6.1 Introduction

Selenium (Se) in irrigation drainage water became a major concern after it was implicated in deaths and deformities of waterfowl at Kesterson Reservoir (Presser and Ohlendorf 1987; Ohlendorf 1989). Subsequently, other areas with irrigation-induced Se problems (or potential problems) have been identified in several western U.S. states (Seiler 1995), reinforcing the status of Se as a U.S. Environmental Protection Agency priority pollutant (Keith and Telliard 1979). Selenium hazards are most often associated with semi-arid climates, geologic sources in proximity to large irrigation projects, and hydrologically-closed basins, all of which lead to significant evapoconcentration of dissolved Se (Seiler 1995). Some 17% of western US land is found on Cretaceous sediments high in Se. When this land is used for irrigated agriculture, Se concentrations in drainage water can reach mg per L levels. Several measures for mitigating these high levels of Se have been proposed, including phytoremediation and phytoextraction of Se-enriched soils and sediments.

Phytoremediation was a newly burgeoning field in the mid-1980s and was being scrutinized as an environmentally benign cleanup technology for a variety of soil contaminants, both inorganic and organic. Researchers affiliated with the UC
Salinity Drainage Task Force began evaluating candidate species for deployment in the San Joaquin Valley (SJV) to help reduce soil burdens of Se (Banuelos et al. 1990; Parker et al. 1991; Duckart et al. 1992). The ecotoxicological history of Se actually dates back almost 200 years, to the first reports from South Dakota concerning “alkali” disease in livestock grazing in certain areas. Much later, in the 1920s, high Se levels in forage were found to be the causative agent of alkali disease (Mayland et al. 1989), and this finding spurred numerous investigations into the “geobotany” of Se, culminating in Rosenfeld and Beath’s (1964) classic treatise on the subject. Among the most notable findings of this early work was the identification of several unique plant taxa, all endemic to the western USA, capable of accumulating Se to hundreds or even thousands of mg per kg. The latter were originally termed “primary accumulators”, while the former were categorized as “secondary accumulators” (Rosenfeld and Beath 1964). The term “hyperaccumulator” was coined much later in reference to plants that accumulate nickel (Ni) to very high concentrations (Brooks et al. 1977) and subsequently extended to a variety of metals and metalloids. Thus, in the mid-1980s, there was actually a large amount of preexisting groundwork concerning the botany and plant physiology of Se accumulation, so that adapting that knowledge base to a phytoremediation context was a logical and facile next step.

Successful phytoremediation using terrestrial plants has always hinged on their tendency to take up Se, predominantly (but not exclusively) as selenate (SeO₄²⁻), from the soil and accumulate the Se in their shoots (Banuelos et al. 1990, 1997a; Parker et al. 1991). The seleniferous plant material could be harvested and landfilled, used as a supplement in low-Se animal diets, or incorporated back into the soil to promote microbial Se volatilization (Thompson-Eagle et al. 1989). Plant-enhanced volatilization of methyl-selenide compounds from leaves and/or the rhizosphere has also been a topic of considerable interest (Terry et al. 1992; Zayed and Terry 1994), because it can augment Se removal in harvested shoots. The search for superior plant taxa has been an ongoing effort (e.g., Banuelos et al. 1997b; Feist and Parker 2001), as the ideal candidate for Se phytoremediation should be tolerant of the wide range of aerial and edaphic conditions found in the western USA, including heat, drought, soil salinity, and high soil boron (B) (Parker et al. 1991). Intuitively, the ideal phytoremediation candidate should also have a rapid growth rate and large biomass production, along with the ability to accumulate high concentrations of Se in shoot tissue under relevant soil chemical conditions.

The plant taxa utilized in the majority of Se phytoremediation-oriented studies fall into one of three broad categories: Some of the true primary accumulators, all from the Astragalus and Stanley genera have been examined in a handful of evaluation studies (e.g., Parker et al. 1991, 2003; Bell et al. 1992; Duckart et al. 1992; Retana et al. 1993). A larger number of studies have focused on certain fast-growing Brassica species that seem to be secondary Se accumulators, probably because they are avid accumulators of sulfur (S) (Banuelos et al. 1997a; Parker et al. 2003). The third group of studies has utilized genetically modified plants,
usually *B. juncea*, in which one or more biochemical/physiological traits is overexpressed in an attempt to boost Se removal rates from soil (e.g., Pilon-Smits et al. 1999; LeDuc et al. 2004).

Our purpose here is to provide an overview of the most salient studies concerning plant uptake, metabolism, and volatilization of Se, framed in the context of phytoremediation approaches to the Se problem in the Central Valley, with an emphasis on field-based validation of the approach.

