Contents

Articles


An Overview of Functional Food in Brunei Darussalam ………………………………………………….. Ibrahim Hj Adb Rahman, Junaidah Abu Bakar, Pg Hjh Rosidah bte Pg Hj Metussin and Pg Hjh Masliati bte PSJ Hj Abd Mumin 9

Biomonitoring and Biosorption Studies on Two Seaweed Species from Brunei Darussalam ………….. Anjie S. Bispat, K.R.Fernando and Hartini Hj Mohd 21

Biochemistry of Vision ……………………… Anjie S. Bispat and Adeline C. Y. Sung 35

Biochemical and Physical Cardiovascular Risk Factors in an Indian Population …………………… Harkirat S. Dhindsa, Margaret A. Bermingham and David R. Sullivan 47

Free and Open Source e-Learning ………………………………………………………………………… Kim Onn Chong, Sei Guan Lim and Chee Ming Lim 57

Abstracts and Reviews

Review of Tan Heok Hui, *The Borneo Suckers* ……………… Zohrah Hj Sulaiman 64

Abstract of “Phylogeny and Biogeography of the South East Asian Rasbora (Pisces, Cyprinidae) and associated species inferred from cytochrome b DNA” …………………… Zohrah Hj Sulaiman, Tan Heok Hui and Peter K.L.Ng 65

Corrigenda

Corrections to *Scientia Bruneiana* 2005 66
EDITORIAL BOARD

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USE OF INDIVIDUAL MARKINGS IN THE STUDY OF THE FOOT-FLAGGING FROG, STAuroIS guttatus

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Abstract: This study reports on the variability in leg markings of the ranid frog Stauois guttatus that can be used for individual recognition. We used leg markings over a period of 20 months to follow seven females along a torrential stream within the Ulu Temburong National Park, Brunei Darussalam. On average, females were re-sighted 9.7 times and moved up to 70 m along the stream. Our preliminary observations suggest that female S. guttatus have small territories but their activity ranges can be much larger. This study shows that leg markings can be used for recognizing individuals and this obviates the use of potentially harmful invasive methods of marking.

Introduction

Studies on the life-history, behaviour and habitat use of Bornean frogs are still in their infancy, since much effort is still consumed by basic species inventories (Inger and Stuebing 2005). Despite these hurdles the need to assess the size and viability of frog populations as well as movement patterns is extremely urgent because of the worldwide decline in amphibian populations (Alford and Richards 1999; Houlahan et al. 2000). An assessment of a species’ rarity and its conservation status also hinges on the ability to measure a species’ abundance. Mark-recapture studies, in which animals are captured, marked, released and re-captured are instrumental in determining population dynamics and provide fundamental data on life-history patterns and activity ranges of free living animals (Donnelly and Guyer 1994). However, to study animal populations under field conditions, animals need to be marked individually to distinguish between individuals. Although many invasive techniques are available for individual marking, such methods have their drawbacks because they can be harmful, may not be useful for small animals, or may influence the behaviour of the animals under study (Henle et al. 1997).

A non-invasive alternative is to use the natural markings of animals for individual recognition. Many amphibians have unique colour patterns that can be used for individual identification (e.g. Jehle 1997) and such patterns have been used to identify individuals in field studies of amphibians and reptiles (Henle et al. 1997).

Here we report on the use of individual markings in female Staurois guttatus (previously S. natator) for use in individual recognition. We are using these markings to investigate the life-history and movement patterns of females in the Ulu Temburong National Park, Brunei.
Darussalam. We present data on re-sighting frequency and activity ranges of individually recognizable females covering 20 months of observations.

Materials and Methods

Digital photos were taken of the hind legs of female *S. guttatus* either after capture or while sitting on their perch sites (Figure 1, lower right panel). While sitting, females expose the dorsal skin of their femur and tibiafibula with their characteristic pattern of dark bands on olive green background. We noticed that these patterns varied and were distinct between individuals.

Between May 2005 until December 2006 females were located in the field visually, often with the aid of binoculars. Females were encountered at specific localities both during daytime and nighttime hours, usually on perch sites which they used to capture prey. We re-visited these sites on a regular basis. In addition, systematic searches were conducted at regular intervals during visits to the study area. Sampling effort to locate individual females differed widely depending on proximity of female activity centres to our other study sites. Three females on the lower section of the Sungai Mata Ikan, near its confluence with the Sungai Belalong, were followed most intensively. In August 2006, perch occupancy was determined on 19 successive days for these females. Other females were only investigated haphazardly. The distance females moved along the stream was estimated to the nearest 2 m by plotting female positions on a detailed, self-generated map of the stream. Means and SD are given as descriptive statistics.

**Figure 1:** Variation in hind leg markings in female *Staurois guttatus* from the Ulu Temburong National Park, Brunei Darussalam. Inset shows method of handling frog for photo documentation.
Results

Figure 1 shows the variation in leg banding patterns encountered in female *Staurois guttatus*. Seven out of 12 females, for which digital photos were available, were encountered more than once. Perches used by females consisted of fallen logs, fallen branches, or branches overhanging the stream. There was considerable variation in the number of times a particular female was re-sighted (Figure 2). One female that showed strong site fidelity (no.1) was observed on 41 different days or nights.

**Figure 2:** Number of times three females were observed during both daytime and nighttime within the 20 month study period.

Females were re-sighted over periods ranging from 1-17 months (Figure 3). The average period in which re-sightings occurred was 9.7 ± 5.1 months. The maximum distance females were seen to move along the stream ranged between 10 - 70 m (39 ± 22 m; Figure 4). In August 2005, female 1 was encountered near a waterfall at which males aggregated and displayed. The waterfall was 70 m from her usual perch site where she had been seen the previous day. She remained at this male aggregation site throughout the night and although her oviduct contained eggs she did not go into amplexus that night. The same female was re-sighted at her usual perch site the next day. Perch occupancy rates, based on 19 days of observation, were 63.2% (12 out of 19 days), 21.1% (4 out of 19 days), and 10.5 % (2 out of 19 days) for females 1, 2, and 3, respectively.
**Figure 3:** Time span in which individual females were re-sighted within the 20 month study period.

**Figure 4:** Maximum distances moved by female *S. guttatus* along a stream in the Ulu Temburong National Park, Brunei Darussalam.

