Biochemical Composition and Heavy Metal Content in the Mussels of Kadiyapattinam, Kanyakumari district, South West Coast of India

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Abstract

Samples of the green mussel, *Perna viridis*, the brown mussel *Perna perna* and the parrot mussel were collected from Kadiyapattinam of Kanyakumari coast and their biochemical composition and the levels of heavy metal contamination were analyzed. The results of this study showed that the biochemical composition of the mussels did not vary much. The specimens of all the three species of mussels were found to be good aquatic invertebrates for human consumption. *P. viridis* contained higher proportion of protein than *Perna perna* and the parrot mussel. *Perna perna* and the parrot mussel contained more lipid, ash, carbohydrate, and fibre; and their caloric values were also more than that of *Perna viridis*. *Perna perna* had the highest moisture content (78.21%) followed by the parrot mussel (19.47%) and *Perna viridis* (20.30%). Concentrations of the heavy metals (Cd, Cu, Pb, Cr, As, Ni and Se) in the edible portions of the samples were within the values recommended by the WHO. Mercury was not detected and so these mussels are safe for the consumers. The order of concentrations of minerals found in the mussel samples was: K > Na > P > Ca > Mg > Fe > Zn > Mn. Potassium content was more than the other elements. It was evident from the study that in the soft tissue of all *Perna* species the concentration of essential minerals was higher than the non-essential metals. Kadiyapattinam mussels are a rich source not only of protein but also of all the other micro and macro minerals. The soft tissues of all the mussels had low levels of heavy metal contamination, and this indicates a comparatively lower heavy metal contamination of Kadiyapattinam coast. Mussels are also a rich source of nutritional components and could be used as human diet in place of other fish so as to reduce the pressure on sea foods.

Introduction

Mussels of the genus *Perna* (Philipsson, 1788) belong to Mytilidae or true mussels (Mollusca; Bivalvia; Lamellibranchia; Mytiloida; Mytilidae). This genus contains green mussels (*Perna viridis*), green lipped mussels (*Perna canaliculus*) and brown mussels (*Perna perna*). The brown mussel of India was named as *Perna indica* [1] but recently it is identified as *Perna perna* [2]. Mussels are well-distributed in the tropical, subtropical, warm temperate and cold temperate regions, but mostly in the southern hemisphere; however they are also found in northern Africa and in the northern coasts of South America [3]. In India both green and brown mussels are widely distributed in the east and west coasts of India along the inter-tidal zones [4]. These mussels are both ecologically and economically important throughout their ranges, and they have long constituted an important source of human food [5]. With an annual production of 10,060 tons, worth of USD 1.79 million, India has found a place among the top 10 mussel producing nations in Asia [6].
Kanyakumari district is situated at the southernmost tip of the Indian peninsula with a total coastline of 68 km, of which 58 km being along the western coast and 10 km along the eastern coast. There are massive mussel beds on the rocky coastal waters [1] and mussel fishery is regularly carried out from September to March at many centers which have mussel beds in submerged or partially submerged rocks in the near shore waters [7]. Distribution of mussels is limited to a coastal stretch of about 150 km, roughly between Kanyakumari and Kollam. The major mussel-fishing centers of the southwest coast of India are located between Kovalam to Pulinkudi in Trivandrum district of Kerala state; and at Enayam, Colachel and Kadiyapattinam in Kanyakumari district of Tamil Nadu state [8]. The Asian green mussel, *P. viridis* and the brown mussel *P. perna* are the two varieties that are much prevalent in the Indian waters, of which the latter species is more abundant. Globally a few more species have been reported such as the green-lipped mussel *P. canaliculus* of New Zealand, *P. dentifera*, the European mussel *P. nucleus*, *M. galloprovincialis* and the black mussel *Choro-mytilus meridionalis* [9]. The most abundant mussel species in Kanyakumari district is the brown mussel *P. perna* and it is found in the rocky beaches of the southwest coast of India [10]. More mussel fishing is practiced in the rocky coastal areas, and the price of mussels varies in different coastal localities and faraway places. The people of the coastal villages and the nearby towns consume the mussel meat but in other regions the benefits of mussel are hardly known.

Knowledge of the nutritional composition of any edible organism is very essential to understand its nutritive values, for these values are but reflection of the biochemical contents of the organism [11]. The demand for protein-rich food is increasing, especially in the developing countries, which in turn stimulates the exploration of unexploited and/or non-traditional resources. Marine molluscs are an inexpensive source of nutrients with a high biological value. The wider application values of these bivalves as food and industrial input, medicine, decoration and in pearl building necessitate the study on their ecology, morphology, growth, adaptation and productivity.