### 6.2 Plant Uptake and Translocation of Selenium

#### 6.2.1 Primary Accumulators

Because Se is so chemically similar to sulfur (S), higher plants (and other organisms) tend to take up and metabolize Se readily via S transporters and pathways. Since replacement of S by Se in proteins and other S compounds disrupts the function of these molecules, Se is toxic at elevated levels to most organisms, and the gap between sufficiency and toxicity is often described as narrow (Mayland et al. 1989; McLaughlin et al. 1999). Unlike animals, many bacteria, certain green algae, and higher plants do not seem to require Se, but they nonetheless readily take it up from their environment and incorporate it into organic compounds.

There is a very broad range in the tendency for higher plants to take up and accumulate Se in their aerial parts, both across species (interspecific variation) and within species (intraspécific variation). The primary accumulators of Se (a synonym for hyperaccumulators) are from the *Brassicaceae*, *Fabaceae* and *Asteraceae* families, are endemic to naturally seleniferous soils, and can exhibit shoot Se concentrations as high as $10 \times 10^3 \text{ mg kg}^{-1}$ dry weight (1% of DW) in field-grown specimens; whereas, in Se nonaccumulators, Se concentrations greater than 100 mg kg$^{-1}$ are relatively rare (Rosenfeld and Beath 1964; Parker and Page 1994; Terry et al. 2000). Selenium hyperaccumulators preferentially take up Se over S, and their Se accumulation to percent levels in leaf tissue does not result in toxicity (Neuhierl and Böck 1996; LeDuc et al. 2004). There is evidence that Se hyperaccumulators can distinguish between S and Se (Bell et al. 1992; White et al. 2007) and have Se-specific metabolism, as discussed below. It has been suggested that Se could be an essential element for hyperaccumulators, since hyperaccumulators sometimes exhibit significantly better growth in the presence of Se (Terry et al. 2000), but there is, to date, no compelling evidence that these plants require Se to complete their life cycle. The observed positive growth responses to Se by hyperaccumulators may instead be due to alleviation of phosphorus (P) toxicity in hydroponic culture, since Se accumulation was considerably less pronounced when plants were grown at lower phosphorus levels (Broyer et al. 1972).
6.2.2 Secondary Accumulators

The secondary Se accumulators include a broad array of selected species from the genera Aster, Astragalus, Atriplex, Castilleja, Comandra, Grayia, Grindelia, Gutierrezia, and Machaeranthera that may accumulate Se to levels between 100 and 1,000 mg kg\(^{-1}\) (Parker and Page 1994); they have been little studied with respect to the physiology or biochemistry of Se uptake and accumulation. Moreover, plants that have a general tendency to accumulate high levels of S compounds, such as members of the *Brassica* genus (mustards and cabbages), also are good accumulators of Se and can rightfully be grouped with the other secondary accumulators (Terry et al. 2000). These plants include the fast-growing agronomic species *B. juncea*, which has been studied extensively and genetically engineered for purposes of phytoremediation (see later sections). The secondary Se accumulator species probably lack any Se-specific transporters or metabolic pathways, but merely take up and metabolize Se and S indiscriminately, simply at elevated rates compared to nonaccumulators. A similar generalization can be made for the nonaccumulators, but there do seem to be a few notable exceptions (see next section). Among the nonaccumulators, the monocot grasses (*Poaceae*) generally have lower shoot Se levels than do dicotyledonous plants (Rosenfeld and Beath 1964; Parker and Page 1994).

Intraspecific variation in Se accumulation has been studied much less, but Feist and Parker (2001) reported that 16 populations of *Stanleya pinnata* (an identified Se hyperaccumulator), collected from around the western USA, exhibited 1.4- to 3.6-fold differences in shoot Se concentration when grown in a “common garden” greenhouse environment utilizing varying selenate and sulfate levels in sand culture. Moreover, shoot levels were positively correlated with soil Se levels at the site where each ecotype was collected. Crop plants may exhibit genotypic differences in Se accumulation, but there is a paucity of common-garden experiments wherein environmental and/or soil factors can be eliminated as possible sources of variation (see Zhu et al. 2009 for references and a recent review).

