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Review

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Review

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Abstract: One of the most concerning global environmental issues is the pollution of agricultural soils by heavy metals (HMs), especially cadmium [1], which not only affects human health by taking Cd-containing foods but also impacts the quality of rice. The soil's nitrification and denitrification processes, coupled with the release of volatile organic compounds by plants, raise substantial concerns. The understanding of three aspects of paddy soil under conditions of heavy-metal pollution and water management is currently limited. In this review, we summarized recent literature related to the deleterious effects of Cd on both soil processes related to the N cycle and rice quality, particularly aroma, in different water management practices. Under both continuous flooding (CF) and alternate wetting and drying (AWD) conditions, cadmium [1] has been observed to reduce both nitrification and denitrification processes. The adverse effects are more pronounced in continuous flooding (CF) compared to alternate wetting and drying (AWD). Similarly, the alteration in rice aroma is more significant in AWD than in CF. The precise modulation of volatile organic compounds (VOCs) by Cd, remains unclear based on the available literature. Nevertheless, HM accumulation is higher in AWD conditions compared to CF, leading to a detrimental impact on volatile organic compounds (VOCs). The literature concluded that AWD practices should be avoided in Cd contaminated fields to decrease accumulation and maintain the quality of rice. In the future, rhizospheric engineering and plant biotechnology can be used to decrease the transport of HMs from the soil to the plant's edible parts.

Keywords: heavy metals; volatile organic compounds; N cycle; paddy soil; water management practices

Rice serves as a staple food for half of the global population, with numerous Asian and African nations, such as Madagascar and Liberia, exhibiting annual per capita rice consumption exceeding 100 kg. In Africa, the consumption of rice is going faster as compared to other commodities because of the growing urban population [2]. In 2023, about 523.1 million tons of rice were produced globally [3]. The majority of rice production comes from Asian countries, with significant contributions from Indonesia, Vietnam, Thailand, Myanmar, China, Bangladesh, India, the Philippines, and Pakistan, as reported by the FAO in 2023. Western and Eastern Asia together account for over 90% of both global

rice production and consumption. China's role in worldwide rice production stands at 41% [3]. This accounts for approximately 23% of China's total cultivated land[3,4].

Water is a crucial element for the growth and development of rice plants. Rice grown throughout the globe by different water management, like continuous flooding among them is the one that is count for the unproductive water loss by seepage, evaporation, and percolation [5–7]. Recent studies showed that continuous flooding conditions are optional for high yields in short-term trails. However, few long-term field studies have been conducted which provide a little information about crop yield during water-saving practices in the fields [5]. Because freshwater is distributed unevenly in terms of both space and time, farmers strive to conserve as much rainwater as they can. Farmers utilize this water for irrigating rice fields when the soil reaches a specific dryness threshold. Currently, the following rice irrigation modes are in use throughout China: Continuous flooding, midseason flooding with intermittent drying, midseason flooding with intermittent irrigation, midseason flooding with rain-fed conditions. The most popular method is flooding-midseason drying-flooding [5].

Nitrogen (N) cycling involves both biotic and abiotic transformations of nitrogen in paddy soils. These transformations encompass processes such as ammonification, N immobilization, nitrification, denitrification, anaerobic ammonium oxidation (anammox), dissimilatory nitrate reduction to ammonium (DNRA), and nitrogen fixation. In paddy soils, the importance of nitrification and denitrification are well documented and a recent addition to this is archaeal ammonium oxidation [8–10]. Reports indicate that the ammonia oxidization to nitrite, carried out by microorganisms encoding the enzymes of ammonia-oxidizing bacteria [11], serves as the initial rate-limiting step in nitrification) and/or ammonia-oxidizing archaea [12,13,14]. Nitrifiers microorganisms are distributed in few functional taxa while denitrifies bacteria are broadly distributed in many taxa which are responsible for the nitrate as an alternative electron acceptor for respiration [15].

Plants interact with plants and other living communities. Therefore, they have developed a variety of strategies. One of them is the diffusion of volatile organic compounds (VOCs); these signals provoke the defense mechanism against biotic and abiotic stresses and several other environmental factors [15]. Angiosperm and gymnosperms release a number of VOCs, such as fatty acids, amino acid derivatives, phenylpropanoids/benenoids, and terpenoids, which are most common [16–18]. Through priming, neighbors, plants developed stress resistance due to VOCs of the stress-exposed plants [19]. This phenomenon was established between tobacco (*Nicotiana attenuate*) and sagebrush (*Artemisia tridentata*) plants [20]. The former was exposed to the VOCs of the later clipped one. The clipped sagebrush mimics insect damage and tobacco-developed herbivore resistance.

Soil acts as a seed bank of microbes, and plant roots recruit the microbes from the soil. The presence of heavy metals in the soil leads to changes in both soil biomass and microbial community structure. In the rhizosphere of contaminated plants, roots exhibit a higher prevalence of heavy metal-resistant bacteria but a lower overall microbial population compared to the rhizosphere of unpolluted plants [21–24]. Within the soil, cadmium [1] induces alterations in microbial community structure, reduces microbial carbon use efficiency, and results in an increase in the microbial C: N ratio [25]. Recent studies in metals-polluted soil have focused on the microbiome of model plants such as *Arabidopsis thaliana,* metal accumulating or metal tolerant and hyper-accumulating plants [26–28]. Both, biotic and abiotic factors shape microbial community assembly in the plant rhizosphere [29]. The ecology of the rice microbial community under metal stress, particularly Cd stress, is the least studied area. The majority of metal-contaminated soil-grown plant rhizosphere consists of metal-resistant bacteria that can maintain metabolic functions [30,31]. *Thiobacillus, Pedobacter,* and *Geobacter* have been reported as high metal resistant bacteria genera responsible for Fe (III) reduction in the Cd contaminated rice rhizosphere [32–34]. However, some PGPRs such as *Dyella* and *Novosphingobium* colonized the rice rhizosphere [34]. Furthermore, the role of VOCs role in soil microbial N transformation also needs to be discussed in detail during HM stress.

Nitrification, denitrification, VOCs, and plant endophytes are very important factors in terms of plant health. In this review, we will focus on Cd detrimental effects on the N transformation, VOCs of rice, and endophytes under different water management practices.

2. Different Water Management Practices for Rice Cultivation

Globally, about 4 billion people are suffering from water scarcity [35]. Furthermore, for sustainable rice production, a shortage of water is a major threat. In order to ensure food security, alternative ways such as less water for rice cultivation are very important. Therefore, in modern agriculture practices water-saving irrigation for rice cultivation must be the point of concentration in future research. Various approaches and technologies can be taken into account, such as alternate wetting and drying (AWD), reducing the outflow of unproductive water, adopting direct seeding, implementing the rice intensification system, employing the rice aerobic system, embracing the ground cover rice production system, and utilizing tensiometers for scheduled irrigation. These technologies can improve water use efficiency and significantly decrease the demand for water for the production of rice.

