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Assessment of Soil Contamination on the Content of Macro and Microelements and the Quality of Grass Pea Seeds (*Lathyrus sativus* L.)

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Abstract**—**Comparative research has been conducted to allow us to determine the content of macro and microelements in the vegetative and reproductive organs of grass pea and the quality of grass pea seeds, as well as to identify the possibility of grass pea growth on soils contaminated by heavy metals. The experiment was conducted on an agricultural field subjected to contamination from the Non-Ferrous-Metal Works (MFMW) near Plovdiv, Bulgaria. The experimental plots were situated at different distances of 0.5 km and 8 km, respectively, from the source of pollution. On reaching commercial ripeness the grass pea plants were gathered. The composition of the macro and microelements in plant materials (roots, stems, leaves, seeds), and the dry matter content, sugars, proteins, fats and ash contained in the grass pea seeds were determined. Translocation factors (TF) and bioaccumulation factor (BCF) were also determined. The quantitative measurements were carried out through inductively-coupled plasma (ICP). The grass pea plant can successfully be grown on soils contaminated by heavy metals. Soil pollution with heavy metals does not affect the quality of the grass pea seeds. The seeds of the grass pea contain significant amounts of nutrients (K, P, Cu, Fe Mn, Zn) and protein (23.18-29.54%). The distribution of heavy metals in the organs of the grass pea has a selective character, which reduces in the following order: leaves > roots > stems > seeds. BCF and TF values were greater than one suggesting efficient accumulation in the above ground parts of grass pea plant. Grass pea is a plant that is tolerant to heavy metals and can be referred to the accumulator plants. The results provide valuable information about the chemical and nutritional composition of the seeds of the grass pea grown on contaminated soils in Bulgaria. The high content of macro and microelements and the low concentrations of toxic elements in the grass pea grown in contaminated soil make it possible to use the seeds of the grass pea as animal feed.

Keywords**—**Grass pea, heavy metals, micro and macroelements, polluted soils, quality.

I. Introduction

GRASS pea (*Lathyrus sativus*) is an annual plant of the Fabaceae family, commonly used for feed or feed components. Grass pea is high in protein (26-32%), highly adaptable to extreme conditions, resistant to disease and low moisture requirement when grown.

Lathyrus sativus is cultivated worldwide as a resistant leguminous plant showing tolerance to various biotic and abiotic factors [1]-[3]. Grass pea is a plant of major economic importance in India, Bangladesh, Pakistan, Nepal and Ethiopia. It is also grown extensively in Central, Southern and Eastern Europe (from Germany to the south to Portugal and Spain and to the east to the Balkans and Russia) in Crete, Rhodes, Cyprus, in West Asia and North Africa (Syria, Lebanon, Palestine, Egypt, Iraq, Afghanistan, Morocco and Algeria). In Europe, the interest in grass pea is increasing because of the need for effective alternative use of the areas used primarily for growing grain crops [4].

It is known that species of the Fabaceae family can be used for phytoremediation of heavy metal contaminated soils, as well as they have the ability to capture and retain organic pollutants in the waxes that cover the leaves of the plants. In addition, plants of this family possess: (a) self-sufficiency for nitrogen; b) high tolerance to drought; and (c) the ability of certain plant species to survive on very poor soils. It was found that herbaceous species such as *Lupinus* sp. and *Vicia* sp., *Sesbania exaltata* [5], *L. albus*, *L. luteus, L. angustifolius* and *L. hispanicus* absorb Mn, Pb, Cr(III), Cr(VI) and CH_3Hg [6].

Medicago sativa has been found to be tolerant to heavy metals [7], [8], *Cytisus striatus* to arsenic [9] and *Melilotus indica* to selenium [10].

The ability of the grass pea to extract lead, cadmium, copper and zinc has also been studied [11], [12]. Nagati [3] found that the above-ground mass and roots of *Lathyrus sativus* absorb cadmium and copper exponentially, indicating that the plant has the capacity of resistance to heavy metals. According to [13], lead is retained by the root system and grass pea has the potential for effective phytoextraction of lead. According to [11] grass pea has the capacity to accumulate lead similar to the root accumulators of *Brassicae juncea* and *Thlaspi rotundifolium*. In a hydroponic experiment conducted by Brunet [11] (0.5 mM of Pb $(NO_3)_2$ for 96 h), grass pea absorbed 153 mg of Pb/g DW in its root system, while the roots of the *Brassica juncea* plants absorbed 138 mg of Pb/g DW after brief exposure to lead (three days with 500 μM of Pb $(NO_3)_2$) [14], and the roots of *Thlaspi rotundifolium* absorbed 28.7 mg of Pb/g DW at longer exposure (12 days) with 20 μM of Pb $(NO_3)_2$ [15].

