CHAPTER 44

Soybean Mineral Composition and Glyphosate Use

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CHAPTER POINTS

- Considering the global importance of GR crops and glyphosate, the small amount of published data on effects of glyphosate on mineral nutrition of GR soybeans is surprising.
- Any herbicide could influence almost all physiological parameters, including mineral nutrition, at a high enough dose in a susceptible species.
- Although glyphosate will chelate mineral cations, it is a weak chelator compared to endogenous, natural chelators found in plants (e.g., review by Duke *et al.*, 2012b).
- The critical question is whether glyphosate significantly affects mineral content of the seed of GR soybean when used at recommended rates in the field. Unfortunately, there are only two studies that address this question directly (Table 44.2), and these studies found no effects on N and several minerals.
- Zobiole *et al.* (2010a–d, 2011, 2012) consistently find that glyphosate is harmful to GR soybeans in many other ways than reducing mineral content, e.g., strong inhibition of photosynthesis and growth. This is inconsistent with the findings of many researchers that glyphosate application to GR soybeans at recommended rates does not influence growth or yield (e.g., Duke *et al.*, 2012a; Bellaloui *et al.*, 2008; Henry *et al.*, 2011; Reddy *et al.*, 2000; Reddy and Whiting, 2000) and that GR soybean is approximately 50-fold less sensitive to glyphosate than non-GR soybeans (Nandula *et al.*, 2007).
- Furthermore, the averaged linear increases in USA soybean yields since GR soybeans were introduced in 1996, argues against harmful effects to GR soybeans by glyphosate.
- Nevertheless, rigorous field studies of the effects of glyphosate on the mineral content of GR soybeans in different soil types and climates are needed to establish with certainty whether glyphosate can significantly alter the mineral nutritional value of GR soybeans under any conditions.

INTRODUCTION

Glyphosate [*N*-(phosphonomethyl) glycine] has become the most widely used herbicide in the world, in large part because of it use in transgenic, glyphosate-resistant (GR) crops (Duke, 2012; Duke and Powles, 2008, 2009). This herbicide is considered by some as the most perfect herbicide ever devised (Duke and Powles, 2008). It is non-selective; that is, it is effective on almost all plant species. It is inactivated by soil, so it can only be used as a postemergence, foliar spray. Once taken up by the plant, it is readily translocated via the phloem, and to a lesser extent the xylem, to metabolically active tissues, such as meristems and any other growing parts of the plant (reviewed by Duke, 1988). Compared to herbicides introduced before glyphosate, it is slower acting, giving time for the herbicide to translocate after being taken up. This allows all growing meristems to be killed, so that regrowth of the weed does not occur.

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Glyphosate is a non-halogenated glycine analogue that kills by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) of the shikimate pathway (reviewed by Duke *et al.*, 2003). The shikimate pathway produces essential aromatic amino acids (phenylalanine, tyrosine, and tryptophan). In addition to causing shortages of these amino acids, glyphosate deregulates the shikimate pathway, drawing in critical carbon backbones from carbon fixation to go into shikimic acid and its derivatives, resulting in inhibited photosynthesis and other metabolic disruption. Furthermore, many of the plant defenses to pathogens are dependent on products of the shikimate pathway, so glyphosate-treated plants are highly susceptible to plant diseases to which they are exposed (reviewed by Duke *et al.*, 2007). Thus, glyphosate predisposes weeds to "biocontrol" by plant pathogens. Glyphosate is less effective in sterile soil and much more effective in the presence of plant pathogens (Schafer *et al.*, 2012).

Animals do not have EPSPS, and glyphosate is considered a very safe herbicide in terms of mammalian toxicity (Williams *et al.*, 2000). In fact, its LD_{50} for rats is higher than that of aspirin or table salt, and the vast preponderance of long-term studies show no chronic effects at doses higher than one might expect to obtain from the highest allowable residues in food. The ecotoxicological risk of glyphosate is also considered to be quite low (Geisy *et al.*, 2000).