The form of soluble Se initially absorbed can profoundly influence its root uptake and subsequent fate. Selenate is the dominant water-soluble form of soil Se, especially under alkaline and aerobic conditions. Although also present in many soils, selenite is generally less bioavailable to plants than is selenate, because the former is more strongly adsorbed. Many nutrient solution studies have utilized selenate (Bell et al. 1992; Feist and Parker 2001), but a few have utilized selenite instead (e.g., Broyer et al. 1972), and some have compared the two inorganic forms (Lewis et al. 1974; Zayed et al. 1998; de Souza et al. 1998; Hopper and Parker 1999). Relatively few studies have examined the uptake of soluble organic forms, such as Se-methionine (Abrams et al. 1990; Zayed et al. 1998). All are “plant available” (Terry et al. 2000), but their comparative bioavailability at equal solution concentrations is confounded by other influential factors, especially the presence of other, “competitive” anions.
Plant uptake of selenate can be inhibited by an assortment of other anions (Khattak et al. 1991), of which sulfate is both the most effective and the most studied (Mikkelsen et al. 1988; Bell et al. 1992; Wu and Huang 1992; Feist and Parker 2001). It is clear that uptake of selenate can be mediated by sulfate transporters, owing to the chemical similarity between selenate and sulfate (Terry et al. 2000). The selectivity of these transporters for selenate and sulfate varies between plant species and with nutritional status (White et al. 2004). It has been suggested that the selectivity of the transport pathway for sulfate over selenate is lower at higher external sulfate concentrations, and that the inducible sulfate transporters have higher selectivity for sulfate over selenate than do the constitutively active sulfate transporters (White et al. 2004). Different sulfate transporters in a single plant may also have somewhat different selectivity for sulfate versus selenate, as had been suggested in studies using *Arabidopsis* (El Kassis et al. 2007; Barberon et al. 2008). Further improvements in our understanding of the selectivity of different sulfate transporters at the molecular level might help the development of plants higher (or lower) in Se via genetic engineering.

The sulfate/selenate transporters in the Se hyperaccumulators have not yet been studied directly, but these taxa are uniquely characterized by generally higher leaf Se concentrations, a higher Se:S ratio, and a higher shoot:root Se concentration ratio (Bell et al. 1992; Feist and Parker 2001; White et al. 2007). These observations are consistent with altered regulation of sulfate/selenate transporters, and/or the presence of specialized Se-specific transporters, perhaps exclusive selenate transporters that have evolved from sulfate transporters. Moreover, a recent study of seasonal fluctuations in Se and S levels in Se hyperaccumulators and related nonaccumulators growing on the same field site suggested independent fluxes of Se and S only in the hyperaccumulators (Galeas et al. 2007). Thus, whole-plant level Se fluxes seem to be rather specialized in Se hyperaccumulators and also distinct from S movement. In the future, it will be particularly instructive to study the properties of the sulfate transporter homologues in Se-hyperaccumulating taxa.

Much less is known about (a) the mechanisms of plant uptake of selenite, which might be more important in neutral-to-acidic soils, especially under reduced soil conditions, and (b) the uptake of organic selenium compounds. Historically, it has been stipulated that selenite uptake by plant roots is not metabolically dependent and does not involve specific ion transporters (Arvy 1993; Terry et al. 2000). But, some more recent results showed that selenite uptake by wheat could be suppressed by a metabolic inhibitor, inhibited by phosphate in the nutrient solution, and enhanced by P deficiency (Li et al. 2008). It was previously argued that inconsistency in the rate of selenite uptake by plants could be ascribed to different phosphate concentrations present in the growth solutions used for different studies (Hopper and Parker 1999). Phosphate is typically present at millimolar levels in almost all nutrient solutions, but at only micromolar levels in soil solutions *in situ* (Parker and Norvell 1999). Increasing P above a basal level of 2 μM in ten-fold increments significantly reduced both Se phytotoxicity and Se uptake in solution-cultured plants supplied with selenite, but the effect was less dramatic.
than a similar, relative increase in sulfate for selenate-grown plants (Hopper and Parker 1999). Thus, quantitative comparisons of selenite and selenate availability to plants require careful consideration of the in-solution phosphate and sulfate concentration levels, respectively.

Translocation of Se from root to shoot depends on which form (or species) of Se is supplied to the plant. In plants exposed to selenate, Se is readily translocated to the shoot, and selenate is the predominant species found in the xylem sap (Li et al. 2008). In contrast, with selenite-treated plants, most of the Se remains in the roots, and little selenite is detected in the xylem sap. Selenite taken up by roots is readily converted to other forms, including selenomethionine (SeMet) and selenomethionine Se-oxide hydrate (SeOMet), but mostly into unidentified and water-insoluble forms (Li et al. 2008). Thus, Se translocation from root to shoot is generally much lower in plants fed with selenite than those fed with selenate (Arvy 1993; de Souza et al. 1998; Hopper and Parker 1999; Li et al. 2008).