References [11], [14] and [15] show that grass peas have the potential to accumulate heavy metals and ability to be used for phytoremediation, but these studies are for hydroponically grown plants treated with $Pb(NO_3)_2$ solutions. There are no studies related to cultivation of grass pea on contaminated soil under field conditions.

The purpose of this article is to conduct a comparative study to enable us to determine the amounts and centers of

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accumulation of heavy metals, micro and macro elements in the vegetative and reproductive organs of grass pea, the quality of the seeds, and the possibilities of using the plant for phytoremediation on heavy metal contaminated soils.

II. Material and Methods

The study included grass pea grown on areas located at different distances from the source of contamination KCM-Plovdiv (0.5 km and 15 km).

Grass pea is grown according to conventional technology. 5 plants of each of the areas were used for the analysis. Upon reaching the stage of technological maturity, grass pea was harvested and the content of heavy metals, micro and macro elements in different parts - roots, stems, leaves, pods and seeds was determined.

Pseudo-total content of metals in soils was determined in accordance with [16]. The available (mobile) heavy metals contents were extracted by a solution of DTPA (1 M NH_4HCO_3 and 0.005 M DTPA, pH 7.8). The contents of heavy metals, micro and macro elements in the plant material (roots, stems, leaves, pods and seeds) were determined by the method of the microwave mineralization. The quantitative measures were carried out by ICP method (Jobin Yvon Emission - JY 38 S, France). Digestion and analytical efficiency of ICP was validated using a standard reference material of apple leaves (SRM 1515, National Institute of Standards and Technology, NIST).

The ash content, sugars, proteins and fats were determined according to standard methods BDS 11374 [17] and BDS 7169 [18].

III. Results and Discussion

A. Soils

In order to clarify the degree of soil contamination with heavy metals and to locate them in the vegetative organs of grass pea, soil samples were taken from areas at different distances from the NFMW (0.5 and 15 km). The physical and chemical properties of the soil samples are presented in Table I. The soils are characterized by a neutral to slightly alkaline reaction, with a medium organic carbon content and a medium nutrient reserve (N, P, K). The total content of Zn, Pb and Cd is high and exceeds the maximum permissible concentrations (MPC) in soils within 0.5 km of the NFMW (S1) (Table I).

TABLE I
Soil Properties for Soil Samples from the Non-Ferrous Metals Plant (KCM) Near Plovdiv

	pH	EC, dS/m	C,%	N, %	P, mg/kg	K, mg/kg	Pb, mg/kg	Zn, mg/kg	Cd, mg/kg
Soil 1 (S1)	7,4	0,15	2,2	0,34	625,6	6960	2509,1	2423,9	64,3
Soil 2 (S2)	7,5	0,15	1,5	0,12	387,3	6780	49,4	172,7	1

MPC (pH 6.0-7.4) – Pb -100 mg/kg, Cd-2.0 mg.kg, Zn-320 mg/kg; MPC (pH >7.4) – Pb – 100 mg/kg, Cd – 3.0 mg/kg, Zn -400 mg/kg

The results presented in Tables I and II show that in soil samples S1 (taken from the area within 0.5 km of the NFMW), the reported Pb values reach 2509.1 mg/kg and exceed the MPCs approved for Bulgaria. In the area located at a distance of 15 km, the Pb content is significantly reduced to 49.4 mg/kg. Similar results were obtained for Cd and Zn. Results for DTPA derived mobile metal forms show that the Cd mobile forms in contaminated soils are the most significant part of its total content, reaching 57.2%, followed by Pb with 33.8% and Zn with 9.8%. In uncontaminated soils, again the Cd mobile forms are the largest part of its total content, followed by Zn and Pb.

TABLE II
DTPA – Available Forms of Pb, Zn and Cd (mg/kg) in Soils

Distance from KCM, km	Pb		Zn		Cd	
	mg/kg x±sd	%*	mg/kg x±sd	%*	mg/kg x±sd	%*
0.5 S1	849.1± 6.0	33.8	236.8± 1.3	9.8	36.8± 0.5	57.2
15 S2	21.5±0.5	43.5	38.9± 0.6	22.5	0.7±0.1	70

x- average value(mg/kg) from 5 repetitions; sd - mean standard deviation, *%DTPA -available/ total content.

B. Content of Heavy Metals, Micro and Macro Elements in Grass Pea

Samples of roots, stems, leaves, seeds and pods were analyzed to clarify the issues of absorption, accumulation and distribution of heavy metals in the vegetative and reproductive organs of grass pea.

