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Development of Plant for Practical Cd Phytoextraction on Agricultural Land

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Abstract

This report shows, the process of developing a phytotechnology-based countermeasure to lower the risk of cadmium in the agricultural land.

The three hazardous substances, cadmium, copper, and arsenic had been determined in Japanese act for an agricultural land.

The risk management for the contaminated agricultural land requires the widely applicable countermeasures including continuous implementation of the existing countermeasures, e.g. soil dressing, well controlled irrigation procedure, maintaining adequate soil pH, and additional introduction of technique recently developed to reduce the concentration of the hazardous sub-stances.

In Japan, the phytoextraction of Cd had been developed as phytotechnology could to meet to farmer's benefit. There are two strategies for the Cd phytoextraction to be developed that the utilization of existing crop cultivar accumulating relatively high Cd or the newly seeking a plant species hyper-accumulating Cd.

In this report, the actual development processes according to these strategies are introduced, that the cultivation method establishment of the Cd hyperaccumulator,.*Arabidopsis helleri* ssp. *gemmifera*. The efficacy criterion of plant selection for the phytoextraction purpose is not only the tolerance to toxic heavy metals, but also the high biomass production to ensure the total removal of metals. Basically, the hyperacumulators are the wild plants living in a natural habitat, thus the practices of utilization must include a process for the domestication of the hyperaccumulators.

New line of *A. h. gemmifera*, named as 'AHG347 winter clean, had been registered by Ministry of Agriculture, Forestry and Fisheries Japan

Keywords: Contaminated agricultural land, Phytoextraction using accumulator plant species, Domestication of wild plants, *Arabidopsis halleri* ssp. *gemmifera* of hyperaccumulator

§1. Introduction: Soil contamination in agricultural land under Japanese regulation

Under the Japanese act, the Act to Prevent Soil Contamination on Agricultural Land (1971, hereafter called as “Prevention Act”), the three hazardous substances, cadmium (Cd), copper (Cu), and arsenic (As), are determined for an agricultural land. The regulatory standards of which listed as the Environmental Quality Standards for Soil Pollution for each substance are the followings:

Cd: less than 0.4 mg kg-1 in rice for agricultural land
Cu: less than 125 mg kg-1 for agricultural land (paddy field only)
As: less than 15 mg kg-1 for agricultural land (paddy field only)

Under the Prevention Act, the designation requirements for the agricultural land are defined for each hazardous substance, as follows:

Cd: An area of which produce a rice exceeding the standard value in cadmium content deter-mined by an acid digestion. Additionally, the nearby area is also designated as the area to be suspected producing the substandard rice, the relevant requirements should be considered that combine Cd content in soil (0.1M HCl extractable) with soil texture for this additional designation.

Cu: An area that a copper content in soil (0.1M HCl extractable) is equal to or more than the standard value.

As: An area that a copper content in soil (1M HCl extractable) is equal to or more than the standard value.

It can be understood that for the Japanese standard of Cd to be determined as a Cd content in rice, the standard should be considered in view of a human health risk caused by Cd intake through a food chain. Then, the designation requirements for Cd would be unavoidable to ambiguously determining due to a Cd content in rice varies depending on the complicated factors (e.g. Cd content in soil, soil pH, chemical forms of Cd in soil, redox condition of soil etc.). Whereas, the other two standards, for Cu and As, had been examined in terms of preventing the decrease of crop production influenced by the elements in soil with the toxic levels.

It would be said that the difference of the criteria reflects many designation cases for Cd according to the survey results of the Ministry of the Environment Japan. The wastewater and smoke discharged from the metal mine and/or smelter processes are the major Cd pollution causes on the agricultural lands. In actual, the publicized handout at fiscal year 2007 by the Ministry of the Environment shows 88.4% (5651.9 ha) of the cumulative areas designated requiring the countermeasures had been impacted by discharge from the metal mine and/or smelter processes [1].

Among these three hazardous substances, as specified by the Act, Cd is different from other two elements that the Cd pollution in the agricultural land was realized from beginning of issue as the hazard risk of an intake of staple food. In general, Cd may be the element of which tends to be relatively easier accumulated in plant body from the media regarding a ratio of Cd concentration in plant to its concentration in soil [2].

