Supporting Information (SI)

Context-Dependent Zinc Regulation of Cadmium Uptake, Transport, and Accumulation in Rice

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Text S1. Literature Search and Evidence Selection

This review adopts a narrative synthesis combined with mechanistic integration. Literature searches were performed using the Web of Science Core Collection, Scopus, PubMed, ScienceDirect, and Google Scholar. The search covered publications from 2000 to 2026 and was updated on 6 October 2026, with priority given to recent reviews, field studies, and mechanistic investigations published during 2022–2026. English-language, peer-reviewed publications were included. Core search terms included "zinc", "cadmium", "rice", "*Oryza sativa*", "Zn–Cd interactions", "paddy soil", "Cd availability", "competitive sorption", "redox cycling", "flooding and drainage", "metal sulfides", "sulfide voltaic coupling", "root uptake", "vacuolar sequestration", "root-to-shoot translocation", "metal transporters", "node redistribution", "phloem transport", "foliar zinc", "grain cadmium", and "zinc biofortification".

Approximately 100 candidate publications were initially screened for relevance. Following relevance assessment and supplementary searches, more than 70 publications were selected for this narrative synthesis, including primary studies, reviews, and a published meta-analysis. The inclusion criteria prioritized studies reporting Zn–Cd interactions, soil Cd availability, root uptake and sequestration, transporter function, vascular redistribution, grain Zn/Cd accumulation, and Zn-management outcomes. Studies were excluded if they focused on water treatment, industrial remediation, or general toxicology without direct relevance to soil–rice systems. Non-rice studies were retained only when they provided directly relevant geochemical mechanisms or explicit cross-species comparisons. Mechanistic support and agronomic applicability were assessed separately. Functional genetic studies and transport assays, soil incubations, and tracer studies informed mechanistic interpretation, whereas pot and field interventions informed treatment responses under specified growing conditions. Transporter expression and stable-isotope fractionation supported pathway interpretation, while transporter-specific causal claims were assessed against functional evidence. Field-survey associations were distinguished from experimental treatment effects, while tissue concentrations, total amounts, and translocation factors were interpreted separately from measured transfer rates. This review did not perform a new quantitative meta-analysis; the cited meta-analysis was interpreted with attention to uncertainty and heterogeneity.

For the mechanistic synthesis, studies were grouped according to soil Cd supply, root uptake and sequestration, root-to-shoot transport, and grain-directed allocation. Contrasting grain-Cd responses were interpreted in relation to the processes that restricted or facilitated Cd transfer under the conditions examined.

Table S1. Detailed study conditions, measured responses, and evidential scope of studies discussed in the review of Zn–Cd interactions in rice.

Study, system, and genotype	Zn/Cd exposure and experimental conditions	Measured responses	Interpretation and evidential scope
A. Soil interfaces, redox cycling, and sulfide interactions			
Ming et al. (2016) [1] Batch sorption; two Australian soils	Single- and dual-metal solutions, 5–200 μM; acidic Ultisol (pH 5.34) and alkaline Alfisol (pH 8.44)	Single-metal Langmuir affinity constants (K_L; $L \cdot kg^{-1}$): alkaline soil, Zn 8.85 and Cd 1.79; acidic soil, Zn 0.19 and Cd 0.07. Competition appeared at lower concentrations in the acidic soil; combined retention was comparable to single-metal retention	Mechanistic soil evidence; competitive sorption can increase Cd mobility. The soils differed in properties other than pH; no rice endpoint
Yang et al. (2020) [2] Regional survey; rice	358 rice fields over three years; variation in soil Zn and Cd; pH groups below and above 5.9	No detectable overall soil-Zn effect on rice Cd accumulation at pH < 5.9; site-dependent relationships at pH > 5.9	Field association, not a Zn-treatment experiment. The pH grouping is study-specific; no universal rice Cd:Zn threshold
Yang et al. (2020) [2] Regional survey; wheat	206 wheat fields over three years; soil Cd:Zn ratios as reported by the authors	Zn-associated inhibition of Cd uptake and translocation at soil Cd:Zn ≤ 0.0083	Wheat-specific field relationship; this threshold requires independent validation before use in rice management
Wang et al. (2018) [3] Southern-China paddy field study	Soil Zn, Cd, and Mn variation; acidic pH range 4.8–5.8. The study also included a separate liming intervention	Higher grain Cd was associated with higher soil Zn and lower soil Mn; the pH–grain Cd relationship was not significant within the examined range	Zn/Mn associations do not establish a causal Zn-fertilization effect. Liming results must be interpreted separately
Wang et al. (2022) [4] Incubation; three contaminated paddy soils	40 days flooding followed by 20 days oxidation; Fe/Mn dynamics and labile Cd monitored	Labile Cd decreased during flooding and remobilized during oxidation; Fe-phase changes and declining pH accompanied these responses	Mechanistic redox evidence; establishes the Cd supply on which Zn acts, rather than testing a Zn-management effect
Yan et al. (2021) [5] Soil incubation and separate rice-pot experiments	Spectroscopic incubation used Cd-spiked soil (165 $mg \cdot kg^{-1}$); separate pots used soil containing 2.1 mg $Cd \cdot kg^{-1}$ and two rice cultivars; flooding followed by drainage	Prolonged flooding decreased Cd availability; drainage increased Cd release, associated with declining pH and oxidation of reduced Cd-bearing phases	Controlled evidence. High-Cd incubation and lower-Cd plant experiments are distinct; spectroscopic exposure is not representative of ordinary field concentrations
de Livera et al. (2011) [6] Soil-suspension reaction cells	Anaerobic incubation for 30 days in laboratory-spiked Griffith soil and 42 days in field-contaminated ChangSha soil, followed by 14 days of aerobic oxidation; sulfate and Zn additions	Without added S, dissolved Cd changed little, while Fe and Zn declined. ≤34% of added S precipitated as sulfides; sulfate reduced Cd solubility. Added Zn lowered dissolved Cd ratio by maintaining higher dissolved Zn	Mechanistic geochemical evidence; a change in Cd:Zn does not necessarily indicate a change in Cd itself. No grain endpoint

