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Review

A Systematic Review on Earthworms in Soil Bioremediation

Francesca Tagliabue †, Enrica Marini †, Arianna De Bernardi *, Costantino Vischetti and Cristiano Casucci *

Department of Agricultural, Food and Environmental Sciences, Polytechnic University of Marche, 60131 Ancona, Italy; f.tagliabue@pm.univpm.it (F.T.); enrica.marini@staff.univpm.it (E.M.); c.vischetti@univpm.it (C.V.)

* Correspondence: a.debernardi@pm.univpm.it (A.D.B.); c.casucci@univpm.it (C.C.)

† These authors contributed equally to this work.

Abstract: Bioremediation techniques are increasingly popular in addressing soil pollution. Despite this, using earthworms as first actors or adjuvants in decontamination is an open and little-discussed field. This paper focuses on vermiremediation effectiveness alone or combined with other bioremediation methods, such as phytoremediation and bioaugmentation. Literature was collected following the PRISMA criteria, setting the search with the following keywords: "(vermiremediation) AND (bioremediation OR phytoremediation OR plant*) AND (bioaugmentation OR bacteria)". The investigation was performed on Google Scholar, Science Direct, SciFinder and Web of Science databases. The article data were collected, compared, elaborated, graphically summarised and discussed to assess if the earthworms' activities play a critical role in tackling several soil pollutions. Furthermore, the review aimed to identify the most promising techniques in the function of the xenobiotic examined: organic, inorganic or both. Any gaps and criticism were highlighted to facilitate future research in this study area.

Keywords: vermiremediation; phytoremediation; bioaugmentation; co-remediation; *Eisenia fetida*; organic contaminant; heavy metal; PHA; pesticide; soil

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Citation: Tagliabue, F.; Marini, E.; De Bernardi, A.; Vischetti, C.; Casucci, C. A Systematic Review on Earthworms in Soil Bioremediation. *Appl. Sci.* **2023**, *13*, 10239. https://doi.org/10.3390/app131810239

Academic Editor: Maximilian Lackner

Received: 10 July 2023
Revised: 7 September 2023
Accepted: 9 September 2023
Published: 12 September 2023

1. Introduction

A contaminated matrix, such as soil, groundwater, or surface water, is characterised by contaminants above those considered safe by regulatory agencies [1]. Soil pollution derives mainly from anthropic activities related to rapid industrial and agricultural development and represents a growing problem on a global scale [2,3]. Different types of contaminants continuously accumulate in the environmental compartment [4], causing adverse effects on the physical, chemical and biological properties of the soil [5] and the health status of plants and terrestrial organisms [6]. The entry of these pollutants into the food chain can have carcinogenic and mutagenic effects on humans, causing widespread public concern [7], the most common and possibly hazardous contaminants found in soil are pesticides, potentially toxic elements (PTEs) and polycyclic aromatic hydrocarbons (PAHs). Organic and inorganic pesticides are synthesised naturally or chemically and used in the agricultural sector to control pests [8], improve the yield and, simultaneously, the quality of crops [9]. The uncontrolled use and their persistent nature make pesticides extremely harmful to ecosystems [10]. According to the latest FAO report [11], agriculture is responsible for the global use of approximately 4.2 million tonnes of pesticides [12]. Countries have a wide disparity in pesticide application rates. The field application increased from 1.55 kg ha^{-1} in 1990 to 2.7 kg ha^{-1} in 2019; however, several countries significantly exceeded this average [11,13]. In particular, developing countries are responsible for a quarter of the global use of pesticides [14], and they are also the largest producers [15,16].

PTEs include metals and metalloids that can be highly toxic to the environment, even at low concentrations, such as lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr) and arsenic (As), and micronutrients such as copper (Cu), nickel (Ni) and zinc (Zn), which become dangerous when they exceed a certain threshold [17]. PTEs have a long residence time in the environmental matrix, and their bioavailability makes them easily assimilated by soil organisms [18]. Moreover, due to their inorganic nature [19], they are not degradable by any biological or physical process [20]. Several soils around the world are contaminated with PTEs; as reported by He et al. [21], there are more than five million contaminated sites with soil concentrations that, in many cases, exceed geo-baseline or regulatory levels. This situation mostly occurs in developed countries such as the United States of America, Australia, European Union member states and China [22]. The main sources of PTEs contamination in agriculture are represented by activities such as the irrigation of fields with wastewater [23], the long-term use of large quantities of fertilisers [24] and pesticides [25]; moreover, the proximity of the crops to traffic routes can increase the levels of these pollutants [26].

Total petroleum hydrocarbons (TPHs) are a mixture of different hydrocarbon compounds in crude oil employed to produce petroleum products [27]. TPHs include aliphatic and aromatic compounds [28]; among the aromatic ones, polycyclic aromatic hydrocarbons (PAHs) are considered the most worrying due to their toxic and mutagenic characteristics [29].

PAHs consist of aromatic hydrocarbons with two or more fused benzene rings configurated in various structures [30]. These organic compounds are hazardous to the environment due to their persistence, molecular stability and hydrophobicity [31,32]. In particular, the soil ecosystem becomes an actual sink for PAHs [33]; according to recent studies, they have phytotoxic effects on the metabolic activity of plants [34] and can alter the physico-chemical properties of the top- and sub-soil [35] as they are strongly adsorbed onto the soil particles [36]. PAHs are equally distributed in aquatic or terrestrial ecosystems and the atmosphere [37]. Natural sources of these compounds are negligible compared to those derived from human activities, which appear to be the most significant [38]. Pyrogenic PAHs are formed through the incomplete combustion of organic materials such as tobacco, fossil fuels, wood and agricultural waste [39,40], while those resulting from the loss of petroleum and its by-products are called petrogenic [38]. Global emissions of PAHs were estimated in 2015 at about 357 million kg [41]; more than 80% of these were attributed to developing countries [42].

The extensive and global spread of the above contaminants leads to a growing concern for environmental and human health. Consequently, effective, inexpensive and eco-friendly strategies to reduce the content of these pollutants in the soil ecosystem are needed to improve its health and fertility.

Bioremediation includes various natural processes in which plants (phytoremediation), microorganisms (bioaugmentation) and soil fauna (vermiremediation) are employed for biodegrading and mitigating soil contamination [43,44].

Phytoremediation is one of the most employed techniques in the panorama of bioremediation [45] and involves plants and their associated microorganisms to extract and remove pollutants or reduce their bioavailability in the environment [46,47]. This approach is particularly suitable for the remediation of PTEs [48,49], but it is also widely used for organic xenobiotics [50]. Phytoremediation techniques can be classified as follows: phytoextraction which uses hyperaccumulator plants to take up contaminants from the soil, concentrating them in aboveground biomass (shoots) [51,52]; phytostabilisation to decrease the bioavailability of xenobiotics, avoiding their mobilisation [53]; phytotransformation which uses the ability of plant enzymes to convert organic pollutants into less toxic forms [54]; rhizodegradation, in which the root exudates improve and promote the activity of the microorganisms in the rhizosphere, transforming organic pollutants [55]; phytovolatilisation which uses plants to volatilise organic contaminants and subsequently release them into the atmosphere by their transpiration process [56,57]; phytofiltration where hydroponically grown and aquatic plants can be used to remove xenobiotics from the water [58].

Bioaugmentation is a green technology that removes organic contaminants by inoculating, in different matrices, bacteria and/or fungi strains or their consortia [59], which have specific degradation abilities to the target pollutants [60]. Microorganisms suitable for bioaugmentation must have specific characteristics, such as rapid growth, easy maintenance in laboratory conditions, adaptability and resistance to strongly contaminated substrates [61]. Microbial strains used in this technique can be indigenous or allochthonous. Autochthonous strains are isolated from the contaminated environment and, once cultivated, are re-inoculated in the original site [59,62]. The other category of microorganisms is taken from areas with similar contaminations and then introduced into the site to be remediated [63].

Most studies have focused on the bioremediation techniques described above, while the use of soil fauna, such as earthworms, as first actors in decontamination is a poorly studied field that has to be explored [64]. Vermiremediation was first defined by Rodriguez-Campos et al. [65] and utilises earthworms' biotic and abiotic interaction, life cycle, burrowing and feeding activities to transform, degrade or remove contaminants from the soil environment [66]. The role of earthworms in the terrestrial ecosystem is pivotal because they positively influence soil's physical, chemical and biological properties [67]. These organisms actively contribute to pedogenesis, soil turnover and the increase in soil porosity; consequently, they are termed ecosystem engineers [68,69]. Thanks to their excavation and soil ingestion activities, earthworms play a pivotal role in organic matter dynamics, redistributing the elements within the soil profile, and can modify the pore system architecture [70]. Furthermore, earthworms are widely used as sentinel organisms by the international scientific community [71]; thanks to their permeable cuticles and large ingestion of surface soil, they are directly in contact with the soil particles and are sensitive to contamination [72,73]. Earthworms are able to remove contaminants both directly, through absorption and digestion, and indirectly by stimulating the growth of beneficial microorganisms for soil remediation [74]. Consequently, there are several mechanisms involved in vermiremediation: vermiextraction is the process in which earthworms remove contaminants from the substrate by dermal and intestinal absorption and accumulate them in their bodies [64,75]; vermitransformation changes organic pollutants, passing through the earthworm's gut, by decomposing into less toxic forms by the microorganisms and enzymes that colonise it [66,75]; the drilodegradation occurs in the 2 mm thick zone around earthworms' burrow walls, called drillosphere [76]. Due to the release of earthworm residues, mucous secretions and casts, this area becomes a microbial hotspot with a great potential for the degradation of organic compounds [77,78].