Mussels are also considered to be good bio-indicator species of aquatic environment and are widely used to determine the metals load. The heavy metals are pollutants which could enormously harm the aquatic ecosystems [12]. Industrialization, urbanization and non-stop human activities along the coasts contribute to the deposition of more heavy metals in the environment [13]. Anthropogenic wastes including toxic metals from earth surface can enter the ocean through runoffs [14]. Such an eventuality is of great concern worldwide [15] for the introduction of more heavy metals into the ocean will alter the properties of marine environments and expose the marine organisms to heavy metal-induced problems, and will render the consumption of seafood a dubious affair [16]. As the content of heavy metals in bivalve could reach an alarming level, the bioaccumulation of heavy metals in bivalve has been extensively studied [17]. According to the World Health Organization (WHO), heavy metals contamination must be controlled in order to ensure public safety [18]. Therefore, during the last four decades the detection of trace elements in living organisms has assumed greater importance [19]. In fact, heavy metals are natural trace components of the aquatic environment, but their levels have increased due to domestic, industrial, mining, and agricultural activities [20][21].

Along the South Indian coasts, two commercially important mussels have been recorded viz. the green mussel *Perna viridis* [22] and the brown mussel *Perna perna*. The brown mussel has a wider distribution and the green mussel has a very restricted distribution along the Kanyakumari coast. Apart from these two, a morphotype of mussel with light yellowish green shell has been observed and it is locally called the parrot mussel [23]. Padi [24] also reported the existence of an intermediate morphotype of mussels from Indian coast. Based on a study using molecular tools [23] reported that the parrot mussel is only a morphotype of the brown mussel and not a true hybrid of *P. viridis* and *P. perna*. Only recently, after the importance of mussel culture in India had been realized, the subject of the biochemical compositions of the mussels began to receive considerable attention. The present study proposes to carry out a nutritional analysis of the mussels *Perna perna*, the parrot mussel and *Perna viridis* in order to understand the biochemical compositions of these species; to give a comparative account of their biochemical makeup so as to bring out the differences and similarities among them; and also to estimate the extent of metal accumulation in the specimens of these species collected from Kanyakumari coasts. In other words the present investigation attempts to provide a complete nutritional picture of *Perna viridis*, *P. perna* and the parrot mussel.
Materials and Methods

Sample collection

Experimental mussel were collected from Kadiyapattinam coast (80° 07’ 57” N 77° 19’ 27” E). Live specimens of *Perna perna*, *Perna viridis* and the parrot mussel were collected with the help of the local fishermen. The size of the collected mussels ranged from 9.0 to 11.5 cm. The collected mussels were wrapped in old newspapers wetted with seawater, and transported to the laboratory in wet condition for further analysis. In the laboratory, the mussel samples were kept at room temperature and the shells were opened with a stainless steel knife. Then the meat was removed from the shells and rinsed in tap water. Then the meat was dried in a hot air oven at 40°C for 3 days. The dried sample was homogenized by grinding in a mortar with a pestle and the meat powder was placed in bottles and stored at -20°C or further analysis.

Biometric parameters

Twenty mussels were randomly selected and the length of each was measured using calipers. The mussels were weighed, and the values were noted. Then the specimens were opened by cutting the adductor muscle with a scalpel and the wet meat and shells were weighed separately and the values noted [25].

Biochemical analysis

Moisture of triplicate samples was determined by drying them in an oven at 105°C for 20 h [26]. The protein and lipid contents of the samples were determined according to the method of Lowry et al. [27] and Folch et al. [28]. Total Carbohydrate was estimated by the phenol sulphuric acid method [29]. Ash content (%) was determined by muffle furnace at 550°C for 8 h. Sulphuric acid solution was added to the sample to estimate the content of crude fiber (%). The sample was heated for about 30 min and then filtered using a vacuum filter. 1.25% NaOH solution was added to the acid extract. The sample was heated again for 30 min and filtered using a vacuum filter and washed with water. All of the material was then transferred to a crucible and dried for 12 h at 120°C. The crucible was placed into a muffle oven at 550°C for 12 h and the weight of the crucible was recorded. The caloric value (kcal/100 g-1) was calculated by the Atwater factors [30] that is, proteins, 4.0, carbohydrates, 4.0 and lipid 9.0.

Minerals and heavy metals

For each mussel species, 2 g of muscle tissue (wet weight) was weighed and placed in a digestion vessel with 5 ml of concentrated (65%) nitric acid (HNO₃) and 2 ml (30%) hydrogen peroxide (H₂O₂), and the tissue was digested in a hot plate system. Atomic Absorption Spectroscope (AAS) was used to determine the content of heavy metal and mineral in the digested samples. The concentrations were expressed as µg/g wet weight. Atomic Absorption Spectrophotometer was used for the analysis of many of the trace metals like cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), and nickel (Ni), and of the essential elements such as zinc (Zn), iron (Fe), manganese (Mn), magnesium (Mg), potassium (K), sodium (Na) and calcium (Ca). The determination of phosphorus was made by vanadomolybdophosphoric acid method following [31]. The values of arsenic (As) and selenium (Se) were determined by hydride generation coupled with an atomic absorption spectrometry, while the content of mercury (Hg) was analyzed with a cold-vapour atomic adsorption spectrophotometer. The calibration curves were prepared separately for all the metals by running different concentrations of standard solutions. A reagent blank sample was analyzed and subtracted from the samples to correct for reagent impurities and other sources of errors from the environment. Average values of three replicates were taken for each determination.

Statistical analysis

The mean values of the measured variables of *Perna viridis*, *Perna perna* and the parrot mussel were compared to determine the significance of difference between them using independent one-way ANOVA test the level of significance is < 0.05.

