Rhizosphere Microbial Communities and Heavy-Metals

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The rhizosphere is a microhabitat where there is an intense chemical dialogue between plants and microorganisms. The two coexist and develop synergistic actions, which can promote plants' functions and productivity, but also their capacity to respond to stress conditions, including heavy metal (HM) contamination.

Keywords: plants ; prokaryotic communities ; microbiome ; chemical dialogue ; exudates ; secondary metabolites ; stress response ; holobiont ; hologenome ; metaorganism

1. Introduction

Agroecosystems provide several ecosystem services [1], such as food and raw materials (e.g., wood, biofuels and fibers), which are essential for human life and activity. The surface covered by arable agriculture is about 13% of the global land surface and another 13% is represented by grassland for grazing [2]. The growth in human population requires an increase in food resources and many types of agriculture have been showing a growing trend over the last decade. For example, tree crops have a global extent of about 10 Mha and a ~20% increase in productivity for many fruit varieties was reported in the decade 2004–2014 [3].

Soil is a resource of enormous importance, but a finite and non-renewable one, and several anthropogenic activities are threatening its quality and long-term use, with loss of its key functions. Soil overexploitation by non-sustainable agriculture and over-grazing, contamination by industry and urbanization are the main causes of its deterioration and more than 24% of global land area is estimated to be degraded [2]. Fertile and unpolluted soils are necessary for ensuring healthy crops destined for human and animal use. In particular, soil contamination (e.g., from heavy metals) can seriously hamper soil biodiversity, fertility and crop productivity [4][5] and make agricultural products toxic. Heavy metals (HMs) are among the most common soil contaminants and their presence in concentrations higher than is natural poses a risk because of their toxicity, [6][7][8], bioaccumulation and biomagnification [9].

Microorganisms are a key soil component, ensuring the soil quality and fertility necessary for high-rate production of crops [4][10]. Soil microorganisms, above all *Bacteria* and *Archaea*, represent the majority of soil biomass and are termed "chemical engineers" [4], because they decompose organic matter and make it possible to recycle nutrients through anaerobic and aerobic reactions, involving up to 90% of soil energy flux. Thanks to their small dimensions and fast reproduction capability, prokaryotic cells adapt promptly to environmental changes. They show homeostatic capabilities versus contaminants and can be considered good biological indicators of soil quality [11][12][13][14]. The rhizosphere is a microhabitat, comprising roots and the 1–2 mm soil immediately surrounding them, where there is an intense chemical dialogue between plants and microorganisms [15][16]. In the rhizosphere, plants release root exudates, which promote bacterial population development. The rhizosphere offers a variety of carbon rich micro-habitats, which can be colonized by beneficial bacterial populations using such substrates [3][15].

Microorganisms communicate with plants through chemical messages and develop synergistic actions which influence plant functions and productivity, in both optimal and stress conditions [15][16][17][18].

Microorganisms have been found both on and inside plant tissues, but especially at root level [19]. The plant microbiome comprises the rhizosphere, phyllosphere and endosphere [20]. Healthy plants host symbiotic and non-symbiotic rhizo-epiphytic and/or endophytic microorganisms, which do not cause diseases, but support the host nutritionally, by stimulating germination and growth, or helping plants to overcome biotic or abiotic stress. Plants can be considered metaorganisms with close relationships with their associated microorganisms [21]. Indeed, according to the holobiont theory, hosts, such as plants and their microbiome, are symbionts [19][22]. Plant life is closely linked to key microbes, which can influence several aspects of plant ecology, such as growth, germination, biotic and abiotic stress resistance and fitness [19][21][22]. This theory suggests which host-microbiome relationships evolve over time, not just during a single host lifetime, but also through a coevolutionary process, leading to very complex relationships in microorganism-root systems

[23]. However, most complex plant-microorganism interactions and chemical dialogues have not so far been understood. Most microorganisms are uncultivable and there are practical difficulties in collecting and separating rhizo-epiphytic and endophytic microorganisms [24].

Developing new technologies and nature-based solutions to prevent deterioration of soil and remediation of contaminated sites, while at the same time maintaining soil functions, is a matter of great interest for science and a challenge for the coming decade. In accordance with the One Health Concept, human, animal and environmental wellness and health are tightly related to each other and it is not possible to take an action concerning one of them without considering the others. This approach is of special importance in guaranteeing food safety and sustainable crop production [25][26][27].