### 6.3 Selenium Metabolism and Volatilization in Terrestrial Plants

The metabolic fate of Se taken up by plants has been reviewed frequently (e.g., Brown and Shrift 1982; Laüchli 1993; Terry et al. 2000; Sors et al. 2005; Pilon-Smits and Quinn 2010), and a similarly detailed review is beyond the scope of this chapter. Instead, we present a brief overview of Se metabolism in plants, both in non-hyperaccumulators and in hyperaccumulators, which is then linked to the issue of plant-mediated volatilization.

In all plants, typically is inorganic selenate is transported to the leaf chloroplast, where it is reduced first to selenite and then further reduced and assimilated into organic Se (Fig. 6.1). Because of the chemical similarities between Se and S, selenate and selenite are readily assimilated by the S-metabolizing enzymes of the plant, and Se can thus be nonspecifically incorporated into almost any S compound (Terry et al. 2000). The first stable, organic form of Se produced is selenocysteine (SeCys). This amino acid can be incorporated nonspecifically into proteins in lieu of cysteine (Cys), leading to phytotoxicity. An alternative fate of SeCys is ultimate conversion to selenomethionine (SeMet), which also can be incorporated mistakenly into proteins, with generally less harmful effects (Fig. 6.1). The SeMet can also be volatilized, converted to volatile dimethylselenide (DMSe), offering a release valve for excess Se from the plant (Lewis et al. 1966 and see below), while SeCys can also be converted in plants to insoluble, elemental Se plus alanine (Pilon et al. 2003). The elemental Se is probably innocuous, as many bacteria use a similar Se detoxification mechanism. Several lines of evidence (de Souza et al. 1998; Pilon-Smits et al. 1999) suggest that the assimilation of Se from selenate is rate-limited by low levels of ATP sulfurylase (APS), the first enzyme needed for the conversion of selenate to selenite (Fig. 6.1).
The forms of Se (Se speciation) that tend to accumulate in plant tissues are important from two perspectives: (a) understanding metabolic pathways and (b) assessing the nutritive value of Se-containing foods; they have been investigated in several plant species. Selenium speciation varies both with plant species and the form of Se provided to the plant (e.g., Wang et al. 1996; Grant et al. 2004; Ximenez-Embun et al. 2004; Kapolna et al. 2007). For example, in *Brassica juncea* (Indian mustard), when the plant is fed with selenate, the main Se species is selenate; whereas, in plants fed with selenite, SeMet and SeOMet tend to dominate (Kahakachchi et al. 2004). This pattern likely applies to many, if not most, nonaccumulators, and to secondary Se accumulators, at least with respect to leaf tissues. In cereal crops, the grain Se seems to be dominated by SeMet (~60–80 % of the total Se) (Stadlober et al. 2001).

Beginning in the late 1980s, the increased emphasis on phytoremediation led to a renewed interest in phytovolatilization of methylated Se compounds, which could represent an “added value” to phytoextraction strategies (Banuelos et al. 1997a, b).
The rates of Se volatilization vary greatly among species, with the hyper-accumulator *A. bisulcatus* often exhibiting the highest rates (Duckart et al. 1992; Terry et al. 1992). Dimethyl selenide (DMSe) is the predominant volatile Se compound produced by nonaccumulators and secondary Se accumulators (Lewis et al. 1974). However, a number of rate-limiting steps must be overcome, including the incremental reduction of selenate by APS described above. In addition, de Souza et al. (2000) found that supplying plants with dimethylselenopropionate (DMSeP) results in a remarkable increase in volatilization, and thus proposed that conversion of SeMet to DMSeP is most likely a key rate-limiting step for Se volatilization. Subsequently, overexpression in *B. juncea* of the first enzyme in the conversion of SeCys to SeMet, cystathionine gamma synthase (CγS), resulted in a two- to three-fold higher volatilization rate compared to untransformed plants (Van Huysen et al. 2003).

In addition to these metabolic “bottlenecks” to DMSe production and release to the atmosphere, Pilon-Smits et al. (1999) further suggested there may often be some complex whole-plant cycling involved. They proposed that after selenate is absorbed and translocated to the leaves, it is converted to organic Se. One (or more) of the soluble organic intermediates is then translocated back to the roots via the phloem, where the majority of the volatilization takes place (Zayed and Terry 1994). Alternatively, the organic Se could be incorporated into proteins in place of their sulfur analogues, perhaps leading to phytotoxicity (Terry et al. 2000).

Perhaps unsurprisingly, the quantities of Se volatilized by non-hyperaccumulators depend on the form of Se supplied to the plants, with SeMet > selenite > selenate (Lewis et al. 1966; Zayed et al. 1998). This result is consistent with the metabolic steps (Fig. 6.1) and rate limitations in the production of DMSe. The observation that increasing sulfate levels decreases volatilization in selenate-supplied plants, but not in those exposed to selenite or SeMet (Zayed and Terry 1994; Zayed et al. 1998), is also consistent with the concept that the most profound effect of sulfate is to inhibit the initial entry of selenate into the plant.