Fig. 1 shows the results obtained for the content of heavy metals, micro and macro elements in the vegetative and reproductive organs of grass pea. The results obtained show that a considerable part of the heavy metals accumulate in the roots of grass pea (Fig. 1). With the removal of the source of pollution, there is a clear tendency for reduction of the content of heavy metals in the vegetative and reproductive organs of grass pea.

Pb content in the roots of grass pea grown at a distance of 0.5 km from the NFMW reaches 842.9 mg/kg, Zn - 680.7 mg/kg, Cd - 52.4 mg/kg. Cu content reaches 96.6 mg/kg, Fe - up to 3251.8 mg/kg and Mn - up to 122.2 mg/kg.

The root of grass pea is known to be spindle-shaped with highly developed root branches, the major part of which is concentrated in the plow layer to a depth of 35 cm. This is probably why grass pea accumulates heavy metals in the roots.

For grass pea grown in the area at a distance of 15 km from the NFMW, the reported values of heavy metals, macro and micro elements (except for K, P and magnesium) in the roots are significantly lower.

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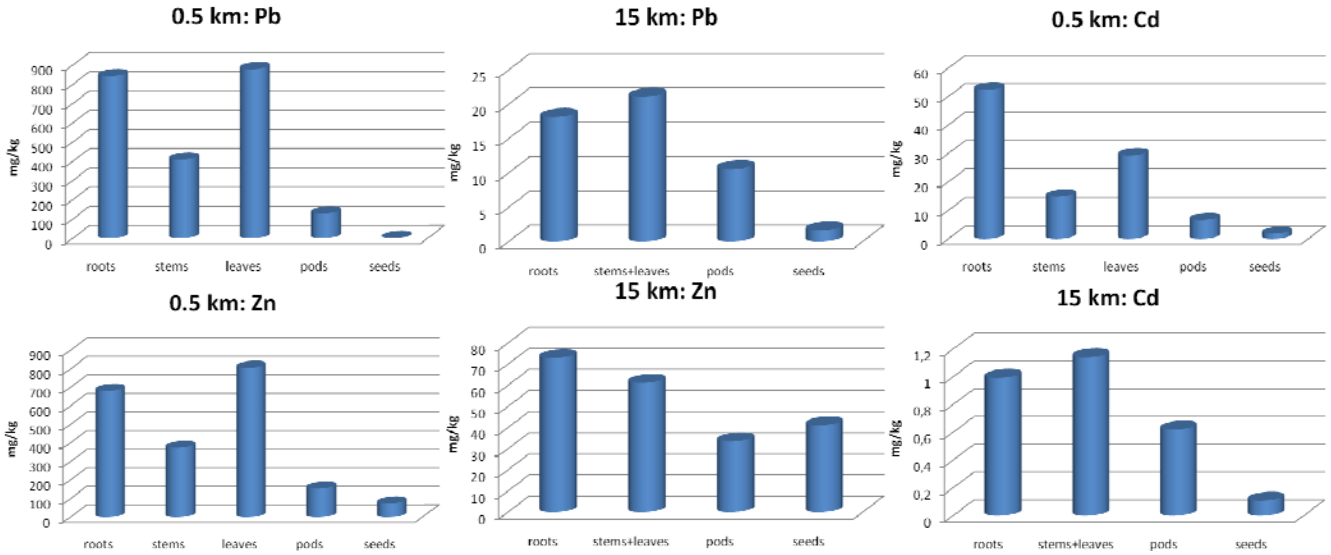