The complex and synergistic actions established in the rhizosphere between tree roots and natural underground microbiota make it possible to remove, convert or contain toxic substances in soils, including trace elements [28][29]. Heavy metals (HMs) are among the most widespread soil contaminants worldwide and their presence is reported in 60% of polluted land [30]. The presence of heavy metals at concentrations higher than natural ones poses a risk because of their toxicity [6][7][8], bioaccumulation and biomagnification [9]. These contaminants are a major risk, especially in the most industrialized and populated regions of the earth, endangering human safety and altering ecosystem functions [29][31].

If heavy metals are present in soils used for agricultural practices, there is a risk of metal uptake by edible plants, with a subsequent possible bioaccumulation in human and animals and detrimental consequences for their health [31][32].

A better knowledge of plant–microorganism interactions is therefore urgently needed to develop correct agronomic management and naturally based solutions, such as phytotechnology for remediation purposes. For this purpose, an ecological approach, taking site-specific biotic and abiotic interactions between plants and microorganisms into consideration, is necessary.

2. Heavy Metals

HMs are generally considered those metals and metalloids with an atomic number of at least 20 and a density higher than 5 g/cm^3 [33]. They are normally present in ecosystems as “trace elements” [29], since many minerals and rocks can contain and release them through erosion and water dissolution.

Some heavy metals are essential micronutrients for plants, microorganisms and organisms at higher trophic levels (e.g., zinc, iron, copper, nickel). Other HMs (e.g., mercury, cadmium, lead and arsenic) are non-essential elements [33]. Unlike organic substances, they are non-biodegradable. Each metal can become toxic if released in higher concentrations than natural ones, or in the case of chronic exposure [33][34][35].

Lead (Pb), Copper (Cu), Cadmium (Cd), Chromium (Cr), Mercury (Hg), Zinc (Zn), Arsenic (As) and Nickel (Ni) are the most common heavy metals found in contaminated soils [33][36][37].

Several anthropogenic activities are responsible for releasing high amounts of heavy metals into natural ecosystems, increasing their concentrations to levels far higher than natural ones and causing, in many cases, serious contamination issues [38][39]. Pollution can be of particular concern when it occurs in agricultural fields, since it can increase the risk of biomagnification and heavy metal uptake, impacting animal and human health [40][41]. Regarding heavy metals (metals and metalloids) in agricultural soils in the EU, Tóth et al. [40] reported that 6.24% of agricultural land shows trace elements (e.g., As, Cd, Cr, Cu, Hg, Pb, Zn, Sb, Co and Ni) exceeding legal limits. In Europe, there are 137,000 km^2 of contaminated agroecosystems requiring remediation actions. However, there is much more contaminated agricultural land in other parts of the world, where the risk of heavy metal contamination is much more alarming [31][38][42][43][44].

Major sources of anthropogenic heavy metal release are mining, industrial activities (especially tannery, smelting and steel mills), atmospheric deposition, sewage from industries and cities, incorrect waste disposal (for example in the case of batteries), fossil fuels and some pesticides and fertilizers [33][36][39][45]. In particular, phosphate fertilizers are a source especially of Cd and can also release Zn, Hg, As, Pb, Cr and U, if they are produced by the acidulation of phosphate rocks naturally containing these metals. Some pesticides can also contain Hg, As and Pb [39][45]. Many pesticides are currently forbidden in some countries, such as the UK [45], while fertilizers in Europe are subjected to regulation in order to limit heavy metal inputs [45][46]. In any case, even if trace element input is limited, their massive use in recent decades has led to HM accumulation in soil [39]. Moreover, crop irrigation with polluted wastewaters can be another heavy metal source [38][45][47].

Heavy metal mobility and bioavailability influence the proportion of metals which can directly interact with living organisms. A part of the total metal concentration in soil is irreversibly linked to or sequestered by the soil matrix and only HMs in a solution are directly available for biota and can be acquired by plant roots [33][35][48]. Zhang et al. [31] reported that heavy metal concentrations found in plant tissue were related to bioavailable metals more than to total ones. Consequently, total heavy metals in soil is not a good parameter for clearly evaluating the possible organism accumulation risk. Many soil properties, such as pH, redox potential, texture, clay content and presence of soil organic matter (SOM), can interact with heavy metals and influence their concentration in a soil solution. In the presence of a low pH, HMs are not specifically adsorbed to soil particles and they are generally more mobile and bioavailable; on the other hand, heavy metals can form stable complexes with SOM, such as humic and fulvic acids [49].

