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Accumulation of Heavy Metals in Safflower (*Carthamus tinctorius* L.)

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Abstract**—**Comparative research has been conducted to allow us to determine the accumulation of heavy metals (Pb, Zn and Cd) in the vegetative and reproductive organs of safflower, and to identify the possibility of its growth on soils contaminated by heavy metals and efficacy for phytoremediation. The experiment was performed on an agricultural field contaminated by the Non-Ferrous-Metal Works (MFMW) near Plovdiv, Bulgaria. The experimental plots were situated at different distances (0.1, 0.5, 2.0, and 15 km) from the source of pollution. The contents of heavy metals in plant materials (roots, stems, leaves, seeds) were determined. The quality of safflower oils (heavy metals and fatty acid composition) was also determined. The quantitative measurements were carried out with inductively-coupled plasma (ICP). Safflower is a plant that is tolerant to heavy metals and can be referred to the hyperaccumulators of lead and cadmium and the accumulators of zinc. The plant can be successfully used in the phytoremediation of heavy metal contaminated soils. The processing of safflower seeds into oil and the use of the obtained oil will greatly reduce the cost of phytoremediation.

Keywords**—**Heavy metals, phytoremediation, polluted soils, safflower.

I. Introduction

PHYTOREMEDIATION is an emerging technology, which should be considered for remediation of contaminated sites because of its cost effectiveness, aesthetic advantages and long term applicability [1], [2]. This technology can be defined as the efficient use of plants to remove, detoxify or immobilize environmental contaminants in soils, waters or sediments through the natural, biological, chemical or physical activities and processes of the plants [3], [4]. Phytoremediation is most effective for low and medium level of pollution with nutrients and heavy metals, since high concentrations of heavy metals are toxic to plants [5]. The use of crop plants for phytoremediation of contaminated soils has the advantages of their high biomass production and capacity to adapt to variable environments [6], [7]. As phytoremediation is a technology based on the plants, its success will substantially depend on the tolerance of the plants to pollutants and their ability to accumulate significant amounts of heavy metals in their tissue. Phytoremediation should be seen as a long time practice need of many cycles of plant breeding to reduce the concentration of metals in the soils, economically viable and environmentally friendly. If the contaminated biomass can be further processed, then this can allow the creation of value-added products, which will further increase the economic efficiency of phytoremediation technology. Industrial crops, i.e. energy crops or crops for production of bio-diesel, are among the preferred candidates as plants for phytoremediation. The use of energy and/or bio-diesel crops as plants for phytoremediation would lead to the production of economically valuable products and reduce the cost of remediation.

Safflower (*Carthamus tinctorius* L.) belongs to family Asteraceae. This oilseed crop has been cultivated on small plots in the world. Safflower is one of the alternative oil crops, particularly in dry land due to tolerance to cold, drought and salinity stress [8], [9]. Traditionally, the safflower is grown for flowers, which are used for coloring and flavoring the food, in medicine, for the preparation of drugs, for the production of red and yellow dyes. Over the past 50 years, the plant is grown mainly for the oil that is extracted from the seeds. The seeds of safflower are mainly used for pet food as a gerbil, hamsters and chinchillas or as feed for wild birds, parrots and other pet birds [10]. According to hull types of seeds, the seed oil content ranges from 20 % to 45 %. The oil is high in linoleic acid, an unsaturated fatty acid that aids in lowering the cholesterol level in the blood. Safflower oil may be used for food purpose - for the preparation of margarine, as an additive in different dressings [11]. The oil can be used for industrial purpose, medicine and cosmetics. Because of rapid drying, the oil is in high demand in paint and emulsion industries [12].

The purpose of this study is to conduct a comparative investigation to allow us to determine the quantities of and depots for the accumulation of Pb, Zn and Cd in the vegetative and reproductive organs of the safflower (*Carthamus tinctorius* L.) and its oil, as well as to assess its potential use in phytoremediation of heavy metal polluted soils.

