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Heavy metal-induced oxidative stress on seed germination and seedling development: a critical review

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Abstract Heavy metal contamination in soils can influence plants and animals, often leading to toxicosis. Heavy metals can impact various biochemical processes in plants, including enzyme and antioxidant production, protein mobilization and photosynthesis. Hydrolyzing enzymes play a major role in seed germination. Enzymes such as acid phosphatases, proteases and α-amylases are known to facilitate both seed germination and seedling growth via mobilizing nutrients in the endosperm. In the presence of heavy metals, starch is immobilized and nutrient sources become limited. Moreover, a reduction in proteolytic enzyme activity and an increase in protein and amino acid content can be observed under heavy metal stress. Proline, is an amino acid which is essential for cellular metabolism. Numerous studies have shown an increase in proline content under oxidative stress in higher plants. Furthermore, heat shock protein production has also been observed under heavy metal stress. The chloroplast small heat shock proteins (Hsp) reduce photosynthesis damage, rather than repair or help to recover from heavy metal-induced damage. Heavy metals are destructive substances for photosynthesis. They are involved in destabilizing enzymes, oxidizing photosystem II (PS II) and disrupting the electron transport chain and mineral metabolism.

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Although the physiological effects of Cd have been investigated thoroughly, other metals such as As, Cr, Hg, Cu and Pb have received relatively little attention. Among agricultural plants, rice has been studied extensively; additional studies are needed to characterize toxicities of different heavy metals on other crops. This review summarizes the current state of our understanding of the effects of heavy metal stress on seed germination and seedling development and highlights informational gaps and areas for future research.

Keywords Antioxidant system · Starch mobilization · Proline · Protein degradation · Chlorophyll · Heavy metal stress

Introduction

Chemical waste resulting from industrial activities and agricultural effluents such as fertilizer, herbicides and pesticide has contributed to the increased accumulation of heavy metals in soil (Fu et al. 2014; Hu et al. 2013; Nicholson et al. 2003; Neilson and Rajakaruna 2015). Since heavy metals are non-degradable, they can cause long-term deleterious effects on ecosystem health. Copper (Cu), As and Cd are the major heavy metals present in agrochemicals while Hg, Cr, Pb and As are rich in industrially contaminated soils (Wuana and Okieimen 2011). Cadmium (Cd), Hg, Pb, Cu and As are some of the most toxic heavy metals or metalloids found in soil (Dago et al. 2014; Clemens 2006; Hadi et al. 2013). Cadmium, for example, is nonessential for plant metabolic activities and a known phytotoxicant (Gratão et al. 2005). The long biological half-life of Cd contributes to its mutagenic, cytotoxic and carcinogenic properties (Sánchez-Virosta et al. 2015; Waalkes 2003). Ore smelting, coal burning, industrial effluents, and As-containing herbicides and insecticides release As, a toxic metalloid, to the environment (Lin et al. 2016). Mercury (Hg), which is one of the most potent toxic heavy metals, is released mainly from mining activities (Lacerda 1997; De Lacerda and Salomons 2012); however, low amounts can also be found in agrochemicals (Saueia et al. 2013; Falkowska et al. 2013). Copper is released from several industrial activities and by the

excess usage of fungicides. Copper-containing fungicides and bactericides are used extensively for disease control in numerous crops. Lead (Pb), a metal that can remain in the soil for thousands of years (Kumar et al. 1995), can be released to the environment via mining activities (Bakırdere et al. 2016) and other industrial processes (Bénard et al. 2014). Although some heavy metals are considered as micronutrients (Cu, Zn, Mn, etc.), other metals and metalloids are highly toxic (Hg, Pb, Cd, As, etc.) and play no role in plant nutrition and their presence in high concentrations is highly toxic (Nagajyoti et al. 2010; Boyd and Rajakaruna 2013).

The organic matter and clay content and pH are the major factors governing the availability of heavy metals in soils (Zeng et al. 2011; Spurgeon and Hopkin 1996). A high proportion of humified organic matter (OM), i.e., mature compost, can also decrease the bioavailability of heavy metals, while increasing the residual fraction of heavy metals in the soils (Castaldi et al. 2005), which is mainly attributed to the precipitation caused by the complex formation (Bolan and Duraisamy 2003). Moreover, the large specific surface area of clay minerals contributes to higher immobilization of heavy metals in soil (Prost and Yaron 2001; Usman et al. 2005). As the soil pH is increased, the immobilization is also increased for many heavy metals, especially for those metals present as cations (i.e., Pb, Cd and Cr). Biochar application to soil, which increases the soil pH, has also demonstrated the effect of pH in heavy metal immobilization (Herath et al. 2015).

High concentrations of heavy metals in soil cause a number of deleterious effects on plants (Khan et al. 2000), such as growth retardation, destruction of chlorophyll, disorders in biochemical activities, mutations and reproductive disorders (Schützendübel and Polle 2002; Sharma and Agrawal 2005; Gall and Rajakaruna 2013). Seed germination, one of the most significant stages in a plant's life, is sensitive to chemical and physical conditions of the rhizosphere (Bewley 1997). Although the seed coat can act as a principle barrier limiting harmful effects of heavy metals, most seeds and seedlings show a decline in germination and vigorin response to heavy metal stress (Adrees et al. 2015), causing a major concern for agricultural and forestry practices. Therefore, the effects of heavy metals on germination and seedling growth are an important research area deserving

extensive study. Recent studies have documented that via inhibition of storage food mobilization, reduction in radical formation, disruption of cellular osmoregulation and the degradation of proteolytic activities, heavy metals cause inhibition of seed germination and seedling development (Adrees et al. 2015; Barceló and Poschenrieder 1990; Perfus-Barbeoch et al. 2002; Karmous et al. 2015; Baszyński 2014) (Fig. 1). However, several management practices have been utilized to minimize heavy metal stress on plants grown in contaminated soils. While physical and chemical technologies are useful in remediation of heavy metal contaminated soils (Dermont et al. 2008; Wu et al. 2010), the use of different amendments is one of the low-cost and efficient methods utilized to minimize the plant heavy metal uptake in contaminated soils; biochar, crab shells and organic matter are a few such amendments (Uchimiya et al. 2010). Amendments are used to immobilize the contaminants, thereby limiting the bioavailable fraction (Bandara et al. 2016, 2017; Herath et al. 2017; Kumarathilaka and Vithanage 2017). In addition to amendments, there are also internal mechanisms in place, which can limit the interaction of heavy metals in plant metabolism. Phytochelatins and metallothioneins are such proteins that may play vital roles in heavy metal detoxicity in microorganisms and plants (Shen et al. 2010).