In certain plant taxa, notably the Se hyperaccumulators, SeCys can be methylated to form Se-methylselenocysteine (SeMeCys) by the enzyme selenocysteine methyltransferase (SMT) (Neuhierl and Böck 1996). This form of Se was first identified in the early 1960s (Trelease et al. 1960; Shrift and Virupaksha 1963), and it has long been believed that this form of Se can safely be accumulated to high concentrations, since it is not incorporated into proteins; SeMeCys likely plays a central role in the detoxification of Se in the hyperaccumulators (Neuhierl et al. 1999). Interestingly, SeMeCys is also a major Se compound found in Se-enriched garlic (*Allium sativum*), onion (*A. cepa*), leek (*A. ampeloprasum*) and broccoli (*Brassica oleracea*), accounting for approximately half of the total Se (Whanger 2002). Recent studies with the hyperaccumulator *Stanleya pinnata* have also found that the soluble Se in the shoots is dominated by amino acid forms (Zhang and Frankenberger 2001). In addition to SeMeCys, the Se hyperaccumulators may also store Se in the leaves as γ-glutamyl-Se-methylselenocysteine, and as selenocystathione (Terry et al. 2000).

While much is known about Se volatilization in secondary Se accumulators, such as *B. juncea*, comparatively little is known about volatilization from the true
hyperaccumulators. An early study of *Astragalus racemosus* collected volatile Se compounds on active carbon and found four compounds, of which only one could be identified – dimethyldiselenide (DMDSe) (Evans et al. 1968). This seminal paper led to repeated speculation that Se hyperaccumulators follow a distinctly different metabolic pathway, wherein DMDSe is produced directly from SeMeCys (e.g., Brown and Shrift 1982; Terry et al. 2000; Sors et al. 2005). Only recently has further credence been given to the significance of DMeDSe. Using sophisticated gas headspace analysis, Kubachka et al. (2007) were able to show the increased significance of DMeDSe (relative to DMeSe) in transgenic *B. juncea* in which the overexpression of SMT led to enhanced accumulation of SeMeCys relative to the wild type. Thus, a *B. juncea* genotype seems to share many of the traits of the true hyperaccumulators (LeDuc et al. 2006). The literature often describes Se hyperaccumulating species as having a characteristic odor, which could be a volatile Se compound, but this remains speculation at present.

To date, studies of plant Se metabolism, including volatilization, have generally been done using nonsterile culture methods. Since all plants live in association with a diverse array of bacteria and fungi, and many microbes can also metabolize and volatilize Se, plant-associated microbes may play a key role in plant Se accumulation, and especially in Se volatilization. For example, in broccoli (*B. oleracea*) 95 % of the Se volatilized by roots was inhibited when roots were treated with antibiotics (Zayed and Terry 1994). Similarly, *B. juncea* plants treated with the antibiotic ampicillin volatilized 30 % less Se, and even accumulated 70 % less Se than the control plants. In addition, *B. juncea* plants grown from surface-sterilized seeds that were subsequently inoculated with rhizospheric bacteria accumulated five-fold more Se and volatilized four-fold more Se than uninoculated controls (Zayed and Terry 1994). The mechanisms for the stimulatory effect of the bacteria appeared to be both an enhancement of root growth, as well as direct stimulation of Se/S uptake and assimilation. Plants inoculated with rhizospheric bacteria had an increased root surface area and the culture media contained nine-fold higher serine levels than control plants. O-acetylserine (OAS) is known to stimulate S uptake and assimilation (de Souza et al. 1999).

While there is convincing evidence that bacteria mediate plant uptake and volatilization of Se, much less is known about a possible role for plant-associated fungi. In one study, the nonaccumulator ryegrass accumulated less Se when inoculated with the mycorrhizal fungus *Glomus mosseae* in comparison to controls lacking the fungal symbiont (Munier-Lamy et al. 2007). Moreover, virtually nothing is known about the role of endophytic microbes in Se uptake and volatilization. These will be interesting areas for further study.

### 6.4 Genetic Engineering for Enhanced Phytoremediation

As discussed previously, all plants can take up inorganic selenate and selenite and further assimilate them to SeCys and other organic compounds, including some volatile forms. Hyperaccumulators of Se may have additional metabolic pathways
for Se, notably the methylation of SeCys, and most likely the conversion of MeSeCys to volatile DMDSe. To further enhance plant selenium accumulation, tolerance, and volatilization, various transgenic approaches have been used, primarily in *B. juncea*.

