Phyto-products may be essential for sustainability and implementation of phytoremediation

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Producing viable products of economical value may help sustain long-term application of field phytoremediation.

Abstract

Interest in selenium pollution and remediation technology has escalated during the past two decades. Although not known to be essential for plants, selenium is essential but could be toxic for humans and animals, depending on its concentration. A major selenium controversy in the 1980's emerged in California when the general public and scientific community became aware of selenium's potential as an environmental contaminant. After extensive research on several strategies to reduce loads of mobile Se for entering the agricultural ecosystem a plant-based technology, defined as 'phytoremediation' received increasing recognition, as a low-cost environmentally friendly approach for managing soluble Se in the soil and water environment. Successful long-term field remediation of Se by plants is, however, dependent upon acceptance and widespread use by growers, who are also concerned about potential commercial value from using the plant-based technology. Obtaining products with economic value from plants used in the cleanup of soil would certainly be an additional benefit to phytoremediation, which could help sustain its long-term use.

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Keywords: Phytoremediation; Selenium; Phytoproduct; Essential micronutrient

1. Introduction

Irrigation of seleniferous soils has produced subsurface drainage that contaminated wetlands and led to deformities in fish and migratory birds in the western United States (Lemly, 1997). The U. S. Geological Survey has identified about 267,000 square kilometers of lands susceptible to irrigation induced Se contamination (Seiler et al., 1999). Soils on the western side of Central California are derived from cretaceous shale rocks that contain high levels of Se, arsenic (As), molybdenum (Mo) and other salts (McNeal and Balisteri, 1989), which are of particular concern because they have been reported to bioaccumulate in many biological ecosystems.

A plant management remediation strategy for Se was developed based upon research from Bañuelos and Meek (1990) and other earlier research that showed that certain exotic plants, e.g., Astragalus, Stanleya, accumulate high concentrations of Se when grown on seleniferous soils (Rosenfeld and Beath, 1964). In this regard, California researchers developed and demonstrated the phytoremediation of Se under a variety of conditions (Bañuelos et al., 2002b; Zayed et al., 2000; Wu et al., 2000; Frankenberger and Karlson, 1995; Lin et al., 2002). Generally, for phytoextraction systems to be practical and sustainable for managing Se in Se-enriched soils, selected plants should be considered as part of a typical crop rotation, and should not result in economic losses for the landowners. Bañuelos (2002) has reported that potential crops used for the phytoextraction of Se in Central California include, moderate Se accumulators within the Brassica family, e.g., broccoli (Brassica oleracea), canola (Brassica napus). He hypothesized that canola and broccoli not only remove soluble Se from soil, but harvesting the Se-enriched crops may produce products of potential economical importance for the grower. These include using Se-rich plant material as
supplemental animal feed or as an edible vegetable. Similarly, other commodity options are presently under consideration, which include using extracted canola oil mixed with diesel fuel for the production of both biofuel for diesel engines and using the seed by-products after oil extraction for animal feed meal. Canola oil, as well as oils from such oleaginous crops, e.g., sunflower (Helianthus annuus), safflower (Carthamus tinctorius), soybean (Glycine max), cotton (Gossypium hirsutum), and peanut (Arachis hypogaea) are considered to be potential alternative fuel stocks for diesel engines, which could perform well as an adequate fuel with chemical and physical properties similar to those of commercial-grade diesel fuel (Dorado et al., 2004). My objective is to report on the derivation and novel utilization of potential phyto-products that were produced from Brassica plants grown for the remediation of Se enriched soils in Central California.

2. Materials and methods

A multi-year field study was conducted between 2002–2004 with B. napus var. Hyola (canola) and with B. oleracea var. Marathon (broccoli) on two different 20-ha field sites at Red Rock Ranch (courtesy of Mr. John Diener, Five Points, CA). Both Brassica crops were selected because Bañuelos et al. (1996) have reported on the ability of this family to accumulate Se under moderately high salinity and B levels. The soil in the experimental area was classified as an Oxic silty clay loam (fine montmorillonitic, thermic Pachic haploxeralf with a well-developed salinity profile). The site was drained by sub-surface 10 cm diameter plastic drains, which were installed at depths ranging from 2.0 to 2.5 m with lateral spacings of 100 m.

