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CENTRO DE CIÊNCIAS DA NATUREZA – CCN

campus LAGOA DO SINO

CURSO DE GRADUAÇÃO EM ENGENHARIA AGRONÔMICA

GABRIEL ANTONIO BORTOLOTI

Estratégias de tolerância da fitorremediação de metais pesados em plantas-modelo: um estudo de caso em espécies do gênero botânico *Brassica*

Buri (SP)

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Trabalho de Conclusão de Curso apresentado ao Curso de Engenharia Agronômica para obtenção do título de Bacharel em Engenharia Agronômica.

Orientação: Prof. Dr. Daniel Baron

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Trabalho de Conclusão de Curso apresentado como requisito parcial à obtenção do título de Bacharel em Engenharia Agronômica pela Universidade Federal de São Carlos.

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RESUMO

BORTOLOTI, Gabriel Antonio. Estratégias de tolerância da fitorremediação de metais pesados em plantas-modelo: um estudo de caso em espécies do gênero botânico *Brassica*. 2021. 56f. Trabalho de Conclusão de Curso (Graduação em Engenharia Agronômica) – Universidade Federal de São Carlos, Buri, 2021.

O aumento da contaminação de solos por metais pesados (MPs) como cádmio (Cd), cromo (Cr), mercúrio (Hg), arsênio (As), chumbo (Pb), níquel (Ni) e zinco (Zn), tem gerado grandes preocupações ambientais, principalmente pela característica de não biodegradabilidade desses MPs, o que faz com que permanecem por longos períodos no ambiente. Algumas técnicas de remediação de solos contaminados são reportadas pela literatura, entre esta se destaca a fitorremediação, uma tecnologia que utiliza plantas capazes de estabilizar ou remediar a contaminação do ambiente por meio de estratégias de absorção e acumulação dos contaminantes. Algumas espécies vegetais apresentam maior tolerância aos efeitos tóxicos dos MPs, à exemplo dos vegetais pertencentes ao gênero botânico *Brassica*. Alguns MPs, como Zn e Ni, são essenciais aos vegetais, entretanto, altas concentrações desses MPs ou de outros não essenciais, como o Cd, podem gerar danos celulares na osmorregulação no metabolismo fotossintético e nas trocas gasosas das plantas. As espécies desse gênero, no entanto, apresentam um eficiente mecanismo de defesa enzimático e não enzimático contra o estresse gerado por esses contaminantes. A literatura reporta que algumas técnicas de manejo agronômico como a utilização de reguladores vegetais, promotores de crescimento de plantas, agentes quelantes e acidificantes, além da seleção de cultivares mais tolerantes e o uso de engenharia genética, podem auxiliar na absorção e tolerância de MPs. Portanto, as espécies do gênero botânico *Brassica* se apresentam como interessante “modelo” de estudos na área de fitorremediação e, desta forma, optamos por redigir a presente monografia na modalidade científica ‘artigo de revisão’ (*paper review*), a partir da atualização contínua do que há de mais atual e promissor na literatura a respeito de fitorremediação de MPs.

Palavras-chave: Estado-da-arte. Fisiologia Vegetal. Fitorremediação. Metais pesados tóxicos. Planta-modelo.

ABSTRACT

BORTOLOTI, Gabriel Antonio. Tolerance strategies of heavy metal phytoremediation in model plants: a case study in species of the botanical genus *Brassica*. 2021. 56f. Course Completion Work (Degree in Agronomic Engineering) – Federal University of São Carlos, Lagoa do Sino *campus*, Buri, 2021.

The increase in soil contamination by heavy metals (HMs) such as cadmium (Cd), chromium (Cr), mercury (Hg), arsenic (As), lead (Pb), nickel (Ni), and zinc (Zn), has generated major environmental concerns, mainly due to the non-biodegradability characteristic of these HMs, which makes them remain in the environment for long periods. Some remediation techniques for contaminated soils are reported in the literature, among them, phytoremediation stands out, a technology that uses plants capable of stabilizing or remediating environmental contamination through contaminant absorption and accumulation strategies. Some plants species are more tolerant to the toxic effects of HMs, such as plants belonging to the botanical genus *Brassica*. Some HMs, such as Zn and Ni, are essential to plants, however high concentrations of these HMs or other non-essential ones, such as Cd, can cause cell damage, osmoregulation, in the photosynthetic metabolism, and the gas exchange of plants. *Brassica* species, however, have an efficient enzymatic and non-enzymatic defense mechanism against the stress generated by these contaminants. The literature reports that some agronomic management techniques such as the use of plant growth regulators, plant growth promoters, chelating and acidifying agents, in addition to the selection of more tolerant cultivars and the use of genetic engineering, can help in the uptake and tolerance of HMs by *Brassica* plants. Therefore, species of the botanical genus *Brassica* are an interesting model for studies in the field of phytoremediation and, therefore, we chose to write this monograph as a scientific review article, based on the continuous update of what is most current and promising in the field literature on phytoremediation of HMs by *Brassica* species.

Key-words: Model plant. Plant Physiology. Phytoremediation. State-of-art. Toxic heavy metals.

SUMÁRIO

1 INTRODUÇÃO E JUSTIFICATIVA PARA ESCOLHA DO TEMA

A contaminação de solos por metais pesados (MPs), como cádmio (Cd), cromo (Cr), mercúrio (Hg), arsênio (As), chumbo (Pb) e zinco (Zn), a partir de práticas relacionadas a industrialização, agricultura e a mineração tornou-se uma séria ameaça à saúde humana e ao meio ambiente nas últimas décadas (CRISTALDI et al., 2017; HORTA et al., 2015; MAHAR et al., 2016). As atividades de mineração podem resultar em contaminação de MPs em áreas circunvizinhas, além de gerarem degradação física e química dos solos (VISCONTI et al. 2020). Outro grande problema atrelado as áreas de mineração está relacionado aos acidentes gerados pelos rompimentos de barragens de rejeitos, como os recentes casos ocorridos em Mariana (2015) e Brumadinho (2019), no estado brasileiro de Minas Gerais.

Os estudos de Ferreira e colegas (FERREIRA et al., 2021) observaram que a lama aluvial contendo altas concentrações de MPs, resultante do rompimento da barragem do ‘Fundão’ em Mariana, apresenta propriedades químicas diferentes dos solos da região e, uma vez em contato com estes solos, pode gerar um material com composição desconhecida e, assim, afetar a sorção/dessorção e biodisponibilidade de nutrientes as plantas. A reportagem de Vergilio e colaboradores (VERGILIO et al., 2020) apresenta um aumento superior ao permitido pela legislação brasileira nas concentrações MPs tóxicos, como Cd, Cr, Ni (níquel), Pb, Zn e U em corpos hídrico e sedimentos na região afetada pelo desastre de Brumadinho. Esses mesmos autores observaram o aumento da concentração desses MPs em todos os níveis tróficos na região avaliada. Além disso, esses MPs não são biodegradáveis e permanecem no meio ambiente por longos períodos (SHAH; DAVEREY, 2020).

Entretanto, a literatura reporta a existência de algumas técnicas de remediação de solos contaminados como vitrificação, lavagem ácida, remoção de solo e fitorremediação (ROSTAMI; AZHDARPOOR, 2019). A fitorremediação consiste na utilização de plantas capazes de estabilizar ou remediar a contaminação do ambiente a partir de diferentes estratégias de absorção e acumulação do MPs tóxicos em seus tecidos (ALI; KHAN; SAJAD, 2013; CAMESELLE; GOUVEIA, 2019; YAN et al., 2019). Algumas espécies de plantas são mais tolerantes aos efeitos tóxicos dos MPs e, portanto, são de interesse para a fitorremediação, como as espécies pertencentes ao gênero *Brassica* (KAUR et al., 2017). *Brassica* spp. podem realizar a fitorremediação de MPs por meio de diferentes mecanismos fisiológicos, como fitovolatilização, fitoestabilização (KUMAR; THAKUR; KUMAR, 2019) e fitoextração (SARWAR et al., 2017).

Alguns MPs são necessários em pequenas concentrações e são essenciais para o crescimento e o ciclo de vida das plantas, como o Zn e o níquel (Ni). No entanto, altas concentrações desses metais e outros não essenciais como o Cd são tóxicos e podem afetar a absorção de nutrientes pelas plantas, principalmente devido à competição por sítios de ligação enzimática comuns (FEIGL et al., 2015). Além disso, é relatado que concentrações tóxicas desses metais podem danificar a estrutura celular (AMARI; GHNAYA; ABDELLY, 2017), o estado da água das plantas (SABIR et al., 2020), o metabolismo fotossintético e de troca gasosa (AYYAZ et al., 2020) e, consequentemente, o crescimento vegetal (DU et al., 2020).

Entretanto, a literatura reporta que espécies de "brássicas" apresentam um eficiente mecanismo de defesa enzimática contra o estresse oxidativo gerado por MPs tóxicos que inclui, por exemplo, as enzimas superóxido dismutase (SOD), catalase (CAT), ascorbato peroxidase (APX), peroxidase (POD), glutationa peroxidase (GPOX) e polifenol oxidase (PPO), glutationa redutase (GR), glutationa s-transferase (GST), desidroascorbato redutase (DHAR) e monodesidroascorbato redutase (MDAR) (SOARES et al., 2019). Este sistema enzimático atua como a primeira linha de defesa antioxidante, porém, em condições de estresse prolongado, outros mecanismos não enzimáticos podem ser utilizados, como a síntese de ácido ascórbico (AsA), glutationa reduzida (GSH), α-tocoferol, carotenóides, poliaminas, flavonóides, fenóis e prolinas (GILL; TUTEJA, 2010; YAN et al., 2016).

Além disso, diversos estudos apontam que algumas técnicas de manejo podem melhorar a absorção e tolerância de MPs tóxicos por brássicas, como o uso de reguladores de crescimento de plantas (PGRs), promotores de crescimento de plantas (PGPs), agentes quelantes e acidificantes e técnicas para seleção de cultivares tolerantes e engenharia genética (RIZWAN et al., 2016; SARWAR et al., 2017).

Portanto, as espécies do gênero botânico *Brassica* se apresentam como um interessante e promissor modelo de estudos na área de fitorremediação de solos contaminados por metais pesados tóxicos. Desse modo, optamos por redigir a monografia de TCC em forma de artigo científico de revisão bibliográfica, a partir do que há de mais atual publicado na literatura a respeito das estratégias de fitorremediação de metais pesados por espécies do gênero botânico *Brassica*.

2 OBJETIVOS

Explorar o potencial fisiológico das espécies do gênero botânico *Brassica* e suas diferentes estratégias de fitorremediação de metais pesados.

3 HIPÓTESE

H0 = Testar se espécies do gênero botânico *Brassica* são potencialmente fitorremediadoras de metais pesados tóxicos.

H1 = As espécies do gênero botânico *Brassica* não são potencialmente fitorremediadoras de metais pesados tóxicos.

