





Ocean Matters: Safeguarding Our Oceans – Project FAQs

1. What is the calendar of project activities?

A: The project activities engaging teachers and students will be carried out in the academic year 2023-24 and 2024-2025. Within each academic year, the project activities will tentatively carried out from June to January.

A. Hybrid Training Programs:

Two hybrid training programs will be carried out to build capacity to use the two protocols-Global Observations to Benefit the Environment (GLOBE) and Microplastic Monitoring Protocol (MMPT)

- The training workshop on the GLOBE protocol will be held in July-August and will spread over 1 working day from 9:30 AM to 1:30 PM. Once the date is fixed it will be communicated to schools
- The training workshop on the MMPT Protocol will be in September-October and spread over 2 working days with a gap of 1-2 weeks between them. The dates will be fixed and communicated.
- **B.** Conducting Experiments:

Training of students: The trained teachers will impart training to a select set of students to carry out experiments and use the protocol.

- *Conducting the experiment:* Students will conduct the experiment under the guidance of their teachers. Project mentors will be assigned for all teachers. Teachers can reach out to them for clarifications or assistance.
- *Analysis and documentation:* Once the experiment is conducted participants will document and upload the findings and analysis under the guidance of TERI.

Schools will have a month-long window to conduct the experiments post training. The schedule will be drawn in consultation with the school so as to not disturb their academic calendar.

C. Campaigns and Outreach

Schools are encouraged to spread awareness in their community through plans that they have devised. TERI will mentor and support schools to execute the campaign (online/offline/hybrid). Timelines for the campaigns will be determined by the school based on their academic calendar.







D. Competition

During the project inter school competitions will be conducted to promote out-of-the box thinking and solutions to address ocean health, to evaluate performance of schools, and to evaluate impact of school-led projects/campaigns.

2. What is the minimum number of students and teachers required to participate from a participating school?

A: A minimum of 10 students (gender balanced) and 2 teachers (preferably science) are required to participate under the project from each participating school. In case more students are interested, we will be happy to accommodate and request you to please share the details.

3. How can schools register the teachers and students for this program?

A: Schools can register names of the students and teachers by logging on to <u>https://forms.gle/2BVLuWDbQSULCats7</u>. Certificates will be issued to the registered participants on completion of training, conducting the experiments, and documenting the same.

4. What grades can participate in the program?

A: Students from grades 8-12 can participate in the program.

5. Does the school need to register students from each of the required grades (8-12)?

A: It is left to the discretion of the school to choose the grade/grades of students participating in the program.

6. How will the project activities be carried out? Will it be online or offline?

A: All programs and activities will be carried out in conjunction with the COVID-19 SOPs for each location. The training program will be carried out through in-person/hybrid mode/ online mode. In the in-person and hybrid training mode, participants will be invited to a central location in their city. For in-person training, the trainers and subject experts will be present in person, whereas in hybrid mode the trainers and subject experts will be present online. In the online mode of training, both trainers and trainees will be online.

7. How does the program benefit the school, students, and teachers?

A: There are several benefits to the schools that will be participating in this internationally acclaimed school program.

- Project schools will be part of a network of schools that are working towards oceanic issues within the country and at an international level be part of the GLOBE network.
- Teachers and students will be trained and mentored to conduct experiments and become peer educators.







- The project will provide schools with lab equipment to conduct the experiments. They will have FREE access to resources and materials that will be made available to the member schools at no extra cost.
- The project offers students and teachers hands-on and real-time experiential education
- The project will positively contribute to generate scientific data of their immediate environment to promote evidence-based solution.
- The project promotes solution-based approach to environmental issues and participating schools will be part of this process.
- Competitions will identify best performing schools and the achievement of these schools will be felicitated and publicized through social and print media to inspire more schools
- All participating students and teachers will receive certificates on completion of project activities issued by U.S. Consulate General Chennai, TERI and other partner institutions.

8. What Lab equipment is required for the project?

A: Lab equipment to measure temperature, salinity, and the presence of microplastic in surface water will be provided under the project to enable schools to undertake experiments. The schools are required to take responsibility for careful storage and safe handling of the equipment.