2.1. Continuous Flooding (CF)

Warm and waterlogged soil is used for rice cultivation. Continuous flooding (CF) is a traditional rice-growing practice in which the rice paddies are flooded by the farmers throughout the rice-growing season. Globally, 70% of the rice cultivation is carried out under CF. CF based rice cultivation is highly productive but some problems related to soil health and environment have been reported. Soil degradation, elevated methane emissions, and the substantial accumulation of harmful substances like arsenic and mercury are primarily associated with rice cultivation using chemical fertilizer-based practices [36].

Lowland rice irrigation covers about 79 million hectares worldwide, with an estimated 75% world's rice production. The global crop irrigation area covered in Asia is approximately 56%, where 40-46% is rice cultivation [37]. About 64-83% of the irrigated area in the Southeast, 46-52% in the East, and 30-35% in the South Asia are occupied by rice cultivation. In comparison to other irrigated crops in the field, rice needs 2-3 times more water [38]. From developed 70% freshwater resources worldwide, rice irrigated receives 24-30% share.

Several factors, such as global warming, rice competition with other seasonal crops, industries, and civic requirements for water, may cause economic or physical water shortages for rice cultivation [39]. In the future, several million hectares of lowland rice irrigated systems will face water shortage by 2050 [5]. In account of all the issues related to the CF rice irrigation system, the development of water-saving irrigation system is compulsory for sustainable rice cultivation [5]. The International Rice Research Institute (IRRI) introduced alternate wetting and drying (AWD) water management technology, among other water-saving rice irrigation systems, to deal with expected water shortages in the future. AWD has got worldwide attention and several research studies are available that are becoming increasingly widespread [40].

2.2. Alternate Wetting and Drying

Developing agronomic practices that minimize water usage for irrigation while sustaining high yields is essential to support the increasing global population. To address the issue of water scarcity in rice agricultural production, alternate wetting and drying (AWD) water management practices are being adopted [36,41]. Under AWD conditions, rice fields are drained out for a certain period in which the field obtains a certain level of moisture and is then re-flooded. It has been reported that using AWD water technology saves about 23% of the water used for rice irrigation when compared to CF practices [6,38]. AWD technology has the potential to reduce greenhouse gas (GHG) emissions, particularly methane (CH_4) gas [36,42]. Alternate wetting and drying conditions decreased by about 45-90% global warming potential (GWP − CH_4 + N_2O) compared to CF. Human activities generate about 48Gt CO_2 annually, which can increase global warming. Therefore, to reduce GHG emissions from paddy fields, it is critical to promote AWD adaptation globally [43]. In the USA, for example, farmers received carbon credits under the project "Voluntary Emission Reductions in Rice Management Systems" involves farmers adopting practices aimed at minimizing greenhouse gas (GHG) emissions, with AWD being one practice [44]. On the other hand, AWD also reduced arsenic

(As) accumulation in rice grains [41,45] and methylmercury concentration in soils, and lowered the consumption of energy/fuel where pumping is in practice for irrigation [36,46,47]. Many efforts have been made to promote AWD in Asia because to its benefits [48]. In China, the "mid-season" drain is widely adopted which is somewhat similar to AWD [49]. However, global AWD technology adoption is limited due to a variety of factors limiting rice productivity [36,46,50].

Since the 1990s, China has embraced water-saving irrigation methods such as thin-shallow-wet-dry irrigation [51] and alternate drying-wetting [52,53]. Soil water management strategies influence oxygen transfer, nitrogen transformation, and soil redox potential [9,10,54]. Soil microbial biomass carbon (MCB) and nitrogen (MBN) have been significantly affected by irrigation practices [55,56]. In paddy soils, MBC and MBN decreased in continuous flooding irrigation as compared to intermitted irrigation [57]. Other studies have reported high N_2O emissions from paddy fields with intermittent irrigation and midseason drainage [58,59].

Soil moisture is very important during the rice grain filling stage. Grain quality and yield are both impacted by water levels during rice reproduction and the filling stage [60,61]. Both CF and AWD effectively enhanced the quality of rice grains [62]. For example, AWD improved grain head recovery, grain weight, and protein contents [63,64]. Similarly, rice kernel opaqueness, abortiveness, and chalkiness were reduced significantly in AWD [60,64]. In addition, AWD significantly increased the nutritional value of the rice grain such as high antioxidants, total tocopherols, γ-oryzanol, flavonoids, zinc and iron [65]. Furthermore, AWD significantly decreased arsenic in the rice grain [66].

2.3. Popular Methods around the Globe

Globally, rice production and greenhouse gas production are under the influence of two important factors i.e., water management and application of nitrogen fertilizer. China is one of the largest rice-producing countries; water shortage threatens rice cultivation. The most popular rice cultivation method is CF. However, due to water shortage and greenhouse gas emissions, nowadays different water-saving irrigation systems are in practice around the globe (**Table 1**). Among different water-saving irrigation, AWD is the most practiced technology for rice production.

However, different studies reported AWD decreased CH_4 and increased N_2O emissions. Moreover, while applying medium nitrogen (N), the optimal rice grain yield has been observed in the case of AWD in contrast to the conventional flooding-midseason drainage-flooding irrigation (PFD) approach. Different studies from Bangladesh, the Philippines, China, and Pakistan reported a 4.52% average increase in rice yield (t/ha) under AWD than CF. Similarly, water use efficiency (WUE) was also increased in AWD as compared to CF.

Another rice production system termed as [46] applied in different regions of the world. In ARS, rice is grown under non-saturated, non-flooded, and non-puddled soil conditions [67]. The yield obtained from ARS was higher than in upland conditions but lower than in flooded conditions. In cool temperate regions, ARS is a successful system for rice production. However, a partial aerobic rice system (PARS) is more suitable alternative for ARS in warm humid regions. There have been reports that ARS reduces rice yield over time, and it has been proposed that this technique may not be ideal for long-term rice agriculture [68]. Several factors among them are Zn, Fe, K, N, and P deficiencies, root-knot nematodes and weeds are responsible for the yield reduction in ARS [69]. However, it save about 50% of irrigation water compared to CF.

Table 1. Different strategies used for Water-Saving irrigation of Rice Production.

