A training manual for assessing pollution (trace/heavy metals) in rivers, estuaries and coastal waters using innovative Artificial Mussel (AM) technology - Bangladesh model
A Training Manual for Assessing Pollution (Trace/Heavy Metals) in Rivers, Estuaries and Coastal Waters Using Innovative Artificial Mussel (AM) Technology - Bangladesh Model

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A Training Manual for Assessing Pollution (trace/heavy metals) in Rivers, Estuaries and Coastal Waters-Using Innovative “Artificial Mussel (AM) Technology” - Bangladesh Model

By

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The opinions, presentation of material and positions expressed in this publication are those of the authors and do not necessarily represent the official views of University of Chittagong or of the supporting agencies

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# 1. Authors Profile

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<th>Research Interests</th>
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Summary

The Artificial Mussel (or AM) is an innovative passive sampling technology/device. The technology (AM) is used to monitor or detect or assess risk of micro-pollutants (such as trace/heavy metals including cadmium, chromium, cobalt, copper, iron, lead, nickel, manganese, mercury, uranium, zinc) in freshwater rivers, brackish estuaries and coastal and marine environments. The technology could also be useful in risk assessment for various water utilities including recycled water, treated wastewaters, groundwater bores, irrigation channels and highly polluted water bodies (anoxic or hypoxic).

AM is a continuous monitoring tool providing a time integrated accumulation of metals and has proved to be a very reliable tool for monitoring spatial and temporal variation/distribution of pollutants and in identifying pollution “hot spots”. As part of ‘Global AM Watch Programme’, the device has been applied in several countries across the globe including Australia, Bangladesh, Brazil, Canada, China, Iceland, Hong Kong, Korea, Philippines, Portugal, Scotland, South Korea, Spain, Portugal, Russia, South Africa, and the USA.

This manual entitled ‘A Training Manual for Assessing Pollution (trace/heavy metals) in Rivers, Estuaries and Coastal waters-Using Innovative “Artificial Mussel (AM) Technology” - Bangladesh Model is the outcome of field and practical experiences gained by the authors, while assessing risk posed by trace/heavy metals to water quality, biodiversity and human health in Bangladesh.

The manual has been prepared to assist scientists, environmental regulators, NGOs and general communities to handle, deploy and retrieve “artificial mussel” passive samplers in freshwater rivers, brackish water estuaries and coastal waters for monitoring or risk assessment. It provides simple but step-wise procedures for deployment, retrieval and handling of AM. Images/photos are included for easy use of the training manual (images included in the appropriate section). The manual also includes procedures for storage of AM, occupational, health and safety (OHS) requirements during deployment and retrieval of AM, shipment of AM, other water quality parameters and information required to be collected during AM watch program.
Introduction

The Institute of Marine Sciences and Fisheries, University of Chittagong, Bangladesh has been using the “Artificial Mussel” (AM) technology since 2013 to assess the risk posed by trace metals/heavy metals on water quality, aquatic biodiversity, and human health in rivers, estuaries and coastal waters of Bangladesh.

AM technology has been used to monitor the spatial and temporal variation of metals in natural waterways and identifying metal pollution “hot spots” in Australia (Kibria et al. 2010; Kibria et al. 2012a), Hong Kong (Wu et al. 2007), Iceland (Leung et al. 2008), Portugal (Gonzalez et al. 2011), Scotland (Leung et al. 2008), South Africa (Degger et al. 2011) and currently being used in South Korea and Russia. As part of ‘Global AM Watch programme’ and international research agreement (research collaboration between the scientists of the University of Chittagong, Bangladesh (IMSF); RMIT University, Australia; the City University of Hong Kong and the University of Hong Kong), IMSF received AM technology and training and analytical support (as part of the research agreement) and has developed a procedure for deployment and retrieval of AM in waterways of Bangladesh. This procedure includes:

- Handling and storage of AM
- Selection of monitoring sites for AM
- Preparation of bags for AM
- Deployment of bags
- Retrieval of AM
- Shipment of AM
- Occupational, health and safety requirements (OHS) during field monitoring with AM

This manual highlights the operations IMSF, Chittagong University undertakes during deployment and retrieval of AM in rivers, estuaries and coastal waters of Bangladesh.
4. What is an Artificial Mussel or AM?

