

Review

Implication of the Plant Species Belonging to the *Brassicaceae* Family in the Metabolization of Heavy Metal Pollutants in Urban Settings

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Received: 2 April 2020

Accepted: 23 May 2020

Abstract

Environmental hazards have motivated the development of a novel perspective of urban environmental quality as a planning strategy prerequisite for the integration of greenery resources in urban infrastructures. The accurate genotype stress response presents a condition for urban adaptation of plants. The goal of this review paper is to present phytoremediation at molecular levels via genes related to transport, accumulation and sequestration/detoxification of heavy metals from the environment. The overexpression/alteration of the native plant-specific gene(s) and transgenic plants whose metal-uptake proteins and metal-efflux proteins enable efficient metal uptake and transport, give rise to transgenic approaches. In a line with this, overexpression/alteration of a gene(s) encoding for phytochelatins and glutathione increase the sequestration of toxic HMs in the vacuoles. Since tolerance to high levels of metals by hyperaccumulators is under genetic control, many studies used genetic approaches to define the genetic determinants of these hyperaccumulators. Many plant species, particularly members of the Brassicaceae family are known heavy metal hyperaccumulators. This paper examines molecular aspects of hyperaccumulator plant species from genera Brassica, Noccea, Alyssum and Arabidopsis in phytoremediation of heavy metal polluted environments, and provides an overview of potential transferable genes that could improve metal tolerance and/or accumulation, as the major targets for phytoremediation. Therefore, plant species identified to have the potential to grow and remediate the heavy metal polluted urban environments require greater attention.

Keywords: heavy metals, environmental pollution, hyperaccumulator genes, *Brassicaceae* for urban settings

Introduction

One of the major global and recurrent environmental issues is the pollution of soil and water with heavy metals (HMs), with negative effects on ecosystems and public health, and subsequent economic losses. Considering that around 75% of periodic table elements are metals, a growth in their use increases the amount of metallic substances in the environment [23]. Minerals weathering, erosion, and volcanic activity are the most important natural sources of these metals in the environment. There are many more anthropogenic sources of heavy metals: industrial discharges, industrial mining, smelting, electroplating, agricultural use of organic and chemical fertilizers, biosolids and pesticides; emissions from incinerators of municipal waste, car exhausts; atmospheric deposition; sludge dumping, etc. Elemental pollutants such as arsenic (As), copper (Cu), cadmium (Cd), mercury (Hg), nickel (Ni), zinc (Zn), and lead (Pb) are toxic elements that cannot be converted by any biochemical reaction, and therefore persist in the ecosystem [39]. The ubiquitously present pollutants found at waste sites are metals and other inorganic contaminants. However, their remediation is one of the most technically challenging, because metals cannot be degraded, unlike organic contaminants [49]. Certain plant species have the genetic potential to remove, degrade, metabolize, or immobilize a wide range of contaminants from soil and water. The ability to tolerate high concentration of toxic heavy metals is related to specific genes related to phytochelatin and glutathione pathway for vacuolar heavy metal sequestration, and antioxidant defense system [2]. These plants belong to more than 50 families, 25% of which are from family Brassicaceae [22].

The Implication of *Brassicaceae* in the Remediation of Urban Environments

Urban soils are known recipients of large amounts of heavy metals from a variety of anthropogenic sources, including industrial wastes, vehicle emissions, coal-burning waste, and other activities. Heavy metals in public areas (such as gardens and parks) are particularly hazardous because of human exposure to significant pollution levels [57]. Plants belonging to the *Brassicaceae* family are suitable for phytoremediation because of considerable growth rate and high biomass [51]. For instance, *Arabidopsis halleri* and *Arabidopsis arenosa* are more tolerant of heavy metals compared to *Arabidopsis thaliana*, therefore this plant gives insight in processes that take place during heavy metal toxicity [67]. Species whose roots contain more than 100 mg Cd x kg⁻¹, 1,000 mg Ni and Cu x kg⁻¹, or more than 10,000 mg Zn and Mn x kg⁻¹ (dry weight) when grown in metal-rich soils are hyperaccumulator plant species [1]. Furthermore, genetic modification/manipulation can greatly increase this potential. These transgenic

strategies involve genes encoding for specific metal uptake and transport proteins. Invoking enhancement of adaptive capacity in the adaptability of transgenic varieties, phytoremediation will be greatly improved [2]. Best-known examples of hyperaccumulator species in the Brassicaceae family are *Nocca caerulea* subsp. *virens* (Jord.) Kerguelen I, *Alyssum* (*Alyssum murale* Waldst. & Kit.; *Alyssum lesbiacum* (Candargy) Rech.f. and *Arabidopsis* (*Arabidopsis halleri* (L.) (O'Kane & Al-Shehbaz) [syn. *Cardaminopsis halleri* (L.) Hayek], *Arabidopsis thaliana* (L.) Heynh). [21] *Coronopus didymus* (L.) Sm. [65, 66] *Barbarea arcuata* (Opiz ex J.Presl & C.Presl) Rchb rorippa palustris (L.) besser, *Brassica campestris* (L.) [19], *Brassica rapa* (L.) [87].