**Discussion**

Observations of female *S. guttatus* revealed that their individual hind leg markings can be used to identify individuals and monitor their activities. Leg markings did not fade or change.
throughout the 20 month duration of this study. Photo documentation allows individual recognition from within 1 m and thus obviates the need for capture and handling.

Recognition of individual female *S. guttatus* revealed that they generally show strong site fidelity. Females were seen to consistently occupy sites both near waterfalls and up to 20 m from the stream on the stream banks. Males in contrast, were seen only in close proximity to waterfalls (Grafe and Wanger unpublished).

One female (no. 1) was seen to repeatedly use the same perch site throughout the study period. These perch sites are used for prey capture and are defended against intruders (Grafe unpublished). Females have been observed to use perches to visually hunt prey and return to them between prey capture attempts. Our observations suggest that females may have several such defended areas or territories and move between them. These feeding territories appear to be very small (< 2 m²).

In addition to documenting site fidelity, females were observed to move up to 70 m along our study stream over short periods of time. In one case, the distance moved could be related to potential reproductive activity. This suggests that females leave their feeding territories to seek reproductive opportunities in areas where males aggregate near waterfalls. Such rapid movements to oviposition sites are well documented for males in other species of anurans (e.g. Spieler and Linsenmair 1998).

New analytical tools allow sophisticated predictions on the factors affecting population fluctuations and allow researchers to assess the conservation status of animals (Cooch and White 2006). These analyses critically hinge on the ability to recognize individuals after release. We hope that the use of natural markers will facilitate the use of mark-recapture analyses for small animals. Photo-documentation is a particularly valuable tool for studying amphibians that are small and difficult to tag. We hope to extend the use of natural marks for individual recognition to other species of frogs in Borneo to better understand their population dynamics and current threats.

**Acknowledgments:** We thank the staff of the Kuala Belalong Field Studies Centre for their support during the many trips to the station. TUG received financial support from a Universiti Brunei Darussalam Research Grant (UBD/PNC2/2/RG/1(58)). TCW, JMP and JP thank the German Academic Exchange Service (DAAD) for travel funding to Borneo. TB acknowledges support from the Bavarian education ministry.

**References**


Introduction

Brunei Darussalam is situated on the northwestern coast of Borneo Island and covers an area of 5765 square kilometres. Brunei Darussalam’s population for 2001 was 348,200. The country has a humid equatorial climate characterised by warm temperatures, high rainfall and high humidity. Soil varies greatly owing to the wide variation in factors influencing soil formation processes. The diversity in soils and microclimate conditions provides a favourable climate for the growth of natural vegetation. An estimated 5000 native species of seed plants consisting of trees, shrubs, liana and creepers can be found in the country (DAMIPR 2000). There is, therefore, a rich source of genetic materials with therapeutically and biologically important substances available in the country. This vast genetic resource should be explored for potential therapeutic, drugs and other useful functional food products.

Functional Food

Functional foods are defined as any modified food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains (Wikipedia, “Functional Foods”). It has been known that functional foods have been associated with the prevention and treatment of various serious diseases such as cancer, diabetes, cardiovascular disease and hypertension (Wikipedia, “Functional Foods”). Some of the cancers are related to diet and the potential impact of functional foods on health is being investigated by a large number of scientists in many parts of the world (Rodríguez-Cabezas 2002; Chearwae 2004).

Status of Development and Production of Functional Foods in Brunei Darussalam

Herbal plant species are important in Brunei Darussalam. Since the early years Bruneians have been using selected plant species – the leaves, roots, stems, flowers, fruits and seeds as ingredients in the preparation of traditional medicines and functional foods. Herbal cures and treatments have remained part of the folklore among the rural communities and are still being used today (DAMIPR 2000).

In 1989, the Research and Development Division of the Department of Agriculture participated in the ASEAN-Australia Biotechnology Project and carried out a project entitled “Development of the Therapeutically and Biologically Important Substances from Plants”. One of the objectives of the project was identifying, documenting and collecting all known indigenous plants for insecticidal, medicinal, culinary and functional food purposes. During the
project period (1989-1993), information on 340 species of plants was recorded. Only 160 plant species were positively identified and described in publications entitled “Medicinal Plants of Brunei Darussalam (Part One and Part Two)”. In the books, explanations for each species are given which include the medicinal use(s) and contents and properties. Medicinal uses include the parts of the plant used, method of preparation and the treatment the plant is traditionally used for. The section on contents and properties refers to the information extracted from the available literature. It provides a brief summary of the chemical constituents of the plant and their properties. It also gives an indication of the extent to which the plant species is being used and investigated by other scientists for medicinal and functional food purposes (DAMIPR 2000).

Antimicrobial screening, phytochemical evaluation and investigation for production of flavours and fragrances were also planned to be carried out on the identified plant species. A few samples (12) were screened for their antimicrobial activities, analysed (22) for their organic compound constituents and investigated (6) for the production of essential oils (DAMIPR 2000).

The numbers of functional foods that have potential benefits for health has grown tremendously all over the world (Rodríguez-Cabezas 2002; Chearwae 2004). However, activities on the development and production of functional foods in Brunei Darussalam are growing slowly due to a lack of manpower, expertise and facilities. Some of the functional foods developed in Brunei Darussalam are produced traditionally in food form as beverages or drinks, powder and dried. Raw materials used are from fruits, vegetables, shrubs, trees, beans, grains, herbs and spices. There is a vast number of plant species available in the country, to be explored for their economic potential uses in medicines and functional foods. Examples of some plant species used as functional food by Bruneians are given in Table 1.