The “Artificial Mussel (AM)” passive sampler is a device that accumulates metals through a diffusion barrier onto a sorbent medium. The device consists of non-permeable Perspex tubing (60 mm x 25 cm) in which 200 mg Chelex-100® resin (50-100 mesh from Bio-Rad) is suspended in 8 mL seawater/freshwater inside the tubing (see Figure 1). Both ends of the tubing are further capped by a layer of polyacrylamide gel (thickness: 1 cm), to protect the gel from possible mechanical damages (Figures 1 and 2). Water diffuses through the polyacrylamide gel into the chelax-100 (metal binding agent) from which the complexed metals can later be extracted (see Figure 1). After several weeks, the chelating agent is sampled to determine its metal content.

**Figure 1:** A schematic diagram showing the design of Artificial Mussel chemical structure of chelex-100 is shown in the inset (Wu et al. 2007).

**Figure 2:** Artificial mussels device used in metals monitoring, Bangladesh (Kibria et al. 2012a)
Traditional monitoring of metals in the aquatic environment involves determining and comparing metals in water, sediment and biota, but each method has its own problems and limitations (see Table 1). For example, temporal variations in metal concentrations in water are typically large, which often require frequent sampling and analysis that are not cost effective. Bio-monitoring has been used extensively to monitor metals in the last two decades, the notable example of which is the ‘Global mussel watch program’ (Goldberg et al. 1978; Phillips 1985, Hossain 1994; Hossain 2004; Hossain 2005; Kimbrough et al. 2008, and other persistent pollutants (e.g. OCPs, PCBs) (Tanabe et al., 1997, Hossain et al., 1999, 2000, 2002).

The AMs can be deployed in a range of situations (e.g. toxic, anoxic waters) where biomonitoring organisms are not available. Moreover, AM is a reliable tool for monitoring metals in waterways and could be useful in risk assessment for various water utilities including recycled water, treated wastewaters, groundwater bores, rivers, and irrigation channels (Kibria et al. 2010). Until recently there were no reliable time integrated techniques to assess metal concentrations in water which could be used to assess the risk concentrations with respect to water quality guidelines. However, recently Wu et al., (2007) developed an “Artificial Mussel” (AM) technology which have been used to monitor different toxic metals (e.g. Cd, Cr, Cu, Hg, Pb, Zn) in different water bodies of Australia, Europe, South Africa and China (Leung et al. 2008; Degger et al. 2011; Gonzalez-Rey et al. 2011; Kibria et al. 2012a). This new device is a cost effective monitoring tool which provides a time-integrated concentration of metals in the aquatic environment during the deployment period. Both laboratory and field studies showed that this newly developed chemical device confers significant advantages compared with traditional monitoring techniques using live mussels, water and sediment (Wu et al. 2007; Leung et al. 2008; Degger et al. 2011; Gonzalez-Rey et al. 2011; Kibria et al. 2012a).
Table 1: A comparison of sampling/monitoring of trace/heavy metals by different techniques (Kibria et al. 2012b).

<table>
<thead>
<tr>
<th>Methods of sampling/monitoring</th>
<th>Pros</th>
<th>Cons</th>
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| Spot water sampling/monitoring | - Quick  
- Analysis straight forward. | - Snap shot.  
- Requires frequent sampling/monitoring  
- Time consuming.  
- Costly.  
- Routine monitoring typically determines total metals but not-bio-available or toxic fractions.  
-Do not provide spatial and temporal variation in pollutant concentration (e.g. metals) |
| Bio-monitoring (using live mussels) (e.g. *Perna viridis*, *Mytilus edulis* etc.) | - Uptake both bio-available and toxic fractions.  
- Indicate both spatial and temporal variations. | - Requires killing of animals.  
- Metals accumulation affected by abiotic and biotic factors.  
- No standard mussel's species are available for worldwide use.  
- Some pollutants can be eliminated by the test animals.  
- Requires translocation of mussels from lab. to field or local mussel species.  
- Requires complex analysis of biological samples. |
| Artificial mussels (AM) technology | - Continuous monitoring.  
- No power or energy required.  
- Metal accumulation/uptake is not affected by biotic and abiotic factors.  
- Less affected by salinity & temperature  
- A standard tool for all waters worldwide (fresh, estuarine & sea)  
- Indicate both spatial and temporal variations.  
- Can be deployed where bioindicator organism are not available  
- Simple to handle, deploy and retrieve  
- Simple to analyse  
- Uptake both bio-available and toxic fractions.  
- Comparisons valid between sites without calibration if water has similar properties.  
- Identify micro-pollutants “hot spots” | - Extensive calibration studies are necessary to characterize the uptake of chemicals into a passive sample. |
6. Selection of Monitoring Sites for AM Deployment

Before deployment of AM in rivers, estuaries and coastal water, the following aspects should be considered as highlighted below:

6.1: The objectives of the investigation should be clearly identified and robust.

