Plants can be used in different ways for the biological depollution of contaminated media.
Organic pollutants, metals and radionuclides can be eliminated with ranging degrees of facility using various methods developed by scientists. Certain bacteria that are resistant to toxics should be able to play a useful role in the depollution of soils and water.

Biodepollution and bioremediation
Plants to clean up soils and water

Ever increasing human activity, whether agricultural, urban or industrial, is a source of environmental pollution by **heavy metals** and organic pollutants. **Biodepollution** refers to an array of biological methods designed to eliminate such pollutants. These methods exploit the abilities of certain organisms and micro-organisms to degrade organic matter and (or) to rid the soil and water of pollutants. **Phytoremediation** is one of these methods: it consists in using plants to "clean up" contaminated soil and water.

Five areas of application

This method is applied in five different ways. **Phytoextraction** uses "hyper-accumulating" plants to extract pollutants from the soil and concentrate them in aerial or underground parts that are then harvested. Photosynthesis makes these plants veritable solar-powered extractors of toxic substances. *Thlaspi*

Ch. Schwartz/Inra

Population of Thlaspi caerulescens that hyper-accumulates cadmium and zinc.

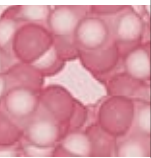Figure 1.
Schematic biotechnological approach involving the over-expression of four enzymes to increase a plant's cadmium resistance. γ-glutamyl cysteine synthase (EC) and phytochelatin synthase (PC) naturally present in the plants and involved in the synthesis of glutathione and phytochelatins, chelate cadmium. Two enzymes (YCF1 and HMT1) of yeasts with different properties, *Schizosaccharomyces pombe* (*Sp*) and *Saccharomyces cerevisiae* (*Sc*), effect the transfer of chelated cadmium to the vacuole, an intracellular compartment that is less sensitive to toxics.
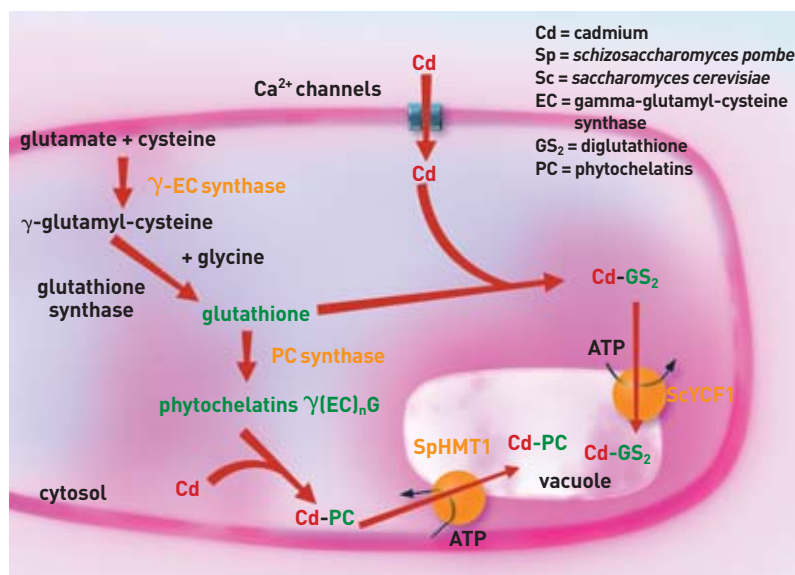
caerulescens, for example, is a metallophyte[1] able to accumulate, in its aerial parts, quantities of zinc amounting to 1.7% of its leaf dry mass. Other plants can concentrate large quantities of cadmium, lead, nickel, etc. **Phytodegradation** uses plants in association with micro-organisms to degrade organic pollutants in the soil. **Rhizofiltration** uses plant roots to absorb and **adsorb** metals in contaminated water. **Phytostabilisation** consists in using plants to reduce the **bioavailability** of pollutants. Lastly, phytovolatilisation uses plants to clear pollutants by making them volatile.