4 METODOLOGIA

O levantamento do referencial teórico foi realizado a partir de publicações de periódicos científicos internacionais, com elevado fator de impacto, obtidos por meio da Plataforma CAFe (Comunidade Acadêmica Federada, site: http://wwwperiodicos-capes-gov-br.ezl.periodicos.capes.gov.br/). As principais bases de dados consultadas foram: Scopus®(https://wwwscopus.ez1.periodicos.capes.gov.br/search/form.uri?display=basic #basic); Science Direct® (https://www-sciencedirect.ez1.periodicos.capes.gov.br/) e SpringerLink (https://link-springer-com.ez31.periodicos.capes.gov.br/). Dessa forma, adotamos a metodologia de Barros e Lehfeld (BARROS; LEHFELD, 1997), ao realizarmos leitura crítica de publicações recentes.

Na busca de conteúdos sobre os temas abordados no trabalho, as combinações de 'palavras-chave' mais utilizadas incluem:

(i) Fitorremediação (*phytoremediation*);

(ii) Metais pesados (*heavy metals*);

(iii) translocação (*translocation*);

(iv) crescimento vegetativo (*plant growth*);

(v) fitoextração (*phytoextraction*);

(vi) fitovolatilização (*phytovolatilization*);

(vii) fitoestabilização (*phytostabilization*);

(viii) Promotores de crescimento vegetal (*plant growth promoters*);

(ix) Reguladores de crescimento vegetal (*plant growth regulators*);

(x) Hiperacumulação (hyperaccumulator);

(xi) Estresse oxidativo (*oxidative stress*).

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6 ARTIGO DE REVISÃO

Conforme estabelecido pelo Projeto Pedagógico do Curso de Bacharelado em Engenharia Agronômica (PPC, 2016 – *link* de acesso: https://www.lagoadosino.ufscar.br/cursos/arquivos/ppcs/ppc_engenharia_agronomica_2018_07.pdf), no item '9.4.2. Regulamento do Trabalho de Conclusão de Curso', estabelece que a monografia do trabalho de conclusão do curso se refere a "*um trabalho acadêmico pode ser monográfico ou de pesquisa o qual poderá ter tema inédito ou advir de pesquisa realizada pelo estudante, no âmbito de sua iniciação científica ou elaborar uma monografia a partir de situações-problema que por ventura vivencie no campo de estágio*".

Considerando as justificativas apresentadas anteriormente ao longo desta monografia, redigimos o presente TCC na modalidade 'artigo de revisão' com os atuais avanços intelectuais, conforme as normas de publicação da revista de alto impacto '*Chemosphere'* (https://www.journals.elsevier.com/chemosphere).

INSIGHTS INTO THE TOLERANCE AND PHYTOREMEDIATION POTENCIAL OF *Brassica* SPECIES GROWN UP UNDER TOXIC HEAVY METALS

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HIGHLIGHTS

- Phytoremediation is a promising bioremediation option
- Brassica shows phytoremediation characteristics
- Agronomic techniques support phytoremediation efficiency

ABSTRACT

Contamination of soils and water bodies by toxic heavy metals (HMs) such as cadmium (Cd), chromium (Cr), mercury (Hg), arsenic (As), lead (Pb), and zinc (Zn), is a major environmental concern. Phytoremediation emerges as an important technique, in which plants are used to decontaminate these areas. The species of the botanical genus *Brassica* are reported as potentially phytoremediators and hyperaccumulators because they present efficient processes that help in this technique, such as phytovolatilization, phytostabilization, and phytoextraction. These species also have physiological processes that aid the absorption, translocation, and accumulation of toxic HMs in low-activity cell organelles, in addition to an efficient enzymatic and non-enzymatic defense mechanism that attenuates the oxidative damage induced by the overproduction of reactive species of oxygen (ROS). In addition to enzymatic and non-enzymatic, our review approach sheds light on other phytoremediation assist techniques potentially used in *Brassica* species such as the use of chelating and acidifying agents, selection of tolerant cultivars, and genetic engineering techniques. Although techniques for the management and disposal of biomass obtained after the phytoremediation process are reported, this issue still lacks studies to present greater consensus between which techniques are safer, more efficient, and economically viable. Phytoremediation of toxic HMs by *Brassica* species is, therefore, a promising technique, however, we speculate the need for further studies aimed at approaching agronomic techniques that assist in structuring the hyperaccumulation of the network of these contaminants so that their applicability and feasibility can reach areas larger (growing areas).

Key-words: brassica*;* phytoremediation pathways; phytoremediation; plant-models; plant physiology; state-of-the-art; toxic heavy metal.

1 INTRODUCTION

Soil pollution, caused by increased levels of heavy metals (HMs) from human activities, has become increasingly a worry (SARWAR et al., 2017). HMs, such as cadmium (Cd), chromium (Cr), mercury (Hg), arsenic (As), lead (Pb), and zinc (Zn) are not biodegradable and remain in the environment for a long time, which can become soils unsuitable for cultivation of crop plants (SHAH; DAVEREY, 2020). Furthermore, some metals are highly toxic to humans, even in trace ions concentrations, such as Cd and Pb (RIZWAN et al., 2018). The literature reports the existence of remediation techniques for contaminated soils such as vitrification, acid washing, soil removal, and phytoremediation (ROSTAMI; AZHDARPOOR, 2019).

Phytoremediation is environmentally friendly biotechnology, capable of preserving the physical characteristics of the soil, and of low effective cost, when compared to other remediation techniques (SHAH; DAVEREY, 2020). The literature reports some crop models and techniques to calculate the cost-effectiveness of using phytoremediation. Corroborating this, some authors observed a high efficiency of removal of toxic heavy metals from the soil (As, Cd and Pb) and, when compared to other remediation techniques, had lower financial costs. For more details, please, we suggest read the paper Wan, Lei and Chen that elucidate the ‘Cost–benefit calculation of phytoremediation technology for heavy-metal-contaminated soil’ (WAN; LEI; CHEN, 2016).

Some plant species belonging to the *Brassica* genus are tolerant to the toxic effects of HMs, and, consequently, interesting to phytoremediation (KAUR et al., 2017). *Brassica* species can perform HMs phytoremediation through physiological mechanisms, such as phytovolatilization (KUMARI et al., 2020), phytostabilization (KUMAR YADAV et al., 2018), and phytoextraction (SARWAR et al., 2017).

Some HMs are essential for plant development and life cycle required at low concentrations, for example, Zn and nickel (Ni) (TAIZ et al., 2015). However, high concentrations of these metals and other non-essentials (e.g., Cd) are toxic to plants and can impair biochemical and morphophysiological processes (THAKUR et al., 2016). The roots ionic uptake is influenced by their bioavailability in the soil solution and can promote organic acids secretion (WANG et al., 2017) and biosurfactants (ARAÚJO et al., 2019). After uptake, these metals can be translocated by xylem vessels to the organs development storage (RUBIO et al., 2020).

Brassica species also present the synthesis of some substances that can help the translocation of these metallic ions to the aerial parts, such as metallothioneins (MTs), glutathiones (SHAH; DAVEREY, 2020), and phytochelatins (PCs), reported as a thiol low molecular weight responsible for transporting metal ions from the PC-metal complex to storage (MOHAMED et al., 2012). Often, plant species stored these toxic ions in parts that cause less damage to biochemical processes, such as in vacuoles (PINTO; AGUIAR; FERREIRA, 2014). Some *Brassica* species have a high capacity for storing these ions, reported as 'hyperaccumulators' (CHAUDHRY et al., 2020). Although some brassicas are reported as hyperaccumulators, high concentrations of toxic HMs impair plant metabolism (DU et al., 2020).

The literature shows that toxic HMs can affect the uptake of nutrients in *Brassica juncea* and *Brassica rapa* (turnip), mainly due to competition for common enzymatic binding sites, as reported by Feigl and collaborators (FEIGL et al., 2016). Furthermore, it is reported that toxic metal concentrations can damage the cell structure (AMARI; GHNAYA; ABDELLY, 2017), plant water status (SABIR et al., 2020), photosynthetic and leaf gas exchange (AYYAZ et al., 2020; MANCILLA-LEYTÓN et al., 2016). However, several investigative studies show that *Brassica* species have an interesting and efficient enzymatic and non-enzymatic antioxidant defense mechanism capable of attenuating the toxic HMs damage effects (DAWOOD; AZOOZ, 2019; RATHIKA et al., 2021).

The main enzymes of the enzymatic defense system include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), glutathione peroxidase (GPOX), and polyphenol oxidase (PPO), glutathione reductase (GR), glutathione s-transferase (GST), dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDAR) (SOARES et al., 2019). The enzymatic system acts as the first line of antioxidant defense, however, under prolonged stress conditions other non-enzymatic mechanisms may be used, such as the synthesis of ascorbic acid (AsA), reduced glutathione (GSH), α-tocopherol, carotenoids, polyamines, flavonoids, phenols and prolines (GILL; KHAN; TUTEJA, 2011; YAN et al., 2016). In addition, several studies aim to manage techniques that can improve the tolerance of toxic metals by *Brassica* (RIZWAN et al., 2016).

The most effective techniques for *Brassica* species phytoremediation are the use of plant growth regulators (PGRs), plant growth promoters (PGPs) chelating and acidifying agents, and plant breeding and genetics techniques (RIZWAN et al., 2016; SARWAR et al., 2017). It is important to notice that, after the accumulation of contaminants, the plant biomass obtained is properly discarded to avoid secondary contamination (RIZWAN et al., 2018). Although many researchers have analysed the removal of environmental pollutants using phytoremediation processes, the removal mechanisms and tolerance have not been comprehensively evaluated yet.

Thus, our paper presents a comprehensive and critical review of physiological strategies for phytoremediation and tolerance to the oxidative effects of toxic HMs in *Brassica* species. We emphasized the phytoremediation sequential processes in the light of recent experimental data and its application in the study of environmental pollutants.

2 A BRIEF CONTEXTUAL TOXIC HEAVY METAL POLLUTION AND PHYTOREMEDIATION

The intensification of anthropogenic activities related to mining, industrialization, incorrect handling of agricultural inputs, urbanization, and their transport processes, civil construction, and incorrect disposal of waste has contributed to the pollution of soils, rivers, and groundwater by heavy metals such as cadmium (Cd), chromium (Cr), mercury (Hg), arsenic (As), lead (Pb) and zinc (Zn) (SARWAR et al., 2017). In addition, other natural processes such as volcanic eruptions and the wear of a rock contribute to the increase in high concentrations of HMs in the agroecosystems (SHAH; DAVEREY, 2020).

Due to the non-biodegradable nature of these HMs, there are persistent in the soil for many years and, in the long term, contamination by HMs impaired the microbiota and soil fertility (SHAH; DAVEREY, 2020). HMs such as Cd, Pb, Cr, and As are highly toxic to animals and can have possible implications for human health, even in low concentration, as they are carcinogenic and harmful to the nervous and endocrine systems (RIZWAN et al., 2018). However, the literature reports some techniques for environmental decontamination of these potentially toxic metals, such as phytoremediation (CAMESELLE; GOUVEIA, 2019).

