Benthic foraminifera as environmental indicators in the lagoon and mangrove environments of Langkawi, Malaysia

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Article

Keywords:

Posted Date: March 30th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1484518/v1

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Abstract

The benthic foraminifera are a single cell protist that are highly abundant in marine and marginal environments. They have been successfully used in many monitoring works to elucidate the status of environmental health conditions. The present study examines the foraminiferal distribution in the surface sediments of the Sungai Kilim, within the Kilim Geoforest Park, in Pulau Langkawi, Malaysia. Information provided by benthic foraminifera were associated with geochemical properties, sediment types, and current speed data to characterise the environment. Twenty-eight genera of benthic foraminifera were identified from the surface samples. *Elphidium* spp. (relative abundance of 13–83%) were the most abundant species in the mangrove and lagoon environments along the Sungai Kilim. Salinity, water depth, and temperature were the important parameters that determined the foraminiferal distribution. Test abnormalities were observed in *Elphidium* spp. (< 10% of tests) at several stations; a higher quantity of abnormal tests (5–6%) were observed at stations with high current speeds, especially in mangrove areas. The sediment in the study areas did not show severe heavy metal contamination; therefore, the low diversity and test abnormalities were associated with natural stress from environmental conditions. This information provides a baseline for future environmental monitoring to preserve the Sungai Kilim and the Kilim Geoforest Park.

Introduction

Benthic foraminifera are single-celled organisms that inhabit the seafloor of modern oceans. Benthic foraminifera have short life and reproductive cycles, from a few weeks for small taxa to one year for some larger forms; thus, they respond quickly to environmental disturbances and form a local record of them. Their unique response to pollution and other changes in environmental parameters has established them as a monitoring tool. The sensitivity shown by selected taxa has proven useful in determining the source of pollution (e.g., chemical or organic). In addition, the development of several indices, such as the Foraminifera in Reef Assessment and Monitoring Index and Foraminifera Stress Index, has allowed the comparison of monitored environments between regions. Furthermore, foraminiferal tests are readily preserved in fossil records and can be used to examine environmental changes over time.

Mangrove and marginal environments typically display far greater anthropogenic influence than open marine ecosystems because of their proximity to pollution sources. Previous studies on benthic foraminifera in mangrove environments have shown that several factors, such as tidal range, vegetation coverage, and salinity, play important roles in determining their distribution. The Kilim mangrove forest is part of the United Nations Educational and Scientific Organization (UNESCO) geopark in Pulau Langkawi, which must be protected from anthropogenic stress and constantly monitored. However, as an ecotourism hotspot in Pulau Langkawi, the number of visitors to the park increases yearly. According to the Langkawi Development Authority, the number of visitors to the island increased from 42,375 visitors in 2006 to 273,450 in 2012. Hence, there is a growing concern that ecotourism may lead to the deterioration of ecosystems by increasing pollutants in the environment. For example, the increased number of recreational boats used to bring tourists through the Kilim Geopark for sightseeing, causes an increased threat of oil and petrol pollution. Oil leakage from the boats may increase the toxicity in the water column, with detrimental effects on aquatic plants and other marine life. In addition, the increased number of uncontrolled tourism activities could further increase pollution issues and adversely affect the environment. Hence, for monitoring purposes, several studies have investigated the geochemistry of the sediments around Sungai Kilim; however, no study had been conducted there using benthic foraminifera as environmental indicators. Thus, this study investigated the distribution, abundance, and diversity of benthic foraminifera and their potential use as environmental indicators in Sungai Kilim. Therefore, this work serves as a baseline study for future environmental monitoring and can be compared to and used in other studies.

Study Area

Sungai Kilim is unique because it is separate from Kilim Geoforest Park and Kilim Karst Geoforest Park. This geopark is one of the geoforest parks associated with the Langkawi Geopark under the UNESCO Global Geopark Network and is comprised of several components, namely river, mangrove, and lagoon environments. Many karst formations and dense vegetation characterize the downstream area of Sungai Kilim. The midstream is connected to two small tributary rivers, and the upstream area is dominated by urbanisation activities.
The environment of Kilim Forest is distinctive because of the combination of several different ecosystems, including mangrove swamps, limestone caves, lagoons and estuaries. Apart from being an interesting site for geological studies, this area is also known for tourist activities, such as mangrove hopping, bird watching, cage fish feeding and cave exploration. Facilities are also available at the park, such as rental boats, the Kilim jetty, and floating restaurants. The only access to the Kilim Geopark is by boat via Sungai Kilim.

Results

Geochemical and physical properties

The current speed model indicated that the opening of the lagoon to the sea was a very high-energy environment (Fig. 2a). The mangrove area along the river possesses strong hydrodynamics energy, while the lagoon is a calm environment. Based on grain size analysis, the sediment in this study was divided into two classes: fine silt and coarse sand (Table 1). Coarser sediments were observed mainly in the mangrove areas (LKS3, LKS4, LKS5, LKS6, LKS8, LKS9, LKS11, LKS12 and LKS13), while fine sediments dominated the lagoon areas (Fig. 2b). The percentage of total organic carbon (TOC) in the sediment ranged from 0.39–6.02%, with an average of 1.91% (Table 1). Higher TOC values were observed in the lagoons (LKS17-20) (Fig. 2c). The percentage of calcium carbonate (CaCO3) had an inverse relationship with TOC (Fig. 2d), and ranged between 0% and 9.54%, with an average of 1.73% (Table 1). The carbonate content was high (>2%) at LKS1, LKS3, LKS8, LKS9, LKS11 and LKS16. However, there was no carbonate present at LKS5, LKS6, LKS15, LKS18, LKS19 and LKS20. The average concentrations of heavy metals in the surface sediment showed the following order: Fe < Al < Cd < Co < Cu < Ni < Pb < Zn < Mn. The analysis accuracy was compared to the Canadian Certified Reference Materials Project standard (NBS 1646a), and the percentage ranged from 76.52–101.60% (Table 1). The average heavy metal concentrations in the sediments were less than the background values. Furthermore, at most stations, all elements were less than background values (20µg/g) except for Pb at LKS17 (27.04µg/g), LKS18 (25.78µg/g), LKS19 (23.06µg/g) and LKS20 (23.87µg/g) (Table 1), which are all in the lagoon areas (Fig. 3). Nevertheless, the Pb concentration was still less than the value suggested by the sediment quality guidelines of the United States Environmental Protection Agency (US EPA) (<40). Furthermore, these guidelines classify sediments as non-polluted, moderately polluted and heavily polluted as shown in Table 1. Based on these guidelines, all sampling stations in this study were not polluted.
Table 1
Mean percentage values of sediment size, total organic carbon, calcium carbonate, and heavy metal concentrations in Sungai Kilim.

