

Selenium Biofortification and Phytoremediation Phytotechnologies: A Review

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Abstract

The element selenium (Se) is both essential and toxic for most life forms, with a narrow margin between deficiency and toxicity. Phytotechnologies using plants and their associated microbes can address both of these problems. To prevent Se toxicity due to excess environmental Se, plants may be used to phytoremediate Se from soil or water. To alleviate Se deficiency in humans or livestock, crops may be biofortified with Se. These two technologies may also be combined: Se-enriched plant material from phytoremediation could be used as green fertilizer or as fortified food. Plants may also be used to “mine” Se from seleniferous soils. The efficiency of Se phytoremediation and biofortification may be further optimized. Research in the past decades has provided a wealth of knowledge regarding the mechanisms by which plants take up, metabolize, accumulate, and volatilize Se and the role plant-associated microbes play in these processes. Furthermore, ecological studies have revealed important effects of plant Se on interactions with herbivores, detritivores, pollinators, neighboring vegetation, and the plant microbiome. All this knowledge can be exploited in phytotechnology programs to optimize plant Se accumulation, transformation, volatilization, and/or tolerance via plant breeding, genetic engineering, and tailored agronomic practices.

Core Ideas

- Plants may be used to clean up excess selenium from the environment.
- Plants may be used to provide dietary selenium in selenium-deficient areas.
- Plants may be used to mine Se from seleniferous soil.

THE PURPOSE of this review is to give an overview of the use of plants (phytotechnologies) to alleviate worldwide problems associated with selenium (Se) deficiency and toxicity. Selenium is an essential element for many species, including humans, but it is toxic at higher levels. The window between Se deficiency and toxicity is very narrow (about one order of magnitude); hence, both Se deficiency and toxicity are problems worldwide (Lyons et al., 2003; Stadtman, 1990). Higher plants do not require Se but readily take it up due to its similarity to sulfur (S) (Anderson, 1993). Although Se accumulation can negatively affect plants, leading to chlorosis and stunted growth, low levels of Se can promote plant growth and stress resistance (Hartikainen, 2005; Pilon-Smits et al., 2009).

Various phytotechnologies make use of the propensity of plants to accumulate Se (Zhao and McGrath, 2009). Plants and their associated microbes may be used to remove excess Se from naturally seleniferous soil or from Se-polluted water or soil (phytoremediation). Because Se is also an essential nutrient, Se-enriched plant material may be considered biofortified food and may be used to alleviate Se deficiency in low-Se areas (Bañuelos and Dhillon, 2011a).

Selenium deficiency has been estimated to affect a billion people worldwide in areas where soil Se levels are naturally low (Lyons et al., 2003), including areas in China, New Zealand, Australia, Africa, and Europe (Oldfield, 2002). Humans and other mammals require Se and incorporate it as selenocysteine (SeCys) in essential selenoproteins (25 in humans), which are involved in detoxifying free radicals (potentially preventing cancer), immune response (disease resistance), thyroid activity, and male fertility (Rayman, 2012). Other areas of the world are Se rich, which can give rise to Se toxicity in livestock and humans; these areas include parts of North America, China, and India (Oldfield, 2002). Naturally Se-rich (seleniferous) soils may contain vegetation that is toxic when ingested by grazers. Agricultural use of seleniferous soils or industrial use of seleniferous fossil fuels can accelerate the release of Se into the environment, often causing toxicity, particularly when the Se gets concentrated by evapotranspiration (Terry et al., 2000).

Plants and their associated microbes may be used in aquatic or terrestrial settings to clean up Se-polluted water or soil (Bañuelos

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Abbreviations: APS, adenosine 5'-phosphosulfate; APSe, adenosine 5'-selenate; ATPS, ATP sulfurylase; CGS, cystathionine-γ-synthase; DMDSe, dimethyldiselenide; DMSe, dimethylselenide; DW, dry weight; GLS, glucosinolate; SeCys, selenocysteine; SMT, selenocysteine methyltransferase; SeMet, selenomethionine.