Some plants species are potentially tolerant to the HMs toxic effects, such as sunflower (*Helianthus annus* L.) (RIZWAN et al., 2016), sorghum [*Sorghum bicolor* (L.) Moench] (FENG et al., 2018), maize (*Zea mays* L.) (RIZWAN et al., 2017), and plants of the botanical family Brassicaceae, especially those belonging to the genus *Brassica*, which is an interesting model for phytoremediation (KAUR et al., 2017).

Phytoremediation is an environmental decontamination technique characterized by the use of plant species performed *in situ* to minimize the toxic effects of pollutants in water, soil, and air. This biotechnology emerges as an alternative mechanism for environmental decontamination, as it is different from physical-chemical remediation techniques, such as vitrification (high temperatures), acid washing, and removal of soil from the area, which have higher costs and affect the soil fertility and biodiversity (YAN et al., 2019).

However, the phytoremediation technique has some limitations, such as slow growth and low dry mass production, longer time required for the process to be effectively carried out, in addition to biochemical and morpho-physiological damage. Due to these conditions, there is a lack of studies on the phytoremediation applicability in crop areas.

3 STRATEGIES OF PHYTOREMEDIATION

The literature indicates that the phytoremediation pathways are carried out from different physiological mechanisms that help plants to remediate metals and/or mitigate toxic damage effects in the environment (SHAH; DAVEREY, 2020). The phytoremediation is carried out by different plants physiological pathways, such as phytovolatilization, phytostabilization, or phytoextraction (Fig. 1) (SARWAR et al., 2017).

3.1 STRATEGIES OF PHYTOREMEDIATION: PHYTOVOLATILIZATION

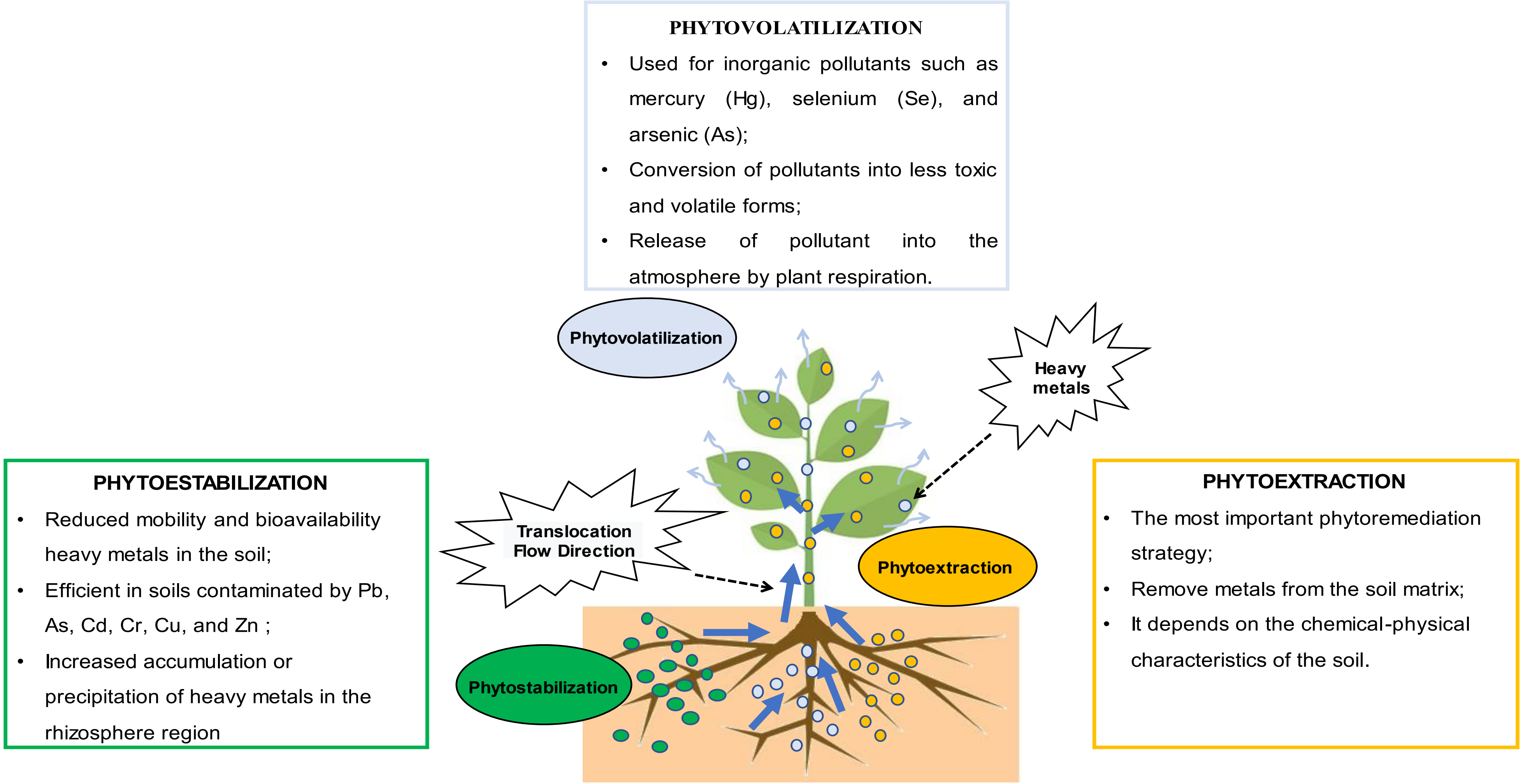
Phytovolatilization refers to the capacity of plants species to uptake and convert pollutants into less toxic and volatile forms, besides, from plant transpiration, release them into the atmosphere (KUMARI et al., 2020). This technique is used for inorganic pollutants such as mercury (Hg), selenium (Se), and arsenic (As) (KUMAR YADAV et al., 2018). According to Moreno and colleagues (MORENO et al. 2008), after supplying different Hg concentrations in hydroponic conditions, the *B. juncea* species accumulated this toxic HM in its root system and, later, released it around the root (external environment), in the vapor form (Hg^0). In this same plant species, the literature also indicates that there is an accumulation of Se in the root and its subsequent release in the rhizosphere (DI GREGORIO et al. 2006).

The oxidation-reduction processes, both Hg and Se, occur in the rhizosphere region, in which there is a formation of volatile and less toxic forms, such as the conversion of bivalent (Hg^{2+}) to Hg^0 which occurs from the commensalism relationship with several bacteria (live in both soil and water) resistant to these pollutants (MORENO et al., 2008). In aquatic environments, studies showed that some algae, depending or not on light, such as *Euglena* species, can help plant roots in the HM reduction processes and conversion in volatile to less toxic forms (DEVARS et al., 2000).

Although some authors indicate that the phytovolatilization strategy does not contribute to a significant increase in the concentration of pollutants in the atmosphere, the literature reported it as a "contradictory" technique, because it transfers the toxic HM from the substrate to the atmosphere, without control over its migration to other areas (CRISTALDI et al., 2017). After this volatilization process, the toxic HM released and dispersed in the atmosphere can precipitate and re-contamination the environment, being, therefore, a technique with 'temporary effects' (SARWAR et al., 2017).

3.2 STRATEGIES OF PHYTOREMEDIATION: PHYTOSTABILIZATION

Phytostabilization or phytoimmobilisation consists in the reduction of the mobility or/and bioavailability of HMs in the soil from sorption, precipitation, complexation, or alteration in the rhizosphere of the metal's valence (ALI; KHAN; SAJAD, 2013). This technique is efficient in soils contaminated by Pb, As, Cd, Cr, Cu, and Zn (KUMAR YADAV et al., 2018).



PHYTOVOLATILIZATION

- Used for inorganic pollutants such as mercury (Hg), selenium (Se), and arsenic (As);
- Conversion of pollutants into less toxic and volatile forms;
- Release of pollutant into the atmosphere by plant respiration.

PHYTOESTABILIZATION

- Reduced mobility and bioavailability heavy metals in the soil;
- Efficient in soils contaminated by Pb, As, Cd, Cr, Cu, and Zn ;
- Increased accumulation or precipitation of heavy metals in the rhizosphere region

PHYTOEXTRACTION

- The most important phytoremediation strategy;
- Remove metals from the soil matrix;
- It depends on the chemical-physical characteristics of the soil.

FIG.1. Schematic representation of some toxic heavy metal phytoremediation strategies used by *Brassica* species: phytovolatilization, phytoextraction, and phytostabilization.

According to Visconti and collaborators (VISCONTI et al. 2020) soils historically explored by mining activities, with high concentrations of As, Cd, Pb e Zn, the *B. juncea* species presents a high efficiency in the phytostabilization of metals As, Cd, and Pb. The same authors analysed the ‘Translocation Factor’ (TF), postulated as the relationship between the amount of toxic HMs ions moved from the soil to de shoots. Although all evaluated metals had TF < 1.0, *B. juncea* species was a greater accumulation of Zn in the shoots. These authors observed that TF of the Zn was higher than the values of As, Cd, and Pb. In this way, plants with lower TF values (FT < 1.0) have less ability to translocate HMs from roots to shoots and may be more efficient in the phytoimmobilisation on HM in the rhizosphere (FEIGL et al., 2016).

It is important to keep in mind that, unlike other phytoremediation techniques, phytostabilization is not intended to remediate soil contaminants, but to improve HMs accumulation or precipitation in the rhizosphere to reduce groundwater contamination (MAHAR et al., 2016).

3.3 STRATEGIES OF PHYTOREMEDIATION: PHYTOEXTRACTION

The literature indicates phytoextraction as the most important phytoremediation strategy, due to the capacity of plant species to uptake, transport, and accumulate toxic HMs in aerial structures, especially in harvesting organs (SHEORAN; SHEORAN; POONIA, 2016). The efficiency of phytoextraction depends on plant morphology and soil physiochemical aspects, such as pH, cation-exchange capacity (CEC), oxi-reduction potential, texture and, HM bioavailability (ALI; KHAN; SAJAD, 2013).

Plant species used for phytoextraction must have specific characteristics, such as high growth rate, biomass production, easy to control cultivation and harvesting process, capacity to hyperaccumulate toxic HMs, higher TF values, and tolerance to the toxic effects generated by the pollutant uptake (CRISTALDI et al., 2017; MAHAR et al., 2016). Several *Brassica* crops are presented with potentially phytoextractors HMs from the environment (Table 1).

TABLE 1. Species belonging to the botanical genus *Brassica*, potentially phytoextractors of toxic heavy metals.

Plant species	Heavy metal	Authors
B. napus	Zn	BELOUCHRANI et al. (2016)
	Cu	ZAHEER et al. (2015)
	Cd	ZHANG; XIAO; WU (2020); ZHANG et al. (2018)
B. juncea	Zn	CHAUDHRY et al. (2020); DU et al. (2020)
	Cu	CHIGBO; BATTY; BARTLETT (2013) FEIGL et al. (2015)
	Cd	BHUIYAN et al. (2011); GURAJALA et al. (2019); IRFAN; AHMAD; HAYAT (2014); NAVARRO-LEÓN et al. (2020).
	Ni	RODRÍGUEZ-VILA et al. (2014)
	U	CHEN et al. (2020).