This review focuses on the influence of heavy metals and metalloids (especially, Cd, Hg, Ag and As) on seed germination and seedling development in plants, focusing particularly on physiological and biochemical effects on germinating seeds and developing seedlings.

Effect of heavy metals on seed germination and seedling growth

Heavy metals are known to negatively influence seed germination and seedling development (Table 1). Cadmium leads to germination inhibition as documented by several studies. For example, when *Sorghum bicolor* (Poaceae) seeds were treated with a Cd concentration series (0.0, 0.5, 1.0, 2.0 and 3.0 mM), a significant reduction in germination was observed in the presence of the metal (Barceló and Poschenrieder 1990). The decrease in germination appears to result from the inhibition of physiological and metabolic activities of the seed. Since Cd is able to decrease the water stress tolerance of plants (Barcelo et al. 1986), it can cause a loss of turgor pressure at a higher relative water content compared to that of non-treated plants. The effect of Cd on plant water status has been examined with germinating rice seeds (Barceló and Poschenrieder 1990), showing that the water status of germinating rice seeds is highly
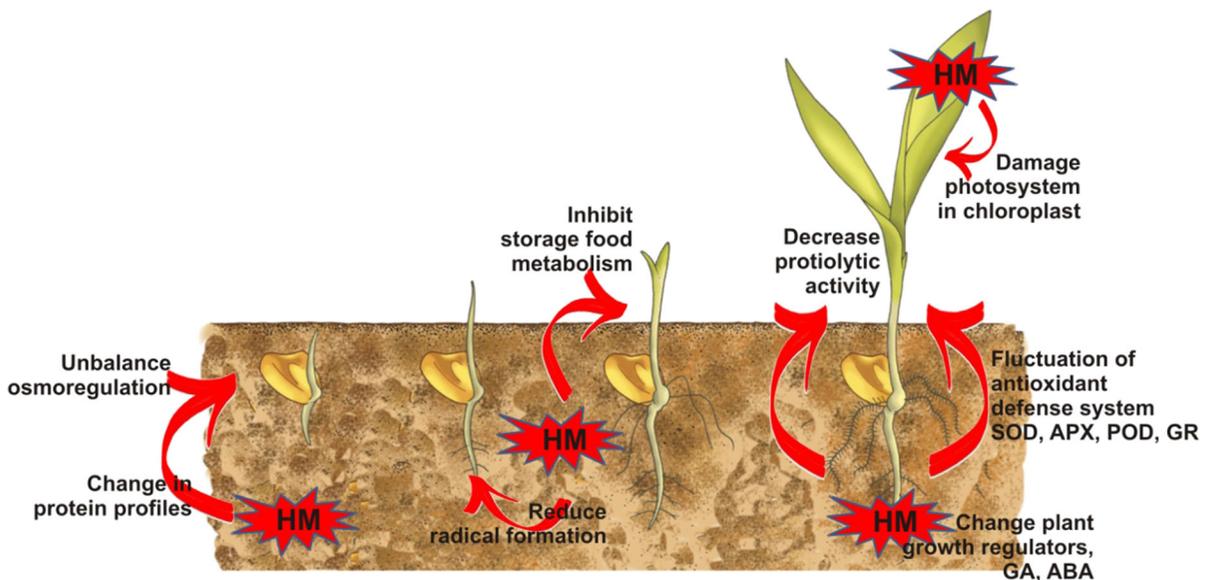

Fig. 1 Different effects of heavy metals on seed germination and seedling development

Table 1 A summary of studies examining the effects of heavy metals on seed germination and seedling development

Heavy metal	Species	Findings	References
Al	*Zea mays*	Reduction in seed germination was observed beyond 50 mg L^{-1}	Nasr (2013)
As	*Brassica oleracea*, *Amaranthus* sp., *Raphanus sativus*, *Daucus carota*	Reduction in germination was observed at 10 mg L^{-1}	Dutta et al. (2014)
Cd	*Triticum aestivum*	Cd showed toxicity at 5 mg L^{-1} in root and shoot growth. Seed germination and germination energy were affected at 20 mg L^{-1} which is aggravated by further addition of Cd from 50 to 80 mg L^{-1}	Ahmad et al. (2012)
	Oryza sativa	Cd shows a significant reduction in rice seed germination index, vigor index, root and shoot lengths as well as fresh weight compared to control at 100 μM	He et al. (2014)
	Picea omorika	1 mM concentration inhibited germination	Prodanovic et al. (2016)
	Solanum nigrum	Germination rate was dramatically reduced at 200–300 μmol L^{-1} Cd	Liu et al. (2012)
	Triticum aestivum	A decrease in germination was recorded from 20 mg Cd L^{-1}	Ahmad et al. (2013)
	Helianthus annuus	Gradual reduction in germination (2–10 mg L^{-1})	Imran et al. (2013)
	Triticum aestivum	Seed germination was stimulated at low concentrations (0–2.5 mg L^{-1}) while a reduction in germination was observed under high concentrations (5–30 mg L^{-1})	Mahdieh et al. (2013)
	Vigna radiata and *Glycine max*	1 mg kg^{-1} As addition stimulated seed germination and increased about 12% of the germination weight, but the seed germination was significantly suppressed when with As addition was over 5 mg kg^{-1}	Wan et al. (2013)
	Festuca rubra	No germination inhibition was observed at 25 mg L^{-1} while a reduction in germination was observed at 25 and 50 mg L^{-1}	Vázquez de Aldana et al. (2014)
	Brassica juncea	No significant effect was observed in seed germination up to 250 μM	Srivastava et al. (2013)
Cd, Pb, Cu	*Arachishypogeae*	Concentrations of 75 and 100 mg L^{-1}, Cd and Pb, respectively, decreased seed germination while Cu condensed seed germination at 100 mg L^{-1}	Abraham et al. (2013)
Cr	*Cucumis melo*	Reduction in seed germination as the concentration increased	Akinci and Akinci (2010)
Co, Cr	*Phaseolus vulgaris*	Reduction in seed germination at high concentrations (10^{-2} M)	(Zeid 2001)