This review deals with current literature on the vermiremediation of inorganic and organic xenobiotics in solid matrices (such as natural or artificial soils, sludges, manure, etc.). Articles that apply vermiremediation alone or in combination with other bioremediation techniques have been analysed in detail. The aims were to collect exhaustive data, identify successful remediation strategies and report the significant criticalities according to the category of contaminants studied.

2. Materials and Methods

2.1. Data Sources and Search Strategies

The authors systematically searched the literature to evaluate the role of earthworms in restoring contaminated matrices and their potential in association with other bioremediation techniques. The research was conducted in four databases (Google Scholar, Science Direct, Web of Science and SciFinder), setting a time range from the year 2018 to 2023. The scientific literature search was performed using keywords combined with Boolean operators as follows: "(vermiremediation) AND (bioremediation OR phytoremediation OR plant*) AND (bioaugmentation OR bacteria)". The search yielded a total number of 693 articles; after the screening work, the papers finally included in this review were 53. Literature collection, screening, inclusion and exclusion criteria are shown in the flow diagram in Figure 1.

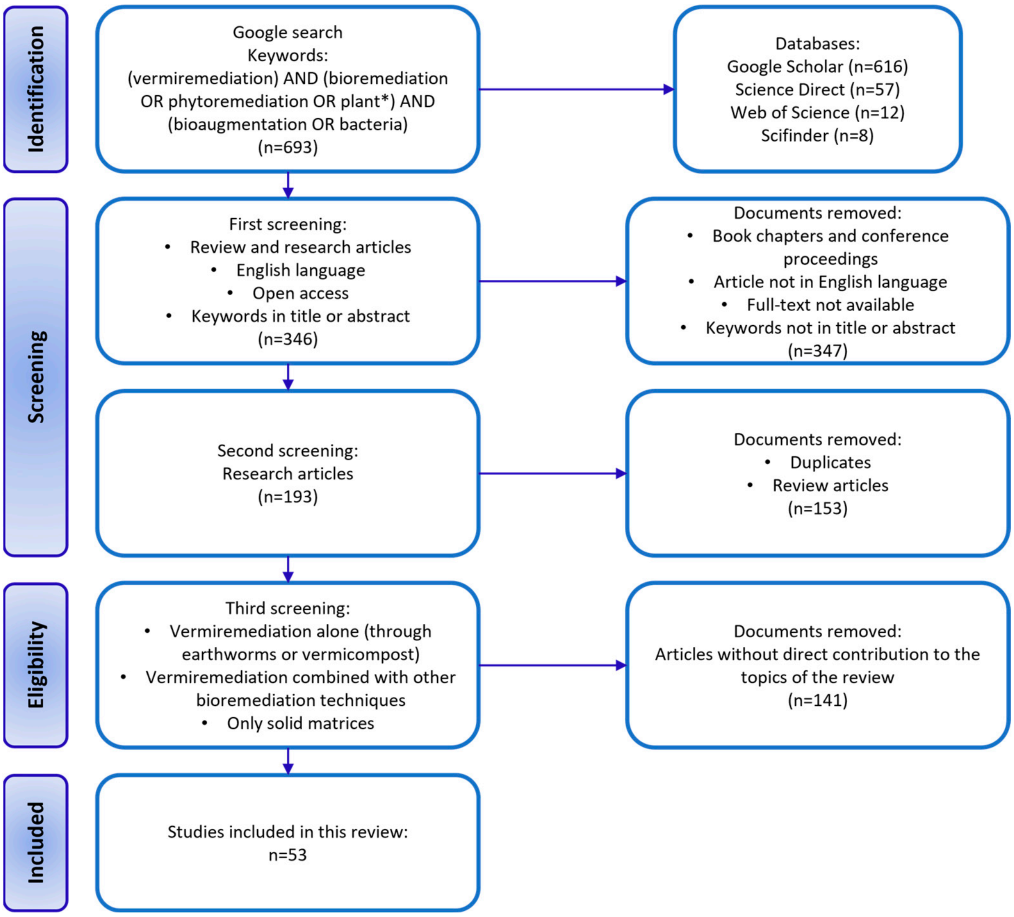

Figure 1. PRISMA study flow diagram from the initial research and screening to the final selection of studies to be included in this systematic review.

2.2. Graphical Construction

To obtain an overall and visually intuitive representation of the data, graphs have been constructed and divided into organic and inorganic contaminants. To produce these graphs, articles that explicitly reported the reduction percentage values and those in which the data allowed their calculation were selected. The reduction percentage was carried out as follows:

$$Reduction\ (\%) = \left(\frac{Ci - Cf}{Ci}\right) \times 100$$

where Ci and Cf represent the initial and final concentration of the xenobiotic understudy, respectively.

Regarding alone vermiremediation, data were elaborated in radar graphs in which reduction percentages were calculated as averages of the same contaminant and earthworm species collected from different articles.

The data on the combined bioremediation techniques were processed by producing two-input graphs: the percentage of reduction of the xenobiotic (bars) and the initial concentration (dash).

In the histograms, capital letters represent the various treatments:

- S refers to the substrate;
- E indicates the presence of earthworms for the application of vermiremediation;
- P indicates the presence of plants for the application of phytoremediation;
- M indicates the presence of microorganisms for the application of bioaugmentation;
- O indicates the presence of other remediation strategies.

The progressive numbers were inserted where more substrates (S1, S2, etc.) or different treatments (i.e., E1 *Eisenia fetida*, E2 *Lumbricus terrestris*, etc.) were tested. The progressive numbers have been coded in order of the appearance of the various cases in each article.

3. Results

3.1. Alone Inorganic Vermiremediation

Vermiremediation is an expanding technology in recent years due to the pivotal role of earthworms in the soil. Of the 53 papers under study, 14 deal with vermiremediation of inorganic contaminants; considering the amount of data collected, it was necessary to carry out a graphic reworking of some (10) of these works to clarify the effects of each species of earthworm on the different PTEs reported in Figure 2.

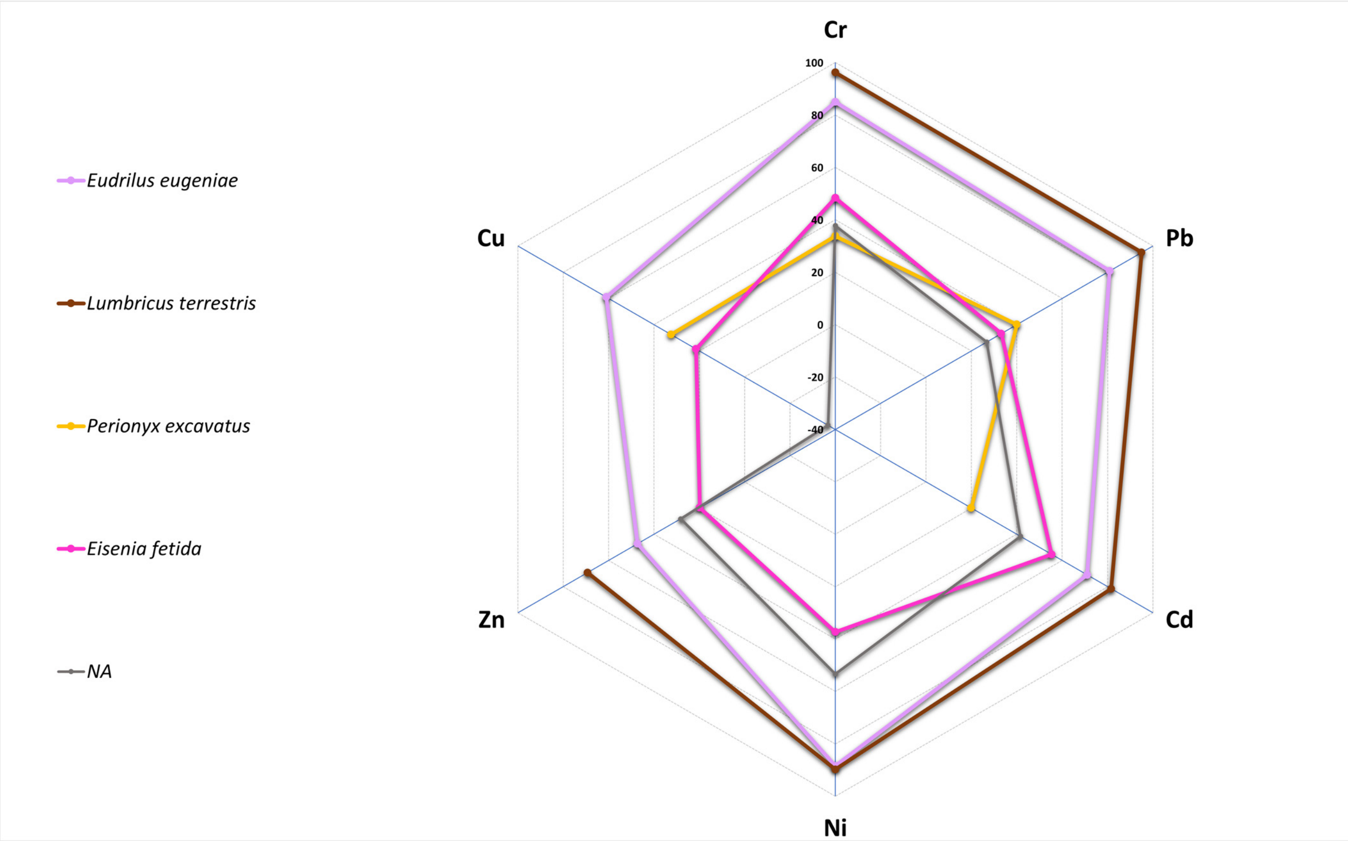

Figure 2. Alone inorganic vermiremediation. Reduction percentages were averages of the same contaminant and earthworm species collected from different articles (Number of articles: 10). The reduction values reported for the NA series refer to the test in the absence of earthworms.