On 200 m long 1 m wide beds, broccoli was planted as 4 to 6 week old transplants to a plant density of 112,000 ha−1 in August, and canola was directly seeded in October of each year to a plant density of 100,000 ha−1. Each bed contained two planted rows spaced 0.3 m apart. Two water sources were available for irrigation; canal water from the California aqueduct and Se-laden drainage water produced from other growing sites on Red Rock Ranch. Each bed was deemed established (between true 2nd or 3rd leaves). Thereafter, irrigation scheduling was based upon weather data reported from California Irrigation Management Information System (CIMIS) located at the University of California Westside Research Station (located less than 5 km from field sites).

Broccoli was generally harvested 95–100 d after transplanting, and separated into floret, stalk, and leaves. Samples were collected, processed as described by Bañuelos and Akohoue (1994), and analyzed for Se by an atomic absorption spectrophotometer (Thermo Jarrell Ash, Smith-Hieftje 1000, Franklin, MA) with an automatic vapor accessory (AVA 880). External quality control standards for soils, soil extract, and plant tissue samples were obtained from the National Institute of Standards and Technology (NIST). The standards used were wheat flour (SRM 1567; Se content of 1.1 ± 0.2 μg g−1 DM, 94% recovery) and internal soil standards (sediment collected from Kesterson Reservoir, CA) with a total Se content of 7.5 and 25 mg kg−1, 94% recovery, respectively. Canola plants were cut generally 150 d after seeding when the pods first began to turn yellow. When 30–40% of the seeds were brownish-red in color (corresponding to 30–35% seed moisture by weight), canola was swath, and allowed to cure and ripen from 10–14 d in the swath before combining. When the seed temperature and moisture content dropped to a safe storage level (<9% by weight) and most seeds were mature with no visible green color, the seeds were collected and stored in tight storage bins. Canola seeds were then processed for their oil with a ‘horizontal press’ and ‘extruder’ (Insta-Pro, Int., Des Moines, IA) at a conservative rate of 2.7–4.5 metric tons of seeds per day at Red Rock Ranch. With this equipment the seeds were put through screw presses or expellers (‘horizontal press’), which mechanically removed the liquid oil from the seeds. The resultant “press cake” was then further processed by the ‘extruder,’ which utilized friction as the sole source of heat accompanied by pressure and attrition. This process ruptured remaining oil cells and results in the additional recovery of oil. The resulting “extrudate” was then pressed one more time for any unrecouped oil with the ‘horizontal press.’  All recovered oil was stored in air-tight tanks, allowing for natural separation of canola oil and any remaining residual particles. At this stage, the resulting cake meal from pressing and extruding canola seeds from the ‘press’ and the ‘extruder’ was now available for use as part of the animal feed ration. Sub-samples were taken from the meal and its quality was analyzed for nutritional parameters (A & L Analytical Laboratory, Modesto, CA; Great Lakes Scientific, Inc. Stevensville, MI; and Barrow-Agee Laboratories; and A. Keck, Illinois State University, IL) for Se (already described by Bañuelos and Akohoue, 1994), and for micro and macronutrients after wet acid digestion by the inductively coupled plasma spectrometer (Perkin Elmer Plasma 2000 Emission Spectrometer).

