	Pahang, Malaysia	0.15-0.47	0.13-0.77	1.69-6.76	0.00-1.14		Ref. 30
	Peninsular Malaysia	2.4	3.8	58.4			Ref. 31
	Langkawee, Malaysia	0.9	0.01	49.39	1.1		Ref. 32
	India	0.17	3.27	8.74	0.37	0.68	Ref. 12
	Malaysia	0.14	1.21	41.84	1.50	0.55	Present study
Crustacean	WHO	2	10	1000	2		Ref. 32
	WHO	0.2	10	1000			Ref. 22
	India	0.095	8.19	17.76	0.50	0.61	Ref. 12
	Ghana		4.81	15.7	0.29		Ref. 33
	Senegal	0.1	4.68	13.9	0.5		Ref. 34
	Cote d'Ivoire	0.25	6.02	17.94			Ref. 35
	Cameroon	0.27	4.85	24.5			Ref. 36
	Sabah, Malaysia	1.6-6.1	12.8–159		4.6-32		Ref. 37
	Malaysia	0.2-49.0	32–99	68.19	1.68–54		Ref. 38
	Malaysia	0.1-0.8	0.8-24	5.0-16.0	0.1-5.9		Ref. 39
	Malaysia	0.10	12.06	42.41	1.00	0.29	Present study
Mollusc	Food standard of Malaysia	1	30	100	2		Ref. 21
	India	0.258	7.22	42.31	0.41	1.82	Ref. 12
	Thailand	0.28	5.6	16.2	0.18		Ref. 40
	Australia	0.2	2	27.7	0.8		Ref. 41
	Australia	2.09	0.73	42.7			Ref. 17
	Malaysia					0.24-0.41	Ref. 42
	North America					0.1–9.6	Ref. 43
	Malaysia	0.82	3.39	51.63	0.97	0.80	Present study

Table 2 Comparison of heavy metal contents ($\mu g/g$ wet wt) in organisms samples with the guidelines and other studies.

for incorporation into the respiratory pigment haemocyanin, a copper-based pigment found in the blood of many species of molluscs and crustaceans⁴⁶. The fish had comparatively lower mean-copper concentration than the invertebrates. Copper concentration may be regulated in fish due to the essential nature of this metal for metabolic process⁴⁷.

No significant difference was observed between *A. maculatus* and *P. merguiensis* (t (73.87) = 1.0, p = 0.32) in the case of zinc concentration. Whereas there was a significant difference between *A. maculatus* and *A. granosa* (t (46.51) = 6.91, p = 0.00), as well as between *P. merguiensis* and *A. granosa* (t (56.23) = 9.27, p = 0.00). *A. granosa* had a zinc concentration of 93 ± 13 µg/g dry wt, which was higher than that

found in *A. maculatus* $(70 \pm 20 \text{ µg/g} \text{ dry wt})$ and *P. merguiensis* $(76 \pm 10 \text{ µg/g} \text{ dry wt})$. Barwick and Maher⁴⁵ also found a lower concentration of zinc in fish. Moreover, Eisler et al⁴⁸ described that the filter feeding bivalve molluscs generally show the highest accumulation level of zinc from marine environment. Zinc concentration in fish is comparatively higher than other reported values but within the range of values in the case reported for Malaysia. However, this value is lower than the safety levels (Table 2). For crustaceans, comparatively higher value was observed in the case of Malaysia but it is still lower than the safety guideline. On the other hand, in the case of molluscs, the measured value was higher than the other reported values but lower than the safety limit,

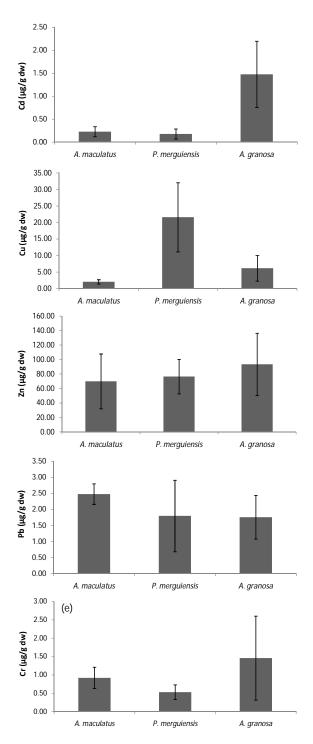


Fig. 2 Mean concentration of cadmium, copper, zinc, lead, and chromium in analysed marine organisms.