Field work has already shown the utility of phytoremediation for the depollution of organic pollutants (TNT, PCB, etc.), metals (Cd, Zn, etc.) and **radionuclides** (^{137}Cs, ^{90}Sr, U)[2]. Phytoremediation possesses obvious advantages (low cost, site-friendliness, concentration of the pollutant before further processing), but its main disadvantage is the long treatment time it needs, which often extends over several crop campaigns. To reduce the impact of this drawback it is necessary to elucidate the factors that limit pollutant uptake. To be efficient, a plant has to possess numerous qualities: a root mass that explores the polluted area, a strong resistance to the pollutant through a capacity for **chelation** and **sequestration**, and an ability to transfer the pollutant to aerial parts to facilitate subsequent processing. Although the metallophytes cited above possess some of these qualities, they generally produce little biomass and offer only a limited yield. Ongoing research is improving accumulation, transfer and resistance of plants to pollutants.

In the framework of the CEA's Nuclear Toxicology Programme, fundamental work is being done on a model plant, *Arabidopsis thaliana*, the **genome** of which was recently fully **sequenced**. Research is being focused on the biological processes that determine the *uptake* of heavy metals or radionuclides through the search for genes that favour root development, the *transfer* of these pollutants from the soil to the roots and then to the aerial parts of the plant through the search for specific membrane carriers, and finally the *sequestration* and *compartmentalisation* of the pollutants by chelating entities. In this framework, empirical studies are being conducted to identify genes and **proteins** involved in these processes. Different methods are thus being used to follow the modifications of the **expression** profile of the genome and the **proteome** in response to exposure to heavy metals or radionuclides. Wide variations in its expression are a sign that a gene or protein may be involved in the response to a pollutant. Such genes can then be deliberately over- or underexpressed[3] and the resulting modified plant tested in the presence of the pollutant. Once relevant genes have been identified in this model plant, they can be transferred by genetic engineering to major crop plants that produce large amounts of biomass (e.g., tobacco, poplar).

Possible strategies

Pending a fuller understanding of the underlying biological mechanisms, various strategies for optimising the abilities of plants to accumulate metals or radionuclides are already possible. They consist either in favouring the synthesis of chelating agents naturally produced in response to a metal stress (phytochelatins, metallothioneins), or in designing and expressing new peptides able to bind certain metals or radionuclides selectively. These two approaches are being studied concurrently in the framework of the Nuclear Toxicology Programme. In response to the presence of heavy metals in the environment, plants express peptides such as phytochelatins (synthesised enzymatically from glutathione by phytochelatin synthase) or small proteins (metallothioneins), involved in the clearance of toxic metals from cells. Experiments conducted in bacteria have shown that the overproduction of these peptides causes a considerable increase in the quantities of

(1) Metallophyte: plant, generally native to a metal-bearing soil, able to accumulate trace elements (its dry matter contains more than 1% Zn or Mn; 0.1% Cu, Co, Pb, Ni; 0.01% Cd).

(2) For more information, consult the following websites:
http://clu-in.com/download/remed/introphyto.pdf
http://www.ensaia.u-nancy.fr/Recherche/solenviro/phytorem.htm
http://www.uga.edu/srel/Fact_Sheets/phytoremediation.htm
http://www.engg.ksu.edu/HSRC/Tosc/metals.pdf

(3) Over- or under-expression of a gene: the expression of a gene results in the synthesis of a variable quantity of a protein. An over-expression exceeds the needs of the cell, while under-expression limits the production of the protein.

cadmium accumulated and generally also lead to high accumulations of other metals or **metalloids** such as mercury, lead, zinc, copper and arsenic. In plants, the over-expression of two **enzymes** in the phytochelatin biosynthesis pathway is envisaged in order to increase the synthesis of phytochelatins and the accumulation of metals. As the vacuole in plant cells is a preferred storage site for toxics, it is also planned to increase the vacuolar storage capacity for metals by over-expressing membrane carriers, such as those involved in yeasts in the vacuolar transport of glutathione-cadmium or phytochelatin-cadmium complexes (Figure).

New peptide structures

Lastly, with a view to lending plants new accumulating properties, especially for radionuclides, it is planned to create new peptide structures able selectively to chelate various elements. The modification, by molecular engineering, of a basic structural motif composed of one of the calcium binding sites of calmodulin yielded peptides binding uranyl or **lanthanides** with high affinity. It is planned to express these peptides in bacteria and later in plants, and to test the abilities of the organisms thus modified selectively to accumulate metals or radionuclides of interest. Eventually it may be possible to create new peptide structures of this type and create organisms dedicated to the selective bioremediation of pollutants.

