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Review

The Response of the Associations of Grass and *Epichloë* Endophytes to the Increased Content of Heavy Metals in the Soil

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Abstract: The rapid development of civilization increases the area of land exposed to the accumulation of toxic compounds, including heavy metals, both in water and soil. Endophytic fungi associated with many species of grasses are related to the resistance of plants to biotic and abiotic stresses, which include heavy metals. This paper reviews different aspects of symbiotic interactions between grass species and fungal endophytes from the genera *Epichloë* with special attention paid to the elevated concentration of heavy metals in growing substrates. The evidence shows the high resistance variation of plant endophyte symbiosis on the heavy metals in soil outcome. The fungal endophytes confer high heavy metal tolerance, which is the key feature in its practical application with their host plants, i.e., grasses in phytoremediation.

Keywords: endophytic fungi; grasses; grass endophyte interaction; heavy metals; soil pollution

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1. Introduction

Technology development and changes in the modern world have a significant impact on the natural environment. Along with the increase of population, demands for energy, consumer goods, machines, devices, etc., are also growing, and this, in turn, affects the environment with pollution from chemicals, pesticides, oils, gasoline, and industrial waste. Contamination of soil with heavy metals and metalloids is currently considered, on a global scale, the main threat to agro-ecosystems, which is associated with food contamination [1].

This is why new solutions are being sought that will allow, in an environmentally friendly way, to eliminate or reduce soil pollution while restoring its productivity. The use of green plants to remove pollutants from the environment to render them harmless (phytoremediation) is the cheapest and the simplest way to use remediation technology [2,3]. However, the use of phytoremediation on a large scale faces some problems, such as slow growth and small biomass, phytotoxicity, and evapotranspiration of volatile contaminants [3,4]. The answer to these problems may come from microbe-assisted phytoremediation in general but with foliar fungal endophytes in particular [2]. The participation of endophytes in the phytoremediation process is based on a positive effect on the growth and development of plants, reducing the phytotoxicity of metals and affecting the translocation and accumulation of metals in the plant.

The total content of chemical pollutants (metals and organic compounds) in soils used for agriculture in Poland does not differ from the levels recorded in neighboring European countries [5]. However, in special cases, i.e., plant cultivation on highly industrialized regions, agriculture lands of high chemicals applications, or in areas close to highways, heavy metals such as lead (Pb), cadmium (Cd), zinc (Zn), nickel (Ni), and copper (Cu) are taken up by plants from the soils [6].

Some of these elements are necessary for the proper functioning of living organisms (e.g., Cu and Zn), while others such as Cd and Pb are the cause of many diseases and disorders. The health effects of systematic consumption of products containing small amounts of heavy metals may become apparent after years due to their cumulative capacity [7]. Heavy metals influence calcium metabolism thereby increasing bone fragility, disturb the functioning of the nervous system causing dementia, intellectual disability, visual disturbances, and coordination of movements, cause damage to the liver and kidneys, and also cause neoplastic changes [7,8].

In soils, heavy metals are commonly found as a result of release from parent rocks in soil-forming processes and during volcanic eruptions [6]. Their natural level does not pose a threat to ecosystems. For example, the average content of heavy metals in agricultural soils of Poland is small and amounts to ($mg \cdot kg^{-1}$), e.g., Cd-0.21, Cu-6.5, Ni-6.2, Pb-13.6, and Zn-32.4 [9]. Over 96% of arable soils are characterized by natural or slightly increased content of heavy metals, which allows us to classify them as high-quality soils, on which all plants can be grown excluding the purpose of growing vegetables for preserves and direct consumption for children [10]. It also follows that over 3% of soils are contaminated with heavy metals to the extent that all grown crops are excluded for consumption. Cationic forms of heavy metals retain in the surface layer of soil and their migration into the soil profile is relatively slow. This is one of the main causes of soil chemical degradation [11]. This type of threat to plant production occurs mainly in industrialized areas and in the vicinity of roads where, together with exhaust fumes, sewage, or industrial dust and heavy metals are collected by plants and introduced in the food chain.

This work aims to present different aspects of the interactions between fungal endophytes in the genus *Epichloë* and their grass host in the response to increased level of heavy metals in soil.

2. Heavy Metals

Due to the degree of danger, heavy metals are divided into the following groups [12]:

- very high level of potential threat, e.g., Cd, Hg, Pb, Cu, Zn,
- high level of potential threat, e.g., Mo, Mn, Fe,
- medium level of potential threat, e.g., Ni, Co,
- low level of potential threat, e.g., Sr, Zr.

Heavy metals that are the most common in contaminated sites are Pb, Cr, As, Zn, Cd, Cu, and Hg [13]. Trace metals such as: Cu, Fe, Zn, Mn, Co, and Se are essential for metabolism and while the other ones do not play an important role in the life cycle of organisms (Pb, Cd, Hg, and As). However, they can be damaging to organisms when the toxicity threshold is exceeded [14].

Heavy metals are often essential for the proper functioning of organisms as they perform biochemical and physiological functions. They are important components of several key enzymes and play an important role in various oxidation-reduction reactions [14]. For example, copper is an essential nutrient that is incorporated into a series of enzymes involved in hemoglobin formation, carbohydrate metabolism, catecholamine biosynthesis, and the cross-linking of hair collagen, elastin, and keratin [15]. Zinc plays an important role in the activity of pyrophosphates and in shaping DNA, and iron is part of various enzymes, regulates the functions of chloroplasts or is involved in oxidation processes [16]. Other heavy metals are enzyme activators and take part in photosynthesis (Mo), the formation of chlorophyll (Co) or show antioxidant activity (Se).