4 IONIC UPTAKE OF HEAVY METALS

Some HMs can be essential to the plant growth and life cycle, mainly because they are related to metabolic functions. For example, the enzymatic activation by zinc (Zn) and manganese (Mn) ions and the presence of nickel (Ni) in the urease enzyme responsible for urea cell homeostasis (TAIZ et al., 2015). However, toxic amounts of essential metals and other non-essential metals, such as Cd, can be harmful to some morphophysiological and biochemical processes in plants (THAKUR et al., 2016). The uptake of these ions by plants occurs at least slightly soluble ions in the soil solution (MARSCHNER, 2011; SHAH; DAVEREY, 2020).

The bioavailability of toxic metals for plants is related to factors associated with the soil, such as texture (clay soils retain more toxic HMs ions when compared to sandy soils), soil moisture, redox potential (E*h*), cation exchange capacity (CEC) and the pH. Literature reports that pH is the most important factor for the bioavailability and absorption of these ions by the roots (SHAH; DAVEREY, 2020). Generally, with a decrease in pH, there is an increase in the bioavailability of HMs in the soil solution due to the desorption of these ions from the surfaces of the colloids (SHEORAN; SHEORAN; POONIA, 2016). Xue and collaborators (XUE et al. 2012) observed that the uptake of toxic HMs in *B. pekinensis* followed the order Cd>Zn>Ni>Cu>Pb, and in *B. oleracea* it was Zn>Ni>Cd>Cu>Pb. These authors concluded that, although the concentration of Pb supplied was 170 times greater than Cd, the absorption of metals by plants depends mainly on their bioavailability.

Initially, for ionic uptake by membrane protein carriers in the roots to occur, chemical elements must penetrate the soil-root interface via symplast (symplast connected between neighbouring cells) and/or via apoplastic (between the free spaces of the cell wall) (LIU et al., 2011). Besides the bioavailability in the soil solution, the literature reports that other factors also influence the uptake of HMs by plants, such as metal toxicity (NASER et al., 2018), and the use of other metabolic strategies, for example, the association with microorganisms.

4.1 ORGANIC ACIDS

Organic acids can be released by microorganisms associated with plant roots (e.g. bacteria) and play an important role in the sequestration, absorption, transport, and tolerance of HM. Examples of organic acids that help to increase the bioavailability of HM are gluconic acid, tartaric acid, oxalic acid, citric acid, humic acid, malic acid, and oxalic acid (SHAH; DAVEREY, 2020). In general, these acids can bind to HMs ions in the soil solution and, from complexation reactions, become more available for root uptake (WANG et al., 2017). Moreover, these compounds acidify the medium and solubilize non-labile fractions of HM, cooperating with greater availability of metal ions in the soil solution (SHAHID et al., 2017).

Afshan and colleagues (AFSHAN et al., 2015) observed in previous studies that the absorption and accumulation of Cr in the roots and shoots of *B. napus* (canola) plants increased proportionally to the different dosages of Cr supplied to the plants. According to these authors, the application of citric acid provided greater uptake of Cr compared to treatments control. Thus, the authors concluded that the increased absorption of Cr by *B. napus* is related to the induction of increased plant biomass, provided by the action of citric acid, which suggests greater accumulation and tolerance to Cr ions. Besides, the decrease in soil pH (increased bioavailability of ions in the soil solution) promotes an effect on the morphological characteristics of the root, such as volume, surface area, and average diameter.

4.2 BIOSURFACTANTS

Biosurfactants are secondary metabolites produced by microorganisms, mainly by bacteria such as *Pseudomonas aeruginosa*, *Bacillus subtilis* and, *Lactobacillus* spp. These organisms help the toxic HMs ions desorption present in the soil matrix, which improves solubilization, mobilization, and the bioavailability of these ions to plants (SHAH; DAVEREY, 2020). The main classes of biosurfactants include glycolipids, lipopeptides, phospholipids, fatty acids, lipoproteins, and particular polymeric compounds (ARAÚJO et al., 2019).

Previously, Ma and colleagues (MA et al., 2015) inoculated *Psychrobacter* sp. SRS8 in *B. juncea* (brown mustard) seedlings cultivated in soils contaminated with Zn, Ni and, Fe, and analysed, the variation of mobility of these metals in the soil and aerial parts. These authors concluded that, regardless of inoculation, there was a decrease in the concentration of the three pollutants in the soil, which is justified by the fact that *B. juncea* is a potentially plant phytoextractor.

However, in treatments with root inoculation, there was a greater reduction in the concentration of Ni and Zn compared to the non-inoculated control. These results suggest that the symbiosis between *B. juncea* and bacteria can occur the uptake of Ni and Zn more efficiently. These authors also concluded that the decrease in Fe absorption in plants inoculated with *Psychrobacter* sp. SRS8 compared to the uninoculated control, was possibly due to the high concentrations of bioavailable Ni and the competition between Ni and Fe for a specific binding site, which can cause a decrease in Fe uptake by plants.

Therefore, all these previously scientific reports allow us to speculate that different phytoremediation strategies are adopted by plants to uptake different types of HM ions. After the absorption of these pollutants, the ions can be translocated to different parts of the plant organ.

5 TOXIC METAL ION TRANSLOCATION

The comprehensive mechanism underlying the toxic HM translocation is of most interest for phytoremediation. This mechanism includes phytovolatilization and phytoextraction techniques, which require the transfer of HM ions from the roots to the shoots part of the plants (RUBIO et al., 2020). The relationship between the amount of toxic HMs moved from the roots to shoots is described by Chen and collaborators (CHEN et al., 2020) as 'Translocation Factor' (TF) (Formula 1).

Formula (1) $$TF = \frac{C_{metal\_shoot}}{C_{metal\_root}}$$

In recent studies, Du and colleagues (DU et al. 2020) provided Zn and Cd HMs to 21 different *B. juncea* cultivars and evaluated the TF of these metals in the plant, which observed a higher TF value for the Zn (TF > 1.0) when compared to Cd (TF = 0.83). These results suggest that, for the studied plant species, the translocation of Cd from roots to shoots is more restricted when compared to Zn and, therefore, *B. juncea* may present satisfactory efficiency in the phytoremediation of Zn compared to Cd. Besides, Chaudhry and colleagues (CHAUDHRY et al. 2020) also found FT values greater than 1.0 for different dosages of the Zn in *B. juncea* plants (160 and 40 mg of Zn/L), which indicates that the aerial parts of the plants accumulated more Zn concerning the roots. Complementing these studies, plants can synthesize metabolites that promote the translocation of these HM to the aerial parts, including chelators, metallothioneins, glutathione, and phytochelatins (PINTO; AGUIAR; FERREIRA, 2014).

5.1 CHELATING

The chelating activity consists of binding an ion that has at least two binding atoms to a molecule with different atoms available for this bond, such as sulfur (S), nitrogen (N), or oxygen (O) (OZYIGIT; CAN; DOGAN, 2020). The chelator molecule gives up its ion with less chemical activity, to form a complex, bonded to another HM ion, thus reducing its potential phytotoxicity. Plants with greater potential for phytoremediation can synthesize some important molecules and/or polypeptides, including metallothioneins, glutathiones, and phytochelatins that will help in the HMs absorption and transport (SHAH; DAVEREY, 2020).

5.1.1 METALLOTHIONEINS (MTs)

Metallothioneins (MTs) are polypeptides formed by high cysteine content, which originate binding with metal ions, such as Cu, Cd, and Zn, from the activation of the antioxidant system, regulation of metalloenzymes, and gene transcription. Plant *MT*s are natural defense and classified into four types, with distinct temporal and spatial expression patterns, in which the type 1 *MT* gene is more abundant in roots than in aerial parts; type 2 *MT* gene are more expressed in aerial tissues; type 3 *MT* gene are expressed at high levels in leaves or fruits at the ripening stage; and the type 4 *MT* gene is restricted to seminiferous development (YU; LIN; ZHANG, 2019).

The chinese cabbage (*Brassica campestris* L. var. *chinensis*) seedlings cultivated with different dosages of Cd and Cu show that the expression of the $BcMT_1$ gene was induced by treatments with both Cd and Cu, while the $BcMT_2$ gene was induced only by the supply of Cd (LV et al. 2013). These authors concluded that the MT1 and MT2 proteins are related to (no)-tolerance to the accumulation of Cd by plants, mainly by the chelation carried out in the cytoplasm, which prevents these HMs from interacting with the cytoplasmic components and/or other organelles.

On the other hand, MTs proteins are related to tolerance and accumulation of Cu in the shoots. Ions Cu is related to catalytic and structural roles in plant growth and development; therefore, greater uptake and accumulation are necessary to maintain the balance between the expression of the MT protein and the availability of the metal to the plant. This relationship is possibly due to the overexpression of these proteins and, consequently, an increase in complexed Cu, which reduces the activity of these free ions in the cytoplasm (LV et al., 2013).

In *B. napus* seedlings exposed to As, Pan and colleagues (PAN et al. 2018) found similar results in the expression of *MT* family genes under As^{3+} availability, in which the *BnaMT3C* gene helped to induce better responses to the generated stress by HM. Therefore, the current approach allow us to speculate that the stimulation of the expression of MT family genes is related to the type of metal ion uptake by the roots and their ability to As tolerate and/or accumulate, to achieve the 'balance' between protein expression and the bioavailability of the metal.

5.1.2 GLUTATHIONE AND PHYTOCHELATINS

Glutathione is a molecule containing tripeptide thiols, whose formation is catalyzed by the enzymes γ-glutamylcysteine synthase and glutathione synthase, which plays an important role in redox homeostasis, through its action against oxidative damage generated by heavy metals (BUCHANAN; GRUISSEM; JONES, 2015). In general, glutathione is a precursor of phytochelatins and plays an important role together chelating agents in the detoxification of free radicals within cells, generated by the presence of toxic HMs(OZYIGIT; CAN; DOGAN, 2020).

In the report by Zlobin and colleagues (ZLOBIN; KARTASHOV; SHPAKOVSKI 2017) the authors revealed that Cu ions reacted quickly with glutathione in *B. napus*, in this same study, the authors observed that glutathione played little role in chelation Zn in canola roots, probably because this ion has other more efficient chelating molecules, for example, the phytochelatins (HAYDON et al., 2012). Phytochelatins (PC) composed of the amino acids glutamate, cysteine, and glycine, synthesized by the phytochelatin synthase-enzyme, with its gene expression (*PCS* genes) expressed constitutively, in which there is greater induction of this expression in the presence of HMs, such as As, Cd, Cu, Hg, Pb and Zn (FILIZ et al., 2019). The literature reports that PCs reduce the concentrations of free toxic HMs ions in the cytosol, from the transport of the PC-metal complex to the vacuoles (MOHAMED et al., 2012). Furthermore, PCs have a high capacity to stimulate the activity of antioxidant enzymes (COBBETT, 2000).

