RSC Advances

REVIEW

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Cite this: *RSC Adv.*, 2020, **10**, 38379

Heavy metal induced stress on wheat: phytotoxicity and microbiological management

Asfa Rizvi, *[a] Almas Zaidi,[a] Fuad Ameen,[b] Bilal Ahmed, *[a] Muneera D. F. AlKahtani[c] and Mohd. Saghir Khan[a]

Among many soil problems, heavy metal accumulation is one of the major agronomic challenges that has seriously threatened food safety. Due to these problems, soil biologists/agronomists in recent times have also raised concerns over heavy metal pollution, which indeed are unpleasantly affecting agro-ecosystems and crop production. The toxic heavy metals once deposited beyond certain permissible limits, obnoxiously affect the density, composition and physiological activities of microbiota, dynamics and fertility of soil leading eventually to reduction in wheat production and *via* food chain, human and animal health. Therefore, the metal induced phytotoxicity problems warrant urgent and immediate attention so that the physiological activities of microbes, nutrient pool of soils and concurrently the production of wheat are preserved and maintained in a constantly deteriorating environment. To mitigate the magnitude of metal induced changes, certain microorganisms have been identified, especially those belonging to the plant growth promoting rhizobacteria (PGPR) group endowed with the distinctive property of heavy metal tolerance and exhibiting unique plant growth promoting potentials. When applied, such metal-tolerant PGPR have shown variable positive impact on wheat production, even in soils contaminated with metals, by supplying macro and micro nutrients and secreting active biomolecules like EPS, melanins and metallothionein (MTs). Despite some reports here and there, the phytotoxicity of metals to wheat and how wheat production in metal-stressed soil can be enhanced is poorly explained. Thus, an attempt is made in this review to better understand the mechanistic basis of metal toxicity to wheat, and how such phytotoxicity can be mitigated by incorporating microbiological remediation strategies in wheat cultivation practices. The information provided here is likely to benefit wheat growers and consequently optimize wheat production inexpensively under stressed soils.

Received 27th June 2020
Accepted 17th September 2020

DOI: 10.1039/d0ra05610c

rsc.li/rsc-advances

1 Heavy metals in soils: source and availability

Soil is considered a natural habitat of heterogenous microbial communities, and may become contaminated by the deposition of heavy metals (HMs): metals and metalloids having densities greater than 5 g cm^{-3}. Hence, the cultivable land around the world is declining due to soil pollution, which has become a serious problem in many countries.[1] Heavy metals occur naturally in soil ecosystems,[2,3] resulting from the pedogenetic processes of weathering of parent materials at levels that are regarded as trace (<1000 mg kg^{-1}) and rarely toxic.[4,5] Other sources of HM pollution to agronomic soils include emissions from volcanoes, dispersal of metal-containing dusts, and the breakdown product of rocks enriched with HM.[6] Apart from these natural sources, HM can also be added extensively to cultivable soils emanating from anthropogenic activities (Fig. 1), such as those involving rapidly expanding industries, mines and smelters,[7,8] disposal of HM wastes, gasoline and paints, land application of fertilizers,[9,10] biosolids (*e.g.* livestock manure, compost, and municipal sewage sludge),[11,12] pesticides for example, copper-containing fungicidal sprays such as Bordeaux mixture (copper sulphate) and copper oxychloride and lead arsenate,[13] unprocessed wastewater used in irrigation,[14] coal combustion residues, spillage of petrochemicals, and atmospheric deposition.[15,16]

Heavy metals also enter the soil through road traffic and road dust,[17–19] and the burning of tires and brake linings.[20,21] Some other notable sources that can also add adequate quantities of HM to soils include fly ash originating from coal-fired power plants,[22] PVC products, colour pigment, and several alloys and chargeable Ni–Cd batteries.[23]

All of the anthropogenic activities causing soil contamination have broadly been grouped into five categories: (i) mining

[a]*Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, India, 202002. E-mail: asfarizvi09@gmail.com; bilalahmed.amu@gmail.com; bilalahmed.rs@amu.ac.in*

[b]*Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia*

[c]*Department of Biology, College of Science, Princess Nourah Bint Abdulrahman University, PO-BOX 102275-11675, Riyadh, Saudi Arabia*

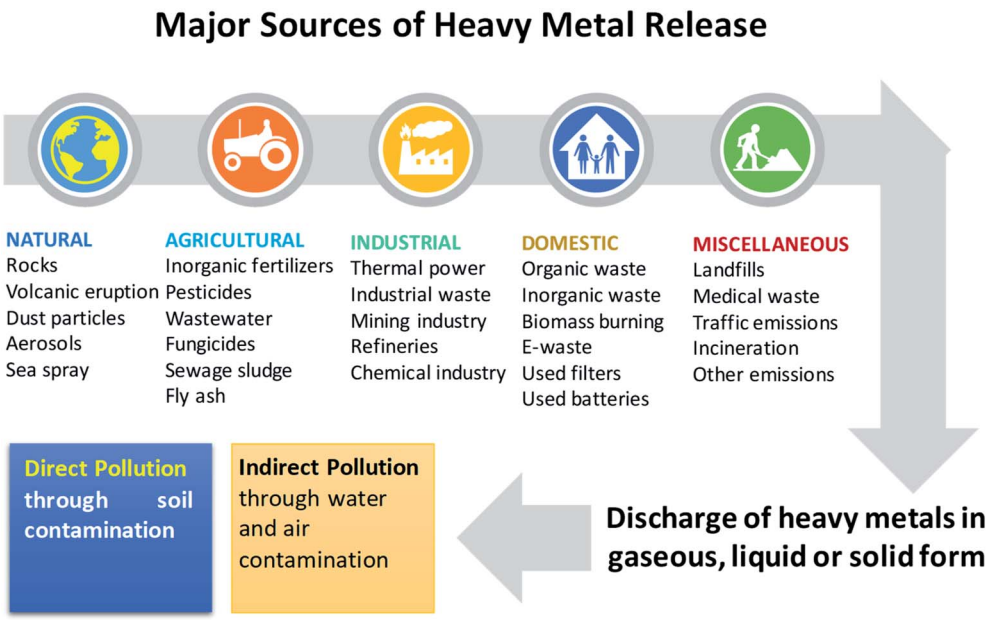

Fig. 1 Sources of heavy metal pollution in the environment.

and smelting, (ii) industries (*e.g.*, As, Cd, Cr, Co, Cu, Hg, Ni and Zn), (iii) agriculture (*e.g.*, As, Cd, Cu, Pb, Se, U and Zn), (iv) atmospheric deposition (As, Cd, Cr, Cu, Pb, Hg and U), and (v) waste disposal (*e.g.*, As, Cd, Cr, Cu, Pb, Hg and Zn). Heavy metals added to soils from anthropogenic sources are generally more mobile and therefore, easily bioavailable.[24,25] However, the total pool of HMs in soils does not provide any information regarding its mobility and availability. Yet, the fate and movement of HMs in soil depends significantly on the composition, concentration and speciation of metals. The distribution of HMs in soils is controlled by: (i) precipitation and dissolution, (ii) ion exchange, adsorption and desorption, (iii) aqueous complexation, (iv) biological immobilization and mobilization, and (v) plant uptake.[26] Based on these properties, soil metals have been grouped into five key geochemical categories: (i) exchangeable, (ii) metal bound to carbonate phase, (iii) metal attached to Fe and Mn oxides, (iv) bound to organic matter, and (v) residual metal. Metals found in one or the simultaneous category in soils, however, differ significantly in bioavailability, mobility and speciation largely due to their ability to react rapidly with low-molecular organic acids, carbohydrates, and enzymes excreted by soil microbiota.[27] In addition, the surface of microbial communities inhabiting the soil has charges which help them to interact very strongly with metal ions in soil solution.[28] For example, symbiotic PGPR have been found to adsorb Cu and Cd in soil, when applied as inoculant.[29] However, how soil organisms affect the speciation and distribution of metals in soils are inadequately explained. In order to better understand these, scientists working in different areas have employed numerous strategies, like soil column leaching experiments, sequential extraction and the single extraction approach. However, none of these methods have been completely successful in predicting/establishing the complexation of variable soil metals. Hence, it did not pinpoint the mobility and bioavailability of elements.[30,31] Still, among these methods, the chemical extraction method is frequently used to determine the bioavailability or mobility of heavy metals,[32,33] which also provides information regarding metal availability and transport of metals to plants.[34] In summary, the availability of heavy metals in soils is affected by factors such as: (1) metal species and influence of environmental factors,[35] (2) structure and compositions of soil, (3) genotypes and plant photosynthates, (4) soil–crop–microbes interactions, and (5) use of agrochemicals in cultivation practices, water management, and crop rotation systems.[36,37]

2 Heavy metal–plant interactions

2.1 Heavy metal toxicity to plants: impact on physiological processes

The rapidly growing industries, uncontrolled and untreated discharge of xenobiotic pollutants, and use of poor-quality waters (wastewater) for irrigation in agricultural practices pose severe unbearable danger to the sustainability of agroecological niches.[38,39] However, the availability of metals for plants is governed by several soil factors, such as pH, cation exchange capacity (CEC), organic matter content and adsorption by clays.[40,41] Heavy metals, following accumulation within soil, enter the food chain[38,42,43] and are subsequently transferred to the end consumers, leading to human health problems.[44] The toxicity of heavy metals that enter vegetal tissues, however, can inhibit multiple physiological processes of plants,[45,46] including wheat[47,48] and eventually human health (Fig. 2). Briefly, metals at exceedingly higher concentrations damage plants by: (i) altering membrane permeability,[49] (ii) inhibiting physiologically active enzymes,[50] (iii) inactivating photosystems,[51] and (iv)