and Dhillon, 2011a; Zhao and McGrath, 2009). Plants use S transporters and metabolic pathways to take up inorganic selenate and selenite and assimilate them into organic SeCys and selenomethionine (SeMet), which may be methylated and stored as methyl-SeCys or methyl-SeMet or further converted into volatile dimethylselenide (DMSe) or dimethyldiselenide (DMDSe) (Anderson, 1993; Shrift, 1969; Sors et al., 2005). When seleno-amino acids nonspecifically get incorporated into proteins, replacing Cys and Met, this results in toxicity because it disrupts protein function (Stadtman, 1990). Inorganic Se oxyanions can also cause toxicity via oxidative stress (Van Hoewyk, 2013). The methylation of seleno-amino acids and Se volatilization may constitute plant mechanisms to prevent Se toxicity. These same mechanisms are found in many bacteria and fungi; in addition, many microbes can reduce selenite to insoluble elemental Se as a detoxification mechanism (Stolz et al., 2006). Different plant species show different levels of Se tolerance, transformation, sequestration, and volatilization and harbor different microbial communities (Sura de Jong et al., 2015; White et al., 2007; Zayed and Terry, 1992). Broad insight into these processes is vital for the optimization of phytotechnology applications. Efficient utilization of the combined plant and microbial processes allows plant-based systems to remove Se from polluted substrates via precipitation in sediments, accumulation in plant tissues, or volatilization (Terry et al., 2000). Volatilized Se has been modeled to precipitate after several days in the atmosphere and can thus constitute a Se input in downwind areas (Blazina et al., 2014). Selenium accumulation in vegetation constitutes a portal for Se into the food chain and may also influence Se cycling (Winkel et al., 2015). When planning large-scale Se phytotechnology projects, ecological effects like these should be taken into consideration.

Se Effects on Ecological Interactions

Several ecological studies have investigated the effects of plant Se accumulation on plant–herbivore, plant–pollinator, plant–plant, and plant–microbe interactions (for a review, see El Mehdaoui and Pilon-Smits [2012]). Selenium-supplied plants were shown to be protected against a wide variety of generalist herbivores with different feeding modes, in some cases already at tissue Se levels below 50 mg Se kg⁻¹ dry weight (DW) (Freeman et al., 2007, 2009; Hanson et al., 2003, 2004; Quinn et al., 2008, 2010a; Vickerman et al., 2002). In agreement with a protective effect of Se, high-Se plants were found to contain a lower invertebrate load in the field and exhibited less herbivory damage (El Mehdaoui et al., 2011a; Galeas et al., 2008). A practical implication of this finding is that high-Se crops such as those used in Se phytoremediation likely have less need for pesticides. Naturally seleniferous habitats, however, contain Se-resistant herbivores that are not deterred by high-Se plants and can withstand high tissue Se accumulation (Freeman et al., 2006a, 2012; Valdez Barillas et al., 2012). A practical implication is that these herbivores may reduce the yield of high-Se crops and form a portal for Se movement up the food chain.

High Se levels in flowers did not affect pollinator visitation, and honey bees and bumble bees were found to carry Se-rich pollen in their pollen baskets and to incorporate Se into their tissues (Quinn et al., 2011). Hladun et al. (2012) reported that there may be a negative effect of Se ingestion on honey bees, depending

on the form of Se. Similar to Quinn et al. (2011), Hladun et al. (2012) found that the bees did not avoid Se-containing food resources, suggesting that Se ingestion by honey bees is likely to happen in fields with Se-rich plants. A practical implication is that the effect on bee health is important to take into account before growing large-scale high-Se crops.

Plants that transform inorganic Se to organic Se and then deposit their leaf litter may cause their surrounding soil to become enriched in Se and to contain a relatively higher fraction of organic Se. This may lead to enhanced Se accumulation in neighboring vegetation and can perhaps also alter the form of Se (El Mehdaoui et al., 2011a,b, 2015). A practical implication is that strategic co-cropping or intercropping may be a way to boost Se accumulation in crops. If crops could also be manipulated to accumulate more organic Se, this would be of interest for bio-fortification because organic selenocompounds are considered healthier for consumers (Rayman, 2012).

Selenium supplementation was shown to protect plants from pathogenic fungi (Hanson et al., 2003), suggesting high-Se crops may require less fungicide. However, in naturally seleniferous areas there are fungal pathogens that can thrive on high-Se plants. Also, a range of endophytic and rhizosphere fungi and bacteria were found to live in association with high-Se plant species (Lindblom et al., 2013; Sura-de Jong et al., 2015; Valdez Barillas et al., 2012; Wangeline et al., 2011). Thus, plant Se accumulation does not appear to impair the associations of plants with beneficial fungi and bacteria; the decomposition of plant litter by micro-arthropods and microbial detritivores was also not negatively affected by high Se content (Quinn et al., 2010b).

What Can Plants Do with Se?

To optimize plant Se accumulation, transformation, volatilization, and/or tolerance in various phytotechnologies, it is helpful to understand the molecular mechanisms through which plants take up, transform, accumulate, and tolerate Se. The current knowledge of these processes is summarized below.