The main thrust of the transgenic work has involved upregulation of key genes involved in S/Se assimilation and volatilization. As previously mentioned, overexpression of the gene for the first enzyme in the reduction of selenate to selenite conversion, ATP sulfurylase (APS), resulted in enhanced selenate reduction, as evidenced by the increase in organic forms of Se when supplied with selenate, while wildtype controls accumulated selenate (Pilon-Smits et al. 1999). The APS transgenics accumulated two- to three-fold more Se than the wild type controls, and 1.5-fold more S. The APS plants also tolerated the accumulated Se better than the wild type controls, perhaps because of the different form of Se accumulated, but the Se volatilization rate was not enhanced in the APS transgenics.

Van Huysen et al. (2003) subsequently showed that overexpression of the first enzyme in the conversion of SeCys to SeMet, cystathionine gamma synthase (CgS), resulted in substantial increases (two- or three-fold) in volatilization rates from *B. juncea*. Probably as a result of their enhanced volatilization, the CgS transgenics accumulated 40% less Se in their tissues than the wildtypes, and were also more Se-tolerant, probably due to their lower tissue Se levels. Another transgenic approach targeted the nonspecific incorporation of SeCys into proteins by overexpressing a mouse selenocysteine lyase (SL), an enzyme that specifically breaks down SeCys into alanine and elemental Se, in both *A. thaliana* and *B. juncea* (Pilon et al. 2003; Garifullina et al. 2003). The SL transgenics showed reduced Se incorporation into proteins, as well as enhanced Se accumulation (up to two-fold) compared to wildtype plants.

Another approach to enhancing Se tolerance was to overexpress SeCys methyltransferase (SMT) from the Se hyperaccumulator *A. bisulcatus* in both *A. thaliana* and *B. juncea* (Ellis et al. 2004; LeDuc et al. 2004). The SMT transgenics showed enhanced Se accumulation in the form of methyl-SeCys, as well as enhanced Se tolerance, and greater production of volatile Se, most likely as DMDSe (Kubachka et al. 2007). While the expression of SMT enhanced Se tolerance, accumulation, and volatilization, the effects were more pronounced when the plants were supplied with selenite, as opposed to selenate, consistent with the perspective that conversion of selenate to selenite was the rate-limiting step for the ultimate production of MeSeCys. To overcome this rate limitation, APS and SMT transgenics were crossed to create double-transgenic plants that overexpress both APS and SMT. The APS × SMT double transgenics accumulated up to nine-fold more Se than the wildtypes, and most of the increase could be ascribed to accumulation of the nontoxic MeSeCys (LeDuc et al. 2006). The APSxSMT plants accumulated almost twice as much MeSeCys as the single SMT transgenics, but Se tolerance was similar in the single and double transgenics.

These various transgenics have been tested further to validate their potential beyond the laboratory, where Se accumulation was up to nine-fold higher and volatilization rates were up to three-fold greater. When grown in a naturally seleniferous soil in greenhouse pots, the APS transgenics accumulated Se to
three-fold higher levels than wildtype *B. juncea*, while the CgS transgenics contained 40% lower Se levels than the wildtypes (Van Huysen et al. 2004), in agreement with the laboratory results. Plant growth was the same for all plant types in this experiment. Subsequently, field experiments were carried out on a Se-contaminated sediment in the San Joaquin Valley (Banuelos et al. 2005a, b, 2007), and again results were obtained that agreed with the earlier laboratory experiments (see last section for details). Thus, the results obtained with the transgenics in the greenhouse or in the field are similar to those obtained under controlled laboratory conditions, and the enhanced Se accumulation, volatilization and/or tolerance exhibited by the transgenics are promising traits for use in phytoremediation.

### 6.5 Other Desirable Plant Traits for Se Phytoremediation

Early on, researchers working as part of the UC Salinity/Drainage Task Force recognized that, if terrestrial plants were to be used to help dissipate Se from high-Se soils or sediments, they would need to be tolerant of an array of adverse aerial and edaphic conditions associated with the relevant semiarid sites in California and elsewhere (Parker and Page 1994). Foremost was concern about high soil levels of both salinity and boron (B), as the substrates in which Se was the highest (such as Kesterson) were also laden with salts. Moreover, plants were needed that could also tolerate heat and drought, and that had agronomic traits useful at the field scale. Initially, these requirements presented something of a fundamental conundrum: the Se hyperaccumulators (e.g., some *Astragalus* and *Stanleya* species) had never been improved via plant breeding, although they are broadly adapted to some rather harsh environments in the western USA. Other candidates included improved crop cultivars of species such as *B. juncea*, but these had not been bred specifically for highly saline soils.

