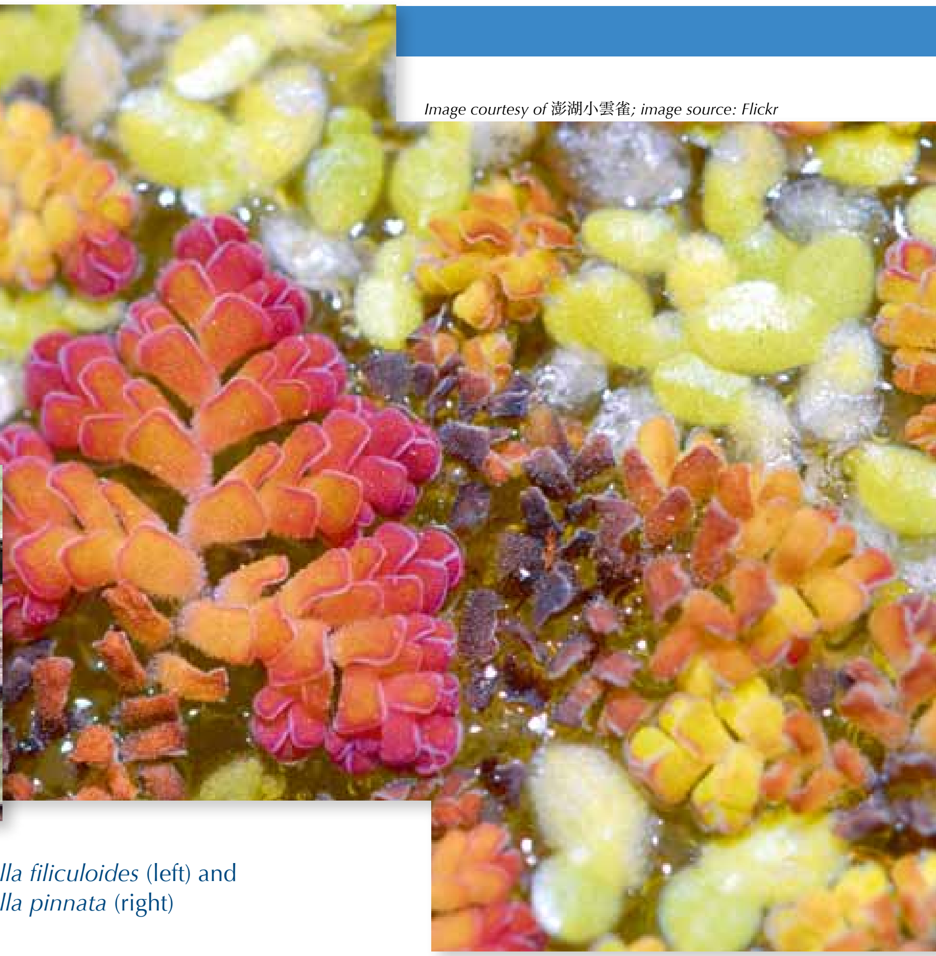


Image courtesy of 澎湖小雲雀; image source: Flickr

Azolla filiculoides (left) and *Azolla pinnata* (right)

Image courtesy of eyeweed; image source: Flickr

Bioremediation in the classroom

Vered Yephlach-Wiskerman introduces a classroom project to investigate the bioremediation powers of the aquatic fern *Azolla*.

When soils or aquatic bodies are contaminated, for example with heavy metals, solvents or oil, one important cleaning option is bioremediation: the use of micro-organisms or plants that will take up the contaminants and either metabolise them into less harmful compounds or accumulate them, allowing them to be removed. Common applications include cleaning up abandoned mining sites or oil spills. Phytoremediation (from the Greek *phyto* – plant) uses a plant's natural ability to contain, degrade or remove toxic chemicals and pollutants from soil or sludge, sediment or ground water.

Azolla is one such plant: this genus of floating freshwater ferns accumulates heavy metals such as nickel, cadmium and mercury (Arora et al., 2006); its biomass is easy to harvest and desiccates very fast (Wagner, 1997). These characteristics make it a perfect candidate for bioremediation systems (Cohen et al., 2004), although it is always important to assess the potential impact of introducing a new species into an ecosystem[w1, w2]. *Azolla* lives in symbiosis with the cyanobacteria *Anabaena azollae*, which can fix atmospheric nitrogen. This independence of a further external source of nitrogen allows the fern to double its biomass every two to three days at room temperature and is the reason that it has been used in China as a biofertiliser in rice paddies for centuries.

Azolla in the science classroom

The plant is a great tool for interdisciplinary school projects involving ecology, environmental studies, biology, chemistry and biotechnology. During such a project, students can develop essential skills for scientific work: formulating a problem and a hypothesis, planning an experiment, writing up the results and drawing conclusions.

