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Bioremediation of Biophilic Radionuclides by Algae

Koji Iwamoto and Ayumi Minoda

Abstract

High amounts of radionuclides were released into the environment by the nuclear power plant accident of 2011 in Japan. Among the radioactive material, cesium, iodine, and strontium were especially dangerous because of their biophilic characteristics that allowed them to accumulate in living organisms, either as essential elements for iodine or analogs of potassium and calcium for cesium and strontium, respectively. As a result, there was a high social demand for decontamination to avoid exposure to these elements. The authors screened around 200 strains of algae and plants for their ability to absorb radioactive nuclides. The eustigmatophycean algae *Vacuoliviride crystalliferum* and the cyanophytes *Stigonema ocellatum* and *Nostoc commune* showed the highest bioaccumulation activity for the removal of cesium, strontium, and iodine from the environment, respectively. In addition to these strains, the authors also found that the extremophilic unicellular red algae *Galdieria sulphuraria* could remove high levels of dissolved cesium from media in mixotrophic growth conditions. In this chapter, the intake mechanism of cesium, iodine, and strontium is reviewed. Recent findings on the absorption of these elements by algae are discussed to highlight the possibility of decontaminating polluted land and water at nuclear sites by phytoremediation.

Keywords: radionuclides, phytoremediation, cesium, strontium, iodine, *Vacuoliviride crystalliferum*, *Galdieria sulphuraria*

1. Introduction

On March 11, 2011, Japan suffered a large earthquake, known as the Great East Japan Earthquake, causing a tsunami that damaged the Fukushima 1st Nuclear Power Plant (F1NPP). This damage led to release of a large amount of radioactive matter into the environment, estimated at 11.6 EBq (exa Bq: 10^{18} Bq) [1]. Radioactive xenon (Xe-133) was the most abundant material discharged, at 11.3 EBq, followed by iodine (I-131, I-132, I-133, and I-135), tellurium (Te-127m, Te-129m, Te-131m, and Te-132), and cesium (Cs-134 and Cs-137). In addition to these, radioactive strontium (Sr-89 and Sr-90), barium (Ba-140), yttrium (Y-91), and plutonium (Pu-238, Pu-239, Pu-240, Pu-241) were also released among others. Among these radionuclides, cesium, strontium, and iodine gained the most attention because of their biophilic properties, in addition to the high amount of the discharged activity, which was 33 PBq (peta Bq: 10^{15} Bq), 200 PBq, and 2.1 PBq in total, 10–37 PBq, 150 TBq (tera Bq: 10^{12} Bq), and 90–500 PBq in the air, and 1.9 PBq, 90–500 TBq, and 2.7 PBq in the ocean, respectively [1–5]. However, eventually the attention focused solely on cesium because iodine and Sr-98 have relatively short

half-lives and so their levels decreased to below detectible levels at an early stage (50.5 days for Sr-98 and 8 days for I-131, which is the longest half-life period among radioactive iodine released by the accident) [1]. For strontium, in addition to the early decrease in the contamination levels of Sr-98, the total discharged activity of Sr-90 was comparatively small, and the polluted area was limited to near the surroundings of the F1NPP due to its low volatility [6]. However, radioactive strontium and iodine still need to be monitored and decontaminated because 140 TBq of Sr-90, with a half-life 29.1 years, was released into environment, and I-129, with a half-life of 16 million years, could be produced from 3.3 PBq of Te-129m in the polluted environment [7, 8]. I-129 is derived from Te-129m, and small amounts of I-129 produced in the reactor were also released during the accident [9].

High-level radiation-contaminated water in the reactor building and the accessory facilities of F1NPP was treated by various chemical and physiological methods including the SARRY system (simplified active water retrieve and recovery system) for cesium removal and the ASPS system (advanced liquid processing system) for multinuclide removal [12, 13]. However, the decontamination of the surrounding area was not as simple because of the wide area of contamination and the low concentrations of the radioactive materials.

However, the total mass of the radioactive material was not as high as the total activity from F1NPP, which was calculated by following equation (Table 1) [10, 11]:

$$W = B \times 8.62 \times 10^{21} \times M \times T \tag{1}$$

where w is the total mass (g), B is the total activity (Bq), M is the radionuclide atomic weight, and T is the half-life period (hour).

Even when calculating the total mass of all the biophilic elements, it did not exceed 6 kg. On the other hand, the polluted area exceeded over 10,000 km², which included forests, field, lakes, rivers, and houses, with a rate of 0.5 μSv h⁻¹ in the air recorded on April 1, 2011 [14]. Ten years later, the area would still cover around 3000 km² due to the half-life period of Cs-137, which is 30 years [14]. The physiological and chemical methods for decontamination are costly and difficult to apply for such a weak and widespread contamination. Hence, the biological method, called bioremediation, is a good candidate for decontamination [10]. Bioremediation is a method that uses living organisms to accumulate or degrade a contaminating material. This method has many advantages because it allows for the possibility to treat various pollutants thanks to its biological diversity. It is effective

Nuclide	Half-life period	Total activity (Bq)	Mass (g)*
Cs-134	2.1 years	1.8 × 10 ¹⁶	373
Cs-137	30.0 years	1.5 × 10 ¹⁶	4753
Sr-89	50.5 days	2.0 × 10 ¹⁵	1.8
Sr-90	29.1 years	1.4 × 10 ¹⁴	277
I-131	8.0 days	1.6 × 10 ¹⁷	35.5
I-132	2.3 hours	1.3 × 10 ¹³	3.4 × 10 ⁻⁵
I-133	20.8 hours	4.2 × 10 ¹⁶	1.0
I-135	6.6 hours	2.3 × 10 ¹⁵	1.7 × 10 ⁻²

*Mass was calculated from the total activity, radionuclide atomic weight, and half-life using the equation in the text below.