In an early study, Parker et al. (1991) used greenhouse sand culture to screen a number of genotypes of the genera *Astragalus*, *Leucaena*, *Medicago*, *Trifolium*, *Elymus*, *Elytrigia*, *Festuca*, *Leymus*, *Oryzopsis*, *Psathyrostachys*, *Puccinellia*, and *Sporobolus* for tolerance to salinity and B. Salinity treatments were specifically designed to mimic the sulfate-rich drainage waters found within the Westside of the San Joaquin Valley. Considerable variation in tolerance to salinity, both within and across species, was observed during seed germination, but B concentrations up to 4.0 mM had little effect. The most promising genotypes, representing some 15 species, were then tested for salinity and B tolerance during the seedling growth stage. Lines of five species (two Se-hyperaccumulators, *Astragalus bisulcatus* and *A. racemosus*, and three grasses, *Elytrigia pontica*, *Puccinellia distans*, and *Sporobolus airoides*) appeared the most promising, as they grew well up to ~15 dS m\(^{-1}\) salinity, and were again unaffected by B at 4.0 mM.

Retana et al. (1993) then attempted to rear four of these salt- and B-tolerant genotypes (along with Indian ricegrass [*Oryzopsis hymenoides*]) in a greenhouse
column study using a soil from Kesterson to assess both growth and uptake of various trace elements (As, B, Mo, Se, U, V). Soil columns were reconstructed to reflect the original depth profile in the field, but subjected to a pre-planting leaching treatment to reduce salinity in the seed zone. All five genotypes were established successfully, with the alkali sacaton and tall wheatgrass yielding the greatest biomass; however, the shoot Se levels were comparatively low in these two grasses. *Astragalus bisulcatus* and *A. racemosus* were much slower-growing, but were able to persist on very saline soil, accumulating by far the greatest amount of Se in their aerial tissues (Retana et al. 1993).

Banuelos et al. (1990) evaluated the effects of Se, salinity, and B on plant growth and elemental accumulation in solution-cultured *B. juncea*. Salinity (chloride-dominated) up to 15 dS m\(^{-1}\) and B up to 1.4 mM both seemed to reduce shoot yield, but by less than 40%, even in concert. In a follow-up study, Banuelos et al. (1996) screened multiple genotypes of *B. napus* (canola), *Hibiscus cannibinus* (kenaf), *Festuca arundinacea* (tall fescue), and *Lotus tenuis* (birdsfoot trefoil) for salinity tolerance (again, chloride-based, up to 20 dS m\(^{-1}\) in soil pots), in conjunction with Se uptake. Overall, canola exhibited the best combination of salinity tolerance, biomass production, and Se accumulation. Wu and Huang (1991) also examined 13 tall fescue lines from around the world and found a correlation between salt tolerance and Se tolerance, although this species is not particularly salt-tolerant, generally. Subsequently, Parker et al. (2003) did a comparative study of *B. juncea* and the hyperaccumulator *S. pinnata* using the same greenhouse methods employed earlier (Parker et al. 1991). Neither species was particularly tolerant of salinity (as compared to alkali sacaton which was grown alongside), although *S. pinnata* exhibited the desired tolerance to excess B. That *S. pinnata* exhibits ecotypic variation in Se accumulation, as well as broad geographical distribution (Feist and Parker 2001), gave rise to some optimism that more salt-tolerant germplasm may exist (Parker et al. 2003). Moreover, *S. pinnata* is a perennial that responded favorably to repeated cutting in the greenhouse, even when subjected to salinity or B stress, a trait that could prove valuable in field-scale phytoremediation. In contrast, *B. juncea* exhibited much poorer survival and regrowth under high salinity and B.

Relatively few other studies have been conducted to screen diverse plant taxa for tolerance to salinity or B, and we know of none that have specifically addressed other traits, such as heat or drought tolerance. There are, however, some field trials from which the ability to withstand these conditions can be inferred, and these are summarized briefly below.

### 6.6 Phytomanagement of Se Under Field Conditions

After more than two decades of extensive research supported by the UC Salinity/Drainage Program, many strategies have been suggested to manage soil Se levels. Removal of Se from soil with conventional physical and chemical techniques is prohibitively expensive, as are excavation and burial. Thus, much of the emphasis
has been upon the “green” technology broadly known as phytoremediation (Pilon-Smits 2005). In its broadest sense, the strategy uses plants to manage contaminated water and/or soil via accumulation (extraction), enhanced volatilization, soil stabilization, rhizofiltration, and degradation/transformation; the first two have been the foci of most of the relevant Se studies. Effective phytoextraction at the field scale requires that plants produce relatively large amounts of biomass, absorb soil Se and translocate it such that it can be harvested and removed from the site, and/or promote volatilization of nontoxic gases (e.g., dimethyl selenide, DMSe).