Several recent papers have claimed that GR soybeans, including both the plant vegetation and the harvested seed, are deficient in certain minerals, particularly Mn (reviewed by Duke *et al.*, 2012a,b). Some of these minerals are important for proper mineral nutrition of the animals (livestock and humans) that consume them. This chapter discusses these claims in the context of the entire body of relevant published research.

SOYBEAN NUTRITIONAL VALUE (IN GENERAL)

Soybean is one of the most widely grown oilseed crops in the world and in particular the USA. In 2010, about 265 million tons of soybean were produced across the world, of which about 91 million tons—nearly a third—were produced in the USA (USDA, 2012a). Soybean is primarily used for the oil and meal products as well as whole bean products. A limited amount of soybean is grown for use of the entire above ground plant as forage for cattle and other ruminants. Soybeans form a very important part of human and animal diet in many parts of the world. Soybean seed consist of about 40% protein, 22% oil, 33% carbohydrates, and 5% ash of the dry weight of soybean seed (USDA, 2011). In addition soybean also contains minerals, vitamins, and isoflavones. Among the major mineral components in soybean, K (17970 ppm) is found in the highest concentration followed by P (7040 ppm), Mg (2800 ppm), Ca (2770 ppm), and S (1690 ppm). The minor minerals present in soybeans are Fe (157 ppm), Zn (49 ppm), Mo (30 ppm), B (26 ppm), Mn (25 ppm), Na (20 ppm), Cu (16 ppm), and Se (0.2 ppm) (Samarah *et al.*, 2004; USDA, 2011).

Soybean seed composition can be affected by genetic diversity, environmental factors, and agronomic production practices as well as interaction between these factors. Pesticides are routinely used for weed, insect, and plant pathogen control in soybean production. Since the introduction of GR soybean, glyphosate has become the most widely used herbicide for weed control in soybean. There has been speculation and some published data to suggest that glyphosate affects accumulation of minerals in GR soybean by affecting plant growth and nutrient uptake and mobility in the plant (summarized by Duke *et al.*, 2012b). The effect of glyphosate on soybean mineral composition is summarized below.

COMMERCIALIZATION OF GLYPHOSATE-RESISTANT SOYBEAN

Since its commercialization in 1974, glyphosate has been used extensively throughout the world as a nonselective, systemic, broad-spectrum, postemergence herbicide. Because of its lack of selectivity, its use in soybean crops was initially limited to preplant, post-directed, and post-harvest applications for weed control. There was limited use of glyphosate as a harvest aid to kill and desiccate the soybean crop before harvest. GR soybean was created by stable integration of a transgene (*cp4 epsps*) from an *Agrobacterium* species that encodes glyphosate-insensitive EPSPS. Expression of insensitive EPSPS enzyme in GR soybean allows maintenance of normal aromatic amino acid levels in GR soybean treated with glyphosate.

In the USA, GR soybean cultivars were first commercialized for planting in 1996. GR soybean, commercially known as 'Roundup Ready®' soybean, remains unaffected when treated with recommended rates of glyphosate for weed management. Transgenic soybean varieties made resistant to the herbicide glufosinate [(±)-2-amino-4-(hydroxymethylphosphinyl) butanoic acid] are also available in the USA, but their adoption rate has been only a small fraction of that of GR soybeans. The most sensitive indicator of an effect of glyphosate on the shikimate pathway is elevated shikimate levels (Singh and Shaner, 1998), and glyphosate-treated soybeans do not have this symptom (e.g., Reddy *et al.*, 2004). GR soybeans are approximately 50-fold less sensitive to glyphosate than non-GR soybeans (Nandula *et al.*, 2007).