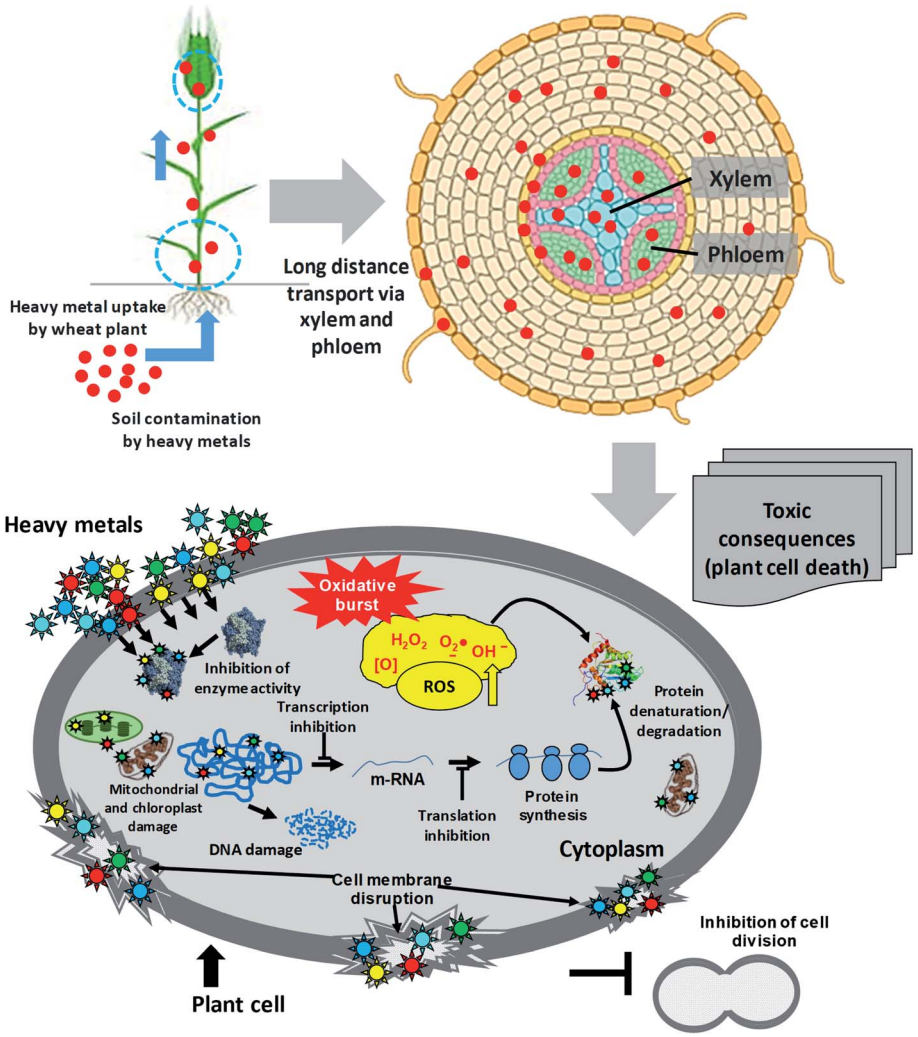

Fig. 2 Sequence of events from metal entry into a plant cell to the death of the plant.

disturbing mineral metabolism.[52] Apart from these, the metal toxicity causes oxidative stress, disruption of pigment function and alteration in protein activity.[53] The hyper generation of ROS under metal pressure may cause significant damage to cell structures in plants, such as: (i) oxidation of proteins and lipids, (ii) nucleic acid damage, (iii) enzyme inhibition, and ultimately (iv) cell death.[54,55] Some of the physiological processes of plants impacted adversely by heavy metals are briefly discussed in the following section.

2.1.1 Germination and seedling growth. The majority of studies have been conducted to assess the impact of different heavy metals on live plants,[56,57] using seedlings or adult plants. However, in some studies, seeds have also been exposed to metals.[58] Hence, the seed germination process becomes an important aspect among many plant physiological processes. Since seed germination is the first physiological process affected by metals, the ability of seeds to germinate in a polluted environment indicates its level of tolerance to metals.[59] Seeds possess certain sensing mechanisms, which allow them to germinate under favourable environmental conditions and to complete the developmental process. Despite these properties, seed germination, growth and the production of many plants have been found to be adversely affected when exposed to metals.[60,61] For example, metals, such as Hg, Cd, Co, Cu, Pb, Zn, Ni, Ag, have shown variable impact on the germination of seeds of many plants,[62] including wheat.[48] In a prior study, the phytotoxic effect of Cr on seed germination and seedling growth of some wheat cultivars (HD2956, HD2932, DBW14, KO512, WH775) individually treated with 25, 50, 75, 100 and 125 ppm of Cr(VI) was variable. A consistent increase in Cr(VI) concentration significantly inhibited seed germination, and the percentage phytotoxicity increased with the gradual increase in the Cr(VI) level for all wheat cultivars. Among the measured parameters, root growth was maximally reduced.[63]

After seeds, the emerging roots are the first organ that comes in direct contact with the various rhizosphere constituents.[64] When a root absorbs water or nutrients from soil, ions (including those of HMs) and molecules move toward this organ both by mass flow (along with soil water) and by diffusion process. Root growth is more sensitive to heavy metal

contamination.[65,66] An immediate effect of the high concentration of metal results in root growth inhibition, which may be shorter, very ramified and without a solid structure.[67,68] The inhibition of root elongation is accompanied by alteration in the architecture and morphology of roots. For instance, many heavy metals, including Pb and Cr, have been reported to rapidly inhibit the root growth, leading to biomass reduction of wheat,[65] probably due to the inhibition of cell division in the root tip.[69,70] In other experiments, wheat[71,72] has shown a decrease in length and in the dry mass of roots when grown under Pb stress.[73] Mechanistically, the distention and lesions in the cell wall of *wheat* roots occur due to the activation of certain wall-degrading enzymes in response to Pb exposure.[74]

2.1.2 Cell wall and plasma membrane. The cell walls are the first structures of the roots that are exposed to metals, if present in soils. From the soil, it enters the root tissues and forms a complex with the carboxylic groups of the pectin constituents of cell walls.[75] The binding of metals in the cell wall and/or to the apoplastic face of the plasma membrane may impair apoplasmic and symplasmic cell metabolism, leading to metal-induced inhibition of root elongation.[76] The inhibition of root elongation is accompanied by changes in the architecture and morphology of the roots. A reduction in the formation of the lateral roots and root hairs, changes in colour, thickening, atrophy and curvature of the roots are common symptoms.[77] The duration of exposure and concentration of metals may influence the cell wall rigidity, causing the rupture of the rhizodermis and outer cortex of the meristem, which may inhibit the elongation of root tips.[78,79] The plasma membrane is yet other site to which any metal can bind and disrupt membrane functions.[80] After interaction, the metal induces changes in the membrane constituents, especially lipids, resulting in an altered structure and physiological functions of the membrane and other cellular processes. For instance, the variation in the composition and fluidity of membrane lipids, oxidation and the cross-linking of protein thiols, and the destruction of some important membrane proteins are some of the toxic consequences of metals.[81] Among different metals, the effect of Cr on the transport activities of the plasma membrane has been reported.[82] The inhibition of ATPase activity is suggested to be due to the disruption of the membrane by free radicals generated under metal stress.[83] The decrease in ATPase activity reduces proton extrusion, and ultimately decreases the transport activities of the root plasma membrane. As a result, the uptake of nutrients by roots is limited. Moreover, it is also reported that Cr interferes with the mechanism controlling intracellular pH.[84] Mechanistically, Cr alters the metabolic activities of plants as it: (i) modifies the production of photosynthetic pigments (like chlorophyll), (ii) increases the production of metabolites (for example glutathione[85] and ascorbic acid[86]) as a direct response to metal stress, which may damage the plants. Moreover, Cd treatment also reduces the ATPase activity of the plasma membrane fraction of roots.[87]

