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Review

Nickel in the Environment: Bioremediation Techniques for Soils with Low or Moderate Contamination in European Union

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Abstract: The review deals with the environmental problem caused by low or moderate nickel concentrations in soils. The main effects of this potentially toxic element on the soil biota and the most common crop species are addressed. Moreover, the paper emphasises biological remediation methods against nickel pollution in European soils. The focus is on the well-accepted phytoremediation strategy alone or in combination with other more or less innovative bioremediation approaches such as microbial bioremediation, vermiremediation and the use of amendments and sequestrants. Results acquired in real field and laboratory experiments to fight against nickel contamination are summarised and compared. The main objective was to evidence the ability of the above natural techniques to reduce the nickel concentration in contaminated sites at a not-risky level. In conclusion, the examined works agree that the efficiency of phytoremediation could be implemented with co-remediation approaches, but further studies with clear and comparable indices are strongly recommended to meet the challenges for future application at a large scale.

Keywords: nickel; agricultural soils; bioremediation; phytoremediation; microbial remediation; metal sequestration; vermiremediation; co-remediation

Citation: Vischetti, C.; Marini, E.; Casucci, C.; De Bernardi, A. Nickel in the Environment: Bioremediation Techniques for Soils with Low or Moderate Contamination in European Union. *Environments* **2022**, *9*, 133. https://doi.org/10.3390/environments9100133

Academic Editors: Manhattan Lebrun, Domenico Morabito, Sylvain Bourgerie and Lukas Trakal

Received: 27 September 2022
Accepted: 19 October 2022
Published: 21 October 2022

1. Introduction

Potentially toxic elements (PTEs) in soils come from natural and anthropogenic sources; in agricultural areas, excessive application of PTE-containing products such as pesticides and fertilisers could lead to soil contamination [1–3]. PTEs include metals and metalloids, among which micronutrients such as nickel (Ni) are fundamental elements for the life of organisms, which, beyond certain thresholds, are toxic [4,5].

Soil contamination by PTEs is a growing global crisis affecting the environment and human health; there are approximately 3,000,000 contaminated sites in the European Economic Area [6].

Following Tóth et al. [7], most European agricultural land can be considered adequately safe for food production, and only 6.24% needs local assessment and eventual remediation action. However, in detail, the threshold value, i.e., the value that indicates the need for further evaluation of an area, is exceeded by more than 70–80% in many places, especially in the Mediterranean countries (Spain, France, Italy and Greece). Therefore, the remediation of PTE-contaminated soils is a priority for governments, scientists and environmental regulatory agencies [8].

At the European level, there is large variability between countries regarding the regulatory standards for heavy metals or metalloids in agricultural soils. For example, the threshold values for cadmium, mercury, lead and nickel range from 0.5 to 20, 0.5 to 80 40 to 750 and 30 to 300 mg kg^{-1}, respectively [9]. Generally, the limit values indicated by the Finnish regulations are used in international assessments on soil contamination by metals, as they have resulted from years of discussions and are considered the most representative [10]. The Ministry of Environment of Finland states the "threshold value" for Ni is 50 mg kg^{-1}, while the "lower guideline value", i.e., the value which presents ecological or health risks in agricultural land, is 100 mg kg^{-1} [11].

The LUCAS Soil Survey is considered Europe's largest soil physicochemical and biological properties dataset [12]. From the LUCAS 2009–2012 sampling campaign and up-to-date maps of heavy metals, the mean Ni concentration in European topsoil was determined to be about 18 mg kg^{-1}, with high variability (sd of 18) [13].

In 2018, LUCAS points with relatively high concentrations of metals in the 2009/2012 sample were re-analysed with additional random points. This time, mean and median Ni concentrations were 88 and 21 mg kg^{-1}, respectively [14].

Looking only at the agricultural land, Toth et al. [7] found that in a considerable percentage of samples from the Mediterranean region (except for most of Spain and South Italy), Ni exceeded 50 mg kg^{-1}, and the density of samples with concentrations between 100 and 150 mg kg^{-1} was the highest in Greece. Considering these reports, the risk for crop production and ecological and human health could be expected to be due to Ni in agricultural soil. The increase in this element in the environment at low or moderate levels could occur in agricultural lands due to the presence of this element in chemical fertilisers, pesticides and sewage and their excessive application in crop fields [15–17]. Assessing the concentration level of PTEs in agricultural soils is still a complex challenge, and the effects of non-hazardous concentrations on crop metabolism and production should be studied more in detail to program the remediation campaigns.

The bioremediation techniques have some advantages, such as good acceptance by public opinion and high efficiency for areas with medium-to-low contamination [18]. Furthermore, they are cost-effective and eco-friendly [19].

This review considers different aspects of low and medium Ni concentrations in soil. The work starts by examining the effects on crops in terms of metabolic responses and the influence on the quantity and quality of agricultural production.

Furthermore, the ecotoxicological risk associated with nickel contamination on soil biota is addressed.

The main aim is to ascertain and collect the bioremediation experiments carried out for low nickel concentrations in European soils.

Phytoremediation alone or in a co-remediation approach with sequestration, microbial remediation and vermiremediation was analysed through the existing literature to evaluate the successes and failures in reducing Ni contamination.