Results

Biometric analysis

The mean and standard deviation values of biometric parameters from the study sites are presented in table 1. The biometric analysis showed that the total weight and length of *P. perna* is greater than *P. viridis* and parrot mussel. The total weight and length of *P. perna* collected from Kadiyapattinam coast varied from 10.17 ± 0.48 g and 6.10 ± 1.00 cm. The length and weight of *P. viridis* was 5.10 ± 0.30 cm 8.22 ± 0.43 g and 5.70 ± 0.65 cm and 8.66 ± 0.73 g for parrot mussel. The length and total weight of the *P. perna* specimens were comparatively higher than those of *P. viridis* and the parrot mussel specimens.

Proximate composition

Comparisons of the proximate compositions of the mussel samples are given in table 2. The biochemical composition of *P. viridis* had 73.45% moisture content,
20.30% protein, 2.30% lipid, 2.39% ash, 2.64% carbohydrate, 0.06% fibre and 124.41 k cal/100g⁻¹ caloric values. *P. perna* had 78.21% moisture content, 17.73% protein, 3.38% lipid, 2.40% ash, 3.20% carbohydrate, 0.139% fibre and 130.14 kcal/100 g⁻¹ caloric values. The parrot mussel has moisture of 74.80%, protein of 19.47%, lipid of 2.53%, ash of 2.47%, carbohydrate of 3.11%, fibre of 0.09% and caloric value of 128.69 kcal/100 g⁻¹. The values of all the nutrient constituents were slightly different among the three samples. *Perna viridis* contained higher protein (20.30%) than the parrot mussel (19.47%) and *Perna perna* (17.73%). *Perna perna* showed higher contents of lipid, fibre, and moisture and caloric value than *P. viridis* and the parrot mussel. Higher ash and carbohydrate contents were noticed in the parrot mussel than the other two species. The results of one-way analysis showed that except moisture content all other parameters showed non-significant variation within the groups.

**Relationship between the proximate with biometric analysis**

*Perna viridis* contains relatively higher percentage of protein than *Perna perna* although it has shorter and lighter shell than the latter. However, lipid content is higher in *Perna perna* than in the other two species. The parrot mussel seems to have more ash content than *Perna viridis* and *Perna perna*. The carbohydrate, fibre and caloric contents were less in *Perna viridis* than the other two.

**Essential elements in mussel**

The occurrences of essential elements such as calcium, iron, manganese, magnesium, phosphorus, potassium, sodium and zinc were noticed during the quantitative analysis were represented in table 3. Of the above mentioned minerals, sodium and potassium contents in mussel samples were higher than the other elements. For example, the values of Na and K in *Perna perna* respectively were 1425 µg/g and 578.73 µg/g; in the parrot mussel the values were 1422 µg/g and 547.57 µg/g; and in *P. viridis* they were 1524 µg/g and 2890 µg/g. Calcium and magnesium content are also important minerals, and the contents of both were high in *P. perna*. Phosphorus content was higher in *Perna viridis* than *P. perna* and the parrot mussel species. Manganese content in the muscle of *Perna perna*, *P. viridis* and the parrot mussel species was found to be 0.19, 0.22 and 0.07 µg/g, respectively. The concentrations of both zinc and iron were the lowest; the values of zinc and iron in *Perna perna*, *P. viridis* and the parrot mussel species respectively were: 12.80, 3.20 and 11.50 µg/g; and 14.27, 17.33 and 17.09 µg/g. The order of mineral concentration in the tissue samples of the three Perna mussel species was: potassium > sodium > phosphorus > calcium > magnesium > iron > zinc > manganese. Of the three mussel species *Perna perna* had higher mineral content (zinc, iron, magnesium, phosphorus, sodium, potassium and calcium) than *P. viridis* and the parrot mussel. One way ANOVA test results for sodium and calcium showed significance between the groups, but the other minerals showed non-significance within the groups.

**Heavy metal contaminants in mussel**

The heavy metal contaminants in mussel samples were shown represented in table 4. The results for the heavy metals showed copper to be in the highest concentration in all the three mussel species with its highest value at 8.70 µg/g in *Perna perna*. Next to copper, cadmium had its maximum value at 2.33 µg/g in *P. perna*; and nickel at 2.25 µg/g in *P. perna*; lead at 2 µg/g in *P. perna*; chromium at 0.58 µg/g in *P. viridis*; and selenium at 0.06 µg/g in *P. viridis*; arsenic at 0.02 µg/g in *P. perna*; while the mercury content was below the detectable limit. The observed concentrations of all heavy metals were within the permissible level proposed by the WHO (1982). The order of metal accumulation in the soft tissues of Perna species collected from Kadiyapattinam coast was: Cu > Cd > Pb > Ni > Cr > Se > As > Hg. ANOVA test results showed significant differences between the mussel groups for nickel and selenium content, but results for the other heavy metals showed non-significance between the groups.

