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# Phytoremediation:
# Searching for plants with high environmental purification capacity

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## Synopsis

Pollution by chemicals from industries, the storage of polluted wastes, and agricultural fertilizer pose a serious threat to human health. These pollutants may pass into the soil where plant uptake or leaching to groundwater can contaminate the food chain. Thus, though most pollution by heavy metals is artificial, natural sources also cause pollution. Natural pollutants include deposits and mother rocks. In a volcanic country such as Japan, in the level of arsenic, cadmium and chromium in soil are high. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants found in air, sea and river water and soil. They occur as common constituents of gasoline, coal tar and shale oil, but are most frequently formed by incomplete combustion of fossil fuels. These actions in the atmosphere are much known, but these actions in soils are little known. Phytoremediation is the process by which contaminants in the environment are removed by plants. The targets of phytoremediation are various pollutants, e.g. heavy metals, NOx, SOx, agricultural chemicals and PAHs. These processes are cost-effective rather than chemical and physical treatments. In this review, we describe the phytoremediation techniques with results of our studies and the status of studies on phytoremediation.

Key Word: Phytoremediation, metal removal, PAHs, environmental purification.

## Introduction

Since the Soil Contamination Countermeasure Law was enforced in February 2003, interest in land soil pollution and clarification of soil pollution have been increasing. This law requires land owners or polluters to remove land soil pollution [1], and restoration of polluted land soil has become an important issue. Currently, physical treatment, such as removal of polluted land soil and bringing earth, and chemical treatment, such as fixation of pollutants and detoxification of pollutants using chemicals, are widely used. However, there remain problems in the elimination of pollution from removed land soil and fixed pollutants. A method that will solve these problems is phytoremediation, which is drawing attention as a technique to be used in this century. Since phyto in Greek means plants, and remediation in Latin means restoration, phytoremediation literally indicates restoration of environmental pollution using plants. Techniques for the restoration of environmental pollution using plants have reached the level of practical application in Europe and America [2]. Current phytoremediation targets clarification of the mechanism of restoration of environmental pollution by plants, identification of the genes, evaluation of the interaction between plants and microorganisms, and application of the system and techniques to various environmental pollution problems. In this review, we describe the phytoremediation techniques with results of our studies and the status of studies on phytoremediation.

## Ranges of application and targets of phytoremediation

Plants absorb and metabolize various chemical compounds including pollutants using solar energy as the energy source, and have self-proliferation ability. An advantage of plants is that they do not utilize pollutants as an energy source. Another advantage is that unlike microorganisms, plants are relatively unaffected by the environment and other organisms. A disadvantage of processing with plants is the necessity of a long time.

Phytoremediation utilizes various plant abilities in applied ranges. Fig. 1 [3] and Table 1 show