Among the research articles, the most used earthworm species involved in the PTEs vermiremediation is *Eisenia fetida*. *E. fetida* is an epigeic earthworm employed in terrestrial ecotoxicology as the standard test organism for the following reasons: it is easy to breed in laboratory, has short generation times and is susceptible to chemicals [79,80].

Seven research articles have been identified [81–87] that employ *E. fetida* for bioremediation of PTEs, using different types of organic waste as the primary substrate with diverse contamination levels. The final aim of these experimental tests was to find a sustainable recycling system for these types of organic waste.

Starting from Cd, one of the elements most analysed by the authors, the mean percentage reduction was 55.38%, with maximum values of 100% [86], 92.86% [85] and 91.27% [83]. The experiment performed by Singh et al. [86] achieved the best reduction (100%), working on pharmaceutical industry sludge, adding from 50% to 75% of cow dung (CD). Also, Paul et al. [85] obtained results close to total removal (92.86%) by mixing silk industrial sludge waste (SIS1) with CD in a 3:1 ratio. However, these matrices were generally poorly contaminated by Cd compared to the starting substrates of Paul et al. [83], which also obtained satisfactory results by adding 50% CD to silk industrial sludge (SIS2), reaching a Cd restoration of 87.30%.

For Cr, the SIS1 and SIS2 matrices gave the greatest reduction percentages, equal to 96.39% without the addition of CD; however, the result reached with the SIS2 matrix [83] is noteworthy as the starting concentration of this metal was considerably higher (16.62 mg kg^{-1}) against 1.66 mg kg^{-1} by SIS1 [85]. By adding 25% or 50% CD to SIS1 and SIS2, respectively, the Cr was reduced by about 93%; also, in this case, Paul et al. [83] started from higher initial concentrations (7.76 mg kg^{-1}). Regarding Pb, the combination of SIS with CD in a 1:1 ratio for both studies [83,85] led to considerable reductions in the contaminant (80.10%, 86.82%).

The most recent work [83] also evaluated vermibeds based on textile sludge (from the cotton industry, CTS1) as a substrate, in which earthworms reduced Cd concentrations by 91.27%. *E. fetida* significantly detoxified Pb (74%); notably, this matrix was characterised by the highest initial Pb concentration (31.21 mg kg^{-1}) among all the articles analysed.

A good Pb reduction equal to 75.91% was obtained in another study [82] on a matrix composed of municipal solid waste. In general, the average reduction calculated for Pb and Cr were 33.14% and 48.35%, respectively, as shown in Figure 2.

Observing the graph (Figure 2), the percentage of reduction of Zn is relatively low (19.3%); this is because in Singh et al. [86] and Sohal et al. [84], the final values of this metal were recorded as higher than the starting concentrations. Despite the ability of *E. fetida* to accumulate metals from the environment, the progressive mineralisation and decomposition of organic matter during vermicomposting can concentrate and increase the PTEs contents [88,89]. As reported by Sing et al. [86], also, Cu has negative percentages of reduction, which affected the final average (Figure 2).

Other papers not shown in the radar graph used *E. fetida* to remedy organic sludge waste [90–93], obtaining ecologically stabilised products rich in nutrients and with PTEs concentrations below the imposed limits.

Lumbricus terrestris, although studied in only one [94] among the twelve articles considered in this subchapter, achieved the greatest reduction percentages compared to the other earthworms for all the metals studied. The authors applied vermiremediation on seven soils collected near welder and auto mechanic workshops with different starting PTEs concentrations. Despite variability in contaminations, *L. terrestris* has shown itself to be particularly efficient, with reduction averages always higher than 80% for all studied metals except for Zn (69%). Averaging the reduction percentages due to earthworms' presence (comparing the PTEs concentrations in the same soils without or with earthworms) from all the soils analysed, the authors found that Pb was the element that best lends itself to vermiremediation with *L. terrestris*. Data reported by Iheme et al. [94] also deal with *Eudrilus eugeniae* and, comparing its remediation capacity with *L. terrestris*, finds it even more efficient.

Both Paul et al. 2022 and 2020 [83,95] evaluated the vermiremediative power of *E. eugeniae* by applying these earthworms to cotton textile sludge (CTS1, CTS2). Regarding Cd, the most newsworthy result was found in the CTS2 matrix; in fact, starting from a high initial concentration (59.03 mg kg^{-1}), a reduction of 70.96% was obtained. Also, in CTS1, earthworms restored the substrate from Cd (84.73%), although the level of contamination was low. More interesting results were found in CTS1 relating to Cr, Pb and Zn. However, the Cr, Zn and Cu removal efficiency was higher in the mixtures CTS2 and CD in a 1:1 or 2:1 ratio.

The primary industrial source of Cr pollution comes from the leather industries because this metal is widely used as a tanning agent [96]. The tannery sludge substrate used in the experiment conducted by Goswami et al. [97] contains high concentrations of Cr (711.6 mg kg^{-1}). In the pure matrix, *E. eugeniae* effectively reduced the amount of that PTE by 63.95%, while in the mixture with 50% CD, the reduction reached 89.45%, starting from a lower initial concentration (467.2 mg kg^{-1}). Aerobic composting, in the absence of earthworms, showed very low and even negative reduction values, indicating the fundamental role of *E. eugeniae* in this work.

The remediation capacity of the epigeic earthworm *Perionyx excavatus* was studied in the article by Yuvaraj et al. [98] on a substrate composed of sludge from paper mill wastewater added with CD in various proportions. The authors measured that earthworms improve the remediation of all PTEs studied (linear regression analysis showed a positive correlation in earthworm treatments). More satisfactory results were obtained when the contaminated matrix was mixed with 50% CD; in this treatment, the health parameters of earthworms (biomass, fertility and histopathological changes) were significantly better, and vermiremediation caused a significant decrease in the level of elements in the following order: Pb (56%) > Cr (46%) > Cu (42%) and Cd (27%).

3.2. Alone Organic Vermiremediation

Regarding vermiremediation for organic xenobiotics, it was found that many species were used only for one contaminant; instead, *E. fetida*, *Amynthas robustus* and the combination of *Drawida modesta* + *Lampito mauritii* have been tested for different types of pollutants (Figure 3).