<table>
<thead>
<tr>
<th>Stations</th>
<th>Mean (ϕ)</th>
<th>Texture</th>
<th>TOC (%)</th>
<th>CaCO3 (%)</th>
<th>Fe</th>
<th>Al</th>
<th>Mn</th>
<th>Zn</th>
<th>Pb</th>
<th>Ni</th>
<th>Cu</th>
<th>Co</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>LKS 1</td>
<td>6.91</td>
<td>Fine silt</td>
<td>0.91</td>
<td>3.57</td>
<td>0.39</td>
<td>0.88</td>
<td>37.38</td>
<td>10.26</td>
<td>11.90</td>
<td>2.17</td>
<td>2.04</td>
<td>0.86</td>
<td>-0.01</td>
</tr>
<tr>
<td>LKS 2</td>
<td>6.70</td>
<td>Fine silt</td>
<td>1.86</td>
<td>1.72</td>
<td>1.02</td>
<td>1.51</td>
<td>72.58</td>
<td>18.46</td>
<td>17.67</td>
<td>3.94</td>
<td>4.93</td>
<td>1.44</td>
<td>0.03</td>
</tr>
<tr>
<td>LKS 3</td>
<td>-0.30</td>
<td>Very coarse sand</td>
<td>1.07</td>
<td>2.00</td>
<td>0.15</td>
<td>1.03</td>
<td>17.11</td>
<td>5.55</td>
<td>8.62</td>
<td>1.40</td>
<td>0.84</td>
<td>0.31</td>
<td>-0.03</td>
</tr>
<tr>
<td>LKS 4</td>
<td>0.21</td>
<td>Coarse sand</td>
<td>1.16</td>
<td>1.37</td>
<td>0.19</td>
<td>1.19</td>
<td>19.93</td>
<td>6.33</td>
<td>7.92</td>
<td>1.94</td>
<td>1.10</td>
<td>0.41</td>
<td>-0.01</td>
</tr>
<tr>
<td>LKS 5</td>
<td>-0.18</td>
<td>Very coarse sand</td>
<td>0.57</td>
<td>1.71</td>
<td>0.12</td>
<td>0.98</td>
<td>9.63</td>
<td>4.94</td>
<td>7.30</td>
<td>0.73</td>
<td>0.72</td>
<td>0.24</td>
<td>-0.03</td>
</tr>
<tr>
<td>LKS 6</td>
<td>0.18</td>
<td>Coarse sand</td>
<td>0.69</td>
<td>0.00</td>
<td>0.17</td>
<td>1.58</td>
<td>20.62</td>
<td>5.40</td>
<td>11.17</td>
<td>1.06</td>
<td>0.86</td>
<td>0.30</td>
<td>-0.02</td>
</tr>
<tr>
<td>LKS 7</td>
<td>6.65</td>
<td>Fine silt</td>
<td>3.38</td>
<td>0.00</td>
<td>0.79</td>
<td>2.55</td>
<td>56.65</td>
<td>17.26</td>
<td>14.89</td>
<td>3.23</td>
<td>2.47</td>
<td>1.18</td>
<td>0.04</td>
</tr>
<tr>
<td>LKS 8</td>
<td>-0.30</td>
<td>Very coarse sand</td>
<td>0.89</td>
<td>5.33</td>
<td>0.27</td>
<td>0.59</td>
<td>89.25</td>
<td>7.76</td>
<td>5.18</td>
<td>1.01</td>
<td>0.97</td>
<td>0.82</td>
<td>-0.03</td>
</tr>
<tr>
<td>LKS 9</td>
<td>0.38</td>
<td>Coarse sand</td>
<td>0.39</td>
<td>9.54</td>
<td>0.21</td>
<td>0.65</td>
<td>83.56</td>
<td>6.59</td>
<td>5.60</td>
<td>0.99</td>
<td>0.64</td>
<td>0.34</td>
<td>-0.01</td>
</tr>
<tr>
<td>LKS 10</td>
<td>6.82</td>
<td>Fine silt</td>
<td>0.43</td>
<td>0.15</td>
<td>0.25</td>
<td>0.83</td>
<td>38.19</td>
<td>8.70</td>
<td>4.98</td>
<td>1.88</td>
<td>0.84</td>
<td>0.51</td>
<td>-0.03</td>
</tr>
<tr>
<td>LKS 11</td>
<td>0.06</td>
<td>Coarse sand</td>
<td>1.61</td>
<td>2.09</td>
<td>0.38</td>
<td>1.20</td>
<td>90.15</td>
<td>10.04</td>
<td>7.25</td>
<td>1.80</td>
<td>1.13</td>
<td>0.96</td>
<td>-0.03</td>
</tr>
<tr>
<td>LKS 12</td>
<td>0.03</td>
<td>Coarse sand</td>
<td>1.17</td>
<td>1.27</td>
<td>0.37</td>
<td>1.00</td>
<td>30.57</td>
<td>9.53</td>
<td>7.40</td>
<td>1.71</td>
<td>1.93</td>
<td>0.65</td>
<td>-0.03</td>
</tr>
<tr>
<td>LKS 13</td>
<td>0.10</td>
<td>Coarse sand</td>
<td>1.74</td>
<td>1.64</td>
<td>0.51</td>
<td>1.53</td>
<td>48.40</td>
<td>20.60</td>
<td>9.64</td>
<td>1.65</td>
<td>1.57</td>
<td>0.82</td>
<td>-0.02</td>
</tr>
<tr>
<td>LKS 14</td>
<td>6.59</td>
<td>Fine silt</td>
<td>0.80</td>
<td>0.27</td>
<td>0.43</td>
<td>1.72</td>
<td>41.68</td>
<td>11.38</td>
<td>8.59</td>
<td>1.50</td>
<td>1.53</td>
<td>0.74</td>
<td>-0.02</td>
</tr>
<tr>
<td>LKS 15</td>
<td>6.82</td>
<td>Fine silt</td>
<td>1.86</td>
<td>0.00</td>
<td>0.65</td>
<td>1.89</td>
<td>44.73</td>
<td>28.43</td>
<td>7.36</td>
<td>2.31</td>
<td>2.03</td>
<td>1.16</td>
<td>-0.01</td>
</tr>
<tr>
<td>LKS 16</td>
<td>7.12</td>
<td>Very fine silt</td>
<td>2.34</td>
<td>3.27</td>
<td>0.79</td>
<td>1.30</td>
<td>77.92</td>
<td>21.34</td>
<td>11.30</td>
<td>3.78</td>
<td>2.76</td>
<td>1.59</td>
<td>-0.01</td>
</tr>
<tr>
<td>LKS 17</td>
<td>6.73</td>
<td>Fine silt</td>
<td>3.58</td>
<td>0.62</td>
<td>1.86</td>
<td>3.27</td>
<td>119.07</td>
<td>40.08</td>
<td>27.04</td>
<td>8.79</td>
<td>7.08</td>
<td>3.00</td>
<td>0.04</td>
</tr>
<tr>
<td>LKS 18</td>
<td>6.46</td>
<td>Fine silt</td>
<td>4.10</td>
<td>0.00</td>
<td>1.72</td>
<td>3.79</td>
<td>92.97</td>
<td>35.16</td>
<td>25.78</td>
<td>7.81</td>
<td>6.68</td>
<td>2.68</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*n.d. (not detected)