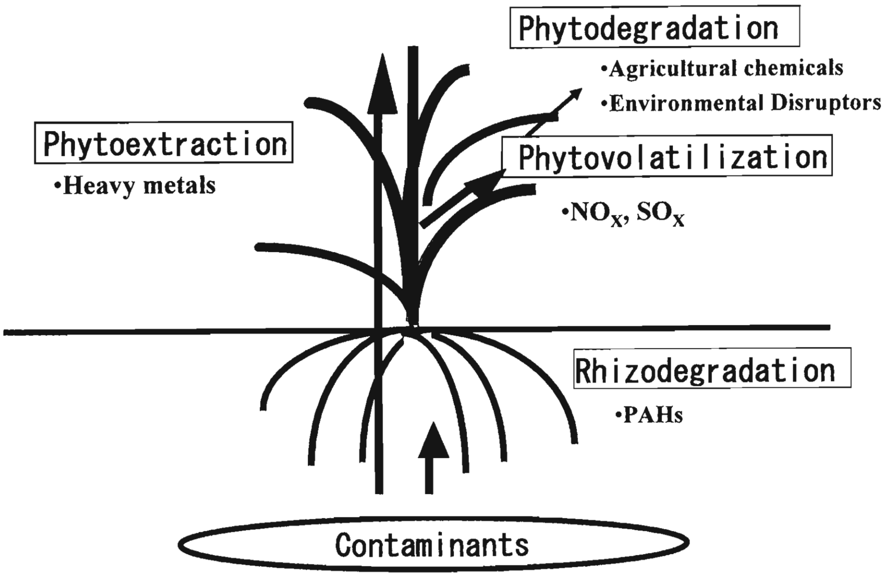


Fig. 1 Process of Phytoremediation [6]

### Table 1 Phytoremediation

| | |
|---|---|
| Phytoextraction(PE) | Concentration of pollutants by plants: Environmental pollutants are accumulated in the plant body. |
| Phytotransformation | Detoxification by plants: Detoxification of pollutants by degradation/metabolization in the plant body or in the rhizosphere by enzymes secreted by plants. |
| Phytovolatilization | Dissipation of pollutants by plants: Pollutants are volatilized in the atmosphere. Air pollutants, such as NOx and SOx, are taken in by plants, and converted into nitrogen and sulfur compounds that can be used as nutrients. |
| Phytoprevention | Prevention of spread of pollutants by plants: Polluted grounds are covered with plants to minimize infiltration of rain water into the ground, and transpiration by the plants causes pumping-up of rain water, which together help prevent the spread of pollutants and soil erosion. (Prevention of desertification) |
| Phytostabilization(PS) | Stabilization of pollution by plants: Pollutants are immobilized to the soil with substances secreted by plants. |
| Phytostimulation | Enhancement of pollutant degradation by plants: Substances secreted by plants activate soil microorganisms, which degrade and detoxify pollutants. |
| Phytomining | Refinement of metals by plants: Precious metals and industrially useful metals are recovered using hyperaccumulator plants that absorb and accumulate these metals at high efficiency. |
| Rhizodegradation | Degradation of pollutants by symbiosis between plants and microorganisms in the rhizosphere. |
| Rhizofiltration(RF) | Absorption and removal of pollutants from the hydrosphere by plant roots: Purification of domestic waste water by reeds and biotopes are examples of rhizofiltration. This technique is most widely spread in Japan. |

cases of phytoremediation [4),5)]. Phytoremediation is applied to various cases with pollutants, such as heavy metals, agricultural chemicals, and nitrogen oxide. This method can also be applied to air pollution [6)] and water pollution as well as land soil pollution. To restore land soil, there are such methods as phytoextraction, in which heavy metals accumulated in the terrestrial parts of plants are removed by collecting the plants, and phytodegradation and phytovolatilization, in which organic pollutants, such as agricultural chemicals, are degraded or metabolized by plants [3)]. To remove hydrocarbons derived from petroleum, such as polynuclear aromatic hydrocarbons (PAH), rhizodegradation, in which degradation is performed not by plants alone but by interaction between plants and microorganisms in the rhizosphere, is utilized [7),8)]. As can be seen in these examples, phytoremediation is useful for the purification of various environmental pollutants. In particular, purification of heavy metals and hydrocarbons derived from petroleum by phytoremediation has been actively studied. With regard to the degradation treatment of air pollutants using plants, Morikawa et al. improved the nitrogen dioxide-degrading ability of plants by genetic engineering [9)].