Table 1: Some of the Local Plant Species that are used as Functional Foods

<table>
<thead>
<tr>
<th>Local Name (s) / Scientific Name / Uses</th>
<th>Pictures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bayam merah / Amaranthus gangeticus L.</strong></td>
<td></td>
</tr>
<tr>
<td>The leaves and young shoots are boiled and drink to alleviate difficulty in urination.</td>
<td>![Picture]</td>
</tr>
<tr>
<td>Local Name (s) / Scientific Name / Uses</td>
<td>Pictures</td>
</tr>
<tr>
<td>----------------------------------------</td>
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</tr>
<tr>
<td><strong>Belimbing buluh</strong> / <em>Averrhoa bilimbi</em> <em>L</em>&lt;br&gt;The whole flowers are pounded and mixed with rock sugar are consumed to relieve coughs and sore throat</td>
<td><img src="image1.png" alt="Picture of Belimbing buluh flowers" /></td>
</tr>
<tr>
<td><strong>Penggaga; pegaga</strong> / <em>Centella asiatica</em> <em>(L)</em> <em>Urb</em>&lt;br&gt;Young leaves are eaten as vegetables either cooked or raw. The leaves can be boiled and consumed to prevent or cure urinary tract infection and stones.</td>
<td><img src="image2.png" alt="Picture of Penggaga leaves" /></td>
</tr>
<tr>
<td><strong>Rancah-rancah</strong> / <em>Cosmo caudatus</em> <em>Kunth.</em>&lt;br&gt;For treating gaseous stomach and mild gastric pains, the young leaves and shoots have been eaten as a raw vegetable.</td>
<td><img src="image3.png" alt="Picture of Rancah-rancah plant" /></td>
</tr>
<tr>
<td>Local Name(s) / Scientific Name / Uses</td>
<td>Pictures</td>
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</tr>
</tbody>
</table>
As a remedy for backache, the roots and shoots are boiled and drunk. | ![Picture](image1.png) |
| **Ciku** / *Manikara achras* L.  
The unripe fruit is pounded, mixed with some warm water and taken orally for diarrhoea. | ![Picture](image2.png) |
| **Penawar teratau** / *Morus alba* L.  
A decoction of the leaves is drunk to treat stones in the kidney or in the urinary bladder. Infusion of the roots has been consumed both as a preventive measure and as an antidote against chemical poisoning. | ![Picture](image3.png) |
<table>
<thead>
<tr>
<th>Local Name (s) / Scientific Name / Uses</th>
<th>Pictures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lingiruh; langiruh / Portulaca oleracea L.</strong>&lt;br&gt;The whole herb is boiled and eaten to relieve high blood pressure.</td>
<td><img src="image1.jpg" alt="Lingiruh" /></td>
</tr>
<tr>
<td><strong>Keramunting / Rhodomyrtus tomentosa (Ait.) Hassk</strong>&lt;br&gt;The leaves are pounded and the juice is taken orally to alleviate anaemia. The fresh fruits are eaten for the same purpose and they are also applied externally to accelerate wound healing.</td>
<td><img src="image2.jpg" alt="Keramunting" /></td>
</tr>
<tr>
<td>Local Name (s) / Scientific Name / Uses</td>
<td>Pictures</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>----------</td>
</tr>
<tr>
<td><strong>Buah delima / Punica granatum L.</strong></td>
<td><img src="image" alt="Buah delima" /></td>
</tr>
<tr>
<td>The remains of the sepals on the fruits are some times ground, infused and drunk as an antidote against any poison ingested, such as poisonous mushrooms, agrochemical or food poisoning.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ubi gadong; ubi belayar / Dioscorea hispida Dennst.</th>
<th><img src="image" alt="Ubi gadong" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>The tuber is washed and then scraped into a small clay-pot of water. An egg is put into the water which is then brought to the boil. The cooked egg is consumed as a traditional remedy for piles.</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Peria / Momordica charnatia</th>
<th><img src="image" alt="Peria" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>It helps to clean blood, reduce blood sugar for diabetes. It was thought to contain plant insulin. It helps to kill nematodes in the stomach of a child by drinking leaf extract after adding salt and water. Skin rashes, burns, and stomach pain and headache can be reduced by crushing leaves in both palms. It removes toxins in the body. Its roots can be used for breathing difficulties such as bronchitis and colds by grinding the roots with honey into paste. It reduces the cholesterol level in the blood.</td>
<td></td>
</tr>
<tr>
<td>Local Name (s) / Scientific Name / Uses</td>
<td>Pictures</td>
</tr>
<tr>
<td>----------------------------------------------------------------------------</td>
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</tr>
<tr>
<td><strong>Serai wangi</strong> / <em>Cymbopogan nardus</em></td>
<td><img src="image1.jpg" alt="Image" /></td>
</tr>
<tr>
<td>Good for killing nematodes in the stomach and colds.</td>
<td></td>
</tr>
<tr>
<td><strong>Serai</strong> / <em>Cymbopogan flexuosus</em></td>
<td><img src="image2.jpg" alt="Image" /></td>
</tr>
<tr>
<td>Aids digestion, increases secretion of bile, prevents flatulence, helps to reduce blood pressure and acts as a sedative.</td>
<td></td>
</tr>
<tr>
<td><strong>Langkuwas</strong> / <em>Lesser Galangal</em></td>
<td><img src="image3.jpg" alt="Image" /></td>
</tr>
<tr>
<td>Fragrant breath, good digestion, relief of flatulence and stimulation of carnal desires. Anti-bacteria and skin diseases.</td>
<td></td>
</tr>
<tr>
<td><strong>Sirih</strong> (piper betel)</td>
<td><img src="image4.jpg" alt="Image" /></td>
</tr>
<tr>
<td>Fatigue, colds, antiseptic, anti-aging (squeeze the juice, soak in hot water and use it for washing the face) contraceptive (root added to black pepper), skin fungus.</td>
<td></td>
</tr>
</tbody>
</table>
**Mengkudo / Morinda citrifolia**

Leaves are heated over a small fire and applied to the chest to relieve cough or to abdomen for enlarged spleen. The fruits (ripe or unripe) are used to brush the teeth of children to prevent tooth decay.

---

**Marketing and Distribution of Functional Foods**

Locally produced and imported functional foods are readily available in the shops (pharmacies, supermarkets and convenience stores) and the open markets. Locally produced functional foods are also sold directly to consumers through practitioners or friends’ recommendation.

**Table 2: Functional Food Products Developed from Local Herbal Plants**

<table>
<thead>
<tr>
<th>Local name / Ingredients or uses</th>
<th>Pictures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Madu kasirat:</strong> Contains jintan manis (aniseed), jintan puteh (cuming), serai (lemon grass), lada sulah (white pepper), bawang merah (red onion), beras (rice powder), kelapa perut (desiccated coconut), gula anau (palm sugar). Mix all the dried ingredients. Heat sugar and water until boiling and add the syrup to the dried ingredient and mix well to form dough and put in mould in diamond shape.</td>
<td><img src="image1.jpg" alt="Madu Kasirat" /></td>
</tr>
</tbody>
</table>

**Table 3: Functional Food Products Developed by Fermentation**

<table>
<thead>
<tr>
<th>Local name / Ingredients</th>
<th>Pictures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tapai:</strong> It is made by fermenting rice to a juicy or a partially liquefied paste with sweet taste. It may contain a small amount of alcohol.</td>
<td><img src="image2.jpg" alt="Tapai" /></td>
</tr>
</tbody>
</table>
**Budu kupang:** It is made from mussels’ flesh (*adula schmididdi*), milled roasted rice, bean sprouts and *kepayang*. It is used as an appetizer eaten raw with chilies and lemon juice.