2. Nickel Toxicity Effects

Ni is an essential micronutrient for several biological functions in plants [20,21] and for selected microorganisms where it participates in various cellular processes [22].

The essentiality of this element for the animal kingdom is debated, as deficiencies rarely occur because it takes little to meet the biological functions; moreover, a metalloenzyme containing Ni has yet to be recovered [23].

Phipps et al. reported that there are no studies on the essentiality of Ni in invertebrates, but it probably acts as an enzymatic cofactor, as observed in vertebrates [24].

This element's potential toxicity depends on many factors, such as its speciations, the way and time of exposure and concentrations. Different effects could occur at cellular and population levels when it exceeds the optimum intake level in organisms [25].

2.1. Effects on Crop

Nickel metabolism in plants is essential for some enzyme activities [26], maintaining the proper cellular redox state and various physiological [27] and growth responses [28].

Ni is considered an essential element for the growth of the majority of plant species with low concentrations (0.05–10 mg kg^{-1} d.w.) [29]; it is involved mainly in nitrogen metabolism, iron uptake and specific enzymatic activities such as urease, hydrogenase and superoxide dismutase [28].

Many authors [30–33] have studied Ni deficiency in plants, as reported in a recent review [34], but actually, cases of Ni deficiency are unusual in agricultural soils [35].

The critical level of Ni toxicity is higher than 10 mg kg^{-1} dry mass in sensitive species [36], more than 50 mg kg^{-1} dry mass in moderately tolerant species [37] and above 1000 mg kg^{-1} dry mass in Ni-hyperaccumulator plants [38]. Ni above certain limits can induce phytotoxicity at multiple levels [39], altering plants' structural and anatomical dynamics [21]. However, it is difficult to establish a threshold of Ni concentration in soils that can be potentially toxic for cultivated plants.

Numerous authors have studied the effects of Ni on European crops such as tomato, spinach, oats, barley, wheat and corn. From the experiment conducted in Poland by Matraszek et al. [40] on cherry tomato *(Lycopersicon esculentum)*, it was found that the plant yield (expressed in dry biomass) does not vary at low Ni concentrations (40 mg kg^{-1}), while it decreased significantly at 100 mg kg^{-1}. In another study on tomatoes, 40 mg kg^{-1} of Ni in the soil affected the plants' development and yield [41].

Other authors found a strong decrease in cherry tomato yield at lower Ni doses caused by this PTE in plant nutrient media (from 5 to 30 mg L^{-1}). Ni probably causes disturbances and imbalances in the absorption and accumulation of other nutrients [42]. More recently, the impact of Ni on *Solanum lycopersicum* was measured throughout the antioxidative enzyme ascorbate peroxidase (APX) when Ni was applied at 50 µM and 15 mg L^{-1} [43,44]. The augmented Ni doses caused a significant increase in APX activity. This effect was also observed in other plants subjected to Ni treatments, such as wheat [45], rice [46] and corn [47].

Poulik [48] studied the toxic effects of Ni on *Avena sativa.* Ni concentrations of 100 mg kg^{-1} resulted in yield depression, while doses higher than 150 mg kg^{-1} caused phytotoxicity and plant mortality. Kumar et al. [49] found that Ni applied to the soil at 10 mg kg^{-1} increased *Hordeum vulgare* yield parameters, but a significant reduction was observed beyond this level. Gupta et al. [50] found similar results in three cereal species (wheat, barley and oats) subjected to doses of 0, 2.5, 5, 10, 20 and 40 mg Ni kg^{-1}. These authors found that the yield of all cereals increased significantly at 2.5–5.0 mg Ni kg^{-1} but decreased at higher levels. In corn plants, Amjad et al. [51] evaluated the mechanisms influencing the growth, physiology and nutrient dynamics after exposure to Ni treatments (0, 20 and 40 mg L^{-1}) in hydroponic conditions. This experiment showed that all the antioxidant enzyme activity tested (SOD, CAT, GR, APX and POX) increased significantly compared to the control after Ni treatments.

Additional experiments [52,53] to test the activity of the antioxidant system in the cells of the spinach plant have shown that Ni doses applied at 50–100 and >25 mg kg^{-1} caused oxidative stress via increased synthesis of ascorbic acid in plant biomass. The author has suggested that ascorbic acid plays a defensive role in Ni stress.

Works regarding Ni toxicity on plant physiological processes almost always refer to laboratory studies with a contaminated solution at different concentrations [51,54,55], but only a few use contaminated soils, so it is difficult to establish when the soil concentration of Ni could be toxic for cultivated plants.

2.2. Effects on Soil Microorganisms and Earthworms

Exposure to excessive Ni concentration in soils could strongly affect living organisms such as microorganisms and soil invertebrates. Until now, the responses of soil organisms to long-term Ni pollution under field conditions has remained largely unknown.

Many microbial processes in the soil are altered by Ni presence at different concentrations, and such alterations are often identified by studying the soil enzymatic activities by the microorganisms that inhabit it. For example, in the study by Helaoui et al. [56], the enzymatic activities of the soil (urease, dehydrogenase, β-glucosidase, arylsulfatase, alkaline phosphatase and FDA) significantly decreased compared to the control at a Ni dose of 50 mg kg^{-1}. However, the most negative effect appears at the high concentration of 500 mg kg^{-1}. Similar results regarding enzymatic activities were previously found by Wyszkowska et al. [57], with maximum doses of 400 mg kg^{-1} of Ni applied.