### Application to pollution of heavy metals

Hyperaccumulator [10)] or accumulator plants, which accumulate several tens- to hundred-fold larger amounts of heavy metals in the terrestrial parts than common plants, are used for phytoextraction. These plants have been found under specific conditions close to mines of heavy metals or in serpentinite areas containing a large amount of Ni, and about 400 kinds of plants have been reported [10)]. Many hyperaccumulator plants belong to the Brassicaceae family, among which *Thlaspi japonicum* [11)] for Ni and *Thlaspi caerulescens* [12)] for Zn and Cd belong to the genus Thlaspi of the Brassicaceae family, and many studies on these plants have been performed. The accumulation of heavy metals in the terrestrial parts of plants is considered to occur by absorption by roots (rhizosphere) and transferring via sieve tubes and vessels. The mechanism of accumulation of a larger amount of heavy metals in hyperaccumulator plants than in common plants has not been clarified, but solubilization of heavy metals by chelation of organic acids exuded from roots, and immobilization of heavy metals as nontoxic forms in the terrestrial parts of plants are considered important in hyperaccumulator plants [13)]. Peptides and proteins, such as glutathione and phytokeratin, may play an important role in transferring heavy metals. It has been reported that accumulation of heavy metals was markedly increased by injecting chelating reagents, such as EDTA, into soil [14)]. However, injection of chelating reagents into soil could cause secondary pollution, such as dissolution of heavy metals in ground water. Since the biomass of hyperaccumulaor plants known so far is small, there are many problems in applying such plants to large polluted areas.

### Phytodegradation of endocrine disruptor [15)]

Degradation-resistant organochlorine aromatic compounds, such as PCB, DDT, and dioxin, and aromatic compounds, such as nonylphenol, which are defined as persistent organic pollutants (POPS), taken in the human body bind to receptors of a female sex hormone, estradiol, impair the endocrine system, and cause demasculinization. Lignin decomposing enzymes secreted by mushrooms include lignin peroxidase, manganese peroxidase, and laccase, and phytodegradation of POPS, such as dioxin, PCB, and DDT, is possible by developing plants carrying genes of mushrooms or microorganisms.

### Degradation of polynuclear aromatic hydrocarbons in the rhizosphere

Petroleum hydrocarbons (TPH) and polynuclear aromatic hydrocarbons (PAH) generated in the burning process of fossil fuels, such as petroleum and coal, are known to have carcinogenicity and environmental hormone activity [16),17)]. The Environmental Protection Agency (EPA) of the

USA published the list of priority pollutants [18] showing 16 hazardous compounds of PAH, which require special attention. These compounds exist in the atmosphere [19], but they are adsorbed by soil with rain. PAH contained in exhaust gas remains in soil by adsorption to road dust. A small amount of oil and gasoline leaked from crude petroleum agents, such as gasoline stations and garages, remains in soil. To purify such organic environmental pollutants, bioremediation is well-known. It has been reported that hydrocarbons were decreased in polluted areas during a relatively short period by herbaceous plants growing in such areas, such as ryegrass [20]. This is due to rhizodegradation, in which plants do not degrade the hydrocarbons on their own but secrete from roots substances that enhance proliferation or protection of microorganisms, which particularly degrade hydrocarbons into plant-absorbable or metabolizable forms, which are used as nutrients by plants. In the rhizosphere, microorganisms often enter the roots of plants, in which they form large colonies more easily than individual microorganism in soil[21]. However, there remain many unknowns, such as the mechanism of links between microorganisms and plants. Purification using various plant spices is being attempted, which the terrestrial biomass does not affect because activity in the rhizosphere is important, differing from removal of heavy metals described above.

In phytoremediation, the selection of plant species to be used is very important. The best plants for phytoremediation would have environmental adaptability, grow rapidly, and produce a large biomass (terrestrial parts particularly for the removal of heavy metals). Plants having such characteristics are weeds and wild grass. Weeds are generally unpopular, but they are very useful for the removal of pollution by phytoremediation.