Heavy metal concentrations in present study are in µg/g, except for Fe and Al, which are %.

Average shale values are in ppm, except for Fe and Al, which are %.

SQGs are in ppm.

Recovery tests are in µg/g, except for Fe and Al, which are %.
The foraminiferal distribution in Sungai Kilim

A total of 28 genera of foraminifera, belonging to 22 families and six orders, were identified from the lagoon and mangrove areas of Sungai Kilim. Of the 20 surface sediment samples collected, only 14 samples contained foraminifera, while the remaining samples (LKS7, LKS10, LKS15, LKS17, LKS18 and LKS19) did not. After data screening, 14 genera with a relative abundance exceeding 2% in at least one sample were used for further analyses (Appendix 1). Elphidium spp. (average relative abundance > 20) dominated foraminiferal assemblages at both mangrove and lagoon stations; additionally, Quinqueloculina spp. and Ammonia spp. (10%) were common genera (Appendix 1). At several stations (LKS9, LKS12 and LKS13) Elphidium spp. composed > 80% of total assemblages. However, at LKS 6 (upper zone of mangrove areas), Elphidum spp. were absent, and species such as Trochaminna spp. (31%), Ammotium spp. (22%), Ammobaculites spp. (19%) and Haplophragmoides spp. (17%) were more abundant.

All diversity indices are valuable for determining diversity and evenness, but the sensitivity of each index differs. Therefore, this study compared similar indices (Table 2). The dominance index ranged from 0.20 to 0.79, while the Fisher's alpha index values ranged from 0.85 to 2.42. A high Fisher's alpha index was observed at LKS1 (2.08), LKS3 (2.41), LKS4 (2.42), LKS6 (2.05), LKS8 (2.08), LKS11 (2.42), and LKS12 (2.05). The dominance index generally showed an inverse pattern to the Fisher's alpha index value (Table 2); a high Fisher's alpha indicates high diversity, while a high dominance is associated with low diversity. The Simpson's index ranged between 0.21 and 0.80, while the Shannon-Wiener index ranged between 0.47 and 1.84. This index was high at most stations except LKS9 (0.47), LKS12 (0.80), LKS13 (0.84), LKS14 (0.77), and LKS16 (0.75). On the other hand, the evenness index ranged between 0.28 and 0.81.

The table below shows the heavy metal concentrations and the SQGs for Sungai Kilim samples:

<table>
<thead>
<tr>
<th>Stations</th>
<th>Mean (ϕ)</th>
<th>Texture</th>
<th>TOC (%)</th>
<th>CaCO3 (%)</th>
<th>Fe</th>
<th>Al</th>
<th>Mn</th>
<th>Zn</th>
<th>Pb</th>
<th>Ni</th>
<th>Cu</th>
<th>Co</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>LKS 19</td>
<td>6.31</td>
<td>Fine</td>
<td>6.02</td>
<td>0.00</td>
<td>1.65</td>
<td>3.43</td>
<td>73.26</td>
<td>34.30</td>
<td>23.06</td>
<td>7.40</td>
<td>5.68</td>
<td>2.46</td>
<td>0.03</td>
</tr>
<tr>
<td>LKS 20</td>
<td>6.46</td>
<td>Fine</td>
<td>3.53</td>
<td>0.00</td>
<td>1.69</td>
<td>2.49</td>
<td>101.38</td>
<td>32.94</td>
<td>23.07</td>
<td>6.53</td>
<td>6.19</td>
<td>2.37</td>
<td>0.03</td>
</tr>
<tr>
<td>Mean (present study)</td>
<td>0.68</td>
<td></td>
<td>1.67</td>
<td>58.52</td>
<td>16.75</td>
<td>12.33</td>
<td>3.08</td>
<td>2.60</td>
<td>1.14</td>
<td>-0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average shale value:

TOC (6.30–6.59) & CaCO3 (73.26–85.00)

SQG

Non-polluted:
- n.d.
- n.d.
- n.d.
- < 90
- < 40
- < 23
- < 25
- n.d.
- < 0.99

Moderately polluted:
- n.d.
- n.d.
- n.d.
- 90–200
- 40–70
- 23–36
- 25–75
- n.d.
- 0.99–3

Heavily polluted:
- n.d.
- n.d.
- n.d.
- > 200
- > 70
- > 36
- > 75
- n.d.
- > 3

Recovery test (NBS 1646a):
- 96.21
- 78.03
- 87.84
- 93.33
- 92.99
- 99.82
- 76.52
- 87.11
- 101.6

* n.d. (not detected)

Heavy metal concentrations in present study are in µg/g, except for Fe and Al, which are %.

