



REVIEW

Heavy Metal Contamination in Soil, Water, and Biota: Sources, Impacts, Remediation Strategies, and the Emerging Role of *Trifolium* spp. with Valorized Eggshell and Bagasse

Biosorbents

[version 1; peer review: 1 approved with reservations]

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Abstract

Heavy metal contamination of soils, water bodies, and food crops represents one of the most persistent and escalating environmental challenges of the twenty-first century. Driven by rapid industrialization, intensive agriculture, urbanization, and mining, metals including lead (Pb), arsenic (As), cadmium (Cd), mercury (Hg), chromium (Cr), nickel (Ni), copper (Cu), and zinc (Zn) have accumulated in ecosystems worldwide, posing severe risks to soil integrity, plant productivity, and human health through bioaccumulation and biomagnification across food chains. This review synthesizes global trends in heavy metal contamination from 1990 to 2025, traces the evolution of remediation strategies from conventional physicochemical treatments toward integrated green and hybrid approaches, and evaluates the role of *Trifolium* spp. (clovers) as phytoremediators. Special attention is given to *Trifolium repens* (white clover) and its documented capacities for metal uptake, rhizosphere enhancement, and nitrogen fixation. The review further examines the emerging waste-to-resource paradigm employing valorized chicken eggshell and sugarcane bagasse as low-cost biosorbents, including their individual and synergistic performance in removing Pb, Cd, As, and Hg from contaminated matrices. Regional findings from Durban,

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South Africa, illustrate how WWTP effluents and riverine contamination translate into tangible soil and crop exposure risks, underscoring the urgent need for locally appropriate, sustainable remediation solutions. The integration of *Trifolium* spp. with eggshell- and bagasse-derived amendments is proposed as a promising low-cost, green technology framework for contaminated land management in resource-constrained settings.

Keywords

heavy metals; phytoremediation; *Trifolium repens*; eggshell biosorbent; sugarcane bagasse; soil contamination; wastewater; Durban; green remediation; circular economy

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1. Introduction

Anthropogenic activities are widely recognized as the foremost driver of soil enrichment with toxic heavy metals, even though these pollutants may also occur as natural constituents of lithogenic origin. Environmental contamination by heavy metals and organic pollutants poses persistent and escalating threats to ecosystem integrity and human health worldwide (Bidar et al., 2007; Lin et al., 2021). Rapid industrialization, mining, intensive agriculture, and urban expansion have accelerated soil degradation, particularly in developing countries where remediation infrastructure, enforcement capacity, and financial resources remain severely constrained (Naicker et al., 2003; Roychoudhury & Starke, 2006).

Heavy metals are metallic elements or metalloids that are toxic at elevated concentrations, environmentally persistent, and capable of accumulating in biological and ecological systems. Common metals of concern include cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), chromium (Cr), nickel (Ni), copper (Cu), and zinc (Zn) (Angon et al., 2024; Rashid et al., 2023; Briffa, Sinagra & Blundell, 2020). Unlike organic contaminants, heavy metals are non-biodegradable and cannot be transformed into harmless compounds. They persist in soils, sediments, and water; bioaccumulate in living organisms; and biomagnify through food chains, posing long-term risks to ecosystems and human health (Li et al., 2019; Timothy & Williams, 2019; Mohammad et al., 2025).

Mining, smelting, industrial processing, fossil fuel combustion, intensive agriculture, urbanization, and vehicular traffic contribute metals to soils, water, and air, creating persistent pollution hotspots and widespread diffuse contamination (Xu, Jin & Zeng, 2024; Wan et al., 2024). Once released, metals are transported through atmospheric deposition, wastewater discharge, soil accumulation, plant uptake, and groundwater leaching, with mobility and bioavailability strongly influenced by soil pH, redox conditions, organic matter content, and microbial activity (Li et al., 2019; Wan et al., 2024).

This review covers: (i) the global trajectory of heavy metal pollution from 1990 to 2025; (ii) the toxicology and environmental behavior of the four priority metals Pb, As, Cd, and Hg; (iii) the evolution of remediation strategies from conventional physicochemical methods to advanced green and hybrid approaches; (iv) the phytoremediation potential of *Trifolium* spp., with emphasis on *Trifolium repens*; (v) the waste-to-resource potential of chicken eggshell and sugarcane bagasse as biosorbents; and (vi) the regional context of heavy metal contamination in Durban, South Africa. The review culminates in a synthesis proposing the integration of *Trifolium* spp. with valorized eggshell and bagasse as a sustainable low-cost green technology for contaminated environments.