### Phytoremediation using weeds and wild grass

Table 2 shows the quantities of major heavy metals in soil samples collected in the suburban areas of Nara City. They do not significantly differ from the mean heavy metal contents in non-polluted areas in Japan reported by Asami et al. [22], but tend to be higher because of the concentration of mineral dust on roads by rain water due to the higher rate of asphalt roads in urban areas. Even in such unfavorable urban environments, many species of weeds and other plants grow to form communities. Table 3 shows the quantities of major heavy metals contained in weeds collected in the suburban areas of Nara City. In most species tested, heavy metal contents were higher in the subterranean parts (roots) than in the terrestrial parts, indicating heavy metals mostly remain in the root. Notably, many of the plants of the buckweed family showed high heavy metal contents [23]. Although these results were obtained by examining the samples collected in non-polluted areas, those plants that accumulated heavy metals 10-fold more than common plants may be used as accelerator plants.

Table 4 summarizes the contents of 16 PAH in soil samples collected from different environments in the suburban areas of Nara City. A close

Table 2 Heavy metal concentrations in soil samples

| | concentration (mg/kg soil) | | | | | | |
|---|---|---|---|---|---|---|---|
| Area | Cr | Cu | Zn | Fe | Ni | Mn | Pb |
| Adjustment pnd (Kinki Univ.) | 11.51±2.52 | 10.22±1.21 | 44.12±1.33 | 2798±31.44 | 63.50±0.01 | 238.70±0.34 | ND |
| Parking Lot (Kinki Univ.) | 24.11±1.19 | 43.02±2.44 | 94.32±3.52 | 2645±11.02 | 39.04±4.91 | 336.23±2.32 | 34.01±1.20 |
| The side of main road (Nara City) | 41.21±2.12 | 40.2±1.25 | 163.01±6.78 | 3248±1.20 | 14.10±6.01 | 285.07±2.21 | 47.09±1.11 |
| The side of street (Nara City) | ND | 13.25±0.94 | 52.75±1.10 | 3168±22.09 | ND | 345.11±2.32 | ND |
| Oile pond (Nara City) | ND | 19.52±2.20 | 70.09±1.21 | 1078±22.22 | 4.51±0.09 | 275.98±1.20 | ND |
| Side of Tomio river (Nara City) | 49.23±1.44 | 70.06±3.45 | 98.24±1.33 | 2160±18.92 | ND | 758.93±2.22 | 97.07±2.20 |
| Non-contaminated soil in Japan [a] | 6.86-106 | 7.95-44.0 | 16.0-105 | 0.82-5.40 [b] | 2.61-37.7 | 62.0-1800 | 9.25-41.8 |

mean±S.D, n=3

ND: not detected

[a] Asami et al. 1988, [b] unit: %

correlation was noted between the total level of PAH and the amount of traffic, and the PAH levels also depended on the density of plants on the sampling points even though the amount of traffic was similar [24]. Probably, the presence of plants reduces the contents of PAH in the soil, especially PAH with 4 or more benzene rings. Most sampling points showing low PAH levels were untended roadsides, which were covered with weeds. This may be important for future studies on the roles of plants in the rhizosphere.

To investigate the degradation mechanism of PAH in the rhizosphere of plants observed on the sides of major roads, superoxide dismutase-like activity (SOD-like activity), which is a well-known index of anti-stress activity, of weed samples were

Table 3 High concentrations in plants of each metal element (Cu, Zn, Mn, Cr and Ni)

| Family | species | part | element | conc.[a] | accum. ratio[b] |
|---|---|---|---|---|---|
| Graminiae | *Alopecurus aequalis* | root | Cu | 142.03±7.20 | 4.08 |
| Polygonaceae | *Persicaria thunbergii* | leaf | Cu | 128.60±6.11 | 38.20 |
| Phytolaccaceae | *Phytolacca esculenta* | root | Cu | 93.98±3.22 | 6.50 |

| Family | species | part | element | conc.[a] | accum. ratio[b] |
|---|---|---|---|---|---|
| Graminiae | *Alopecurus aequalis* | root | Zn | 248.03±20.36 | 4.77 |
| Polygonaceae | *Rumex japonicus* | flower | Zn | 159.03±5.12 | 3.06 |
| Polygonaceae | *Persicaria thunbergii* | leaf | Zn | 146.70±5.83 | 6.97 |