Average shale values are in ppm, except for Fe and Al, which are %.

SQGs are in ppm.

Recovery tests are in µg/g, except for Fe and Al, which are %.
Table 2
Diversity indices and abnormalities of foraminiferal tests in the surface sediment samples.

<table>
<thead>
<tr>
<th>Station</th>
<th>Dominance index</th>
<th>Simpson's index</th>
<th>Shannon-Wiener index</th>
<th>Fisher's alpha index</th>
<th>Evenness</th>
<th>Abnormal tests (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LKS1</td>
<td>0.35</td>
<td>0.65</td>
<td>1.36</td>
<td>2.08</td>
<td>0.49</td>
<td>0.00</td>
</tr>
<tr>
<td>LKS2</td>
<td>0.39</td>
<td>0.61</td>
<td>1.20</td>
<td>1.73</td>
<td>0.47</td>
<td>0.00</td>
</tr>
<tr>
<td>LKS3</td>
<td>0.20</td>
<td>0.80</td>
<td>1.84</td>
<td>2.41</td>
<td>0.70</td>
<td>3.03</td>
</tr>
<tr>
<td>LKS4</td>
<td>0.22</td>
<td>0.78</td>
<td>1.74</td>
<td>2.42</td>
<td>0.63</td>
<td>1.92</td>
</tr>
<tr>
<td>LKS5</td>
<td>0.21</td>
<td>0.79</td>
<td>1.74</td>
<td>1.71</td>
<td>0.81</td>
<td>0.00</td>
</tr>
<tr>
<td>LKS6</td>
<td>0.21</td>
<td>0.79</td>
<td>1.68</td>
<td>2.05</td>
<td>0.67</td>
<td>0.00</td>
</tr>
<tr>
<td>LKS8</td>
<td>0.41</td>
<td>0.59</td>
<td>1.19</td>
<td>2.08</td>
<td>0.41</td>
<td>5.20</td>
</tr>
<tr>
<td>LKS9</td>
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<td>0.21</td>
<td>0.47</td>
<td>0.85</td>
<td>0.40</td>
<td>0.00</td>
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<tr>
<td>LKS11</td>
<td>0.29</td>
<td>0.71</td>
<td>1.60</td>
<td>2.42</td>
<td>0.55</td>
<td>2.70</td>
</tr>
<tr>
<td>LKS12</td>
<td>0.68</td>
<td>0.32</td>
<td>0.80</td>
<td>2.05</td>
<td>0.28</td>
<td>1.10</td>
</tr>
<tr>
<td>LKS13</td>
<td>0.64</td>
<td>0.36</td>
<td>0.84</td>
<td>1.71</td>
<td>0.33</td>
<td>0.00</td>
</tr>
<tr>
<td>LKS14</td>
<td>0.65</td>
<td>0.35</td>
<td>0.77</td>
<td>1.11</td>
<td>0.43</td>
<td>0.00</td>
</tr>
<tr>
<td>LKS16</td>
<td>0.63</td>
<td>0.37</td>
<td>0.75</td>
<td>1.12</td>
<td>0.42</td>
<td>6.30</td>
</tr>
<tr>
<td>LKS20</td>
<td>0.38</td>
<td>0.62</td>
<td>1.17</td>
<td>1.71</td>
<td>0.46</td>
<td>0.00</td>
</tr>
<tr>
<td>Mean</td>
<td>0.43</td>
<td>0.57</td>
<td>1.22</td>
<td>1.82</td>
<td>0.50</td>
<td>1.45</td>
</tr>
</tbody>
</table>

Test abnormalities in foraminifera

Among the 14 genera identified in the study area, only *Elphidium* spp. showed morphological abnormalities (Fig. 4). Abnormal tests in *Elphidium* spp. were observed in surface sediments, especially at LKS3 (3%), LKS4 (1.9%), LKS8 (5.25%), LKS11 (2.7%), LKS14 (1.1%), and LKS16 (6.3%) (Table 2).

Canonical correspondence analysis (CCA) was used to relate foraminiferal assemblages to environmental parameters and geochemical properties, as shown in Fig. 5. Two factors were identified (eigenvalue < 1), which explained 0.5% and 0.3% of the eigenvalue, respectively. In particular, 27% of the variance between species and the environment was analysed.

As shown in Fig. 5, environmental variables are represented by arrows that extend in both directions from the center. CCA separates the foraminiferal assemblages into two groups, where the first group is located on the positive side and the second group is located on the negative side (Fig. 5). In the first group, *Ammotium* spp. were associated with salinity, while *Eponides* spp. and *Quinqueloculina* spp. were associated with dissolved oxygen. In addition, *Textularia* spp. were associated with heavy metals, such as Al and Pb. In the second group, opportunistic genera such as *Ammonia* spp. and *Elphidium* spp. were associated with temperature, and *Triloculina* spp. were associated with CaCO$_3$. In addition, *Buliminia* spp. were associated with TOC and all heavy metals except Al and Pb. In contrast, other taxa in the second group, such as *Bolivina* spp., *Miliammina* spp., *Ammobaculites* spp., *Trochammina* spp., *Reophax* spp., and *Haplophragmoides* spp. were not associated with any parameters or geochemical properties. Overall, *Elphidium* spp. was the dominant genus in this study. CCA demonstrated that *Ammonia* spp. and *Elphidium* spp. were positively correlated with temperature and depth (Fig. 5). This was supported by a Monte Carlo permutation test, which showed a strong correlation ($p < 0.005$) between temperature and depth and *Ammonia* spp. and *Elphidium* spp.