S. No	Technologies for Conserving Water in Rice Cultivation.	Reference
1	1. Surface Irrigation Techniques in Rice Cultivation.	[70–72]
	2. Drip Irrigation Methodology in Rice Cultivation.	[68]

	3. Sprinkler Irrigation Approach for Rice Cultivation.	[73]
2	1. Alternate wetting and drying (AWD)	[10,74]
	2. Soil water potential (SWP)	[36,75]
	3. Mulching Cultivation without Flooding	[76,77]
	4. Aerobic rice system [46]	
	5. Optimal Irrigation Regimen (OIR)	[73]
	6. Saturated soil culture (SSC)	[78]
	7. Field water level (FWL)	[73]
	8. Intermittent drainage (ID)	[79]
	9. Leaching and flushing methods (LFM)	[73]
	10. Conventional flooding-midseason drainage-flooding irrigation (FDF)	[1]

2.4. Heavy Metal Pollution in Paddy Soil

Cadmium [1] is considered one of the hazardous heavy metals that permeates the soil through various sources [9]. Its pollution is a severe global issue since it can build in agricultural soil and edible sections of crops, and eating those contaminated components can cause cancer in humans [10]. Various natural and anthropogenic sources like rock weathering, atmospheric deposition, urbanization, and wastewater irrigation, sewage sludge application, overuse of phosphate fertilization, mining, and smelting originate Cd contamination in the soil [80]. Geological parent material decides the background of Cd level. However, generally it has been reported a range from (0.06-1.1 mg kg^{-1}) with a 0.01 mg kg^{-1} lower and 2.7 mg kg^{-1} higher value [80]. However, due to human activities, the Cd level (up to 14 mg kg^{-1}) has been reported in several regions of the world. Several factors such as cultivars, crop species, and soil properties contribute to the accumulation of Cd in plants.

2.5. Sources

2.5.1. Natural Ways of Cd Origination (Geogenic)

Naturally, Cd is existing in all rocks in entire soils. Among the top 20 priority hazardous metals, Cd ranked 7th [81]. Different rocks have different Cd concentrations. For example sedimentary deposits rocks have higher Cd than igneous and metamorphic rocks [82]. Thus, the soils originating from sedimentary phosphatic rocks have more Cd due to high Cd concentration [83,84]. Where the soils develop on phosphorites, shales or carbonates then the geogenic soil Cd concentration range from 0.01-10 or 100 mg kg^{-1} [85,86]. Cd exhibits weak sorption to the surfaces of both organic matter and minerals in the soil. Cadmium from mineral lattices releases slowly by weathering of high-Cd parent rocks [87].

Soil Cd is hard to distinguish either via geogenic or anthropogenic origin. Generally, Cd enriched topsoil is assumed to occur via farm and atmosphere inputs. Nevertheless, this is not the case, as Cd is transported to subsoils through the biological cycle [88]. Furthermore, via vegetation, Cd in agricultural brings from subsoils to topsoil [89]. At a continental scale, the concentration of Cd in European soil is primarily influenced by geological factors rather than anthropogenic inputs, despite the widespread use of intensive fertilizers, urban wastes, and manures in agriculture [90].

2.5.2. Anthropogenic Sources of Cd Input

Human activities are the leading cause of worldwide Cd deposition in the environment, accounting for 13, 000 tons of the 30, 000 tons of Cd inclined to the environment each year [10,91]. In the agriculture soils, Cd had been introduced by various sources and its concentration is generally

lower than 0.2 mg kg^{-1}. The level of Cd in the agriculture soils increased due to long-term phosphate fertilizer application along with sewage water used for irrigation purposes [92]. Discharged wastewater, mining activities (exhaust and solid waste), the smelting sector, and atmospheric deposition, on the other hand, all contribute to Cd contamination of agricultural soil. Blast furnaces sludge and flue dust contain a relatively high content of Cd [93]. The oxidizable and residual fraction of textile dyeing plants, sludge, has been reported to consist of an average 3.72 mg kg^{-1} Cd [94]. In Changsha City which is the capital of Hunan Province of China, dust analysis showed that 50% Cd was insoluble and reducible fractions in PM 2.5 from re-suspension of dust, fuel combustion, vehicular emissions, and other pollution sources, speciation analysis during spring [95]. Nevertheless, the predominant source of Cd input into the soil arises from agricultural activities, such as the irrigation of wastewater. Unnecessary phosphate and organic fertilizer applications, and random disposal of untreated urban sewage sludge and garbage [96]. The issue of Cd input via phosphate fertilizer application in agriculture soils of China is controllable because naturally phosphate and Cd contents are low in the native phosphate rocks of China, which lead to import high-quality phosphate fertilizers which contain a high level of Cd [97]. The Cd content was about 7.5-156 mg kg^{-1}, 84-144 mg kg^{-1}, 9.5 mg kg^{-1}, and 24.5 mg kg^{-1} in imported ammonium phosphate, single superphosphate, normal superphosphate, and triple-superphosphate, respectively, which exceeded the standards [97]. Atmospheric deposition of Cd in paddy soil have been reported about 65-70% [98]. Similarly, fertilizers, particularly phosphate fertilizers and biosolid contribute 10-50% each to Cd accumulation in paddy field [99]. However, in China, animal manure about 55% contributed to total soil Cd inputs [100].

2.6. Chinese Paddy Fields and Heavy Metal (HM)

In china, paddy soil Cd contamination is a widespread problem. The Cd paddy soil issue is more sever in some mining and industrial regions of China. The range of paddy soil Cd contamination range from 0.01-5.50 mg kg^{-1}, with an average value of 0.23 mg kg^{-1} [101]. Hunan was reported with highest Cd (0.73 mg kg^{-1}), whereas Guangxi and Sichuan provinces were recorded 0.70 and 0.46 mg kg-1 paddy soil Cd. However, in 1990s, the background value of Cd in Chinese paddy soil was 0.1 mg kg^{-1} [98]. Paddy Cd contamination across China following the trend like Northwest > South central > East > Northeast > North [98]. In China, paddy fields are mostly contaminated by Cd due to the smelting of the ores of Cu, Pb, and zinc [91,102]. Cd contents of rice grains are greatly varying. Rice grains sampled from the field and market were reported to have raised levels of Cd, up to 7.0 and 1.24 mg kg^{-1}, respectively [102]. A study containing 100 rice grain samples in Hunan, one of the large nonferrous metals production zones, reported only 15% of rice grains meet the standard level of As, Cd and Pb [103]. Another study randomly collected 63 rice grain samples from the open markets of Jiangxi and Anhui, along with 7 samples from Guangdong., Hunan, and Jiangxi, and Cd level in about 70% of grain samples was found to be higher than the standard 0.2 mg kg^{-1} [104].