II. Material and Methods

The experiment was performed on an agricultural fields contaminated by Zn, Pb and Cd, situated at different distances (0.1, 0.5, 2.0 and 15.0 km) from the source of pollution, the NFMW near Plovdiv, Bulgaria.

Characteristics of soils are shown in Table I. The soils were slightly acidic to neutral, with moderate content of organic matter and essential nutrients (N, P and K) (Table I). The psedo-total content of Zn, Pb and Cd is high and exceeds the maximum permissible concentrations (MPC).

The test plant was saflower (*Carthamus tinctorius* L.). Safflower seeds were sown in each plot; between row and

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within row distances were 60 and 20 cm, respectively. Each hole was 5–6 cm deep, containing three seeds. After safflower had grown for 15 days, it was thinned to one plant per hole.

TABLE I
CHARACTERIZATION OF THE SOILS

Parameter	Soil 1 (S1) 0.1 km	Soil 2 (S2) 0.5 km	Soil 3 (S3) 2.0 km	Soil 4 (S4) 15 km
pH	7.4	7.6	6.5	7.5
EC, dS/m	0.15	0.2	0.2	0.15
Organic C,%	3.99	4.95	2.22	1.54
N Kjeldal,%	0.15	0.34	0.24	0.12
P, mg/kg	631.5	823.2	642	387.3
K, mg/kg	6988.1	5467.6	5517.5	6780.0
Pb, mg/kg	1983.5	2428.6	876.5	24.6
Zn, mg/kg	2029.0	2475.5	1430.7	33.9
Cd, mg/kg	50.1	43.5	31.4	2.7

MPC (pH 6.0-7.4) – Pb -60 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

Upon reaching commercial ripeness, the safflower plants were gathered and the concentrations of Pb, Zn and Cd in their different parts (roots, stems, leaves and seeds) were determined. The oil from safflower was derived under laboratory conditions through an extraction method with Socksle's apparatus. Total content of heavy metals in soils was determined in accordance with ISO 11466 [13]. The mobilisable heavy metals contents in soils, considered as a "potentially bioavailable metal fraction", were extracted by a solution of DTPA (1 M NH_4HCO_3 and 0.005 M DTPA, pH 7.8) [14].

The contents of heavy metals (Pb, Zn and Cd) in the plant material (roots, stems, leaves and seeds) and in the oils of safflower were determined by the method of the microwave mineralization. The quantitative measurements were carried out with inductively coupled plasma emission spectrometry (ICP) (Jobin Yvon Emission - JY 38 S, France).

The content of crude oil in safflower was determined by weight after the extraction method. The composition of fatty acids in the oils was determined by preparing methyl esters of the fatty acids in accordance with the procedures described in [15]. The analysis of the methyl esters of the fatty acids was carried out by gas chromatography according to [16].

Statistical analyses were conducted with Statistica v. 7.0.

III. RESULTS AND DISCUSSION

A. Soils

The results presented in Tables I and II show that the soil contamination in the area of NFMW-Plovdiv is on the torch of contamination and with the moving away from NFMW - Plovdiv a notable trend to reduce the total content of heavy metals in the soil is observed. In the soil samples S1, S2, S3 (taken from the area situated at the distance of 0.1 km, 0.5 and 2.0 km from NFMW), the reported values for Pb were exceeding MPC approved for Bulgaria and varied from 876.5 mg/kg to 2428.6 mg/kg. In the area located at a distance of 15 km, the contents of Pb significantly reduce to 24.6 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 range from 63.1 to 69.4%, followed by Pb with 35.6 to 58.4% and Zn with 11.5 to 17.0%. It is notable that the content of the mobile forms of heavy metals is significantly higher in soil located at a distance of 2.0 km from NFMW (S3), as it is characterized by slightly acid reaction.

