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Review

Advances in Cotton Tolerance to Heavy Metal Stress and Applications to Remediate Heavy Metal-Contaminated Farmland Soil

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Abstract: Heavy metal-contaminated soil is one of the major environmental pollution problems of agricultural production and human health in the world. Remediation of heavy metals in soil is one of the most popular research subjects. Different remediation strategies have been reported to remove heavy metals from contaminated soil, among which phytoremediation is the most important one. Compared with other major crops, cotton shows the strongest and most widespread resistance to abiotic stresses, such as heavy metals. Although heavy metal stress adversely affects the growth and development of cotton, cotton possesses a set of sophisticated stress-resistance strategies. As the main product of cotton is nonedible fibers, which have a large biomass and strong heavy metal absorption and enrichment capacities, cotton is an ideal crop to restore heavy metal-contaminated soils and has unique advantages in terms of both ecological and economic benefits, with great application prospects. In this review, based on domestic and foreign research results in recent years, the effects of heavy metals on cotton growth and product quality were analyzed, the heavy metal absorption, accumulation, translocation and enrichment characteristics of cotton plants were summarized, and the adaptation and tolerance mechanisms of cotton to heavy metals were explored. Furthermore, the view that cotton is an effective crop to remediate heavy metal pollution in farmland soil has been proposed, and popularization and application suggestions for planting cotton to repair heavy metal pollution have been put forward to provide a reference for the comprehensive evaluation of the economic feasibility of cotton to repair heavy metal pollution in farmland soil.

Keywords: Cotton plant; heavy metal; farmland soil; tolerance mechanism; application

1 Introduction

In the past 100 years, with the development of global industrialization and urbanization, the fertility and production capacity of farmland soil has been improved by sewage irrigation and the application of chemical fertilizers and pesticides. However, heavy metal elements contained in sewage, fertilizers and pesticides may be concentrated in farmland soil, endangering normal ecological environment function and causing heavy metal pollution in the soil, affecting the growth and development of plants and animals, and then harming agricultural production and human health [1,2]. Heavy metal-contaminated farmland exceeds

20 million hm^2, accounting for nearly 1/5 of the total cultivated land in China [3]. The main heavy metal pollutants are cadmium (Cd), nickel (Ni), copper (Cu), and lead (Pb), and Cd pollution has the highest level exceeding the standard rate (7.0%), as shown in a bulletin on the survey of national soil pollution issued by the Ministry of Environmental Protection and the Ministry of Land and Resources in China on April 17, 2014. Therefore, it is the key target for the comprehensive prevention of heavy metal pollution in soil. The contaminated soil cannot be repaired and utilized and must rely on its self-purification ability in the short term, due to the characteristics of long-term, cumulative, hidden, irreversible and morphological diversity of heavy metal pollution [4,5]. Therefore, it is necessary to reduce soil heavy metal pollution to ensure the safety of agricultural products and the sustainable and efficient use of land resources.

Regarding the remediation technology of heavy metal-contaminated soil, many scholars have proposed physical remediation, chemical remediation and bioremediation. Compared to the other two methods, phytoremediation technology, belonging to bioremediation, has the advantages of low cost, suitability for large-scale treatment, ability to maintain soil biological activity and physical properties, and does not easily cause secondary pollution. A hyperaccumulator has the strong ability to absorb, transport, detoxify and isolate heavy metals [6]. It can complex and accumulate 10-500 times the amount of heavy metals and isolate them regionally in the cell wall or vacuole, etc., reduce their free ion concentrations in the cell and reduce their toxicity to plants [7]. At present, approximately 570 kinds of plants have been identified as heavy metal hyperaccumulation plants, mostly Ni-accumulating plants, while there are relatively few Pb, chromium (Cr), and Cd hyperaccumulator plants, and they are mostly wild type with strong regional distribution and narrow ecological adaptability, small biomass, and slow growth rate, most of which are difficult to effectively apply in actual restoration [8]. Cotton is a nonedible economic crop, and cotton fiber, the main product, does not enter the food chain. It is one of the best crops to repair heavy metal-contaminated farmland soil due to cotton plants having a strong ability to absorb heavy metals and possessing a large biomass [9,10]. The effects of heavy metals on cotton growth and development and the putative adaptation and tolerance mechanism are shown in Fig. 1.