3. HM and Soil N Transformation

Rice plants prefer ammonium to nitrate. When both ammonium and nitrate are present, rice seedlings exhibit a faster uptake of NH_4^+ compared to NO_3^- [105]. From last half-century, due to the NH_4^+ preference of the rice, farmers used to cultivate rice in paddy fields, ammonium-based fertilizers like ammonium sulfate and urea are applied [105]. In addition, organic fertilizers for rice cultivation are also commonly applied in the fields, in which the organic N is converted to ammonium through a biological process called ammonification (mineralization of N). Due to the low level of oxygen or depletion in paddy soils, the rate of ammonification is slower compared with upland soils [106]. Depletion of O_2 in soils not only slow down the ammonification but also limits the process of ammonium oxidation (NH_4^+ to NO_3^-) and immobilization of N (microbial assimilation of NH_4^+ i.e., the conversion of inorganic N to organic N), which lead to the accumulation of NH_4^+ in soils [107].

3.1. The Significance of Nitrification and Denitrification Processes in Paddy Soils

The most limiting nutrient for rice production is generally known as N. Denitrification in paddy soils was initially studied in order to make the efficient use of fertilizers and link the gaseous products such as NO, N_2O, and N_2 with the microbial process involved in the reduction of nitrate [108]. The thin oxidized layer of paddy soils is the region where nitrification occurs while denitrification takes place below the oxidized layer [109]. The loss of N from paddy soils included ammonia volatilization, nitrate leaching and nitrification [110]. The denitrification occurred just following nitrification in paddy soils. It is proposed that ammonium fertilizers should be deeply applied before waterlogging (4-5 days) in paddy fields to overcome the N loss from the paddies [111]. N transformation on a microscale in the flooded soil surface layer reported a high number of nitrifiers and denitrifiers bacteria in the above 2 cm layer than the below-reduced layer of paddy soil [11,112]. After one to two weeks of waterlogging low-molecular-weight organic molecules (acetate) accumulated in the top layer which increased the denitrifiers bacteria. The correlation of organic carbon and denitrifiers bacteria in another environment along with lake sediments and river waters also have been reported [113]. The most important factor for the controlling of denitrification is the availability of organic C since it provides electrons donor for the denitrifiers. Rice plant roots release O_2 in the rhizosphere, therefore nitrification and denitrification activities were also reported for rice rhizosphere [114,115] and green algae which grown in rice fields [116], as well as in the bulk soil between the oxidized and reduced layers. The process of nitrification-denitrification in rice paddy soil may cause the loss of N from the applied fertilizer. Globally, 40% of the total greenhouse gas emission recorded from paddy soils. Different studies have been reported the little emission of N_2O from the paddy soil under control condition than the upland field crops [117,118]. Denitrification is a strong activity of rice paddy soils, as a result, little emission of N_2O occurred by conversion of N_2O into N_2 [119]. Furthermore, several studies on dissolved N_2O in the rice surface and groundwater have been conducted [120]. These findings suggested that little emission of N_2O gas from rice paddy soil occurred due to the N_2O reducers, present in the rice paddy fields [121]. However, recent findings suggested that iron plague in the rhizosphere contribute largely to N_2O emission that was previously ignored [122].

3.2. Nitrification

Nitrification is a totally microbial mediated process in which nitrate (NO_3^-) formation takes place by ammonium (NH_4^+) oxidation via nitrite (NO_2^-) [9,123,124] (Figure 1). This is the energy acquiring process for autotrophic nitrifiers. The process in which NO_3^- or NO_2^- formation takes place from inorganic N (NH_4^+) and organic N without energy acquired by microbes is known as heterotrophic nitrification and the microbes termed as heterotrophs [123].

Nitrification has been divided into two steps: the oxidation of ammonia ($NH_4^+ \rightarrow NO_2^-$) and nitrite oxidation ($NO_2^- \rightarrow NO_3^-$). *Nitrosospira spp.* and *Nitrosomonas spp.* of *Betaproteobacteria* and *Nitrosococcus spp.* of *Gamma proteobacteria*, these bacteria are known as ammonia-oxidizing bacteria [11] can carry out the oxidation of ammonia. Archaea, along with bacteria can also take place in ammonia oxidation which is termed as ammonia-oxidizing archaea [12] [125,126]. Different studies have been reported a higher number of AOA than AOB in the ocean [127]. A positive correlation of AOB abundance was observed with rice paddy nitrification activity soil which indicates that AOB could be the major players of nitrification than AOA [128]. However, with the application of urea fertilization increased the abundances of both AOA and AOB in the rice rhizosphere [129,130]. Recent reports showed, comammox relative contribution to nitrification is higher than AOA and AOB in the rice paddy soil [128].

The diversity of ammonia-oxidizing bacteria [11] communities has been investigated in rice paddy soils [131]. The rice genotypes can also influence the diversity of AOB [132]. In China, rice paddy soil the *Nitrosospira spp.* are the most dominated AOB [133–135], while biochar amended rice paddy soil favored *amoA* gene containing AOB just related to *Nitrosomonas spp.*, [136]. Among the other factors, fertilizer practices may change the AOB community in the soil because of the environments with high ammonia concentration (like wastewater) *Nitrosomonas spp.* predominates

while in the soil *Nitrosospira spp.* is the major AOB [137]. Nitrite-oxidizing-bacteria (NOB) form the genera of *Nitrobacter*, *Nitrospina*, *Nitrococcus,* and *Nitrospira* perform the nitrite oxidation which is the second step of nitrification [138,139].
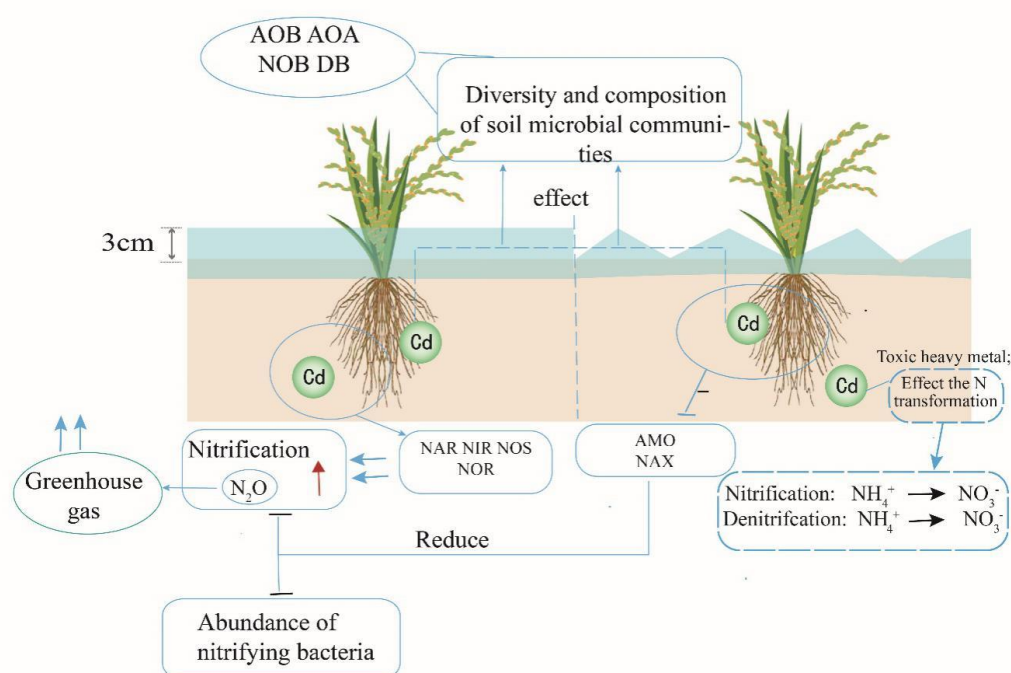