In biological systems, heavy metals influence cell organelles and components such as the cell membrane, mitochondria, lysosomes, endoplasmic reticulum, nuclei, and some enzymes involved in metabolism, detoxification, and damage repair [17].

2.1. Occurrence in the Soil

The development and extensive application of plant protection chemicals in agriculture, mineral and organic fertilizers, e.g., compost and sewage sludge produced from municipal waste, has led to the excessive accumulation of heavy metals in soils and then in plants. Intensive use of transportation routes emits large quantities of harmful substances into the environment. The main components of traffic pollution are nitrogen oxides, sulfur, polycyclic aromatic hydrocarbons, and heavy metals, such as Pb, Cr, and Cd [18]. A major source of lead is petrol and sources of cadmium include vehicle construction components, tires, or moisturizers. Chrome can come from corrosive parts of metal vehicles [19]. The highest concentrations of heavy metals are recorded in the so-called street dust, collected from the surface directly in the lane. These values can reach even 15,000 $mg \cdot kg^{-1}$ for lead, 196 $mg \cdot kg^{-1}$ for chromium, and 72 $mg \cdot kg^{-1}$ for cadmium [19,20]. This most often applies to the Far East or Africa, where the increase in the number of transport means occurs very quickly, which is not covered by the applicable regulations, which are changed more slowly.

Over 90% of the total content of cadmium, copper, zinc, and lead in soils and sediments of rivers and other water reservoirs comes from anthropogenic pollutants [21]. A particularly high content of heavy metals in the soil was found in the regions adjacent to the smelters of copper [22–28], of zinc smelters [29–33] and mines [34,35]. Thus, soil pollution of heavy metals has a local character and mainly concerns the areas near the smelters, the landfills of slag or mines where long-term emission of gaseous pollutants and metal-bearing dusts is the source of the enrichment of soils with metals around the emitters of pollutants.

The main carriers of heavy metals were previously active substances of pesticides (e.g., arsenic, copper, mercury, zinc, or lead compounds) [36], but they have since been replaced with different types of organic compounds. Concerning the content of heavy metals in the soil environment, commonly the term "background" or "baseline" are often used as synonymously [18,37]. The natural abundance of an element in rock, sediment, or soil with reference to a particular area is the most common definition of background, and will be used in this meaning in below article.

Higher values from the background are considered as evidence of environmental pollution. European countries have several approaches to define the risk level associated with different concentrations of heavy metal in soil [38,39]. For example, for lead content in soil, background values in Poland are 20–40 $mg \cdot kg^{-1}$, for cadmium-0.05–0.7 $mg \cdot kg^{-1}$ and for chromium-15–60 $mg \cdot kg^{-1}$ [19]. The guideline value standards of the Ministry of Environment of Finland [40] in this regard for lead-60 $mg \cdot kg^{-1}$, cadmium-1 $mg \cdot kg^{-1}$, and chromium-100 $mg \cdot kg^{-1}$. The Finnish standard values represent a good approximation of the mean values of different national systems in Europe [38] and India [41] and they have been applied in an international context for agricultural soils as well [42].

However, we must be aware that the few metals, e.g., Cu, Zn, Co, and Fe in trace amounts are essential for various metabolic activities of plants. However, excess of all kinds of metals (both, essential and non-essential) adversely affect plant metabolism [43]. The acceptable concentrations of heavy metals in the soil are different for individual countries and designated by relevant state offices. For example in Poland limit values for individual metals depend on the soil or soil quality standard and its current and planned function (Table 1).

Agricultural land on which increased heavy metal content has been found should be excluded from cultivation for consumption purposes. It should be proposed to introduce such crops that would not lead to soil degradation (e.g., due to erosion) and would allow the systematic binding of harmful elements.

Table 1. Permissible levels of substances (in mg·kg^{-1} of dry soil, soil surface layer: 0–0.2 m) causing risk for given land categories based on the Annex to the Regulation of the Minister of Environment from 1 September 2016).

Metal	Land Categories *					
	I	II			III	IV
		II-1	II-2	II-3		
Arsene (As)	**25**	10	20	50	50	100
Bar (Ba)	400	200	400	600	1000	1500
Chrome (Cr)	200	150	300	500	500	1000
Zinc (Zn)	500	300	500	1000	1000	2000
Cadmium (Cd)	2	2	3	5	10	15
Cobalt (Co)	50	20	30	50	100	200
Copper (Cu)	200	100	150	300	300	600
Molybdenum (Mo)	50	10	25	50	100	250
Nickel (Ni)	150	100	150	300	300	500
Lead (Pb)	200	100	250	500	500	600
Mercury (Hg)	0.5	2	4	5	10	30

* Land categories: I-residential and other built-up areas, developed agricultural land, recreational and leisure areas, areas with entertainment functions, such as: amusement parks and amusement parks, zoological and botanical gardens; water intakes and their protection zones. II-arable land and areas of family allotment gardens arranged on orchards, permanent meadows, pastures, land under ponds and ditches and areas of family allotment gardens arranged on land; subdivided into following 3 groups: II-a: very light and light mineral soils, granulometric fraction (GF) below 0.02 mm between 10- and 20%); II-b mineral soil from light to heavy, GF 0.02 mm between 10- and 35%, mineral-organic soil with 3.5–6.0% of C org.; II-c mineral and mineral-organic soils of GF 0.02 mm above 20%, pH higher than 5.5, more than 6.0% of C org. National parks and nature reserves. III-forests, wooded and shrubby land, wastelands, recreational and leisure areas of a historic nature (castle ruins, fortified settlements, burial mounds, natural monuments), unmanaged green areas, ecological lands. IV-industrial and urbanized areas, communication areas (roads, and railway areas).

