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Assessment of arsenic and heavy metal contents in cockles (*Anadara granosa*) using multivariate statistical techniques

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Abstract

Cockles (*Anadara granosa*) sample obtained from two rivers in the Penang State of Malaysia were analyzed for the content of arsenic (As) and heavy metals (Cr, Cd, Zn, Cu, Pb, and Hg) using a graphite flame atomic absorption spectrometer (GF-AAS) for Cr, Cd, Zn, Cu, Pb, As and cold vapor atomic absorption spectrometer (CV-AAS) for Hg. The two locations of interest with 20 sampling points of each location were Kuala Juru (Juru River) and Bukit Tambun (Jejawi River). Multivariate statistical techniques such as multivariate analysis of variance (MANOVA) and discriminant analysis (DA) were applied for analyzing the data. MANOVA showed a strong significant difference between the two rivers in term of As and heavy metals contents in cockles. DA gave the best result to identify the relative contribution for all parameters in discriminating (distinguishing) the two rivers. It provided an important data reduction as it used only two parameters (Zn and Cd) affording more than 72% correct assignations. Results indicated that the two rivers were different in terms of As and heavy metal contents in cockle, and the major difference was due to the contribution of Zn and Cd. A positive correlation was found between discriminate functions (DF) and Zn, Cd and Cr, whereas negative correlation was exhibited with other heavy metals. Therefore, DA allowed a reduction in the dimensionality of the data set, delineating a few indicator parameters responsible for large variations in heavy metals and arsenic content. Taking into account of these results, it can be suggested that a continuous monitoring of As and heavy metals in cockles be performed in these two rivers.

Keywords: Cockles; MANOVA; Discriminant analysis; Arsenic; Heavy metals; GF-AAS; CV-AAS

1. Introduction

Malaysia is presently undergoing rapid industrial development and there have been incidences of toxic pollution from industry. Solid and liquid wastes emanating from the industrial activities are the inevitable by products of manufacturing process. These wastes contain toxic chemicals such as chromium salts, sulfides and other substances including heavy toxic trace metals [1]. A number of natural and anthropogenic sources produce heavy metals. People are becoming more aware of the complexity of the nature and the delicate balance that exist within the global ecosystem [2]. The discharge of effluents and associated toxic compounds into aquatic systems represents an ongoing environmental problem due to their possible impact on communities in the receiving aquatic water and a potential effect on human health [3]. Especially in highly polluted and industrial areas, point and non-point sources of anthropogenic chemicals and metals have polluted rivers with highly complex mixtures of chemicals and other anthropogenic perturbations to degree where life in rivers is severely impacted [4]. Urbanization, increases in population density and the intensification of agricultural activities in certain area are among the main causes of water pollution [5].

The blood cockle *Anadara granosa* is a bivalve mollusc in the family Arcidae, subfamily Anadarinae. The bivalves in this family are of considerable importance as a source of cheap protein in tropical areas, especially in the Indo-Pacific region [6]. Therefore, semi-culture of marine bivalves particularly *A. granosa* is of considerable economic importance in Malaysia. For the past 19 years up to 5000 ha of mudflats along the west coast of Malaysia have been utilized for this purpose [7]. Since *A. granosa* is a filter feeding organisms contamination of the highly productive mudflats with heavy metals tend to be accumulated

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in their whole body tissue. This could serve as an important environmental sinks of heavy metals [8] and provide an indication of river pollution.

The application of multivariate methods has increased tremendously in recent years for analyzing environmental data [7,8]. These methods are useful where several dependant variables are measured on each sampling unit. Multivariate analysis of variance (MANOVA) can be used to test the significant differences, while discriminant function (DF) has been used to identify the relative contribution of all variables to the separation of the groups [9,10].

The objectives of this study have been to determine whether the concentrations of arsenic and heavy metals in cockles sampled from two different rivers are different based on the concentrations of arsenic and six heavy metals (Cu, Pb, Cd, Cr, Zn, and Hg). In addition, it is important to identify the relative contribution for all parameters in distinguishing the cockles according to the above selected parameters contribution. This study may assist the evaluation of the impact of industry and agricultural discharge on aquaculture products of the two areas of choice.

