POTENTIAL OF *SILYBUM MARIANUM L.* FOR PHYTOREMEDIATION OF SOILS CONTAMINATED WITH HEAVY METALS

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Abstract

Comparative research has been conducted to allow us to determine the accumulation of heavy metals, micro and macroelements in the vegetative and reproductive organs of milk thistle, the quality of milk thistle oil, as well as the possibilities to use the plant for phytoremediation of heavy metal contaminated soils. The experiment was performed on an agricultural field contaminated by the Non-Ferrous-Metal Works (NFMW) near Plovdiv, Bulgaria. The experimental plots were situated at different distances (0.5, and 15 km) from the source of pollution. The contents of heavy metals in plant materials (roots, stems, leaves, seeds and head seeds) were determined. The quality of milk thistle oils (heavy metals and fatty acid composition) was also determined. The quantitative measurements were carried out with inductively-coupled plasma (ICP). Mllk thistle is a plant that is tolerant to heavy metals and can be referred to the hyperaccumulators of lead and the accumulators of cadmium and zinc. The plant can be successfully used in the phytoremediation of heavy metal contaminated soils. Heavy metal soil contamination has no significant effect on the oil content of the milk thistle seeds and the fatty acid composition of the oil. Oil of milk thistle grown in contaminated soils is characterized by a lower content of saturated acids (with the exception of arachidonic acid) and polyunsaturated acids, and a higher monounsaturated acid content compared to the oil from the uncontaminated area. The processing of milk thistle seeds into oil and the use of the obtained oil will greatly reduce the cost of phytoremediation.

Keywords: *heavy metals, milk thistle, phytoremediation, polluted soils*

1. INTRODUCTION

Environmental pollution with heavy metals is a global problem, and therefore the development of phytoremediation technologies for plant-based clean-up of contaminated soils is therefore a significant interest. In recent years, an idea has been imposed of the use of heavily contaminated land for the cultivation of non-food crops (technical, medicinal and aromatic plants, etc.) and the production of end products with high economic value. This is an opportunity to turn a serious problem (contaminated land) into a potentially profitable source of income (Lydakis-Simantiris et al., 2016). The products obtained by the processing of aromatic plants are used in food and cosmetic industry, etc. (Tapsel et al., 2006). Aromatic and medicinal plants have shown to absorb and accumulate toxic heavy metals from polluted areas and could be used as biomonitors or accumulators of pollutants (Abu-Darwish et al, 2009; Angelova, 2012). The use of aromatic plants in the phyto-extraction of contaminated soils is also possible, but according to McGrath & Zhao (2003) neither their hyperacumulation capacity, nor their biomass can justify such use.

Preliminary studies (Brunetti et al., 2009; Farrag et al., 2012; Perrino et al., 2014) show that plant species such as Carduus pycnocephalus L. subsp. pycnocephalus, Dasypyrum villosum (L.) P. Candargy, Ferula communis L., Silybum marianum (L.) Gaertner, Sinapis arvensis and S. Austroitalica, which grow naturally in different regions of the world, are able to survive on heavy metal contaminated soils. The plants studied are well adapted to the environment and to the unfavourable conditions of water stress and nutrient deficiency, and can be used to create a vegetation coating on heavy metal contaminated terrains (Perrino, 2014) as well as used in phytostabilization of the soil (Brunetti et al., 2009; Farrag et al., 2012).

Silybum marianum L. Gaertner, is an annual or biennial plant of the Asteraceae family (Wianowska & Wiśniewski, 2015). It originates in the Mediterranean region. As a weed it occurs in Southern and Western Europe, Asia, Australia and America. The milk thistle is also cultivated in Bulgaria due to its demand from the pharmaceutical industry. The milk thistle seeds containing 0.08% of essential oil, resins, biogenic amines /tyramine, histamine/, flavones, mucus, 16-18% of oil, microelements (predominantly Se), vitamins (F, E and B) and other components are used (Lavreneva and Lavrenev, 1997).

The milk thistle has been used for medical purposes for 2000 years, most commonly for the treatment of liver diseases (cirrhosis and hepatitis) and for liver protection from toxic substances (Wianowska & Wiśniewski, 2015; Khan et al., 2007). The therapeutic effects of the milk thistle are closely related to the presence of a flavonoid complex called silmarin. The mix consists of silybin A and silybin B, isosilibin A and B, silicocrystine and silidianin. The largest part of the complex is present in the fruit of the plant (Hussain et al., 2011; Wianowska & Wiśniewski, 2015).

The conditions under which the milk thistle is grown have a great impact on the economic qualities of the raw material. It is recommended that the crop be sown on sunny, southern slopes, because when milk thistle growm in shady places the content of silymarin - the valuable pharmaceutical ingredient, is reduced to 1%.

Milk thistle can also be used as a dietary supplement, animal feed, cosmetics, and for the production of bioenergy. In recent years, milk thistle fruit has been marketed in Europe and the United States as food supplements in various forms: whole seeds, tea bags, alcohol-based seed extracts, oil seed extracts, capsules and soft gels (Tournas et al., 2012; 2013; Andrzejewska et al., 2015). There is evidence of the use of milk thistle seeds when making cookies, cakes and pizzas, with 3% of wheat flour being replaced with milk thistle flour. The resulting dough is more even, drier and less sticky. Baked products have better nutritional quality, bark colour, texture and symmetry and remain fresh for longer time compared to traditional products.

Silybum marianum oil can be used for producing biogas, as well as transport and heating fuel (Ahmad et al., 2014; Takase et al., 2014; Andrzejewska et al., 2015). According to Vachleva et al. (2001), the ethanol extraction from milk thistle seeds has insecticidal properties and can be used to produce biodegradable pesticides for organic farming. Elhaak et al. (2014) offers the use of extracts from seeds, flowers, stems, leaves and roots of milk thistle as a bioherbicide.

Insufficient is the information available on the potential of milk thistlefor accumulation of heavy metals and its potential for use for phytoextraction. The studies connected with growing the milk thistleon polluted soils are too limited. In the scientific literature, related to the determination of the fatty acid composition of oils predominate, whereas data on the chemical composition of plant biomass (leaves, stems and heads) of S. marianum are limited.