Discussion

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According to\textsuperscript{29}, the distribution of marine sediments is controlled mainly by marine dynamics. The predominance of coarser sediment is associated with sediment transport via tidal currents and wave action\textsuperscript{30}, especially in mangrove areas. Thus, the tidal currents that are responsible for establishing the circulatory systems in Sungai Kilim can carry sediments and pollutants into and out of the area during high and low tides. This is supported by\textsuperscript{31}, who demonstrated that tides could control the water flow and sediment transport within a mangrove area. This phenomenon was observed in the present study, where debris was carried into the channels by tidal activities. Unlike the Sungai Kilim tributary where mangrove can be found, the lagoon area of Sungai Kilim is protected from tidal currents and wave action. Thus, it has very low hydrodynamics energy and is dominated mostly by fine sediment. Besides the accumulation of fine sediment, the low energy tidal currents usually permit the accumulation of organic matter which become food source to abundant benthic fauna\textsuperscript{32,33,34}.

In this study, the sediment quality analysis revealed that the lagoon environment (LKS17-20) has finer sediments (mean > 6), higher organic concentration, and low carbonate (< 1\% CaCO\textsubscript{3}). This indicates that finer sediments trap more organic carbon\textsuperscript{36}, owing to the similar settling velocity of both the organic constituents and fine sediment particles\textsuperscript{37}. In addition, both the relatively rapid accumulation rate of fine-grained inorganic carbon and the low oxygen concentration of the waters immediately above the sediments favoured high organic carbon in the bottom sediments\textsuperscript{38}. The organic carbon in Sungai Kilim most likely originated from nearby sewage outlets and the accumulation of mangrove plant litter. Meanwhile, the higher CaCO\textsubscript{3} at the mangrove stations, especially LKS8 (5.33\%) and LKS9 (9.54\%) (Table 1), was due to the presence of shelled organisms in the environment (dead foraminifera, bivalve, and gastropods\textsuperscript{39}). The absence of these shells from the lagoon environment could explain the carbonate deficiency in those areas.

The distribution of heavy metals in sediments can be influenced by carbonates, organic carbon, and sediment size\textsuperscript{40}. High heavy metal concentrations are usually found in fine sediments\textsuperscript{41}. Fine particles have large specific surface areas and can therefore act as a site for the accumulation and transport of metals\textsuperscript{42,43}, which explains the high heavy metals concentrations recorded at LKS17, LKS18, LKS19, and LKS20, especially Zn (> 30µg/g) and Pb (> 20µg/g).

Heavy metals in water are derived from both natural and anthropogenic sources. Natural processes, such as the weathering of rocks and soil and atmospheric inputs, are directly released to surface waters\textsuperscript{44}. These processes are the largest natural source of heavy metals; however, they also originate from anthropogenic activities, such as industry, agriculture, mining, burning of fossil fuels, and vehicular emissions\textsuperscript{45}. After entering the aquatic system, heavy metals accumulate in the sediment. The concentration of heavy metals in the surface sediments in Sungai Kilim were low compared to the average background value (20µg/g) except for Pb at LKS17 (27.04µg/g), LKS18 (25.78µg/g), LKS19 (23.06µg/g), and LKS20 (23.87µg/g) (Table 1). The heavy metals in the sediments in this study were primarily derived from the weathering of the sedimentary rocks in the surrounding Setul Formation. Nonetheless, these rocks were not the only source of Pb. The excessive Pb most likely resulted from human activities because samples were taken in pathways with heavy boat traffic. The Sungai Kilim is one of the main entrances to tourists visiting the limestone karst of the Kilim Geopark. Therefore, boating activities resulted in the burning of leaded gasoline and causes oil leaks along the study area. Furthermore, floating restaurants and aquaculture cages which also serve the tourism activities were situated near the study area.

Foraminifera as a bio-indicator in mangrove and lagoon environments

Monitoring and evaluating coastal ecosystem risks associated with heavy metal pollution are highly complex issues that require interdisciplinary teams with expertise in the biology and ecology of lagoonal biotas\textsuperscript{46}. Natural (i.e., storms) or human activities (i.e., boating) that disturb sediments can mix mobile heavy metals from hypoxic waters into the water column, where the heavy metals can readily affect the local biota\textsuperscript{11}. For the past 50 years, foraminifera have been used for pollution studies in various environments.

The foraminifera in Sungai Kilim changed according to the depositional environment. Of the 28 genera identified, 	extit{Elphidium} was the dominant genus and was distributed mostly in the mangrove areas but only selected lagoon stations. Other genera such as 	extit{Ammonia} and 	extit{Quinqueloculina} were common in the mangrove areas too. However, our observation showed that benthic foraminifera were absent from the inner lagoon environment (LKS17-19), except at LKS 20, where 	extit{Ammonia} spp. (45\%) and 	extit{Elphidium} spp. (43\%) co-dominated. We suspect that the absence of carbonate, the presence of very fine substrate, and the higher heavy metal concentrations may have prevented any foraminifera from inhabiting this area. Benthic foraminifera were also absent from three mangrove stations. Two of these stations (LKS 7 and 15) were situated at the deeper end of the tributary channels, away from the opening to the sea.
Both stations reported a deficiency of calcium carbonate (Table 1), and the absence of benthic foraminifera at LKS10 was also associated with the absence of CaCO₃.