2. Materials and methods

2.1. Description of study sites

The study site is located on the North West coast of Peninsular Malaysia, in the state of Penang and within a coastal mudflat in the Juru and Bukit Tambun district (Fig. 1). The sites are located adjacent to industrial areas which were reclaimed from mangrove. The types of industry presently in operation include: electronics; textiles; basic and fabricated metal products; food

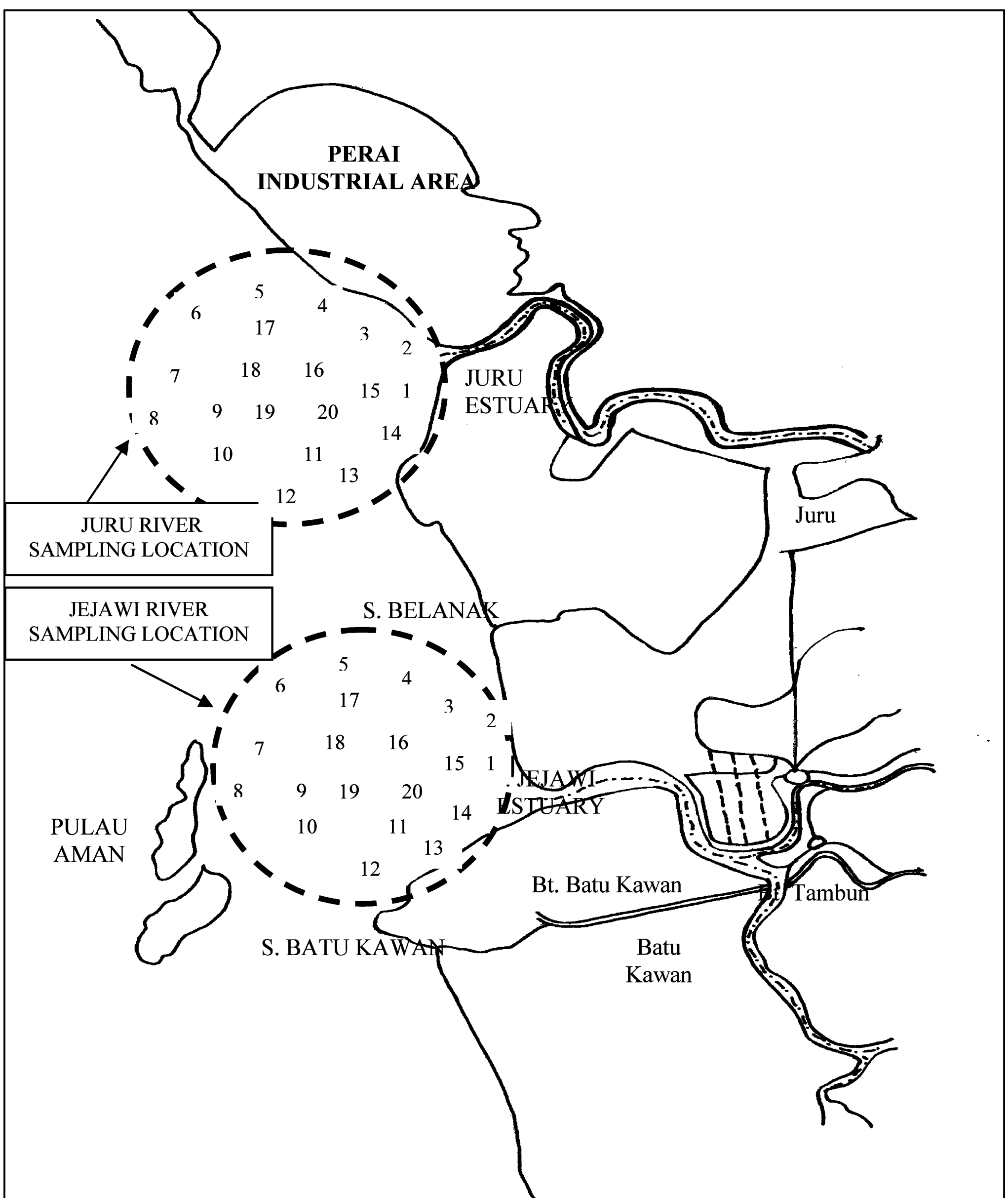

Fig. 1. Map of sampling locations for study areas.

processing and canning; processing of agricultural products; feed mills; chemical plants; rubber based industry; timber based wood products; paper products and printing works; transport equipment. Other main activities that are operating in vicinity of the cultured area are a ships' harbour with petroleum unloading and a red earth quarry which extends right up to the coastline. There are three main rivers flowing into the area, Sungai Juru, Sungai Semilang and Sungai Jejawi where some fishing villages are situated.