2.2. The Importance of Heavy Metals for Plants, Animals, and Humans

Heavy metals are common in soils. Their natural level does not pose a threat to ecosystems. However, the concentration above the permissible norms already means a serious problem. Through plants, heavy metals enter the human body, where they can contribute to the occurrence of many diseases, including cancer [8,44–47].

The metals in the form of free ions are most easily absorbed by plants from the soil, while metals in the form of complexes can be mobilized by active substances secreted by plant roots and then taken up by plants [48,49]. The uptake of heavy metals by plants depends on the type of metal, their content in the soil, the forms in which they occur, and the plant species [50]. The heavy metal content of various plant organs is reduced in the following order: root, leaves, stem, flowers, and seeds. The toxic effect of heavy metals on most plants often occurs only in soil with high levels of contamination. On the other hand, humans are particularly sensitive to the presence of increased amounts of heavy metals, especially cadmium and lead (Table 2) [51]. Heavy metals in animal and humans primarily cause changes in protein synthesis and disturbances in ATP production, which may cause serious pathological changes, including cancer [12].

Heavy metals are systemic toxicants known to induce adverse health effects in humans. Exposure to toxic metals causes long-term health problems, mainly cardiovascular disease, developmental abnormalities, neurological and neurobehavioral disorders, diabetes, hearing loss, hematological and immune disorders, and various types of cancer. The severity of the adverse health effects is related to the type of heavy metal and its chemical form, and is also time and dose dependent [52]. The results of studies on the effects of heavy metals on human health indicate that simultaneous exposure to several heavy metals produced more severe effects even at lower dose levels than for one metal [53]. Nordberg et al. [54] showed that simultaneous human exposure to cadmium and inorganic arsenic caused more pronounced kidney damage than exposure to each of these elements separately.

Table 2. The most important heavy metals and their toxic effect on humans and animals (more details: Tchounwou et al. [52], Siemiński [55], Ware [56], Walker et al. [57], Kołacz and Brodak [58], Szymański [59], Zwoźniak [60], Brandys [61], Albińska et al. [62], Hong et al. [63], Davidson et al. [64]).

Metal	Effect	Accumulation
Cadmium	- disturbs protein metabolism - interferes with the transformation of vitamin B1 - affects metabolism of calcium and phosphorus compounds - increases bone fragility - carcinogenic, embryotoxic and teratogenic - anemia, hypertensive disease, changes in the circulatory system, pregnancy complications - changes in pulmonary function, increased risk of emphysema - Itai-itai disease	- liver, kidneys, pancreas, intestines, lungs
Lead	- poisoning of the digestive, nervous, blood, respiratory, kidney and liver systems - embryotoxic, carcinogenic and mutagenic effects - disorders of reproductive functions and calcium metabolism - impaired development, lower IQ, shortened attention span, hyperactivity, and mental deterioration	- skin and brain, - blood, where it reaches all parts of the body and accumulates in almost every tissue
Arsenic	- affects virtually all organ systems, including the cardiovascular, dermatological, nervous, gastrointestinal and respiratory system - a relationship with diabetes and reproductive organs	- absorbed into the body and metabolized, expelled mainly through the urine
Zinc	- carcinogenic - metabolic disorders (causes anemia), susceptibility to infections, immune and mental development disorders - disorders of the digestive and respiratory systems	- liver, kidneys
Chrome	- allergic factor - damages the digestive system - carcinogenic, embryotoxic and teratogenic	- liver, kidneys, lung tissue, blood, bone marrow and spleen
Mercury	- brain damage - cell damage - paralyzes sensory nerve endings, - visual, hearing, speech disorder and limb muscle paralysis - severe poisoning of the whole organism and even death.	- kidneys, liver, nervous system

In crop and wild species, there is considerable variability in the ability to accumulate different metal ions from contaminated soils. Within plant species, genotypes characterized by increased ability to accumulate a given metal can be distinguished. In general, plants can grow on land with the content of most heavy metals two or even three times exceeding the limit values. The growth and development of the plant stops in the stage of a few-leafed seedling, only after reaching the content of metals in the order of (in $mg \cdot kg^{-1}$) ca. 1400 Pb, 90 Cd, or over 4500 Zn [65]. These exceedances of heavy metals are, however, very rare and most often encountered in the vicinity of non-ferrous metallurgy plants.

Some plant species, called metallophytes, have the ability to grow and develop on metal-rich habitats. These plants possess the special mechanisms for coping with higher heavy metal concentrations in soil and are divided into three categories: excluders, indicators, and hyperaccumulators [6]. Hyper accumulative plants accumulate heavy metals in their tissues in an amount of metals, often exceeding their concentration in the soil [66].

During the search and selection crop genotypes for phytoremediation, the greatest number of candidates was found among *Brassicaceae*, *Poaceae*, and *Fabaceae* plants. In the grass family, the commonly grown maize is most useful in the phytoextraction process. Other species and varieties in this family come from the genera *Festuca*, *Agrostis,* and *Lolium*. Although they accumulate much less biomass, the cost of their cultivation is much lower and they have lower soil requirements. It seems a good idea to use *Agropyron repens* L., which accumulates the largest amounts of toxic substances in the soil in its roots [67].