B. Safflower

Fig. 2 presents the distribution of the heavy metals in the vegetative and reproductive organs of the studied oilseed crop. Significant differences were found in the distribution of metals in different parts of safflower in its cultivation on contaminated and uncontaminated soils. In all three elements, considerably lower values were established in the roots of safflower compared to the above-ground parts. The obtained results do not confirm that the majority of the heavy metals was retained in the roots of safflower, which is in contrast to the results found from [17]-[19] that heavy metals accumulate mainly in the root system of safflower.

TABLE II
DTPA - EXTRACTABLE PB, ZN AND CD (MG/KG) IN SOILS SAMPLED FROM NFMW

Soils	Pb		Cd		Zn	
	mg/kg	%*	mg/kg	%	mg/kg	%
S1	942.2	47.5	31.6	63.1	233.3	11.5
S2	863,4	35.6	27.8	63.9	379.8	15.3
S3	512,1	58.4	22.0	69.4	242.9	17.0
S4	2.1	8.5	0.67	24.8	2.4	7.1

*DTPA -extractable / total content

The content of Pb in the roots of safflower grown on contaminated soils (S1-S3) ranged from 142.8 mg/kg to 850 mg/kg, Zn from 436 mg/kg to 846.9 mg/kg and Cd from 19.3 mg/kg to 36.0 mg.kg. Safflower developed a thickened taproot that can extend down to 2-3 m, with a strong ability to uptake the nutrients. Considerably lower values were established in the in the roots of safflower grown on uncontaminated soil (S4). The content of Pb in the roots reached to 4.1 mg/kg, Zn 24.4 mg/kg and Cd 0.3 mg/kg.

The heavy metal contents in the stems of the safflower were considerably lower compared to those in the root system. The content of Pb in the stems of safflower grown on contaminated soils (S1-S3) ranged from 86.3 mg/kg to 454.3 mg/kg, Zn – from 207.5 mg/kg to 371.9 mg/kg and Cd – from 17.6 mg/kg to 64.3 mg/kg. Considerably lower values were established in the stems of safflower grown on uncontaminated soil (S4). The content of Pb in the stems reached to 0.74 mg/kg, Zn – 19.4 mg/kg and Cd – 0.18 mg/kg.

The highest accumulated levels of heavy metals were those of Pb, Zn and Cd in the leaves of safflower, where Pb ranged from 580.5 mg/kg to 5226.7 mg/kg, Zn – from 651.9 mg/kg to 2558.9 mg/kg, and Cd – from 148.1 mg/kg to 236.6 mg/kg. There were no signs of Cd toxicity despite high concentrations of cadmium in leaves. Considerably lower values were established in the leaves of safflower grown on uncontaminated soil (S4). The concentrations of heavy metals in the leaves were: Pb up to 5.2 mg/kg, Zn up to 47.6 mg/kg, and Cd up to 0.5 mg/kg.