Regarding the effect of Ni on soil microbial biomass, some studies showed a strong decrease at Ni doses of 100 and 200 mg kg^{-1} [58,59]. A similar trend was observed up to 250 mg kg^{-1}, although an increase in microbial biomass at the higher dose of 500 mg kg^{-1} probably indicates an integrated defence system was observed [56].

Several authors have found that soil microbial respiration is stimulated at low Ni concentrations (50–150 mg kg^{-1}) but declines with increasing Ni levels (> 200 mg kg^{-1}) [58,60,61]. This tendency reflects a mechanism of "hormesis", in which a small concentration of xenobiotics stimulates certain bodily functions [61].

In some neocaledonian soils with high levels of Ni (from 800 to 5000 mg kg^{-1}), Héry et al. [62] found different Ni-resistant bacteria that adapted due to the long-time exposure to these high concentrations. The addition of $NiCl_2$ at 30,000 mg kg^{-1} to these soils and a reference soil (20 mg kg^{-1} Ni) had an initial negative effect on bacterial growth, regardless of the soil or population considered, and this result was surprising, as the Caledonian soils had adapted to long-term exposure to high concentrations of Ni. However, the bacterial community of the reference soil was highly disturbed by the addition of Ni, while only a few changes occurred in the bacterial structure (shifts in the genetic profiles) of the neocaledonian soils, suggesting a good adaptation to Ni of these microorganisms.

In recent decades, some studies have been reported the effect of Ni concentrations (low, medium and high) on soil invertebrates such as earthworms. Scott-Fordsmand et al. [63] reported the toxic effects of Ni on the earthworm *Eisenia veneta*, in sandy-clay soil, at a concentration above 85 mg kg^{-1}. Reproduction and lysosomal membrane stability showed a dose–response relationship and were already altered at 85 mg kg^{-1}, while adult survival was reduced only at concentrations above 245 mg kg^{-1}.

Lock and Janssen [64] examined the chronic toxicity of this metal at different concentrations, in OECD soil, for three soil invertebrates: *Eisenia fetida*, *Folsomia candida* and *Enchytraesus albidus*. At the highest Ni concentration of 1000 mg kg^{-1}, no mortality occurred in *E.fetida*, while *F.candida* showed a mortality of 10%, and all *E.albidus* died. The reproduction test showed a significant effect on cocoons, and juvenile production in *E.fetida* started to be evident from a concentration of 320 mg kg^{-1}.

E.fetida did not show an increased tolerance toward Ni despite being exposed to elevated levels for more than ten generations: worms exposed to Ni for several years showed an increased sensitivity towards this element [65].

Other authors [66] analysed the effects of the addition of nickel at concentrations ranging from 0 to 1000 mg kg^{-1} to 13 Chinese soils on growth, cocoon and juvenile production in the earthworm *E.fetida*. The body weight of *E.fetida* was insensitive to Ni until 320 mg kg^{-1}, while a significant decrease in growth was observed at 560 and 1000 mg kg^{-1}. Juvenile production, compared to cocoon output, was a more sensitive end-point for Ni, and the two parameters did not show a significant correlation with the properties of the 13 soils studied, probably due to the narrow range of properties of the selected soils.

More recently, a study examined the toxic effect of Ni-spiked farmland at concentrations from 0 to 800 mg kg^{-1} on *E.fetida*. A low mortality rate (10%) was observed only in earthworms exposed to the higher dose (800 mg kg^{-1}) on day 14, while the avoidance response reached 100% at this concentration [67].

Depending on the end-point and substrate type, there is a broad range of Ni limit values, evidencing that the soil and substrate characteristics greatly influence Ni's availability and toxicity [59,68,69], as well as those of other PTEs [70].

3. Bioremediation Techniques

As described in previous sections, the potentially toxic effect of Ni in soil on some crops imposes a strategy of intervention to reduce Ni contamination. Cost-effective and potentially environmentally dangerous physical and chemical traditional treatments are more often indicated for large-scale and high-concentration cases of PTE contamination [6,71–73].

Instead, non-impacting bioremediation strategies are emerging, as well as eco-friendly techniques that can be simultaneously used with other methods to reduce hazardous pollutant contamination in the environment to undetectable, non-toxic or acceptable levels [74].

Bioremediation treatments use indigenous or exogenous microorganisms, organic substrates and several organisms (animals or plants) to restore the soil or other environmental matrices [75].

3.1. Phytoremediation

Among several bioremediation techniques, phytoremediation is one of the most well-accepted.

The term phytoremediation includes all those environmental remediation techniques that use plants to reduce or in some way "inactivate" a contaminant. Phytoremediation is considered an integrated multidisciplinary approach [76]. It is possible to classify six main modalities of application [77]: phytodegradation (phytotransformation), where the absorbed organic contaminant is broken down, metabolised or mineralised through a series of enzymatic reactions and metabolic processes [78]; phytostabilisation (phytoimmobilization), in which the purpose is to avoid mobilisation of organic and inorganic contaminants, which are locked into the humus or the lignin of the cell wall of the roots [79]; phytovolatilisation, when plants can volatilise certain metals/metalloids and also some organic compounds from the root absorption and convert these into non-toxic forms [80], phytofiltration, as seen in plants in aquatic environments that absorb, concentrate or precipitate the contaminants through their submerged organs, and if the filtering activity is performed by the roots, the term rhizofiltration is used [81]; rhizodegradation (phytostimulation), when the exudates from the root system enhance and promote the activity of the microorganisms in the rhizosphere, which can transform organic pollutants; and finally phytoextraction (phytoaccumulation, phytoabsorption or phytosequestration), a technique applied against organic and inorganic contaminants (especially metals) that uses mainly hyperaccumulator species [82,83].