Fig. 1 (a) Content of heavy metals (mg/kg) in grass pea

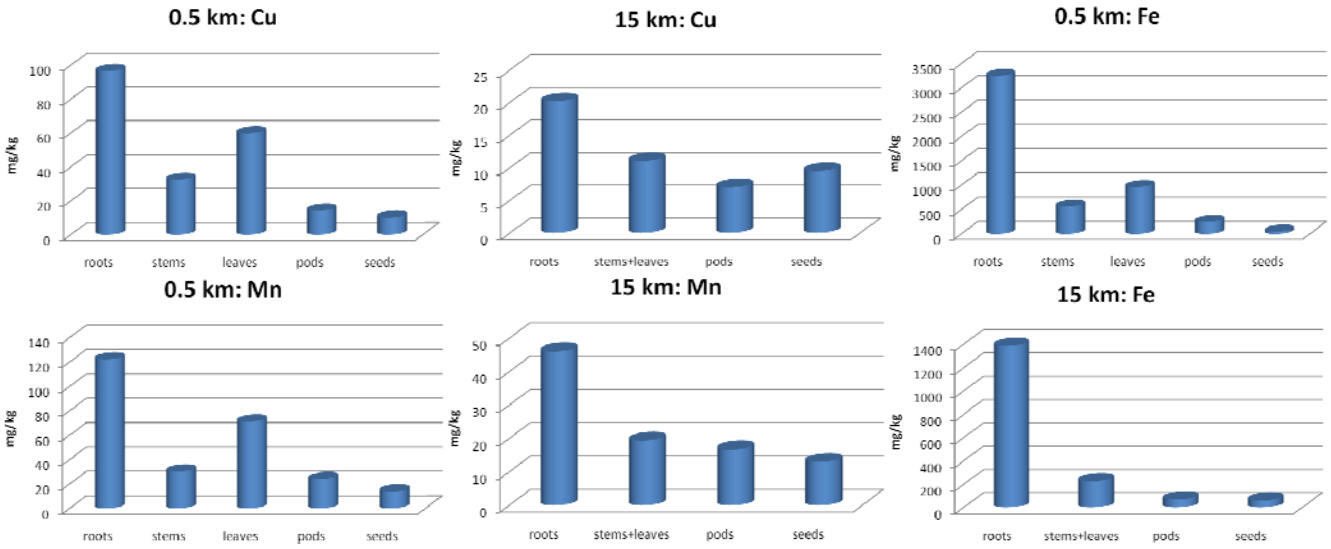
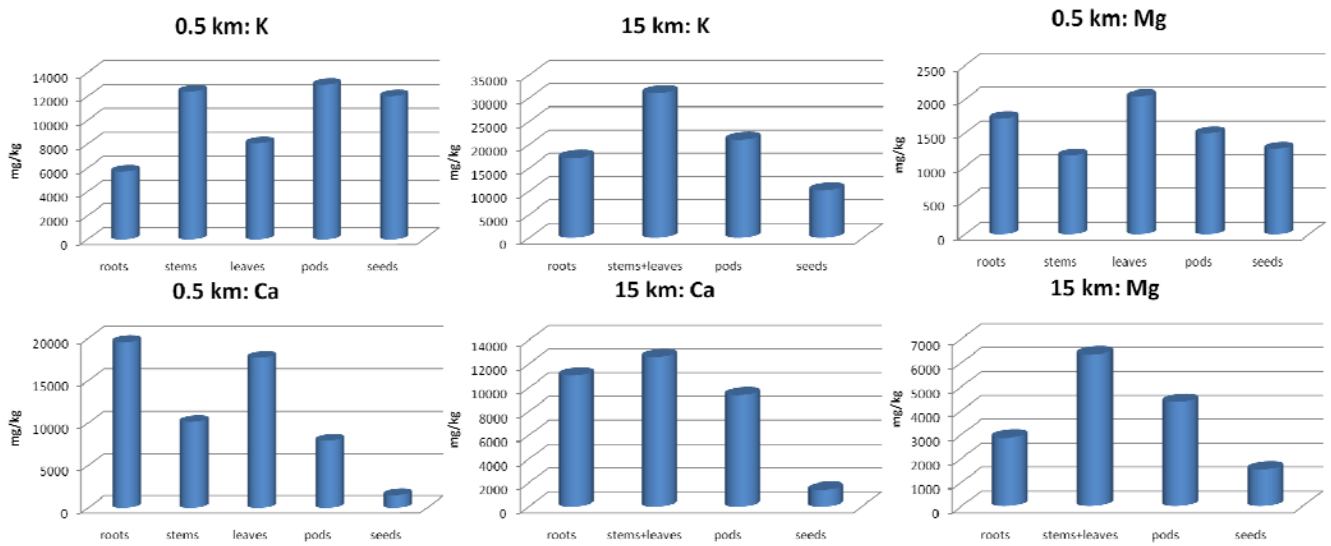