| Family | species | part | element | conc.[a] | accum. ratio[b] |
|---|---|---|---|---|---|
| Polygonaceae | *Persicaria scabra* | root | Mn | 1554.33±10.97 | 6.34 |
| Polygonaceae | *Persicaria thunbergii* | leaf | Mn | 1192.00±10.15 | 4.86 |
| Polygonaceae | *Persicaria bydropiper* | root | Mn | 937.50±9.34 | 3.82 |

| Family | species | part | element | conc.[a] | accum. ratio[b] |
|---|---|---|---|---|---|
| Graminiae | *Alopecurus aequalis* | root | Cr | 142.03±2.90 | 5.92 |
| Graminiae | *Alopecurus aequalis* | flower | Cr | 56.00±1.75 | 2.33 |
| Polygonaceae | *Rumex japonicus* | root | Cr | 46.07±1.31 | 1.92 |

| Family | species | part | element | conc.[a] | accum. ratio[b] |
|---|---|---|---|---|---|
| Compositae | *Bidens pilosa* | root | Ni | 185.00±5.14 | 12.3 |
| Compositae | *Artemisia montana* | root | Ni | 125.03±12.45 | 3.79 |
| Graminiae | *Setaria viridis* | leaf | Ni | 66.50±1.47 | 1.14 |

[a] conc. mg/kg DW, mean±S.D. , n=3
[b] accum. Ratio=(average conc. in plant)/(average conc.in soil), n=3

Table 4 Concentration of total and rings number of PAHs (mean±S.D.) at different sites (S.No.1-8) [24]

| | | | Concentration (mg/kg soil) | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sample No. | Traffic* | Vegetation** | Total PAHs | 2-ring | 3-ring | 4-ring | 5-ring | 6-ring |
| 1 | +++ | - | 4.25±1.18 | 0.29±0.07 | 1.06±0.29 | 0.53±0.17 | 1.21±0.27 | 1.16±0.39 |
| 2 | +++ | - | 3.86±1.31 | 0.29±0.05 | 1.13±0.32 | 0.66±0.44 | 0.90±0.15 | 0.90±0.36 |
| 3 | +++ | + | 2.59±1.53 | 0.38±0.22 | 0.82±0.69 | 0.50±0.29 | 0.54±0.30 | 0.35±0.25 |
| 4 | ++ | + | 0.51±0.13 | 0.11±0.02 | 0.26±0.04 | 0.10±0.03 | 0.03±0.04 | 0.01±0.01 |
| 5 | ++ | + | 0.37±0.12 | 0.03±0.01 | 0.07±0.02 | 0.08±0.01 | 0.15±0.05 | 0.05±0.01 |
| 6 | + | ++ | 0.25±0.12 | 0.06±0.05 | 0.08±0.02 | 0.04±0.04 | 0.05±0.09 | 0.02±0.03 |
| 7 | + | ND | 1.07±0.38 | 0.35±0.01 | 0.47±0.20 | 0.12±0.08 | 0.10±0.09 | 0.03±0.05 |
| 8 | + | - | 1.05±0.26 | 0.20±0.16 | 0.63±0.04 | 0.10±0.01 | 0.07±0.00 | 0.04±0.04 |

* +++: >5000cars/day, ++: 500-5000cars/day, +: <500cars/day

** ++: It is covered the whole surface vegetation, +: 70% are covered vegetation, -: Less than 50% are covered vegetation, ND: No vegetation measured (Table 5). Entire plants collected including roots were immersed in a pyrene solution (0.35 mg/L in distilled water) or distilled water for 72 hours, and the extracts were adjusted to 1,000 ppm, and subjected to SOD-like activity assay. Most plants showed higher activity in the presence of pyrene [25], suggesting that pyrene enhanced the effusion of substances with high SOD-like activity. This is probably due to a defense reaction of the plants, which may be noteworthy for future studies although it is not directly related to degradation and metabolism of pollutants.

