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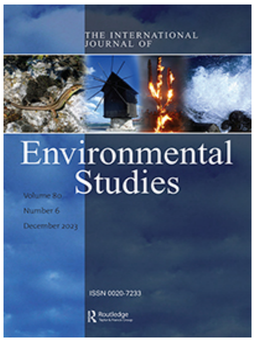

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Phytoremediation of heavy metal contamination by perennial legumes

Serhii Razanov[a,b], Oleksandr Tkachuk[b], Natalia Lebedieva[c], Yuri Shkatula[b], Mikhaylo Polishchuk[b], Maryna Melnyk[b], Bohdan Krektun[a] and Alla Razanova[a]

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ABSTRACT

Uptake of heavy metals from the soil by perennial legumes was measured over two- and four-year periods, and their accumulation measured in succeeding crops of winter wheat. Of the legumes tested: lucerne, red clover, sand sainfoin, white sweet clover, bird's foot trefoil, and eastern goatweed, sainfoin exhibits the greatest bioaccumulation over 2 years but all the studied legumes are effective. Moreover, we observe optimisation of the environment for the following crops, including the conversion of mobile forms of heavy metals in the soil into hard-to-reach forms that cannot migrate into the plant under simultaneous influence on the biological and mineral components.

KEYWORDS
Phytoremediation; heavy metals; soil; perennial legumes

Introduction

Heavy metals have an atomic mass of more than 40 and a density of more than 5 g/cm^3. Those exhibiting toxicity, in particular Zn, Cu, Pb and Cd, attract particular attention. In addition to their innate properties (valency, ionic radius, ability to form complex compounds), phytotoxicity also depends on soil and climatic conditions and specific characteristics of the plants themselves [1].

In soils, heavy metals assume different degrees of solubility and mobility: insoluble (included in soil minerals), exchangeable (in dynamic equilibrium with their ions in the soil solution), mobile and soluble forms; and transformation of one form into another can occur. The greatest hazard is presented by mobile forms that can accumulate to high concentrations that are toxic to both soil biota and plants [2]. Important factors affecting the mobility of heavy metals in soils are the content of clay minerals, pH, and redox potential [3].

The ecological impact of heavy metals on the soil-plant system depends on the forms of heavy metal compounds in soils and their transformation, the composition and properties of the soil, the physiology of plants and their stage of growth [4]. Plants accumulate heavy metals in different ways, usually through the root system but some elements, in particular cadmium, are also absorbed through the aerial parts. Lead and

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copper are less mobile than other heavy metals; mostly, they accumulate in the upper layer of the soil [5]. Lead is weakly accumulated and weakly retained; copper and cadmium are weakly accumulated and strongly retained; zinc is actively accumulated by plants and retained [6,7]. The strength of retention of heavy metals by soils depends on the pH, so control of soil reaction can be an effective way to mitigate the effects of heavy metals.

An alternative to chemical treatment is phytoremediation by plants that can accumulate heavy metals [8]. This research aims to substantiate bioremediation of heavy metals by perennial legumes – lucerne *(Medicago sativa)*, red clover *(Trifolium pratense)*, sand sainfoin (*Onobrychis arenaria*), white sweet clover (*Melilotus albus)*, bird's foot trefoil (*Lotus corniculatus)*, and eastern goatweed (*Galega orientalis)* – according to the changes in the content of mobile forms of lead, cadmium, copper and zinc in the soil, as well as the extent of uptake into the grain of winter wheat grown after the legume crop.

Materials and methods

Field research was conducted during 2013–2019 in the Forest-Steppe zone of Ukraine at the Agronomic Research Farm of the Vinnytsia National Agrarian University on *Luvic greyzemic phaeozem/Dark grey podzolised* medium loam, located at lat. 49°11′31″ N, long. 28°22′16″ E. The area of the accounting plot was 25 m^2 with four replicates.

Winter wheat was the predecessor of the perennial legumes. Before main tillage, lime was applied at the rate of 3.8t$CaCO_3$/ha and double superphosphate and potassium chloride fertilisers at the rate of $P_{45}K_{45}$. The main cultivation of the soil was ploughing to a depth of 28 cm. Perennial legumes were sown without cover in early spring. In the first-second leaf phase, imazethapyr herbicide (100 g/l water-soluble concentrate) was applied at the rate of 1.0 l/ha against annual grass and dicotyledonous weeds. The legume crops were mown at the beginning of the flowering phase and, depending on the intensity of growth, different species were mown more or less often in the growing season: white sweet clover once or twice, red clover and eastern goat weed 2–3 times; sand sainfoin 3 times, lucerne 4 times, bird's foot trefoil 5 times. All the harvested biomass was removed.

