



How heavy metal stress affects the growth and development of pulse crops: insights into germination and physiological processes

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Abstract

The current work is an extensive review addressing the effects of heavy metals in major pulse crops such as Chickpea (*Cicer arietinum* L.), Pea (*Pisum sativum* L.), Pigeonpea (*Cajanus cajan* L.), Mung bean (*Vigna radiata* L.), Black gram (*Vigna mungo* L.) and Lentil (*Lens culinaris* Medik.). Pulses are important contributors to the global food supply in the world, due to their vast beneficial properties in providing protein, nutritional value and health benefits to the human population. Several studies have reported that heavy metals are injurious to plants causing inhibition in plant germination, a decrease in the root and shoot length, reduction in respiration rate and photosynthesis. Properly disposing of heavy metal wastes has become an increasingly difficult task to solve in developed countries. Heavy metals pose one of the substantial constraints to pulse crops growth and productivity even at low concentrations. This article attempts to present the morphological, biochemical and various physiological changes induced on the pulse crops grown under various heavy metal stress such as As, Cd, Cr, Cu, Pb, and Ni.

Keywords Heavy metals · Pulse crops · Anthropogenic activities · Toxic effects · Plant response

Introduction

Heavy metal (HM) refers to a group of metals with a high atomic number, i.e. above 20 and having a higher density (5 g/cm^3), such as cadmium (Cd), mercury (Hg), lead (Pb), arsenic (As), nickel (Ni), chromium (Cr), copper (Cu) and zinc (Zn) (Mishra et al. 2019). These metals directly pollute the environment and cause biological toxicity when excess amounts of HMs are introduced into the environment. However, some of these metals including Zn, Cu and Ni are necessary micronutrients and only required in trace levels since they serve as cofactors for certain enzymes (Ghori et al. 2019). Organic pollutants are easily degradable, while the toxic HMs are unmodifiable by biochemical reactions and cause environmental pollution worldwide. Therefore, these metals are difficult to remediate from the water and soil by natural means (Ramesh kumar and Anbazhagan 2018). When these HMs enter agricultural lands they not only cause

soil contamination, but also affect food quality, production and human health. Crops grown in HM polluted sites have been reported to display altered metabolism, biochemical and physiological processes leading to growth reduction, lower biomass production and HM accumulation (Edelstein and Ben-Hur 2018). One such important agricultural crops are pulses, which are globally very important and play a key role in dietary diversity to eliminate malnutrition and hunger.

Pulse crops belong to the Fabaceae family their importance ranks second to that of the Poaceae family in the agriculture system. Moreover, pulse crops can arrange as a substitute for animal protein and thus become an essential dietary protein required especially in developing countries (Farooq et al. 2018). Pulse crops are a source of rich protein in comparison to various other cultivated crops. In addition, pulses belong to a subgroup of legumes that harbors nitrogen-fixing bacteria in their root system which improves soil fertility (Schwember et al. 2019). Legume plants have an important role as health enhancers due to the fact these bioactive peptides possess various properties such as antioxidant activities, antimicrobial effects, immunomodulation, enhancing of mineral bioavailability/absorption, lowering of blood pressure and cholesterol (Çakir et al. 2019). Edible

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seeds provided by the legume plants have shown anticarcinogenic properties. Many potential bioactive components provided by pulses are protease inhibitors, saponins, lectins, phytosterols and phytates which act as anticancer agents (Mathers 2002). Furthermore, the absence of gluten proteins in seeds of pulse crops is critical in meeting the need of gluten-free diets for the population suffering from celiac disease (Mlyneková et al. 2014).

Heavy metals

Chromium

Naturally, Cr is present on the Earth having an electronic configuration $[Ar] 4d^5s^1$ and is the seventh most abundant element found in the Earth's crust having concentrations varying from 100 to 300 $\mu\text{g/g}$. Cr is found in nature in its compound states, and chromite (Fe, Mn) Cr_2O_4 is the most relevant Cr ore (Focardi et al. 2013). Table 1 summarises the sources of HMs/metalloids. Cr(III) is an important trace element for having a particular role in maintaining normal carbohydrate metabolism in yeast and mammals (Dębski et al. 2004). For normal insulin function

Cr is required, insulin resistance have been reported at low levels of Cr. Extreme deficiency of Cr is followed by symptoms that mimic diabetes mellitus (Liu et al. 2015). In nature, Cr occurs in two distinct and stable oxidation states i.e. hexavalent Cr[Cr(VI)] and trivalent Cr[Cr(III)], in both Cr(VI) and Cr(III), there is a difference in toxicity, bioavailability and mobility (Panda and Choudhury 2005). Hexavalent Cr is more toxic than the trivalent form, which generally occurs in association with oxygen as dichromate ($\text{Cr}_2\text{O}_7^{2-}$) or chromate (CrO_4^{2-}) (Shanker et al. 2005). Cr(VI) is more mobile in comparison to Cr(III) and in aquatic and soil environments Cr(III) is mostly found confined to organic matter which is thought to take place due to the reduction of Cr(VI) to Cr(III) (Becquer et al. 2003). The International Agency for Research on Cancer (IARC) has classified Cr(III) compounds as Group 3 and Cr(VI) compounds as Group 1 (IARC 2012). In humans, Cr(VI) exposure can induce irritations in the skin and nose, nasal ulcer, contact dermatitis, nasal ulcer, lung cancer and respiratory tract disorders (Shrivastava et al. 2002). Exposure to Cr can cause serious phytotoxicity in plant cells, Cr causes a reduction in chlorophyll (Chl) content, breakdown of carotenoids and an increase in lipid peroxidation which hampers the growth of the plant (Panda and Patra 2000).

