sustainability

Review

Vermibiochar: A Novel Approach for Reducing the Environmental Impact of Heavy Metals Contamination in Agricultural Land

Sami ur Rehman [1], Federica De Castro [1], Paolo Marini [2], Alessio Aprile [1], Michele Benedetti [1,*] and Francesco Paolo Fanizzi [1,*]

[1] Department of Biological and Environmental Sciences and Technologies (DiSTeBA), University of Salento, Via Monteroni, I-73100 Lecce, Italy; sami.urrehman@unisalento.it (S.u.R.); federica.decastro@unisalento.it (F.D.C.); alessio.aprile@unisalento.it (A.A.)
[2] Compost Natura s.r.l., Via Mallacca Zummari 32, I-73010 Arnesano, Italy; info@compostnatura.com
* Correspondence: michele.benedetti@unisalento.it (M.B.); fp.fanizzi@unisalento.it (F.P.F.)

Abstract: Environmental pollution has become a pressing concern worldwide due to the accumulation of pollutants from industries and agricultural sectors in soil and water environments. Heavy metals pose severe hazards to the environment, plants, and human health. Consequently, an eco-friendly technique is needed to combat environmental pollutants. Vermibiochar, a product prepared through the combined action of earthworms and biochar, demonstrates great potential in reducing heavy metal concentrations in contaminated soil. Its large surface area and high cation exchange capacity enhance the sorption of contaminants onto the vermibiochar surface, reducing their bioavailability. This review highlights the roles played by earthworms and biochar in heavy metal detoxification and immobilization. It discusses the current methods of remediation, vermibiochar production, its effects on soil properties and plant growth, and biochar's impact on earthworm growth and reproduction. The studies reviewed suggest that vermibiochar is a novel strategy for addressing heavy metal contamination.

Keywords: vermibiochar; heavy metals; immobilization; vermicompost; biochar

check for updates

Citation: Rehman, S.u.; De Castro, F.; Marini, P.; Aprile, A.; Benedetti, M.; Fanizzi, F.P. Vermibiochar: A Novel Approach for Reducing the Environmental Impact of Heavy Metals Contamination in Agricultural Land. *Sustainability* **2023**, *15*, 9380. https://doi.org/10.3390/su15129380

Academic Editor: Jia Wen

Received: 2 May 2023
Revised: 6 June 2023
Accepted: 8 June 2023
Published: 10 June 2023

1. Introduction

Soil contamination is a significant environmental issue originating from either lithogenic (natural processes, such as weathering) or anthropogenic (human) activities [1]. Organic and inorganic toxins released into the soil through immediate or secondary exposure can cause toxic effects on plants and the environment leading to severe environmental risks [2,3]. Anthropogenic activities responsible for soil contamination include atmospheric deposition (gasoline combustion, smelting, industrial activities), extensive use of chemicals (pesticides, fertilizers), and land application of waste materials (animal manure, sewage sludge) [4]. Moreover, these contaminants are transferred from the pedosphere to the biosphere and hydrosphere, affecting human health and ecosystems. Heavy metal (HMs) contamination is a major environmental concern among soil contaminants, and its mitigation is urgently needed. HMs, even at lower concentrations, can affect the immune and nervous systems, causing various problems such as lung cancer, and liver and kidney issues, due to their ability to form relatively stable and toxic coordination and organometallic compounds [5–10]. Currently, industries release considerable concentrations of HMs. Reports indicate that in recent decades, significant amounts of Cd ($\approx 2.2 \times 10^4$ metric tons), Cu ($\approx 9.4 \times 10^5$ metric tons), Pb ($\approx 7.4 \times 10^5$ metric tons), and Zn ($\approx 3.5 \times 10^5$ metric tons) have been released into water and soil, leading to reduced soil fertility [11] and consequently slowing the plant growth due to impaired nutrient transport and metabolic processes [12–14]. Numerous studies show that plant responses to heavy metal toxicity involve complex mechanisms at physiological, biochemical, cellular, and molecular levels. Recent technological advancements have helped identify stress-inducible proteins, transcription factors, and metabolites involved in HM tolerance [15–18]. Different techniques are currently in use to remediate soil contaminated with heavy metals. For the remediation of industrial wastewater, plant-based activated carbon has been found effective in HM sequestration. The corncob and groundnut husk-based activated carbons were observed to have optimum adsorption capacities for Cr, Cu and Zn [19]. However, there are limitations on the use of these adsorbents for the removal of all metal ions from wastewater as one type of absorbent is effective for specific metal ions, thus making the remediation method ineffective. Similarly, the use of nanoporous materials such as metal-organic frameworks (MOFs) has gained utmost attention for their ability to adsorb metal ions from polluted water. The Co(II)-based phosphonate MOFs showed excellent adsorption capacity in the removal of Cr(VI) [20] and Cd ions from polluted water. Some metal terminals in MOFs are expensive and, in a few cases, poisonous, which increases the production cost and harms the environment. The instability of MOFs and metal ion leakage into the water result in secondary pollution that poses serious threats to the environment and public health [21]. Correspondingly, chitosan is also used as an adsorbent for contaminants due to its high adsorption potential and low cost. Chitosan is abundant with amino and hydroxyl groups that can bind metal ions. However, it is sensitive to pH and has low mechanical strength and thermal stability, which limits its application in the remediation process [22]. Similarly, for the treatment of acid mine drainage (AMD) water, there are several chemical processes, but none of them have been capable of solving the problem completely and are very costly, and their by-products might be harmful for living organisms [23].

Consequently, there is a need to adopt eco-friendly techniques to address heavy metal contamination. Recent research emphasizes bioremediation techniques, such as microbial remediation and phytoremediation. However, these methods have some drawbacks, as factors such as pH, temperature, and soil nutrient types can alter heavy metal contamination patterns. Additionally, microbes possess limited binding sites and generally exhibit low absorption capacities. Studies on using animals in HM remediation are scarce, and only a few reports provide comparative analyses of plant, microbial and animal remediation. Nevertheless, the use of earthworms in metal detoxification garners increased interest, and they have been employed as indicators of soil contamination [24,25]. Earthworms, during vermicomposting, modify the soil properties by ingestion, excretion, and mixing organic matter (OM) in the soil, which enhances fertility by converting mineral nutrients into plant-available forms [26]. In addition, recent research has revealed that earthworms affect HMs and their availability in soil [27].

Biochar is a carbonaceous substance produced by the thermochemical transformation of organic material in an anaerobic environment at relatively low temperatures (below 700 °C) [28]. While it is recognized as a means of carbon sequestration, its primary application lies in environmental remediation. For instance, it can be used to improve soil and water quality, serve as a contaminant sorbent, remediate polluted soil, act as a fertilizer component, and function as an additive in composting [29–32]. Biochar binds with HMs through precipitation, electrostatic binding, complexation, and ion exchange processes [33]. In the past two decades, there has been an increase in the number of studies exploring biochar's potential for adsorbing contaminants [34]. The sorption of pollutants to biochar is linked to processes that regulate the concentration of organic toxins in contaminated soils. The large surface area of biochar regulates its interactions with soil, and the adsorption of contaminants in soil is significantly influenced by factors such as feedstock material, production conditions, and temperature [35]. The decomposition time of biochar in soil varies with pyrolysis temperature and nature of the substrate. Wood biochar (pyrolysis temperature: 550 °C) is highly stable in soil, with a mean lifetime of more than 1000 years [36]. The adsorption capacity of aged biochar for Cd^{2+} and Pb^{2+} is enhanced due to oxygen-containing functional groups and the specific surface area of biochar. However, the stability of aged biochar to immobilized metals is considerably reduced [37].

Among biological techniques aimed at improving contaminated soils, the application of earthworms in combination with biochar has emerged as an environmentally friendly approach [38]. Studies have shown that certain enzymes, such as alkaline phosphatase, catalase, and superoxide dismutase, which are secreted by earthworms' guts and other symbionts, can bind to biochar flakes in the presence of earthworm mucus. Therefore, earthworms and biochar (organic matter + biochar + earthworms) can be used in vermicomposting processes (conversion of organic materials into fertilizer by earthworms), resulting in the formation of enzyme-coated vermibiochar. At present, combined investigations (biochar + earthworms) are not extensively addressed. According to available literature, earthworms consume organic substrate with biochar and release vermibiochar, which contains high molecular weight ligands that can be employed to remediate HM-contaminated soils [39].

This review focuses on (i) HM contamination and potential risks to soil, water, and plants, (ii) the effective role of earthworms and biochar in HM immobilization, and (iii) earthworms' response to biochar amendment. Additionally, it highlights the future perspectives for using this novel method to reduce HM contamination. This review focuses on the heavy metal's remediation of agricultural land, not considering specifically radionuclides.

2. Sources of Heavy Metals in Agricultural Land

Heavy metals occur in the ecosystem at varying concentrations. Human activities have contributed to the massive introduction of HMs into the environment. HMs, such as Mn, Fe, Co, Cu and Zn, are considered essential microelements when present in small amounts, but exert negative impacts at higher concentrations [40]. HMs in agricultural land from human-based activities are generally more bioavailable than those from lithogenic and pedogenic activities [41]. Metal-containing soil from polluted areas can originate from human-based sources such as metal mine tailings, the accumulation of metal-bearing wastes into landfill sites, fertilizers, petrochemicals, and coal combustion [42]. The use of phosphatic fertilizers accidentally introduces Fe, Cd, Hg and Pb into agricultural land [43]. Many pesticides add HMs to soil; for example, applying a copper oxychloride and Bordeaux mixture (copper sulfate) to control diseases in plants adds these metals to the agroecosystem [44]. Herbicides such as Ordram and Saturn-G, which are used to control crop weeds, contain higher concentrations of Mn, Fe, Ni, Zn and Pb [45].

HMs such as Cu and Zn are added to biosolids to promote growth in pig farming, while As is added to health products for the poultry industry [46]. HMs are released from the waste of various industrial processes, as summarized in Figure 1, and induce toxicity through environmental accumulation.

Soil contamination by HMs is site-dependent, resulting in high variability of average HM concentrations in different countries, as shown in Table 1. The root cause of this contamination is the rapid pace of industrialization and urbanization, especially in developing countries with higher populations [47]. Developed countries such as the USA, Belgium, and England release HM-polluted sludge that could be toxic to plant and animal health [48]. Some countries, including India, China, Brazil, and Russia, use animal dung as a soil amendment to improve crop production, which can lead to HM accumulation in agricultural land. Local industrial activities, laws, and practices strongly influence these aspects.

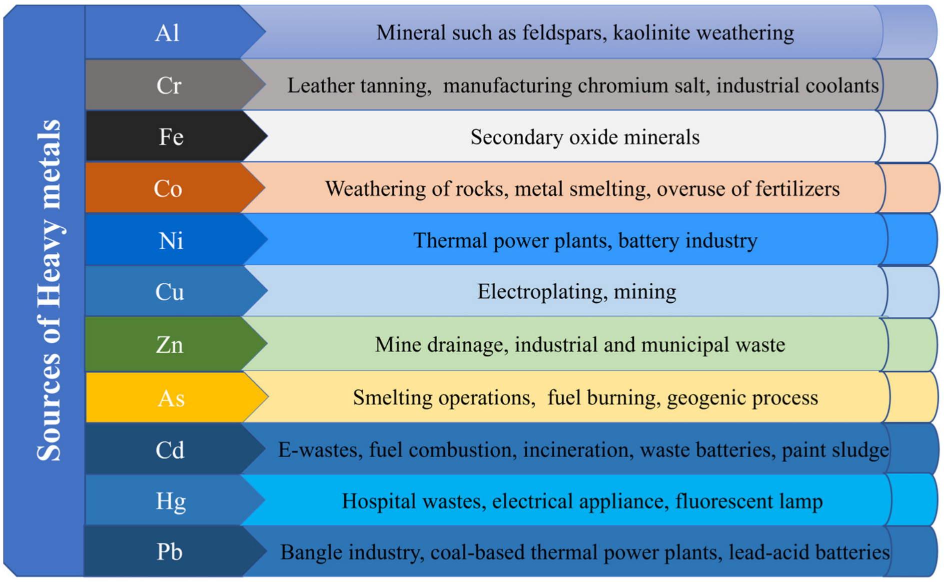

Figure 1. Heavy metals are commonly released from the waste of various industrial processes, inducing toxicity through environmental accumulation.

Table 1. Averaged heavy metal concentration (ppm) in the soil of different countries.

Country	Heavy Metal Concentrations (ppm) in Contaminated Soil									References
	Cr	Fe	Co	Ni	Cu	Zn	As	Cd	Pb	
USA	13.83	-	-	0.7–269	0.6–495	3–264	5.14	0.30	95.82	[49,50]
Turkey	342	68,200	86.4	755	647	529	89.8	-	-	[51]
Germany	76.7	-	11.8	0.5	28.6	123.3	-	0.60	41.6	[52]
France	58.5	-	16.3	37.1	116.6	3677	19.0	18.8	1023	[53]
Italy	34	-	5.2	17.8	63	138	-	0.68	202	[54]
Malaysia	0.1	6.6	0.2	-	0.77–1.03	6.90–9.90	9.38–57.05	0.05–0.08	8.8–10.84	[55–57]
China	40	-	-	33.65	46.98	119.82	20.49	0.44	43.85	[58]
Pakistan	-	3.9	-	8.8	12.9	56.7	-	0.8	36.5	[59]
India	82.7	10,344	8.5	92.9	48.7	76.2	14.8	1.6	15.7	[60]
Australia	0.6	-	-	2.9	13	20	0.7–62.5	0.15	11.5	[61,62]

2.1. Potential Risks from Heavy Metals in Soil, Plants, and Aquatic Ecosystems

Soil pollution by HMs is primarily due to Cr, Fe, Ni, Cu, Zn, As, Cd, and Pb accumulation. Certain minerals (HMs), such as Zn, Fe, Ca, Cu, and Mg, are essential and are recommended in human diets due to their positive impact on health. However, ensuring that their concentrations remain within safe and permitted levels is important. In comparison, other HMs (e.g., As, Cd, Hg and Pb) have no biological importance for human health and can produce noxious effects even in minute quantities [63]. These HMs harm soil microbial activity, population, and composition [64]. HMs also show detrimental and severe effects on plants' growth, development, and yield capacity. HMs cause oxidative stress, damage cells, inhibit the bioavailability of essential micronutrients, and disrupt photosynthetic activities [65]. Agrochemicals containing HMs can enter the aquatic environment through leaching and surface runoff, harming the aquatic environment.