Figure 1. Paddy soil N transformation under different water management practices and Cd contamination.

3.3. Denitrification

Denitrification is a process in which gaseous form of N (NO, N_2O and N_2) forms via the stepwise reduction of (NO_3^- and NO_2^-) by microbial respiration (Figure 1). Microbes utilize nitrogen oxides as electron acceptors in lieu of oxygen when operating under anaerobic conditions for respiration.

Nitrous oxide also acts as an electron acceptor but non-denitrifiers reduce N_2O [140]. Two types of nitrite reductases are responsible for denitrification i.e copper-containing NirK encoded by gene *nirK*, and NirS cytochrome cd1-containing enzyme encoded by *nirS* gene [12]. These enzymes transform oxidized ammonia to NO, and N_2O. Finally nitrous oxide reductase enzymes encoded by *nosZ*, reduces N_2O into N_2. Nitrite reductases (*nirK* and *nirS*) gene related community has been studied in detail [12,141]. In the rice paddy soil, the average gene copy number of *nirK*, *nirS*, and *nosZ* were 7.65×10^{-7}, 3×10^{-7}, and 8.71×10^{-7}, respectively [9] 16S sequencing approaches in paddy soil, revealed that denitrifiers community genes are distributed in *Betaprotobacteria*. The phylum was further divided in to *Azospira* and *Burkholderiales*, belonging to the genus *Herbaspirillum*, and *Rhodocyclales* in the rice paddy soil under denitrifying conditions [142].

The soil type, plant genome, pH, Eh, organic matter, temperature, substrate, and fertilizers affects the response of microbes towards denitrification. For example, with increasing temperature, increases the denitrification rate in paddy soil [143,144]. Similarly, high moisture also increased denitrification [144]. However, opposite is true for N fertilizer. N fertilizers increased the *nirK* gene abundances and decreased *nirS* in paddy field [141].

3.4. Production of N_2O

Nitrous oxides production occurs through different sources in the soil, incomplete denitrification can lead to the production of N_2O ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$). The lack of N_2O reducing enzymes in the denitrifiers can also lead to the production of N_2O [145], one such example

is the denitrification activities of fungus, in which N_2O is the major end product. Not only denitrifiers are the only source of N_2O, but some ammonia oxidizers are also the source of N_2O production either through hydroxylamine oxidation or nitrifier denitrification [146]. Both AOA and AOB have been reported for the nitrifier denitrification because they are positive for nitrite reductase gene (*nirK*). These microbes first perform the activity of ammonium oxidation then oxygen-depleted environment converts the product into N_2O and N_2 which is termed as nitrifier denitrification or the reduction of nitrite [147]. One of the intermediate products of ammonia oxidation is hydroxylamine, during the oxidation of hydroxylamine, N_2O produced as a byproduct. A study reported both AOA and AOB for the N_2O is production via the hydroxylamine oxidation pathway. In addition, dissimilatory nitrate reduction to ammonium (DNRA) bacteria has been reported for N_2O [148]. Non-denitrifiers can also produce N_2O by the reduction of nitric oxide as detoxification of toxic NO molecule. In this process, quinol-type nitric oxide reductase (qNor) has been reported instead of denitrifiers containing cytochrome c-type nitric oxide reductase (cNor) [140,149]. Chemodenitrification has also been reported in which nitrite is reduced to NO and N_2O [150].

3.5. Reduction of N_2O

Though different way N_2O produced in the soil can be used as electron acceptors by different N_2O reducer's microbes for respiration which contains the N_2O reductase (*nosZ*) [151,152]. The ability of N_2O reduction into N_2 had been reported in a phylogenetically diverse group of bacteria and archaea. This reduction of N_2O is the part of denitrification but some denitrifiers lack the enzymes to reduce it into N_2 gas. In addition, the reduction of N_2O can also be done by some DNRA bacteria in the rice paddy soil [153]. Both culture-dependent (FSC) and culture-independent [90] analyses were used for the detection of N_2O reducers in the rice paddy soil [154,155]. *Herbaspirillum sp.* strains were grown under the conditions which exist during rice paddy soil denitrification and were reported as the active player of N_2O reduction step of the denitrification [156]. *Herbaspirillum* and *Azospirillumcan* had been reported as denitrifiers recently, depending on the environmental condition these bacteria may be acting like N fixers or N losers from the soil just like legume symbiont *Bradyrhizobium* which perform both processes (N fixation and denitrification) [157,158].

3.6. Effect of Cd on N Cycle in Paddy Soils

For bioavailability, different definitions exist but it can be defined as the chemical availability to the living receptors either directly or through uptake (Figure 1). The main concern during metal contamination in the soil is the bio-available portion of metal not the total metal concentration because only the bio-available portion uptake is possible for plants from the soil [159]. The most important factors which control the availability of the metals in the soil are pH and organic matter [160,161]. In the rhizosphere of the plant, heavy metals bioavailability depends on soil type, plant genotype, metals speciation, and the change in biological and physicochemical of the soil induced by roots [162,163]. Spatially and temporally rhizosphere varies which acts as a dynamic habitat [164]. The rhizosphere is the most complex hot spot which is comprised of a diverse plant-associated microbial community, responsible for the metal mobilization and immobilization in the soil either directly or indirectly [165,166]. The mobilization of metals occurs through the release of chelators or redox changes done by the microbial processes in the rhizosphere such as chelation, chemical transformation, and soil pH reduction via protonation, while the immobilization of metals occurs in the rhizosphere by biosorption or bioprecipitation via microbes [167,168]. In a recent study reported higher metals-tolerant bacteria in the rhizosphere microbial communities [169].