3. Results and discussion

A Se management strategy with crops like canola and broccoli is dependent on the ability of the crops to accumulate Se under increasing salinity conditions and to tolerate increasing soil B concentrations in the soil (Rhoades, 1984). Stand establishment for both crops was excellent. Vegetative yields for canola were comparable to yields reported with good quality water (Bañuelos et al., 2002a), while fresh weight floret yields for broccoli were on the average, 35% lower than typical yields of broccoli grown in Central California. Since sulfate and selenate are biogeochemical analogs in soil, the high sulfate concentration in the drainage water likely inhibited plant uptake of selenate and kept the plant Se concentrations under 7 mg kg−1 DM (Table 1). The phytoextraction and biovolatilization of Se (Zayed et al., 2000) by canola and broccoli apparently not only removed Se that has accumulated on the soils after irrigation with Se-laden drainage water, but harvesting the Se-enriched crops, e.g., broccoli florets, produces cash value crops for the grower. In this regard, the Se concentrations measured in the broccoli florets (<5 mg kg−1 DM) were well below potentially toxic concentrations for human consumption (note: total Se content cannot be used as an indication of selenium’s efficacy, but knowledge of the individual seleno-compounds is necessary to fully assess its significance). Because Se is also an essential trace nutrient for humans (the US Food and Drug Administration) recommends approximately 200 μg Se on a daily basis), and it is known as an antioxidant, capable of depressing anticancer activity (Clark et al., 1996; Whanger, 2004), using broccoli as a recipient for Se-laden effluent may render this high value crop a potential source of supplemental dietary Se for humans. Extensive research on metabolism and potential, health, benefits of Se-enriched broccoli is being conducted by J.W. Finley at the Human Research Center in Grand Forks, ND. These products include using the Se-enriched tissue, e.g., broccoli floret, leaves, and canola seed, as supplemental sources of Se for humans, animals, plants, and canola oil, to be used as a blend with diesel fuel.

As an essential trace element in animal nutrition, Se deficiencies are generally a far greater problem than Se toxicities.
in livestock animals in the United States. Canola has long been used as a forage crop for grazing or silage (Bell, 1995). In this regard, Bañuelos and Mayland (2000) substantially improved the Se status of lambs (Ovis aries), cattle (Bos taurus), and rabbits (Bañuelos et al., 2002c) by mixing Se-enriched vegetative material (plant tissue from canola plants previously used as recipients of Se-laden drainage water) with other animal feedstuffs. Although the 6 mg ha⁻¹ (Table 1) (mg Se kg⁻¹ DM) recipients of Se-laden drainage water) with other animal feedstuffs. Although the 6 mg ha⁻¹ DM of broccoli leaves produced in this study were not used in any animal feed study (Table 1), Wiedenhoeft and Barton (1994) have also shown that broccoli leaves were more comparable to a concentrate than traditional forage because of their low fiber and high protein content. Hence, Se-enriched broccoli leaves could also be considered for blended use in animal forage.

Canola seed yields were generally 2 mg ha⁻¹ (Table 2). Under ideal conditions, canola seeds yield between 35–40% oil. Using the ‘horizontal press/extruder’ we managed to extract 50% of the available oil (more fine tuning of new equipment is required before we can extract more of the potentially available oil). Table 2 shows yields of seeds, cake meal, and canola oil available for blending with diesel fuel for the production of biofuel at different oil to diesel fuel ratios typically used within the biofuel industry. Percentages of canola oil (%) mixed with diesel fuel are designated as B100 (100% canola oil), B10 (10% canola oil), and B5 (5% canola oil). Since the canola oil was not transesterified (a process whereby the triglyceride fraction of the oil was converted to methyl ester, with the co-production of glycerine), the oil is not certifiable ASTM quality biofuel. In place of the transesterification process, a DFX (diesel fuel extender) amendment (Maxi-Lube, Inc) was added on-site to the blend of canola oil and diesel fuels to improve lubricity and prevent coking or plugging in the diesel engines. The canola oil is mixed with diesel fuels at varied ratios (ranging from 5–20%), and will be used to power 125 HP John Deere tractors on Red Rock Ranch (presently in progress).

Presently Red Rock Ranch consumes 760,000 liters of diesel annually on its 1875 ha ranch. The use of blended biofuels may result in a reduction in certain atmospheric pollutants (e.g., particulate matter, carbon monoxide, volatile organic compounds), as well as extend the life of engines due to increased lubricity (future investigations will examine both performance and emission quality from different ratios of blended biofuel). Clearly a partial switch from conventional diesel fuels to blended biodiesels within the agro-industry in Central California gives growers using canola as a Se phytoremediation crop, the first-hand ability to improve air quality in sensitive air quality regions, e.g., San Joaquin Valley, CA. Because of mild climate and warm temperatures occurring in Central California, we can expect less “cold-temperature behavior”, e.g., gelling and hardening, of the blended biodiesel. Biodiesel must flow easily at ambient temperatures from the tank, through filters and pumps, to the engine injection nozzles. Hence, preference is given to oils like canola oil with low saturation and low polyunsaturation, and a favorable fatty acid profile. With the introduction of newly-developed specifications for biofuels and the eventual desire to use or sell the blended biofuel for all types of engines off the farm site, it will be imperative that the best quality biofuels be produced with the transesterification process (Dorado et al., 2004).

