



Cultivation of genetically modified tobacco in an isolated greenhouse

Cleaning of cadmium contaminated soil using plants for phytoremediation

- 1. Environmental remediation technique utilizing plants**
- 2. Accumulation of cadmium by genetically modified tobacco**
- 3. Use of genetic modification technique and natural plant functions in combination**

● **Brief Note:** Toshihiro Yoshihara, Research Scientist, Bioscience Department, Abiko Laboratories

Environmental remediation technique utilizing plants

The “Law on Countermeasures for Contaminated Soil” came into force on February 15, 2003 in Japan, the result of moves toward global enforcement of environmental regulations and greater awareness of the impact of contaminated soil on health and residential environments. Cleaning contaminated soil is now obligatory under the Law. At the Central Research Institute of Electric Power Industry, genetically modified tobacco was prepared to target cadmium, a heavy metal and soil contaminant, and the accumulation of cadmium confirmed.

The Institute has also set up a tissue culture experiment system for *Athyrium yokoscense*, a cadmium-accumulating plant in nature, and is clarifying its various functions, and carrying out research into practical applications of the environmental remediation technique (phytoremediation) utilizing plants.

Phytoremediation

Phytoremediation is a method of remedying environments contaminated with hazardous substances, utilizing the innate capabilities of plants. It is composed of four functions: (1) absorbing and accumulating the hazardous substance, (2) degrading and detoxifying it, (3) stabilizing it around the roots, and (4) activating microbes around the roots to degrade and detoxify it.

The merits of the method include that specific tools are not required, the effects are long-lasting, and the impact of the method itself on the environment is low. On the other hand, its demerits include that it can be applied only under the environmental conditions of plant growth, the reach of plant roots is limited, and contaminants of high concentration cannot be promptly treated.

For treatment of various contaminants

The targets for which the method is best suited are heavy metals that cannot be neutralized and degraded by bacteria and chemical procedures, however research is underway on using the method to tackle also various compounds that cause problems at present such as organic chlorides (PCBs etc.), nitrates (TNT explosives etc.) and environmental hormones.

The Institute is conducting research on cadmium, the regulation of which has been tightened in recent years, due to an expected reduction in permitted intake of cadmium by the FAO/WHO of the heavy metals found in comparatively large quantities in the soil of Japan, that until now could only be treated by a conventional physical removal method.

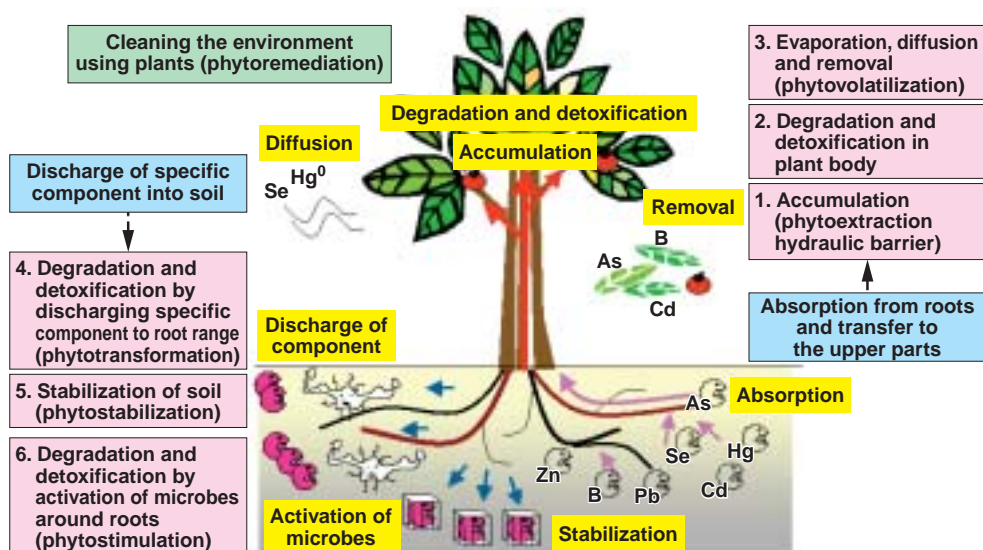


Figure. How phytoremediation works

Accumulation of cadmium by genetically modified tobacco

Utilization of pores of cells

Heavy metals are classified into two sorts: essential elements necessary for plant growth (iron, copper, zinc etc.), and non-essential elements that inhibit growth (lead, mercury, cadmium etc.). Almost all contaminants are non-essential elements.

Plants are equipped with a pump to absorb the required doses of essential elements, but have no pump for non-essential elements. Accordingly, a new pump must be installed or an alternative arrangement made to actively absorb the non-essential elements and carry out remediation.

The Institute thought of using the pores between the cell membrane of plants and cell wall (apoplasma zones). The transportation of heavy metals to these zones is occurred by transferring flow and diffusion, but does not require power such as a pump. Accordingly, if a large quantity of receptacles can be provided to bond the target heavy metals in the zones, a notable improvement in accumulation of the heavy metals in question can be expected.

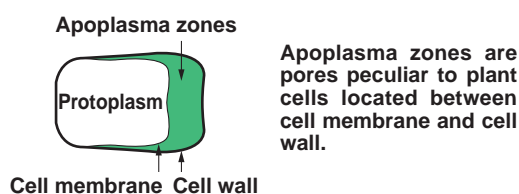
Preparation of gene recombination tobacco

Gene recombination was carried out in order to bestow the capacity of accumulating cadmium to the fore-mentioned Apoplasma zones using tobacco as a plant.