Fig. 1 (b) Content of microelements (mg/kg) in grass pea



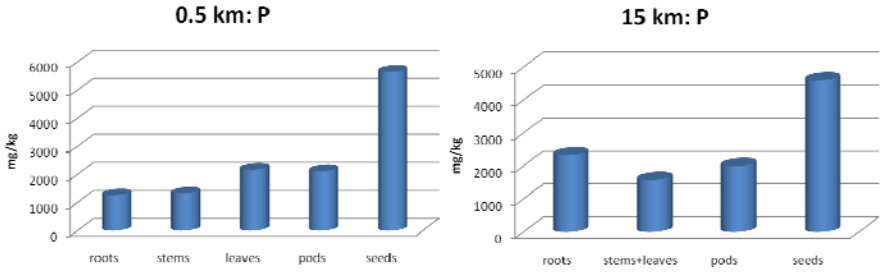


Fig. 1 (c) Content of macroelements (mg/kg) in grass pea

According to [13], lead is retained by the root system and grass pea has the potential for effective phytoextraction of lead. Brunet [11] found that an average of 153.0 mg/g hydroponically grown and treated with 0.5 mM $Pb(NO_3)_2$ was accumulated in the root of grass pea for 96 hours. The authors also found up to 6 times less absorption of calcium, 2 times less zinc and 3.5 times less copper and less influence of lead on Zn, Cu, K content compared to plants not treated with lead. Lower levels of calcium in lead-treated plants have also been observed in other species, such as rye, maize, tomatoes and mustard [19], which may result from the inhibition of Ca transporters from toxic lead ions [20]-[24] and/or substitution of Ca ions with Pb ions due to their high affinity for Ca binding sites [25]-[28]. The higher amount of Pb ions is possible to compete with Ca ions [20], [24] which are preferentially transported inside and outside the roots of grass pea. Despite the mineral imbalance due to the higher absorption of lead in the roots, grass pea plants have no visible symptoms and can survive in poor quality soils [1].

The movement of heavy metals and their accumulation in the vegetative organs of grass pea is specific for the individual elements. Significant accumulation of lead in the leaves and stems of grass pea was found. The content of this element reaches 408.9 mg/kg in the stems and 878.9 mg/kg in the leaves and is significantly higher than the toxic values for animals - 30 mg/kg [29]. However, the results we have obtained show a significant ability of grass pea to accumulate lead in leaves. This is probably due to the anatomical and morphological features of the crop.

The content of Cd in the stems and leaves of grass pea reaches 14.8 mg/kg and 29.3 mg/kg respectively, values considered toxic for plants (5.0 mg/kg [30]). The results we have obtained show the significant ability of grass pea to accumulate Cd in its above ground mass.

Zn content in the stems and leaves of grass pea reaches 373.3 mg/kg and 806.4 mg/kg, respectively, and these values are higher than the critical values for plants - 100 - 400 mg/kg.

For grass pea grown in the area at a distance of 15 km from the NFMW, the reported values for heavy metals in the stems and leaves are significantly lower. The increased concentrations of heavy metals in the leaves of grass pea grown at a distance of 0.5 km from the NFMW are largely due to aerosol contamination.

The amount of Cu in the stems and leaves of grass pea grown at a distance of 0.5 km from the NFMW reaches 32.6 mg/kg and 59.9 mg/kg, Fe - up to 561.1 and 964.9 mg/kg, Mn - up to 30.2 and 71.3 mg/kg. For grass pea grown at a distance of 15 km from the NFMW, the values for Cu in the above ground mass reach 11.1 mg/kg, Fe - up to 227.0 mg/kg, Mn - up to 19.4 mg/kg.

Ca content in the stems and leaves of grass pea grown at a distance of 0.5 km from the NFMW reaches 10146.2 mg/kg and 17705.7 mg/kg, Mg - up to 1168.9 and 2046.7 mg/kg, K - up to 12422.5 and 8074.7 mg/kg and P - up to 1326.8 and 2168 mg/kg. For grass pea grown at a distance of 15 km from the NFMW, the values for Ca in the above ground mass reach 1576.1 mg/kg, Mg - up to 6344.4 mg/kg, K - up to 31026.8 mg/kg and P - up to 1576.1 mg/kg. With the removal of the source of contamination, there is a clear tendency for increasing the content of K and Mg in the leaves of grass pea.

According to Madejon [31], no significant difference has been observed between the content of macro elements in the cultivation of plants on contaminated and uncontaminated soils. However, according to [30], heavy metals can lead to a change in the absorption of nutrients from plants. The results obtained confirm the less absorption of K and Mg nutrients from the leaves of grass pea when grown on contaminated soils.

Based on the content of the elements in the plants and the calculated factors (BF, TF), comparisons can be made between the behaviour of the plants when grown in different places around the world. In order to give a definite answer to the question what is the ability of grass pea to extract heavy metals from the soil and to assess the potential of grass pea for phytoextraction, a TF was calculated (TF = Heavy metal content in the above ground mass/ Heavy metal content in the roots) [32] and BCF (BCF = Heavy metal content in the above ground mass/Heavy metal content in the soil). The results we have obtained indicate that the TF for Pb is greater than 1, regardless of the degree of soil contamination (Table III).

With respect to Pb, BCF for grass pea grown on contaminated soil (0.5 km from the NFMW) ranges from 0.99 for BCF roots to 1.52 for BCF shoots, Zn from 2.88 to 4.98 and cadmium from 1.42 to 1.2. BCF roots ratios are close to 1 when growing grass pea on heavy metal contaminated soils (S1). The low bioconcentration factors (BCF) make the plants unsuitable for phytoextraction of lead.