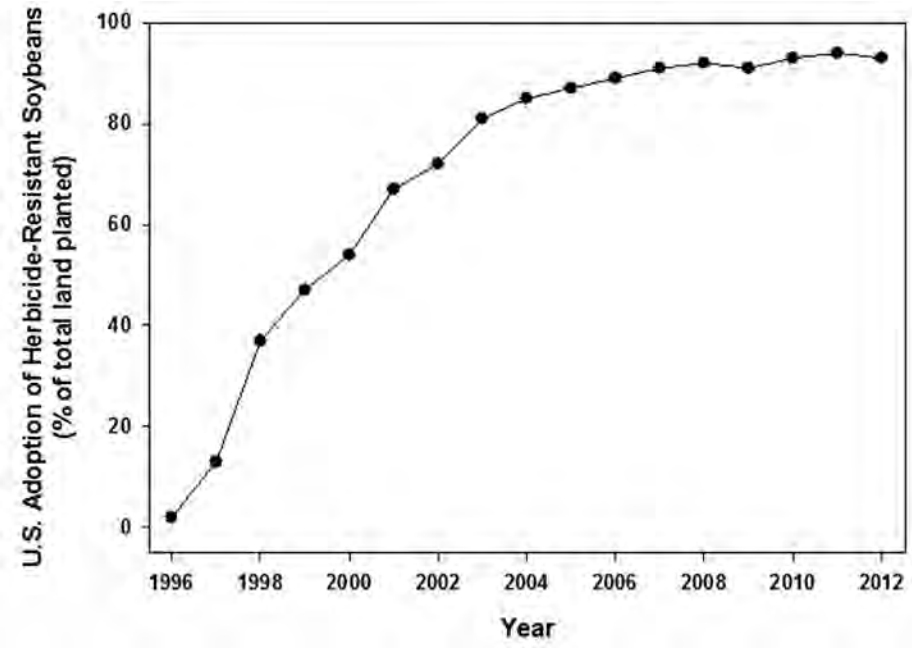

FIGURE 44.1 Percent of herbicide-resistant soybeans planted since GR soybeans were introduced in 1996. *Data are from the U.S. Economic Research Service of the United States Dept. of Agriculture (http://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us.aspx).*

GR soybean has been widely adopted by farmers in North America and in several other countries, particularly Brazil and Argentina. The soybean area planted with GR cultivars has increased from 2% in 1996 to 93% in 2012 (USDA, 2012a) (Figure 44.1). Globally, 75.4 million ha or 75% of soybean area was planted to GR soybean cultivars in 2011 (James, 2011). GR soybeans cover more area than any other transgenic crop.

Where available, this technology was adopted more rapidly than any other agricultural technology in history. There were multiple reasons for this, including economic gains, superior weed control, and simplicity and flexibility of the glyphosate/GR soybean technology (Gianessi, 2008; Duke and Powles, 2009; Hurley *et al.*, 2009; Fernandez-Cornejo *et al.*, 2012). Furthermore, in addition to other environmental benefits (Brookes and Barfoot, 2010) adoption of this postemergence herbicide technology allowed farmers to reduce or eliminate tillage, leading to the environmental benefits of reduced soil erosion by wind and water (Cerdiera and Duke, 2006; Givens *et al.*, 2009) and reduced expenditure of fossil fuels (Brookes and Barfoot, 2012). Soybean yields in the USA have continued to increase since GR soybeans were introduced in 1996 (Duke *et al.*, 2012b).