Table 1 continued

Heavy metal	Species	Findings	References
Cu	*Oryza sativa*	The germination of seeds decreased as the concentration increased (0.2–1.5 mM)	Ahsan et al. (2007a)
	Triticum aestivum	The germination of seeds decreased from 5 mg L^{-1}	Singh et al. (2007)
	Zea mays	Increased seed germination at 0.1 mM	Bashmakov et al. (2005)
	Triticum aestivum and *Oryza sativa*	Reduced germination by more than 35 and 60%, respectively, with 10 µM	Mahmood et al. (2007)
	Medicago sativa	Inhibit seed germination by 39% with 40 mg L^{-1}	Aydinalp and Marinova (2009)
	Eruca sativa	0.3–1.2 mM did not decrease seed germination while lower Cu concentration (<0.7 mM) increased seed germination	Zhi et al. (2015)
Hg	*Vignaradiata*	No significant reduction in seed germination was shown at 1 mM; however, beyond 3 mM, a reduction in germination was observed	Muhammad et al. (2015)
	Albizia lebbeck	A significant gradual reduction in seed germination was observed beyond 1 mM	Iqbal et al. (2014)
	Cajanus cajan	Reduction in seed germination was observed beyond 5 mg L^{-1}	Patnaik and Mohanty (2013)
	Brassica napus	Reduction in seed germination was observed beyond 1 mM	Rezaei et al. (2013)
Ni	*Medicago sativa*	40 ppm inhibited seed germination by 24%	Aydinalp and Marinova (2009)
	Zea mays	Reduction in seed germination was observed beyond 50 mg L^{-1}	Nasr (2013)
Pb	*Triticum aestivum*	Increased germination rate and root mass with Pb concentration less than 50 mg L^{-1}	Kang et al. (2009)
	Lens culinaris	A significant reduction in germination was observed from 0.5 mM	Cokkizgin and Cokkizgin (2015)

affected by Cd. Since water imbibition is a major requirement for seed germination, the negative influence of Cd on the water content of the seed is significant (Barceló and Poschenrieder 1990). Similarly, an inhibition of root and coleoptile growth has been observed in seedlings under Cd stress (Kuriakose and Prasad 2008). Cadmium appears to cause an inhibition of carbohydrate hydrolysis and the translocation of hydrolyzed sugars, resulting in a reduction in seedling growth. Mercury (Hg) is also known to negatively influence seed germination and seedling growth (Muhammad et al. 2015). As *Vigna radiata* (Fabaceae) was treated with a Hg concentration series (1, 3, 5 and 7 mM), there was no significant reduction under 1 mM; however, the 7-mM treatment showed the highest reduction among all treatments, resulting in 42, 70, 66 and 47% reduction in seed germination, seedling length, root length and seedling dry weight, respectively. Though silver (Ag) has often being used in nanofertilizers, only a handful of studies have been conducted to assess the effects of Ag with respect to seedling growth. A study examining the effects of polyvinylpyrrolidone-coated silver nanoparticles (PVP-AgNP) on 11 wetland plants (*Loliummultiflorum, Panicumvirgatum, Carexlurida, C. scoparia, C. vulpinoidea, C. crinita, Eupatorium fistulosum, Phytolacca americana, Scirpus cyperinus, Lobelia cardinalis* and *Juncus effusus*) showed that Ag

nanoparticles had no effect on germination, while Ag 40 mg L^{-1} gum arabic coated silver nanoparticle (GA-AgNP) exposure significantly reduces the germination rate in three species (*S. cyperinus, J. effusus* and *P. americanum*) and enhances the germination rate of one (*E. fistulosum*) species (Yin et al. 2012). Arsenic (As), another toxic metalloid, is known to suppress plant growth (Han et al. 2015), showing significant negative effects on root and shoot length and root and shoot biomass of wheat seedlings; the growth of root and shoot was inhibited by high concentration of As (5–20 mg kg^{-1}), where the inhibition of root was stronger than that of the shoot (Wang et al. 2002). A reduction in starch granules in seeds reflects the mobilization of insoluble sugars; scanning electronic microscopic images can be used to analyze the immobilized starch content in seedlings (Singh et al. 2003); Kuriakose and Prasad (2008) have observed that under 0.5 mM Cd, starch granules further decrease, indicating more mobilization of starch. Whereas most of the starch were in seeds treated with 2.0 mM Cd, a significant reduction of acid phosphates was observed in both seed and seedling with 2.0 and 3.0 mM of Cd, respectively. Activity staining using densitometric scanning showed the reduction of an isozyme in all Cd-treated seedlings, whereas a reduction in total protease activity was observed in both embryonic axes and seeds compared to the control. Further, a significant decrease in rice seed germination index, vigor index, root and shoot lengths as well as fresh weight compared to control was observed in plants exposed to Cd (He et al. 2014). The study showed that the exogenous nitric oxide donor sodium nitroprusside (SNP) can balance the inhibitiry effects of rice seed germination and seedling growth.