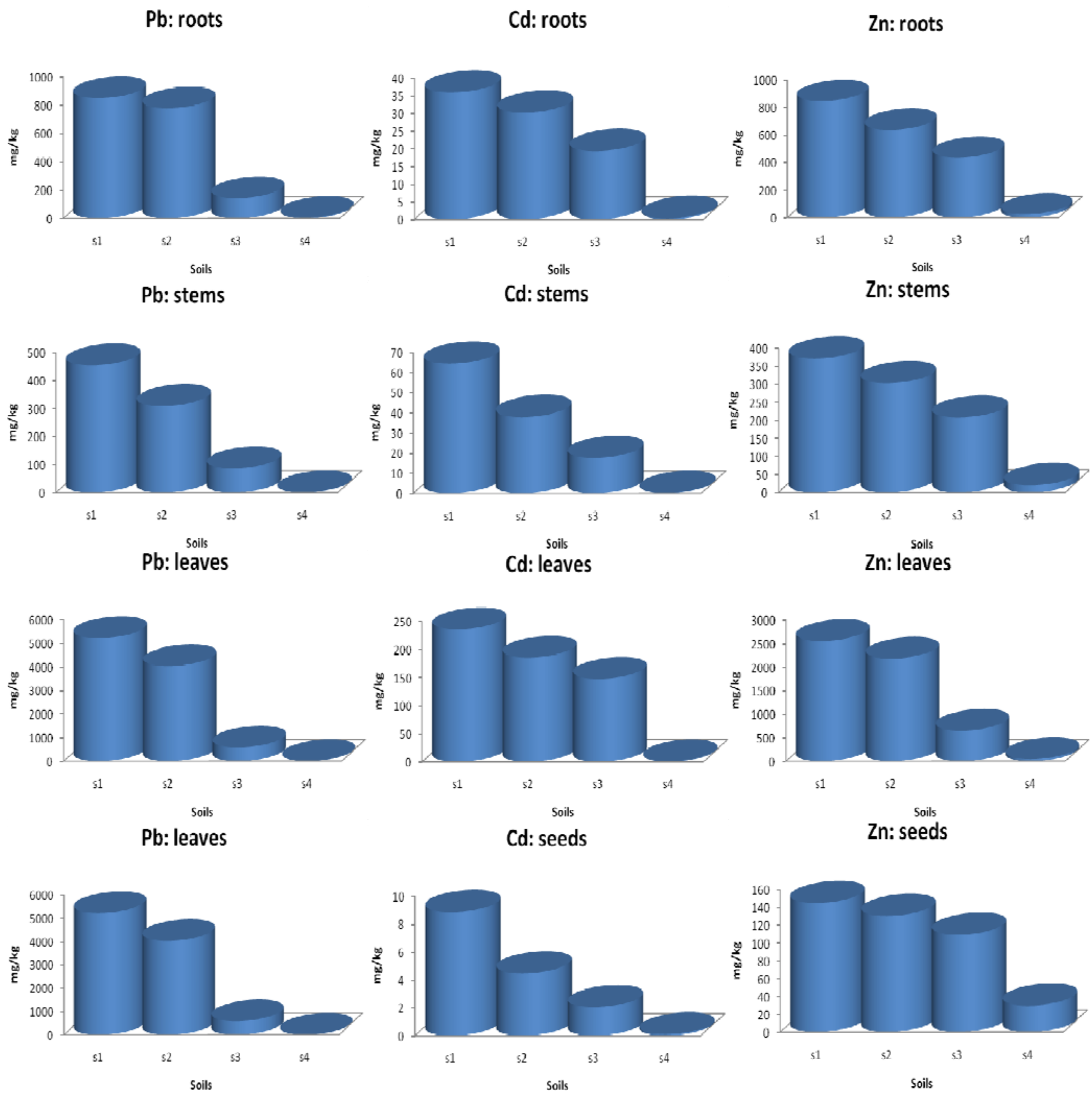

Fig. 1 Content of heavy metals in vegetative and reproductive organs of safflower

Their stronger accumulation in safflower grown on contaminated soils (S1-S3) probably due to the fact that the leaves of safflower were waxy, shiny, oval in shape and had spiny-edged leaves alternating around the stem, which contributed to the fixing of the aerosol pollutants and for their accumulation.

The content of Pb, Zn and Cd in leaves is higher compared to the root system, which shows absorption and movement of metal in the vascular system. According to [20], the safflower plant accumulates a moderate amount of heavy metals in its aboveground biomass. Our results do not confirm those found by [17], [18], and [20] that the content of cadmium is lower in the aboveground biomass compared to the roots, and Cd partially moves from the roots to the aboveground biomass. Symptoms of Cd toxicity are not observed in spite of the high concentrations of cadmium in the leaves.