Table 1 Sources of HMs/metalloids

Metals/ metalloids	Sources	References
Cr	Volcanic eruptions, weathering of parent rocks, fossil fuel combustion, waste incineration, industrial processes, pulp and paper mills, leather tanning, chromium plating, metal fabrication, wood preservatives, printing inks, paints and anti-corrosive materials	Bielicka et al. (2005), Biradar et al. (2012), Cheng et al. (2014) and Owlad et al. 2009)
As	Earth crusts as ores and minerals, industrial sites, mining, smelting, manufacturing alloys, burning fossil fuels, leather industry, wood preservatives, pesticides	Bissen and Frimmel (2003), Melamed (2005), Morin and Calas (2006) and Bencko and Slámová (2007)
Cd	Weathering of cadmium-rich rocks, volcanic activities, industrial processes, nickel-cadmium batteries, PVC products, electroplating, electrodes, phosphate fertilizer production, nuclear reactors, fossil fuel combustion and smoking	Genchi et al. (2020a) and Suhani et al. 2021)
Pb	Volcanic explosions, forest fire, mining, smelting, coal burning, batteries, painters, pigments, car radiators, cable wires, ammunitions, cement industry, automobile exhaust and oil combustion	Cheng and Hu (2010), Hou et al. (2015), Karrari et al. (2012) and Wang et al. (2000)
Cu	Volcanoes, windblown dust, forest fires, mining, milling, refining and smelting of ores, concentrating, electroplating industries, electrical wastes, fuel combustion, wood preservatives, copper biocides and pesticides	Alvarado et al. (2002), Freeman and McIntyre (2008), Kuehne et al. (2017), Lamichhane et al. (2018), Rehman et al. (2019) and Shrivastava (2009)
Ni	Wind-blown dust, weathering of rocks, forest fires, volcanic activities, mining, refining, nickel alloys, petroleum industries, smelting, paint, batteries, combustion of coal, diesel and fuel, incineration of waste and sludge	Ahn et al. (2019), Genchi et al. (2020b) and Iyaka 2011)

Cadmium

Cd is most commonly found as a divalent cation that is complexed with other elements (e.g., CdCl_2). Cd is found in the Earth's crust at roughly 0.1 parts per million, found as an impurity in Pb or Zn deposits; it is generally generated as a byproduct of Pb or Zn smelting (Bernhoft 2013). Cd is a soft, ductile, silvery-white metal belonging to the group 12 element, present in d block and period 5 with atomic number 48 and electronic configuration $[\text{Kr}] 4d^{10}5s^2$ (Sharma et al. 2015). Normally +2 oxidation state is present in most of its compounds although the +1 oxidation state is found in rare instances (Morrow 2010). Cd is a widespread pollutant that persists in nature and pollutes soil and water. In addition, it is a non-essential metal for both plants and humans (Meysam Hoseini and Zargari 2013). Cd is considered to be a Group 1 human carcinogen (IARC 2012). Cd is a common HM contaminant that is extremely hazardous to living organisms. Exposure to Cd leads to shortness of breath, acute respiratory distress syndromes, lung edema, mucous membrane destruction due to pneumonitis, acute gastrointestinal disorder (vomiting and diarrhea), kidney damage and Cd induce deleterious effects in the reproductive system (Godt et al. 2006).

Arsenic

Arsenic, a metalloid, is the 20th most abundant element in the Earth's crust, 1.5–3 mg/kg As is present in the soil (Mandal and Suzuki 2002). Arsenic like nitrogen, phosphorus, antimony and bismuth, belong to group V element. It has the electronic configuration $[\text{Ar}] 3d^{10}4s^24p^3$ with an atomic weight of 75 (Garelick et al. 2008). Only 3 compounds of 150 species of As containing minerals found in nature are regarded as As ore, due to their high amount of As, namely realgar or As sulfide (As_2S_2), orpiment or As tri-sulfide (As_2S_3) and arsenopyrite or ferrous As sulfide (FeAsS_2) (Hossain 2006). As can be found in both organic and inorganic forms in nature, while pentavalent arsenate As(V) and trivalent arsenite As(III) valence states of As are most commonly seen in environments contaminated with As (Dhankher et al. 2012). According to Environmental Protection Agency (EPA) and IARC, As is one of the most extremely toxic and carcinogenic element present on the planet. As and its compounds have been placed in Group 1 human carcinogen (IARC 2012). As is a potent carcinogenic metalloid pollutant with negative health effects in humans. Moreover, As can cause non-carcinogenic consequences, including weakness, edema, cardiovascular disease, hypertension, diabetes mellitus, respiratory problems, conjunctival congestion and neurological deficits (Björklund et al. 2018).

Lead

Pb is a notable hazardous HM, which has attracted a lot of interest due to its extensive distribution and potential environmental hazard (Zeng et al. 2007). Pb is slightly bluish to gray in color, easily molded and shaped, and has a melting point of 621.43 °F. Additionally, it can be combined with different metals to create alloys. Pb is placed in group 14 in the periodic table and has an atomic weight and an atomic number of 207.2 and 82, respectively, and having electronic configuration of $[\text{Xe}] 6s^24f^{14}5d^{10}6p^2$ (Abd El-Hack et al. 2019). Pb is the most common HM pollutant in the environment according to EPA, IARC has classified Pb as a possible human carcinogen in Group 2B and its inorganic compounds in Group 2A (IARC 2012). In humans, Pb causes severe damage to the brain, kidneys, central nervous system, cardiovascular system, immune system. In addition, other notable effects caused by Pb exposure are depression, problems with sleep, fatigue, headaches, slurred speech, stupor, nausea, abdominal pain, lack of coordination, numbness, blood pressure increases, anaemia and miscarriage (Wani et al. 2015). Pb is not necessary for plant growth; a higher concentration of Pb causes a magnitude of negative effects causing inhibition of growth and germination, suppressing photosynthesis, altering membrane structure and permeability (Li et al. 2012). Pb toxicity destroys the chloroplast substructure and mitochondria, reduction in ascorbic acid and Chl contents, production of reactive oxygen species (ROS) and decline in the activity of antioxidative enzymes causing an overall reduction in plant growth and development (Xu et al. 2007).