Changes in antioxidant system

Cadmium is a toxic metal influencing seedling growth and development. A concentration series of Cd was used to determine Cd stress on the antioxidant system of rice seedlings (Ali et al. 2002). The fluctuation of antioxidant concentrations was different at varying Cd levels. Super oxide dismutase (SOD) and glutathionine reductase (GR) showed an increasing pattern in both leaves and roots. Ascorbate peroxidase (APX) and peroxidase (POD), however, showed an increase at low concentrations and a decline at high concentrations. Further, catalase (CAT) activity showed contrasting effects, with low concentrations in leaves and high concentrations in roots. A similar pattern of non-enzymatic and enzymatic antioxidants was detected with oilseed rape treated with Cd (Yan et al. 2015). Interestingly, interactive effects of Cd and Pb in rice seedlings cause a reduction in glutathione and ascorbate activity and their redox ratios, while causing an increase in activities of antioxidative enzymes, superoxide dismutase (SOD) and guaiacol peroxidase (Srivastava et al. 2014).

Acetyl salicylic acid (ASA) is another protective measure utilized by plants for removing oxygen free radicals. Under low As concentrations, the ASA content increases, whereas with an increase in As concentrations, the amount of oxygen free radicals increases (Li et al. 2007). With further increase in As concentration, the ASA content decreases in wheat seedling leaves in order to eliminate the oxygen free radicals (Li et al. 2007). Ascorbate peroxidase (APX) activity decreased slightly at low concentrations of As (0–5 mg kg^{-1}) and then increased as the concentration of As increased. The CAT activity displayed an increasing trend at lower concentrations (0–1 mg kg^{-1}) and an decreasing trend with increasing As concentrations (5–20 mg kg^{-1}). However, POD activity increased significantly with an increase in As concentrations. The enhanced production of antioxidant enzymes represents typical defense mechanism against As accumulation and toxicity (Li et al. 2007). Although many studies have shown changes in antioxidant systems with respect to heavy metals, the mechanisms of heavy metal influence on the antioxidants have not been explored. Additional studies should be carried out for a better understanding of antioxidant production under oxidative stress. Table 2 summarizes studies examining the effects of heavy metals on antioxidant properties of germinating seed.

Production of plant growth regulators

Plant growth regulators play an important role in seed germination. Gibberellic acid (GA) is a plant hormone controlling seed germination (Groot and Karssen 1987). Abscisic acid (ABA) reduces this effect while cytokinins are able to reverse the effect of ABA (AA Khan 1968). Heavy metals can influence endogenous plant growth regulator (PGRs) levels in seed

Table 2 Summary of studies showing heavy metal-induced antioxidant production in germinating seeds and seedlings

Metal	Species	Changes in antioxidant system	References
As	*Triticum aestivum*	The ascorbate peroxidase and glutathione *S*-transferase activities increased with the increase in As concentration, while glutathione reductase (GR) activities increased only at 0.25 mM. The monodehydroascorbate reductase and catalase activities were not changed upon exposure to As. The activities of dehydroascorbate reductase and glyoxalase I decreased at any level of As, while glutathione peroxidase and glyoxalase II activities decreased only upon 0.5 mM As	Hasanuzzaman and Fujita (2013)
As–Se	*Vigna radiata*	As severely inhibited the growth at 10 μM. The As-treated (10 μM) plants supplemented with 5 μM Se showed improved growth indicating an antagonistic interaction between the two elements. This likely resulted from elevated levels of enzymatic superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase	Malik et al. (2012)
As	*Oryza sativa*	Superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase increased in arsenite treated seedlings, while dehydroascorbate reductase activity declined initially during 5–10 days and then increased	Mishra et al. (2011)
As	*Oryza sativa*	Up-regulation of superoxide dismutase, ascorbate peroxidase and glutathione reductase with exposure to As	Shri et al. (2009), Rai et al. (2011)
	Oryza sativa	Up-regulation of superoxide dismutase, ascorbate peroxidase and glutathione reductase with exposure to As	Shri et al. (2009), Rai et al. (2011)
Ag	*Ricinus communis*	The peroxidase activity and superoxide dismutase activity increased with increasing Ag concentration	Yasur and Rani (2013)
Ag–Cd	*Vicia faba*	In leaves, catalase activity declined, but peroxidase activity increased with time. In roots, catalase activity and peroxidase activity increased during early stages and then declined. Superoxide dismutase activity showed an upward trend with an increase in the duration of treatment after 3 and 6 days and then declined both in leaves and in roots (at 9 days) under 1–50 μM	Zhang et al. (2009)
Cd	*Picea omorika*	1 mM inhibited the activities of catalase, superoxide dismutase and peroxidase	Prodanovic et al. (2016)
	Trigonella foenum-graecum	With concentrations greater than 0.1 mM, catalase, superoxide dismutase and ascorbate peroxidase were increased in different parts of the plant	Zayneb et al. (2015)
	Brassica napus	From 100 μm catalase, superoxide dismutase, glutathione reductase and ascorbate peroxidase are reduced	Mohamed et al. (2012)
	Oryza sativa	Marked elevation in superoxide dismutase and peroxidase activities was observed with increase in Cd. The activity of catalase increased in seedlings grown at moderately toxic Cd (100 μM) level, whereas a highly toxic Cd (500 μM) level inhibited catalase activity.	Shah et al. (2001)
	Brassica juncea	Peroxidase, catalase and superoxide dismutase in the leaves of plants increased significantly in parallel with an increase in the concentration of the metal	Irfan et al. (2014)
Cd, Pb	*Oryza sativa*	Superoxide dismutase and guaiacol peroxidase increased in metal-treated seedlings. Catalase activity increased during early stages (8 days) of metal exposure and declined by 16 days	Srivastava et al. (2014)
Cr	*Glycine max*	The shoots contain higher catalase activity than roots. The reduction of catalase was observed from 5 mg L^{-1} onwards, and it was severely affected at 200 mg L^{-1}. The peroxidase and super oxide dismutase activity increased with the increase in Cr	Sankarganesh et al. (2015)
Cr	*Brassica juncea* and *Vigna radiata*	Higher activities of superoxide dismutase, ascorbate peroxidase, catalase and glutathione reductase were observed in shoot of *B. juncea* and *V. radiata* at all levels of Cr treatments (0, 50, 100, 200 μM)	Diwan et al. (2010)
Cu, Zn	*Cumumis sativus*	Significant increase in superoxide dismutase, peroxidase and catalase activities at 100 mg L^{-1} metal concentrations	Kim et al. (2012)