The peoples who, like Japanese, eat rice as their staple diet that Cd-induced risk in rice should have been great concern for all persons on a food-chain including a farmer, distributor, and consumer. The Japanese government, the Ministry of Agriculture, Forestry and Fisheries (2016), had implemented the monitoring for the Cd concentrations in domestically produced crops from fiscal year 2009 to

2014. The purpose of this monitoring to verify the effectiveness of means for reducing the Cd risk in farm produce, that to be assessed by comparison with previous survey results of Cd in crops. This latest monitoring results publicized at February 2016, concluded that the health risks induced by dietary intake of Cd have been decreased with reducing the Cd concentrations in crops as low as no practical impact under ordinary eating habits [3].

Whereas, for the farmer working on the moderately contaminated agricultural land, the possibility would remain that of crop production with relatively higher concentrations of the hazardous substances potentially or incidentally.

The risk management for the moderately contaminated agricultural land needs the widely applicable countermeasures including continuous implementation of the existing countermeasures, e.g. soil dressing, well controlled irrigation procedure, maintaining adequate soil pH, and additional introduction of technique reducing the concentration of the hazardous substances.

The agricultural lands are places basically to be developed for plant production. Thus, using plants technology should be reasonably practicable for the rehabilitation of the mine-impacted agricultural land.

This report reviews mainly the developed phytotechnologies reducing Cd risk to be most significant issue on the agricultural land in Japan.

§2. Strategy of reducing Cd risk; Phytoextraction removing Cd from soil

The basic concept is clear that for the recovery procedure for the Cd contaminated agricultural lands to be successful for all participants, a farmer's benefit should be considered primarily. The purposely designed methods to recover must be practicable for farmer, i.e., restarting easier a crop production or reducing risk to be promoted simultaneously with a crop production. Thus, the development of using plants technology for this purpose, two strategies can be driven that removing the specified substances from soils and causing decreased absorption into crops. The former for-instance appears that as the phytoextraction is assumed adaptable technique for the purpose, that the hazardous substances are removed by plant functions of which absorption from soil and accumulation in plant body. Then, the latter include the means of biological and physicochemical methodologies that choosing or breeding the low absorption crop varieties, optimizing soil properties to restrict the absorption, i.e., soil pH, water condition.

§3. Phytoextraction for agricultural land

As is emphasized by Ebbs et al. [4], the requisites of phytoextraction procedure to be successful that should be the rapid identification of the effective plant species and establishment the agronomic practices increasing metal uptake and accumulation into plant body. This concept leads two policies to develop the phytoextraction procedure (Figure 1), that the utilization of existing crop with the ability to accumulate metals and the finding a favorable wild plant species with the ability to produce high biomass.

Table 1 shows the Cd removal on field trials of phytoextraction researches in Japan. These results are selected from which the reports describe the basic data set, Cd removal, soil Cd concentration, and shoot Cd concentration, directly in its tables or text. Basically, it is difficult to evaluate the performance of plant species comparing solely the Cd removals between the experiments under different conditions. Then a calculation of ratio of Cd concentrations in shoot to its in soil should be helpful index to represent an effectiveness of accumulation comparing the results in combination with the Cd removals.