This gave us the grounds to carry out a comparative research, which to allow us to determine the quantities and the centers of accumulation of heavy metals, micro and macro elements in the vegetative and reproductive organs of milk thistle, the quality of milk thistleoil, as well as the possibilities to use the plant for phytoremediation of heavy metal contaminated soils.

2. MATERIALS AND METHODS

2.1. Materials

The experiment was performed on an agricultural fields contaminated by Zn, Pb and Cd, situated at different distances (0.5, and 15.0 km) from the source of pollution, the Non-Ferrous-Metal Works (NFMW) near Plovdiv, Bulgaria. Characteristics of soils are shown in Table 1. The soils were neutral to slightly calcaric, with moderate content of organic matter and essential nutrients (N, P and K). The pseudo-total content of Zn, Pb and Cd is high and exceeds the maximum permissible concentrations (MPC) in soil 1 (from 0.5 km from the NFMW) (Table 1).

The test plant was *Silybum marianum L*. (milk thistle). Milk thistle seeds were sown on depth 3-4 cmin each plot; between row and within row distances were 70 and 30 cm, respectively. On reaching commercial ripeness the plants of *Silybum marianum L*. were gathered.

2.2. Methods

The oil from *Silybum marianum L* was derived under laboratory conditions through an extraction method with Socksle's apparatus, allowing the extraction of the oil from the preliminarily peeled and ground seeds of *Silybum marianum L*. by using petroleum ether and the subsequent liberation of the latter through distillation.

The concentrations of contents of heavy metals, micro and macroelemensin different parts of milk thistle (roots, stems, leaves, seedsand head seeds), and oils were determined by the method of the microwave mineralization.Total content of heavy metals in soils was determined in accordance with ISO 11466. The mobilisable heavy metals contents in soils, considered as a "potentially bioavailable metal fraction", were extracted by a solution of DTPA (ISO 14870). The quantitative measurements were carried out with inductively coupled plasma emission spectrometry (ICP) (Jobin Yvon Emission - JY 38 S, France).

Statistical analyses were conducted with Statistica v. 7.0.

Parameter	pH	EC, dS/m	Organic C,%	N Kjeldal,%	P, mg/kg	K, mg/kg	Pb, mg/kg	Zn, mg/kg	Cd, mg/kg
Soil 1 (S1)									
0.5 km	7.4	0.15	2.2	0.34	625.6	6960	2509.1	2423.9	64.3
Soil 2 (S2)									
15 km	7.5	0.15	1.54	0.12	387.3	6780	49.4	172.7	1.0

MPC (pH 6.0-7.4) – Pb -100 mg/kg, Cd - 2.0 mg.kg, Zn-3 20 mg/kg

MPC (pH >7.4) – Pb – 100 mg/kg, Cd – 3.0 mg/kg, Zn - 400 mg/kg

Table 1. Characterization of the soils

3. RESULTS

The results presented in Tables 1 and 2 show that in the soil samples S1 (taken from the area situated at the distance of 0.5 km from NFMW), the reported values for Pb were exceeding MPC approved for Bulgaria and reached to 2509.1 mg/kg. In the area located at a distance of 15 km, the contents of Pb significantly reduce to 49.4 mg/kg. Similar results were obtained for Cd and Zn. The results for the mobile forms of the metals extracted by DTPA show that the mobile forms of Cd in the contaminated soils are the most significant portion of its total content and reached to 57,2%, followed by Pb with 33,8 % and Zn with 9,8%.

In the soil located at a distance of 15 km from NFMW the mobile forms of Cd are the most significant part of its.The results should be clearly and concisely presented. It is necessary to provide a logical explanation of the obtained results.

Figures 1, 2 and 3 presents the results for heavy metal, micro- and macroelements in milk thistle's vegetative and reproductive organs. The results obtained show that the major part of the heavy metals, micro- and macroelements are accumulated in the upper parts of milk thistle.By moving the source of contamination away, there is a clear tendency of reduction of the heavy metal content in milk thistle's vegetative and reproductive organs.

Soils	**Pb**		**Cd**		**Zn**	
	mg/kg	%*	mg/kg	%	mg/kg	%
S1	849.1	33,8	36.8	57,2	236.8	9,8
S2	21.5	43.5	0.7	70	38.9	22.5