2. Global trajectory of heavy metal pollution, 1990–2025

From 1990 to 2025, heavy metal pollution has remained a persistent and escalating global environmental challenge, largely driven by mining and smelting, industrial expansion, urbanization, transportation, and intensive agricultural practices (Timothy & Williams, 2019; Selvi et al., 2019; Adnan et al., 2022; Angon et al., 2024). The non-degradable nature and long environmental persistence of metals such as Cd, Pb, Hg, As, Cr, Ni, Cu, and Zn have facilitated their accumulation in soils, water, air, sediments, and biota, with redistribution occurring only through physical, chemical, or biological processes (Rashid et al., 2023; Angon et al., 2024).

During the 1990s and early 2000s, rapid industrialization—particularly in developing regions—combined with limited regulatory oversight led to elevated metal concentrations in surface waters, agricultural soils, and sediments surrounding mines, smelters, and industrial corridors. Concurrently, intensified agricultural practices—including widespread use of phosphate fertilizers, sewage sludge, pesticides, and wastewater irrigation—contributed to long-term contamination reservoirs of Cd, Pb, As, and other metals in cultivated soils (Rashid et al., 2023; Angon et al., 2024).

Between the 2000s and 2010s, scientific awareness of geoaccumulation, bioaccumulation, and biomagnification expanded, enabling more accurate identification of contaminated sites and the development of pollution risk maps. Despite growing regulatory attention, ongoing inputs from industrial effluents, vehicular emissions, atmospheric deposition, and wastewater reuse sustained progressive accumulation of metals in topsoils, sediments, and irrigation zones, particularly near transport corridors and industrial belts (Adnan et al., 2022; Matei et al., 2025).

From the 2010s through 2025, global syntheses confirmed that heavy metal pollution remains widespread and unresolved, with Cd, Pb, and Zn frequently exhibiting high geoaccumulation indices in polluted regions (Yu et al., 2025). Emerging evidence also indicates that microplastics and airborne particulate matter act as secondary vectors enhancing metal transport and plant uptake, while chronic metal exposure has reshaped soil microbial communities toward metal-tolerant taxa, altering nutrient cycling and ecosystem functions (Angon et al., 2024; Mi et al., 2025).

3. Priority heavy metals: environmental behavior and toxicology

3.1. Lead (Pb)

Lead occurs mainly as Pb (II) compounds bound to airborne particulates, dissolved Pb^{2+} in aquatic systems, and strongly adsorbed mineral- or organic-bound forms in soils, where it exhibits high persistence and limited mobility (Angon et al., 2024; Kumar et al., 2020). Major anthropogenic sources include mining and smelting, legacy leaded gasoline, batteries, paints, contaminated fertilizers, sewage sludge, industrial effluents, and urban dust (Bouida et al., 2022; Gupta et al., 2024).

In soils, Pb contamination leads to long-term fertility degradation and suppression of microbial activity, while in plants it disrupts germination, photosynthesis, nutrient balance, and growth through oxidative stress. In humans and animals, Pb is a potent neurotoxin causing cognitive impairment, developmental disorders, renal dysfunction, and cardiovascular damage, with food-chain transfer via crops and forage representing a major health concern (Angon et al., 2024; Bouida et al., 2022).

3.2. Arsenic (As)

Arsenic is predominantly found as arsenite (As (III)) and arsenate (As(V)), with environmental speciation controlled by redox potential, pH, and microbial activity, strongly influencing its solubility, mobility, and toxicity in soils and water (He et al., 2025; Patel et al., 2023; Sinha et al., 2023). Sources include natural mineral weathering, mining and smelting, fossil fuel combustion, arsenical pesticides, wood preservatives, fertilizers, and industrial effluents, contributing to widespread groundwater and agricultural soil contamination.

Arsenic impairs plant growth by disrupting nucleic acid synthesis, enzyme activity, and antioxidant defense systems, leading to reduced biomass and yield. In humans, chronic exposure via contaminated water and food is associated with skin lesions, cancers, cardiovascular disease, and neurological disorders, establishing arsenic as a major global groundwater contaminant (He et al., 2025; Rajendran et al., 2024; Sinha et al., 2023).

3.3. Cadmium (Cd)

Cadmium primarily exists as Cd^{2+} in soil and water, forming soluble complexes with chlorides, sulfates, carbonates, and organic ligands, which enhance its mobility and bioavailability relative to less mobile metals such as lead (Angon et al., 2024; Haider et al., 2021). It is released through phosphate fertilizers, Ni-Cd batteries, PVC stabilizers, pigments, mining and smelting, coal combustion, wastewater discharge, and sludge application in agriculture (Genchi et al., 2020; Zulficar et al., 2022).