Table S1. *(Continued)*.

Study, system, and genotype	Zn/Cd exposure and experimental conditions	Measured responses	Interpretation and evidential scope
Fulda et al. (2013) [7] Contaminated-soil incubation	40 days reduction followed by 28 days reoxidation; reducible sulfate and Cu supply varied	Adequate sulfate favored Cd-sulfide formation and lower dissolved Cd; limited sulfate or greater Cu constrained immobilization; reoxidation increased Cd solubility	Sulfur availability relative to competing chalcophile metals is a boundary condition; no direct Zn-fertilization or grain endpoint
Hashimoto et al. (2016) [8] Reduced-soil spectroscopy	Zerovalent Fe amendments with contrasting S contents; selected soil particles analyzed by Cd K-edge μ-XAFS	Microscale CdS fractions were 64–98% with high-S zerovalent Fe, compared with 20–87% with low-S material	Mechanistic evidence for S-dependent CdS formation. These are selected microscale fractions, not whole-soil CdS percentages; not a Zn trial
Furuya et al. (2016) [9] Incubation; two Japanese paddy soils	Contrasting S levels; soil spiked to 150 mg $Cd \cdot kg^{-1}$; 29 days reduction followed by 20 days oxidation	CdS reached approximately 90% in the high-S soil but did not exceed 35% in the low-S soil. Reoxidation dissolved CdS and increased Cd solubility above soil-specific Eh thresholds	Mechanistic speciation evidence at high experimental Cd loading; S status and redox history condition Cd immobilization and release
Khaokaew et al. (2012) [10] Cd–Zn-contaminated soil; XAFS and release kinetics	Alkaline and acidified soils; contrasting flooding/drainage conditions; 2 h desorption tests	Little change in Zn speciation or release; Zn-layered double hydroxides and Zn-phyllosilicates predominated; less than 22% of soil Zn desorbed	Boundary evidence: total Zn can include stable pools that contribute little to dissolved-metal competition or sulfide formation
Huang et al. (2023) [11] Paddy-soil-derived SRB enrichment cultures	Cd-containing cultures with Zn^{2+} and/or Fe^{2+}; anaerobic growth followed by reoxidation; Eh fell below −100 mV and pH approached neutrality	CdS formed near cells; Zn/Fe additions enhanced sulfide production, polymetallic-sulfide formation, and Cd removal from solution; little Cd reappeared upon reoxidation	Controlled microbial–geochemical evidence. High pH and sulfide interactions were proposed contributors to retention; no field or grain endpoint
Zhang et al. (2025) [12] Soil-slope incubations; three contaminated paddy soils	Drainage-induced reoxygenation; ROS imaging, DGT, and elemental mapping resolved a transient front approximately 1–5 cm below the water–soil interface	Fe(II) oxidation generated •OH hotspots and accelerated CdS oxidation; Cd solubility increased by up to 2.6-fold	Mechanistic evidence for a competing Cd-remobilization pathway; not a Zn trial or direct test of whether •OH overrides ZnS protection
Huang et al. (2021) [13] Soil microcosms and electrochemical assays	CdS oxidation during reoxygenation in the presence of ZnS or CuS	Coexisting ZnS suppressed CdS oxidation and Cd release, whereas CuS promoted them	Mechanistic evidence for sulfide voltaic coupling; mineral identity matters. Grain response requires plant or field validation
Huang et al. (2022) [14] Rice-pot experiments	One-time soil $ZnSO_4$ amendment equivalent to 75 kg elemental $Zn \cdot ha^{-1}$; flooding followed by drainage; sulfate controls included	Drainage-stage $CaCl_2$-extractable soil Cd decreased by 32–64%; brown-rice Cd decreased by 74–87%	Pot intervention supporting soil-mediated protection; experimental remediation dose, not a routine nutritional fertilizer recommendation
Huang et al. (2022) [14] Field trials	One-time 75 kg elemental $Zn \cdot ha^{-1}$ as $ZnSO_4$; drainage/grain filling; second season without additional Zn	$CaCl_2$-extractable Cd fell 16–30% and brown-rice Cd 60–72%; suppression persisted into season 2; no significant yield effect	Field intervention supporting sulfide-related protection under tested conditions; two-season persistence does not establish long-term safety