9. Is there any registration fee for the schools to participate in the program?

There is no registration fee for schools to participate in the program. Participation is FREE. The project aims to help promote scientific temperament to positively address environmental issues through citizen scientists.







Ocean Matters: Safeguarding Our Oceans – GLOBE Protocol FAQs

Sampling

1. How do I collect water samples from the sea?

At low tide, wade/walk out a distance of 10-15m, or up to knee depth before completely immersing the collecting vessel under water. Ensure there are no bubbles and avoid scooping the water or disturbing the sandy seabed before collecting. Remember to rinse the vessel/container with water from the water body (rinse with seawater and discard) before collecting the sample. Please ensure that teachers or adults only collect water samples, and heed is paid to the seabed in case of a sudden decline.

2. Can I collect water samples from a lake/ freshwater stream?

It is preferable to sample from a saline water body like seawater and estuary unless the lake is saline in nature.

3. Can turbid water be used for taking the measurement?

Although it is preferred to collect clear water, it is likely you will collect water that is turbid. Allow sediments and particles to settle down in the water before salinity measurements. Temperature measurements can be taken immediately and directly without water collection (by immersing the probe/thermometer into the water body).

4. Can I collect water samples at any time?

Samples are to be collected as per the tidal chart. Although you can select either the high tide or low tide, low tides are preferable due to accessibility and safety reasons. Once established (for example, you have opted for low tide), you must ensure your subsequent samples will be collected at low tide as per the timings provided in the tidal chart. The tides follow the lunar day, not the solar day. It takes half a lunar day, on average 12 hours and 25 minutes, from one high tide to the next, so we have high and low tides nearly twice a day. If you collect water the next day, your sampling timing will be separated by 24 hours and ~50 minutes.

5. What is the minimum number of observations (how many times should I sample and record)?

Sampling frequency will depend on how often you, the teachers, and students can visit the sampling site. Although there is no minimum number of observations, monthly samplings would be ideal considering feasibility (provided the samples are collected as per the tidal chart one month apart at the exact tide timing). Collecting samples spanning all seasons (pre-monsoon, monsoon, post-monsoon) is preferred. We urge schools to create a schedule for sample collection and observation based on their academic calendar







with equal intervals separating them and share it with their respective TERI coordinators/mentors.

- 6. Do I need to collect the following samples after a regular interval at the same time? You will be required to follow the tide chart and collect your sample at the exact timing mentioned for the low tide.
- 7. Can I use one sample for conducting the GLOBE experiments (salinity, and temperature measurements)?

One sample is sufficient, provided enough water is collected. Once the temperature is recorded, you will need 250ml for salinity measurements. It is ideal if you can collect 1-1.5l of the water sample.

8. Will sampling location details be provided to schools?

A map representing all the schools has been shared with the registered participants. Sampling locations are identified based on teacher inputs, expert opinion, and your school's location. You can select the sampling site closest to you based on the map provided.

9. Can I choose sampling locations other than those shared by TERI?

Yes, you can select a sampling location other than what TERI has shared. Please ensure the site is accessible and student friendly. Please also record the lat./long coordinates and elevation of the site you selected. Please share the details with TERI coordinators via the link: <u>https://forms.gle/ov49jxD3NSbeCEND9</u>.

10. My school is located far away from the sea/water body. What are my alternatives?

It is essential to collect water samples from a saline water body to fully appreciate the hydrometer's application. An alternative would be to send a single person to the site, take temperature measurements on-site and collect a sufficient water sample for salinity measurements. The salinity measurements can be completed in the classroom amongst the students. We suggest delegating a teacher or adult for sample collection.

11. Can multiple schools collect water samples from the same location?

Yes, multiple schools can collect water from the same location provided in the sampling map. We can opt for a sampling schedule if required where schools can collect and share samples with each other.

12. How many students can participate in one sampling session?

The lab equipment kit allows only one group of 5-8 students to carry out the activity. But the kit can be rotated amongst student groups for wider participation.







13. Where can I acquire the tidal chart for my location?

TERI has shared tidal charts for each month with the registered schools. You can also directly obtain your local tidal chart from <u>https://surveyofindia.gov.in/pages/tidal</u>. Port authorities in the respective cities also maintain tidal records.