2.1.3 Peroxidation of membrane lipids. The alteration in membrane functions due to metal action leads to changes both in the structure and peroxidation of membrane lipids.[88,89] For example, Pb contamination has led to the excessive production of ROS (radical O^{2-}) and hydrogen peroxide (H_2O_2) in plant cells.[90,91] However, the excessive generation of ROS under metal stress is a common feature of plants, which may react with lipids, proteins, photosynthetic pigments, and other cellular organelles. It can cause lipid peroxidation and membrane damage, eventually leading to cell death.[92,93] Malondialdehyde (MDA) is one of the ultimate products of lipid peroxidation, whose level is influenced by the degree of membrane lipid peroxidation.[94] Malondialdehyde, synthesized in plants as a result of the oxidation of polyunsaturated fatty acids (PUFAs), has a potential role in damaging the cell membrane.[95] However, the concentration of MDA increases when plants suffer from excessive oxidative stress, and are unable to scavenge the ROS.[96] Since lipid peroxidation may serve as a biomarker for oxidative stress in plants, the MDA content in plants (including those in wheat leaves) is generally determined to assess the extent of metal toxicity. Some ROS even alter gene expression and modulate the activity of specific proteins involved in the plant defense system.[97] The increased lipid peroxidation also changes the membrane properties, such as fluidity and permeability, and modulates the activities of membrane-bound ATPases.[98] Undeniably, peroxidation is a chain reaction, wherein unsaturated fatty acids are transformed into different small hydrocarbon fragments, such as malondialdehyde.[99] The lipid peroxidation processes and ensuing substances, in effect, sternly affect the physiological functions of the plasma membrane, leading eventually to the death of the cells.[100,101] In a similar study,[45] confirmed Cr-induced membrane damage, lipid peroxidation reduction in plant growth and yield, emulation of oxidative stress, and anti-oxidative defense. In addition, the overproduction of ROS exhausts ATP, reduces respiration rates and affects growth.[45,102] In another experiment, the gradual accumulation of Cu in the plant tissues caused certain specific changes in the composition of lipids, for instance: (i) the content of sulfolipids in chloroplasts was declined, (ii) the content of monogalactosyl diacylglycerols, digalactosyl diacylglycerols and phosphatidyl glycerols in chloroplasts and mitochondria grew after an hour of Cu exposure, (iii) and the content of all lipids except phosphatidic acids decreased after 3 h of exposure.[100] Other metals (like Fe and Cu compounds) have been found to generate more free radicals, and consequently increased the peroxidation.[103]

2.1.4 Photosynthesis. Among various metabolic processes, photosynthesis is one of the most significant physiological traits of plants. However, it has been reported to be deleteriously impacted by numerous heavy metals.[104] The toxic metals attack different photosynthetic apparatus and cause: (i) the deposition of metals in the plant foliage,[105] (ii) a change in the physiological activity of the chloroplast membrane and distribution of metals in leaf tissues, such as the stomata, mesophyll and bundle sheath,[106] (iii) reduction in the formation of photosynthetic pigments,[107,108] (iv) alteration in the cytosolic enzymes and organics,[109] (v) variation in the supra-molecular level action, especially on photosystem I, photosystem II, membrane acyl liquids and the carrier proteins of the vascular tissues,[110] and (vi) the destruction of enzymes associated with photosynthetic carbon reduction (PCR) and the xanthophyll

cycle.[50,110] The decrease in the chlorophyll ratio under metal stress could possibly be due to the destabilization and destruction of peripheral proteins.[111] Among metals, Cd and Zn, for example, have been reported to induce various components of photosynthesis, such as pigments and light capture centre, thylakoid ultrastructure and photosynthetic electron transport, stomatal conductance and access of CO_2, and activities of the Calvin cycle enzymes.[112,113] Both Cd and Zn significantly decreased the activities of photosystem II (PSII) and, to a lesser extent, also of photosystem I (PSI), as well as the rate of photosynthetic electron transport.[114] However, the effects of high Cd and excess Zn concentrations on the light-dependent photosynthetic processes are still not fully understood. Due to the altered photosynthetic activity, plants generally exhibit leaf chlorosis[115] and stunted growth.[116] As a result, HMs decrease the plant biomass by disturbing photosynthesis, respiration and other metabolic processes.[51] As an example, the exposure of wheat to high Cd^{2+} (50 μM) and Zn^{2+} (600 μM) concentrations resulted in similar relative growth rate (RGR) inhibitions by about 50% and comparable retardations of the CO_2 assimilation rates (about 30%) in the second developed leaf of the wheat seedlings. Moreover, the fluorescence analysis of chlorophyll a indicated that both metals disturbed photosynthetic electron transport processes, causing a 4- to 5-fold suppression of the efficiency of energy transformation in photosystem II. The non-specific toxic effects of Cd and Zn, which prevailed, were an inactivation of part of the photosystem II reaction centres and their transformation into excitation-quenching forms, as well as disturbed electron transport in the oxygen-evolving complex.[104]

Since heavy metals trigger the oxidative damage within the plant cell, it is very likely that the metals could inhibit the synthesis of certain enzymes involved in chlorophyll synthesis. Hence, the chlorophyll concentration could be reduced under heavy metal stress.

2.1.5 Antioxidant defense system in plants. Plants, while growing in the metal-enriched environment, suffer heavily from oxidative damage.[117] In addition, the increased concentrations of heavy metals decrease the activity of antioxidant enzymes.[118,119] Mechanistically, auto-oxidation and the Fenton reaction may cause the oxidative loss of the defense enzymes. For example, in a previous study, O_2^- was found to directly inhibit the catalase activity.[120] However, the overproduction of ROS under metal stress is a common reaction of plants toward stressor molecules, which sequentially lead to the death of plants by first altering the photosynthetic pigments, lipids, proteins, and other cellular organelles, resulting in lipid peroxidation and membrane damage.[93,94] The excessive secretion of ROS also depletes ATP and hence, adversely affects the respiration rates, eventually leading to weakened plant growth. However, to protect cells and tissues from injury and dysfunction, plants have evolved to have a broad range of antioxidative defense systems (both constitutive or induced one) to quench ROS and consequently, to prevent oxidative damage.[121,122] Some notable antioxidants providing protection to plants include guaiacol peroxidase (GPx), ascorbate peroxidase (APx), superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and glutathione reductase.[123,124] Apart from these antioxidants, plants also synthesize and secrete low molecular weight non-enzymatic antioxidants, such as proline,[125] cysteine,[126] non-protein thiol,[127] ascorbic acid,[128] and glutathione,[129] which reduce oxidative stress by scavenging ROS[130,131] and protect the cellular structures.[132] However, the release of these active molecules differs with plant genotypes, types of tissues and metal speciation.[133] For example, an increase in the H_2O_2 content upon Pb exposure caused a substantial increase in CAT activity in *Triticum aestivum*.[74] However, ref. 134 and [74] reported a decline in the activity of POXs in *Elsholtzia argyi* and *Triticum aestivum* roots, respectively, upon Pb exposure. Therefore, from these studies, it was concluded that a higher concentration of

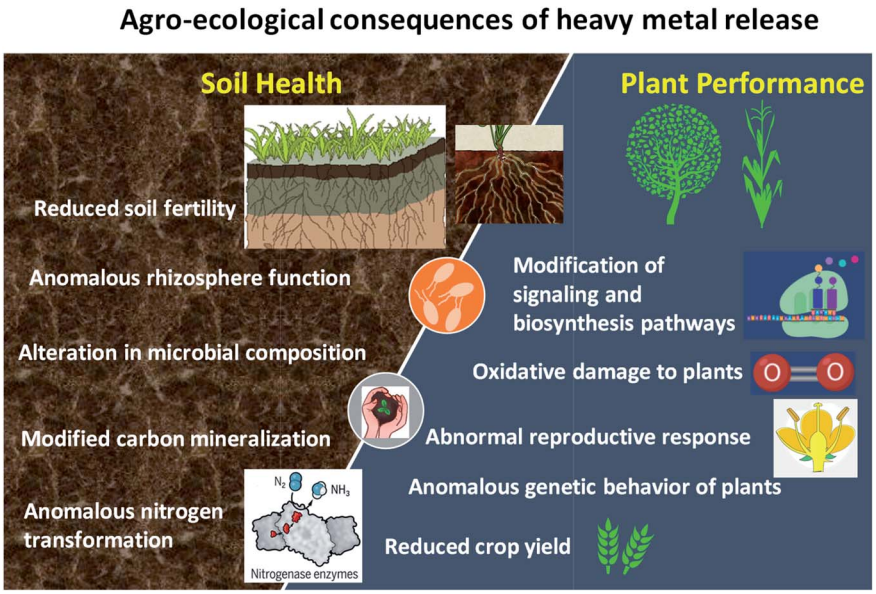

Fig. 3 Agro-ecological consequences of heavy metals on soil and plant health.

Pb or its longer exposure inhibited cell metabolism and H_2O_2 production, which in turn, decreased the CAT activity.[130]

2.1.6 Induction of proline. Among various non enzymatic molecules, proline (a multifunctional amino acid) is reported to accumulate in higher concentrations in plant tissues exposed to various stressor molecules.[135,136] Physiologically, proline helps plants maintain osmotic regulation and homeostasis.[137] Proline also protects plants from the destructive effects of ROS[138] since proline acts as a singlet oxygen quencher and as a scavenger of OH radicals.[139,140] Thus, proline is not only an important molecule in redox signaling, but also an effective quencher of reactive oxygen species formed in all plants against abiotic stress. In other plants, proline was produced as an indicator of stress.[132] Similarly, ref. 141 reported that proline did not contribute to a great extent in conferring tolerance to plants against Cd stress. This could have been due to the fact that endogenous levels of proline were negatively correlated to the shoot ($r = -0.397$) and root dry weights ($r = -0.432$), chlorophyll a ($r = -0.361$) and H_2O_2 ($r = -0.194$). In summary, the damaging effects of toxic heavy metals on the performance of plants and soil health are illustrated in Fig. 3.