**Statistics**

The mean values of proximate components, minerals and heavy metals of *P. viridis*, the parrot mussel and *P. perna* were statistically analyzed using Independent One-Sample ANOVA test at < 0.05 level of difference. As the values are less than the test value for all variables, it can be confirmed at significance that the species differ from each other significantly, with regard to their biochemical component concentration. The values greater than the test value are non-significant.

**Discussion**

The rocky coastal areas of Enayam, Enayamputhenthurai and Kadiyapattinam in district have abundant mussel beds [8]. Mussel availability is high during the period from August to December and it declines from January to March. This may be due to the long fishing season and favorable conditions. The spawning of mussels commences during May and lasts till September and so mussel fishing was
Table 1: Biometric parameters of *Perna viridis*, *Perna perna* and Parrot mussel.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total weight (g)</th>
<th>Meat weight (g)</th>
<th>Shell weight (g)</th>
<th>Shell length (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. viridis</em></td>
<td>8.22±0.43</td>
<td>2.51±1.34</td>
<td>5.01±0.91</td>
<td>5.1±0.30</td>
</tr>
<tr>
<td><em>P. indica</em></td>
<td>10.17±0.48</td>
<td>4.41±0.97</td>
<td>7.34±0.71</td>
<td>6.1±1.00</td>
</tr>
<tr>
<td>Parrot mussel</td>
<td>8.66±0.73</td>
<td>2.67±0.71</td>
<td>4.77±0.28</td>
<td>5.7±0.65</td>
</tr>
</tbody>
</table>

Values are given as mean percentage ± standard deviation

Table 2: Proximate composition of *Perna viridis*, *Perna perna* and Parrot mussel.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th><em>Perna viridis</em></th>
<th><em>Perna perna</em></th>
<th>Parrot mussel</th>
<th>ANOVA P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (%)</td>
<td>20.3±1.56</td>
<td>17.73±2.01</td>
<td>19.47±2.01</td>
<td>0.302</td>
</tr>
<tr>
<td>Lipid (%)</td>
<td>2.3±0.68</td>
<td>3.38±0.70</td>
<td>2.53±0.52</td>
<td>0.17</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>2.39±0.73</td>
<td>2.4±0.56</td>
<td>2.47±0.16</td>
<td>0.98</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>2.64±0.34</td>
<td>3.2±0.56</td>
<td>3.11±0.7</td>
<td>0.462</td>
</tr>
<tr>
<td>Fibre (%)</td>
<td>0.06±0.04</td>
<td>0.139±0.06</td>
<td>0.09±0.04</td>
<td>0.179</td>
</tr>
<tr>
<td>Caloric values (kcal/100g⁻¹)</td>
<td>124.41±5.99</td>
<td>130.14±4.44</td>
<td>128.69±1.65</td>
<td>0.323</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>73.45±1.25</td>
<td>78.21±1.69</td>
<td>74.8±1.31</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD of triplicate measurements. P value < 0.05 significance.

Table 3: Essential elements content (μg/g) of *Perna viridis*, *Perna indica* and Parrot mussel

<table>
<thead>
<tr>
<th>Essential elements</th>
<th><em>Perna viridis</em></th>
<th><em>Perna perna</em></th>
<th>Parrot mussel</th>
<th>ANOVA P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc</td>
<td>12.8±1.41</td>
<td>13.2±1.09</td>
<td>13.2±1.10</td>
<td>0.323</td>
</tr>
<tr>
<td>Iron</td>
<td>14.27±2.02</td>
<td>17.33±2.18</td>
<td>17.33±2.19</td>
<td>0.163</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.22±0.09</td>
<td>0.19±0.03</td>
<td>0.19±0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>Magnesium</td>
<td>357.23±9.58</td>
<td>366.48±31.99</td>
<td>366.48±31.100</td>
<td>0.081</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1524±205.47</td>
<td>1425±112.53</td>
<td>1425±112.54</td>
<td>0.694</td>
</tr>
<tr>
<td>Potassium</td>
<td>2890±165.45</td>
<td>2973±158.46</td>
<td>2973±158.47</td>
<td>0.249</td>
</tr>
<tr>
<td>Sodium</td>
<td>1244±48.75</td>
<td>1579±99.02</td>
<td>1579±99.03</td>
<td>0.004</td>
</tr>
<tr>
<td>Calcium</td>
<td>438.16±36.44</td>
<td>547.57±32.57</td>
<td>547.57±32.58</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD of triplicate measurements. P value < 0.05 significance.

Table 4: Heavy metal contaminants in *Perna viridis*, *Perna perna* and parrot mussel