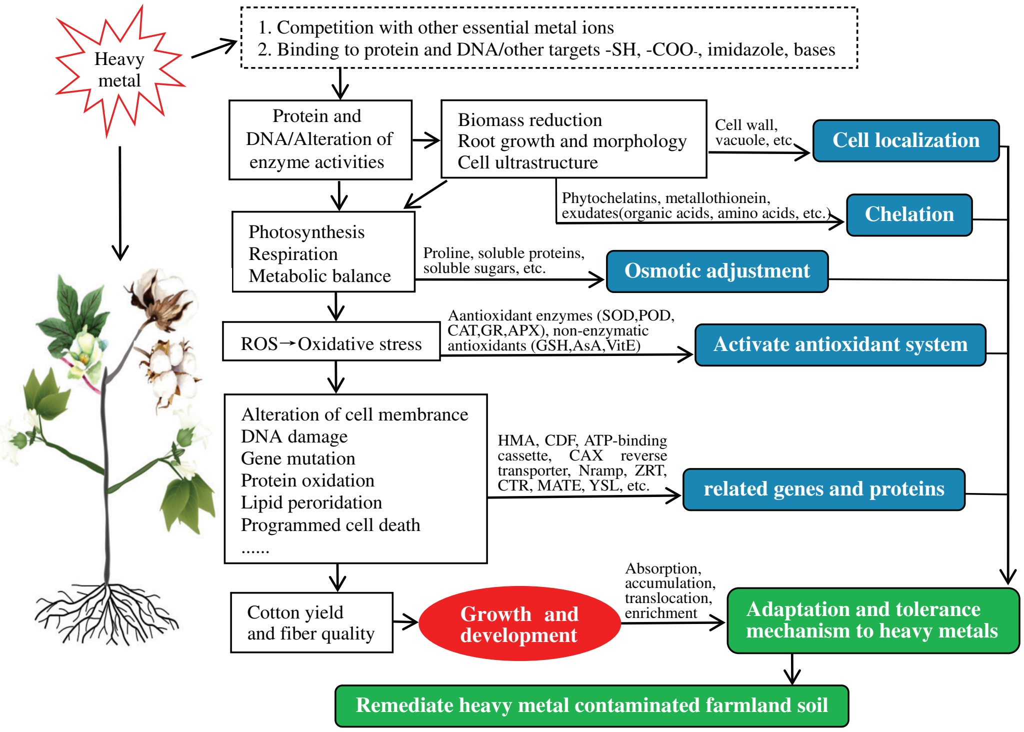

Figure 1: The putative adaptation and tolerance mechanism of cotton growth and development to heavy metals

2 Effects of Heavy Metals on the Growth and Product Quality of Cotton

2.1 Plant Growth and Physiological Characteristics

Heavy metal pollutants, such as Cd, Ni and Pb, are nonessential elements of cotton growth and development. Although they may have a positive effect on cotton growth and development at low concentrations, excessive amounts will damage cotton plants and inhibit their growth and development [11]. Simulated field experiment research showed that with the increase in Cd concentration, cotton root length shortened, the number of fruit branches decreased [12], chlorophyll content and the number of stomata decreased, the content of reactive oxygen species (ROS) and malondialdehyde (MDA) increased significantly [13], the activities of antioxidant enzymes (POD, SOD, CAT, APX) and the net photosynthetic rate of leaves first increased and then decreased, and the transpiration rate and stomatal conductance gradually decreased [10]. Pb stress caused a decrease in the germination rate of cotton seeds [14], shortened root length, induced decay of seedlings, decreased biomass and chlorophyll content, and increased MDA and hydrogen peroxide (H_2O_2) contents [15]. The ratio of the underground part to the aboveground part of cotton seedlings, the number of secondary roots, and the quality of root dry mass and fresh matter decreased significantly under Cr stress, while the MDA and H_2O_2 contents increased [16]. Cotton seedling tolerance to Pb stress is greater than that to Cd stress [14].

The ultrastructural changes in cotton cells were found to be dose dependent. With elevated levels of Cd, the damage to the cell structure became serious [17,18]. Cd stress changed the organ structure and cell structure of cotton root tips greatly, increased the number and volume of nucleoli and vacuoles, caused shrinkage of the cytoplasm, increased chloroplast numbers and decreased their volume, caused grana thylakoid expansion, destroyed the membrane structure, and increased the number of starch granules [17]. The changes that occurred in the cottonseed cells included detrimental plasmolytic shrinkage, disintegration of the nucleus, chromatin condensation, shrinkage of the cytoplasm, abnormal structures of organelles, thickening and constriction of the cell wall, cell collapse and disintegration, etc., which caused irreversible fatal damage to the cells [18]. Cd existed in the form of rings, crystals, and electron-dense granules, which were distributed in the protein vacuole, intercellular space, cytoplasm, and cell wall [17,18]. Pb stress caused severe shrinkage and deformation of cotton cells, and even rupture and ulceration; the cell gap disappeared, and the cell layer was broken [15].

Heavy metal ions can compete with certain essential metal ions in cotton, affecting the absorption and accumulation of essential metal ions and resulting in the loss of essential elements [5]. The absorption, transport and distribution of Zn, Cu and Fe were affected by Cd stress in cotton plants [19]. A study showed that Cd stress promoted the absorption and accumulation of Mg, Mn, Cu, and Zn in the fiber and cottonseed hull and affected the absorption and accumulation of Mn and Zn in the leaf parts, especially Mn. Additionally, the absorption of Mn and Cu in seeds and stems was affected, while the absorption of Mg, Mn and Zn in roots was promoted by Cd stress [20]. The effect of heavy metals on the absorption and accumulation of essential metal ions varies with different organs in cotton plants. Low concentrations of Cd and other heavy metals have little effect on the physiological characteristics and growth of cotton plants, showing a certain degree of tolerance.