*DTPA -extractable / total content

Table 2. DTPA-extractable Pb, Zn and Cd (mg/kg) in soils sampled from NFMW

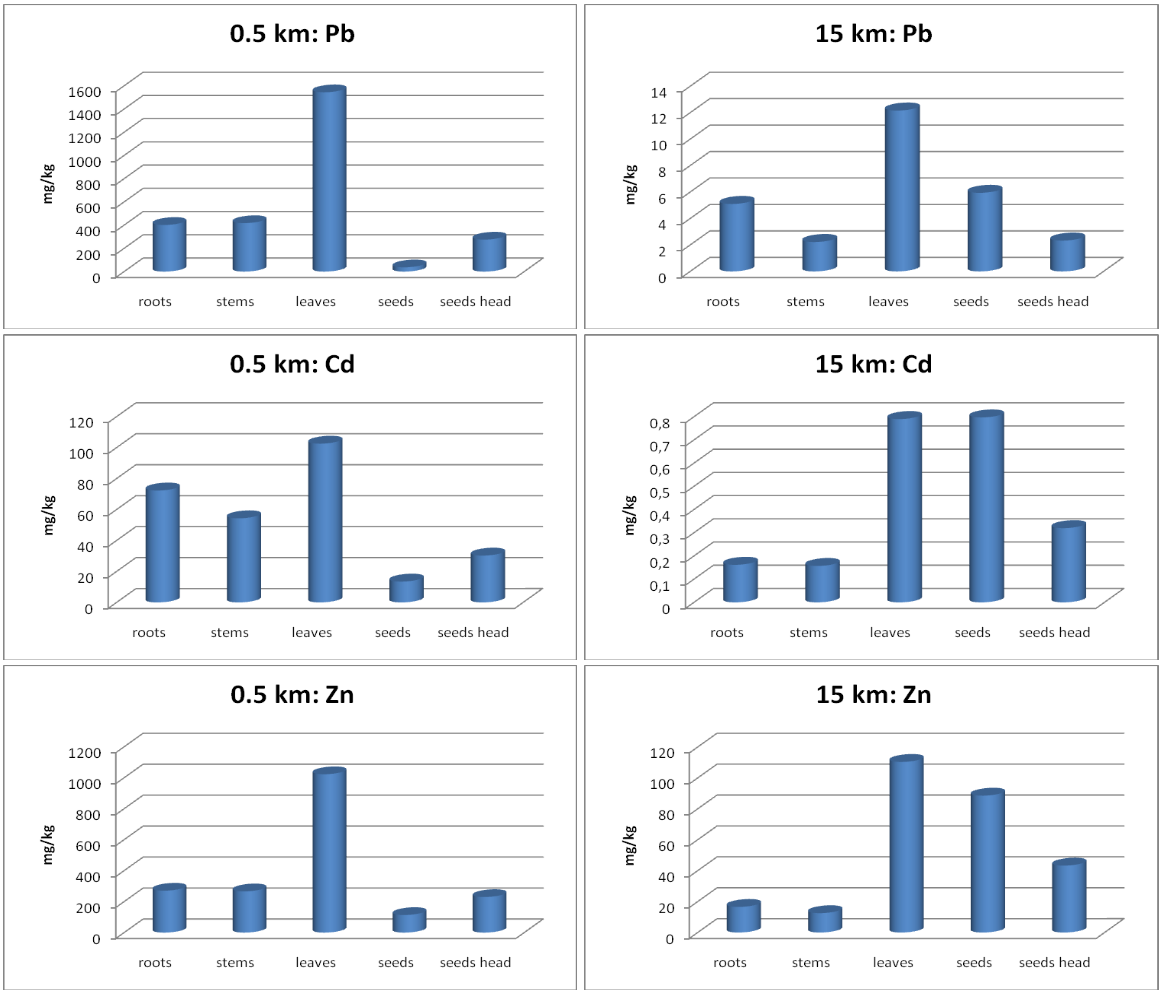

Fig. 1. Content of heavy metals (mg/kg) in milk thistle

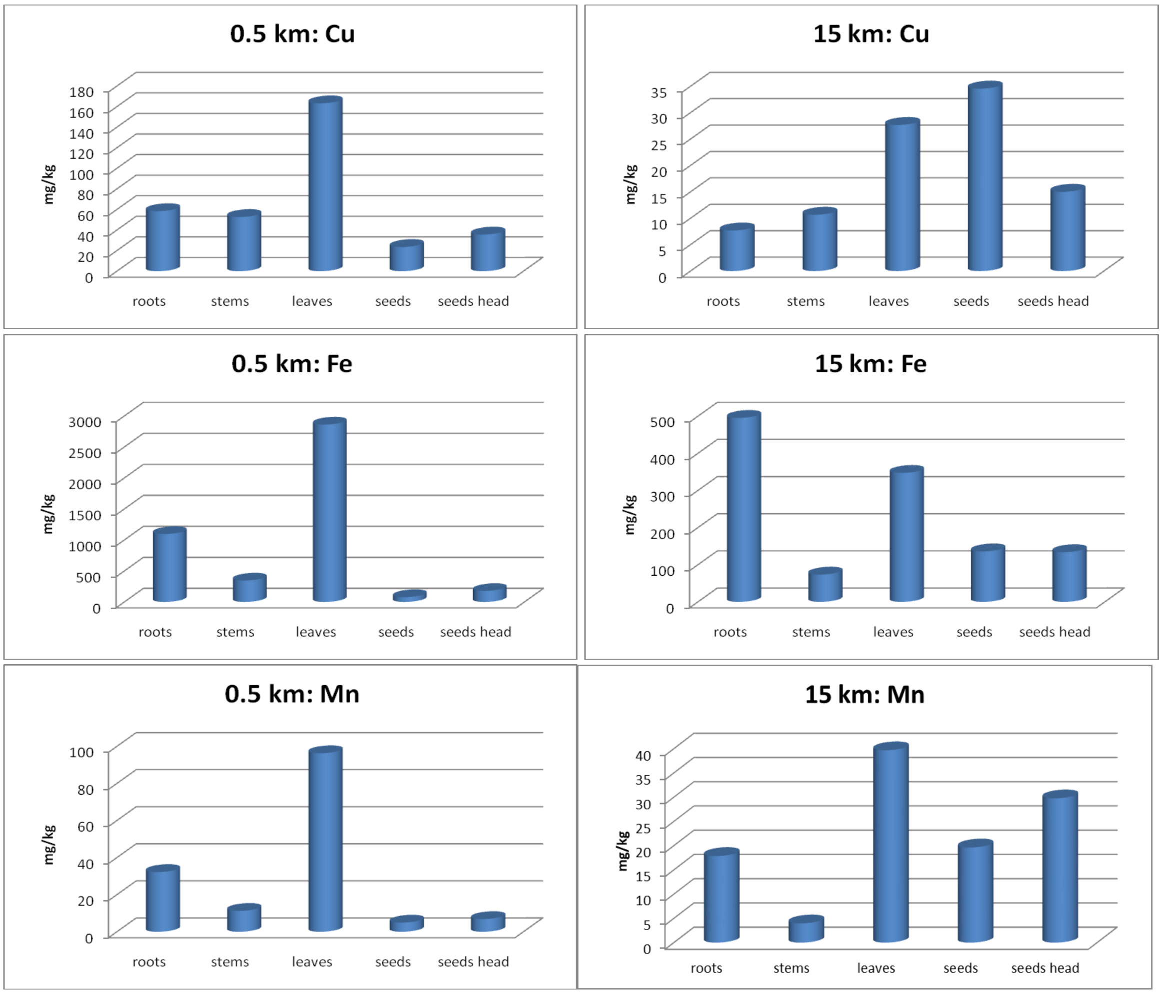

Fig. 2. Content of microelements (mg/kg) in milk thistle

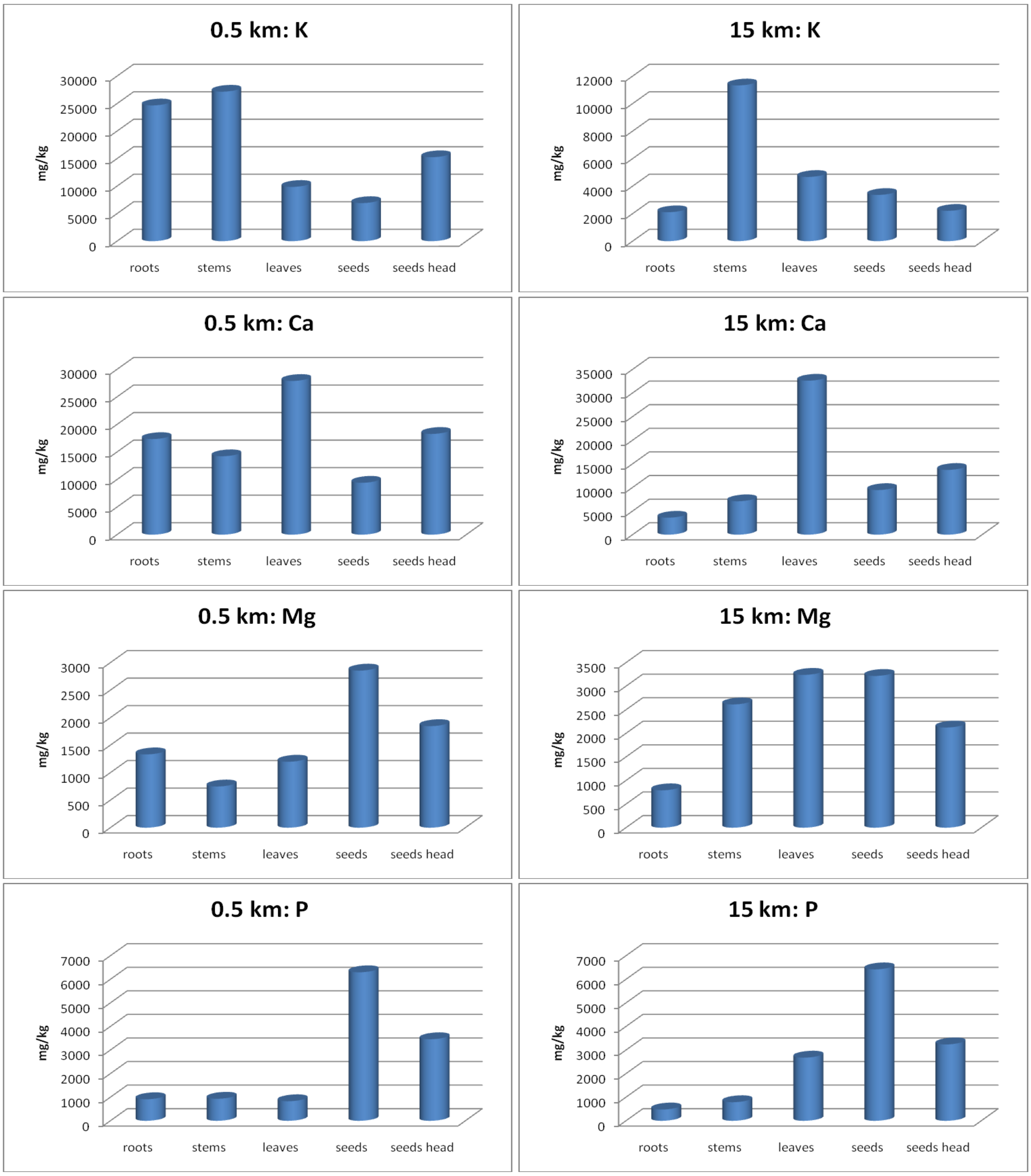

Fig. 3. Content of macroelements (mg/kg) in milk thistle

4. DISCUSSION

4.1. Accumulation of heavy metals, micro and macroelements in vegetative ad reproductive organs of milk thistle

Pb content in the roots of milk thistle grown at 0.5 km from the NFMW reaches 401.8 mg/kg, Zn - 270.9 mg/kg, Cd - 72.3 mg/kg. Cu content reaches 58.4 mg/kg, Fe - up to 1095.8 mg/kg and Mn – up to 32.2 mg/kg. The resulting values for heavy metals (Cd, Pb and Zn) and microelements (Cu and Fe) (except for manganese) in the roots are much higher than the values considered by Liphadzi & Kirkham, 2005) to be toxic to plants (0.1 mg/kg Cd, 30 mg/kg Pb, 100 mg/kg Zn, 20 mg/kg Cu, 1000 mg/kg Fe and 300 Mn).

It is known that milk thistle is distinguished by a well-developed root system, which consists of a highly developed, deeply penetrating main spindle root and lateral branches. It reaches a depth of up to 2 m but the main root mass is concentrated in the top layer at a depth of up to 35 cm. Probably this is the reason why milk thistle accumulates significant amount of heavy metals in the roots.

The movement of heavy metals and their accumulation in the vegetative organs of milk thistle is specific to the individual elements. Significant accumulation of lead in the leaves and stems of milk thistle was found. The content of this element reaches 417.6 mg/kg in the stems and 1547.7 mg/kg in the leaves and is significantly higher than the toxic values for animals - 30 mg/kg, (Chaney, 1989). However, the results we obtained show the considerable ability of milk thistle to accumulate lead in the leaves. This is probably due to the anatomical and morphological features of the crop. Its greater accumulation in the leaves is probably due to the fact that they are fleshy and spiky, i.e. they have a xerophytic nature, which determines their drought tolerance and less evaporation of the water and salts contained.