Cadmium readily accumulates in soils and is efficiently taken up by crops, posing threats to food safety and ecosystem integrity. In humans, Cd has no biological function and exhibits a prolonged biological half-life of 10 to 30 years, causing chronic kidney damage, bone demineralization, pulmonary toxicity, and increased cancer risk with long-term exposure (Genchi et al., 2020; Suhani et al., 2021; Xu et al., 2024).

3.4. Mercury (Hg)

Mercury occurs as elemental Hg^0 , inorganic Hg^{2+} , and organic methylmercury (MeHg), with MeHg being the most toxic and bioaccumulative species, formed via microbial methylation in aquatic and anoxic environments (Beckers & Rinklebe, 2017; Gworek et al., 2020). Major sources include coal combustion, metal smelting, artisanal gold mining, chlor-alkali industries, waste incineration, industrial effluents, and mercury-containing products.

Mercury biomagnifies in aquatic and terrestrial food webs, causing phytotoxicity through reduced photosynthesis, enzyme inhibition, and oxidative damage in plants. In humans and animals, MeHg is a potent neurotoxin linked to cognitive impairment, developmental defects, and sensory dysfunction, while inorganic and vapor forms induce renal and neurological damage (Beckers & Rinklebe, 2017; Ray et al., 2025).

4. Evolution of heavy metal remediation strategies

4.1 Conventional physicochemical approaches

Early heavy-metal remediation relied predominantly on physicochemical technologies such as chemical precipitation, coagulation-flocculation, membrane filtration, ion exchange, adsorption, soil washing, solidification/stabilization, and electrochemical treatments (Jadaa & Mohammed, 2023; Xu, Jin & Zeng, 2024; Zamora-Ledezma et al., 2021). These methods offer rapid and effective contaminant removal, particularly in water treatment systems and industrial effluent management.

Despite their effectiveness, long-term sustainability is constrained by high capital and operational costs, intensive energy consumption, extensive chemical usage, declining efficiency at low metal concentrations, secondary waste generation

(e.g., metal-rich sludge), and disruption of soil structure during ex situ soil treatments. Critically, these approaches do not destroy heavy metals; they transfer contaminants into other matrices—sludge, concentrates, or spent sorbents—creating ongoing disposal and re-contamination risks (Zamora-Ledezma et al., 2021; Xu, Jin & Zeng, 2024).

4.2 Biological and nature-based remediation

In response to the environmental and economic constraints of conventional technologies, biological and nature-based remediation strategies have gained increasing attention. Phytoremediation techniques—including phytoextraction, phytostabilization, rhizofiltration, phytovolatilization, and phytodetoxification—employ hyperaccumulator plants to uptake or immobilize metals in contaminated soils and waters (Nedjimi, 2021; Sharma et al., 2023; Yan et al., 2020). Microbial bioremediation involving plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi further enhances metal bioavailability or immobilization while improving plant stress tolerance (Raklami et al., 2022; Karnwal et al., 2024).

Recent advances emphasize plant-microbe consortia and amendment-assisted phytoremediation, where biochar, compost, chelating agents, microbial inoculants, and plant root exudates improve metal uptake, stabilization, and detoxification while enhancing soil health (Tamma et al., 2025; Wan et al., 2024; Wei et al., 2025). Emerging biochemical strategies such as enzyme-induced carbonate precipitation (EICP) have demonstrated effective immobilization of Cd, Pb, and Zn through biomineralization into stable carbonate phases (Xu et al., 2025).

However, biological approaches are often limited by slow remediation rates, sensitivity to environmental conditions, root-zone constraints, and the requirement for safe management of metal-enriched biomass to prevent secondary contamination (Alengebawiy et al., 2021; Srivastava et al., 2017).

4.3 Advanced and emerging technologies

To overcome the scalability limitations of both physicochemical and biological methods, advanced remediation strategies increasingly integrate engineered materials, nanotechnology, and biotechnology (Jadaa & Mohammed, 2023; Mitra et al., 2022; Xu, Jin & Zeng, 2024). Biochar-based remediation represents a major technological advancement, with engineered and functionalized biochars immobilizing heavy metals through adsorption, ion exchange, surface complexation, and mineral precipitation, while simultaneously improving soil fertility and microbial activity (Tamma et al., 2025; Wang et al., 2021; Wei et al., 2025).

Nanomaterial-assisted remediation—including magnetic nanoparticles, metal oxides, zeolites, polymers, chitosan, and metal-organic frameworks (MOFs)—offers high sorption capacity and selective metal removal, particularly in water treatment systems (Babu et al., 2025; Mohamed et al., 2025). Nevertheless, concerns remain regarding production cost, regeneration efficiency, long-term field stability, and potential nanotoxicity under large-scale deployment.