which is stated for the Malaysian population. Thus zinc consumption from organisms from Kapar coastal area poses no threat to humans. This study found that *A. granosa* had a statistically significant lower lead value $(1.76 \pm 0.62 \ \mu\text{g/g})$ dry wt) than that of *A. maculatus* $(2.47 \pm 0.16 \ \mu\text{g/g})$ dry wt; t(82.31) = 5.62, p = 0.00). There was a significant difference between *A. maculatus* and

P. merguiensis (t (59.22) = 3.40, p = 0.00). However, no significant difference was found in the case of P. merguiensis and A. granosa (t(68.61) = 0.19)p = 0.84). The higher concentration of lead in fish is probably because of the food source. Comparatively lower lead concentrations were observed by Barwick and Maher⁴⁵, which supports the present study. These lower concentrations in molluscs and crustaceans, are thought to be due to the partitioning of lead to shells and exoskeleton, as lead has been shown to accumulate via the same process as calcium⁴⁹. In the case of fish and crustaceans, the lead concentrations are higher compared to other reported values, except for the value reported in Malaysia (Table 2). However, these values are lower than the safety limits. On the other hand, the measured value for molluscs in the present study demonstrated higher values than the literature but the value did not cross the limit of safety which has been declared for the Malaysian population. However, all organisms analysed have lead concentrations below the maximum permitted lead concentration for human consumption (2.5 µg/g drv wt)⁵⁰.

The filter feeding species, A. granosa, had the highest concentration of chromium $(1.5 \pm 1.1 \,\mu\text{g/g} \,\text{dry})$ wt) followed by the A. maculatus $(0.79 \pm 0.27 \ \mu g/g)$ dry wt), and *P. merguiensis* $(0.53 \pm 0.04 \ \mu g/g \ dry$ wt). An independent sample *t*-test revealed significant differences between A. maculatus and P. merguiensis (t(77.76) = 7.32, p = 0.00), between A. maculatus and A. granosa (t(50.23) = 3.13, p = 0.00), and P. merguiensis and A. granosa (t(46.92) = 5.52)p = 0.00). The chromium concentration observed in fish is within the range of other reported values but lower than the reported guideline (Table 2). There are very limited studies about the concentration of chromium in crustaceans. However, the value in this study is lower than the value reported in India. On the other hand, the chromium concentration in molluscs is also within the range of other places. Although there is no deleterious health effect from molluscs, the biologically available Cr (VI) is known to be carcinogenic to man and other species⁵¹.

The hierarchical cluster analysis and biotasediment accumulation (BSAF) factor were used to classify the analysed species in different groups. Hierarchical cluster analysis is a procedure which attempts to identify relatively homogeneous groups of cases Dendrogram using Ward Method

		Rescaled	Distance	Cluster	Combine	
CASE Label Num	0	5	10	15	20	25
Fish 1 Shrimp 2 Cockle 3]					

Fig. 3 Dendrogram for hierarchical cluster analysis of the three marine species based on the concentration of heavy metals during different sampling periods.

or variables based on selected characteristics. Fig. 3 shows the dendrogram derived by the ward method of the three species analysed in this study based on heavy metal accumulation. Results of the cluster analysis revealed two groups. The first group includes fish (A. maculatus) and shrimps (P. merguiensis), while the second group included cockles (A. granosa). Generally, the major differences are in the clustering of cockles and another two species, whereas the major similarities (the minimum distance) can be observed between fish and shrimp. Finally, the two groups are merged into a single cluster at the distance of 0.2. The differences in between the analysed species in the case of heavy metal accumulation may have occurred from species-specific feeding habits such as uptake of food which plays an important role⁵².