Cd content in the stems and leaves of milk thistle reaches 54.4 mg/kg and 102.6 mg/kg, respectively, considered to be toxic to plants (5.0 mg/kg) (Kabata-Pendias & Pendias, 2001). Zn content in the stems and leaves of milk thistle reaches 266.3 mg/kg and 1023.0 mg/kg, and these values are higher than the critical values for plants - 100-400 mg/kg.

For milk thistle grown in an area 15 km away from the NFMW, the heavy metal values in stems and leaves are significantly lower. Increased heavy metal concentrations in the leaves of milk thistle grown at a distance of 0.5 km from the NFMW are largely due to aerosol contamination.

Cu amount in the stems and leaves of milk thistle grown at a distance of 0.5 km from the NFMW reaches 52.7 mg/kg and 163.3 mg/kg, Fe up to 345.5 and 2861.4 mg/kg, Mn up to 11.2 and 96.3 mg/kg. In the case of milk thistle grown at a distance of 15 km from the NFMW, the Cu values in the stems and leaves reaches 10.7 mg/kg and 27.7 mg/kg, Fe up to 73.4 mg/kg and 347.2 mg/kg, Mn up to 3.98 mg/kg, up to 397.0 mg/kg.

The heavy metal content in the above ground mass of the milk thistle is higher than that in the root system, which is consistent with the results of other authors. Brunetti et al. (2009) found less accumulation of most heavy metals (Cr, Ni, Pb, Zn) in the roots of milk thistle. Similar results were obtained from Zehra et al. (2009), according to whom in Sylibum marianum Mn and Zn are accumulated in the above-ground parts of the plant. However, the values obtained from them are significantly lower than those obtained by us in the present study. According to Brunetti et al. (2009), the Cd content in milk thistle plants ranges from 0.35 to 0.56 mg/kg in the above-ground mass and 0.15-0.35 mg/kg in the roots, Pb from 0.6 to 0.9 mg/kg in the above-ground mass and from 0.4 to 1.8 mg/kg in the roots, and Zn - from 22.9 to 44.4 mg/kg in the above-ground mass and from 19.9 to 118.3 mg/kg in the roots. The values we obtained for heavy metals in the above-ground mass of milk thistle significantly exceed the results obtained by Del Rio et al.(2002),Wei et al.(2005), García-Herrera et al. (2014) in the cultivation of milk thistle in contaminated soils.

By moving the source of contamination away, there is a clear tendency for increase in the content of macroelements in the leaves of milk thistle (except for K and P). Ca and Mg concentration is increased accordingly: for Ca from 27899.7 mg /kg (S1) to 32601.3 mg/kg (S2), for Mg from 1199.2 mg/kg (S1) to 3245.2 mg/kg (S2).

According to Madejon et al. (2014), there is no significant difference between the content of macroelements in plant growing in contaminated and uncontaminated soils, while Kabatta-Pendias & Pendias (2001) found that heavy metals can lead to a change in the absorption of nutrients from plants. The results we obtained confirm that the high heavy metal content in the soil leads to less absorption of the nutrients Ca, Mg and P from the milk thistle leaves.

Based on the element content in plants and calculated factors (BCF, TF), comparisons can be made between the behaviour of plants growing in different places around the world. Our results show that the translocation factor for all metals is greater than 1 regardless of the degree of contamination of the soil (Table 3).Similar results have been obtained by Brunetti et al. (2009) for TF> 1. According to the authors milk thistle can not be referred to the accumulators (Baker and Brooks 1989), although there is a relatively high translocation factor (TF), and can not be used for phytoextraction of contaminated soils.

In terms of Pb the BCF in milk thistle grown on contaminated soils (0.5 km from NFMW) ranged from 0.47 for roots to 2.3 for shoots, in Zn from 1.1 to 5.4, and in cadmium from 1.96 to 4.2. BCF coefficients for root are lower than 1 in the cultivation of the milk thistle on heavy metal polluted soils (S1) and uncontaminated soils (S2). Similar results have been obtained by Brunetti et al.(2009), according to whom all BCF values are <1, although the heavy metal content is quite high in soils. According to the authors, the low ability of plants to accumulate large amounts of metals in the above-ground parts and the increased heavy metal content in soils are the cause of a low bioconcentration factor (BCF) which makes plants unsuitable for phytoextraction. According to the authors, milk thistle may be more suitable for phytostabilization.

Soils	**Pb**			**Cd**			**Zn**		
	TF	BAC root	BAC shoot	TF	BCF root	BCF shoot	TF	BCF root	BCF shoot
S1	4.9	0.47	2.3	2.2	1.96	4.23	4.8	1.1	5.4
S2	2.8	0.23	0.67	5.8	0.23	1.35	7.4	0.43	3.2

TF=[Metal]shoots/[Metal]roots), BCFroots=[Metal]roots/[Available metal]soils, BCFshoots=[Metal]shoots /[Available metal]soils

Table 3. Translocation (TF) and bioconcentration factor (BCF) factors in milk thistle

A tolerance mechanism for Carduus Pycnocephalus, Silybum marianum and Sinapis arvensis has been offered because of the capacity of milk thistle to move and accumulate some of the metals in the above-ground parts (Baker, 1981). Milk thistle refers to heavy metal tolerant plants, which have developed mechanisms that allow growth and development in highly contaminated soils.

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

The heavy metal content in seeds is significantly lower compared to the root system and the above-ground mass of the plants. Their accumulation in milk thistle is probably done through the conductive system. The Pb and Zn content in milk thistle grown at a distance of 0.5 km from the NFMW reaches 36.8 mg/kg and 113.7 mg/kg, respectively, and does not reach the critical values of 30 mg/kg Pb and 300 mg/kg Zn (Hapke, 1991). Cd accumulates in seeds (13.5 mg/kg) at levels well above the recommended maximum levels tolerated by the animals (0.5 mg/kg Cd, Chaney, 1989) and the recommended food values (1 mg/kg, Hapke, 1991). Cu content in milk thistle seeds reaches 23.5 mg/kg and is below the critical 25 mg/kg Cu (Hapke, 1991).