Copper

Cu is present in group 11, period IV in the periodic table with the electronic configuration $[\text{Ar}] 3d^{10}4s^1$, having atomic number 29 and an average molecular weight of 63.55 (Barber et al. 2021). All biological species, from bacteria to humans, require Cu as an essential trace element. Cu participates in redox reactions through cycling between Cu(I) and Cu(II) oxidation states, making it an important component of metalloenzymes and using coordination chemistry maintains higher order structure which serves as an essential component of macromolecules (Stern et al. 2007) Cu toxicity disturbs redox properties leading to a significant increase in ROS, damaging lipids, mitochondria, proteins and nucleic acids (Mehta et al. 2006). Excess Cu accumulation in humans causes Wilson disease, abdominal pain, headache, dizziness, nausea, vomiting, diarrhea, tachycardia, respiratory problems, hemolytic anemia, hematuria, substantial gastrointestinal bleeding, kidney and liver failure (Stern et al. 2007). In plants, Cu plays key roles in photosynthesis, respiration, cell wall metabolism and hormone perception. Multiple Cu transporters regulate the transport of Cu

in a tightly controlled fashion, such as Copper transporter (COPT), Zrt/Irt-like protein (ZIP), HM ATPase (HMA), yellow stripe-like (YSL) transporters and natural resistance-associated macrophage protein (Nramp), directly maintaining Cu homeostasis (Liu et al. 2017).

Nickel

Ni is one of the major metal pollutants of concern due to its toxic effects at a higher concentration, which is increasing in the soil every year in various parts of the world. Ni is a long-term potential environmental hazard, ranking 22nd among the most common elements in the Earth's crust (Rizwan et al. 2019). Ni has a silvery-white color, hard and ductile, belonging to group VIII B of the transition metal series in the periodic table and having an atomic number 28 with the electronic configuration $[Ar] 3d^8 4s^2$. Although Ni can exist in numerous different oxidation states, Ni(II) i.e. in the +2 valence state is the most prevalent oxidation state in the environment. Other valence states (-1, +1, +3, and +4) are also found in nature and are less frequent (Cempel and Nikel 2006). Many plant species require small amounts of Ni (0.01–5.0 mg/kg) to complete their life cycle, Ni also plays an important role in urease enzyme (Rizwan et al. 2017). Glyoxalases, hydrogenases, peptide deformylases, methyl-coenzyme reductase and superoxide dismutases, are among the enzymes that use Ni as a key component. Furthermore, Ni participates in various metabolic processes, including ureolysis, methane biogenesis, hydrogen metabolism and acetogenesis (Chen et al. 2009). Ni is carcinogenic to humans, Ni compounds belong to Group 1 and metallic Ni belong to Group 2B (IARC 2012). Ni is a known immunotoxic, haematotoxic, neurotoxic, pulmonary toxic, hepatotoxic, nephrotoxic, reproductive toxic, genotoxic and carcinogenic agent (Das et al. 2008). In humans, acute toxicity of Ni causes nausea, vomiting, irritation, vertigo, stiffness in chest, constant cough, cyanosis, dyspnea, tachycardia, palpitations, visual disturbances, sweating, weakness, cardiac arrest and death due to respiratory distress syndrome have been reported, whereas chronic toxicity leads to asthma and bronchitis (Das et al. 2018). Exposure to a high concentration of Ni disrupts many morphological and anatomical

processes in plants causing growth reduction, chlorosis, wilting and necrosis (Jamil et al. 2014).

Pulse crops

Chickpea

Chickpea is an annual grain legume, a self-pollinated diploid ($2n = 2x = 16$) plant with a genomic size of roughly 738 megabase pair (Mbp)/1C (Arumuganathan and Earle 1991). Chickpea plant in association with effective nitrogen-fixing symbiotic bacteria improves soil fertility by fixing atmospheric nitrogen. The amount of nitrogen fixed in the soil by different pulse crops are provided in Table 2. Chickpeas were first domesticated in Southeastern Turkey and have since adapted to a variety of environmental and climatic factors all over the globe from East Africa to South Asia in subtropical conditions to regions of North America in temperate conditions (Kozlov et al. 2019). Two distinct types of chickpeas are cultivated based on seed size, shape and color. Specifically, the *desi* form, which is characterized by pink or purple flowers, seeds are small, angular in shape, brown in color containing a high proportion of fiber primarily cultivated in East Africa and the Indian subcontinent and central Asia, while type *kabuli*, having white flowers, seeds are large, beige, containing a low proportion of fiber, cultivated in Central Asia and Mediterranean basin (Iruela et al. 2002). To increase chickpea productivity, *Mesorhizobium* is used for symbiotic nitrogen fixation where the beneficial microorganisms are traditionally applied either to the soils or to the seeds. To boost the nodulating capacity of the crop and the effectiveness of the symbiosis (Wani et al. 2007a), Chickpea is an essential source of protein for millions of people in India and other South Asian countries. Chickpeas have high fiber content, unsaturated fatty acids, β -carotene and are rich in minerals (calcium, phosphorus, magnesium, zinc and iron) (Gaur et al. 2010). Chickpea ranks third after beans in terms of production worldwide with a mean production of more than 10 million tons annually, with the majority of production concentrated in India. Chickpea plantation has begun to increase in land area in recent years. Reaching an estimated 13.5 million hectares, the rate of increase in per

Table 2 Amount of nitrogen fixed by different pulse crops in the soil

Pulse crops	Fixed nitrogen in the soil (kg/ha/year)	References
<i>Cicer arietinum</i>	31–186	Aslam et al. (2003) and López-Bellido et al. (2011)
<i>Cajanus cajan</i>	32–117	Adu-Gyamfi et al. (2007) and Wezi et al. (2017)
<i>Lens culinaris</i>	41–154	Schmidtke et al. (2004)
<i>Pisum sativum</i>	53–286	Gollner et al. (2019) and Kumar and Goh (2000)
<i>Vigna radiata</i>	25–112	Shah et al. (2003) and Delfin et al. (2008)
<i>Vigna mungo</i>	13–91	Hayat et al. (2008)

unit area production have been slow but steady. The global yield, production and area of major pulse crops data estimated from 2018 to 2020 based on Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) are provided in Table 3. Every year, more than 1.3 million tons of chickpea arrive in world markets where India, Pakistan, Iran, Turkey and Australia are the leading chickpea producing countries (Muehlbauer and Sarker 2017).