4.4 Modern green and hybrid remediation frameworks

Modern remediation increasingly favors integrated green hybrid systems that combine biological processes with advanced materials. In soil remediation, leading strategies include plant-microbe phytoremediation, biochar-assisted immobilization, iron-based stabilization, enzyme-mediated biomineralization, and nano-enabled sorbents deployed within in situ stabilization frameworks to minimize soil disturbance. For aquatic and water remediation, aquatic plant phytoremediation, biochar and natural zeolite sorbents, functionalized nanoadsorbents, and electrochemical or membrane-based systems have demonstrated high removal efficiency with reduced sludge production and chemical demand (Ali et al., 2020; Pang et al., 2023).

A consistent finding across all remediation categories is that heavy metals are rarely destroyed; they are transferred into secondary matrices—sludge, biomass, or immobilized soil fractions—that require secure long-term management. Consequently, the field is shifting toward integrated, low-impact, circular remediation models prioritizing in situ stabilization, plant-microbe synergies, engineered biochars, and resource-recovery concepts (Jadaa & Mohammed, 2023; Zamora-Ledezma et al., 2021).

5. *Trifolium* spp. as phytoremediators: systematics, ecology, and remediation potential

5.1 Taxonomy, evolutionary diversity, and systematics

The genus *Trifolium* L. (clovers), belonging to the family Fabaceae, comprises approximately 250–260 herbaceous annual and perennial species distributed mainly across temperate regions of Europe, the Mediterranean, western Asia, North Africa, and the highlands of eastern Africa (Uslu & Babac, 2019; Ellison et al., 2006). Molecular phylogenetic analyses support the monophyly of *Trifolium* and indicate a probable Mediterranean origin during the Early Miocene, followed by dispersal to the Americas and sub-Saharan Africa. Classical taxonomic systems divided the genus into

approximately eight sections based primarily on morphological characters; however, recent molecular systematics recognize two principal subgenera: subgenus *Trifolium* and subgenus *Chronosemium* (Ahmed et al., 2021).

Chromosomal evolution has played a significant role in diversification within the genus. Cytogenetic reconstructions suggest an ancestral basic chromosome number of $2n = 16$, with repeated events of aneuploidy and polyploidy contributing to speciation and adaptive radiation (Ellison et al., 2006; Ahmed et al., 2021). Although more than 200 species have been described worldwide, only approximately 25 are of significant agricultural value; several, notably *T. repens* (white clover), *T. pratense* (red clover), and *Trifolium alexandrinum* (berseem clover), have achieved global prominence (McKenna et al., 2018).

5.2 White clover: agricultural importance and biological properties

T. repens L. stands out as one of the most economically and ecologically important species within the genus. Native to Europe, this perennial stoloniferous legume has attained a cosmopolitan distribution following agricultural introduction and naturalization across temperate regions worldwide (Carlsen & Fomsgaard, 2008). Its global success is attributed to grazing tolerance, vegetative propagation, environmental plasticity, and efficient symbiotic nitrogen fixation—commonly ranging from 100 to 300 kg N ha⁻¹ year⁻¹ under field conditions (Rodríguez-Navarro et al., 2021). In pasture-based livestock systems, white clover enhances forage quality, increases crude protein content, and reduces reliance on synthetic nitrogen fertilizers.

Several *Trifolium* species are also rich in proteins, flavonoids, isoflavones, saponins, and phenolic compounds, supporting their use in human nutrition, herbal medicine, and dietary supplements (Sabudak & Guler, 2009; Kołodziejczyk-Czepas, 2016). *Trifolium pratense* in particular is widely incorporated into nutraceutical formulations for managing menopausal symptoms and cardiovascular risk.

5.3. Phytoremediation capabilities

White clover has received substantial attention in phytoremediation research due to its documented heavy-metal uptake capacity, including cadmium, chromium, and lead, through rhizosphere-mediated mechanisms (Lin et al., 2021; Liu et al., 2021). It has also demonstrated effectiveness in the bioremediation of petroleum hydrocarbons and polycyclic aromatic hydrocarbons (PAHs) (Sawicka et al., 2023; Ahmad et al., 2025). Importantly, white clover stimulates soil enzyme activities including dehydrogenases, ureases, phosphatases, and catalases, while compatibility with plant growth-promoting endophytes such as *Pseudomonas putida* enhances performance under metal stress (Lin et al., 2021; Liu et al., 2021).