3 Wheat: production, nutritional composition and importance in human health

Common wheat (*Triticum aestivum* L. em Thell.), also known as bread wheat, is a major strategic cereal crop that plays an important role in the traditional human health system, particularly in developing countries. Wheat is a major staple food crop for about 36% of the world population, including those of people from Asian countries. Among various wheat producing countries, India ranks second after China followed by United States, Russian Federation, France, Australia, Germany, Ukraine, Canada, Turkey, Pakistan, Argentina, Kazakhstan and United Kingdom.[142] India contributes approximately 11.9% to the world wheat production from about 12% of global area.[143] Nutritionally, wheat is a major source of energy (carbohydrate), but it also contains a significant amount of other important nutrients, including proteins, fiber, and minor components such as lipids, vitamins, minerals, and phytochemicals.[144,145] Worldwide, wheat provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally.[146] Even though wheat acts as a major constituent of human dietary systems, about 10% of wheat is retained for seed and industry (for production of starch, paste, malt, dextrose, gluten). Due to the nutritive ingredients, consumption of wheat is increasing globally as wheat-based foods provide a range of essential and beneficial components to the human diet. In regards to health, cereal dietary fiber reduces the risk of cardiovascular disease, type 2 diabetes and forms of cancer, notably colorectal cancer.[147]

4 Heavy metal–wheat interaction: an overview

Like many plants, wheat is also sensitive to heavy metals.[148,149] Once heavy metals enter the plants, the stress triggers different responses in plants, ranging from germination to growth,[150] to biochemical responses[104] and yield losses in wheat.[151,152] Among various toxic metals, Cd for instance has been found highly dangerous to wheat,[153,154] resulting in stunted growth and altered membrane permeability, leading eventually to the production of ROS.[155] The accumulation of ROS within plant tissues, in turn, causes the leakage of electrolytes and disrupts the integrity of the cell membrane.[156] As a result, the oxidation of membrane proteins and lipids is usually disrupted, which results in cell death.[157] In a similar study, ref. 158 evaluated the toxicity of six experimental concentrations (0.03; 0.06; 0.12; 0.6; 1.2; 2.4; 4.8 mM) of Cd on seed germination and seedling growth [index of velocity of germination (IVG)], length of aerial section and root of seedlings (green and dry mass of the seedlings) of wheat. Results suggested that Cd induced a decrease in the normal germination percentage and IVG. The inhibitory effect of Cd on the initial growth of seedlings influenced the growth of the roots and aerial parts, and also reduced the production of green and dry mass of seedlings. Conclusively, the accumulation of Cd in the soil affected the viability and production of wheat largely due to the absorption of this metal by the plant roots. Ref. 159, in a pot experiment conducted at the Old Botanical Garden, University of Agriculture Faisalabad, assessed the effect of $ZnSO_4$ on the morphological, physiological and yield attributes of two wheat varieties (W-141 and W-142) grown in sandy loam textured field soils. The toxic concentration of Zn adversely affected the morphological, physiological and yield attributes of wheat. Of the two varieties, W-141 was less sensitive to Zn than W-142. In a similar study, ref. 104 assessed the effects of varying rates of Cd^{2+} (50 μM) and excess Zn^{2+} (600 μM) on the photosynthetic contents of hydroponically grown durum wheat seedlings. The results revealed that 7 days after exposure, both Cd and Zn had similar inhibitory effect and reduced the relative growth rate (RGR) and CO_2 assimilation rates by 50% and 30%, respectively, in the second developed leaf of the wheat seedlings. Furthermore, both metals disrupted the photosynthetic electron transport processes and caused a four- to five-fold decrease in the energy transformation efficiency of photosystem II.

Chromium is yet another highly toxic metal that hampers the growth and yield of wheat.[160] Some of the toxicity symptoms associated with chromium include: (i) chlorosis of leaves, (ii) impaired growth of both roots and shoots, and (iii) wilting.[161] Once chromium enters plant tissues, it also disrupts the lamellar system. As an example, ref. 160 reported the inhibition of growth parameters due to alteration in the metabolism of plant cells in response to Cr toxicity. Chromium toxicity reduced the active reaction centres of PSII, rate of electron transport, and change in PSII heterogeneity. However, Cr did not cause any change in the heterogeneity of the reducing side, but a significant change in the antenna size heterogeneity of PSII was observed due to Cr toxicity. Ref. 162, in a study, evaluated the effects of interactions between high temperature and Cr(VI) and Cu on wheat (cv. Dagdas 94) seedlings. High concentrations of Cr and Cu at 40 °C decreased the root and shoot length and dry biomass, while the total chlorophyll content was decreased at 30 μM Cr at 40 °C. In contrast, the higher rates of Cr and Cu

increased the carotenoid and proline contents, but decreased the soluble protein relative to the control plants. Among the metals, Cr exhibited greater toxic effects on the growth and biochemical parameters compared to Cu. Similar reduction in the chlorophyll a and b contents of leaves and protein content of two wheat varieties (WH-711, C-306) while growing under different regimes of arsenic (As) has been reported.[151] Of the two varieties, WH-711 was found more sensitive to heavy metal than C-306. Cobalt is another metal that had less mobility in the leaf tissues compared to the vascular system, but had greater inhibitory impact on wheat.[163] For example, different concentrations of Co (100, 200, 300, 400 and 500 ppm) have shown a variable influence on the wheat performance when grown in a sand culture medium.[164] Cobalt at 200 ppm reduced the germination percentage, while the vigour index decreased with increasing Co concentration. However, 300 ppm of Co did not show any negative effect on the germination index, while the seed germination decreased beyond 300 ppm. Interestingly, at Co concentrations up to 200 ppm, Co enhanced the plant height, leaf number, leaf area and dry matter accumulation. Conversely, higher Co concentrations resulted in the destructive effect on the measured parameters. Also, chlorophyll a/b increased while the chlorophyll stability index decreased with Co concentration from 300 ppm onwards. Cobalt was accumulated in both above ground plant biomass and wheat grains. It was concluded from these findings that lower concentrations of Co (up to 200 ppm) had stimulatory effect on the growth of wheat. Hence, it could serve as a good phyto-extracting agent, allowing the wheat to grow under mild Co concentrations. In contrast, nickel adversely affects plant growth at higher concentrations,[165] yet it regulates N metabolism at lower concentrations,[166,167] and consequently affects germination and other vital physiological plant processes. Similarly, different concentrations (0, 40 and 60 ppm) of Pb had variable adverse effects on the growth and development of two different varieties, *i.e.*, Chakwal-97 and Sehar-2006, of wheat when tested in a pot trial experiment.[168] In general, Pb reduced the morphological parameters, such as length (shoot and root), fresh and dry biomass of shoots and number of tillers per plant. The photosynthetic pigments (chl a, chl b) also decreased under Pb stress, while the carotene contents of the wheat plants were considerably increased. Sodium and K contents were also decreased by Pb.

Mercury (Hg) is yet another major toxicant that persists in soils and hence, is a major global problem.[169] Mercury mainly remains in the solid phase, and the predominant form of Hg in agricultural soils is the ionic form (Hg^{2+}).[170] The interaction of Hg with plant systems is extremely vital since Hg has largely been used as seed disinfectants, in fertilizers and in herbicides.[171] Following interaction with plants, Hg has been found to generate ROS (such as superoxide radical, H_2O_2 and hydroxyl radicals) in plants.[169,172] From a toxicity point of view, Hg has the greatest inhibitory effects on seed germination, root elongation, and hypocotyl and coleoptile growth in wheat compared to other heavy metals.[73] Due to these properties, ref. 90, in an experiment, investigated the effect of Hg on chlorophyll content in winter wheat var. jinan no. 17. Also, the level of Ca and the bioaccumulation of Hg in wheat leaves were assayed, employing an inductively coupled plasma sector field mass spectrometer (ICP-SF-MS). The results revealed that both low and high Hg concentrations stimulated chlorophyll formation at the early stages, while it inhibited chlorophyll generation at the later stages of wheat growth. Also, the Ca and Hg concentrations in the wheat foliage enhanced with consistently increasing Hg concentration with advancing age of plants, as demonstrated by ICP-SF-MS.

5 Wheat–microbe interactions: impact on growth and yield

The constantly increasing costs of synthetic fertilizers, together with its harmful influence on soil nutrient pool (fertility) and indirectly to human health, is a major threat to wheat cultivation across the globe.[173,174] In order to solve such daunting problems, microbial formulations often called "biofertilizers" have provided sound and inexpensive solutions to the toxic chemicals in sustainable wheat production systems.[175–177] Indeed, soil microbiota that have been applied once will aggregate in the cultivable habitat, and following colonization, enhances wheat production by different mechanisms.