Pigeonpea

Pigeonpea (*Cajanus cajan* L.) is an important legume crop that is widely grown in tropical and subtropical regions for its nutritive seeds. Pigeonpea is extensively versatile, hardy, fast-growing and can withstand drought conditions (Bekele Tesemma 2007). These species are diploid in nature ($2n = 2x = 22$) having an estimated genome size of about 858 Mbp/1C (Greilhuber and Obermayer 1998). Due to its resistance to drought conditions, it is considered highly relevant for food security in areas where rainfall is uncertain and the prevalence of drought is common (Cox 2014). Cultivated varieties can be divided into two types, the cultivar *arhar* (*C. cajan* var. *bicolor*) is a large, bushy, late-maturing perennial plant whose dorsal side of the standard is either red or purple. Another variety is three seeded, shorter plant having a yellow standard and are earlier maturing known as *tur* cultivars *C. cajan* var. *flavus* (Sharma et al. 2008). Despite some postulation for African origin, it has been proved beyond a reasonable doubt that the most likely wild progenitor of pigeonpea was *Cajanus cajan ifolius* found in Eastern India

today, comprising the present state of Odisha and neighboring states as an origin (Fuller et al. 2019). The most popular use of pigeonpea is to make *dhal* (hulled, soaked dried, and split seeds) (Shinde et al. 2017). In certain regions of Southeast Asia, the seeds are used to make *tempe* (Shurtliff and Aoyagi 2013). 20–22% of protein can be found in the seeds of pigeonpea along with a significant amount of minerals and essential amino acids. Pigeonpea is commonly consumed as whole grain, green peas or split peas (Saxena et al. 2002). India is the world's largest pigeonpea growing country. The primary pigeonpea producing countries are India, Myanmar, Tanzania, Malawi, Uganda, Mozambique and Southern Africa (Sharma et al. 2019). In terms of area and production in India, the following states comprise Maharashtra, Madhya Pradesh, Uttar Pradesh, Gujarat, Karnataka, Telangana, Andhra Pradesh and Bihar (Sameer Kumar et al. 2017).

Pea

The pea (*Pisum sativum* L.) plant was the first model organism utilized in Mendel's (1866) discovery of the laws of inheritance, laying the groundwork for modern plant genetics (Smýkal et al. 2012). Pea is grown all over the world having a temperate climate, it is a herbaceous, annual, self-pollinated diploid ($2n = 2x = 14$) plant with an enormous genome size estimated to be 4397 Mbp/1C (Arumuganathan and Earle 1991). Archaeological evidence indicates its cultivation in Greek settlements and the Near Eastern region in early 6000 BC, but there is no strong consensus on its actual

Table 3 Global yield, production and area of major pulse crops

Crop	Scientific name	Year	Yield (Mg/ha)	Production (Mt)	Area (Mha)
Chickpea	<i>Cicer arietinum</i>	2018	1.05	16.94	16.18
		2019	1.03	14.18	13.79
		2020	1.02	15.08	14.84
Pigeonpea	<i>Cajanus cajan</i>	2018	0.98	5.38	5.48
		2019	0.78	4.36	5.59
		2020	0.82	5.01	6.10
Lentils	<i>Lens culinaris</i>	2018	1.19	6.57	5.51
		2019	1.19	5.78	4.85
		2020	1.30	6.54	5.01
Pea (dry)	<i>Pisums ativum</i>	2018	1.80	13.41	7.45
		2019	1.96	14.00	7.14
		2020	2.40	14.64	7.19
Mung bean	<i>Vigna radiata</i>	2018	–	–	–
		2019	–	–	–
		2020	–	–	–
Black gram	<i>Vigna mungo</i>	2018	–	–	–
		2019	–	–	–
		2020	–	–	–

Data source: (FAOSTAT 2022)

origin. Primitive forms flourished throughout Central Asia, including Afghanistan, the Near East, the Mediterranean and Ethiopia, all of which are rich in genetic diversity (Wrigley et al. 2015). Pea is valuable due to their nutritional quality of protein having amino acids containing low levels of sulfur, methionine and cysteine, but lysine-rich and possessing other essential amino acids (Dahl et al. 2012). Furthermore, peas include substantial amounts of vital minerals such as calcium, iron and phosphorus, which are absent in cereals (Haque et al. 2015). Peas are frequently used as a break crop in continuous cropping systems and also used as forage for cattle (Borreani et al. 2007). Peas are grown extensively in India and the Indian subcontinent and are consumed as vegetables, *dhal* (pulses), *chhola* (whole grain), *chat* (spicy dish) and flour; thus making a major contribution to the business economy (Choudhury et al. 2007). The immature seeds of this crop are eaten as raw, frozen, or packaged vegetables in many countries around the world. Pea is a starchy plant with high protein, vitamins (A, B6, C, and K), elements (Cu, P, Mg, Fe, and Zn), fiber and lutein (El-Amier et al. 2019). Pea seeds, which contain 270–560 g/kg starch, 200 g/kg dietary fibers and 130–300 g/kg protein are an excellent source of plant protein for human and cattle feeds (Park et al. 2010). It is also consumed to avoid cardiovascular disorders due to its low fat, cholesterol and sodium contents (Shahid et al. 2018). Canada is the largest producer of pea, other prominent pea producing countries are France, Russia, China, India and Ukraine (Janzen et al. 2014).

Mung bean

The *Vigna* genus is pantropical consisting of about 170 species, Africa comprising 120 and 22 in Indo-Pak subcontinent and Southeast Asia, and few from other countries. Since ancient times, mung bean [*Vigna radiata* (L.) R. Wilczek] has been a major pulse crop in Asia (Ghafoor et al. 2002). Mung bean is an annual, self-pollinated diploid legume plant with 22 chromosomes ($2n = 2x = 22$) with approximately 579Mbp/1C genome size (Arumuganathan and Earle 1991). Mung beans are thought to be originated and domesticated in the Indian subcontinent around 2500 BC, data on the domestication of mung bean, morphological research, and archaeological findings suggest they might have originated from places like minor hill groups between the Krishna and Godavari rivers, near the upper Ganges in the Eastern Harappan zone and the Western Himalayan foothills (Fuller and Harvey 2006). *V. radiata* var. *sublobata* are the nearest wild relatives of the cultivated *V. radiata*, and are considered to be their wild progenitors (Singh and Jauhar 2006). Domestication and selection from *V. radiata* ssp. *radiata* resulted in the cultivation of mung bean which is widespread across Eastern and Southern Asia, Austronesia and Africa (Lambrides and Godwin 2007). Mung bean protein

is easily digestible, dried grains can be eaten whole or split after cooking to make soup or *dhal*, deep-fried delicious cakes, noodles, and also flour for making biscuits and bread (Tomooka et al. 2005). On a dry weight (DW) basis, a mung bean seed contains 59–65% carbohydrate, 24–28% protein, 4.5–5.5% ash 3.5–4.5% fiber and 1.0–1.5% fat and provides energy in the range of 334–344 kcal (Mehandi et al. 2019). The majority of the mung bean production (90%) takes place in Asia where India is the world's largest producer, accounting for more than half of the global output. In India, mung beans are grown on approximately 4.2 million hectares, with an estimated annual production of 1.3 million tons in 2008 (Isemura et al. 2012).