2.1.1. Impact on Soil Ecology

HM-contaminated soil is deemed a "chemical time bomb" that may initiate severe ecological damage. Soil contamination due to HMs is an alarming situation in industrial zones worldwide. Pollution triggered by HMs affects soil fertility and modifies microbial flora size and diversity. HMs such as Cr, Cu As, and Cd have an enormous impact on clay content, organic matter, pH, and soil biochemistry. The accumulation of some HMs in the soil has a detrimental impact on microbial growth [66]. Soil microbes are effective in biochemical reactions to maintain soil quality, organic matter formation and decomposition, soil structure formation, detoxifying toxic elements, and controlling pests. Many investigations have shown that HM-contaminated soil is conducive to CO_2 release, thus inhibiting soil microbial activity and soil respiration, and threatening the soil ecosystem. Cr(VI) is a powerful oxidizing agent, thus extremely toxic [67]. It is known to modify the structure of communities of microorganisms, and it is recognized to have severe impacts on cell metabolism at higher intensities [68]. The primary effect of HMs is the disruption of populations of bacteria, fungi, and actinomycetes. In contaminated soil, bacterial diversity and biomass are reduced, but the proportion of actinomycetes grows [69]. The more organic carbon in contaminated soil, the poorer the ability of microbial populations to mineralize organically, which can hint at HMs toxification on microbial communities.

Along with microbes, soil enzymes are also influenced by numerous HMs due to their chemical attraction in the soil system [70]. HMs hinder enzyme reactions by forming complexes with substrates, attaching protein groups to the enzymes, and reacting with enzyme–substrate complexes [71]. Cd^{2+} binds with the sulfhydryl group of enzymes and impedes or deactivates the enzyme's activity [72]. As ion inactivates the enzymes by forming arsenic sulfide [73]. Pb is less noxious to enzymes due to its binding with soil colloids. It reduces urease, invertase, and catalase, whereas As inhibits phosphatase and sulfatase. Cr harms the alkaline phosphatase, urease, and protease activity. A higher concentration of Zn (>25 ppm in soil) shows a noxious effect on microbial biomass and several types of flora [74]. The schematic description of heavy metals' impact on the soil microbes and enzymes is shown in Figure 2.

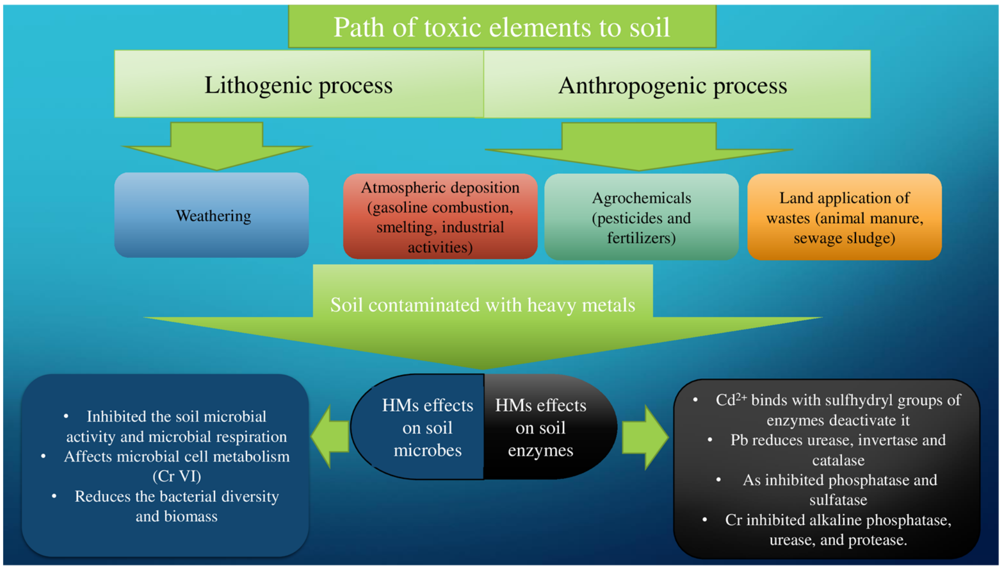

Figure 2. Schematic description of the pathway of heavy metals into the soil and their impact on soil microbes and enzymes.

2.1.2. Impact on Plants

Heavy metals such as As, Cd, Hg, Pb, and Se have no function in plant physiology and are thus unnecessary for plant growth. On the other hand, metals such as Fe, Cu and Zn are important for plant metabolism and growth; however, when their concentrations rise above the recommended limits, they can lead to toxicity [75,76]. Several studies elaborated on different mechanisms involved in the induction of heavy metal toxicity in plants [77,78]. Among these, oxidative stress represents the main physiological damage to plants caused by heavy metals (Figure 3).

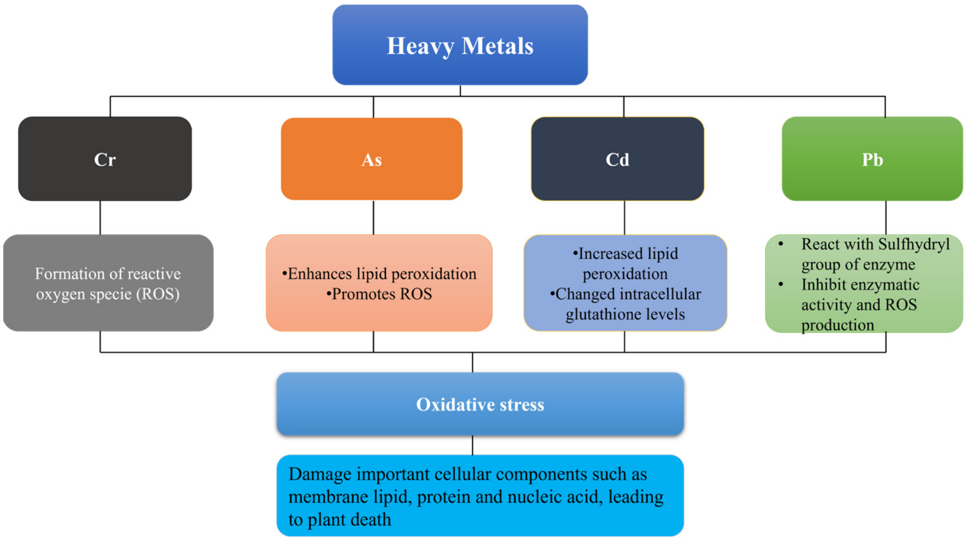

Figure 3. Summary of main physiological damage to plants caused by heavy metals, such as Cr, As, Cd and Pb at their higher concentrations.

Cd is a very mobile element in soil and is freely transported through the plant, circulating in all its organs [79–81]. In addition to its effects on water balance, Cd influences plants' absorption, transport, and use of various elements (P, K, Ca, Mg) [82]. At higher concentrations, plants show destructive symptoms such as growth inhibition, chlorosis, and root browning, ultimately leading to death [83,84]. Pb affects the plant morphology, germination, seedling growth, mineral nutrition, water content, and enzymatic activities within the plants [85,86]. Pb reacts with the sulfhydryl group, which causes inhibition of enzymatic activity and reactive oxygen species (ROS) production, resulting in oxidative stress [87]. As inhibits root proliferation, biomass production, and metabolic processes, and exacerbates the plant reproductive ability [88,89]. Higher concentrations of As(III) and As(V) stimulate ROS in plants [90]. Its toxicity boosts lipid peroxidation and harms cellular membranes due to electrolyte outflow [91]. As toxicity decreases nodule formation in roots, it causes wilting and necrosis of leaves.

Zn is an indispensable element and performs a vital role in the biosynthesis of auxins, enzymes, and other proteins. However, its higher concentration can cause oxidative damage, chlorosis at an earlier stage of plant growth, and changes in metabolic processes. Fe is a key element in the plant life cycle; however, its surplus accumulation can cause an oxidative burst, which damages thylakoid membrane energization, decreasing photosystem-II efficacy, enzyme activity, and protein synthesis [92–94]. The toxicity of Cu largely affects the growth and morphology of roots, as it accumulates in root tissues. Excessive quantities of Cu threaten membrane integrity, photosynthesis, and growth retardation in plants [95].

2.1.3. Impact on Aquatic Ecosystem

Large amounts of HMs move into the aquatic ecosystem through several sources, such as erosion from mines, wind, volcanic activity, and groundwater. Other sources of aquatic environment contamination include ore mining industries, which empty huge amounts of HMs into the aquatic system during mining activities. Aquatic environment pollution by drainage water coming from sulfur-containing rocks is referred to as acid mine drainage (AMD). AMD water might be contaminated with HMs present in the adjacent environment, which become soluble at lower pH and enter the water bodies [23].

The pH of drained water is marginally acidic, increasing the solubility of metal compounds. Nevertheless, HMs are not usually naturally removed, remaining in the ecosystem for a long time [96]. Water pollution disturbs the balance of aquatic ecosystems, thus reducing the population of marine creatures due to the magnitude of HMs [97]. The pollutants in aquatic environments remain insoluble/suspended in the water and/or taken by marine organisms [98]. The acidity and toxicity of HMs in drainage water makes it unfit for consumption. HMs accumulate in aquatic fauna, such as fish, through their body, gills, and digestive tract. However, the immediate uptake of metals is done by the gills [99]. These metals alter the activities of metabolites and enzymes, causing physiological and biochemical changes in fish. The presence of metals in the kidney, gills, and liver of fish causes skeletal lesions and functional disorders [100]. Zn contamination has a dispiriting impact on respiration, leading to hypoxia, which results in death. Excess Cr has noxious effects on blood changes, such as bronchial lesions and anemia. Hg causes neurotoxicity in fish by damaging the brain and causing congenital deformations. HMs bioaccumulate in the human body and can cause cramps, hypertension, and renal damage. HMs can be vitally dangerous to humans because of their cancerous effect and oxidative deterioration of macromolecules.

3. Existing Methods of HMs Contaminated Soil Remediation

Among various remediation technologies used for removing metal contamination in soil, in situ techniques are considered more effective than ex situ processes [101]. In situ remediation of polluted soil consists of physical (e.g., activated carbon, clay, and zeolite), chemical (e.g., hydrolysis reaction and ionization) and biological (e.g., bioremediation, compost addition, phytoremediation and bioaugmentation) [102]. However, many of these techniques are not frequently used on agricultural land due to soil erosion, leaching, high environmental hazards, and high costs [103,104]. Physio-chemical remediation methods such as washing, electrokinetic remediation, nanomaterial remediation, surface caping and solidification require less time, but they affect soil ecosystems. Biological techniques, however, are highly feasible and promising opportunities at a large scale but have some downsides regarding the time needed to complete the removal of pollutants from soil and metals' toxicity to microorganisms [105]. Among these methods, bioremediation and bioaugmentation are considered feasible techniques for the remediation of the affected soil [100]. Bioremediation carried out by microorganisms (microbial remediation) depends on the metabolic ability of microbes [106]. It can also be attained using plants that bind, isolate, and remediate soil from environmental contaminants (phytoremediation) [107]. Microbes and plants from polluted soil having bioremediation capacity can be artificially improved. The eventual aim of bioremediation is the reuse of soil by immobilization and/or transformation of pollutants into less toxic forms. The organisms with the abilities of noxiousness resistance, metals immobilization, adsorption, and degradation of pesticides are responsible for the redress of HMs and pesticide-contaminated soil [108,109]. The biodegradation and biotransformation process by actinobacteria is useful for the removal of lindane and reduction of Cr(VI) to Cr(III), respectively [110]. Microbial remediation is used to alleviate soil contamination with HMs except for Cr(VI) and pesticides. Numerous plants can mitigate HM- and pesticide-polluted soil such as *Medicago sativa*, *Spinacia oleracea*, and *Lolium perenne* [111,112]. However, phytoremediation is sometimes not as effective as expected. The growth of Lupinus luteus is inhibited and endophytes do not colonize plants with HMs-contaminated landfill soil even at the lowest pollution [113]. The collapse of phytoremediation is primarily due to the combined stress caused by organic contaminants and HMs. This combined stress may reduce plant growth, biomass, inadequate uptake, and transfer of contaminants by plants, changes in rhizosphere microbes' activity and population, and failure of colonization of endophytic bacteria. Moreover, the soil properties, plant species, plant growth stage during phytoremediation and trial conditions also affect phytoremediation.