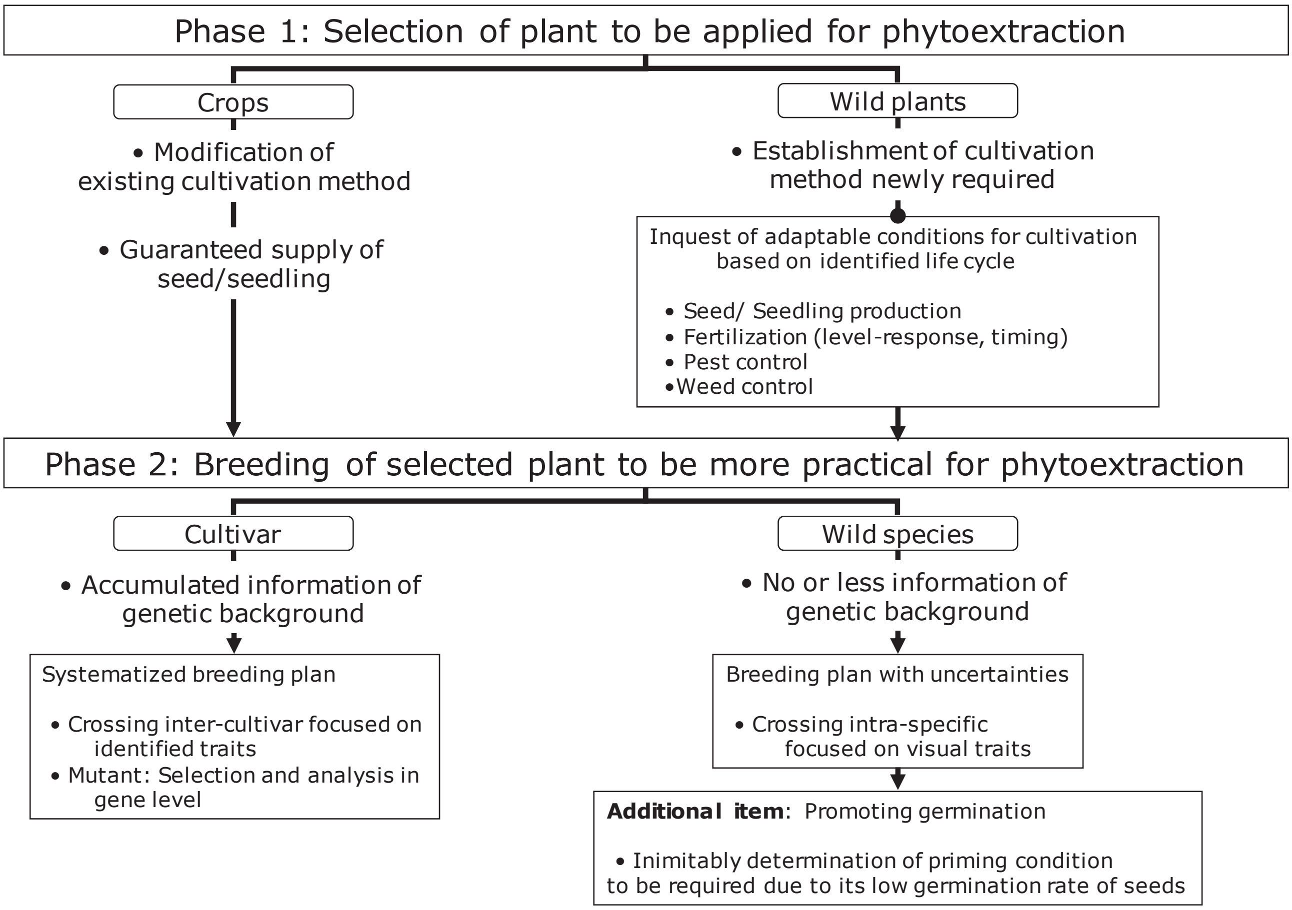

Figure 1. Two strategies for development of plant for phytoextraction

Table 1. Cd removal on field trials of phytoextraction researchers in Japan

	Plant species		Cd removal	Soil Cd concentration	Shoot Cd concentration	Shoot to soil ratio	References
	Scientific name	Cultivar name	g ha^{-1}	mg kg^{-1} DW	mg kg^{-1} DW		
Crop	*Oriza sativa*	MORETSU	516	2.91	40.0	13.7	[5]
		IR-8	657	2.52	49.7	19.7	[5]
		Chokoukoku	377	0.67	33.7	50.3	[6]
		Chokoukoku*	204	0.66	28.6	43.3	[7]
		IR-8*	70	0.69	6.7	9.7	[7]
		Chokoukoku	153	0.64	13.5	21.1	[7]
		IR-8	63	0.76	7.0	9.2	[7]
Wild plant	*Hibiscus moscheutos*	-	347	2.1	9.6	4.6	[8]
	Crassocephalum crepidioides	-	184	2.9	28.6	9.9	[9]
	Solidago altissima	-	2820	30.1	14.5	0.5	[10]
			3687	4.9	18.0	3.7	[10]
	Arabidopsis halleri ssp. *gemmifera*	-	1408	3.1	354	114.2	[11]
			259	1.2	91	75.8	[11]
			1560	3.6	421	118.6	unpublished
			3000	3.5	908	263.2	unpublished

* upland condition
For a multi-year experiments, the maximum Cd removal is trascribed. The soil Cd concentrations are 0.1M HCl extractable.