Wheat, among cereals, is highly sensitive to N and insufficiency of this element results in yellowing (chlorosis) of leaves, which is attributed to poor chlorophyll formation, reduced tillering, disturbance of normal cell growth division, and a reduction in the rate and extent of protein synthesis.[178] To obviate such deficiency, numerous microflora have been used in wheat cultivation practices. Chief among them is *Azotobacter*, which asymbiotically supply approx. 20 kg N/ha per year to wheat. Apart from N, inoculation of free living *Azotobacter* benefits wheat plants by providing various physiologically active growth hormones, like gibberellin, auxin and cytokinin,[179,180] ammonia, vitamins and other substances that influence seed germination,[181] protection against root pathogens,[182,183] stimulation of beneficial rhizospheric microorganisms and plant yield.[184] For example, *Azotobacter* inoculation has been reported to replace up to 50% of urea-N for wheat grown in a greenhouse trial under aseptic conditions,[185] and has been found to increase the plant height, tillers, ear length and grain yield over the non-inoculated control.[186] The grain and straw yield of wheat markedly increased from 39.4 q ha^{-1} to 41.8 q ha^{-1} and 54.3 q ha^{-1} to 57.2 q ha^{-1}, respectively, because of seed inoculation with *Azotobacter*.[187] Ref. 176 in a pot experiment reported that *Azotobacter* used either alone or in combination with NPK (nitrogen, phosphorous and potassium) and FYM (farm yard manure) significantly enhanced the root length, biomass of roots, shoots and whole plants, height, panicle weight and grain yield and other biological yields of wheat (var. Gautam). The sole application of *Azotobacter* increased the grain yield by 16.5–19.42% over the control, while with other fertilizers, the grain yield was augmented between 19–63%. The increase in yield was 23% with NPK alone relative to the control. So, *Azotobacter* was suggested as a biofertilizer, which could effectively be used to optimize the yield of wheat substantially, along with FYM and

NPK. In yet another study, ref. 188 investigated the response of mineral phosphatic fertilizer and phosphate solubilizing bacteria [PSB (*Pseudomonas* sp. and *Klebsiella* sp.)] alone or as a mixture on the growth, yield, nutrient uptake and P use efficiency of wheat grown in the field soils treated with varying levels of inorganic P (triple super phosphate: TSP) fertilizer. The maximum grain and straw yield (2.13 and 2.84 t ha^{-1}) were recorded when *Pseudomonas* sp. was applied with 15 kg P ha^{-1} and *Klebsiella* sp. with 15 kg P ha^{-1}. *Pseudomonas* sp. for Pabna and *Klebsiella* sp. for Rajshahi, along with TSP, showed better performance than other applications in terms of the yield, nutrient uptake and quality of soil. When used alone, the PSB increased the P use efficiency during crop production. A positive significant correlation was observed between the yield-contributing characters and the grain yield of wheat. From this study, it was suggested that the increase in the nutrient uptake and yield of wheat occurred largely due to the inoculation of PSB, which supplied enough of the available P to the growing wheat plants. In another experiment, ref. 177 observed a significant increment in the growth and nutrient uptake of *Pseudomonas* inoculated wheat plants. Following *Pseudomonas* sp. inoculation, the dry biomass of shoots increased significantly over the un-inoculated control. Additionally, the maximum concentration of macronutrients, like N, P, and K, in root and shoot tissues were found in the inoculated wheat plants. The application of the sulfur oxidizing bacterium *Thiobacillus thiooxidans* in the presence of Tilemsi rock phosphate (TRP) caused an increase in wheat yields. The formulation of RP fertilizers, along with *T. thiooxidans* AHB411 and *T. thiooxidans* AHB417, increased the yield upto 33.3% and 11.9%, respectively. Other biological parameters, like the number of tillers per plants, panicle length and seed attributes (such as grains per panicle and 1000 grain weight) were dramatically enhanced. A mixed inoculation of *T. thiooxidans* and Bio TRP1 increased the grain yield of wheat by 46%, whereas the straw yield was enhanced by 74% relative to the control.[189] In a follow up study, the plant growth-promoting rhizobacterial strains *Bacillus amyloliquefaciens* GB03 (BamGB03), *B. megaterium* SNji (BmeSNji), and *A. brasilense* 65B (Abr65B) significantly increased the biomass and N content of wheat plants.[190] In addition, elongation in the roots and shoots, and the increased root and shoot dry biomass of wheat plants inoculated with *Piscibacillus salipiscarius* E5 and *Halomonas* sp. G11 compared to uninoculated control plants was reported.[191] Moreover, a significant increase in the plant height, root length, leaf area, total dry matter, total chlorophyll content, and relative water content of wheat plants was observed following inoculation with *Pseudomonas aeruginosa* strain 2CpS1.[192] Inoculation of PSB, together with RP, optimized the shoot height, shoot and root dry biomass, grain yield and total P uptake of wheat plants relative to other treatments. Also, soil available P, enzyme activities and PSB populations were significantly improved due to the inoculation of PSB with RP compared to DAP (diammonium phosphate) treatment alone. Still, the mixture of PSB and RP was found to be more economical compared to fertilizer application.[193] Some examples of how PSB inoculants facilitate wheat production are summarized in Table 1.

Apart from a traditional and unstressed environment, plant growth promoting rhizobacteria have been reported to enhance the performance of wheat, while growing under stressed conditions.[203] As an example, endophytic strain *Bacillus subtilis* 10-4, capable of producing IAA and siderophores exhibited a protective effect on wheat plants grown under salinity (2% NaCl) stress. As expected, the exposure to salt stress resulted in a considerable increase in the proline (Pro) and MDA levels in wheat seedlings. However, inoculation of *B. subtilis* 10-4 decreased the level of stress-induced Pro and MDA contents. Moreover, both *B. subtilis* 10-4 inoculation and salinity caused

Table 1 Inoculation effects of P-solubilizing bacteria on the performance of wheat

PSB inoculants	Growth promotory effect	References
Bacillus strain MWT14	Increased growth rate, higher chlorophyll contents, straw yield and grain yield	194
B. polymyxa	Enhancement in plant height, number of spikelets per spike, grain yield, grains per spike, 100-grain weight	195
Enterobacter cloacae strain B1	Increased plant height, fresh and dry weight, flag leaf area, chlorophyll content, spike length, spikelets number number of grains per spike, 1000 grain weight, spike weight, biological weight	196
Pantoea allii strain BD 390, *Stenotrophomonas maltophilia* strain IAM 12423, *Pseudomonas frederiksbergensis* strain DSM 13022	Shoot length and dry weight	197
Azotobacter + phosphobacteria	Plant height, number of tillers per plant, number of spikes per plant, spike length, number of grains per spike, grain yield, straw yield	198
Pseudomonas fluorescens	Growth traits and yield	199
B. megaterium BHU1 + *Arthrobacter chlorophenolicus* BHU3	Plant height, grain yield, straw yield and nutrient acquisition	200
Pseudomonas sp.	Nutrient uptake and seedling growth	177
B. megaterium var. phosphaticum	No. of kernels per spike, grain yield, grain protein ratio	201
PSB strain MR1	Grain and straw yield	202

Table 2 Plant growth promoting active biomolecules released by soil microbiota affecting wheat growth

Soil microbiota	Source	PGP activities	References
Pseudomonas fluorescens, *P. putida*	Wheat rhizosphere and rhizoplane	IAA, siderophore, P solubilization	204
Pantoea sp.	Wheat seeds	IAA, siderophore, N_2 fixation	205
Burkholderia sp., *Enterobacter* sp.	Wheat rhizosphere	IAA, siderophore	206
P. fluorescens	Wheat rhizosphere	Siderophore, IAA	207
Serratia marcescens, *P. aeruginosa*	Vegetables rhizosphere	IAA production, NH_4, HCN production	208
Psychrobacter maritimus, *S. proteomaculans*, *Bacillus anthracis*	Wheat rhizosphere	IAA, siderophore production	209
S. grimessii, *S. marcescens*	Wheat rhizosphere	N_2 fixation, zinc solubilization, EPS activity, ACC deaminase, biocontrol activity, IAA production	210
Stenotrophomonas rhizophila, *Acetobactor pasteurianus*	Wheat rhizosphere and endosphere	N_2 fixation, IAA production, Zn and P solubilization	211
Azotobacter sp.	Rhizospheric soil	IAA production	200

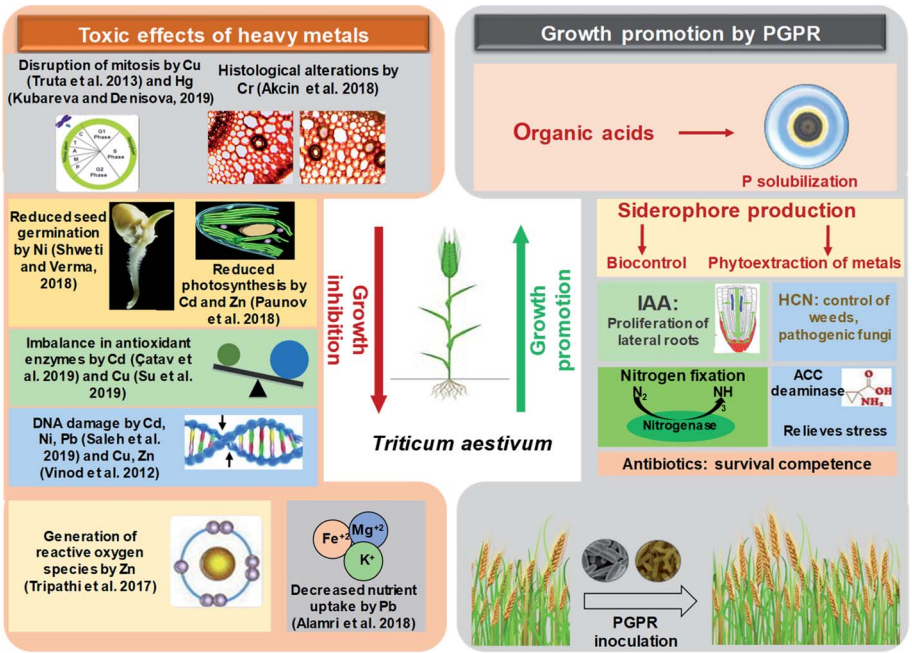

Fig. 4 A schematic representation depicting the toxicity of heavy metals to wheat plants and growth improvement by plant growth promoting rhizobacteria.