Several studies report the efficacy of phytoremediation in reducing PTE concentration in soils [84–88].

Among PTEs, Ni is one of the most studied, as extensively reported in recent reviews [21,89,90]. Obviously, among the six techniques listed above, some (phytodegradation and rhizodegradation) cannot be applied to inorganic contaminants as they refer to the transformation of organic compounds into their less toxic metabolites. Phytovolatilisation is applied above all for volatile organometals such as Se, As and Hg and not for Ni [90,91]. The remainder can be used for soil decontamination from Ni.

Ni-hyperaccumulator plants can have at least 1000 mg kg^{-1} d.w. of Ni in their shoot tissues without showing any signs of suffering [92]. There are many examples in the literature of metallophytic plants; for nickel, we can mention the genera *Alyssum* and *Noccaea* as the most interesting hyperaccumulators [93–95].

Sometimes, the use of crop species is preferred to hyperaccumulators because they can extract equal or more significant quantities of metals as they have high biomass production, are easier to grow, are available and are economically attractive [96–101].

Several indices are commonly used in phytoremediation works to quantitatively describe the plant performance for decontamination purposes. Unfortunately, these indices are not expressed in a univocal and coherent way in the bibliography.

Backer (1981) reported using bioconcentration (BCF) and translocation (TF) factors to evaluate the phytoremediation potential of plants [102]. BCFs and TFs were obtained as the ratio between the metal (loid) concentration in the root to the soil, and leaf to roots, respectively. In this context, a TF > 1 indicates the suitability for phytoextraction, while when the TF is under 1, but the BCF reaches a high value, the plants could be used for phytostabilisation.

Some authors calculate TFs and BCFs for each aerial organ of the plant studied. In contrast, others tend to evaluate the metal(loid) concentration of the overall above-ground part of the plant.

The present chapter refers to the translocation factor (Tf) and the bioaccumulation factor (Bf) as follows:

$$Tf = \frac{C_{Aboveground\ tissues}}{C_{Roots}}$$

$$Bf = \frac{C_{Roots}}{C_{Soil}}$$

where C means concentration of nickel.

The most recent European papers dealing with phytoremediation from Ni at low or moderate concentrations (no more than 200 mg kg^{-1}) were analysed. Translocation and bioaccumulation factors were reported for each species. Where these indexes were not provided or obtained differently, they have been recalculated as *Tf* and *Bf* to standardise and facilitate the comparison (Figure 1).

Some spontaneous species (non-cultivated species) showed a *Tf* above 1, indicating their functionality for phytoextraction: *Alyssum saxatile* [103], *Chenopodium album*, *Tripleurospermum inodorum* [104] *Achillea millefolium*, *Arrhenatherum elatius*, *Artemisia vulgaris*, *Bromus inermis*, *Silene vulgaris* and *Urtica dioica* also mention *Holcus lanatus* with 0.99 [105]. Efficient Ni translocation in the epigeal portion in the crops was found for *Brassica juncea* and *napus*, sunflower, corn [100,106] and aromatic thyme [107]. Among these, exceptionally high values ($\geq$2) were found in the recent works of Tőzsér et al., Antoniadis et al. and Salinitro et al. for the species *C.album*, *B.napus*, *A.vulgaris*, *H.annus* and *T.inodorum* [104–106].

If we take into account all the studies analysed, *Brassica* species have given highly variable results with *Tf*s even lower than 1 in the studies conducted in the laboratory on urban [108] and agricultural [109] soils and in the greenhouse with sewage sludge [106]. The same can be said for corn and sunflower [106,108,110].

The ability to concentrate the metal in the roots (*Bf* > 1), and therefore the possibility of phytostabilisation, was noted in the plants *Cannabis sativa* [111,112], *Avena sativa* [113], *Trifolium alessandrinum* [114] and *Zea mais* [110]. Despite a certain variability in most cases, the *Phalaris arundinacea* species also showed a good range of Bf, from 0.69 to 0.93 [110].

Among the studies analysed, some obtained interesting results for PTEs other than Ni [115,116], highlighting how the choice of species must be calibrated according to the type of contaminants under study.

Although phytoremediation is widely used, some limitations of the technique must be taken into account, such as the need for extended times or more vegetative seasons before obtaining valid results, as reported by several authors [110,116,117]. Moreover, if this technique is applied outdoors, it strongly depends on climatic and geological conditions, and there could be a risk in transporting the contaminant in the food chain. Finally, phytoremediation action is limited to the radical depth and generally used for low level of contamination [118,119].

One step following the phytoextraction to be considered is undoubtedly the plant biomass's fate. If the plants used have concentrated the PTE in their epigeal portion, mowing is usually carried out. Instead, suppose the concentration occurs in the roots or generally in the root zone; in that case, a mechanical eradication of the entire plant or at worst the removal of the first layers of soil plus the plant could be proposed. All this material must be sent to special landfills from which it is possible to produce energy and recover the PTE (phytomining) [120].