3. Heavy Metal Toxicity

Heavy metals can be toxic for biota at all trophic levels, including human. Despite large differences in organization and complexity, HM action mechanisms are similar among organisms, since they act primarily at a cellular level in highly conserved systems. Metals are generally cytotoxic and genotoxic with direct effects on cellular activities [18], which can have several consequences depending on the target organism. Heavy metal characteristics which are responsible for their primary toxicity are:

- high affinity for negatively charged cellular groups, such as sulfhydryls, phosphates and hydroxyls;
- generation of reactive oxygen species (ROS), causing oxidative stress;
- competition with essential ions acquisition;
- disturbance of cellular ion balance and osmotic regulation.

Heavy metals can interfere with normal cellular processes and metabolic functions, causing cellular damage. The affinity for negatively charged groups acts at all trophic levels, causing cell membrane alteration and lipidic peroxidation, with consequences for cell growth and division [41]. Eukaryote membranes of organelles, such as mitochondria, peroxisomes and chloroplasts, can be altered, causing severe damage. For example, in plants, HMs can alter chloroplast inner membrane organization, essential for photosynthesis, potentially leading to a decrease in photosynthetic rates [41][50]. At the same time, heavy metals can bind thiol groups of proteins causing alteration of their structure and leading to protein denaturation and/or loss of functionality [50]. Similarly, metal cations binding to the catalytic sites of enzymes are responsible for direct alteration of their activity. There is great concern about damage to nucleic acids; metal cations can cause the inhibition of transcription and replication and changes in DNA structure, i.e., mutagenic effects, and hinder cell division and cellular cycle [34].

Heavy metals can interfere with cellular redox systems, with the generation of ROS; the disturbance of mitochondrial activity can also cause a lowering in respiration rates in plant tissues. ROS can cause further stress and damage to DNA or other biomolecules, potentially resulting in inhibition of photosynthesis in plants [18][41].

Moreover, heavy metals can interfere with the consumption of essential plant nutrients because of competition with their uptake systems. They can act as antagonists of other ligands of biomolecules and can disturb nutrient translocation systems [37]; a deficiency in micronutrients can have numerous effects, because they are involved in a wide number of biological activities. In different plant species, Cr^{+3} and Cr^{+5} can interfere with uptake of Mg, Mn, Fe, Cu, Zn, but also P and K [37]. Cd, Ni and Pb can replace other ions, such as Zn, in enzymes involved in chlorophyll and other pigment production, lowering photosynthetic rates [41].

Moreover, cellular ionic balance is an issue for both plants and microorganisms, since alteration of cation consumption can generate osmotic problems. In plants, for example, Zn and Pb contamination can contribute to altering the plant water balance [34][41]. It is important to highlight that such effects can also be caused by essential elements if they are in excessive quantities (**Table 1**), such as in the case of Ni [41].

Table 1. Heavy metals (HMs), which are micro-essential nutrients for plants but toxic in excessive concentrations. The roles and toxicity of zinc, copper, iron and nickel are reported. The legal limits refer to agricultural soils in accordance with Italian Decree 46/2019. Currently, at the EU level, there is no Directive on soil.

Essential HMs for Plants	Role	Toxicity	Legal Limits mg kg^{-1}
Zn^{+2}	**Cofactor in many enzymes, present in protein–DNA domain interaction (zinc finger proteins); role in plant defense response; response to oxidative stress [51].**	**It competes with other essential ion adsorptions (Fe^{+2}; Mn^{+2}, Mg^{+2}), it can substitute Mg^{+2} in chlorophyll inhibiting photosynthesis [52].**	**300**
$Cu^{+}/^{+2}$	**Cofactor of many enzymes necessary in electron transport chain; involved in iron mobilization and in cell wall metabolism; it has a role in plant stress responses [53].**	**It substitutes Mg^{+2} in chlorophyll, inhibiting photosynthesis; it can cause malfunctioning of photosystems (PSI and PSII); it can cause oxidative stress at higher concentrations and alter root morphology and biomass [52].**	**200**
$Fe^{+2}/^{+3}$	**Essential in electron transport chain; cofactor of many enzymes; involved in photosynthesis and chlorophyll synthesis [22].**	**It can cause severe oxidative stress and ROS generation; Fe^{2+} can be responsible for photosystem damage and inhibition of photosynthesis; Fe^{+2} e Fe^{+3} can interact with transport systems for other essential elements [52].**	**--**
Ni^{+2}	**Necessary for plant growth at low concentration; involved in enzymatic functions necessary for plant redox state maintenance; involved in nitrogen metabolism [54].**	**It can cause inhibition of growth and biomass accumulation; it can interfere with water and nutrient acquisition; it can cause lipid peroxidation and interfere with pigment production [54].**	**120**