Further, tobacco expressing a large quantity of Metallothionein was prepared by recombining Metallothionein gene which was modified so as to be carried to Apoplasma zones, using the protein of Metallothionein which has a property of coupling cadmium, as a receiver of coupling cadmium.

As a result of analyzing the capacity of accumulating cadmium by the gene recombination tobacco, roots having the highest accumulation capacity exhibited a cadmium content of about 2.5-fold in comparison to tobacco which was not treated with gene operation(non-transformation), at the culture medium of a cadmium concentration of 10 μ M(micromor). The priority of the tobacco prepared by gene recombination was cleared.

1) What are apoplasma zones?



2) Utilization of apoplasma zones (effectiveness verified by this study)

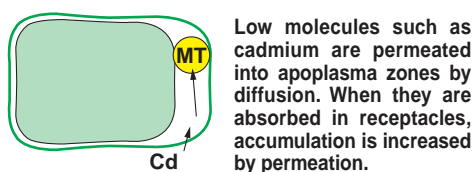


Figure. Apoplasma zones (MT: metallothionein, Cd: cadmium)

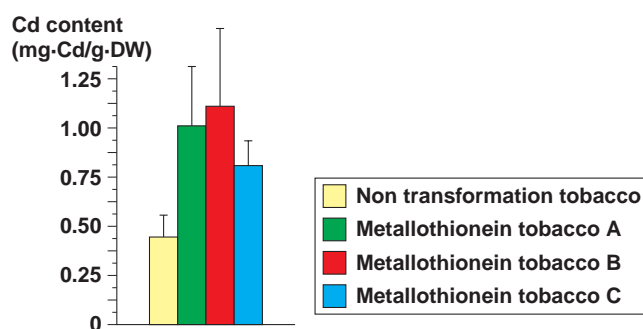


Figure. Accumulation of cadmium in roots of metallothionein tobacco (When the concentration of cadmium in a medium is 10 μ M, the accumulation of cadmium is significantly higher for metallothionein series tobacco in comparison with the non transformation series ($P < 0.05$), and in particular, the accumulation of cadmium increases up to 2.5 times for metallothionein series B.)

Use of genetic modification technique and natural plant functions in combination

Learning to plant “*Athyrium yokoscense*” in nature

A plant that has powerful durability against the heavy metals of contaminants and accumulates them at high concentration (hyper accumulator) is necessary for practical use in phytoremediation.

Accordingly, the Institute focused on “*Athyrium yokoscense*” of Pteridophyta, known as a hyper accumulator of cadmium in nature, to utilize it for further modification, and studied its genetic background, control mechanisms etc.

However, it is necessary to obtain a large quantity of stable plant tissues under uniform conditions in order to do this. Accordingly, the Institute established a tissue culture experiment system for *Athyrium yokoscense* using plant hormone (kinetin +2,4D). The Institute also clarified that this system can be used to research characteristics and identify the mechanism in a similar manner to a grown plant body using the culture tissue obtained.

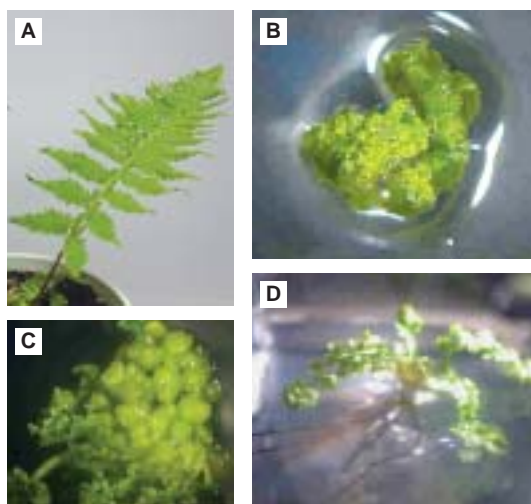


Figure. Tissue culture of *Athyrium yokoscense* and neogenesis of plant body

A. Feathery leaves of polarization article before callus induction
 B. Callus vigorously proliferated
 C. Forest leaf body generated from callus
 D. Seeding plant generated from callus
 (Callus: wound-healing tissue. A cell group generated when a plant was damaged, that becomes callus or flesh-chunk. Rooting starts after appearance of callus.)

Future developments

A plant with greater capabilities of absorption, accumulation and durability is required to reduce the length of treatment and make practical use of the phytoremediation technique.

The Institute will continue to study the cadmium receptacle and absorption by a genetic modification technique, and clarify the absorption and accumulation mechanisms of cadmium in natural hyper accumulators, using the tissue culture experiment system for *Athyrium yokoscense*, to develop and make practical use of a plant for phytoremediation as soon as possible.

● Brief Note



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When I first encountered the concept of cleaning contaminated soil using the power of plants, I was reminded of the “Fukai Forest” that is a naturally occurred restoration site for heavily contaminated soil in Miyazaki Hayao’s film “Nausicaa”. Since my original specialty is the study of how plants absorb and utilize heavy metals, I was confident that this knowledge would prove useful in the establishment of a new technique. Making practical use of the technique will be hard work requiring a long time, and will not be easy, but I see it as my life work.