14. Why are tides important to salinity and temperature measurements?

Tides influence salinity and temperature by mixing water layers, bringing cooler, saltier water from deeper areas to the surface during high tides, and allowing warmer, fresher water to dominate during low tides. This mixing causes daily fluctuations in both salinity and temperature, which must be considered when taking measurements.

15. How and when do I take readings?

Temperature readings are to be taken *on-site before sample collection* to ensure an accurate representation of surface water temperature. As far as possible avoid collecting water in a vessel and then measuring the temperature. In the eventuality when there is no direct access to the water sample as in the case of collection of water sample from a pier or a place where direct access to the water is not available and the water sample needs to be drawn with the help of a bucket or sampling container, then in such cases the reading can be taken immediately on site from the vessel. Salinity measurements require sufficient water to immerse the hydrometer fully. Once water is collected and safely transported, salinity measurements (specific gravity measurements) can be performed at school. Measurement of the presence of microplastics also can be performed at school.

16. What is the timeframe for data collection and completion of experiments?

Sampling can commence immediately per your academic calendar and should end by December 2024. Please plan sampling frequency and intervals accordingly and share the same with your respective TERI coordinator /mentor. Given that this project builds capacity of students and teachers to monitor environmental parameters, we urge teachers to make monitoring a part of their school curriculum and carry forward the same in the subsequent academic year.

Hydrometer handling

1. What is a hydrometer?

A hydrometer is an instrument used to measure the density of a liquid as compared to that of water. Hydrometers usually consist of a calibrated glass tube ending in a weighted glass sphere that makes the tube stand upright when placed in a liquid. The lower the density of the liquid, the deeper the tube sinks.

Depending upon the intended use hydrometers can vary in size and will feature different types of scales







2. What are the different kinds of hydrometers and when do you use them?

• <u>Specific Gravity</u> hydrometers can be used for almost any liquid. Specific Gravity is a dimensionless unit defined as the ratio of density of the material to the density of water. If the density of the substance of interest and the reference substance (water) are known in the same units (e.g., both in g/cm³ or lb/ft³), then the specific gravity of the substance is equal to its density divided by that of the reference substance (water =1 g/cm³)

Sodium Chloride hydrometers measure saturation and concentration of sodium chloride.

Salt Brine hydrometers are graduated in percent of either saturation of sodium chloride in water, or by weight of sodium chloride.

Calcium Chloride Salometers (a hydrometer for indicating the percentage of salt in a solution) are for determining the percentage of saturation, specific gravity or freezing point of CaCl2.

Draft Survey hydrometers determine the apparent density of sea/fresh water.

• <u>Baume hydrometers</u> are calibrated to measure specific gravity on evenly spaced scales; one scale is for liquids heavier than water, and the other is for liquids lighter than water.

• <u>Brix (Bx) hydrometers</u> are for determining the percentage of weight by sucrose. One degree Brix is 1 gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by weight (% w/w) (strictly speaking, by mass). If the solution contains dissolved solids other than pure sucrose, then the °Bx only approximates the dissolved solid content. The °Bx is traditionally used in the wine, sugar, fruit juice, and honey industries.

• <u>API hydrometers</u>, also known as The American Petroleum Institute index, is a measure of how heavy or light a petroleum liquid is compared to water. If its API gravity is greater than 10, it is lighter and floats on water; if less than 10, it is heavier and sinks. API gravity is thus an inverse measure of the relative density of a petroleum liquid and the density of water, but it is used to compare the relative densities of petroleum liquids. For example, if one petroleum liquid floats on another and is therefore less dense, it has a greater API gravity. Although mathematically, API gravity has no units (see the formula below), it is nevertheless referred to as being in "degrees". API gravity is gradated in degrees on a hydrometer instrument. The API scale was designed so that most values would fall between 10 and 70 API gravity degrees.