2.2. Sampling and analytical procedure

Samplings were carried out during a rainy season in the year 2005. Five samples of cockles (*A. gradosa*) were collected from each of the 40 estuarine sites in Juru and Jejawi rivers (20 sites from each location). The cockles were collected manually at low tide from the inter-tidal plats (slikkes) of the two areas. Cockles were cleaned externally by washing thoroughly through a 1 mm mesh sieve with deionized water before being transferred into a high density polyethylene (HDPE) sampling bag. After a 36 h purging period, during which time sediment-bound metals were voided from the gut, the bivalves of approximately similar size (32.6 mm) were rinsed in deionized water. Soft parts (7–10 g of wet tissue) were digested in 10 mL of boiling concentrated nitric acid (Analar grade) to near dryness. Additional digestion was accomplished by the addition of 10 mL of 1:1 nitric acid/deionized water. The resulting residue was diluted to 50 mL in deionized water. The solution mixture was then filtered through a 0.45 μm cellulose nitrate filter prior to arsenic and heavy metals analysis.

Graphite furnace atomic absorption spectrometer (GF-AAS; Perkin-Elmer HGA-600) was employed for the analysis of arsenic and heavy metals (Cr, Cd, Zn, Cu and Pb) and cold vapor atomic absorption spectrometer (CV-AAS) method was employed for Hg analysis after sample digestion in acid solution. Calibration curves from standard mixtures of 0.05, 0.1, 0.2, 0.5 and 1.0 mg L^{-1} of Cu, Zn, Cd, Cr and Hg and 0.5, 1.0. 2.0. 5.0 and 10 mg L^{-1} of As and Pb were prepared in nitric acid solution. The accuracy of the methods was determined by preparing digestion mixture blanks and by spiking the sample with known concentrations of As, Cu, Cd, Cr, Zn, Pb and Hg with mean recoveries of $90.5 \pm 1.2\%$.

3. Statistical analyses

3.1. Multivariate analysis of variance (MANOVA)

Multivariate analysis of variance is used where several dependent variables (p) are measured on each sampling unit instead of one variable. The objective of MANOVA is to compare the mean vectors of k groups for significant difference. Equality of the mean vectors implies that the k means are equal for each variable, and if two means differ for just one variable then we conclude that the mean vectors of the k groups are different.

Table 1
Mean concentrations (standard deviations) arsenic and heavy metals in *A. granosa* obtained from Juru River (mg kg^{-1} wet weight)

Parameter	Minimum	Maximum	Mean	Standard deviation
Cr	0.14	0.20	0.17	0.01
As	2.38	2.94	2.67	0.15
Cd	0.82	0.92	0.89	0.03
Zn	0.18	0.29	0.22	0.03
Cu	0.16	0.24	0.19	0.02
Pb	0.11	0.14	0.11	0.01
Hg	1.19	1.46	1.33	0.08

3.2. Discriminant function

Discriminant analysis is a multivariate technique used for two purposes, the first purpose is description of group separation in which linear functions of the several variables (discriminant functions (DFs)) are used to describe or elucidate the differences between two or more groups and identifying the relative contribution of all variable to separation of the groups. Second aspect is prediction or allocation of observations to group in which linear or quadratic functions of the variable (classification functions (CFs)) are used to assign an observation to one of the groups [10,9]. SPSS Version 12 software was used for carrying out the statistical analysis of the data.

4. Result and discussion

4.1. Descriptive statistics

Descriptive statistics including the mean, standard deviation, maximum, and minimum values for Juru and Jejawi Rivers are shown in Tables 1 and 2, respectively. The average arsenic and heavy metal contents in cockles was arranged in the order; As > Hg > Cd > Zn > Cu > Cr > Pb and As > Hg > Zn > Cd > Cu > Cr > Pb for Juru and Jejawi River, respectively. Therefore, the As and Hg contents exhibited higher levels in cockles, whereas Cr and Pb exhibited the lowest. The maximum values of As and Hg content of cockles from Juru river were 2.67 and 1.33 mg kg^{-1} wet weight, respectively, while the minimum values of Cr and Pb were 0.17 and 0.11 mg kg^{-1} wet weight, respectively. The maximum values of As and Hg content of cockles from Jejawi river were 2.69 and 1.36 mg kg^{-1} wet weight, respectively, while the minimum values of Cr and Pb were 0.17 and 0.12 mg kg^{-1} wet weight,