<table>
<thead>
<tr>
<th>Contaminants (µg/g)</th>
<th><em>Perna viridis</em></th>
<th><em>Perna perna</em></th>
<th>Parrot mussel</th>
<th>ANOVA P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>1.79±0.49</td>
<td>2.33±0.74</td>
<td>1.39±0.16</td>
<td>0.164</td>
</tr>
<tr>
<td>Copper</td>
<td>6.03±0.92</td>
<td>8.7±0.60</td>
<td>3.6±0.62</td>
<td>0</td>
</tr>
<tr>
<td>Chromium</td>
<td>0.58±0.22</td>
<td>0.39±0.09</td>
<td>0.427±0.12</td>
<td>0.351</td>
</tr>
<tr>
<td>Lead</td>
<td>1.72±0.84</td>
<td>2±0.12</td>
<td>1.22±0.70</td>
<td>0.377</td>
</tr>
<tr>
<td>Mercury</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>0</td>
</tr>
<tr>
<td>Arsenic</td>
<td>0.008±0.0</td>
<td>0.02±0.01</td>
<td>0.013±0.01</td>
<td>0.336</td>
</tr>
<tr>
<td>Nickel</td>
<td>1.34±0.45</td>
<td>2.25±0.42</td>
<td>1.06±0.38</td>
<td>0.03</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.06±0.04</td>
<td>0.03±0.02</td>
<td>0.03±0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD of triplicate measurements. P value < 0.05 significance.
banned during this period. The mussel is fished from the rocks at a depth of 5 to 30 feet employing skin diving, and the monthly average quantity of mussel collected per fishing trip varies from 12 kg to 83 kg in the coastal areas of Kanyakumari district. The length of the mussel harvested during the season ranges between 1.30 cm to 11.30 cm and the weight between 2.40 g to 62.20 g. The consumer preferred the average sized mussels with the length of 7 cm and above.

According to Siddall [32], specific identification of living specimens of Perna species can be based on their distinct geographical distribution. Shells of P. viridis, P. perna and the parrot mussel species are thick, equivalved, in equilateral, elongate and triangularly ovate in outline. Muscle scars are deeply impressed on the inner side of the shell and the anterior adductor muscle is absent. Thick, white and highly pitted resilial ridge and two short and thick bundles of posterior byssal retractors are common to the species of Perna. The distinguishing conchological feature of P. viridis is the green or bluish green external colour of its shell. The differentiating features of Perna perna is its light or dark brown coloured shell, with pointed anterior side of the shell. In these localities, a third morphotype of mussel with light yellowish green shell colouration occurs, which is locally called the parrot mussel. Divya et al. [23] reported that the parrot mussel is only a morphotype of P. perna and not a true hybrid of the two (P. perna and P. viridis). In the present study nutritive evaluation of these three types of mussel species were analyzed.

The biometric analysis of the present study showed that P. perna is comparatively larger than P. viridis. The average total weight and length of P. perna was found to be 10.17 g and 6.10 cm respectively. But the corresponding values of P. viridis were only 8.22 g and 5.10 cm. The average total weight and length of the parrot mussel was 8.66 g and 5.70 cm. The total weight and length of the P. perna specimens were comparatively more than those of P. viridis and the parrot mussel.

Knowledge of the macronutrient contents of the mussels namely moisture, protein, lipid, carbohydrate, fibre, ash and minerals enables a better understanding of their nutritional values, which in turn leads to improvement of diet for human consumption. Salasker and Nayak [33] reported that shell fishes are known to be rich in protein, but with low fat content and calories. In the present study, the proximate composition analysis did not show any significant differences between the three species. Protein is the major organic constituent and a source of energy reserve in bivalves; it plays a more important role than glycogen and other intermediaries of carbohydrate metabolism [34]. Our proximate analysis illustrated that the three mussel species contained more than 18% protein, which is very close to the 20% of protein content of fishes [35]; far higher than the values of shrimps which have a protein content of 10% [36]; and more than the values of crab at 14% [37]. The higher value of protein content in the mussel specimens studied might be mainly due to the increased food availability; and secondly, the specimens were collected just prior to the spawning period. Another possible reason for the elevated protein content could be the increased feeding efficiency associated with food availability thereby resulting in proper assimilation of food and better metabolic conditions at that time [38].

The quantum of lipid content of mussel samples depends on their feeding efficiency and the biomass of chlorophyll and phytoplankton in the environment [39]. This study shows an inverse relationship between the contents of lipids and carbohydrates. As the value of lipid content increases, the value of carbohydrate content decreases [40]. This finding, thus, coincides with the results of the present work. The moisture content in the meat of P. perna was 78.21% while those of P. viridis and the parrot mussel were 73.45 and 74.80%, respectively. The moisture content of the three mussel species significantly varied and they were lower than the values reported by Thamnoon [40] for the brown mussel (78.65%) and the green mussel (81.48%) from Panay Island. The differences in the moisture content of the mussel species could be due to the differences in their habitats.

The ash content of P. viridis and P. perna were 2.39 and 2.40% respectively while the value for the parrot mussel was 2.47%. No definite pattern of variation was discernible in the ash content of these mussels during the present study. However they were lower than the values reported by Thamnoon [40] for the green mussel (11.49%), and the brown mussel (12.51%). Of the three species studied the parrot mussel had the highest ash content. This indicates that the parrot mussel species had a relatively high content of mineral salts, mainly potassium and calcium [41]. In younger mussels, values of ash content were higher [42] and this had a parallel in the case of the parrot mussel of this study. In all the three species ash content showed inverse relation with protein content and this trend coincided with the results.
The pattern of variation in carbohydrate content registered its higher values in the *P perna*. The carbohydrate content of the mussel samples depends on the chlorophyll and phytoplanktonic biomass in the water [44]. The fluctuation in the carbohydrate content is directly related to the reproductive cycle [45]. Unfavorable environmental condition causes considerable stress to the mussels and it reduces the nutrient level in mussel species [46]. According to Rivonker and Perulekar [47] carbohydrate content was higher in large-sized mature mussels than in small-sized immature mussels. This fact coincided with our results. The low value of carbohydrate in *P. viridis* of this study coincided with the low level of their caloric content. In the present investigation, *Perna perna* of Kadiyapattinam coast shows very high calorific values during the study. The high fat content of the species has a direct relationship with its caloric value [48]. This fact too was borne out by our results.