The use of non-hyperaccumulating plants often leads to plant biomass with low (below the permitted limits) concentrations of PTE; in these cases, the harvested plants can be destined for animal or human consumption or composted. The phytoremediation approach alone may not be sufficient when facing complex contamination [121,122]. A possible solution to remediating PTE-contaminated soils could be phytoremediation together with other biological remediation strategies, or a co-remediation approach with sequestrants and fertilisers.

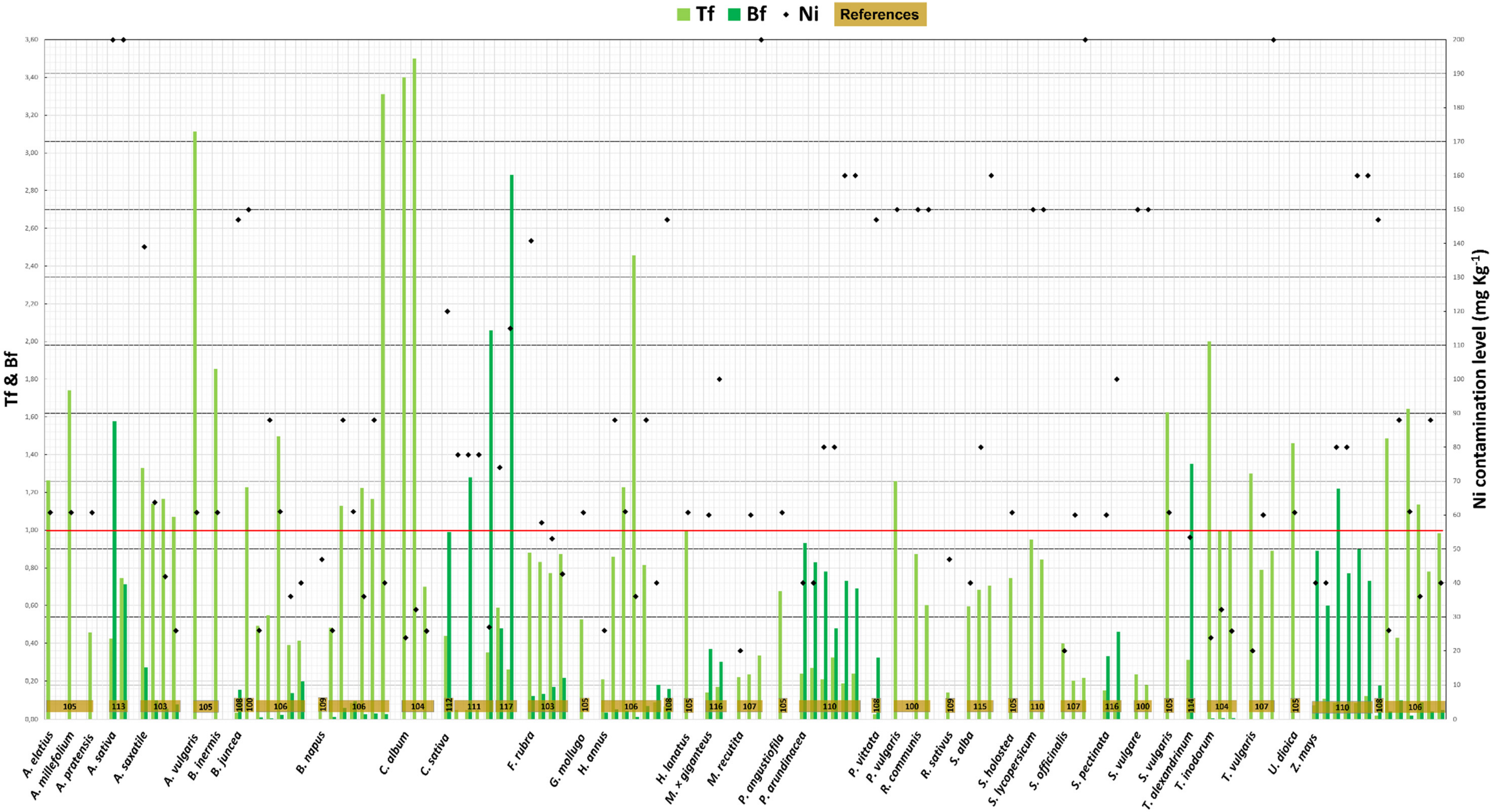

Figure 1. European phytoremediation experiments with Ni at low/moderate contamination. The red line indicates level 1 of the evaluated phytoremediation parameters: *Tf* (translocation factor, light-green bars) and *Bf* (bioconcentration factor, dark-green bars). Black diamond points indicate the level of Ni contamination in the substrate studied (secondary vertical axis.

3.2. Aided Plant-Based Bioremediation

In this work, aided phytoremediation refers to combined techniques using sequestering agents, soil improvers, microbial communities or earthworms. Table 1 presents the summary of the European papers analysed.

Table 1. European aided phytoremediation experiments with low/moderate Ni contamination. (FC: field contamination; LC: laboratory contamination).