Specific Genes in Similar Species Involved in Heavy Metal Tolerance and Accumulation

Uptake by Metal Transporters in the Plasma Membranes

Plants possess two classes of heavy metal transporter proteins categorized into metal-uptake proteins and metal-efflux proteins. Metal-uptake proteins can transport essential heavy metals into the cytoplasm. Metal-efflux proteins catalyze the efflux of toxic heavy metals from the cytoplasm, or move these metals into the vacuole, helping plant detoxification [36]. These proteins include cation diffusion facilitator (CDF) family, heavy metal CPx-ATPases, Nramp (natural resistance-associated macrophage protein) family and ZIP (Zinc-regulated transporter iron-regulated transporter Proteins) family [29]. Enhanced metal uptake from the roots and translocation to the shoots in hyperaccumulators is a tightly controlled process mediated by membrane transport proteins. *N. caerulea* contains at least three different expressed genes: ZTP1 that is highly similar to the *Arabidopsis* ZAT-Zn gene, and ZNT1 and ZNT2 that resemble the *Arabidopsis* AtZIP4 gene, especially ZNT1 with 90% cDNA and 87% amino acid overlap [44]. In Zrt this sequence is HDHDHD and in Irt₁, this motif is GHGHGH. These histidine-rich motifs are part of the putative cytoplasmic domain, which may define a putative metal binding site for the transporter [52]. According to Salome (2019), lime-induced chlorosis/ iron-deficient plants showed limited expression of Fe⁺² IRT1 transporter to roots. Also, the potential for cobalt and cadmium uptake was observed [60]. Compared to the wild type, overexpression of NcZNT1 in transgenic *Arabidopsis* plants indicated increased Zn and Cd tolerance and accumulation, when compared to non-transformed wild-type plants. Also, Zn deficiency activation of NcZNT1 and AtZIP4 promoters in *A.thaliana* revealed the usual cis-regulatory sequences in both promoters associated with gene regulation. Furthermore, both AtZIP4 and NcZNT1 promoters were involved in the Zn deficiency response.

However, under low zinc regimes, the activity of the *N. caerulescens* NcZNT1 promoter expression was higher [45]. As reported by Teng et al. (2019) *N. caerulescens* Ni exposure activated NcIRT1 expression to higher Fe accumulation, at the same time, Fe deficiency increased Ni accumulation. Increased Zn uptake is driven by overexpression of members of the ZIP family of transporters. Additionally, this research showed that Ni exposure triggers the Zn transporter gene NcZIP10, and the Fe transporter gene NcIRT1, indicating their role in Ni uptake [73]. Also, *A. thaliana* Ni exposure led to increased Fe accumulation in plants by the Fe(II) transporter gene IRT1 activation [73]. Some heavy metal transporter cDNAs are very likely involved in both root metal uptake and metal partitioning between the root and shoot tissues in *A. halleri* (e.g., ZIP6), and *N. caerulescens* (e.g., ZIP4). Other candidate genes, HMA2, and HMA4 encode for a plasma membrane protein of P-type ATPase family (HMAs) involved in hyperaccumulation and hypertolerance to Zn and Cd in *A. halleri* [8, 71]. The *AtHMA4/ NcHMA4* highly expressed in roots and shoots of both *A. halleri* and *N. caerulescens* evidence the role of HMA4 in xylem metal loading in roots, in metal exclusion from metal-sensitive tissues (root tip, cambium), and metal distribution within leaves [30]. *Brassica rapa* (*L*) is another candidate plant with high Cd accumulation potential. The heavy metal ATPase gene BrHMA3 was identified as a gene that encodes a tonoplast-localized Cd transporter, with the role in the Cd root to shoot translocation [55, 87, 88].

Transport in plants is also mediated by the NRAMP (natural resistance-associated macrophage protein) family transporters. In *Arabidopsis* six of seven NRAMP proteins are divided into two groups. *AtNRAMP1* and *AtNRAMP6* belong to the first group, and *AtNRAMP2* through 5 comprise the second group [61]. While *AtNRAMP1* acts as a physiological Mg transporter, the *AtNRAMP3*, and *AtNRAMP4* mediate the remobilization of Fe from the vacuolar store [60]. Besides, *AtNRAMP3* functions as Fe, Mn, Cd, and Zn transporter between the vacuole and the cytosol, and its function is linked with Fe. While under Fe sufficient conditions, overexpression of *AtNRAMP3* does not change metal content; under Fe starving condition overexpression of *AtNRAMP3* reduces Zn and Mg in the cell [29]. *AtNRAMP4* is responsible for Fe, Mn Cd and Zn transport, while *AtNRAMP6* transports Cd and regulates the distribution of Fe and Mn within the cell [13]. Many members of ATPase and NRAMPs were identified in both *A. halleri* and *A. thaliana*. Similarly, *N. caerulescens* NcNRAMP3 and NcNRAMP4 can transport various metal cations, including Fe, Mn, Cd, Ni, and Zn [61].