Black gram

The *Vigna* genus is a leguminous plant consisting of a large taxon that is found in tropical and subtropical regions of America, Asia, Africa and Australia. Black gram is a self-pollinated diploid ($2n = 2x = 22$), short duration annual plant species with a genomic size estimated to be 574 Mbp/1C (Arumuganathan and Earle 1991). Cultivated black gram [*Vigna mungo* (L.) Hepper var. *mungo*] (known also as *urad*, *urd* or *mash*). It is believed in India that the domestication of black gram originated from *Vigna mungo* var. *silvestris* as its wild progenitor (Fuller et al. 2004). *Vigna mungo* seeds are a staple food in South Asia, and the split and de-hulled seeds (*dhal*) are a common dish (Joyner and Yadav 2015). Many traditional products are prepared from black gram like *papad*, *wari*, *idli*, *halwa*, *dosa* and *imrati* (Zia-Ul-Haq et al. 2014). Owing to its relatively short life cycle (75–90 days) and drought tolerance, the ability to fix nitrogen from the atmosphere with the help of soil bacteria *Bradyrhizobium* and *Rhizobium*, black gram is grown as part of a variety of cropping systems, although it is most commonly grown after wheat and rice (Kaewwongwal et al. 2015). Black gram is primarily grown in India, Afghanistan, Bangladesh, Pakistan, Myanmar, Nepal, Philippines, Thailand, Sri Lanka, South and Southeast Asian countries. India is the world's leading producer as well as consumer of black gram, owing to its major contribution to its production. In 5.44 million hectares, it is grown with an annual production of roughly 3.56 million tons in 2017–2018 and a productivity of 655 kg/ha (Raizada and Souframanien 2019).

Lentil

Lentil (*Lens culinaris* Medik.) is one of the oldest legume crops, dating back to 7000–6000 BC according to archeological evidence (Ersikine et al. 2016). The lentil plant is present in the clade Hologalegina, tribe Vicieae, subfamily Faboideae as a member of the Fabaceae (Leguminosae) family (Chahota et al. 2018). Lentil plant is an annual diploid

($2n = 2x = 14$), a self-pollinated crop having genome size of 4063 Mbp/1C (Arumuganathan and Earle 1991). There are many wild lentils but *L. orientalis* is thought to be the progenitor of the cultivated lentil (Yadav et al. 2007). The lentils which are chiefly cultivated have been split into two commercial classes, acrosperma and microsperma, with seed diameters ranging from 6 to 9 mm and 2 to 6 mm, respectively (Koul et al. 2017). Lentil is a popular staple food crop in many parts of the world, having protein (20–30 g/100 g), healthy fats (<2 g/100 g), dietary fiber, and a variety of micronutrients (selenium, zinc, beta-carotene and folates) (Saygılı and Saygılı 2019). In India, large-seeded macrosperma type of lentil varieties are chiefly grown in Madhya Pradesh and neighboring districts of Uttar Pradesh, covering about 35% of the total area, whereas small-seeded microsperma type varieties are cultivated mainly in Haryana, Punjab, Uttar Pradesh and Bihar covering 65% of the total area (Kishor et al. 2020). The lentils are usually grown with cereals in a rotation system to help break up the cycle of cereal disease and simultaneously fix atmospheric nitrogen. Lentil contains more than twice as much dietary protein as cereals due to their high protein content. Farmers in many developing countries prefer it because of its potential to survive in drought-prone environments and marginal lands where they can grow in very little rainfall of 250–300 mm (Solh and Van Ginkel 2014). In South Asia, lentils are commonly consumed as fried or boiled (*dhal*) which has a soupy texture and is traditionally served with unleavened bread (*roti*). Boiled rice is also popularly served as a staple along with lentil *dhal*. *Khichuri* is a dish made with split or de-hulled lentils and split rice or wheat. Lentils may also be eaten as a snack after being deep-fried or mixed with cereal flour to make foods like bread and cake (Sarker et al. 2004). After threshing, plant residues such as stalks, dried leaves, husk and podwall are used as an excellent source of animal feed (Maneepun 2003). Lentils are grown all over the world, from North and South America, Australia, Northern Africa, Middle East to the Indian subcontinent and Southern Europe (Rubeena et al. 2003). Global lentil production being led by Canada, India, Turkey and the United States (Johnson et al. 2020).

Mode of action of HMs in plant system

Contamination of the soil is the leading cause of the entry of HMs into the plant system. The root surface absorbs the bioavailable metal, which is then transported through the cellular membrane into the root cells. There are two primary pathways for the uptake of HMs by roots, the apoplastic pathway (passive diffusion) and the symplastic pathway (which involves concentration across the plasma membrane and active transport against electrochemical potential gradients) (Yan et al. 2020). The accumulation of HMs in plants

involves a series of processes which include mobilization of the HMs, uptake by the roots, xylem loading, transportation from roots to shoots, compartmentalization within cells, sequestration and distribution to aerial parts (Dalvi and Bhalerao 2013). HMs are commonly absorbed through the symplastic pathway, which is an energy-dependent process facilitated by metal ion carriers or complexing agents (Okon et al. 2020). To enter the xylem, HMs are thought to use membrane pumps or channels, which also transport essential elements to the plant system (Peer et al. 2005). HMs activate distinct signaling cascades in plants, including but not limited to, calcium-dependent signaling, ROS signaling, mitogen-activated protein kinase (MAPK) signaling and hormone signaling pathways. These pathways subsequently augment the expression of transcription factors (TFs) and/or stress-responsive genes, thereby leading to a physiological response in the plant system (Dubey et al. 2014). Plants contain multiple Ca^{2+} sensors, e.g., calmodulins (CaMs), calcineurin B-like proteins (CBLs), CaM-like proteins and Ca^{2+} -dependent protein kinases (CDPKs) that detect, decode and transmit alteration in cytosolic Ca^{2+} levels to facilitate stress response (Steinhorst and Kudla 2014). HM stress has a significant impact on MAPK signaling pathways. The activation of MAPKs occurs in response to the recognition of particular metal ligands and ROS generated during metal stress (Jalmi et al. 2018).