**Cincaluk:** It is made by fermenting tiny prawns called shrimp of the *Acetes* species which are known locally as *bubok*.

**Belacan:** It is made by fermenting shrimp with added salt. The fermented shrimp is pounded to form a cohesive pasty material called *belacan*. 
Belutak: The intestine of either a cow or a buffalo is stuffed with meat trimmings which is mixed with salt and sugar. Belutak is produced after drying the stuffed intestine in the sun.

Budu pakis: It is a fermented Bruneian food made from pakis (Diplazium esculentum) which is a kind of vegetable that grows in the wild. This food contains lactic acid bacteria which has a variety of health benefits such as probiotic, anticarcinogenic, hypocholesterolemic and antagonistic actions against enteric pathogens and other intestinal organisms.

Imported Functional Food Products

Some imported functional food products include:

Tongkat ali (Eurycoma, longi folia): Combats fatigue, increases energy and stamina, antioxidant, improves blood circulation, treats skin diseases, hypertension, diabetes, ulcers and relieves fever.

Propolis: Antiseptic, antibiotic and anti-bacterial and anti-fungal.

Garlic (Allium sativum): The purported health benefits of garlic are numerous. These include cancer chemo preventive, antibiotic, anti-hypertensive, and cholesterol-lowering properties. It also stimulates secretion of digestive juices and bile, soothes the intestine muscle, affects respiratory passages, regulates bacteria in the large intestine, and increases blood flow in the arteries.

Tea: The functional property of tea is due to the presence of polyphenolic constituents, particularly in green tea. The polyphenols comprise up to 30% of the total dry weight of fresh tea leaves. Catechins are the predominant and most significant of all tea polyphenols. The four major green tea catechins are epigallocatechin-3-gallate, epigallocatechin, epicatechin-3-gallate, and epicatechin.
Honey: Digestive system.

Habbatus sauda (*Nigella sativa*): asthma.

**Legislation and Regulation on the Production and Distribution of Functional Food**

In Brunei Darussalam, functional food in the Public Health (Food) Order, 1998 and Public Health (Food) Regulations, 2000 is regarded as food additives and special purpose food. The Department of Health Services within the Ministry of Health, Brunei Darussalam is the lead agency responsible for enforcing these food legislation and regulations.

In these food order and regulations, food additives are regulated by the following:

1. Subject to sub-regulations (2) and (3), no person shall import or manufacture for sale or sell any article of food which contains any food additive which is not permitted by these Regulations.

2. Notwithstanding sub-regulation (1), any food may have in it or it may permit food additive of the description and in the proportion specified under these Regulations.

3. Notwithstanding sub-regulation (1), any food containing as an added ingredient any specified food may contain any such permitted food additive of the description for and of an amount appropriate to the quantity of such specified food in accordance with these Regulations.

4. No person shall import, sell, advertise, manufacture, consign or deliver any permitted food additive unless the purity of that food additive conforms with the specifications as provided in this Part. Where it is not so provided, the purity of the permitted food additive shall conform with the specifications as recommended by the Joint Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO) Expert Committee on food additives.

In addition, special purpose food is defined as food named or described as particularly suitable for consumption by persons belonging to a particular class requiring a special diet. It shall be composed of food substances modified, prepared or compounded so as to possess nutritive and assimilative properties, which render it suitable for use as food by these persons requiring a special diet. In these regulations, special purpose food shall include diabetic food, low sodium food, gluten-free food, low protein food, carbohydrate-modified food, low calorie food, energy food, infant formula food and formulated food. The special purpose food, unless otherwise prohibited under these regulations, may contain vitamins, minerals, amino acids and other nutrient supplements.

Labeling requirements in the Public Health (Food) Order, 1998 and the Public Health (Food) Regulations, 2000 for the food additives and special purpose food, state that every package of special food, unless otherwise exempted, shall bear a label containing a nutrition information panel in the form specified in the Fourth Schedule of the Regulations and adequate information to support any claim made for that food.

**Human Resources in R&D Staff Training in Functional Food**

The Brunei Agricultural Research Centre (BARC) and the Department of Agriculture and University of Brunei Darussalam (UBD) have conducted research and development (R&D)
activities that included the area of functional food. However, R&D activities in this area need to be improved and manpower increased. At present researchers and technical staff in both institutions, BARC and UBD, are continuously being encouraged and trained in functional food related areas through participation in workshops, seminars, conferences and short courses held in the country and outside Brunei Darussalam both in the region and at international forums. Some students at UBD (in the Chemistry and Biology Departments) are also encouraged to conduct research projects in functional foods so as to increase awareness and exposure among students in this field.

In Brunei Darussalam’s 8th National Development Plan, human resource development (HRD) will continue to be one of the main thrusts of the Plan in line with the goals and aspirations for stronger, stable, sustainable, integrated and balanced socio-economic development. In an effort to expand the capability of science and technology (S&T), including in the field of functional food, education and training will continue to be widened and strengthened through various measures such as:

- gradually increased intake of manpower and expertise in R&D of functional food,
- better incentives for local researchers, technical staff and students to take up R&D activities in functional food,
- provision of more vocational/technical institutions to overcome the shortage of technical and vocational workers in both the public and private sectors
- upgrading and increase of R&D facilities,
- upgrading career guidance and counseling to give advisory services to students on career opportunities, particularly in the field of science and technology,
- strengthening the participation of students, researchers and technical staff at science exhibitions, conferences, workshops etc. in functional food and related areas.
- strengthening capabilities by participation in collaborative projects and research linkages within and outside the country

References


Department of Agriculture, Ministry of Industry and Primary Resources, Brunei Darussalam. 2000. Medicinal Plants of Brunei Darussalam.