TABLE III
UPTAKE OF HEAVY METALS FROM GRASS PEA

Element	TF		BACroots		BACshoots	
	0.3 km	1 km	0.3 km	1 km	0.3 km	1 km
Pb	1.5	1.2	0.99	8.6	1.52	10.0
Cd	0.84	1.2	1.42	1.48	1.20	1.70
Zn	1.73	0.84	2.88	30.4	4.98	25.5

$BACroots=[Metal]_{roots}/[Available\ metal]_{soil}$, $TF=[Metal]_{shoots}/[Metal]_{roots}$), $BACshoots=[Metal]_{shoots}/[Available\ metal]_{roots}$

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The content of heavy metals, micro and macro elements, in the pods of grass pea is lower than that of the above ground mass of grass pea. The content of lead, cadmium, manganese, iron, potassium, calcium and magnesium in the pods is higher than the seeds of grass pea grown at a distance of 0.5 km from the NFMW, the quantities of copper and zinc are comparable, while in the case of phosphorus the opposite tendency is observed - 5152.7 mg/kg is accumulated in the seeds, while -2062.6 mg/kg in the fruiting shell.

The results obtained strongly indicate that the fruit shell plays the role of a selective filter in the path of heavy metals to seeds and depends primarily on the element under consideration.

In the seeds, macroelements (Ca, K, Mg, P) predominate, followed by Fe and Zn. The content of Cu and Mn is significantly lower. The seeds also contain the toxic metals lead and cadmium.

The content of heavy metals in the seeds is significantly lower compared to the root system and the aboveground mass of the plants. Their accumulation in the seeds of grass pea is probably by the conduction system. The content of Pb and Zn in the seeds of grass pea grown at a distance of 0.5 km from the NFMW reaches 1.7 mg/ kg and 74.4 mg/kg, respectively, and does not reach the critical values of 30 mg/kg Pb and 300 mg/kg Zn [33]. Cd is accumulated in seeds (0.4 mg/kg) in amounts lower the recommended maximum levels tolerated by animals (0.5 mg/kg Cd) [29] and the recommended nutritional values (1 mg/kg) [33]. The content of Cu in the seeds of grass pea reaches 10.2 mg/kg and is below the critical values of 25 mg/kg Cu [33].

Legume seeds are known to contain important nutrients like Mn, Cu, P, Zn, Mg, Ca and K [34], [35]. The results we have obtained show that Ca, K and P are the predominant elements in the seeds of grass pea, followed by Mg. The content of Ca reaches 1481.7 mg/kg, K - up to 12029.0 mg/kg, P - up to 6253.6 mg/kg, and Mg up to - 1273.4 mg/kg.

The content of Pb in the seeds of grass pea when grown at a distance of 15 km from the NFMW reaches 1.6 mg/kg, Zn - up to 41.1 mg/kg, Cd - up to 0.05 mg/kg, Cu - up to 9.5 mg/kg, Fe up to 59.3 mg/kg, Mn - up to 13.0 mg/kg, K - up to 10157.4 mg/kg, Ca - up to 1403.2 mg/kg, Mg - up to 1531.2 mg/kg and P - up to 5152.7 mg/kg.

It seems that the seeds of grass pea, when grown at a distance of 15 km from the NFMW, contain more magnesium, there is no significant difference in the content of phosphorus and calcium, and the content of potassium is lower.

Comparison was made of the data of the content of elements in the seeds of grass pea with the results published by other authors. According to [36], the seeds of grass pea contain 8.75-9.23% K, 1.14-1.24% Mg, 0.97-1.03% mg/kg Ca, 4.68-5.13% P, 6.98-7.95 mg/kg Cu, 43.52 mg/kg Fe, 29.57-31.24 mg/kg Zn, 13.29-15.31 mg/kg Mn, seeds of grass pea can be a good source of minerals due to the high content of calcium and magnesium in them. Cabrera [37] and Özcan [38] report that the levels of macro elements in plants of the Fabaceae family are within the following range: 7426-16.558 mg K/kg; 269.75-445.81 mg Na/kg; 2719-5556 mg P/kg; 1309-2781 mg Ca/kg; and 2083-2900 mg Mg/kg. The micro element levels were as follows: 2.1-22.0 mg Cu/kg; 22.5-152.80 mg Fe/kg; 31-109 mg Zn/ kg; and 17.53-2277.16 mg Mn/kg, which is consistent with the values obtained in this study.

The values for manganese and magnesium do not differ significantly from the results of Sadowska [39]. The contents of zinc, copper, potassium and calcium are slightly lower, while higher values are obtained for the other elements studied. The variation between the results may be due to growing conditions, genetic factors, varietal characteristics and other factors. Micro elements are not only an important component in the diet of animals and humans, but also in plant nutrition. Varieties and crops with higher micro element content are more resistant and require less irrigation [36].