Table 1.
Total activity and the mass of radioactive cesium, strontium, and iodine released from F1NPP.

for trace levels of pollutants; it can reduce the cost of remediation because of the low energy usage. However, in addition to the disadvantage of it being both temperature- and weather-dependent, the speed of the remediation method is limited. Despite this, bioremediation is considered to be the remediation technology for the next generation.

2. Uptake of cesium, strontium, and iodine by organisms

Cesium, strontium, and iodine were regarded as the most hazardous of the radionuclides diffused into the environment by the F1NPP accident. These elements are easily absorbed by living organisms through water and air or indirectly through food and result in an increased health risk by internal exposure. The indirect uptake of these nuclides through food is more serious because the strength of the activity may be increased by biological concentration via food chain [10]. Therefore, the number of radionuclides, such as Cs-137, Sr-90, and I-131, as well as Ru-106 and K-40, has been monitored by the United States (Food and Drug Administration), Japan (Ministry of Health, Labor and Welfare), and other countries in addition to toxic metals, pesticides, and chemicals [11].

2.1 Cesium

Of the different radionuclides, radioactive cesium has attracted the most attention for the following reasons: (i) the total amount of released radioactivity for Cs-134 and Cs-137 was very high, (ii) the long half-life of cesium (2.1 and 30.0 years for Cs-134 and Cs-137, respectively) means it remains in environment for many years, (iii) the area contaminated by cesium was considerably large with respect to its volatility, and (iv) cesium is easily absorbed and accumulated in the body of living organisms.

Cesium is absorbed by the body as an analog of potassium, which is one of the essential elements of living cells, and is accumulated in the parenchyma of the muscles and organs [12]. However, the biological concentration factor of cesium is not very high, unlike for heavy metals, and is in the range of one to two orders of magnitude in large predatory fish [13–19]. The effective half-life is considered to be the same level as biological half-life in humans, about 100 days, because of the long half-life of Cs-134 and Cs-137 [20]. Cesium is thought to be taken up by the potassium assimilation system, which includes the potassium transporter, the sodium/potassium pump, and the potassium channel. In animals, cesium is absorbed in the intestine, stored partially in the liver, and used in the cells and organs across the whole body, including muscles, bones, brain, etc. In plants, potassium also has important physiological roles, such as the extension and division of the cell, the opening and closing of stoma, and signal transduction between organelles. On the other hand, it has been previously reported that over 200 μM of cesium can inhibit plant growth by disturbing the potassium uptake and jasmonate signaling [21, 22]. Many potassium transporting systems have been identified, and cation and/or calcium-transporting systems are thought to be involved in cesium transport in plants [23].

Figure 1 is a schematic diagram of a charging and discharging system of cesium in a plant root cell [23, 24]. Plant roots have several reported systems for potassium intake, including K^+/H^+ symporters (KUP) and inward-rectifying K^+ channels (KIRCs) [25]. Cesium is transported into the cell by these systems. The KUP system belongs to the KT/KUP/HAK family. AtHAK5 and AtKUP/HAK/KT9 were identified in *Arabidopsis thaliana* [26]. The KUP system is thought to be involved in the

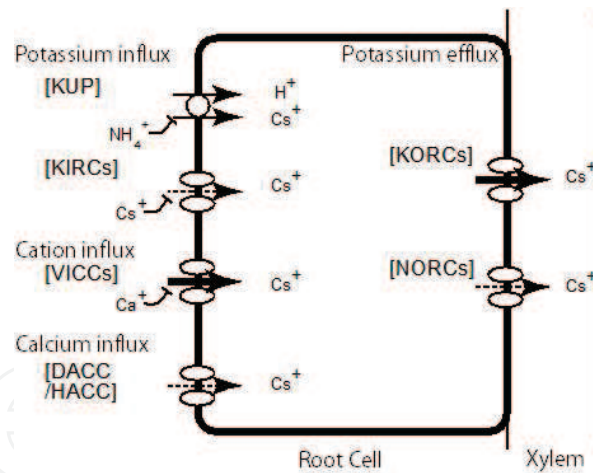


Figure 1.

Cesium charging and discharging system in a plant root cell. DACCs: depolarization-activated Ca^{2+} channel, HACC: hyperpolarization-activated Ca^{2+} channel, KIRC: inward-rectifying K^+ channels, KORC: outward-rectifying K^+ channels, KUP: K^+ uptake, NORC: nonselective outward-rectifying K^+ channel, VICC: voltage-independent cation channel. The arrows and their widths indicate the direction of transport and their relative activity, respectively.

uptake of cesium when potassium levels are low because the gene expression of AtHAK5 has been reported to increase in potassium or NH_4^+ -deficient conditions. Moreover, KUP has been previously shown to transport cesium by an AtHAK5-deficient *Arabidopsis* mutant and an AtHAK5-producing yeast whose phenotypes showed lower Cs^+ accumulation and higher cesium uptake, respectively [26]. The role of KIRCs in cesium uptake is not clear because these potassium channels are severely inhibited by the transport of Cs ions, which act as potassium channel blockers and close the channel [23, 27].

The importation of Cs in plants is also carried out by other cation efflux systems. One of these are the voltage-independent cation channels (VICCs) [23]. The AtCNGC (*A. thaliana* cyclic-nucleotide gated channel) and AtGLR (*A. thaliana* glutamate receptor) gene families are involved in the VICCs identified in *A. thaliana* [24]. Other cation efflux systems also considered to be involved in cesium transport are HACCs (hyperpolarization-activated Ca^{2+} channel for calcium efflux), DACCs (depolarization-activated Ca^{2+} channel systems for calcium efflux), KCO (the cation-transporting channel to vacuole), KEAs (K^+ efflux antiporter as potassium-proton antiporters), and AtNHX1 (*A. thaliana* Na^+/H^+ antiporter classified in cation-proton antiporters) [23]. For discharging of cesium from cells to bundle, KORCs (outward-rectifying K channel) are believed to play a fundamental role among the potassium efflux system such as NORCs (nonselective outward-rectifying K channel) [23].