BIOMONITORING AND BIOSORPTION STUDIES ON TWO SEAWEED SPECIES FROM BRUNEI DARUSSALAM

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*This project was undertaken as a partial fulfilment of the requirement for the Bachelor of Science Education Degree.
**Email address: rodney@fos.ubd.edu.bn

Abstract: The brown seaweed Padina and the green seaweed Acetabularia were harvested from the eastern coastline area known as Tanjung Batu in the Brunei – Muara district (see figure 2). Copper (Cu), zinc (Zn), cadmium (Cd), iron (Fe), and lead (Pb) content of these two species were determined using atomic absorption spectrophotometry, as a prelude to using them as potential biomonitors of heavy metal pollution. In both seaweeds, metal content sequence was observed to follow the trend Fe > Zn > Cu > Cd and Pb was not detected. Biosorption characteristics of the dried biomass were investigated under hydrodynamic conditions. It was found that adsorption of cadmium from dilute aqueous solutions by the native biomass resulted in the adsorption of hydrogen ions as well and the release of other non-toxic light metal ions. The brown seaweed Padina showed a higher potential for adsorption of cadmium compared to the green seaweed Acetabularia, with a maximum uptake capacity of 191.1 µg g\(^{-1}\) of dried weight of Padina. The adsorption data were interpreted using the Langmuir and Freundlich adsorption isotherms. This study clearly shows that the readily available local seaweeds such as Padina may be used as a potential biomonitor as well as a cadmium adsorbent from aqueous industrial waste effluents.

Keywords: Biosorption; Biomonitoring; Seaweed; AAS; Heavy metal; Isotherm

1. Introduction

Seaweeds, which are considered as marine macro algae, have been classified into three broad groups according to their pigmentation: brown (Phaeophyta), red (Rhodophyta), and green (Chlorophyta). The brown and red seaweeds are exclusively found in seawaters whereas the green algae are marine as well as freshwater and terrestrial. The colour of the brown seaweeds can be attributed to the dominance of the xanthophyll pigment fucoxanthin over other pigments. The pigment phycoerythrin of the red seaweeds absorbs the blue radiation while reflecting red. Chlorophyll a and b are responsible for the green colour of Chlorophyta.

Seaweed has been used as food by the Japanese since the fourth century (FAO, 2004). At present, Japan, China, and Korea are the biggest consumers of seaweed as food. It is estimated that six million tonnes of wet seaweeds are harvested annually for human and animal consumption with an annual turn-over value of around US dollars five billion. Seaweeds are also used in cosmetics, fertilizers, and in the production of hydrocolloids such as agar, alginate, and carrageenan. Seaweeds are ecologically important as well, as they supply oxygen to the sea, and act as one of the primary producers in the marine food chain.
Marine organisms have been commonly used as bioindicators for heavy metal pollution, which is considered as an environmental problem of worldwide concern (Rainbow 1995; Chan et al 2006). Historically, fish and invertebrates have been used to monitor contaminants in the aquatic environment. Recent reports show however that algae and molluscs are also utilized in biomonitoring exercises (Rainbow 1995; Conti and Cecchetti 2003). Seaweeds are able to bioaccumulate heavy metals up to concentrations that are many times higher than the corresponding concentrations in sea water and do not undergo short term concentration fluctuations (Bryan and Langston 1992). Lead (Pb), copper (Cu), cadmium (Cd), zinc (Zn), and nickel (Ni) can be regarded as the most common heavy metal pollutants. The toxicity of these metals to organisms, including humans, is well documented (ATSDR Public Health Statements). Some of these metals are considered to be toxic even at ppb (parts per billion) concentrations. For example, lead poisoning can damage the reproductive and nervous systems, particularly in children. Cadmium poisoning can cause renal dysfunction and bone degradation. Copper and zinc are required by humans at trace levels but higher concentrations can cause harmful health effects (ATSDR Public Health Statements).

Seaweeds are known to bioaccumulate only free metal ions, which could come from solution, suspended particulate matter, sediment, and phytoplankton. The suspended particulate matter could be organic or inorganic complexes. The uptake of metals may differ between seaweed species e.g. brown seaweeds are considered to be better bioaccumulators of metals than their green counterparts. It is quite evident that this metal regulation is species-specific and it is highly unlikely that it is possible to identify a particular seaweed species which could be used as a universal bioindicator, capable of assessing heavy metal contamination under all natural conditions (Volterra and Conti 2000). The advantages of using seaweeds as biomonitors clearly out number the few disadvantages as mentioned above. Hence seaweeds fulfill all the important criteria necessary for suitable biomonitoring of metal pollution in the marine environment. Also seaweeds are sedentary, large, robust, easy to handle in laboratory experiments, easy to collect from different geographical locations, and further, biosorption and desorption kinetics of some seaweed species have been well established (Rainbow et al 2002). Another major advantage of using seaweeds as biomonitors is the fact that they are widely distributed and hence any studies can be readily compared with data from various regions. Objectives of this project were: (i) to compare the heavy metal content of Padina and Acetabularia which are distributed in the coastal environment of Brunei Darussalam, (ii) to compare these findings with the data from studies conducted elsewhere in the Asean region as no such studies have been reported on Bruneian seaweeds, and (iii) to assess the capability of the two seaweeds involved in this study as possible biomonitors.

The second component of this project was to investigate the adsorption and desorption characteristics of the dried biomass of the two seaweeds Padina and Acetabularia in order to estimate the effectiveness of these two seaweeds as possible biosorbents of heavy metals. The removal of heavy metals from polluted aquatic environments is of immense importance to producing ‘clean’ drinking water. Heavy metal toxicity is a major threat to plant and animal life on earth as well as in the aquatic environment. This objective of removing of metal ions is generally achieved by using treatment methods such as precipitation, ion exchange, and adsorption. On the other hand, recovery of certain essential and precious metals from industrial waste for recycling purposes is becoming increasingly important. Even though Brunei Darussalam is virtually free from anthropogenic sources of heavy metals such as electroplating, semiconductor manufacturing, etc., it is difficult to rule out the possibility of future industrialization and the need to manage heavy metal pollutants. Another aspect which requires much attention is the cost-effectiveness as well as the efficiency of the treatment methods for
the removal of heavy metals. Biosorption, which can be considered as the ability of living and non-living, both microbial and plant, biomass to sequester metal ions from aqueous medium by physicochemical means, has been widely researched as a possible technology for sequestering trace level of heavy metals from dilute aqueous solutions. Particularly, the use of dead marine macro algae biomass, which was shown to be more effective than living cells, for removal of metal ions has attracted much attention over the last two decades (Sheng et al 2004). The seaweed biomass has a relatively high adsorption capacity for metals and also they can be harvested in abundance from many parts of the world’s marine environment.