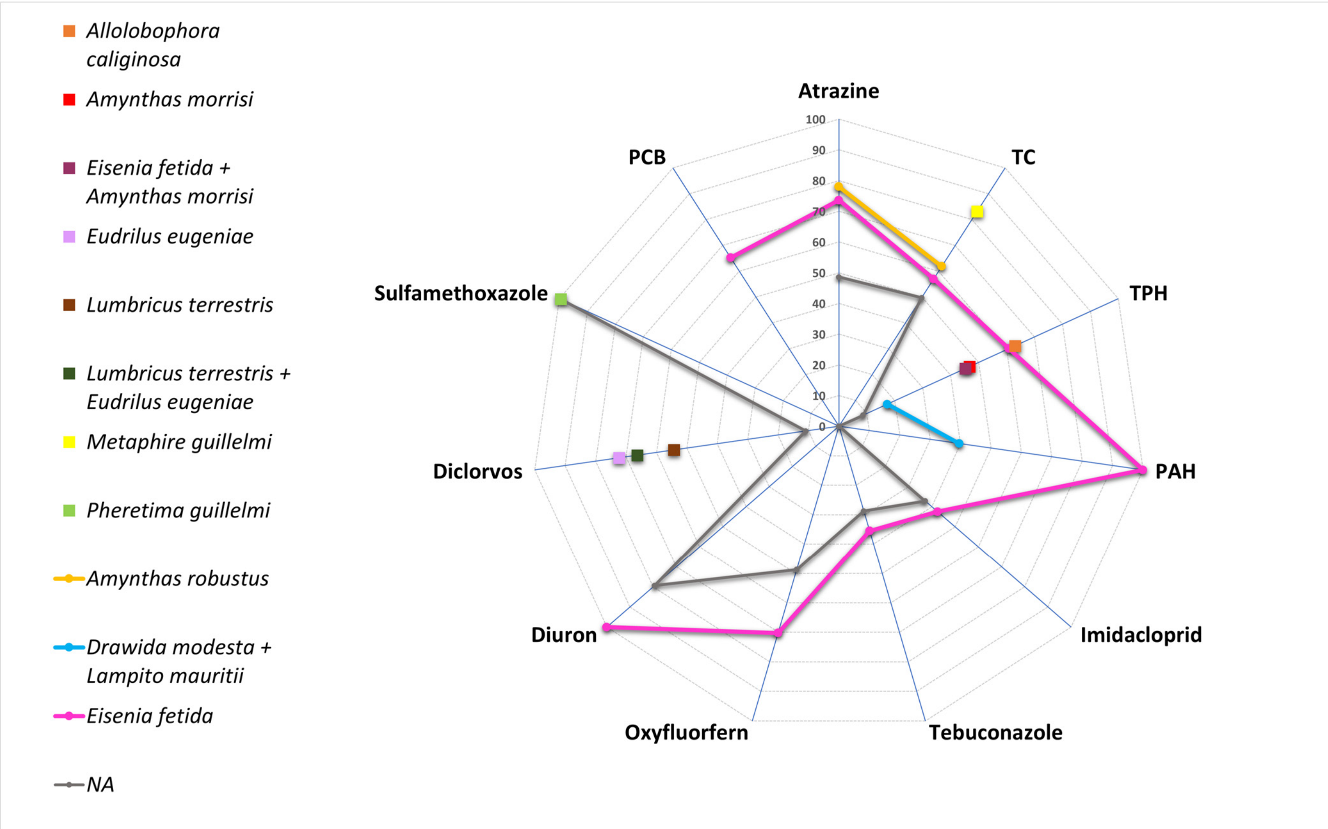

Figure 3. Alone organic vermiremediation. Reduction percentages were averages of the same contaminant and earthworm species collected from different articles (Number of articles: 11). The reduction values reported for the NA series refer to the test in the absence of earthworms.

Six articles [99–104] of the seventeen deal with alone vermiremediation versus organic contaminants and were not included in Figure 3 due to the absence of explicit reduction values; however, they are discussed below.

Several authors examined the vermiremediation efficiency and the tolerance of different earthworm species in substrates contaminated by TPHs.

Nassar et al. [105] investigated the bioremediation of crude oil at two concentrations (45.2 and 62.4 mg kg^{-1}) using *Allopophora caliginosa*, a common species found in Egypt. These endogeic organisms consume large quantities of soil during burrowing; this feeding way might increase the contact of soil particles with the worm gut and result in more TPHs degradation. At the end of the study, the authors found that TPHs concentrations followed a time- and dose-dependent pattern and significantly decreased by 54.9% and 71% in soil samples at 45.2 and 62.4 mg kg^{-1}, respectively. The maximum reduction was observed at the highest TPHs concentration, highlighting that *A. caliginosa* had a good remedial capacity for these contaminants. Despite this, some changes in worms' metabolic and haematological parameters were observed.

In a recent study, Rubiyatno et al. [101] evaluated *E. fetida*'s tolerance to pyrene, a very soil-persistent PAH. Authors have chosen this earthworm for its ability to survive in highly contaminated soils; it is also known that *E. fetida* promotes the degradation of crude oil in soils thanks to its ability to stimulate and synergise with other beneficial microorganisms [64]. For this purpose, three different soil conditions were utilised to cultivate the earthworms: sterilised, unsterilised and soil mixed with CD. The worst pyrene degradation was recorded in sterilised soil (23.2%), while the best was in soil supplemented with CD (31.2%). This information indicates that sterilised soil inhibited pyrene decomposition due to the absence of indigenous pyrene-degrading microbes, emphasising the crucial symbiotic relationships between soil biota and earthworm enzymes. Furthermore, the study demonstrated that *E. fetida* is extremely resistant and can live and thrive in PAHs-contaminated environments, although survival rates followed a dose- and time-dependent trend.

The PAHs soil restoration ability of *E. fetida* was also studied by Fawole et al. [106]. The contaminants used in this experiment were Acenaphthylene (AcPY), Benzo(e)pyrene (BeP) and Benzo(ghi)perylene (BP), which differ in their structural configurations. The data showed that the PAHs vermiremediation in soil was 100% efficient; the authors [106] suggested that these contaminants were degraded into various metabolite states. In particular, *E. fetida* accelerates the successful removal of PAHs, leading to their removal and disappearance [65]. Earthworms release soil-bound organic contaminants and prevent new binding; in this way, PAHs are more easily available for degrading microorganisms [107].

Nobili et al. [108] experimented with vermiremediation using *E. fetida* and *Amynthas morrisi*, alone or in combination in a precomposted matrix artificially contaminated with TPHs; the incorporation of both species was performed to assess a synergistic effect. *A. morrisi* is an epi-endogeic earthworm that lives on the soil–litter interface and produces large amounts of cast, improving soil proprieties. The increment of TPHs degradation was evident and significant in substrates with earthworms compared to the negative control. More in detail, the treatment which implied the highest reduction is the one with *E. fetida* (60.81%), followed by *A. morrisi* (46.74%) and, finally, the treatment with the incorporation of the two species (45.2%). *E. fetida* seems to be the most effective species in the vermiremediation process; furthermore, it is also more resistant, with higher survival rates than *A. morrisi*.

Another study [109] explored the ability of two earthworm species used together, *D. modesta* (epigeic) and *L. mauritii* (anecic), to restore a soil polluted by PAHs and TPHs. The species selected by the authors are commonly isolated from fields and are widely known for their ability to detoxify heavy metals in industrially contaminated substrates; however, they are underutilised in studies with crude oil contaminations. Authors found that co-inoculating these worms can alter the contamination level till the intermediate dose (7.5 mL), with a PAHs and TPHs reduction of 68.6% and 34.3%, respectively. This capacity undergoes a substantial breakdown at higher doses of oil contamination (10 mL), indicating suffering in both earthworms associated with low activity and histopathological damage observed on cross sections under a compound microscope such as degradation of cells, irregular surface epidermis or detected cellular debris.

Vermiremediation is also suitable for matrices contaminated by pesticides: a pilot study [110] was conducted to vermiremediate soil contaminated by organophosphate insecticide dichlorvos, using *L. terrestris* and *E. eugeniae* alone and in combination. As shown in Figure 3, the best results were obtained by *E. eugeniae*, with an average reduction percentage of 72.29%, followed by the co-inoculated treatment (66.27%) and *L. terrestris* (54.23%). Njoku et al. [110] concluded that *E. eugeniae* is the better remediator agent in this research case.

Owagboriaye et al. [103] evaluated in their study three different species of earthworms, including *E. eugeniae*, *Alma millsoni* and *Libyodrilus violaceus*, against the glyphosate-based herbicides (GBHs).

At the end of the experiment, all organisms significantly reduced the GBHs concentration in soil; however, the bioaccumulation factor (ratio between xenobiotic concentration in earthworm tissue and substrate) demonstrated that *E. eugeniae* (1.11) and *L. violaceus* (1.15) are better accumulators than *A. millsoni* (0.40). Moreover, the enzymatic analyses showed that *E. eugeniae* and *L. violaceus* responded to the exposure with physiological and biochemical adjustments, activating antioxidant defence mechanisms as a compensatory response.

Other authors who worked with glyphosate were Lescano et al. [99], who tested the capacity of *E. fetida* in a biobed system. They studied different biomixtures in the presence or absence of earthworms, concluding that *E. fetida* leads to a 90% reduction of the pesticide compared to the test without worms (80%). This earthworm species can tolerate elevated glyphosate levels without altering its life characteristics.

Also, Delgado-Moreno et al. [111] set up a vermiremediation test in a biobed system contaminated with four different pesticides: Diuron, Imidacloprid, Tebuconazole and Oxyfluorfern. As can be seen in Figure 3, *E. fetida* successfully reduced the amount of Diuron (100%) and Oxyfluorfern (70.25%). At the same time, the other two pesticides were not significantly reduced compared to uninoculated controls, maybe due to the high levels of pesticides and the composition of the biomixtures, which could limit the vermiremediation.

The same epigeic earthworm versus the endogeic *A. robustus* was evaluated against atrazine, a triazine herbicide used worldwide at low cost for agricultural production. Lin et al. [112] found that both species coupled with soil microbes can decrease atrazine contamination, performing trials with sterilised and non-sterilised soil.

The presence of earthworms and soil microorganisms resulted in a statistically higher reduction rate (94.9% and 95.7%) than sterilised treatments with earthworms alone (52.3% and 60.3%). *A. robustus* showed significantly higher atrazine accumulation in tissues than *E. fetida*.

Antibiotics are still used worldwide for bacterial infection treatments; because of the poor absorption rate in the intestines of animals and humans, these substances are discharged through excretions and can enter into water or soil ecosystems [113,114].

A group of harmful antibiotics widely and globally consumed is represented by Tetracyclines (TCs) [115]. These substances are widely used because of their broad-spectrum activity and low cost [116]. In Europe, from 2010 to 2020, despite their consumption in the community sector having decreased (while in the hospital sector, it has remained unchanged), attention is still high on the dangers of TCs, above all due to antimicrobial resistance [117].

The study conducted by Lin et al. [118] investigated the effects of two earthworms with different behaviours and ecological functions (*E. fetida* and *A. robustus*) on TCs degradation in sterilised and natural soil. The authors demonstrated that indigenous soil microbes have weak power to mineralise TCs; no significant differences were found between treatments with sterilised and natural soil alone. On the contrary, earthworms in sterile treatment showed a good remedial capacity, which significantly increased in association with soil bacteria. Looking at Figure 3, on average, slightly higher reduction rates were observed for *A. robustus* (61.9%) than for *E. fetida* (57%).