Damage at cell level is related to that at an organism level. Heavy metal effects on plants are of particular concern, because their stress and toxicity can directly affect crops, decreasing their productivity. Moreover, contamination can be a serious threat for food security in the case of bioaccumulation [35][50]. In fact, heavy metal toxicity can influence plant growth, root elongation and seed germination [50]. It is also reported that heavy metal toxicity can lower plant resistance to other stresses, e.g., pest invasion. Lakshmanan et al. [55] reported that rice plants subjected to arsenic contamination showed higher vulnerability toward rice blast infection.

The sensitivity of plants to heavy metals can be different. There are several species able to survive and grow with a high level of heavy metals; they are termed hyperaccumulating plants and can accumulate up to 100 μg/g of each metal, such as Cd and Cu, and much higher concentrations of other metals [56]. More than 500 taxa are considered hyperaccumulating plants. These species can be very useful for remediation purposes; however, it is not desirable to use them for edible crops in contaminated sites [29][56].

Zhang et al. [31] reported concentrations of Pb in rice crops up to 10 times higher than Chinese legislative limits and cases of Cd contamination of wheat, which is one of the most widespread cereal crops worldwide, are of great concern [57]. Unfortunately, heavy metal contamination has been reported in a great number of crops (e.g., brassica, soybean, sugar beet, potatoes and lettuce) all over the world, demonstrating that this problem is here and now and widespread [44].

If heavy metals are accumulated in plants, they can be directly toxic even for animals and humans, through ingestion of contaminated food. Cd poisoning is also reported for tobacco smoking [35] and HM skin contact can cause irritation and allergic reactions in humans. Several illnesses are reported to be linked to heavy metal poisoning. In fact, many heavy metals, such as Hg, Pb and As, are known to be toxic for the renal, cardiovascular, gastrointestinal and nervous systems. Lead poisoning, for example, can cause headache, mental confusion and disorientation [50]. Permanent damage can occur after long-term exposure and some metals are thought to be carcinogenic [18].

References

1. Jónsson, J.Ö.G.; Davídsdóttir, B. Classification and valuation of soil ecosystem services. Agric. Syst. 2016, 145, 24–38.
2. Karlen, D.L.; Rice, C.W. Soil degradation: Will humankind ever learn? Sustainability 2015, 7, 12490–12501.
3. Mercado-Blanco, J.; Abrantes, I.; Barra Caracciolo, A.; Bevivino, A.; Ciancio, A.; Grenni, P.; Hrynkiewicz, K.; Kredics, L.; Proença, D.N. Belowground microbiota and the health of tree crops. Front. Microb. 2018, 9, 1006.
4. Turbé, A.; De Toni, A.; Benito, P.; Lavelle, P.; Lavelle, P.; Ruiz, N.C.; Van der Putten, W.H.; Labouze, E.; Mudgal, S. Soil Biodiversity: Functions, Threats and Tools for Policy Makers; Report for European Commission (DG Environment); Bio I ntelligence Service, IRD, and NIOO: Paris, France, 2010.