### Preventive phytoremediation

As described above, phytoremediation is a potentially effective method of environmental purification. It may also be useful for the prevention of pollution as "preventive phytoremediation". Although large-scale heavy environmental pollution is rare, environmental pollution of smaller scale is wide-spread not only in industrialized areas but also in newly urbanized areas which have been developed in the past 20 years. To minimize or permanently prevent pollution, it will be effective to conduct risk assessment at the time of city planning and to introduce plants selected based on the assessment. In some urban areas, trees with the purification ability of air pollution, such as poplars and round-leaf holly, have been planted [26]. To further extend such measures, the market of phytoremediation will grow in Japan where physical and chemical means of environmental purification have so far been given priority because of its small land and consideration of surrounding environments. Planting trees on rooftops, which has become common in cities, will contribute to the reduction of load on the environment if the kinds of trees to be planted are selected based on prior risk assessment.

### Conclusions

The selection of most effective species of plants is most important in phytoremediation. Worldwide researchers are searching polluted areas and mining areas for plants with high activity of environmental purification. However, soil diversity is a major obstacle to practical application in actual environments, and in many cases, plants fail to manifest their abilities demonstrated in pot experiments in laboratories. In addition, many plants that grow in specific environments are often unable to grow on common soil. Therefore, weeds, which have strong vital force, are very attractive candidates. Since it would be tragic to destroy the ecosystem by introducing plants for phytoremediation, species that would not destroy the ecosystem, such as those endemic to Japan, must be selected.

Table 5 SOD activity of plant exudates (n=3) [25]

| plant | solution type | SOD activity (%)* |
|---|---|---|
| *Artemisia princeps* | distilled water | 9.1±1.2 |
| | pyrene dissolved | 68.3±3.6 |
| *Chenopodium album* | distilled water | 22.2±1.5 |
| | pyrene dissolved | 11.9±2.2 |
| *Solidago altissima* | distilled water | 16.1±3.4 |
| | pyrene dissolved | 36.8±3.3 |
| *Fatoua villosa* | distilled water | 7.8±2.3 |
| | pyrene dissolved | 13.1±1.5 |
| *Amaranthaus viridis* | distilled water | 6.1±0.2 |
| | pyrene dissolved | 15.3±1.3 |

* This activities indicate superoxide scavenging activities that was determined by NBT reduction each sample concentration was 1000 $\mu$ g/1mL

Molecular mechanisms of dehalogenation, aromatic ring cleavage, and resistance to and accumulation/detoxification of heavy metals as well as the genes involved will be elucidated in the future. The creation of more effective plants on the basis of such mechanisms is expected.

Phytoremediation has been successfully used against pollution by single causes, but in the future, development of phytoremediation as a system that can treat multiple pollution is necessary.

In conclusion, phytoremediation is an ideal technique for restoration of natural environment that has been destroyed in the twentieth century. Our results will considerably contribute to the improvement of the efficiency of phytoremediation and its practical application.

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# ファイトレメディエーション
# —環境浄化能力の高い植物を求めて—

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要　約

近年、生産され排出される化学物質の数は飛躍的に増大している。その中でも、重金属、多環芳香族炭化水素類（PAHs）、アルキルフェノール類およびダイオキシン類などといった環境ホルモンといわれる物質は残存性や難分解性が高く、大気–土壌–水域底質間、いわゆる土壌圏を循環していると考えられる。これらの土壌圏における環境汚染物質の修復法の一つとして植物を用いて、汚染物質の蓄積、吸収や分解などを行うファイトレメディエーションがある。この技術は、対抗技術である物理的、化学的除去、封じ込め技術に比べ、コストが安い、大型機器が必要ないなどの利点があり、欧米を中心に研究されている技術である。

本稿では、このファイトレメディエーション技術について、著者らの研究実例を挙げながら、ファイトレメディエーション研究の現状を紹介する。