Table 2 continued

Metal	Species	Changes in antioxidant system	References
Cu	*Helianthus annuus*	In the presence of 0.5 mM metal concentration, superoxide dismutase activity was increased while catalase, ascorbate peroxidase, glutothionine reductase were decreased	Gallego et al. (1996)
Mn	*Oryza sativa*	3 and 6 mM Mn increased superoxide dismutase guaiacol peroxidase and catalase in 10 day old seedlings, but declined by 20 days under Mn treatment. Moreover, ascorbate peroxidase monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase increased significantly with Mn	Srivastava and Dubey (2011)
Pb, Cd and Hg	*Lycopersicon esculentum*	50 μM decreased catalase activity in roots. The activity of ascorbate peroxidase was unchanged in roots and in stems, while it was diminished in leaves. However, guaiacol peroxidase activity was increased only in roots and stems	Mazhoudi et al. (1997)
Ni and Zn	*Cajanus cajan*	Increased the superoxide dismutase, peroxidase and glutathione reductase and reduced catalase activity in all Zn and Ni treatments	Rao and Sresty (2000)

germination. The mevalonic acid pathway is the main biosynthetic pathway for GA production (Arteca 1996). Several studies report an increase in GA content during seed germination (Debeaujon and Koornneef 2000; Nambara et al. 1991). The GA content showed a reduction in chickpea seeds in the presence of Zn and Pb (1.0–10 mM) and Cd. Although GA is reduced under high Zn concentrations, it showed an increase at low Zn (0.1 mM) concentrations (Atici et al. 2005). The mechanism behind heavy metal-caused reduction in GA production is also not well understood.

Abscisic acid is another PGR governing the seed germination process. It is largely responsible for seed dormancy and germination (Fujii et al. 2007). The role of ABA on inhibition of seed germination been well studied (Koornneef et al. 1984). It is known that ABA levels in plants are enhanced with exposure to environmental stress (Jiang and Zhang 2002). An increase in ABA concentration was observed in Cd-tolerant *Oryza sativa* (Poaceae) seedlings while an exogenous application of ABA increased Cd tolerance in *O. sativa* seedlings (Hsu and Kao 2003). Further, ABA causes a reduction in transpiration rates, helping to reduce shoot Cd accumulation, thereby reducing heavy metal-caused oxidative stress (Salt et al. 1995). Similarly, both GA and ABA are able to reduce translocation of heavy metals in *O. sativa* although an external application of these hormones was unable to overcome oxidative stress (Rubio et al. 1994). However, ABA appears to mediate paclobutrazol-induced Cd tolerance in *O. sativa* (Hsu and Kao 2005).

Effect of heavy metals on α-amylase activity

Hydrolyzing enzymes which are produced by the aleurone layer of seed play a major role in seed germination (Yan et al. 2014; Taiz and Honigman 1976). Enzymes such as acid phosphatases (ACPs), proteases and α-amylases facilitate both seed germination and successful seedling establishment and growth via mobilizing food reserves in the endosperm (Yan et al. 2014). In the presence of Cd and Cu, α-amylase, acid phosphatase and alkaline phosphatase activities were reduced in the endosperm of barley seeds (Kalai et al. 2014). Similarly, as germinating barley seeds were soaked under various concentrations of arsenite and arsenate, a reduction of α-amylase was detected (Sanal et al. 2014).

Starch, proteins and fat are the reserved food sources in a seed, with starch as the most abundant food source of most angiosperm seed (Dua and Sawhney 1991). Starch hydrolyzation can occur either by means of an amylolytic or phosphorolytic pathway. Amylases are the major enzymes responsible for the starch mobilization process. As pea (*Pisumsativum*; Fabaceae) seeds were treated with a Cd concentration series, both α- and β amylase activities were suppressed (Chugh and Sawhney 1996). After 7 days of germination, the total amylase activity with 0.25, 0.50 and 1.00 mM of Cd was 56, 42 and 33%, respectively. This suppression was more pronounced in the α-amylase activity compared to the β-amylase activity. However, the net effect was due to the synergistic effect of both enzymes, with α-amylase degrading

starch into smaller oligosaccharides, creating non-reducing ends for exo-hydroxylation of β-amylase (Fulton et al. 2008). Therefore, the starch degradation can be impeded even at low Cd levels due to the reduction in α-amylase activity.

Lead is another toxic heavy metal released by various industrial activities. A 50% reduction of α-amylase was observed in pea seedlings treated with Pb, resulting in high starch content. The decline in 6-phosphogluconate dehydrogenase (G6PDH) production and an increase in hexokinase (HXK) production in roots and shoots of stressed seedlings signify that hexoses could be utilized for glycolysis more than the pentose phosphate pathway (Devi et al. 2013). Similarly, starch immobilization by Pb via reduction of starch hydrolyzing enzymes has been shown in *Brassica campestris* (Singh et al. 2011).

Chromium also reduces seed germination by causing deleterious effects on hydrolyzing enzymes (Dua and Sawhney 1991). With the increase in Cr concentrations, the total amylase activity showed a reduction in germination pea seeds; however, on the 8th day since exposure, the level of activity was comparable to that of the control (Dua and Sawhney 1991). Therefore, it was hypothesized that the toxic effect can be mitigated with a prolonged exposure to the metal. Similarly, α-amylase showed a more pronounced reduction in concentrations than β-amylase with an increase of Cr, suggesting that the recovery activity of β-amylase plays an important role in the restoration of total enzyme activity. Furthermore, Cr has inhibitory effects on acid phosphatases phytase and ribonuclease, with no significant effects on amylase and protease (Dua and Sawhney 1991).