Oil crop seeds are rich in P and Ca, necessary for bones and teeth (Brody, 1994). The results we obtained show that Ca, K and P are predominant elements in milk thistle seeds, followed by Mg. Ca content reaches 9419.0 mg/kg, K - to 6928.1 mg/kg, P - to 6300.2 mg/kg, and Mg up to - 2855.4 mg/kg.

Pb content in milk thistle seeds at a distance of 15 km from the NFMW reaches 5.9 mg/kg, Zn up to 88.6 mg/kg, Cd up to 0.79 mg/kg, Cu up to 34.6 mg/kg, Fe up to 135.9 mg/kg, Mn up to 19.6 mg/kg, K up to 3363.3 mg/kg, Ca up to 9447.6 mg/kg, Mg up to 3218.6 mg/kg and P up to 6424.7 mg/kg.It is noteworthy that milk thistle seeds cultivated as a distance of 15 km from the NFMW contain more copper, iron, manganese and magnesium, and there is no significant difference in the phosphorus and calcium content, and the potassium content is significantly lower.

A comparison was made of the content of the elements in milk thistle seeds with the results published by other authors. According to the literature, milk thistle seeds contain Mg 2225 mg/kg, Ca 778.5

mg/kg, Cu 108.3 mg/kg, Fe 74.3 mg/kg, Zn 69.4 mg/kg, Pb 44.3 mg/kg, Ni 35.5 mg/kg, Mn 23.5 mg/kg, Cr 6.8 mg/kg and Cd 3.2 mg/kg. Milk thistle can be a good mineral source due to the high content of calcium and magnesium in them. The results we obtained are similar to the results of Khalil (2015). The values for Fe, Magnesium and P do not differ significantly from the results of Sadowska et al. (2011). The zinc, copper, potassium and calcium content is slightly lower, while for manganese significantly higher values are obtained. According to Abu Jadayil et al. (1999) and Sadowska et al.(2011) milk thistle seeds are characterized by high iron content ranging from 81 to 375 mg/kg. The variation between the individual results may be due to conditions of cultivation, genetic factors, varietal peculiarities and other factors. The heavy metal distribution in the milk thistle organs is selective in nature, observing the following order: leaves> roots> stems>head seeds> seeds.

There is a distinct feature in the accumulation of heavy metals in the vegetative organs of the milk thisle. The accumulation and distribution of metals in plant tissue are important aspects to evaluate the role of plants in remediation of heavy metal contaminated sites (Garbisu and Alkorta 2001). The success of phytoremediation process depends on adequate plant yield and the plant having an ability to take up significantly high amount of heavy metals in their shoots (Chand et al., 2015). The milk thistle accumulates heavy metals through its root system, but a small portion of the heavy metals are retained by the roots, and most of them move and accumulate in the above-ground parts (stems, leaves). Our results strongly suggest that milk thistle is a crop which is tolerant to heavy metals and can be grown in contaminated soil. It can be assigned to the hyperaccumulators of lead and to the accumulators of zinc and cadmium, and therefore, it can be successfully used for phytoremediation of soils contaminated with Pb, Cd and Zn.

From the milk thistle seed, oil was obtained in laboratory conditions by an extraction method with the Soxhlet apparatus, allowing extraction of the oil from the pre-milled seeds with petroleum ether and subsequent distillation of the latter.

Pb content in milk thistle oil reaches 0.19 mg/kg (Table 4). The maximum permissible concentrations for Pb in vegetable oil is 0.1 mg/kg. The results obtained show that the major portion of Pb contained in the milk thistle seeds does not go into the oil obtained, but its oil content is higher than the maximum permissible concentrations and can not be used for food purposes.

Soils	Pb	Cd	Zn	Cu	Fe	Mn	P	Ca	Mg	K
S1	0.19	0.025	1.9	0.68	4.9	0.04	24.3	78.7	8.3	24.1
S2	0.05	nd	1.5	0.36	5.6	0.05	38.4	81.8	9.8	35.7

nd- non detected

Table 4. Content of heavy metals, micro and macroelements in milk thisle seeds

Cd content is below the limits of quantitative measurement with the method used. According to the current standard, the Cd content should not exceed 0.05 mg/kg. Although Cd is contained in the seed, it does not pass into the oil when processed.

The maximum permissible concentrations for the Zn content in vegetable oils is 10 mg/kg. The results obtained clearly show that the major portion of Zn contained in milk thustle seeds does not pass into the oil obtained and its content in oil is lower than the maximum permissible concentrations.

The maximum permissible concentrations for the Cu content in refined oils is 0.1 mg/kg, and in the unrefined - 0.4 mg/kg. In our studies, Cu content in oil reaches 0.68 mg/kg and its amount is above the maximum permissible concentrations. It is noteworthy that even though the soil in the NFMW area is not contaminated with Cu, the Cu content in seeds is low, the Cu contained in oil is above the limit values (LV). Probably the reason for this is the way of extracting the oil from the milk thistle seeds. In the context of our experiment, the oil was derived by extraction. However, there is evidence in the literature that the oils obtained by solvent extraction contain higher values of Cu and heavy metals than the cold-pressed oils. It is not desirable for the oil to contain significant amounts of microelements, in particular Cu. It is known that Cu ions are effective in oxidizing lipids, so they are unwanted components in terms of the oil's resistance to oxidation. Probably this is the reason for the higher criteria regarding the Cu content in the oils, although Cu is not a toxic element for human health in a relatively wide range.

Fe content in milk thistle oil reaches 4.7 mg/kg and is within the limits for oils (for crude oils 5 mg/kg, for refined oils 1.5 mg/kg). Fe content in crude oils is due to Fe contained in oilseed crops (mainly bound to proteins, phospholipids and other components).It is known that Fe has a prooxidant activity (Karaali, 1985) and can act as a catalyst that can initiate oxidation of the free radicals of unsaturated fatty acids. For this reason, Fe content in oils should be controlled.

Of the nutrients in the content of milk thistle oil grown at a distance of 0.5 km from the NFMW, Ca (78.7 mg/kg) prevails, followed by P (24.3 mg/kg), K (24.1 mg/kg) and Mg (8.3 mg/kg) (Table 3). There is no evidence of the negative impact of these elements on oil stability. It is known that P and Ca form salts which are insoluble in oil and can easily be removed by refining the oil.