Soil Type	Ni Level (mg kg^{-1})	Plant Species	Aided-Phytoremediation	Main Results	Country	Reference
Natural	36.4 54.3 48.2 (FC)	*T.durum*, *H.vulgare*	*B.licheniformis* BLMB1	Increased Ni concentration in wheat and barley roots after the application of *B.licheniformis*	IT	[123]
Urban	147 (FC)	*B.juncea*, *H.annuus*, *Z.mays*, *P. vittata*	Florawiva FW	Plant growth as stimulated by FW, but BF and TF were not enhanced significantly	IT	[108]
Natural	53.4 (FC)	*T. alexandrinum*	Biochar	Biochar did not affect Ni accumulation in above-ground tissues, but significantly increased Ni in roots compared to the control	IT	[114]
Artificial	100 (LC)	*C. sativa*	*G.mosseae*	Fungi enhanced the translocation from root to shoot	IT	[112]
Natural	200 (LC)	*H. annuus*	*B.weihenstephanensis* SM3	Bacteria increased the plant's weight compared to the non-inoculated control. There was a decrease in Ni accumulation of 14% and 48% in the root and shoots, respectively	PT	[124]
Natural	18.9 (FC)	*B. juncea*	compost 95% + biochar 5% (holm oak wood)	The 40% amendment was the most advantageous treatment for the Ni phytoextraction	ES	[125]
Natural	100 (FC)	*M.sativa*, *C.sativus*, *L.sativa*	*E.fetida*, *B.xenovorans* LB400, *Paenibacillus* sp.	The best Ni elimination yields were obtained after P+B+E treatment	ES	[126]
Agricultural	152.8 (FC)	*A. pintodasilvae*	PGPR	*A. nicotinovorans SA40* was able to promote plant growth and improve Ni phytoextraction	ES	[127]
Natural	100 200 (LC)	*A. sativa*	Zeolite	The reduction in Ni accumulation in *A.sativa* is limited to sandy-silty loam	PL	[113]
Natural	73.1 168.4 (FC)	*F. arundinacea*	Mineral fertiliser (Azofoska)	Ni was few translocated from the root to shoot; BF (roots/shoots) was > 1, showing that *F.arundinacea* accumulates metals mostly in roots	PL	[128]

Table 1. *Cont.*

Soil Type	Ni Level (mg kg^{-1})	Plant Species	Aided-Phytoremediation	Main Results	Country	Reference
Artificial	40 100 (LC)	*L.esculentum*, *C.sativus*	Ion-exchange substrates (Biona-312)	Biona 312 application significantly decreased Ni in tomato plants, while in cucumber, it increased and decreased in roots and above-ground, respectively	PL	[40]
Natural	91.3 (FC)	*M.×giganteus*	*S.maltophilia* KP-13; *B.altitudinis* KP-14; *P. fluorescens* KP-16	Ni was accumulated in the roots. The treatments with *M.× giganteus + P. fluorescens* KP-16 significantly increased the root absorption	CZ	[129]
Agricultural	100 (LC)	*B. napus*	EDTA	The Ni amount in root and shoot increased with increasing EDTA application	TR	[130]

The use of chelators simultaneously with plants can facilitate the soil detoxification process by increasing the immobilisation of PTEs (chemophytostabilisation) [98,131,132] or their absorption and translocation in plant tissues [133–135]. In the presence of synthetic or natural chelators (EDTA, EDDS, bentonite, zeolite), the accumulation of metals and plant biomass yield are significantly improved [136,137]. Several European studies have extensively studied the efficiency of phytoremediation assisted by chelators and other sequestering material. Adiloğlu et al. [130] proposed an original, easily applicable and cheap method to clean up Ni pollution in agricultural soils using *B.napus* and EDTA (5, 10 and 15 mmol kg^{-1}). The study showed a dose-dependent trend: increased EDTA significantly improved the amount of Ni in the roots and shoots. The authors affirmed that the chelator increases the solubility of Ni, and thus the absorbability of the soil.

Matraszek et al. [40] assayed ion-exchange substrates (Biona-312) for Ni detoxification on tomato and cucumber. After introducing Biona-312 in the polluted soil, a significant decrease in Ni (at both doses of 40 and 100 mg kg^{-1}) was found in the tomato aerial parts and roots. Similar results were observed for cucumber plants, but this reduction was seen only at higher doses.

Boros-Lajszner et al. [113] reported that the addition of zeolites (clinoptilolite) causes an improvement in Ni accumulation in *A.sativa* (11.69%) only in sandy-silty-loam soil, so the efficiency of this mineral in soil remediation by the PTE is reduced.

Another approach used for the detoxification of soil contaminated with Ni is the combined use of plant–biochar. Biochar is a carbon-rich material prepared from various organic waste feedstocks [138] that plays an important role in the bioavailability of heavy-metal-polluted soil, resulting in biotransformation and bioremediation [139]. In a recent study conducted in Italy by Pescatore et al. [114], the Ni concentration in the above-ground tissues of plants grown in soil amended with biochar at 0.8% was not significantly different from that of the control, while in the trial with biochar at 1.8%, they found a significant reduction in Ni in aerial tissues (37.2%). Regarding Ni in roots, there was a significant increase from the control to biochar at 0.8% (30.66%) and to biochar at 1.8% (18.92%). This resulted in a TF reduction and an increase in BCFr for berseem clover treated with biochar amendments. Rodríguez-Vila et al. studied phytoremediation of *B.juncea* assisted by different percentages of compost and biochar (holm oak wood): mustards accumulated PTEs well, and the best phytoextraction combination was BC40P (8% compost + 2% biochar) [125].