Plants absorb Se primarily as selenate (SeO₄²⁻) or selenite (SeO₃²⁻), which are the two predominant bioavailable forms of Se in natural oxic and anoxic environments, respectively (White et al., 2007). Selenate is generally more soluble and bioavailable than selenite (Fordyce, 2012; Mikkelsen et al., 1989). The uptake of selenate and selenite into plants is mediated by active transport mechanisms (Hawkesford et al., 1993; Lass and Ullrich-Eberius, 1984; Li et al., 2008; Sors et al., 2005; Terry et al., 2000; Zhao et al., 2010; Zhang et al., 2014). Specifically, selenite uses phosphate carriers (Hopper and Parker, 1999; Li et al., 2008; Zhang et al., 2014) and aquaporins (Zhao et al., 2010) to enter the plants, whereas selenate movement throughout the plant involves the activity of sulfate transporters (Shinmachi et al., 2010; Sors et al., 2005; Terry et al., 2000; White et al., 2004, 2007). In addition to Se oxyanions, plants can take up organic Se compounds such as SeCys and SeMet by means of amino acid permeases (White and Broadley, 2009). The uptake rate of these amino acids was shown to be much higher than that of selenate or selenite in various plant species (Kikkert and Berkelaar, 2013; Zayed and Terry, 1992).

Because Se shares high chemical similarity with S, selenate can access the sulfate assimilation pathway to be assimilated into the Se-amino acids SeCys and SeMet (Sors et al., 2005). Initially,

selenate is activated by the enzyme ATP sulfurylase (ATPS), which catalyzes the conversion of sulfate/selenate into adenosine 5'-phosphosulfate/selenate (APS/APSe) (Leustek, 1994; Sors et al., 2005). This step has been identified as rate-limiting Se accumulation and tolerance because transgenic ATPS-overexpressing *Brassica juncea* plants supplied with selenate accumulated more Se in organic form compared with the wild type and were also more tolerant to Se (Pilon-Smits et al., 1999). In the field, these transgenic APS plants were four to five times more efficient in taking up Se from contaminated sediments (Bañuelos et al., 2005).

The APSe produced by the activity of ATPS can be further reduced to selenite in a reaction promoted by APS reductase (Anderson, 1993). This enzyme was shown to play a pivotal role in the control of selenate assimilation, as inferred from studies using *Arabidopsis thaliana* transgenics overexpressing APS reductase, where the enhancement of both Se flux through the plant and selenate reduction into organic forms were observed (Sors et al., 2005; Suter et al., 2000).

Selenite can be further converted to selenide (Se^{2-}) by the enzyme sulfite reductase or non-enzymatically after the interaction between selenite and reduced glutathione (Anderson, 1993; Terry et al., 2000; White, 2016). Selenide can be coupled to O-acetylserine, forming SeCys; this step is mediated by the enzyme O-acetylserine thiol lyase (Sors et al., 2005; Terry et al., 2000). The formation of O-acetylserine is mediated by serine acetyltransferase.

The enzyme Cys desulfurase, which liberates elemental S from Cys for the formation of iron-sulfur clusters, can also function as SeCys lyase, releasing elemental Se from SeCys (Van Hoewyk et al., 2005). Overexpression of this enzyme led to a significant decrease of Se misincorporation into proteins and enhanced Se tolerance and accumulation (Van Hoewyk et al., 2005). The transgenic SeCys lyase plants also accumulated more Se in a field phytoremediation experiment (Bañuelos et al., 2007).

The amino acid SeCys may also be converted to SeMet in a three-step process. Initially, SeCys is converted to Se-cystathionine by the activity of cystathionine- γ -synthase (CGS) (Pilon-Smits, 2012; Sors et al., 2005). This reaction is reported as a rate-limiting step for the conversion of SeCys to volatile DMSe (Van Huysen et al., 2003). *Brassica juncea* transgenics overexpressing CGS showed elevated rates of Se volatilization, reduced Se accumulation in plant tissues, and improved Se tolerance compared with wild-type tissues (Van Huysen et al., 2003, 2004). Selenium-cystathionine can be converted to Se-homocysteine via cystathionine- β -lyase and then transformed to SeMet in a reaction catalyzed by methionine synthase (Cossins and Chen, 1998). Selenomethionine is a precursor for volatilization of Se in the form of DMSe (Terry et al., 2000). Some plant species also methylate SeCys, which may be stored or further converted to DMDSe (Terry et al., 2000).

A special category of plant species are the so-called Se hyperaccumulator plants, which accumulate Se to levels two orders of magnitude higher than surrounding vegetation on seleniferous soils and specifically accumulate Se over S (Beath et al., 1939; Cappa and Pilon-Smits, 2014; White et al., 2007). Around 50 species of Se hyperaccumulators have been described, mostly from the Fabaceae (in the genus *Astragalus*), Asteraceae (genera *Xylorhiza*, *Oonopsis*, *Symphyotrichum*), and Brassicaceae (genus *Stanleya*), natives to seleniferous North American areas. Selenium hyperaccumulators sequester Se in all plant organs at

levels 0.1 to 1.5% of dry weight, mainly in the form of methyl-SeCys (Freeman et al., 2006b). This nonprotein amino acid is produced by the enzyme SeCys methyltransferase (SMT). Hyperaccumulators are acutely toxic to herbivores and likely sequester Se as a protection from biotic stresses (El Mehdawi and Pilon-Smits, 2012). The unique properties of these wild Se hyperaccumulator species make them (or their genes) interesting material for Se phytoremediation and biofortification.