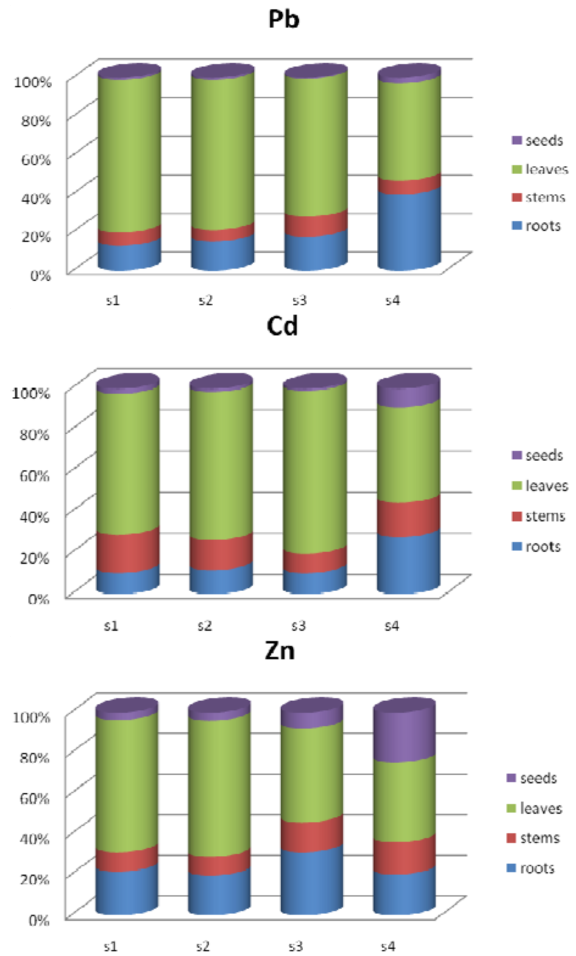

Fig. 2 Distribution of heavy metals in vegetative and reproductive organs of safflower

The ratio of the heavy metals in the above-ground mass and the roots of the safflower grown on the heavy metal polluted soils under field conditions is higher than 1, which means that the safflower is an accumulator of heavy metals and can be used for phytoextraction of heavy metals from the soils.

The heavy metal content in the seeds of the safflower was lower in comparison to that in the roots and leaves. The heavy metal accumulation in safflower seeds was likely caused by the conductive system. The content of Pb in the seeds of the safflower grown on contaminated soils (S1-S3) ranged from 3.6 to 68.9 mg/kg and exceeded the critical value of 30 mg/kg for Pb [21]. The content of Zn in the seeds of the safflower grown on contaminated soils (S1-S3) ranged from 109.6 to 145.3 mg/kg and did not reach the critical value of 300 mg/kg for Zn [21]. The content of Cd in the seeds of safflower grown on contaminated soils ranged from 2.0 to 8.87 mg/kg, which was significantly higher than the recommended maximum level tolerated by animals (0.5 mg Cd /kg) [22] and higher than the recommended level for food (1 mg/kg) [21].

Considerably lower values were established in the seeds of the safflower grown on uncontaminated soils - 0.297 mg/kg Pb, 29.8 mg/kg Zn and 0.098 mg/kg Cd.

The distribution of the heavy metals in the organs of the safflower has a selective character that in safflower decreases in the following order: leaves > roots> stems > seeds (Fig. 2).

C. Safflower Oil

Data illustrated in Table III showed the effect of soil contamination on heavy metal content in safflower oil, oil content of safflower plants, and fatty-acids composition. As shown in Table III, heavy metals content, oil content in seeds and fatty acid composition varied among safflower grown on contaminated and uncontaminated soils. The content of heavy metals in the oil is significantly lower compared with the seeds of safflower. Despite the high total content and DTPA-extractable quantities of metals in the rhizosphere of the safflower, their content in the oils are extremely low. The obtained results showed that the main part of the heavy metals contained in the seeds of safflower was not transferred in the oil during the seed processing, due to which their content in the oil was considerably lower. Lead in safflower oil obtained from safflower grown on contaminated soils (S1-S3) ranged from 0.21 to 0.89 mg/kg, Zn from 5.98 to 14.18 mg/kg, and the content of Cd is below the limits of detection of the apparatus. Although the contents of heavy metals in the oil was lower compared with the seeds, the quantities of Pb and Zn in the safflower oil, were higher than the accepted MPCs (0.1 mg/kg Pb and 10.0 mg/kg Zn).