The selection of soil samples was carried out by the diagonal method, collecting samples from the plough layer, 0–25 cm. The agrochemical composition of the topsoil was characterised by: humus content – 2.0%; easily hydrolysed nitrogen (Kornfield) – 133 mg/kg; mobile phosphorus (Chirikov) – 390 mg/kg; mobile potassium (Chirikov) 64 mg/kg; hydrolytic acidity – 2.53meq/100 g; reaction – pH_{salt}5.0. The content of mobile heavy metals was determined by atomic absorption spectrophotometry following DSTU 4362:2004, DSTU 4770 (2, 3, 9):2007 [9]. The plant biomass of the perennial legumes was selected for laboratory analysis in proportion to the number of leaves, stems, and buds. It was dried to an air-dry state and ground. The determination of mobile forms of heavy metals in the soil, physico-chemical and agroecological indicators of soil fertility, the content of heavy metals in winter wheat grain and in the green mass of legumes was carried out in accredited laboratories of the Vinnytsia branch of the State Soil Protection of the Ministry of Agrarian Policy and Food of Ukraine, and the Agrochemical Laboratory of the Department of Ecology and Environmental Protection of the Vinnytsia National Agrarian University.

According to standard methods, the coefficient of accumulation of heavy metals in the grain of winter wheat was calculated as the ratio of the content of heavy metals in the grain to the content of their mobile forms in the soil. Correlation-regression analysis was applied using Excel, Sigma, Statistics program packages [10].

Results

Bioremediation depends on the capacity of the remedial plants to transform, redistribute or accumulate heavy metals in their biomass. This is manifested in a decrease in the content of mobile forms of heavy metals in the soil and a decrease in the coefficient of accumulation of heavy metals by the vegetative mass of the following crops. Our data (Table 1) establish that during a two-year spell of perennial legumes, the content of mobile forms of lead, cadmium, copper and zinc in the soil *decreased most significantly under sainfoin*. During a four-year spell: lead decreased most *after sand sainfoin*; cadmium and copper decreased significantly after *all studied perennial legumes*; zinc, most *after bird's foot trefoil*.

The lowest coefficient of accumulation of lead, cadmium, copper and zinc in winter wheat grain is observed *following lucerne*, and the highest *after bird's-foot trefoil* (Table 2).

We determined correlational dependences between the coefficient of accumulation of lead, copper, zinc and cadmium in winter wheat grain and the content of their mobile forms in the soil before growing winter wheat, depending on the legume predecessors (Table 3).

The ecological effect of reducing the coefficient of accumulation of heavy metals by the next crop in the rotation following the perennial legumes may be attributed to 1)

Table 1. Mobile heavy metals in the topsoil depending on the term of perennial legumes, M±m.

		Mobile forms of heavy metals, mg/kg			
Perennial legumes	Vegetation year	Pb	Cd	Cu	Zn
Before growing perennial legumes	×	5.9 ± 0.22	0.60 ± 0.08	6.8 ± 0.16	9.1 ± 0.08
Lucerne *(Medicago sativa)*	2	5.7 ± 0.19	0.05 ± 0.01	6.8 ± 0.14	9.1 ± 0.08
	4	3.6 ± 0.19	0.02 ± 0.01	0.1 ± 0.01	1.1 ± 0.14
Red clover *(Trifolium pratense)*	2	3.0 ± 0.22	0.03 ± 0.01	6.7 ± 0.22	6.6 ± 0.14
Sand sainfoin (*Onobrychis arenaria*)	2	1.5 ± 0.08	0.02 ± 0.01	6.0 ± 0.22	2.8 ± 0.08
	4	1.5 ± 0.07	0.01 ± 0	0.4 ± 0.08	2.4 ± 0.16
White sweet clover (*Melilotus albus)*	2	3.6 ± 0.08	0.60 ± 0.01	6.4 ± 0.08	4.3 ± 0.08
Bird's foot trefoil (*Lotus corniculatus*)	2	2.3 ± 0.14	0.50 ± 0.02	6.6 ± 0.08	4.0 ± 0.08
	4	3.4 ± 0.08	0.02 ± 0.01	0.2 ± 0	0.9 ± 0.08
Eastern goatweed (*Galega orientalis*)	2	5.9 ± 0.22	0.60 ± 0.01	6.5 ± 0.22	5.4 ± 0.08
	4	2.6 ± 0.08	0.01 ± 0	0.1 ± 0	1.1 ± 0.14

Table 2. Coefficient of accumulation of heavy metals in winter wheat depending on the precursors, M±m.