2.2 Cotton Yield and Fiber Quality

The effect of heavy metal stress on plant growth and physiological characteristics will eventually cause a decline in growth and yield. Li et al. [10] found that low Cd treatment concentration had little effect on cotton yield or certain promotion effects on some special cotton genotypes, while a high Cd concentration significantly reduced cotton yield, especially seed cotton yield, lint yield, boll weight, and boll number per plant, which were significantly reduced. The effect of Cd stress on fiber yield was mainly determined in the early growth period, and in particular, the transportation of assimilation products and nutrients was affected in the stage during which the volume of cotton bolls increases and during internal filling; then the development of cotton bolls was hindered, and the yield of seed cotton and lint cotton was significantly reduced eventually after boll opening, while it had a small effect in the late growth period of cotton, which was related to the metabolic characteristics of cotton plants at different growth stages and the adaptability to Cd [21]. When cotton was planted in mixed pollution soil, it produced cotton bolls and open bolls under treatment with a Cd concentration of 5 mg/kg, and the concentrations of Pb and Zn were 400 mg/kg and below, while cotton cannot produce cotton bolls at a Cd concentration of 10 mg/kg when the concentrations of Pb and Zn are 800 mg/kg and above [22]. Low or high contamination of heavy metals, that is, the low or high heavy metal treatment concentration in the simulation experiment, corresponded to the low or high content of one or several heavy metals in the contaminated farmland soil. Low heavy metal pollution can increase the cotton biomass, hardly affects the economic output of cotton (seed cotton) or promotes increased yield, but the reproductive process is significantly inhibited under high heavy metal pollution [10,22]. The effect of Cd stress on cotton fiber quality is not significant, and the fiber quality is decreased somewhat, but the difference is not obvious. Fiber length, uniformity and micronaire value performed better at low Cd concentrations and decreased at high concentrations, while fiber length and strength increased with increasing Cd concentration, but elongation decreased [10]. Therefore, from the assessment of cotton fiber yield and quality, cotton is suitable for cultivation on low heavy metal-contaminated farmland soil, which can not only produce cotton fiber for economic benefits but also absorb and accumulate heavy metals to repair contaminated soil.

2.3 Cotton Byproduct Output and Quality

Cottonseed is the main byproduct of cotton. The upland cottonseed contains a very high nutritional value, such as 27.83–45.6% protein and 28.24–44.05% oil, so it is an important resource for high-quality edible oil and potential protein food [23]. Nutritional quality traits, such as the content of starch, protein, oil, and amino acids, and seed sowing quality traits, such as grain maturity and shell thickness, all inform cottonseed quality. Studies have shown that the physical qualities of cottonseed, such as seed index, kernel index, kernel percentage and kernel-to-shell ratio, decreased with increasing Cd concentration, so the fullness and maturity of cotton seeds and the growth and development of young embryos may be affected by Cd stress [24]. High concentrations of Cd caused a decrease in the number of seeds and a higher proportion of empty grains, which may result in decreased fertility or difficulty in the fertilization of male and female gametes in cotton [21]. The protein content of the kernel is lower under a high Cd concentration than a low Cd concentration but higher than that of the control, and the opposite change is observed in the oil content [18]. The amino acid contents of Asp, Thr, Ser, Glu, Gly, Ala, Ile, Leu, Lys, His, Arg, and Pro in cottonseed kernels increased at low Cd concentration and decreased at high Cd concentration, which were lower than those of the control, but the difference is not significant [20]. Palmitic acid content decreased significantly with increasing Cd concentration, and there was no significant difference in linoleic acid content between different Cd treatment concentrations [20].

Gossypol is a characteristic chemical substance in cotton, with a wide range of antiviral, anticancer and antifungal functions [25], and is another important byproduct of cotton. It is synthesized in the roots and then transported to the aerial parts of the plant and stored in the pigment glands of cotton, and it is an important natural protection substance because of the antagonistic effect on pathogens and pests, while it is poisonous to humans and monogastric animals. The gossypol content in upland cotton seeds is usually 0.6–2% [26]. Cd induced a reduction in the gossypol content and pigment gland density, and a negative correlation was observed between the gossypol content and Cd concentration in cottonseed kernels [27]. Due to the ability to transport Cd from the root to the aerial part of cotton plant, it is speculated that the decrease in gossypol content may be caused by Cd absorption and translocation, which affects the synthesis or transportation ability of gossypol.

3 Absorption, Translocation and Enrichment Characteristics of Heavy Metals in Cotton

In general, the heavy metal content in plants increases with the increase in heavy metal content in the soil. The results of Li et al. [10] clearly demonstrated that Cd accumulation enhanced with the increase in Cd concentration; its accumulation was in the order of fiber < seed < seed shell < root < leaf < shoot < boll shell < petiole, and the lowest Cd content in cotton fiber did not affect its use. The highest Cd accumulation in the petiole might be due to the presence of carrier proteins in vascular tissues and the formation of chelators and metallothioneins in these vascular tissues [28]. While the content of Cd in leaves is higher than that in shoots and roots, Cd content in roots decreased with the growth process [29], and the mass fractions of Cd and Pb in upland cotton leaves were higher than those in roots, stems and fibers [30]. The Cd absorption value of 59 cotton varieties (lines) was 29.2–248.0 mg·kg^{-1} at the seedling stage planting in a culture solution with 2 mg·L^{-1} Cd, the average absorption value was 82.2 mg·kg^{-1}, and there were 10 varieties with a Cd absorption value of more than 100 mg·kg^{-1}, indicating that cotton has a better ability to absorb environmental Cd [31]. Under the combined pollution of heavy metals, the average mass fractions of Cu, Ag and Au were respectively 0.8–10.5 mg·kg^{-1}, 26.45–57.4 $\mu\text{g·kg}^{-1}$, 2.99–4.89 $\mu\text{g·kg}^{-1}$ in the root, shoot, boll shell, seed, fiber and other tissues of upland cotton plants, which have a strong absorption capacity for Cu, Ag and Au elements in farmland soil [2]. The Cd content in cotton leaves under the combined pollution of Cd, Pb and Zn was significantly lower than that with only Cd pollution, indicating that the absorption of heavy metals in cotton has an antagonistic effect under the combined pollution of heavy metals [22,31].