Considerably lower values were established in the oil from safflower grown on uncontaminated soil (S4) and their content is significantly lower than the received MPC - 0.02 mg/kg Pb and 2.98 mg/kg Zn. The content of Cd is below the limits of detection of the apparatus.

Safflower oil quality is high due to its fatty acids composition [23]. As known, the fatty acid composition of vegetable oil is a main factor affecting on its commercial uses. Standard safflower oil contains about 5-8% palmitic acid, 2-3% stearic acid, 8-20% oleic acid, and 68-83% linoleic acid [24].

Safflower oil naturally contains the lowest levels of total saturated fatty acids among cultivated oil crops [25]. Oil quality is a significant concern of consumers, particularly for the contents of oleic and linoleic acids which are proven as healthy sources of oil for human body. Safflower is thought to be one of the highest quality vegetable oils and its oil consists of mainly palmitic, stearic, oleic and linoleic acids [26], [27]. Thus, we performed oil analyses to determine the content of aforesaid fatty acids in the present study. As shown in Table III, oil content in seeds and fatty acid composition varied among safflower grown on contaminated and uncontaminated soils. There are no significant difference between fatty acid composition of safflower grown on contaminated and uncontaminated soils ($P < 0.01$). In all tested oils the following prevail: linoleic, followed by oleic, palmitic, and stearic acids, and these fatty acids together comprise from 99.1 to 100.0 % of the total amount of fatty acids in all the analyzed oils (Table III).

TABLE III
CONTENT OF HEAVY METALS AND THE FATTY ACID COMPOSITION OF SAFFLOWER OIL (EXPRESSED AS % OF TOTAL FATTY ACID COMPOSITION)

	Contaminated soils (S1-S3)	Uncontaminated soil (S4)	Codex Standard
Pb, mg/kg	0.21-0.89	0.02	0.1
Cd, mg/kg	nd	nd	nd
Zn, mg/kg	5.98-14.2	2.98	10.0
Oil content, %	24.87-28.59	30.86	
Lauric acid C 12:0	0.09-0.10	Nd	nd
Palmitic acid C 16:0	6.04-6.83	5.89	5.3-8.0
Palmitoleic acid C 16:1	nd-0.08	nd	nd-0.2
Stearic acid C 18:0	1.73-1.91	2.35	1.9-2.9
Oleic acid C 18:1	14.03-14.93	12.46	8.4-21.3
Linoleic acid C 18:2	76.22-76.70	79.30	67.8-83.2
α- Linolenic C 18:3	nd-0.08	nd	nd-0.1
Arachidic acid C 20:0	0.28-0.33	nd	0.2-.4
Eicosenoic acid C 20:1	nd-0.15	nd	0.1-0.3
Erucic acid C 22:1	nd-0.21	nd	nd-.0
Saturated: Unsaturated	8.0-8.8:91.2-92.0	8.24:91.76	

nd- not detected

The mean stearic acid and palmitic acid in safflower oil from contaminated soils (S1-S3) ranged from 1.73 to 1.91% and 6.04 to 6.83%, respectively. Very low levels of lauric (nd-0.09%) and arachidic acids (0.28-0.32%) were recorded in oil from contaminated soils. Thus, the mean saturated fatty acid ratio was found to range from 8.0 to 8.8%. The safflower oil is high in linoleic acid. The oleic acid ratio (14.3-14.93%) was lower than the linoleic acid ratio (76.22-76.70%). Very low levels of C18:3 (nd-0.08%), C20:1 (nd-0.15%) and C22:1 (nd-0.21%) acids were recorded in safflower oil. Thus, the mean unsaturated fatty acid ratio was found to range from 91.2 to 92.0%.

Oil content of seeds is a very important economic trait for safflower cultivars and considered one of the most important factors affecting the success of safflower introduction in new areas [28]. Oil content is known to chance depending on factors like cultivar, soil characteristics and climate [29]. Oil content of safflower cultivars from different production areas of the word was reported as 23.86-40.33% [30], 26.72-35.78% [9], 26.3-28.5% [31] and 31.3-36.3% [32]. Evaluating our results of oil content measurements, it can be established that our results are in accordance with those of previous reports.