an increase in the endogenous salicylic acid (SA) content in wheat seedlings as compared to the SA content of the control, while *B. subtilis* 10-4 suppressed the stress-induced SA accumulation. The water storage capacity (WSC) in the leaf tissues was increased. In addition, the stress-induced hydrolysis of the statolite starch in the root cap cells of the germinal roots was reduced by *B. subtilis* 10-4. The data indicated that the activation of the defense reactions induced by *B. subtilis* 10-4 might be related to their ability to decrease the level of stress-induced oxidative damage and osmotic stress in seedlings. The increased level of endogenous SA might also have played an important protective role against salinity stress, *vis-à-vis* improving plant growth. Overall, the enhancement in the growth and yield of wheat due to microbial inoculations could be attributed to the expression and signaling of various growth promoting activities: (i) P-solubilization, (ii) N_2 fixation, (iii) phytohormone secretion, (iv) secretion of antagonist secondary metabolites (*e.g.*, HCN, siderophores, antifungal antibiotics), and (v) secretion of polymeric substances (EPS). The impact of different active biomolecules synthesized by PGPR on the growth of wheat is depicted in Table 2.

The toxic impacts of heavy metals on wheat and the subsequent growth improvement following PGPR inoculations through various direct and indirect mechanisms of plant growth promotion have been demonstrated in Fig. 4.

Table 3 Different metal detoxifying strategies adopted by metal tolerant bacteria

Mechanism	Organism	Description/effectiveness	References
Bioaccumulation	*Delftia* sp. B9	Intracellular dissolution of Cd, reduction of Cd and accumulation in rice grain	243
Biotransformation and bioaccumulation	*Micrococcus* KUMAs 15	Arsenite oxidation and accumulation	244
Biosorption	*Bacillus* sp. MC3B-22, *Microbacterium* MC3B-10	EPS mediated sorption of Cd^{2+}	245
Bioreduction	*Bacillus* sp. MNU16	Reduction of Cr(VI) and growth improvement of plants	246
Bioadsorption	*Pseudomonas aeruginosa* PSK1, PSK2, PSK3 and PSK4	Removal of Cd from contaminated soil and industrial wastewater	247

6 How microbial communities overcome metal stress?

Microbial communities inhabiting various environmental habitats have developed many efficient strategies to clean up the metal contaminated environment.[222–224] Approaches adopted by useful soil microflora to combat metal toxicity include: (a) exclusion, (b) active transport of metals away from the cell, (c) intracellular and extracellular sequestration, (d) enzymatic degradation of toxic metals to lesser toxic forms, and (e) reduction in metal sensitivity of cellular targets. One or more simultaneous mechanisms can be adopted at one time by soil microbes to detoxify the contaminated environment. However, the metal clearing approaches might depend on factors, such as type of microorganisms,[225] concentration and species of metals, and environmental variables. Many workers have isolated and identified different species of HM-resistant bacteria from various sources, like water and soil.[226,227] As an example, the resistance of *Bacillus subtilis* isolated from different water resources in Taif towards different concentrations (100–1200 g mL^{-1}) and metal species, such as Pb, Cd and Ag, was variable.[228] Likewise, ref. 229 revealed that 12 strains of *A. chroococcum* isolated from wheat, corn and asparagus rhizospheres were sensitive to 50 ppm concentration of Zn, and that the optimum concentration of Zn for the growth of these bacteria was 20 ppm. Accordingly, it was observed that the maximum activity and growth of *A. chroococcum* strains was achieved when they were grown in the presence of Zn (6–20 ppm). All *A. chroococcum* strains also carried the nifH gene, an indicator of the nitrogenase enzyme system, and nitrogen fixing capability. Genetic determinants of HMs can be localized both on bacterial chromosomes and on extra-chromosomal genetic elements,[230,231] which could provide resistance to toxic HMs. Of these, plasmid-mediated HM resistance determinants have been found to be inducible.[232] For example, a Pb-tolerant *E. faecalis* showed resistance towards many HMs and antibiotics. The metal-tolerant characters were found located on four plasmids of *E. faecalis*, which were 1.58, 3.06, 22.76 and 28.95 kb in size. Interestingly, the Pb resistance ability of *E. faecalis* was retained even when all the plasmids were eliminated, as demonstrated by the plasmid profile of the cured bacterial derivatives.[233] Similarly, free living nitrogen fixing Gram negative *A. chroococcum* recovered from contaminated soils exhibited higher resistance to Hg, Cd, Cu, Cr, Co, Ni, Zn and Pb.[234] Likewise, the HM resistance characteristics have also been found on bacterial chromosomes.[235] For example, Hg^{2+} resistance in *Bacillus*, Cd^{2+} efflux in *Bacillus* and As efflux in *E. coli* have been reported.[236] Efflux pumps, determined by plasmid and chromosomal systems, are either ATPases or chemiosmotic systems. Both can sometimes function in an identical manner even in different bacterial species. As an example, Cd resistance may involve: (i) an efflux ATPase in Gram-positive bacteria, (ii) cation-H^+ antiport in Gram-negative bacteria, and (iii) intracellular metallothionein (MT) in cyanobacteria.[237] Likewise, the As-resistant Gram-negative bacteria have an arsenite efflux ATPase and an arsenate reductase.[238] Similar systems for Hg^{2+} resistance were found located on plasmids of Gram-positive and Gram-negative bacteria. Those genes located on plasmids were transcribed to produce the detoxifying enzyme, mercuric reductase, which reduces Hg^{2+} to elemental Hg^0.[239] Conclusively, the identification of a metal resistant/tolerant feature might be a useful strategy to develop inexpensive potential bacterial cultures as bioremediation agents for detoxifying the metal contaminated sites.

7 Bioremediation of heavy metal toxicity: a general perspective

In order to make derelict soils cultivable again, various methods such as coagulation, chemical precipitation, electrodialysis, evaporative recovery, floatation, flocculation, ion exchange, nanofiltration, reverse osmosis, and ultrafiltration as well as physico-chemical methods such as extraction, stabilization, immobilization, soil washing of landfills, and excavation have been used. Most of these methods are, however, generally expensive, troublesome for the soil ecosystem, and cannot be used over a large area.[240,241] Therefore, to overcome these issues, bioremediation, has emerged in recent times as a magical alternative method to make polluted soils cultivable again. Broadly, bioremediation is "a process used to treat contaminated media, including water, soil and subsurface material, by altering environmental conditions to stimulate the growth of microorganisms and degrade target pollutants", and has been

categorized into two types: (a) *in situ* bioremediation: the treatment of xenobiotics at origin site and (b) *ex situ* bioremediation: transportation of contaminated soil from a poisoned site and then treating it.[242] Of these, the *ex situ* approach is expensive, environmentally unsafe and labour-intensive. Due to these issues, the *in situ* approaches are generally preferred. The commonly employed microbiological metal remediation strategies are summarized in Table 3.

8 Microbe-based metal detoxification strategies: current perspectives

Soil microbial populations generally belonging to the metal-tolerant PGPR group have been found to be the most suitable choice for alleviating metal toxicity.[248] The metal tolerant bacterial strains evolved to have multiple strategies[249] to remediate metal-contaminated soils. Among many bioremediation strategies, about 35% people prefer the use of microbial remediations, whereas only 16% people prefer phytoremediation approach for metal clean-up.[250,251] For example, biosorption, extracellular precipitation, conversion of toxic metal ions into less toxic forms and flush out (efflux pumping) of metals to an exterior environment are some of the approaches adopted by bacteria to thrive well even under metal-stressed conditions.[252,253] Microbes also enhance the bioavailability of metals from the soil by chelation, acidification, and precipitation. For example, organic acids released by microbes and plant roots lower the soil pH and help in sequestration of metal ions.[254]

Broadly, the determination of the metal toxicity and detoxification of metals by microbes are indeed the real challenge for the scientists. It is always interesting to see the extent of damage caused by metals and the level of remediation of contaminated environment by microbial communities. To answer these questions, various sensitive techniques have been developed, which very precisely reveal the extent of damage caused by metals to microbes, and also decipher the location and stabilization of metals in various structural components of bacterial cells (Fig. 5). Certain metal detoxification mechanisms adopted by microbes are discussed briefly in the following section.