2.2 Strontium

Radioactive strontium received the least attention of the three biophilic elements released by the F1NPP because the total discharged radioactivity was the lowest, at 2.1 PBq, that is, 1/10th of that of radioactive cesium. Moreover, Sr-89, with a half-life of 50.5 days, made up about 2 PBq of this, and hence the majority of the strontium in the environment decayed early on. The area polluted by radioactive strontium was limited because of its nonvolatility, and detection was difficult because both Sr-89 and Sr-90 have low-range radiation as low-energy beta-ray nuclides [1, 10]. Strontium is taken up by cells in the intestine as an analog of calcium and is stored in the bones [28]. Once strontium is absorbed, it is not easily

excreted from the body due to its long biological half-life of 30–50 years. This poses a serious health risk due to long-term internal exposure to radiation. Furthermore, high levels of strontium were reported in predatory freshwater fish, with a concentration factor of 10^3 [29]. Therefore, radioactive strontium should receive equal if not greater attention than the other radionuclides. Calcium is the most abundant metal element and mostly exists in bones in animals. In plants, calcium is the second most abundant metal element after potassium and exists in the extracellular space in its insoluble form. It binds to cell wall components to form part of the support system of the plant structure. In the cell, calcium is stored in organelles such as mitochondria and endoplasmic reticulum, or chloroplasts and vacuoles in plants. The concentration of calcium in the cytosol is kept in at very low levels (10^{-7} M) because calcium plays an important role for signal transduction in cells and acts as a secondary messenger. Therefore, there are constitutive calcium excretion systems on the surface of the cell, including CAX1 and CAX2 as the H^+/Ca^{2+} antiporter, ECA and ACA as the calcium pump, and TPC1 and VICCs as the ion channels [30].

Figure 2 is a schematic diagram of the absorption mechanism of calcium in the small intestine of animals. There are two types of absorption, paracellular absorption and transcellular absorption, which function to transport calcium passively under high calcium concentrations and actively under low calcium concentrations, respectively [31]. In the paracellular pathway, small molecules and ions such as Ca^{2+} are selectively allowed to pass through a very narrow space between the cells of the intestinal epithelium, i.e., the tight junctions [32]. For transcellular absorption, there are two active pathways for Ca^{2+} absorption. One is mediated by a calcium channel called Ca_v 1.3, and the other is mediated by a calcium transporter called TRPV6. The calcium transported into the intestinal epithelium cell by Ca_v 1.3 is thought to be released into the bloodstream vesicles. On the other hand, calcium incorporated into the cell is secreted into the plasma by the vesicular transport pathway or via PMCA1b, a calcium pump (Ca^{2+} ATPase). Calcium ions are delivered to the pump by simple diffusion of free Ca^{2+}

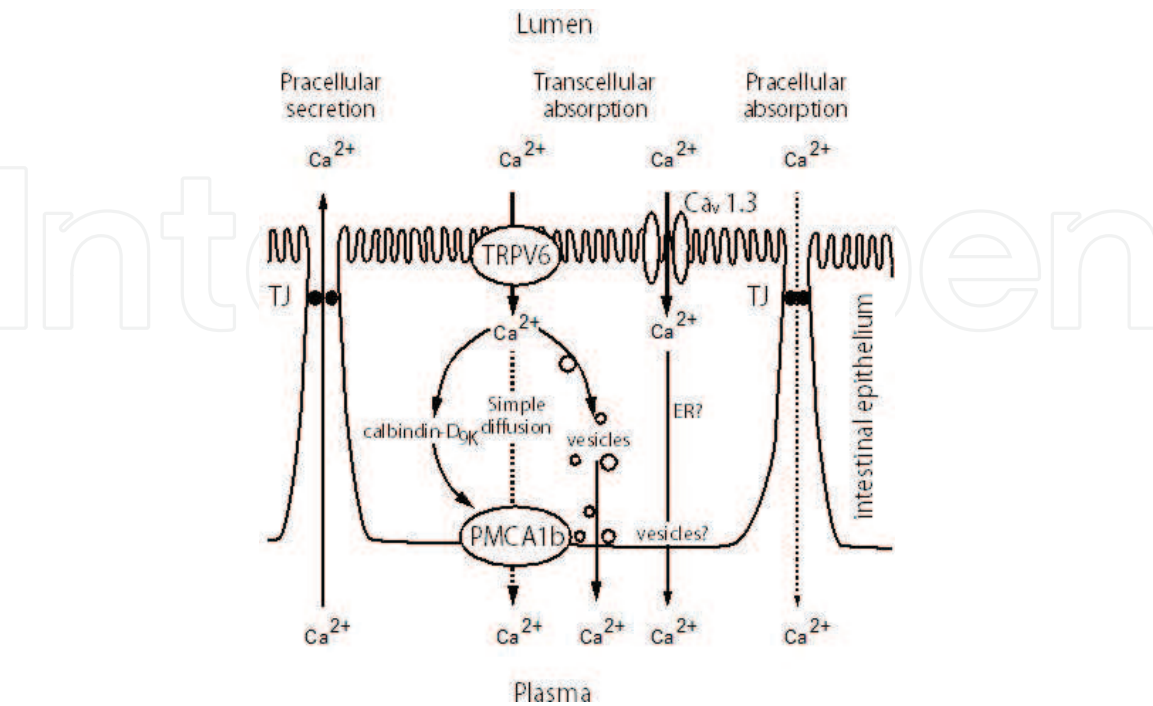


Figure 2.
The calcium uptake system in the small intestine of animals. The arrows and their widths indicate the direction of transport and their relative activity, respectively. ER: endoplasmic reticulum, TJ: tight junction. Solid and dashed lines indicate active transport and diffusion, respectively.

or by the facilitated diffusion pathway with a vitamin D-dependent calcium-binding protein, calbindin-D9K [31].