5. Tiwari, S.; Lata, C. Heavy metal stress, signaling, and tolerance due to plant-associated microbes: An overview. Front. Plant. Sci. 2018, 9, 452.
6. Kara, Y. Bioaccumulation of Cu, Zn and Ni from the wastewater by treated Nasturtium officinale. Int. J. Environ. Sci. Tec hnol. 2005, 2, 63–67.
7. Arora, M.; Kiran, B.; Rani, S.; Rani, A.; Kaur, B.; Mittal, N. Heavy metal accumulation in vegetables irrigated with water f rom different sources. Food Chem. 2008, 111, 811–815.
8. Memon, A.R.; Schroder, P. Implications of metal accumulation mechanisms to phytoremediation. Environ. Sci. Pollut. R es. 2009, 16, 162–175.
9. Alloway, B.J. Heavy Metals in Soils, Trace Metals and Metalloids in Soils and Their Bioavailability, 3rd ed; Springer: Dor drecht, The Netherlands, 2013.
10. Adhikari, K.; Hartemink, A.E. Linking soils to ecosystem services—A global review. Geoderma 2016, 262, 101–111.
11. Pulleman, M.; Creamer, R.; Hamer, U.; Helder, J.; Pelosi, C.; Pérès, G.; Rutgers, M. Soil biodiversity, biological indicato rs and soil ecosystem services-an overview of European approaches. Curr. Opin. Environ. Sustain. 2012, 4, 529–538.
12. Oves, M.; Saghir Khan, M.; Huda Qari, A.; Nadeen Felemban, M.; Almeelbi, T. Heavy metals: Biological importance and detoxification strategies. J. Bioremediat. Biodegrad. 2016, 7, 334.
13. Zhang, C.; Nie, S.; Liang, J.; Zeng, G.; Wu, H.; Hua, S.; Liu, J.; Yuan, Y.; Xiao, H.; Deng, L.; et al. Effects of heavy meta ls and soil physicochemical properties on wetland soil microbial biomass and bacterial community structure. Sci. Total Environ. 2016, 557–558, 785–790.
14. Barra Caracciolo, A.; Grenni, P.; Garbini, G.L.; Rolando, L.; Campanale, C.; Aimola, G.; Fernandez-Lopez, M.; Fernand ez-Gonzalez, A.J.; Villadas, P.J.; Ancona, V. Characterization of the belowground microbial community in a poplar-phyt oremediation strategy of a multi-contaminated soil. Front. Microb. 2020, 11, 2073.
15. Badri, D.V.; Weir, T.L.; van der Lelie, D.; Vivanco, J.M. Rhizosphere chemical dialogues: Plant-microbe interactions. Cu rr. Opin. Biotechnol. 2009, 20, 642–650.
16. Sasse, J.; Martinoia, E.; Northen, T. Feed your friends: Do plant exudates shape the root microbiome? Trends Plant. Sc i. 2018, 23, 25–41.
17. Ma, Y.; Rajkumar, M.; Zhang, C.; Freitas, H. Beneficial role of bacterial endophytes in heavy metal phytoremediation. J. Environ. Manage 2016, 174, 14–25.
18. Manoj, S.R.; Karthik, C.; Kadirvelu, K.; Arulselvi, P.I.; Shanmugasundaram, T.; Bruno, B.; Rajkumar, M. Understanding t he molecular mechanisms for the enhanced phytoremediation of heavy metals through plant growth promoting rhizoba cteria: A review. J. Environ. Manage 2020, 254, 109779.
19. Hassani, M.A.; Durán, P.; Hacquard, S. Microbial interactions within the plant Holobiont. Microbiome 2016, 6, 58.
20. Compat, S.; Samad, A.; Faist, H.; Sessitsch, A. A review on the plant microbiome: Ecology, functions, and emerging tre nds in microbial application. J. Adv. Res. 2019, 19, 29–37.
21. Berg, G.; Rybakova, D.; Grube, M.; Köberl, M. The plant microbiome explored: Implications for experimental botany. J. Exp. Bot. 2016, 67, 995–1002.
22. Schmidt, R.; Saha, M. Infochemicals in terrestrial plants and seaweed holobionts: Current and future trends. New Phyto l. 2020, 229, 1852–1860.
23. Bordenstein, S.R.; Theis, K.R. Host biology in light of the microbiome: Ten principles of Holobionts and Hologenomes. PLoS Biol. 2015, 13, e1002226.
24. Zilber-Rosenberg, I.; Rosenberg, E. Role of microorganisms in the evolution of animals and plants: The hologenome th eory of evolution. FEMS Microbiol. Rev. 2008, 32, 723–735.
25. Boqvist, S.; Söderqvist, K.; Vågsholm, I. Food safety challenges and One Health within Europe. Acta Vet. Scand. 2018, 60, 1.
26. Garcia, S.N.; Osburn, B.I.; Jay-Russell, M.T. One health for food safety, food security, and sustainable food production. Front. Sustain. Food Syst. 2020, 4, 1.
27. Lammie, S.L.; Hughes, J.M. Antimicrobial resistance, food safety, and one health: The need for convergence. Annu. Re v. Food Sci. Technol. 2016, 7, 287–312.
28. Aken, B.V.; Correa, P.A.; Schnoor, J.L. Phytoremediation of biphenyls: New trends and promises. Environ. Sci. Technol. 2010, 44, 2767–2776.
29. Ancona, V.; Barra Caracciolo, A.; Campanale, C.; Rascio, I.; Grenni, P.; Di Lenola, M.; Bagnuolo, G.; Uricchio, V.F. Hea vy metal phytoremediation of a poplar clone in a contaminated soil in southern Italy. J. Chem. Technol. Biotechnol. 202