So far, research has focused on endophytes, fungal symbionts of grasses, associated with hyperaccumulators because of their resistance to metals associated with long-term adaptation to high metal concentrations in plants [68]. Many such endophytes have been isolated from hyperaccumulating plants, such as *Alyssum bertolonii*, *Alnus firma*, *Brassica napus*, *Nicotiana tabacum*, *Thlaspi caerulescens*, *Thlaspi goesingense*, and *Solanum nigrum* [2].

Plant growth in contaminated soils is usually associated with the accumulation of these elements in their biomass, which in turn significantly limits its further use. The suitability of such biomass for energy conversion is related to the reduction of metal emissions in the energy production process and the management of leftovers after processing (e.g., ashes after combustion, post-concession deposit). The presence of heavy metals for plants can be critical especially in metabolic processes that can be inhibited (Table 3).

Table 3. A negative effect of some heavy metals on plants.

Metal	The Symptoms and Effect	References
Lead	- lower yielding, stunted leaves often with necrotic spots and shortened roots with less hair density, - photosynthesis, cell division, nitrogen metabolism and water management disorders,	- Kabata-Pendias and Mukhejee, 2007 [51]
Cadmium	- chlorotic and brown spots on leaf blades, reddening of veins, twisted leaves, shortening of roots, - disturbances in photosynthesis, transformations of nitrogen compounds, changes in the permeability of cell membranes, changes in DNA structure,	- Kabata-Pendias and Pendias, 2001 [11], Kabata-Pendias and Mukhejee, 2007 [51]
Copper	- growth inhibition, chlorosis, necrosis, and leaf depigmentation, - decrease in fluorescence, - decline in photosynthesis,	- Küpper et al., 1996 [69], Vidaković-Cifrek et al., 2015 [70]
Zinc	- both the deficiency and the excess of this element limit the growth and development of plants, - excess causes the inhibition of plant growth and reproduction, chlorotic and necrotic changes on leaves, reduction of photosynthesis, - the deficiency disturbs the metabolism of proteins, phosphates, carbohydrates and the synthesis of RNA and DNA.	- Kabata-Pendias and Mukhejee, 2007 [51]

However, the presence of some heavy metals is necessary for the course of some life processes, e.g., photosynthesis. Both deficiency and elevated concentration of heavy metals affects both light and dark reactions of photosynthesis directly or indirectly. For example, iron is necessary for the synthesis of chlorophyll, and manganese is required as a cofactor for several enzymes involved in photosynthesis, particularly decarboxylase and dehydrogenase enzymes [69].

3. The Symbiosis of Grass-*Epichloë*

3.1. Occurrence and Importance

Endophytic fungi are species of the genus *Epichloë*, which live their whole life in symbiosis in the tissues of their hosts plants. The most common grass endophytes include: *E. festucae* var. *lolii* co-living with *L. perenne*, *E. coenophiala* co-occurring with tall fescue (*F. arundinacea*), and *E. uncinata* occurring in meadow fescue (*F. pratensis*) [71]. Previously, the *Epichloë* genus contained only sexual forms (teleomorph), but now also includes asexual forms (anamorph), which were previously classified as *Neotyphodium* [72], and before that, *Acremonium* [73] (Table 4).

Table 4. Some *Epichloë* species and their select host.

Grass Species	*Epichloë* Species	Reference
Achnatherum inebrians	*E. gansuensis*	Li et al. 2004 [74]
Achnatherum sp.	*E.chisosum, E. inebrians, E. funkii*	Moon et al. 2004 [75]; Chen et al. 2015 [76]; Leuchtmann et al. 2014 [72]
Agropyron repens	*E. bromicola*	Lembicz et al., 2010 [77]
Agrostis spp.	*E. baconii, E. amarillans*	Cagnano et al., 2019 [78], Clay and Brown, 1997 [79], White, 1993 [80]
Ammophila breviligulata	*E. amarillans*	Drake et al. 2018 [81]
Anthoxanthum sp.	*E. typhina*	Cagnano et al., 2019 [78]
Brachyelyrtum sp.	*E. brahyelytrei*	Cagnano et al., 2019 [78]
Brachypodium sp.	*E. sylvatica, E. typhina*	Cagnano et al., 2019 [78]
Bromus aleuticus	*E. pampeana; E. tembladare*	Leuchtman et al. 2014 [72]
Bromus erectus	*Epichloë bromicola*	Leuchtmann and Schardl, 1998 [82]
Bromus laevipes	*E. cabralii, E.* spp.	Charlton et al. 2014 [83]
Bromus setifolius	*E. tembladerae*	Leuchtman et al. [72]
Bromus setifolius	*E.typhina* var. *aonikenhana*	McCargo et al. 2014 [84]
Bromus setifolius	*E. typhina*	Gentile et al. 2005 [85]
Calamagrostis sp. *Cinna arundinacea*	*E. stromatolonga* *E. schardlii*	Song et al. 2016 [86] Ghimire et al. 2011 [87]
Dactylis glomerata	*E. typhina*	Clay and Brown, 1997 [79]
Elymus canadensis	*E. canadensis*	Leuchtman et al. 2014 [72]
Elymus repens	*E. elymi, E. bromicola*	Cagnano et al., 2019 [78], Leuchtmann and Schardl, 1998 [82]
Festuca argentina	*E. tembladerae*	Cabral et al. 2007 [88]
Festuca brevipila	*E. festucae*	Clarke et al. 2006 [89]
Festuca arizonica	*E. huerfanum, E. tembladare*	Moon et al. 2004 [75]
Festuca arundinacea	*E. coenophialum*	Cagnano et al., 2019 [78]
Festuca gigantea	*E. festucae*	Leuchtmann et al., 1994 [90]
Festuca hieronymi	*E. tembladerae*	Cabral et al. 2007 [88]
Festuca longifolia	*E. festucae*	Niones and Takemoto 2014 [91]
Festuca pratensis	*E. uncinatum, E. siegelii*	Craven et al., 2001 [92], Gams et al., 1990 [93]
Festuca pulchella	*E. festucae*	Niones and Takemoto 2014 [91]
Festuca rubra	*E. festucae*	Clay and Brown, 1997 [79], Leuchtmann et al., 1994, [90]