Table 2
Mean concentrations (standard deviations) arsenic and heavy metals in *A. granosa* obtained from Jejawi River (mg kg^{-1} wet weight)

Parameter	Minimum	Maximum	Mean	Standard deviation
Cr	0.15	0.19	0.17	0.01
As	2.43	2.90	2.69	0.14
Cd	0.79	0.93	0.87	0.04
Zn	0.17	0.24	0.20	0.02
Cu	0.15	0.23	0.19	0.02
Pb	0.11	0.14	0.12	0.01
Hg	1.21	1.50	1.36	0.08

Table 3
Mean concentrations arsenic and heavy metals in *A. granosa* obtained from Juru and Jejawi River with comparison to Malaysian Food Act 1983 and Food Regulations 1985 Fourteen Schedule (in mg kg^{-1} wet weight)

Parameter	In this work		Standard deviation
	Juru	Jejawi	
Cr	0.17	0.17	Not mentioned
As	2.67	2.69	1
Cd	0.89	0.87	1
Zn	0.22	0.20	100
Cu	0.19	0.19	30
Pb	0.11	0.12	2
Hg	1.33	1.36	0.5

respectively. Zinc and cadmium have interchanged their content ranking in cockles in the two rivers. The observed spread around the mean metal content was low and random. However, Pb showed the lowest content in cockles' samples from both rivers.

The reported mean concentration of As and heavy metals in *A. granosa* is tabulated in Table 3 with comparison to Malaysian Food Act 1983 and Food Regulations 1985 Fourteen Schedule [11]. The mean concentration of As in *A. granosa* was 2.67 mg kg^{-1} wet weight for Juru River and 2.69 mg kg^{-1} wet weight for Jejawi River. These values were higher than the permissible limit (1 mg kg^{-1} wet weight) established by the Malaysian Food Act 1983 and Food Regulations 1985 Fourteen Schedule [11]. However, the concentration level of As in *A. granosa* in the present investigation was within the mean concentration observed in the previous study on *A. granosa* derived from retail outlets in the city of Kuala Lumpur [11] and as observed in various edible molluscs in Hong Kong [12].

The mean concentrations of Cr in *A. granosa* for both rivers are similar (0.17 mg kg^{-1} wet weight). These mean concentrations are slightly lower than those mean concentrations (0.24–0.41 mg kg^{-1} wet weight) obtained from various retail outlets in Kuala Lumpur [13] and within the lower range of commercially important North American molluscs where their concentrations varied from 0.1 to 0.6 mg kg^{-1} wet weight [14]. Although there is no deleterious health effect of molluscs, the biologically available Cr(VI) is known to be carcinogenic to man and other mammals [12].

An excessive level of Cd in the body has been shown to result in kidney and liver damages as well as deformation of bone structures [15]. *A. granosa* in Juru and Jejawi Rivers accumulated Cd in the range of 0.82–0.89 mg kg^{-1} wet weight and 0.79–0.87 mg kg^{-1} wet weight, respectively. The mean concentrations of Cd for both rivers were slightly lower than the legislative limit in Malaysia (1 mg kg^{-1} wet weight) as in Malaysian Food Act 1983 and Food Regulations 1985 Fourteen Schedule [11]. This concentration level is comparable to the same species of bivalve molluscs [16,12] as well as other *Anadara* spp. [17,18,12]. Although the degree of coastal water pollution is not prevalent in Malaysia, the presence of 'hot-spots' cannot be ruled out [19]. Therefore, it is of major concern to monitor the level of contamination of Cd especially in the muddy coastal environment. This is due to the fact of extensive cultures of cockles are being carried out in the areas. The excessive contamination of sea foods of this nature will not render safe for consumption [13].

Filter feeding bivalve molluscs are generally shown to accumulate highest level of Zn from marine environment [20]. Therefore, it was not surprised to find that Zn is in third order after arsenic and mercury for trace element and heavy metal accumulation in *A. granosa* for both studied rivers. Previous study from various retail outlets in Kuala Lumpur showed that *A. granosa* and *P. undulata* accumulated Zn from 12.85 to 14.73 and 7.40 to 8.11 mg kg^{-1} wet weight, respectively [13].