0, 95, 940–949.

30. Panagos, P.; Van Liedekerke, M.; Yigini, Y.; Montanarella, L. Contaminated sites in Europe: Review of the current situati on based on data collected through a European network. J. Environ. Public Health 2013, 2013, 158764.
31. Zhang, J.; Li, H.; Zhou, Y.; Dou, L.; Cai, L.; Mo, L.; You, J. Bioavailability and soil-to-crop transfer of heavy metals in far mland soils: A case study in the Pearl River Delta, South China. Environ. Pollut. 2018, 235, 710–719.
32. Deng, L.; Zeng, G.; Fan, C.; Lu, L.; Chen, X.; Chen, M.; Wu, H.; He, X.; He, Y. Response of rhizosphere microbial com munity structure and diversity to heavy metal co-pollution in arable soil. Appl. Microbiol. Biotechnol. 2015, 99, 8259–82 69.
33. Ali, H.; Khan, E.; Ilahi, I. Environmental chemistry and ecotoxicology of hazardous heavy metals: Environmental persist ence, toxicity, and bioaccumulation. J. Chem. 2019, 6730305.
34. Igiri, B.E.; Okoduwa, S.I.R.; Idoko, G.O.; Akabuogo, E.P.; Adeyi, A.O.; Ejiogu, I.K. Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater: A review. J. Toxicol. 2018, 2018, 2568038.
35. Vardhan, K.H.; Senthil Kumar, P.; Panda, R.C. A review on heavy metal pollution, toxicity and remedial measures: Curr ent trends and future perspectives. J. Mol. Liq. 2019, 290, 111197.
36. Fashola, M.O.; Ngole-Jeme, V.M.; Babalola, O.O. Heavy metal pollution from gold mines: Environmental effects and ba cterial strategies for resistance. Int. J. Environ. Res. Public Health 2016, 13, 1047.
37. Shahid, M.; Shamshad, S.; Rafiq, M.; Khalid, S.; Bibi, I.; Niazi, N.K.; Dumat, C.; Rashid, M.I. Chromium speciation, bioa vailability, uptake, toxicity and detoxification in soil-plant system: A review. Chemosphere 2017, 178, 513–533.
38. Dixit, R.; Malaviya, D.; Pandiyan, K.; Singh, U.B.; Sahu, A.; Shukla, R.; Singh, B.P.; Rai, J.P.; Sharma, P.K.; Lade, H.; et al. Bioremediation of heavy metals from soil and aquatic environment: An overview of principles and criteria of fundame ntal processes. Sustainability 2015, 7, 2189–2212.
39. Selvi, A.; Rajasekar, A.; Theerthagiri, J.; Ananthaselvam, A.; Sathishkumar, K.; Madhavan, J.; Rahman, P.K.S.M. Integr ated remediation processes toward heavy metal removal/recovery from various environments—A review. Front. Enviro n. Sci. 2019, 7, 66.
40. Tóth, G.; Hermann, T.; Da Silva, M.R.; Montanarella, L. Heavy metals in agricultural soils of the European Union with i mplications for food safety. Environ. Int. 2016, 88, 299–309.
41. Amari, T.; Ghnaya, T.; Abdelly, C. Nickel, cadmium and lead phytotoxicity and potential of halophytic plants in heavy me tal extraction. S. Afr. J. Bot. 2017, 111, 99–110.
42. Sun, W.; Xiao, E.; Krumins, V.; Häggblom, M.M.; Dong, Y.; Pu, Z.; Li, B.; Wang, Q.; Xiao, T.; Li, F. Rhizosphere microbia l response to multiple metal(loid)s in different contaminated arable soils indicates crop-specific metal-microbe interactio ns. Appl. Environ. Microbiol. 2018, 84, e00701-18.
43. Zhao, X.; Huang, J.; Lu, J.; Sun, Y. Study on the influence of soil microbial community on the long-term heavy metal pol lution of different land use types and depth layers in mine. Ecotoxicol. Environ. Saf. 2019, 170, 218–226.
44. Rai, P.K.; Lee, S.S.; Zhang, M.; Tsang, Y.F.; Kim, K. Heavy metals in food crops: Health risks, fate, mechanisms, and m anagement. Environ. Int. 2019, 125, 3365–3385.
45. Nicholson, F.A.; Smith, S.R.; Alloway, B.J.; Carlton-Smith, C.; Chambers, B.J. An inventory of heavy metals inputs to ag ricultural soils in England and Wales. Sci. Total Environ. 2003, 311, 205–219.
46. Hukari, S.; Hermann, L.; Nättorp, A. From wastewater to fertilisers-Technical overview and critical review of European l egislation governing phosphorus recycling. Sci. Total Environ. 2016, 542, 1127–1135.
47. Ali, H.; Khan, E. Trophic transfer, bioaccumulation, and biomagnification of non-essential hazardous heavy metals and metalloids in food chains/webs—Concepts and implications for wildlife and human health. Hum. Ecol. Risk Assess. 201 9, 25, 1353–1376.
48. Liu, Y.; Du, Q.; Wang, Q.; Yu, H.; Liu, J.; Tian, Y.; Chang, C.; Lei, J. Causal inference between bioavailability of heavy m etals and environmental factors in a large-scale region. Environ. Pollut. 2017, 226, 370–378.
49. Kim, R.Y.; Yoon, J.K.; Kim, T.S.; Yang, J.E.; Owens, G.; Kim, K.R. Bioavailability of heavy metals in soils: Definitions an d practical implementation-a critical review. Environ. Geochem. Health 2015, 37, 1041–1061.
50. Kushwaha, A.; Rani, R.; Kumar, S.; Thomas, T.; David, A.A.; Ahmed, M. A new insight to adsorption and accumulation o f high lead concentration by exopolymer and whole cells of lead-resistant bacterium Acinetobacter junii L. Pb1 isolated f rom coal mine dump. Environ. Sci. Pollut. Res. 2017, 24, 10652–10661.
51. Cabot, C.; Martos, S.; Llugany, M.; Gallego, B.; Tolrà, R.; Poschenrieder, C. A role for zinc in plant defense against path ogens and herbivores. Front. Plant. Sci. 2019, 10, 117.