The main factor limiting the absorption of nutrients in the consumption of leguminous seeds is the presence of phytic acid, which reduces the bioavailability of the elements by chelation of the cations of zinc, copper, cobalt, iron, calcium, potassium and magnesium and the formation of non-digestible phytates [40]. Phytic acid content is below 1% in grass pea and cultivated lupines, up to 1.6% in soybeans and up to 2% in bean seeds [41] and has the potential to influence nutrient absorption. Seed treatment such as soaking, sprouting, fermenting, grinding or cooking effectively inactivates or reduces the effects of anti-nutrient components such as tannins, trypsin inhibitors and phytic acid, which limit the absorption of nutrients [42].

The distribution of heavy metals in the organs of grass pea is selective, as the following order can be observed: with lead, zinc and copper – leaves > roots > stems > pods > seeds, with cadmium, iron and manganese – roots > leaves > stems > pods > seeds, with P – leaves > pods > stems > roots > seeds, and with K – pods > stems > leaves > roots > seeds (Fig. 2).

Significant differences are observed in the accumulation of heavy metals in the vegetative organs of grass pea. The accumulation and distribution of metals in plant tissue are important aspects for assessing the role of plants in the rehabilitation of heavy metal contaminated sites [43]. The success of the phytoremediation process depends on the yield of the plants and the plant, which has the ability to absorb a significant amount of heavy metals in its above ground mass [44].

Grass pea accumulates heavy metals through its root system, but a small portion of the heavy metals is retained by the roots and most of them move and accumulate in the aboveground parts (stems, leaves). Our results strongly prove that grass pea is a crop that is tolerant to heavy metals and can be grown on contaminated soils. It can be attributed to the low accumulator crops, and therefore it cannot be used for effective phytoextraction of soils contaminated with Pb, Cd and Zn.

C. Nutritional Composition of Grass Pea Seeds

Fig. 3 shows data on the content of dry matter, sugars, proteins, fats and ashes in seeds of grass pea.

No differences were observed in the dry matter content in the seeds grown at a distance of 0.5 and 1 km from the NFMW (ranged from 90.83% (point 2) to 90.89% (point 1), which is consistent with the results obtained by Urga [45] and

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Heuze [46]. According to the authors, the dry matter content in the seeds varies from 89.5 to 92.5%.

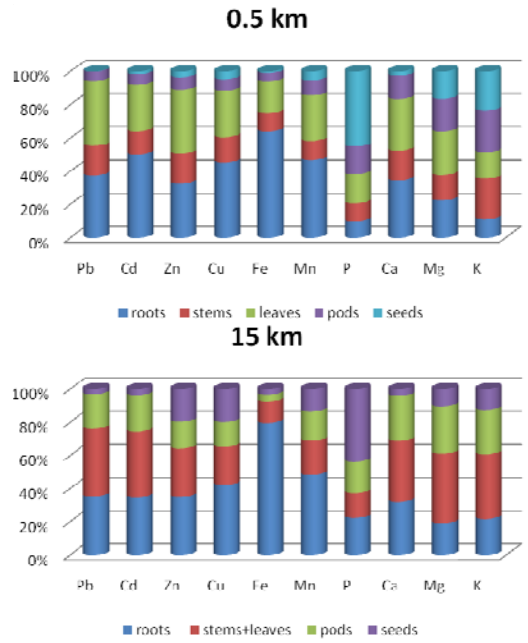

Fig. 2 Distribution of heavy metals, micro and macroelements (mg/kg) in grass pea

No significant differences were observed in the fat content in the seeds of grass pea grown on contaminated and uncontaminated soils. The amount of sugars in the seeds of grass pea varies from 5.78% (point 1) to 5.98% (point 2). Similar results were obtained by Heuze [46], who found an average sugar content of m6.3% (ranged from 4.7 to 8.9%). Urga [45] (average sugar content of 5.4% (ranged from 5.1 to 6.2%)).

Fat content in seeds varies from 1.02% (point 2) to 1.10% (point 2). No significant differences were observed in the fat content in the seeds of grass pea grown on contaminated and uncontaminated soils (Fig. 3). According to Clayton [47], the total fat content in the seeds varies from 0.58 to 0.8%, while Urga [45] found 0.92-1.47% fat.

Differences in protein content were observed in the seeds of grass pea grown on highly contaminated and slightly contaminated soils (Fig. 3). The protein content in the seeds of grass pea grown on contaminated soil (point 1) is lower (23.18%) compared to the protein content in the seeds of grass pea grown on unpolluted soil (29.54%) (point 2) (Fig. 3). According to Clayton [47], the protein content in seeds varies from 25.67 to 28.4%, according to Heuze [46] it is in the range between 26.44-33.8%, and according to Urga [45] it ranges from 27.29% to 31.98%.