Protein catabolism

Proteins are another source of energy for seed germination. Proteins in seeds are found as both storage substances and enzymes. Germinating seeds growing under heavy metals stress show alterations and behavior of many enzymes. Extensive studies have been conducted on the effect of As, Cd, Al and Ni on enzyme activities (Maheshwari and Dubey 2008, 2009; Mishra and Dubey 2006; Mishra et al. 2011; Shah and Dubey 1995, 1998; Shah et al. 2001). During seed development and maturation, proteins are synthesized and placed within membrane-bound protein bodies. During seed germination, the storage proteins are hydrolyzed

and transported to the growing points of the embryo. Proteases and peptides hydrolyse proteins into a mixture of free amino acids and small peptides (Ranki et al. 1990). The profile of a cell can be altered by external stress signals. Proteolytic enzyme changes with respect to Cd stress have been studied in rice seeds (Shah and Dubey 1998). Cadmium concentration were increased in the rice seeds with a reduction in protease activity that were subjected to heavy metals and at the same time, enhanced uptake of Cd in embryo axes than in the endosperm (Shah and Dubey 1998). Additionally, there was a 30–50% decline in leucine amino peptidase activity, which catalyzes the hydrolysis of leucine residues, and a marked increase in carboxypeptidase activity, which catalyzes the release of arginine or lysine residues from polypeptides in endosperm. However, there was always a reduction in peptidase activity in embryo axes of germinating seeds (Shah and Dubey 1998).

Karmous et al. (2012, 2014a) examined the effects of Cu on protease activity in seeds. The effects of Cu on bean (*Phaseolus vulgaris*) cotyledons were assessed with respect to protease activity (Karmous et al. 2012). The enzymes involved in storage protein mobilization during bean germination have also been studied via electrophoresis. Copper influenced both embryo development and total protease activity. Moreover, cysteine, aspartic and metalloprotease activities were all decreased under Cu stress, with an increase in the serine-protease activity. Hence, excess Cu appears to disturb the release of nitrogen from storage tissue during seed germination. It has been suggested that there may be a relationship between defense capacity and proteolytic responses in Cu-treated embryos (Karmous et al. 2014b).

Similar results were obtained with rice seedlings treated with As (Mishra and Dubey 2006). RNA and protein degradation are key processes in cellular biochemistry. RNase is one of the key marker enzymes for stress conditions. The changes in the activity of RNase are an indicator of stress conditions (Shah and Dubey 1995). Since rice is sensitive to As, the effects of As_2O_3 on activities of RNase, protease, peptidases as well as on the levels of RNA, proteins, free amino acids and proline in growing rice seedlings were tested Compared to the control, RNA proteins and proline were increased. A reduction in hydrolysis of RNA and proline in rice seeds could be due to As induced inhibition of RNase and protease activity. Moreover,

protease and ribonuclease inhibition of rice seedling has been documented under Ni stress (Maheshwari and Dubey 2008). There is acellular level balance of macromolecules such as RNA and proteins within their synthesis and degradation. A possible suppression of RNA hydrolysis in rice plants under Cd toxicity has also been documented (Shah and Dubey 1995). This may be due to the reduced activity of RNase under metal stress, hindering the degradation of RNA. Since the cellular RNA levels and protein synthesis are main processes that maintain cellular development, metal stress can disturb the cellular biochemistry (Shah and Dubey 1995).

Abiotic stresses can induce or suppress different forms of proteases. A relationship between the enzyme activities of protein metabolism and the level of free amino acids/proteins was reported in As-treated plants (Mishra and Dubey 2006). There was a suppression of both protease and leucine aminopeptidase activity while an enhanced activity was observed in carboxypeptidase in rice under As stress, showing the variation in the response of different enzymes under heavy metal stress. Moreover, carboxypeptidase activity showed a considerable tolerance under the concentration range of As used.

Protein damage is one of the crucial effects of oxidative stress. Oxidation of residual amino acid side chains and formation of covalent cross-linkages between proteins can denature cellular proteins. The rapid elimination of those proteins is necessity to avoid toxicities during cell metabolism. The ubiquitin (Ub) proteasome system is an important non-lysosomal proteolytic pathway involved in the degradation of intracellular proteins under biotic and abiotic stress (Davies 2001; Jackson and Durocher 2013). An increase in protein carbonylation and an decrease in the reduced S–H pool reflect protein damage in bean seeds under heavy metal stress (Kranner and Colville 2011). Moreover, Cu showed an induced inactivation of Ub-proteasome, which limits the removal of oxidatively damaged proteins in germinating bean seeds (Karmous et al. 2014a).

Protective role of proline against heavy metal stress

Proline is an amino acid which plays an important role in maintaining cellular homeostatis via osmotic regulation (Fariduddin et al. 2013; Iqbal et al. 2016).

Studies show that the proline content is increased under different environmental stresses. An accumulation of proline was observed under numerous biotic stresses (Fabro et al. 2004). It was hypothesized that proline is able to scavenge free radicals produced by a variety of abiotic stresses, including heavy metal stress. The potential of proline to scavenge the free radicals was assessed with in vitro assay systems, namely graft copolymerization and auto-oxidation of pyrogallol (Kaul et al. 2008).

Although the relationship between proline accumulation and abiotic stress tolerance in crops is always not obvious, studies have shown that there is a correlation between the plant proline accumulation and stress conditions (Ashraf and Foolad 2007). The accumulation of proline was tested in *Cajanus cajan, Vigna mungo* and *Triticumae stivum* under Cd, Co, Pb and Zn stress (Saradhi 1991). According to the phenotypic data obtained, Cd was the most toxic metal among the heavy metals used. An accumulation of proline was observed in all seedlings under heavy metal stress. The proline level was inversely proportional to the fresh weight of the plant, irrespective of the heavy metal, suggesting that materials used for plant growth were used for proline production. Cadmium appeared to be the most effective heavy metal in stimulating proline production (Saradhi 1991).