According to Sun and collaborators (SUN et al. 2020), these authors observed that the Cd-induced the production of PC in malaysian cabbage (*Brassica parachinensis* var. *Lvbao-701*) seedlings mainly in the root region. Recently, another study (AGNIHOTRI; SETH, 2020) reported that the supply of Pb in *B. juncea* seedlings cultivation increased the content of PCs in the roots and leaves, proportionally to the increase Pb supply, which allowed the authors to claim that exposure to HMs stimulates the synthesis of PCs. After chelation, HMs can be compartmentalized in inactive parts of the root cells or exported to the shoot, via xylem vessels (PINTO; AGUIAR; FERREIRA, 2014).

6 COMPARTMENTALIZATION OF HEAVY METALS

Generally, toxic HMs ions are stored in parts/organelles that cause less damage to essential cell processes, for example, in plant tissues like epidermis, trichomes, cuticles and, vacuole. The vacuoles represent about 60 to 95% of the volume of epidermal and parenchymal cells and are pointed out as the main storage place for HM (TAIZ et al., 2015). The storage of toxic HM ions in the vacuole of roots or shoots can be considered an interesting strategy induced by plants since this organelle has limited metabolic activity (SHAH; DAVEREY, 2020).

The literature reports that metallic ions are sequestered by the vacuole after chelation, mediated by *ABC* type (*ATP-Binding Cassette*) transporter proteins, located in the vacuole membranes. The *ABC* transporters belong to a very numerous family of carrier proteins, whose members are responsible for sequestering several substrates in vacuoles, using the energy of ATP hydrolysis, therefore, these transporters are not dependent on the primary electrochemical gradient (HUANG et al., 2021).

The studies by Huang and colleagues (HUANG et al., 2021) showed that the presence of Cd ions significantly induced the expression of *BcABCC1* and *BcABCC2* in *B. campestris* var. *Chinensis* (field mustard). Consequently, increase the sequestration of the PC-Cd complex in vacuoles, which suggests the presence of HM as a stimulus for phytoremediation strategies to toxic effects of HM mitigation. In summary, some plant species express characteristics that give them greater absorption, accumulation, and defense capabilities against toxic HM ions in high concentrations. These plant species are described as 'hyperaccumulators' (CHAUDHRY et al., 2020).

7 PLANT HYPERACCUMULATION

The ability of a plant phytoremediator to hyperaccumulated one or more toxic HM ions differs between species and, even, populations and ecotypes of the same species. In this way, characteristic depends on three factors: (i) high capacity to toxic HM uptake in concentrations 100 to 1000 times greater than those found in non-hyperaccumulating species; (ii) toxic HM ions translocate from the roots to the shoots, and compartmentalization, especially in the leaves and other parts destined for harvesting; and (iii) greater capacity to defend the phytotoxicity of these ions in their metabolism (Figure 2) (RASCIO; NAVARI-IZZO, 2011). These three factors related to the capacity of a plant phytoremediator to hyperaccumulating toxic HMs can be expressed from the

'Bioaccumulation Factor' (BAF) and the 'Tolerance Index' (TI) (CHAUDHRY et al., 2020).

BAF is defined as the amount of HM accumulated in the root and aerial parts of plants about the concentration of this metal available in the soil (Formula 2) (CHAUDHRY et al., 2020). This factor establishes, therefore, the efficiency of a plant in accumulating HMs and, therefore, BAF values greater than 1.0 (BAF > 1.0) are indicative that the plant is a hyperaccumulating species (AUDET; CHAREST, 2007).

Formula (2) $$BAF = \frac{C_{metal\_shoot\ or\ root}}{C_{metal\_soil}}$$

TI is reported as the ability of plants to grow, develop and, tolerate high concentrations of HMs when exposed for long periods in contaminated soil (CHAUDHRY et al., 2020). TI is established by the relationship between plant length and roots and shoots biomass exposed and not exposed (control) to HMs (AUDET; CHAREST, 2007). In general, TI values lower than 1.0 (TI < 1.0) indicate a reduction in biomass production by the plant and suggest that the plants are under stress conditions. On the other hand, TI values greater than 1.0 (TI > 1.0) indicate an increase in biomass production and can hyperaccumulate HMs without impairment plant development (BELOUCHRANI et al., 2016). The literature points out that several plant species have these characteristics and, therefore, are potentially hyperaccumulators phytoremediators.

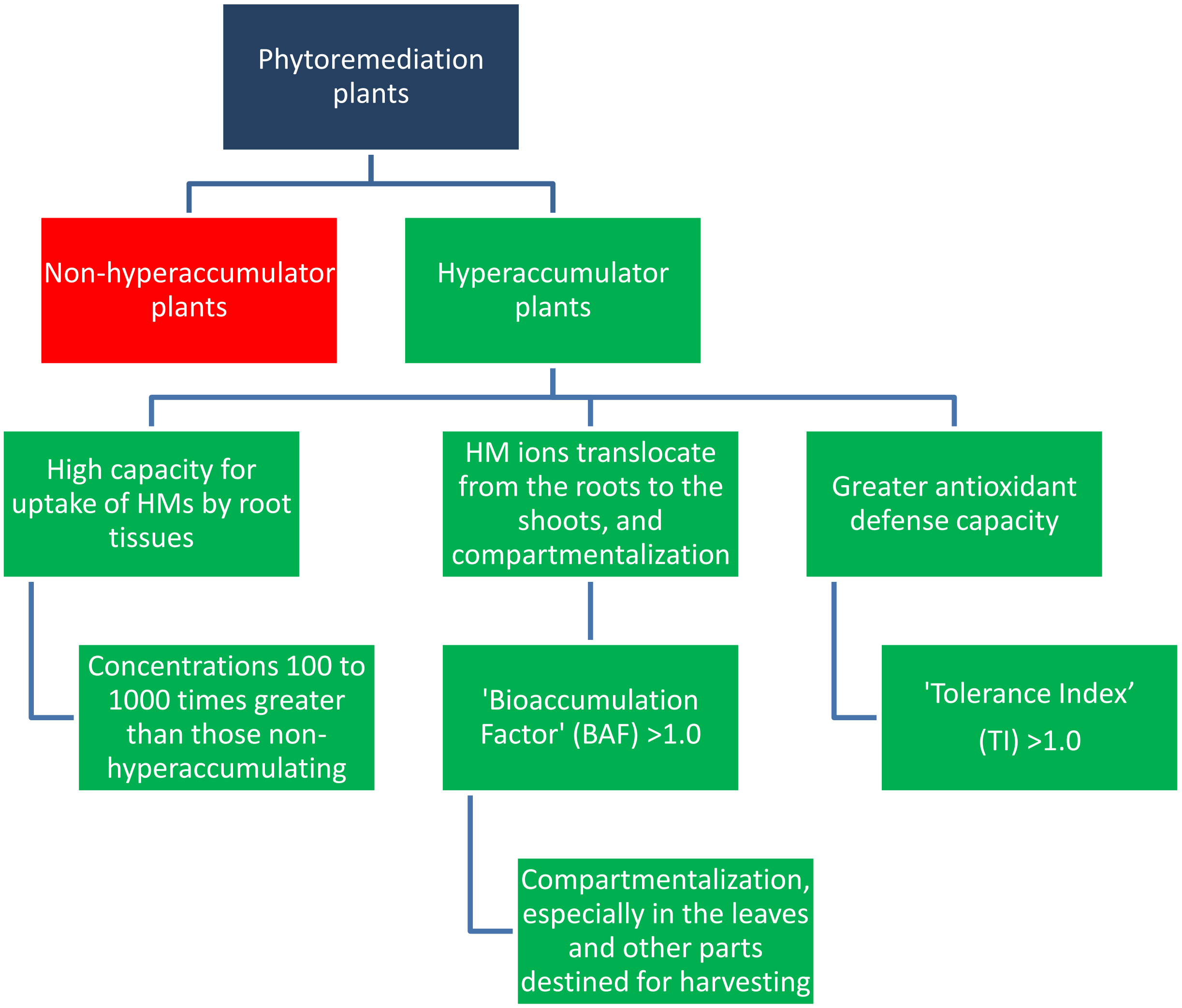


FIG 2. Logical flowchart of characteristics of hyperaccumulating plants of toxic heavy metals.

The plant species candidate capable of hyperaccumulate toxic HMs ions are those belonging to the genera *Alyssum*, *Thlaspi*, and *Berkheya*, however, the biggest disadvantage of using these genera for phytoremediation is the slow growth rate and low biomass production, conditions that should be reversed in potentially phytoremediation species. On the other hand, the use of species belonging to the genus *Brassica* is presented as a promising strategy, not only for phytoremediation but also for the hyperaccumulation of toxic metals, mainly because they present faster growth, greater biomass production, and tolerate the toxic effects of heavy metals (THAKUR et al., 2016).

Belouchrani and collaborators (BELOUCHRANI et al., 2016) performed different Zn dosages to *B. napus* seedlings and observed that up to the 8th week, after the setup of the experimental design, there was a fluctuation in TI values, which indicates that the plants suffered some type of stress, however, between the 8th and the 12th week, the TI values for all treatments were greater than 1.0. In this way, these results helped the authors to confirm the hypothesis that *B. napus* are a hyperaccumulators plant Zn. Besides, Dhiman and collaborators (DHIMAN et al., 2016) also corroborate the hyperaccumulation potential of Zn for this same species, that the *B. napus* accumulated approximately 95% of the heavy metal Zn available in the soil.

8 EFFECTS OF TOXIC HEAVY METALS ON PRIMARY METABOLISM IN *BRASSICA* SPECIES

Brassica species can accumulate concentrations of HMs in non-metabolic parts of plants and, consequently, alleviate or prevent toxic effects on plant growth and metabolism. However, high HMs concentrations may inhibit plant growth, mainly by affecting nutrient uptake, limiting cell development, and reducing photosynthetic assimilation (DU et al., 2020; REDONDO-GÓMEZ; MATEOS-NARANJO; ANDRADES-MORENO, 2010).

8.1 EFFECT OF TOXIC HEAVY METALS ON PLANT IONIC UPTAKE

The presence of toxic HMs can affect plant nutrient uptake. The literature reports that one of the possible mechanisms responsible for dysregulation of uptake is related to competition for common enzymatic binding sites between the HMs and mineral nutrients ionic rays, which suggests an antagonistic relationship between the ions due to competition for *B. juncea* and *B. rapa* plasmalemma protein carriers (FEIGL et al., 2016).

Different dosages of toxic HM, Cd, and Cu were supplied to *B. napus* seedlings decreased potassium (K), Fe, Zn and, Mn contents, which elucidated a clear interference hypothesis (dose-response) (MWAMBA et al., 2016). According to Jiang and collaborators (JIANG et al., 2004), the concentrations of K and P ions in the roots were significantly higher in *B. juncea* subjected to higher levels of Cd (170 mg.kg^{-1}), possibly because high doses of Cd alter the permeability of the plasma membrane and facilitate the nutrients uptake. On the other hand, Amari and collaborators (AMARI et al., 2014) observed a decline in K ion absorption in this same species (*B. juncea*) when subjected to Ni stress, possibly because this metal can generate metabolic disturbances and reduce the ATPase activity and, consequently, decrease the energy production necessary for the absorption of this nutrient. In addition to nutritional problems, ionic dysregulation in plants causes structural changes in cells.