In the studies using crop, the experiments conducted with rice plant have a predominantly high number of study case, inevitably have been carried out an inter cultivar evaluation of performances. Japanese researchers who oriented in the rice plant utilization for Cd phytoextraction illustrate the advantages to meet appreciably the requirements of which Ibaraki and Taniguchi [12] pointed, as followings:

- Stability of transplanting cultivation
- The consistent cultivation method had been established with increased mechanization.
- Most of the farmers are proficient in rice cultivation i.e. without hesitation to cultivate.

In actual, as shown in Table 1 rice cultivar like 'Chokoukoku' with high Cd accumulation ability must be fit to practical phytoextraction for the agricultural lands.

On the other hand, using wild plant trials, the amount of Cd removal achieved by *Solidago altissima* cultivation is extremely high. However, the Cd concentrations in shoot and its ratios shoot to root represent clearly that the large amount of Cd removal is brought by remarkably high biomass production that makes post-harvest process difficult to collect and treat plenty of plant residue.

Arabidopsis halleri ssp. *gemmifera* performed high amount of Cd removal as shown in Table 1, would be considered plant species to be ideal for phytoextraction that is known as a hyperaccumulator of Cd and Zn [11], [13], [14]. Hyperaccumulator plants have peculiarly high ability to absorb and storage toxic heavy metal in its body. The amount of the hazardous substrate removal of phytoextraction is expressed as the multiplication of a plant biomass by the hazardous substrate concentration of plant body. Thus, the primal benefit is that for phytoextraction which used hyperaccumulator performed likewise for *A. h. gemmifera*, lesser plant residual can bring out bigger hazardous risk from the agricultural production.

The development for wild plants requires a huge effort to establish the cultivation method maximizing the biomass production. Meanwhile, if it has been achieved, the results of field trials utilizing *A. h. gemmifera* mean that the hyperaccumulators can became strong candidates for the practical phytoextraction.

§4. Development of wild plants for practical Cd phytoextraction

4.1 Established cultivation method for *Arabidopsis halleri* ssp. *gemmifera*

As described above, the utilizing wild plants species needs a huge effort to establish the cultivation method to domesticate wild plants for phytoextraction procedure. Then, the behavior of that species growing in the natural habitat should be observed and used as a reference to build a cultivation schedule.

Arabidopsis halleri ssp. *gemmifera*, a member of the Brassicaceae, is perennial, wintering ability herbal plant living in Russian Far East, north-eastern China, Korea, and Japan.

A. h. gemmifera spends the winter season in the form of rosette, then moves into bolting and flowering phase at the next spring season. Thus, the basic cropping pattern was built up to be started from midsummer or early autumn then harvested by the end of flowering season, typically by the middle of May.

Transplanting of seedling was selected as the start of cultivation for *A. h. gemmifera* due to its low germination rate being inherent in wild plants. The usual germination rate of its seed is of around 30% by two weeks on wetted filter paper and is not practically high to start the cultivation with direct seed sowing on a field. The systematic way was employed that produce plug-shaped seedlings grown on the standardized plastic tray for approx. 45 days growing.

The response to fertilization of *A. h. gemmifera* indicates that the favorable nitrogen application level is 15 to 20 gN per m^2 within the cropping period using ordinal chemical fertilizer, then the density of cropping is standardized on 16 to 20 plants per m^2.

An insecticide treatment for seedlings just before transplanting would be effective not only avoiding an insect damage but also labor-saving. For the weed control using the selective herbicide to Poaceae, is predictably effective that combines a soil-applied herbicide prior cropping with a foliage treatment herbicide for early spring.