In the lesson, introduce the topic of bioremediation and have the students collect information about *Azolla*[w1, w3, w4, w5, w6, w7] (Arora & Saxena, 2005), such as the morphology of water plants as op-

- Chemistry
- Biology
- Applied science
- Environmental science
- Earth science
- Ages 16-18

REVIEW

Keeping the environment clean and pollution free as well as monitoring the environment are now major concerns, and these subjects are studied in school science. Natural ways of cleaning up the environment, such as bioremediation by plants and microbes, are ideal and have been studied for many years. This article shows how a common aquatic plant, *Azolla*, can be used to demonstrate bioremediation in the classroom. The activities foster scientific thinking skills, an essential part of the 'how science works' element of the curriculum. A simplified version of this activity could be used with younger students.

The experiments could be linked to chemistry – in tests for cations and anions and titrations. The activity also has links to microbiology and biotechnology, as the students could learn the basics of how microbiological water quality can be monitored. As an extension activity, students could use the Internet find out how microbes can be used to clean up oil spills and how micro-organisms can be selected or genetically engineered to deal with particular contamination problems.

Shelley Goodman, UK

posed to land plants, the importance of symbiosis, the nitrogen cycle, the use of *Azolla* in agriculture (Pabby et al., 2004), and *Azolla*'s ability to absorb heavy metals.

Help the students to formulate the research questions and hypotheses that they would like to investigate. Possible topics include measuring the gain in biomass depending on growth conditions (e.g. CO_2 level, iron level in the water, amount of light), or the effect of *Azolla* on water quality.

Teams of two to three students work well, and each experiment should be repeated three times for confirmation. Different teams can work on different aspects of the plant or on the same topic to replicate a single experiment.

In the final session, the results can be presented and discussed in class.

Table 1 can help students plan their experiment. The table can also be downloaded from the *Science in School* website[w8].

Next, the students should tabulate their experimental results and represent them graphically. They should consider the most appropriate type of graph to use (e.g. line graph or bar chart).

Below is an example of a project suitable for students in Grades 10-12 (aged 16-18). We used *Azolla filiculoides*, but any of the seven *Azolla* species will do. They can easily be obtained in plant nurseries or garden centres, aquarium shops or online.

The effect of *Azolla* on water quality

Hypothesis / research question: *Azolla* will lower water's conductivity because the plant will absorb available metal ions. Does *Azolla* influence the water quality in other ways?

To test this hypothesis and answer the question, several parameters that indicate water quality will be monitored over the course of 2 weeks. Apart from measuring the conductivity, we also decided to investigate some aspects of water quality that were easy to test and for which the equipment was readily available.

Students should be familiar with basic plant physiology and the use of the instruments / methods.

	Details of the experiment	Your answer
1	Formulate your hypothesis.	
2	What is the biological basis of your hypothesis?	
3	What is the dependent variable you want to measure in your experiment? How is it measured? In what units?	
4	What is / are the independent variable(s) you want to change to study its / their effects on the variable in Step 3? How will you change it / them?	
5	What are the fixed variables in the experiment – those that will not change?	
6	Detail the controls you intend to include and explain their importance.	