6.2: A reconnaissance survey should be conducted to identify the potential high, medium and low risk/impacted sites (e.g. most impacted sites could be sites close to industrial, domestic and agricultural wastes discharge points).

6.3: A survey should be conducted to identify reference/control/least impacted sites (e.g. least impacted or pristine sites such as upstream of a river without agriculture farming, or domestic or industrial waste discharge points, mining or human habitation; a natural forest area could be a good reference site).

6.4: If the objectives are to identify "hot spots", a mix of potential high, medium and low risk sites plus at least one reference/control/least impacted sites should be included in the AM watch programme.

6.5: If the objectives are to protect the ‘most ecologically sensitive sites’ or high conservation values, such as, RAMSAR sites, Ecologically Critical Areas (ECAs) and Protected water bodies, Marine Protected Areas (MPAs) or ‘Sanctuary’ as declared by Govt. of Bangladesh should be the prime sites for such monitoring. (http://www.mincos.gov.au/__data/assets/pdf_file/0019/316126/wqg-ch3.pdf)

6.6: If the objectives are to protect the aquatic biodiversity (e.g. native fish/biota) or the environment, slightly to moderately disturbed systems and highly disturbed systems should be included in AM watch program (see http://www.mincos.gov.au/__data/assets/pdf_file/0019/316126/wqg-ch3.pdf).

7. Duration of Monitoring in AM Watch Programme

The duration of AM monitoring/watch programme can be for a short or long periods (pilot or extended monitoring):

7.1: Pilot Monitoring: If the objectives are to collect some preliminary or baseline data and information, a pilot study can be conducted for at least for 3 months by selecting few possible risk sites (possible most impacted, medium impacted, less impacted and least impacted). This pilot study will help to test the AM technology and provide information as to whether trace/heavy metals are a potential problem in an area and whether any further or long term investigations is required etc.

7.2: Extended Monitoring: If the objectives are to assess the effects of pollutants (metals) in an aquatic environment or ecologically sensitive sites (Ramsar sites in Bangladesh such as Tanguar haor, Sunamganj, Sundarbans, Khulna) or St Martins coral island, Bay of Bengal, Bangladesh or aquatic biodiversity (e.g. native fish, other native aquatic organisms), or human health - a long term AM watch programme should be run for at least one to two consecutive years or more. Such a study will also generate data on climate variability (dry vs. wet years / or between six seasons of Bangladesh such as summer (14 April-14 June), monsoon/rainy (15 June-17 August), Autumn (18 August-18 October), Late Autumn (19 October-16 December), Winter (17 Decemebr-13 February) and Spring (14 February-13 April) impacts on inputs, transport and bioavailability of trace (toxic metals) including spatial and temporal variations.

8. Storage of AM

AMs should be stored in MilliQ (deionized) water or distilled water until deployment in the field in ambient temperature (see Figure 3).

Figure 3: AMs are stored in MilliQ (deionised) water before deployment in the field.
It is essential to conduct a site specific risk assessment and take appropriate OHS measures during deployment and retrieval of AM. Accordingly, a site specific risk assessment be conducted and safe work instructions (risk control measures) is to be developed for all the AM watch/sampling sites. This may include working several people together with life jackets (4-5) during deployment and retrieval of AM as experienced during deployment and retrieval of AMs in estuarine and coastal monitoring sites of Bangladesh, because of strong tidal action/current and rough sea. Furthermore, mechanismed good quality boat/ fibre glass boat, carrying a first aid kit, a snake bite kit, sunscreen, insect repellent and personal protective clothing as and when required (sun hat, high visibility vest, swimming life jacket, waders, PVC gloves, lab gloves, safety glass, gum boots, long sleeved shirt and trousers, sturdy footwear, mobile phone would be essential. Weather forecast information/radio (sea rough or calm), tide information are very important for study in estuary- coastal sites.