An additional ecological and inexpensive approach for the decontamination of PTE-polluted soils is using soil improvers or fertilisation. Fertilisers can improve plant growth, thus favouring their capacity to produce specific proteins for detoxification. This mechanism positively influences PTE's absorption, dissociation and migration into the soil. Steliga and Kluk studied the performance of phytoremediation assisted by the fertilisation process (mineral fertiliser "Azofoska") involving *F.arundinacea* grown on soils with Ni at different concentrations (73 and 168 mg kg^{-1}) [128]. Nickel is not translocated well from root to shoot (TF = 0.29–0.31), while the bioconcentration factor of roots/shoots is >1, showing that *F.arundinacea* accumulates metals mainly in roots. In the same year, the uptake of PTE by four plants (*B.juncea, H.annuus, Z.mays and P. vittata*) with the addition of a soil improver (green waste and anaerobically digested organic materials) in urban soil [108] was evaluated. The study revealed that the soil improver is a growth stimulator that increases the Ni BF slightly in *P.vittata*; this value was augmented following treatment with FW from 0.33 to 0.49. The TF of plants is not affected by the presence of the soil improver.

Gentle Remediation Options (GROs) such as microbial remediation and vermiremediation have received large acceptance in recent years as effective risk-management strategies for polluted soil [140]. Biological treatments can offer an original, economical and ecological solution to soil co-polluted with PTEs [141] and are increasingly employed in place of the traditional remediation techniques [142].

In a recent review, Saha et al. analyse the recent developments in microbe–plant-based bioremediation to reduce PTE concentrations in polluted soils [143].

The microbial communities have evolved Ni detoxification mechanisms utilising efflux systems, sequestration, accumulation and reduction [144]. Bacteria are important biosorbents due to their ubiquity, dimension and capacity to grow under controlled conditions and resist environmental conditions [145]. In particular, some bacteria of the rhizosphere, called PGPR (plant-growth-promoting bacteria), can assist the phytoremediation due to their capacity to enhance plant growth [123] and biomass production [146]. Furthermore, PGPR can solubilise unavailable forms of metals by producing organic acids [147].

Numerous studies have investigated the aptitude of selected bacterial strains to assist in phytoremediation processes [123,124,127,129]. A pot experiment was conducted in 2008 [124] to elucidate the effects of *Bacillus weihenstephanensis* SM3 on plant growth and the uptake of Ni, Cu or Zn by *H. annuus*. *B. weihenstephanensis* SM3 has a high degree of resistance to Ni (1500 mg L^{-1}), and the strain showed the ability to solubilise phosphate, produce indole-3-acetic acid and increase the weight of the plant (fresh 47%, dry 23%) compared to the non-inoculated control. Despite this, bacteria did not improve phytoremediation and decreased Ni accumulation by 14% in the root and 48% in the shoots. This may be due to *H.annuss*' low translocation capacity. On the contrary, an Italian study conducted in 2012 in the Apulia region observed a positive response after the application of *B.licheniformis*, with an increase in Ni concentration in wheat and barley roots [123]. Another good performance was proven by Cabello-Conejo et al. with *Arthrobacter nicotinovorans* SA40- *A.pintodasilvae*, which improved Ni phytoextraction significantly compared to the non-inoculated plants [127]. A recent experiment highlighted different effects on the phytoremediation of *Miscanhus* × *giganteus* with three PGPB bacteria tested individually or in combination [129]. In treatments with only *Pseudomonas fluorescens* KP-16, an increase in Ni accumulation in the roots (by 144%) emerged. In contrast, treatments with consortia (where *P.fluorescens* KP-16 was present) showed a significant Ni decrease (by 54% and 67%, respectively).

Regarding fungi populations, they are widely used as bio-sorbents for their ability to detoxify toxic metals; many studies have shown that arbuscular mycorrhizal fungi (AMF) play a significant role in the adhesion of inorganic chemicals [148]. Citterio et al. evaluated the effect of *Glomus mosseae* on heavy metal uptake and translocation in *C.sativa* grown in soil contaminated with Ni at 100 mg kg^{-1}. *C.sativa* accumulated Ni mainly in the hypogeal organs, and mycorrhisation significantly enhanced the translocation of the PTE from the root to shoot [112].

Vermiremediation is an innovative method that exploits earthworms' biotic and abiotic interactions to transform, degrade or remove contaminants from the soil through accumulation in their tissues [149,150] because earthworms can activate specific detoxification systems to tolerate certain levels of PTEs in soil [151,152]. Considering that inorganic contaminants such as PTEs cannot be degraded, the strategy of vermiremediation in these cases is almost always considered together with phytoremediation.

As described by Zeb et al. [153], the multiple efficiencies and processes involved in vermiremediation are closely interconnected and highly influenced by many factors, i.e., the species of earthworms and PTE studied.