Table 4. *Cont.*

Grass Species	*Epichloë* Species	Reference
Festuca sinensis	*E.* sp.	Zhou et al. 2015 [94]
Festuca sp.	*E. sinofestucae*	Song et al. 2016 [86]
Glyceria sp.	*E. glyceriae*	Schardl and Leuchtmann 1999 [95]
Holcus lunatus	*E. clarkii*	Clay and Brown, 1997 [79], Leuchtmann et al., 2014 [72]
Holcusmollis	*E. mollis*	Clay and Brown,1997 [79], Morgan- Jones and Gams, 1982 [73]
Hordelymus sp.	*E. disjuncta, E. danica, E. hordelymi, E. sylvatica* subsp. *pollinensisi*	Leuchtmann and Oberhofer, 2013 [96]; Leuchtmann et al., 2014 [72]
Hordeus comosum	*E. tembladerae; E. amarillans; E. typhina hybrids*	Iannone et al. 2015 [97]
Koeleria cristata	*E. festucae*	Niones and Takemoto 2014 [91]
Leymus chinensis	*E. bromicola*	Wang et al., 2016 [98]
Lolium canariense	*E. typhinum* var. *canariense*	Moon et al., 2000 [99]
Lolium multiflorum	*E. occultans*	Moon et al., 2000 [99]
Lolium perenne	*E. festucae* var. *lolii, E. typhina, E. lolii, E. hybrida*	Latch et al., 1984 [100], Morgan-Jones and Gams, 1982 [73]
Lolium rigidum	*E. occultans*	Leuchtmann et al., 2014 [72]
Melica ciliata	*E. guerinii*	Leuchtman et al. 2014 [72]
Melica decumbens	*E. melicicola*	Moon et al. 2004 [75]; Moon et al. 2002 [101]
Phleum alpinum	*E. tembladerae*	Leuchtman et al. 2014 [72]
Phleum alpinum	*E. cabralii*	McCargo et al. 2014 [84]
Phleum sp.	*E. typhina*	Cagnano et al., 2019 [78]
Poa alsodes	*E. alsodes*	Shymanovich et al. 2017 [102]
Poa secunda ssp. *junicolia*	*E. poae*	Tadych et al., 2012 [103]
Poa spp.	*E. typhina, E. typhina* subsp. *poae,*	Tadych et al., 2012 [103]
Poa spp.	*E. liyangensis*	Li et al. 2006 [104]
Roegneria sp.	*E. sinica, E yangzii*	Song et al. 2016 [86]; Li et al. 2006 [104]
Sphaenopholis sp.	*E. amarilians*	Cagnano et al., 2019 [78]

Many studies indicate the frequent presence of these fungi in grass plants, both cultivars and wild ecotypes [86,105–109], as well as in the seeds of commercially available varieties [110]. However, maintaining the viability of endophytes in seeds varies depending on the storage conditions, and at the same time it is possible to eliminate them from the seed when necessary, e.g., due to the production of harmful alkaloids. It should be mentioned that seed is not the only source of endophytes spreading, but they can migrate from plant to plant during mowing (i.e., horizontal transmission) [111].

3.2. *The Effects of Epichloë Endophytes on Plants*

The effects of this symbiosis can be both negative and positive. Endophytes confer benefits to grasses, such as mechanisms of tolerance to drought and regeneration of damage after long-term drought, as well as the more effective management of nitrogen and improved phosphorus assimilation [112]. In addition, grasses infected by endophytes are resistant to pests, nematodes, and certain diseases [105]. However, the presence of endophytes in plants can pose a threat to herbivores. It has been found that some of the alkaloids produced by these fungi are toxic and that their accumulation in feed can affect the health status of farm animals [113]. In cattle is fed with grass containing ergovaline or lolitrem B, there may be a decrease in milk production, a decrease in body weight, a decrease in activity (animals are not active, and avoid the sun). In extreme cases, severe diseases such as "ryegrass staggers syndrome" and "fescue toxicosis" can occur [105]. Previous studies have shown that endophytes induce drought tolerance mechanisms in grass plants; however, these effects appear to depend on the species, variety, and developmental phase of plants [107,114]. This favorable aspect of the presence of endophytic fungi in grasses has been best documented for tall fescue [115–119]. In other grass species, e.g., perennial ryegrass, the results of drought resistance studies are not so clear [120–123]. Gundel et al. [124] indicates that the results obtained in most of the studies on the impact of endophytes on drought stress are inconclusive, often they do not describe any experiments on drought or related information. They argue that there is an unreasonable extrapolation that attributes the overall positive effect of fungal endophytes on drought tolerance of host plants in general and in the long term, and that it has spread in the literature to predict the effects of endophytes on wild species in natural, arid ecosystems. It has also been found that endophytes affect the nitrogen management by both assimilatory and basic nitrogen metabolism and may be correlated with mechanisms of its utilization by endophyte mycelium and better phosphorus assimilation [112], as well as improve the viability of seedlings and tiller number [125]. In addition, grass inhabited by endophyte are resistant to pests, nematodes, and some diseases such as tall fescue with increased resistance to fungus *Rhizoctonia zeae* [105,123,126].