10.1: Safeguard of the Site: It is essential that a preliminary survey be conducted to select and identify the risk sites/monitoring sites (see section 5). Once we selected a site in Bangladesh, we had several meetings with the local communities and stakeholders (including govt. authority and local elected representative/s) to inform them about the objectives of monitoring/research, their cooperation in accessing the sites during sampling and to be vigilant of the site. In addition, we have also hired a local man at each site to take care of AM containing deployed baskets (The duration of each deployment of AM was for 4 weeks).

10.2: Equipment Required: boat (access to a boat), buoy (access to a buoy), bamboo poles (could be 12-16 feet or 366 cm-488cm, depending tidal heights and current etc.), cable ties, deionised water/distilled water, cotton wool/ sponge, metal wire, measuring tape, name tags, nets, permanent marker, plastic bottles, plastic boxes/baskets with mesh, pen & pencil, pliers, red flags, resealable bags, rubber bands, small rope (cords-polypropylene braded), rocks, red flags, rope (thick), rope (thin), rope (cords-polypropylene braded), strong structures (such as culvert to attach AM baskets), side cutter.

10.3: Procedure: Baskets containing AMs to be deployed should be attached/tied into a buoy or culvert or bamboo poles (digging deeply into the mud of water (in the bottom) of river, estuary or coastal water in a distance from the land mark, not to be affected by tide and AM always remain at least 1 meter below the water surface (see section 11- deployment of AM).
11. Deployment of AM

Field deployment of AM procedures and protocols include placing of AM (at least three (03) replicate AM per site) in a plastic basket (17.8 x 16.8 x 15.6 cm) or cage or nylon mesh bag with mesh opening of 5-10 mm minimal for exchange of water (Figure 4a). A piece of rock should be attached under the basket to facilitate sinking of the basket containing AMs (Figure 4b). Strong nylon rope should be passed through the top corners of the AM containing box (Figures 4c and 4d). After that, the AM box should be deployed at 1m depth of water (1m below the lowest low tide line level) by attaching the basket either onto a structure of a bridge or culvert or buoy or jetty pillars or bamboo poles (depending on the site locations and availability of the structure at the selected sites).

11.1 Deployment of AM (steps)

- **4a.** Replicate AMs were placed in the basket tied with cable ties
- **4b.** Weight/rock attached at the bottom of the basket to facilitate sinking at least 1 meter below the surface of the water column.
- **4c.** Nylon rope passed through the top corners of the AM basket
- **4d.** Nylon rope passed through the top corners of the AM basket (see also Figures 5a to 5c)
- **4e.** Bridge structure can be used to attach AM containing baskets
- **4f.** Bamboo pole can be used to attach AM containing baskets (see also Figures 5a to 5c)

*Figure 4: Steps in deployment of AM*
11.2: Deployment of AM in Creeks/Canals/Rivers/Sea

Figure 5a. AM can be deployed in a shallow and narrow creek/canal/river/drain by fixing wooden/bamboo poles on both side of the creek/river/drain and passing a rope in between the poles and hanging down the basket at a desired point and operating from the shore.

Figure 5b. AM can be deployed in a tidal river/sea by fixing strong bamboo poles/wooden logs at a desired location or attaching to a behind net. Care should be taken that deployed AM basket is always 1m below the depth of low tide level.

Figure 5c. AM can be deployed by using floating buoys
12. Retrieval of AM

AM retrieved from the field needs to be properly labelled for easy identification, storage and shipment, which requires the following materials: cotton wool/sponge, resealable bags, name tags (showing the source, date, water type etc.) and rubber bands.

12: Retrieval Process

Each batch of AM deployed in rivers, estuaries and coastal waters should be retrieved at the end of a four weeks (28 day) interval. It is important to retrieve, label and store AMs from each site separately (DO NOT retrieve in bulk and then attempt to accurately label each AM). Baskets containing AM may be covered with silt and algae that should be rinsed with the site water. After rinsing, each AM is wrapped within a wet sponge, with identification tags included inside each whirl pack bag/resealable bag before shipment to a laboratory. The following procedures should be followed:

a. The surface of the AM should be rinsed with the site water to remove attached fouling organisms such as algae or other debris (if any) (Figures 6a and 6b).

b. Occupational Health and Safety (OHS) equipment should be used when handling AM (globes, apron, masks) to reduce cross contamination of AM samples and for safety of researcher (Figure 6d).

c. Sponge/cotton pads soaked with water from the site and should be wrapped separately around each individual AM, and held in place using rubber bands (Figure 6e).

d. Each AM is placed in an individual resalable bag (Figure 6f).

e. Each bag should be double labelled (with pencilled card inside + water proof label on each resealable bag) (Note: Texta® or pen labels on plastics are easily rubbed off and are not recommended).

f. The name tag should include date, site name, AM replicate 1, 2, 3 and other information as appropriate etc.