In addition to utilizing the vegetative parts of the plant for animal forage, Se-enriched seed meal, the major by-products resulting from the oil extraction processes of seed crushing,

Table 1
Fresh and dry weights and tissue Se concentrations for canola and broccoli irrigated with drainage water during two growing seasons

<table>
<thead>
<tr>
<th>Crop/organ</th>
<th>Fresh weight yield (mg ha⁻¹)</th>
<th>Dry weight yield (mg ha⁻¹)</th>
<th>Se concentration (mg Se kg⁻¹ DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st year</td>
<td>2nd year</td>
<td>1st year</td>
</tr>
<tr>
<td></td>
<td>1st year</td>
<td>2nd year</td>
<td>1st year</td>
</tr>
<tr>
<td>Canola</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stems</td>
<td>44.7 (1.6)ab</td>
<td>52.2 (1.8)b</td>
<td>5.1 (0.32)a</td>
</tr>
<tr>
<td>Leaves</td>
<td>70.1 (2.7)a</td>
<td>78.2 (2.9)b</td>
<td>7.6 (0.39)a</td>
</tr>
<tr>
<td>Roots</td>
<td>8.5 (0.71)a</td>
<td>10.1 (0.83)a</td>
<td>2.3 (0.36)a</td>
</tr>
<tr>
<td>Broccoli</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stalks</td>
<td>34.7 (1.2)a</td>
<td>36.5 (1.6)a</td>
<td>3.4 (0.36)a</td>
</tr>
<tr>
<td>Leaves</td>
<td>62.7 (2.4)a</td>
<td>68.3 (1.1)b</td>
<td>6.0 (0.29)a</td>
</tr>
<tr>
<td>Florets</td>
<td>10.2 (0.42)a</td>
<td>12.5 (0.51)a</td>
<td>1.3 (0.21)a</td>
</tr>
<tr>
<td>Roots</td>
<td>10.1 (0.89)a</td>
<td>11.8 (0.78)b</td>
<td>2.1 (0.33)a</td>
</tr>
</tbody>
</table>

a Mean values and standard error in parenthesis were computed based on population density and plant yields described in Section 2.
b Within each row for each respective fresh weight yield, dry weight yield, and Se concentration, means followed by the same letter are not significantly different at the P < 0.05 level by Duncan’s multiple range test.

c B20 is 20% canola oil blended with 80% diesel.
d B5 is 5% canola oil blended with 95% diesel.

d B5 is 5% canola oil blended with 95% diesel.

e B10 is 10% canola oil blended with 90% diesel.

Table 2
Projected production of biofuel (without transesterification) from blending canola oil with diesel fuel based upon average seed yield and extracted canola oil

<table>
<thead>
<tr>
<th>Seed yield</th>
<th>Yields after oil extraction:</th>
<th>Projected production of biofuel</th>
</tr>
</thead>
<tbody>
<tr>
<td>kg ha⁻¹</td>
<td>Seed meal</td>
<td>Canola oil</td>
</tr>
<tr>
<td>4000</td>
<td>B100</td>
<td>B20b</td>
</tr>
<tr>
<td>2600</td>
<td>B100</td>
<td>B20b</td>
</tr>
<tr>
<td>1400</td>
<td>B100</td>
<td>B20b</td>
</tr>
<tr>
<td>1538</td>
<td>B1538</td>
<td>15,380</td>
</tr>
<tr>
<td>7690</td>
<td>15,380</td>
<td>30,760</td>
</tr>
<tr>
<td>15,380</td>
<td>15,380</td>
<td>30,760</td>
</tr>
<tr>
<td>30,760</td>
<td>30,760</td>
<td>30,760</td>
</tr>
</tbody>
</table>