Among biological remediation techniques, bioaugmentation seems to be the most auspicious way to detoxify heavy metals by adding local and exogenic microbes that can endure and diminish the toxicity of HMs [114]. Local microbes are separated from polluted soil and injected back into that corresponding polluted soil after multiplication, whereas exogenous microorganisms are separated from fertile soil and inoculated into contaminated soil [115–117]. Bioaugmentation depends upon the improvement of the catabolic ability of soil microbes for the contaminant's degradation. Further, genetically modified microorganisms, which exhibit the degradation capability of toxic pollutants, are potent in bioaugmentation. The selection of microbial strains for bioaugmentation should have the ability to grow faster, be easy to cultivate, have a high potential for degradation, and be able to live in a broad range of environmental conditions [118]. Several processes involved in this technique are bio-chelation, biomineralization, biotransformation and biodigestion [119]. However, the bioaugmentation method has several limitations to use for the detoxification of large-scale contaminated soil. Separation of HMs from large-scale contaminated soil by centrifugation is not feasible due to the unavailability of large centrifugation equipment, high energy consumption and time-consuming processes. The separation efficiency of HMs and clean soil report is currently limited, for instance, only 5.5% of stable HMs can be retrieved by bioaugmentation of aluminum-polluted soil [120].

Combined remediation technologies could be excellent alternative approaches for detoxifying HMs and organic pollutants. These refer to the combined application of two or more remediation techniques to accomplish contaminants remediation and enhance the remediation rate. For example, plant-microbe remediation is extensively used as a combined bioremediation technique for contaminated soil bioremediation. However, its efficiency is also affected by many factors: soil physicochemical properties (pH, EC, water retention), interactions with plant microbes, species of plants, type of contaminants and bioavailability potential of microbes. Unfortunately, this approach is not generally applicable to soil polluted with bio-resistant molecules, as in the case of Polycyclic Aromatic Hydrocarbons (PAH). PAH is firmly attached to the soil organic matter, and their low bioavailability constitutes the main limitation of this technique [121]. In this scenario, the addition of biochar could offer synergistic options improving the soil remediation efficiency due to its peculiar physio-chemical properties, enhancing both the physical adsorption of PAH and active soil microbial population [122].

4. Vermibiochar as Bio-Conditioner to Immobilize Heavy Metals

As detailed above, the combined remediation methods are limited to cultured and/or natural microbial strains and plants. However, the combined remediation with the use of effective biological vectors for microbes, such as earthworms with biochar activation, has not been investigated much. Earthworms have a great influence on soil microbiota. Vermicomposting and drilosphere (soil under the impact of worms) are two environments with high populations and a diversity of microorganisms and exoenzyme production [123–125]. Various earthworm species, such as *Eisenia fetida*, *Eudrilus eugeniae*, *Eisenia Andrei* and *Lumbricus rubellus* have been assessed for their potential application in vermicomposting processes. However, the most frequently used species in vermi-technology is *E. fetida*. Earthworms can alter the metal concentrations in soil by accumulating them in their tissues [126], thus reducing their presence in the food chain. The HMs biotransformation and detoxification ability of earthworms is due to the influence of gut microflora, chloragocyte cells, enzymes, and active metabolic system [127]. This mutual action of earthworms and microbes confirms the effectiveness of vermi-technology for diminishing the genotoxicity of wastes. It is also noted that the alteration in dissolved organic carbon (DOC) in the soil solution can overwhelm the availability of metals by forming complexes with metals [128,129]. During the activity of earthworms, humic acid is produced. This can increase the HMs availability to plants by forming organo-metal complexes [130]. A considerable correlation has been observed between the effect of earthworms boosting DOC and metals concentration (Cr, Co, Ni, Cu, Cd, and Pb) [131]. The specific site of metal absorption in earthworms is chloragogenous tissue encircling the dorsal alimentary canal. Two pathways exist in this tissue for the binding of heavy metals. Pathway-1 is involved in the binding of metals in chloragosomes (non-soluble phosphate-rich chloragocytes granules). Pathway-1 is considered to sequester Zn and Pb in an insoluble form by replacing Ca, thus immobilizing the metals in toxic concentrations [132]. Pathway-2 involves the metal binding to cadmosome (cysteine-rich protein, such as metallothionein in mammals, enclosed in vesicular organelle) [133,134] as explained in Figure 4. Pathway-2 is deemed as a distinct function reliant on metal concentration in soil.

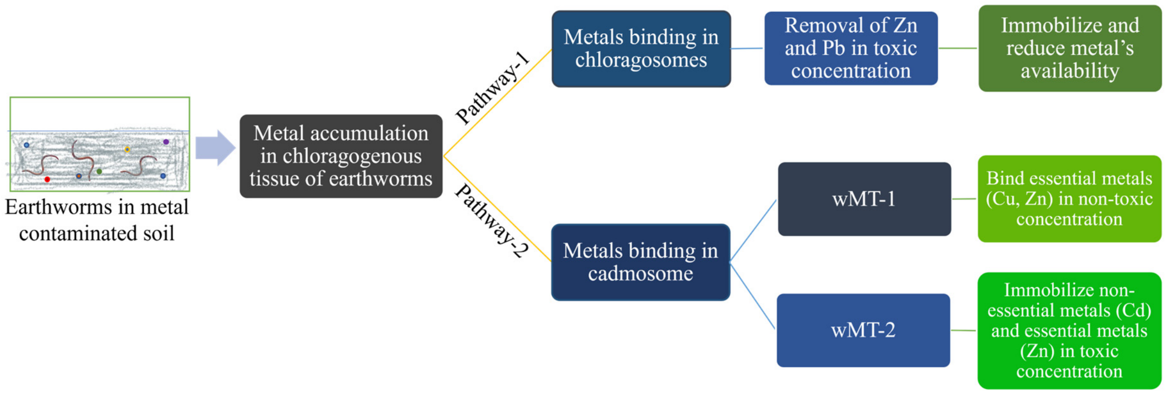

Figure 4. Mechanism of metal accumulation in chloragogenous tissue of earthworms. Pathway-1 sequesters Zn and Pb in an insoluble form in toxic concentrations, while Pathway-2 involves the metals Zn and Cd binding to cadmosomes, similar to metallothionein in mammals.

Two variants of metallothionein protein, wMT-1 and wMT-2, have been identified in earthworms and are induced when they are exposed to any stress, such as contaminants. The former variant (wMT-1) acts as a transporter for essential metals in non-toxic concentrations, while the latter helps to immobilize Zn and Cd in high concentrations [132]. The HMs in the polluted waste materials may cause toxicity in earthworms, as they cannot survive in high concentrations of HMs. The LC_{50} (the concentration of HMs in waste material that kills 50% of earthworms) of different metals is shown in Table 2. Therefore, a bulking agent, such as biochar, can be used to immobilize metals in waste materials. Moreover, the immobilizing potential can be enhanced through the bioactivation of bulking agents, such as biochar, due to the binding of exoenzymes on the biochar surface [135]. Adding such bulking agents can have a constructive effect during vermicomposting process. The mobility and bioavailability of metal elements are reduced when fly ash and phosphoric rock are added to sewage sludge vermicomposting [136].

Table 2. LC_{50} values of various heavy metals on earthworms.

Heavy Metal	LC_{50} of Metals on Earthworms (mg kg^{-1})	Reference
Cu	400–450	[137]
Zn	1500–1900	
Cd	900	
Pb	2350–2400	
Cr(III)	1656–1902	[138]
Cr(VI)	222–257	
Hg	170	[139]

HM bioavailability in sewage sludge is decreased by the addition of biochar during vermicomposting [140], and a reduction in the degradation of OM is also observed [31]. Till now, little investigation has been made to demonstrate the effect of biochar addition in vermicomposting, the earthworms' response, and the final product quality. Previous investigations have focused on the impact of biochar on earthworms' growth and activity in soil [141]. This review elaborates on the combined potential of earthworms and biochar in the HMs detoxification process, as shown in Figure 5. Vermicompost containing biochar as a constituent that is bioactivated during vermicomposting is termed vermibiochar [142].

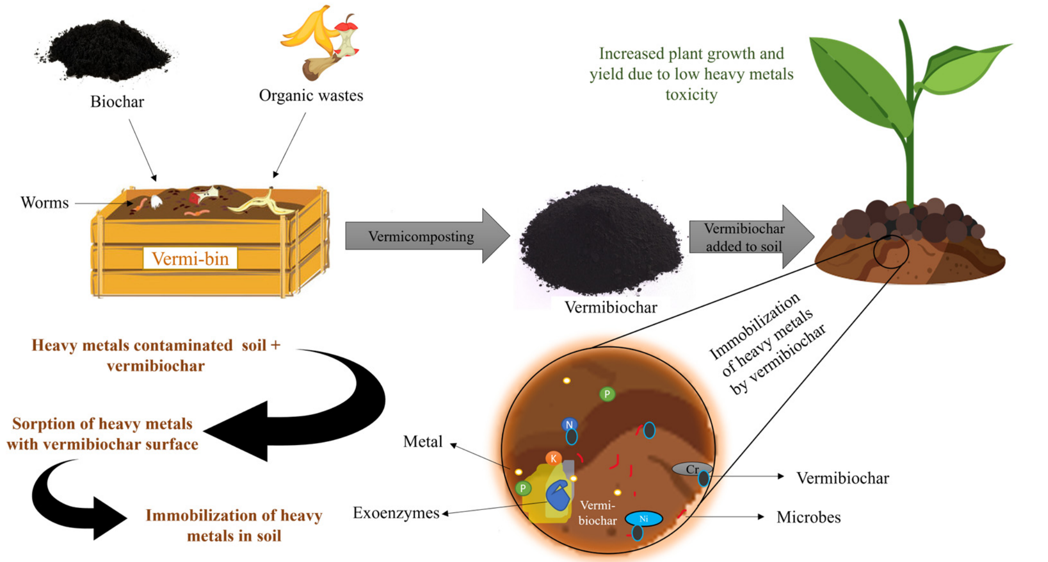

Figure 5. The combined potential of earthworms and biochar in the bioaccumulation and immobilization of HMs during the vermicomposting process and in HMs contaminated soil, respectively.

Biochar could be a valuable amendment in vermicomposting as it reduces ammonia emissions [143], and nitrogen losses [144], and improves compost quality [145]. The combined application of compost and biochar has excellent immobilizing potential for Pb and Cu from the soil of copper mines [146]. The reduction in heavy metals' bioavailability after biochar addition to composted material can be due to physical sorption, precipitation, and complex formation on the biochar surface [28,147]. Many studies indicated that adding biochar during sewage sludge composting reduced the Ni, Cu, Zn and Pb availability [148,149]. Phosphorus-rich biochar significantly reduced the available Pb contents in metal-contaminated soil [150]. The available Cu concentration in soil is reduced up to 29% with biochar application [151]. Soil amendment of rabbit manure-derived biochar reduced plant-available Cu in roots (38%) and shoots (82%) of *Brassica napus* [152]. *Oenothera picensis* roots accumulated more Cu in the presence of biochar, and this might be due to the complexation of Cu and biochar in soil [153]. Additionally, the Zn concentration in biochar-corrected soil elute was strongly reduced from 270 µg/L to 10 µg/L, due to Zn immobilization by irreversible adsorption of Zn on the biochar surface along with alkalinization [154]. The joint action of biochar with earthworms increases Zn bioavailability [155]. Moreover, the addition of biochar in serpentine soil immobilizes Ni and decreases Ni toxicity in tomato plants [156]. Biochar immobilizes Cd in soil by adsorption, complexation, and ion exchange processes [157].

As described, modification of biochar during the pre-digestion phase of vermicomposting is effective for HMs immobilization in the final vermicompost [158] and boosts vermicomposting efficiency [140]. The annexation of the biochar surface by microbes explains the synergistic effect of biochar for HM-reduction processes. Moreover, the presence of earthworms in the decomposing vermicomposting process can offer further benefits, particularly through the earthworms' mucus, which contains amino acids, mucopolysaccharides, and glycoproteins secreted by the gastrointestinal epithelial cells of worms. This mucus assists in the movement of worms through the soil and in the transfer of consumed materials through the worm's digestive system [159–161]. Indeed, it has been proven that the epidermis mucus of *E. fetida* can hasten the decomposition and humification of the ingested material by the potential of modifying microbial communities [162]. The worm gut secretes a wide variety of enzymes as catalysts that boost biochemical reactions [163]. Various studies elaborate that enzyme activity is at its peak during the first 2–3 weeks of vermicomposting, then their activity is gradually reduced due to a decrease in the activity of microbes and earthworms as vermicompost stabilizes [164]. In this perspective, the addition of amendments, such as biochar, boosts the stability of enzymes. The biochar surface comprises binding sites and different functional groups that can occupy the enzymes during vermicomposting and become activated. The adsorption of enzymes to the biochar surface depends on the type of enzymes, biochar, and substrate pH [165]. These studies indicated that activated biochar adsorbed the metals in the contaminated soil, thus reducing HM toxicity. The literature also indicated that the earthworms reduced HM mobility and bioaccumulation in their tissues. The potential of vermicompost and biochar in metal detoxification and their effect on vermibiochar properties are explained in Table 3.

Vermibiochar can adsorb several heavy metal ions and its adsorption depends on the selection of biochar. Descriptively, the biochar prepared by dairy waste can eliminate several HM ions such as Ni^{+2}, Cu^{+2}, Cd^{+2}, Pb^{+2} [166] and Zn^{+2} [167]. The adsorption of Cd^{+2} and Pb^{+2} increases during the vermicomposting of the cow manure process [168]. Vermibiochar produced from kitchen waste and sewage sludge mixed with pine tree, poplar plant, wetland plant, and yard waste biochar significantly reduced the Cr (7.3–10.8%), Mn (3.2–8.4%), Cu (3.1–7.4%), Zn (1.1–5.7%), Cd (0.2–5.1%) and Pb (9.0–45.9%) [169].