The results indicate that the levels of Zn in *A. granosa* from various retail outlets in Kuala Lumpur were two orders of magnitude higher than those obtained in the research findings. The difference in Zn concentration was due to different industrial activities from different states (about 300 km in distance between Penang and Kuala Lumpur) of Malaysia. The similar situation can be observed in another bivalve's species name as *Cerastoderma edule* from two lagoon ecosystems on the Moroccan Atlantic coastline, Moulay Bou Selham and Sidi Moussa. It was found that zinc concentrations were higher ($P < 0.001$) in cockles from Sidi Moussa (115.4 mg kg^{-1} dry weight) than at Moulay Bou Selham (58.3 mg kg^{-1} dry weight) [21].

Kuala Lumpur was a pioneer area for most of industrial activities in Malaysia compare to Penang State where all industrial activities tend to be grouped together in places that combine a series of facilities: transport, communication, energy and water supplies, etc. Thus, many of the major industrial centers in coastal area of Kuala Lumpur and Klang Valley are located along estuaries and close to the main cities and ports. Therefore, its coastal areas have been accumulated more with heavy metals compared to Penang coastal areas. Its habitats have been, and continue to be, altered, disturbed or destroyed by industrial development. Other environmental impacts arise as a result of discharges, emissions and losses to land, air and water.

The mean concentration levels (Table 3) of Cu in *A. granosa* from both rivers were very much lower than permissible limits of 30 mg kg^{-1} wet weight. These concentrations were lesser as those observed in various *Anadara* spp. [22,17,18,12,23].

Phase of industrialization and other anthropogenic activities in the vicinity of coastal areas are always positively associated with elevated level trace metals such as Pb in marine biota [20,24]. The mean concentrations of 0.11 mg kg^{-1} wet weights of Pb from Juru River and 0.12 mg kg^{-1} wet weights of Pb from Jejawi River in *A. granosa* were below the permissible limit compare to Malaysian Food Act 1983 and Food Regulations 1985 Fourteen Schedule [11] that 2 mg kg^{-1} wet weights.

The green-lipped mussels *Perna viridis* that was collected along the West Coast of Peninsular Malaysia and analyzed for Cd, Cu, Pb, Zn and Hg showed a similar trend in the heavy metals concentration as per present studied for *A. gradosa*. The level of heavy metals analyzed for *P. viridis* was ranged from 0.68 to 1.25, 7.76 to 20.1, 2.51 to 8.76 and 75.1 to 129.0 mg kg^{-1}

Table 4
Linear correlation coefficient matrix for arsenic and selected heavy metals in Juru River

Metal	Cr	As	Cd	Zn	Cu	Pb	Hg
Cr	1						
As	0.04	1					
Cd	−0.30	0.25	1				
Zn	−0.26	0.14	−0.09	1			
Cu	−0.14	−0.07	0.10	0.09	1		
Pb	0.07	**0.46***	0.02	0.32	0.08	1	
Hg	0.04	0.17	−0.12	−0.24	0.06	**0.50***	1

* Correlation is significant at the P-value < 0.05.

Table 5
Linear correlation coefficient matrix for arsenic and selected heavy metals in Jejawi River

Metal	Cr	As	Cd	Zn	Cu	Pb	Hg
Cr	1						
As	−0.07	1					
Cd	−0.25		1				
Zn	0.23	**−0.63****	**−0.48***	1			
Cu	**−0.46***	0.01	**0.47***	−0.16	1		
Pb	0.00	−0.41	−0.42	0.25	−0.18	1	
Hg	0.12	**−0.47***	−0.24	0.43	0.02	−0.15	1

* Correlation is significant at the P-value < 0.05.
** Correlation is significant at the P-value < 0.01.

wet weights for Cd, Cu, Pb and Zn, respectively and 3.89 to 50.0 mg/kg wet weight for Hg [25] Cu and Zn concentrations are below the reference values for human consumption set by Malaysian Food Act 1983 and Food Regulations 1985 Fourteen Schedule [11]. A high level of As could be related to some industrial activities, such as semiconductors, wood preservatives, and also from oil-based products such as petrol, diesel and motor oil. Arsenic level in marine products is higher than other foods, and due to environmental factors such as pollution [26]. The use of coastal waters as a convenient receptacle for domestic and industrial wastes threatens the quality of seafood rivers and coastal waters are presently exposed not only to increasing quantities of natural materials such as metals and nutrients, but also to cocktails of industrial derived contaminants, many of which exhibit significant persistence and capabilities for bioaccumulation [27].