Table S1. *(Continued)*.

Study, system, and genotype	Zn/Cd exposure and experimental conditions	Measured responses	Interpretation and evidential scope
B. Root uptake, vascular transport, and redistribution			
Cai et al. (2019) [15] EGTA-buffered nutrient solution; four indica cultivars: Zhongjiaozao17 (YK17), 14ZFan-15 (14Z), Xianwanxian12 (XWX12), and XZY	Zn^{2+} activity gradient spanning deficiency to phytotoxicity; Cd activity selected to represent field exposure	Root Cd concentrations decreased and shoot Cd concentrations generally decreased as Zn activity increased, although shoot Cd in YK17 did not change significantly. The shoot-to-root Cd concentration ratio increased across the four cultivars. Both deficient and toxic Zn impaired growth, and shoot Fe/Mn concentrations declined at higher Zn	Controlled physiology. Uptake competition and biomass dilution both contribute; concentration and transfer-ratio responses are distinct. No mature-grain endpoint
Chang et al. (2023) [16] Hydroponic wild types and transporter mutants	Zn dose series 0–5 μM with 0.5 μM Cd for 3 or 7 days; key comparisons used 0 versus 2.5 μM Zn. Wild types included Nipponbare, ZH11, Zhongjiazao17, W0, and W4	Zn promoted root-to-shoot Cd transfer in wild types, but not *oshma2* mutants; estimated OsHMA2-dependent contribution increased from about 43% to 81%. At 2.5 μM Zn, *osnramp5* root Cd was 89.9–94.1% below wild type	Functional mechanistic evidence under defined seedling conditions; 2.5 μM Zn is not a universal excess-Zn threshold. No mature-grain or field Zn-treatment endpoint
Zhong et al. (2024) [17] Soil–rice pots; stable isotopes and gene expression	Zn addition under contrasting flooding and drainage regimes	Zn isotope fractionation and transporter expression changed more markedly than Cd isotope fractionation; results supported partially overlapping Zn/Cd uptake and transport routes	Controlled soil–plant evidence; isotope/expression patterns support pathway interpretation but do not independently demonstrate transporter-specific causation
Fontanili et al. (2016) [18] Hydroponics, Roma; ^{107}Cd tracing, Nipponbare	Roma: 10 days exposure to 0.1, 1, or 10 μM Zn with 0.1 μM Cd; reciprocal series used 0–1 μM Cd at 1 μM Zn. Separate short-term tracer assays	Increasing Cd inhibited shoot Zn accumulation more completely than increasing Zn inhibited shoot Cd accumulation; xylem/tracer measurements supported a residual Zn-insensitive Cd-transfer component	Mechanistic kinetic evidence; reciprocity is incomplete. The transport-pathway interpretation is conditional and is not a field fertilization response
Yamaguchi et al. (2012) [19] Soil-grown Koshihikari; node μ-XRF/μ-XANES	Nonflooded greenhouse pots; 0.1 M HCl-extractable soil Cd 1.8 $mg \cdot kg^{-1}$; tissues sampled one week after heading; no Zn-treatment comparison	Cd: node I 440 ± 39 and panicle 19 ± 0.3 $\mu g \cdot g^{-1}$ DW; Zn: 2204 ± 455 and 55 ± 5 $\mu g \cdot g^{-1}$ DW, respectively. Cd was 84–95% S-coordinated at analyzed EVB-xylem positions	Spatial evidence for selective nodal retention and redistribution. Tissue/local-site measurements under Cd exposure are not a Zn-treatment effect or mature-grain prediction
Tanaka et al. (2007) [20] ^{109}Cd tracing; rice cv. Kantou	Cd supplied through different plant positions during grain filling; phloem contribution estimated from tracer distribution	An estimated 91–100% of grain-deposited Cd arrived through the phloem	Mechanistic allocation evidence; identifies the delivery route under the tested conditions, not a Zn-treatment effect