Vermibiochar contains several organic acids, such as humic acid, and enzymes that can bind with heavy metal ions and improve soil and water quality. Indeed, biochar activation through vermitechnology (vermibiochar) has developed a new role in industrial sludge and wastewater treatment, as well as the remediation of agricultural land [36]. Vermibiochar is a cost-effective method of heavy metal remediation compared to activated carbon and MFOs due to its low production cost and environmentally friendly remediation. Moreover, vermibiochar can replace all types of peat used in greenhouse production of horticultural crops [142].

Table 3. Literature-reported efficiency of vermicomposting and biochar in detoxifying/immobilizing heavy metals and their effects on plant growth.

Material	Earthworm Specie	Metal Detoxified	Metal Removal Efficiency/Adsorption Capacity and/or Bioavailability Reduction	Physico-Chemical and Plant Growth Characteristics	Reference
BC + VC	-	Cd	BC and VC reduced Cd uptake by plant	Combined VC and BC improved the biochemical status of *B. integerrima*	[170]
BC prepared from SS	*E. fetida*	Cu, Zn	Biochar immobilized Zn and Cd for *E. fetida*	Biochar-amended vermicompost proved an excellent fertilizer with pH of 5.27–5.61	[30]
SS + ST	*E. fetida*	Mn, Fe, Cu, Zn, Pb	Concentration reduction: Cu (4.98–30.5%); Fe (5.08–12.64%); Mn (3.31–18.0%); Zn (2.52–15.90%); Pb (2.38–20.0%)	Decrease organic C (4.8–12.7%) and exchangeable K (3.2–15.3%) content. Increased total N (5.9–25.1%), available P (1.2–10.9%), exchangeable Ca (2.3–10.9%) and exchangeable Mg (4.5–14.0%) contents	[171]
CS + CD	*Eudrilus eugeniae*	Cr, Zn, Cd, and Pb	Heavy metal concentration reduced by 50–60%	N and P availability was significantly increased, whereas pH, Ca, S, and organic C reduced	[172]
Sewage sludge + spent mushroom compost	*Lumbricus rubellus*	Cr, Cu, Zn Cd, Pb	90–98.7% removal of Cd, Cr, and Pb, but Zn and Cu concentrations slightly increased, but it is 10–200-times below the EU and USA biosolid compost limits	Produced vermicompost is good as a biofertilizer	[173]

Table 3. *Cont.*

Material	Earthworm Specie	Metal Detoxified	Metal Removal Efficiency/Adsorption Capacity and/or Bioavailability Reduction	Physico-Chemical and Plant Growth Characteristics	Reference
Soil leaching with EDTA	*Lumbricus rubellus, E. fetida*	Zn, Pb	As consequence of *E. fetida* digestion, the bioavailability of Pb in casts was higher than in soil.	-	[174]
Spent pleurotus sajor-caju compost + livestock manure	*Lumbricus rubellus*	Cr, Cd, Pb	99.81% removal of Cr, Cd, and Pb	C/N ratio of vermicompost ranges 20.65–22.93 and it is a valuable tool for soil conditioner	[175]
Sewage sludge vermicompost	Iranian and Australian *E. fetida*	Cr, Cu, Zn, Cd, Pb	Iranian species bioaccumulated higher Cr, Cu, Cd, Pb, and Zn. Whereas, Australian species accumulated more Cr, Cd, and Pb	-	[176]
Sewage sludge	*E. fetida, E. eugeniae, P. excavatus*	Mn, Cu, Zn, Pb	Heavy metals amount reduced below the permissible limits	Reduced C/N ratio 25.6 to 6–9, TOC (25%) but increased EC (47–51%), total N (2.4–2.8 times), K (45–71%), Ca (49–62%), Na (62–82%) and total P (TP) (1.5–1.8 times)	[177]
Sewage sludge + fly ash (FA) + phosphoric rock (PR)	*E. fetida*	Cu, Zn, As, Cd, Pb	Addition of 20% FA mitigates the Cu and Zn toxicity, whereas 20% PR addition reduces the Pb, Cd, and As toxicity	The total organic carbon (TOC) and total metal concentrations in the mixtures decreased	[178]
Milk Processing Industry Sludge	*E. fetida*	Cr, Mn, Cu, Pb	Metal concentration reduction: Cr (30.9–40.6%), Cu (32.7–44.6%), Mn (23.9–36.3%), Pb (32.6–42.9%)	Significant increase in TKN (23–46%), and TAP (39–47%), and a decrease in pH (6.2–6.8%), EC (24.6–37.2%), TOC (16.8–37.9%), C/N ratio (23.8–97.9%), TK (26.6–40.6%), and Total Na (31.3–53%)	[179]
Rice Straw Biochar	-	Cu	Adsorption capacity of BC600 was higher (Q_m = 43.75 mg g^{-1}) in Cu concentration of 0~300 g kg^{-1}	Reduced Cu uptake by ryegrass	[180]
Sewage sludge + (soil, straw, fly ash and sawdust)	*E. fetida*	Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Pb,	The vermicomposting process lowered the total heavy metals concentration	pH, TOC decreased, while the EC and germination index improved	[181]
Vermicompost (VC) and biochar (BC)	-	Cd	Cd concentration reduction: VC, 5.2–6.8%; BC, 9.0–13.5%; VC+BC, 7.9–12.1%	Soil conditioners indicated potentials to enhance soil fertility	[182]
Silk processing effluents and sludge + cow dung	*E. fetida* and *E. eugeniae*	Cr, Zn, Cd and Pb	*E. fetida* reduced the metals by 60–70%	Nutrient fortification of vermicompost increased	[183]
Dairy Manure Derived Biochar	-	Pb	Pb sorption was higher (up to 680 mmol Pb kg^{-1}) with the application of BC200 (prepared at 200 °C)	-	[184]

Table 3. *Cont.*

Material	Earthworm Specie	Metal Detoxified	Metal Removal Efficiency/Adsorption Capacity and/or Bioavailability Reduction	Physico-Chemical and Plant Growth Characteristics	Reference
Sewage sludge and urban plant litter	*E. fetida*	Cd	The Cd content in vermicompost with urban plant litter addition was decreased by about 31%	TOC increased significantly with UPL addition. In vermicompost, macroaggregates (0.25–2 mm) increased by 119.11–165.29%, whereas in silt and clay particles (<0.053 mm), macroaggregates decreased by 64.90–75.67%	[185]
Silk and cotton processing sludge	*E. fetida* and *E. eugeniae*	Cr, Zn, Cd, Pb	HMs accumulation efficiency of earthworms was higher in silk-based vermibed	-	[186]

BC: Biochar; VC: Vermicompost; SS: Sewage sludge; ST: sugarcane trash; CS: Cotton textile sludge; CD: cow dung, TKN: Total Kjeldahl nitrogen, TAP: Total available phosphorus, N: Nitrogen, P: Phosphorus, K: Potassium, Ca: Cal-cium, Mg: Magnesium, Zn: Zinc, S: Sulfur, Fe: Iron, OM: Organic matter, TOC: Total organic carbon.

5. The Response of Earthworms to Biochar Amendment

The plant-based biochar is epitomized as a soil conditioner and thus may affect the bio-interactions in the soil ecosystem. However, previous findings have revealed different responses of earthworms to biochar in contaminated soil. In some instances, earthworms have preferred the soil amended with biochar, which might be due to the liming characteristics of biochar [187,188]. Zhang et al. [189] performed an experiment to study the response of earthworms (*E. fetida*) to biochar amendment (at the rate of 0, 1, 3, and 10%) in pesticide-contaminated soil. He observed no earthworm mortality in any of the treatments. The average weight of *E. fetida* increased in the soil mixed with a low level of biochar (1–3%) and decreased with a higher level (10%). This positive effect of biochar can be ascribed to improved nutrients, microbial activity, and water-holding capacity [190]. The impact of biochar amendment on earthworms has also been studied by Malinska et al. [140]. Their results depicted a positive effect of biochar on the number of cocoons and juveniles, but no significant effect on earthworm biomass was reported. The effect of adding biochar to a composting system depends on the concentration of biochar added. In the mixture containing biochar (4% and 8%), the number of cocoons and juveniles was enhanced by 13% and 66%, respectively, after 4 weeks. The decrease in the number of cocoons after 18 weeks can be due to an increase in the worm population and low organic matter. However, a significant rise in the number of juvenile worms was detected after 18 weeks, as illustrated in Figure 6. Gong et al. [191] confirmed that the biochar addition enhanced the earthworm biomass, juveniles, and cocoons of *E. fetida*, in addition to increasing the dehydrogenase, urease, alkaline phosphatase, and cellulase activity. In contrast, the findings of Tammeorg et al. [140] show that biochar and earthworms have antagonistic effects in terms of pH, CEC, enzymatic activity and earthworm growth. The presence of *E. fetida* has been shown to decrease soil pH and CEC, whereas biochar has been found to increase them. However, biochar also suppresses earthworm growth and enzyme activity. As such, further research should be conducted to examine the practical effects of amending vermicomposting systems with biochar on earthworm growth and reproduction.

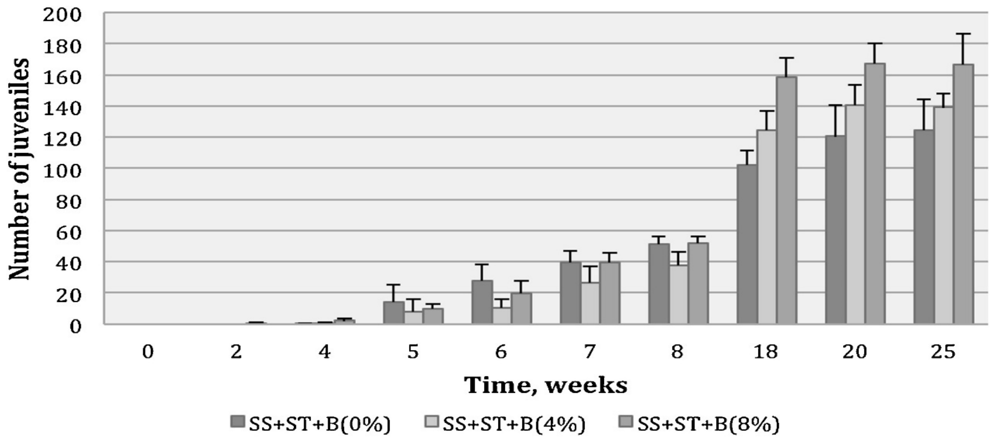

Figure 6. The effect of adding varying amounts of biochar (B) to a composting system based on a sewage sludge and wheat straw mixture (SS + ST) on the number of juveniles of *E. fetida* during 25 weeks of vermicomposting. This figure is reprinted from [137] with permission from Elsevier.

6. Prospects of Vermibiochar

Vermibiochar is an effective substitute for HMs immobilization in polluted soil due to its binding with HMs through adsorption, ion exchange, and complexation [157]. As described in this review, biochar has a large surface area with active binding sites for HMs, which increases its binding with heavy metals and boosts metal immobilization. Some contaminants may already be present on the surface of biochar before it is added to the vermicomposting process. The ongoing use of earthworms with biochar that has retained pollutants during vermi-remediation could lead to the death of these organisms. Consequently, further investigation is needed to evaluate the toxicity of contaminants retained on biochar concerning earthworm gut health. The biochar prepared from different wastes at different pyrolysis temperatures and its concentration in pre-composted material will affect earthworms differently. Thus, the dose and type of biochar and specific earthworm species suitable to produce vermibiochar also need the scientific community's attention. Some valuable characteristics of biochar that have been revealed to diminish HMs mobility can also immobilize essential plant nutrients in the soil. Engaging soil remediation techniques primarily aims to improve soil quality favorable for plant growth. Therefore, extensive research is needed to assess the use of biochar with other bio-stimulants, such as products derived from earthworms, which contain microbial and nutritional characteristics. Although a few studies have been conducted in the lab on the remediation of contaminated soil through vermibiochar, its efficacy on soil properties and plant growth under field conditions is still lacking. In this review, we have attempted to provide an insight into current knowledge covering the use of vermibiochar for the remediation of polluted soil, along with implying the direction for future needs.

7. Conclusions

Soil pollution is a significant environmental issue originating from either lithogenic or anthropogenic activities and HM contamination is a major concern among these. HMs show detrimental effects on plants' growth, development, and yield by causing oxidative stress, cell damage, inhibiting the bioavailability of essential micronutrients, and disrupting photosynthetic activities.

The joint action of biochar and earthworms can be a sustainable choice for the remediation of contaminated soil, improving soil biodiversity, and soil health. The large surface area and high cation exchange capacity enhance the sorption of contaminants (such as HMs) onto the vermibiochar surface, reducing their bioavailability. The present investigations indicate the effectiveness of biochar through exoenzymes produced by earthworms. The enzymes on the vermibiochar surface increase their affinity for metal ions in contaminated soil, and thus the soil contaminants. This review highlights the possible use of vermibiochar for a new soil bioremediation and soil quality improvement technique in the coming future.

Author Contributions: Conceptualization: S.u.R. and M.B.; data curation: S.u.R., F.D.C., P.M. and M.B.; writing—original draft preparation: S.u.R. and F.D.C.; active discussion: P.M.; writing—reviewing and editing: S.u.R., A.A., M.B. and F.P.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the project "National Biodiversity Future Center—NBFC" (project code: CN00000033).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data are available upon request.