2.3 Iodine

Radioactive iodine is easily absorbed by the body as the main component of the thyroid hormone. Iodine is focally accumulated and concentrated at the thyroid gland, which increases the risk of developing goiter, especially in infants and small children. As a result of the F1NPP accident, radioiodide was spread into a wide area due to its high volatility. Radioactivity was detected not only in many places and food but also in the drinking water, because of its high solubility. For these reasons, it attracted the most attention immediately after the F1NPP accident. After several months, the attention shifted to radioactive cesium due to the early decay of iodine as a result of its short half-life (half-life of 8.0 days for I-133). However, radioactive iodine I-129 still poses a great health risk as it was both directly released from the reactor and can be formed indirectly via Te-129m [7–9]. Even though the release of I-129 was small and the concentration is currently not at a level that would pose a risk to human health [33, 34], because of its extremely long half-life of 16 M years, it could be deposited in the long-term by its continuous release from the nuclear power station or nuclear fuel reprocessing facilities, by another serious accident in the nuclear facility, or by the use of the nuclear weapons.

In higher animals, iodine is concentrated and located in the thyroid glands. Iodine is incorporated into the thyroid glands during the biosynthesis of thyroid hormone, namely triiodothyronine (T3) and thyroxine (T4). Iodide ions (I^-) brought to thyroid epithelial cells by the blood are oxidized into iodinium ions (I^+) by thyroperoxidase. This is activated by the thyroid-stimulating hormone (TSH). The subsequent iodination of tyrosyl residues of the thyroglobulin protein produces iodotyrosines, monoiodotyrosine (MIT), and diiodotyrosine (DIT) by an electrophilic substitution reaction. T4 and T3 are then synthesized and stored in thyroglobulin following an MIT and DIT coupling reaction. Finally, the thyroid hormone-binding protein is hydrolyzed into water-soluble amino acids by the activation of TSH [35].

Brown algae are known to accumulate iodine at rates 10,000 times more than that in seawater. **Figure 3** shows the schematic diagram of the absorption mechanism of iodine by brown algae [36]. Iodide ions (I^-) in the seawater are oxidized

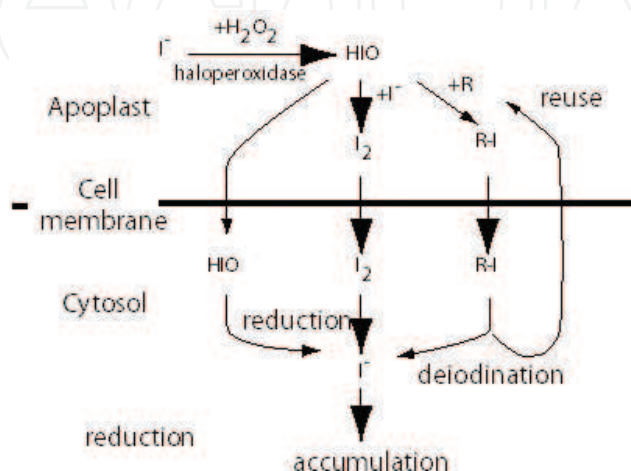


Figure 3.
Iodine uptake system in the brown algae.

into hypiodous acid (HIO) by haloperoxidase in the apoplastic space in the cell wall. Oxidized iodine passes through the cell membrane, by facilitated diffusion in HIO form, or reacts with other I^- or organic substances to produce molecular I_2 or organic iodine, respectively, and crosses the membrane by diffusion. The iodine transported as HIO, I_2 , or organic iodine is then reduced or deiodinated in the cytosol and stored in the cell in I^- form, binding noncovalently with carbohydrates, polyphenols, and proteins [33].

3. Phytoremediation by higher plants for decontamination of a radioactive element

Bioremediation using plants is called phytoremediation, and its use for the decontamination of radioactive elements has been previously reported. For cesium decontamination, Dushenkov et al. reported on the bioaccumulation of cesium, strontium, and uranium by sunflowers in which over 90% of the polluting substances were removed from a solution within 24, 28 and 24 hours, respectively [37]. Broadley et al. showed that dicotyledons (*Magnoliopsida*) possessed a higher activity of bioaccumulation than monocotyledons (*Liliopsida*). Specifically, from the comparison of data extracted from 14 reports regarding cesium bioaccumulation in the plant shoots of 136 species among Magnoliophyta taxa, using a residual maximum likelihood (REML) analysis, they found that a redroot pigweed (*Amaranthus retroflexus*) in Amaranthaceae had the highest bioaccumulation ability [38]. The authors then found that a sugar beet (*Beta vulgaris*) in Chenopodiaceae, a turnip (*Brassica napus*) in Brassicaceae, quinoa (*Chenopodium quinoa*) in Chenopodiaceae, and a swede (*Brassica napus*) had the highest abilities for cesium accumulation after *A. retroflexus*.