Also, Liu et al. [100] tested the efficacy of *E. fetida* versus TCs, obtaining statistically different results compared to the treatment alone at the higher dose applied (100 mg kg^{-1}).

According to Yin et al. [119], using *Metaphire guillelmi* in soil vermiremediation experiments is a more suitable choice as it is commonly isolated in farmland and is more sensitive to some contaminants than *E. fetida* [120]. *M. guillelmi* significantly accelerated the TCs degradation, with an average value of 83.17% compared to soil without earthworms (63.55%). At the same time, the enzyme activity established no oxidative damage due to TCs, demonstrating the efficient detoxification ability of the antioxidant system in this species.

Microcosm experiments were conducted by Zhang et al. [121] to study the effects of *Pheretima guillelmi* on the fate of Sulfamethoxazole (SMX), an antibiotic commonly detected in almost all environmental compartments. The authors found that the *P. guillelmi* activities significantly reduced the SMX concentration in soil (99.55%) compared to the treatments without them due to their gut detoxification and stimulation of soil indigenous microbes.

Polychlorinated biphenyls (PCBs) are a group of organic chemicals comprising carbon, hydrogen and chlorine atoms of anthropogenic origin, belonging to chlorinated hydrocarbons. These compounds are a source of concern because they are globally distributed, highly persistent and toxic [122,123].

Zenteno-Rojas et al. [102] studied the *E. fetida* removal ability of decachlorobiphenyl (DCB) in the vermicomposting process. Data in Figure 3 showed that earthworms and their symbiotic bacteria could significantly reduce high DCB concentrations, with a value of 65.24%.

Among all the research articles (53) analysed in this review, only two experiments used a product of earthworms, the vermicompost, as an alternative method to the earthworms' presence.

Luo et al. [124] used *E. fetida*'s casts to accelerate the atrazine biodegradation pathway, testing combinations of sterilised and unsterilised soils and casts. The contribution of vermicompost both in physical (sterilised cast 44.23%) and biological (unsterilised 60.77%) terms was decisive for reducing the contaminant compared to sterilised soil alone. However, the synergistic relationship between soil and cast microorganisms led to the highest reduction percentage of 93.97%. In conclusion, the vermicast enhanced the soil's physical properties and provided extra nutrients, promoting the chemical hydrolysis of atrazine and the activity of indigenous soil microorganisms.

Also, Mohammadi-Moghadam et al. [104] evaluated the vermicompost capacity for bioremediation. The experimental soil was artificially contaminated with phenanthrene (PHE) and pyrene (PYR) at three doses (100, 200, 300 mg kg^{-1}). These compounds provided a good source of carbon and energy for the microorganisms, which compensated for their toxic effects up to the intermediate dose. In fact, this treatment showed a greater removal efficiency; balance tended towards toxicity at the highest dose tested. Treatments with PYR manifested more variation in the microbiota, but in general, microbes adapted to the toxic conditions and began their decomposition activities.

3.3. Combined Vermiremediation for Inorganic Pollution

Recently, the scientific community's attention has grown on combining different bioremediation techniques to obtain better restoration results.

Phytoremediation is a technique widely used in soil restoration from PTEs; however, it has limitations, such as low biomass of accumulator plants and poor bioavailability of these contaminants in soil [125]. The co-application of other biological techniques, such as vermiremediation, can enhance the efficacy of phytoremediation.

Due to their life activity and ingestion processes, earthworms improve the soil's physical, chemical and biological fertility, creating an ideal environment for plant fitness and enhancing its phytoremediation power [126,127]. Moreover, earthworms augment the bioavailability of metals in the soil and influence pH and dissolved organic carbon [128].

Nine articles were discussed regarding the use of vermiremediation together with other bioremediation techniques with respect to inorganic contaminants, four of which were reworked, as shown in Figure 4.

In a microcosm experiment, Tibihenda et al. [129] evaluated the influence of two species of earthworms (*E. fetida* and *Amynthas aspergillum*) on the growth and metal accumulation of *Brassica campestris*. The substrate consists of an agricultural topsoil artificially contaminated with three different Pb concentrations (100, 500 and 1000 mg kg^{-1}).

Different ecological earthworm species may have dissimilar effects on the cycle of PTEs and soil proprieties. Indeed, this study showed that *E. fetida* had a superior impact on cation exchange capacity, tissue accumulation and availability of Pb, while *A. aspergillum* affects more soil C and N contents. At the end of the trial, soil bioavailable Pb was generally higher in the treatments with earthworms, independently from the species used. Although the presence of *E. fetida* and *A. aspergillum* increases the bioavailable Pb and, therefore, its potential toxicity towards plants, they improve the physicochemical properties of the soil, bypassing the adverse effects. In fact, *B. campestris* subjected at the higher Pb level with the presence of earthworms, significantly increasing some parameters such as aboveground fresh biomass, leaf area and chlorophyll concentration.

At the same plant biomass condition, a higher Pb concentration was observed in treatments with *A. aspergillum*; however, *E. fetida* enhanced the aboveground biomass production, leading to a higher accumulation of this metal in the plant. Earthworm activity favours Pb accumulation and concentration; nevertheless, the translocation factor (ratio between xenobiotic concentration in aboveground and root plant tissues) indicates that the metal remains at the root level, so *B. campestris* is not a good hyperaccumulator.

The addition of organic amendments is suitable for bioremediation processes as it improves the soil's physical, chemical and biological properties by supplying nutrients and organic matter; consequently, these increase the survival and growth performance of the organisms involved in the restoration. Naseer et al. [130] tested the vermiremediation (*E. fetida*) and phytoremediation (*Spinacea oleracea*), alone or combined, with or without the addition of cow and buffalo dung. An artificial soil was prepared for the tests and contaminated with Pb, Cd and Cr. Cow dung is known for extending the phytoremediation potential of plant species by supplying the necessary plant nutrients [131]. Furthermore, CD creates favourable habitats for earthworms, improving parameters such as growth rate, body weight and reproduction compared to buffalo dung, which has a reduced content of organic matter. In general, the presence of *E. fetida* in soil reduced or mitigated the effect of PTEs on *S. oleracea*. In particular, the strong influence of earthworms was observable in trials with Cr contamination where treatment with plant alone showed no germination, while the combination of plant + amendment + earthworm enhanced the plant growth and seed germination. It was observed that the *E. fetida* bioaccumulation factor was higher when vermiremediation and phytoremediation were jointly applied; also, the metals' accumulation efficiency of the plant increased in the combined treatments.

Additionally, Guo et al. [132] explored the interactive effects of *E. fetida* and CD on the uranium (U) phytoextraction efficiency of two plant species (*Brassica juncea* and *Helianthus annuus*). The combined application of the amendment and *E. fetida* resulted in a major and significant increase of 53% in sunflower shoot biomass. Statistically, earthworms have increased the U solubility, thus making it more bioavailable for plant uptake and accumulation. Indeed, the total metal accumulation increased by 68.5% for Indian mustard and 85% for sunflowers compared to treatments with plants alone.

Soil microorganisms are used in bioremediation because they bind, immobilise, oxidise, transform and volatilise inorganic contaminants [133].

There is a symbiotic relationship between soil microorganisms and earthworms, which, thanks to their burrowing activities, mucus and cast production, positively impact soil microbial properties [134,135]. The gut microbiota represents a unique ecological niche [136], and it is the origin of the restoration capacity of earthworms [137]. Consequently, several authors have recently investigated the effectiveness of bioaugmentation combined with vermiremediation. El-Hassanin et al. [138] investigated the remediation capacity of *E. fetida* and fungal bio-accelerators (*Trichoderma harzianum, viride* and *Phanerochaete chrysosporium*), alone or in combination; the substrate was composed of sewage sludge and rice straw in different percentages, contaminated with Cd, Ni and Pb. As can be seen from Figure 4, the use of *E. fetida*, individually or in combination, gave promising results; the highest percentage reductions for all analysed contaminants were found in treatment where the sewage sludge and rice straw ratio was 1:1 (S3 + E + M) where (Cd 72%, Ni 67%, Pb 62%). Furthermore, authors found a primary role of earthworms in increasing the metals' available fraction compared to the concentrations measured in the treatments with microorganisms alone.

Xiao et al. [139] evaluated the influence of *Bacillus megatherium* on the remediation process of earthworms in Cd removal from artificially contaminated soils. In this study, the combined treatment (S + E + M) had a Cd content at the end of the experiment statistically lower than the soil treated with earthworms alone (S + E) (Figure 4), contradicting what was observed by El-Hassanin et al. [138]. Microorganisms can enhance the metals' bioavailability in soil by altering pH, oxidation/reduction reactions and chelator ions [140]. The use of *B. megatherium* probably stimulated the accumulation of Cd in earthworms, making it more assimilable as a food source and leading to a 23.6% increase in tissue content.

