

What's **toxic** to ecosystems?

One of the first steps in site remediation involves defining the nature of the problem. It's a bit like going to the doctor. An effective medicine cannot be prescribed unless the ailment is properly diagnosed. And having prescribed and applied the treatment, assessing its success is equally important.

A contaminated site may contain hundreds of pollutants. One approach is to take samples at regular intervals and subject them to a barrage of chemical tests. It's expensive, but at least the tests reveal what's there. Or do they?

Chemical tests alone cannot offer a comprehensive diagnosis of site contamination. This is because:

- the detection of toxic elements by chemical methods may not indicate whether the elements found are in a form that can be taken up by organisms and cause them harm;
- the tests may react only to the specific elements they are designed to reveal, leaving unknown toxicants undetected; and
- chemical tests do not indicate when relatively harmless elements are interacting to produce something more hazardous, or, conversely, when two toxic compounds are neutralising each other.

A further complication is that observed ecological disturbances at contaminated sites are not always caused by toxic chemicals. They may be the result of natural variability or another form of habitat alteration.

To compensate for these shortcomings, chemical analyses are supported by a separate suite of 'ecotoxicological' tests based on living organisms. When both kinds of tests are used, agents with the potential to adversely affect ecosystems can be pin-pointed. Such information is essential to successful site remediation.

A range of species, selected from different levels in the ecosystem, is used to assess the toxicity of media collected from contaminated sites. Many species are needed, because no single organism can be considered the most sensitive to all chemicals in all situations.

For example, plants are sensitive to herbicides, but may be unaffected by relatively large concentrations of pesticides. In contrast, soil insects may be adversely affected by low concentrations of pesticides, but may tolerate high herbicide concentrations. Other organisms may have an intermediate response to toxic chemicals. By using a number of organisms, a clear

picture of the range of responses that can occur at different levels in an ecosystem can be obtained.

Research staff at the CSIRO Centre for Advanced Analytical Chemistry (part of CSIRO's Division of Coal and Energy Technology) at Lucas Heights in New South Wales are using earthworms, soil insects (the survival and reproduction of springtails), terrestrial plants (the success of seed germination) and bacteria (Microtox solid phase test) to test soils. Algal growth, plant root elongation, bacteria survival, duckweed growth and an earthworm contact test are used to test groundwaters and surface waters, and to assess the potential for toxic substances to be leached from soils. Earthworms are an obvious choice as an indicator. They make up the major part of the animal biomass in a healthy soil; they decompose organic matter, and they increase the fertility of the soil by making nutrients readily available to plants. Through these actions they improve the aeration, drainage and water-holding capacity of the soil.

Ecologically, earthworms are towards the bottom of the terrestrial food web but, as they concentrate pesticides and heavy metals, they may transfer them to other

Dip sites a test of tests

Since the late 1800s Australian graziers have used 'plunge dips' filled with pesticide to rid cattle of ticks. Recent studies of contamination around these dips highlights the need for a range of tests to characterise soil toxicity.

For many years the pesticide used in cattle-tick dips contained mainly arsenic. In 1955, arsenic was replaced with DDT which, seven years later, also was banned. A range of pesticides, predominantly carbamates and organophosphates, has since been used.

In New South Wales some 1600 dips were built, with 1041 still in operation. The disposal of unwanted chemicals around these dips has resulted in high levels of soil contamination, some of the chemicals being highly persistent and able to be passed on up the food chain.

In one study, soils from four dip sites were screened for toxicity using the soil-phase Microtox bioassay (a commercial toxicity bioassay based on monitoring changes in the light output of the luminescent bacterium *Photobacterium phosphoreum*). One of the most toxic soils contained more than 300 milligrams per kilogram of arsenic and more than 3000 mg/kg of DDT: well above the 1992 ANZECC guidelines.

Soils spiked with pure arsenic compounds are toxic to plants at concentrations 10 times lower than the level in this soil. Yet when this soil was subjected to standard tests which use lettuce seed germination and root elongation as a measure of toxicity, the high level of arsenic seemed to have little or no effect on seed germination or growth. In this soil the arsenic appears to be in a form unavailable to plants, possibly adsorbed strongly to hydrous iron and aluminium oxides. Nevertheless the soil was toxic to other organisms.

In one test 50% of earthworms died when exposed to a 65% concentration of the soil solution. High levels of toxicity were also found using aqueous extracts on algae and Microtox. Further tests, however, showed this toxicity was not attributable to the high DDT levels. Instead the toxicity appeared to result from some other constituent—as yet unidentified—which has the capacity to contaminate surface and groundwater, making pollutants at the dip site a potential hazard to the surrounding environment.

These studies illustrate the need for care in determining the toxicity in any soil. Neither straight chemical testing, nor a single ecotoxicological test, reveal the whole picture. Without this whole picture, measures cannot be tailored to treat the contamination.