4.2 Selection and crossing for *A. h. gemmifera* to be uniformed

Non-uniformity among the individuals must be great concern of wild plants to be utilized commercial basis. The policy for *A. h. gemmifera* to be uniformed was built upon the selection focused on visually apparent traits and then observation of reproducibility after crossing due to no information of genetic background. The processes of selection and crossing were as follows;

・ Selection of relatively bigger individuals from community of over a thousand of individuals cultivated on the same field conditions

・ Secondary selection for a hundred individuals to identity the line with bigger size and surviving through summer season

・ Selected ten lines were tested to certify the reproducibility of visual property focused on

・Crossing and observation of uniformity for the combinations of selected three lines

Figure 2 and 3 show the process and result of selection and crossing for *A. h. gemmifera* to be uniformed, respectively. New line of *A. h. gemmifera*, named as ‘AHG347 winter clean, had been registered by Ministry of Agriculture, Forestry and Fisheries Japan.

4.3 Treatment for promoting germination

For the purpose of more labor-saving, *A. h. gemmifera* cultivation starting directly sowing of seed should be meet to former’s desirability. However, inherent germination rate must be too low for this purpose. Then, the conditions of priming treatment had to have been investigated for seeds of bred *A. h. gemmifera* line. It has been found that the priming treatment in an original way should be effective but the protocol for priming must be modified appropriately for each lot of seed production (Figure 4).

The germination ability of primed seeds is certified as practical for the field trials, in fact the emergence of *A. h. gemmifera* could be observed at two weeks after sowing on the actual field as shown in Figure 5.