The bioaccumulation of strontium by higher plants was surveyed comprehensively among 670 species in 138 families [39], although the individual species names were not specified. Sasmaz and Sasmaz selected *Euphorbia macroclada* in Euphorbiaceae, *Verbascum cheiranthifolium* in Scrophulariaceae, and *Astragalus gummifer* in Fabaceae from a polluted field in a mining area in Eastern Turkey for potential use in phytoremediation [40]. These reports indicate that these plants have a great potential for the decontamination of radiostrontium from polluted areas. Specifically, redroot pigweed and a tepary bean (*Phaseolus acutifolius*) have shown rates of 4.5% and 3.1% removal of Sr-90 from soil, respectively [41].

There are few reports on the uptake of iodine by higher plants because iodine is not an essential element for growth. Instead, it is considered to be a toxic element that causes growth inhibition. For example, type III Akagare disease is caused by the inhibition of photosynthesis due to high concentrations of iodide (>1 ppm) [42]. Therefore, the screening of iodine tolerant plants may be required for the effective phytoremediation and subsequent decontamination of radioactive iodine, unlike cesium and strontium.

Subsequently, decontamination was tested using higher plants, including sunflowers and 12 plant species in Fabaceae and Poaceae, to remove radioactive cesium from contaminated soils in the area polluted by the F1NPP accident [43]. However, a significant rate of removal was not achieved because only 1/2000 of the radioactive cesium in the soil was removed by sunflower [44]. Phytoremediation by higher plants is considered to be an effective method for the decontamination of low- and middle-level radio-polluted soils. However, the screening and breeding of a radionuclide hyperaccumulator is required, in addition to the optimization of the culture conditions and application, according to the localization of the target nuclides [43].

4. Phytoremediation of biophilic radionuclides by algae

4.1 Advantages of microalgae for decontamination of water

Phytoremediation using algae, also called phycoremediation, has the following advantages, in particular when using microalgae [45]:

1. Fast remediation because of the high growth rates of microalgae.
2. Remediation with lower energy costs because of autotrophy.
3. High and effective remediation because of the biological concentration function.
4. Volume reduction of polluted material/water because of the single cellular or simple structure.

In particular, volume reduction by microalgae is considered to be an important advantage in the F1NPP case.

4.2 Algae absorb radioactive cesium, strontium, and iodine

The ability to remove the radioactive cesium, strontium, and iodine from the solution was comprehensively examined to cover broad phylogenetic variation by Fukuda et al. [46]. These aquatic plants and algae were consisted from 188 strains of algae and aquatic plants including 91 seawater, 86 freshwater, and 11 terrestrial strains, which covered almost all phylogenetic group, i.e., 45 classes, 21 divisions, and 7 super groups such as cyanobacteria, Opisthokonta, Excavata, Archaeplastida, Rhizaria, Alveolata, and Stramenopiles in two domains (**Table 2**).

These organisms show various advantageous features in their morphology, physiology, biochemical properties, and nutritional properties, namely autotrophy and/or heterotrophy. Most of these strains were obtained from the culture collections of the Laboratory of Plant Diversity and Evolutionary Cell Biology, University of Tsukuba, Japan, and the Microbial Culture Collection at the National Institute for Environmental Studies (NIES Collection, Tsukuba, Japan). Several other strains were collected from the surrounding area of the University of Tsukuba or were purchased from the local market.

The strains were inoculated in a 70 mL scale plastic culture bottle containing 15 mL of medium under a fluorescent light at 20°C. The strains were inoculated in 15 mL of fresh medium in the plastic culture bottle 1 day before the addition of artificial radionuclide, Cs-137, Sr-85, or I-125, with a concentration of 1 kBq/mL. The same medium was used for the preparative and the experimental culture, except for the cesium removal assay in which potassium was excluded. An aliquot was obtained, and the radioactivity contained in the medium fraction and the cell fraction was assayed using a gamma-ray counter by silicone oil layer methods [47]. The medium fraction was only assayed for the macroscopic algae and aquatic plants, which do not fit the silicone oil layer methods.

For the comprehensive examination of the radionuclide elimination ability of algae and aquatic plants, 167 strains out of 188 strains showed this activity (**Figure 4a**). From the 167 strains, the 15 strains that showed an elimination activity of over 40% were reexamined in a second screening (**Figure 4b**). As a result of the second screening, four strains possessing an elimination activity of 30% were selected as high radioactive cesium eliminators. These were the freshwater eustigmatophycean algae *Vacuoliviride*

Domain	Super group	Divisions	Nutrient condition
Prokarya	Bacteria	Cyanobacteria	A
Eukarya	Opisthokonta	Fungi	A/H
		Choanozoa	H
	Excavata	Metamonada	H
		Euglenozoa	A/H
		Percolozoa	H
	Archaeplastida	Rhodophyta	A
		Chlorophyta	A
		Mesostigmatophyta	A
		Chlorokybophyta	A
		Klebsormidiophyta	A
		Zygnematophyta	A
		Charophyta	A
		Land plants	A
		Cryptophyta	A/H
	Hacrobia	Haptophyta	A
	Rhizaria	Cercozoa	A/H
	Alveolata	Dinophyta	A/H
	Stramenopiles	Bicosoecacea	H
		Pseudofungi	H
		Ochrophyta	A/H

A, H, and A/H, nutrient conditions indicating “mostly autotrophic,” “mostly heterotrophic,” and “a mixture of A and H,” respectively.

Table 2.
The phylogenic position of experimental organisms (phylum level).

crystalliferum (strain nak 9, 90% elimination), the freshwater florideophycean algae *Batrachospermum virgato-decaisneanum* (NIES-1458, ca. 38% elimination), and two strains of aquatic plants (tracheophytes) *Lemna aoukikusa* (TIR 2 and TIR 3, ca. 45 and 66% elimination, respectively). Of these five strains, *V. crystalliferum* showed the highest elimination ability, removing over 90% of the cesium-137 from the liquid within 2 days. This alga is considered to be a candidate strain for the decontamination of radiocesium by phytoremediation. The floating weed *L. aoukikusa* is also considered to be useful for the decontamination of water because it is easy to harvest using a net.