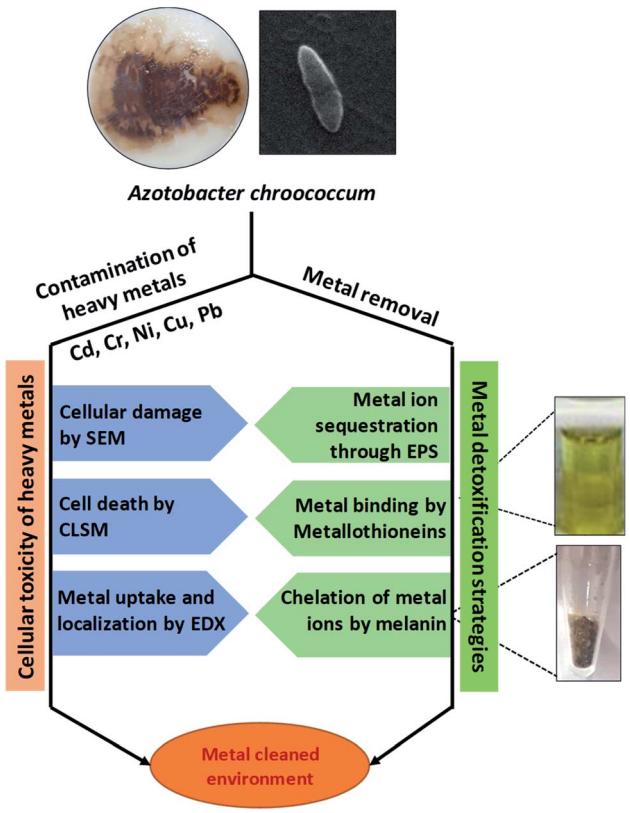

Fig. 5 Examples of most sensitive tools to detect metal toxicity and strategies adopted by soil bacteria to clean up metal contaminated environment [This figure has been adapted and modified from Rizvi *et al.* (2019)[267] with permission from Ecotoxicology, Springer, Copyright 2019].

9 Metal biosorption: biosorbents used in metal clean up

Extracellular polymeric substances (EPSs) are a complex mixture of macro-molecular electrolytes excreted by bacteria that play critical roles in the adsorption of heavy metals.[255] For example, *Azotobacter*,[256] *Bacillus*,[257] *Achromobacter*[258] and *Pseudomonas*[259] have been found to secrete EPS, which allows them to: (i) survive even in the presence of stressor molecules, like heavy metals[260] and pesticides[261] by masking their toxic impact[262,263] and (ii) form complexes with stressor molecules (chelation/sequestration). While doing these functions, the EPS positive strains serve as an important detoxifying agent.[264] In this context, as an example, the EPS secretion by the Cd-resistant strain *P. aeruginosa*[265] and *A. chroococcum* strain XU1 (ref. 266) have been reported.

9.1 Understanding the role of EPS in metal sequestration by SEM, EDX and FTIR

The elemental analysis of EPS, after binding with metal ions and various functional groups involved in metal binding, can be determined using microscopic techniques like SEM (scanning electron microscopy), EDX (energy dispersive X-ray spectroscopy), FTIR (Fourier-transform infrared) spectroscopy and three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy.[268,269] Generally, the adsorption of metal ions by EPS is energy-independent and occurs due to the proteins, polysaccharides and humic substance fractions found in EPS.[270] In addition, the shift/disturbances in the wavenumber of a particular spectrum corresponds to the metal binding process taking place either on the bacterial cell surface or the surface of EPS. This could be attributed to the fact that the oxygen of the polysaccharides complexes with metal ions during adsorption in order to reduce the electron cloud density of the functional groups containing oxygen, and to change the vibration frequency and intensity of the participating electrons.[271] Due to these properties, the EPS released by many aerobic soil bacteria have been reported to participate in metal removal from polluted environment.[245] As an example,[272] explained the

structural properties and metal biosorption behaviour of a novel EPS secreted by a thermophilic bacterium *Anoxybacillus* sp. R4-33. This study further revealed the heteropolysaccharide nature of EPS-II, which principally comprised monosaccharide (D-mannose and D-glucose) units present in the ratio of 1 : 0.45. Furthermore, it has been established that EPS secreted by asymbiotic N_2 fixers, like *Azotobacter* and *Pseudomonas*, also participate in the binding and immobilization of metals.[273] After binding, the EPS of *Azotobacter* creates a microenvironment in the soil, which is composed of metal ions essentially required for regulating the soil ecology. It thereby promotes the normal growth and development of plants.[274] In a similar experiment, EPS secreted by *Pseudomonas* sp. W6 formed a complex with Pb. This relieved the Pb pressure from the contaminated soils, which in turn allowed the crops to grow normally in metal polluted regions.[275] Furthermore, SEM coupled with EDX can help to better understand the morphological architecture and composition of bacterial cells besides EPS.[276] In this context, ref. 262 reported the tolerance of *P. agglomerans* towards various metals (for instance, Hg, Cu, Ag, and As), wherein EDX analysis revealed the metal accumulation, while FTIR showed the presence of diverse functional groups influencing the metal attachment. Also, the effect of metals on the surface of bacterial cell employing SEM and TEM showed that heavy metals damaged the cell surface, and accumulated on cell-bound EPS with some intracellular deposition. Thus, this study established that EPS-producing *P. agglomerans* exhibited the remarkable potential of metal sequestration, and could be used as a potential candidate for heavy metal bioremediation.

9.2 Bacterial biomass

Heavy metal biosorption is an innate and entirely non-enzymatic process found among microorganisms, which they employ to remove metals from contaminated environments by binding/chelating the metal ions even from very dilute solutions.[277,278] The biosorption by living or dead microbial biomass involves ion exchange, chelation, adsorption and diffusion through cell walls and membranes. The heavy metal ions are adsorbed on the bacterial surface by an active or passive process,[279] both of which may either work independently or in unison. The active process of biosorption is quite slow, and depends mainly on cellular metabolism of the microorganisms involved. It is also affected by various factors like metabolic inhibitors, uncouplers and temperature. In contrast, the passive process does not involve the cellular metabolism of microbes. Instead, metals bind to the cell walls through an ion exchange process. Additionally, the exchange process is not influenced by environmental variables such as pH and ionic strength. However, this process is swift and the complete adsorption of metals occurs within 5–10 min. The passive process of biosorption is also reversible, and involves the biomass of both living and dead microbial cells. However, whatever may be the mode of uptake of metal ions, the adsorption occurs as a result of nonspecific binding of heavy metal ions to the microbial cell surface or extracellular polysaccharides and proteins.[264] While comparing both Gram negative and Gram positive bacteria, the cell wall of Gram positive bacteria binds larger quantities of toxic metals in general as compared to the cell envelopes of Gram-negative bacteria.[280]

Ref. 277, in an experiment, demonstrated the biosorptive ability of live and dead biomass of a novel strain of *Bacillus* for chromium. Here, both live and dead bacterial biomasses exhibited the monolayer biosorption, where the best fit adsorption isotherm model was the Langmuir isotherm. The results showed that the maximum biosorption potential for Cr was 20.35 mg g^{-1}, which was achieved at 25 °C, pH 3, and the contact time was 50 min. Moreover, SEM and FTIR studies of the metal-loaded bacterial biomass demonstrated the maximum impact of dead bacterial cells on the biosorption of Cr. Also, about 92% and 70% desorption efficiencies were obtained using dead and live cells, respectively. Similarly, in another study, the maximum removal efficiency of Zn(II) by live and dead cell biomasses of *V. paradoxus* was found to be 92.7 and 91.3%, respectively. In contrast, the live and dead cells of *A. viscosus* showed a maximum Zn(II) removal efficiency computed as 89.4 and 90.8%, respectively. The results also showed that the biosorption process followed a pseudo-second-order reaction, wherein the best-fit isotherm was the Freundlich isotherm model.[281] The FTIR analysis of the bacterial biomass showed that the functional groups present on the bacterial cell surface, such as hydroxyl, amino, carboxylate, and phosphoryl, contributed towards metal-complexing, thereby assisting the process of metal biosorption.[282] Therefore, when used as inoculants, such microbes endowed with the property of metal biosorption are likely to circumvent metal toxicity *vis-à-vis* enhancing the production of agronomically important crops, including cereals grown in soils variously contaminated with heavy metals.

9.3 Metal-induced synthesis of metallothioneins: insights into heavy metal clean up

Metallothioneins (MTs) are a group of low molecular weight (approx. 3500–14 000 Da), cysteine-rich proteins. Chemically, MTs are composed of small polypeptide chains with approximately 30% cysteine residues, which have a strong binding affinity for metals. As a result, MTs sequester various toxic heavy metals by means of thiolate bonds of cysteine groups, and thus make the metal ions unavailable to microbes.[283,284] The suggested roles of MTs include: (a) regulation of homeostasis of essential metal ions within cells, (b) chelation of toxic metal ions, and (c) protection of bacterial and plant cells against oxidative damage induced by metal stress.[285–287] The synthesis of MTs by soil microbiota under stressed environment has been reported. For example, the induction of MTs in *Bacillus cereus* cells while growing in the presence of varying concentrations of Pb[288] and *P. aeruginosa* and *P. putida* cells upon exposure to Cu and Cd has been reported.[283] It has therefore been argued that MTs synthesized by bacterial communities under a metal-stressed environment could be valuable in the efficient detoxification of metals.[289] Hence, the bacteria possessing this property of secreting MTs could be explored as a cost-effective

approach in bioremediation strategies, which could eventually be employed in the growth enhancement of cereal crops growing in metal-contaminated soils.