12.1: Retrieval of AM (steps)

Figure 6: Steps in retrieval of AM
During deployment and retrieval of AM, some basic water quality parameters of each site should be recorded including surface temperature, water temperature, salinity, dissolved oxygen, electrical conductivity (EC), hardness, pH, rainfall etc. (see Tables 2 and 3). In addition, if the objective is to assess the climate variability impacts on trace/heavy metals inputs, transport and bioavailability, rainfall data in the monitoring site would be required (such as upper catchment), such information can be obtained from local Bureau of Metrological Station. The condition of AM during retrieval (such as whether it is broken or lost) or condition of the basket (e.g. attachment of algae or other aquatic organisms) (e.g. score 1-3, with 1 for minimal algae and 3 to severe) and site information (e.g. access damaged during a recent rainfall or flood or other causes such as vandalism) should be recorded.

Table 2: Some examples of site specific average water quality data of 21 sampling sites. Such data should be collected during deployment/retrieval of AM

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<th>Air temp. (°C)</th>
<th>Water temp. (°C)</th>
<th>Soil temp (°C)</th>
<th>Soil temp (°C)</th>
<th>Water pH</th>
<th>Salinity (ppt)</th>
<th>Conductivity (ms)</th>
<th>TDS (g/L)</th>
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<td>Hulda (Site 1) Near Madunaghat Bridge</td>
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<td>28.6</td>
<td>28.1</td>
<td>6.6</td>
<td>7.7</td>
<td>0.4</td>
<td>0.77</td>
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<td>7.5</td>
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<td>0.77</td>
<td>0.34</td>
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<td>7.1</td>
<td>11.49</td>
<td>18.33</td>
<td>12.47</td>
<td>701.8</td>
</tr>
<tr>
<td>Salimpur (Site 8) Fauzdarhat coastline</td>
<td>28</td>
<td>29.2</td>
<td>29.2</td>
<td>6.4</td>
<td>7.2</td>
<td>11.71</td>
<td>18.18</td>
<td>12.33</td>
<td>657.5</td>
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<tr>
<td>Bansbaria (Site 9) Sitakundu coast line</td>
<td>32.66</td>
<td>30.4</td>
<td>31.8</td>
<td>6.1</td>
<td>7.2</td>
<td>11.6</td>
<td>17.87</td>
<td>12.68</td>
<td>657.53</td>
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<tr>
<td>Uttar Patenga (Site 10) patenga coastline</td>
<td>34</td>
<td>30.33</td>
<td>32</td>
<td>6.4</td>
<td>7.3</td>
<td>15.88</td>
<td>24.58</td>
<td>17.0</td>
<td>948.71</td>
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<tr>
<td>Jahaighata (Site 11) Anwara coastline</td>
<td>34.6</td>
<td>30.5</td>
<td>32.23</td>
<td>6.3</td>
<td>7.3</td>
<td>16.6</td>
<td>25.72</td>
<td>17.95</td>
<td>1053.2</td>
</tr>
<tr>
<td>Mosheshkhali Channel (Site 12), Cox's Bazaar</td>
<td>29</td>
<td>28</td>
<td>30</td>
<td>6.4</td>
<td>7.4</td>
<td>27</td>
<td>43.38</td>
<td>29.27</td>
<td>1656.48</td>
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<tr>
<td>Near hatchery zone (Site 13), Sonapara, Cox's Bazaar</td>
<td>29.5</td>
<td>28</td>
<td>30</td>
<td>6.2</td>
<td>7.1</td>
<td>28</td>
<td>44.21</td>
<td>30.23</td>
<td>1765.43</td>
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<tr>
<td>Rejukhal (Site 14), Cox's Bazaar</td>
<td>28.5</td>
<td>27</td>
<td>29</td>
<td>6.3</td>
<td>7.2</td>
<td>23</td>
<td>35.23</td>
<td>24.82</td>
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### Dhaka (sites 15-17)