The biosorption capability of seaweeds has been related to the presence of charged polysaccharides in the cell wall matrix, which contain alginates and sulphated fucans, and also to the polyphenolic substances in the brown seaweed physodes. The polyphenols could be significant when assessing the metal adsorption characteristics as they have shown different affinities to different metals and also there is a notable increase in polyphenol content particularly in brown seaweeds with age and salinity (Foresberg 1987). It has been reported that alginates are found both within the cell wall and in the intercellular substance. The mass composition of alginates in dry biomass is estimated to be about 40% (Sheng et al 2004). Alginates and alginic acid have a high affinity for divalent cations (Chan et al 2002). The biosorption of trivalent metal ions has been linked to the presence of sulphated polysaccharides in the cell walls of the seaweeds, particularly in brown seaweeds (Figueira et al 1999).

Alginic acid for commercial purposes is extracted from brown seaweeds and is a hydrophilic colloidal polysaccharide. It is a linear copolymer consisting mainly of $\beta - 1,4$-linked D-mannuronic acid and $\alpha - 1,4$-linked L-glucuronic acid with the chemical formula $(C_6H_8O_6)_n$ and an average $n$ value of 200. Alginate absorbs water, which makes it useful as an additive in dehydrated products such as slimming aids, and is used in the manufacture of paper and textiles. It is also used for waterproofing and fireproofing fabrics, as a gelling agent, thickening agent for drinks, ice cream and cosmetics, and as a detoxifier that can adsorb poisonous metals from the blood.

This project was conducted to assess the biomonitoring and biosorption potentials of two local seaweeds. The biosorption capacity and capability of the dried biomass was investigated with cadmium (II) as the adsorbate at a pH of 5.8 under hydrodynamic conditions. An equilibrium isotherm was constructed from the results obtained with different concentrations of cadmium nitrate. Langmiur and Freundlich isotherms have been used to model the adsorption data obtained for the brown seaweed Padina.

2. Materials and Methods

2.1 Sampling and sample pre-treatment

The seaweeds Padina and Acetabularia were collected on the August 08, 2006 from coastal rocks at Tanjung Batu in the Brunei – Muara district (figure 2). It was noted that there had been hardly any anthropic activities in this coastal area prior to the collection date. Padina (figure 1(a)) samples were hand picked from rock surfaces during a low-tidal period. Acetabularia (figure 1(b)) appears to grow virtually on any surface (e.g. wood, rubber, small scattered rocks). The samples were washed in seawater at the point of collection, transported in a closed plastic container, and refrigerated in pre-cleaned polyethylene bags. The seaweed samples, particularly Padina, were thoroughly cleaned and any epibionts and sediments were manually (using rubber gloves to avoid contamination) removed under tap water within 3-5 days of collection. Cleaned seaweed materials were thoroughly rinsed (at least three times) in excess deionized water to
remove extraneous materials and common ions such as Na$^+$ and Ca$^{2+}$ present in sea water. This was followed by cleaning in an ultrasonic bath (Walker Ultrasonics) for 12 minutes. The samples were then oven-dried at 50°C for 24 hours before pulverizing to particles of size fraction 355 – 850 μm. Seaweed particles in this range were thoroughly mixed in order to make the sample homogeneous. The ground biomass was stored in a vacuum desiccator in sealed polyethylene bags. The mass of the algal samples were recorded before and after drying and the water content was determined.

**Figure 1:** Pictures of algae, (a) *Padina* and (b) *Acetabularia.*
(Taken from Google Images: [http://jrscience.wcp.muohio.edu/Photos/Padina.jpeg](http://jrscience.wcp.muohio.edu/Photos/Padina.jpeg) and [http://www.biol.tsukuba.ac.jp/~inouye/ino/g/ulv/Acetabularia5.jpg](http://www.biol.tsukuba.ac.jp/~inouye/ino/g/ulv/Acetabularia5.jpg))
Figure 2: Map of Brunei Darussalam (inset) and a close-up map of the Tanjung Batu area showing the sampling site indicated by the arrow.
2.2 Sample digestion

All glassware were pre-cleaned in an ultrasonic bath for 30 minutes and rinsed in deionised water. All chemicals used were of AnalaR grade. The biomass was digested using various concentrated acid ratios as given below: (a) 5:1 mixture of HNO₃:HClO₄, (b) 3:1 mixture of HNO₃:HClO₄, and (c) 5:1:0.5 mixture of HNO₃:HClO₄:H₂SO₄. The mixture (c) was found to give the best results, digesting the samples completely and vigorously. Algal samples (about 0.2000 g) were digested, and the resulting solutions were diluted with deionized water, filtered (Whatman No. 42) and the volume adjusted to 50.00 mL with 6 M nitric acid.

2.3 Determination of heavy metals

The solutions resulting from 2.2 were analysed for Cd, Cu, Fe, Pb and Zn using a Shimadzu Atomic Absorption Flame Emission Spectrophotometer (AA-6701) in absorption mode using BGC-SR (background correction – self reversal). The calibration curve for Cd, Cu, Fe and Zn were carried out using twelve, six, seven and five points respectively. The concentration ranges of the standard solution were between 0 – 3.00 mg L⁻¹ for Cd, 0 – 2.00 mg L⁻¹ for Cu, 0 – 10 mg L⁻¹ for Fe and 0 – 2.00 mg L⁻¹ for Zn, which are in the linear dynamic range of the instrument. The characteristic wavelength and sensitivity for the various metals are shown in Table 2.1.

Table 2.1: The characteristic wavelengths and detection limits for various metals used for AAS measurements.