Conflicts of Interest: The authors declare no competing interests.

References

1. Kalyvas, G.; Gasparatos, D.; Liza, C.A.; Massas, I. Single and combined effect of chelating, reductive agents, and agro-industrial by-product treatments on As, Pb, and Zn mobility in a mine-affected soil over time. *Environ. Sci. Pollut. Res.* **2020**, *27*, 5536–5546. [CrossRef] [PubMed]
2. Abrahams, P. Soils: Their implications to human health. *Sci. Total Environ.* **2002**, *291*, 1–32. [CrossRef] [PubMed]
3. Asai, H.; Samson, B.K.; Stephan, H.M.; Songyikhangsuthor, K.; Homma, K.; Kiyono, Y.; Inoue, Y.; Shiraiwa, T.; Horie, T. Biochar amendment techniques for upland rice production in Northern Laos: 1. Soil physical properties, leaf SPAD and grain yield. *Field Crops Res.* **2009**, *111*, 81–84. [CrossRef]
4. Gasparatos, D.; Barbayiannis, N. The Origin of Nickel in Soils. *Nickel Soils Plants* **2018**, *3600*, 105.
5. Shaheen, S.M.; Antoniadis, V.; Kwon, E.; Song, H.; Wang, S.-L.; Hseu, Z.-Y.; Rinklebe, J. Soil contamination by potentially toxic elements and the associated human health risk in geo-and anthropogenic contaminated soils: A case study from the temperate region (Germany) and the arid region (Egypt). *Environ. Pollut.* **2020**, *262*, 114312. [CrossRef] [PubMed]
6. De Castro, F.; Vergaro, V.; Benedetti, M.; Baldassarre, F.; Del Coco, L.; Dell'Anna, M.M.; Mastrorilli, P.; Fanizzi, F.P.; Ciccarella, G. Visible light-activated water-soluble platicur nanocolloids: Photocytotoxicity and metabolomics studies in cancer cells. *ACS Appl. Bio Mat.* **2020**, *3*, 6836–6851. [CrossRef]
7. De Castro, F.; Stefàno, E.; Migoni, D.; Iaconisi, G.N.; Muscella, A.; Marsigliante, S.; Benedetti, M.; Fanizzi, F.P. Synthesis and Evaluation of the Cytotoxic Activity of Water-Soluble Cationic Organometallic Complexes of the Type $[Pt(\eta^1\text{-}C_2H_4OMe)(L)(Phen)]^+$ ($L = NH_3$, DMSO; Phen = 1, 10-Phenanthroline). *Pharmaceutics* **2021**, *13*, 642. [CrossRef]
8. Benedetti, M.; Girelli, C.R.; Antonucci, D.; Fanizzi, F.P. $[PtCl(\eta^1\text{-}CH_2\text{–}CH_2OR)(N\hat{}N)]$ and $[PtCl(\eta^2\text{-}CH_2{=}CH_2)(N\hat{}N)]^+$, *N^N* = dinitrogen ligand, complexes. Sterical and electronic effects evidenced by NMR analysis. *J. Organomet. Chem.* **2014**, *771*, 40–46. [CrossRef]
9. Benedetti, M.; Barone, C.R.; Girelli, C.R.; Fanizzi, F.P.; Natile, G.; Maresca, L. H/D exchange at sp^3 carbons in the coordination sphere of platinum(ii). *Dalton Trans.* **2014**, *43*, 3669–3675. [CrossRef]
10. Benedetti, M.; Antonucci, D.; Girelli, C.R.; Capitelli, F.; Fanizzi, F.P. Reactivity of $[PtCl(\eta^2\text{-}C_2H_4)(N\text{-}N)]^+$, *N-N* = diimine ligand, with phenol derivatives and first comparison between single crystal X-ray structures of *syn*-and *anti*-$[Pt(N\text{-}N)(phenolate)_2]$ rotamers in the solid state. *Inorg. Chim. Acta* **2014**, *409*, 427–432. [CrossRef]
11. Ahmadpour, P.; Ahmadpour, F.; Mahmud, T.; Abdu, A.; Soleimani, M.; Tayefeh, F.H. Phytoremediation of heavy metals: A green technology. *Afri. J. Biotechnol.* **2012**, *11*, 14036–14043.
12. Hossain, Z.; Komatsu, S. Contribution of proteomic studies towards understanding plant heavy metal stress response. *Front. Plant Sci.* **2013**, *3*, 310. [CrossRef] [PubMed]
13. Ovečka, M.; Takáč, T. Managing heavy metal toxicity stress in plants: Biological and biotechnological tools. *Biotechnol. Adv.* **2014**, *32*, 73–86. [CrossRef] [PubMed]
14. Sharma, R.K.; Agrawal, M. Biological effects of heavy metals: An overview. *J. Environ. Biol.* **2005**, *26*, 301–313. [PubMed]
15. Singh, S.; Parihar, P.; Singh, R.; Singh, V.P.; Prasad, S.M. Heavy metal tolerance in plants: Role of transcriptomics, proteomics, metabolomics, and ionomics. *Front. Plant Sci.* **2016**, *6*, 1143. [CrossRef]
16. Benedetti, M.; De Castro, F.; Fanizzi, F.P. Square-Planar Pt^{II} versus Octahedral Pt^{IV} Halido Complexes: ^{195}Pt NMR Explained by a Simple Empirical Approach. *Eur. J. Inorg. Chem.* **2016**, *2016*, 3957–3962. [CrossRef]
17. Benedetti, M.; De Castro, F.; Antonucci, D.; Papadia, P.; Fanizzi, F. General cooperative effects of single atom ligands on a metal: A ^{195}Pt NMR chemical shift as a function of coordinated halido ligands' ionic radii overall sum. *Dalton Trans.* **2015**, *44*, 15377–15381. [CrossRef]

18. Benedetti, M.; De Castro, F.; Fanizzi, F. ^{73}Ge, ^{119}Sn and ^{207}Pb: General cooperative effects of single atom ligands on the NMR signals observed in tetrahedral [MX_nY_{4-n}] (M = Ge, Sn, Pb; 1 ≤ n ≤ 4; X, Y = Cl, Br, I) coordination compounds of heavier XIV group elements. *Dalton Trans.* **2017**, *46*, 2855–2860. [CrossRef]
19. Ugwu, E.I.; Agunwamba, J.C. A review on the applicability of activated carbon derived from plant biomass in adsorption of chromium, copper, and zinc from industrial wastewater. *Environ. Monit. Assess.* **2020**, *192*, 240. [CrossRef]
20. Lupa, L.; Maranescu, B.; Visa, A. Equilibrium and kinetic studies of chromium ions adsorption on Co (II)-based phosphonate metal organic frameworks. *Sep. Sci. Technol.* **2018**, *53*, 1017–1026. [CrossRef]
21. Nabipour, H.; Rohani, S.; Batool, S.; Yusuff, A.S. An overview of the use of water-stable metal-organic frameworks in the removal of cadmium ion. *J. Environ. Chem. Eng.* **2022**, *11*, 109131. [CrossRef]
22. Zhang, Y.; Zhao, M.; Cheng, Q.; Wang, C.; Li, H.; Han, X.; Fan, Z.; Su, G.; Pan, D.; Li, Z. Research progress of adsorption and removal of heavy metals by chitosan and its derivatives: A review. *Chemosphere* **2021**, *279*, 130927. [CrossRef] [PubMed]
23. Das, P.K. Phytoremediation and nanoremediation: Emerging techniques for treatment of acid mine drainage water. *Def. Life Sci. J.* **2018**, *3*, 190–196. [CrossRef]
24. Benedetti, M.; Antonucci, D.; De Castro, F.; Girelli, C.R.; Lelli, M.; Roveri, N.; Fanizzi, F.P. Metalated nucleotide chemisorption on hydroxyapatite. *J. Inorg. Biochem.* **2015**, *153*, 279–283. [CrossRef]
25. Gu, X.; Liu, Z.; Wang, X.; Luo, J.; Zhang, H.; Davison, W.; Ma, L.Q.; Xue, Y. Coupling biological assays with diffusive gradients in thin-films technique to study the biological responses of *Eisenia fetida* to cadmium in soil. *J. Hazard. Mater.* **2017**, *339*, 340–346. [CrossRef]
26. Rehman, S.u.; Aslam, Z.; Aljuaid, B.S.; Abbas, R.N.; Bashir, S.; Almas, M.H.; Awan, T.H.; Belliturk, K.; Al-Taisan, W.a.A.; Mahmoud, S.F. Reduction in the Allelopathic Potential of *Conocarpus erectus* L. through Vermicomposting. *Sustainability* **2022**, *14*, 12840. [CrossRef]
27. Wang, K.; Qiao, Y.; Zhang, H.; Yue, S.; Li, H.; Ji, X.; Liu, L. Bioaccumulation of heavy metals in earthworms from field contaminated soil in a subtropical area of China. *Ecotoxicol. Environ. Saf.* **2018**, *148*, 876–883. [CrossRef]
28. Lehmann, J.; Joseph, S. *Biochar for Environmental Management: Science, Technology and Implementation*; Routledge: New York, NY, USA, 2015; pp. 111–121.
29. Inyang, M.I.; Gao, B.; Yao, Y.; Xue, Y.; Zimmerman, A.; Mosa, A.; Pullammanappallil, P.; Ok, Y.S.; Cao, X. A review of biochar as a low-cost adsorbent for aqueous heavy metal removal. *Crit. Rev. Environ. Sci. Technol.* **2016**, *46*, 406–433. [CrossRef]
30. Shaaban, M.; Van Zwieten, L.; Bashir, S.; Younas, A.; Núñez-Delgado, A.; Chhajro, M.A.; Kubar, K.A.; Ali, U.; Rana, M.S.; Mehmood, M.A. A concise review of biochar application to agricultural soils to improve soil conditions and fight pollution. *J. Environ. Manag.* **2018**, *228*, 429–440. [CrossRef]
31. Malińska, K.; Golańska, M.; Caceres, R.; Rorat, A.; Weisser, P.; Ślęzak, E. Biochar amendment for integrated composting and vermicomposting of sewage sludge–the effect of biochar on the activity of *Eisenia fetida* and the obtained vermicompost. *Bioresour. Technol.* **2017**, *225*, 206–214. [CrossRef]
32. Ngo, P.-T.; Rumpel, C.; Ngo, Q.-A.; Alexis, M.; Vargas, G.V.; Gil, M.d.l.L.M.; Dang, D.-K.; Jouquet, P. Biological and chemical reactivity and phosphorus forms of buffalo manure compost, vermicompost and their mixture with biochar. *Bioresour. Technol.* **2013**, *148*, 401–407. [CrossRef] [PubMed]
33. Li, H.; Dong, X.; da Silva, E.B.; de Oliveira, L.M.; Chen, Y.; Ma, L.Q. Mechanisms of metal sorption by biochars: Biochar characteristics and modifications. *Chemosphere* **2017**, *178*, 466–478. [CrossRef] [PubMed]
34. Yuan, P.; Wang, J.; Pan, Y.; Shen, B.; Wu, C. Review of biochar for the management of contaminated soil: Preparation, application and prospect. *Sci. Total Environ.* **2019**, *659*, 473–490. [CrossRef] [PubMed]
35. Joseph, S.; Lehmann, J. *Biochar for Environmental Management: Science and Technology*; Earthscan: London, UK, 2009.
36. Singh, B.; Cowie, A.; Gilkes, R.; Prakongkep, N. The mean turnover time of biochar in soil varies depending on biomass source and pyrolysis temperature. In Proceedings of the 19th World Congress of Soil Science, Soil Solutions for a Changing World, Brisbane, Australia, 1–6 August 2010; pp. 1–6.
37. Zhang, R.-H.; Shi, L.-F.; Li, Z.-G.; Zhou, G.-L.; Xie, Y.-L.; Huang, X.-X.; Ye, A.-H.; Lin, C.-F. *Aged Biochar for the Remediation of Heavy Metal Contaminated Soil: Analysis through an Experimental Case the Physicochemical Property Changes of Field Aging Biochar and Its Effects on the Immobilization Mechanism for Heavy Metal*; Intechopen: London, UK, 2022.
38. Rodriguez-Campos, J.; Dendooven, L.; Alvarez-Bernal, D.; Contreras-Ramos, S.M. Potential of earthworms to accelerate removal of organic contaminants from soil: A review. *Appl. Soil. Ecol.* **2014**, *79*, 10–25. [CrossRef]
39. Yuvaraj, A.; Thangaraj, R.; Karmegam, N.; Ravindran, B.; Chang, S.W.; Awasthi, M.K.; Kannan, S. Activation of biochar through exoenzymes prompted by earthworms for vermibiochar production: A viable resource recovery option for heavy metal contaminated soils and water. *Chemosphere* **2021**, *278*, 130458. [CrossRef]
40. Dimari, G.; Abdulrahman, F.; Akan, J.; Garba, S. Metals concentrations in tissues of tilapia gallier, crarias lazera and osteoglossidae caught from Alau Dam, Maiduguri, Borno State, Nigeria. *Am. J. Environ. Sci.* **2008**, *4*, 373. [CrossRef]
41. Kaasalainen, M.; Yli-Halla, M. Use of sequential extraction to assess metal partitioning in soils. *Environ. Pollut.* **2003**, *126*, 225–233. [CrossRef]
42. Zhang, M.-K.; Liu, Z.-Y.; Wang, H. Use of single extraction methods to predict bioavailability of heavy metals in polluted soils to rice. *Commun. Soil Sci. Plant Anal.* **2010**, *41*, 820–831. [CrossRef]