- <u>Alcohol Proof hydrometers</u> are used for distilling and rectifying and for waste liquors.
- <u>Isopropyl Alcohol hydrometers</u> measure percent by volume of isopropyl alcohol.
- 3. What is the difference between a "calibrated" and a "standardized" hydrometer? All hydrometers are standardized, but not all hydrometers are calibrated. In order to manufacture a hydrometer, the instrument must be compared to a traceable reference







standard. Standardization during manufacturing establishes the guidelines for scale placement, thus the hydrometer has been standardized. If a hydrometer is calibrated (in the past the term certified may have been used) this means that after the instrument is manufactured, it is compared to an instrument that was certified by an outside body at a specific point or points along the scale. The results of this calibration are recorded on an official report. This report of calibration accompanies the calibrated instrument after the calibration process.

Using a hydrometer

The size jar needed depends on the size of the hydrometer. The jar should be at least 1" taller than the hydrometer, and should have a minimum diameter of 50mm.





When measuring with a hydrometer

you should have an idea of what reading you are looking for. If the reading does not fall within the range of the hydrometer you should use a hydrometer that includes the expected reading. Adjust the range up or down depending on if the hydrometer sinks or is overly buoyant.

We have provided you with a hydrometer that is suitable for salinity measurements

5. What is the proper way to store hydrometers?

Hydrometers should be stored in clean, dry, safe places. They also should not be subjected to extreme temperatures.

6. What is the metal ballast in hydrometers?

The metal pellets act as ballast (to help stabilize the buoyant hydrometer). Older hydrometers use lead ballast, but these hydrometers are being phased out due to environmental concerns. Newer, more environmentally friendly hydrometers use steel shot ballast.

Cleanliness and Handling:

Cleanliness increases the measuring accuracy. For uniform and reproducible readings, the hydrometer must be free of fingerprints, from handling the body, and free of test liquids or







materials. The jar must be clean and free of all impurities. Clean hydrometers and measuring cylinders with de-ionized or distilled water and dry them with a lint-free cloth.

After cleaning, you can only touch the measuring instrument at the stack point above the scale reading. Hold the hydrometer carefully between thumb and forefinger, at the top of the stem only, when immersing in the jar for testing.

Handling and Packing hydrometers

Hydrometers are extremely delicate glass instruments. We recommend placing the instruments back into the packaging after each measurement or placing frequently used instruments in a rack. We pack delicate hydrometers individually to avoid breakage. When packing or handling, before and after use, hold the hydrometer by the body to protect the instrument, shielding the stem from shock or impact.

Important points

- Check your hydrometer's temperature calibration
- Measure the temperature of the liquid
- Pour a sample into a clean container
- Bring the water to the correct temperature The hydrometer's calibrated temperature should be marked on the hydrometer label or the package instructions
- **Insert the hydrometer into the liquid** (Make sure the hydrometer is dry, then lower it into the liquid just below the point at which it would float naturally. Be sure the hydrometer bulb does not touch the sides or bottom of the jar when it settles)



- Spin the hydrometer gently. This will dislodge air bubbles clinging to the tool, which would disrupt the measurement. Wait for the hydrometer and the liquid to stop moving, and for bubbles to dissipate
- **Read the hydrometer scale at the lowest point of the liquid's surface.** The surface of the fluid may cling to the hydrometer and the container walls, forming a curve called a meniscus. Look for the scale mark on the hydrometer level with the *lowest* point on the fluid's surface. Do not use the mark where the fluid touches the hydrometer
- Understand the measurement. The most common scale on hydrometers is "specific gravity." This is the ratio of the liquid's density to the density of water. Pure water should give a reading of 1.000 (*lowest measurement point in the hydrometers provided*). A higher reading means the liquid is denser (heavier) than water, and a lower reading means it is lighter.







Data recording

1. What is the website for registering the data?

TERI has shared the details of registered schools along with the name of the coordinating teacher and school email ID with the GLOBE Asia Pacific Office. With this information, the GLOBE office will register the schools into their database and the coordinating teachers will receive an email on their school email ID from GLOBE. This email will contain a registration link to enable the schools to approve their registration and sign in accordingly to register your data.

2. Are two teachers required to register separately?

TERI has shared the names of one coordinating teacher with a school email ID with the GLOBE Asia Pacific Office. Each school will receive a registration link sent directly by GLOBE on the school email. ID Hence two teachers are not required to register separately. It can be a common sign-in password and username for the school which both teachers can use.

3. Do we use a personal email ID or a school ID?

School email ID is provided to GLOBE partners. Hence only the school email ID will be used.