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength (nm)</th>
<th>Bandwidth (nm)</th>
<th>Sensitivitya (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>228.8</td>
<td>0.5</td>
<td>0.032</td>
</tr>
<tr>
<td>Cu</td>
<td>324.8</td>
<td>0.5</td>
<td>0.041</td>
</tr>
<tr>
<td>Fe</td>
<td>248.3</td>
<td>0.2</td>
<td>0.060</td>
</tr>
<tr>
<td>Pb</td>
<td>217.0</td>
<td>0.5</td>
<td>0.100</td>
</tr>
<tr>
<td>Zn</td>
<td>213.9</td>
<td>0.5</td>
<td>0.013</td>
</tr>
</tbody>
</table>

a recommended by the manufacturer

2.4 Biosorption studies

Cadmium nitrate solutions of varying concentrations in the range 0.02 – 3.11 mmol dm⁻³ were prepared. 50.00 mL of cadmium nitrate solutions were pipetted into 250 mL conical flasks with lids, and 0.1000 g of dried biomass was added to these solutions. These flasks were shaken at 300 rpm (Edmund Buhler KL-2) for 4 hours at room temperature (22 ± 1)°C. The cadmium concentrations of the agitated solutions were determined using AAS as described in 2.3. The initial and final pH values were recorded.

The amount of the cadmium ions biosorbed (q/mmol g⁻¹ of dried biomass) for the system described above was calculated using the mass balance equation
\[ q = \frac{V(C_i - C_f)}{w} \]

where \( V \) = volume of solution (mL) [Note: it was assumed that there is no change in volume during the experiment], \( C_i \) = initial concentration, \( C_f \) = final concentration, \( w \) = amount of dried biomass used.

3. Results and Discussion

3.1 Water content in seaweed samples

The mean (± 1 S.D.) water content in the Padina and Acetabularia samples were (86.8 ± 1.3) % and (87.2 ± 0.5) % respectively.

3.2 Heavy metal content in seaweeds

The concentrations of Cd, Cu, Fe and Zn, in the solutions obtained by digestion of seaweed samples, were determined by AAS. The amount of each metal per gram of dried seaweed can be calculated using the equation

\[ C = \frac{C_{\text{AAS}} \times V}{m} \]

where \( C \) = concentration of metal ion (μg g⁻¹ d.w.), \( C_{\text{AAS}} \) = concentration determined from calibration curve (μg mL⁻¹), \( V \) = volume of solution (mL) and \( m \) = mass of dried seaweed sample.

The mean (± 1 S.D.) concentration of Cd, Cu, Fe and Zn for Padina and Acetabularia are presented in Tables 3.1. and Figure 3. The concentration of Pb is not listed in the Tables since it was below the detection range of the AAS instrument.

Table 3.1: Average concentration of metals (Mean ± Standard deviation) in Padina and Acetabularia.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Padina</th>
<th>Acetabularia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average concentration (μg g⁻¹ d.w.)</td>
<td>N*</td>
</tr>
<tr>
<td>Cd</td>
<td>10.8 ± 1.1</td>
<td>8</td>
</tr>
<tr>
<td>Cu</td>
<td>151.5 ± 12.8</td>
<td>7</td>
</tr>
<tr>
<td>Fe</td>
<td>4121 ± 124</td>
<td>6</td>
</tr>
<tr>
<td>Zn</td>
<td>223.1 ± 48.7</td>
<td>8</td>
</tr>
</tbody>
</table>

* number of samples analysed

In the Padina species, the following trend was observed where the mean metal content of Fe >> Zn > Cu > Cd. Also, in Acetabularia a similar trend was observed and the mean metal
content of Fe > Zn > Cu > Cd. The different algae species generally have different capacities to accumulate metals. The concentration of copper and zinc in the brown seaweed *Padina* was greater than the green seaweed *Acetabularia*, and the concentration of Fe in the *Padina* was much greater than in *Acetabularia*. However, the concentration of cadmium in both seaweeds did not differ by a significant margin (Figure 3). The high concentration of Fe in both seaweeds investigated in this study, particularly the *Padina*, could be attributed to the scavenging from particulate materials as was suggested elsewhere (Malea et al 1995). In general, the concentration of trace metals in *Padina* is greater than that of *Acetabularia* according to our study as well as by others (Campanella et al 2001). Therefore it may be generalized that the Phaeophyta showed a greater tendency to concentrate heavy metals compared to the Chlorophyta, indicating that brown seaweeds would be a better biomonitor compared to green macro algae (Malea et al 1995; Campanella et al 2001). The values given in table 3.1 have not been presented to correct number of significant figures as the scatter of experimentally determined values could well be due to the inhomogeneous nature of the samples used for analysis as discussed below, rather than entirely due to experimental shortcomings.

The complete removal of all the epibionts and epiphytes present, particularly in *Padina*, was very difficult and this could have contributed to the fluctuation in the concentrations of the heavy metals observed, particularly zinc. Another possible reason may be the ages of the seaweeds collected. It has been reported that the metal content in the brown seaweed *Ascophyllum nodosum* is directly related to the age since the phenol content increases with the age of the plant (Foresberg et al 1987). If this is also applicable to the seaweed samples collected locally, especially *Padina*, then the concentration of certain metals would be expected to be higher in some of the older species collected than the younger ones and thereby causing variation in metal contents in different samples. In addition, the degree of exposure to the seawater can also lead to some variation since during low tide and high tide some of the seaweed may not be exposed to seawater whereas others are submerged. Other factors which may cause fluctuations in metal content may be to salinity and dynamic factors such as water movement, currents, and winds which may result in differing degrees of metals accumulation. This could be tested by comparing seaweeds from different geographical locations, however since the species were all collected from the same general area, these factors may not play such an important role compared to the others mentioned.

Although metal uptake and the use of macro algae as bioindicators have been widely investigated in various parts of the world, no such information could be found in the literature for the South Asean region, despite the fact that ‘seaweed as food’ industry has been growing in countries such as Thailand, Indonesia, and Philippines. We believe that the determination of the metal content of various seaweeds, particularly the ones used as food, is imperative to health concerns. In addition to this aspect, biomonitoring by seaweeds along the coastal line of Brunei Darussalam can be undertaken as a baseline study and for comparison purposes for the future industrialization possibilities. The present study needs to be extended to include geographical and seasonal parameters as well as the types of species. Another notable feature to come out of this study is that no lead was detected at the detection limit of the instrument. The presence of lead in seaweeds, particularly in *Padina*, has been reported for contaminated (13.2 to 525 µg g⁻¹ of dry weight) and uncontaminated (0.07 µg g⁻¹ of dry weight) seawater (Malea et al 1995; Campanella et al 2001). The lead content of various seaweeds of Brunei Darussalam needs to be established beyond any reasonable doubt before initiating any seaweed based industries, even at a cottage level.
3.3 Biosorption studies

In general brown macro algae have shown a greater capacity to biosorb cadmium (II) as well as other bivalent metal ions, compared to green and red algae (Sheng et al 2004). As a consequence of this, biosorption characteristics of Padina were investigated using cadmium (II) as the adsorbate. The solid-liquid equilibrium that exists between the dilute cadmium solutions and the biomass can be modelled using the Langmuir and Freundlich isotherms.