Responses and effects of HM stress on pulse crops

Growth attributes

Medda and Mondal (2017) clearly showed that the structure of the xylem and phloem both in root and shoot were gradually distorted at a higher concentration of Cr ranging from 20 to 100 mg/L in *C. arietinum*. The root and shoot length significantly decreased at 33.3 mg As/kg dry weight (DW) contaminated soil after 12 days, while after 32 days, the root and shoot fresh weight (FW) and DW were significantly decreased at 73.3 mg As/kg DW in soil (Päivöke and Simola 2001). *P. sativum* grown at 30 mg/kg As soil had a higher As content in the tissue system of root than in shoot and least in grains (Alam et al. 2020). As (25 and 250 μM) caused mitotic aberrations, DNA fragmentation, degeneration of spindle and disrupted the microtubule architecture at different stages of cell cycle in the root apices (Dho et al. 2010). Mineral contents in seeds were found to decline under As stress, exhibiting decreased accumulation of Cu, Fe, Se, Zn, Mn, Ni and Co in different plant parts. Total amino acid contents also decreased by 46% in treated *C. arietinum* (Tripathi et al. 2015a, b). Hydroponically grown *P. sativum* exposed to Pb, accumulated the highest Pb content in the root system followed by stem and leaves. Moreover, Pb stress induced impairment in the meristematic

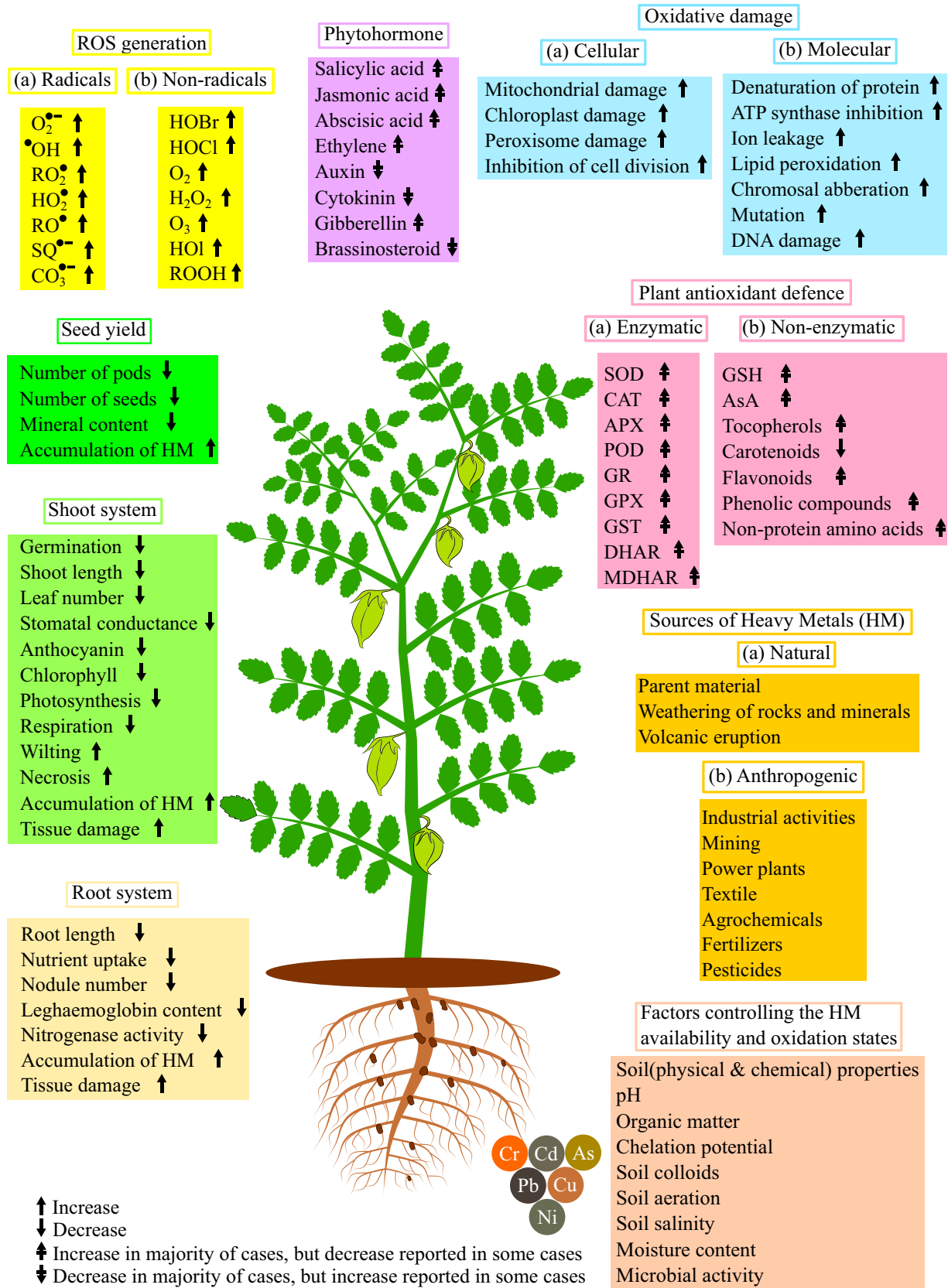


Fig. 1 Schematic diagram showing responses and effects of HM on plant (aliphatic, aromatic or heterocyclic group R, alkoxy radical RO[•], ascorbate AsA, ascorbate peroxidase APX, carbonate CO₃²⁻, catalase CAT, dehydroascorbate reductase DHAR, glutathione peroxidase GPX, glutathione reductase GR, glutathione S-transferase GST, heavy metal HM, hydrogen peroxide H₂O₂, hydroperoxides ROOH, hydroxyl radical [•]OH, hypobromous acid HOBr, hypochlorous acid HOCl, hypiodous acid HOI, monodehydroascorbate reductase MDHAR, oxygen O₂, ozone O₃, perhydroxy radical HO₂[•], peroxidase POD, peroxy RO₂[•], reduced glutathione GSH, semiquinone SQ^{•-}, superoxide anion O₂^{•-}, superoxide dismutase SOD)