4. For passwords, do we need to use an existing email password or create a new password? Teachers can generate a new password of their choice. We recommend that you write down the login and password and share it with the school and the other coordinating teacher so that there is no chance of forgetting or losing the same.

5. Despite registering on GLOBE, I cannot upload data on the GLOBE website. What can I do?

Please contact your TERI coordinator or send an email to the Globe Help desk with a copy to the TERI coordinator so that the problem can be resolved at the earliest.







Ocean Matters: Safeguarding Our Oceans– Microplastics Monitoring Protocol Trial (MMPT) FAQs

Sampling

1. Can I collect water samples from a lake/ freshwater stream?

For microplastic measurements, you can collect water samples from any water body. Sea and saline or brackish water from estuaries is preferred when aligning your water samples with salinity measurements, which will be covered in the GLOBE protocol. Sticking to one sampling site (that will allow you to conduct both sets of investigations) will benefit your sampling scheduling. We request that teachers contact their respective TERI coordinators/mentors for further clarification.

2. How do I collect water samples from the sea?

At low tide, wade/walk out a distance of 10-15 m, or up to knee depth before completely immersing the sampling bottle underwater. Ensure there are no bubbles and avoid scooping the water or disturbing the sandy seabed before collection. Remember to rinse the bottle with water from the water body (rinse with water and discard) before collecting the sample, preferably downstream, down current and downwind from where you will sample. Please ensure that students are supervised by teachers or adults when collecting water samples. Once water is collected and safely transported, measurement of the presence of microplastics can be performed at school.

3. Can I collect water samples at any time?

For *microplastic measurements*, water samples can be collected at any time at your convenience. Please refer to the GLOBE protocol for collecting samples for the *salinity and temperature measurements*

4. What is the ideal sampling frequency (how many times should I sample and record)?

Sampling frequency will depend on how often you, the teachers, and students can visit the sampling site. There is no minimum number of observations. Monthly samplings would be ideal considering feasibility (provided the samples are collected as per the tidal chart one month apart at the exact tide timing). Collecting samples spanning all seasons (pre-monsoon, monsoon, post-monsoon) is preferred. We urge schools to create a schedule for sample collection and observation based on their academic calendar with equal intervals separating them and share it with their respective TERI coordinators/mentors. Please use the Google link (<u>https://forms.gle/ov49jxD3NSbeCEND9</u>) for sharing the sampling site and







frequency details. This will enable TERI to streamline mentoring support to schools accordingly.

5. How many samples should I take per sampling event?

Three water samples each time you sample (sampling event) will be ideal.

6. Why do I have to collect more than one sample per site?

Microplastics are solid objects that are dispersed in water and throughout the water column. Given the nature of large volumes of water, their concentration may not be homogenous. Three or more samples will ensure a more 'accurate' representation of the sampling site and reduce the chances of contamination in the analysis.

7. What precautions are to be kept in mind when collecting water samples?

- Rinse sample bottles and sampling equipment using water from close to the study site (sampling and disposing of the same downstream from the same sampling location)
- Collect samples by facing the direction of the water flow (to prevent potential contamination from the operator collecting the water sample)
- Samples collected should be processed as early as possible.
- If processing the samples beyond 4-6 hours from collection, refrigerate the samples.

8. Can I use one sample for conducting all three experiments (microplastic, salinity, and temperature measurements)?

One sample is sufficient, provided enough water is collected. You will need 600-800 ml for microplastic measurements and around 500-1000 ml for salinity measurements. It is ideal to collect 1500-2000 ml of the water sample. If you are collecting water for only microplastic measurements, 1000 ml should be sufficient. However, please keep in mind that more than one sample in triplicate is required per site (*refer to question 6*).

9. Will the sampling location details be provided to schools?

A map representing all the schools has been shared with the registered participants. Sampling locations are identified based on teacher inputs, expert opinion, and your school's location. You can select the sampling site closest to you based on the map provided.

10. Can I choose sampling locations other than those shared by TERI?

Yes, you can select a sampling location other than what TERI has shared. Please ensure the site is accessible and student friendly. Please also record the lat./long coordinates and elevation of the site you selected. Please share details with TERI coordinators via the link: <u>https://forms.gle/ov49jxD3NSbeCEND9</u>.