Szentmihályi (1998) explores the chemical composition of milk thistle oil and establishes heavy metal content in all tested oils. Amounts of elements vary depending on the extraction conditions, with the highest values found in solvent extraction oils compared to cold-pressed oils. According to Szentmihályi (1998), Ca content ranges from 81.32 to 879.2 mg/kg, Cr from 12.84 to 248 mg/kg, Fe from 27.85 to 189.28 mg/kg, K from 6.59 to 149.74 mg/kg), Mg from 22.06 to 173.46 mg/kg, Na from 564.1 to 4634 mg/kg and S from 378.0 to 587.2 mg/kg and Pb concentration of is below the detection limit.

4.2. Fatty acid composition of milk thistle oil

According to the literature, milk thistle seeds contain 12-26% of oil, depending on genotype, environmental factors and growing conditions (Růžičková et al., 2011). According to Harrabi et al. (2015) the fat content in milk thistle seeds may reach 30.5%. Malekzadeh et al. (2011) report that the total oil content in milk thistle seeds decreases with drought.

The results we obtained show that the oil content of milk thistle seeds varies from 14.55 to 15.51%. Higher results were obtained from Růžičková et al. (2011) for the oils from the Czech Republic (17,5-21,6%) and KHAN et al., 2007 for the oils from Pakistan (26,5%). Milk thistle seeds have a higher oil content compared to corn (4%) (Harrabi et al., 2009), similar to soybean seeds (17-21%), cottonseed (15-24%), olives (20-25%) (Matthaus and Ozcan, 2006), and much lower compared to argan nuts (50%), sesame seeds (54%), sunflowers (44%) (Ismaili et al., 2016). According to Hadolin et al. (2001) and El-Mallah et al., 2003 milk thistle oil may be used for food purposes but the lower oil content in milk thistle for oil production is not economically profitable for industrial production.

Fatty acid composition in oil is the main factor determining the use of oil for food, industrial or pharmaceutical purposes, as variety, climate and production area have major influence (Velasco et al., 2005). According to Zhelev (2014), in milk thistle oil, the following predominate: palmitic acid (C 16: 0) of the saturated fatty acids and oleic (C 18: 1) and linoleic (C 18: 2) acids of the unsaturated acids.

The unsaturated fatty acids predominate in the fatty acid composition of the oil obtained from the extraction of milk thistle seeds from the NFMW region, reaching 82.17%, respectively. The oil composition is dominated by linoleic acid (C18: 2, 43.85%), followed by oleic acid (C18: 1, 37.95%). Linolenic (C18: 3, 0.17%), palmitic (C16: 1, 0.13%) and gadoleic (C20: 1, 0.07%) acids are also found.

Of the saturated fatty acids, the following predominate: palmitic acid (C16: 0) in the amount of 8.4%, followed by stearic acid (5.03%). The oil also contains myristic (C14: 0, 0.07%) and arachidonic (C20: 0, 0.08%) acids. The content of saturated fatty acids in milk thistle oil grown at a distance of 0.5 km from the NFMW reaches 17.82% (Table 5).The results we obtained confirm that linoleic acid prevails in S. marianum oil (El-Mallah et al., 2003, Malekzadeh et al., 2011; Ruóžicková et al., 2011; Sadowska et al., 2011). According to the literature, the linoleic acid content ranges from 45.36% to 66.4%. Oils from Iran contain up to 45.36% of linoleic acid (Hasanloo et al., 2008), while oils from

Serbia and the Czech Republic contain higher levels of linoleic acid (50.58-66.4%) (Růžičková et al., 2011). Large amounts of linoleic acid make the milk thistle seed oil more susceptible to oxidation).

It has been found that the oleic acid content ranges from 16.26% to 31.58% (Růžičková et al., 2011), (Mirzaeva et al., 2011; Ismaili, et al., 2016; Mhamdi et al, 2016; Meddeb et al, 2017). The oil from Iran contains up to 31.58% (Hasanloo et al., 2008), while the oils from Serbia and the Czech Republic contain lower amounts of oleic acid (16.26-25.44%) (Růžičková et al., 2011).

Parameter	S1 (0.5)	S2 (15 km)	Reference
Oil content,%	15.51	14.55	12 - 30.5
Saturated (S)			
Lauric acid (C12:0)	0.07	0.11	nd
Palmitic acid (C16:0)	8.4	8.62	7.56 – 11.40
Magaricacid (C17:0)	-	0.19	nd - 0.07
Stearic acd (C18:0)	5.03	5.21	2.90 – 5.26
Arachidic acid (C20:0)	0.08	0.063	1.80 – 3.99
Behenic acid (22:0)	4.24	4.18	0.92 – 3.85
Monounsaturated (MUFA)			
Palmioletic acid (C16:1)	0.13	0.13	0.049 – 0.056
Oleic acid (C18:1)	**37.95**	31.52	16.21 - 31.58
Gadoleic acid (C20:1)	0.07	0.78	nd - 1.11
Polyunsaturated (PUFA)			
Linoleicacid(C 18:2)	43.85	**48.11**	45.36 - 66.4
Linolenic acid (C 18:3)	0.17	0.23	0.12 - 0.59
Total unsaturated (U)	82.17	81.07	64.71 – 89.94
MUFA	38.15	32.73	16.23 – 29.63
PUFA	44.02	48.34	48.48 – 60.31
SFA	17.82	18.94	16.26 – 21.67
Unsaturated:saturates (U/S)	17.82:82.17	18.94:81:07	
P/S index	2.47	2.57	2.20

Table 5. Fatty acid composition of milk thistle oil (expressed as % of total fatty acid composition)

Linoleic acid content in oil is less than 1%. According to Ismaili (2016), the low percentage of linoleic acid, less than 1%, has a positive effect on the thermal and oxidation stability of oil.