zone, reduction of mitochondrial cristae, swelling of mitochondria, alterations in peroxisomal membrane, shrunken protoplasts and in severe cases degeneration was observed in the root system (Małacka et al. 2008). *P. sativum* seedlings showed a significant reduction in alkaline invertase and acid invertase activities under Pb stress. In Pb-treated seedlings, sucrose-6-phosphate synthase (SPS) and sucrose synthase (SS) enzyme production were inhibited in the root, whereas SS activity was upregulated in the cotyledons. Moreover, Pb increased glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) in cotyledons, while downregulation of G6PDH and upregulation of hexokinase in the root and shoot parts were reported in stressed seedlings (Devi et al. 2013). The growth attributes like leghemoglobin, carbohydrate and nitrogen content in the nodules, Chl content, photosynthetic rate, leaf water potential, transpiration rate, activities of carbonic anhydrase, nitrate reductase (NR) and nitrogenase decreased proportionately at higher concentrations of Ni at 45-day stage pot grown *V. radiata* (Yusuf et al. 2014). Similarly, enzyme activities like α -amylase, protease and H⁺ ATPase decreased with increasing As exposure to cotyledons of germinating seeds (Ismail 2012). According to Garg and Kashyap (2017) As stress resulted in a significant decrease in seed yield, number of pods, seed development, seed weight, harvest index, nutrients uptake, plant biomass and productivity.

Photosynthetic performance

HM toxicity can remarkably damage the plant's photosynthetic machinery. HM in particular, can have a severe impact on the photosynthetic rate, Chl content, and intracellular CO₂ concentration of plants (Nikalje and Suprasanna 2018). 50 μ M Cd stress greatly altered the physiological functions of leaves like net photosynthetic rate, internal CO₂ concentration, stomatal conductance, transpiration rate and maximum quantum yield of photosystem II (PSII) showed a significant decrease in their photosynthetic attributes (AS and Tahir 2019). Application of Pb interfered with the plant growth and metabolism leading to a significant reduction in the leaf area, harvest index and assimilation rate (Hussain et al. 2007). At 200 μ M Cd, nicotinamide adenine

dinucleotide (NADH) oxidase, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, 6-phosphogluconate dehydrogenase (6PGDH) and glucose-6-phosphate dehydrogenase (G6PDH) activities in root and shoot of *C. arietinum* seedlings increased considerably in treatment sets when compared to that of control (Sakouhi et al. 2016).

ROS and antioxidative system

One of the major outcomes of being exposed to HMs stress is the excessive generation of various reactive oxygen species (ROS) such as hydroxyl radicals (OH[•]), superoxide (O₂^{•-}), hydroperoxyl radicals (HOO[•]), the peroxyionite (OONO⁻) ion, paramagnetic singlet oxygen (¹O₂), hydrogen peroxide (H₂O₂), nitrogen oxide radical (NO), ozone (O₃) and hypochlorous acid (HOCl) (Sharma et al. 2020a, b). The functioning of different antioxidative enzymes undergoes significant alterations in the plant system when they are exposed to HM stress. To counteract the harmful effects of ROS, plant tissues increase the expression and activity of enzymatic antioxidants, superoxide dismutase (SOD), guaiacol peroxidase (GPOX), catalase (CAT) and enzymes belonging to ascorbate–glutathione (AsA–GSH) cycle like ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR) and non-enzymatic glutathione reductase (GR), phytochelatin (PCs) along with total glutathione, oxidised glutathione (GSSG), glutathione-S-transferase (GST) (Saroy and Garg 2021; Garg and Aggarwal 2012; Hossain et al. 2020). Schematic diagram on the effects of HM stress on plant system is shown in Fig. 1. SOD, CAT and peroxidase (POD) increased under Cd stress during HM uptake, showing clear symptoms of Cd stress that were significantly higher than that of control (Garg and Aggarwal 2012). Similarly, Ni stress enhanced the activities of antioxidant enzymes such as NADH-oxidase, SOD, APX, CAT, GR and GST in comparison to control (Gajewska and Skłodowska 2005; El-Amier et al. 2019). Under As (50 μ M) stress, NADP-dependent isocitrate dehydrogenase and NADP-dependent malic enzyme activities increased in roots, but in leaves remained unaffected. S-nitrosoglutathione reductase (GSNOR) activity, nitric oxide (NO) and peroxyionite (ONOO⁻) content decreased in roots, while in leaves, NO content increased, whereas no significant changes were registered for GSNOR activity and ONOO⁻ content (Rodríguez-Ruiz et al. 2019). Polyamine contents (free polyamine, soluble-conjugated and insoluble-bound) such as spermine, spermidine and putrescine, along with activities of polyamine-biosynthetic enzymes such as arginine decarboxylase, S-adenosylmethionine decarboxylase and ornithine decarboxylase in *P. sativum* leaves increased under the influence of Ni stress (Shahid et al. 2014).

Table 4 Effects and response of different pulse crops under HM stress

Parameters studied	Alteration in plant parameters	HMs	References
Growth parameters and yield components of the respective plants were studied, like root and shoot length, root and shoot FW and DW, number of pods, seeds and grain yield	Root and Shoot length decreases	<i>C. cajan</i>	<i>V. mungo</i>
	Root and shoot FW and DW decreases	Dotaniya et al. (2014)	Shanker et al. (2004)
	No. of pods, seeds and grain yield decreases	(Pamaik and Mohanty 2013)	Ghani (2010)
		Mondal et al. (2013) and Ullah et al. (2020)	Saleh (2007)
		Bhattacharya et al. (2012), Gupta et al. (2004) and Malik et al. (2011)	Päivöke and Simola (2001) and Alam et al. (2020)
		Naz et al. (2015)	Alam et al. (2019) and Ahmed et al. (2012)
		(Gandhi et al. 2020)	Borah and Devi (2012) and Anwar et al. (2020)
		Askari and Khurshid (2018)	Cokkizgin and Cokkizgin (2010)
		Khan and Khan (2010)	Janas et al. (2010)
		Oves et al. (2013)	Saad et al. (2016)
Photosynthesis and related components, nodule development and number, leghemoglobin content and nitrogenase activity	Total carotenoid and Chl (a,b) content decreases	–	Jabeen et al. (2016)
	Nodule number, nodule DW, NR and leghemoglobin content decreases	(Garg and Singh 2018), (Sharma et al. 2020a, b)	Sharma et al. (2021)
		Garg and Kashyap (2017)	Shamshad et al. (2018)
		Nautiyal and Sinha (2012), Garg and Aggarwal (2011)	Sharma (2013)
		Singh et al. (2010)	Upadhyaya et al. (2014)
		Çanakci and Dursun (2011)	Arif et al. (2019)
		Garg and Saroy (2020)	Chaturvedi et al. (2021)
		Sharma et al. (2017b)	Hattab et al. (2009)
		Saad et al. (2016)	(Wani et al. 2007b), (Ahmad et al. 2008)
		Garg and Saroy (2020)	(Yusuf et al. 2014)
		Gurpreet et al. (2012)	