Moisture, lipid, ash, carbohydrate and protein were the major components that presented values within the variation band cited in the literature for fish [49][50]. The value reported in the literature for the proximate composition and caloric value is less, especially for the *P. perna* and the parrot mussel species. Very few articles like [51] reported a value of protein content for *Perna perna* at 9.90%; Saritha et al. [52] reported that the mean protein content of *Perna viridis* as 36%. Salasker and Nayak [33] in their studies (from November 2003 to March 2004) on nutritional quality of bivalves in Kali estuary, Karnataka, reported the composition of *P. viridis* as: crude protein 57.39 to 66.51%; carbohydrate 14.69 to 26.81%; lipid 8.09 to 12.62%; and ash 6.18 to 9.02%. Shafee [53] in his study on the variations in biochemical composition of the green mussel of Ennore estuary, Chennai, noted a yearly average of 59.26% of crude protein, 22.81% of carbohydrate, 6.13% of lipids, 11.81% of ash and 5.432 kcal of energy content. The mussels studied in the present work had protein content higher than that obtained by Marques and Pereira [51], and lower than that reported by Saritha [52]. Also the value is higher than that of the species *Perna viridis* (12.80%) harvested elsewhere in India, as reported by George et al. [54], that of *Mytilus edulis* of the Atlantic, and that of *Mytilus Californianus* of the Pacific (14.40%) as reported by Watt and Merrill [41]. Based on the results of this study, biochemical composition of *P. viridis* and parrot mussel species had not much varied between them, but lower lipid, ash, fibre, moisture and caloric value than with *Perna perna* species.

Mussels are good source of pure protein for they have relatively moderate to high percentage of protein. However the differences observed in the protein content among the species, may be attributed to animal consumption or absorption capability and conversion potentials of essential nutrients from their diet or their local environment into such biochemical attributes needed by the organisms’ body [55]. The variations recorded in the concentration of the different nutritional components in the mussels species examined might most probably depend on the rate in which these components are available in the water body, and the ability of the mussels to absorb and convert the essential nutrients from the diet or the water bodies where they live. This is supported by the findings of Windom et al. [56], Prapasri et al. [57], Wilson [58], and Adewoye et al. [59]. To analyze the significance of the differences between *P. viridis*, the parrot mussel and *P. perna* with regard to their nutritional composition, ANOVA test was conducted with the mean values of their entire variables. The values were found to be bigger than the test values. The ANOVA test revealed that biochemical results did not showed significant variation among three selected mussel species.

**Essential Elements**

In the present study a total of eight minerals were detected and of them the macro minerals such as potassium, sodium, phosphorus, calcium and magnesium were observed in high concentrations whereas the other macro minerals viz. iron, zinc and manganese were in negligible amounts. Mineral components such as sodium, potassium, magnesium, calcium, iron and phosphorus are important in human nutrition [60]. Further, Rajagobal et al. [61] and Devadas [62] reported that bivalves in general are excellent sources of K, Ca, P, Na, Fe, and Zn, and that shellfish can absorb minerals directly from the aquatic environment through the gills and body surface. In the present study *P. viridis*, *P. perna* and the parrot mussels showed higher level of sodium, potassium, phosphorus and calcium and a good deal of other minerals too. The nutritional value of the bivalves has not been brought to the limelight so far, so consumption of these nutrient-rich bivalves has not become much prevalent.

Mineral contents in shellfish depend first and foremost on their availability in the environment; second on the capability of shellfish to absorb diet; and thirdly on the preferential accumulation of the nutrients by the fish [56][59][63][64]. Calcium and phosphorus together help in skeleton formation. Phosphorus plays a vital part in the metabolism of adenosine polyphosphates and
phospholipids [65]. The health benefits of potassium include relief from stroke, high blood pressure, heart and kidney disorders, and anxiety and stress. It helps enhance muscle strength, metabolism, water balance, electrolytic functions, and the nervous system. Copper is an important component of several enzyme systems, including cytochrome oxidase and tyrosinase. With iron, copper catalyses oxidation-reduction mechanisms and tissue respiration. Numerous aspects of cellular metabolism are zinc-dependent. Iron prevents anemia [66]. Seafood is one of the best sources of iron for adults and children. Manganese is needed for growth and good health, and its deficiency can cause nervous problems [66]. Iron is among the major minerals found in all species, and it performs vital functions such as carrying oxygen in hemoglobin of vertebrates [67].