White clover exhibits a conservative accumulation profile with physiological regulation favouring phytostabilization, rather than hyperaccumulation. Its designation as a certified reference material (BCR-402) supports its widespread use as an analytical standard in biomonitoring studies (Ghani et al., 2014). Among indigenous South African *Trifolium* species, comparative data indicate stronger accumulation tendencies: *Trifolium burchellianum* recorded elevated cadmium concentrations (0.5 mg kg⁻¹), while *T. dubium* accumulated high lead levels (up to 7.57 mg kg⁻¹), suggesting phytoextraction potential in regional remediation contexts (Gounden, 2017). *T. africanum* presents intermediate accumulation behaviour, and *T. pratense* exhibits a very conservative profile with arsenic, cadmium, and lead frequently below detection limits.

Collectively, white clover's combination of regulated metal accumulation, strong rhizosphere stabilization, perennial stoloniferous growth, nitrogen fixation, and effectiveness against both inorganic and organic contaminants positions it as a versatile and low-risk species for integrated phytoremediation programmes. Nevertheless, indigenous South African *Trifolium* species remain substantially understudied at the rhizosphere level, representing a key knowledge gap for regional applications.

6. Waste-derived biosorbents: eggshell and sugarcane bagasse

6.1 Rationale: the waste-to-resource paradigm

South Africa and other developing regions struggle with solid waste management, especially in rural and low-income communities where poor collection systems lead to illegal dumping and open burning. Turning organic waste into functional biosorbents supports both circular economy principles and waste reduction objectives. Chicken eggshells constitute approximately 11% of an egg's weight and are composed predominantly of calcium carbonate (94–97%), with small amounts of magnesium carbonate, calcium phosphate, and organic material (Mignardi et al., 2020). Sugarcane bagasse, the fibrous residue after juice extraction, contains 32–45% cellulose, 20–32% hemicellulose, 17–32% lignin, and 1–9% ash, conferring a porous fibrous structure rich in hydroxyl groups suitable for adsorption and surface modification (Alokika and Singh, 2020).

6.2 Eggshell as a biosorbent: structure, mechanisms, and performance

Eggshell exhibits a complex hierarchical structure comprising cuticle, prismatic, palisade, and mammillary layers, with inner and outer membranes and a shell thickness of 280–400 μm featuring approximately 17,000 pores facilitating gas exchange (Mignardi et al., 2020). The predominant mineral phase is calcite (CaCO_3), providing reactive carbonate groups for metal binding. Functional groups, including hydroxyl ($-\text{OH}$), carboxyl ($-\text{COOH}$), and amino ($-\text{NH}_2$) groups, drive ion exchange, precipitation, and electrostatic attraction. While native shells may exhibit limited porosity, calcination, pyrolysis, or chemical modification substantially increases adsorption capacity (Table 1).

Eggshell-mediated metal removal operates through multiple complementary mechanisms: (i) ion exchange, where Ca^{2+} from CaCO_3 exchanges with metal cations (Pb^{2+} , Cd^{2+} , Co^{2+}) on surface sites; (ii) surface complexation through interactions with carboxyl and carbonate functional groups; and (iii) precipitation and co-precipitation, particularly in eggshell-derived hydroxyapatite systems where metal-phosphate minerals form (Mignardi et al., 2020; Harripersadth & Musonge, 2022) (Table 1). The elevated pH induced by CaCO_3 dissolution promotes the formation of low-solubility metal hydroxides and carbonates (Table 1).

6.3 Sugarcane bagasse: structure, mechanisms, and performance

Bagasse's fibrous lignocellulosic structure forms a porous network that facilitates contaminant diffusion and entrapment. Abundant hydroxyl groups on polysaccharide chains serve as active sites for metal binding via surface complexation and physical adsorption. Chemical or thermal treatments expose more cellulose and hemicellulose while disrupting lignin resistance, increasing metal binding capacity. Bagasse-derived biochar demonstrates enhanced metal immobilization capacity, particularly when converted at 300–600 $^{\circ}\text{C}$, developing a mesoporous structure (1.4–4.3 nm pore diameter) with surface areas of 180–198 m^2/g (Jamilatun et al., 2022).

Comparative studies show that bagasse generally removes less Pb^{2+} than eggshell (31.45 vs. 277.8 mg/g), but performs equally well or better for Cd^{2+} (19.49 vs. 13.62 mg/g), highlighting the complementarity of combining both materials. Metal removal mechanisms on bagasse-based materials include complexation with oxygen-containing functional groups, π -electron interactions with aromatic structures, electrostatic attraction to charged surfaces, and mineral-associated precipitation.