3.6.1. Nitrification

Soil physical and chemical properties of soil influence the microbial-mediated processes rates in the field environments [170–172]. Nitrification and denitrification processes are not only affected by the soil pH but a recent study reported that soil NH_4^+-N and C/N also responsible while changing the abundances of soil microbial communities [173]. The effect of metals on ammonia oxidation depends

upon the specific metal or combination of metals in the environments like no change in the abundance of AOA was reported in mercury-spiked soil used for vegetable cultivation while Cu spiked up to 1000 mg kg^{-1} changed the AOA community in the grassland and arable soils [174,175]. A study has reported that Cu was more toxic to the ammonia oxidizer community as compared to Ni. For example, Cu strongly affects the AOB community in the sludge-amended soil and adaptability was more common to Zn and Ni [176]. However, general microbial community was shown more vulnerable to Cu and Cr contamination as compared to Zn and Ni as well [177]. Heavy metals pollution had a negative effect on the abundance, diversity, activity, and composition of AOA and AOB in the natural soil [10]. Another study reported a higher copy number of AOA than AOB in the rice paddy field, contaminated with heavy metals and the ratio of AOA to AOB copy number was in the range of 2.5 to 59 [175].

3.6.2. Denitrification

Denitrification is a heavy metal sensitive process and the heavy metals concentration in the soil directly proportional to its inhibition [178]. Heavy metals change the denitrifiers in the soil which reflect the inhibition of denitrification (Figure 1). A recent study reported a significant change in the composition of the denitrifiers in the metals polluted paddy soil via denaturing gradient gel electrophoresis (DGGE) [179]. In addition, in the rice paddy soil, the microbial community has been reported to adopt the metal stress by changing the abundances of certain microbial taxa. Not only the abundance but also the functional potential has been reported altered in the metals polluted paddy soil. The replacement process is the main factor for the adaptability of heavy metals of the microorganisms while soil pH variation is totally responsible for the community richness[180]. The negative effect of metals on the abundance of denitrifiers was also varied with the elements. The copy of *nirK* and *nosZ* genes sharply decreased with the spiked Cu in a 6 days lab incubation experiments but a recovery in copy number of the denitrifiers genes has been reported for long time incubation [181]. The heavy metals pollution especially the Cd decreased the abundance, diversity, and structure of both nitrifiers and denitrifiers in the rice paddy soil [182].

3.6.3. Greenhouse Gases from Paddy Fields under Metals Contamination

The application of N fertilizers is essential for rice production with high yield. However, due to the overuse of N fertilizers, the production of GHG such as nitrous oxide (N$_2$O) is one of the major concerns [183,184]. Different studies have addressed these problems by specifying which soil types are more prone to N losses by describing many environmental and management factors linking to biological nitrogen transformation [185,186]. However, the question, how heavy metals pollution could affect the biological transformation in the soil is still on the list to answer, especially, the introduction of heavy metals through atmospheric deposition versus spiked metals into the soil which suddenly increases the heavy metals concentration in the soil [176,187]. Another issue is the linking between changes in N transformation genes abundances and heavy metals contamination in soils. It will become more difficult to address this issue in rice paddy soils where significant changes occur in the redox potential due to wet/dry cycles which affect the heavy metals bioavailability, structures of microbial community and rate of biological transformations of N [180,188].

Among all the green GHG, N$_2$O is the most radiative GHG increasing 0.26% in the atmosphere per year [180]. Incomplete denitrification results in the production of nitrous oxide in which the last reduction step fails to reduce the N$_2$O into N$_2$ in the soil. It had been reported that about 60% of the total N$_2$O globally is produced by anthropogenic sources with the major players is known as paddy fields [189,190]. China's paddy fields produced up to 29.0 Gg of N$_2$O emissions which are about 7-11% of total GHG emission from China's croplands [51,191]. Recent studies have shown that in metal-polluted paddy soils, microbial biomass and fungal to bacterial ratio are declined with an increase in the concentration of heavy metals, which could lead to changes in carbon (C) cycling. Heavy-metal contamination in soil could affect the rates of microbial-mediated biogeochemical processes. For example, soil nitrification rates were suppressed by metal salts such as CdCl$_2$ and ZnCl$_2$, both in spiked soil samples in short-term studies and in polluted fields where metals typically accumulated

at a slower rate [192–194]. In a study using surface wetland sediments spiked with multiple metals, total denitrification activity was inhibited by Cd (30.9%), Zn (24.9%) and Cu (18.9%) over a period of 7 days of incubation, with Cd being the strongest inhibitor[195]. Heavy-metal pollution was found to affect the activities of denitrifying bacteria and ammonia oxidizers in soil [196].

3.7. Different Forms of Cd and Its Mineralization under the Change in Redox Conditions of the Soil

Various forms of heavy metals are consistently found in the soil, each exhibiting distinct levels of toxicity, bioavailability, and solubility such as 1) insolubly precipitated with other soil, 2) The structural elements of the lattices within clay minerals, 3) Substitutable in both organic and inorganic components, and, 4) dissolved (in soil solution) [197]. The properties of soil such as pH, soil redox potentials (Eh), cation exchange capacity, and organic matter contents, Mn and Fe oxides, clay minerals, and calcium carbonate [9]. Changes in soil Eh impact the bioavailability of Cd due to alterations in redox status and variations in electron acceptors. In aerobic, oxidizing conditions, Cd commonly exists as a soluble salt and cationic form, specifically Cd^{2+}. However, in anaerobic situations with a low redox potential, Cd tends to exist as precipitated species like CdS and $CdCO_3$ [198]. Conversely, alterations in redox potential influence both the organic matter and mineral components of the soil [100]. Furthermore, soil redox conditions regulate the microbial community characteristics and functions [199,200].

The soil moisture regime regulates both soil Eh and biological activity. Paddy soil typically experiences saturated conditions. Heavy metals are found in solid-phase components, and the introduction of heavy metals to paddy soil leads to their transition from more labile fractions to less labile fractions [201,202]. At field capacity moisture, Cd exhibited lower stability when compared to saturated conditions [203]. The saturated regime resulted in more complete transformation of metals toward the stable fractions than the field capacity regime [203]. Soil kept dry exhibited significantly higher soluble concentrations of Cd, Cu, and Zn than the field capacity and the saturated treatment [204].