<table>
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<tr>
<th>Location</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bastuha boropol (Site 15), Buriganga River</td>
<td>31.6</td>
<td>27.83</td>
<td>27.5</td>
<td>5.2</td>
<td>6.9</td>
<td>0.56</td>
<td>1.10</td>
<td>0.75</td>
<td>176</td>
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<tr>
<td>Near babubazarbridge (Site 16), Buriganga River</td>
<td>33.16</td>
<td>31.5</td>
<td>30</td>
<td>5.7</td>
<td>6.8</td>
<td>0.61</td>
<td>1.15</td>
<td>0.78</td>
<td>160.11</td>
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<tr>
<td>Kamringir Char (Site 17), Buriganga River</td>
<td>33.33</td>
<td>29.33</td>
<td>32</td>
<td>5.66</td>
<td>6.8</td>
<td>0.63</td>
<td>1.25</td>
<td>0.84</td>
<td>186.83</td>
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### Khulna (sites 18-21)

<table>
<thead>
<tr>
<th>Location</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mirerdanga Kheyaghat (Site 18), Bhairav River</td>
<td>35.7</td>
<td>31.25</td>
<td>33.5</td>
<td>6.22</td>
<td>7.5</td>
<td>6.75</td>
<td>9.76</td>
<td>8.78</td>
<td>459.89</td>
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<td></td>
<td></td>
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<tr>
<td>Hardboard mill Kheyaghat (Site 19), Bhairav River</td>
<td>36.5</td>
<td>31.3</td>
<td>34.37</td>
<td>6.27</td>
<td>7.43</td>
<td>7.27</td>
<td>11.68</td>
<td>8.29</td>
<td>514.63</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mathavanga (Site 20), Rupsa River</td>
<td>34.7</td>
<td>33</td>
<td>37.67</td>
<td>6.27</td>
<td>7.5</td>
<td>8.32</td>
<td>13.42</td>
<td>9.43</td>
<td>581.44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Near Mongla port (Site 21), Passur River</td>
<td>37.25</td>
<td>33</td>
<td>37.67</td>
<td>6.4</td>
<td>7.2</td>
<td>10.22</td>
<td>16.8</td>
<td>11.36</td>
<td>685.55</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Average rainfall in Chittagong during 2013 ([www.weather-and-climate.com](http://www.weather-and-climate.com))

### 14. Sampling Frequency in AM Watch Programme

Three to six replicate AM should be placed at each site in a basket or cage or nylon mesh bag and be retrieved after four weeks interval.

### 15. Use of Native Biota in AM Watch Programme

In addition to deployment of AM passive samplers at a site, a similar number of live native mussels (LM) can also be stocked (matching AM numbers; an example 3 to 6) in separate baskets or cages or nylon bags at the same site, where AM were deployed (see Figures 7.1-7.4). This is called bio-monitoring or active monitoring and such strategy may or may not be feasible or possible because of the non-availability of native mussels or poor survival of live mussels in basket/cages. In addition, live mussels require depuration of toxic metals prior to deployment (at least 1-2 weeks) which can be done by keeping them in tanks.

Native mussels (LM) should be selected based on biological characteristic such as abundance, hardy in nature (e.g. tolerance to low dissolved oxygen). Inclusion of biota will help to assess the bioaccumulation potential of metals to native biota, threats (metals) it poses to native biota and risks to human consumers (public health risk) via eating seafood contaminated with metals. It will also allow comparing the time integrated accumulation of heavy metals in artificial mussel (passive samplers) with that in native mussels (biota) (i.e. accumulation of metals in biota, i.e LM vs. AM device).

It is essential that approval is received for translocations of live aquatic organisms before such live mussels stocking can be done (from appropriate authorities since there could be some restriction in translocation of native species into new areas). It is further suggested that native mussels to be stocked should originate from the same river or estuaries or coastal areas to avoid any genetic variability between populations and transfer of any diseases etc. into a new location.
(a) **Use of novel AM technology:** Will provide the most accurate spatial and temporal estimations of pollutants. AM will also provide information relating to bio-available and toxic fractions of pollutants. AM will enable identification of pollutants “hot spots” (Kibria et al. 2012b).

(b) **Use of live native (LM) mussel:** Will provide both spatial and temporal variations of pollutants in aquatic biota but not as accurate as AM (since accumulation of pollutants in biota can be significantly affected by chemical and biological factors). However, bioaccumulation in LM can provide an indication of threats posed to local biota as well as to human consumers (from eating seafood contaminated with metals). LM is also an additional check of results obtained with AM (Kibria et al. 2012b).