6.4 Combined and co-pyrolyzed eggshell-bagasse systems

The combination of eggshell and bagasse exploits complementary adsorption mechanisms while valorizing two abundant waste streams. Eggshell provides CaCO_3 -rich mineral phases that drive precipitation and ion exchange with strong affinity for Pb, Co, and As, while bagasse contributes lignocellulosic structures with hydroxyl and carboxyl groups particularly effective for Cd. Fixed-bed column studies demonstrated that all eggshell:bagasse mixing ratios (1:0 to 0:1) achieved 93–99% Pb^{2+} removal, with the 1:3 eggshell:bagasse mixture at 12 cm bed depth achieving 91% removal

Table 1. Selected eggshell-based biosorbent performance data for heavy metal removal.

Metal/System	Eggshell form	Key findings
Pb^{2+} , Cd^{2+} in water	Raw eggshell	q_{max} Pb 277.8 mg/g , Cd 13.62 mg/g ; Langmuir isotherm, pseudo-2nd-order kinetics
Co^{2+} in water	Eggshell-derived hydroxyapatite	70–80% removal; q_{max} 457 mg/g via $\text{Ca}^{2+}/\text{Co}^{2+}$ exchange and precipitation (Mignardi et al., 2020)
As(V) in water	Calcined eggshells	q_{max} 91.05 mg/g ; phosphate competition observed
Pb, Cd, Fe in river water	Raw eggshell waste	94.4% Pb, 64.7% Cd, 51.4% Fe removal; complete <i>E. coli</i> elimination
Pb^{2+} in column systems	Eggshell-bagasse binary	93–99% removal across mixing ratios; 91% at 1:3 ratio, 12 cm depth (Harripersadth & Musonge, 2022)

capacity at 28.27 mg Pb/g, while extending column service life through improved hydrodynamics (Harripersadth & Musonge, 2022).

True composite materials advance beyond physical mixtures: developed an eggshell-sugarcane bagasse composite that removed >90% of Cu^{2+} and Zn^{2+} from synthetic wastewater, with equilibrium data fitting the Freundlich isotherm and pseudo-second-order kinetics. Co-pyrolysis of eggshell and bagasse offers a route to engineered biosorbents with tunable properties; at 600 °C, the resulting biochar contains CaO distributed throughout an aromatic carbon matrix, yielding higher pH, greater surface area, and enhanced metal sorption through both carbon- and calcium-based mechanisms compared to physical mixtures alone (Jamilatun et al., 2022) (Table 2).

6.5 Metal-specific removal efficacy and research gaps

Lead and cadmium removal by eggshell-bagasse systems represent the most extensively characterized application. Batch studies consistently demonstrate Pb removal capacities of 87–99.9% with maximum adsorption capacities up to 461 mg/g for eggshell-enhanced biochar composites. Cadmium removal efficiencies of 94–100% are achieved across various eggshell-based systems, with maximum capacities ranging from 125–265 mg/g.

Arsenic removal remains less thoroughly investigated. Calcined eggshells achieve maximum As(V) capacities of 91.05 mg/g, though phosphate ions compete strongly for binding sites. Fe_3O_4 /bagasse-activated carbon magnetic composites exhibit As (III) capacity of 6.69 mg/g, outperforming plain bagasse but still modest compared to Pb/Cd systems. Mercury removal represents the most significant research gap; sparse data show low capacities for these specific materials, and methylmercury—the most toxic and bioaccumulative form—receives minimal attention. Future research should prioritize development of eggshell-bagasse carbons modified with iron or sulfur to improve mercury capture and stabilization.

7. Regional context: heavy metal contamination in durban, south africa

Building on global patterns of heavy metal persistence and toxicity, local studies in and around Durban illustrate how these contaminants manifest in regional ecosystems. Sewage and wastewater are significant contributors of heavy metals—including As, Cd, Cr, Fe, Mn, Ni, Pb, and Zn—to rivers and, indirectly, to soils and crops via irrigation and sludge application (Bakare & Adeyinka, 2022; Chollom et al., 2023). Investigations of Durban wastewater treatment plants (WWTPs) report influent metal concentrations ranging from approximately 0.122 to 1.808 mg/L, with effluent levels reduced but often remaining above both international and South African guidelines for irrigation and surface water (0.118–0.854 mg/L). Hazard assessments indicate a medium non-carcinogenic risk for As, Pb, Cr, and Cd, alongside potentially elevated lifetime cancer risk for exposed populations (Bakare & Adeyinka, 2022).

Along the uMgeni River, water, soil, and sediment analyses reveal elevated As, Mn, Cd, and Cr concentrations exceeding WHO and USEPA limits, with water quality indices classifying the river as unfit for domestic or irrigation purposes. Both carcinogenic and non-carcinogenic risks are particularly pronounced for children, with contamination sources traced to treated and untreated municipal wastewater, industrial effluents, and stormwater runoff, highlighting the direct influence of WWTP performance on river health (Chollom et al., 2023).