Se Phytotechnologies: Phytoremediation, Phytomining, and Biofortification

Both Se toxicity and deficiency are problems for humans and other mammals worldwide, and plants may be used to alleviate both, as illustrated in Fig. 1. Plants may be used to remove excess Se from soil or water, thus preventing Se toxicity to affected life forms (Terry et al., 2000; Zhu et al., 2009). To battle Se deficiency in susceptible human populations and livestock, biofortification can be performed where crops are enriched with Se either supplied in fertilizer or by growing crop plants on Se-rich soil (Bañuelos and Lin, 2009). Combining the two technologies, Se-enriched plant material from phytoremediation could be used as green fertilizer in biofortification practices (Bañuelos et al., 2015; Yasin et al., 2015a). If the Se levels are high enough, Se could also be mined from the harvested plant material, a process called “phytomining” or “agromining” (van der Ent et al., 2015). To optimize the efficacy of these phytotechnological applications, a broad understanding is needed of the physicochemical, biological, and ecological processes that affect soil Se bioavailability, plant uptake, organ distribution, and transformation. Using this knowledge, limiting factors can be pinpointed and targeted to enhance phytotechnology efficiency via classical plant breeding, genetic engineering, or management of agronomic practices (Pilon-Smits and LeDuc, 2009; Wu et al., 2015; Zhao and McGrath, 2009; Zhu et al., 2009).

Phytoremediation Studies: Terrestrial Systems

Certain soils defined as “seleniferous” can contain up to 100 mg kg^{-1} Se. When these soils are used for cultivation of crops or when fossil fuels from seleniferous areas are used, Se may accumulate in the environment to levels that are toxic to organisms (Terry et al., 2000). As an example of Se toxicity, in the district of Enshi in Hubei Province, China, soils are extremely rich in Se, and humans and animals frequently experience toxicity symptoms (Fordyce et al., 2000). Some of the most common toxic symptoms include hair loss and nail deformation, damage to the nervous system, and cardiovascular disorders (Li et al., 2012; Wilber, 1980). A well-known case of selenosis in the United States was due to the high levels of Se in the subsurface agricultural drainage water conveyed to the Kesterson reservoir by the San Luis Drain (Ohlendorf et al., 1990). This phenomenon led to extensive studies on the behavior, transformation, and fate of Se in aquatic and terrestrial ecosystems. At Kesterson, phytoremediation approaches were used to prevent the movement of soluble Se forms from irrigated areas with seleniferous soils and to dissipate already accumulated Se through plant bioaccumulation and volatilization (Bañuelos and Meek, 1990). Similar

