

# Water & a Healthy Environment

## 4. METHYLENE BLUE TEST



An indication of oxygen demand in a river



### What do we need?

- Nearby river/stream/pond
- Protective gloves
- Two bijou bottles (in pack)
- Prestik
- Methylene blue (in pack)
- Tin foil

### Questions we are asking, and how we plan to take our investigation further:

We know that oxygen combines with the 'indicator' methylene blue. If oxygen is used up, it comes out of this indicator, and the color changes. We also know that 'aerobic' micro-organisms (e.g. some bacteria in sewage) use up oxygen as they multiply. So if our sample became lighter, we could assume that there were microbes in the water - with high 'oxygen demand'.

If the sample stayed blue, we might assume that there was plenty of oxygen, and not much microscopic life in the water (low 'oxygen demand'). What about microbes that do *not* use oxygen (i.e. 'anaerobic')? Could the Methylene blue test indicate their presence?

We know that many other microbes are useful to us, e.g. *Lactobacillus* is used for making soured milk, yeast for bread-making. How could we be sure that microbes are useful or harmful?

Where do micro-organisms come from? How do they get into the water? Do we know if there is untreated sewage contaminating the water? Are there cattle nearby? What is going on in the area?

How much can the Methylene blue test tell us? Is it enough to give us a sense of how safe the water is to drink? To wash in? To water our plants? Do we know enough about what is there in the water?

What have we learned so far? Where could we take our investigation from here?

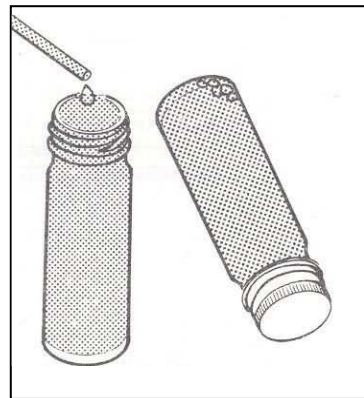
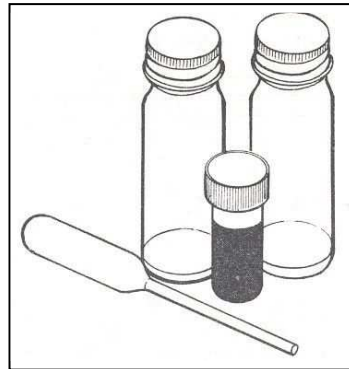
## How do we use the kit?

Here is a suggested activity to help get a better sense of what is actually there in the water - has oxygen been used up by microscopic life in the water? This will give us a sense of what environmental issues and risks there may be.

**Methylene blue test:** an indication of oxygen demand in a river

Micro-organisms (e.g. bacteria in sewage) use up oxygen when they multiply. We can check if these microbes have used up oxygen in the water by doing a Methylene blue test.

- Label one bijou bottle TEST and the other CONTROL.
- Inside each lid, press a 'cone' of Prestik - this will help displace any air bubbles (which contain oxygen) when you close the lid.
- Fill the 'test' bottle with water from the pond/stream water (almost overflowing).
- Add 1 small drop of methylene blue - do not to add too much.
- Close the lid tightly and check that there are no air bubbles.
- Fill the 'control' bottle with sterile/boiled water (that has cooled down), add 1 small drop of methylene blue, and close the lid, making sure there are no air bubbles.
- Wrap each bottle in tin foil (to keep



them in darkness).

- Keep at room temperature for not more than 2 days.
- Check every 12 hours to see if there is a colour change.

If there is still plenty of oxygen in the water, the sample will remain blue for a long time. If there is little oxygen in the water, it will turn light blue or clear. The quicker the colour changes, the less oxygen there is.

- Did the CONTROL bottle stay blue? If not, what would this tell us about our methods?
- Did the TEST bottle go clear? If so, how quickly did it change, and what might this tell us about the amount of microbes in the water?

### Sterilising plastic equipment with Jik

Pour 25ml Jik into 1 cup clean water. Immerse open plastic bijou bottles & lids in the solution for 10 minutes. Remove, shake out excess water, and place upside down on clean paper towel. Jik will kill most microbes.

N.B. Re-use the bijou bottles once they are completely dry!

### Sterilising equipment with a Spirit Burner:

Pour some methylated spirits into a *spirit burner*, shut the top firmly. Make sure the string is protruding from the spout on top, Use a match to light the string (which will have soaked up some of the 'meths'). Using the wire holder, hold metal item or glass bijou bottle (with lid on, not tightly shut) above the flame for a few minutes (not too close or it will heat too quickly and crack). The heat will kill most microbes.

N.B. Handle hot equipment with care!