Table S1. *(Continued)*.

Study, system, and genotype	Zn/Cd exposure and experimental conditions	Measured responses	Interpretation and evidential scope
Wang et al. (2018) [21] Field foliar experiment	Cd-contaminated acidic soil; 0.3% or 0.5% (w/v) $ZnSO_4 \cdot 7H_2O$ during early grain filling	Cd concentrations decreased and Zn concentrations increased in rice tissues, including brown rice; grain yield was not significantly affected	Field intervention; demonstrates a tested foliar benefit, with applicability conditional on dose, soil, and growth stage
Zheng et al. (2023) [22] Rice-pot foliar experiment	Early grain filling; $ZnSO_4$ sprays reported as 0.2% and 0.4% Zn treatments	Mature-grain Cd decreased by 24% and 31%, respectively; xylem Cd decreased by up to 26%; at 0.4%, Cd increased in husks, rachises, first internodes, and roots by 60%, 69%, 23%, and 22%	Pot intervention: lower grain Cd coexisted with greater retention elsewhere. Expression changes are supportive, not proof of transporter-specific causation
Zheng et al. (2024) [23] Field trial; TY390, ZLY189, and XWX12	Separate foliar Zn, Se, and combined ZnSe treatments; Zn-only results distinguished from mixture effects	Zn alone decreased grain Cd by 13.9–21.8%; ZnSe decreased it by 11.9–29.5%. Flag-leaf cell-wall/pectin Cd retention and antioxidant responses increased	Field intervention with physiological support; mixed-treatment effects must not be attributed to Zn alone, and expression changes do not establish causation
C. Grain responses, genotype, and Zn management			
Tavarez et al. (2022) [24] Nutrient solution; rice lines 546, 428, and 667	1 µM Cd; Zn increased from 2 to 10 µM; plants grown to grain maturity	Grain Cd increased by 348% in line 546 and 102% in line 428; the change in line 667 was not significant	Controlled genotype-dependent grain response. Defined nutrient-solution exposure and percentage increases should not be extrapolated to field magnitudes
Wissuwa et al. (2008) [25] Field trials; five rice genotypes at four sites	0 versus 15 kg elemental $Zn \cdot ha^{-1}$; native soil Zn ranged from severely deficient to high availability	Within one genotype, grain Zn ranged from 8 to 47 $mg \cdot kg^{-1}$ across soils; fertilizer did not fully compensate for low native Zn availability	Field evidence for soil/genotype constraints on Zn enrichment; Cd response was not assessed in this comparison
Johnson-Beebout et al. (2016) [26] Two pot experiments; IR69428 and IR68144	Zn fertilization and contrasting water management; uptake and allocation followed through grain filling	IR69428 maintained stronger root Zn uptake during grain filling, whereas IR68144 relied more on redistribution from vegetative tissues	Controlled evidence for genotype-dependent Zn loading and water effects; does not establish the direction of grain Cd response
Liu et al. (2026) [27] Two consecutive field trials	2023–2024; soil $ZnSO_4 \cdot H_2O$, 0–180 kg $fertilizer \cdot ha^{-1}$; independent MnO_2 treatments	Zn decreased grain Cd by 51.9–78.8% without significant changes in the reported indicators of root Cd uptake or root-to-straw transfer; later straw-to-grain remobilization was restricted. Mn decreased grain Cd by 69.6–84.4% through broader uptake/transport inhibition	Field intervention separates Zn and Mn effects. The Zn rate is fertilizer mass; Zn suppression can be dominated by grain-directed allocation

Table S1. *(Continued)*.