Translocation factor (TF) is the ratio of the heavy metal content in the plant aboveground or an organ to that in the root, representing the ability of a plant to absorb and transport heavy metals [32]. The higher the TF, the stronger the ability to absorb and transport heavy metals. Studies have shown that plants with the TF greater than 0.5 have better tolerance to heavy metals [33], while heavy metals can be fixed in the root, limiting their transfer to the ground when plants had a transport coefficient less than 1, so the toxic effects of heavy metals could be reduced [34]. The TF of Cd in the aboveground organs of cotton plants were greater than 1, between 6.010 and 13.638, indicating that the accumulated Cd content of cotton aboveground was much higher than that in the root, which meant that cotton had good tolerance and a high transportation ability for Cd [35].

The bioconcentration factor (BCF) is the ratio of the heavy metal content in the plant organs to that in the soil, representing the enrichment ability of the plant for the heavy metal in the soil and reflecting the migration degree of heavy metal in the soil-plant system to some extent [36]. Hyperaccumulators have the characteristics that the heavy metal content in the plant reaches the critical standard, the heavy metal content in the plant aboveground is greater than that in the root, the tolerance to heavy metals is strong, and the BCF of the plant aboveground is greater than 1 [10]. Hu et al. [37] showed that upland cotton had significant enrichment of Cd in soil, and the BCF of Cd in various organs from large to small followed the order leaf, shoot, root, boll shell, seed, and fiber. The BCF of vegetative organs, plant aboveground parts and whole plants of cotton were all greater than 1, and the Cd content in the plant aboveground was much greater than that in the root, which may be related to the existing form and translocation mechanism of Cd in cotton [10]. The root, shoot, boll shell, seed and fiber of upland cotton had strong absorption and enrichment effects on Cu, Ag, and Au in farmland soil [2]. Cotton has better absorption, enrichment and tolerance for low concentrations of Pb, Cd, Zn, etc., than for high concentrations, and it can have a better effect on the remediation of lightly heavy metal-contaminated soil. Additionally, high heavy metal pollution caused cotton production to decline, but the cotton can still absorb and enrich heavy metals [38].

4 The Adaptation and Tolerance Mechanism of Cotton to Heavy Metals

4.1 Cell Localization

The entry of metal ions into the cell walls of plant roots from the external environment is a passive nonmetabolic process, which is simple diffusion or mass flow. Most heavy metals exist in the form of ions or bind to the cellulose and lignin of the cell wall, so they cannot reach the cytoplasm to affect physiological and metabolic activities. Bringezu et al. [39] reported that a series of heavy metals were accumulated in the epidermal cell wall of resistant plants (*Silene vulgaris*), and these heavy metals were either bound to proteins or existed in the form of silicates. Cd existed in the form of rings and crystals as well as electron-dense granules and occurred in the intercellular space, the cytoplasm, and the cell wall of cottonseeds under Cd stress [18]. The structure of the cell wall itself was modified under heavy metal stress, the precipitation of divalent and trivalent heavy metals increased by increasing the content of low-methylated colloids of polysaccharides in the cell wall, and then heavy metal ions were restricted to the cell wall and could not enter into the cell [40]. Therefore, the cell wall of the root is the first barrier to prevent heavy metal ions from entering the protoplasm, and it has a certain adsorption and storage effect on heavy metal ions.

Excess heavy metals ions will enter the cytoplasm through the cell membrane when they bind to the cell wall to reach saturation. However, there is no heavy metal ion transporter on the plant cell membrane; it can only enter the cytoplasm through Mg^{2+}, Ca^{2+}, Fe^{2+}, etc., transporters [41,42]; and the cell plasma membrane has the function of actively excluding heavy metal ions. When a small amount of heavy metal enters the cytoplasm, it exists in the form of free ions. When there are too many free ions, they combine with amino acids, organic acids, peptides, or inorganic substances in the cytoplasm and pass through a carrier on the vacuole membrane (such as an ABC carrier located on the cell membrane) or a channel protein into the vacuole [43]. Heavy metals are detoxified and confined to a limited area of the vacuole by combining with proteins, sugars and organic acids in the vacuole, which prevents excessive heavy metals from entering the protoplast and reduces the damage to physiological and metabolic activities in the cytoplasmic matrix and organelles [44]. Khan et al. [45] and Daud et al. [46] found that there were heavy metal deposition areas in the vesicles of cotton leaves, but the deposition areas are different in terms of metal concentrations, genotypes and tissues. Therefore, these areas play an important role of vacuole region fixation in eliminating the toxicity and tolerance of heavy metals.