From the selection of research articles for this review, only one work mixed the three main bioremediation techniques, i.e., phytoremediation, vermiremediation and bioaugmentation, on soil contaminated with PTEs. Wang et al. [141] investigated the separate and combined effects of *Rhizophagus irregularis* (arbuscular mycorrhizal fungi, AMF) and *E. fetida* on the Cd restoration ability of *Solanum nigrum*. The positive effect of earthworms was influenced by the different levels of Cd contamination (15, 30, 60, 120 mg kg^{-1}), as *E. fetida* improved plant growth by enhancing phosphorus absorption at high Cd concentrations compared to low ones. The shoot biomass yield was greater in the presence of both AMF and *E. fetida*, maybe because earthworm activities mitigate metal damage to these microorganisms. Interestingly, AMF alone caused an increase in plant tissues' Cd content only at low and moderate contamination levels, while earthworms increased it at moderate and high levels. Lastly, the results of this study demonstrated that the treatment with the most significant impact on the phytoremediation abilities of *S. nigrum* is the combined one, followed by *R. irregularis* and earthworm inoculation.

Biochar is a carbon-rich material obtained from biomass pyrolysis at high temperatures (200 to 700 °C) under limited oxygen conditions [142]. Biochar can bind PTEs through precipitation, complexation and ion exchange mechanisms [143]. To obtain a high-quality organic fertiliser, some authors add different amounts of this amendment to improve the vermicomposting process, achieving a product with an acceptable PTEs concentration.

A recent study by Ameen and Al-Homaidan [144] investigated the combined effects of biochar and *E. fetida* on some PTEs (Cd, Cr, Cu and Pb) removal from sewage sludge. Different concentrations of biochar were evaluated (O1 2%, O2 4% and O3 6%); however, the 4% biochar treatment with earthworms (S + E + O2) appeared to be the most efficient in lowering the PTEs concentration, with a reduction of Cd 55%, Cr 28%, Cu 30% and Pb 21% (Figure 4). The substrate with the highest percentage of biochar (S + E + O3) was adverse to earthworm fitness and remediation efficiency despite this amendment being recognised as a stabiliser and sequester [145]; furthermore, treatments with only biochar (S + O1) did not improve the reduction processes. Consequently, this study demonstrated that earthworms are the real protagonists in the bioremediation of these inorganic contaminants.

Also, Khan et al. [146] evaluated the effect of different types of biochar applied at 10% in a vermicomposting process on a base matrix composed of sewage sludge and kitchen waste. The biochar from the poplar plant residues (PPB) was the most effective since the concentration of all the metals analysed (Cr, Cu, Pb, Zn) decreased significantly compared to the treatment without biochar, except for Cd. *E. fetida* bioaccumulated these elements within its tissues, bringing to that decrease.

Xiao et al. [139] instead used agricultural topsoil as substrate, artificially contaminated with Cd, to evaluate co-remediation effectiveness with 2% biochar and *E. fetida* (S + E + O). In this case, the removal rate in the combined treatment (34.4%) did not statistically differ from the one with earthworm alone (30.5%), as reported in Figure 4. However, Cd accumulation in earthworm tissue increased by 13.1% in S + E + O, probably due to the ingestion of metal-bound biochar particles.

In conclusion, from the papers examined, it emerges that biochar helps the earthworm in the bioremediation activity; however, attention must be paid to the application rate because a high dose of biochar could be toxic for these organisms. Badhwar et al. [147] performed an experimental study using paper mill sludge (PMS) by adding cow dung (CD) and tea waste (TW) at different combinations with *E. fetida.*

For the authors, the best mixture in PTEs removal was PMS:CD in equal ratio (S2 + E), followed by S1 + E (PMS:CD ratio 1:2) and S2 + E + O1 (PMS: TW:CD ratio 1:1:1). In fact, as observable from Figure 4, the S2 + E treatment showed the highest reduction percentage for Cd (58.21%), Cu (79.83%), Ni (81.9%) and Pb (83.48%).

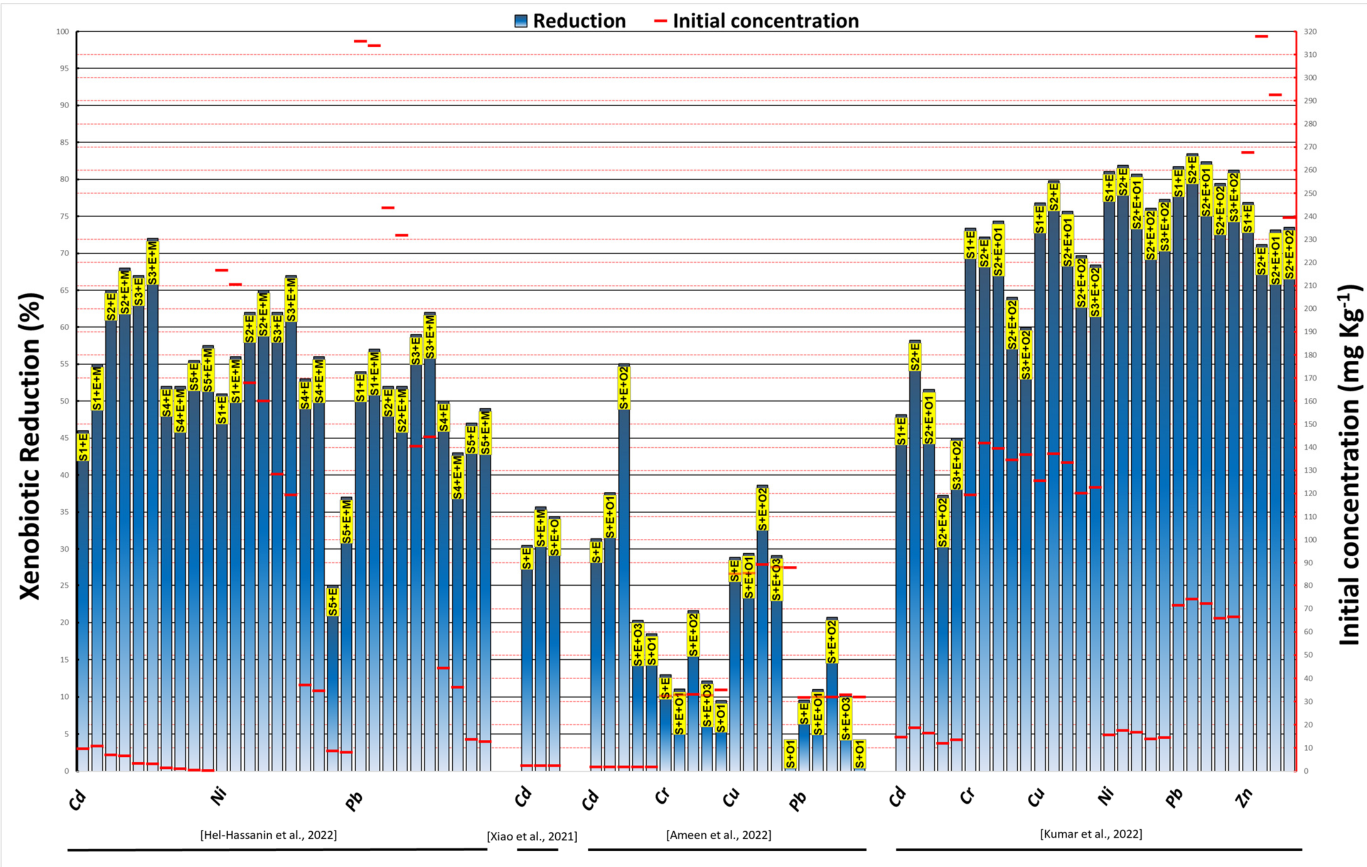

Figure 4. Combined vermiremediation for inorganic pollution. Xenobiotic reduction percentage (blue bars) and initial concentration (red dash) for each treatment. Treatments (yellow labels) legend: S (substrate), E (earthworm), M (microorganisms), O (other treatment). When progressive numbers appear on the label, different substrates (i.e., S1, S2, etc.) or experimental designs (i.e., E1, E2, etc.) were tested. The square brackets show the bibliographic references of the articles taken into consideration (Number of articles: 4, in order of comparison, Hel-Hassanin et al., 2022 [138]; Xiao et al., 2021 [139], Ameen et al., 2022 [144]; Kumar et al., 2022 [147]).

3.4. Combined Vermiremediation for Organic Pollution

In this chapter, ten articles were analysed, six of which were reworked, as shown in Figure 5.

Ghavidel et al. [148] studied the efficiency of the co-application of *E. fetida* and *Lolium perenne* in agriculture topsoil contaminated in a laboratory test with anthracene, a model compound for PAHs. Earthworms alone reduced the amount of contamination by 40%, while plants gave significantly higher results (81%, S + P). However, as seen in Figure 5, earthworm-assisted phytoremediation gives the best results, decreasing 92% (S + E + P) of the anthracene in the soil.

This research article reported that ryegrass and *E. fetida* have a mutualistic relationship. The plant presence was beneficial in contaminated treatments for the earthworm survival rate, which was statistically more relevant than in the S + E treatment. The excavation activity of these organisms raised soil porosity and aeration, favouring plant dry biomass in general; specifically, the root extension demonstrated a significant increase with or without contamination.