While the ZIP, P-type ATPases (HMAs), and NRAMPs influx protein families are responsible for uptake of metals such as Zn and Cd from the soil, transport into the cells and distribution throughout the plant, the efflux CDF proteins are involved in metal

vacuolar sequestration [32]. Within the *Arabidopsis* genome, 12 nucleotide sequences encode members of the CDF transporter family [84]. The ZAT/ZTP1/MTP1 gene belonging to the CDF family of cation transporters identified in *N. caerulescens* bears sequence homology to the *A. thaliana* transporter *AtMTP1* (At2g46800) [41]. *A. halleri* *AtMTP1* gene highly expressed in leaves and roots [19] encodes a vacuolar membrane Zn transporter, responsible for metal sequestration in leaf vacuoles [42]. However, differential expression of MTP1 genes variations in *A. halleri*, and different Zn tolerance levels when expressed in yeast, indicate different evolutionary fates for different copies of MTP1 genes [20, 63] Also, it was found that up-regulation of Fe-deficiency response genes, such as *AtMTP3*, contributes to metal homeostasis in non-accumulator *A. thaliana* [5]. ZTP1 shares high sequence homology with *NgMTP* isolated from the Ni hyperaccumulator, *Nocca goesingense*. Like *NcZTP1* and *AtMTP1*, *NgMTP1* is located in vacuolar membrane [46]. In nonaccumulator *A. thaliana*, *AtMTP1*, *PtdMTP1*, *AtMTP3*, and *NgMTP1* genes were involved in increased Zn accumulation [31]. The source is not in the list of reference. Transgenic *Arabidopsis thaliana* (SULTR3 quintuple mutant) shows increased sulfur uptake via sulfate transporter SULTR3 subfamily encoded by the Sultr gene. Placed into chloroplast membrane, SULTR 3 transporter increased sulfate influx into the chloroplast for more than than 50% [6, 11]. Also, *Arabidopsis* basic helix-loop-helix transcription factors FIT, AtbHLH38, and AtbHLH39 are involved in plant Cd-Fe interactions [81]. Furthermore, there is a link between over co-expression of FIT with AtbHLH38 or AtbHLH39 and triggering HMA3, MTP3, IREG2, IRT2, NAS1, and NAS2 which has a role in vacuole HM sequestration as well in the synthesis of NA [81].

Tolerance to heavy metals, especially Mg and Ni, plays a crucial role in plant adaptation to serpentine soils. The well-known role of Ca is not only in growth and metabolic regulation but also Ca regulate the function of membrane protein transport systems and gene expression. Detoxification system is also based on a Ca role in glutathione and phytochelatin synthesis, thereby prevention metal ions entering the cell [26]. Therefore, genes involved in *Arabidopsis* and *Alyssum* Ca: Mg and Ca: Ni homeostasis are key factors associated with the toxic concentration of essential metals [72, 86]. A vacuolar membrane transporter, cation/proton antiporters, and calcium exchanger 1 CAX1 is Ca²⁺/H⁺ antiporter that reduces cytosolic calcium and enhances Mg and Ni tolerance. According to Ghasemi et al. (2018), the enhanced concentration of calcium in the cytosol was related to increasing Ni and Mg tolerance. Additionally, *A. thaliana* mutation of CAX 1 causes increase Cd sensitivity and decreased Ni tolerance. Taking into consideration that in serpentine soil Ca is very low compared to Mg and Ni, without a minimum cytosolic Ca concentration, Ni tolerance through Ni detoxifying mechanisms would be successful

in *Alyssum inflatum* [24, 25]. Furthermore, the *Alyssum inflatum* and *Alyssum lanceolatum* genetic structure indicate HM tolerance in *Alyssum*, thus, identifying candidate genes such as *CAX1* was essential for understanding plant adaptation to serpentine soil [53].

Chelation and Sequestration

Chelation and sequestration of heavy metals by particular ligands happen both at cellular and sub-cellular levels, in leaf epidermal cells and/or in cell walls and vacuoles from hyperaccumulator plants [2, 90]. By binding the PCs in complexes with heavy metals and sequestering the complexes inside their cells, plants avoid metal toxicity [2, 83]. Plants also produce a range of ligands for Cd, Cu, Ni, and Zn. The ligands include carboxylic acids, such as citric, malic, etc., and amino acids, such as histidine (His) [90].

Nitrogen Donor Ligands

Nicotianamine (NA) is a nonproteinogenic amino acid that forms strong complexes with most transition metal ions, and its role seems to be metal hyperaccumulation, both in *A. halleri* and in *N.caerulescens* [5]. Enzyme NA synthase (NAS) is involved in chelation of many transition metal ions [9]. Consider to be one of the major ligands, NA is involved in hyperaccumulation of Zn, Cd, and Ni [15] as well as iron (Fe), copper (Cu), and manganese (Mn) [33, 64].