Table 1: Planning your experiment

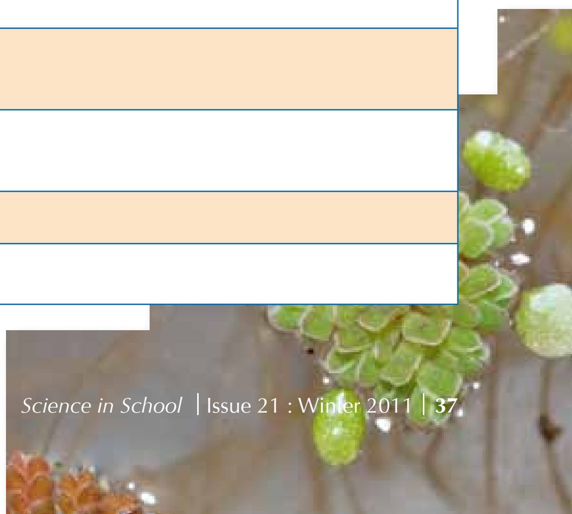
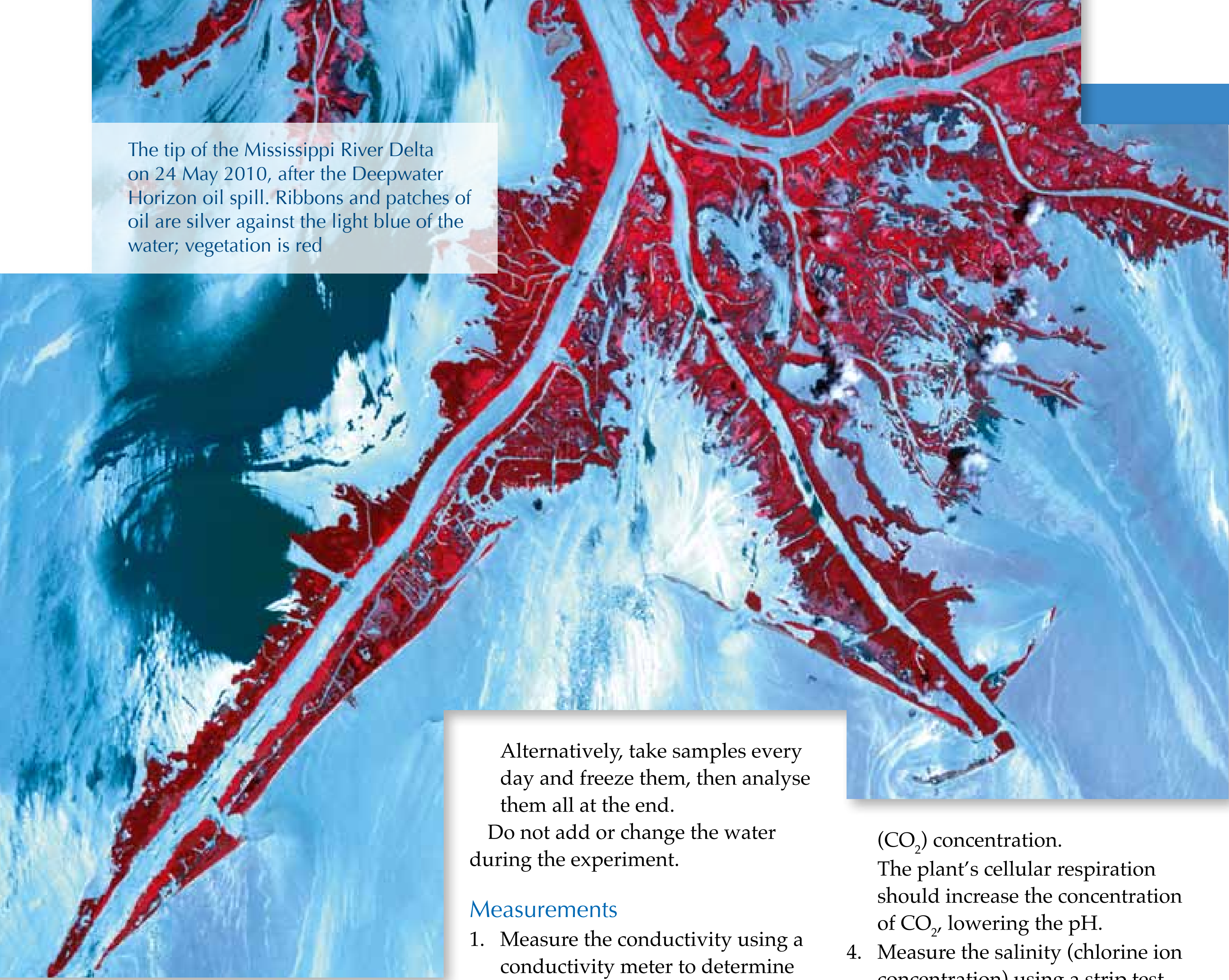


Image courtesy of 澎湖小雲雀; image source: Flickr



The tip of the Mississippi River Delta on 24 May 2010, after the Deepwater Horizon oil spill. Ribbons and patches of oil are silver against the light blue of the water; vegetation is red

Image courtesy of NASA Goddard Space Flight Center

1. Pour 250 ml tap water into each of two glass containers, then place them on the window sill.
   Ideally, we would use contaminated water so that the plant can demonstrate its bioremediation, but this would not be safe. However, you could add metals from the school collection to a sample of tap water, and use this mixture.
2. Measure your parameters in both containers.
3. To one of the containers, add about 50 g *Azolla* (add more if the plant is very wet). The second container should be left untreated.
   To extend the investigation, a third aquatic plant – one that does not absorb heavy metals, such as duckweed (*Lemna spp.*) – could be used in a third container.
4. Repeat your measurements every 1-2 days.
   Alternatively, take samples every day and freeze them, then analyse them all at the end.

Do not add or change the water during the experiment.