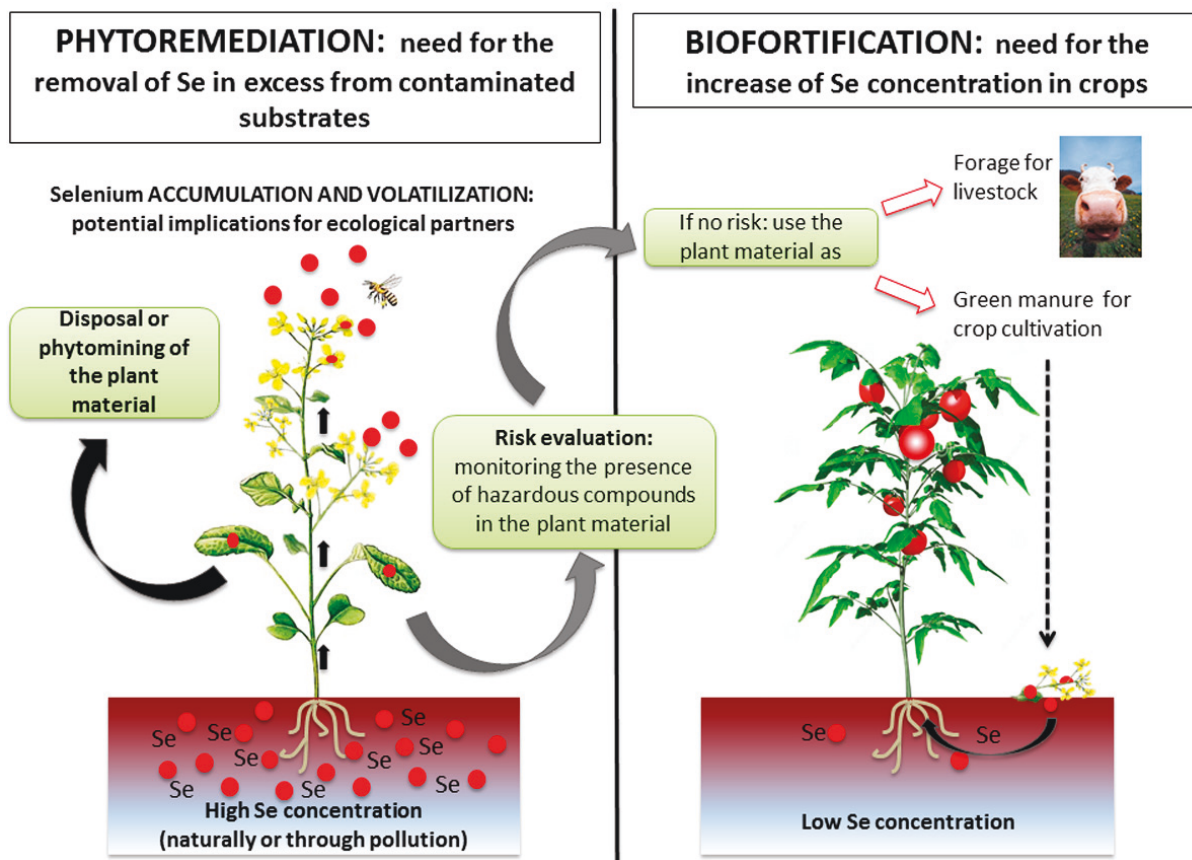


Fig. 1. Phytotechnologies may phytoremediate Se in areas where it is present in excess, either naturally or due to pollution (left side), or may provide Se-enriched plant material to alleviate Se deficiency in low-Se areas (right side). The two technologies may also be combined when Se-enriched plant material is transferred to a low-Se area. Another possible fate of high-Se plant material is phytomining, where the Se is recycled from harvested plant material. High-Se plant material will need to be carefully monitored to avoid negative ecological impact and toxicity to potential consumers.

phytotechnology measures are taken in other seleniferous areas in the western United States, for instance in Colorado and Idaho (Gunnison Basin Selenium Task Force, 2009).

Selenium contamination of sediments, soils, and drainage water frequently occurs in arid and semiarid seleniferous areas with intensive crop irrigation. In these cases, it is difficult and costly to remove Se via traditional physical and chemical techniques. Instead, plants can be used to remove Se from agricultural and industrial wastewaters and soils through less invasive and less expensive phytoremediation techniques (Bañuelos and Meek, 1990; Hansen et al., 1998; Lin and Terry, 2003; Pilon-Smits, 2005). Different plant processes may be exploited for the remediation of Se-contaminated soils. In phytoextraction, Se is absorbed by plant roots and translocated to the shoot, where it may be harvested and removed from the contaminated site. In phytovolatilization, Se can be completely removed from the site in volatile forms (DMSe/DMDSe) and released into the atmosphere (Wu, 2004).

Plant species of choice for Se phytoremediation may include terrestrial or aquatic species that grow well under the local conditions, are hardy and competitive, grow fast and produce much biomass, are tolerant to Se and good at accumulating or volatilizing Se, and ideally also have economic value. For terrestrial applications, *Brassica* species (e.g., Indian mustard [*Brassica juncea* L.] and canola [*Brassica napus* L.]) are very popular, and for aquatic applications cattail (*Typha angustifolia* L.) and bulrush (*Scirpus acutus* Muhl. ex Bigelow) have been used.

Although Se hyperaccumulator species are relatively slow growing, they exhibit high capacity to tolerate, take up, translocate to the shoot, and volatilize Se (Freeman et al., 2006b). They are wild species and therefore not as easy to obtain and grow. Because of their high Se concentration, their use at a large scale would require a careful assessment of possible ecological implications; whereas most herbivores would likely avoid high-Se plants, honey bees do not, and high Se ingestion might affect bee health (El Mehdawi and Pilon-Smits 2012; Quinn et al., 2011). Even if hyperaccumulators themselves are not practical to use, the study of the molecular mechanisms responsible for Se hyperaccumulation and hypertolerance can identify new target genes that could be manipulated via genetic engineering to create new Se-accumulating, fast-growing, high-biomass producing species, with high potential for Se phytoremediation. Hyperaccumulator sulfate/selenate transporters, for instance, could represent potential targets for transfer to crop species if demonstrated to exhibit higher specificity for selenate over sulfate. High sulfate levels are currently often a hindrance for effective uptake of selenate by plants (Zayed and Terry, 1992). Genetic engineering approaches have already been successful for several S assimilation-related enzymes (reviewed by Pilon-Smits and LeDuc [2009]). Overexpression of ATPS, CGS, and SMT in nonhyperaccumulator *B. juncea* have led to significant increases in Se accumulation (ATPS, SMT), volatilization (CGS), and/or tolerance (ATPS, CGS, SMT) (LeDuc et al.,

2004; Pilon-Smits et al., 1999; Van Huysen et al., 2003). The practical applicability of ATPS and SMT transgenic *B. juncea* lines to clean up Se-polluted sediments has been demonstrated in field experiments in California (Bañuelos et al., 2005, 2007). *Brassica juncea* APS and CGS transgenics also showed promising results when grown on naturally seleniferous soil in a greenhouse pot experiment (Van Huysen et al., 2004).