Management practices such as air drying cycles are pertinent to soil moisture conditions, influencing the exchangeable fraction of heavy metals in the soil. Air drying enhanced Cd desorption in soil and the leachability of metals from dredged canal sediment increased during drying and oxidation processes [205,206]. Drying the soil in an oven resulted in notable rises in the concentrations of dissolved copper (Cu) and nickel (Ni). This outcome can be attributed to the complexation of these metals with increased levels of humic acid and fulvic acid in the soil [207]. Air drying enhances the extractability of manganese [118], iron (Fe), copper (Cu), and zinc [91] at low-incubation moisture levels, while reducing the extractability of metals at high-incubation moisture levels [208]. In paddy soil incubated under three distinct moisture regimes, the exchangeable fraction of copper (Cu), lead (Pb), and cadmium [1] exhibited the following order: 75% field capacity > wetting–drying cycle > flooding. Conversely, the reducible fraction followed the reverse order [209,210]. Eh stands as a crucial factor influencing the mobility of metals in soil or sediment, and is closely tied to soil moisture regulation [211]. Under oxidizing conditions, there is a discernible likelihood of significant heavy metal release [212].

4. Toxic Effects of Cd on Rice

Elevated cadmium [1] concentrations have the potential to impede plant growth, reduce grain yield and quality [212]. This can result in modifications to plant tissues, and disrupt the process of photosynthesis [212], and regulating expressions of at least 36 proteins in rice [213]. Accumulation and transport of Cd occur in various organs and cell organelles, once it enters into the rice [214]. Different researchers have reported the toxic effects of Cd on plant growth [215,216]. Stunted growth and chlorosis are the most typical Cd toxicity symptoms [217]. Due to the direct and indirect interaction of the high level of Cd in the leaves, chlorosis (chlorophyll loss) takes place. The change in physiological activities of plant-like respiration, cell proliferation, and photosynthesis indirectly [217], nitrogen metabolism, plant water relationship, and mineral nutrition under direct Cd stress had been reported, which resulted in poor growth and low biomass of plants [218]. Sensitivity to

drought, enzymes inactivation, and damages to membranes of plants have been well-establishing facts of Cd toxicity [219]. It stops indirectly the photosynthesis of the plant by closing the stomata due to water conductance which reduces the access of CO_2 to the plant [220]. Directly, Cd prevents the photosynthesis by affecting electron transport, chloroplast organization, chlorophyll biosynthesis, and activity of Calvin Benson Bassham cycle enzymes [221,222].

The general symptoms of Cd toxicity in rice plants include growth inhibition, chlorosis of leaves and sheaths, brown root system, and wilted leaves [203,223]. Different metals-sulfide complexes make brown pigment which deposits on the surface of rice roots [224]. The production of lipid peroxides is a response to Cd toxicity in rice plants. In addition, the uptake of plant essential elements such as Fe, Zn, Mn, and Cu has also been reported to be affected by Cd toxicity in rice plants [225,226]. In addition, the light-harvesting center and photosystem II along with chlorophyll metabolism in rice leaves are affected by Cd [227].

5. HM and VOCs in Paddy Soil under DWMP

Various water management practices influence the Cd uptake in rice (Figure 2). Volatile organic compounds (VOCs) emitted by vegetation encompass a wide array of chemical molecules, predominantly comprised of isoprene, monoterpenes, and methanol [15,228]. In any case, the variety of plant-emitted VOCs depends on the plant species, the distinctive parts of the plants, or the growth conditions [229]. Plants can emit higher quantities of these molecules by directing a greater amount of carbon into stress-triggered volatile organic compounds (VOCs) when subjected to attacks by pathogens or herbivores. Additionally, exposure to various abiotic stresses such as UV radiation, ozone, drought, eutrophication, warming, and overall oxidative stress can also contribute to the increased release of these compounds [230–233]. Actually, many VOCs of the family of terpenes, such as isoprene and monoterpenes, are antioxidants and can protect cells from oxidative stress[233,234].
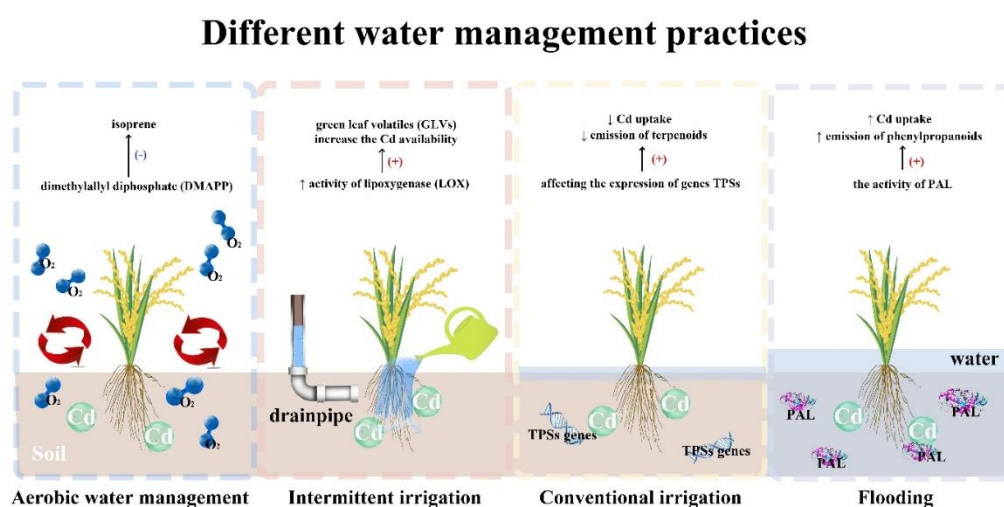

Figure 2. Effects of SWMP on rice VOCs under Cd contamination.

Under aerobic irrigation of rice, Cd uptake and isoprene emission decreased [235,236]. Isoprene (2-methyl-1, 3-butadiene) is an important plant VOC known for its plant protection role against numerous abiotic stimuli, including heat [237] and drought stress [238]. Cd decreases the activity of isoprene synthase, an enzyme responsible for conversion of dimethylallyl diphosphate (DMAPP) to isoprene [239,240]. Similarly, One class of VOCs, that Cd amplified was green leaf volatile (GLVs), which play a part in plant defense [239,241]. Furthermore, Cd contamination modulating the emission of terpenoids and expression of genes encoding terpene synthases (TPSs). TPSs is responsible for different terpenoids formation utilizing isopentenyl diphosphate (IPP) and DMAPP [239].

5.1. Volatile Terpenoids

The most abundant (more than 40,000 individual compounds) and structurally diverse group of floral volatiles are called terpenoids [242]. Terpenoids are essential for the growth and development of plants. Terpenoids are the components of abscisic acid (C15), membrane structures (sterols C30), photosynthetic pigments (carotenoids C40), involves in mitochondrial electron transport chain, and gibberellins (C20). Several plant secondary metabolites are terpenoids, such as mono-, sesqui- and diterpenes play important role in plant-plant interactions and plant-environment interaction [243].