Moreover, the expression of *NAS1* and *NAS2* (nicotianamine synthases) genes is Cd up-regulated, because of high concentrations of metal-chelator nicotianamine (NA), indicating NA involvement in Cd-tolerance [5]. Higuchi et al. (1999) purified *NAS* protein and isolated, cloned, and characterized *NAS1*, *NAS2*, *NAS3*, *NAS4*, *NAS5-1*, *NAS5-2*, and *NAS6* genes encoding *HvNAS* and *HvNAS*-like proteins from Fe-deficient barley (*Hordeum vulgare* L. cv Ehimehadaka no. 1) roots [34]. To identify the gene encoding *NAS* in *Arabidopsis thaliana*, Suzuki et al. (1999) used the nucleotide sequence of the *NAS* gene from barley (*HvNAS*). Searching *A. thaliana* databases, they found several ESTs and three genomic sequences highly homologous to *HvNAS*. The authors isolated the *NAS* orthologues in *A. thaliana* *AtNAS* (*AtNAS1*, *AtNAS 2*, and *AtNAS 3*). The expression of *AtNAS1* was detected in both shoots and roots of *A. thaliana*, *AtNAS3* expression was only detected in the shoots, while *AtNAS2* expression was not detected in any organs [70]. Increase NA synthesis in *A.thaliana* roots was Zn, and Cd hyperaccumulation indicator [16]. However, decreased synthesis of NA has the opposite effect on Ni accumulation. Investigating, Zn tolerance of *A.halleri* wild type and *AhNAS2*-RNAi interference plants, Cornu et al. (2015) showed a strong relation between increase NA synthesis in both plants exposed to elevated Zn concentration. Also, it was shown that *A.halleri* *NAS* genes are involved in

the adaptation of *AhNAS2*-RNAi transgenic plants. Zinc-induced Facilitator 1 (ZIF1) is an important Zn related protein placed in the vacuolar membrane of *A.thaliana*. However, this protein family is highly tight to NA when it comes to Zn root to shoot translocation. Thus, ZIF is not able to transport Zn-NA complexes or Zn alone, but with coexpression of ZIF1 and a *NAS* gene [13]. Furthermore, there is one more membrane-localized protein family, yellow strip-like (YSL) gene family related to Fe(II)-NA yeast transporter [17]. *Brassica juncea* *BjYSL7* encodes for this group of transporters, that has been involved not only in Fe but also in Cd and Ni root to shoot translocation. Moreover, with a 90% sequence identity with *N. caerulescens* *NcYSL7* and *A.thaliana* *AtYSL7* [78]. YSL members in *Arabidopsis*, *AtYSL1* and *AtYSL3* predominantly mediate Fe(II)-NA and Fe(III)-citrate, *AtYSL2* besides transporting Fe(II)-NA transport Cu-NA in yeast *AtYSL4* and *AtYSL6* are reported to localize in the vacuole membrane and chloroplast envelope in response to the detoxification of excessive metals in plant cells although there is no transport capacity for Fe(II)-NA in the yeast [10, 15, 18]. Additionally, Chen et al. (2018) have cloned the YSL1 gene from *M. sacchariflorus* *MsYSL1* is a transporter involved in the creation of metal-NA complexes in vivo. The overexpression of *MsYSL1* in transgenic *Arabidopsis* increased Cd detoxification and translocation [10].

Sedum alfredii *Hance SaNAS1* encoded protein responsible for enhanced Cd and Zn roots and shoots uptake. Transgenic *Arabidopsis* plants expressing *SaNAS1* showed the same pattern associated with increased Cd and Zn accumulation, tolerance and nicotianamine production in plants [7, 12]. Also, microarray analysis in *N. caerulescens* showed that all four *NAS* genes were highly expressed in *N. caerulescens* compared to non-hyperaccumulator *A. thaliana* when exposed to high levels of Zn, Cd, and/or Ni [75].

Amino Acids (AA) is also known as a group of nitrogen donor ligands as Changed pathways of nitrogen utilization of tested plants were noted. The glutamic acid and glutamate contents in plant biomass were decreased under Cd content more than 60mg/kg soil [89]. According to Zemanova et al. (2013), under Cd stress for *N. caerulescens* glutamate were main for nitrogen transport, while for *A. halleriare* asparagine were noted. Also, under Cd stress, the content of aspartic acid and proline was determined only in *N. caerulescens*, while *A.halleriare* didn't show any changes. At the molecular level, heavy metal tolerance of plants is under the regulatory genes, that encode various transcription factors (TFs) and/or functional genes encoding metabolic compounds such as amines, etc. [67]. In *A. thaliana* exposed to Cd, various TFs families, AP2/ERF superfamily and ERF1 and ERF5, as well as the dehydration-responsive element-binding protein (DREB) transcription factor were induced [4]. However, *Brassica oleracea* AP2/ERF-like genes

haven't had an expression profile exposed to abiotic stress (74). Furthermore, 5-aminolevulinic acid (ALA), is a well-known growth regulator, that is proved to be involved in Cd tolerance in *Brassica napus* L. Moreover, ALA-induced Cd²⁺ tolerance in *B. napus* is related to gene expression of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) as well as glutathione, carotenoids, and ascorbate [3].