a B100 is 100% canola oil. Canola seed contains 35% oil with a density of 0.91 g/mL.
b B20 is 20% canola oil blended with 80% diesel.
c B10 is 10% canola oil blended with 90% diesel.
d B5 is 5% canola oil blended with 95% diesel.
pressing, and extruding, is of high nutritional quality for use as part of a feed ration. Canola meal is one of the most widely traded protein ingredients around the world. Dairy cows readily consumed the Se-enriched cake-like meal as part of their daily feed ration in a preliminary feed trial in Central California (unpublished, Bañuelos). Results in Table 3 show that nutrient concentrations, except Ca, are more than adequate for the meal when compared to other reported values for grass and or legume forage (National Research Council, 1985). More importantly, the Se concentration in the meal was less than 2 mg kg$^{-1}$ DM. Dietary requirements for Se generally range from 0.1 to 0.3 mg kg$^{-1}$ DM (National Research Council, 1985). The ADF (acid detergent fiber; 13%) and NDF (neutral detergent fiber; 23%) values are lower than typical values for good quality legume, grass and legume grass hays and silages (National Research Council, 1985). However, the CP (crude protein; 27%) and CF (crude fat; 21%) values are slightly better than legume hays. The low glucosinolate content in the meal of 21 μmol/g is an important quality of the seed meal (Table 3), because the glucosinolates break down into toxic aglucones and their bitter taste results in reduced feed intake.

An additional potential use of the resultant meal cake from the oil extraction process is its application as a soil conditioner or organic source of N (work is currently in progress). With a total N content greater than 4% (by weight) in the seed meal (Table 3), the slow release of N makes the plant ideal as a source of green manure for supplying crops with N. Similarly, earlier research by Bañuelos and colleagues demonstrated that incorporating Se-enriched vegetative Brassica material to soils as an organic source of Se, increased plant Se concentrations in alfalfa and other forages under greenhouse and field conditions, respectively (Bañuelos et al., 1991, 1992; see Table 4). By incorporating the Se-enriched Brassica material into soils supporting known forage crops, the livestock grower almost guarantees that the animals would readily feed upon the Se-enriched forage.

### 4. Conclusion

Developing successful phytoremediation strategies is dependent on selecting plants or crop rotations that are most effective for removing or stabilizing the potential contaminant, e.g. Se, from the soil or waters over a long period of time. When possible, potential plant candidates should also be evaluated for the ability to realistically produce products that may have economical value as a food supplement, soil conditioner or fuel additive economical resource. Chances for widespread acceptance and usage of phytoremediation could exacerbate if there are any marketable products from the harvested plant. Using Brassica plants like canola and broccoli for the remediation of Se under field conditions could result in phyto-products enriched in an essential trace element in broccoli, feed meal, organic fertilizer, and produce oil that can be used as a biofuel additive.
Table 4
Projected dry matter yield and selenium concentration of different plant species planted in field soils incorporated with Se-enriched Brassica material

<table>
<thead>
<tr>
<th>Species</th>
<th>Yield (mg ha(^{-1}))</th>
<th>Average Se concentration (mg kg(^{-1}) DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tall fescue</td>
<td>1.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>4.4</td>
<td>7.7</td>
</tr>
<tr>
<td>Birdsfoot trefoil</td>
<td>4.7</td>
<td>3.9</td>
</tr>
<tr>
<td>Canola</td>
<td>4.9</td>
<td>51.0</td>
</tr>
</tbody>
</table>

\(^{a}\) Tall fescue (*Festuca arundinacea*, Shreb. Var. Alta), alfalfa (*Medicago sativa* L. var.), birdsfoot trefoil (*Lotus corniculatus* L. Var. Empire), and canola (*Brassica napus* L. var. *Westar*).

\(^{b}\) Values based upon actual yield and projected plant density of 100,000 plants.

\(^{c}\) Values for perennial species consist of only two clippings.

\(^{d}\) Values based upon whole plant.

\(^{e}\) Selenium analysis was performed on leaf.

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References