4.2 Chelation

The chelation of heavy metals by plants is a major way of detoxifying heavy metals in soil. Phytochelatin, metallothionein, organic acids, amino acids, etc., in plants chelate with heavy metal ions to avoid the contact of heavy metal ions with organelles and reduce the concentration of free ions in the cell, thereby reducing the toxicity of heavy metals to plants [47]. Phytochelatins (PCs) exist widely in plant cells that can effectively chelate heavy metal ions, such as Cd^{2+}, Pb^{2+}, and Cu^{2+}, and have a highly conserved primary structure: $(\gamma\text{-Glu-Cys})n\text{ -Gly}(n = 2{\sim}11)$ [48]. PCs can enrich and detoxify free heavy metal ions due to their structure, which is rich in cysteine (Cys) sulfhydryl (-SH) groups [49]. PCs are peptide compounds synthesized with reduced glutathione (GSH) as substrate and catalyzed by phytochelatin synthase (PCS) [48,50], and they are also induced by various heavy metals, such as Cd, Pb, Cu, Zn, Mn, etc.; Cd is the strongest inducing factor in the cell, and its activation efficiency on the PCS enzyme is 50 times that of Pb [51–53]. The toxicity of Cd and other heavy metal ions to cells is mainly removed or reduced by PC complexation and compartmentalization [49]. Mei et al. [54] showed that 2, 2 and 4 PCS genes were identified in G. *raimondii* (D5), G. *arboreum* (A2) and G. *hirsutum* ([AD]1), respectively, and G. *hirsutum* and its donor G. *arboreum* probably have higher catalytic activity and are more heavy metal-tolerant than G. *raimondii*.

Metallothionein (MT) is a low molecular weight and highly conserved protein rich in cysteine (Cys) residues. Under heavy metal stress, the thiol group on the Cys residue of MT can combine with heavy metal ions to form low toxicity or nontoxic complex, especially Hg^{2+}, followed by Au^{+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, etc., and then detoxify heavy metals in plants [55]. MT type I was identified and isolated from upland cotton by Hudspeth et al. [56], and it is expressed in roots in a large amount, with little or no expression in other organs, which may be related to roots being the first line of defense against heavy metal stress in plants. GhMT3 identified from upland cotton can be induced by $CuSO_4$ and $ZnSO_4$, chelating with metal ions and scavenging ROS, and its expression is regulated by reactive oxygen signals under abiotic stress [57].

Organic acids, amino acids, sugars, etc., in the root exudates can also chelate heavy metals, change the form of heavy metals in the plant rhizosphere, and reduce their damage to plants. The amount of organic acids secreted by plant roots increased under heavy metal stress, caused acidification of the rhizosphere soil, changed the morphology and bioavailability of heavy metals in soil, and promoted the absorption and activation of heavy metals in soil by plants [58]. The content of Cd^{2+} in plant tissues was directly proportional to the content of low molecular weight organic acids in rhizosphere soil [59]. Citric acid or acetic acid secreted by plant roots can activate heavy metals in soil and promote the absorption of heavy metals by plants and had the greatest improvement in Pb^{2+} desorption at higher concentrations ($>10^3$ mol·L^{-1}), followed by malic acid; the smallest improvement was seen with oxalic acid, so it plays an important role in the accumulation of heavy metals in plants [60,61]. Oxalic acid, malic acid, and tartaric acid were the predominant low molecular weight organic acids in rhizosphere soil solution of the hyperaccumulating ecotype *S. alfredii*, and cadmium accumulation was promoted by the exudation of tartaric acid, which was highly efficient at Cd solubilization due to the formation of soluble Cd-tartrate complexes [62]. The amino, carboxyl, hydroxyl, etc., functional groups in amino acids (such as histidine and proline) can combine with heavy metal ions to form stable compounds to deactivate and detoxify heavy metals [63]. The glycine derived from root exudates or protein degradation products provided sufficient sorption sites for Cd^{2+} and Pb^{2+}, resulting in enhanced metal fixation via complexation, and increased the sorption of heavy metals to soils, thus reducing their toxicity to plants [64].

4.3 The Defense Function of the Antioxidant System

The production and removal of ROS are in a dynamic balance when cotton plants are in normal growth. When subjected to heavy metal stress, the production and conversion of a large amount of ROS are induced, including superoxide anion ($O_2\cdot^-$), hydroxyl radical (·OH) and hydrogen peroxide (H_2O_2), etc.; ROS are the main mechanism of cotton damage caused by heavy metal stress [65,66]. The antioxidant system contains antioxidant enzymes and nonenzymatic antioxidants [66]. Antioxidant enzymes include superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione reductase (GR) and ascorbate peroxidase (APX), while nonenzymatic antioxidants include reduced glutathione (GSH), ascorbic acid (AsA) and tocopherol (VitE), etc., and the free radical scavenging system composed of these substances works together to relieve the damage to cotton plant cells [67].