Also, Alves et al. [149] investigated the effect of joint application of bio-techniques, using plant (*Pennisetum clandestinum*) and earthworm (*E. andrei*) versus hydrocarbon contamination. The substrate employed was a compost obtained after six months of aerobic composting process of hydrocarbon-contaminated waste food industry sludge and shredded plant material. The bioassay results with plants and earthworms are reported in Figure 5. In the S + P treatment, a significant TPH reduction of 9.3% was observed, which becomes superior (15.2%) in the joint treatment (S + E + P). The reduction observed in the combined treatment may be due to the transfer of TPHs in the earthworm body to the plant's rhizosphere, where these pollutants were degraded in the best way [150].

Another recent study conducted in Italy [151] used the earthworm–plant strategy as a restoration technique. This combined process was tested on soil taken from the national priority site of Brescia-Caffaro, which is over-limit contaminated by polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs). The study found that plants and earthworms had a weak bioremediation effect compared to treatments with only soil; moreover, in this case, earthworms and plant interaction seems to reduce the positive impact of each other alone. These negative results may be related to the low bioavailability of PCDD/Fs and PCB, the insufficient experiment length and the number of *E. fetida* individuals. Furthermore, due to their excavation activity, earthworms may contribute to a higher contaminant concentration in percolated water; accordingly, attention must be paid to the fate of these pollutants under field conditions.

The purpose of Ahmed et al. [152] was to investigate the effect of *L. terrestris* (at 5 and 10 earthworms kg^{-1} soil, E1 and E2, respectively in Figure 5) and a consortium of three bacteria (*Corynebacterium* sp., *Sphingobacterium gobiense* and *Kocuria flava*) on the percentage removal of chlorpyrifos insecticide from the soil. The bacteria consortium alone (S + M) showed the highest effect on the reduction percentage (73.83%), followed by their combination with a high density of earthworms (71.22%, S + E2 + M). The lowest rates of bioremediation were recorded in treatments with *L. terrestris* alone, either at high or low densities; however, both organisms employed gave promising results in the degradation of chlorpyrifos.

Koolivand et al. [153] inoculated an immature compost with hydrocarbon-degrading bacteria (S + M) and *E. fetida* (S + E), individually and in combination (S + E + M). The matrix was artificially contaminated with different amounts of petroleum oily sludge to obtain three levels of TPHs pollution (5, 10 and 20 g kg^{-1}). As can be seen from Figure 5, treatments with the microbial consortium were effective for all concentrations tested, demonstrating high tolerance and resistance. Earthworms, instead, were more sensitive to this contaminant and showed a dose-dependent mortality; the reduced removal rate is statistically visible in S + E compared to S + M at 20 g kg^{-1}. The constantly higher reduction percentages (91.2%, 90.9%, 85.35%) in combined treatments proved the synergistic effect of *E. fetida* and microorganisms.

The nanomaterials are defined as particles of 100 nm or less in at least one dimension and represent an innovative strategy suitable for remediating either organic or inorganic contaminated sites [154,155]. A 28-day microcosm experiment [156] was conducted using the nano zerovalent iron (nZVI) and earthworms to remediate an agricultural soil contaminated by a mixture of three representative polychlorinated biphenyls (PCBs): 2,4,4′-Trichlorobiphenyl (PCB28), 2,2′,5,5′-tetrachlorobiphenyl (PCB52) and 2,2′,4,5,5′-pentachlorobiphenyl (PCB101). The bioaccumulation of PCBs in *E. fetida* reached equilibrium after 14 days of exposure; the accumulation was rapid, and no mortality was recorded, indicating a high endurance of earthworms to these compounds. The addition of nanomaterials to the substrate significantly increased the concentration of PCBs in *E. fetida*, reaching over 60 mg kg^{-1} in tissues, compared with a maximum accumulation of 55.3 mg kg^{-1} in the absence of nZVI. In conclusion, nanomaterials could be a good solution for enhancing earthworms' vermiremediation potential.

The efficiency of vermiremediation is limited by the low bioavailability of PAHs and their biotoxicity [157]. Therefore, surfactants could be a promising method to improve the earthworms' accessibility to these low-soluble and recalcitrant compounds [158]. Surfactants are amphipathic substances capable of absorbing and altering the conditions of the interfaces [159] and are widely used in remediation technologies. However, the role of these helpers in vermiremediation has rarely been studied. Shi et al. [160] used Tween-80 (20, 100 mg kg^{-1}) and rhamnolipid (25, 100 mg kg^{-1}) surfactants in contaminated fluoranthene soil (25, 50 mg kg^{-1}). Both Tween-80 and rhamnolipid significantly increased the bioconcentration of fluoranthene in *E. fetida* by 35–64.1% and 34.5–44.2%, respectively. These results proved that surfactants promote the earthworms' pollutant uptake, intensifying fluoranthene bioavailability. Surfactants statistically reduced residual PAH in the soil in all treatments; however, it emerged that this attitude is closely related to the surfactant level, which should be added at an optimal concentration. For example, at 50 mg kg^{-1} of fluoranthene, the lowest dose of rhamnolipid gave better removal results than the highest dose.

Zhen et al. [161] added biochar as a helper to vermicompost, contaminated with di-(2-ethylhexyl) phthalate (DEHP), to verify its impact on the earthworm restoration process. In the present study, biochar and *E. fetida*, either alone or combined, significantly enhanced the DEHP degradation compared to treatment with only soil. The best results were obtained by treatment with earthworm alone, followed by combined and biochar ones.

Also, Cuevas-Díaz [162] used an organic soil amendment, palm oil bagasse, to increase the bioremediation activity of *Pontoscolex corethrurus*, in soil artificially contaminated by TPH from heavy crude "Maya" oil. All biological-added treatments showed significant percentages of TPH reduction compared to S (Figure 5). The higher removal was found in the combined treatment (S + E + O) with a 39.6% value, followed by S + O (32.6%) and finally S + E (29.7%). In the presence of the amendment, *P. corethrurus* final biomass was 2.7 times higher, probably due to the richest nutrients availability. However, attention must be paid to the number of earthworms used since this species can lead to soil compaction, producing large coalescent aggregates if present at high densities [163].

From 2018 to today, only one study has evaluated the synergistic effects of the three main bioremediation techniques on soils contaminated by organic pollution. Rodriguez-Campos et al. [164] performed an experiment to evaluate the TPHs and its components (PAHs and alkanes) removal testing the earthworm *P. corethrurus*, a bacterial consortium and the plant *Panicum maximum*, individually or combined. The S + E + M combination had the highest TPHs reduction efficiency of 86.4%, followed by the S + E + P + M combination, which removed 82.7% in 112 days (Figure 5). Microorganisms improved the effects of earthworms and grass on hydrocarbons. Most alkanes and PAHs removal occurred within 28 days and then slowed down and generally followed the restoration trend observed for TPHs. The total plant biomass resulted significantly superior (2.6 times) in the joint application of bioremediation techniques compared to *P. maximum* grown on non-polluted soil, demonstrating that soil organisms exert a solid, beneficial effect, even under contaminated conditions. Interestingly, the earthworms and plants used in this study were taken directly from the contaminated site, showing original adaptability to TPHs; especially, Hernández-Castellanos et al. [165] found that *P. corethrurus* is the dominant earthworm in oil-contaminated sites, suggesting tolerance and potential in soil remediation.

Furthermore, the authors conducted a parallel experiment sterilising the soil; no earthworms survived at the end of the experiment, showing the pivotal, mutual interactions between soil microorganisms and earthworms.