Broader South African evidence indicates that long-term irrigation with municipal or industrial wastewater, along with sludge application, typically elevates Cd, Cr, Cu, Ni, Pb, Zn, and other metals in topsoils beyond guideline thresholds, with transfer to crops often exceeding WHO/FAO food safety limits. Soil-to-plant transfer factors are highest for Cd and Zn, with Pb accumulation in leafy vegetables and cereals presenting substantial dietary exposure risk (Atta et al., 2023; Wydro et al., 2021; Kidd et al., 2006).

Table 2. Temperature-dependent transformations in eggshell-bagasse co-pyrolysis.

Temperature	Bagasse transformations	Eggshell transformations	Composite properties
450 °C	Higher char yield; O-rich bio-oil; moderate gas release; lower carbon content	Remains largely CaCO_3 ; limited CaO formation	C- CaCO_3 composite; moderate surface area; more functional groups
600 °C	Less char; higher gas; more aromatic/deoxygenated carbon; higher HHV	Significant $\text{CaCO}_3 \rightarrow \text{CaO}$ conversion; CO_2 release	C-CaO composite; higher pH and basicity; enhanced surface area; superior metal sorption

Collectively, these findings indicate that Durban's WWTP effluents and riverine systems constitute ongoing sources of heavy metal exposure with strong potential for soil enrichment and crop uptake if reused for irrigation. Metals of greatest regional concern—As, Cd, Cr, and Pb—consistently exceed guideline values and pose the highest ecological and human health risks. These observations underscore the critical need for stringent monitoring, management, and targeted remediation strategies tailored to the region (Bakare & Adeyinka, 2022; Chollom et al., 2023; Atta et al., 2023).

8. Integrated low-cost green technology: trifolium spp. with valorized eggshell and bagasse

The persistent presence of As, Cd, Cr, and Pb in Durban's rivers, soils, and crops underscores the urgent need for effective, affordable, and locally applicable remediation strategies. The integration of *Trifolium* spp. with valorized eggshell and bagasse under controlled conditions represents a compelling low-cost advanced green technology framework combining biological phytoremediation with waste-derived chemical amendments.

This integrated approach leverages the complementary strengths of each component: *T. repens* contributes rhizosphere-mediated metal uptake and stabilization, nitrogen fixation for soil fertility restoration, stimulation of beneficial soil microbial communities, and long-term vegetative cover; eggshell-derived CaCO_3 and hydroxyapatite phases provide alkalinity, elevate soil pH, and immobilize metals through ion exchange, surface complexation, and carbonate/phosphate precipitation; and bagasse-derived lignocellulosic or biochar amendments supplement organic matter, increase porosity and water retention, and offer additional sorption sites through hydroxyl and carboxyl functional groups.

Co-amendment of soils with eggshell and bagasse prior to or concurrent with *Trifolium* establishment could: (i) reduce bioavailable metal fractions to levels tolerable for plant establishment on heavily contaminated substrates; (ii) promote phytostabilization by chemically immobilizing metals in the rhizosphere; (iii) improve soil structure and fertility to support productive clover growth; and (iv) simultaneously valorize two locally abundant waste streams, aligning with circular economy objectives and reducing costs relative to commercial amendments.

The framework is particularly relevant in peri-urban and agricultural contexts around Durban, where WWTP effluent irrigation and sludge application have elevated soil metal concentrations, resources for expensive engineered remediation are limited, and there is an existing agricultural infrastructure capable of supporting forage legume cultivation. Indigenous *Trifolium* species such as *T. burchellianum* and *T. dubium* warrant further investigation given their apparent stronger accumulation tendencies, potentially enabling phytoextraction alongside phytostabilization strategies within the same genus.

Key research priorities to advance this integrated framework include: field-scale trials combining *Trifolium* spp. with eggshell and bagasse amendments on Durban-area contaminated soils; optimization of amendment ratios, application timing, and pyrolysis temperature for co-pyrolyzed eggshell-bagasse biochars; elucidation of rhizosphere-level interactions between clover root exudates, soil microbiota, and amendment-stabilized metal fractions; mercury-specific studies exploring sulfur- or iron-modified eggshell-bagasse carbons in conjunction with phytovolatilization; and lifecycle cost and environmental assessment to guide practical implementation and policy recommendations.

9. Synthesis and conclusions

Heavy metal contamination poses interconnected threats to soil integrity, plant productivity, ecosystem stability, and human health that will persist for decades given the non-degradable nature of these pollutants. The global scientific consensus from 1990 to 2025 confirms that no single universally effective remediation solution exists; rather, sustainable, site-specific, and integrated approaches are required that combine pollution prevention, ecological restoration, and sound regulatory enforcement.