SOD catalyzes the combination of $O_2\cdot^-$ and H^+ to form H_2O_2 and O_2, and POD and CAT further catalyze the conversion of H_2O_2 to H_2O under low heavy metal stress [68]. GR and APX are the two key enzymes of the AsA-GSH circulation system, which can directly remove H_2O_2 in the chloroplast and cytoplasm under high heavy metal stress, thereby reducing the damage of ROS to plant cells [69]. Daud et al. [46] found a decrease in SOD activity and a slight increase in POD and APX activity caused by 50 μmol·L^{-1} Cd treatment, and an increase in SOD and CAT activity induced by 500 μmol·L^{-1} Cd stress in upland cotton leaves. The activities of POD, SOD and CAT increased rapidly in the early stage of cotton growth or under low Cd concentration, but the enzyme activity was inhibited with the increase in Cd concentration and the extension of stress time [10]. A similar change was found using Pb, Cr, etc., to treat cotton leaves and roots, indicating that there is a certain universality to antioxidant enzyme system activation under heavy metal stress [16]. Using exogenous GSH at a concentration of 50 $\mu mol \cdot L^{-1}$ to detoxify heavy metal stress in upland cotton TM-1, the activities of POD, SOD and APX increased significantly under 50 $\mu mol \cdot L^{-1}$ Cd stress [46], and SOD and CAT activity increased significantly under 500 $\mu mol \cdot L^{-1}$ Pb stress [45]. GSH, as a nonenzymatic substance, has an important alleviating effect on cotton heavy metal stress, which is related to the role of GSH as both a major intracellular antioxidant and a precursor substance of phytochelatin [70].

4.4 Osmotic Adjustment

Osmotic adjustment is a positive strategy for cotton plants in response to environmental changes. Proline, as an important osmoregulatory substance in cells, has the functions of regulating cell osmotic balance, enhancing cell structural stability and preventing the generation of oxygen free radicals [71,72]. The content of proline in the leaves of cotton seedlings increased generally under the stress of Ni, Pb and Cd [14,15]. Plant cells may actively accumulate soluble proteins, soluble sugars, etc., to reduce intracellular osmotic potential, ensure the normal supply of water under heavy metal stress, and maintain normal physiological function [73]. The accumulation of soluble sugar in the seedling stage of cotton was highest under Pb (50 $mg \cdot L^{-1}$) and Cd (30 $mg \cdot L^{-1}$) stress, and it continued to decline in other periods [14]. The content of soluble protein in cotton seedling leaves generally showed an upward trend under Ni and Pb treatments, indicating that the resistance to heavy metal stress occurred mainly by reducing cell osmotic potential and improving tolerance to dehydration, thereby providing a relatively stable internal environment for normal cell life activities [15,74,75].

4.5 Heavy Metal Transport-Related Genes and Proteins

Heavy metal ATPase (HMA) is a type of protein that can transport Zn^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, etc., across the membrane and transport heavy metal ions from roots to stems and leaves [76,77]. The protein AlHMA1 is related to Cu^{2+} transport, and AlHMA2 is related to Zn^{2+} transport and highly expressed in leaves and roots [77]. AtHMA3 located on the vacuole membrane is highly expressed in guard cells, drainage holes, vascular tissues and root tips; participates in the storage and isolation of Cd^{2+} in the vacuole; and plays a detoxification role [78]. AtHMA4 on the plasma membrane is overexpressed to improve root growth in environments containing metals such as Zn^{2+}, Cd^{2+}, Co^{2+}, etc. [77].

Cation diffusion facilitator (CDF) family members are located in the vacuole membrane and promote the secretion of heavy metal ions, such as Zn^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, etc., from the cytosol, including discharge to the apoplast and endoplasmic reticulum and isolation in the vacuole, and control its reverse transport, thereby improving the resistance of plant cells to heavy metals [79]. AtMTP1 is a Zn^{2+}/H^{+} reverse transport protein and is highly specific for Zn^{2+} [80]. The overexpression of AtMTP1 and AtMTP3 improved the tolerance to Zn^{2+} and promoted Zn^{2+} enrichment in roots and rosette leaves in *Arabidopsis* [80,81]. Mn^{2+} can be transported specifically by AtMTP11 [82]. The expression of OsMTP1 can enhance the tolerance to Zn^{2+}, Cd^{2+}, and Ni^{2+}, and OsMTP1 dsRNAi caused rice seedlings to be sensitive to heavy metals and changed the accumulation of heavy metals in different organs of rice [83].

An ABC-type cassette (ATP-binding cassette) is a type of transmembrane transporter that can transport metal chelates to the vacuole for isolation and storage and alleviate the toxicity of heavy metals [84]. The stronger the expression of AtPDR12 is, the stronger the tolerance of *Arabidopsis* plants to Pb^{2+} [85]. AtMRP3 participated in detoxification of Cd^{2+} in *Arabidopsis* [84]. The expression of AtAMT3 can clear Fe^{2+} enriched in the mitochondria of the atm1 yeast mutant across the membrane and restore mitochondrial respiratory function [86]. Furthermore, the CAX reverse transporter [87], natural resistance associated macrophage protein family (Nramp) [88], Zinc-iron regulatory protein family (ZRT, IRT-like protein ZIP) [79], copper transporter family (CTR) [89], multidrug and toxic compound extrusion family

(MATE) [90] yellow stripe1-like family (YSL) [91], etc., play important roles in the absorption, transport, enrichment and detoxification of heavy metal ions.