43. Srivastav, A.L. Chemical fertilizers and pesticides: Role in groundwater contamination. In *Agrochemicals Detection, Treatment and Remediation*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 143–159.
44. Jones, L.; Jarvis, S.; Green, D.; Hayes, M. *The Fate of Heavy Metals in the Chemistry of Soil Processes*; John Wiley and Sons: New York, NY, USA, 1981.
45. Sumner, M.E. Beneficial use of effluents, wastes, and biosolids. *Commun. Soil Sci. Plant Anal.* **2000**, *31*, 1701–1715. [CrossRef]
46. Rai, P.K.; Lee, S.S.; Zhang, M.; Tsang, Y.F.; Kim, K.-H. Heavy metals in food crops: Health risks, fate, mechanisms, and management. *Environ. Int.* **2019**, *125*, 365–385. [CrossRef]
47. González-Martín, M.; Revilla, I.; Betances-Salcedo, E.; Vivar-Quintana, A. Pesticide residues and heavy metals in commercially processed propolis. *Microchem. J.* **2018**, *143*, 423–429. [CrossRef]
48. Duruibe, J.O.; Ogwuegbu, M.; Egwurugwu, J. Heavy metal pollution and human biotoxic effects. *Int. J. Phys. Sci.* **2007**, *2*, 112–118.
49. Jones, D.H.; Yu, X.; Guo, Q.; Duan, X.; Jia, C. Racial disparities in the heavy metal contamination of urban soil in the Southeastern United States. *Int. J. Environ. Res. Public Health* **2022**, *19*, 1105. [CrossRef]
50. Holmgren, G.; Meyer, M.; Chaney, R.; Daniels, R. Cadmium, lead, zinc, copper, and nickel in agricultural soils of the United States of America. *J. Environ. Qual.* **1993**, *22*, 335–348. [CrossRef]
51. Özen, Y. Spatial distribution of heavy metals and sources of soil contamination in southern Konya (Turkey): Insights from geochemistry, Pb and Sr–Nd isotope systematics. *Environ. Earth Sci.* **2022**, *81*, 285. [CrossRef]
52. Martin, C. Heavy metal concentrations in floodplain surface soils, Lahn River, Germany. *Environ. Geol.* **1997**, *30*, 119–125. [CrossRef]
53. Douay, F.; Pruvot, C.; Roussel, H.; Ciesielski, H.; Fourrier, H.; Proix, N.; Waterlot, C. Contamination of urban soils in an area of Northern France polluted by dust emissions of two smelters. *Water Air Soil Pollut* **2008**, *188*, 247–260. [CrossRef]
54. Manta, D.S.; Angelone, M.; Bellanca, A.; Neri, R.; Sprovieri, M. Heavy metals in urban soils: A case study from the city of Palermo (Sicily), Italy. *Sci. Total Environ.* **2002**, *300*, 229–243. [CrossRef]
55. Ong, G.; Yap, C.; Maziah, M.; Suhaimi, H.; Tan, S. An investigation of arsenic contamination in Peninsular Malaysia based on Centella asiatica and soil samples. *Environ. Monit. Assess.* **2013**, *185*, 3243–3254. [CrossRef]
56. Yuswir, N.S.; Praveena, S.M.; Aris, A.Z.; Syed Ismail, S.N.; De Burbure, C.; Hashim, Z. Heavy metal contamination in urban surface soil of Klang district (Malaysia). *Soil Sediment Contam.* **2015**, *24*, 865–881. [CrossRef]
57. Alrawiq, N.; Khairiah, J.; Latif, T.; Ismail, S. The concentration and distribution of selected heavy metals (Pb, Cd, Cu and Zn) in soils collected from the rice fields of MADA in Kedah, Malaysia. In *AIP Conference Proceedings*; American Institute of Physics: College Park, MD, USA, 2013; pp. 576–582.
58. Su, C.; Meng, J.; Zhou, Y.; Bi, R.; Chen, Z.; Diao, J.; Huang, Z.; Kan, Z.; Wang, T. Heavy metals in soils from intense industrial areas in south China: Spatial distribution, source apportionment, and risk assessment. *Front. Environ. Sci.* **2022**, *10*, 23. [CrossRef]
59. Khan, M.N.; Wasim, A.A.; Sarwar, A.; Rasheed, M.F. Assessment of heavy metal toxicants in the roadside soil along the N-5, National Highway, Pakistan. *Environ. Monit. Assess.* **2011**, *182*, 587–595. [CrossRef] [PubMed]
60. Kumar, V.; Sharma, A.; Kaur, P.; Sidhu, G.P.S.; Bali, A.S.; Bhardwaj, R.; Thukral, A.K.; Cerda, A. Pollution assessment of heavy metals in soils of India and ecological risk assessment: A state-of-the-art. *Chemosphere* **2019**, *216*, 449–462. [CrossRef] [PubMed]
61. Kachenko, A.G.; Singh, B. Heavy metals contamination in vegetables grown in urban and metal smelter contaminated sites in Australia. *Water Air Soil Pollut* **2006**, *169*, 101–123. [CrossRef]
62. Smith, C.; Hopmans, P.; Cook, F. Accumulation of Cr, Pb, Cu, Ni, Zn and Cd in soil following irrigation with treated urban effluent in Australia. *Environ. Pollut.* **1996**, *94*, 317–323. [CrossRef] [PubMed]
63. Uddin, M.M.; Zakeel, M.C.M.; Zavahir, J.S.; Marikar, F.M.; Jahan, I. Heavy metal accumulation in rice and aquatic plants used as human food: A general review. *Toxics.* **2021**, *9*, 360. [CrossRef]
64. Ashraf, R.; Ali, T.A. Effect of heavy metals on soil microbial community and mung beans seed germination. *Pak. J. Bot.* **2007**, *39*, 629–636.
65. Reddy, A.M.; Kumar, S.G.; Jyothsnakumari, G.; Thimmanaik, S.; Sudhakar, C. Lead induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) and bengalgram (*Cicer arietinum* L.). *Chemosphere* **2005**, *60*, 97–104. [CrossRef]
66. Gülser, F.; Erdoğan, E. The effects of heavy metal pollution on enzyme activities and basal soil respiration of roadside soils. *Environ. Monit. Assess.* **2008**, *145*, 127–133. [CrossRef]
67. Garnier, J.; Quantin, C.; Martins, E.; Becquer, T. Solid speciation and availability of chromium in ultramafic soils from Niquelândia, Brazil. *J. Geochem. Explor.* **2006**, *88*, 206–209. [CrossRef]
68. Huang, S.-H.; Bing, P.; Yang, Z.-H.; Chai, L.-y.; Zhou, L.-c. Chromium accumulation, microorganism population and enzyme activities in soils around chromium-containing slag heap of steel alloy factory. *Trans. Nonferrous Met. Soc. China* **2009**, *19*, 241–248. [CrossRef]
69. Chen, G.-Q.; Chen, Y.; Zeng, G.-M.; Zhang, J.-C.; Chen, Y.-N.; Wang, L.; Zhang, W.-J. Speciation of cadmium and changes in bacterial communities in red soil following application of cadmium-polluted compost. *Environ. Eng. Sci.* **2010**, *27*, 1019–1026. [CrossRef]
70. Karaca, A.; Cetin, S.C.; Turgay, O.C.; Kizilkaya, R. Effects of heavy metals on soil enzyme activities. In *Soil Heavy Metals*; Springer: Berlin/Heidelberg, Germany, 2010; pp. 237–262.
71. Tejada, M.; Moreno, J.; Hernández, M.; García, C. Soil amendments with organic wastes reduce the toxicity of nickel to soil enzyme activities. *Eur. J. Soil Biol.* **2008**, *44*, 129–140. [CrossRef]

72. Lorenz, N.; Hintemann, T.; Kramarewa, T.; Katayama, A.; Yasuta, T.; Marschner, P.; Kandeler, E. Response of microbial activity and microbial community composition in soils to long-term arsenic and cadmium exposure. *Soil Biol. Biochem.* **2006**, *38*, 1430–1437. [CrossRef]
73. Bhattacharyya, P.; Tripathy, S.; Kim, K.; Kim, S.-H. Arsenic fractions and enzyme activities in arsenic-contaminated soils by groundwater irrigation in West Bengal. *Ecotoxicol. Environ. Saf.* **2008**, *71*, 149–156. [CrossRef] [PubMed]
74. Owojori, O.J.; Siciliano, S.D. The potentiation of zinc toxicity by soil moisture in a boreal forest ecosystem. *Environ. Toxicol. Chem.* **2015**, *34*, 600–607. [CrossRef]
75. Garrido, S.; Campo, G.; Esteller, M.; Vaca, R.; Lugo, J. Heavy metals in soil treated with sewage sludge composting, their effect on yield and uptake of broad bean seeds (*Vicia faba* L.). *Water Air Soil Pollut* **2005**, *166*, 303–319. [CrossRef]
76. Rascio, N.; Navari-Izzo, F. Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? *Plant Sci.* **2011**, *180*, 169–181. [CrossRef]
77. Küpper, H.; Andresen, E. Mechanisms of metal toxicity in plants. *Metallomics* **2016**, *8*, 269–285. [CrossRef]
78. Ghori, N.-H.; Ghori, T.; Hayat, M.; Imadi, S.; Gul, A.; Altay, V.; Ozturk, M. Heavy metal stress and responses in plants. *Int. J. Environ. Sci. Technol.* **2019**, *16*, 1807–1828. [CrossRef]
79. Gruenhage, L.; Jaeger, H. Effect of heavy metals on growth and heavy metal content of *Allium porrum* L. and *Pisum sativum* L. *Angew. Bot.* **1985**, *59*, 59.
80. Kabato, P.; Pendias, A. *Trace Element in Soils and Plants*, 2nd ed.; CRC Press: Boca Raton, FL, USA, 1992.
81. Schulze, E.-D.; Beck, E.; Müller-Hohenstein, K. *Plant Ecology*; Springer Science & Business Media: Berlin/Heidelberg, Germany, 2005.
82. Das, P.; Samantaray, S.; Rout, G. Studies on cadmium toxicity in plants: A review. *Environ. Pollut.* **1997**, *98*, 29–36. [CrossRef] [PubMed]
83. Yli-Pirilä, T.; Huttunen, K.; Nevalainen, A.; Seuri, M.; Hirvonen, M.R. Effects of co-culture of amoebae with indoor microbes on their cytotoxic and proinflammatory potential. *Environ. Toxicol.* **2007**, *22*, 357–367. [CrossRef] [PubMed]
84. Sharma, C. Effect of heavy metals Co^{2+}, Ni^{2+} and Cd^{2+} on growth and metabolism of cabbage. *Plant Sci.* **2002**, *163*, 753–758.
85. Miller, R.; Bittell, J.; Koeppe, D. The effect of cadmium on electron and energy transfer reactions in corn mitochondria. *Physiol. Plant.* **1973**, *28*, 166–171. [CrossRef]
86. Küpper, H.; Küpper, F.; Spiller, M. Environmental relevance of heavy metal-substituted chlorophylls using the example of water plants. *J. Exp. Bot.* **1996**, *47*, 259–266. [CrossRef]
87. Pallavi, S.; Rama, S.D. Lead toxicity in plants. *Braz. J. Plant. Physiol.* **2005**, *17*, 35–52.
88. Meharg, A.; Macnair, M. Suppression of the high affinity phosphate uptake system: A mechanism of arsenate tolerance in *Holcus lanatus* L. *J. Exp. Bot.* **1992**, *43*, 519–524. [CrossRef]
89. Garg, N.; Singla, P. Arsenic toxicity in crop plants: Physiological effects and tolerance mechanisms. *Environ. Chem. Lett.* **2011**, *9*, 303–321. [CrossRef]
90. Gupta, D.; Inouhe, M.; Rodríguez-Serrano, M.; Romero-Puertas, M.; Sandalio, L. Oxidative stress and arsenic toxicity: Role of NADPH oxidases. *Chemosphere* **2013**, *90*, 1987–1996. [CrossRef]
91. Singh, N.; Ma, L.Q.; Srivastava, M.; Rathinasabapathi, B. Metabolic adaptations to arsenic-induced oxidative stress in *Pteris vittata* L and *Pteris ensiformis* L. *Plant Sci.* **2006**, *170*, 274–282. [CrossRef]
92. Pandey, N.; Verma, L. Nitric Oxide Alleviates Iron Toxicity by Reducing Oxidative Damage and Growth Inhibition in Wheat (*Triticum aestivum* L.) Seedlings. *Int. J. Plant Animal Env. Sci.* **2019**, *5*, 16–22. [CrossRef]
93. Ahammed, G.J.; Wu, M.; Wang, Y.; Yan, Y.; Mao, Q.; Ren, J.; Ma, R.; Liu, A.; Chen, S. Melatonin alleviates iron stress by improving iron homeostasis, antioxidant defense and secondary metabolism in cucumber. *Sci. Hortic.* **2020**, *265*, 109205. [CrossRef]
94. Xing, W.; Li, D.; Liu, G. Antioxidative responses of *Elodea nuttallii* (Planch.) H. St. John to short-term iron exposure. *Plant Physiol. Biochem.* **2010**, *48*, 873–878. [CrossRef] [PubMed]
95. Abraham, K.; Sridevi, R.; Suresh, B.; Damodharam, T. Effect of heavy metals (Cd, Pb, Cu) on seed germination of *Arachis hypogeae* L. *Asian J. Plant Sci.* **2013**, *3*, 10–12.
96. Woo, S.; Yum, S.; Park, H.-S.; Lee, T.-K.; Ryu, J.-C. Effects of heavy metals on antioxidants and stress-responsive gene expression in Javanese medaka (*Oryzias javanicus*). Comparative Biochemistry and Physiology Part C: Toxicol. *Appl. Pharmacol.* **2009**, *149*, 289–299.
97. Ay, T.; Fawole, O.; Adewoye, S.; Ogundiran, M. Bioconcentration of metals in the body muscle and gut of Clarias gariepinus exposed to sublethal concentrations of soap and detergent effluent. *J. Cell Anim. Biol.* **2009**, *3*, 113–118.
98. Olaifa, F.; Olaifa, A.; Onwude, T. Lethal and sub-lethal effects of copper to the African catfish (*Clarias gariepinus*) juveniles. *Afr. J. Biomed. Res.* **2004**, *7*, 65–70. [CrossRef]
99. Romoea, M.; Siaub, Y.; Sidoumou, Z.; Gnassia-Barelli, M. Heavy metals distribution in different fish from the Mauritiana coast. *Sci. Total Environ.* **1999**, *232*, 169–175. [CrossRef]
100. Jezierska, B.; Witeska, M. The metal uptake and accumulation in fish living in polluted waters. In *Soil and Water Pollution Monitoring, Protection and Remediation*; Springer: Berlin/Heidelberg, Germany, 2006; pp. 107–114.
101. Morillo, E.; Villaverde, J. Advanced technologies for the remediation of pesticide-contaminated soils. *Sci. Total Environ.* **2017**, *586*, 576–597. [CrossRef]