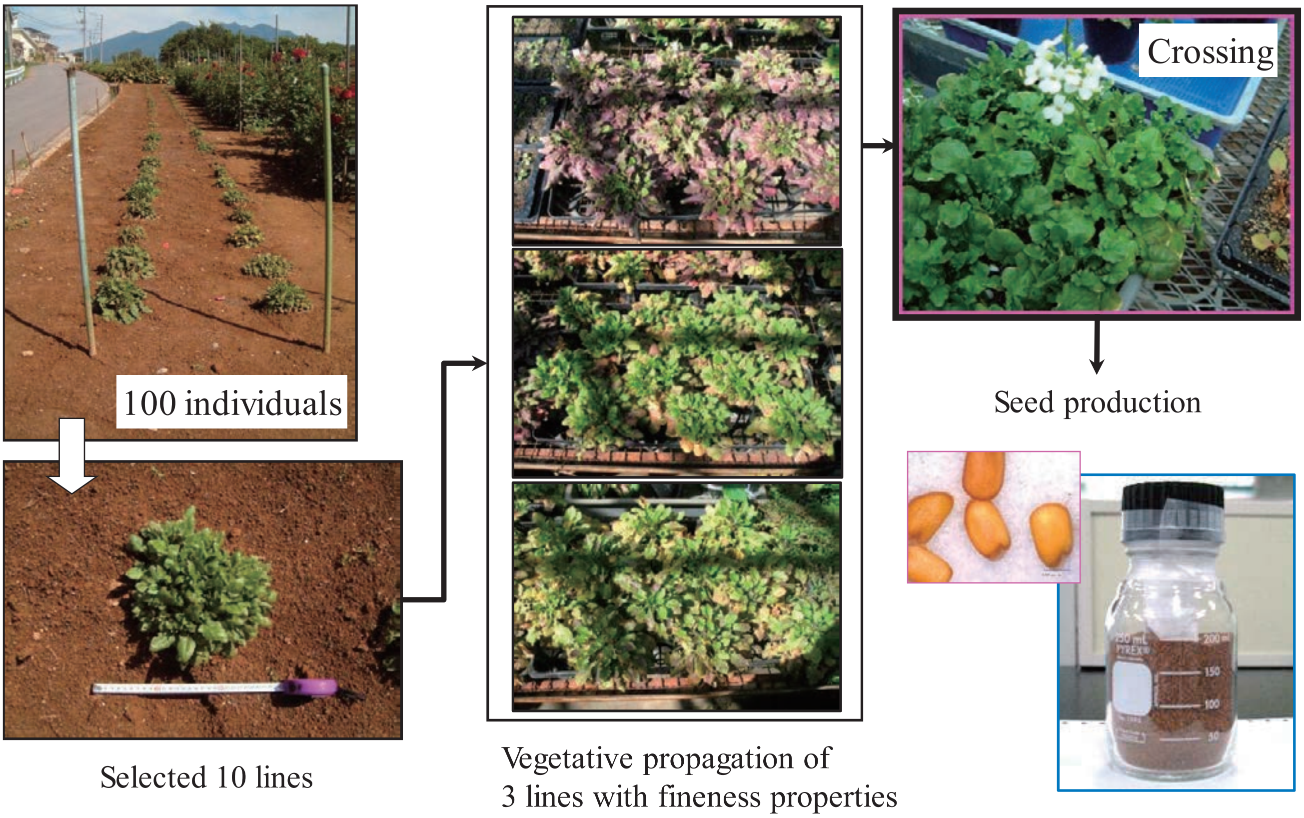

Figure 2. Breeding process of *A. h. gemmifera* to be uniformed

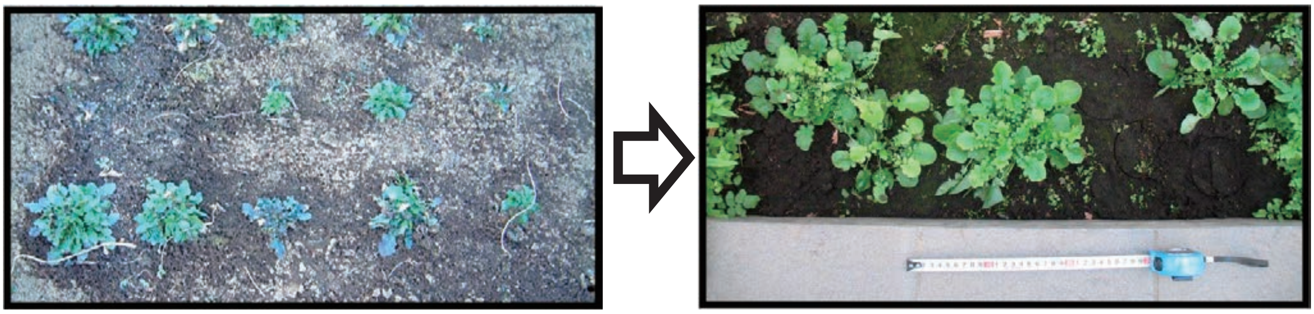

Figure 3. Improved uniformity of *A. h. gemmifera* , AHG347 winter clean (right), compared with inbred lines (left)

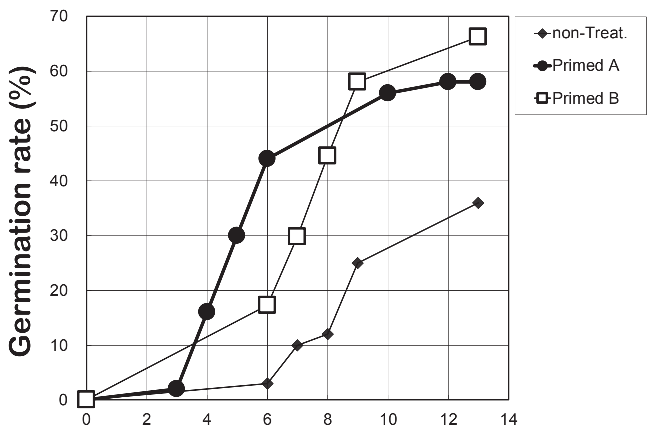

Figure 4. Effectiveness of priming treatment

Figure 5. Emergence of primed seed on the actual contaminated field, 2 weeks after sowing

§5. Overview

The status of pollution in the agricultural lands had been monitoring and controlling under Japanese regulation. Whereas, the possibility would remain that of crop production with relatively higher concentrations of the hazardous substances.

The efforts of the people involved in this issue maintain lowering the risks of hazardous substances. The phytoextraction, one of the such trials, based on plant-technologies could provide an agricultural solution for the industrial generation source of pollution. Then, the countermeasures developed are resource should to be taken depending on its features. If the phytoextraction is applied for the contaminated agricultural lands in accord with the crop production, it brings the sustainable and safe crop production.

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Short comment

The developed plant for practical phytoextraction on the agricultural land, had been registered as a variety of "AHG347 winter clean". It means symbolic milestone of the domestication of wild plant to be completed, and also of the product commercialization.