The two rivers seemed to be polluted with Hg and As and this is in agreement with previous studies at Juru River, Malaysia on metal accumulation on aquaculture [28,29]. According to report by DANCED [28] the electroplating, pulp and paper, textiles, food and beverages and auto-workshops industries were closely linked to industrial pollution in the Prai industrial area. This is in line with the report conducted by department of environment [30] that the four predominant industries of Penang are electronics/electrical, textiles, fabricated metal products, plastic and plastic products. Other industries include rubber based, paper and paper products/printing works, chemical/fertilizers and basic metal industries.

4.2. Correlation coefficient analysis

The correlation matrixes of arsenic and heavy metal contents in cockles obtained from the two rivers were examined. The arsenic and heavy metals correlation data for Juru and Jejawi River are presented in Tables 4 and 5, respectively. For Juru river, a strong positive relationship was exhibited between Pb, As and Hg, while other heavy metal contents did not show significant relationship between them. For Jejawi river As, Zn, Pb, and Hg were significantly negatively correlated. This indicates that As has a strong association with other heavy metals and it shares a common origin with them. Other significant relationship was shown between Cd and Zn and Cu, while Cr correlated with Cu negatively.

Table 6
Multivariate test (MANOVA) for both locations

Test	Value	F	P-value
Pillai's Trace	0.38	2.80	<0.05
Wilks' Lambda	0.62	2.80	<0.05
Hotelling's Trace	0.61	2.80	<0.05
Roy's Largest Root	0.61	2.80	<0.05

4.3. Multivariate statistics

The results of MANOVA for arsenic and heavy metal contents are shown in Table 6. According to these results, the contents of arsenic and heavy metals in cockles from the two rivers exhibit strong significant difference in terms of selected parameters (arsenic and heavy metals). Variation in arsenic and heavy metal contents was evaluated through DA. The DA applied on raw data consisted of seven parameters. Only one DF was found to discriminate the two rivers as shown in Table 7. Wilk's Lambda test showed that DF is statistically significant as shown in Table 8. Furthermore, 100% of the total variance between the two locations was explained by only one DF. The relative contribution for each parameter is given in Eq. (1).

$$Z = -0.221\,\mathrm{Cu} - 0.298\,\mathrm{Pb} + 0.901\,\mathrm{Zn} + 0.732\,\mathrm{Cd} + 0.227\,\mathrm{Cr} - 0.137\,\mathrm{As} - 0.143\,\mathrm{Hg} \quad (1)$$

Zn and Cd exhibited strong contribution in discriminating the two locations and account for most of the expected variations in heavy metal contents, while other parameters showed less contribution in explaining the variation between Juru and Jejawi River.

Table 7
Eigen-value of DF for the two locations

Function	Eigen-value	% of Variance	Cumulative %
1	0.61	100	100

Table 8
Wilks' Lambda for testing discriminant function validity

Test of function	Wilks' Lambda	P-value
1	0.62	<0.05

Table 9
Correlation between all metals and standardized discriminant function

Parameter	Correlation
Zn	0.62
Cd	0.41
Hg	−0.25
Pb	−0.24
As	−0.08
Cu	−0.06
Cr	0.02

Table 10
Classification results for discriminant analysis of the two rivers

Locations	% Correct	Predicted group membership	
		1	2
Juru River	70	14	6
Jejawi River	75	5	15

A 72.5% of original grouped cases correctly classified.

The relative contribution for arsenic and heavy metal contents can be arranged in the order; Zn > Cd > Pb > Cr > Cu > Hg > As.

Correlation data between arsenic and heavy metal contents and Eq. (1) are summarized in Table 9. It can be seen that Zn was strongly correlated with Eq. (1), while arsenic and heavy metals such as Cu and Cr was not strongly correlated. The classification matrix (Table 10) showed that more than 72% of the cases were correctly classified to their respective groups. The results of classification also showed that significant differences existed between these two rivers, which are expressed by in term of one discriminant function.