Sesquiterpenes, Isoprenes and monoterpenes are known as volatile terpenoids and the largest class of plant volatile compounds (PVCs). They exist in almost every part of the plants including seeds, fruits, leaves, and roots. However, the predominantly PVCs are floral volatiles called lipophilic liquids. Floral volatiles are low molecular weight and high vapor pressure at ambient temperatures [243,244]. There is a link between the PVCs and plants pollinators [244,245]. They attract pollinators such as bats, birds, mammals, insects, and other animals as vectors for pollens transfer [246]. For Long range communication and evolutions of flowering plant, floral scent is an important source of communication [247]. Floral volatiles changes the behavioral response in respective pollinators. Floral volatiles upon emission guiding the pollinators towards the flowers. For example, more volatile terpenoids emitting by *latifolia* and *Petunia axillaris*, moths pollinated than the same genus *Petunia integrifolia* and Silene dioica a day-emitting bee-pollinated [243,248]. Cd inhibit terpenoids by interfering with enzymes responsible for terpenoid production production, such as geranylgeranyl diphosphate synthase (GGPPS), 1-deoxy-D-xylulose-5-phosphate synthase (DXS), and 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR). In rice, Cd reported to induce oxidative stress and damage the cellular membranes. Cellular membranes are mostly composed of sterols and carotenoids i.e., terpenoids. Furthermore, Cd in rice disrupting the hormonal balance and signaling pathways responsible for terpenoids metabolism [249].

5.2. Effects of Heavy Metals on Rice Fragrance

Aromatic 2-acetyl-1-pyrroline is the main ingredient in rice fragrance (2AP). Two genes, betaine aldehyde dehydrogenase (*BADH1*) and *BADH2*, functioned in the 2AP biosynthetic pathway. The exact mechanism of Cd affecting these genes is not well studied. However, studies reported that Cd may interfere with the expression and functions of *BADH1* and *BADH2*, limiting rice yield and quality. For example, upon exposure to Cd, the expression of *BADH1* and *BADH2* in rice seedlings. Similar Cd lowered the activity of enzymes encoded by these genes *BADH* and betaine aldehyde synthase (Figure 3) [26,250]. These enzymes are in responsible for converting the aldehyde betaine into glycine, a precursor of 2AP. Glycine is a compatible solute which protect plants from stress. The decreased activity of these enzymes lower the levels of glycine betaine and higher levels of betaine aldehyde leads to plant toxicity. Furthermore, the accumulation of 2PA accumulation due to Cd in the grain also affected the rice fragrance [250].

The duplication of the *BADH2* gene in rice decreased Cd accumulation in rice root and shoots under salt and Cd stress. Duplication increased the expression and activity of *BADH2*, which uptake more manganese, a micronutrient and competitor with Cd for transport in rice cell [251]. Furthermore, the introduction of the *BADH2* gene duplication into various rice cultivars reduced Cd accumulation without impacting yield or grain quality [251]. Similarly, 96 rice accessions were found to have 12 single nucleotide polymorphisms (SNPs) in six cadmium-related genes, comprising *BADH1* and *BADH2* [99]. SNPs are linked with the Cd accretion in rice grains. In rice breeding practices, SNPs might be used as molecular markers for low-Cd accumulating rice cultivars.
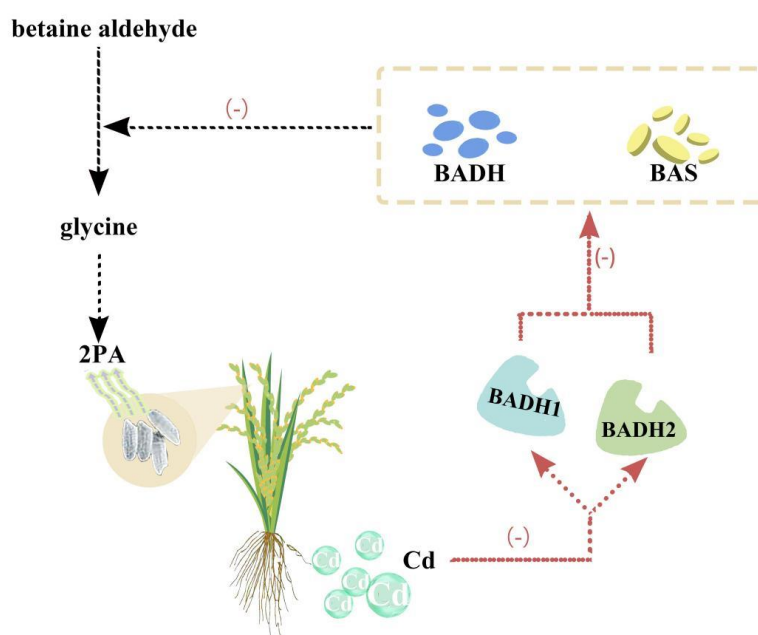
Figure 3. The effect of Cd on rice genes responsible for fragrance.

6. Conclusions and Future Directions

Cadmium contamination poses serious threats to rice agriculture and paddy soil N transformation. The N transformation is severely affected in AWD practices than CF. Similarly, Cd interferes with the signaling and synthesis of plant cells that are responsible for VOC production and aroma. High Cd deposition in rice cells inhibits VOC formation; nevertheless, the precise mechanism by which VOC synthesis is modulated by DWMP remains unknown. This may compromise plant defense, induced by VOCs and rice aroma as well. The issue of rice aroma, quality, and yield can be mitigated in future breeding procedures by introducing low-Cd absorption rice cultivars and utilizing water-saving technology.

Currently, Cd accumulation in grains is reduced via agronomic parameters (soil improvement, water, fertilizer, tillage management) and applying silicon and abscisic acid externally. Another strategy is microbial and phytoremediation. However, these processes are cost-effective and laborious with low efficiency. Therefore, screening and breeding low Cd accumulation rice varieties is the only choice considered economical and effective. Crossbreeding is very effective in transferring low Cd gene pool to offspring. However, molecular marker assistant breeding can reduce the screening process in breeding and save time. In addition, mutation breeding also helps to develop low Cd accumulating variety quickly. However, molecular marker assistant breeding can reduce the screening process in breeding and save time. CRISPR and hybridization technologies can also be used to develop new low-Cd accumulator rice verities. Adaptations of such techniques by the breeders may develop rice variety that can be grown in Cd-polluted paddies but will maintain the aroma and grain quality.

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