(c) **Spot water samples:** It is a validation and quality control exercise. Data of spot water will be an additional check-up of data obtained with AM and LM of a site (i.e. whether it matches or differs). Spot water sampling data will also enable comparisons with the recommended aquatic ecosystems guideline values for metals. There will be a comparison amongst spot, LM and AM in determining risk. However spot water sampling is a snap shot of pollutants, determines total metals but not-bio-available or toxic fractions and do not provide spatial and temporal variation in metals (Kibria et al. 2012b)

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**Figure 7:** Locally available live mussels (LM), *Perna viridis* (collected from Moheshkhali, Coxs Bazar, Bangladesh) were deployed at selected coastal areas of Bangladesh with artificial mussels (AM).

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**16. Significance of Three Ways of Monitoring- AM, LM and SW**
17. Shipment of AM

After retrieval, each AM is wrapped with a wet sponge with identification tags and custom declaration form included inside each whirl pack bag before shipment to Hong Kong for analysis. Any reliable couriers can be used for example; we have shipped AM to Hong Kong via Fedex express courier (Figure 8).

![AM finally packed in Cartoon and ready for shipment to Hong Kong for analysis via courier service (FedEx).](image)

18. Analysis

18.1: Analysis of Artificial Mussels (AM)

The contents of each individual AM (chelex resins) will be emptied into a sintered glass filter followed by eluting twice with 12.5 mL 6 M HNO3 (analytical grade). The elutriant will be made up to a known volume with deionized double distilled water and the concentrations of Cd, Cr, Cu, Pb and Zn will be determined by flame atomic absorption spectrophotometry (FAAS; Shimadzu 6501S) and inductively-coupled plasma atomic emission spectrometry (ICP-AES; Perkin-Elmer Plasma 1000). Concentrations of metals in AM will be expressed as μg/g of chelex (Wu et al. 2007; Degger et al. 2011; Kibria et al. 2012a).

18.2: Analysis of Live Mussels (LM)

Live mussels will be dissected with a knife, and their byssal threads will be removed. The soft tissue will be rinsed with Milli-Q-water, weighed and subsequently dried in an oven at 60°C to a constant weight. The dry weight will then be determined before acid digestion in a block digester, using 30% hydrogen peroxide and 70% nitric acid (1:1 v/v). Metal concentrations in the soft tissue of the digested samples will be determined using FAAS or ICP-AES as appropriate. Concentrations of metals in mussel tissues will be expressed as μg/g dry tissue (Wu et al. 2007; Degger et al. 2011).


Hossain, M.M (2004). An assessment of the ability of natural stocks of Green mussels (P. viridis), clam (Meretrix meretrix) & oyster (Crassostrea sp.) to meet international standards of food safety regarding unnatural contaminants. Presented in technical session (post-harvest technology sect.) of the Nat. Seminar on the ‘Fisheries Education & Research Fair’, 04, in BIAM Auditorium, Dhaka, Bangladesh, 19-20 July, 2004, organized by Bangladesh Fisheries Research Forum (BFRF) & supported by DFID (SUFER Project), UK & UGC, Bangladesh (Key findings of the SUFER Funded research project).