Measurements

1. Measure the conductivity using a conductivity meter to determine the concentration of electrolytes.
   Because metal ions are taken up by *Azolla*, the conductivity in the water should decrease over time.
2. Measure the concentration of several specific ions. This can be done easily using commercial strip tests. We tested the nitrate and iron content because we had kits for them.
   Levels of iron should decrease over time as it is absorbed by *Azolla*, because it is an essential element for nitrogenase activity. If the iron supply is too low, *Azolla* fronds will turn yellow and wilt.
   Due to their symbiotic cyanobacteria, *Azolla* can live without a further source of nitrogen apart from the air, but the plant's growth rate will drop. Thus a small decrease in nitrate levels in the water would be expected.
3. Measure the pH using either a pH meter or a strip test, to get an indication of the carbon dioxide (CO_2) concentration.
   The plant's cellular respiration should increase the concentration of CO_2, lowering the pH.
4. Measure the salinity (chlorine ion concentration) using a strip test.
   If the salinity increases, it should do so equally in both water samples – it will be the result of evaporation, because *Azolla* does not take up chlorine ions.

As a control, you could determine bacterial content by measuring turbidity with a spectrometer or turbidity meter, or by measuring the concentration of colony-forming units using the dilution method, seeding isolation, and counting colonies of bacteria on a rich agar medium. Differing levels of bacteria in the initial water samples may influence the water quality and could falsify results, as bacteria may also take up nutrients and metals.

Safety note: The cyanobacteria in *Azolla* produce a neurotoxin, so the plants should not be eaten. In addition, some *Azolla* species are considered a weed and are an invasive species in many countries, so the plants should be disposed of safely after use.

Polluted water

Image courtesy of Chesapeake Bay Program; image source: Flickr

Azolla filiculoides (left) and *A. pinnata* (right)

Image courtesy of eyeweed; image source: Flickr

See also the general *Science in School* safety note on page 65.

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Arora A, Saxena S, Kumar Sharma D (2006) Tolerance and phytoaccumulation of chromium by three *Azolla* species. *World Journal of Microbiology and Biotechnology* **22(2)**: 97-100. doi: 10.1007/s11274-005-9000-9

Cohen MF, Yamasaki H, Mazzola M (2004) Bioremediation of soils by plant-microbe systems. *International Journal of Green Energy* **1(3)**: 301-312. doi: 10.1081/GE-200033610

Pabby A, Prasana R, Singh PK (2004) Biological significance of *Azolla* and its utilization in agriculture. *Proceedings of the Indian National Science Academy* **B70(3)**: 299-333. www.new.dli.ernet.in/rawdataupload/upload/insa/INSA_1/2000c954-299.pdf or use the direct link: http://tinyurl.com/62wq6mp

Wagner GM (1997) *Azolla*: A review of its biology and utilization. *The Botanical Review* **63(1)**:1-26. doi: 10.1007/BF02857915

Web references

w1 – To learn more about *Azolla*, its distribution, its status as invasive species, its biology and its uses, see the UK's Natural History Museum website (www.nhm.ac.uk) or use the direct link: http://tinyurl.com/azolla

w2 – To learn more about *Azolla* as an invasive species, see the website of NOBANIS, the European Network on Invasive Alien Species (www.nobanis.org) or use the direct link: http://tinyurl.com/65hhueu

w3 – The United States Environmental Protection Agency has compiled a wealth of information on remediation technologies in English and Spanish, including citizen's guides with useful background information. See: www.epa.gov or use the direct link: http://tinyurl.com/5rg3yfk

w4 – For a comprehensive overview on bioremediation, see the website of the Earth Sciences Division of the Lawrence Berkeley National Laboratory (http://esd.lbl.gov/research/projects/ersp/generalinfo/primers_guides/03_NABIR_primer.pdf) or use the direct link: http://tinyurl.com/6jchus7

w5 – To learn more about phytoremediation from the Ecological Engineering Group, see: www.ecological-engineering.com/phytorem.html

w6 – For more information on the biology of *Azolla* and its use in agriculture, see Wayne's Word, 'an online textbook of natural history': http://waynesword.palomar.edu/plnov98.htm

w7 – For an overview of *Azolla*'s preferred growth conditions, see the website of the University of Hawaii (www.ctahr.hawaii.edu) or use the direct link: http://tinyurl.com/6gojelj

w8 – To download Table 1 in Word or PDF format, visit the *Science in School* website: www.scienceinschool.org/2011/issue21/azolla#resources

Resources

The World Water Monitoring Day offers a number of resources in English and Spanish on how to monitor a range of water-quality parameters, as well as kits. See: www.worldwatermonitoringday.org

For further resources on testing water quality, see the website of Lifewater Canada: www.lifewater.ca/Section_16.htm

For downloadable water-testing manuals for schools, see the website of the Massachusetts Water Resources Authority (www.mwra.state.ma.us) or use the direct link: http://tinyurl.com/6jerkan

To learn about a school project to test water quality in the local environment, see:

Harwood R, Starr C (2006) Environmental chemistry: water testing as part of collaborative project work. *Science in School* **2**: 34-37. www.scienceinschool.org/2006/issue2/envt

If you enjoyed reading this article, why not browse the full collection of science education projects published in *Science in School*? See: www.scienceinschool.org/projects

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To learn how to use this code, see page 65.