Sulfur Donor Ligands

Phytochelatin (PCs) and metallothioneins (MTs) belong to different classes of cysteine-rich, heavy metal-binding proteins [8, 14]. PCs are enzymatically synthesized peptides, from the tripeptide glutathione (γ -Glu-Cys)-Gly [62, 68]. PCs are not genetically encoded but are non-ribosomal peptides [7]. Also, PC peptides are not primary translation products of mRNAs but are synthesized enzymatically [63]. The synthesis of PC is followed by a decrease in cellular levels of glutathione (GSH) [50]. As products of an enzymatic reaction involving the enzyme PC synthase, PCs are positively correlated with metal accumulation in plant tissues [50], i.e., plants naturally produce phytochelatin when exposed to Cd, Pb, Zn, Ag, Hg, As and Cu. Because of the structural similarity between PCs and GSH, GSH is thought to be the substrate for PCs biosynthesis [8, 14]. As a precursor of PCs, GSH-dependent pathway takes place in Cd sequestration [5, 9]. Also, GSH has a crucial role as a cellular antioxidant, as a reactive oxygen species (ROS) signaling molecule, and as a metal chelator [42]. In comparison to nonaccumulators, high concentrations of cysteine O-acetylserine and GSH were found in Ni-hyperaccumulating *Noccaea* species and hyperaccumulators *N. caerulescens* and *N. goesingense*. Increased serine acetyltransferase (SAT) activity and higher steady-state GSH levels were in correlation with high expression levels of genes encoding SAT and glutathione reductase in *N. goesingense*. High expression of NgSAT in *A. thaliana* was reported to confer Ni, Co, and Zn tolerance, and to a small extent Cd tolerance [42]. According to Chen et al., (2015), *AtMAN3*, which encodes an endo- β -mannanase regulates Cd tolerance of *Arabidopsis thaliana* through the GSH-dependent PC synthesis pathway. In a line with this exposure to Cd trigger MAN3 expression followed by increase mannanase activity and a larger amount of mannose in cell walls [5, 9]. Furthermore, exposure to Cd triggers an ABC-type transporter *AtABCC3* known as a transporter of PC-Cd complexes. This finding showed that overexpression of *AtABCC3* increases Cd tolerance and vacuolar Cd content in protoplast [6]. Song et al. (2017) investigated Cd stress-regulation pathway in transgenic *Arabidopsis*, which involves Ferrochelataase-1 (FC1, EC4.99.1.1) as the terminal enzyme encoded by *AtFC1* gene. It was noted that activation of *AtFC1* by Cd exposure exceeded the

primary root by PCs-dependent detoxifying pathway, and increased biomass and chlorophyll content [69].

As a metal chelator, GHS acts through its thiol groups, which have high metal-binding affinity [43]. GSH1 and GSH2 genes involved in GSH synthesis were identified in *A. thaliana* when exposed to Cd [43]. The expression of GSH1 and GSH2 contributed to Cd tolerance, while a decrease in GSH levels reduced Cd tolerance [43]. Furthermore, *Arabidopsis AtFC1* regulate Cd stress response in hematin triggering GSH/PCs-synthesized gene expression, GSH1, GSH2, PCS1, and PCS2 [69]. It was also found that coexpression of PCS1 and GS in transgenic *Arabidopsis* increased PC production, Cd, and As tolerance and accumulation [28].

Also, GSH is essential in Fe-mediated Zn tolerance in *A. thaliana* [43]. Xiang and Olive (1998) have treated *Arabidopsis* plants with Cd or Cu. The plants responded by increased transcription of the genes for glutathione synthesis, γ -glutamylcysteine synthetase, and glutathione synthetase, as well as glutathione reductase. The study showed that the response was specific for metals whose toxicity is thought to be mitigated through phytochelatin, and for toxic and nontoxic metals, which did not alter mRNA levels. It was also reported that neither oxidative stress (as a result of exposure to H₂O₂) nor oxidized or reduced glutathione levels were responsible for activating transcription of these genes. Unlike H₂O₂, oxidized or reduced glutathione, jasmonic acid treatment increased mRNA levels and the capacity for glutathione synthesis but did not increase the glutathione content in unstressed plants, which indicated that the glutathione concentration was controlled at multiple levels. Interestingly, jasmonic acid activated the same suite of genes for GA synthesis, which suggests that it might be involved in the signal transduction pathway for copper and cadmium [82]. Sidhu et al. (2018b) have pioneered the applicability of EDDS (chelant ethylenediamine disuccinic acid) to boost Ni-phytoextraction by *Coronopus didymus*. The results of the study indicated that EDDS treated soil increased antioxidant activities of superoxide dismutase, catalase, and glutathione peroxidase, raising of H₂O₂ content and MDA levels and production of superoxide anion. The increased antioxidant enzyme activity led to increased binding affinities to Ni, as well as enhanced Ni translocation in plant tissues [66]. Also, it was found that *Coronopus didymus* has the potential to ameliorate Pb-contaminated soils. The study showed greater Pb accumulation potential in roots compared with the shoots, with the bioconcentration factor less than 1, and the translocation factor greater than 1 [65].