The progressive evolution from conventional physicochemical treatments—limited by cost, secondary waste generation, and poor sustainability—toward biological, nature-based, and advanced hybrid strategies reflects a genuine paradigm shift in environmental science and engineering. Plant-microbe phytoremediation, biochar-based immobilization, enzyme-driven biomineralization, and advanced adsorption or electrochemical water treatment represent the most promising current pathways (Sharma et al., 2023; Wei et al., 2025; Xu et al., 2025).

Within this landscape, the proposed integration of *Trifolium* spp.—particularly *T. repens* and locally relevant indigenous species—with valorized eggshell and bagasse biosorbents offers a synergistic, cost-effective, and ecologically compatible framework for contaminated land management. This approach simultaneously addresses metal immobilization, soil fertility restoration, and waste valorization, making it particularly appropriate for resource-constrained developing-country contexts such as the Durban metropolitan region.

Long-term field validation, lifecycle cost analysis, nanomaterial risk assessment, safe handling of contaminated residuals, and deeper investigation of mercury-specific remediation pathways remain critical challenges and priorities for future research before these integrated technologies can be widely deployed for real-world environmental restoration.

Data availability

No data is associated with this article.

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Overall recommendation: Approved with reservations

This manuscript reviews heavy metal contamination in soil, water and biota, with emphasis on sources, environmental and health impacts, remediation strategies, the phytoremediation potential of *Trifolium* spp., and the use of valorized chicken eggshell and sugarcane bagasse as low-cost biosorbents. The topic is relevant and timely, and the manuscript brings together important themes such as green remediation, circular economy and locally applicable solutions for resource-constrained settings.

Overall, the review is useful but requires substantial revision before it can be considered scientifically sound. I answered "Partly" to several of the assessment questions because the article covers relevant literature, but it lacks a clear review methodology, a sufficiently critical synthesis, and direct evidence for some of its main conclusions.

The main limitation is the absence of a transparent literature-search methodology. Since the manuscript claims to review literature from 1990 to 2025, the authors should explain which databases were searched, which keywords were used, what inclusion and exclusion criteria were applied, and how the selected studies were evaluated. Without this information, it is difficult to assess whether the review is comprehensive and reproducible.

The scope of the manuscript is also very broad. It covers global heavy metal pollution, toxicology, conventional and emerging remediation methods, phytoremediation, *Trifolium* taxonomy, eggshell and bagasse biosorbents, co-pyrolysis and the Durban regional context. These topics are relevant, but the manuscript would benefit from a clearer central focus and better organization.

The authors should clarify whether the article is primarily a general review of heavy metal remediation or a focused review on the proposed *Trifolium*-eggshell-bagasse remediation framework.

Many factual statements are supported by citations, but some broad claims need stronger and more direct evidence. In particular, statements about global trends, removal efficiencies and the integrated use of *Trifolium* spp. with eggshell and bagasse amendments should be carefully verified. The authors should ensure that each key claim is directly supported by an appropriate citation.

The proposed integration of *Trifolium* spp. with eggshell and bagasse biosorbents is promising,

but the manuscript should present it more cautiously. At present, the evidence appears stronger for the individual components —*Trifolium* phytoremediation, eggshell adsorption and bagasse-based sorption— than for the combined system under field conditions. Therefore, this integrated approach should be described as a promising conceptual framework that requires further validation, rather than as an already established remediation technology.

The review is generally understandable, but the language and presentation need improvement. Some sections are dense and descriptive, and there are typographical, formatting and consistency issues. The authors should revise the manuscript for clarity, reduce repetition, standardize chemical notation and scientific names, and improve the flow between sections.

To make the article scientifically sound, the authors must address the following points: add a clear literature-review methodology; verify and strengthen citations; moderate unsupported conclusions; clarify the scope and central objective of the review; provide a more critical comparison of the evidence; and discuss practical limitations such as field validation, biomass management, long-term stability of immobilized metals, and risks associated with contaminated plant material or spent biosorbents.

In conclusion, the manuscript addresses an important environmental issue and has potential value, but it requires major revision. I recommend approval with reservations, provided that the authors strengthen the methodological transparency, improve the critical synthesis and present the proposed integrated remediation strategy with appropriate caution.

Is the topic of the review discussed comprehensively in the context of the current literature?

Partly

Are all factual statements correct and adequately supported by citations?

Partly

Is the review written in accessible language?

Partly

Are the conclusions drawn appropriate in the context of the current research literature?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Environmental geology, applied geochemistry, mining environmental management, soil and water contamination, heavy metal assessment, geophysical and geochemical characterization, and remediation strategies for contaminated environments.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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