D.Safflower – Accumulator or Hyperaccumulator?

To be able to give a categorical answer to the question what are the abilities of the safflower to extract heavy metals from the soil and to assess the potential of safflower for phytoextraction, the translocation factor (TF=Cshoots/Croots) [33] and bioaccumulation factor (BF=Cshoots/Csoils) [34] were calculated. Our results show that the translocation factor for all metals is greater than 1 regardless of the degree of contamination of the soil (Table IV).

In terms of Pb the bioconcentration factor in safflower grown on contaminated soils (0.1 km, 0.5 km and 2.0 km from NFMW) ranged from 4.67 to 6.03, in Zn from 1.97 to 3.89, and in cadmium from 1.97 to 3.89. Both TF and BF coefficients are greater than 1 in the cultivation of the safflower on heavy metal polluted soils. It can be claimed that by increasing the level of contamination the potential of safflower to translocate and accumulate Cd, Pb and Zn in its above-ground parts increases as well. The safflower may be referred to the group of hyperaccumulators of Pb and Cd, and the accumulators of zinc, as the content of lead and Cd in the above-ground mass exceed 1000 mg/kg Pb and 100 mg/kg Cd, respectively. The above-ground mass of the safflower in its cultivation on heavy metal polluted soils accumulates Zn, but does not reach the values of 10,000 mg/kg for Zn so that the castor oil plant can be referred to the Zn hyperaccumulators.

TABLE IV
TRANSLOCATION (TF) AND BIOACCUMULATION (BF) FACTORS IN SAFFLOWER

Soils	Pb		Cd		Zn	
	TF	BF	TF	BF	TF	BF
S1	6.68	6.03	8.34	9.52	3.46	12.56
S2	5.58	5.03	7.41	8.06	3.90	6.55
S3	4.67	1.30	8.59	7.53	1.97	3.54
S4	1.45	2.83	2.27	1.01	2.75	27.92

Our results strongly suggest that safflower 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 cadmium and to the accumulators of zinc, and therefore, it can be successfully used for phytoremediation of soils contaminated with Pb and Cd. It is necessary to carry out further research in order to clarify the real potential of safflower when grown on larger areas because there is no practical experience from the cultivation of this crop in our country. Experts will need to offer additional management practices in order to increase the potential and efficiency of safflower for cleansing soil of heavy metal contaminants. The cultivation of the plant, planting, irrigation, pests and diseases, crop rotation, cycle duration and harvesting should be further studied. For now there is no experience in the cultivation of safflower in Bulgaria under field conditions. These issues need further clarification and a significant amount of research.

IV. CONCLUSION

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

1. A clearly distinguished species peculiarity existed in the accumulation of heavy metals in the vegetative and reproductive organs of safflower. The distribution of heavy metals in the organs of safflower decreases in the following order: leaves> stems> roots> seeds.
2. The main part of the heavy metals contained in the seeds, during their processing does not enter in oil, however their content in oil is significantly higher than the accepted MPC, and it cannot be used for food purposes.
3. Heavy metals do not affect the development of safflower as well as on the quality of the oil (oil content and fatty acids composition).
4. The safflower is a crop tolerant to heavy metals and can be grown on the highly heavy metal polluted soils. The

plants are characterized by a great capacity to absorb and accumulate Pb, Cd and Zn, but show no signs of toxicity (chlorosis and necrosis) in content of 50.1mg/kg Cd, 2428.6 mg/kg Pb and 2475.5 mg/kg Zn in the soil. It can be referred to the group of hyperaccumulators of Pb and Cd and the accumulators of zinc and can be successfully used for phytoremediation of heavy metal polluted soils. The processing of the seeds to oil and the use of the obtained oil will significantly reduce the cost for phytoremediation.

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