To summarise and simplify the mechanisms that can achieve vermiremediation of PTEs (schematically shown in Figure 2), it is possible to identify two modes of action. One is a direct remediation pathway in which earthworms actively (dietary uptake) or passively (dermal uptake) assimilate PTE [149]. The other way can be defined as indirect as the excavation and excretion activities (cast, mucus, calcium compounds, urine) of earthworms improve the soil's physical, chemical and biological fertility, thus favouring the health of the plants and their possible phytoextraction or phytoimmobilisation ability [154–157].

Moreover, earthworms increase the pH and dissolved organic carbon, raising the phytoavaiability of most metals [158].

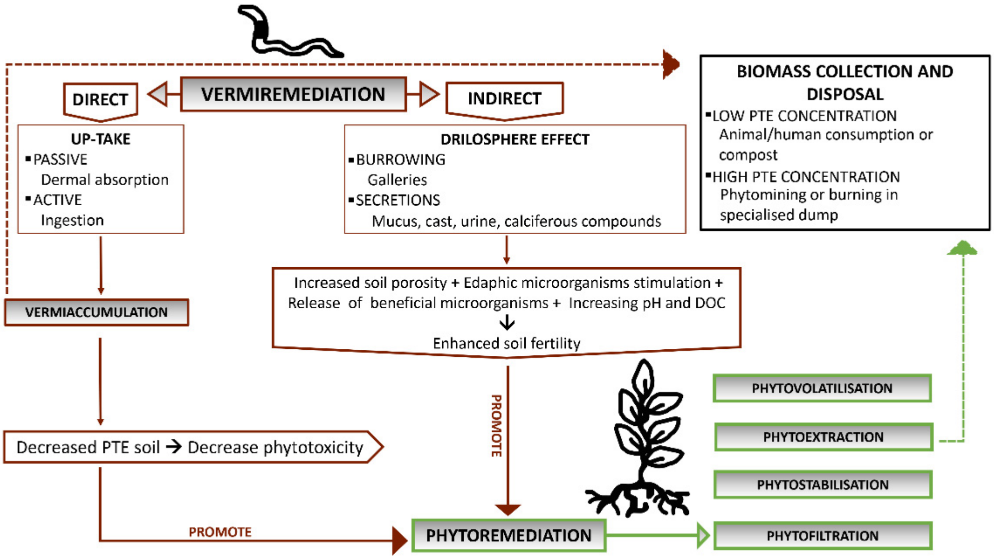

Figure 2. Schematic representation of the processes involved in vermiremediation for PTEs with correlation to phytoremediation and the possible fate of biomass.

Regarding vermiaccumulation, also called vermiextraction (storage of PTEs in earthworms' bodies, reducing soil contamination) [159], the fate of these PTE-rich earthworms is described in a recent review by Shi et al. [150], which lists different methods of collection of earthworms (the use of chemical substances or electrical methods). As for plant biomass in phytoextraction, it is proposed collected enriched earthworms are burned in special landfills [82,160].

For Ni, it seems that earthworms increase the availability of the element in the soil and accumulate it in a short time, but this accumulation easily reaches a steady state without being dose-dependent [157,158,161,162].

The application of vermiremediation against Ni has been reported in many studies worldwide [8,158,163], but only in a few European countries [162,164].

An interesting study was conducted in Spain regarding the combination of micro-, vermi- and phytoremediation techniques [126]. The authors tested the effectiveness of the methods applied individually or in combination (bacteria + earthworms, bacteria + plants, plants + earthworms, plants + bacteria + earthworms) in a multipollutant-contaminated soil with a Ni concentration ranging from 28 to 128 mg kg^{-1}. The study showed that the cultivation of *M.sativa* as an individual treatment was ineffective in soil decontamination.

On the contrary, when phytoremediation was combined with vermiremediation or the three treatments were combined (plant, bacteria and earthworms), an increase in the root elongation of *C. sativus* and *L. sativa* was observed. This implies a soil health improvement.

4. Conclusions

Considering the high variability of the experimental designs (type of substrate, plant species and variety, formulation of applied Ni), defining a Ni toxicity threshold and unique values for the crops is challenging. Different thresholds are measured depending on the cereal species and type of growth substrate (soil or hydroponic). Regarding microorganisms, several studies report response mechanisms (enzymatic activities, increased biomass and respiration) when exposed to high concentrations of Ni in the soil, indicating their adaptability. On the contrary, resistance mechanisms in earthworms when there are high concentrations of Ni are not activated. In fact, adverse effects on their fertility are measured at even low Ni doses, being a more sensitive end-point, and then the death of adult individuals occurs when concentrations increase.

The existing literature and monitoring campaign on a European scale confirm that Ni contamination is not entirely under control, and the study of low-environmental-impact techniques to manage and detoxify soils is strongly requested at the community level.

Unfortunately, it is difficult to compare the effectiveness of bioremediation in terms of mass balance, which is often omitted in the results because it can only be calculated in experimental designs with a closed system. More frequently, authors refer to efficacy in a different manner, such as the mass of PTE extracted per surface unit or percentage of removed metal.

The comparison of the bioremediation works analysed shows that using techniques in combination with phytoremediation can be a winning strategy in combating Ni contamination. At the European level, there is a lack of knowledge especially regarding the use of vermiremediation together with plants. Therefore, it is proposed the promising field of aided phytoremediation is explored in view of a holistic approach in which organisms and microorganisms improve the extraction efficiency of plants; thus, the goal of remediation is achieved more easily.

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

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

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

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