11. Can multiple schools collect water samples from the same location?

Yes, multiple schools can collect water from the same location provided in the sampling map. You can opt for a sampling schedule if required where schools can collect and share samples with each other.

12. What is the timeframe for data collection and completion of experiments?

Sampling can commence immediately per your academic calendar and should end by December 2024. Please plan sampling frequency and intervals accordingly and share the same with your respective TERI coordinator /mentor. Given that this project builds capacity of students and teachers to monitor environmental parameters, we urge teachers to make monitoring a part of their school curriculum and carry forward the same in the subsequent academic year.

13. How many students can participate in one sampling session?

The lab equipment kit allows only one group of 5-8 students to carry out the activity. But the kit can be rotated amongst student groups for wider participation.

Microplastics Monitoring Protocol Trial

MMPT Kit



1. What are the contents of the Microplastic Monitoring Protocol kit?







Contents of the MMPT Kit

Sr. No.	Item	Description
1	Sampling water bottles- plastic	500 ml
2	Filtering Unit	250 ml for both
3	Plastic Petri dish with lid	55 mm diameter
4	Tweezers	Stainless steel
5	Syringe	50 ml
6	Three way connector (1 Nos)	Segment w=5.0mm, Segment J=5.3 mm
7	One way valve (2 Nos)	Segment Z=4.7 mm, Segment X=5.7 mm
8	Larger tubing (Latex Silicon PVC tubing)	250 -300 mm length Dia-inter=6.5 mm, Dia-ext=10.0mm
9	Smaller tubing (Latex Silicon PVC tubing)	250 -300 mm length Dia-inter=4.0 mm, Dia-ext=5.5mm
10	Syringe air filter	WHATMAN PURADISC SYRINGE FILTERS 4/0.2 NYLON







Sample preparation

1. Why should I shake the bottle vigorously before filtering?

Thoroughly shaking/mixing the bottle before filtration will help you have a stillsignificant sample. Remember to let the bottle settle for 30 seconds, to allow for sand and other dense debris to settle.

2. How do I ensure I have no contamination?

- Wash all your equipment before use (air-dry only, don't wipe-dry using cloths, etc.)
- Follow the procedure (below)
- Prepare, filter and analyse a "blank" control sample to see what level of contamination you are likely to have

3. What is a blank sample or a control sample?

Deionised water can be used as the blank or control sample.

Unit setup and sample filtration

https://drive.google.com/file/d/1jr5A7DNy60nsoHs3iLAY07OlZ6Pk20HL/view?usp=dri ve_link

4. How do I position the membrane?

- Wear gloves
- Unscrew the locking ring and remove the upper compartment
- Wet the surface of the filtering body using deionized water.
- The filter membranes are individually packed and will be protected between two thin sheets of paper. The two sheets used to sandwich the membrane filter can be discarded (or you can hold on to them to store the membrane into when dry). The side with grid lines is to be used 'facing up' when filtering. Carefully handle the membrane using a pair of forceps and place the membrane on to the filtration unit stage 'facing upwards.' Remember to mark the membrane with cardinal points (N, S, E, W) using a marker before handling.
- Let the membrane wet.
- The membrane should be manipulated or moved gently using the forceps and placed as centrally as possible
- Make sure that the two support plate O-rings are positioned correctly. One fits into a groove on the underside of the upper compartment, and the other fits onto the underside of the support plate. Leakage will result if the O-rings are not seated properly
- Once you have placed the upper compartment on the lower compartment, screw in the locking ring