Pressure exerted on plants by disease factors is the most important abiotic stress that grasses are subjected to during the entire growing season. The most dangerous pathogens of perennial ryegrass in turf maintenance are pink snow mold (*Microdochium nivale*), powdery mildew (*Erysiphe graminis*), leaf spot (*Drechslera* spp. and *Bipolaris* spp.), stem rust (*Puccinia graminis*), brown patch (*Rhizoctonia* spp.), and crown rust (*Puccinia coronata*) [127]. The ability to control plant diseases by infecting more desirable *Epichloë* endophytes, which did not affect flowering, was investigated in vitro by White and Cole [128] and Siegel and Latch [129]. They showed that *Epichloë* isolates grown on agar plates inhibited the growth of colonies of many pathogenic fungi including *Rhizoctonia solani*, *R. zeae*, *Bipolaris sorokiniana*, and *Colletotrichum graminicola*. Siegel and Latch [129] also found that the antifungal activity of endophyte differed between strains. Often for many microorganisms there is a low correlation between the effect of antibiosis between fungal cultures under laboratory conditions and the effect on the disease in the field [130]. It was confirmed in field experiments conducted by Burpee and Bouton [131], which showed that the presence of endophytes did not protect tall fescue plants against *R. solani*, the main pathogen occurring in soil. Moreover, endophyte-infected tall fescue seedlings were not protected against *B. sorokiniana* infection [132] and pathogens frequently observed on lawns: *Magnaporthe poae* and *Pythium aphanidermatum* [133]. The presence of endophyte in tall fescue or perennial ryegrass does not appear to affect the occurrence or severity of root rot caused by *Fusarium oxysporum*, *F. equiseti*, and *Pythium acanthicum*, as reported by Hume et al. [134].

Studies on abiotic factors such as water deficit, mowing, shading, low nitrogen fertilization, or low soil pH have demonstrated varying plant responses, depending on the plant genome–endophyte genotype structure [135–137]. It was found that the stress tolerance conferred by some endophytes involves also habitat-specific fungal adaptations, and this concept has been confirmed with different fungal and plant species, and different environmental stresses [138]. Redman et al. [139] studied a plant species *Dichanthelium lanuginosum* from the geothermal soils colonized by one dominant endophyte *Curvularia protuberata*. They found that this fungus confers heat tolerance to the host plant, and neither the fungus nor the plant can survive separate from one another when exposed to heat stress more than 38 °C. A comparative study was done by Rodriguez et al. [140] which revealed that the ability to confer heat tolerance was specific to isolates from geothermal plants hence and the ability to confer heat tolerance is a habitat-adapted phenomenon.

In the variable European climatic and soil conditions, the beneficial effects of endophytes in grasses compared to plants without these fungi are not clear. There are reports of plants with an endophyte that have accumulated higher amounts of metals from the soil and even used them to produce more biomass [141–144]. Our preliminary research shows the possibility of increasing the uptake of cadmium, for example by 25% in plants infected by endophyte versus non-infected plants [145]. In an abiotic and biotic stress conditions, major losses in plant yield take place. Throughout the life cycle, plants can adjust their physiology and metabolism, including the synthesis of a range of defensive proteins to overcome the stress [146]. Further, endophytes that colonize plants can improve plant growth and health through better mineral absorption or increased resistance to biotic and abiotic stresses [147].

4. Response of the Endophyte—Grass Association on the Heavy Metals in the Soil

The results of many scientific studies indicated that heavy metals are toxic to plants, but many plants are metal tolerant and some of them are metal hyperaccumulators [148–150]. Irdis et al. [68] found that the plants with metal hyper-accumulative properties and associated with endophytes could be metal resistant, due to long-term adaptation to the high concentration of metals accumulated in the plants. The phytoremediation efficiency of contamination of heavy metals is mainly dependent on metal uptake and accumulation in shoots. It has been demonstrated that some heavy-metal resistant and plant-growth-promoting endophytes can improve uptake and accumulation in plants of nickel [151], cadmium [152], or lead [153].

Results of studies [152,154–157] confirm the key role of endophytes in the adaptation of plants to the conditions of a polluted environment by immobilizing contaminants in the soil, promoting plant growth, decreasing phytotoxicity, and improving plants' metal tolerance. Some endophytic bacteria can produce indole-3-acetic acid, which stimulates plant growth and enhances phytoremediation [158], or affects the plant's root elongation compared to plants in which these bacteria were not observed [159]. Other endophytes can produce cytokinins or gibberellins, which under both stress and non-stress conditions can stimulate plant growth and modify their morphology [160]. Many authors [158,161,162] also claim that some of the endophytes exhibit features that can alter contaminants' toxicity for example through the production of iron chelators, siderophores, organic acids, and various degrading enzymes.