The *Elphidium* spp. were highly abundant (>80%) in the mangrove stations (e.g., LKS9, LKS12, and LKS13), where the water depth was >3 m, the salinity was close to marine levels (33‰), and the sediment was much coarser (mean <1). Meanwhile, *Ammonia* spp. dominated stations with low CaCO₃ but >1% TOC. Moreover, along the Malacca Straits, *Ammonia* sp. has been documented as a resilient species that favours a marginal hypersaline environment with abundant food⁴⁷, ¹⁷. The CCA indicated that genera such as *Elphidium*, *Ammonia* and *Bulimina* prefer deeper water to prevent exposure to high temperatures during low tide periods.

Fisher's alpha index is a relationship between the number of species and the number of individuals in a group and represents the richness of the benthic associations⁴⁸. High Fisher's alpha indices indicate high diversity in foraminiferal biotas⁴⁹. Fisher's alpha index generally increases as the number of species increases, from estuarine towards the seas. Fisher's alpha index in this study had low values (<6) (Table 2). The higher observations were mainly due to the existence of both porcelaneous-miliolid and hyaline-perforate taxa and are similar to those commonly found in coastal nearshore environments, estuarine, tropical lagoons, or back reef environments, where the diversity of foraminifera is much higher than of mangrove forest⁵⁰, ¹⁵, ¹⁶, ¹⁷, ¹⁸. The Shannon-Wiener index considers the number of individuals of each taxon and the number of taxa present in each sample⁴⁸. Generally, lower values (Shannon-Wiener <1) are recorded under stressful environmental conditions or the presence of opportunistic or pioneer species. In terms of faunal diversity, the Shannon-Wiener index was low (<1) at LKS9, LKS12, LKS13, LKS14, and LKS16 (Table 2), indicating environmental stress at those sites. Additionally, the evenness index is a measure of uniformity in the distribution of different taxa⁵⁷. As stated by⁴⁸, the evenness value is high when all taxa are equally abundant in a sample. The evenness values in this study were low (<1), indicating that all taxa were not equally abundant; *Elphidium* spp. and *Ammonia* spp. dominated this study (Appendix 1).

The changes in species diversity in foraminiferal assemblages can indicate environmental stress, whereas higher diversity indicates more stable and less polluted environments⁵⁸, ⁵⁹. In general, the diversity increased with increased water depth and decreased pollution. Low diversity was recorded at the stations that had high heavy metal concentrations (LKS16 and LKS20) (Table 1 and Table 2). ⁶⁰ showed that moderate concentrations of heavy metals influenced foraminiferal density and species richness. Only certain opportunistic species can survive in highly polluted sediments⁶¹, ⁶. However, in Sungai Kilim, heavy metal concentrations did not exceed the sediment quality guideline values (Table 1), which indicates non-polluted conditions. In addition, the CCA showed a weak correlation between genera and selected metal concentrations. Instead, salinity was a more prominent parameter, for foraminiferal distribution (Fig. 5). *Ammotium* spp. and *Reophax* spp., for instance, prefer a more saline environment (34‰), whereas *Elphidium* spp. and *Ammonia* spp. can tolerate lower salinity (33‰).

**Test abnormalities**

Benthic foraminifera are effective for the assessment and monitoring of coastal and shelf environments because of their taxonomic diversity, wide distribution, abundance, relatively small size, and short reproductive cycles, and well-preserved tests in sediments⁵. Foraminifera have specific ecological niches, and populations respond quickly to environmental changes⁶². As a further indicator, foraminifera often develop deformed testes in stressful environments (Fig. 4). Most studies have used foraminifera to examine pollution by analysing their assemblages in sediments. The presence or absence of key taxa, as well as their abundance and distribution, are usually associated with the source of the pollutants. The responses of foraminiferan assemblages to pollutant gradients include drastic changes in the assemblages⁶³, step-wise faunal changes⁶⁴, or fluctuations in faunal assemblages and species abundance⁶⁵.

Numerous studies relating to foraminifera and pollution have shown that heavy metals affect ecosystems and foraminiferal distribution⁶⁶, ⁵⁹, ⁶⁷. In many cases, where the environment is severely polluted with heavy metals, foraminiferal species show abnormalities in their tests⁶⁶, ⁶. These abnormalities can occur under natural conditions as well⁵⁸, ⁶⁹, ⁷⁰. In natural environments, test abnormalities may result from environmental stresses such as low pH⁷¹, hypersalinity (e.g., ⁷², ⁷³, ⁷⁴), or high hydrodynamic energy⁶⁹. Meanwhile, in polluted environments, the number of morphological deformations and abnormal tests may increase dramatically in areas with high heavy metal pollution⁵⁸. In this study, test abnormalities were observed in *Elphidium* spp., with most of the tests showing signs of regeneration or repair⁶⁹ (Fig. 4). The highest proportions of the abnormal tests were found mainly in stations...
(Table 2) with low heavy metal content (Table 1). Further, the highest number of abnormal tests were recorded in LKS8 (5.25%) and LKS16 (6.3%), a mangrove area that is affected by strong hydrodynamic energy (Fig. 2a).

Therefore, based on the morphological conditions of the abnormal tests of *Elphidium* spp. (Fig. 4), we suspect that strong hydrodynamics energy from tidal currents in the mangrove areas may have caused the abnormalities. A similar phenomenon has been observed in some abnormal foraminiferal tests in paralic environments. These studies found that the percentage of abnormal tests was higher in non-polluted areas subjected to strong natural stress. Even with significant anthropogenic activity, morphological abnormalities may be related to natural environmental stress. In this study, the high proportion of abnormal tests in the mangrove areas was probably due to environmental stress from natural factors such as strong hydrodynamics.

**Conclusions**

This study was based on the quantitative analyses of the foraminiferal assemblages, together with geochemical, physical, and current speed data. The study indicates that calcareous hyaline taxa (i.e., *Elphidium* and *Ammonia*) is very abundant along the mangrove and lagoon area of Sungai Kilim. The fine sediment, high organic matter concentration and adequate calcium carbonate permitted the occurrence of a higher diversity of foraminifera assemblages. The heavy metal concentration does not affect the foraminifera assemblage due to its low concentration values. Additionally, the morphological abnormalities in benthic foraminiferal tests in Sungai Kilim that were observed in *Elphidium* spp. were due to the very strong hydrodynamic energy in the mangrove areas. This was also reflected in the foraminiferal distribution and diversity. Thus, this study showed that the lower abundance and test abnormalities in Sungai Kilim were solely due to natural stress rather than anthropogenic causes. In addition, this study showed that the morphological abnormalities in foraminiferal tests could be utilised in environmental monitoring.