Precursors	Pb	Cd	Cu	Zn
Lucerne *(Medicago sativa)*	1.07 ± 0.02	0.73 ± 0.01	16.72 ± 0.05	23.66 ± 0.16
Red clover *(Trifolium pratense)*	1.18 ± 0.01	0.85 ± 0.01	19.40 ± 0.26	26.05 ± 0.43
Sand sainfoin *(Onobrychis arenaria)*	1.43 ± 0.04	1.70 ± 0.13	22.95 ± 0.12	34.63 ± 0.27
White sweet clover *(Melilotus albus)*	1.42 ± 0.02	1.14 ± 0.01	21.50 ± 0.09	28.17 ± 0.16
Bird's foot trefoil *(Lotus corniculatus)*	1.82 ± 0.03	1.82 ± 0.03	25.62 ± 0.17	34.71 ± 0.75
Eastern goatweed *(Galega orientalis)*	1.27 ± 0.02	1.00 ± 0.01	20.25 ± 0.27	31.16 ± 0.76

Table 3. Correlation-regression dependences between ecological and agrochemical soil parameters and the accumulation of heavy metals in winter wheat grain.

Correlation dependences	Correlation coefficient, r	Coefficient of determination, R^2	Connection level
Coefficient of accumulation of Pb in grain/content of mobile forms of Pb in soil	−0.617	0.38	average
Coefficient of accumulation of Cu in grain/content of mobile forms of Cu in the soil	−0.500	0.25	average
Coefficient of accumulation of Zn in grain/content of mobile forms of Zn in the soil	−0.880	0.25	strong
Coefficient of accumulation of Cd in grain/hydrolytic acidity of the soil	0.530	0.28	average
Coefficient of accumulation of Zn in grain/hydrolytic acidity of the soil	0.530	0.28	average
Coefficient of accumulation of Cd in grain/reaction of soil solution, pH	−0.520	0.26	average
Coefficient of accumulation of Zn in grain/reaction of soil solution, pH	−0.550	0.30	average
Pb content in grain/humus content	−0.825	0.68	strong
Cd content in grain/humus content	−0.823	0.68	strong
Cu content in grain/humus content	−0.803	0.64	strong
Pb content in grain/mobile P content in the soil	−0.630	0.39	average
Cu in grain/mobile P content in the soil	−0.600	0.36	average
Zn in grain/mobile P content in the soil	−0.520	0.27	average
Pb in grain/mobile K content in the soil	−0.690	0.48	strong
Cu in grain/mobile K content in the soil	−0.730	0.53	strong
Cu content in the soil/mobile P content in the soil	−0.795	0.63	strong
Zn content in the soil/mobile P content in the soil	−0.600	0.36	average
Pb content in the soil/humus content	−0.990	0.99	strong
Cd content in the soil/humus content	−0.900	0.82	strong
Pb content in the soil/mobile P content in the soil	−0.970	0.96	strong
Cd content in the soil/mobile P content in the soil	−0.950	0.73	strong
Cd content in the soil/mobile K content in the soil	−0.730	0.45	strong
Pb content in the soil/mobile K content in the soil	−0.940	0.88	strong
Cu content in the soil/coefficient of soil structure	−0.640	0.42	average
Zn content in the soil/coefficient of soil structure	−0.510	0.26	average
Cu content in the soil/soil moisture	−0.940	0.88	strong
Zn content in the soil/soil moisture	−0.67	0.45	strong

bioaccumulation of the most easily accessible forms of heavy metals in the tissues of the legume, which may be carried off-site; 2) redistribution of mobile forms of heavy metals in the soil or, even, their transformation to less-available forms with simultaneous transformation of the biological and mineral composition of the soil. Beyond these explanations, Samokhvalova et al. [11]. believe, and our previous studies [12] have confirmed, that crops respond to the optimisation of soil conditions manifest in the increase in humus, nutrients and moisture; normalisation of hydrolytic acidity and soil reaction; and improvement of accessible soil volume, soil structure and the vitality of the whole soil system – not least soil microorganisms that enhance the bioremediation of heavy metals. Antisari et al. [13] have established that the specified methods of bioremediation can function separately or in combination.