GLYPHOSATE USE ON GR SOYBEAN

The rapid adoption of GR soybean by farmers has shifted herbicide use patterns in favor of glyphosate. Glyphosate is used either alone or with other herbicides for managing weeds in GR soybean. As glyphosate use in soybeans increased, the use of other herbicides decreased concomitantly in the USA (Reddy, 2001). In the USA, the total active ingredient of glyphosate use has increased in soybean from 2.9 million kg/year in 1995 (the year before GR soybean was commercialized) to 41.7 million kg/year in 2006 (USDA, 2012b). This represents a 14-fold increased use of glyphosate in GR soybean since commercialization of GR technology. Also, the average amount of glyphosate use has increased from 0.68 kg ai/ha/year in 1995 to 1.49 kg ai/ha/year in 2006, and the average number of glyphosate applications has increased from 1 in 1995 to 1.7 in 2006 in soybean production (USDA, 2012b). Increased use (both frequency and amount) of glyphosate in GR soybean has been of concern to some scientists because glyphosate is a metal ion chelator (reviewed by Duke, 1988; Duke *et al.*, 2012b), and, being a systemic herbicide, it could potentially affect mineral uptake and mobility in plants. Several researchers have examined the effects of glyphosate on mineral composition in soybean and their results are summarized below.

GLYPHOSATE DRIFT

Glyphosate is usually applied multiple times in a year, both as a preplant treatment to kill existing vegetation before planting spring-seeded crops and as one or more postemergence treatments in GR crops using ground or aerial equipment. As with all pesticide applications, a small fraction of glyphosate can drift downwind and can be deposited on non-target plants. Glyphosate drift is of particular concern because it is a non-selective herbicide and can cause toxic effects on sensitive plant species at low doses. In 2012, 93% of soybean hectareage was planted to GR soybean in the USA. Seventeen years after introduction of GR soybean, about 7% of soybean area is still planted to non-GR cultivars. Glyphosate drift complaints are common in the Mississippi delta; 55 cases of glyphosate drift onto non-target crops were reported in Mississippi in 2011 (Vollor, L. T. Mississippi Department of Agriculture and Commerce, personal communication, 2012). In a simulated drift study, 12.5% of the common use rate of 0.84 kg ae/ha glyphosate caused chlorosis and decreased nitrogen fixation and assimilation, but had no affect on yield of non-GR soybean (Bellaloui *et al.*, 2006). This study did not examine effects on mineral nutrition of the crop.

There has always been a problem with drift of herbicides from sites of intended use to sensitive crops. The mode of action of no herbicide is directly associated with plant mineral nutrition (Fedtke and Duke, 2005), but any herbicide to which a crop is sensitive will secondarily influence almost all physiological functions of the crop, including mineral nutrition, at a high enough dose. The next section examines the potential effect of glyphosate at drift concentrations on non-GR soybean.

MINERAL COMPOSITION IN NON-GR SOYBEAN

We should emphasize that glyphosate is not applied directly to non-GR soybean for weed management. Thus, non-GR soybean exposure to glyphosate is limited only to off-target drift from ground and aerial applications. Several researchers have examined the direct application of glyphosate at potential drift rates on soybean and observed physiological and metabolic disturbances, however, soybean recovered over time without reduction in yields (Bellaloui *et al.*, 2006; Cakmak *et al.*, 2009; Reddy *et al.*, 2000). Effects of foliar-applied glyphosate on mineral content of non-GR soybean are summarized in Table 44.1. Glyphosate applied at a drift rate of 105 g ae/ha had no effect on leaf N content in non-GR soybean under field conditions (Bellaloui *et al.*, 2006). In a greenhouse study, glyphosate applied at 189 μM had no effect on P, K, Ca, Zn, and Cu content, but decreased Mg and Mn in non-GR soybean (Cakmak *et al.*, 2009). Glyphosate effect on leaf Fe was mixed, with no effect in a greenhouse study (Cakmak *et al.*, 2009), but with decreases in a field study (Bellaloui *et al.*, 2009).