Free proline was also measured in response to Cu, Cd and Zn in non-tolerant and metal-tolerant *Silene vulgaris* (Schat et al. 1997). The constitutive proline concentration in the leaves of the metal-tolerant ecotype was 5–6 times greater than in the non-tolerant ecotype. Similarly, Ni-induced proline was also documented in rice (Maheshwari and Dubey 2009) and wheat (Gajewska and Skłodowska 2008). In addition to the metal, the plant species is also an important consideration in biochemical processes of plants. In *Silene vulgaris,* Cu was the most effective proline inducer (Schat et al. 1997). Complete inhibition of proline accumulation under heavy metal reduced transpiration explains the dependence of metal-induced proline accumulation on the development of metal-induced water deficit in leaves.

However, contradictory results have also been recorded on the water deficit hypothesis of proline. The accumulation of proline was tested in detached rice leaves treated with excess Cu (10 mM) (Chen et al. 2001). Both $CuSO_4$ and $CuCl_2$ were effective in simulating proline accumulation in rice leaves.

Interestingly, there was no effect on the relative water content of detached rice leaves with excess Cu, suggesting that there is no relationship between Cu-induced proline accumulation and water deficit condition in plants (Chen et al. 2001). Moreover, there was an increase in abscisic acid (ABA) concentration in detached rice leaves under Cu stress, which indicates the possibility of ABA to increase proline in stressed tissue. Similarly, an increment in proline content was observed with increasing Cu concentration in germinating wheat seedlings. There was also an increase in proline accumulation in the root of wheat seedlings even at a non-toxic concentration of Cu (1 µM) (Chen et al. 2001).

Proline provides diverse protective functions in mitochondria. During cellular stress conditions, proline helps via supplying energy which is needed to recover from stress. It enhances reducing potential of mitochondria by donating electrons to the respiratory chainvia oxidation by proline dehydrogenase (PDH) and proline-5-carboxylatedehydrogenase (P5CDH) proline (Szabados and Savouré 2010). The high proline accumulation under heavy metal stress has also been hypothesized as leading to a reduction in proline degradation, an increase in proline biosynthesis, a decrease in proline utilization in protein synthesis and the hydrolysis of proteins (Charest and Ton Phan 1990).

Heavy metal-induced protein changes

Proteins play an important role in all living systems. Any physiological or biochemical change, whether negative or positive, will be reflected by alterations in protein activity. Globulin is a major protein in plants, containing a high content of sulfur-containing amino acids (Kriz 1989; Xiang et al. 2013). Compared to that of untreated seeds, the storage protein accumulation was comparatively higher under heavy metal treatment. Seed proteome analysis reveals the presence of globulin and its precursor in cereals in that these proteins play an important role in basic cellular processes in rice embryogenesis and germination (Komatsu et al. 2005). The up-regulation of globulin-like proteins with their precursors was observed in the presence of excessive Cu in germinating rice (Ahsan et al. 2007a). Similarly, an up-regulation of the globulin precursors in germinating maize seeds was also detected with potassium dichromate, indicating

the activation of storage proteins (Labra et al. 2006). These results suggest that heavy metals may hinder the glycolytic pathways in germinating seeds, since the accumulation of seed storage proteins was higher relative to that of the untreated seeds.

The physiological, biochemical and proteomic changes in germinating rice seeds have also been tested in response to Cu stress. The total proteins were extracted and subjected to one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE). Differences were observed in the protein band patterns between the control and Cu-treated samples. The protein pattern changes were further analyzed by two-dimensional electrophoresis for better separation and characterization of germination-related proteins (Østergaard et al. 2004). The Cd-induced protein profile change was also examined in rice seeds with SDS–PAGE. Changes in protein patterns due to heavy metal binding proteins were visualized (Ahsan et al. 2007b), revealing results similar to those shown in previous studies with rice and maize (Ahsan et al. 2007a; Labra et al. 2006). Since the SDS–PAGE is not sensitive enough to determine the pin point changes of polypeptide patterns, the 2-DE analysis was used to investigate the changes in proteins (Zhang and Riechers 2004). Apre-fractionation techniquewas used to extract the rare proteins. The selected spots were subjected to MALDI-TOF mass spectrometry for peptide mass mapping, and 21 upregulated proteins were identified. These proteins were involved in RNA processing, signal transduction, protein biosynthesis, antioxidant defense and germination processes (Ahsan et al. 2007b).

Further, as *Lepidium sativum* plantlets were exposed to Cd, considerable amounts of unmodified or minimally modified storage proteins were detected in parallel to a reduction in plantlet elongation (Gianazza et al. 2007). This suggests the inhibition of storage protein catabolism with metal exposure during germination and seedling elongation. Since the *L. sativum* plantlets exhibit changes in protein expression with low concentrations of heavy metal, they are effective bio-indicators for assessing environment pollution.

Heat shock proteins in photosynthesis protection

Heat shock proteins are general stress proteins implicated in the protection of physiological processes

during most abiotic stresses (Basile et al. 2013). Chloroplast small Hsp (smHsp) is not a typical protein produced by plants in the absence of stress. Studies have confirmed the protective role of smHsps on photosynthetic electron transport (Ph_{et}), during heat, oxidative and photoinhibition stresses (Shakeel et al. 2012). As *Zea mays* (corn) plants were treated with varying soil concentrations of Cu, Ni, Pb and Zn, net photosynthesis was decreased with all heavy metals, with a greater decline shown under higher concentrations and during extended exposure times (Heckathorn et al. 2004). However, chloroplast small heat shock protein content was increased to levels comparable to levels induced by severe acute heat stress in the presence of Cu, Ni, Pb and Zn (Heckathorn et al. 2004). Further, the production of chloroplast small Hsp is a primary response in plants to heavy metal accumulation and its mode of protection is the limitation of photosynthesis damage, rather than repair or recovery from heavy metal damage (Downs et al. 1999; Waters et al. 1996). Hsp70 and small Hsp are induced in response to heavy metals, with Hsps protecting the cell membrane during heavy metal stress (Hall 2002).