5. What is the next step once I have positioned the membrane?

- Assemble the tubing on one outlet on the lower compartment and place one rubber stopper on the other outlet.
- Ensure there is an air filter on one of the little tubes (port) on the lid of the upper compartment, and two rubber stoppers on the other ports (refer images below).
- Immediately before use, remove the upper compartment lid and rinse with deionised water the upper compartment and the lid of the filtering unit.
- Before pouring, shake/agitate the sample inside the sampling bottle to homogenise its contents and let it sit for 30 sec.
- Open the upper compartment lid and pour the water sample (the lid should be lifted as briefly as possible to avoid contamination). Take note of the volume being filtered.
- Replace the lid and apply vacuum gently (push-pull) to avoid tearing the membrane or accidentally tipping the bottle
- If you are filtering more water using the same membrane than the lower compartment can hold, gently remove the rubber stopper on the lower compartment to stop vacuum application. Lift the top of the unit including the membrane holder and empty the lower compartment by pouring the filtrate (collected water) through the open port. Reattach the rubber stopper once emptied. Attach the lower unit to the upper unit once again.
- Add the remaining sample volume into the upper compartment and filter the sample as described
- Once filtration of the sample volume is completed, release the vacuum by removing the stopper from the lower compartment, lift the lid, and rinse the sides of the filtering unit using DI water from a spray bottle
- Attach the lid and stopper on the lower unit and apply vacuum to remove the DI water.
- Once filtration and rinsing is completed, unscrew the locking ring and remove the upper compartment.
- Carefully remove membrane filter with forceps and place the membrane in a Petri dish.
- Close the Petri dish and label it with the relevant information.
- Rinse the filtration unit with DI water before filtering another sample









The correct platform is highlighted in the red box. The other platform is not applicable for the MMP

The platform sits securely on top of the lower compartment (the rubber stoppers are positioned on each end)



Positioning of Air filter on one of the ports of the upper compartment lid

You can refer to this MMPT installation video: <u>https://drive.google.com/file/d/1jr5A7DNy60nsoHs3iLAY07O1Z6Pk20HL/view?usp=dr</u> <u>ive_link</u>

6. What should I keep in mind while preparing the samples for filtration?

• Turbidity of the sample

If the water sample is turbid, it is recommended that 500 mL of sample water is spread over 2-3 membranes. This would mean filtering smaller amounts of water (50-100 ml depending on the turbidity) each time (instead of the recommended 500 ml).







This prevents the membrane from being overloaded (in which case you would not be able to see microplastics clearly) and rupturing from excessive force.

Operators need to assess the suction process with the syringe and filter appropriate amounts of water accordingly.

7. Why is it recommended to use deionised water after filtering the sample water?

In addition to removing particles which could have adhered to the walls of the upper compartment, thorough washing with deionised water will also prevent salt deposition on the membrane. Lift the lid of the filtering unit and rinse the sides of the upper compartment using a spray bottle (right) to add deionised water (this will dislodge particles on the walls as well as prevent direct contact with membrane which may be disturbed by direct contact with the water stream).

8. How long does it take for the membrane to dry before it is ready to visualize? Depending on certain factors, it would be ideal to allow the membrane to dry in a Petridish overnight.

9. How do I visualize dried membranes that have curled?

To look at a membrane under the microscope you can use two glass slides stuck together with sticky tape to form a base for the membrane (the membrane sits on top of these). However, this may be insufficient to hold the membrane. If a membrane curls, then you will also need to hold it down, because curling may hamper visualizing the membrane. Hence it is recommended to use one or two clean, wiped, glass slide to hold the sides down when watching. Place the glass slides on the sides of the membrane, so that they don't interfere with observation (see figure below).









Sample observation

1. How do I observe the membrane through a microscope?

- Prepare the microscope on the lower magnification.
- Using forceps, take the membrane from its petri dish and place it in the middle of the glass slide, which you will place on the sample stage. Close the petri dish to prevent contamination
- Rotate the membrane in the field of view, so as to see the lines on the membrane parallel and perpendicular to the observer, the centre point in the middle of the view, and such as to have N up, S down. This will facilitate the recording of item coordinates and facilitate navigation of the sample
- Quickly scan the membrane to gain an understanding of its contents, using the grid lines on the membrane as a guide. Start from the topmost point, and move from left to right, then move down by one cell at the end and proceed from right to left (see diagram below).



- After the first scan, restart the observation from the top as described. Increase the magnification if required
- Take note of the observations following the datasheet. Refer to the reference images, <u>Microplastics Recognition Guide</u>, and datasheets provided for item recognition
- Photograph objects observed to keep a record for data validation
- At the end of the observation, place the membrane into the Petri dish for safekeeping
- **2.** What microscope is required to view and analyze the filter membranes? A standard light microscope with a 100X Objective will be sufficient.