The BSAF is a parameter which describes the accumulation of sediment-associated organic compounds or metals into tissues of ecological receptors. In this study, the efficiency of metal bioaccumulation in three types of marine organisms was evaluated by calculating the BSAF, which is defined as the ratio between the metal concentration in organisms and that in the sediment. The mean concentrations of Cd, Cu, Zn, Pb, and Cr in sediment collected during the three sampling periods from the different stations of the study area have been calculated as $0.29 \pm 0.02, \ 9.34 \pm 0.12, \ 118.6 \pm 1.0, \ 35.21 \pm 0.18,$ and $14.07 \pm 0.09 \,\mu\text{g/g}$, respectively. On the basis of the calculated BSAF, the A. maculatus was ranked in the following order, Cd > Zn > Cu > Pb >Cr (Fig. 4). None of the studied metals were bioconcentrated in the tissues of A. maculatus to the levels higher than those in sediments, hence the BSAF were less unified. These findings suggest that the levels of contamination of these metals in Kapar coastal area do not exceed the fish capacity to regulate them. On the other hand, the trend of BSAF in *P. merguiensis* was Cu > Zn > Cd > Pb > Cr. The mean concentrations of heavy metals in this species were generally lower than the sediment, except Cu,

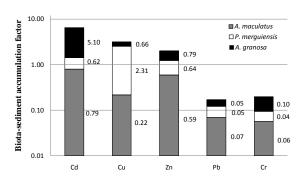


Fig. 4 The pattern of BSAF in analysed marine species collected from Kapar coastal area.

which indicates that this metal is readily accumulated in P. merguiensis. In the case of A. granosa, the pattern of BSAF is Cd > Zn > Cu > Cr > Pb. The calculated value of BSAF for Cd is much higher than 1, suggesting a higher rate of Cd accumulation in this species. In this case, water probably acts as an additional source of Cd in A. granosa. Moreover, as Cd is known to be mobile in soils⁵³, thus the A. granosa is more likely to accumulate higher concentrations of Cd relative to the sediment. Cheggour et al⁵⁴ also calculated higher BSAF values for Cd in cockle tissue. Moreover, Bryan and Langston⁵⁵ have suggested that Cd was solubilized from the sediment, rather than the solid-phase itself, and could be the main source of Cd to a number of benthic organisms. The BSAF values for other metals in A. granosa were lower than the unity. Accumulation factors were also reported to be around unity or below, generally, in soft tissues of the mussel Mytella strigata from a mangrove area of the northwest coast of Mexico⁵⁶.

However, Adjei-Boateng et al⁵⁷ stated that the relationship between the concentration of metal in the clam tissues and the sediment was not distinct. This study also failed to find any stable pattern of metal bioaccumulation between the three species analysed species, which supports the fact that the several variables control both bioavailability and accumulation of heavy metals in individuals exposed to contamination. Based on the values calculated, the different species of marine organisms could be classified into a few groups such as macro concentrator (BSAF > 2), micro concentrator (1 < BSAF < 2) or deconcentrator (BSAF < 1) as proposed by Dallinger⁵⁸. Therefore, based on that proposal, A. maculatus (mean BSAF 0.34) and P. merguiensis (mean BSAF 0.73) could be classified as a deconcentrator while the A. granosa (mean BSAF 1.34) follows the group of micro concentrator. Therefore, among the species analysed in this study, A. granosa possesses a greater capacity for metal bioaccumulation than A. maculatus and P. merguiensis. This finding suggests that A. granosa is a potential indicator for pollution in a marine environment. In a previous study, it was found that molluscs have the potential to be used as bio-indicators for the contamination of Cd and Zn in water and sediment of an estuarine environment¹⁴ and it was proposed as a simple model for predicting the bioaccumulation of sediment-associated natural organic contaminants by in faunal invertebrates⁵⁹⁻⁶¹. Heavy metal BSAF in Perna viridis reflects the degree of industrialization and population density in coastal areas of Hong Kong⁶². Similarly, the bivalve possessed high BSAF for heavy metals mainly due to the wastewater and waste-residue drainage from the industries nearby⁴. Although several studies on molluscs associated with heavy metal pollution have been done, few studies reports the BSAF values. Therefore, the findings of the present study are quite significant from the environmental pollution viewpoint.

CONCLUSIONS

The higher metal concentration observed in *A. granosa* is due to their mode of life and feeding habit. Similarly, the calculated values of BSAF and MPI were also higher in the case of *A. granosa*. Therefore, it is suggested that these species are used as a bio-indicator of marine pollution. Moreover, the heavy metal concentrations in the analysed samples were well within the approved limits set by the guidelines. As a result, it can be assumed that the seafood from this region is safe for human consumption.

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