Palmitic acid content in oil varies from 7.2% to 10.40% while stearic acid content in oil varies from 2 to 6% (Khan et al., 2007; Ben Rahal et al., 2011; Rusičková et al., 2011; Mirzaeva et al., 2011 and Ismaili et al., 2016).

Heavy metal soil contamination does not significantly affect the unsaturated acid content in oil. The amount of linoleic acid slightly increases from 43.85% (contaminated soil, S1) to 48.11% (uncontaminated soil, S2) while the linoleic acid content decreases from 37.95% to 31.52%. It has been found that there is a strong negative relationship between the linoleic and oleic acid content, with the increase of one leading to the reduction of the other (Seiler, 1986). The results we received fully confirm this relation.

Linoleic acid is one of the major fatty acids that can not be synthesized by the human body and should be obtained through complete nutrition or in the form of supplements (Malekzadeh et al., 2011). Recently, the use of this fatty acid is popular in cosmetics and pharmaceuticals.

Heavy metal soil contamination has little effect on the composition of saturated acids. The amount of saturated acids is higher in the oil from uncontaminated soils (except for arachidonic acid). The palmitic acid content is higher in the oil from uncontaminated area. The lower palmitic acid content makes the oil suitable for dietary purposes, while its higher content reduces its quality and increases the level of cholesterol in the blood. Similar results are obtained for stearic acid.

There are also minor changes in the arachidonic, palmitoleic and linolenic acid content in oil. It should be noted that the oil from the contaminated area contains C17:0, whereas in the oil from the uncontaminated area it is not detected.

The distribution of fatty acids is shown in Figure 4. The unsaturated to saturated fatty acids ratio in milk thistle oil from the NFMW region is 82.17:17.82 and in the uncontaminated oil from 81.07:18.94. Similar results were obtained from Zhelev (2014) for milk thistle oils from Bulgarian 80.3:19.7. Similar data on the unsaturated to saturated fatty acids ratio for S. marianum (75.1: 24.9) were found by El-Mallah et al. (2003).

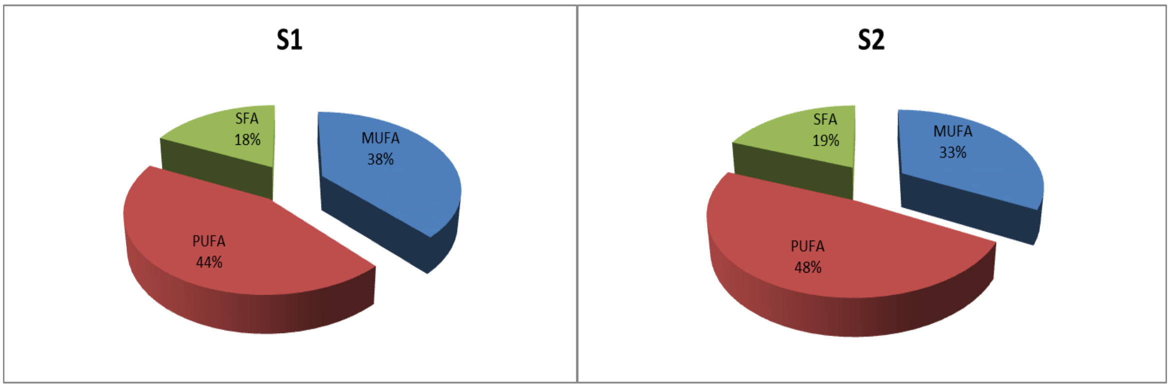

Fig. 4. Distribution of fatty acids in milk thustle oil

The total content of saturated fatty acids (SFA) in white thistle oil ranges from 17.82% to 18.94% of the total fatty acids and is comparable to the amounts of these acids in the oils from Iran (19-21%) (Fathi-Achachlouei and Azadmard-Damirchi, 2009). The content of monounsaturated fatty acids (MUFA) varies from 32.73 to 38.15% and the content of polyunsaturated fatty acids - from 44.02 to 48.43%. The high PUFA content in milk thistle oil makes it useful for therapeutic purposes in cardiovascular diseases. PUFAs are useful for reducing the risk of certain chronic diseases such as coronary heart disease, stroke and rheumatoid arthritis (Calder, 2008) and are used in the treatment of certain chronic diseases such as diabetes, cardiovascular diseases, inflammatory processes, atherosclerosis (Finley & Shahidi, 2001).

The relationship between the saturated and polyunsaturated fatty acid content is expressed as P/S index. This value is an important parameter for determining the nutritional value of certain types of oils. Oils with a P/S index greater than 1 are considered valuable food oils. The results of Ismaili (2016) show that milk thistle oil has a P/S index higher than 1 (2.23), which is in line with the results obtained (2.47-2.57). These values indicate that milk thistle oils can have a good effect on human health and are suitable for mass consumption.

The quality of the milk thistle oil is determined by its fatty acid composition. The milk thistle oil from the contaminated and uncontaminated area is rich in polyunsaturated fatty acid (linoleic acid), which determines oil as extremely valuable for human consumption (for nutritional or therapeutic purposes), as oil can be used to prevent diseases such as coronary heart diseases, atherosclerosis and high blood pressure.

5. CONCLUSIONS

Based on the obtained results for the content of heavy metals in the studied milk thistle plants from family Asteraceae, the following conclusions can be made:

1. There is a distinct feature in the heavy metal accumulation in the vegetative organs of milk thistle. Milk thistle accumulates heavy metals through its root system, but a small portion of the heavy metals are retained by the roots, and the bulk of them is moved and accumulated in the above-ground parts (stems, leaves).
2. Milk thistle is a heavy metal tolerant crop that can be applied to the Pb hyperaccumulators and the Cd and Zn accumulators and can be successfully used in the phytoremediation of heavy metal contaminated soils.
3. Heavy metal soil contamination has no significant effect on the oil content of the milk thistle seeds and the fatty acid composition of the oil.
4. Oil of milk thistle grown in contaminated soils is characterized by a lower content of saturated acids (with the exception of arachidonic acid) and polyunsaturated acids, and a higher monounsaturated acid content compared to the oil from the uncontaminated area.

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