<table>
<thead>
<tr>
<th><strong>Artificial Mussel</strong>:</th>
<th>or AM (see passive sampling).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bio-available</strong>:</td>
<td>the degree and rate at which a substance (chemical, toxicant) is absorbed into a living organism or is made available at the site of physiological activity.</td>
</tr>
<tr>
<td><strong>Bio-monitors</strong>:</td>
<td>a species that is sensitive to, and shows measurable responses to, changes in the environment, such as changes in pollution levels.</td>
</tr>
<tr>
<td><strong>Biotic</strong>:</td>
<td>relating to living organisms.</td>
</tr>
<tr>
<td><strong>Calibration</strong>:</td>
<td>to check, adjust, or determine by comparison with a standard.</td>
</tr>
<tr>
<td><strong>Chelex-100 resin</strong>:</td>
<td>is a chelating material which has the ability to bind transition metal ions.</td>
</tr>
<tr>
<td><strong>Chelating agent</strong>:</td>
<td>certain organic compounds which are capable of forming coordinate bonds (with metals through two or more atoms of the organic compound); such organic compounds are called chelating agents. The compound formed by a chelating agent and a metal is called a chelate. Ethylenediaminetetraacetate, ((-\text{O}_2\text{CH}_2)\text{NCH}_2\text{CH}_2\text{N(CH}_2\text{CO}_2\text{-})\text{-2, (EDTA) is a common chelating agent.})</td>
</tr>
<tr>
<td><strong>CityU</strong>:</td>
<td>City University of Hong Kong.</td>
</tr>
<tr>
<td><strong>Concentration</strong>:</td>
<td>The strength of a solution; number of molecules or mass (e.g. in μg/l) of a substance in a given volume.</td>
</tr>
<tr>
<td><strong>Deployment</strong>:</td>
<td>placing of AM.</td>
</tr>
<tr>
<td><strong>Electrical conductivity or EC</strong>:</td>
<td>Electrical conductivity (EC) estimates the amount of total dissolved salts (TDS), or the total amount of dissolved ions in the water.</td>
</tr>
<tr>
<td><strong>Heavy metals</strong>:</td>
<td>Are a group of metallic elements with an atomic number greater than 20 e.g. mercury, chromium, cadmium, and lead characterized by the ability to form co-ordination bonds with organic chelates and anions, and can damage living things at low concentrations and tend to accumulate in the food chain.</td>
</tr>
<tr>
<td><strong>HKU</strong>:</td>
<td>the University of Hong Kong.</td>
</tr>
<tr>
<td><strong>Limit of detection</strong>:</td>
<td>is defined as the concentration at which an analyte can be identified but not quantified.</td>
</tr>
<tr>
<td><strong>Limit of reporting (LOR)</strong>:</td>
<td>is defined as the concentration at which an analyte can be identified and quantified with 99% certainty.</td>
</tr>
<tr>
<td><strong>Milli-Q or Type 1 water</strong>:</td>
<td>also called high purity water, refers to water that has been purified and deionized to a high degree by water purification systems.</td>
</tr>
<tr>
<td><strong>Micro-pollutants</strong>:</td>
<td>are compounds which are detected in the concentration range of ng/L up to μg/L in the environment.</td>
</tr>
<tr>
<td><strong>Passive sampling</strong>:</td>
<td>A device that collects or accumulates pollutants (e.g. Heavy metals) by diffusion onto a receiving phase usually separated from water by a membrane.</td>
</tr>
<tr>
<td><strong>Polyacrylamide</strong>:</td>
<td>Polyacrylamide (poly(2-propenamide) is a polymer ((-\text{CH}_2\text{CHCONH}_2\text{-})) formed from acrylamide subunits that can also be readily cross-linked.</td>
</tr>
<tr>
<td><strong>RAMSAR sites</strong>:</td>
<td>Wetlands that is important for conserving biological diversity. The Convention on Wetlands of International Importance (the RAMSAR Convention) was signed in Ramsar, Iran on 2 February 1971. The Ramsar Convention aims to halt the worldwide loss of wetlands and to conserve, through wise use and management, those that remain.</td>
</tr>
<tr>
<td><strong>Retrieval</strong>:</td>
<td>removal of AM from the rivers</td>
</tr>
<tr>
<td><strong>Spatial</strong>:</td>
<td>of or relating to space</td>
</tr>
<tr>
<td><strong>Temporal</strong>:</td>
<td>of, relating to, or limited by time</td>
</tr>
<tr>
<td><strong>Toxic</strong>:</td>
<td>of, pertaining to, affected with, or caused by a toxin or poison</td>
</tr>
<tr>
<td><strong>Trace metals</strong>:</td>
<td>Metals with a concentration in water between μg/L to mg/L.; e.g. calcium and magnesium associated with essential elements</td>
</tr>
<tr>
<td><strong>Translocation</strong>:</td>
<td>Translocation is the movement of living organisms from one area with free release in another. This includes intentional and unintentional 8</td>
</tr>
</tbody>
</table>
Bangladesh, India, Indonesia, Malaysia, Maldives, Myanmar, Sri Lanka and Thailand are working together through the Bay of Bengal Large Marine Ecosystem (BOBLME) Project to lay the foundations for a coordinated programme of action designed to better the lives of the coastal populations through improved regional management of the Bay of Bengal environment and its fisheries.

The Food and Agriculture Organization (FAO) is the implementing agency for the BOBLME Project.

The Project is funded principally by the Global Environment Facility (GEF), Norway, the Swedish International Development Cooperation Agency, the FAO, and the National Oceanic and Atmospheric Administration of the USA.

For more information, please visit www.boblme.org