The class of polyhydroxy steroids called Brassinosteroids (BRs) is found to have protective activity in the plants exposed to various stresses by the synthesis of antioxidant compounds in plants. One of the roles is in the syntheses of PC in plants exposed to lead [54] Although, at very low concentrations up to 120 ng/kg, BRs can be found in the roots, the leaves,

and the stem. Within the *Brassicaceae* family these steroidal plant hormones are present in *Brassica napus* L., *Brassica campestris* var. *pekinensis* L., *Raphanus sativus* L. and *Arabidopsis thaliana* L. but not in *Brassica juncea* plants [37].

To prevent biotic and abiotic stress caused by HMs, 70 BRs analogs have been identified in different cultivated plants, and among these, 24-epibrassinolide (24-eBL) [85]. Application of 24-eBL reduced uptake and accumulation of copper in *B. juncea*, and regulated developmental processes, such as biomass production [54]. Genetic analyses clearly showed the signal transduction pathway of BR, from the Brassinosteroid insensitive 1 (BRI1) receptor kinase to transcriptional regulation by the Brassinazole resistant (BZR) family, involved in BR-mediated stress tolerance in *Arabidopsis* [79]. Based on the Geneinvestigator *Arabidopsis thaliana* Cd-regulated genes database, there is a similarity in gene expression in response to Cd and brassinosteroids (BR). Furthermore, Cd-induced activation of the BR-signalling pathway reflects in the expression of genes able to influence the Cd response [77]. Since the plasma membrane is the first line of defense, from Cd stress, activation of the plasma membrane protection enzymes H⁺-ATPase and NADPH oxidase by BR gives a new perspective. Therefore, *Arabidopsis thaliana* transmembrane receptor BR insensitive 1 (BRI1) and the co-receptor BRI1-associated receptor kinase 1 (BAK1) regulate BR signaling processes and have a key role in transcription and gene regulation related to Cd stress regulation [55]. Also, *B. juncea* treated with EBL and 28-homobrassinolide (HBL) showed lower Ni exposed stress, by BRs stimulated the activity of some antioxidant enzymes [76]. Furthermore, Huang et al. (2018) showed that *Brassica rapa* BRI1 proteins BrBRI1-1, BrBRI1-2, and BrBRI1-3 had sequence similarity to *Arabidopsis thaliana* BRI1 (AtBRI1) [35].

Application of EDTA, EDDS chelants to increase Cd, Cu, and Zn uptake and accumulation by *Brassica napus* L. showed increased Cd accumulation in stems and leaves, Zn concentration was higher in stems with leaves, while higher Cu concentration was found in roots [38]. Meyer et al. (2011) compared PCs between Cd-hyperaccumulators *A. halleri* and *N. caerulescens*, and non-hyperaccumulator *A. thaliana*, and found that PCS1 gene from *A. halleri* and *N. caerulescens* showed lower expression than its orthologue from *A. thaliana* [47].

Unlike PCs, MTs are gene-encoded polypeptides [8;14]. (Metallothioneins (MTs) are a class of small cysteine-rich proteins with high binding affinity to metals via metal-binding motifs that provide sulfhydryl for interacting with divalent metal ions [43]. Based on the type of cysteine residues, MTs in higher plants are classified into four types: MT1, MT2, MT3, and MT4 [56]. There are some general trends in the tissue-specific expression of MT genes, with type-1 MTs expressed predominantly in roots, type-2 MTs in leaves, type-3 MTs in fruits, and type-4 MTs in seeds

[56]. According to Guo et al. (2008a), most active MT genes confer Cu tolerance and accumulation, MT4 types confer Zn tolerance and accumulation, and MT1, 2, and 3 types enhance tolerance to Cd, but often not Cd accumulation. Hence, some difference in the gene expression levels between hyperaccumulators and non-accumulators has been found. In non-accumulator plants, such as *A. thaliana*, MT1a and MT1b are expressed at high levels in roots during exposure to Cd, Cu, and Zn, while in hyperaccumulator *N. caerulescens* the levels of MT1 mRNA were found in leaves constitutively higher than in roots [59]. As a consequence, NcMT1 and NcMT2 are expressed at much higher levels in *N. caerulescens* compared to *A. thaliana*. However, MT2 is constitutively expressed in both *A. halleri* and *N. caerulescens* [59, 75]. According to Roosens et al. (2004), in *N. caerulescens*, NcMT3 confers much greater levels of tolerance to Cu than to Cd and increases the intracellular Cd concentrations. NcMT3 and AtMT3 expressed in the yeast mutant both showed similar Cd tolerance. However, better growth of yeast expressing NcMT3 than AtMT3 under Cd exposure, implies that NcMT3 can chelate more Cu than AtMT3 [58]. Furthermore, expression of *B. juncea* BjMT2 gene in *A. thaliana*, increased not only Cu but also Cd tolerance [90]. A member of type-1 MT genes, MT1, is found to be expressed in leaves of *Brassica napus*, *Brassica rapa* L. and *Arabidopsis* sp. exposed to Cd. Also, BrMT1 isolated from *Brassica rapa* has shown resistance to Cd, in part, because its genome, has on average, 50% DNA similarity in coding regions with the genome of hyperaccumulator *B. juncea* [40]. *Arabidopsis* has seven active MT genes belonging to four types (MT1a, MT1c; MT2a, MT2b; MT3; MT4a and MT4b) and one pseudogene (MT1b) [56]. MT gene expression is highly induced by Cu exposure, and a correlation between type 2 MT gene expression and Cu tolerance has been concluded from studies of *Arabidopsis* ecotypes [27]. Ren et al. (2012) investigated the functions of *Arabidopsis* AtMT4a and AtMT4b genes, in seed development, germination, and early seedling growth. This study showed that AtMT4a and AtMT4b are responsible for the accumulation of some important metal ions in late embryos [56]. As well, phytohormones abscisic acid (ABA) and gibberellic acid (GA) was found to be important in regulating the expression and function of AtMT4a and AtMT4b during seed development. In many *Brassicaceae* species, including metal hyperaccumulators *A. halleri*, non-hyperaccumulator relatives *A. lyrata*, *A. thaliana*, and metal hyperaccumulators *N. goesingense* and *N. caerulescens*, MT gene expression is strongly induced by Cu treatment and, to a lesser degree, by Cd and Zn [43]. Additionally, MT genes protect the plant from heavy metal toxicity by their MT genes expression, which presents the main difference between hyperaccumulator plants and non-hyperaccumulator plants. Significantly, this reflects their important role in phytoremediation [8].