5 Cotton is an Effective Crop to Remove Heavy Metal Pollution from Farmland Soil

Heavy metal pollution in the soil directly affects the quality and safety of agricultural products, and it is a common problem in all countries in the world. Using the accumulation and transfer ability of hyperaccumulators for heavy metals, the heavy metals in the soil are transferred to the plants in large quantities, especially the aerial parts, and harvested at the appropriate time to repair and use contaminated soil, which has good comprehensive ecological benefits. Given its large biomass and strong enrichment capacity for heavy metals, cotton has a large advantage over other major crops. The absorption of Pb and Cd in cotton plants is greater than that in rice at the same treatment level [92]. The export of heavy metals by cotton slows the accumulation of thallium (Ti) in the soil, which can effectively reduce soil Ti pollution [93]. The elements Cu, Ag and Au in farmland soil can be effectively absorbed and enriched by cotton plants [2]. The Cd accumulation was the lowest in the fiber and the highest in the petiole of cotton, and the BCF of vegetative organs, aerial parts and the whole plant were all greater than 1, so cotton plants had a strong ability to absorb, accumulate and transport Cd and a good Cd transport mechanism, which show the basic characteristics of being hyperaccumulators and can be used to repair Cd-contaminated soil [35].

Based on the research results, cotton has the following advantages as a remediation plant for heavy metal-contaminated soil. First, it has a certain tolerance to heavy metals due to its ability to grow and develop normally in most polluted farmland, and the yield and fiber quality are basically not affected. Second, cotton is one of the main economic crops, with a large planting area, wide adaptability, large biomass and heavy metal absorption, and cotton plants are easy to pull out and transfer, which is convenient for centralized treatment and will not cause secondary pollution. Third, cotton does not directly enter people's food chain, fiber as the main product is nonedible and has low Cd enrichment, and Cd content does not exceed the standard and meets national food safety standards of cottonseed as the byproduct, so these two products are safe. Finally, transgenic varieties have a stronger ability to absorb heavy metals than nontransgenic varieties, so we can consciously cultivate transgenic cotton varieties that are hyperaccumulators. Therefore, cotton can be considered the first choice for plants to repair heavy metal-contaminated soil. By planting cotton in heavy metal-contaminated farmland soil, it can help solve the problems of not only low biomass, difficult commercialization and large-scale promotion faced by current plant remediation technologies but also overcome the need for more people and less land, the contradiction between grain and cotton, the limited area of cotton planting, and the insufficient demand for cotton production and further result in eventual economic benefits.

6 Proposal for Popularization and Application of Planting Cotton to Repair Heavy Metal-Contaminated Farmland Soil

6.1 Screening Varieties with High Accumulation Tolerance and Supporting Planting Techniques and Patterns

The higher the biomass of cotton plants is, the stronger the ability to absorb, transport and accumulate heavy metals, and the better the ecological restoration effect on the contaminated soil. To date, although some studies have initially screened some cotton varieties (lines) that were tolerant to some heavy metals and have strong enrichment capacity, they are still not sufficient for the cultivation and restoration of contaminated farmland at a large scale. Based on the analysis of various physiological indexes before and after Cd stress, Li et al. [35] selected SGK3 and GK44 with strong tolerance to Cd from the tested cotton varieties, and the cotton hybrid Xiangzamian 15 showed relatively good capacities of Cd tolerance and adsorption [29]. Cotton varieties (lines) with high Cd accumulation (FZ09, Xiangzamian 23, X-7 and

FZ06) were screened, and all the tested varieties (lines) grew normally in Cd-contaminated soil, indicating that cotton itself had a strong adaptability to Cd-contaminated soil [94]. Therefore, the selection and breeding of cotton varieties that are tolerant to heavy metals and have high enrichment capacity and population biomass are the basis for the use of cotton to repair soil contaminated by heavy metals. When breeding varieties with a high biomass per plant, hybrid cotton can be selected, focusing on fine quality and high yield. When breeding varieties with a low biomass per plant, early maturing cotton can be selected for dense planting, focusing on simplicity and efficiency.

Simple and efficient planting techniques and models are necessary supporting measures for the promotion of planting alternative crops in heavy metal-contaminated soil. The study of alternative cultivation techniques for cotton in Cd-contaminated soil is advisable to select the varieties with high yield, fine quality, high Cd enrichment and short growth periods, and standardized production technology has been applied using presowing rotary tillage, mechanical direct seeding, centralized fertilization, safe weeding, regulation and chemical capping, specialized pest control, and centralized removal of cotton stalks [94]. The method of intercropping, such as cotton-rapeseed continuous cropping mode with a density of 60,000 plants·hm^{-2}, row spacing of 0.76 m, and plant spacing of 0.22 m, resulted in a significant decrease in the Cd content of soil after cotton planting, with a decline of 30% per year [94]. The Cd accumulation and seed cotton yield were significantly higher at a planting density of 4000 plants per acre than that at 1500 plants per acre, so increasing the planting density of cotton had an obvious effect on the removal of Cd in soil and increased the yield of cotton, and the ecological restoration effect and economic benefit were more significant [94]. Therefore, the use of cotton planting to repair Cd-contaminated soil is relatively simple in terms of technology and low in cost and can be implemented in large areas. The promotion and application of cotton varieties with high Cd accumulation in contaminated soil should heed the following aspects. First, the Cd-contaminated alternative cotton planting area was mostly rice fields, with thick soil and poor air permeability, and it was difficult for cotton to emerge, while some of the plants did not grow even to emergence. Second, years of rice cultivation have made it difficult for soil nutrients and physicochemical properties to meet cotton planting needs. Third, the high density direct seeding of cotton requires a large amount of seeds and a delay in seeding stage, and early maturing cotton varieties should be selected to ensure maturity before frost and to reduce seed costs and stabilize cotton production. Finally, the cotton stalks must be removed in time after the harvest to clean up heavy metals from the cultivated land. Therefore, planting cotton in heavy metal-contaminated areas should be based on the principles of ecological restoration, quality improvement and efficiency enhancement, focusing on the research on seedling emergence, field weeding and balanced fertilization.