Glyphosate increased concentrations of K, Zn, and Cu, reduced concentrations of Ca, Mg, Fe, and Mn, and had no effect on P in non-GR soybean seed (Bellaloui *et al.*, 2009; Cakmak *et al.*, 2009) (Table 44.1). Glyphosate effects on non-GR soybean seed N were mixed, with no effect in a field study (Bellaloui *et al.*, 2006) and increases in a greenhouse study (Cakmak *et al.*, 2009). The decreased concentrations of Fe, Mg, and Mn in glyphosate treated non-GR soybean leaves and seeds were attributed to reduced root uptake and reduced translocation, possibly due to formation of weak glyphosate-metal complexes, although this mechanism is not supported by what we know of the relatively weak chelating properties of glyphosate, compared to endogenous, natural phytochemical chelators found in plants (summarized by Duke *et al.*, 2012b). Foliarly-applied glyphosate translocates rapidly to roots, where it inhibits growth and other processes. Since young, growing roots are responsible for mineral uptake from soil (e.g., Harrison-Murray and Clarkson, 1973), it is not surprising to find that glyphosate can have significant effects on mineral nutrition in sensitive plants. Because glyphosate is not directly used in non-GR soybean, literature is sparse on glyphosate effects on mineral content in non-GR soybean.

MINERAL COMPOSITION IN GR SOYBEAN

The published literature on this topic is conflicting and inadequate. The first information available to the public on this topic was that generated as part of the required compositional equivalence studies for GR crops by a company that sells GR soybean seed. Such studies, using good laboratory practices and at multiple locations, are required by USA regulatory agencies to assess potential effects of the glyphosate resistance transgene on the crop. None of these studies found any effect of the transgene conferring glyphosate resistance on mineral nutrition of GR crops (reviewed by Duke *et al.*, 2012b). An university study by Loecker *et al.* (2010) found no effect of the transgene on Mn uptake or any response to Mn in the absence of glyphosate. Unfortunately, the two such published studies on GR soybean treated with glyphosate do not provide mineral data (Harrigan *et al.*, 2007; Lundry *et al.*, 2008), making it impossible to determine whether any exposure of GR crops to glyphosate affects mineral composition of the crop. In studies with other crops (e.g., maize; Ridley *et al.*, 2011) treated with glyphosate, mineral levels (Ca, Cu, Fe, Mg, Mn, P, K, and Zn) were within the 99% tolerance level of conventional, non-GR hybrids.

There are several studies that only dealt with the effects of glyphosate on potted GR soybeans. Such studies cannot be extrapolated to field conditions because the root system of potted plants is limited by the pot, resulting in mineral uptake that is unlike what occurs in the field. Results of such studies are variable. For example, Bott *et al.* (2008) found that glyphosate reduced Mn and Zn in vegetative GR soybean tissues when applied at 0.9 and 1.8 kg ae/ha. Similarly, Zobiole *et al.* (2010c, d) found that application of 0.6 kg ae/ha applied twice or 1.2 kg ae/ha applied once reduced Zn, Mn, Fe, B, Ni, and even Ca in leaves of greenhouse-grown GR soybeans. Zobiole *et al.* (2010b)

TABLE 44.1 Glyphosate Effect on Components in Non-Glyphosate-Resistant (Non-GR) Soybean Leaves and Seed

	Non-GR Soybean Leaves			Non-GR Soybean Seeds			
Components	**Non-treated**	**Glyphosate treated**	**Glyphosate effect on component**	**Non-treated**	**Glyphosate treated**	**Glyphosate effect on component**	**Reference**
N (%)	5.48	5.56	No effect	5.97	6.02	No effect	Bellaloui *et al.* (2006)
N (%)	NA	NA		4.6	6.2	Increased	Cakmak *et al.* (2009)
P (g/kg)	2.3	2.3	No effect	5.9	6.1	No effect	
K (g/kg)	26.3	25.2	No effect	14.1	14.7	Increased	
Ca (g/kg)	22.3	15.6	No effect	3.9	2.9	Decreased	
Mg (g/kg)	8.8	5.8	Decreased	2.4	2.1	Decreased	
Fe (mg/kg)	49	40	No effect	71	36	Decreased	
Mn (mg/kg)	232	121	Decreased	56	31	Decreased	
Zn (mg/kg)	93	65	No effect	44	58	Increased	
Cu (ppm)	5.5	4.1	No effect	11	13	Increased	
Fe (mg/kg)	150	57	Decreased	85	54	Decreased	Bellaloui *et al.* (2009)