Table 1. Genes and proteins contributing to heavy metal tolerance or accumulation found in species of family *Brassicaceae*.

Gene name	Plant	Protein families	Effect	Reference
IRT1	<i>Arabidopsis thaliana</i>	Iron-regulating transporter	Fe(II) transporter	[60]
SULTR3	<i>Arabidopsis thaliana</i>	SULTR subfamily transporter	S transporter	[11]
ZTP1, MTP1	<i>Arabidopsis thaliana</i> <i>Noccea goeingense</i> , <i>Noccea caeruleascens</i> ,	CAD	Vacuolar sequestration of Zn and Cd	[5, 19, 31, 32, 41, 52, 63, 87]
HMA1-8,	<i>Arabidopsis thaliana</i> , <i>Arabidopsis halleri</i>	P-type ATPases family (HMAs).	Cd, Cu, Zn, transporter	[8, 88]
<i>CAX1 and CAX 3</i>	<i>Arabidopsis thaliana</i>	CAX	Vacuolar membrane transporter Cd, Mn, and Zn; Mg and Ni tolerance	[26, 53]
CAX1 from <i>A. thaliana</i>	<i>Transgene Alyssum inflatum</i>	CAX	Vacuolar membrane transporter Ni tolerance	[26]
<i>CAX1</i>	<i>Alyssum inflatum</i> , <i>Alyssum lanceolatum</i>	CAX	Vacuolar membrane transporter Mg and Ni tolerance	[24, 25]
ZAT-Zn gene and ZNT1 and ZNT2 resemble the <i>Arabidopsis ZIP4</i> gene	<i>N. caeruleascens</i> , <i>N. arvensis</i>	Zinc transporter (ZAT) family	Zn accumulation and transport	[84]
Zinc-induced Facilitator 1 <i>A. thaliana</i> (ZIP1)	<i>A. thaliana</i>	Zinc-induced Facilitator 1 (ZIP) super-family	Zn translocation	[13]
AthMA1-8; <i>BtHMA3</i>	<i>A. thaliana</i> , <i>A. halleri</i> , <i>B. rapa</i>	P-type ATPases family (HMAs).	Cd, Cu, Zn, transporter	[61, 71, 87]
<i>AtNRAMP1-6</i> , <i>AtNRAMP3</i> <i>NcNRAMP3</i> and <i>NcNRAMP4</i>	<i>Arabidopsis thaliana</i> , <i>Arabidopsis halleri</i> , <i>Noccea caeruleascens</i>	NRAMP family	Fe, Cd transporter Fe, Mn, Cd, Ni, and Zn transporter	[29, 71]
<i>AtZIP1-12</i> <i>NcZIP10</i>	<i>Arabidopsis thaliana</i> , <i>Noccea caeruleascens</i>	ZIP family	Zn transporter, Ni uptake	[73, 80]
<i>AtIRT1</i> , <i>NcIRT1-2</i>	<i>Arabidopsis thaliana</i> , <i>Noccea caeruleascens</i>	IRT family	Zn and Cd transporter Ni uptake	[52, 73]
<i>AtIRT2</i>	<i>Arabidopsis thaliana</i>	IRT family	Retention of Cd in vacuoles and intracellular vesicles	[81]
<i>AtMTP1</i> , <i>AtMTP1</i> , <i>NgMTP1</i>	<i>Arabidopsis thaliana</i> , <i>Arabidopsis halleri</i> , <i>Noccea goeingense</i>	CDF family	Zn transporter	[20, 40]
<i>AtMRP3</i> , <i>AtABCCC3</i>	<i>Arabidopsis thaliana</i>	ABC transporter	Confers Cd tolerance	[6]
<i>AtNAS1</i> , <i>AtNAS 2</i> , and <i>AtNAS 3</i>	<i>Arabidopsis halleri</i> and <i>Noccea carulences</i>	enzyme NA synthase (NAS)	Forms strong complexes with Ni	[75]
<i>AhNAS2-RNAi</i>	<i>Arabidopsis halleri</i>	enzyme NA synthase (NAS)	Forms strong complexes with Zn, Cd, and Ni	[16]

Table 1. Continued.