The Langmuir isotherm may be expressed mathematically as

\[
q = \frac{Kdc}{1 + Kc}
\]

where \( q \) = amount of the cadmium ions biosorbed, \( c \) = equilibrium concentration, and \( k \) and \( d \) are Langmuir parameters. \( K \) provides an indication of the affinity between the adsorbent and the adsorbate (cadmium ion) and \( d \) reflects the maximum biosorption capacity of the biomass at monolayer level. A plot of \( q \) against \( c \), generally produces a curve which is linear at low equilibrium concentrations then begins to curve (concave to the x-axis) with an asymptotic tendency towards saturation which corresponds to monolayer coverage of the surface. The Langmuir equation may be arranged into the linear form

\[
\frac{1}{q} = \frac{1}{Kdc} + \frac{1}{d}
\]
Whereby a plot of $\frac{1}{q}$ versus $\frac{1}{c}$ can be used to determine the parameters, $K$ and $d$ which can be calculated from the angular and linear coefficients respectively.

The Freundlich isotherm is an empirical formula which can be expressed as

$$ q = Kc^{1/n} $$

where $q$ and $c$ has their usual meaning and $K$ and $n$ are parameters. The Freundlich equation can also be expressed linearly as

$$ \log q = \frac{1}{n} \log c + \log K $$

A plot of $\log q$ versus $\log c$ is a straight line where the parameter $n$ and $K$ can be determined from the angular and linear coefficients respectively.

In order to evaluate the Langmuir and Freundlich parameters, the linear forms of both isotherms were plotted and are shown in Figures 4 and 5. The fitting equation and the parameters for the Langmuir and Freundlich are shown in Tables 3.6 and 3.7 respectively.

![Figure 4: Linear plot for Langmuir isotherm.](image-url)
Figure 5: Linear plot for Freundlich isotherm.

Table 3.6: Langmuir linear fitting equation and parameters for Padina.

<table>
<thead>
<tr>
<th>Langmuir linear fitting Equation</th>
<th>$K$ / mol$^{-1}$ L</th>
<th>$d$ / mmol g$^{-1}$ d.w.</th>
<th>Correlation ($R^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\frac{1}{q} = (0.40 \pm 0.03) \frac{1}{c} + (600 \pm 200)$</td>
<td>$1500 \pm 100$</td>
<td>$1.7 \pm 0.6$</td>
<td>0.9859</td>
</tr>
</tbody>
</table>

Table 3.7: Freundlich linear fitting equation and parameters for Padina.

<table>
<thead>
<tr>
<th>Freundlich linear fitting Equation</th>
<th>$K$</th>
<th>$n$</th>
<th>Correlation ($R^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\log q = (0.57 \pm 0.07) \log c - (1.3 \pm 0.3)$</td>
<td>$0.05 \pm 0.01$</td>
<td>$1.8 \pm 0.2$</td>
<td>0.9558</td>
</tr>
</tbody>
</table>
The purpose of this biosorption study is to assess the suitability of the locally harvested and readily available seaweed *Padina* as a possible heavy metal scavenger. Even though our study at the moment is limited to the cadmium (II) adsorption work, this can be easily extended to include other divalent (e.g. lead, zinc, nickel, etc.), trivalent (e.g. lanthanum), and hexavalent (e.g. chromium) metals, as these metals are also likely to be discharged in industrial waste effluents. It was reported recently (Royal Society of Chemistry 2006) that waste seaweed material (leftover material after alginates are acid and alkali extracted) from the alginate industry could be used as an efficient biosorbent of many heavy metal ions. Another positive aspect of this heavy metal removal procedure is the fact that desorption of these adsorbed metal ions can be achieved by a simple change of pH. Hence these adsorption-desorption cycles present a simple and reliable yet inexpensive and time-efficient method of recovering and concentrating trace and precious metals present in industrial waste matter.

Our work is still at a preliminary stage and the desorption aspects of cadmium biosorption by *Padina* is yet to be explored under controlled pH conditions. It was noted that during the cadmium uptake process, the pH of the solution changed from 5.8 to about 7.2, indicating the loss of H\(^+\) either due to adsorption by the biomass or due to the release of OH\(^-\). A mechanism for the release of OH\(^-\) from the biomass is very difficult to justify. This is another aspect that will be investigated further. The biosorption under controlled acidic conditions would shed some light on this problem. It is likely that positively charged hydrogen ions could be competing with metal ions for the available adsorption sites in the biomass and these adsorption sites are generally the surface functional groups on the cell walls of the biomass such as weakly acidic carboxyl groups.

The biosorption equilibrium data obtained for cadmium adsorption on *Padina* biomass was modelled using the Langmuir and Freundlich adsorption isotherms and the derived parameters are shown in Tables 3.6 and 3.7. The interpretation of Freundlich constants have not been considered in this communication as more data are required for the system under investigation. The maximum adsorption capacity, d, was found to be \((1.7 \pm 0.6)\) mmol g\(^{-1}\) of dry weight from the Langmuir plot. This constant depends on certain experimental parameters such as temperature and pH. This value for *Padina*, collected from the Singapore coast, was reported to be 0.75 mmol g\(^{-1}\) (Sheng et al 2004). It appears that the Bruneian *Padina* somehow has better adsorption capacity for cadmium than its counter parts in Singapore. The cadmium content of *Padina*, for that matter any metal content of any seaweeds of Singapore could not be found from the literature.

As a concluding remark, it would be justifiable to state that the seaweeds harvested from the coastal stretch of Tanjung Batu area could be utilized for fingerprinting any possible heavy metal distribution along the Bruneian coast. This aspect of biomonitoring requires the current project to be extended to other geographical locations as well as to other marine algae. The second objective of this work, to use the Bruneian seaweed biomass as a possible biosorbent for heavy metal scavenging, also needs further exploration, even through our results clearly show encouraging signs.

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References