102. Cycoń, M.; Mrozik, A.; Piotrowska-Seget, Z. Bioaugmentation as a strategy for the remediation of pesticide-polluted soil: A review. *Chemosphere* **2017**, *172*, 52–71. [CrossRef] [PubMed]
103. Kumpiene, J.; Lagerkvist, A.; Maurice, C. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments—A review. *Waste Manag.* **2008**, *28*, 215–225. [CrossRef] [PubMed]
104. Kong, L.-L.; Liu, W.-T.; Zhou, Q.-X. Biochar: An effective amendment for remediating contaminated soil. *Rev. Environ. Contam. Toxicol.* **2014**, *228*, 83–99.
105. Megharaj, M.; Ramakrishnan, B.; Venkateswarlu, K.; Sethunathan, N.; Naidu, R. Bioremediation approaches for organic pollutants: A critical perspective. *Environ. Int.* **2011**, *37*, 1362–1375. [CrossRef]
106. Jan, A.T.; Azam, M.; Ali, A.; Haq, Q.M.R. Prospects for exploiting bacteria for bioremediation of metal pollution. *Crit. Rev. Environ. Sci. Technol.* **2014**, *44*, 519–560. [CrossRef]
107. McIntyre, T. Phytoremediation of heavy metals from soils. In *Phytoremediation*; Springer: Berlin/Heidelberg, Germany, 2003; pp. 97–123.
108. Ghosh, M.; Singh, S. A review on phytoremediation of heavy metals and utilization of it's by products. *Asian J. Energy Environ.* **2005**, *6*, 18.
109. Balazs, H.E.; Schmid, C.A.; Podar, D.; Hufnagel, G.; Radl, V.; Schröder, P. Development of microbial communities in organochlorine pesticide contaminated soil: A post-reclamation perspective. *Appl. Soil Ecol.* **2020**, *150*, 103467. [CrossRef]
110. Aparicio, J.D.; Saez, J.M.; Raimondo, E.E.; Benimeli, C.S.; Polti, M.A. Comparative study of single and mixed cultures of actinobacteria for the bioremediation of co-contaminated matrices. *Environ. Chem. Eng.* **2018**, *6*, 2310–2318. [CrossRef]
111. Zhang, M.; Wang, J.; Bai, S.H.; Zhang, Y.; Teng, Y.; Xu, Z. Assisted phytoremediation of a co-contaminated soil with biochar amendment: Contaminant removals and bacterial community properties. *Geoderma* **2019**, *348*, 115–123. [CrossRef]
112. Mattina, M.I.; Lannucci-Berger, W.; Musante, C.; White, J.C. Concurrent plant uptake of heavy metals and persistent organic pollutants from soil. *Environ. Pollut.* **2003**, *124*, 375–378. [CrossRef]
113. Gutiérrez-Ginés, M.; Hernández, A.; Pérez-Leblic, M.; Pastor, J.; Vangronsveld, J. Phytoremediation of soils co-contaminated by organic compounds and heavy metals: Bioassays with *Lupinus luteus* L. and associated endophytic bacteria. *J. Environ. Manag.* **2014**, *143*, 197–207. [CrossRef] [PubMed]
114. Hassan, A.; Periathamby, A.; Ahmed, A.; Innocent, O.; Hamid, F.S. Effective bioremediation of heavy metal–contaminated landfill soil through bioaugmentation using consortia of fungi. *J. Soils Sed.* **2020**, *20*, 66–80. [CrossRef]
115. Purwanti, I.F.; Kurniawan, S.B.; Titah, H.S.; Tangahu, B.V. Identification of acid and aluminium resistant bacteria isolated from aluminium recycling area. *Int. J. Civ. Eng.* **2018**, *9*, 945–954.
116. Huang, H.; Ye, L. Biological technologies for cHRPs and risk control. In *High-Risk Pollutants in Wastewater*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 209–236.
117. Rehman, S.u.; Bellitürk, k.; Buyukfiliz, F.; Rehmat, R.; Jaffar, M.T.; Benedetti, M. *Bioaugmentation: A Novel Strategy to Cope with Environmental Impacts of Agrochemicals*; Iksad Publications: Ankara, Turkey, 2022; pp. 7–70.
118. Thompson, I.P.; Van Der Gast, C.J.; Ciric, L.; Singer, A.C. Bioaugmentation for bioremediation: The challenge of strain selection. *Environ. Microbiol.* **2005**, *7*, 909–915. [CrossRef] [PubMed]
119. Nwaehiri, U.L.; Akwukwaegbu, P.I.; Nwoke, B.E.B. Bacterial remediation of heavy metal polluted soil and effluent from paper mill industry. *Environ. Anal. Health Toxicol.* **2020**, *35*, e2020009. [CrossRef]
120. Purwanti, I.F.; Kurniawan, S.B.; Ismail, N.I.; Imron, M.F.; Abdullah, S.R.S. Aluminium removal and recovery from wastewater and soil using isolated indigenous bacteria. *J. Environ. Manag.* **2019**, *249*, 109412. [CrossRef]
121. Ouvrard, S.; Leglize, P.; Morel, J.-L. PAH phytoremediation: Rhizodegradation or rhizoattenuation? *Int. J. Phytoremediation* **2014**, *16*, 46–61. [CrossRef]
122. Zhu, X.; Chen, B.; Zhu, L.; Xing, B. Effects and mechanisms of biochar-microbe interactions in soil improvement and pollution remediation: A review. *Environ. Pollut.* **2017**, *227*, 98–115. [CrossRef]
123. Edwards, C.A.; Arancon, N.Q.; Sherman, R.L. *Vermiculture Technology: Earthworms, Organic Wastes, and Environmental Management*; CRC Press: Boca Raton, FL, USA, 2010.
124. Hoang, D.T.; Razavi, B.S.; Kuzyakov, Y.; Blagodatskaya, E. Earthworm burrows: Kinetics and spatial distribution of enzymes of C-, N-and P-cycles. *Soil Biol. Biochem.* **2016**, *99*, 94–103. [CrossRef]
125. Rehman, S.u.; De Castro, F.; Aprile, A.; Benedetti, M.; Fanizzi, F.P. Vermicompost: Enhancing Plant Growth and Combating Abiotic and Biotic Stress. *Agronomy* **2023**, *13*, 1134. [CrossRef]
126. Karaca, A.; Kizilkaya, R.; Turgay, O.C.; Cetin, S.C. Effects of earthworms on the availability and removal of heavy metals in soil. In *Soil Heavy Metals*; Springer: Berlin/Heidelberg, Germany, 2010; pp. 369–388.
127. Srivastava, R.; Kumar, D.; Gupta, S. Bioremediation of municipal sludge by vermitechnology and toxicity assessment by Allium cepa. *Bioresour. Technol.* **2005**, *96*, 1867–1871. [CrossRef]
128. Antoniadis, V.; Alloway, B. The role of dissolved organic carbon in the mobility of Cd, Ni and Zn in sewage sludge-amended soils. *Environ. Pollut.* **2002**, *117*, 515–521. [CrossRef]
129. Steenbergen, N.T.; Iaccino, F.; de Winkel, M.; Reijnders, L.; Peijnenburg, W.J. Development of a biotic ligand model and a regression model predicting acute copper toxicity to the earthworm Aporrectodea caliginosa. *Environ. Sci. Technol.* **2005**, *39*, 5694–5702. [CrossRef]

130. Halim, M.; Conte, P.; Piccolo, A. Potential availability of heavy metals to phytoextraction from contaminated soils induced by exogenous humic substances. *Chemosphere* **2003**, *52*, 265–275. [CrossRef] [PubMed]
131. Wen, B.; Hu, X.-Y.; Liu, Y.; Wang, W.-S.; Feng, M.-H.; Shan, X.-Q. The role of earthworms (*Eisenia fetida*) in influencing bioavailability of heavy metals in soils. *Biol. Fertility Soils* **2004**, *40*, 181–187. [CrossRef]
132. Morgan, J.; Morgan, A. Zinc sequestration by earthworm (Annelida: Oligochaeta) chloragocytes. *Histochemistry* **1989**, *90*, 405–411. [CrossRef] [PubMed]
133. Morgan, A.; Morris, B. The accumulation and intracellular compartmentation of cadmium, lead, zinc and calcium in two earthworm species (Dendrobaena rubida and Lumbricus rubellus) living in highly contaminated soil. *Histochemistry* **1982**, *75*, 269–285. [CrossRef]
134. Demuynck, S.; Grumiaux, F.; Mottier, V.; Schikorski, D.; Lemière, S.; Leprêtre, A. Metallothionein response following cadmium exposure in the oligochaete Eisenia fetida. *Comp. Biochem. Physiol. Part-C Toxicol. Pharmacol.* **2006**, *144*, 34–46. [CrossRef] [PubMed]
135. Sanchez-Hernandez, J.C. Biochar activation with exoenzymes induced by earthworms: A novel functional strategy for soil quality promotion. *J. Hazard. Mater.* **2018**, *350*, 136–143. [CrossRef]
136. Wang, L.; Zheng, Z.; Zhang, Y.; Chao, J.; Gao, Y.; Luo, X.; Zhang, J. Biostabilization enhancement of heavy metals during the vermiremediation of sewage sludge with passivant. *J. Hazard. Mater.* **2013**, *244*, 1–9. [CrossRef]
137. Song, Y.; Zhou, Q.; Xu, H.; Ren, L.; Sun, T.; Gong, P. Acute toxicological effects of heavy metal pollution in soils on earthworms. *Ying Yong Sheng Tai Xue Bao J. Appl. Ecol.* **2002**, *13*, 187–190.
138. Sivakumar, S.; Subbhuraam, C. Toxicity of chromium (III) and chromium (VI) to the earthworm Eisenia fetida. *Ecotoxicol. Environ. Saf.* **2005**, *62*, 93–98. [CrossRef]
139. Jiménez, E.; Cabañas, B.; Lefebvre, G. *Environment, Energy and Climate Change I: Environmental Chemistry of Pollutants and Wastes*; Springer: Berlin/Heidelberg, Germany, 2015; Volume 32.
140. Malińska, K.; Zabochnicka-Świątek, M.; Cáceres, R.; Marfà, O. The effect of precomposted sewage sludge mixture amended with biochar on the growth and reproduction of Eisenia fetida during laboratory vermicomposting. *Ecol. Eng.* **2016**, *90*, 35–41. [CrossRef]
141. Tammeorg, P.; Parviainen, T.; Nuutinen, V.; Simojoki, A.; Vaara, E.; Helenius, J. Effects of biochar on earthworms in arable soil: Avoidance test and field trial in boreal loamy sand. *Agric. Ecosyst. Environ.* **2014**, *191*, 150–157. [CrossRef]
142. Karki, R.; Solberg, S.Ø. activated biochar with vermicompost as an alternative peat substitute. *Int. J. Res. Biosci. Agric. Technol.* **2018**, *9*, 64–70.
143. Malińska, K.; Zabochnicka-Świątek, M.; Dach, J. Effects of biochar amendment on ammonia emission during composting of sewage sludge. *Ecol. Eng.* **2014**, *71*, 474–478. [CrossRef]
144. Chen, Y.-X.; Huang, X.-D.; Han, Z.-Y.; Huang, X.; Hu, B.; Shi, D.-Z.; Wu, W.-X. Effects of bamboo charcoal and bamboo vinegar on nitrogen conservation and heavy metals immobility during pig manure composting. *Chemosphere* **2010**, *78*, 1177–1181. [CrossRef] [PubMed]
145. Zhang, J.; Lü, F.; Luo, C.; Shao, L.; He, P. Humification characterization of biochar and its potential as a composting amendment. *J. Environ. Sci.* **2014**, *26*, 390–397. [CrossRef] [PubMed]
146. Karami, N.; Clemente, R.; Moreno-Jiménez, E.; Lepp, N.W.; Beesley, L. Efficiency of green waste compost and biochar soil amendments for reducing lead and copper mobility and uptake to ryegrass. *J. Hazard. Mater.* **2011**, *191*, 41–48. [CrossRef]
147. Ahmad, M.; Rajapaksha, A.U.; Lim, J.E.; Zhang, M.; Bolan, N.; Mohan, D.; Vithanage, M.; Lee, S.S.; Ok, Y.S. Biochar as a sorbent for contaminant management in soil and water: A review. *Chemosphere* **2014**, *99*, 19–33. [CrossRef]
148. Awasthi, M.K.; Wang, Q.; Huang, H.; Li, R.; Shen, F.; Lahori, A.H.; Wang, P.; Guo, D.; Guo, Z.; Jiang, S. Effect of biochar amendment on greenhouse gas emission and bio-availability of heavy metals during sewage sludge co-composting. *J. Clean. Prod.* **2016**, *135*, 829–835. [CrossRef]
149. Borchard, N.; Prost, K.; Kautz, T.; Moeller, A.; Siemens, J. Sorption of copper (II) and sulphate to different biochars before and after composting with farmyard manure. *Eur. J. Soil Sci.* **2012**, *63*, 399–409. [CrossRef]
150. Netherway, P.; Reichman, S.M.; Laidlaw, M.; Scheckel, K.; Pingitore, N.; Gascó, G.; Méndez, A.; Surapaneni, A.; Paz-Ferreiro, J. Phosphorus-rich biochars can transform lead in an urban contaminated soil. *J. Environ. Qual.* **2019**, *48*, 1091–1099. [CrossRef] [PubMed]
151. Chen, D.; Liu, X.; Bian, R.; Cheng, K.; Zhang, X.; Zheng, J.; Joseph, S.; Crowley, D.; Pan, G.; Li, L. Effects of biochar on availability and plant uptake of heavy metals—A meta-analysis. *J. Environ. Manag.* **2018**, *222*, 76–85. [CrossRef] [PubMed]
152. Gasco, G.; Alvarez, M.L.; Paz-Ferreiro, J.; Mendez, A. Combining phytoextraction by Brassica napus and biochar amendment for the remediation of a mining soil in Riotinto (Spain). *Chemosphere* **2019**, *231*, 562–570. [CrossRef] [PubMed]
153. Meier, S.; Curaqueo, G.; Khan, N.; Bolan, N.; Cea, M.; Eugenia, G.M.; Cornejo, P.; Ok, Y.S.; Borie, F. Chicken-manure-derived biochar reduced bioavailability of copper in a contaminated soil. *J. Soils Sed.* **2017**, *17*, 741–750. [CrossRef]
154. Beesley, L.; Marmiroli, M. The immobilisation and retention of soluble arsenic, cadmium and zinc by biochar. *Environ. Pollut.* **2011**, *159*, 474–480. [CrossRef]
155. Gomez-Eyles, J.L.; Sizmur, T.; Collins, C.D.; Hodson, M.E. Effects of biochar and the earthworm *Eisenia fetida* on the bioavailability of polycyclic aromatic hydrocarbons and potentially toxic elements. *Environ. Pollut.* **2011**, *159*, 616–622. [CrossRef]