**Methods**

**Sample collection**

Twenty surface sediment samples were retrieved from Sungai Kilim, Pulau Langkawi, in March 2016 (Fig. 1). The water depth at all stations along the river and lagoon ranged between 0.2 and 4.7 m. Sixteen sediment samples (LKS 1 to LKS 16) were collected from mangrove areas, which were dominated by *Rhizophora apiculata* and *R. mucronata*. They were also surrounded by alluvium and in close proximity to the Setul formation. Five sediment samples (LKS 16 to LKS20) were collected from the semi-enclosed lagoons of Sungai Kilim, which were also surrounded by the Setul formation.

Measurements of pH, salinity, temperature, and dissolved oxygen (mg/L) were recorded at each station (Table 3) using the HydroLab Quanta Multiparameter. Surface sediment samples were collected from the top 1 cm of the lagoon or river floor using a ponar grab and were then split into subsamples of approximately 30 mL for foraminifera analyses and 50 g for geochemical analyses (heavy metal, particle size, and organic carbon). Foraminiferal samples were then transferred into 200 mL pre-labelled containers. Sample preservation was performed following the procedure suggested by. The samples were fixed with 70% buffered ethanol to reduce test degradation. The sediment samples for the geochemistry analyses were stored in labelled and sealed plastic bags. All samples were returned to the laboratory for further analyses.
Table 3
The physical and chemical parameters (dissolved oxygen (DO), pH, salinity, and temperature (Temp.)), water depth, and coordinates of each sampling station in Sungai Kilim, Langkawi.

<table>
<thead>
<tr>
<th>Station</th>
<th>Latitude</th>
<th>Longitude</th>
<th>DO (mg/L)</th>
<th>pH</th>
<th>Salinity (‰)</th>
<th>Temperature (ºC)</th>
<th>Depth (m)</th>
</tr>
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<tr>
<td>LKS 1</td>
<td>6° 27' 28.3&quot; N</td>
<td>99° 49' 47.6&quot; E</td>
<td>5.74</td>
<td>7.93</td>
<td>33.13</td>
<td>30.43</td>
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<td>6° 27' 21.7&quot; N</td>
<td>99° 49' 41.1&quot; E</td>
<td>4.96</td>
<td>7.83</td>
<td>33.21</td>
<td>30.5</td>
<td>4.4</td>
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<td>6° 27' 14.76&quot; N</td>
<td>99° 49' 32.01&quot; E</td>
<td>5.19</td>
<td>6.46</td>
<td>33.65</td>
<td>30.36</td>
<td>0.5</td>
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<tr>
<td>LKS 4</td>
<td>6° 27' 0.73&quot; N</td>
<td>99° 49' 17.95&quot; E</td>
<td>6.05</td>
<td>7.06</td>
<td>33.65</td>
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<td>LKS 5</td>
<td>6° 26' 59.02&quot; N</td>
<td>99° 49' 8.01&quot; E</td>
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<td>7.24</td>
<td>33.65</td>
<td>30.3</td>
<td>0.2</td>
</tr>
<tr>
<td>LKS 6</td>
<td>6° 26' 50.7&quot; N</td>
<td>99° 49' 02.2&quot; E</td>
<td>5.83</td>
<td>7.33</td>
<td>34</td>
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<td>99° 49' 54.3&quot; E</td>
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<td>7.95</td>
<td>33.14</td>
<td>30.53</td>
<td>3</td>
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<tr>
<td>LKS 8</td>
<td>6° 26' 35.2&quot; N</td>
<td>99° 49' 54.4&quot; E</td>
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<td>7.92</td>
<td>33.14</td>
<td>30.56</td>
<td>3.6</td>
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<tr>
<td>LKS 9</td>
<td>6° 26' 28.0&quot; N</td>
<td>99° 49' 54.8&quot; E</td>
<td>5.82</td>
<td>7.92</td>
<td>33.14</td>
<td>30.56</td>
<td>4.2</td>
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<tr>
<td>LKS 10</td>
<td>6° 26' 18.1&quot; N</td>
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<td>7.62</td>
<td>33.52</td>
<td>30.76</td>
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<tr>
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<td>LKS 12</td>
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<td>5.31</td>
<td>7.88</td>
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<td>30.7</td>
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</table>

Sample pre-treatment (Sediment analyses)

The sediment samples were transferred into Petri dishes and oven-dried for 48 h. After drying, large particles (gravel, pebbles, and rocks) were removed and a portion of each sample was set aside for particle size analysis. The remaining samples were crushed to a powder using a porcelain mortar and pestle. The powdered samples were used for the heavy metals analysis. For the organic carbon analysis, the powdered samples were sieved using a 150 µm screen-size sieve.

Grain size analyses were carried out using dry sieving and laser diffraction methods. The dry sieving method is suitable for sizes ranging between 63 µm and 250,000 µm. The laser diffraction method is appropriate for determining the grain size of sediments that have > 10% silt and clay. The sediments were described and classified according to the Wentworth size classification. TOC analysis was conducted based on the methods of to obtain an accurate value of the total carbon content in the bulk sediment. TOC was analysed using a carbon analyser (Shimadzu TOC-VCPH with a solid sample module SSM-5000A) and calculated by subtracting the total inorganic carbon from the total carbon. Glucose (C₆H₁₂O₆) and sodium carbonate (Na₂CO₃) were used as standards to calibrate the Shimadzu analyser. The standard calibration curve showed that R² > 0.999, indicating the machine was in good condition.