Selenium phytoremediation research was first conducted through greenhouse experiments, especially for identifying potential plant species that can naturally accumulate Se (Bañuelos, 2001). However, field studies have been further recognized as much more important to develop manageable and realistic phytoremediation strategies for remediating soils, given the complex nature and influence of environmental factors in high-Se soils. These studies were initially performed in central California. In a multiyear field study, Bañuelos et al. (1997) tested the efficacy of crop rotation for Se removal using four plant species: Indian mustard, tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.], birdsfoot trefoil (*Lotus corniculatus* L.), and kenaf (*Hibiscus cannabinus* L.). After 4 yr and various rotations among these crops, the soil Se level was reduced by 60%. Tall fescue, which is able to perform Se volatilization, was used in another multiyear field study and was found to decrease soil Se concentrations by 25% (Bañuelos et al., 1995). In addition to plant Se accumulation, processes like biological volatilization of Se, lateral movement, and leaching can contribute to the loss of Se from soils.

Phytoremediation Studies: Aquatic Systems

In addition to terrestrial systems to remove Se from soil or sediments, plants may also be used in aquatic systems. A substantial number of field studies have shown the efficacy of constructed wetlands to remove selenate or selenite from agricultural or industrial wastewaters. Hansen et al. (1998) studied the fate of selenite in oil refinery wastewater in a 36-ha constructed wetland in Richmond, CA. The predominant species in the wetland were cattail (*Typha angustifolia* L.) and bulrush. The inflow was 20 to 30 $\mu\text{g Se L}^{-1}$, and the outflow was $<5 \mu\text{g Se L}^{-1}$, with on average 89% of the Se removed. The removed Se was in part precipitated in the sediments and in part incorporated into plant tissues, and 10 to 30% of the Se was removed through volatilization. The plant (shoot) Se levels were around 15 mg kg^{-1} DW, and Se volatilization rates were around 150 $\mu\text{g Se m}^{-2} \text{d}^{-1}$. In another study, Lin and Terry (2003) studied the efficacy of constructed wetlands for removing selenate from agricultural drainage water in Corcoran, CA. The 10 wetland cells vegetated with different plant species removed on average 70% of the Se from the drainage water. Most of the Se was precipitated in the sediments, and minor fractions were accumulated in plant tissues (5%) or volatilized (5–10%). Thus, constructed wetlands can efficiently trap inorganic Se from wastewater streams, and although around 10% may be volatilized, most is captured in sediment and plant biomass, which will have to be periodically harvested (plant biomass) and replaced (sediment). If managed properly and if no other contaminants preclude it, the Se-rich plant biomass from constructed wetlands may have applications as green manure or animal feed. Biofortification applications like these are discussed below.

Biofortification Studies

Globally, plants represent one of the main dietary sources of Se for humans and animals, and crop enrichment in this element is desirable in areas with low Se. Many countries worldwide, including Finland, United Kingdom, New Zealand, Malawi, and parts of China, Tibet, and Brazil, possess soils that are poor in Se and where the population might suffer from Se deficiency-related health problems, such as Keshan disease and Kashin-Beck disease or elevated risk of infections and cancer (Combs, 2005; Rayman, 2005; Renwick et al., 2008; Tan et al., 2002). The dietary Se intake in these areas is lower than the recommended daily dose of 55 to 200 μg for adults, which is required to ensure a healthy metabolism and the full expression of essential selenoproteins (USDA-ARS, 2012; WHO, 2009). As an example, almost two thirds of the Chinese dietary Se intake is below 40 $\mu\text{g d}^{-1}$ (Wu et al., 2015).

In recent years, many Se-biofortified crops have been generated (Ávila et al., 2014; Bachiega et al., 2016; Bañuelos et al., 2015; Brummell et al., 2011; Poblaciones et al., 2014; Rodrigo et al., 2014; Schiavon et al., 2013; Thavarajah et al., 2008). Among them, those enriched in SeMet and MetSeCys may exert a broad variety of beneficial effects on human health (Combs, 2005; Fernandes and Gandin, 2015; Jackson and Combs, 2008; Sepúlveda et al., 2013). There is increasing evidence suggesting that higher intake of SeMet and MetSeCys is associated with enhancement of the immune response, alleviation of thyroid disorders, improvement of male fertility, and reduced cancer risk (Hatfield et al., 2014; Rayman, 2005, 2012; Roman et al., 2014).