For the elimination of strontium, activity was confirmed in 181 of 188 strains (**Figure 5a**). By the second screening, 10 strains were identified, which had an elimination ability over 30% (**Figure 5b**). From these, three strains were selected as the highest strontium eliminators, namely the freshwater cyanobacterium *Stigonema ocellatum* (NIES-2131, ca. 41% elimination), the freshwater chlorophycean alga *Oedogonium* sp. (nak 1001, ca. 36% elimination), and the freshwater Magnoliopsida *Egeria densa* (We2, ca. 34% elimination).

For the elimination of radioactive iodine, all strains except the green alga *Stigeoclonium aestivale* (NIES-531) showed activity at the global screening. However, the level varied between 0% and 40% depending on the strain (**Figure 6a**). After the second screening, 14 strains that showed an iodine elimination activity of over 40% were found, and finally, four high radioiodine eliminator strains were selected

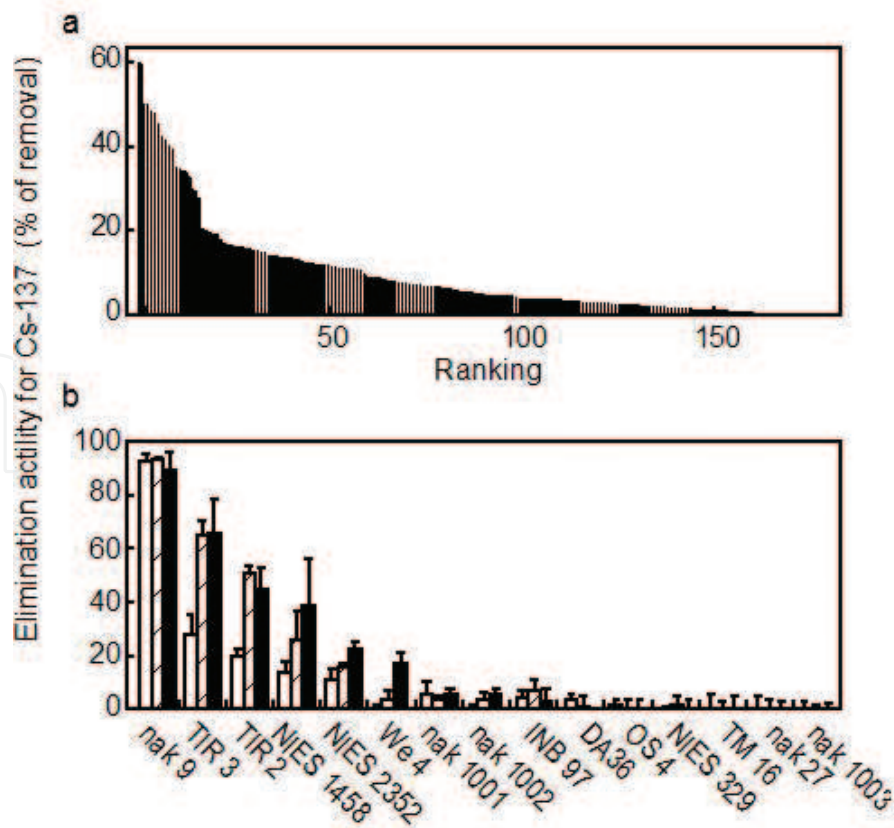


Figure 4. Elimination activity of Cs-137 from the culture medium by algae and aquatic plants. (a) Global screening. Average values were ranked in descending order. (b) Second screening by selected algae and aquatic plants.

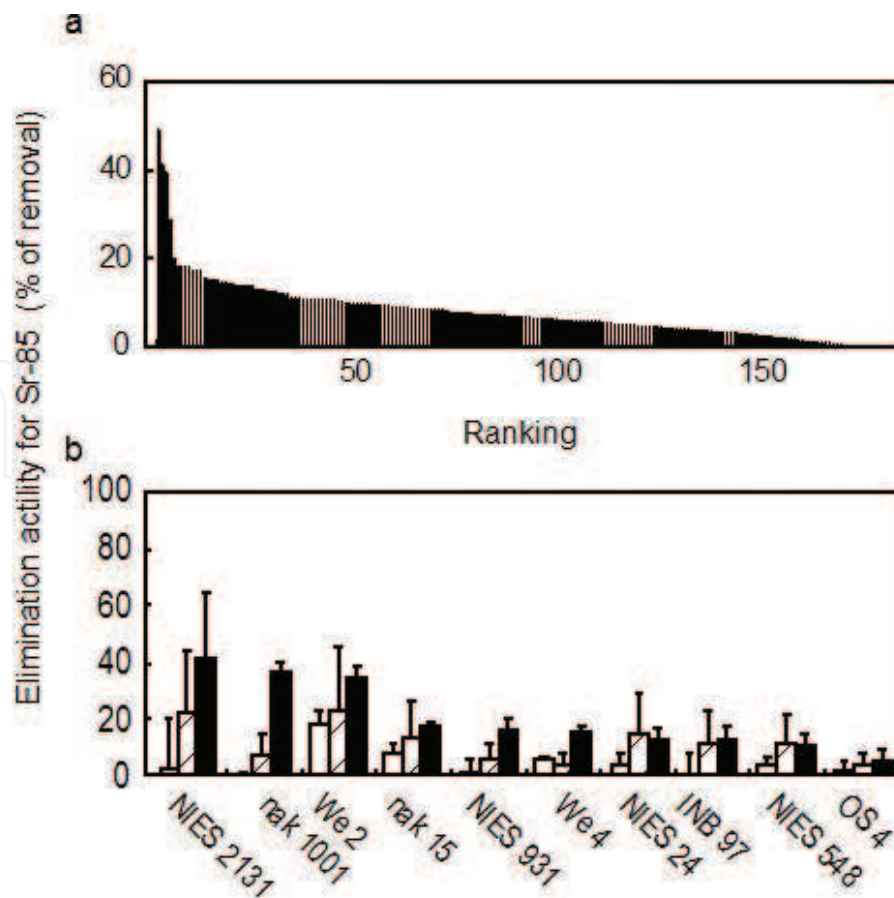


Figure 5. The elimination activity of Sr-85 from the culture medium by algae and aquatic plants. (a) Global screening. Average values are ranked in descending order. (b) Second screening by selected algae and aquatic plants by the global screening.