156. Herath, I.; Kumarathilaka, P.; Navaratne, A.; Rajakaruna, N.; Vithanage, M. Immobilization and phytotoxicity reduction of heavy metals in serpentine soil using biochar. *J. Soils Sed.* **2015**, *15*, 126–138. [CrossRef]
157. Hamid, Y.; Tang, L.; Sohail, M.I.; Cao, X.; Hussain, B.; Aziz, M.Z.; Usman, M.; He, Z.-L.; Yang, X. An explanation of soil amendments to reduce cadmium phytoavailability and transfer to food chain. *Sci. Total Environ.* **2019**, *660*, 80–96. [CrossRef]
158. Godlewska, P.; Schmidt, H.P.; Ok, Y.S.; Oleszczuk, P. Biochar for composting improvement and contaminants reduction. *A review. Bioresour. Technol.* **2017**, *246*, 193–202. [CrossRef] [PubMed]
159. Pan, X.; Song, W.; Zhang, D. Earthworms (*Eisenia foetida*, Savigny) mucus as complexing ligand for imidacloprid. *Biol. Fertility Soils* **2010**, *46*, 845–850. [CrossRef]
160. Zhang, D.; Chen, Y.; Ma, Y.; Guo, L.; Sun, J.; Tong, J. Earthworm epidermal mucus: Rheological behavior reveals drag-reducing characteristics in soil. *Soil Tillage Res.* **2016**, *158*, 57–66. [CrossRef]
161. Brown, G.G.; Doube, B.M.; Edwards, C. Functional interactions between earthworms, microorganisms, organic matter, and plants. *Agric. Ecosyst.* **2004**, *2*, 213–239.
162. Huang, K.; Xia, H. Role of earthworms' mucus in vermicomposting system: Biodegradation tests based on humification and microbial activity. *Sci. Total Environ.* **2018**, *610*, 703–708. [CrossRef]
163. Garvín, M.; Lattaud, C.; Trigo, D.; Lavelle, P. Activity of glycolytic enzymes in the gut of *Hormogaster elisae* (Oligochaeta, Hormogastridae). *Soil Biol. Biochem.* **2000**, *32*, 929–934. [CrossRef]
164. García-Sánchez, M.; Taušnerová, H.; Hanč, A.; Tlustoš, P. Stabilization of different starting materials through vermicomposting in a continuous-feeding system: Changes in chemical and biological parameters. *Waste Manage.* **2017**, *62*, 33–42. [CrossRef]
165. Noritomi, H. Application of biochar to enzyme carrier for stress tolerance of enzymes. In *Biochar-An Imperative Amendment for Soil and the Environment*; IntechOpen: London, UK, 2018; p. 75.
166. Inyang, M.; Gao, B.; Yao, Y.; Xue, Y.; Zimmerman, A.R.; Pullammanappallil, P.; Cao, X. Removal of heavy metals from aqueous solution by biochars derived from anaerobically digested biomass. *Bioresour. Technol.* **2012**, *110*, 50–56. [CrossRef]
167. Xu, X.; Cao, X.; Zhao, L.; Wang, H.; Yu, H.; Gao, B. Removal of Cu, Zn, and Cd from aqueous solutions by the dairy manure-derived biochar. *Environ. Sci. Pollut. Res.* **2013**, *20*, 358–368. [CrossRef]
168. He, X.; Zhang, Y.; Shen, M.; Tian, Y.; Zheng, K.; Zeng, G. Vermicompost as a natural adsorbent: Evaluation of simultaneous metals (Pb, Cd) and tetracycline adsorption by sewage sludge-derived vermicompost. *Environ. Sci. Pollut. Res.* **2017**, *24*, 8375–8384. [CrossRef]
169. Khan, M.B.; Cui, X.; Jilani, G.; Lazzat, U.; Zehra, A.; Hamid, Y.; Hussain, B.; Tang, L.; Yang, X.; He, Z. Eisenia fetida and biochar synergistically alleviate the heavy metals content during valorization of biosolids via enhancing vermicompost quality. *Sci. Total Environ.* **2019**, *684*, 597–609. [CrossRef] [PubMed]
170. Khosropour, E.; Weisany, W.; Tahir, N.A.-R.; Hakimi, L. Vermicompost and biochar can alleviate cadmium stress through minimizing its uptake and optimizing biochemical properties in Berberis integerrima bunge. *Environ. Sci. Pollut. Res.* **2022**, *29*, 17476–17486. [CrossRef] [PubMed]
171. Suthar, S. Vermistabilization of municipal sewage sludge amended with sugarcane trash using epigeic *Eisenia fetida* (Oligochaeta). *J. Hazard. Mater.* **2009**, *163*, 199–206. [CrossRef] [PubMed]
172. Paul, S.; Goswami, L.; Pegu, R.; Bhattacharya, S.S. Vermiremediation of cotton textile sludge by *Eudrilus eugeniae*: Insight into metal budgeting, chromium speciation, and humic substance interactions. *Bioresour. Technol.* **2020**, *314*, 123753. [CrossRef]
173. Azizi, A.; Lim, M.; Noor, Z.; Abdullah, N. Vermiremoval of heavy metal in sewage sludge by utilising Lumbricus rubellus. *Ecotoxicol. Environ. Saf.* **2013**, *90*, 13–20. [CrossRef]
174. Udovic, M.; Lestan, D. The effect of earthworms on the fractionation and bioavailability of heavy metals before and after soil remediation. *Environ. Pollut.* **2007**, *148*, 663–668. [CrossRef]
175. Azizi, A.B.; Choy, M.Y.; Noor, Z.M.; Noorlidah, A. Effect on heavy metals concentration from vermiconversion of agro-waste mixed with landfill leachate. *Waste Manag.* **2015**, *38*, 431–435. [CrossRef]
176. Shahmansouri, M.; Pourmoghadas, H.; Parvaresh, A.; Alidadi, H. Heavy metals bioaccumulation by Iranian and Australian earthworms (*Eisenia fetida*) in the sewage sludge vermicomposting. *J. Environ. Health Sci. Eng.* **2005**, *2*, 28–32.
177. Khwairakpam, M.; Bhargava, R. Vermitechnology for sewage sludge recycling. *J. Hazard. Mater.* **2009**, *161*, 948–954. [CrossRef]
178. Wang, L.; Zhang, Y.; Lian, J.; Chao, J.; Gao, Y.; Yang, F.; Zhang, L. Impact of fly ash and phosphatic rock on metal stabilization and bioavailability during sewage sludge vermicomposting. *Bioresour. Technol.* **2013**, *136*, 281–287. [CrossRef]
179. Singh, S.; Bhat, S.A.; Singh, J.; Kaur, R.; Vig, A.P. Earthworms converting milk processing industry sludge into biomanure. *J. Waste Manag.* **2017**, 10. [CrossRef]
180. Wang, X.; Ma, S.; Wang, X.; Cheng, T.; Dong, J.; Feng, K. The Mechanism of Cu^{2+} Sorption by Rice Straw Biochar and Its Sorption–Desorption Capacity to Cu^{2+} in Soil. *Bull. Environ. Contam. Toxicol.* **2022**, *109*, 562–570. [CrossRef] [PubMed]
181. He, X.; Zhang, Y.; Shen, M.; Zeng, G.; Zhou, M.; Li, M. Effect of vermicomposting on concentration and speciation of heavy metals in sewage sludge with additive materials. *Bioresour. Technol.* **2016**, *218*, 867–873. [CrossRef]
182. Wang, Y.; Xu, Y.; Li, D.; Tang, B.; Man, S.; Jia, Y.; Xu, H. Vermicompost and biochar as bio-conditioners to immobilize heavy metal and improve soil fertility on cadmium contaminated soil under acid rain stress. *Sci. Total Environ.* **2018**, *621*, 1057–1065. [CrossRef] [PubMed]

183. Paul, S.; Das, S.; Raul, P.; Bhattacharya, S.S. Vermi-sanitization of toxic silk industry waste employing *Eisenia fetida* and *Eudrilus eugeniae: Substrate* compatibility, nutrient enrichment and metal accumulation dynamics. *Bioresour. Technol.* **2018**, *266*, 267–274. [CrossRef]
184. Cao, X.; Ma, L.; Gao, B.; Harris, W. Dairy-manure derived biochar effectively sorbs lead and atrazine. *Environ. Sci. Technol.* **2009**, *43*, 3285–3291. [CrossRef]
185. Wu, D.; Yu, X.; Chu, S.; Jacobs, D.F.; Wei, X.; Wang, C.; Long, F.; Chen, X.; Zeng, S. Alleviation of heavy metal phytotoxicity in sewage sludge by vermicomposting with additive urban plant litter. *Sci. Total Environ.* **2018**, *633*, 71–80. [CrossRef] [PubMed]
186. Paul, S.; Goswami, L.; Pegu, R.; Chatterjee, S.K.; Bhattacharya, S.S. Epigenetic regulations enhance adaptability and valorization efficiency in *Eisenia fetida* and *Eudrilus eugeniae* during vermicomposting of textile sludge: Insights on repair mechanisms of metal-induced genetic damage and oxidative stress. *Bioresour. Technol.* **2022**, *345*, 126493. [CrossRef]
187. Topoliantz, S.; Ponge, J.-F. Charcoal consumption and casting activity by *Pontoscolex corethrurus* (*Glossoscolecidae*). *Appl. Soil Ecol.* **2005**, *28*, 217–224. [CrossRef]
188. Liesch, A.M.; Weyers, S.L.; Gaskin, J.W.; Das, K. Impact of two different biochars on earthworm growth and survival. In *Annals of Environmental Science*; USDA: Washington, DC, USA, 2010; Volume 4, pp. 1–9.
189. Zhang, Q.; Saleem, M.; Wang, C. Effects of biochar on the earthworm (*Eisenia foetida*) in soil contaminated with and/or without pesticide mesotrione. *Sci. Total Environ.* **2019**, *671*, 52–58. [CrossRef]
190. Li, D.; Hockaday, W.C.; Masiello, C.A.; Alvarez, P.J. Earthworm avoidance of biochar can be mitigated by wetting. *Soil Biol. Biochem.* **2011**, *43*, 1732–1737. [CrossRef]
191. Gong, X.; Cai, L.; Li, S.; Chang, S.X.; Sun, X.; An, Z. Bamboo biochar amendment improves the growth and reproduction of Eisenia fetida and the quality of green waste vermicompost. *Ecotoxicol. Environ. Saf.* **2018**, *156*, 197–204. [CrossRef] [PubMed]