8.2 EFFECTS OF TOXIC HEAVY METALS ON PLANT CELL STRUCTURE

Toxic HMs ions binding to cell wall components induce changes in their properties, especially to plasticity that interferes with other mechanisms such as cell division and elongation (AMARI; GHNAYA; ABDELLY, 2017). The literature reports that the plasma membrane, as the first cell component to come into contact with HM ions, has its functionality severely affected concerning its lipid composition (GILL et al., 2015). It is also reported that HMs induce the lipid peroxidation of layers present in cells, which considerably alters the structure of membranes, enzymatic, and transport activities (YAN; KE; TAM, 2010).

Ali and colleagues (ALI et al., 2013) reported that the Cd-induced chloroplast deformation and the dispersion and elongation of thylakoid membranes. Besides increasing the number of lipid bodies in the cytoplasm membrane lipid peroxidation. In *B. napus* seedlings changes ultrastructural were observed in different parts of the cells as a dose-response to Cr, such as mesophyll and root cells rupture and extravasation of cellular content, in addition to the disappearance of different organelles (GILL et al., 2015). As reported, the effects on cell structure affect several plant water status, due to changes in the structure of the mesophyll and stomata complex.

8.3 EFFECT OF TOXIC HEAVY METALS ON PLANT WATER STATUS

The water status of plants depends on the balance between water transpiration and the presence of certain toxic HMs ions concentrations affects the plants hydration by impairing water uptake and changing stomatal conductance (AMARI et al., 2014). The plant growth rate decrease generated by the presence of toxic HMs results in smaller leaves and, consequently, a smaller area of its main transpiring organ (SABIR et al., 2020). These authors report that *B. napus* plants exposed to Cd presented decreased transpiration rate and lower biomass production in the shoots. Furthermore, the presence of HM ions in cells can affect osmotic balance.

Mahamud and collaborators (MAHMUD et al., 2018), reported that Cd stress resulted in a water content reduction, as indicated by relative leaf water content (RWC) of *B. juncea*. Similar results were found in *B. juncea* exposed to Cr (MAHMUD et al., 2017). In previously published paper, Amari and collaborators (AMARI et al., 2014) observed that, under high concentrations of the Ni, *B. juncea* plants showed a drastic reduction in leaf water content. Possibly, toxic HMs ions altered root system cellular osmoregulation and damaged, and, consequently, tissues content water reduced in (AMARI; GHNAYA; ABDELLY, 2017).

8.4 EFFECT OF TOXIC HEAVY METALS ON PLANT LEAF GAS EXCHANGES AND PHOTOSYNTHETIC METABOLISM

The total chlorophyll content and stomatal conductance reduction, caused by the presence of toxic HM ion concentrations, directly affect CO_2 assimilation by plants (AMARI et al., 2014). The literature reports that toxic HMs ions induce changes in the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), responsible for carbon-fixation during light-dependent reactions (first stage of photosynthesis) (MANCILLA-LEYTÓN et al., 2016). Previously papers showed that *B. juncea* seedlings exposed to Ni showed a significant decline in A_{net}, g_{s}, E, and C_{i} (AMARI et al., 2014). Furthermore, similar data (reduction in g_{s} in A_{net}) were found in studies by Sabir et al. (SABIR et al., 2020) by submitting *B. napus* plants to the stress generated by Cd.

Toxic HMs ions can significantly imply structural and ultrastructural modifications of the photosynthetic machinery (AYYAZ et al., 2020). These HM can, for example, replace the magnesium (Mg^{2+}) present in chlorophyll molecules and become unsuitable for photosynthesis, due to the unstable excitation state that interferes with resonance and energy transfer from the pigment-antennas to the reaction centers, which will generate electron transport chain rupture and impaired the photosynthetic process (SOURI et al., 2019).

The presence of toxic HMs ions can also inhibit chlorophyll biosynthesis, by limiting the synthesis of its precursor, the amino acid δ-aminolevulinate (ALA), and interfering with the content of photosynthetic pigments (PARMAR; KUMARI; SHARMA, 2013). According to Chen and collaborators (CHEN et al., 2020) and Taamalli and collaborators (TAAMALLI et al., 2014), there is a decrease in the total chlorophyll content subjected to stress by Cd and U investigated in *B. juncea* seedlings.

Although the literature reports that the Cd affects photosynthetic pigments, Navarro-León and collaborators (NAVARRO-LEÓN et al., 2019) observed that *B. rapa* plants submitted to the TILLING mutagenesis technique (*Targeting Induced Local Lesions in Genomes*), with the *HMA_4* gene (Cd and Zn transporters) showed no differences between chlorophyll *a* and chlorophyll *b* concentration compared to control treatment. HMs concentrations in chloroplasts can also damage Photosystem II (PSII) and, consequently, chlorophyll fluorescence (SHARMILA et al., 2017).

8.4.1 EFFECTS OF TOXIC HEAVY METAL ON CHLOROPHYLL FLUORESCENCE

The chlorophyll fluorescence assessment provides important data on the functioning of the photosynthetic apparatus, so that, under conditions of stress by disturbance and/or blocking of photosynthetic electron transport, which will lead to a fluorescence increase (NAVARRO-LEÓN et al., 2019). The literature reports the routine use of fluorescence quantification methodologies that assess initial fluorescence (Fo), maximum fluorescence (Fm), variable fluorescence (Fv = Fm-Fo), and maximum quantum yield for primary photochemistry (ΦPo = Fv/ Fm) that indicate the functioning of the PSII *in vivo* (CHEN et al., 2021).

In the study of Ayyaz and colleagues (AYYAZ et al., 2020), *B. napus* plants were treated with different Cr dosages. From the analysis of chlorophyll fluorescence, these authors observed that the Cr presence reduced the initial fluorescence (Fo) and the maximum fluorescence (Fm), in addition to the reduction in electron transfer to the PSII receptor site, which induced changes in the reduction rate of Quinone A (QA) and, consequently, reduced the electron flow to Photosystem I (PSI). Zhang and Liu (ZHANG; LIU, 2018) observed in *B. juncea* seedlings that excess toxic metal cesium (Cs) significantly decreased chlorophyll fluorescence parameters, indicating that this metal damages PSII leaves and attenuates the energy conversion rate. According to these authors, Cs blocks the electron transport from QA to quinone B (QB) and decrease the release of oxygen in the PSII reaction center.

8.5 EFFECTS OF TOXIC HEAVY METALS ON PLANT GROWTH

Plants exposure to toxic HM techniques causes a decrease in plant growth, mainly due to damage caused to the photosynthetic apparatus (AYYAZ et al., 2020; RIZWAN et al., 2018). Du and colleagues (DU et al., 2020) evaluated the growth response of different *B. juncea* cultivars seedlings exposed to different Zn and Cd and observed that toxic HM concentration affected the plant growth, biomass production, and reduction in the length of the shoot at the root. Recent studies indicate that *B. napus* plants are exposed to stress due to reduced production of plant biomass, but no number of leaves (AYYAZ et al., 2020). These authors concluded that the lower biomass production is related to damage to the photosynthetic apparatus.

However, Belouchrani and collaborators (BELOUCHRANI et al., 2016) observed that *B. napus* plants, subjected to Zn, increased stem height and root length, proportionally to the increase in Zn dosage. Biomass productivity also progressed according to the metal availability, achieving a minimum value in the highest dosage of Zn treatment, and maximum in the control treatment. The promotion of the plant growth exposed to sub-lethal doses of toxic metals leads us to suppose that there is a 'hormesis' effect in these experimental conditions, that is, some ‘toxic metals’ concentration, such as Zn, can stimulate plant development. In addition, plants possess an antioxidant defense mechanism based on the activity of enzymes and other non-enzymatic compounds that help to attenuate the stress generated by toxic metals in plant tissues (SOARES et al., 2019).

9 PLANT ANTIOXIDANT DEFENSE MECHANISMS

Reactive oxygen species (ROS) are natural products generated by various processes aerobic plant biochemicals, such as singlet oxygen (production of $^1O_2$ by PSII O_2) superoxide anion ($O2^{\bullet-}$ by PSI and PSII), hydrogen peroxide (H_2O_2), hydroxyl (HO^-) and peroxyl (HO_2^-), and $O_2^{\bullet-}$ by mitochondria. In addition, abiotic stresses such as high concentrations of toxic metals stimulate ROS overproduction in plant organisms that can alter the redox balance and cell death (RIZWAN et al., 2018). However, plants shows developed strategies for ROS detoxification from an efficient enzymatic and non-enzymatic antioxidant defense system (BUCHANAN; GRUISSEM; JONES, 2015).

9.1 ENZYMATIC ROS SCAVENGING SYSTEM

Enzymatic ROS scavenging systems include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD), glutathione peroxidase (GPOX), and polyphenol oxidase (PPO). SOD is the first enzyme activated by the plant antioxidant defense mechanism and acts in the detoxification of ROS from the dismutation of $O_2^{\bullet-}$ to H_2O_2 and CAT, APX and POD, subsequently converting H_2O_2

into H_2O and O_2 (SOARES et al., 2019). According to the literature, other enzymes also help to attenuate the ROS stress but do not directly participate, such as glutathione reductase (GR), glutathione s-transferase (GST), dehydroascorbate reductase (DHAR), and monodehydroascorbate reductase (MDAR), which are antioxidant enzymes of the ascorbate-glutathione cycle (RIZWAN et al., 2018).

Rathika and collaborators (RATHIKA et al., 2021) show that *B. juncea* seedlings exposed to Pb show an increase in the ROS concentration and activity of the antioxidant enzymes SOD, POD, CAT as a defense mechanism against oxidative stresses. In *B. napus* exposed to Pb, Bilal-Shakoor and collaborators (BILAL-SHAKOOR et al., 2014) observed an increase in the concentration of H_2O_2 and, in parallel, an increase in the SOD, POD, CAT, and APX enzymes activity, in leaves and roots, however, higher levels of Pb ions decrease the enzymatic activity. Farid and collaborators (FARID et al., 2015) also reported an increase in the production of ROS in *B. napus* seedlings exposed to different Cd concentrations with increase SOD, CAT, APX, and POD activity in root and aerial tissues at less toxic concentrations of Cd. On the other hand, higher levels of this toxic metal decrease enzymes activity.