Table 4 (continued)

Parameters studied	Alteration in plant parameters	HMs	References
Antioxidant defense system (enzymatic and non-enzymatic)	SOD, CAT, POD, APX, GR, GPX, GSH, GSSG and GST activities mainly increases with the increase in HMs concentration	Cr	Saif and Khan (2018) Battana and Ghanta (2014) Tripathi et al. (2015a, b) and Gangwar and Singh (2011) Gautam et al. (2020), Karuppanapandian and Manoharan (2008) and Jabeen et al. (2016)
		Cd	Ahmad et al. (2016) Garg and Aggarwal (2012) Talukdar (2012) Anjum et al. (2001) and El-Amier et al. (2019) Mansoor (2017) Dutta et al. (2018), Molina et al. (2008)
		As	Tripathi et al. (2017) Garg and Kashyap (2019) Mitra and Paul (2020) Pandey and Bhatt (2016) Jha et al. (2021), Srivastava and Sharma (2013)
		Pb	Khwaza and Verma (2015) Garg and Aggarwal (2012) Haider and Azmat (2012) Singh et al. (2017), Arif et al. (2019) and Aly (2013) Yasin et al. (2020)
		Cu	Sharma and Singh (2013) Sharma et al. (2017a) Chaoui and El Ferjani (2014) and Hossain et al. (2020) Malecka et al. (2012) and Chaoui et al. (2004) Fariduddin et al. (2014) and Mao et al. (2018) Akhtar et al. (2016)
		Ni	Saif and Khan (2018) Madhava Rao and Sresty (2000) El-Amier et al. (2019) and Balal et al. (2016) Mahmood et al. (2016)
Stress response/indicators components and enzymes	EL, MDA, H ₂ O ₂ and proline contents increases in response to HMs stress. Likewise, DHAR, MDHAR activities also increases in presence of HMs stress among different plant species	Cr	Singh et al. (2020) Battana and Ghanta (2014) Shanker et al. (2009) and Jabeen et al. (2016) Karuppanapandian and Manoharan (2008)
		Cd	Kumari et al. (2010), Ahmad et al. (2016) Anjum et al. (2008) and Hassan and Mansoor (2017) Dutta et al. (2018) and Molina et al. (2008)
		As	Chandrakar et al. (2020), Adhikary et al. (2019) Shabnam et al. (2019) and Singh et al. (2007) Srivastava and Sharma (2013)
		Pb	Bhagyawant et al. (2019) and Reddy et al. (2005) Aly (2013) Gurpreet et al. (2012)
		Cu	Nair and Chung (2015) Islam et al. (2016) Gopalakrishnan Nair et al. (2014) Akhtar et al. (2016)
		Ni	Saif and Khan (2018) Saroy and Garg (2021) Yusuf et al. (2014) Mahmood et al. (2016)

According to reports, when plants are exposed to HM toxicity, there is an increase in the production of ROS which can trigger an oxidative burst. This burst can damage various components of the plant such as lipids, proteins and pigments as well as stimulate the process of lipid peroxidation and electrolyte leakage (EL) (Kumari et al. 2010). The presence of Cd (0.3 mM)-induced oxidative stress and membrane injury which enhanced significant levels of total soluble protein and malondialdehyde (MDA) contents (Hassan and Mansoor 2014). EL increased significantly in both the root and shoot systems of pea upon As treatment (Rahman et al. 2017). At 100 μ M Cr, along with an increase in activities of DHAR, MDHAR and H₂O₂ increased, respectively, in course of time (12–120 h) with respect to control in *V. mungo* (Karuppanapandian and Manoharan 2008). Under Cu stress, proline content and total phenolic content increased to variable levels in all treated sets (Akhtar et al. 2016). Table 4 represents HM induced phytotoxic effects and response on different plant species in their oxidative and biochemical traits.

Conclusion

In conclusion, HMs pose a great threat to pulse production. Most of the HMs get accumulated in the plant tissues and adversely affects plant growth and metabolism. This review article provides quick access to information about the effects of HM toxicity on important pulse crops in their development and physiological processes. To feed the ever increasing population, solving food demand and increasing crop production is a difficult task due to climate changes, biotic and abiotic stresses. The presence of HMs reduces plant yield and production, which negatively impacts the health and economy. Therefore, it is imperative to properly manage HM usage, release and distribution into the environment. Hence, environmental agencies and government bodies should take necessary steps to ensure keeping HM pollution to a minimum. Plants have developed mechanisms to prevent the toxic effects of HMs, but in excess concentration, even the plant defense mechanisms fail to recover from the toxic effects of HMs. Additionally, attempts should be undertaken to decipher the precise biochemical pathways and molecular mechanisms of HM toxicity in plants. Moreover, further research in genomics, transcriptomics, proteomics, metabolomics and signaling pathways are required to explore the intricate crosstalk of HM tolerance and modulation in the plant system. So that in the future, HMs do not cause any significant constraints limiting crop productivity worldwide.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical statements

Not applicable.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

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Data availability The data presented in Table 3 were collected from FAOSTAT and are available in <https://www.fao.org/faostat/en/#home>. The schematic diagram, i.e., Fig. 1 is a tiff file which was generated

electronically in .emf format via Inkscape software (<https://inkscape.org/release/inkscape-1.2.1/>) and later converted to .tiff file.

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