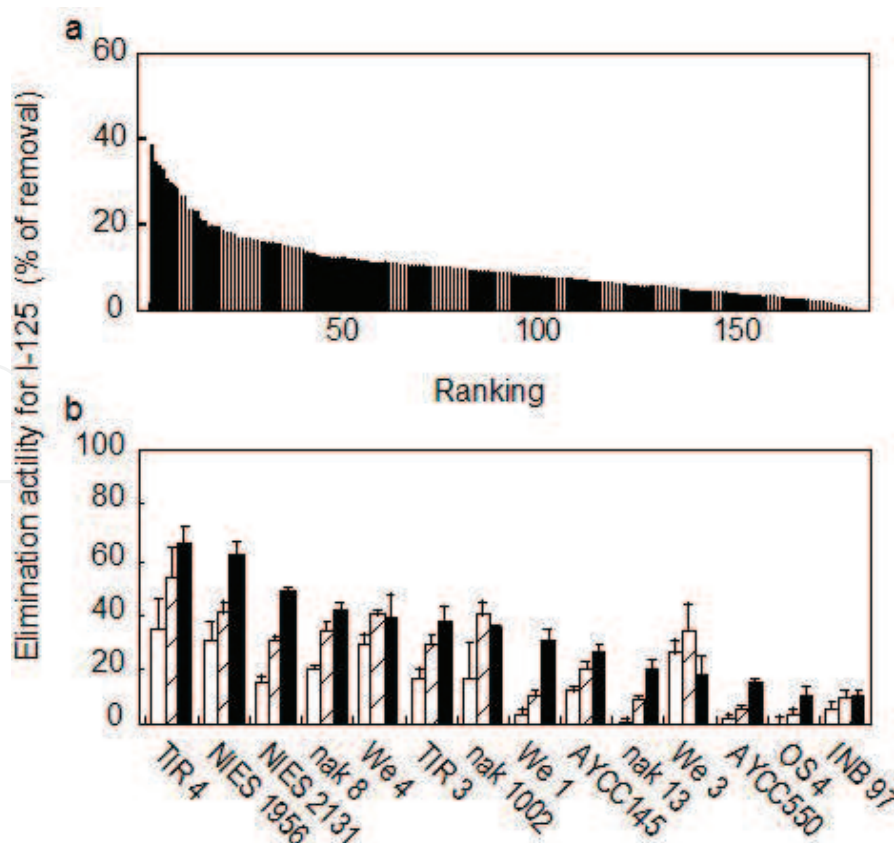


Figure 6. Elimination activity of I-125 from the culture medium by algae and aquatic plants. (a) Global screening. Average values are ranked in descending order. (b) Second screening by selected algae and aquatic plants by the global screening.

(**Figure 6b**). These strains were the terrestrial cyanobacteria *Nostoc commune* (TIR 4, ca. 66% elimination) and *Scytonema javanicum* (NIES-1956, ca. 62% elimination), the freshwater cyanobacterium *Stigonema ocellatum* (NIES-2131, ca. 49% elimination), and the freshwater xanthophycean alga *Ophiocytium* sp. (nak 8, ca. 42% elimination).

All the selected strains were either freshwater or terrestrial strains. This is apt because of the competitive inhibition of the stable elements in the seawater medium, which contained 10, 10 mM, and 0.5 μ M of potassium, calcium, and iodine, respectively [48, 49]. Likewise, for heterotrophic algae, the absorption and elimination of radionuclides were inhibited by the potassium and calcium in the yeast extract used for the medium (data not shown). Interestingly, the cyanobacterium *S. ocellatum* (NIES-2131) possessed a high eliminating activity for both Cs-137 and I-125. This may suggest that the decontamination of both elements occurs simultaneously. These data suggest that algae are a key organism for the phytoremediation of a polluted environment, especially for water polluted by radioactive cesium.

4.3 High-cesium bioaccumulating alga *V. crystalliferum* and *Galdieria sulphuraria*

The high-cesium bioaccumulating alga *V. crystalliferum* was isolated and recently identified as a new species and genus [50]. At the cellular level, the cells of this alga possessed a typical structure, which is a large reddish globule and crystalline. The uptake of Cs-137 was quite significant. The time course of Cs-137 elimination from the medium is shown in **Figure 7**. Over 60% of radioactive cesium was eliminated within 15 min and 90% within 1 hour. The maximum velocity for uptake was calculated as 63 mg Cs-137 mg^{-1} Chl h^{-1} . It has been reported that sunflowers and the vetiver *Vetiveria zizanioides* absorbed 150 μ g and 61% of cesium after 100 and