Ash content varies from 3.88% (point 2) to 4.35% (point 1). According to Clayton [47], the ash content in the seeds varies from 2.9 to 4.6%, according to Heuze [46] it is in the range between 1.8-5.1%. According to Urga [45] the ash content can vary from 1.28 to 4.14% depending on the variety and the location of cultivation.

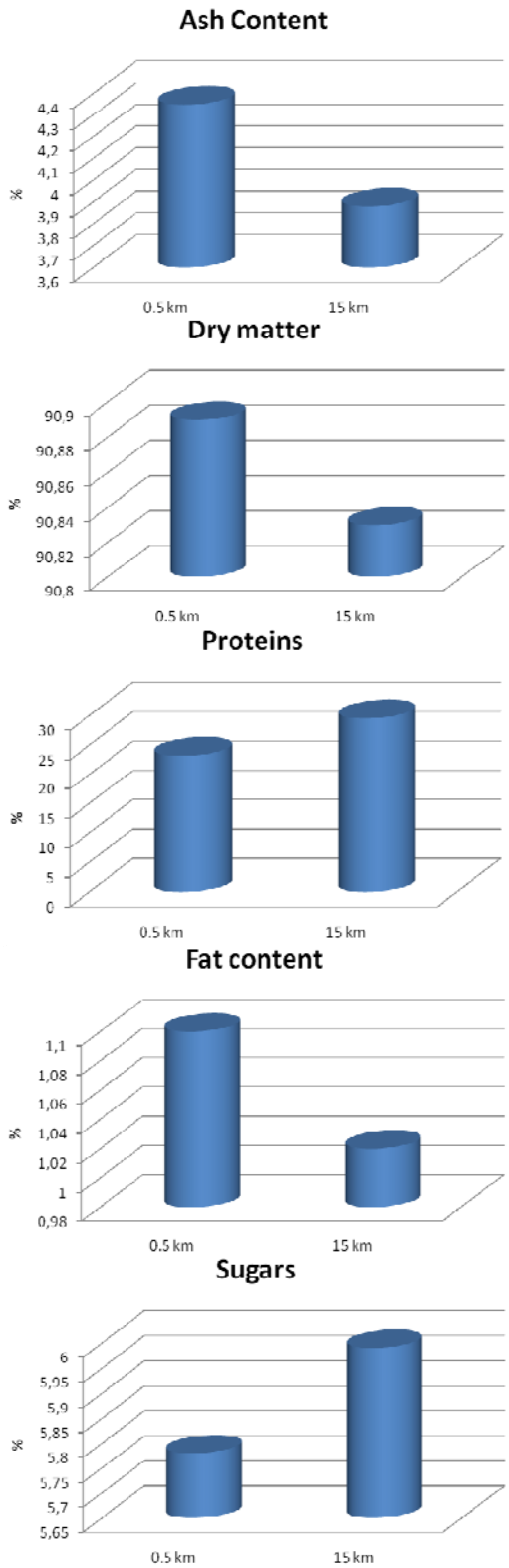

Fig. 3 Content of dry matter, ash content, protein content, sugars, fat content (%) in grass pea

The results obtained provide valuable information on the chemical and nutritional composition of the seeds of grass pea

grown on contaminated and uncontaminated soils in Bulgaria. It is confirmed that the seeds of grass pea contain significant amounts of nutrients (K, P, Cu, Fe Mn, Zn). The low content of toxic metals in growing grass pea in contaminated soil (point 1) makes it possible to use the seeds of the grass pea as animal feed.

IV. Conclusion

Based on the obtained results, the following conclusions can be made:

1. Grass pea is a crop that is tolerant to heavy metals, it can be attributed to accumulators of Pb, Cd and Zn and can be successfully grown in heavy metal polluted soils.
2. There is a clear distinction in the accumulation of heavy metals in vegetative organs of grass pea. The distribution of heavy metals in the organs of grass pea is selective, as the following order can be observed: with lead, zinc and copper – leaves > roots > stems > pods > seeds, with cadmium, iron and manganese – roots > leaves > stems > pods > seeds, with P – leaves > pods > stems > roots > seeds, and with K – pods > stems > leaves > roots > seeds.
3. The seeds of grass pea contain significant amounts of nutrients (K, P, Cu, Fe Mn, Zn) and low content of toxic metals in growing grass pea in contaminated soil (point 1) makes it possible to use the seeds of the grass pea as animal feed.
4. No significant differences were observed in the ash content, sugars and fat content in the seeds of grass pea grown on contaminated and uncontaminated soils.
5. Soil pollution with heavy metals does not affect the quality of the grass pea seeds.

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