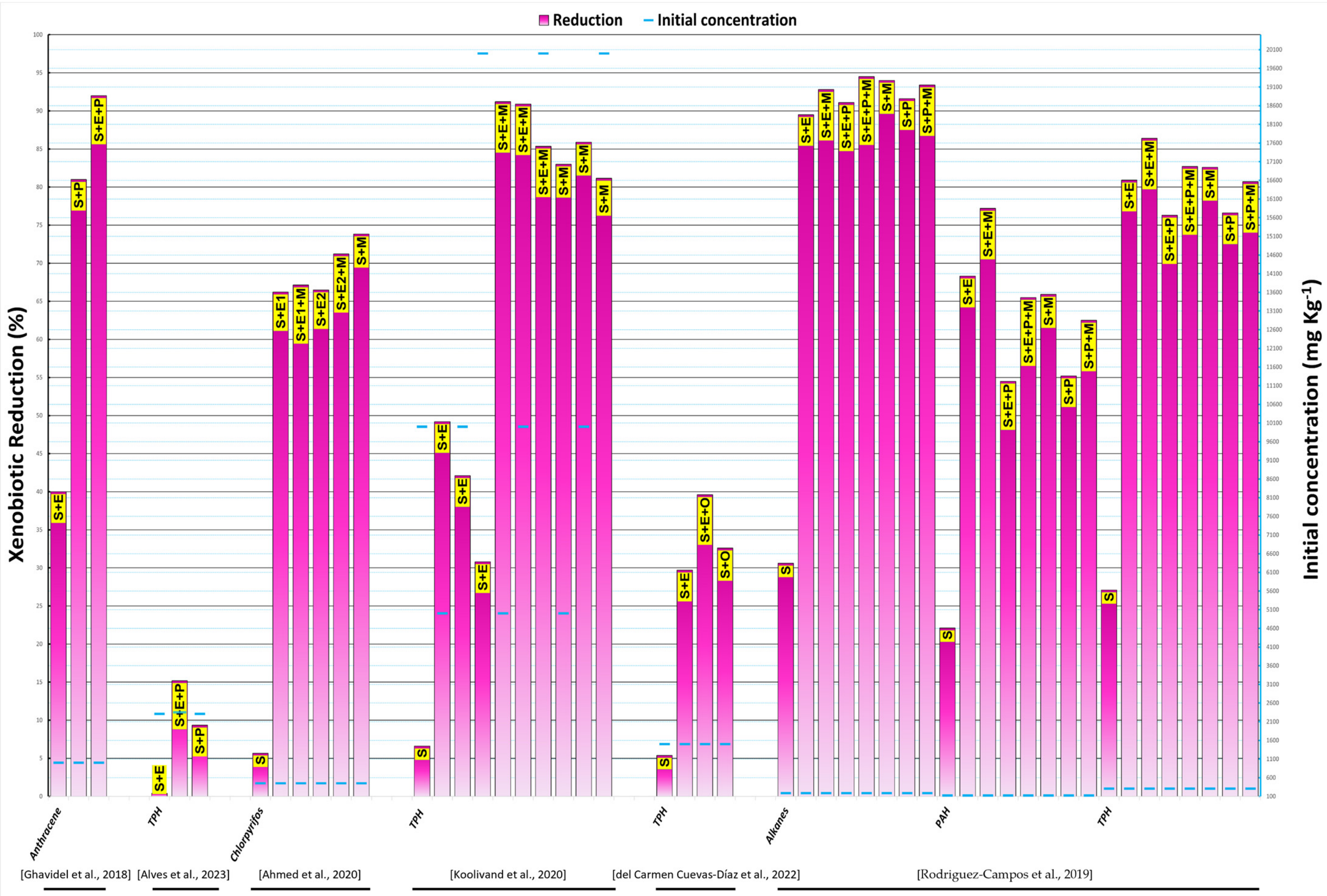

Figure 5. Combined vermiremediation for organic pollution. Xenobiotic reduction percentage (pink bars) and initial concentration (blue dash) for each treatment. Treatments (yellow labels) legend: S (substrate), E (earthworm), P (plant), M (microorganisms), O (other treatment). When progressive numbers appear on the label, different substrates (i.e., S1, S2, etc.) or experimental designs (i.e., E1, E2, etc.) were tested. The square brackets show the bibliographic references of the articles taken into consideration (Number of articles: 6, in order of comparison, Ghavidel et al., 2018 [148]; Alves et al., 2023 [149]; Ahmed et al., 2020 [152]; Koolivand et al., 2020 [153]; del Carmen Cuevas-Díaz et al., 2022 [162]; Rodriguez-Campos et al., 2019 [164]).

3.5. Combined Vermiremediation for Co-Contamination Pollution

Unfortunately, cross-contamination with inorganic and organic xenobiotics of several environmental matrices is a widespread and dangerous condition that needs to be studied in depth.

Urionabarrenetxea et al. [166] conducted an in-depth study on natural soil contaminated by PTEs (Cd, Cr, Pb and Ni) and organic compounds (Dieldrin and Benzo(α)pyrene).

Ecotoxicological tests were first conducted on this matrix to understand the applicability of the proposed bioremediation techniques, which were then applied, i.e., vermiremediation (with earthworms *E. fetida*), phytoremediation (using *Medicago sativa*) and bioaugmentation (*Burkholderia xenovorans LB400* and *Paenibacillus* sp. *Burkholderia xenovorans LB400* strain). These techniques were applied individually and in all possible combinations (double or triple) in situ.

From integrating the ecotoxicological endpoints and the chemical characterisation of the substrate after the twelve-month treatment, the authors measured the better remediations in combined treatments and, above all, in the triple application (E + P + M) for all the contaminants studied. More important reduction percentages were found for Dieldrin (50–78%), followed by PTEs (maximum reduction for Cd, Cr, Pb and Ni of 35%, 39%, 33%

and 37%, respectively) and, finally, to a lesser extent for the PAH Benzo(α)pyrene (maximum reduction of 28%). The E + P + M combination proved to be the winning strategy, showing results with less variability.

Another study that considers co-remediation techniques on soil always contaminated by an organochlorine insecticide (lindane) and Cr is that of Lacalle et al. [167]. In this work, several bioremediation approaches (vermiremediation with *E. fetida*, phytoremediation with *Brassica napus* and Bioaugmentation with *Streptomyces* sp. *M7*, *Streptomyces* sp. *MC1*, *Streptomyces* sp. *A5* and *Amycolatopsis tucumanensis* strain) were tested alone or in combination on two natural soils that differed mainly in organic matter content (OM): 1% (unamended soil; U) and 2.6% (amended soil; A). Regarding the organic contaminant, it has been noted that this was generally less degraded in soil A, where it has been less bioavailable for biodegradation due to its links with organic substances and probably because the microorganisms prefer to consume the OM rather than the pesticide. On the other hand, regardless of the concentrations tested (100 and 300 mg kg^{-1}), OM decreased the toxicity of hexavalent chromium, reducing it to Cr (III). The authors [167] observed how combining *E. fetida* or *B. napus* with the consortium of actinomycetes gave better results in reducing both studied xenobiotics. Still, the highest efficiency was found if the three bioremediation techniques were combined.

The same soils (U and A) and contaminants (lindane and Cr) were employed by Aparicio et al. [168] to evaluate the remediation capacity of nano remediation techniques with nZVI alone or in aid of the combined previously discussed (E + P + M + nZVI).

Again, the tests confirmed how much organic matter (OM) affects the fate of contaminants in the soil, together with the intrinsic characteristics of the xenobiotics, favouring the reduction of inorganic contaminant, while it disadvantages the biodegradation of the insecticide beyond the tested concentrations.

Treatment with nZVI, either alone or applied in combination with other bioremediation techniques, was found to be the most efficient in reducing Cr(VI) to its less toxic form (Cr(III)), with protecting effects on plants and earthworms. The most efficient degradation of lindane was obtained with combined bioremediation (E +P + M). Still, this efficiency can mainly be attributed to the activity of the microbial consortium, considering that Cr exerted toxicity on plants (i.e., 100% mortality in soil U) and earthworms (50 and 100% in tests with Cr at 100 and 300 mg kg^{-1}, respectively). The authors recommend the use of the complete combination (E + P + M+ nZVI) in the case of high contamination of Cr and lindane, while at moderate levels of chromium pollution, they suggest the application of bioremediation techniques as more economically and environmentally sustainable.

A synergy of help between plants and their root exudates, earthworms and microorganisms activities was assessed by the studies regarding the health status (i.e., biomass and survival rate) of all the organisms. Earthworm fertility indices (i.e., the cocoons and juveniles' production and vitality) or root elongation and photosynthetic pigments measurements in plants and specific microbial parameters such as functional diversity and respiration were stimulated by their contemporary presence in E + P + M treatments.

4. Conclusions

To summarise considerations on the bioremediation efficiency and highlight critical issues, a careful analysis of the literature dealing with vermiremediation was performed.

It was found that vermiremediation alone to remediate inorganic contamination is conducted on sludge waste from industrial activities or waste management processes. Regarding remediation from organic contaminants (mostly TPHs, pesticides and antibiotics), different species of earthworms were tested, often selected from among the autochthonous ones. Noteworthy, only two papers used vermicast instead of the earthworm directly, obtaining good results (improves soil fertility by promoting biodegradation of pollutants). When the reviewed articles tested various combinations of bioremediation, the most used earthworm species was *E. fetida*, regardless of the nature of the contaminant (inorganic or organic).

From the evaluation of the articles, the following main conclusions emerged:

- Vermiremediation alone should be considered, especially in conditions of limited contamination, which allows the survival of earthworms regardless of the species and type of contaminant.
- In the case of vermiremediation to restore PTEs pollution, the solutions in which manure is added to the substrate as an organic amendment gave better remediation results.
- When vermiremediation is applied for organic pollutants, the results are highly variable and extremely dependent on the contaminant and the species studied.
- The combination of several strategies improves the effectiveness of remediation and allows working with high contamination. Despite the contaminant, plants, earthworms and microbes stimulate each other and establish symbiotic relationships even in decontamination processes.

To conclude, some gaps and prospects in this research area have emerged:

- The use of native species (earthworms, plants and microorganisms) adapted to the contamination of the study site should be increased.
- To understand and identify the most promising combinations, experimental designs should consider comparisons between different species and levels of contamination.
- Few works deal with substrates affected by simultaneous contamination of organic and inorganic pollutants, a very frequent situation; therefore, implementing these studies is hoped for.
- The addition of amendments as a nutrient supply must be carefully evaluated in the case of contamination by organic xenobiotics, seeking the proper dosage to favour earthworm activity but, at the same time, not affect the biodegradation capacity.

Author Contributions: Conceptualisation, A.D.B. and C.C.; methodology, F.T., E.M. and A.D.B.; validation, C.C. and C.V.; formal analysis, F.T., E.M. and A.D.B.; investigation, F.T., E.M. and A.D.B.; data curation, F.T., E.M. and A.D.B.; writing—original draft preparation, F.T. and E.M.; writing—review and editing, F.T., E.M., A.D.B. and C.C.; visualisation, F.T., E.M. and A.D.B.; supervision, C.C. and C.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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