TABLE 44.2 Reported Effects of Glyphosate Treatment on Mineral Content of Glyphosate-Resistant (GR) Soybean Seed in Field Studies

Glyphosate Treatment	**Effects**	**Reference**
1.12 or 3.36 kg ae/ha at 4 and 6 weeks after planting	No effect on seed N content	Bellaloui *et al.* (2008)
0.86 kg ae/ha at both 3 and 6 weeks after planting	No effects on Mg, Ca, K, Sr, Ba, Mn, Fe, Ni, Cu, Zn, Cd, Cr, Co, or Se content in soybean seed	Duke *et al.* (2012a)

found 1.2 kg ae/ha of glyphosate applied 30 days after sowing GR soybean in the greenhouse to reduce P (−16%), Ca (−18%), Mn (−22%), Zn (−4%), and Cu (−13%) in immature seed. There were no effects on N, K, Mg, S, Fe, or B. In an almost identical study Zobiole *et al.* (2010a) found linear decreases in Fe, Zn, Mn, Fe, Cu, Mo, Co, B, Cu, Mg, S, Ca, P, N, and K in foliage of greenhouse-grown, GR soybeans treated with linear increases in glyphosate dose (0.6 to 2.4 kg ae/ha). Serra *et al.* (2011) found no effect on the efficiency of uptake or translocation of N, Mn, Cu, Zn, and Fe in vegetation of soybean plants treated with up to 2.6 kg ae/ha glyphosate, but growth reductions resulted in reductions in the amount of mineral per plant. N, P, K, Ca, Mg, S, Zn, Mn, Fe, and Cu were reported to be reduced in foliage of second generation GR soybeans with doses of 0.8 to 2.4 kg ae/ha in the greenhouse (Zobiole *et al.*, 2011). In an extensive greenhouse study, six different formulations of glyphosate, each applied at 0.96 kg ae/ha to two different GR cultivars, gave mixed results on N, P, K, S, B, Ca, Mn, Fe, Mg, Zn, and Cu content of vegetative tissues, with inconsistent increases and decreases in mineral concentrations (Cavalieri *et al.*, 2012). In another greenhouse study, there were no effects of glyphosate on any of the minerals measured, including Ca, Mg, Zn, Fe, Cu, Sr, Ba, Al, Cd, Cr, Co, Ni, Mn, and Zn, in vegetative or harvested, mature seeds of plants treated twice with 0.86 kg ae/ha (Duke *et al.*, 2012a). Likewise, in studies done in Brazil (Rosolem *et al.*, 2010; Andrade and Rosolem, 2011) found no effect of glyphosate at 0.75–0.96 kg ae/ha on Mn in foliage of greenhouse-grown plants.

Unfortunately, there are only two field studies of glyphosate effects on mineral content of mature GR soybean seeds, and only one of these deals with minerals that form divalent cations. Results of these studies are summarized in Table 44.2. The details of the only study on metallic mineral content are provided in Figure 44.2. There were no effects of glyphosate in either study. Other field studies have only examined effects of glyphosate on mineral content of GR soybean vegetative tissue. In one of these studies, Henry *et al.* (2011) found 0.84 kg ae/ha to cause no consistent effects on N, P, K, S, Mg, Ca, B, Zn, Mn, Fe, Cu, and Al. They concluded that there was no evidence of mineral deficiencies. Similarly, Stefanello *et al.* (2011) found no effect of three applications of glyphosate totaling 2.4 kg ae/ha on foliar content of Mn, Fe, Zn, or Cu. Ebelhar *et al.* (2006) found in a two-year field study that applications of glyphosate up to four times (3.46 kg ae/ha) the recommended rate had no effect on foliar Mn content. In the only field study reporting an