6.2 Effective Use of Cotton Byproducts and Safe Disposal of Cotton Biomass

The main product of cotton is fiber (lint cotton), and cotton seed is the main byproduct of cotton production. For every 100 kg of cotton lint produced, there are approximately 200 kg of cotton seed output. Cotton byproducts, such as cottonseeds and cotton stalks, can produce more than 130 products in the light, textiles, chemicals, food, and medicine industries and are a major source of cotton production and income. Heavy metal is enriched in all parts of the plant when cotton is planted on heavy metal-polluted farmland. Cottonseed is used for oil extraction, production of ruminant feed and edible fungus culture medium, and from a safety point of view, if the heavy metal content exceeds the standard, development and application are not recommended. Cotton linter can be spun by roving and woven into cotton blankets, and velvets; can be processed with man-made fibers or for papermaking, etc.; and can be made into nitrocellulose and smokeless gunpowder or as a solid fuel for propelling rockets. Cotton stalks are rich in cellulose, lignin and polypentose and can replace wood to make board and building materials, such as particleboard, fiberboard and sound-absorbing board, and they can also be used as raw materials for papermaking and culture medium for edible fungus [95]. Therefore, the development and utilization of cotton planting and cotton byproducts in heavy metal-polluted areas will reap both ecological and economic benefits, which will help realize the promotion of cotton planting restoration models and further increase cotton planting profits.

The use of plants to remediate soil contaminated by heavy metals requires not only plants to have a high absorption and transport capacity but also plants to be removed and disposed of safely from the area to be repaired because the plants absorb pollutants. At present, research on the safe disposal of phytoremediation plants from contaminated soil is still in the initial stage, especially for heavy metal-contaminated phytoremediation plants; the disposal is relatively difficult, the technology is immature, and there are relatively few experiences to draw from. Under the principles of comprehensive utilization and coordinated development, cotton biomass can undergo disposal and utilization followed by industrial papermaking, use as a new energy material for extracting alcohol and oil, and recycled and degraded by composting technology using the metabolism of microorganisms, landfills and recovery of ashed biomass [96]. Among them, the recovery of ashed biomass can avoid secondary pollution of heavy metal accumulation because it consists of the incineration and storage of plant biomass; extracting heavy metals from ashed biomass for recovery turns waste into treasure and completely reduces heavy metal pollution [96].

7 Conclusion and Prospects

Heavy metal pollution is one of the new environmental pollution problems faced by farmland soils that needs to be resolved. It not only affects the growth and development of plants, resulting in the reduction of crop yield and quality but also enters the human body through the food chain, posing a serious threat to human food safety and health. Cotton has a large biomass and shows stronger and widespread resistance to abiotic stresses. It has a strong ability to absorb and accumulate heavy metal ions in the soil. Its main product, fiber, and byproduct, cottonseed used for edible oil, have low heavy metal content. The low heavy metal content in fiber and cottonseed does not affect the use of fiber and does not enter the food chain, providing new possibilities for the phytoremediation of soils contaminated by heavy metals. Therefore, cotton planting in heavy metal-contaminated areas can have both ecological and economic benefits, is one of the best measures to control heavy metal pollution, and has very important development prospects.

In-depth study of the adaptation mechanism and improving the adaptability of cotton to heavy metals are of double importance to ensure high yield of cotton and control soil contaminated by heavy metals. The toxic effects of heavy metals on cotton manifest in many ways, and the detoxification mechanism of cotton for heavy metals is also very complicated. It is a comprehensive effect of various physiological processes regulated by multiple genes, and further exploration is needed. On the one hand, in-depth study of the toxicity mechanism of heavy metals in cotton and the adaptation and tolerance mechanisms of cotton to heavy metals will reveal potential processes at multiple levels, such as morphology, physiology, molecular biology, and breeding new “repair type” varieties, and from multiple perspectives, such as genetics and cultivation, allow use for both contaminated soil remediation and cotton production. On the other hand, this knowledge will allow the cloning of key genes for hyperaccumulation and tolerance and the cultivation of genetically modified new heavy metal hyperaccumulation varieties using transgenic or genome-editing techniques with the help of synthetic biology methods. In short, the treatment and restoration of heavy metal pollution in farmland soil is a long-term process.

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