52. Kupper, H.; Andresena, E. Mechanisms of metal toxicity in plants. Metallomics 2016, 8, 269.

53. Shabbir, Z.; Sardar, A.; Shabbir, A.; Abbas, G.; Shamshad, S.; Khalid, S.; Natasha, N.; Murtaza, G.; Dumat, C.; Shahid, M. Copper uptake, essentiality, toxicity, detoxification and risk assessment in soil-plant environment. Chemosphere 2020, 259, 127436.

54. Shahzad, B.; Tanveer, M.; Rehman, A.; Cheema, S.A.; Fahad, S.; Rehman, S.; Sharma, A. Nickel; whether toxic or essential for plants and environment—A review. Plant. Physiol. Biochem. 2018, 132, 641–651.

55. Lakshmanan, V.; Cottone, J.; Bais, H.P. Killing two birds with one stone: Natural rice rhizospheric microbes reduce arsenic uptake and blast infections in rice. Front. Plant. Sci. 2016, 7, 1–12.

56. Ojuederie, O.B.; Babalola, O.O. Microbial and plant-assisted bioremediation of heavy metal polluted environments: A review. Int. J. Environ. Res. Public Health 2017, 14, 1504.

57. Han, H.; Wu, X.; Yao, L.; Chen, Z. Heavy metal-immobilizing bacteria combined with calcium polypeptides reduced the uptake of Cd in wheat and shifted the rhizosphere bacterial communities. Environ. Pollut. 2020, 267, 115432.