The iron content was the highest in *Perna perna* (17.33 µg/g) and the parrot mussel was a rich source of K. The recommended daily intake (RDI) for potassium (K) is 3 g [68], which corresponds to the more than 1000 µg/g of potassium content of mussel species. The lowest concentration of Mn (0.07 µg/g) was measured in the parrot mussel, while the highest concentration (0.22 µg/g) was measured in *Perna viridis*. Concentrations of Mn were generally lower in all the mussel samples analyzed vis-à-vis the other essential minerals, and the Mn concentration was lower than those of several other studies. According to previous literature, the Mn concentrations in the shellfish samples ranged from 0.54 to 79.08 µg/g [69]. The results of this study suggest that *Perna viridis* is a rich source of zinc, which is good for human health. The reported values of zinc in the tissues of *Perna viridis* in Peninsular Malaysia were 90 - 135 µg/g dry wt. [70]. The maximum value as per FAO guidelines for Zn is 30 µg/g [71]. The concentrations of Zn in the present study are not more than the guidelines value. Toxicity due to excessive intake of Zn has been reported to cause electrolyte imbalance, nausea, anemia, and lethargy [72]. The results of the present study not only provide information about the mineral content, but also recommend the consumption of these bivalves. Even though most elements found in seafood are necessary for several human metabolic processes they must be taken from the diet in balanced doses, as low intakes result in nutritional deficiencies, and high intakes can result in toxicity. FAO/WHO/UNU [73] reported that an adult may eat the equivalent to 100 g of mussel meat per meal (approximately, 13 mussels).

### Heavy metal contaminants

Marine organisms especially the filter feeders are known to accumulate toxic materials including heavy metals in their bodies [74] a phenomenon called bioaccumulation. The toxicity of heavy metals in this study has been reported to follow the general order: Cu > Cd > Pb > Cr > Se > As > Hg. This order may vary depending on the environmental conditions and on the species involved [75]. Heavy metals present some of the major environmental hazards due to their affinity for metal sensitive groups, such as the thiol groups. Heavy metals block functional groups of proteins, displace and/or substitute essential metals, induce conformational changes, denature enzymes and disrupt cells and organelle integrity [76].

Toxic heavy metals accumulations in mussel are of great economic importance. Their presence in higher concentrations in the edible soft tissues is of great health concern. Higher concentrations of heavy metals like Pb, Cd, Cr and Cu in the green mussel *Perna viridis* were reported by Shin et al. [77], Blackmore and Wang [78], Wang and Wong [79], Yap et al. [80], Pan and Wang [81], Liu and Kueh [82], Yap et al. [83]. According to Fung et al. [84] due to industrial activity the concentration levels of heavy metals such as Cd, As, Cr, Ni, Pb, Se, Zn, Cu, Fe and Hg have increased in the body of *Perna viridis* and *Mytilus edulis* in the east coast of China. In the present study limited concentration of metals has been found, and this indicates that the environment is free from the heavy metals.

Copper is the most abundant metal present in the body fluid of mollusks. According to the WHO [85], the daily requirement of copper is 18 µg/g bw/day. In this study copper content varied between the species (6.03 µg/g in *P. Viridis*; 8.70 µg/g in *P. perna*; and 3.60 µg/g in the parrot mussel). This might be due to various mechanisms which include homoeostatic processes in the body in response to varying metabolic demands; and to the entrapment of Cu under certain conditions by additional mucilage production / extrusion by the animal Pyatt et al. [86]. The copper content of the mussel did not exceed the acceptable limit of 30 µg/g in seafood as recommended by the WHO [87].

Cadmium is present in the edible tissues of *P. viridis* (1.79 µg/g), *P perna* (2.33 µg/g) and the parrot mussel (1.39 µg/g). It is observed that the cadmium concentration is increasing with growth and aging of the animal. The acceptable range of cadmium in food specified by Food
and Drug Administration (USA) is 7 μg/g per body weight. Low level of cadmium has been reported in the tissues of *Perna viridis* of Peninsular Malaysia (0.68-1.25 μg/g dry wt [70], Gulf of Thailand (1.20 mg kg⁻¹ dry weight) [88], and the east coast of China (5.31 μg/g dry wt) [84].

Lead concentrations in edible soft tissues of *P. viridis*, *P. perna* and the parrot mussel respectively were 1.72, 2.33 and 1.39 μg/g. It was also observed that the concentration of lead was increasing with age and growth of the animal. The values were well below the permissible limit of 5 μg/g proposed by the WHO [87]. However the lead concentration in the edible tissues of *Perna viridis* in the west coast of Peninsular Malaysia ranged from 75.10 to 129 μg/g [70][80], and in Hong Kong from 2.02 to 4.36 μg/g [89]. Blackmore and Wang [78] have reported that factors like salinity, temperature and planktonic diversity and richness influenced the uptake of lead in bivalves.

The chromium concentration in the edible soft tissues of the three samples studied were 0.58 μg/g (*Perna viridis*), 0.39 μg/g (*P. perna*) and 0.427 μg/g (parrot mussel). The reported values were slightly above the range of the permissible limit of 0.1 mg/L water [90]. The normal level of blood chromium concentration in human beings is between 20 - 30 μg/L. The intake of chromium at about 50 - 200 μg/day is regarded as safe and adequate. The value of chromium concentration in the tissues of *Perna viridis* in Peninsular Malaysia was 0.82-4.89 μg/g [70]; it was 15.72 mg kg⁻¹ in the gulf of Thailand [88] and 5.31 μg/g in the east coast of China [84]. In the present study, chromium concentration was found to be within the acceptable limits.