9.4 Melanin: importance in metal detoxification

Melanin, the name derived from melons (Greek dark), is a dark brown to black coloured indolic polymer and amorphous substance that is usually attributed to the Swedish chemist Berzelius in 1840. Melanins are synthesized by many prokaryotic organisms, including nitrogen-fixing organisms.[290] Based on the colour and structural classes, there are primarily three types of melanin: (i) eumelanins: black to brown colour pigments produced by melanization by classic Mason–Rapper pathway, which produce tyrosine intermediates or metabolites by the action of tyrosinases, (ii) pheomelanins: brown, red or yellow colour pigments, which are produced due to the oxidation of tyrosine and/or phenylalanine to dihydroxyphenylalanine (DOPA) and dopaquinone. Pheomelanin results from cysteinylation of DOPA and these are sulphur containing compounds, and (iii) allomelanins: include nitrogen free heterogeneous group of polymers formed from catechol precursors.

The production of melanin by some bacterial strains, for example *A. chroococcum*,[291] and its ability to chelate metals is yet other mechanism by which the toxicity of metals can be reduced.[292–294] Melanin also protects organisms from UV radiation, chemical stresses and high temperatures,[295] thermoregulation and camouflage.[296] Melanin is an amorphous substance with no definite structure. The structure and property of metal sequestration by melanin could be confirmed by SEM and EDX analysis, respectively. In an experiment, ref. 297 confirmed similar features of melanin extracted from *Pseudomonas* sp. The adsorption of toxic hexavalent Cr onto melanin pigment isolated from the ink sac of squids was confirmed by SEM and EDX.[298] Furthermore, FT-IR spectroscopy of melanin extracted from *Bacillus weihenstephanensis* displayed a broad peak in the range of 3268–3278 cm^{-1}, which is attributed to –OH stretching. The absorbance peaks detected in the region 1511–1729 cm^{-1}, 2926–2970 cm^{-1}, 1045 cm^{-1} and 1220 cm^{-1} indicated the bonding vibration of the C=C and C=O aromatic ring stretching, the presence of double bonds in the COOH group, and the presence of saturated carbon and stretching vibrations in the carbonyl, alcoholic and phenolic groups, respectively.[299] Due to these features, bacterial melanin could serve as an excellent metal detoxification mechanism adopted by metal-tolerant microbes. Such melanin synthesizing bacteria, when applied to derelict soils as bioinoculants, could substantially improve the growth of plants, including cereal crops like wheat and maize growing in soils polluted or deliberately treated with toxic heavy metals by sequestrating metal ions and rendering them unavailable for uptake by plants. Heavy metal detoxification by soil bacteria employing various mechanisms has been summarized in Fig. 6.

10 PGPR assisted growth improvement of wheat plants under metal stress

The viability, colonizing efficiency and functionality of metal-tolerant PGPR determines the success of metal removal microbiological strategies used to alleviate contamination, and consequently to enhance the production of wheat growing in polluted soils. Plant growth-promoting rhizobacteria, apart from their normal growth promoting traits, facilitates plant

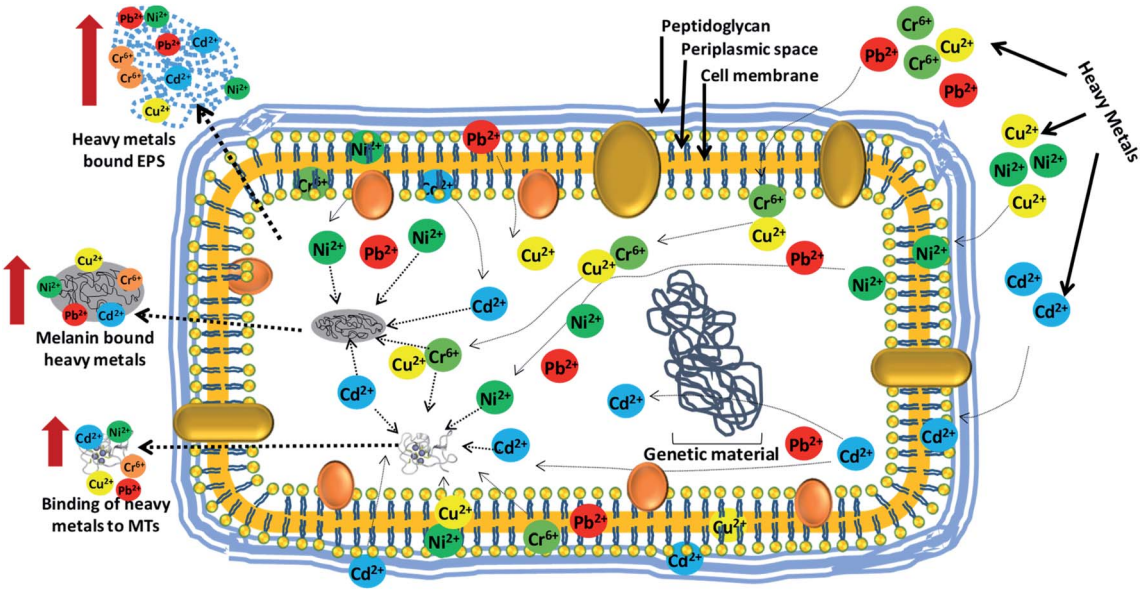

Fig. 6 Interaction of bacterial cells with heavy metal ions and their removal/detoxification by active biomolecules (EPS, MTs and melanin) secreted by bacterial strains when exposed to metal stress [This figure has been adapted and modified from Rizvi *et al.* (2019)[267] with permission from Ecotoxicology, Springer, Copyright 2019].

growth by mitigating the toxic impact of metals.[300,301] Considering these features, the effects of some of the metal-tolerant PGPR on the overall growth and yield of wheat plants grown in metal polluted soils are discussed briefly in the following section.

In an experiment, an improvement in the germination rate, root and shoot length and other growth parameters of wheat plants grown in Cd-treated soils was recorded following inoculation with *Pseudomonas* strains SNA5 and PBB1.[302] Similarly, the growth of roots (208%), shoots (67%) and root (140%) and shoot (71%) dry biomass of wheat plants grown under Cr stress following inoculation with ACC deaminase positive and P-solubilizing *Pseudomonas fluorescens* Q14 and *Bacillus thuringiensis* strain KAP5 has been reported.[303] Likewise, ref. 304 found a substantial increase in leaf photosynthetic pigments and other vital growth parameters of *P. aeruginosa* inoculated wheat plants grown under the influence of various doses of Zn. Furthermore, lowered MDA levels and antioxidant enzyme activity were observed following inoculation with *P. aeruginosa* under Zn stress. Similarly, the grain yield and membrane integrity of various wheat genotypes were found to be enhanced following inoculation with *Azotobacter* and *Azospirillum* strains even under Pb stress. Conversely, such bioinoculants significantly declined the production of MDA, proline and H_2O_2 under the influence of heavy metal stress.[305] The stress alleviation and a simultaneous growth promotion of wheat plants by IAA-synthesizing *Bacillus* sp. strain USTB-O grown under Cu stress was also reported.[306] The beneficial bacterium also improved the antioxidant defense mechanism in metal-stressed wheat plants. In this context, a decline in the levels of proline and other stresses within *A. chroococcum* and other PGPR-inoculated wheat plants grown under metal stress has been reported.[307,308] Moreover, a decline in oxidative stress in PGPR inoculated wheat plants was recorded. Also, *Bacillus subtilis* SU47 and *Arthrobacter* sp. SU18 has been found to minimize the accumulation of antioxidant enzymes in wheat plants by alleviating the stress.[309] In another study, the dry biomass of wheat plants was increased and a reduction in oxidative stress was recorded following inoculation with *Pseudomonas gessardii* and *Brevundimonas intermedia* when wheat was grown in As-polluted soil.[310] Also, a similar reduction in antioxidant enzyme activity was recorded in wheat plants inoculated with *Planomicrobium chinense* and *Bacillus cereus* under stressed conditions.[311]

11 Conclusion

Despite the toxicity conferred by heavy metals onto various parameters of plants in general, the introduction of metal-tolerant PGPR in polluted soils greatly diminishes the ruinous effects of metals, and enhances the growth and yield of wheat. The metal-tolerant bacteria, through biosorptive ability and capability to secrete EPS, can remove significant amounts of metal ions from the contaminated environment. The release of EPS by metal-tolerant strains also protects plants from other challenges, like pathogen attacks and desiccation. This consequently allows them to survive, and perform normal physiological and biochemical activities in stressed environments. Moreover, the secretion of MTs and melanin by viable cells under the influence of heavy metals could be considered another vital strategy evolved within bacterial strains to mitigate metal toxicity. Overall, the microbial management strategy to detoxify/remediate the contaminated environment through biosorption, secretion of MTs, melanin and EPS secretion makes metal-tolerant bacterial strains a promising and most suitable choice for heavy metal clean up from contaminated soils. The novel and fascinating traits of metal-tolerant bacteria could serve as an inexpensive yet environmentally viable approach in the metal clean-up program *vis-a-vis*, the growth and yield enhancement of wheat growing in metal-enriched soils.

Conflicts of interest

The authors declare no competing interests.

Acknowledgements

This research was funded by the Deanship of Scientific Research at Princess Nourah Bint Abdulrahman University through the Fast-track Research Funding Program. One of the authors, Asfa Rizvi, is also thankful to Department of Science and Technology, New Delhi, India for providing financial assistance through the INSPIRE fellowship.

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