Heavy metal-induced photosynthesis damage in seedlings

Heavy metals are often described as destructive substances for photosynthesis (Prasad and Strzałka 1999). They are involved in disrupting enzyme activities, oxidizing PS II and disorganizing the electron transport chain and mineral metabolism. A reduction in chlorophyll and heme levels was observed when germinating seedlings of *Phaseolus vulgaris* were treated with Cd (Somashekaraiah et al. 1992). There was a significant increase in lipid peroxide levels in the presence of Cd. However, a dose-dependent induction of lipoxygenase activity was observed with the Cd^{2+} treatment. Moreover, a reduction in antioxidant enzyme activity was also seen with the Cd treatment. Inhibition of free radical scavenging enzymes via lipid peroxide accumulation may be a reason for the reduction in the chlorophyll and heme levels.

Similarly, a growth reduction, inhibition of chlorophyll content and photosynthesis have been reported in wheat in the presence of Cd (Ouzounidou et al. 1997). The indirect effect of Cd on essential nutrient content in plants is one reason for photosynthesis inhibition. Moreover, structural damage of chloroplasts was evident by the distorted shape and the dilation of the thylakoid membranes. These ultrastructural changes suggest the possibility of Cd-induced premature senescence. However, Sheoran et al. (1990) demonstrated that the reduction in photosynthesis in the presence of Cd was due to the effect on stomatal conductance and not due to decreased enzyme activities. They show that Cd^{2+} inhibited the leaf O_2 evolution alongside a reduction in photosynthesis.

The chlorophyll molecule with Mg has the highest capacity of electron release from a single excited state (Küpper et al. 1996). As the central Mg atom of chlorophyll is substituted with heavy metals such as Hg, Cu, Cd, Ni, Pb, it prevents photosynthetic light-harvesting, contributing to a decline in photosynthesis (Mishra and Dubey 2005). Under low light irradiance, some of the chlorophyll substituted with heavy metals is more stable than Mg-containing chlorophyll. Consequently, crops can remain green even when they are dead. Even though several studies have been carried out to determine the effects of heavy metals on photosynthesis of plants, limited studies have been carried out on the effects of heavy metals on the photosynthesis of seedlings.

Reduction in heavy metal toxicity in seeds

The reduction in heavy metal toxicity in seeds can be indirectly achieved by immobilization of heavy metals in soil. This is mainly done with the use of different organic and inorganic amendments. Biochar, green waste manure, compost are some of the organic amendments that can be used to immobilize heavy metals (Branzini and Zubillaga 2012; Gadepalle et al. 2007; Park et al. 2011). Composted organic soil amendments have the ability to bind with heavy metals and can cause rapid immobilization. Due to the high surface area of carbonaceous sorbents such as black carbon and activated carbon with a high affinity for heavy metals, there can be an increased immobilization of heavy metals. Numerous studies have shown the efficiency of heavy metal adsorption of by biochar (Uchimiya et al. 2010; Inyang et al. 2012). Further, the addition of organic matter can also play a major role in heavy metal adsorption in soil (Davis 1984). Several studies have shown that the sorption power of organic matter is mainly due to its cation exchange capacity

(Basta et al. 1993). Therefore, the addition of these soil amendments can indirectly reduce the potential toxicity of heavy metals on seed.

Future perspectives

Heavy metal release to the environment is pronounced growing environmental problem, leading to detrimental effects at the ecosystem level. When early life cycle stages such as seed germination and seedling establishment are affected by heavy metals, there can be lasting consequences at the individual plant, population and community levels. The ways in which heavy metals can cause toxicities, including in storage food mobilization, photosynthesis and plant osmoregulation, can vary depending on the type of heavy metal, its speciation, soil pH, plant species and the mobility of the metal. In order to overcome heavy metal-induced toxicities, plants employ numerous mechanisms, including the activation of the antioxidant system, proline production, production of heat shock proteins and acetyl salicylic acid, among others. There are many informational gaps relating to the study of heavy metal influence on seed germination and seedling establishment. Some of these areas are summarized below to generate interest for further investigation.

- Protein hydrolysis and mobilization are key processes in seed germination. The interference of heavy metals with key germination-related proteins has not been well documented at the proteomic level. The study of proteomics under heavy metal stress can be a fruitful area for future study.
- The specific processes or cell components protected by Hsps remain largely unknown. Additionally, the specific Hsps induced by heavy metals and their cellular location also deserve further investigation.
- GA production by the embryo is a major step in seed germination. More studies are needed in the area of heavy metal influences on GA production.
- Ubiquitin (Ub) proteasome system is an important non-lysosomal proteolytic pathway involved in the degradation of intracellular proteins under biotic and abiotic stress. However, more research is necessary to document the involvement of the Ub system in the degradation of specific cellular proteins. There are numerous studies carried out on the effect of heavy metals on hydrolyzing enzymes; however, there is a lack of studies on the effects of heavy metals on the seed's aleurone layer where the hydrolyzing enzymes are produced.

- Although it is known that heavy metals are able to reduce the water uptake in germinating seeds, the exact mechanisms for heavy metal-induced osmotic stress are unclear. Additional studies are needed to explore the mechanisms of heavy metal-induced osmoregulation in germinating seed.
- Most of the studies to date have been carried on cereal crops, especially on rice. Hence, it is imperative that additional studies are carried out on a broad range of plants, including fruit and vegetable crops and wild plants. Research on wild plant tolerance of heavy metals can also provide useful information on understanding heavy metal-induced stresses on germination and early seedling growth.
- The effects of understudied metals including Ag are important to examine in the laboratory as these metals are now commercially used as nano fertilizer.

Seed germination and early seedling growth are two important stages of plant development. An understanding of the multitude of ways in which heavy metals influence these critical stages in plant growth and development is fundamental for advancing plant science and developing better practices in agriculture, forestry and restoration. This review highlights the primary ways in which heavy metals impact critical stages of plant development in the hope of fostering additional research for improving our knowledge on the deleterious effects heavy metals have on seeds and seedlings and how such effects could be minimized with additional research in phytotechnologies.

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