4.4. *Source identification*

Relationship between the scores of discriminant function and the samples from various locations (Fig. 2) corresponded to the scores of discriminant function for various samples. The samples nos. 1–20 corresponded to cockle samples along 20 sampling points of each river. It could be seen from Fig. 2 that most points of Juru River showed positive contribution to discriminant function, whereas a number of points showed negative contribution. For Jejawi river most of the points showed negative contribution, while five points showed positive contribution. This could be due to differences in industrial and agricultural activities operating close to the study areas. In general, the heavy metal contents such as Zn and Cd were higher in cockles obtained from Juru than Jejawi river.

The positive contribution was attributed to a high content of Zn and Cd in cockles, while the negative contribution was attributed to a high content of other heavy metals and arsenic.

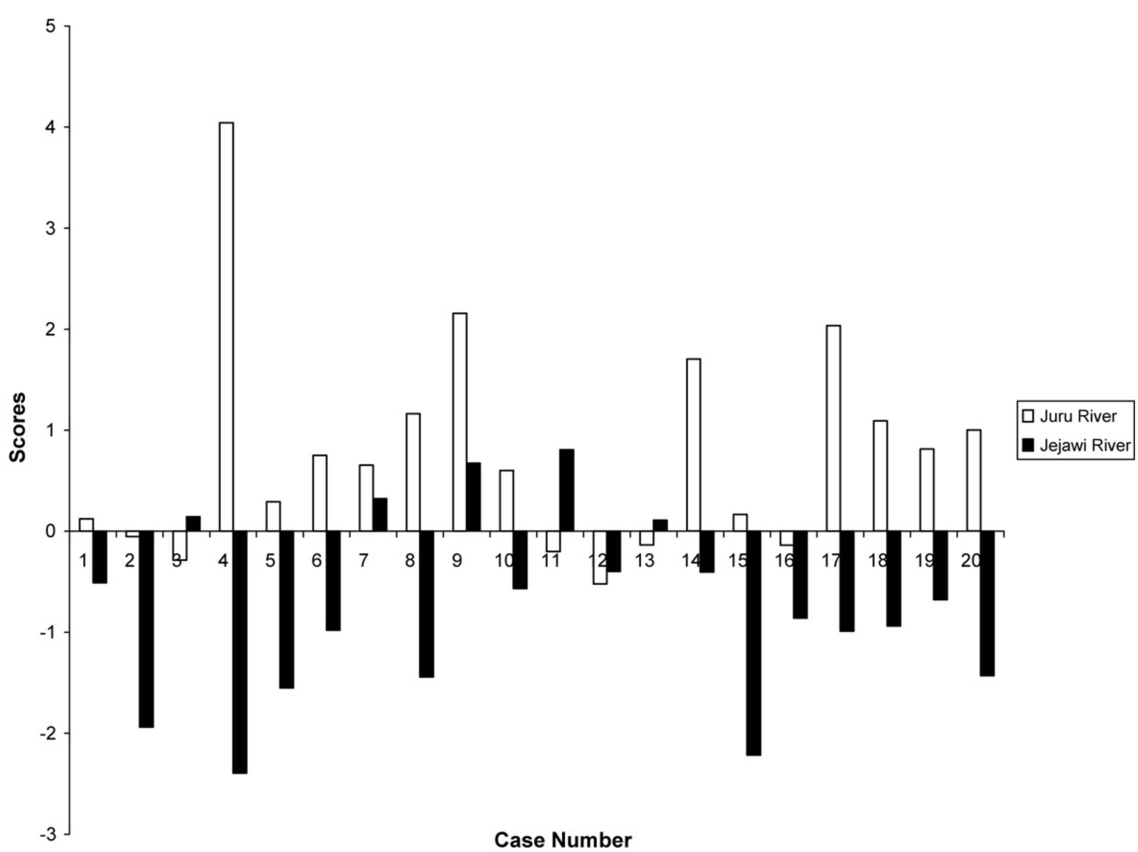

Fig. 2. Scores for the discriminant function.

5. Conclusion

Based on the results of arsenic and heavy metal contents as well as multivariate statistical technique for identifying the relative contribution of arsenic and heavy metals, it may be concluded that the arsenic and heavy metal contents in cockle obtained from Juru and Jejawi Rivers are different according to MANOVA result. It can also be concluded that out of seven parameters Zn and Cd were responsible for large variations in the data, according to their coefficient with discriminant function affording more than 72% correct assignations. Thus, DA helped to identify and understanding the source of variations. Therefore, the application of multivariate statistical techniques has been proven to be an effective tool for analyzing a huge and complex environmental data matrix.

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