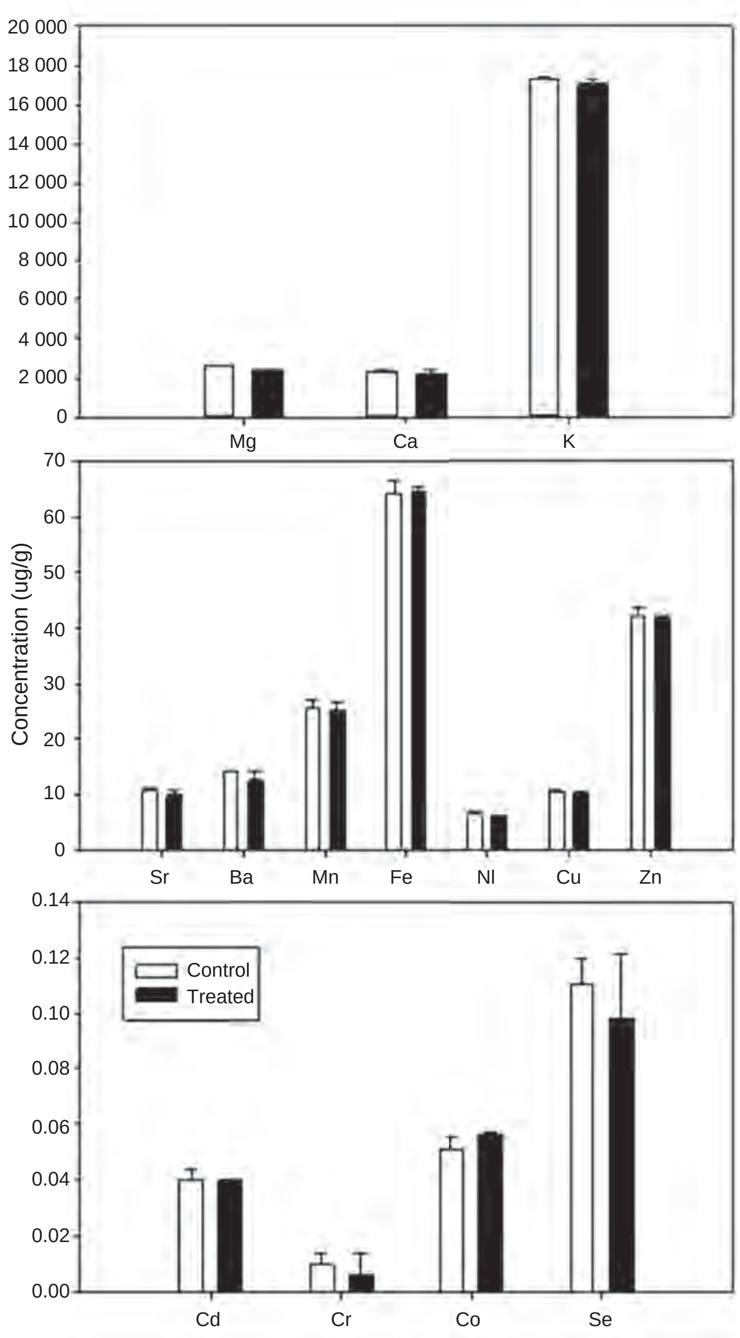

FIGURE 44.2 Effects of different two glyphosate treatments (0.86 kg ai/ha at both 3 and 6 weeks after planting) on metal content of mature seed of field-grown GR soybean plants. Error bars are ±1 SE of the mean. *Reprinted with permission from* Duke et al. (2012a)*, Copyright 2012, American Chemical Society.*

effect, Zobiole *et al.* (2012) reported that 0.8 to 2.4 kg ae/ha of glyphosate applied at 10, 20, and 34 days after sowing reduced N, P, K, Ca, Mg, S, Zn, Mn, Fe, Cu, and B in vegetative tissue.

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