Many studies on the use in the phytoremediation process have been made for endophytic bacteria [163–166] but also among the fungi, some species have been found that can be successfully used in this process [167–169]. Soleimani et al. [167] investigated the effect of two endophyte-infected grass species (*Festuca arundinacea* and *Festuca pratensis*) on the degradation of petroleum hydrocarbons. They found that endophyte-infected plants contained more root and shoot biomass and created higher levels of water-soluble phenols and dehydrogenase activity in the soil. Song et al. [86] show that *Epichloë* endophytes conferred stress tolerance to native grasses in China and played a significant role in the survival of some plants in high-stress environments, such as cadmium contaminated soils. *Achnatherum inebrians* [170,171] and *Elymus dahuricus* [172] had higher germination rates, more tillers, longer shoots and roots, and more biomass compared to endophyte-free plants in high concentration of cadmium ions. There was no significant difference between endophyte-infected and endophyte-free plants under low cadmium concentration, indicating that *Epichloë* infection was only beneficial to the growth and development of *A. inebrians* and *E. dahuricus* exposed to the high concentration of cadmium ions. Further, endophytic fungi inhabiting perennial grasses were the subject of research, the results of which showed that they can be successfully used in the process of phytoremediation [173,174].

4.1. Aluminum

Malinowski and Belesky [175] reported that *Epichloë coneophialum* infection had no effect on root and shoot development in tall fescue (*Festuca arundinacea*) grown in elevated concentration of Al. A higher concentration of Al (35%) and P (10%) has been observed in E+ plants, but no differences were found in roots. Elevated concentration of Al gave a variable effect on root and shoot dry weight (from positive to negative) in red fescue (*Festuca rubra*) and *Poa ampla* inhabited by *Epichloë festucae* and *Epichloë* sp., respectively [176]. However they claim that endophyte infection alone is not enough to confer aluminum tolerance in fine fescues, but in certain plant–fungus combinations, endophyte infection can contribute to enhanced aluminum tolerance.

4.2. Cadmium

Cadmium (Cd) is one of the most toxic environmental pollutants for plants [177]. Cadmium can interfere with numerous biochemical and physiological processes including photosynthesis, respiration, nitrogen and protein metabolism, and nutrient uptake [178].

Generally, the presence of endophytes had a positive effect on plants grown in elevated Cd concentrations. *Epichloë* endophyte presence in the seed of *Elymus dahuricus* promoted germination range and index, as compared to endophyte-free seed, in higher (i.e., from 100 to 300 μmol L^{-1}) Cd concentrations [172]. Higher values of shoot and root length and dry biomass were also observed in E+ plants of mentioned grass species.

Presence of endophytes in tall fescue (*Epichoë* sp.) and *Achnatherum inebrians* (*Epichloë gansuensee*) grown in the presence of an elevated concentration of Cd yielded also increased tiller number and higher biomass [167,179]. Plants inhabited by endophytes were able to accumulate more Cd ions in roots and shoots [167,179]. This is the result of improved transport of Cd ions from roots to shoots and resulting higher (2.4-fold) phytoextraction efficiency of E+ plants [179]. The effects of cadmium on germination, and antioxidative enzyme activity present within *Achnatherum inebrians*, were determined for plants infected and non-infected by *Neotyphodium gansuense* [171]. They found that under high Cd concentrations, endophyte-infected plants exhibited a higher germination rate and index, and higher values for shoot length, root length, and dry biomass, but there was no significant difference under low Cd concentrations. Endophyte infection was concluded to be beneficial to the germination and anti-oxidative mechanisms within *A. inebrians* under plant exposures to high cadmium concentrations. In the case of this plant increased anti-oxidative enzyme activity, H_2O_2 content and increased levels of chlorophyll *a* and *b*, as well as declined proline and malondialdehyde content, were observed in E+ plants vs. E-plants [172]. All these results indicate that the presence of endophyte may ameliorate the effect of cadmium toxicity on plant.

4.3. Copper

Copper (Cu) is an essential element to plant growth but toxic in higher concentrations. The presence of endophyte (*Epichloë coenophialum*) in tall fescue was associated with lower Cu concentrations in plants, which may further contribute to lowered Cu status in grazing animals, thus contributing to the etiology of fescue toxicosis [180]. However, plants were grown on pasture or pots without artificially increased Cu levels, which was one from many micronutrients in soil. Other results suggest that the effects of endophyte infection on Cu acquisition in perennial ryegrass (*Lolium perenne*) is quite different from those found in tall fescue [181]. This was explained that *Epichloë* endophytes present in perennial ryegrass often triggers sets of physiological, biochemical, and morphological responses in host plant different from those found for tall fescue infected with *N. coenophialum* [112]. To our knowledge, no research is available concerning the effect of artificially increased, high concentrations of Cu in growing medium on the grass performance and endophyte interaction. Most of recent research concerns natural Cu deficiency in forage where endophyte-induced decrease of Cu in stems and leaves was an additional negative effect for animals.

4.4. Nickel

Studies of Mirzahosseini et al. [182] have shown that endophytes also affect the presence of nickel in the soil. The obtained results indicated that some E+ genotypes were characterized by better growth and tolerance to this chemical element. However, the results were inconclusive, because otherwise endophyte free genotypes showed greater tolerance to Ni stress compared to E + plants. This revealed that the effect of endophyte infection in *Festuca* plants depends on the host genotype [182].