Phytomanagement of Se both with enhanced phytoextraction and/or volatilization goals requires cogent use of the most appropriate plant species. Plants with differing properties may be needed to obtain an effective overall technology, especially under field conditions, and may involve the use of hyperaccumulator plants, agronomically improved crops, particular plant cultivars, or even genetically-modified plants (Terry et al. 2000; Banuelos et al. 2007). Although plant selection is critical for successful phytoremediation, understanding the following factors is also essential when considering phytomanagement strategies under field conditions:

1. Remediation site: soil vs. water; adverse growing conditions, presence of shallow water tables, field variability.
2. Agronomics, e.g., cultivation, management practices, crop rotation, including availability of water for irrigation.
3. Crop disposal or utilization of harvested material, economic sustainability.
4. Time frame: how long-term is the process?
5. Parameters used to measure success: fraction removed, fraction converted to less toxic forms.
6. Grower acceptance of alternative use crops

It is beyond the scope of this chapter to review all of the published field studies of Se phytomanagement. Instead, we review key examples that help illustrate the foregoing, while also demonstrating the application of the more fundamental knowledge gain in the laboratory and greenhouse, which we have reviewed previously.

In an early study, Banuelos et al. (1993) examined the ability of B. juncea to dissipate soil Se in a field being irrigated with saline drainage water (Se = 154 μg L⁻¹). Although Se was taken up by the pant and volatilized, the quantities removed were small, and, in fact, insufficient to offset the annual Se inputs from the irrigation water. Similar results were later obtained by Lin et al. (2002). Even with the “best” volatilizer of Se (pickleweed [Salicornia bigelovii]), only about 7 % of the annual total Se input was volatilized.

Van Mantgem et al. (1996) studied the ability of two forage species to dissipate Se from Kesterson Reservoir sediments, using “clean” irrigation water. The plants removed a measurable fraction of the more labile forms of Se in the sediment, but these comprised only 10 % of the total Se inventory; there was thus no measurable reduction in total Se in a year-long time frame.

More recently, Banuelos et al. (2005a) looked at phytovolatilization of Se from the heavily contaminated sediments in the San Luis Drain, using several salt-tolerant species and/or genotypes. Volatilization rates were generally low
(<40 μg m⁻² day⁻²), which the authors attributed to the high sulfate levels present and to competitive inhibition of uptake and/or metabolism. They concluded that the volatilization process required enhancement in order to be effective. In a follow-up study, amendment of the same sediments with casein or methionine increased volatilization by up to 17-fold (Banuelos and Lin 2007), but with greatly increased operating costs.

Some of the transgenic lines of B. juncea described previously have now been tested under field conditions in the San Joaquin Valley. Banuelos et al. (2005b) reported that APS transgenics accumulated Se to four-fold higher levels than did the wildtypes, which is similar to the earlier laboratory and greenhouse results. In a second field experiment on the same high-Se sediment, the SL and SMT transgenics showed two-fold greater Se accumulation relative to the wildtype (Banuelos et al. 2007), also in agreement with earlier laboratory experiments. In both field experiments, biomass was comparable for the different genotypes. Thus, the results obtained with the different transgenics using Se-enriched soils in both greenhouse or field are similar to those obtained under controlled laboratory conditions. The various transgenics showed enhanced Se accumulation, volatilization and/or tolerance, all promising traits for use in phytomanagement as in phytoremediation.

In closing, it is apparent that, under field conditions, phytomanagement of Se will require time (months/years) for effective removal of soluble Se from the soil. The efficacy of phytomanagement is generally greater under controlled greenhouse or microplot conditions, especially if Se is added as soluble selenate. Under field conditions, naturally-occurring soil Se usually exists as a complex melange of forms (e.g., elemental Se, sorbed selenite, organic Se) that are not immediately available for plant uptake and/or for volatilization. When biogeochemical changes occur within the soil profile, which may be achieved partially through growing crops, the bioavailability of Se may be enhanced. Moreover, irrigation cycles (wetting and drying) may enhance the release of Se, such that uptake and/or volatilization are also enhanced. However, lowering the overall concentration of total soil Se with plant-induced and associated microbiological processes will always take time. Under some circumstances, the best we can do under field conditions is to phytomanage soluble Se by promoting both plant uptake and biological volatilization. Unless plants take up Se faster than evapotranspiration occurs, the net effect of irrigating land with any Se-enriched water may be a gradual increase in the soil Se level (Parker and Page 1994), unless dissipation processes can be greatly accelerated.

References