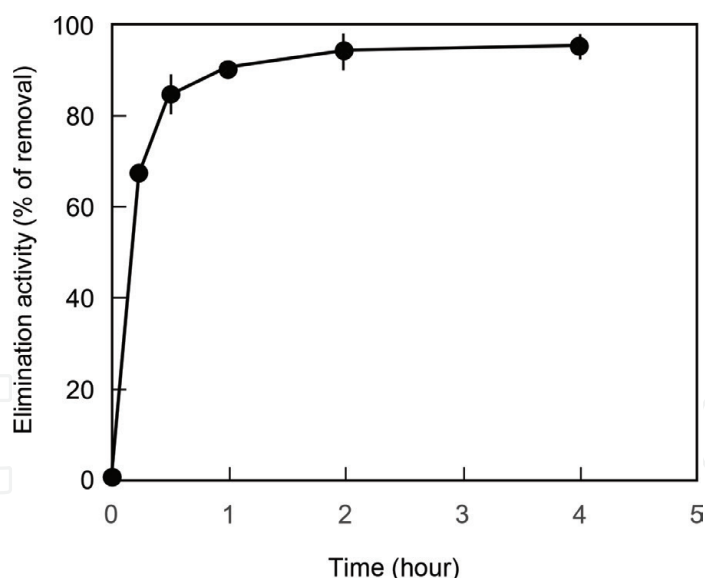


Figure 7.
Elimination of Cs-137 from the medium by *V. crystalliferum*.

168 hours, respectively [37, 51]. The partition coefficient (K_d) was calculated at around $4 \times 10^5 \text{ L kg}^{-1}$. This value was higher than the value of cesium absorption by zeolite and Prussian blue, the ferrocyanide pigment [10]. Therefore, this alga could be used for the effective elimination and absorption of radioactive cesium. Another advantage was in the physiological toughness of the cell. It can be grown in severe environmental conditions, such as high osmotic pressure, dim light, and high and low temperatures, because it was originally isolated from sediment found in a bottle of glue [10, 50]. As such, the use of this organism could help to reduce the cost of phytoremediation for decontamination of radioactive cesium because the energy needed to maintain the culture conditions of this strain is minimal.

In addition to *V. crystalliferum*, Fukuda and his coresearchers found that an extremophilic unicellular red alga *Galdieria sulphuraria* eliminated ca. 50% of cesium in the medium in 10 days [52]. Interestingly, the elimination did not take

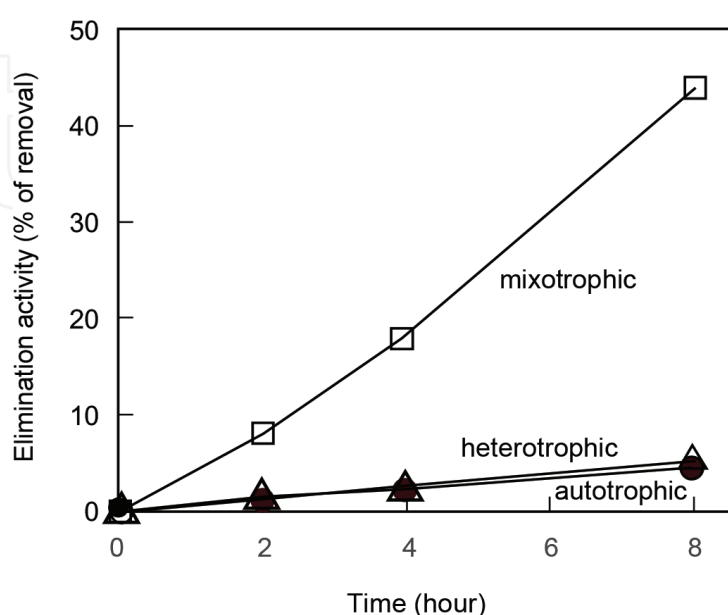


Figure 8.
Elimination of Cs-137 from the medium by *G. sulphuraria*. Cells were incubated without 25 mM glucose under light condition (autotrophic condition, closed circle), with 25 mM glucose under light condition (mixotrophic condition, open square), or with 25 mM glucose under dark condition (heterotrophic condition, open triangle).

place under autotrophic and heterotrophic conditions but only under mixotrophic conditions (**Figure 8**). Even though the elimination velocity was lower than that of *V. crystalliferum*, this alga may be useful for acidic contaminated polluted water because this alga prefers acidic conditions below pH 4.0.

5. Conclusion


In this chapter, we introduced a biological mechanism for the uptake of cesium, strontium, and iodine. Phytoremediation is a method that utilizes biological functions such as substance accumulation by biological concentration and biodegradation. Screening for a hyperaccumulator was carried out to apply phytoremediation technology in the decontamination of polluted land and water by radioactive substances released by the F1NPP accident. Global and secondary screening was used to identify several candidates of algae and aquatic plants for the phytoremediation and elimination of cesium, strontium, and iodine, respectively. In particular for cesium decontamination, the high-cesium bioaccumulating alga, novel eustigmatophytes *V. crystalliferum*, and an extremophilic unicellular red alga, *G. sulphuraria*, were found to possess high activity for elimination. Notably, the *V. crystalliferum* activity was particularly high. This suggests that these algae possess a specific transport system for cesium. One alternative could be to identify the transport system and introduce it into another plant or organism. In addition to the high activity, both algae are able to grow in severe environmental conditions, which is one of the required characteristics for phytoremediation. However, the decontamination of soil polluted by radioactive cesium would require more than only phytoremediation technology since the radioactive cesium found in and around the Fukushima area is firmly bound to the soil [53]. Therefore, further developments in the technology for the removal and solubilization of radioactive cesium in soils are essential. For this, the collaboration between scientists in different scientific disciplines is essential.

Author details

Koji Iwamoto* and Ayumi Minoda
Malaysia Japan International Institute of Technology, University Technology
Malaysia, Kuala Lumpur, Malaysia

*Address all correspondence to: k.iwamoto@utm.my

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