Jan Forness

Soil toxicological tests have been developed in Europe using the collembolan (or springtail) species, *Folsomia candida*. Dr Penelope Greenslade of CSIRO's Division of Entomology is working with research staff at the Centre for Advanced Analytical Chemistry to develop tests using this and other Collembola which are more appropriate for the Australian environment. Collembola are convenient test organisms because they are easy to keep in culture in large numbers, reproduce fast, are small, and can be fed exclusively on yeast. Also, they are common in soils everywhere, occurring in densities of a few thousand to as many as 100 000 per square metre.

animals which are higher up the food chain. Worms share many metabolic pathways with humans. Thus the effect of soil pollution on earthworms can provide a good indication of potential harm to terrestrial ecosystems and to human health.

The centre breeds its own worms: tigers and red wigglers. At the moment soils are being tested using mortality as an end point, but a research aim is to develop new tests, based on the earthworm's reproductive cycle and weight loss.

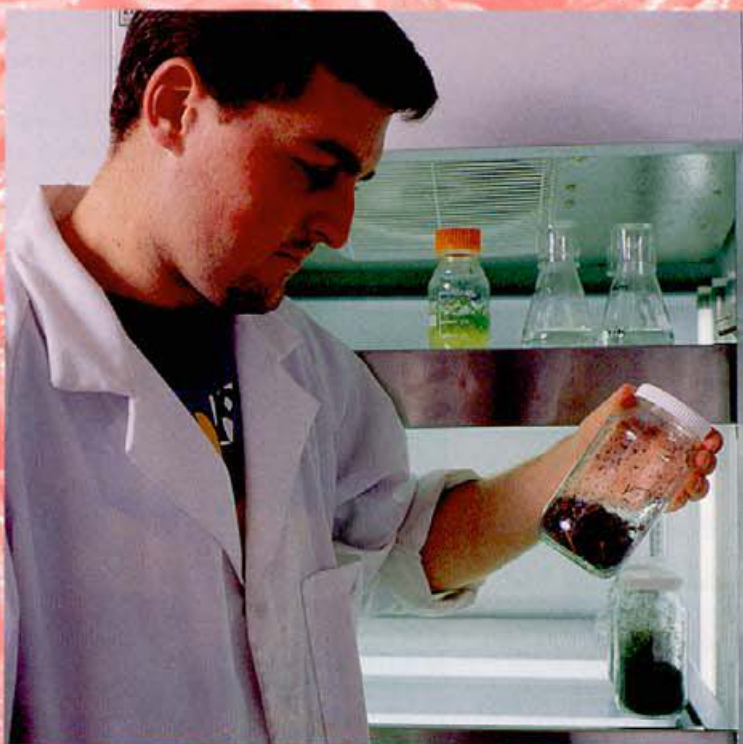
A major goal is to develop toxicity tests that are rapid, ecologically relevant and require minimal amounts of sample. Reducing sample amounts lessens the need to collect, transport, test and dispose of large amounts of toxic materials, thereby minimising health, safety and environmental risks and cost.

In line with this aim, tests using single-celled algae have been miniaturised. Instead of a test volume of 50 millilitres, as little as 1 mL is required. A result is that these tests can now be applied to samples that were previously difficult to test, such as the water found between soil and sediment particles. In other research a rapid (one hour) algal assay is being developed.

Ecotoxicology offers a range of tests for assessing the toxicity and bioavailability of both organic and inorganic pollutants. The value of these tests is that they integrate the toxicity of the many chemicals which may be present and measure the net effect of their interactions on the environment. They ensure that subsequent remediation is well-targeted and cost-effective.

*Chris Thompson and
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Keeper of the WORMS



Paul de Serres

Adam Beasant, a 'year in industry' student from University of Technology Sydney, is 'keeper of the worms' at CSIRO's Centre for Advanced Analytical Chemistry.

The worms are bred in artificial soil, made up of industrial sand, kaolin and sphagnum peat inside black polystyrene boxes that are about 40 centimetres long and 20 cm deep and wide.

Beasant feeds them once a week on a mixture of chicken pellets with small amounts of bran, wholemeal flour, powdered milk and agricultural lime. For the rest of the time they live in the boxes, covered by moist newspaper in a warm room with temperatures of about 20°C.

It takes approximately two months for the worms to grow to full size, when they weigh between 300 and 600 milligrams and can be used for testing.

For the simplest of the tests, Beasant first makes up a series of containers with measured amounts of the artificial soil mix. To these he adds different percentages of a solution obtained from the soil sample being tested. Into each bottle go 10 of the adult worms. They are incubated inside a chest with constant fluorescent light, to ensure the worms bury into the soil.

After 14 days the jars are opened and the number of survivors counted. Scrupulous presentation is essential to ensure none of the samples are contaminated from 'outside' sources, and uniform treatment.