Yan and colleagues (YAN et al., 2016) observed that *B. napus* exposed to toxic concentrations of the Cd increased the production of $O_2^{\bullet-}$ in their leaves and, as a defense mechanism, overproduction of the SOD, CAT and POD enzymes at the stress onset. In *B. juncea* seedlings exposed to Cd stress Kaur and collaborators (KAUR et al., 2017) observed that the toxic metal altered the antioxidant defense mechanism and inhibited the activities of the enzymes SOD, POD, GST, and PPO, while increasing CAT, APX, GPOX, DHAR and GR levels. According to these authors, occurs the inhibition of the synthesis of the first group of enzymes generated by Cd toxic concentrations. ROS, however, under conditions of prolonged stress may be insufficient and thus other non-enzymatic defense mechanisms are used by plants.

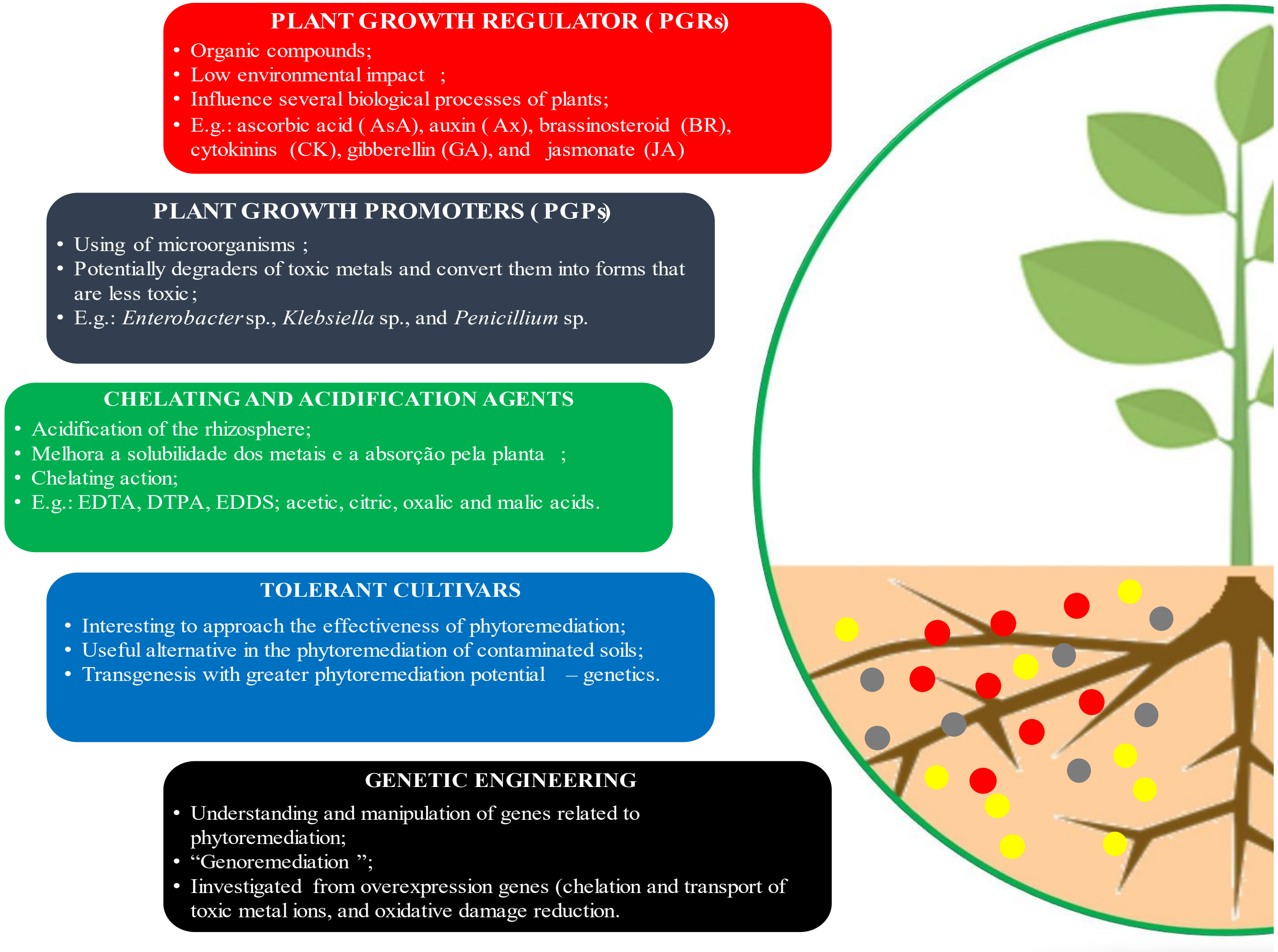
9.2 NON-ENZYMATIC ROS SCAVENGING SYSTEM

The main plants non-enzymatic antioxidant compounds include ascorbic acid (AsA), reduced glutathione (GSH), α-tocopherol, carotenoids, polyamines, flavonoids, phenols, and prolines. The literature reports that non-enzymatic systems operate ROS scavenging antioxidant defense in long term (YAN et al., 2016). Sharmila and collaborators observed that *B. juncea* seedlings exposed to Cd showed a three to six-time increase in the proline level, an osmoprotectant, and antioxidant, which helps to protect various cellular components (eg.: nucleic acids) (SHARMILA et al., 2017). Similar results were found by Ahmad and colleagues (AHMAD et al., 2021) also observed a proline content increase, especially between 30 and 90 days after the application of the Cd metal treatment.

Dawood and Azooz reported that broccoli (*Brassica oleracea* L. var. *italica*) seedlings exposed to tungsten metal (W) different dosages showed ROS overproduction (DAWOOD; AZOOZ, 2019). These same authors identified an increase in the production of AsA, α-tocopherol, phenols, and flavonoids up to a dosage of 50 mg of W kg^{-1} of soil. Other authors, such as Kaur et al. (KAUR et al., 2018) and Ahmad et al. (AHMAD et al., 2016), observed that *B. juncea* seedlings exposed to Cd showed ROS overproduction and increased total carbohydrates production, anthocyanins, flavonoids, and polyphenols as a defense mechanism. Therefore, the tolerance of *Brassica* plants to the toxic effects of some metals is related to its ability to induce antioxidant defense responses through enzymatic and non-enzymatic mechanisms.

10 IMPROVING PHYTOREMEDIATION OF TOXIC HEAVY METALS

In general, the literature reports that some techniques improve responses to the oxidative effects generated by toxic metals (RIZWAN et al., 2016). Although attractive to solve problems of contamination by toxic metals in the soil, the bioremediation technique approach has limitations related to slow plant growth, low plant biomass production, reduced species adaptation, and/or intolerance to certain conditions environmental factors to HMs toxic concentrations (SARWAR et al., 2017). In general, to minimize these limitations and ensure phytoremediation large-scale applications (*in situ*), the literature reports plant growth regulators (PGRs), plant growth promoters (PGPs), chelating and acidifying agents, and techniques for selection of tolerant cultivars and genetic engineering (Fig. 3) (RIZWAN et al., 2016).



PLANT GROWTH REGULATOR (PGRs)

- Organic compounds;
- Low environmental impact ;
- Influence several biological processes of plants;
- E.g.: ascorbic acid (AsA), auxin (Ax), brassinosteroid (BR), cytokinins (CK), gibberellin (GA), and jasmonate (JA)

PLANT GROWTH PROMOTERS (PGPs)

- Using of microorganisms ;
- Potentially degraders of toxic metals and convert them into forms that are less toxic;
- E.g.: *Enterobacter* sp., *Klebsiella* sp., and *Penicillium* sp.

CHELATING AND ACIDIFICATION AGENTS

- Acidification of the rhizosphere;
- Melhora a solubilidade dos metais e a absorção pela planta ;
- Chelating action;
- E.g.: EDTA, DTPA, EDDS; acetic, citric, oxalic and malic acids.

TOLERANT CULTIVARS

- Interesting to approach the effectiveness of phytoremediation;
- Useful alternative in the phytoremediation of contaminated soils;
- Transgenesis with greater phytoremediation potential – genetics.

GENETIC ENGINEERING

- Understanding and manipulation of genes related to phytoremediation;
- "Genoremediation ";
- Iinvestigated from overexpression genes (chelation and transport of toxic metal ions, and oxidative damage reduction.

FIG.3. Some techniques improve responses to the oxidative effects generated by toxic metals in *Brassica* species.

10.1 PHYTORREMEDIATION ASSISTED BY PLANT GROWTH REGULATORS (PGRs)

Phytoregulators (PGRs) are organic compounds with low environmental impact applied exogenously to the plant which, in low concentrations, influence several biological processes of plants (ALI et al., 2013). According to the literature, PGRs such as AsA, auxin (AX), brassinosteroid (BR), cytokinins (CK), gibberellin (GA), and jasmonate (JA) are widely used in scientific research related to pollutants phytoremediation (CHEN et al., 2020). PGRs play significant roles in signalling biochemical pathways and plant defense against stress generated by toxic metals, by regulating membrane permeability, stimulating enzymatic activity, secondary metabolites, and plant growth (ROSTAMI; AZHDARPOOR, 2019; SYTAR et al., 2019).

AsA, AX, CK, and GA metabolites are related to ascorbate-glutathione cycle regulation, transpiration rate, cell division, plant growth, and N assimilation metabolism, while BRs, ET, and JA stimulate the antioxidant system, attenuate ROS levels, lipid peroxidation, and improve photosynthesis in plants exposed to HMs stress (SYTAR et al., 2019). There is evidence that the PGRs exogenous application during the initial stage of plant development can help plants to enhance the antioxidant defense mechanism and assist in the accumulation of toxic metals in plant tissues (NEDJIMI, 2021).

B. juncea seedlings treated with AX and BR, when subjected to Cd and U stress, showed greater uptake and translocation of these toxic metals (CHEN et al., 2020). The BR exogenous application stimulated higher production of CAT and POD and increased the Pb content uptake by *B. juncea* seedlings without impaired their growth (SOARES et al., 2020). Per and collaborators (PER et al., 2016) showed that the application of JA to *B. juncea* plants under Cd stress resulted in higher GSH production and protected the chloroplast structure against the toxic HMs oxidative action. The application of 24-epibrassinolide (BR) together with JA in *B. juncea* seedlings increase the length of the stem and roots photosynthetic pigments content, GSH, AsA, α-tocopherol, CAT, POD, GR, DHAR, and GST even when exposed to Pb toxic concentrations (KOHLI et al., 2018). In addition to phytoregulators, plant growth promoters (PGPs) can also stimulate plant metabolism against toxic effects.

10.2 PHYTOREMEDIATION ASSISTED BY PLANT GROWTH PROMOTERS (PGPs)

Phytoremediation assisted by plant growth promoters (PGPs) consists of using microorganisms capable of colonizing the rhizosphere and stimulating plant growth and nutrition. In addition, PGPs are potentially degraders of toxic metals and convert them into forms that are less toxic (NEDJIMI, 2021). Recent studies report that PGPs are a biotechnology tool for phytoremediation, as these soil microorganisms secreted chelators and organic acids that, by decreasing the pH of the rhizosphere, increase the bioavailability of toxic metal ions for plants (JING et al., 2014).