3. How can I take pictures of the membrane through the microscope eye piece?

- Your smartphone can be used as part of the microscope eyepiece. Certain microscope attachments can clamp your phone over the microscope's eyepiece in order to capture the vital images. However, in the case you do not possess a specially adapted microscope with a camera port, a camera with removable lens system, or an adapter to attach the camera to the port-you can manually position a smartphones camera above the microscope's eye piece and take pictures as outlined below¹:
 - Hold the camera lens against the microscope eyepiece. A rubber cup over the eyepiece helps to hold the camera steady. A small circle of light will be seen on the phone's screen.
 - Use the phone's zoom function to increase the size of the circle as required. The most difficult step is moving the phone lens small distances across the eyepiece to center the circle. The camera's autofocus should then self-adjust to give a clear image.
 - Adjust the fine focus of the microscope to maximise image clarity.
 - If the image is too dark or grainy, the phone's ISO setting can be increased (usually 100 or 200 will suffice) or the "darkness" or "nighttime" setting selected. You can increase the microscope's light source to maximum intensity or use a reading light to provide more illumination from the side. *Note that generally, the higher the ISO, the more difficult it is to obtain a clear image.*
 - While holding the phone very still, a photograph can be taken, and the image can be examined to see if it is satisfactory. If there is an excessive blur from the phone shake, retake the image.
 - You can take a picture of the membrane inside the petri dish if you wish to showcase it to other students and teachers.

4. How many microplastic particles should we find?

Difficult to say as it depends from sample to sample. Our experts have found no less than 10 objects of interest (fibres, microplastics, etc.) across ~100 samples. This equates to 20,000 particles per m³.

5. How do the numbers relate to existing literature?

Typically, we find more than the literature. The literature using neuston nets (trawler approach) reports around 1-200 items per km². It is not surprising that we find many more, considering we are using a much finer net.

¹ Maude RJ, Koh GC, Silamut K. Taking photographs with a microscope. Am J Trop Med Hyg. 2008 Sep;79(3):471-2. doi: 10.4269/ajtmh.2009.08-0256. PMID: 18784246







Data recording

1. Is there a format in which the data needs to be recorded?

A copy of the datasheet has been provided at the workshop. It is also available at https://docs.google.com/spreadsheets/d/1cFZh79hR0uApP_oAlDLznb33h35eRXy_/edit ?usp=drive_link&ouid=104645035211350112365&rtpof=true&sd=true. Each school will need to record the data along with the images on the digital datasheet and upload it.

2. How many datasheets are there for recording the data? What is the use of these?

- Sampling Site Data Sheet to record details regarding the sampling site
- The Microplastics Recognition Guide and datasheet for item recognition
 - Paper Data Sheet
 - Excel Spreadsheet
- Summary Datasheet

3. How do we upload the data that is collected?

Each school has received an email from TERI with their individual link to access the MMPT cloud where the data can be uploaded. This link provides schools the right to upload or delete the data. Request schools to carefully use the link.

4. Why is that some images do not get uploaded?

Care should be taken to ensure that the images are saved in the same folder when uploading them into the datasheet. This will ensure that the image link is maintained in the datasheet.

5. How do I document the student training sessions and field experiments?

Documentation can be done through:

- Taking vibrant and engaging pictures that are descriptive of the activity being performed
- Recording testimonials (30-60s videos) of students, fellow teachers and school management outlining their experiences with the trial and any inputs and what more can be done.

These can be shared on school social media handles using the **#oceanmatters2022** hashtag and tagging TERI and the U.S. Consulate General Chennai.

6. Where can I find the tutorial for data recording?

You can find the tutorial for data recording on YouTube: https://www.youtube.com/watch?v=v2JSLjX7c70







7. What are the social media handles I can use to increase coverage and promote my school?

Organization	Twitter handle	Instagram handle	Facebook handle
TERI	@teriin	@teriindia	@TERIIN
US Consulate	@USAndChennai	@Usconsulatechennai	@U.S. Consulate General Chennai
Partners' tags for MMPT	@Labtercrea_MN @DeakinIFM @AleSutti @future_fibres @GlobeItalia		