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Use of heavy metal resistant bacteria for restoration of polluted environments

Some bacteria are naturally resistant to toxic compounds such as uranium or selenium oxides. They can be found in fossil nuclear reactors (e.g., Oklo, Gabon) or in some american wetlands (e.g. the Kesterson Reservoir) for instance. The metal resistant bacteria could play an efficient role in restoration of soil and water. The detailed knowledge of the molecular mechanisms of resistance could lead to the production of optimized microorganisms used for bioremediation processes targeting **heavy metals**-polluted sites.

Among the different mechanisms conferring to bacteria the ability to resist to high concentrations of heavy metals, the scientists from CEA are particularly interested in those leading to **redox** changes after uptake of the toxic compounds. The entire process could result in an immobilization by the cell, which could accumulate and concentrate the toxic metal. The pollutant could be recovered simply by harvesting the biomass. Selenium and uranium oxides are concerned by such an approach.

The researchers want to delineate the molecular mechanisms (uptake and electron transfers) leading to the storage of the toxic compounds under their elemental, non- or less toxic forms. This is particularly important since bacteria are at the beginning of the food chain and can transmit these toxic compounds to other living organisms.
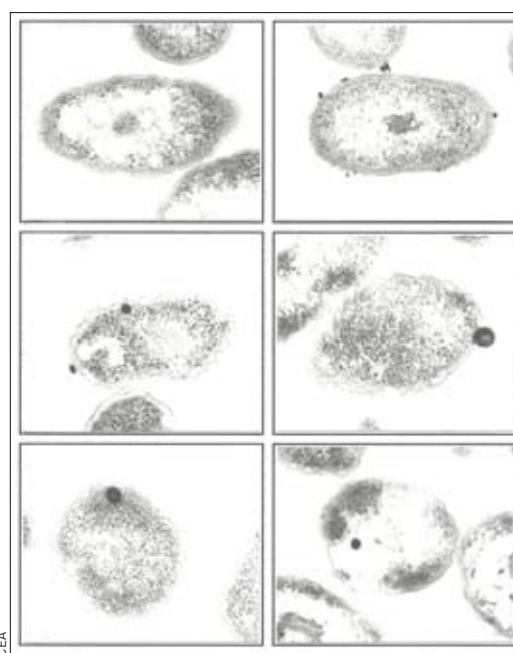
Certain bacteria deal with selenium oxides by reducing them to elemental selenium, which is much less toxic. This results in the appearance of a characteristic red colour (culture on the left: control without selenite; culture on the right: experiment in the presence of selenite). The red bacteria all possess dense inclusions made up of accumulated selenium. The top left image shows a control culture without selenite, the others different possible localisations for selenium accumulation.
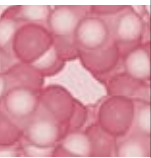
Three main approaches

Three different approaches are developed to study these mechanisms[1]. The first approach consists in an empirical screening of thousands of mutants in which certain **genes** were randomly modified. This strategy can lead to identify the genes of interest involved in the redox changes of the toxic compounds issued from the nuclear domain such as the uranium, an **actinide**, or the technetium, a **fission product**. Second, researchers develop the study of the metagenome. This method consists in extracting the total DNA present in a sample from a polluted site. This DNA, supposed to arise from several different resistant bacteria is subsequently transferred into the sensitive bacteria used in the study, conferring them new resistance properties. Finally, the third approach, the so-called **proteomic** approach, is based on the comparison of the total **protein** content of bacteria exposed or not to the toxic compounds.

(1) The model microorganisms used in this study are representative of the bacterial biodiversity. They originate soil (*Ralstonia metallidurans*, *Shewanella oneidensis*, *Rhodobacter sphaeroides*), or water (*Synechocystis*). The well-known laboratory strain *Escherichia coli* is also used.

The proteins demonstrated to be involved in these resistance mechanisms are characterized by structure-function studies.

The scientists from CEA have already demonstrated that selenium oxides induce an **oxidative stress** in the cells. Moreover, they have identified the translocators involved in the uptake of the selenium oxides (selenate and selenite).

The identification and the characterization of the **enzymes** involved in the redox mechanism are currently under progress.

The working bioremediation technology derived from these studies will be based upon the use of mixtures of optimized bacteria, able to transform soluble, bioavailable and toxic pollutants into an insoluble, immobilized and essentially non-toxic form. These highly insoluble states could represent a non-toxic storage form for the bacteria, thus easily recoverable by simple harvesting of the biomass.

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