Ma and colleagues (MA et al., 2015) showed that the inoculation of *Pseudomonas libanensis* TR1 and *Pseudomonas reactans* Ph3R3 in *Brassica oxyrrhina* treated with toxic metals significantly helped in plant growth and the photosynthetic concentration pigments. The inoculation of *Serraria* PRE01 and *Arthrobacter* PRE05 promote vanadium (V) phytoremediation by *B. juncea* (WANG et al., 2020). The association of arbuscular mycorrhizal fungi (AMF) helped *Brassica indica* to reduce lipid peroxidation and H_2O_2 production, in addition to stimulating the activity of antioxidant enzymes (HASHEM et al., 2019).

B. napus seedlings exposed to metals Cd, Pb, and Zn were inoculated with *Enterobacter* sp. and *Klebsiella* spp. and showed greater plant growth, increased IAA production, and greater toxic metals uptake (JING et al., 2014). The use of *Fusarium* sp. CBRF44 and *Penicillium* spp. CBRF65 increased biomass production in *B. napus* and improved Pb and Cd uptake in contaminated soils (SHI et al., 2017). The findings of Wang and colleagues (WANG et al., 2019) show that inoculations of *Burkholdria* SaMR10 and *Sphingomonas* SaMR12 in *B. juncea* plants improved root morphology (volume, length, surface area) and Cd uptake. The literature reports the use of chelating and acidifying agents is another tool presented that improved toxic metals uptake and translocation by roots to the leaf.

10.3 PHYTOREMEDIATION ASSISTED BY CHELATING AND ACIDIFICATION AGENTS

The uptake of toxic metals by plants is directly related to bioavailability and root access rate to these metal ions. The use of chelating and acidifying agents such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), ethylenediamine succinic acid (EDDS), acetic acid, citric acid (CA), oxalic acid (OA), malate, and tartaric acid affects metals solubility and plant uptake, mainly by acidification of the rhizosphere (RATHORE et al., 2019). In addition, the chelating action of these compounds helps in the translocation of these toxic metals from the roots to the plant aerial part (ROSTAMI; AZHDARPOOR, 2019).

The literature reports that CA exogenous application plays an important role in the toxic metals phytoremediation by *Brassica* species, by aiding phytoextraction and increasing stress tolerance through the antioxidant defense system upregulation (MAHMUD et al., 2018). The CA provided greater absorption of Cr in *B. napus* and attenuated the oxidative effects on plant metabolism (AFSHAN et al., 2015). Zaheer and colleagues (ZAHEER et al., 2015) observed that CA provided greater absorption and accumulation of Cu in the tissues of *B. napus* plants and alleviated the toxic effects of this metal. Besides, Mahmud and collaborators (MAHMUD et al., 2018) reported that the application of CA on *B. juncea* exposed to Cd, resulted in higher biomass production and less oxidative damage, mainly by stimulating the antioxidant enzymes activities (APX, MDHAR, GR, and CAT).

The application of organic acids in soils contaminated by Cd improved the Cd uptake by *B. napus* plants, mainly by increasing the mobility of these metallic ions in the rhizosphere (QIAO; LU; ZHANG, 2020). Mahmud and collaborators (MAHMUD et al., 2017) observed in their studies that the malic acid exogenous application in *B. juncea* seedlings, exposed to the metal Cr, stimulated the activity of enzymatic antioxidants (APX, MDHAR, DHAR, GR, and CAT) and non-enzymatic (AsA and GSH) which provided an increase in the photosynthetic pigments content and plant biomass.

According to Guo and colleagues (GUO et al., 2019), the application of EDTA chelate alone and combined with CA and OA, in soils contaminated with Cd and Zn, show greater bioavailability of toxic metals in the rhizosphere due to the complexation of these ions and, consequently, higher accumulation of these contaminants in the root and aerial parts of *B. juncea*. Besides, EDTA application increased the growth of *B. napus* plants exposed to Cd, in addition to improving the net photosynthetic rate, leaf gas exchange, and antioxidant enzymes activity (FARID et al., 2015).

10.4 PHYTOREMEDIATION ASSISTED BY SELECTION OF TOLERANT CULTIVARS

Several hyperaccumulation pathways are available between plant species of the same genus and differ, depending on the toxic metals and concentrations to which plant species are exposed (RASCIO; NAVARI-IZZO, 2011). The literature reports that the selection of *Brassica* cultivars tolerant to toxic metals is interesting to the phytoremediation effectiveness (RIZWAN et al., 2018). Gill and colleagues (GILL et al., 2015) evaluated the tolerance of four different cultivars of *B. napus* to the metal Cr (ZS758, Zheda 619, ZY50, and Zheda 622) and observed that the cultivar ZS758 was more tolerant to metal toxic effects, as it showed greater effectiveness in enzymatic and non-enzymatic antioxidant defense compared to the other cultivars.

Gill, Khan, and Tuteja (GILL; KHAN; TUTEJA, 2011) evaluated the response of five *B. juncea* cultivars (Alankar, Varuna, Pusa Bold, Sakha, and RH30) to the stress generated by Cd and observed less reduction in biomass production, net assimilation rate (NAR) and increased antioxidant activity in Alankar cultivar. Farooq and collaborators (FAROOQ et al., 2021) evaluated two cultivars of *B. napus* (ZS758 and ZD622) exposed to As and concluded that, the cultivar ZS758 showed greater tolerance to metal toxicity, possibly dependent on efficient multilevel defense coordination, although oxidative stress resulted in extensive damage to both. Thus, the use of cultivars that are more tolerant to toxic HMs can be a useful alternative in the phytoremediation of contaminated soils. The selection of tolerant cultivars sheds a critical light related to genetic engineering, to achieving plant transgenesis with greater phytoremediation potential.

10.5 PHYTOREMEDIATION ASSISTED BY GENETIC ENGINEERING APPROACHES

In recent decades, the literature reports that biotechnological advances have been highlighted through the understanding and manipulation of genes related to phytoremediation (KUMAR et al., 2015). According to Rai and collaborators (RAI et al., 2020) to overcome phytoremediation limitations through genetic engineering can be called “genoremediation”. The genetic mechanisms addressed in phytoremediation are investigated from overexpression genes related to the chelation and transport of toxic metal ions, oxidative damage reduction in plant metabolism, and increased biomass production (BILAL SHAKOOR et al., 2014; KUMAR et al., 2015).

Several studies aimed to select new plants genotypes capable of absorbing and tolerating higher concentrations of toxic metals using different biomolecular techniques. In this sense, Navarro-León and colleagues (NAVARRO-LEÓN et al., 2019) investigated the role of the *HMA*$_4$ gene, a Cd and Zn transporter candidate intolerance to Cd toxicity to mutant plants of *B. rapa* (*BraA.hma4a-3*) and observed that these mutants produced low biomass, increased production of photosynthetic pigments contents and higher accumulation of Cd in the aerial parts, which can be explained by the increased production of PCs that attenuate the toxic effects of the metal. The literature shows that genes related to CAXs proteins (cation exchangers) can also improve phytoremediation.

CAXs proteins are antiporter transporters located in the plasma membranes of organelles, play a fundamental role in Ca^{2+} homeostasis, and possess potentially interesting to Cd phytoremediation since both elements have similar physical characteristics and compete with each other by ion channels (PITTMAN; HIRSCHI, 2016). Navarro-León et al. (NAVARRO-LEÓN et al., 2020) evaluated three *B. rapa* mutants for the CAX_1 transporter obtained by TILLING subjected to stress generated by Cd and concluded that it improved the absorption and attenuated the ROS caused by Cd.

Other genoremediation investigate are reported by Bhuiyan and collaborators (BHUIYAN et al., 2011a) that introduced the *AtATM*$_3$ gene, (*ATP-Binding Cassette* transporter family) into *B. juncea* seedlings and observed greater tolerance to oxidative stresses generated by Cd and Pb and higher accumulation of these metals. In another study, Bhuiyan and collaborators (BHUIYAN et al., 2011b) introduced the *YCF*$_1$ transporter genes from *Saccharomyces cerevisiae* into *B. juncea* plants and observed that the transgenic plants showed greater tolerance and greater accumulation of Cd and Pb. Plant biomass obtained after the phytoextraction process is highly contaminated by toxic metals and, therefore, important that its disposal management is correctly carried out, to avoid environmental recontamination.

11 OPPORTUNITIES IN MANAGEMENT OF DISPOSAL OF PLANTS BIOMASS USED FOR PHYTOREMEDIATION

From the phytoextraction, it is expected that toxic HMs are transferred to the aboveground plant biomass and, therefore, their concentrations are reduced in the environment

(RIZWAN et al., 2018). Posteriorly, the handling and disposal of plant residues enriched with toxic components must be properly carried out, to avoid environmental recontamination (GONG et al., 2018).

Some studies point to techniques that can be used in the treatment and disposal of contaminated biomass such as biomass incineration, heavy metal leaching from different solubilizing agents (H_2O, sodium hydroxide or acetic, nitric, sulfuric, or hydrochloric acids), composting (with control of toxic metal concentrations), combustion, biofuel production, and pyrolysis. According to Rizwan and colleagues (RIZWAN et al., 2018) point to incineration is an efficient alternative technique, as it reduces the volume of biomass by up to 99%, generating ash or fly ash. Gong and colleagues (GONG et al., 2018) report that toxic metals can be stabilized in biomass residues after pyrolysis, in addition to the conversion of plant residues into biochar. The hydrolysed biomass of *B. napus* cultivated in soil contaminated with toxic metals enable satisfactory bioethanol production (DHIMAN et al., 2016). Although the literature presents some alternatives for the disposal of the contaminated biomass obtained, it is necessary to elucidate further studies (subjects) in the area, to assess which technique is more environmentally sustainable, generates less toxic waste and mitigates the risk of recontamination.

12 CONCLUSIONS AND FUTURE PERSPECTIVES

Contamination of soils and water bodies by toxic HMs is a major environmental concern. Phytoremediation emerges as an important technique to solve this problem, in which plants are used to remove contaminants from the environment. *Brassica* species are potentially phytoremediators because they are efficient in physiological processes for phytoremediation strategies describing as phytovolatilization and/or phytostabilization and/or in the toxic metals phytoextraction, which will improve uptake, translocation, and accumulation of toxic HMs ions in low activity cellular organelles. This botanical genus possess an efficient enzymatic and non-enzymatic defense mechanism and attenuation of oxidative damage generated by toxic HMs in metabolism, induced by the formation of reactive oxygen species.

In addition to enzymatic and non-enzymatic defense mechanisms, our review shed light approach of other phytoremediation assistance techniques potentially used in *Brassica* species, such as plant growth regulators and promoters, chelating and acidifying agents, selection of cultivars tolerant, and genetic engineering techniques. Although biomass management and disposal techniques obtained after the phytoremediation process are reported, this subject is scarce to present greater consensus between which techniques are security, more efficient, and economically viable.

The toxic HMs phytoremediation by *Brassica* species presents itself as a promising technique in recent times, although, we speculate the need for more studies so that its applicability and feasibility go for larger areas (crop areas), from the agronomic techniques approach to assist metals hyperaccumulation-regulation framework for phytoremediation.

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