The effectiveness of bioaccumulation of heavy metals in the biomass of perennial legumes may be determined directly (Table 4). The greatest concentration of lead has been found *in sand sainfoin*; of cadmium and zinc, *in goatweed*; and of copper, *in red clover*.

The lowest content of mobile forms of lead in the soil is observed *after the cultivation of sainfoin* and the biomass of sainfoin is characterised by the highest

Table 4. Content of heavy metals in the green mass of perennial legumes, mg/kg, M±m.

Perennial legumes	Lead	Cadmium	Copper	Zinc
Lucerne *(Medicago sativa)*	0.80 ± 0.03	0.08 ± 0.01	5.2 ± 0.3	15.7 ± 0.3
Red clover *(Trifolium pratense)*	0.90 ± 0.03	0.07 ± 0.01	7.3 ± 0.1	20.0 ± 0.7
Sand sainfoin *(Onobrychis arenaria)*	0.95 ± 0.01	0.06 ± 0.01	7.0 ± 0.3	16.5 ± 0.6
White sweet clover *(Melilotus albus)*	0.60 ± 0.04	0.06 ± 0.01	4.7 ± 0.1	17.0 ± 0.4
Bird's foot trefoil *(Lotus corniculatus)*	0.70 ± 0.04	0.05 ± 0.01	5.1 ± 0.01	18.0 ± 0.3
Eastern goatweed *(Galega orientalis)*	0.80 ± 0.01	0.09 ± 0.01	7.2 ± 0.3	24.0 ± 0.6

content of lead among all perennial legumes studied. Other legumes do not show such dependencies so we may conclude with Burghardt *et al.* [14] and Arora *et al.* [15] that all kinds of perennial legumes operate by a combination of bioaccumulation and redistribution. Correlations observable in our data (Table 3) enable us to tease out some of these indirect actions and interactions. Thus, the significant decrease in the content of mobile heavy metals in the soil achieved by by *sowing sand sainfoin and lucerne* as precursors of winter wheat, and the coefficient of accumulation of heavy metals in its grain, are probably connected to the improved soil structure, drainage and water supply; and, very probably, related to enhanced microorganic activity promoted by the legumes. This would explain why the lowest content of mobile forms of heavy metals in the soil was found after growing *sand sainfoin*, which produced the greatest green mass in the second year of growth amongst the species trialled.

Discussion

Of course, statistical correlation does not demonstrate cause-and-effect. Kour *et al.* [16] and Massadeh *et al.* [17] suggest *phytostabilization* – accumulation of heavy metals by plants owing to their absorption and binding by plant roots or retention in the rhizosphere with their subsequent deposition by root secretions [18,19]. This is the most common pathway of phytomelioration – transformation of heavy metals from the soil by plants during metabolic processes after absorption or under the influence of root secretions before absorption. Such root exudates are characteristic of sand sainfoin and this may account for the low content of mobile forms of heavy metals in the soil where sand sainfoin was grown. Kocira *et al.* [20] and Jach *et al.* [21] suggest *rhizodegradation* of heavy metals in the soil by rhizosphere microorganisms which, promoted by root secretions, are able to transform the state of heavy metals. This may be a powerful factor in the case of the rhizopsphere of legumes [18,22]. It also follows that the greater the consumption of water from the soil by plants, the greater the removal of mobile forms of heavy metals from the soil.

Ugulu *et al.* [19] and Mazur *et al.* [23] note that an effective phytoremediant of heavy metal-contaminated soil should be characterised by resistance to the pollutant, the ability to accumulate high levels of heavy metals in its biomass, the presence of a branched root system capable of absorbing a large amount of water and nutrients from the soil, and high growth potential. Perennial legumes possess all these characteristics [24,25] in particular their contribution to soil nitrogen, soil organic matter and soil structure, *optimising conditions for following crops.*

Conclusions

- Perennial legumes are effective phytomeliorants on agricultural land contaminated with heavy metals. The greatest ecological effectiveness is observed during the first two years of cultivation.
- Reduction of the coefficient of accumulation of heavy metals during the winter wheat grain is the result of bioaccumulation of heavy metals and their redistribution in the soil during the cultivation of perennial legumes as predecessor crops. The highest ecological efficiency of phytoremediation is observed when winter wheat is grown after sand sainfoin.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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