The window between dietary deficiency ($<40 \mu\text{g d}^{-1}$) and detrimental ($>400 \mu\text{g d}^{-1}$) Se concentrations is very narrow (WHO, 2009). The threshold toxic concentration in livestock feed is 2 to 5 mg kg^{-1} Se (Wilber, 1980; Wu et al., 1996). Selenium at high dosages can become toxic for organisms that need it, likely because of Se misincorporation into proteins (Kieliszek and Blazejak, 2013; Misra et al., 2015; Vinceti et al., 2001; Wilber, 1980). Therefore, biofortification practices must be carefully conducted to prevent plant-derived food products from having Se levels that may be harmful to the organisms that feed on them (Finley, 2006). In this respect, there are studies that meticulously describe Se biofortification technologies, prospective health effects, and food safety regulations (White and Broadley, 2009; Zhao and McGrath, 2009; Zhu et al., 2009).

Among the possible strategies for Se biofortification is the selection of plant cultivars that take up moderate Se levels (Broadley et al., 2006; Zhu et al., 2009). This approach, also called “genetic biofortification,” is based on varietal differences in Se absorption, which may be associated with the differential expression and/or affinity for Se over S of root sulfate transporters (White, 2016; White et al., 2004). Plants can also be selected based on the main chemical form of Se they accumulate, with a preference for those accumulating SeMet and/or MetSeCys. When Se levels in the soils are very low, the selection of crop cultivars should be combined with the application of selenate- or selenite-containing fertilizers to plants or soils to enhance Se concentration in crops (Alfthan et al., 2015; Broadley et al., 2006; Wu et al., 2015). In Finland for instance, foliar Se fertilization has been a promising practice since the 1980s for increasing crop Se levels and, with that, the blood Se levels in

the population (Alfthan et al., 2015). The application of soil Se fertilizers can increase the total and bioavailable Se in soil for plant uptake (Broadley et al., 2010) and substantially increase Se concentration in grains, fruits, and vegetables (Bañuelos and Lin, 2009), but, compared with foliar Se application, fertilizers have the disadvantage of functioning only when soil Se distribution is homogeneous and soil conditions are favorable for Se uptake. Selenium bioavailability is affected by soil organic matter, soil pH and oxygenation, and levels of other elements. Particularly, fertilizers containing nitrogen (N), phosphorus (P), and S fertilizers may affect Se speciation and accumulation in plants via competitive inhibition (Cabannes et al., 2011; Duncan et al., 2016; Li et al., 2008; Liu et al., 2004; Schiavon et al., 2012; Schiavon et al., 2015).

As an alternative to Se-enriched fertilizers, the use of phytoremediation-derived plant material has been recently proposed for Se biofortification (Bañuelos et al., 2015). The Se-laden plant materials obtained through phytoremediation may be further used as green manure to increase Se soil concentration or as supplemental forage for livestock. Of course, this plant material should not contain other toxic elements in addition to Se; this needs to be verified because polluted soils frequently contain mixtures of contaminants. Plants grown on naturally seleniferous soils, including Se hyperaccumulators, also form a potential source of Se-rich green manure for crops (Bañuelos et al., 2015; Yasin et al., 2015a).

Optimizing Effects of Plant–Microbe and Plant–Plant Interactions for Se Phytotechnologies

A novel area of research is focusing on the role of plant–microbe and plant–plant interactions to enhance Se phytotechnologies (Yasin et al., 2015b). Soil microbes in general, or plant-associated rhizosphere or endosphere microbes (both bacteria and fungi), have been shown to affect plant Se uptake, translocation, and metabolism, including volatilization. Selenium-rich habitats, including polluted sites, as well as Se hyperaccumulators are potentially interesting sources of microbes with favorable properties to inoculate to plants or soil to improve plant growth and Se enrichment. In a recent study, Se hyperaccumulator endophytic bacterial isolates were shown to be characterized by high Se resistance, by the capacity to produce elemental Se, and by plant growth–promoting properties (Staicu et al., 2015; Sura de-Jong et al., 2015). Furthermore, because different plant species exhibit the capacity to influence Se accumulation and perhaps speciation in their neighboring plant species (El Mehdawi et al., 2011a, 2012), future research could be addressed toward the investigation of the potential of an array of co-cropping techniques to optimize crop Se biofortification and the nutritional quality of plant-derived food products.

Interactions of Plant Se Uptake with Other Pathways and Relevance for Phytotechnologies