The nickel concentration in the edible soft tissues of *P. viridis* (1.34 μg/g), *P. perna* (2.25 μg/g) and the parrot mussel (1.06 μg/g) were within the permissible limit (5 μg/g) proposed by FDA [90]. Nickel is not a cumulative toxin in animals or in humans. Soluble nickel is excreted through urine, and a small amount is excreted through feces. Hair is also excretory tissue for nickel. It is reported that the concentration of nickel in the tissues of *Perna viridis* was 3.25 - 6.87 μg/g dry wt. in Peninsular Malaysia [70], 1.54 mg kg⁻¹ dry wt. in the gulf of Thailand [88] and 4.78 μg/g dry wt. in the east coast of China [84]. Nickel concentrations of 2.3 μg/g or greater, may cause reproductive impairment and lack of recruitment in seafoods [91]. None of the samples in this study approached these levels of concern. Hence nickel concentrations in the entire species of mussels do not constitute any threat upon its consumption.

Although Se is an essential micronutrient, it can be toxic at high levels [92]. A concentration of about 1 μg/g in prey is the threshold for Se toxicity in seafood, while muscle concentration of 2.60 μg/g is adverse for the seafood itself [93][94]. However, selenium concentration observed in *Perna viridis* was 0.06 μg/g, 0.03 μg/g in *P. perna*, and 0.03 μg/g in the parrot mussel. A correlation has also been found between the levels of Hg and Se in the mussel, which confirms selenium's protective role against the toxic effects of mercury. Mercury is bound by selenium to form the non-toxic compound HgSe in mammalian livers [95][96]. Se concentrations reported for organisms from uncontaminated environments vary from 0.50 to 4 mg/g dry mass [97]. The estimates of concentrations of selenium in small and large shrimps ranged respectively from 0.14 to 0.16 and from 0.19 to 0.23 μg/g wet weight [98]. These values are higher than those of the present study.

Arsenic is present in our food in different chemical forms, but inorganic arsenic is more toxic than organic arsenic. It is difficult to reliably measure the forms of arsenic. Inorganic arsenic is estimated to be 10 % of total arsenic USFDA [99][100][101]. The results of the present study showed a higher arsenic concentration in *P. perna*. The maximum allowable level of arsenic contaminants in fish, shellfish, and crustaceans are 2, 1, and 2 mg/kg, respectively, FSANZ [102]. Thus, our result indicated the as concentration is still below the maximum allowable level of arsenic recommended by FSANZ. However, a recent research has suggested that as acts as an endocrine disruptor at extremely low concentrations [103].

Mercury (Hg) is one of those heavy metals in the marine ecosystem which are of great concern at an elevated level. Hg could have hazardous impacts owing to its toxicity [83]. However, in this study the concentration of Hg was found to be below the detectable level in the mussels. The low concentration of Hg observed in the soft tissues of the organisms clearly indicates the general tendency of organisms to accumulate lower concentration of Hg in soft tissue. Usero et al. [104] revealed that when compared to the other metals Hg was in concentrations below the detectable levels in molluscs from the Atlantic coast of southern Spain.

Generally, the toxic potential of heavy metals is not very high and their concentrations in the mussel are within the permissible level. The concentrations of metals vary between the mussels due to several processes that
affect metal flux rates in mussels; due to variations in environmental concentrations; and due to the conditions of an individual associated with its growth, gonadal development, spawning, sex, and age [105]. Overall, the findings of the present study demonstrate that the bivalves are safe for human consumption, and that the ecosystems where they were harvested do not pose any hazard to man in terms of health risk. However, caution should be taken when the bivalves are continuously used; especially important is the fact that the rate of gastrointestinal absorption of metals in children is five times more than adults. The data from this study revealed that the mussel species sampled are of high nutritive value and are good sources of protein. However, the levels of heavy metals obtained for all the mussel samples have not reached level of concern since the values were found to be low when compared with WHO allowable limits in seafood. Thus the mussel species sold in the Kadiyapattinam coastal water at the time of study were safe for consumption.

Conclusion

*P. perna* is far more superior to *P. viridis* in terms of biometric and nutritional features. The results indicate that the compositional values of the major biochemical constituents are greatly influenced by the quality and quantity of food available; they are also influenced by such other exogenous parameters as affect the rate of metabolism and the reproductive cycle. Overall, the findings demonstrate that the bivalves are safe for human consumption, and that the ecosystems where they are harvested do not pose any hazard either to humans or to the marine life. The reported heavy metal concentrations in Kadiyapattinam coast are well below the permissible limits and hence do not pose a threat to human health. There is much demand for these species as food and they are consumed in many parts of the country. If they are systematically cultured, then the surplus harvest can be exported to foreign markets also. Mussels farming under controlled condition can generate employment as well as revenue.

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