SaNAS	<i>Transgenic Arabidopsis</i>	enzyme NA synthase (NAS)	Accumulation and tolerance to Cd and Zn	[9]
<i>BjYSL7</i> , <i>NcYSL3</i> , <i>NcYSL5</i> , and <i>NcYSL7</i> <i>NcYSL7</i> , <i>AtYSL7</i> , <i>AtYSL1</i> and <i>AtYSL3</i> , <i>AtYSL2</i> , <i>AtYSL4</i> and <i>AtYSL6</i>	<i>Brassica juncea</i> <i>Noccea caemulescens</i> <i>A.thaliana</i>	Yellow stripe like (YSL) gene family	Fe(II)-NA Ni-NA translocation <i>Fe(II)-NA</i> and <i>Fe(III)-</i> <i>citrate</i> , <i>Cu-NA</i>	[10, 15, 18, 78]
<i>MsYSL1</i> , from <i>Miscanthus sacchariflorus</i> .	<i>Transgenic Arabidopsis thaliana</i>	Yellow stripelike (YSL) gene family	<i>Cd detoxification and translocation</i>	[10]
CAD1, <i>NrPCS1</i> , <i>AtPCS1</i>	<i>Arabidopsis</i> , <i>Noccea aestivum</i>	Phytochelutins (PCs)	Tolerance to Cd	[47]
GSH1 and GSH2,	<i>Arabidopsis thaliana</i>	Glutathione (GSH)	Strong Cd tolerance, in Fe-mediated Zn tolerance	[43, 80]
FIT, <i>AtbHLH38</i> , and <i>AtbHLH39</i>	<i>Arabidopsis thaliana</i>	Basis helix-loop-helix transcription fac- tors (bHLH family)	Fe transportation and homeostasis, Cd toler- ance	[81]
<i>NgSAT</i>	<i>Noccea goesingense</i> and <i>trans-</i> <i>genic Arabidopsis thaliana</i>	Serine acetyltransferase (SAT)	Confer Ni, Co, Zn tolerance, and to a small extent Cd tolerance	[42]
<i>MT1</i> , <i>MT2</i> , <i>MT3</i> , and <i>MT4</i> , <i>BjMT2</i> , <i>BrMT1</i>	<i>Arabidopsis</i> , <i>Noccea</i> , <i>Brassica juncea</i> , <i>Brassica napus</i>	Metallothioneins (MTs)	confer Cu, Zn and Cd tolerance	[27, 40, 56, 58, 59, 75, 90]
<i>AtBRI1</i> , <i>BrBRI1-1</i> , <i>BrBRI1-2</i> , and <i>BrBRI1-3</i>	<i>Arabidopsis thaliana</i> <i>Brassica rapa</i>	BRI1 family	Cd tolerance	[35]
<i>AtBAK1</i>	<i>Arabidopsis thaliana</i>	RLKs receptor-like kinases	Abiotic stress, Cd tolerance	[55]
AP2/ERF	<i>Arabidopsis thaliana</i>	AP2/ERF superfamily (ERF1 and ERF5)	Cd stress tolerance	[4]
DDREB	<i>Arabidopsis thaliana</i>	DDREB family	Cd stress tolerance	[4]

Conclusion

The myriad of physicochemical methods for removal of the pollutants through effective is very expensive and not suitable where the city budgets are limited [48]. To tackle the problem of the metal-contaminated environment in the most economically end ecologically friendly way, construction of transgenic plants, based on the introduction of foreign genes, which are connected with uptake, transport, and accumulation of heavy metals, present a solution to clean up the urban environment. The review highlighted the remarkable results obtained from different studies regarding molecular aspects of proved accumulators of heavy metals in the *Brassicaceae* family. Most of the discovered hyperaccumulators belong to the genera *Noccaea*, *Brassica*, *Alyssum* and *Arabidopsis* [21]. The suitable candidates for the bio-removal of a wide range of heavy metals from the environment are shown in Table 1.

Table 1. Genes and proteins contributing to heavy metal tolerance or accumulation found in species of family *Brassicaceae*. Based on the results reviewed in this paper, the differences between species genetic blueprints in the same family provide more significant insights into the specific genes, their regulatory mechanisms, proteins, and informational pathway responses connected to phytoremediation. Variations on molecular levels within plants of one family are a way for populations to adapt to changing environments, landscapes, etc. The implication of this research is in the direct insertion of identified genes to enhance the metabolic capabilities and metal accumulating potential of *Brassicaceae* and other plant species.

Conflict of Interest

The authors declare no conflict of interest.

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