Depending on the strategy used to generate crops fortified with Se and the level and species of Se accumulated in plant tissues, this element may interfere with some plant primary and secondary metabolic pathways, thus influencing the content of other nutraceuticals (Bachiega et al., 2016; Robbins et al., 2005; Schiavon et al., 2013). Selenium is available to plants mainly as selenate or selenite. Selenate can compete with sulfate for the

absorption by root sulfate transporters and further assimilation into the S analogs Se amino acids, SeCys, and SeMet (Sors et al., 2005). In *Brassicaceae* species, the S amino acid methionine (Met) is a precursor of the anticarcinogenic aliphatic glucosinolates (GLSs) (Kumar et al., 2015). As a result of Se competition with S for assimilation in plants, Se fertilization might be expected to reduce the amounts of Met-derived GLSs in these plants. Contrasting results have been reported in this respect. For instance, a decrease in aliphatic GLSs and sulforaphane was observed by Barickman et al. (2013) and Robbins et al. (2005) after supplying broccoli (*Brassica oleracea* L.) with high Se doses, whereas Sepúlveda et al. (2013) did not measure any variation in the content of GLSs, sulforaphane, and myrosinase activity in the same plant species treated with 100 μ M selenate. In the presence of a lower Se dosage (10 μ M) or high S concentration, broccoli could maintain elevated levels of GLSs in its tissues (Barickman et al., 2013), perhaps because of higher S uptake rates and promoted synthesis of S organic compounds by low Se dosages (Harris et al., 2014).

The Se species and the method of supplementation play a crucial role for the success of biofortification. For instance, when Se as selenium dioxide (SeO_2) was applied via root irrigation to *Brassica rapa* plants, GLS accumulation increased (Thiruvengadam and Chung, 2015). Selenium fertilization can also induce different effects on GLS content, depending on the plant organ (Ávila et al., 2014; Tian et al., 2016). Glucosinolates in the florets of broccoli treated with selenate were decreased, but GLS levels in the sprouts were enhanced, including the potent anticarcinogens glucoraphanin and SeMetCys. *Brassicaceae* species can also produce Se-glucosinolates (Matich et al., 2012, 2015), especially (methylseleno)glucosinolates and their Se-containing aglycons, which seem to be more potent anticancer compounds than their S analogs (Emmert et al., 2010).

Selenium biofortification may also affect N metabolism because Se is known to reduce molybdenum (Mo) uptake by plants (Harris et al., 2014), which is an essential cofactor for the activity of the enzyme nitrate reductase that mediates the conversion of nitrate to nitrite. As a result, the synthesis of amino acids and N-containing compounds could decrease in the presence of Se. Some of these amino acids function as precursors of GLSs (Agerbirk and Olsen, 2012) and other important metabolic compounds synthesized via the shikimate pathway, including phenylpropanoids (phenols and flavonoids). Although Robbins et al. (2005) observed a reduction in the content of these compounds in broccoli, most studies to date indicate a positive effect of Se on accumulation of phenolics, including GLSs. In *B. rapa*, the application of SeO_2 enhanced phenol and flavonoid accumulation and induced the expression of genes related to their biosynthesis (Thiruvengadam and Chung, 2015). Similar results were obtained in broccoli (Bachiega et al., 2016), in tomato (*Solanum lycopersicum* L.) (Schiavon et al., 2013), and in radish (*Raphanus sativus* L.) (Schiavon et al., 2016).

Future Prospects

There is increasing interest in Se compounds, both for industry (e.g., Se nanoparticles) and as food supplements. Selenium may be harvested from plants grown on seleniferous soils, a process called phytomining or agromining (van der Ent et al., 2015).

For this purpose, Se hyperaccumulators are of particular interest because they accumulate methyl-SeCys, one of the preferable forms of Se for biofortification (Rayman, 2012), to levels that can exceed 1% of DW (Galeas et al., 2007). In addition to use of Se as dietary supplement, industrial uses of plant-harvested Se may include the synthesis of pigments, glass making, and photocells. Selenium is one of the few elements that has a high enough value (\$52,000 Mg⁻¹ in 2015) to make phytomining economically viable (van der Ent et al., 2015).

It will likely be possible to effectively harvest this soluble form of Se, which is accumulated in the vacuoles of these plants (Freeman et al., 2010). Moreover, red elemental Se nanoparticles may be effectively produced using bacterial symbionts of hyperaccumulators from Se accumulated in plants or from selenite in refinery waste water (Staicu et al., 2015). That way Se bioremediation can be combined with production of a valuable selenocompound. The feasibility of Se remediation from agricultural soil and wastewater and from refinery wastewater has already been demonstrated by the work of Hansen et al. (1998), Lin and Terry (2003), and Bañuelos et al. (2015). The resulting Se-rich plant material can be successfully used for biofuel (canola oil), for animal feed, and for Se-enriched agricultural products for human consumption (e.g., cactus fruit) (Bañuelos et al., 2011b). These phytotechnologies may be further optimized for enhanced efficiency. Depending on how and where the plants are to be used, optimization efforts may focus on enhancing Se tolerance, Se accumulation, and/or Se volatilization. Approaches may include (i) selection of varieties that have the best genetic potential; (ii) testing different agronomic practices, such as soil amendments, that affect Se bioavailability or plant growth (pH regulators, fertilizer), optimizing plant density, inoculation with microbes, or co-cropping with hyperaccumulators; or (iii) genetic engineering, for instance to express a selenate-specific transporter or a master switch gene from a hyperaccumulator in crop species.

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