Study, system, and genotype	Zn/Cd exposure and experimental conditions	Measured responses	Interpretation and evidential scope
Zhang et al. (2026) [28] Pots; two contrasting rice genotypes	Soil $ZnSO_4$ amendments, 50 or 100 mg fertilizer·kg^{-1}; separate $MnSO_4$ comparisons under Cd stress	Zn decreased grain Cd by 43.67–52.60%, although the lower Zn dose increased the root-to-shoot translocation factor by 1.54–1.76-fold; soil-available Cd was unchanged	Pot intervention: an increased transfer factor can coexist with lower grain Cd. Tissue endpoints and expression cannot be treated as measured transport fluxes
Liu et al. (2025) [29] Flag-leaf manipulation with ^{111}Cd	Constant Cd supplied directly to flag leaves with varying Zn concentrations; ionic Zn compared with nonionic ZnO nanoparticles	Higher ionic Zn increased leaf-associated Cd release and allocation to grain; ZnO nanoparticles mitigated these effects	Mechanistic leaf-source scenario. Direct Cd application does not reproduce ordinary root-derived exposure or demonstrate a typical field foliar-Zn outcome
Zhao et al. (2023) [30] Published meta-analysis	Soil Zn application in pot and field studies; 53 grain-Cd treatment–control comparisons	Grain Cd: −25.95% overall (95% CI below zero); −52.74% under continuous flooding; +35.99% in the highest reported rate category (>0.2 t·ha^{-1}; n = 10)	Exact CI limits and Zn-specific I^2 not reported; no universal Zn-dose threshold
Phattarakul et al. (2012) [31] 17 field trials in five countries	China, India, Lao PDR, Thailand, and Turkey, 2008–2010; soil, foliar, or combined Zn; soil pH 4.8–8.8 and DTPA-extractable Zn 0.5–6.5 mg·kg^{-1}	Brown-rice Zn increased on average by 25% with foliar, 32% with combined, and 2.4% with soil application; postflowering foliar supply was more effective	Multi-environment field evidence for Zn biofortification; Cd reduction was not tested in this comparison and cannot be inferred
Mabesa et al. (2013) [32] Field biofortification breeding lines	Moderately and severely Zn-deficient sites; foliar 4 kg elemental Zn·ha^{-1} as $ZnSO_4$ at active tillering, heading, or both	Heading-stage foliar Zn increased grain Zn by approximately 1–10 mg·kg^{-1}, depending on genotype; foliar supply did not overcome agronomic Zn deficiency	Field evidence for genotype/timing effects on Zn enrichment; does not establish a Cd-reduction benefit
Zhen et al. (2021) [33] Field trial; five late-rice cultivars	0.4% $ZnSO_4$ foliar treatment; JLYHZ, FYY272, JY284, CLY7, and LXY130	Grain Cd decreased and grain Zn increased in all five cultivars; yield decreased significantly in JY284, CLY7, and JLYHZ	Field intervention with cultivar-dependent yield costs; leaf cell-wall retention and antioxidant responses support mechanistic interpretation

Table S1. *(Continued)*.

Study, system, and genotype	Zn/Cd exposure and experimental conditions	Measured responses	Interpretation and evidential scope
Wang et al. (2020) [34] Field foliar experiment	0.3% or 0.5% (w/v) Zn-EDTA during early grain filling	At 0.5%, grain Cd/Pb decreased and grain Zn/Fe increased, but grain yield decreased significantly	Field evidence for a nutrition–contamination–yield trade-off; grain-Cd reduction alone does not define an optimal treatment

Notes: (1) Percent changes are relative to the controls or comparison groups in the cited studies; they are not estimates calculated in this review. Concentrations, contents, translocation factors, tracer allocation, and kinetic measurements are distinct endpoints. Rates retain their reported basis. Elemental Zn doses, fertilizer-mass doses, foliar percentages, and free-ion activities are not directly interchangeable. Qualitative treatment descriptions indicate the comparison summarized and do not imply a complete dose inventory. (2) Survey pH groupings and the wheat soil Cd:Zn ratio are study-specific; neither is a validated universal rice-management threshold. A lack of a grain-Cd endpoint is stated explicitly where the cited comparison cannot support a grain-Cd conclusion. (3) Mechanistic, observational, pot-intervention, field-intervention, and meta-analytic evidence are distinguished by what they establish, rather than ranked on one quality scale. Transferability depends on soil properties, Zn/Cd status, water regime, genotype, and application route and timing. (4) Abbreviations: DW, dry weight; DGT, diffusive gradients in thin films; EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; EVB, enlarged vascular bundle; K_L, Langmuir affinity constant; SRB, sulfate-reducing bacteria; XAFS, X-ray absorption fine structure; XANES, X-ray absorption near-edge structure; XRF, X-ray fluorescence; ROS, reactive oxygen species; •OH, hydroxyl radical.

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