Calcium carbonate was calculated for all samples according to the following formula proposed by:

\[
CaCO_3 = (TC\% - TOC\%) \times 8.33
\]
where TC is the percentage of total carbon and TOC is the percentage of total organic carbon.

The heavy metal concentrations in the sediment were determined based on the modified method of Refs. 82 and 77. Approximately 0.05 g of each powdered homogenized sediment sample was placed into a Teflon beaker. Samples were then digested in a sealed Teflon vessel with 1.5 mL of concentrated mixed acid (2HF: 3HCl: 3HNO$_3$). The Teflon vessel was heated in an oven at 100°C for 7 h. The samples were cooled to room temperature and transferred into a centrifuge tube. Selected metals (Fe, Al, Mn, Zn, Pb, Ni, Cu, Co, and Cd) were analysed using inductively coupled plasma mass spectrometry. The analysis accuracy was compared to the Canadian Certified Reference Materials Project standard (NBS 1646a). The chemical contamination in the sediments was evaluated by comparison with the sediment quality guidelines proposed by the US EPA. Average shale values of Refs. 83 and average crustal abundance of Refs. 84 are often used as global background concentration values, therefore, the average shale values of Refs. 83 were used as the background elemental concentrations.

**Foraminifera analysis**

For the foraminiferal analysis, sediment samples were gently washed under running tap water on 250 µm and 63 µm sieves of Refs. 89. Sediment retained on these sieves was then carefully transferred into a weighing boat and dried overnight at 60°C of Refs. 112. Once dried, the samples were stored in small plastic bags until further analysis. A total of 200 foraminiferal specimens were obtained from each sample using a picking tray and a stereo microscope of Refs. 90. If a sample had less than 200 foraminifera, all specimens were selected of Refs. 90. The foraminifera were sorted, mounted on micropaleontology slides, and identified to the genus level based on Refs. 91, 92, 93, and other regional taxonomic manuscripts of Refs. 94, 90, 47. All foraminiferal data in this manuscript are expressed as relative abundance.

**Species distribution and correlation with environmental parameters**

The raw foraminiferal abundance data was normalized to relative abundance. The relative abundance of each assemblage was defined using the limits of Refs. 95: dominant (> 20%), common (10–20%), accessory (5–10%), and rare or accidental (1–5%). All statistical analyses in this study used this relative abundance data. Genus counts were square-root transformed, and only the genera that had a relative abundance > 2% in at least one sample were used to reduce potential taphonomic bias or the influence of chance occurrences of rare genera of Refs. 96, 97.

The foraminiferal species diversity indices, specifically the Shannon-Wiener, Simpson's, Fisher's alpha, Dominance, and Evenness indices, were calculated using the Paleontological Statistics Data Analysis (PAST) software of Refs. 98. To understand the correlations between the species and the environment, multivariate analysis and CCA were performed using CANOCO software.

**Current modelling**

A simulation model for the current speed was conducted using a hydrodynamic module in the MIKE 21 software to estimate the current speed in Sungai Kilim. The governing equations for this model can be found in the scientific documentation for MIKE 21 of Refs. 99. The model specification for this study used the hydrodynamic module user guide and followed the same setup as Refs. 30, 34, 100, and 83, with a root mean square error below 0.2. The model simulation period was 31 days, simulating the month of March 2016.

**Declarations**

**Acknowledgments**

This research would not have been possible without financial support from the Long-Term Research Grant Scheme (LRGS) (LRGS/1/2020/UMT/01/1/2), Joseph A. Cushman grant award for student research (Cushman Foundation for Foraminiferal Research), and MyMaster scholarship (Ministry of Education Malaysia). We would like to thank Editage (www.editage.com) for English language editing.

**Author Contributions**

A.R. N. S. carried out analysis, interpretation of results and drafted the manuscript. S. H. participated in the design of this study and helped to draft the manuscript. M. F. I. contributed to major revisions of the manuscript and statistical interpretations. A. E. H. and S. F contributed for current modelling and interpretations. Y. S and and S. R. contributed to the final version of the manuscript.
Additional Information

Competing Interests

The author(s) declare no competing interests.

Data availability

All data described in this study are available within the article and its Supplementary material.

Additional Information

“Supplementary Information is available for this paper.”

“Correspondence and requests for materials should be addressed to S.H.”

“Reprints and permissions information is available at www.nature.com/reprints.”

References


Figures
Figure 1

Map showing the locations of sampling stations in Sungai Kilim, Langkawi, Malaysia. The Pulau Langkawi is marked by the black circle on the map of Peninsular Malaysia. The mangrove areas are LKS1-LKS16, and the lagoon areas are LKS17-LKS20.
Figure 2

(a) Current speed data in Sungai Kilim, very strong to strong energy is notable in the mangrove areas and low energy in the lagoon areas. (b) Distribution of mean particle size. (c) Distribution of total organic carbon (TOC) and (d) Distribution pattern of calcium carbonate (CaCO$_3$). The mangrove areas are LKS1-LKS16, and the lagoon areas are LKS17-LKS20.
Figure 3

Distribution patterns of heavy metals (a) Fe, (b) Al, (c) Cd, (d) Co, (e) Cu, (f) Ni, (g) Pb, (h) Zn, and (i) Mn in the surface sediments in Sungai Kilim. The mangrove areas are LKS1-LKS16 and the lagoon areas are LKS17-LKS20.
Figure 4

Various test deformities in *Elphidium* spp. in surface sediment samples from Sungai Kilim, Langkawi (Scale bars = 100 μm).
Figure 5

Canonical correspondence analysis of the relative abundance of foraminiferal genera in the surface sediments in relation to environmental variables and geochemical properties. The species are abbreviated as follows: Miliammi = Miliammina spp., Quinquel = Quinqueloculina spp., Trilocul = Triloculina spp., Textular = Textularia spp., Ammobacu = Ammobaculites spp., Trochamm = Trochammina spp., and Haplophr = Haplophragmoides spp.

Supplementary Files

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