4.5. Zinc

Endophytes infecting tall fescue and perennial ryegrass have been found to improve chlorophyll functions (i.e., Fv/Fm) under high concentrations of Zn in growing medium [174]. Shoots of endophyte-infected tall fescue had 82% greater concentration of Zn than endophyte-free plants. The opposite relation was found for perennial ryegrass plants: endophyte-free plants exposed higher (29%) concentration of Zn in shoots than endophyte-infected. Other authors suggested no statistically significant differences between endophyte-free and endophyte-infected plants of tall fescue in Zn content in plant tissues [141].

The increase in zinc concentration induced some reduction in photosystem II (PSII) activity of perennial ryegrass plants but not enough to account for the total drop in the net photosynthetic rate [143]. In plants with endophyte, the quenching of the reaction center and antenna complexes rose simultaneously and at a constant rate, as zinc concentrations increased. The same authors examining the concentration of Zn in the leaves stated that it increases with the increase in Zn in the medium and the number of days after which this study was carried out. At the same time, they found that the concentration of Zn in the leaves was 24–32% lower in the presence of *N. lolii* [142]. Leaf dry weight was higher in the presence of endophyte, particularly at 5 mM Zn. It is a known fact that the effect of endophyte for its host is the production of a greater number of tillers [183]. It has been confirmed by Monnet et al. [144] for Zn stress from 1 to 10 mM. Zamani et al. [174] have shown that the number of plant tillers grown under different Zn concentrations was greater in endophyte-infected *Festuca* and *Lolium* compared to their endophyte-free plants. Roots and shoots dry weights in infected *Festuca* plants were also greater than non-infected. Endophyte infected *Festuca* and *Lolium* improved chlorophyll fluorescence as Fv/Fm at high concentrations of Zn, showing their better chlorophyll functions and significant reduction of Zn stress in endophyte-infected plants.

Some interactions of fungal endophytes on heavy metals are shown in Table 5.

Table 5. Grass–fungal endophyte association and their influence on heavy metals.

Host	Fungi	Effect	References
Achnatherum inebrians	*Epichloë gansuensis* (=*Neotyphodium gansuense*)	better germination rates and index in the high concentration of cadmium, increased tolerance to cadmium by improving the antioxidant defense system	Zhang et al. 2010a [170], Zhang et al. 2010b [171]
Elymus dahuricus	*Epichloë* spp. (=*Neotyphodium* spp.)	positively affected seed germination and seedling growth exposed to high Cd concentration	Zhang et al. 2012 [172]
Festuca arundinacea	*Epichloë coenophiala* (=*Neotyphodium coenophialum*)	increased exudation of phenolic-like compounds from roots improved Al tolerance	Malinowski and Belesky 1999 [175]
Festuca arundinacea, *F. pratensis*	*Epichloë* spp.	improved cadmium tolerance and bioaccumulation and showed better germination potential	Soleimani et al. 2010a [167]
Festuca arundinacea, *Lolium perenne*	*Epichloë* spp. (=*Neotyphodium* spp.)	accumulation and transport more Zn in aboveground parts under Zn-stress, a significant effect on the photochemical efficiency of photosynthesis	Zamani et al. 2015 [174]
Lolium arundinaceum (*Festuca arundinacea*)	*Epichloë coenophiala* (=*Neotyphodium coenophialum*)	enhanced Cd accumulation in plant and improved its transport from the root to the shoot	Ren et al. 2011 [184]
Lolium perenne	ryegrass endophytes	increase Cd transport and accumulation in shoots	Ren et a. 2006 [179]
Lolium perenne	*Epichloë* spp.	the increase of accumulation of cadmium and copper in aerial parts of the host plants, better plant growth and photosynthesis in the elevated concentration of Cu	Żurek et al. (in press) [145]
Lolium perenne	*Epichloë lolii* (=*Neotyphodium lolii*)	a limitation of the Zn concentration in the leaves	Monnet et al. 2001 [142]

Weyens et al. [3] claim that endophyte infection not only improves plant growth but also to reduces phytotoxicity of contaminants and increase metal translocation to the above-ground plant parts. Moreover, endophytes that can break down organic pollutants in soil and facilitate the uptake and accumulation of metals may be a way to improve the phytoremediation process [167,185]. However the process of optimal phytoremediation requires the presence of such associations, where endophyte isolates have both tolerance to the accumulation of pollutants and the production of phytohormones, which can also affect plant growth and metabolism [186].

5. Conclusions

Endophytes of the genus *Epichloë* are well-known associates of grasses and research shows their potential use in the phytoremediation of areas excluded from agricultural activities due to heavy metal contamination. There are many known associations of grasses with fungi of the *Epichloë* genus, which can be an excellent basis for research on their potential use in areas excluded from agricultural activities due to the increased content of heavy metals.

Grasses inhabited by endophytes are sometimes characterized by a much higher ability to accumulate heavy metals from the soil. The cultivation of grass-endophyte symbiotes in areas contaminated with heavy metals has a positive effect on the quality of the soil, significantly reducing its phytotoxicity. Monnet et al. [142] claim that the action of the endophyte remains unclear, but different mechanisms are possible. The fungus may accumulate heavy metals in mycelia or influencing on the mechanism to the limit metals concentration in the leaves through a restriction in its transport to the leaf tissues [187,188].

In some cases, plants inhabited by endophytes may have ecological and evolutionary advantage over uninfected, but there exist great variability of plant reactions, even concerning the same host species and the same fungus in uniform growth conditions. The choice of most effective grass–endophyte association for phytoremediation should be based on the earlier evaluation of the efficiency of the symbiosis. Summarizing, the fungal endophytes pass high metal tolerance capacities suggesting their future utilization in endophyte-assisted phytoremediation applications [189].

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